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How Both Hormonal Factors and Social Experience can Affect Emotional Processing
By Zi Lan Alice Chen

Abstract

The social distinction between sexes has been a common topic of interest and debate among civilizations for centuries. Because of this, developments in women's health have not been as consistent as developments in men's health have been. It was only recently that female risk-factors for cardiovascular diseases were considered, when for decades cardiovascular research had been based on the male anatomy and hormonal levels. Thus, it is important to consider how factors that affect personality and cognitive functioning affect the male and female brain differently. The aging process has been shown to affect men and women differently because of their sexual dimorphism and their hormonal differences, and has been shown to increase the rate of decline in women's cognitive functioning more than the rate of decline in men's cognitive functioning. Specifically due to the differences in their ability to produce sex gonadal hormones such as estrogen, progesterone, and testosterone that affect their unique neurochemistry. However, there are also social factors to consider when comparing how men and women process psychological and cognitive functioning. Due to women's specific neurochemistry and their unique social experience, they might adapt to factors such as processing childhood trauma in group therapy sessions better than men would.

Introduction

Traumatic experiences can often result in severe, long-term chronic disabilities, secondary pathological conditions, and psychiatric changes that can cause cognitive decline and personality changes. Childhood trauma, in specific, is a form of psychological trauma experienced by many, and can lead to lasting cognitive impacts on people far into their adulthood

However, the conversation around how men and women experience and process childhood trauma and trauma in general is nuanced because they have very different neurochemistries. One of the major underlying reasons for the difference in sexes are the ability to produce certain sex gonadal hormones. These are defined as hormones that create a unique and "different endocrine environment" (Lauretta, R., Sansone, M., Sansone, A., Romanelli, F., & Appetecchia, M., 2018). The specific 3 hormones that cause these environments are estrogen and progesterone, which females produce more than males, and testosterone, which males produce more of. These hormones control the body's ability to regulate temperature, control reproductive ability, and can affect how the body responds to mechanisms related to aging. To better understand the discrepancy that exists between the effects of aging on women and men, an analysis of how both sexes experience and adapt to traumatic events and injury will be performed. Gerontological research has so far explored how the deterioration of certain mechanisms affect men and women differently, but there is a lack of evidence on how that can affect their adaptability to traumatic experiences, events, or injuries.

Because of the difference in production of sex hormones, experience in aging, and rate of cognitive decline that men and women experience, previous research can help explain this

knowledge gap. Especially because the long-term effects of physical trauma can often be amplified with the progression of age as they include a heightened vulnerability to neurodegenerative diseases that are common amongst older populations. Specifically, conditions such as Alzheimer's disease, Parkinson's syndrome, and CTE are often cited to be overlapping conditions in older patients and those who have experienced a severe TBI (Jost, Z., & Kujach, S., 2025). Because of differences in production of sex gonadal hormones between male and females, factors that affect personality and cognitive functioning such as traumatic experiences more dramatically affect women than they do men.

Furthermore, from an epigenetic standpoint, women and men experience drastically different social environments and respond differently to them because of this. The history of how men and women have been able to respond to changes in their social environments, often linked with psychological changes, may also affect how they respond to external stimuli and traumatic experiences. Thus, to properly examine the differences in response to stimuli, the socio-cultural perspective must be studied (Hägg, S., & Jylhävä, J., 2021). In this study, an examination of just how differently men and women experience and respond to external stimuli and traumatic events will be performed. To fully understand how men and women adapt to psychological trauma best, the biopsychosocial model should be used. This model was introduced by Dr. George Engel in 1977, as a means to remedy the knowledge deficit left by the previously popular biomedical model (Bolton D., 2023). This model introduces how patients experience pain or recovery through a biological standpoint, a psychological standpoint, and a social standpoint. Because of the discrepancy in men and women's social behaviors, this is the model in which men and women's response to psychological trauma should be analyzed.

The theories of aging and how aging affects cognition

There are many theories for why humans age, and what happens during the aging process. The two main biological theories include the programmed theory of aging and the senescent theory of aging (Hägg, S., & Jylhävä, J., 2021). Both theories attempt to explain on a cellular level why aging occurs. The programmed theory describes the aging process as the process in which the body's biological clock, or hypothalamic clock controls the body's growth and development (Hägg, S., & Jylhävä, J., 2021). The senescent theory of aging describes that the accumulation of errors, drift, and damages to certain cells is behind how the body ages (Hägg, S., & Jylhävä, J., 2021). Due to these theories, the aging process takes a physical toll on the human brain. Aging is consistent with cognitive decline in humans, as with the deterioration of certain neuromechanisms, the brain is more susceptible to experiencing conditions such as Alzheimer's disease and Parkinson's disease (Bishop, N. A., Lu, T., & Yankner, B. A., 2010).

As humans age, the brain shows more localization in the brain regions when performing certain higher-order tasks such as learning and memory; previous studies have shown that individuals who have higher cognitive functioning exhibit more delocalized brain activity than those who have lower or regressing cognitive functioning. This study highlights that both diseases and the aging process can significantly damage the delicate circuitry of the brain's

physiology (Bishop, N. A., Lu, T., & Yankner, B. A., 2010). The 3 main causes behind the localization of brain functioning are: the brain regions that interact to perform higher-order functioning tasks become less-coordinated with a large loss of sensory input and analysis known as integrative function, the degeneration of cognitive regions, and neural activity being less localized in the prefrontal cortex, a region responsible for the execution of executive functions such as focus and impulse control (Bishop, N. A., Lu, T., & Yankner, B. A., 2010). Cortical regions, which are the distinct regions of the brain that control sensory input, motor activity, and language ability, are also physically affected by the aging process. The myelinated fibers that connect neurons between these regions tend to physically deteriorate with disruptions in mechanisms that would usually repair and maintain their function (Bishop, N. A., Lu, T., & Yankner, B. A., 2010).

Genetics also play a role in the aging process. Genetically linked mitochondrial dysfunction can cause accelerated aging due to disruptions in the electron transport chain in the cell membranes of mammals, that strongly affect brain and muscle function (Bishop, N. A., Lu, T., & Yankner, B. A., 2010). While the disruption of mitochondrial function can rapidly encourage cellular deterioration, men who receive half of their genetic information from their mother are more susceptible to sex-chromosome defects (Hägg, S., & Jylhävä, J., 2021).

How women's cognitive function is affected by internal and external stimuli

The discrepancy between the neuroanatomy and neurochemistry of men and women can be explained by the difference in production of sex gonadal hormones (Lauretta, R., Sansone, M., Sansone, A., Romanelli, F., & Appetecchia, M., 2018). Women produce larger amounts of estrogen from the gonads of their pituitary glands, leading to a difference in how they regulate their central processes. While this may manifest in more phenotypic differences such as higher BMIs, wider hips, and other adiposity traits, they are credited for impacting the cognitive function of women as they grow older (Lauretta, R., Sansone, M., Sansone, A., Romanelli, F., & Appetecchia, M., 2018). Estradiol, a specific kind of estrogen, is what ultimately regulates cognitive processes such as cell migration patterns and even neurogenesis (Lauretta, R., Sansone, M., Sansone, A., Romanelli, F., & Appetecchia, M., 2018). Because these processes occur in the hypothalamus and the corpus callosum of the brain, estradiol is partially credited for the unique neurochemistry of the female brain.

The differences in production of sex hormones are related to how men and women experience the aging process in many ways, but when considering the impact of their lifespan, hormones are specifically important. Because the experience of metabolic processes are linked with the presence of certain hormones, life expectancy in women can actually be linked to their reproductive lifespan. A reproductive lifespan is determined by the estrogen exposure a woman has. With a shorter estrogen exposure and reproductive lifespan, women in their 60s are known to experience more cardiovascular diseases and symptoms. This was shown in a study where both women who experience early menopause, and late menopause's health records were shown to contain more frequent cardiovascular problems. This is significant because a disrupted

experience in the menopause mechanism is associated with disruptions in hormone production. However, because of the constant fluctuations of hormones during the aging process, women have been shown to actually process physical changes, and withstand neurological trauma better than men.

In a study conducted to observe how women respond and adapt to emotionally and physically traumatic events, women have been shown to respond much differently than men (Stige, S. H., Binder, P. E., Rosenvinge, J. H., & Træen, B., 2013). When they learn their symptoms of trauma, they seem more prone to emotional openness and are more in-tune with their emotions and their bodies (Stige, S. H., Binder, P. E., Rosenvinge, J. H., & Træen, B., 2013). Furthermore, they experience a higher sense of agency over their own experiences going forward (Stige, S. H., Binder, P. E., Rosenvinge, J. H., & Træen, B., 2013).. This may be due to the social and epigenetic factors of womanhood. In social settings women are more likely to exhibit emotional openness because it is expected of women, especially when considering female dominated fields such as psychology and therapy (Stige, S. H., Binder, P. E., Rosenvinge, J. H., & Træen, B., 2013).. Thus, it can be inferred that how women respond to traumatic events can be a product of both genetic and epigenetic factors.

How men's cognitive function is affected by internal and external stimuli

Men produce higher amounts of testosterone than women do, though similarly by the leydig cells in their pituitary glands. In men, the estradiol that affects their cognitive functioning is differently sourced than those in women because they are converted from testosterone (Lauretta, R., Sansone, M., Sansone, A., Romanelli, F., & Appetecchia, M., 2018). The aromatase enzyme synthesizes testosterone into estradiol that is similarly responsible for certain cognitive processes in the hypothalamus and corpus callosum of the male brain (Lauretta, R., Sansone, M., Sansone, A., Romanelli, F., & Appetecchia, M., 2018). However, their production of hormones differently impacts how they experience the aging process on a physiological level than women do.

Unlike women, high levels of endogenous testosterone levels are directly correlated with lower risk of cardiovascular diseases as a man gets older. This is likely due to the absence of hormone fluctuations that women experience due to the menstrual cycle and eventually the menopausal period. However, because of this, men are shown to be more susceptible to lasting effects of neurological damage than women are.

In a study conducted on how men process and recover from both physically and emotionally traumatic experiences, their reactions and symptoms were entirely opposite to the traits that their female counterparts exhibited (Røberg, L., Nilsen, L., & Røssberg, J. I., 2018). They were noted to show very little emotional openness towards the female therapists and their male peers in their experiment, and instead were shown flirting with their female therapists as a way to connect instead (Røberg, L., Nilsen, L., & Røssberg, J. I., 2018). Furthermore, the cultural implications of male-victimization could have impacted how they responded to the tests and treatments they were given (Røberg, L., Nilsen, L., & Røssberg, J. I., 2018). Men in the

study were shown to experience performance anxiety when answering questions during psychological tests because of the overarching presence of cultural shame. Again, men may experience internal and external stimuli differently than women due to both genetic and epigenetic factors such as cultural norms. The male-vicitimization cultural norm may suggest that men are less likely to share emotional openness with their peers because of the patriarchal view of masculinity. While many view masculinity as a performance, it clearly has real impacts on men who still try to perform it in times where they should instead prioritize psychological treatment.

Thus, men's unique social experience with how masculinity is often presented may impact their ability to effectively process trauma in group therapeutic sessions, or even be open about their display of alexithymia: trouble identifying and processing their own emotions.

Conclusion and discussion

Men and women experience and process both physical and emotional trauma differently. This is due to both genetic and epigenetic factors. Because of their differences in hormone production, how they experience the aging process, and the preexisting cultural expectations they experience, their response to psychological and social treatment vary dramatically. The biopsychosocial model thus explains how due to the combination of men and women's unique neurochemistry and social experiences, they both process emotional traumas differently.

In both studies, the discrepancy is made abundant with the quantitative and qualitative data collected by the researchers. Although the two studies used different methods of data collection and sample sizes, the data collected from both studies were both quantitative and qualitative. In the study regarding women's emotional processing, the hermeneutical-phenomenological approach was used, and women's in-depth interviews were collected and analyzed against their response in group therapy. In the study regarding men's emotional processing, the qualitative explorative approach was used, in which more structured interviews were conducted and analyzed against the men's therapy benchmarks.

This thus introduces the idea that women generally respond to therapy regarding processing deeply rooted psychological, childhood trauma better than men are able to, potentially due to their unique neurochemistry, and social experience. It should be noted, however, that future research on the importance of the gender of the therapists and sexual orientation of the participants may factor into consideration on how effective group therapy is, as performance anxiety may skew the results. Overall, however, it is important to note that the social construct of gender and the performance of it, may be damaging to both psychological, and physical well-being.

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Works Cited

Al-Shargie, F., Taresh, S. M., & Al-Ezzi, A. (2024). Mental stress and cognitive deficits management. Brain Sciences, 14(4), 316. https://doi.org/10.3390/brainsci14040316

Bishop, N. A., Lu, T., & Yankner, B. A. (2010). Neural mechanisms of aging and cognitive decline. Nature, 464(7288), 529–535. https://doi.org/10.1038/nature08983

Bramlett, H. M., & Dietrich, W. D. (2015). Long-Term consequences of traumatic brain injury: Current status of potential mechanisms of injury and neurological outcomes. Journal of Neurotrauma, 32(23), 1834-1848. https://doi.org/10.1089/neu.2014.3352

Charles, S. T., Turiano, N. A., & Almeida, D. M. (2016). Personality and stressor-related affect. Journal of Personality and Social Psychology, 111(6), 917-928. https://doi.org/10.1037/pspp0000083

DeYoung, C. G., Beaty, R. E., Genç, E., Latzman, R. D., Passamonti, L., Servaas, M. N., Shackman, A. J., Smillie, L. D., Spreng, R. N., Viding, E., & Wacker, J. (2022). Personality neuroscience: An emerging field with bright prospects. Personality Science, 3(1). https://doi.org/10.5964/ps.7269

Donnellan, M. B., Blonigen, D. M., Krueger, R. F., McGue, M., Iacono, W. G., & Burt, S. A. (2011). Genetic and environmental influences on personality trait stability and growth during the transition to adulthood: A three-wave longitudinal study. Journal of Personality and Social Psychology, 100(3), 545-556. https://doi.org/10.1037/a0022409

Gray, J. C., MacKillop, J., Chen, C., & Palmer, A. A. (2017). The genetics of human personality. Genes, Brain and Behavior, 17(3). https://doi.org/10.1111/gbb.12439

Hägg, S., & Jylhävä, J. (2021). Sex differences in biological aging with a focus on human studies. ELife, 10. https://doi.org/10.7554/elife.63425

Jirout, J., LoCasale-Crouch, J., Turnbull, K., Gu, Y., Cubides, M., Garzione, S., Evans, T. M., Weltman, A. L., & Kranz, S. (2019). How lifestyle factors affect cognitive and executive function and the ability to learn in children. Nutrients, 11(8), 1953. https://doi.org/10.3390/nu11081953

Jost, Z., & Kujach, S. (2025). Understanding cognitive decline in aging: Mechanisms and mitigation strategies – A narrative review. Clinical Interventions in Aging, Volume 20, 459-469. https://doi.org/10.2147/cia.s510670

Kang, W., Steffens, F., Pineda, S., Widuch, K., & Malvaso, A. (2023). Personality traits and dimensions of mental health. Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-33996-1

Lauretta, R., Sansone, M., Sansone, A., Romanelli, F., & Appetecchia, M. (2018). Gender in Endocrine Diseases: Role of Sex Gonadal Hormones. International journal of endocrinology, 2018, 4847376. https://doi.org/10.1155/2018/4847376

Røberg, L., Nilsen, L., & Røssberg, J. I. (2018). How do men with severe sexual and physical childhood traumatization experience trauma-stabilizing group treatment? A qualitative study. European journal of psychotraumatology, 9(1), 1541697. https://doi.org/10.1080/20008198.2018.1541697

Stige, S. H., Binder, P. E., Rosenvinge, J. H., & Træen, B. (2013). Stories from the road of recovery - How adult, female survivors of childhood trauma experience ways to positive change. Nordic psychology, 65(1), 3–18. https://doi.org/10.1080/19012276.2013.796083

Zhang, Y., Tang, Y., & Yang, Y. (2019). Brain differences between men and women: Evidence from deep learning. Frontiers in Neuroscience, 13. https://doi.org/10.3389/fnins.2019.00185

To What Extent do the Technical Challenges of CRISPR- Cas9 Limit Its Viability As A Mainstream Cancer Therapy? By Ada Runyi Ma[1]

Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR)- associated protein 9 (CRISPR-Cas9) was initially discovered in prokaryotes. CRISPR-Cas system is the most advanced gene-based biomedical engineering tool, which is applied in medical treatments such as cancer therapy.

The usage of CRISPR system has become mature in cancer treatments, and the limitations are moderate. CRISPR system is widely used as a primary treatment, and is applied in therapeutic immune cells, such as construction of chimeric antigen receptor T (CAR-T) cell-based immunotherapy and programmed cell death protein 1 knockout. It is able for CRISPR/Cas9 to achieve gain or loss-of-function mutations and help to target causative genes in multiple cancers, such as brain cancer, renal cell carcinoma, and hepatocellular carcinoma. Several types of clinical trials have been performed to examine the security and effectiveness of CRISPR/cas9 on refractory cancer. This review critically evaluates the mechanism, and the application of CRIPSR-Cas system for cancer treatment, the extent to which these technical barriers limit clinical translation.

CRISPR/Cas9 gene editing is usually promising, which opens the door for additional testing to ensure that genetic editing does not lead to any inadvertent changes. However, its transition from a powerful research tool to a mainstream cancer therapy still has interrelated technical challenges.

Keywords CRISPR-Cas9 system, therapeutic treatments, cancer, biomedical engineering tool, challenges.

Introduction

The clustered regularly interspaced short palindromic repeats (CRISPR)- associated protein 9 (CRISPR-Cas9) genome editing has fundamentally altered the landscape of cancer research and therapeutic. Unlike conventional treatments that exert broad cytotoxicity, CRISPR-based offers molecular specificity, enabling direct targeting of oncogenes, tumor suppressors, and regulatory elements implicated in cancer progression. The technology has demonstrated remarkable preclinical efficacy. It is essential for scientists to embrace new technologies that results in more accurate data, faster discoveries, and better patient outcomes. As a vital research tool for creating animal models, CRISPR offers potential cures and has broad applications beyond cancer. The scientists are keen on applying such technologies to improve life qualities of patients.

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The technology has demonstrated remarkable preclinical efficacy: adenoviral delivery of SpCas9 targeting the KRAS G12S mutation achieved a 46% reduction in tumor volume in lung cancer xenografts.[1] However, the clinical adoption of CRISPR-Cas9 remains limited. As of 2026, only a handful of early-phase trials have been completed, predominantly in ex vivo T cell engineering contexts.[2] This review examines the mechanism, and the application of CRIPSR-Cas system for cancer treatment, the extent to which these technical barriers limit clinical translation.

The mechanism of the CRISPR-Cas system

CRISPR, which stands for "Clusters of Regularly Inter-spaced Palindromic Repeats" refers to regular and repetitive nucleotide sequences that are universally present in bacterial DNA. Asmamaw, M and Zawdie, B (2021) describe CRISPR/Cas-9 system in National Library of Medicine, Mechanism and Applications of CRISPR/ Cas-9-Mediated Genome editing, as "divided into Class1(type1,2,3, roman numerals) and Class2 (type 4,5,6,roman numerals).[3] It was found out to be the microorganism's defense system for fighting against viral invasion, but it can be applied to cancer treatments in patients. Bacteriophages kill bacteria primarily through the lytic cycle. When the phage infects a bacterium equipped with a CRISPR-Cas system, a new sequence can be added to the CRISPR array. In this process, two Cas proteins called Cas1 and Cas2 proteins work together to capture segments of injected phage DNA. After that, the Cas1-Cas2 complex acts as a ruler and hold onto the phage DNA and inserts it into one end of the CRISPR array as a new spacer (a piece of foreign DNA chopped off by the bacterium).[4] The bacterium thereby stores a memory of the phage. This phage DNA is converted into RNA in CRISPR RNA biogenesis, and the whole CRISPR array of repeats and spacers is transcribed into a precursor CRISPR RNA (pre-crRNA). Cas proteins then cut the long RNA into shorter segments containing one spacer and parts of the repeats. The final trimmed RNA copies of the phage memories are called CRISPR RNAs. The Cas proteins will use each crRNA to hunt for matching phage DNA. As a result, when a phage injects its DNA into the cell, the surveillance complex searches for sequence that is complementary to the crRNA. After that, the surveillance complex cuts the DNA strand, which leads to the breakdown of the rest of the proteins.

According to the illustration shown in Figure 1, a single guide RNA detects Cas9 endonuclease to a specific region of the genomic DNA, resulting in a double-strand break. By providing a donor DNA in trans, a transgenic DNA can be created, and the double strand break will be replaced by the host cell, resulting in the insertion or deletion of a sequence of bases, thus disrupting the original reading frame of a gene. Similarly, in cancer patients, single guide RNAs (also called cas9 nuclease) encode a guide DNA and double-stranded breaks at a particular location of the DNA sequence. The binding of the Cas9 nuclease and a target DNA occurs, causing the double strand to break. One advantage of using CRISPR is that it is able to target multiple DNA sites at a time through multiplexing. This allows scientists to edit genes efficiently in mammalian cells effieciently. Examples will be given in the next section.

CRISPR system not only eliminates or edits the genes of affected cells, but also inactivates the oncogenes which leads to malignant gene proliferation by using a guide DNA to

direct the Cas protein to the normal gene. First of all, the Cas9 and Cas12a protein is sent into the cell dividing abnormally via vectors. Followed by that, the guide DNA finds the abnormal sequence, so that the enzyme separates the strand, forming a double strand break. In order to fix the DNA strand, the cell inserts more random DNA bases, which permanently destroys the oncogene.

The Application of CRIPSR-Cas system for cancer treatment

CRISPR system is responsible for genetic editing of cells that deal with killing cancer cells in the laboratory. CAR-T cells, changed in the laboratory, are responsible for recognizing and killing cancer cells with special receptors. Yet, cancer patients only have CAR-T cells in their body only after receiving CAR-T cell therapy.[5] T cells store memories of past diseases so that the immune system is able to react faster in the future. However, sometimes T cells don't recognize cancer cells accurately, a phenomenon also known as T cell exhaustion. This is when scientists add modified genes to the CAR-T cells that target specific cancer markers, so that the recognition is more precise. Inhibitory receptors such as PD-1, CTLA-4, LAG3, and TIM3 have their ligands bound which leads to inhibition of cell activation and the decrease in the release of cytotoxic cytokines.[6] The chemical substance released by T cells is responsible for the killing of abnormal cells, so cell exhaustion would lead to the uncontrollable division of cancer cells. However, the disruption of such inhibitory receptors by using the CRISPR system can enhance its resistance as well as tumor control. Thus, Cas9 mediated PD-1 disruption could suppress CAR-T cell exhaustion, and enhance cancer patients' immune system.[7] Overall, the entire process of extracting the white blood cells from the patients, to genetic engineering, to inserting them back to the patient's body takes about three to five weeks. Patients with transferred T cells often show stronger antileukemic function, depicting the effectiveness of the therapy.

Here are some other examples of application of the CRISPR system in treating different types of cancers. For instance, scientists see the CRISPR system as a possible method to cure Hepatitis B virus, which results in hepatocellular carcinoma. The proteins encoded in its genome, HBV protein X, is believed to be responsible for liver cancer.[8] Chronic Hepatitis B involves episomal persistent DNA, also known as cccDNA. Consequently, CRISPR can be applied to the treatment of eradication of cccDNA from infected cells by causing double-stranded breaks. Other types of cancers such as nasopharyngeal cancer caused by Epstein Virus that affect cellular transformation can be targeted by CRISPR-Cas9 without any off-target cleavage.

By knocking out certain oncogenic mutants, the CRISPR system is able to inhibit the proliferation of cancer cells. The epidermal growth factor receptor, also known as EGFR, is responsible for the development of lung cancer. Yet, Cas9 is able to knock out the oncogenic EGFR allele.[9] CTNND2 gene, responsible for the production of delta-catenin protein which helps cell stick together and disrupts E-cadherin based adherens junctions (ensures intercellular adhesions upon epithelial cells, essential for the maintenance of organ and tissue structure), can result in cancers. Yet, CRISPR system is able to knock out the CTNND2 gene, stating its ability to reduce abnormal cell proliferation.[10]

Beside treatment, the CRISPR system is also widely applied to diagnosis. Here, both protein Cas 12 and protein Cas 13 play an important role in developing CRISPR-based molecular diagnostics（Figure 2）. There are multiple Cas13 that lack photospacer flanking sites, which means that there aren't any nucleotides adjacent to the tRNA that help guide DNA target specific DNA sequences. As a result, some Cas 13 can target any random mutate sequences without specific nucleotide motif next to the target sequence. This allows immediate recognition of mutated DNA sequences during early diagnosis of abnormal cells.

The two systems DETECTOR and SHERLOCK involve CRISPR for diagnosis of cancer by helping us to identify tumor DNA or cancer-related viruses.[11] DETECTOR, shortform of DNA endonuclease-targeted CRISPR trans reporter, can be applied to detect any oncogenes by targeting mutated sequences with the help of the Cas12a protein, which has a low sensitivity. After Cas12a reacts with complementary target crRNA, its collateral activity would be turned on which destroys DNA probes. Similarly, SHERLOCK is able to detect any target nucleic acids with the help of Cas13 (doesn't require accurate order bias at target cells, which indicates a wider range but lower sensitivity) proteins and Csm6 (type 3 CRISPR effector nuclease), which recognizes and cleaves RNA. Both systems involve fluorophores which show fluorescent signals, indicating the amount of target in the sample.[12] Moreover, SHERLOCKv2 can detect cancer mutations with single-base mismatch sensitivity and enable live cell RNA tracking, which helps visualize RNA while providing profiles of patients. Despite their low sensitivity, the fact that there is an alternative to detect oncogenes is a great step into prevention during the early stage of treatment of cancer.

Advantages and limitations of the CRISPR system

In the last few decades, researchers have been trying to apply gene-editing technology to treat the defective gene. The CRISPR-cas9 system consists of the Cas9 protein and guide, RNA(gRNA). While the gRNA determines the specific sections that need to be removed, the Cas9 protein acts as a "scissors" that cuts and isolates the section of DNA. Therefore scientists can alter the sequence of an organism's DNA via such technology to produce certain types of proteins they want.

What are the advantages of this system? CRISPR is widely applied to medical treatments due to its simplicity, efficiency, as well as accuracy. It is said that CRISPR is able to " edit virtually any segment of DNA within the 3 billion letters of the human genome, and it's more precise than other DNA-editing tools." By NCI Staff, July 27, 2020. For instance, scientists can produce mutant mice via direct zygote injections of Cas9, which does not require much preparation(Brendan Doe, Ellen Brown, Katharina Boroviak,2018). Secondly, it allows the rearrangements of sequence of the bases. For instant, scientists used CRISPR system to engineer a specific chromosomal translocation in an adult mouse vivo in order to induce cleavage of murine endogenous Eml4 for gene rearrangement.[13] CRISPR system, acting as a lentiviral vectors, induced such arrangement in the lung cells of the mice, causing them to develop Eml4-Alk arranged lung tumors two months after the inoculation. This allows the study of such

tumor cells in mice viva rather than in human bodies. As a result, it depicts the fact that Cas9 system is a simple and effective method for rearrangement of chromosomes in vivo. Such somatic chromosomal rearrangement can be applied in other mammals as well, involving in the deletion of chromosome regions. When it comes to multi-gene disorders, CRISPR is much smaller in size compared to other delivery tools, which makes it much more convenient to target a specific region.

One major concern for implementing CRISPR for gene therapy is the relatively high risk of off-target effects, with a frequency of greater than 50%. One strategy of reducing such risks is to exhibit improvements in on-target specificity. This utilizes Cas9 nickase, which induces single-strand breaks, targeting both strands of the DNA at the intended location to produce a double-strand break. SpCas9-HF1 is a widely recognized variant of the Streptococcus pyogenes Cas9 protein which minimizes off-target effects.[14] Its ability to render all off-target editing events indicates its high precision. Compared to other common Cas9 nucleases, the off-target efficiency is exceptionally low. Moreover, technical advancements such as high throughput genome -wide next-generation sequencing (NGS) can also play a significant role in minimizing off-target effects. NGS is a massively parallel laboratory technology that sequences millions of DNA or RNA fragments and is able to map the entire genome with extraordinarily high accuracy and speed. It is able to map protein-binding sites and detect rare genetic orders, which further reduces the off-target risks.

Other challenges include risks in delivering CRISPR-Cas through viral vectors into cells. Due to the low loading and packaging efficiency of adeno-associated viruses, risk of carcinogens, immunogenicity, and narrow host range, systematic delivery have limited practical applications. Components of the CRISPR-Cas system are often delivered through lentiviral vectors which show less impact on transduced cells. Its ability to carry large molecules and sustain a prolonged expression in cells while able to minimize its toxicity and pathogenicity to a significant extent makes it a choice for the transformation of CRISPR systems into cells. Functional lentiviruses often integrate without active protein integrase enzymes in its life cycle, and it reduces the possibility of activating the host cell by accident. However, this prolonged expression usually leads to off-target effects and increased chances of mismatches in the guide-matching region. To mitigate such issues, scientists have come up with ideas of using non-viral vectors such as nanoparticles, which are cost-effective, ease of chemical modification, and have larger packaging capacity. Most important of all, it has better protection over the CRISPR-Cas9 genome editing system in vivo.

Conclusion

CRISPR-Cas9 demonstrates broad capability across a spectrum of oncological therapeutic strategies. Although the technical challenges of CRISPR-Cas9—delivery inefficiency, off-target editing, and immunogenicity—mainly limit its use as a mainstream cancer therapy at present. This technology has demonstrated remarkable potential in preclinical models and early-phase trials, the transition from bench to bedside remains incomplete.

CRISPR-Cas9 is best understood not as a current therapy but as an evolving therapeutic platform for cancers. Its eventual clinical impact will depend on translational barriers. The technology is likely to achieve mainstream status in ex vivo engineered cell therapies, with in vivo tumor editing remaining a longer-term prospect.

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Works Cited

1.Gao QQ, Ouyang WJ, Kang B, et al. Selective targeting of the oncogenic KRAS G12S mutant allele by CRISPR/Cas9 induces efficient tumor regression. Theranostics. 2020;6;10(11):5137-5153.

2.Kim JH, Cho HJ, Lee HM. Leveraging CRISPR/Cas9 for optimized adoptive T cell therapies: From molecular engineering to clinical manufacturing. Biochem Biophys Res Commun. 2026;13:826:154003.

3.Asmamaw M, Zawdie B. Mechanism and Applications of CRISPR/ Cas- 9 - Mediated Genome Editing. Biologics. 2021; 21:15:353-361.

4.Hofmann R, Herman C, Mo CY, et al. Deep mutational scanning identifies Cas 1 and Cas 2 variants that enhance type II-A CRISPR - Cas spacer acquisition.Nat Commun. 2025;1;16(1): 5730.

5.Allehyani N, Alissa M, Alghamdi A, et al.CRISPR-Engineered CAR-T Cell Therapy for Epstein-Barr Virus-Associated Nasopharyngeal Carcinoma: A Review of Emerging Therapeutic Prospects.Rev Med Virol. 2026;36(5):e70199.

6.Hsu CY, Abdelgawwad El-Sehrawy AAM, Alshkarchy SS, et al. Innovative approaches in the treatment of hematologic malignancies: the role of CRISPR-engineered microbiomes along the gut-immune axis in immunotherapy development. Cancer Cell Int. 2026;27;26(1):228.

7.Mohammad SI, Kareem AK, Vasudevan A et al. Genome editing of immune checkpoints: CRISPR-mediated PD-1 inhibition in cancer. Semin Oncol. 2026;53(1):152438.

8.Peng C, Lu M, Yang D.CRISPR/Cas9-based tools for targeted genome editing and replication control of HBV. Virol Sin. 2015;30(5):317-25.

9.Koo T, Yoon AR, Cho HY, et al. Selective disruption of an oncogenic mutant allele by CRISPR/Cas9 induces efficient tumor regression. Nucleic Acids Res. 2017;27;45(13):7897-7908.

10.Vaz R, Edwards S, Dueñas-Rey A, et al. Loss of ctnnd2b affects neuronal differentiation and behavior in zebrafish. Front Neurosci. 2023;3;17:1205653.

11.Mustafa M, Makhawi A. SHERLOCK and DETECTR: CRISPR-Cas Systems as Potential Rapid Diagnostic Tools for Emerging Infectious Diseases. J Clin Microbiol. 2021;18;59(3):e00745-20.

12.Ramadan NK, Gaber N, Ali NM, et al. SHERLOCK, a novel CRISPR-Cas13a-based assay for detection of infectious bursal disease virus. J Virol Methods. 2025;337:115185.

13.Peng R, Zhang R, Lin G, et al. CRISPR/Cas9 Technology-Based Xenograft Tumors as Candidate Reference Materials for Multiple EML4-ALK Rearrangements Testing. J Mol Diagn. 2017;19(5):766-775.

14.Chen JS, Dagdas YS, Kleinstiver BP, et al. Enhanced proofreading governs CRISPR-Cas9 targeting accuracy. Nature. 2017;19;550(7676):407-410.

Figure 1. reprinted with permission from Innovative Genome Intuition et al.

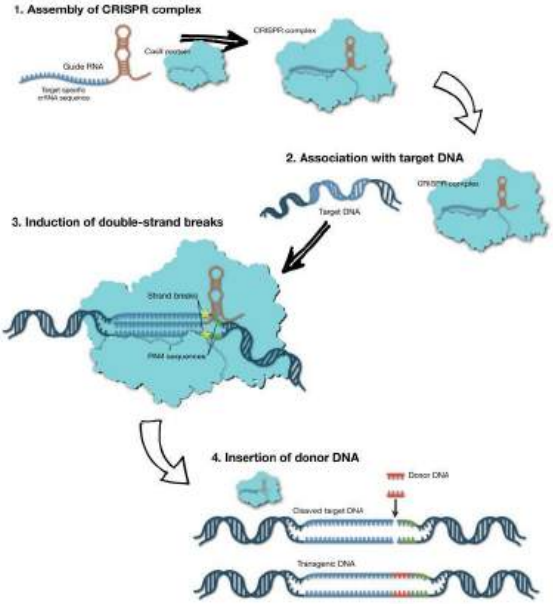
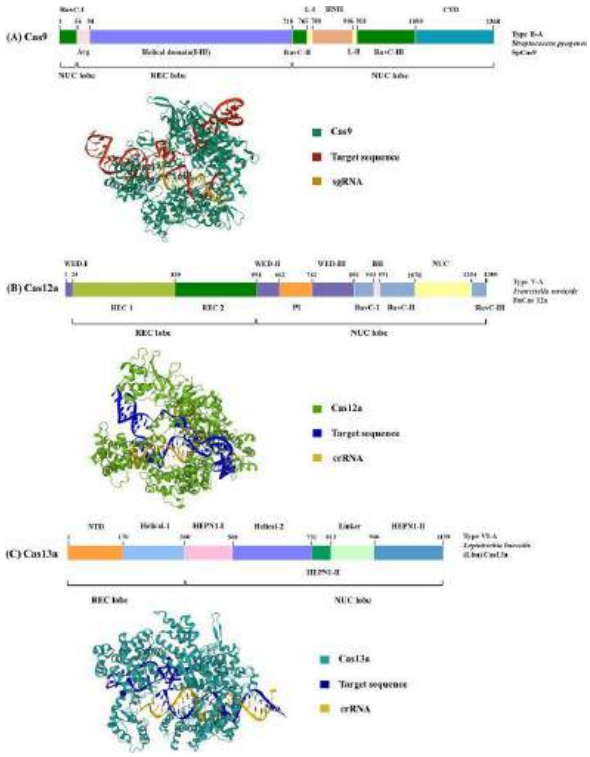


Figure 2. Displays the general structure of Cas9, Cas12a, and Cas13a. Reprinted with permission from National Library of Medicine et al.



Participating Animals' Mental Health During Animal Therapy Based on Cortisol Levels
By Shiho Itagaki

Abstract

Animal-assisted therapy has gained popularity in recent years worldwide. It has shown significant benefits for human patients in terms of improving their mental health. There are many academic papers about the benefits of animal therapy for humans showing the evidence for its usefulness; however, those research papers tend to focus only on benefits for humans and not on the impact on participating animals, and the welfare and psychological impact on participating animals remain critical areas of inquiry. An abundant body of research implies that animals have a capacity to feel complex emotions. Consequently, animal therapy should not be only beneficial to animals. Humans should consider animals' mental health during animal therapy. Based on academic research papers, this paper investigates animals' perspectives on animal therapy. Specifically, this study discusses their mental health, looking at their cortisol levels, which could indicate stress, and concluding that animals are actually not under stress, but humans have to consider individual differences, overwork, and environment, and finally indicates the importance of looking at animals' experience in animal-assisted therapy. This paper specifically focuses on animal therapy by dogs.

Introduction

Animals and humans have been strongly bonded for a long time as great friends and coworkers in human history. Dogs and cats are popular as domestic pets; however, the use of those animals for therapeutic purposes dates back to at least the early Middle Ages (Cantanzaro, 2003a). Recently, animal therapy exists in a wide range of occasions including prisons, juvenile homes, hospices, retirement homes, treatment centers, homeless shelters, schools, and hospitals. In animal therapy, there are participating animals for humans to relax with. Commonly, dogs, cats, horses, and rabbits are used. Dogs are commonly used in animal-assisted therapy because they are generally well suited to interacting with humans.

During animal therapy, animals can provide calmness, comfort, or safety and divert attention away from their stress. Scientifically, interaction can lower blood pressure(Beck and Katcher, 1983); aiding in the development of motor skills like coordination, balance, and posture (McCowan, 1984); have a variety of psychosocial effects like decreasing feelings of loneliness, despair, isolation, and fear (Muschel, 1984), and decreasing symptoms of depression (Jessen, Cardiello, and Baun, 1996). By interacting with animals, humans can feel safe and connected. People can talk more easily when animals are around because animals facilitate communication among residents, staff, and the human volunteers according to research(Granger and Carter, 1991; Bernstein, Friedmann and Malaspina,
2000).

A study involving 822 undergraduate and graduate students at a private university found that 23% were diagnosed with mental illness stemming from stress(Gibbons et al., 2019). The

stress experienced by university students can lead to non-graduation or negative outcomes(Auerbach et al., 2016). Animal therapy is effective as a treatment for students' stress. The study provided 10 minutes of interaction between students and dogs, resulting in self-reported decreases in stress levels. (Machová et al., 2020)

Although the benefits of animal therapy for humans were well researched in this field, the psychological impact on animals themselves received little attention. A recent study shows that animals have distinct selves and the ability to feel and display a variety of emotions (Arluke and Sanders 1996; Alger and Alger 1997, 1999, 2003; Sanders 1999; Irvine, 2004). These findings question the mental harm to the participating animals in animal therapy, including stress, fear, and discomfort, and whether the animals actually enjoy participating.

In this paper, we will investigate the animals' stress levels mainly based on cortisol levels affected by animal therapy. Cortisol levels explain stress levels because when we are stressed, the adrenal glands manufacture cortisol. Cortisol data suggest therapy animals experience minimal acute stress, but welfare still depends on individual variation, workload, and environmental context. Filling this gap in the literature makes animal welfare a critical area for further inquiry.

Body 1: Stress

Because we don't share a language, it is difficult to understand the animals' emotions fully. Academic papers reviewed in this paper measure stress in animals based on cortisol levels, which could indicate stress. In conclusion, in many studies, large stresses are not observed, leading to the conclusion that animal therapy does not generally cause strong immediate stress in dogs.

The cortisol level(stress indicator hormone) didn't change during the therapy. Also, from analyzing the behaviour, highly stressed behaviour was rarely shown(3.12%), and most of the behaviour of dogs was friendly(80.67%)(Hatch, 2007). The finding indicates that dogs do not feel tension during therapy sessions. In another study, it was even revealed that animals were relaxed. Cortisol levels didn't increase, and oxytocin didn't decrease, showing that it was not negatively affecting the animals. Moreover, heart rate actually decreased, and right ear temperature decreased, showing that they were at ease(Clark et al., 2020). It suggests that animal therapy is not only beneficial to humans but also to animals, making the therapy sessions justifiable. The study conducted on a large scale also supports that participating animals are not stressed.It took place in five different hospitals across the country, making this claim stronger and generalizable. Saliva cortisol didn't increase during the session, and no increase in stress/problematic behaviour was observed(McCullough et al., 2018). This evidence suggests that animals are not harmed by therapy generally. Those studies should be spread because conducting therapy knowing that they are not stressed and not knowing or thinking anything about an animal's mental state during therapy is different. All the pet owners and therapy organizers need to understand this mutual relationship.

Dogs are not under stress by participating in animal therapy. However, it is necessary to consider long-term effects (explained in the next paragraph)

Body 2: Long-term Stress / Workload→fatigue

Low immediate stress does not mean there is no long-term burden. They are living animals, so they get tired if they work too hard, just like humans. It is important to consider them as an organism rather than a tool to comfort people.

One study revealed that saliva cortisol was unchanged, but fecal cortisol increased. Specifically, the fecal cortisol level during the therapy was high, but in the week after the therapy ended, the fecal cortisol level was low. Fecal cortisol shows long-term stress in animals, unlike saliva cortisol, which measures immediate stress. The authors suggested that this could indicate positive stimulation, but further research is needed to distinguish positive stimulation from distress. (Yotanyamaneewong et al., 2025). This study might mean that therapy dogs did not feel immediate stress from the therapy, but because the therapy continued for 1 week, they might have felt simply tired from it. That tiredness is shown as a fecal cortisol increase later. But an increase in cortisol level doesn't necessarily mean "an unpleasant thing". It might be a thing that therapy dogs did hard work on because they liked it. Handlers should be cautious not to tire the dogs too much and consider the long-term stress.

They are, in a positive way, "Volunteers", but themselves have no choice but to attend therapy because of humans directing them. Humans should recognize that animals have rights to do what they actually want to do.

Body 3: Individual differences, experience

It is important to consider individual differences, the same as humans. There are several factors to decide the dog's fitness for therapy.

Although it is true from the data that, on average, therapy dogs don't feel stress, not all the dogs feel the same, as one study suggests(Giuliano et al., 2025). Surprisingly, during the therapy, those who showed stress behaviour also showed more friendly behaviour. Of course, those who showed stress behaviour showed an increase in cortisol(McCullough et al., 2018). This might mean that these dogs were highly expressive and showed friendly behaviour while also displaying stress-related behaviour. This study reveals animals' reactions depend on their scale of expression. It is not accurate to conclude only by their behaviour whether they are stressed or not because their reaction depends. Checking their cortisol level in addition to observing each animal to see the signs of tiredness and unpleasantness might be effective.

Adding on to individual differences, in terms of species, different types of animals respond differently to the therapy. Cats tend to feel more stress than dogs; sheltered/adapted animals are not the best fit for therapy activity because we are not sure about their past life(trauma), and they don't have enough experience and training(Hatch, 2007). It is difficult to understand each other fully because humans and dogs can't talk, so we have to check if the

animal is a good fit for therapy, including meeting new people and a new environment, by observing each of them carefully and checking scientifically.

A difference in the amount of experience is one factor. Experienced dogs showed less stress during the therapy. The study measured adrenaline and noradrenaline in the dogs, and found that if dogs are experienced, they are less likely to feel stress, because they get used to stimuli(Tanaka et al., 2005). Humans should let the dogs get used to stimuli slowly.

Although this paper tried to discuss the effect on animals depending on breed, this topic was not well researched in this field. This is a research gap we should work on in the future.

To summarize, the stress the animals are feeling is determined by multiple factors. It is important to let them get used to it slowly and see if they look like they actually want to participate in it. We should measure cortisol levels to know if they have hidden stress that is not clear only by the behaviour due to expressiveness differences.

Body 4: Environment, rather than therapy itself

The environment of the therapy should be considered too.

In the study, it discusses that although the dogs are experienced, they still feel stress if they are in an unfamiliar environment. Rather than human interaction or therapy itself, the new environment is stressful for them(Tanaka et al., 2005). It reveals that it is also the environment they should be familiar with, not only the therapy.

In another study, conducted in Ukraine and Germany, cortisol levels were compared between countries. Surprisingly, it concluded that animals in Germany felt more stress than in Ukraine, although there was a war going on in Ukraine(Foltin et al., 2026). This reveals that a bad environment for humans doesn't necessarily mean stressful for animals, and further research is needed on what is considered a stressful environment for animals.

Body 5: Suggestion

It is important to choose a way that both humans and animals benefit. Virtual therapy might be good as an alternative to animal therapy because, physically, dogs are not present. It is only the video recording, so dogs don't have to meet a lot of strangers in an unfamiliar place. This study tested whether virtual dog therapy is effective or not using 4 trained therapy dogs. The process was like this: A handler and a dog appear in the video, and he explains which part of the body people should touch to make the dog feel pleasant by showing his movement as an example, he asks the audience to imagine themselves petting the dog, and finally he asks a question which make audience think about well being and give a little time to think about it. As a result, there was a stress reduction among participants, including students and community members. It is interesting that even only a 5-minute video of dogs was effective in reducing stress. (Tardif-William et al., 2025)

Conclusion

The studies show that animals are not stressed by the therapy, but be careful about individual differences, overwork, and environment. Animals also feel relaxed by attending therapy. However, it is important to think about animal welfare during animal therapy. Humans should consider whether we are not exploiting them for human benefit.

I suggest, to ensure the animal's mental health: Observing if the dog is fit for therapy and understanding the body language, basic freedom/need of the animals(Hatch, 2007).
Virtual therapy also might be good as an alternative to animal therapy because, physically, dogs are not present.

While researching, papers about questioning the effect of animal therapy were also found. Some point out that the benefits of a pet visitation program may depend on the personality of the human participants(Colby and Sherman, 2002). Another study also found that companion animals provide only little long-term therapeutic benefits for human health and well-being (Beck and Katcher, 1984)

Considering the potential positives against the potential negatives during animal therapy, concern about the use of animals for animal therapy is justified. Future research on stress levels depending on the specific dog breeds, the potential effect of Virtual animal therapy, and the potential method or device to understand animals' emotions is needed to ensure the animals' mental health during animal therapy.

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Works Cited

Alger, J. and Alger, S. 1997. Beyond Mead: Symbolic interaction between humans and felines. Society & Animals 5(1): 65–81.

Alger, J. and Alger, S. 1999. Cat culture, human culture: An ethnographic study of a cat shelter. Society & Animals 7(3): 199–218.

Alger, J. and Alger, S. 2003. Cat Culture: The Social World of a Cat Shelter. Philadelphia, PA: Temple University Press.

Arluke, A. and Sanders, C. 1996. Regarding Animals. Philadelphia, PA: Temple University Press.

Auerbach, R. P., Alonso, J., Axinn, W. G., Cuijpers, P., Ebert, D. D., Green, J. G., Hwang, I., Kessler, R. C., Liu, H., Mortier, P., Nock, M. K., Pinder-Amaker, S., Sampson, N. A., Aguilar-Gaxiola, S., Al-Hamzawi, A., Andrade, L. H., Benjet, C., Caldas-de-Almeida, J. M., Demyttenaere, K., … Bruffaerts, R. (2016). Mental disorders among college students in the World Health Organization World Mental Health Surveys. Psychological Medicine, 46(14), 2955–2970. https://doi.org/10.1017/S0033291716001665

Beck, A. and Katcher, A. 1983. Between Pets and People: The Importance of Animal Companionship. New York: G.P. Putnam's Sons.

Beck, A. and Katcher, A. 1984. A new look at pet facilitated therapy. Journal of the American Veterinary Medical Association 184: 414–421.

Bernstein, P. L., Friedmann, E. and Malaspina A. 2000. Animal-assisted therapy enhances resident social interaction and initiation in long-term care facilities. Anthrozoös 13(4): 213–224.

Cantanzaro, T. 2003a. Section introduction: Human–animal bond and primary prevention. American Behavioral Scientist 47(1): 29–30.

Clark, S. D., Smidt, J. M., & Bauer, B. A. (2020). Therapy Dogs' and Handlers' Behavior and Salivary Cortisol During Initial Visits in a Complex Medical Institution: A Pilot Study. Frontiers in Veterinary Science, 7. https://doi.org/10.3389/fvets.2020.564201

Colby, P. and Sherman, A. 2002. Attachment styles impact on pet visitation effectiveness. Anthrozoös 15(2): 150–165.

Foltin, S., Kostenko, S., Hartwig, A.-D., & Glenk, L. M. (2026). War Exposure and Canine Cortisol Responses: Country Differences in Cortisol Profiles of Therapy Dogs. Animals, 16(3), 381. https://doi.org/10.3390/ani16030381

Gibbons, S., Trette-McLean, T., Crandall, A., Bingham, J. L., Garn, C. L., & Cox, J. C. (2019). Undergraduate students survey their peers on mental health: Perspectives and strategies for improving college counseling center outreach. Journal of American College Health: J of ACH, 67(6), 580–591. https://doi.org/10.1080/07448481.2018.1499652

Giuliano, V. O., Sacchettino, L., Rusu, A. S., Ciccarelli, D., Gazzano, V., de Cesare, M., Visone, M., Mizzoni, V., Napolitano, F., & d'Angelo, D. (2025). Well-Being Indicators in Autistic Children and Therapy Dogs During a Group Intervention: A Pilot Study. Animals, 15(14), 2032. https://doi.org/10.3390/ani15142032

Granger, B. and Carter, D. 1991. The use and nonuse of companion animals by volunteers in nursing homes: A comparative study. Anthrozoös 4(4): 237–246.

Hatch, A. (2007). The View from All Fours: A Look at an Animal-Assisted Activity Program from the Animals' Perspective. Anthrozoös, 20(1), 37–50. https://doi.org/10.2752/089279307780216632

Irvine, L. 2004. If You Tame Me: Understanding Our Connection with Animals. Philadelphia, PA: Temple University Press.

Jessen, J., Cardiello, F. and Baun, M. 1996. Avian companionship in alleviation of depression, loneliness, and low morale of older adults in skilled rehabilitation units. Psychological Reports 78: 339–348.

Machová, K., Procházková, R., Vadroňová, M., Součková, M., & Prouzová, E. (2020). Effect of Dog Presence on Stress Levels in Students under Psychological Strain: A Pilot Study. International Journal of Environmental Research and Public Health, 17(7), 2286. https://doi.org/10.3390/ijerph17072286

McCowan, L. L. 1984. Equestrian therapy. In Dynamic Relationships in Practice Animals in the Helping Professions, 237–256, ed. P. Arkow. Alameda, CA: Latham Foundation.

McCullough, A., Jenkins, M. A., Ruehrdanz, A., Gilmer, M. J., Olson, J., Pawar, A., Holley, L., Sierra-Rivera, S., Linder, D. E., Pichette, D., Grossman, N. J., Hellman, C., Guérin, N. A., & O'Haire, M. E. (2018). Physiological and behavioral effects of animal-assisted interventions on therapy dogs in pediatric oncology settings. Applied Animal Behaviour Science, 200, 86–95. https://doi.org/10.1016/j.applanim.2017.11.014

Muschel, I. 1984. Pet therapy with terminal cancer patients. Social Casework: The Journal of Contemporary Social Work 65: 451–458.

Sanders, C. 1999. Understanding Dogs: Living and Working with Canine Companions. Philadelphia, PA: Temple University Press.

Tanaka, T., Ota, M., & Uetake, K. (2005). Effects of contents of animal-assisted activities and habituation on stress level of companion dogs. 麻布大学雑誌, 9, 113–117.

Tardif-William, C. Y., Willcox, A., Rousseau, C. X., Singal, A., Godard, R. J. P., Green, F. L. L., Binfet, J.-T., & Roma, R. P. S. (2025). Beyond the campus context: Reducing stress among students and community members through virtual canine comfort modules. Human-Animal Interactions. https://www.cabidigitallibrary.org/doi/10.1079/hai.2025.0015

Yotanyamaneewong, S., Junla, D., Brown, J. L., Siriapaipant, N., Yodkamol, N., Prachasilchai, W., Saengthong, A., Sanguansak, P., Kankonsue, T., Punyapornwithaya, V., Sansamur, C., Nganvongpanit, K., & Khonmee, J. (2025). The impact of canine-assisted intervention on stress reduction among university students in Thailand. PLOS ONE, 20(3), e0318777. https://doi.org/10.1371/journal.pone.0318777

Physical and Mental Health Benefits of Dance Relative to Traditional Sports: Evidence from CDC NHANES By Suhana Bhattacharya

Abstract

Dance is one of the most prevalent forms of leisure-time physical activity (LTPA) among women, yet studies comparing its health benefits with those of traditional sports remain relatively limited. Existing studies have largely examined dance and sport separately, while the few studies that directly compare these approaches are mostly short-term, randomized trials examining a single athletic activity in comparison to dance rather than several athletic activities. Using data from the 2005–2006 National Health and Nutrition Examination Survey (NHANES), this study compares the physical and mental health outcomes associated with dance, soccer, basketball, tennis, swimming, softball, and running. Unlike prior intervention studies, the analysis examines naturally occurring participation in real-world settings using a nationally representative dataset. Least-squares regression, instrumental-variable estimation, and propensity score matching reveal no statistically significant differences between the health benefits associated with dance and those associated with traditional sports. Participation in LTPA is found to be associated with 2.25 fewer adverse physical health days and 2.1 fewer adverse mental health days per month. Dance is also found to be the most prevalent LTPA among adult women and is found to be associated with lower rates of osteoporosis and hypertension among older women.

Introduction

While the health benefits of traditional sports are well documented, a growing body of research has established dance as a health-promoting form of leisure-time physical activity (LTPA). Yet studies of sport and dance have largely evolved as separate research streams, with most investigations examining either traditional athletic activities or dance participation in isolation. Consequently, although both dance and sport are known to improve physical, psychological, and cognitive health relative to inactivity, less is known about the effectiveness of dance relative to other active leisure pursuits. The few studies that have directly compared dance with specific athletic activities have typically relied on short-term randomized interventions involving a limited set of comparison activities. Less is known about how dance compares with other leisure activities in naturally occurring, real-world settings. The following quotation from *The Baylor Lariat*, the award-winning student-run newspaper of Baylor University, captures the motivation underlying the present study:

"*When you think of an athlete, the same stereotypical sports come to mind: football, basketball, baseball and soccer. In reality, physical activity comes in many forms outside of these major sports. I grew up as a competitive dancer, and I constantly dealt with people defining dance as an 'art form' or a 'hobby.' This always offended me, because the level of dedication I poured into dance takes the same amount of skill that is required of other athletic activities*" (Frisby).

Despite increasing recognition of dance as a physically demanding activity, empirical evidence directly comparing its health benefits with those of traditional sports remains limited. This gap is particularly important because dance appears to promote long-term participation through its combination of physical activity, music, enjoyment, social interaction, and self-expression (Haynes et al.; Lu et al.).

This study contributes to the literature in three ways. First, it moves beyond active-versus-inactive comparisons by examining dance relative to a broad range of alternative leisure activities. Second, unlike prior studies that have relied primarily on short-term randomized interventions, it examines naturally occurring participation using a nationally representative dataset and a common econometric framework. Third, it considers broader preventive health outcomes, including hypertension and osteoporosis, that are difficult to examine in short-term intervention studies.

Using NHANES data, two-stage least squares (2SLS) and propensity score matching methods are employed to test three hypotheses: (1) dance provides physical and mental health benefits comparable to traditional sports, (2) dance becomes more prevalent among older women, and (3) dance is associated with improved age-related health outcomes.

Literature Review

There is extensive evidence that leisure-time physical activity (LTPA) improves physical and mental health. LTPA includes activities undertaken voluntarily during leisure time, such as sports, exercise, walking, gardening, and dance. Participation has been associated with improved physical functioning, reduced chronic disease risk, enhanced psychological well-being, and better quality of life (Fong Yan et al., "Physical Health Outcomes"; Tao et al.; Lu et al.).

A substantial literature has also documented the benefits of sport participation in non-clinical settings. Observational studies show that sport participation is associated with higher psychological well-being, lower levels of depression and anxiety, and stronger social connectedness than non-participation (Eather et al.; Eime et al.). Team sports may provide additional benefits through social support and interpersonal relationships (Eather et al.; Eime et al.).

Likewise, dance provides important physical, psychological, and social benefits (Alpert; Keogh et al.; Malkogeorgos et al.; Quiroga Murcia et al.; Ward). Dance is one of the most common forms of LTPA among women and frequently remains popular throughout later adulthood (Fan et al.; Marquez et al.; Marquez and McAuley). Its combination of physical activity, music, self-expression, and social interaction appears to promote long-term engagement (Haynes et al.; Pines & Giles; Thøgersen-Ntoumani et al.). Dance programs also demonstrate high rates of participation and retention among older adults (Britten et al.; Clifford et al.).

Although many forms of exercise improve health relative to inactivity, the key question is whether dance differs from other active leisure pursuits. Existing meta-analyses comparing the separate studies of dance and athletic activities indicate that dance produces physical, psychological, and cognitive outcomes comparable to traditional exercise programs (Fong Yan et

al., "Physical Health Outcomes"; Fong Yan et al., "Psychological and Cognitive Health Outcomes"; Lu et al.; Waugh et al.; Zhang and Ma).

However, direct comparisons between dance and traditional athletic activities are relatively few and have primarily relied on randomized controlled trials (RCT). Findings have been mixed, with some studies favoring sports and others finding little difference across activities (Barene and Krustrup; Bass and DiBrezzo; Johnston et al.). Similar results have been reported among older adults and clinical populations, where dance often performs comparably to walking, physical therapy, and structured exercise programs (Bisbe et al.; Kaltsatou et al.; Merom et al.; Müller et al.; Rawson et al.; Rodziewicz-Flis et al.; Volpe et al.).

Although RCTs are valuable for evaluating short-term outcomes such as mood, cognition, and functional capacity, they provide limited insight into the long-term health consequences of sustained participation and whether individuals continue engaging in an activity once an intervention concludes. Consequently, less is known about whether long-term engagement in dance as a lifestyle behavior is associated with broader preventive health outcomes, including hypertension and osteoporosis. The present study addresses this gap by examining naturally occurring participation in dance and other leisure activities using a nationally representative dataset and a common econometric framework.

Data Section

Data were obtained from the 2005–2006 National Health and Nutrition Examination Survey (NHANES), the most recent wave containing detailed information on specific leisure-time physical activities. The data collected in this survey was retrospective, and the dataset did not identify any participants.

Name of Data set **Dependent variables**	**Name of Relevant variables**
Health Status	HSQ470 - Number of days physical health was not good HSQ480 - Number of days mental health was not good
Blood Pressure & Cholesterol	BPQ020 - Ever told you had high blood pressure
Osteoporosis	OSQ060 - Ever told had osteoporosis/brittle bones
Main Explanatory variable	
Leisure Time Physical Activities	PADACTIV - Leisure time activity
Control Variables	
Demographic Variables	RIAGENDR – Gender RIDAGEYR - Age at Screening INDHHINC – Annual Household Income
Potential Instrumental Variables	
Housing Characteristics	HOQ070- Source of tap water (Proxy for Urban/Rural)
Demographic Variables	RIDEXMON- Six Month Period of Interview

Table 1: Merging of CDC-NHANES Datasets Based on Unique Person-ID

Note: This table lists the variables used for this study and the data set they originated from in the National Health and Nutrition Examination Survey by the CDC in the 2005-2006 cycle. Created by author, 2026.

Multiple NHANES files were merged using the respondent identifier (SEQN), as summarized in **Table 1**. The merged dataset contained 16,359 observations. Restricting the analysis to women reduced the sample to 7,871 observations. A further restriction to respondents reporting participation in dance, soccer, basketball, softball, tennis, swimming, running, or no LTPA yielded a final sample of 3,173 women. **Figure 1** shows the breakup of the sample across the more important LTPA categories, as well as including the share of people who do not engage in any physical activity. These latter individuals form my control group.

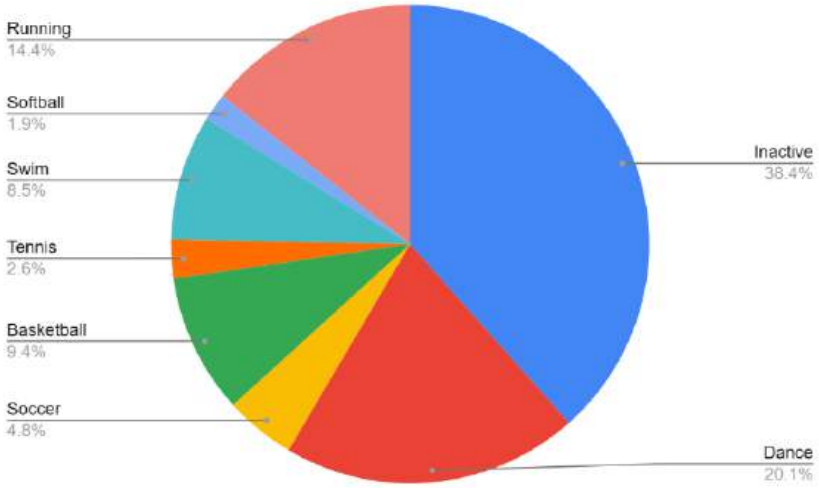

Figure 1: Proportion of Each Major LTPA in the Sample

Note: This figure illustrates the breakdown of the different recreational sport activities that the participants in this study's sample participated in. Created by author, 2026.

For the primary analysis, the dependent variables are the number of adverse physical health days and adverse mental health days reported during the previous month. For the analysis of osteoporosis and hypertension, the sample is restricted to women aged 20 years and older because these questions were only asked of adults.

Methods

To examine whether dance provides health benefits comparable to traditional sports, the study first estimates ordinary least squares models relating health outcomes to participation in specific leisure-time physical activities, controlling for age, age squared, and household income.

Because healthier individuals may be more likely to participate in physical activity, the study also estimates two-stage least squares (2SLS) models. Source of tap water to proxy for

location and month of interview to proxy for season (Angrist and Krueger) are used as instruments for LTPA participation. Instrument validity is evaluated using first-stage F-statistics and Sargan overidentification tests. Age, age squared, and household income are included as controls in both stages.

As a robustness check, the analysis employs propensity score matching to compare individuals with similar observable characteristics who differ in their choice of leisure-time physical activity.

Results

	(1) Adverse Physical Health Days	(2) Adverse Mental Health Days
Dance	-1.796***	-1.586***
	(0.000)	(0.000)
Soccer	-2.342***	-1.429**
	(0.000)	(0.038)
Basketball	-2.428***	-1.955***
	(0.000)	(0.000)
Softball	-2.019**	-2.600**
	(0.035)	(0.012)
Tennis	-2.281***	-2.116**
	(0.004)	(0.014)
Swim	-1.957***	-1.535***
	(0.000)	(0.003)
Run	-2.101***	-2.184***
	(0.000)	(0.000)
Age	0.063*	0.179***
	(0.055)	(0.000)
Age_sq	-0.000	-0.002***
	(0.773)	(0.000)
Annual Family Income	-0.011	-0.028**
	(0.318)	(0.018)
Constant	2.834***	2.730***
	(0.000)	(0.000)
Observations	2805	2805
R^2	0.070	0.030

Table 2: Relation Between LTPA and Number of Adverse Physical or Mental Health Days.

Note: p-values in parentheses, * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$The table lists the estimated coefficients to show association between each physical activity and negative physical and mental health days compared to the control group of inactive females. A negative coefficient indicates a reduction in negative health days, indicating positive impact. Created by author, 2026.

In **Table 2**, all activity coefficients are negative and statistically significant relative to inactive respondents, indicating that participation in dance, soccer, basketball, softball, tennis, swimming, and running is associated with fewer adverse physical and mental health days. While coefficient magnitudes differ across activities, pairwise tests presented in **Table 3** indicate that these differences are not statistically significant.

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Test	p-values for Adverse Physical Health Days	p-values for Adverse Mental Health Days
Dance vs. Basketball	0.417	0.515
Dance vs. Softball	0.707	0.883
Dance vs. Soccer	0.341	0.370
Dance vs. Tennis	0.505	0.581
Dance vs. Swim	0.941	0.936
Dance vs. Running	0.477	0.200

Table 3: Test for Difference in Effect Between Traditional Sports and Dance Participation

Note: The table lists the p-value estimated as a result of each t-test comparing the association between dance and other recreational physical activities. Notice that all p-values exceed 0.05, indicating that the null hypothesis of equal effects cannot be rejected. Created by author, 2026.

The inability to reject equality of coefficients across activities provides no evidence that dance yields different physical or mental health benefits than traditional sports, supporting Hypothesis 1.

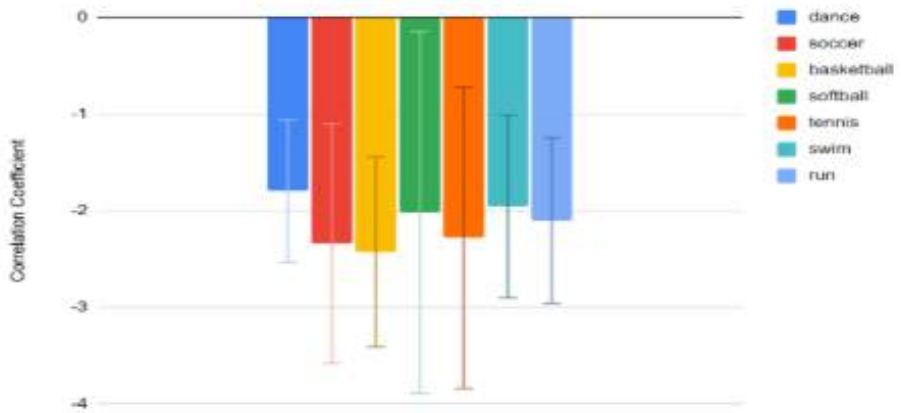

Figure 2: Association Between leisure time physical activity (LTPA) and Adverse Physical Health Days ± 1.96 Standard Deviations.

Note: This figure visually depicts the estimated coefficients for the association between poor physical health days and each sport with their 95% confidence interval. Because all the intervals overlap, the figure illustrates how the associations of each sport are not statistically different from one another. Created by author, 2026.

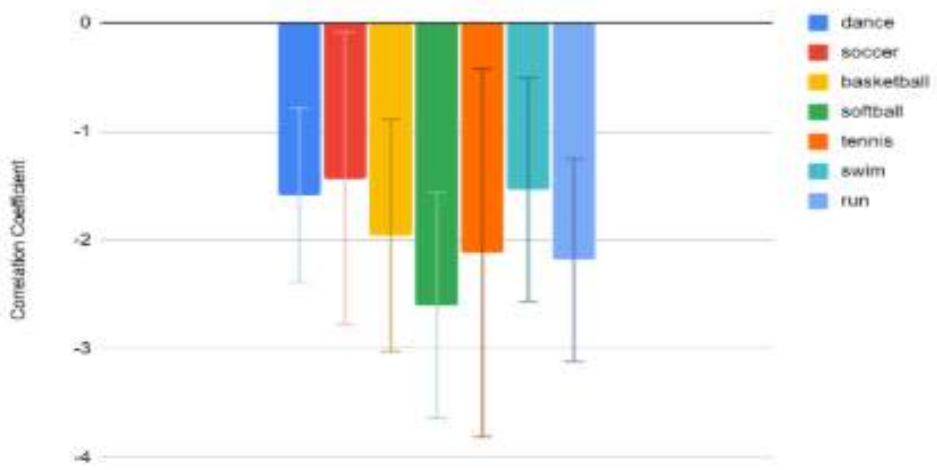

Figure 3: Association Between LTPA and Adverse Mental Health Days ± 1.96 Standard Deviations.

Note: This figure visually depicts the estimated coefficients for the association between poor mental health days and each sport with their 95% confidence interval. Because all the intervals overlap, the figure illustrates how the associations of each sport are not statistically different from one another. Created by author, 2026.

Figure 2 and **Figure 3** present the above results visually. The substantial overlap of confidence intervals across activities is consistent with the pairwise tests and reinforces the conclusion that health benefits do not differ significantly across activities.

The study next employs a two-stage least squares approach to identify the effects of physical activity on health outcomes. Women who engaged in any of the above physical activities were coded as active=1, whereas those who did not engage in any of these activities were coded as active =0. The instrumental variables for LTPA engagement: season of interview and source of tap water to proxy for rural versus urban residence, satisfy the relevance condition (first stage F-statistic has p-value of 0.000), and the overidentification tests (Sargan test p-values) fail to reject the validity of the exclusion restrictions.

Each of the specific activities was included as further controls to examine whether that activity leads to any additional effects that are different compared to being physically active in general. The results from using this technique are presented in **Table 4**. It shows that physical activity reduces the number of days lost due to physical and mental health reasons by 2.25 days and 2.1 days respectively. For the coefficients capturing further effects corresponding to each specific activity, we fail to reject the null hypothesis that these coefficients equal zero. Consequently, the instrumental variable estimates provide no evidence that participation in dance leads to either better or worse physical or mental health outcomes than participation in the other sports activities considered in this study. These findings are consistent with the first hypothesis that dance provides health benefits comparable to those associated with traditional sports

participation. Note that tennis serves as the omitted activity category in the specification and therefore does not receive a separate coefficient estimate.

	(1) Adverse Physical Health Days	(2) Adverse Mental Health Days
Active	-2.248***	-2.099**
	(0.005)	(0.015)
Dance	0.470	0.464
	(0.553)	(0.593)
Soccer	-0.086	0.652
	(0.926)	(0.519)
Basketball	-0.184	0.138
	(0.827)	(0.881)
Softball	0.229	-0.529
	(0.844)	(0.677)
Swim	0.295	0.492
	(0.730)	(0.599)
Run	0.143	-0.103
	(0.860)	(0.907)
Age	0.067**	0.170***
	(0.043)	(0.000)
Square of Age	-0.000	-0.002***
	(0.610)	(0.000)
Annual Family Income	-0.008	-0.025**
	(0.500)	(0.042)
Constant	2.779***	2.854***
	(0.000)	(0.000)
Observations	2757	2757
R^2	0.067	0.029
IV Statistics		
First stage F-statistic	26.09 (p-value:0.000)	15.37 (p-value:0.000)
Underidentification test	51.319 (p-value:0.000)	30.471 (p-value:0.000)
Sargan Overidentification	0.102 (p-value: 0.7495)	1.547 (p-value: 0.2136)

Table 4: Effect of LTPA on Number of Adverse Physical or Mental Health Days, using Instrumental-Variable estimation

Note: *p*-values in parentheses; * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$. The instrumental variables for LTPA that satisfied the criteria for both relevance and the exclusion restriction were month of interview and source of tap water. The instrumental-variable estimates provide evidence consistent with a causal interpretation between each LTPA and physical and mental health days

compared to the control group of inactive females. A negative coefficient indicates a reduction in negative health days, indicating positive impact. Created by author, 2026.

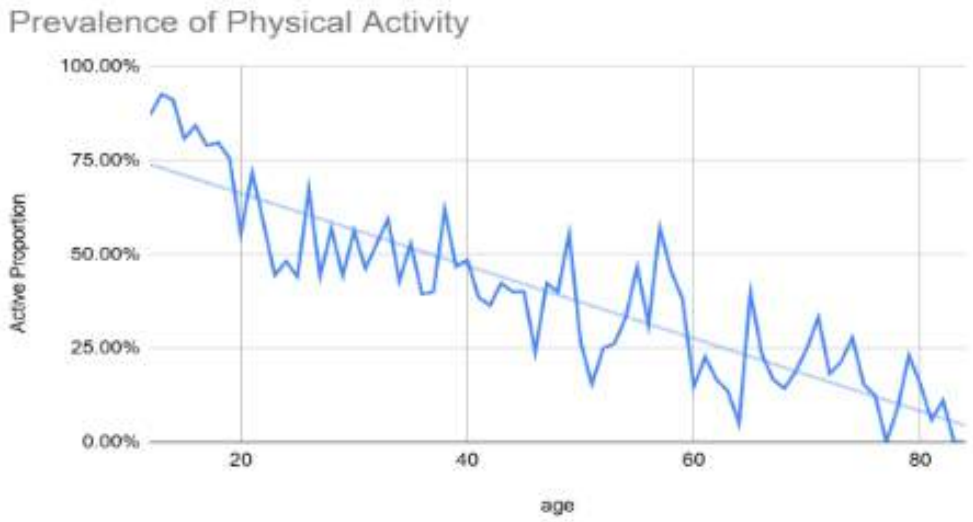

Figure 4 : Leisure time physical activity (LTPA) engagement as age increases.

Note: Figure 4 presents the relationship between age and activity participation with a best fit line calculated. The figure shows a clear decline in activity participation as age increases. Created by author, 2026.

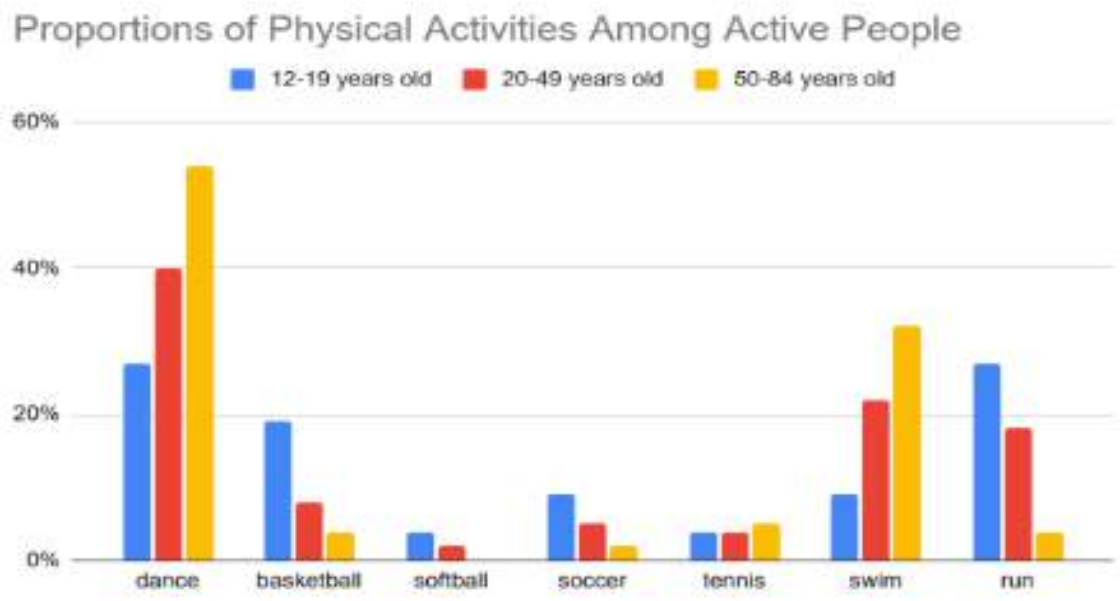

Figure 5: Leisure time physical activity (LTPA) engagement over life stages.

Note: Figure 5 shows the breakdown of participation in selected recreational sports between three different age groups. Notice how the figure consistently shows dance to have the highest

percentage of participation among every age group, and the figure shows the percentage of participation to increase as the age groups increase. Created by author, 2026.

Figure 4 shows that participation in major leisure-time physical activities declines sharply after adolescence and continues to decrease with age, highlighting the importance of identifying activities that remain attractive throughout adulthood and later life. **Figure 5** shows that among women who participate in LTPA, dance accounts for an increasing share of activity participation as age rises, supporting Hypothesis 2 and suggesting that dance may be particularly sustainable across the life course.

Because dance becomes increasingly prevalent among older women, the paper next examines whether it is associated with health outcomes particularly relevant to this population: osteoporosis and high blood pressure (hypertension). Using women aged 20–84 years, the study estimates 2SLS models controlling for age, income, and an indicator for respondents aged 50 years and older.

Table 5 reports the instrumental variable estimation results for the sample of female respondents aged 20–84 years. The instruments satisfy the relevance condition and pass the overidentification tests. The results indicate that dance participation is associated with a 16.6% lower probability of reporting osteoporosis and a 19.3% lower probability of reporting hypertension, supporting Hypothesis 3.

	Osteoporosis	Hypertension
Dance participation	-0.166*	-0.193*
	(0.054)	(0.053)
Annual Household Income	0.001*	-0.000
	(0.066)	(0.946)
Age	0.003***	0.007***
	(0.002)	(0.000)
Age more than 50 years	0.022	0.143***
	(0.456)	(0.002)
Constant	-0.048	-0.009
	(0.327)	(0.920)
Observations	1194	1482
R^2	-0.063	0.058
IV Statistics		
First stage F-statistic	21.41	29.40
	(0.000)	(0.000)
Underidentification test	64.540	83.575
	(0.000)	(0.000)
Sargan Overidentification	2.461	1.506
	(0.292)	(0.471)

Table 5: Effect of dance participation on health for females aged 20–84 years, based on Instrumental Variable estimation

Note: This table shows the estimated coefficients between dance participation (only using data from people 20 years old and older) and the effect on the probability of having osteoporosis or hypertension. The negative coefficients in dance participation illustrate a reduction in the probability of reporting osteoporosis or hypertension. Created by author, 2026.

Robustness Analysis

	Adverse Physical Health		*Adverse Mental Health*	
	Coefficient of difference	**p-value**	**Coefficient of difference**	**p-value**
Effect of dance versus basketball	0.427	0.294	0.737	0.137
Effect of dance versus soccer	0.999	0.100	1.111	0.114
Effect of dance versus softball	0.735	0.452	1.658	0.111
Effect of dance versus tennis	-0.233	0.779	0.330	0.712
Effect of dance versus swim	-0.201	0.697	-0.591	0.309
Effect of dance versus run	0.461	0.147	0.629	0.127

Table 6: Propensity Score Matching: Effects of dance relative to other LTPA

Note: This table reports propensity score matching estimates comparing dance participants with participants in specific sports activities. All p-values exceed 0.10, indicating no statistically significant differences in adverse physical or mental health outcomes between dance and the comparison activities. Created by author, 2026.

Propensity score matching produced results (**Table 6**) consistent with the instrumental variable analyses, with no statistically significant differences found between dance participation and participation in traditional athletic activities. These findings reinforce the conclusion that dance provides physical and mental health benefits comparable to those associated with traditional sports. The relatively small number of pairwise matches across activities among older women prevented the use of propensity score analysis to examine age-related health outcomes.

Conclusion

Direct comparisons between dance and traditional athletic activities remain relatively limited and have relied primarily on randomized controlled trials (RCTs) examining a single athletic activity rather than comparing dance with several athletic activities. Although RCTs are useful for evaluating short-term outcomes such as mood, cognition, and functional capacity, they provide limited insight into the long-term health consequences of sustained participation or whether individuals continue engaging in an activity after an intervention concludes. The present study addresses this gap by examining naturally occurring lifestyle behavior in the form of leisure-time physical activity (LTPA) participation in dance and other athletic activities using a nationally representative dataset and a common econometric framework.

This study examined whether dance provides physical and mental health benefits comparable to those associated with traditional sports using nationally representative data from NHANES. Consistent across ordinary least squares, instrumental-variable, and propensity score matching analyses, the findings provide no evidence that dance differs significantly from soccer,

basketball, softball, tennis, swimming, or running in its association with adverse physical or mental health days. Participation in leisure-time physical activity was associated with approximately 2.25 fewer adverse physical health days and 2.1 fewer adverse mental health days per month, while the specific type of activity did not appear to meaningfully alter these benefits.

The results also indicate that dance occupies a unique position among women's leisure-time physical activities. Participation in most activities declines substantially with age, whereas dance accounts for an increasing share of activity participation among older women. In addition, dance participation was associated with lower probabilities of reporting osteoporosis and hypertension among adult women, suggesting that its sustained popularity across the life course may contribute to important preventive health outcomes.

Taken together, the findings support the view that dance should be regarded as a legitimate health-promoting form of physical activity rather than merely an artistic or recreational pursuit. Because dance combines physical exertion with music, enjoyment, social interaction, and self-expression, it may offer a particularly sustainable pathway to lifelong physical activity. From a public health perspective, promoting dance alongside traditional sports may help increase physical activity participation among women and support healthy aging across the population.

A limitation of NHANES is that it records participation during the previous month but does not capture long-term participation histories. Consequently, it is not possible to distinguish between occasional and long-term participants within the data. Collection of longitudinal data in the future would allow studies to provide stronger evidence regarding whether sustained dance participation generates cumulative benefits for chronic conditions such as hypertension and osteoporosis and would further clarify the role of dance as a lifelong health-promoting activity.

Works Cited

Alpert, P. T. "The Health Benefits of Dance." Home Health Care Management & Practice, vol. 23, no. 2, 2010, pp. 155-57, doi.org/10.1177/1084822310384689.

Angrist, J. D., and A. B. Krueger. "Does Compulsory School Attendance Affect Schooling and Earnings?" Quarterly Journal of Economics, vol. 106, no. 4, 1991, pp. 979-1014, doi.org/10.2307/2937954.

Barene, S., and P. Krustrup. "Football and Zumba Training in Female Hospital Staff: Effects after 12 and 40 Weeks on Self-Reported Health Status, Emotional Wellbeing, General Self-Efficacy and Sleep Problems." International Journal of Environmental Research and Public Health, vol. 19, no. 3, 2022, p. 1101, doi.org/10.3390/ijerph19031101.

Bass, M. A. E., and R. DiBrezzo. "Comparison of Two Exercise Programs on General Well-Being of College Students." Psychological Reports, vol. 91, no. 3, pt. 2, 2002, pp. 1195-1201, doi.org/10.2466/pr0.2002.91.3f.1195.

Bisbe, M., et al. "Comparative Cognitive Effects of Choreographed Exercise and Multimodal Physical Therapy in Older Adults with Amnestic Mild Cognitive Impairment: A Randomized Clinical Trial." Journal of Alzheimer's Disease, vol. 73, no. 2, 2020, pp. 769-83, doi.org/10.3233/JAD-190552.

Britten, L., et al. "Dance On: A Mixed-Method Study into the Feasibility and Effectiveness of a Dance Programme to Increase Physical Activity Levels and Wellbeing in Adults and Older Adults." BMC Geriatrics, vol. 23, 2023, p. 48, doi.org/10.1186/s12877-022-03646-8.

Clifford, A. M., et al. "Social Dance for Health and Wellbeing in Later Life." Complementary Therapies in Clinical Practice, vol. 37, 2019, pp. 6-10, doi.org/10.1016/j.ctcp.2019.07.006.

Eather, N., et al. "The Impact of Sports Participation on Mental Health and Social Outcomes in Adults: A Systematic Review and the 'Mental Health through Sport' Conceptual Model." Systematic Reviews, vol. 12, 2023, p. 102, doi.org/10.1186/s13643-023-02264-8.

Eime, R. M., et al. "A Systematic Review of the Psychological and Social Benefits of Participation in Sport for Adults: Informing Development of a Conceptual Model of Health through Sport." International Journal of Behavioral Nutrition and Physical Activity, vol. 10, 2013, p. 135, doi.org/10.1186/1479-5868-10-135.

Fan, J. X., et al. "Walking or Dancing: Patterns of Physical Activity by Cross-Sectional Age among U.S. Women." Journal of Aging and Health, vol. 25, no. 7, 2013, pp. 1182-1203, doi.org/10.1177/0898264313495561.

Fong Yan, A., et al. "The Effectiveness of Dance Interventions on Physical Health Outcomes Compared to Other Forms of Physical Activity: A Systematic Review and Meta-Analysis." Sports Medicine, vol. 48, no. 4, 2018, pp. 933-51, doi.org/10.1007/s40279-017-0853-5.

Fong Yan, A., et al. "The Effectiveness of Dance Interventions on Psychological and Cognitive Health Outcomes Compared with Other Forms of Physical Activity: A Systematic

Review with Meta-Analysis." Sports Medicine, vol. 54, no. 5, 2024, pp. 1179-1205, doi.org/10.1007/s40279-023-01990-2.
Frisby, J. "Dance Should Be Considered a Sport." The Baylor Lariat, 21 Jan. 2021, baylorlariat.com/2021/01/21/dance-should-be-considered-a-sport/.
Haynes, A., et al. "'It Doesn't Feel like Exercise': A Realist Process Evaluation of Factors That Support Long-Term Attendance at Dance Classes Designed for Healthy Ageing." Frontiers in Public Health, vol. 11, 2023, article 1284272, doi.org/10.3389/fpubh.2023.1284272.
Johnston, S. A., et al. "Effects of Team Sports on Anxiety, Depression, Perceived Stress, and Sleep Quality in College Students." Journal of American College Health, vol. 69, no. 7, 2021, pp. 791-97, doi.org/10.1080/07448481.2019.1706530.
Kaltsatou, A. C., et al. "Functional and Psychosocial Effects of Either a Traditional Dancing or a Formal Exercising Training Program in Patients with Chronic Heart Failure: A Comparative Randomized Controlled Study." Clinical Rehabilitation, vol. 28, no. 2, 2014, pp. 128-38, doi.org/10.1177/0269215513492988.
Keogh, J. W. L., et al. "Physical Benefits of Dancing for Healthy Older Adults: A Review." Journal of Aging and Physical Activity, vol. 17, no. 4, 2009, pp. 479-500, doi.org/10.1123/japa.17.4.479.
Lu, J., et al. "The Effects of Dance Interventions on Physical Function and Quality of Life among Middle-Aged and Older Adults: A Systematic Review." PLOS ONE, vol. 19, no. 4, 2024, p. e0301236, doi.org/10.1371/journal.pone.0301236.
Malkogeorgos, A., et al. "The Social-Psychological Outcomes of Dance Practice: A Review." Sport Science Review, vol. 20, no. 5-6, 2011, pp. 105-26, doi.org/10.2478/v10237-011-0067-y.
Marquez, D. X., and E. McAuley. "Gender and Acculturation Influences on Physical Activity in Latino Adults." Annals of Behavioral Medicine, vol. 31, no. 2, 2006, pp. 138-44, doi.org/10.1207/s15324796abm3102_5.
Marquez, D. X., et al. "Physical Activity of Urban Community-Dwelling Older Latino Adults." Journal of Physical Activity and Health, vol. 8, suppl. 2, 2011, pp. S161-S170, doi.org/10.1123/jpah.8.s2.s161.
Merom, D., et al. "Cognitive Benefits of Social Dancing and Walking in Old Age: The Dancing Mind Randomized Controlled Trial." Frontiers in Aging Neuroscience, vol. 8, 2016, p. 26, doi.org/10.3389/fnagi.2016.00026.
Müller, P., et al. "Evolution of Neuroplasticity in Response to Physical Activity in Old Age: The Case for Dancing." Frontiers in Aging Neuroscience, vol. 9, 2017, p. 56, doi.org/10.3389/fnagi.2017.00056.
Pines, R., and H. Giles. "Dancing While Aging: A Study on Benefits of Ballet for Older Women." Anthropology & Aging, vol. 41, no. 1, 2020, pp. 83-94, doi.org/10.5195/aa.2020.209.

Quiroga Murcia, C., et al. "Shall We Dance? An Exploration of the Perceived Benefits of Dancing on Well-Being." Arts & Health, vol. 2, no. 2, 2010, pp. 149-63, doi.org/10.1080/17533010903488582.

Rawson, K. S., et al. "Exercise and Parkinson Disease: Comparing Tango, Treadmill, and Stretching." Journal of Neurologic Physical Therapy, vol. 43, no. 1, 2019, pp. 26-32, doi.org/10.1097/NPT.0000000000000245.

Rodziewicz-Flis, E. A., et al. "The Positive Impact of 12 Weeks of Dance and Balance Training on the Circulating Amyloid Precursor Protein and Serotonin Concentration as Well as Physical and Cognitive Abilities in Elderly Women." Experimental Gerontology, vol. 162, 2022, p. 111746, doi.org/10.1016/j.exger.2022.111746.

Tao, D., et al. "The Physiological and Psychological Benefits of Dance and Its Effects on Children and Adolescents: A Systematic Review." Frontiers in Physiology, vol. 13, 2022, p. 925958, doi.org/10.3389/fphys.2022.925958.

Thøgersen-Ntoumani, C., et al. "'Shall We Dance?' Older Adults' Perspectives on the Feasibility of a Dance Intervention for Cognitive Function." Journal of Aging and Physical Activity, vol. 26, no. 4, 2018, pp. 553-60, doi.org/10.1123/japa.2017-0203.

Volpe, D., et al. "A Comparison of Irish Set Dancing and Exercises for People with Parkinson's Disease: A Phase II Feasibility Study." BMC Geriatrics, vol. 13, 2013, p. 54, doi.org/10.1186/1471-2318-13-54.

Ward, S. A. "Health and the Power of Dance." Journal of Physical Education, Recreation & Dance, vol. 79, no. 4, 2008, pp. 33-36, doi.org/10.1080/07303084.2008.10598161.

Waugh, M., et al. "The Use of Dance to Improve the Health and Wellbeing of Older Adults: A Global Scoping Review of Research Trials." PLOS ONE, vol. 19, no. 10, 2024, p. e0311889, doi.org/10.1371/journal.pone.0311889.

Zhang, Y., and H. Ma. "The Impact of Dance on the Mental Health of Older Adults: A Network Meta-Analysis of Anxiety, Depression, and Well-Being." Frontiers in Psychology, vol. 16, 2025, p. 1733911, doi.org/10.3389/fpsyg.2025.1733911.

The Hidden Burden of Surgery: Prevalence, Mechanisms, and Management of Preoperative Anxiety in Pediatric Patients By Olivia Park

Abstract

Preoperative anxiety is one of the most common psychological challenges experienced by pediatric patients undergoing surgery and has been associated with adverse perioperative and postoperative outcomes, including increased pain, delayed recovery, and greater emotional distress. Despite growing recognition of its clinical importance, research on pediatric preoperative anxiety remains less extensive than that involving adult populations. It can arise from uncertainty about the procedure, fear of complications, and the perceived loss of control associated with medical environments. While some degree of anxiety before surgery is expected, elevated levels of preoperative anxiety may negatively affect both psychological well-being and surgical recovery. This review examines the definition and prevalence of preoperative anxiety, with particular attention to pediatric patients, as well as the biological and psychological mechanisms that contribute to its development. In addition, this paper highlights certain gaps in the current literature, particularly the limited number of studies focusing specifically on pediatric patient populations and cultural differences in anxiety experiences. Current management strategies and gaps in pediatric patients-focused research are also discussed. A better understanding of preoperative anxiety in pediatric patients may help improve perioperative psychological support and patient outcomes for young surgical patients.

Introduction

Preoperative anxiety has become an increasingly important focus within perioperative research due to its impact on patient experience and surgical outcomes. Elevated levels of anxiety prior to surgery may negatively affect both psychological well-being and surgical outcomes, and have been associated with an increased postoperative pain, delayed recovery, and greater emotional distress following surgery (Kain et al., 2000; Stamenkovic et al., 2018).

Despite the growing recognition of preoperative anxiety as a factor that can affect surgical outcomes, systematic reviews of perioperative anxiety literature indicate that while preoperative anxiety is documented in adult populations, research involving pediatric patients remains comparatively less extensive (Kain et al., 2006). This gap in literature is essential because individuals under eighteen represent a population undergoing a unique developmental stage characterized by increased autonomy, identity formation, and heightened sensitivity to medical stressors, which may influence the anxiety present in surgical settings (Sawyer et al., 2018). Overall, this review aims to analyze preoperative anxiety in pediatric patients in comparison with adult populations, examining differences in its prevalence and underlying psychological and biological mechanisms, as well as similarities in its clinical presentation and management, in order to develop a more comprehensive understanding of anxiety across age groups and improve perioperative care.

What is preoperative anxiety?

Preoperative anxiety is generally described as a state of discomfort or tension caused by disease, the ward environment, or worries about anesthesia and surgery before the operation (Ni et al., 2023). The preoperative period includes the time prior to surgery when patients are evaluated, prepared, and informed about the upcoming operation. During this period, many patients experience anxiety related to uncertainty about the procedure, anesthesia, possible complications, or postoperative outcomes (Caumo et al., 2001; Stamenkovic et al., 2018). Preoperative anxiety may take several forms, including general worry, anticipatory anxiety about the surgery, or more acute symptoms such as panic or fear associated with medical procedures (Shafer et al., 1996). Crucially, preoperative anxiety does not necessarily require that anxiety develop for the first time before surgery. Patients who already have anxiety disorders or anxious temperaments may experience an intensification of symptoms during the preoperative period (Chow et al., 2019). While some degree of anxiety before surgery is considered a normal emotional response to a stressful medical event, excessively high levels of anxiety may become clinically significant and require medical or psychological attention.

Prevalence

Previous reviews have reported that the prevalence of preoperative anxiety among pediatric patients ranges from 41.7% to 75.44% across studies (Liu et al., 2022). Such a wide range likely reflects underlying inconsistencies in how preoperative anxiety is defined and measured across studies, making it difficult to establish a single, reliable prevalence estimate for pediatric populations. This variability is also evident across countries: a multicenter survey conducted in China reported that 25.9% of surgical patients experienced high levels of preoperative anxiety, highlighting potential cultural and clinical differences in reported prevalence rates (Li et al., 2021), while a Brazilian study reported a prevalence of 42.0% among pediatric surgical patients (Moura et al., 2016), and studies from Turkey reported rates ranging from 60% to 80% among children undergoing anesthesia and surgery (Topalel et al., 2020). These cross-country discrepancies may be related to differences in patient populations, healthcare settings, family-related factors, and methods used to assess anxiety (Vieco-García et al., 2021).

Prevalence also appears to shift depending on the clinical setting and stage of the surgical process: one pilot study found that over 80% of adolescents reported significant anxiety at the time of anesthesia induction specifically, a rate above the pooled range reported elsewhere, likely reflecting both the narrower, adolescent-only sample and the fact that anxiety tends to peak at induction rather than earlier in the preoperative period (Fortier et al., 2011). However, because this finding is limited to adolescents and drawn from a small pilot sample, it may not generalize to the broader pediatric population, underscoring the limited amount of age-stratified prevalence data available across the full pediatric age range.

Taken together, these findings suggest that preoperative anxiety is a widespread psychological response among young surgical patients across diverse clinical and cultural

settings, with reported prevalence shaped by both where a study was conducted and when during the surgical process anxiety was measured.

Risk factors

Preoperative anxiety in pediatric patients arises from a complex interaction of psychological, developmental, and environmental factors (Stamenkovic et al., 2018). Rather than resulting from a single cause, it is shaped by both individual vulnerability and situational stressors associated with surgery (Stamenkovic et al., 2018). These contributing elements can be broadly categorized into risk factors, which increase susceptibility to anxiety and underlying mechanisms which explain how and why anxiety develops during the preoperative period (Stamenkovic et al., 2018).

One important individual factor is temperament (Chow et al., 2019). A systematic review and meta analysis examining 23 studies involving 4,527 participants aged 1-18 years found that certain temperament characteristics were significantly associated with higher levels of preoperative anxiety. Specifically, traits such as negative emotionality, greater intensity of reaction, and withdrawal behaviors were positively associated with increased preoperative anxiety, whereas higher activity levels were associated with lower levels of anxiety (Chow et al., 2019).

Related individual characteristics include preexisting anxiety (Chow et al., 2019; Caumo et al., 2001). A range of individual and psychological characteristics have been identified as significant risk factors for preoperative anxiety. Temperament plays a particularly important role, as demonstrated by a systematic review and meta-analysis conducted by Chow et al. (2019), which found that traits such as negative emotionality, high intensity of reaction, and behavioral withdrawal were associated with increased levels of preoperative anxiety. In contrast, higher activity levels were associated with lower anxiety. In addition, pediatric patients with preexisting anxiety disorders or anxious tendencies are more likely to experience heightened anxiety prior to surgery, suggesting that preoperative anxiety may represent an exacerbation of underlying psychological vulnerability rather than a completely new condition (Chow et al., 2019; Caumo et al., 2001).

Beyond individual characteristics, family and social factors also contribute significantly to the development of preoperative anxiety (Kain et al., 1996). Parental anxiety has been consistently identified as a key predictor in pediatric patients, with higher levels of parental distress associated with increased anxiety during critical moments such as anesthesia induction (Kain et al., 1996). This relationship suggests that anxiety may be transmitted or reinforced through family dynamics and emotional modeling. Although pediatric patients are more developmentally independent than younger children, family influence remains relevant, particularly in stressful medical contexts. Developmental stage also influences anxiety responses; adolescents may experience tension between their increasing desire for independence and the dependence imposed by hospitalization and surgery, while parental anxiety may further amplify their emotional distress (Breuner et al., 2023). Situational factors, including unfamiliar hospital

environments, previous negative medical experiences, and inadequate communication or preparation before surgery, can also intensify preoperative anxiety by increasing uncertainty and reducing perceived control (Dziadzko et al., 2022).

Finally, medical and situational factors can also increase susceptibility to preoperative anxiety. Fear of anesthesia, concerns about postoperative pain, and uncertainty regarding surgical outcomes are among the commonly reported sources of anxiety (Stamenkovic et al., 2018). Previous negative medical experiences may further increase susceptibility to anxiety, while inadequate information about the procedure may contribute to feelings of uncertainty and loss of control (Caumo et al., 2001).

Mechanisms

Preoperative anxiety develops when children perceive surgery as a threatening event characterized by fear of anesthesia, pain, surgical complications, and uncertainty regarding the procedure (Kain & Caldwell-Andrews, 2005). These fears generate anticipatory anxiety and emotional distress before surgery, which can subsequently activate physiological stress-response pathways.

At the psychological level, preoperative anxiety can be understood through cognitive appraisal processes in which individuals evaluate a situation as potentially threatening and assess their ability to cope with it. Anxiety is characterized by an intense sense of worry, tension, or apprehension related to anticipated negative events (Saviola et al., 2020). A key mechanism underlying this process is maladaptive conscious threat appraisal, including catastrophizing and worry. Catastrophizing involves overestimating the likelihood or severity of negative outcomes, while worry refers to repetitive negative thinking about uncertain future events (Kalisch & Gerlicher, 2014). These cognitive patterns are particularly relevant in the preoperative context, where individuals may anticipate pain, complications, or loss of control despite limited objective information. Within this framework, a distinction can be made between state anxiety and trait anxiety. State anxiety refers to a transient emotional response to a specific stressor, such as an upcoming surgical procedure, whereas trait anxiety reflects a more stable personality disposition toward perceiving situations as threatening and responding with heightened worry and vigilance (Saviola et al., 2020). Individuals with higher trait anxiety may be more likely to interpret medical settings and surgical procedures as threatening, resulting in amplified state anxiety responses when facing perioperative stressors. Thus, preoperative anxiety may arise from the interaction between stable individual predispositions and situational threat appraisal, with maladaptive cognitive processes reinforcing anticipatory worry and emotional distress.

After a surgical situation is appraised as threatening at the psychological level, coordinated neurobiological stress systems become activated to prepare the body for a perceived challenge. This response involves large-scale brain networks that regulate emotional salience and self-referential processing, shifting the brain from a resting state toward heightened environmental monitoring and emotional responsiveness (Saviola et al., 2020). Within these networks, the rostral dorsal anterior cingulate cortex and dorsomedial prefrontal cortex play

important roles in the conscious evaluation and sustained monitoring of perceived threats. Hyperactivation of these regions has been associated with heightened anxiety and maladaptive threat processing, contributing to persistent attentional focus on potential danger (Kalisch & Gerlicher, 2014). This sustained neural activity engages downstream autonomic pathways, resulting in activation of both the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (Benarroch, 1993). Sympathetic activation produces the classic "fight-or-flight" response, characterized by increased heart rate, elevated blood pressure, pupil dilation, inhibition of digestive and urinary activity, and increased sweating (Bates et al., 2022). Concurrently, activation of the HPA axis stimulates cortisol release, prolonging the physiological stress response and maintaining heightened vigilance during periods of perceived threat (Benarroch, 2025). Together, these neurobiological processes translate psychological threat perception into the physiological manifestations commonly observed in patients experiencing preoperative anxiety.

Given the variability in preoperative anxiety and its potential effects on perioperative outcomes, accurate identification and assessment are important in pediatric patients. Preoperative anxiety can be assessed using validated tools such as the Modified Yale Preoperative Anxiety Scale (m-YPAS), an observational measure designed to assess anxiety in children in the perioperative setting (Kain et al., 1997). The State-Trait Anxiety Inventory for Children (STAIC) can also be used to evaluate state and trait anxiety in pediatric patients (Vieco-García et al., 2021). These screening tools can help clinicians identify children experiencing elevated anxiety and determine appropriate psychological support before surgery (Vieco-García et al., 2021).

Management: Non-pharmacological Methods

Non-pharmacological interventions are commonly used approaches for managing preoperative anxiety in pediatric patients and have been shown to be effective in reducing anxiety across multiple psychological and behavioral strategies (Chen et al., 2025).

In clinical practice, various non-pharmacological interventions are frequently used by anesthesiologists for both pediatric and adult patients. For example, a survey of Korean anesthesiologists reported that 46.3% preferred non-pharmacological methods, with 14.6% reporting no clear preference for pediatric anesthesia (Wang et al., 2022). Similarly, a study from the United Kingdom found that 95% of anesthesiologists reported using communication and reassurance as their primary strategy to reduce preoperative anxiety in adult patients (Wang et al., 2022). These findings highlight the prominence of effective communication in anxiety management and suggest that developmentally appropriate reassurance may also play a valuable role in reducing anxiety among pediatric cases.

These approaches include a range of psychological, behavioral, and complementary techniques such as abdominal or deep breathing, progressive muscle relaxation, guided imagery, mindfulness-based strategies, and music therapy (Wang et al., 2022). These interventions are thought to reduce physiological arousal by decreasing sympathetic nervous system activity and promoting parasympathetic activity. This shift in autonomic nervous system activity promotes a

more relaxed physiological state and may help reduce anxiety-related symptoms such as increased heart rate, elevated blood pressure, and rapid breathing (VA.gov | Veterans Affairs, 2016).

Cognitive Behavioral therapy (CBT) is a time-limited, goal-oriented form of psychotherapy that has been extensively researched across multiple psychiatric disorders, including anxiety and depression. It focuses on identifying and modifying maladaptive patterns of thinking and behavior that contribute to psychological distress, with the goal of improving emotional regulation and reducing functional impairment (Coffey et al., 2015). In the perioperative setting, CBT-based interventions have been adapted to help patients manage anxiety related to surgery, anesthesia, and postoperative outcomes (Wang et al., 2022).

CBT targets maladaptive cognitive and behavioral processes underlying psychological distress through the identification and restructuring of dysfunctional thoughts and the reduction of avoidance behaviors using exposure-based strategies (Coffey et al., 2015). CBT integrates both cognitive and behavioral principles, where cognitive therapy addresses misperceptions and maladaptive beliefs about the self and environment, while behavioral therapy focuses on modifying learned patterns of avoidance and reinforcing more adaptive behaviors (Hendriks et al., 2024). Exposure, a core component of CBT for anxiety, is based on extinction learning and helps patients reduce fear responses by directly confronting anxiety-provoking stimuli rather than avoiding them (Hendriks et al., 2024).

The effectiveness of CBT in reducing anxiety has also been demonstrated in perioperative populations, where it has been associated with improvements in preoperative anxiety, physiological stress responses, and postoperative outcomes. *A meta-analysis found that perioperative psychological interventions reduced postoperative anxiety (d=-0.33, 95% CI -0.54 to -0.11) and pain intensity (d= -0.45, 95% CI -0.77 to -0.13); Moderator analyses showed that cognitive behavioral therapy was among the most effective psychological modalities for surgical patients*, *and interventions delivered by psychologists produced larger effect sizes compared to those delivered by other professionals* (Castaño-Asins et al., 2*025).* Evidence from adult surgical populations also supports the potential effectiveness of CBT in reducing preoperative psychological distress. In patients undergoing coronary artery bypass graft surgery, a brief CBT intervention targeting anxiety and depression was found to be feasible and effective in improving preoperative emotional symptoms (Dao et al., 2011). Similarly, in adult bariatric surgery candidates, a ten-week CBT program significantly reduced preoperative anxiety and depressive symptoms compared with standard care (Gade et al., 2015). However, these findings may not be directly generalizable to pediatric patients, highlighting the need for further research examining CBT-based interventions specifically in pediatric preoperative populations.

Cognitive-behavioral play therapy, an adapted form of CBT incorporating behavioral activation, modeling, and cognitive engagement, significantly reduced both subjective and physiological measures of preoperative anxiety in children, outperforming audiovisual distraction and conventional tell-show-do techniques (Rajeswari et al., 2019). Play-based cognitive-behavioral interventions have been shown across randomized trials to significantly

reduce anxiety in children undergoing invasive procedures compared to routine care, with effects observed across multiple standardized anxiety measurement scales (Halemani et al., 2022). In a randomized controlled trial of children aged 6–10 undergoing dental procedures, CBT-based play therapy involving structured play, drawing, and modeling of cooperative patient behavior produced the greatest reductions in preoperative anxiety (Rajeswari et al., 2019).

Digital CBT refers to technology-based adaptations of traditional cognitive behavioral therapy delivered through digital mental health technologies, such as internet-based interventions, smartphone applications, telehealth systems, and other digital platforms that extend psychological care beyond in-person settings (Torous et al., 2021). One major form of digital CBT is internet-delivered cognitive behavioral therapy (iCBT), a structured, online adaptation of CBT that delivers standardized treatment modules, often with asynchronous therapist guidance through digital platforms (Carlbring et al., 2018). Unlike traditional CBT, which is typically delivered through in-person sessions, digital CBT allows psychological interventions to be delivered remotely through digital platforms. Digital CBT may be particularly relevant for adolescents, who are highly familiar with smartphones and online platforms. Although most evidence for iCBT comes from studies of general anxiety disorder rather than preoperative anxiety specifically, these interventions have demonstrated anxiety-reducing effects comparable to face-to-face CBT when adequate therapist support is provided (Rūta Bakanaitė et al., 2025). Higher dropout rates remain a limitation of self-guided programs, suggesting that guided or hybrid digital formats may be more effective for younger patients. Because adolescents routinely engage with digital technologies, adapting CBT-based interventions for perioperative preparation represents a promising direction for future research in preoperative anxiety management.

Pharmacological Methods

Pharmacological approaches to preoperative anxiety can be broadly divided into medications used for immediate perioperative anxiolysis and sedation and medications used for the long-term management of underlying anxiety disorders. Benzodiazepines and α2-adrenergic agonists generally have relatively rapid effects and are commonly used as preoperative medications shortly before anesthesia, whereas selective serotonin reuptake inhibitors (SSRIs) have a slower onset of therapeutic effect and are primarily used for long-term anxiety management rather than immediate preoperative anxiolysis (Kulikov et al., 2018; Martins Esteves et al., 2026).

Benzodiazepines are the most commonly used pharmacological agents given prior to the induction of anesthesia. Midazolam serves as the standard premedication in many clinical settings; it can be given orally, intranasally, rectally, or intravenously, making it practical for pediatric use (Martins Esteves et al., 2026). Midazolam enhances γ-aminobutyric acid (GABA-A) receptor activity, producing anxiolytic, sedative, and amnestic effects. Despite its effectiveness, concerns remain regarding adverse effects such as paradoxical reactions, transient

respiratory depression, excessive sedation, and drowsiness (*DailyMed - MIDAZOLAM-Midazolam Injection, Solution*, 2023), which may limit its use in some pediatric patients.

Other pharmacological agents such as dexmedetomidine, an α2-adrenergic agonist with sedative and anxiolytic properties, has emerged as an alternative to benzodiazepines for premedication (Martins Esteves et al., 2026). Clinical evidence supports its effectiveness, demonstrating significant reductions in preoperative sedation scores and physiological stress responses, such as decreased cortisol levels following tracheal intubation. Additionally, patients receiving dexmedetomidine exhibit more stable hemodynamic profiles, with reductions in heart rate and mean arterial pressure during the peri-induction period, showing effective anxiolysis and attenuation of stress response (Xiong et al., 2022).

Beyond benzodiazepines and α2-adrenergic agonists, several alternative pharmacological agents have been explored for preoperative anxiolysis, including melatonin, ketamine, and clonidine (Martins Esteves et al., 2026). Melatonin, a melatonergic agent acting on MT1 and MT2 receptors, has been investigated as a premedication option due to its mild sedative and anxiolytic effects with a favorable safety profile. Ketamine, an NMDA receptor antagonist, has also been explored in sub-anesthetic doses for preoperative anxiolysis. While it does provide analgesia, its use may be limited by psychomimetic side effects including hallucinations and dissociative emergence reactions (Martins Esteves et al., 2026). Finally, clonidine, another α2-adrenergic agonist, has been investigated as a pediatric premedication because it provides anxiolysis and sedation with minimal respiratory depression, although its slower onset and potential for hypotension and bradycardia may limit routine use (DailyMed - CLONIDINE HYDROCHLORIDE Tablet, 2021).

Other pharmacological classes are not used for acute preoperative sedation but may be relevant in patients with pre-existing anxiety disorders or chronic psychiatric conditions rather than for immediate perioperative anxiolysis. SSRIs are primarily used for long-term management of generalized anxiety disorder and other chronic anxiety conditions in pediatric patients (Connolly & Bernstein, 2007). Due to their delayed onset of therapeutic effect, they are not utilized for immediate anxiolysis (Kulikov et al., 2018). Similarly, agents such as beta-adrenergic blockers and gabapentinoids have been explored as adjunctive options for perioperative anxiety management (Boyce et al., 2021; Hong et al., 2022).

Limitations

This review has several limitations. First, preoperative anxiety was examined broadly across surgical contexts without differentiating between operation types, generalizing experiences under a single "preoperative" category that may differ meaningfully depending on procedure invasiveness, anesthesia type, and recovery expectations. Second, the majority of studies reviewed were conducted in Western populations, with comparatively limited representation of Asian pediatric populations, restricting the generalizability of these findings across cultural and healthcare contexts. Third, this review followed a narrative rather than a systematic methodology, meaning sources were selected thematically rather than through a

standardized search protocol; this approach supports broad synthesis across psychological, biological, and clinical themes but does not offer the same reproducibility as a systematic review.

Future Directions

Despite advances in the management of pediatric preoperative anxiety, several gaps in the current evidence remain. First, there is no single intervention that is universally effective, as anxiety responses vary considerably according to a child's age, developmental stage, temperament, previous medical experiences, parental anxiety, and the type of surgical procedure (Fortier et al., 2011). Consequently, selecting the most appropriate intervention may require an individualized approach. Future research should prioritize developing personalized, evidence-based strategies that account for individual patient characteristics rather than relying on a one-size-fits-all approach.

Second, expanding research on iCBT specifically for pediatric surgical populations may enhance access to psychological interventions while reducing barriers related to geography, scheduling, and healthcare resources (Carlbring et al., 2018). Moreover, optimizing therapist-guided and hybrid digital delivery models may improve treatment adherence and patient engagement, as these interventions have demonstrated higher completion rates than self-guided formats (79.09% vs. 48.17%) (Rūta Bakanaitė et al., 2025).

Third, further randomized controlled trials are needed to directly compare pharmacological and non-pharmacological interventions, as well as multimodal approaches that combine the two, to determine which strategies are most effective for different pediatric populations and surgical settings. Lastly, research should extend beyond immediate preoperative outcomes by evaluating long-term effects on postoperative pain, behavioral recovery, healthcare utilization, and overall patient and family well-being.

Collectively, these findings suggest that optimizing preoperative anxiety management in pediatric patients will likely require individualized, evidence-based approaches that integrate both pharmacological and psychological interventions while expanding research on accessible, scalable strategies such as digital CBT.

Clinical Implications and the Importance of Routine Screening

The high prevalence of preoperative anxiety and its association with adverse perioperative outcomes underscore the importance of identifying pediatric patients with clinically significant anxiety before surgery (Kassahun et al., 2022). Because anxiety is influenced by individual, developmental, and environmental factors, relying solely on clinical observation may fail to identify patients who would benefit from additional support (Liu et al., 2022). Standardized screening during preoperative assessment can facilitate early identification and allow healthcare providers to tailor interventions according to individual needs (Goebel & Mehdorn, 2018; Shepardson et al., 2026).

Several validated tools are available for assessing anxiety in pediatric patients (Vieco-García et al., 2021; Topalel et al., 2020). The m-YPAS is specifically designed to assess

preoperative anxiety in children through behavioral observation (Kain et al., 1997). Other measures, such as STAIC and the Spence Children's Anxiety Scale (SCAS), assess broader anxiety symptoms rather than anxiety specifically related to surgery (James et al., 2020). The GAD-7, in comparison, is primarily used to screen for generalized anxiety symptoms and is not specifically designed to assess pediatric preoperative anxiety (O'Connor et al., 2023). Therefore, selecting a screening tool based on the child's age, developmental level, and clinical context may improve the identification of clinically significant anxiety (Vieco-García et al., 2021).

Routine screening can also facilitate communication among surgeons, anesthesiologists, nurses, psychologists, child life specialists, patients, and families. Identifying anxiety before surgery allows healthcare teams to provide developmentally appropriate explanations, address misconceptions, involve caregivers in preparation, and establish individualized care plans (Turunen et al., 2017; Romito et al., 2020). Early identification may therefore support timely intervention and improve the perioperative experience for pediatric patients and their families.

Future research should determine which screening tools are most practical, reliable, and effective for routine use across different pediatric age groups and surgical settings. Standardized screening protocols could also establish when elevated anxiety scores should prompt additional psychological, behavioral, or pharmacological interventions. Such approaches may improve early identification while supporting more individualized and efficient perioperative care.

Conclusions

Preoperative anxiety is a common and clinically significant concern among pediatric patients undergoing surgery, shaped by individual factors such as temperament and preexisting anxiety, family-related factors such as parental anxiety, and situational factors such as previous medical experiences and the nature of the surgical procedure. As discussed above, preoperative anxiety has been associated with increased postoperative pain, delayed recovery, and greater emotional distress (Kain et al., 2000; Stamenkovic et al., 2018), underscoring the importance of early recognition and appropriate management in pediatric surgical care.

Several non-pharmacological interventions, such as cognitive behavioral therapy and play-based approaches, and pharmacological agents, such as midazolam and dexmedetomidine, have shown promise in reducing preoperative anxiety; however, evidence specific to pediatric populations remains more limited than in adults, and no single intervention has been established as universally effective (Fortier et al., 2011). Individualized management based on each child's developmental and psychological needs therefore remains important. Routine screening using developmentally appropriate assessment tools, such as the m-YPAS, may help identify patients who require additional support and guide the selection of appropriate interventions.

Future research should focus on developing standardized pediatric-specific screening protocols, directly comparing and combining pharmacological and non-pharmacological interventions across different age groups and surgical settings, and evaluating digital CBT and other scalable interventions, as well as the long-term effects of anxiety management on postoperative recovery and well-being.

Works Cited

Bass, V., Brown, F., Beiser, D. G., Peterson, T., Gibbons, R. D, & Nagele, P. (2022). Preoperative Assessment of Anxiety and Depression Using Computerized Adaptive Screening Tools: A Pilot Prospective Cohort Study. Anesthesia and Analgesia, 134(4), 853–857. https://doi.org/10.1213/ANE.0000000000005844

Bates, R. A., Militello, L., Barker, E., Villasanti, H. G., & Schmeer, K. (2022). Early childhood stress responses to psychosocial stressors: The state of the science. Developmental Psychobiology, 64(7). https://doi.org/10.1002/dev.22320

Benarroch, E. (2025). What Is the Role of Glucocorticoids in the Effects of Stress on the Brain? Neurology, 105(2). https://doi.org/10.1212/wnl.0000000000213874

BENARROCH, E. E. (1993). The Central Autonomic Network: Functional Organization, Dysfunction, and Perspective. Mayo Clinic Proceedings, 68(10), 988–1001. https://doi.org/10.1016/s0025-6196(12)62272-1

Boyce, T. G., Ballone, N. T., Certa, K. M., & Becker, M. A. (2021). The Use of β-Adrenergic Receptor Antagonists in Psychiatry: A Review. Journal of the Academy of Consultation-Liaison Psychiatry, 62(4), 404–412. https://doi.org/10.1016/j.jaclp.2020.12.009

Breuner, C. C., Alderman, E. M., Jewell, J. A., Breuner, C. C., Alderman, E. M., Jewell, J., Berlan, E., Chung, R. J., Colburn, M., Grubb, L. K., Lee, J., Wallace, S. B., Powers, M., Upadhya, K. K., Amies, A.-M., Hua, L. L., Vyver, E., Menon, S., Zapata, L. B., … Alexander, S. N. (2023). The Hospitalized Adolescent. Pediatrics, 151(2). https://doi.org/10.1542/peds.2022-060647

Carlbring, P., Andersson, G., Cuijpers, P., Riper, H., & Hedman-Lagerlöf, E. (2018). Internet-based vs. face-to-face cognitive behavior therapy for psychiatric and somatic disorders: an updated systematic review and meta-analysis. Cognitive Behaviour Therapy, 47(1), 1–18. https://doi.org/10.1080/16506073.2017.1401115

Castaño-Asins, J. R., Barceló-Soler, A., Estíbaliz Royuela-Colomer, Sanabria-Mazo, J. P., García, V., Neblett, R., Bulbena, A., Víctor Pérez-Solà, Montes-Pérez, A., Urrútia, G., Feliu-Soler, A., & Luciano, J. V. (2025). Effectiveness of peri-operative psychological interventions for the reduction of postsurgical pain intensity, depression, anxiety and pain catastrophising. European Journal of Anaesthesiology. https://doi.org/10.1097/eja.0000000000002157

Caumo, W., Schmidt, A. P., Schneider, C. N., Bergmann, J., Iwamoto, C. W., Bandeira, D., & Ferreira, M. B. C. (2001). Risk factors for preoperative anxiety in adults. Acta Anaesthesiologica Scandinavica, 45(3), 298–307. https://doi.org/10.1034/j.1399-6576.2001.045003298.x

Chen, H., Zhang, J., Li, S., Zhang, H., & Wei, L. (2025). Non‐pharmacological Interventions for Preoperative Anxiety in Children: A Systematic Review and Network Meta‐Analysis. Journal of Clinical Nursing, 34(4). https://doi.org/10.1111/jocn.17582

Chow, C. H. T., Rizwan, A., Xu, R., Poulin, L., Bhardwaj, V., Van Lieshout, R. J., Buckley, N., & Schmidt, L. A. (2019). Association of Temperament With Preoperative Anxiety in

Pediatric Patients Undergoing Surgery. JAMA Network Open, 2(6), e195614. https://doi.org/10.1001/jamanetworkopen.2019.5614

Coffey, S. F., Banducci, A. N., & Vinci, C. (2015). Common Questions About Cognitive Behavior Therapy for Psychiatric Disorders. American Family Physician, 92(9), 807–812. https://pubmed.ncbi.nlm.nih.gov/26554473/

Connolly, S. D., & Bernstein, G. A. (2007). Practice Parameter for the Assessment and Treatment of Children and Adolescents With Anxiety Disorders. Journal of the American Academy of Child & Adolescent Psychiatry, 46(2), 267–283. https://doi.org/10.1097/01.chi.0000246070.23695.06

DailyMed - CLONIDINE HYDROCHLORIDE tablet. (2021). Nih.Gov. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=c83e4e4f-bd0e-449d-958a-dc0d570a01ba

DailyMed - MIDAZOLAM- midazolam injection, solution. (2023). Nih.Gov. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=9221894f-d840-46f2-a11c-6b71358d3b5b#Section_5

Dao, T. K., Youssef, N. A., Armsworth, M., Wear, E., Papathopoulos, K. N., & Gopaldas, R. (2011). Randomized controlled trial of brief cognitive behavioral intervention for depression and anxiety symptoms preoperatively in patients undergoing coronary artery bypass graft surgery. The Journal of Thoracic and Cardiovascular Surgery, 142(3), e109–e115. https://doi.org/10.1016/j.jtcvs.2011.02.046

Dziadzko, M., Mazard, T., Bonhomme, M., Raffin, M., Pradat, P., Forcione, J.-M., Minjard, R., & Aubrun, F. (2022). Preoperative Anxiety in the Surgical Transfer and Waiting Area: A Cross-Sectional Mixed Method Study. Journal of Clinical Medicine, 11(9), 2668. https://doi.org/10.3390/jcm11092668

Fortier, M. A., Martin, S. R., MacLaren Chorney, J., Mayes, L. C., & Kain, Z. N. (2011). Preoperative anxiety in adolescents undergoing surgery: a pilot study. Pediatric Anesthesia, 21(9), 969–973. https://doi.org/10.1111/j.1460-9592.2011.03593.x

Gade, H., Friborg, O., Rosenvinge, J. H., Småstuen, M. C., & Hjelmesæth, J. (2015). The Impact of a Preoperative Cognitive Behavioural Therapy (CBT) on Dysfunctional Eating Behaviours, Affective Symptoms and Body Weight 1 Year after Bariatric Surgery: A Randomised Controlled Trial. Obesity Surgery, 25(11), 2112–2119. https://doi.org/10.1007/s11695-015-1673-z

Goebel, S., & Mehdorn, H. M. (2018). Assessment of preoperative anxiety in neurosurgical patients: Comparison of widely used measures and recommendations for clinic and research. Clinical Neurology and Neurosurgery, 172, 62–68. https://doi.org/10.1016/j.clineuro.2018.06.036

Halemani, K., Issac, A., Mishra, P., Dhiraaj, S., Mandelia, A., & Mathias, E. (2022). Effectiveness of Preoperative Therapeutic Play on Anxiety Among Children Undergoing Invasive Procedure: a Systematic Review and Meta-analysis. Indian Journal of Surgical Oncology, 13(4). https://doi.org/10.1007/s13193-022-01571-1

Hendriks, G.-J., Janssen, N., Robertson, L., van Balkom, A. J., van Zelst, W. H., Wolfe, S., Oude Voshaar, R. C., & Uphoff, E. (2024). Cognitive behavioural therapy and third-wave approaches for anxiety and related disorders in older people. Cochrane Database of Systematic Reviews, 2024(7). https://doi.org/10.1002/14651858.cd007674.pub3

Hong, J. S. W., Atkinson, L. Z., Al-Juffali, N., Awad, A., Geddes, J. R., Tunbridge, E. M., Harrison, P. J., & Cipriani, A. (2022). Gabapentin and pregabalin in bipolar disorder, anxiety states, and insomnia: Systematic review, meta-analysis, and rationale. Molecular Psychiatry, 27(3), 1339–1349. https://doi.org/10.1038/s41380-021-01386-6

James, A. C., James, G., Cowdrey, F. A., Soler, A., & Choke, A. (2020). Cognitive behavioural therapy for anxiety disorders in children and adolescents. Cochrane Database of Systematic Reviews, 2(2). https://doi.org/10.1002/14651858.cd004690.pub4

Julian, L. J. (2011). Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). Arthritis Care & Research, 63(S11), S467–S472. https://doi.org/10.1002/acr.20561

Kain, Z. N., & Caldwell-Andrews, A. A. (2005). Preoperative Psychological Preparation of the Child for Surgery: An Update. Anesthesiology Clinics of North America, 23(4), 597–614. https://doi.org/10.1016/j.atc.2005.07.003

Kain, Z. N., Mayes, L. C., Caldwell-Andrews, A. A., Karas, D. E., & McClain, B. C. (2006). Preoperative Anxiety, Postoperative Pain, and Behavioral Recovery in Young Children Undergoing Surgery. Pediatrics, 118(2), 651–658. https://doi.org/10.1542/peds.2005-2920

Kain, Z. N., Mayes, L. C., Cicchetti, D. V., Bagnall, A. L., Finley, J. D., & Hofstadter, M. B. (1997). The Yale Preoperative Anxiety Scale. Anesthesia & Analgesia, 85(4), 783–788. https://doi.org/10.1097/00000539-199710000-00012

Kalisch, R., & Gerlicher, A. M. V. (2014). Making a mountain out of a molehill: On the role of the rostral dorsal anterior cingulate and dorsomedial prefrontal cortex in conscious threat appraisal, catastrophizing, and worrying. Neuroscience & Biobehavioral Reviews, 42, 1–8. https://doi.org/10.1016/j.neubiorev.2014.02.002

Kassahun, W. T., Mehdorn, M., Wagner, T. C., Babel, J., Danker, H., & Gockel, I. (2022). The effect of preoperative patient-reported anxiety on morbidity and mortality outcomes in patients undergoing major general surgery. Scientific Reports, 12(1). https://doi.org/10.1038/s41598-022-10302-z

Kisielewska, W., Kościółek, M., Kowalczyk, W., Mitura, B., Mitura, L., Rogula, S., Leszczyński, P. K., Antosik, K., & Mitura, K. (2025). Decreasing Preoperative Anxiety in Patients with Newly Available Multimodal Approaches—A Narrative Review. Journal of Clinical Medicine, 14(9), 2940. https://doi.org/10.3390/jcm14092940

Kulikov, A. V., Gainetdinov, R. R., Ponimaskin, E., Kalueff, A. V., Naumenko, V. S., & Popova, N. K. (2018). Interplay between the key proteins of serotonin system in SSRI antidepressants efficacy. Expert Opinion on Therapeutic Targets, 22(4), 319–330. https://doi.org/10.1080/14728222.2018.1452912

Li, X.-R., Zhang, W.-H., Williams, J. P., Li, T., Yuan, J.-H., Du, Y., Liu, J.- De, Wu, Z., Xiao, Z.-Y., Zhang, R., Liu, G.-K., Zheng, G.-R., Zhang, D.-Y., Ma, H., Guo, Q.-L., & An, J.-X. (2021). A multicenter survey of perioperative anxiety in China: Pre- and

postoperative associations. Journal of Psychosomatic Research, 147, 110528. https://doi.org/10.1016/j.jpsychores.2021.110528

Liang, Y., Huang, W., Hu, X., Jiang, M., Liu, T., Yue, H., & Li, X. (2021). Preoperative anxiety in children aged 2–7 years old: a cross-sectional analysis of the associated risk factors. Translational Pediatrics, 10(8), 2024–2034. https://doi.org/10.21037/tp-21-215

Lin, C.-J., Liu, H.-P., Wang, P.-Y., Yu, M.-H., Lu, M.-C., Hsieh, L.-Y., & Lin, T.-C. (2018). The Effectiveness of Preoperative Preparation for Improving Perioperative Outcomes in Children and Caregivers. Behavior Modification, 43(3), 311–329. https://doi.org/10.1177/0145445517751879

Liu, W., Xu, R., Jia, J., Shen, Y., Li, W., & Bo, L. (2022). Research Progress on Risk Factors of Preoperative Anxiety in Children: A Scoping Review. International Journal of Environmental Research and Public Health, 19(16), 9828. https://doi.org/10.3390/ijerph19169828

Lööf, G., & Lönnqvist, P. A. (2022). Role of information and preparation for Improvement of Pediatric Perioperative Care. Pediatric Anesthesia, 32(5). https://doi.org/10.1111/pan.14419

Marquez, J. L., Wang, E., Rodriguez, S. T., O'Connell, C., Munshey, F., Darling, C., Tsui, B., Caruso, J., & Caruso, T. J. (2020). A retrospective cohort study of predictors and interventions that influence cooperation with mask induction in children. Pediatric Anesthesia, 30(8), 867–873. https://doi.org/10.1111/pan.13930

Martins Esteves, I., Pires, R., Pestana-Santos, M., Sampaio, F., & Reis Santos, M. (2026). Preoperative interventions for reducing anxiety in school-age children undergoing surgery: a network meta-analysis. Cochrane Database of Systematic Reviews, 2026(2). https://doi.org/10.1002/14651858.cd016330

Moura, L. A. de, Dias, I. M. G., & Pereira, L. V. (2016). Prevalence and factors associated with preoperative anxiety in children aged 5-12 years. Revista Latino-Americana de Enfermagem, 24(0). https://doi.org/10.1590/1518-8345.0723.2708

Ni, K., Zhu, J., & Ma, Z. (2023). Preoperative anxiety and postoperative adverse events: a narrative overview. Anesthesiology and Perioperative Science, 1(3). https://doi.org/10.1007/s44254-023-00019-1

O'Connor, E. A., Henninger, M. L., Perdue, L. A., Coppola, E. L., Thomas, R. G., & Gaynes, B. N. (2023). Anxiety Screening. JAMA. https://doi.org/10.1001/jama.2023.6369

Rajeswari, S. R., Chandrasekhar, R., Vinay, C., Uloopi, K., RojaRamya, K. S., & Ramesh, M. V. (2019). Effectiveness of Cognitive Behavioral Play Therapy and Audiovisual Distraction for Management of Preoperative Anxiety in Children. International Journal of Clinical Pediatric Dentistry, 12(5), 419–422. https://doi.org/10.5005/jp-journals-10005-1661

Romito, B., Jewell, J., & Jackson, M. (2020). Child Life Services. Pediatrics, 147(1), e2020040261. https://doi.org/10.1542/peds.2020-040261

Rūta Bakanaitė, Indrė Bakanienė, & Kanyeredzi, A. (2025). Long-Term Effectiveness of Internet-Based Versus Traditional Cognitive Behavioral Therapy Across Psychiatric Disorders: A Systematic Review and Meta-Analysis. PubMed. https://doi.org/10.1177/15305627251358855

Saviola, F., Pappaianni, E., Monti, A., Grecucci, A., Jovicich, J., & De Pisapia, N. (2020). Trait and state anxiety are mapped differently in the human brain. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-68008-z

Sawyer, S. M., Azzopardi, P. S., Wickremarathne, D., & Patton, G. C. (2018). The Age of Adolescence. The Lancet Child & Adolescent Health, 2(3), 223–228. https://doi.org/10.1016/s2352-4642(18)30022-1

Shafer, A., Fish, M. P., Gregg, K. M., Seavello, J., & Kosek, P. (1996). Preoperative Anxiety and Fear. Anesthesia & Analgesia, 83(6), 1285–1291. https://doi.org/10.1097/00000539-199612000-00027

Shepardson, R. L., Khan, J. S., Buckheit, K. A., & Funderburk, J. S. (2026). Treatment of Anxiety for Adults in Primary Care Settings. JAMA Internal Medicine. https://doi.org/10.1001/jamainternmed.2026.0395

Topalel, S., Orekici Temel, G., & Azizoglu, M. (2020). Evaluation of Preoperative Anxiety in Turkish Paediatric Patients and Validity and Reliability of the Turkish Modified Yale Preoperative Anxiety Scale. Turkish Journal of Anaesthesiology and Reanimation, 48(6), 484–490. https://doi.org/10.5152/tjar.2020.116

Torous, J., Bucci, S., Bell, I. H., Kessing, L. V., Faurholt‐Jepsen, M., Whelan, P., Carvalho, A. F., Keshavan, M., Linardon, J., & Firth, J. (2021). The Growing Field of Digital psychiatry: Current Evidence and the Future of apps, Social media, chatbots, and Virtual Reality. World Psychiatry, 20(3), 318–335. https://doi.org/https://doi.org/10.1002/wps.20883

Turunen, E., Miettinen, M., Setälä, L., & Vehviläinen-Julkunen, K. (2017). An integrative review of a preoperative nursing care structure. Journal of Clinical Nursing, 26(7–8), 915–930. https://doi.org/10.1111/jocn.13448

VA.gov | Veterans Affairs. (2016). Va.Gov. https://www.va.gov/WHOLEHEALTHLIBRARY/tools/diaphragmatic-breathing.asp?ut

Vieco-García, A., López-Picado, A., Fuentes, M., Francisco-González, L., Joyanes, B., Soto, C., Garcia de la Aldea, A., Gonzalez-Perrino, C., & Aleo, E. (2021). Comparison of different scales for the evaluation of anxiety and compliance with anesthetic induction in children undergoing scheduled major outpatient surgery. Perioperative Medicine, 10(1). https://doi.org/10.1186/s13741-021-00228-x

Wang, R., Huang, X., Wang, Y., & Akbari, M. (2022). Non-pharmacologic Approaches in Preoperative Anxiety, a Comprehensive Review. Frontiers in Public Health, 10(854673). https://doi.org/10.3389/fpubh.2022.854673

Xiong, J., Gao, J., Pang, Y., Zhou, Y., Sun, Y., & Sun, Y. (2022). Dexmedetomidine premedication increases preoperative sedation and inhibits stress induced by tracheal intubation in adults: a prospective randomized double-blind clinical study. 22(1). https://doi.org/10.1186/s12871-022-01930-z

The Role of Amyloid-Beta Plaques in Disrupting Neural Communication in Alzheimer's Disease By Aditya P. Reddy

Abstract

Alzheimer's Disease (AD) is defined as a particular onset and course of cognitive and functional decline. Central to AD pathology is the abnormal processing of amyloid precursor proteins (APPs), which produces amyloid-beta (Aβ) peptides prone to misfolding and aggregation. Soluble Aβ oligomers are now also recognized as the earliest and most potent disruptors of synaptic communication, leading to the impairment of receptor function and long-term potentiation. These amyloidogenic events serve as an upstream trigger for the hyperphosphorylation and aggregation of tau. As Aβ accumulation progresses, it activates kinases that drive tau hyperphosphorylation, misfolding, and neurofibrillary tangle formation. These effects destabilize microtubules and interrupt axonal transport. The combined effect of Aβ-induced synaptic toxicity, tau-mediated structural breakdown, and chronic neuroinflammation leads to the progressive loss of neural connectivity characteristic of Alzheimer's Disease. This paper will examine how Aβ plaques create disruptions in neural signaling, enabling the transition of tau from a microtubule-stabilizing protein to a neurotoxic aggregate. By evaluating Aβ as the initiator of synaptic stress and tau as the primary driver for structural neurodegeneration and clinical decline, this review aims to show that the loss of neural connectivity in AD is the result of this interdependent proteinopathy, which is further exaggerated by chronic neuroinflammation.

Keywords Psychology, Neuroscience, Cognitive Decline, Alzheimer's Disease, Amyloid-Beta, Tau

Introduction

AD is a neurodegenerative disease that progressively destroys memory, cognitive skills, and characteristic behavior. The disease was first described by Alois Alzheimer in 1906 based on post-mortem findings of extracellular amyloid-beta plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau (Alzheimer, 1907; Kraepelin, 1910). Currently, AD is considered one of the most prevalent causes of dementia, wherein impaired synaptic signaling, along with disrupted neural networks, forms the core of its pathophysiology (Selkoe & Hardy, 2016).

Amyloid precursor protein (APP) is a transmembrane protein concentrated at synapses, where it participates in normal neuronal communication and signaling. APP cleavage via the amyloidogenic pathway results in the production of Aβ peptides of variable length and stability, and among these, soluble Aβ oligomers are the most synaptotoxic. Given its known interference with neurotransmission, weakening of long-term potentiation (LTP), and increased susceptibility to tau-related cytoskeletal breakdown, clarifying the relationship between Aβ and tau is essential

to understanding AD pathophysiology and the early disruptions in neural communication that drive Alzheimer's disease, helping shape future therapeutic approaches.

Discussion

Section 1: Formation of Amyloid-Beta Plaques

1.1 - Origins of Amyloid Pathology: The Amyloid Precursor Protein

Amyloid Precursor Proteins (APPs) are considered the first step to the formation of Amyloid-Beta (Aβ) Plaques. APP is synthesized in the endoplasmic reticulum (ER), where it is subject to initial folding and post-translational modifications before being transported via the secretory pathway to the trans-Golgi network (TGN), where it undergoes maturation before being transported (Lai et al., 1995). From the TGN, mature APP is delivered to the plasma membrane. Still, it is only transiently retained at the cell surface, as the majority of APP is rapidly internalized into early endosomes through regulated endocytic mechanisms. Within early endosomes, the APP enters a critical sorting phase where it may be recycled to the plasma membrane, directed toward lysosomal degradation, or retrieved to the TGN via "retromer-mediated retrograde transport" (Zhang & Song, 2013). This sorting step is crucial because it determines the exposure of APP to secretases (enzymes that cleave APP) and, consequently, whether APP will follow a non-pathogenic or potentially pathogenic pathway. In fact, alterations in APP trafficking, such as defective endosome-to-TGN retrieval, have been shown to increase the likelihood that APP is processed via the amyloidogenic pathway (i.e., leading eventually to Aβ production) in cell and animal models of AD (Small et al., 2005), underscoring the importance of trafficking processes before any cleavage events (in-text citation). Taken together, these data demonstrate that APP's cellular journey, from synthesis, maturation, membrane insertion, endocytosis, and sorting, is an essential upstream determinant of whether APP remains essentially harmless or is overprocessed into pathological Aβ peptides in AD.

1.2 - Secretases

Another step vital to understanding the production and structure of AB Plaques is the secretases that are produced. The critical step in the production of Aβ concerns how APP is cut by secretase enzymes. Which secretase cuts APP and where this happens inside the cell determines whether APP is broken down into innocuous fragments or into Aβ peptides with the potential to drive disease. In the non-amyloidogenic pathway, α-secretase cuts in the middle of the Aβ region, which prevents Aβ from forming at all. This action releases sAPPα, a soluble fragment often linked to protective effects in neurons (Selkoe & Hardy, 2016; Postina, 2008). In the amyloidogenic pathway, β-secretase (BACE1) makes the first cut at the start of the Aβ sequence, producing the membrane-bound C99 fragment. Then, γ-secretase cuts C99 within the membrane to release Aβ peptides of different lengths (Vassar et al., 1999; De Strooper &

Annaert, 2010). The γ-secretase complex, whose core includes presenilin, also determines exactly how long the final Aβ peptide will be, and disease-related presenilin mutations tend to increase production of longer, more aggregation-prone forms such as Aβ42 (De Strooper & Annaert, 2010). Which pathway dominates is greatly dependent on the cellular location of APP and the secretases. For instance, when APP is pulled into acidic endosomes, the function of BACE1 is more efficient, which in turn shifts the processing toward the production of Aβ (Nixon, 2005; Yan & Vassar, 2014). Consequently, relatively small changes in secretase levels, activity, or localization can strongly affect how much Aβ accrues during aging or disease (Vassar et al., 1999; Yan & Vassar, 2014).

1.3 - Beta-Amyloid Peptides

One important thing to keep note of when discussing Aβ peptides is that they aren't a single, uniform species across the entire system. Rather, they are a species where their length and chemical features govern characteristics such as their solubility, propensity to misfold, and their interactions with other cellular components. These are all factors that determine the neurotoxicity of the peptide and, hence, are important to understand.

The two major forms of Aβ in the brain are Aβ40 and Aβ42. Aβ42 has about two additional hydrophobic amino acids at its tail end, making it more prone to β-sheet formation, a structural pattern that favors aggregation (Selkoe & Hardy, 2016; Benilova et al., 2012). While being amphipathic, that is, having water-loving and a water-repelling part, a single molecule of Aβ interacts with cellular membranes, metal ions such as copper and zinc, as well as other Aβ molecules, strongly favoring how the peptide folds and how fast it forms oligomers and fibrils (Knowles et al., 2014; Haass & Selkoe, 2007).

Chemical modifications to Aβ, such as oxidation or pyroglutamate formation, and conditions in the environment, such as pH, salt concentrations, or presence of lipid membranes, are additional factors that can further drive the peptide toward shapes more disposed to aggregation. The fact that certain mutations to APP or presenilin increase the relative production of Aβ42 underlines how important peptide length and its chemistry are in defining disease.

1.4 - Oligomerization

The next step in the formation of Beta-Amyloid Plaques is the formation of oligomers, which are small soluble Aβ aggregates that form first as the main early drivers of synaptic problems in Alzheimer's disease. Not only do they interfere with learning by blocking long-term potentiation, but they also lead to tau hyperphosphorylation (later discussed in this review). Studies in experimental models and human tissue indicate that soluble Aβ oligomers, from dimers and trimers to larger assemblies, disrupt synaptic signaling and correlate much better with cognitive decline than the amount of plaque in the brain (Shankar et al., 2008; Lambert et al., 1998). Their formation follows nucleation dynamics, with a small, unstable "seed" first forming, then recruiting additional Aβ molecules to grow (Cohen et al., 2013; Knowles et al., 2014). These oligomers are structurally diverse. Whereas some oligomers go on to become fibrils,

others never form fibrils but are still highly toxic (Kayed et al., 2003). Environments that concentrate Aβ, such as lipid raft membrane regions, cell surfaces, or areas rich in metal ions, can accelerate oligomer formation by stabilizing aggregation-prone shapes (Benilova et al., 2012; Kayed et al., 2003). Importantly, Aβ oligomers extracted from Alzheimer's brains block long-term potentiation and impair memory when introduced into experimental systems, providing direct evidence for their pathogenic role (Shankar et al., 2008; Lambert et al., 1998).
1.5: Fibril formation, β-sheet assembly, and Plaque Deposition

With further assembly, Aβ oligomers may transition into more ordered β-sheet–rich fibrils. These large structures are highly stable and rigid in nature, thus providing the cores for amyloid plaques. The mechanism of fibril formation involves a process termed nucleation-dependent polymerization. If a small seed is present, there can be rapid addition of monomers, while secondary nucleation on the surface of existing fibrils can further accelerate aggregation (Cohen et al., 2013; Knowles et al., 2014). Such fibrils adopt the characteristic cross-β architecture and are resistant to normal protein-clearing mechanisms, thus enabling persistence in the extracellular space (Knowles et al., 2014).

Although fibrils diffuse less readily than soluble oligomers, they can act as long-term reservoirs, slowly releasing small toxic assemblies and altering the local environment example, by binding metal ions or disturbing ionic balance-which can maintain or amplify neurotoxicity (). Over the years, these fibrils accumulate as macroscopic amyloid plaques, typically consisting of a dense fibrillar core surrounded by reactive glia and damaged neurites. Neuropathological studies consistently show both diffuse and dense-core plaques in AD brains, usually accompanied by dystrophic neurites and clusters of activated microglia and astrocytes. Although plaque burden is one of the two hallmarks of AD pathology, cognitive impairment tends to correlate more strongly with soluble oligomer levels than with plaque load, suggesting that plaques reflect chronic deposition, while oligomers are the more immediate drivers of synaptic injury.
Importantly, plaques also act as local hubs of inflammation: microglia and astrocytes accumulate around them, releasing cytokines and reactive species that can influence both APP processing and Aβ clearance to create a feedback loop further intensifying local pathology.

Section 2: Tau Pathology + Interaction with Aβ

2.1 - Tau Protein Dysfunction and Neurofibrillary Tangle Formation

Along with amyloid-beta plaques, Alzheimer's disease is also characterized by significant alterations inside neurons caused by the abnormal actions of tau, a protein that normally stabilizes the cell's transport system. In normal times, tau attaches to microtubules and assists in shuttling nutrients, organelles, and signaling molecules up and down long neuronal pathways. In the case of Alzheimer's, however, tau becomes hyperphosphorylated. In this condition, it has received too many phosphate groups and can no longer attach to the microtubules (Bloom, 2014). When tau lets go, microtubules break down, axonal transport stutters, and the internal

framework of the neuron starts to collapse. As hyperphosphorylated tau amasses, it misfolds and aggregates into paired helical filaments. Eventually, neurofibrillary tangles (NFT) are formed; these are a key pathological feature of Alzheimer's disease (Nelson et al., 2012). These tangles physically block the cell's interior and cause needed functions to malfunction. Unlike amyloid-beta plaques, which exist outside of neurons and would not necessarily kill the neuron right away, NFT buildup is indicative that the neuron is degenerating. Moreover, the extent and distribution of tau tangles significantly correlate with memory loss and cognitive decline. In fact, studies have shown it correlates more than plaque burden (Arriagada et al., 1992). All this evidence points to tau's major role in Alzheimer's symptoms.

2.2 - The Synergistic Interaction between Aβ and Tau Pathology

Although Aβ plaques and tau tangles were once viewed as separate processes, studies show they are closely linked. Aβ accumulation appears to come first, usually decades before symptoms, while tau tangles appear to be induced by the Aβ oligomers through activation of various kinases such as "GSK-3β" and "CDK5". These places phosphate groups on the tau at irregular sites and further push tau into a pathological state. Once tau is misfolded, it can spread between neurons in a prion-like manner, helping explain the stepwise progression of Alzheimer's pathology: from the entorhinal cortex, the hippocampus, and then the neocortex.

This can be an interaction that works both ways. For example, studies show that reducing tau in mouse models with high Aβ levels protects neurons from synaptic damage and memory impairment (Roberson et al., 2007). What this data suggests is that while Aβ may initiate the disease, tau is required for Aβ to exert its full toxicity. More recently, many researchers have started to think of Aβ and tau as part of a single reinforcing network. The idea goes that Aβ sparks the problem, while tau spreads and amplifies it.

2.3 - Impact of Tau on Neuronal Communication + Cognitive Decline

Once tau becomes dysfunctional, its impact on neuronal communication is far-reaching. With microtubules destabilized, axonal transport fails, preventing key molecules such as mitochondria, synaptic vesicles, and growth factors from reaching their destinations. Even small disruptions in this transport system can quickly amplify, weakening the synapses. It may also prevent long-term potentiation, a biological process that is very important for forming new memories from occurring (Hoover et al., 2010).

Tau pathology first impacts brain regions critical for memory, including the entorhinal cortex and hippocampus. These impacts are consistent with the early symptoms of Alzheimer's disease. Deficits in language, reasoning, and daily function emerge as tangles spread. Eventually, the far-reaching neuronal loss driven by tau leads to brain atrophy, characteristic of late-stage cognitive disease. Given that tau deposition strongly correlates with symptom severity, many consider tau to be a stronger pathological predictor of clinical decline alongside than Aβ.

2.4 - Comparing the Roles of Aβ and Tau in Neurodegeneration

Both Aβ and tau are critical factors in understanding the onset of Alzheimer's disease, but they do different things at different times within the sequence of the disease. On one hand, Aβ accumulation begins early and silently, thus often serving as a trigger for downstream pathology. Still, many studies have shown that a lot of people with extensive deposition of Aβ remain cognitively normal for years, according to Jack et al. in 2013. This data supports the idea that plaque alone is not enough to cause symptoms. Tau pathology, on the other hand, closely tracks cognitive decline. Once tau tangles begin spreading, neuronal dysfunction and memory loss become far more predictable and additionally difficult to reverse.

This distinction shapes current therapeutic strategies for Alzheimer's Disease. Early-stage interventions currently focus on reducing Aβ accumulation with the aim of preventing tau from becoming abnormal. Later in the intervention, treatments become progressively targeted at tau phosphorylation, tangle formation, and mechanisms that allow tau to spread. Considering Aβ as an upstream instigator and tau as a direct driver of neurodegeneration gives a clearer basis for explaining Alzheimer's progression and for designing stage-specific therapies.

Section 3: Aβ Plaques, Tau Pathology & Disruption of Neural Communication

3.1 Aβ Plaques to Tau Dysregulation

Aβ plaques mark one of the earliest disturbances in the brain and help set in motion the changes that eventually destabilize neurons. Although plaques form outside cells, the soluble Aβ oligomers associated with them interfere with normal synaptic signaling and place neurons under stress (Selkoe & Hardy, 2016; Haass & Selkoe, 2007). This stress, in turn, activates kinase enzymes like GSK-3β and CDK5 that add excessive phosphate groups to tau. Once hyperphosphorylated, tau loses its ability to stabilize microtubules and begins to detach, weakening the cell's internal structure (Ittner et al., 2010; Roberson et al., 2007). This allows Aβ plaques to initiate the molecular changes that push tau toward dysfunction.

3.2 Plaques Promote Tau Aggregation and Spread

After tau is hyperphosphorylated, neurons are far more vulnerable to tau misfolding and aggregation. Aβ plaques, along with the inflammation and oxidative stress they provoke, generate an environment that promotes tau aggregation into neurofibrillary tangles (Serrano-Pozo et al., 2011; Heneka, Golenbock & Latz, 2015). These tangles disrupt axonal transport, cutting off delivery of critical cargo, including mitochondria and neurotransmitter receptors. Aβ-induced synaptic stress and glial activation also contribute to the cell-to-cell dissemination of misfolded tau, further weakening communication between brain regions and hastening network failure (Roberson et al., 2007; Ittner et al., 2010).

3.3 Disruption of Synapses Directly by Aβ

Studies also show that Aβ disrupts synaptic function independent of plaque deposition. Specifically, soluble Aβ oligomers bind to the key synaptic receptors, interfering with calcium

homeostasis and blunting long-term potentiation, a cellular process underlying learning and memory (Haass & Selkoe, 2007; Selkoe & Hardy, 2016). This leads to weaker synaptic signaling, reduced stability of dendritic spines, and early disconnection of neural circuits. Consequently, plaques function not only as a long-term repository but also as a source of diffusible Aβ species that continue to impair synapses even before extensive tau pathology develops.

3.4 Sustained Neuroinflammation and Compromised Neural Homeostasis

Aβ plaques attract microglia that attempt to clear them, but, in the process, become chronically activated. Over time, these microglia release inflammatory cytokines and reactive oxygen species that damage synapses and stress neurons. This inflammatory environment further promotes tau misfolding and reduces the brain's ability to maintain and repair synaptic connections. As inflammation persists, neurons lose resilience, and communication between neural networks becomes increasingly compromised.

Conclusion

A huge question that has been brought up in the study of Alzheimer's Disease concerns the role Aβ plaques play in disrupting neural communication, specifically in Alzheimer's disease. In two decades of research, it has become clear that Aβ plaques disrupt neural communication by driving a series of molecular changes that weaken the foundations of healthy signaling. That process starts with how cells handle APP and whether it leads to the formation of longer, aggregation-prone Aβ peptides. These peptides assemble into soluble oligomers that interfere with synaptic function long before plaques appear. As these oligomers build up, they disturb receptor activity, impair long-term potentiation, and weaken the structural stability of dendritic spines.

Meanwhile, Aβ exposure turns on intracellular signaling pathways that induce tau to let go of microtubules and start to aggregate into tangles. Tau pathology now contributes prominently to neuronal dysfunction, crumbling transport systems within the cell, and diminishing the neuron's capability to sustain synaptic contacts. In this way, the interrelationship between Aβ and tau forms a reinforcing loop in which each process accelerates the injury caused by the other.

Where plaques have formed, they continue to emit soluble Aβ species and attract microglia and astrocytes into a chronic state of inflammation. Chronic inflammation further degrades synapses, promotes the spread of tau pathology, and diminishes the brain's capacity for repair. Over time, these combined effects wear down connectivity across neural networks and directly contribute to the progressive cognitive decline that characterizes Alzheimer's Disease.

In sum, Aβ plaques have a pivotal and multifaceted role in causing the breakdown of neural communication. They produce toxic oligomers, initiate tau-dependent structural collapse, promote inflammation, and compromise the synaptic systems on which healthy brain function depends. These mutually reinforcing mechanisms contribute to how Aβ pathology drives the

incremental and diffuse impairment of neural communication that characterizes Alzheimer's Disease.

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Works Cited

Alzheimer's disease. (2019). In Handbook of Clinical Neurology (Vol. 167, pp. 231–255). Elsevier. https://doi.org/10.1016/B978-0-12-804766-8.00013-3

Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory | Nature Medicine. (n.d.). Retrieved December 12, 2025, from https://www.nature.com/articles/nm1782

Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T., & Hyman, B. T. (n.d.). Neurofibrillary tangles but not senile plaques parallel duration and severiti of azheimer's disease.

Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T., & Hyman, B. T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology, 42(3), 631–631. https://doi.org/10.1212/WNL.42.3.631

Benilova, I., Karran, E., & De Strooper, B. (2012a). The toxic Aβ oligomer and Alzheimer's disease: An emperor in need of clothes. Nature Neuroscience, 15(3), 349–357. https://doi.org/10.1038/nn.3028

Benilova, I., Karran, E., & De Strooper, B. (2012b). The toxic Aβ oligomer and Alzheimer's disease: An emperor in need of clothes. Nature Neuroscience, 15(3), 349–357. https://doi.org/10.1038/nn.3028

Bloom, G. S. (2014a). Amyloid-β and Tau: The Trigger and Bullet in Alzheimer Disease Pathogenesis. JAMA Neurology, 71(4), 505–508. https://doi.org/10.1001/jamaneurol.2013.5847

Bloom, G. S. (2014b). Amyloid-β and Tau: The Trigger and Bullet in Alzheimer Disease Pathogenesis. JAMA Neurology, 71(4), 505. https://doi.org/10.1001/jamaneurol.2013.5847

Cohen, S. I. A., Linse, S., Luheshi, L. M., Hellstrand, E., White, D. A., Rajah, L., Otzen, D. E., Vendruscolo, M., Dobson, C. M., & Knowles, T. P. J. (2013). Proliferation of amyloid-β42 aggregates occurs through a secondary nucleation mechanism. Proceedings of the National Academy of Sciences, 110(24), 9758–9763. https://doi.org/10.1073/pnas.1218402110

Common Structure of Soluble Amyloid Oligomers Implies Common Mechanism of Pathogenesis | Science. (n.d.). Retrieved December 12, 2025, from https://www.science.org/doi/abs/10.1126/science.1079469

De Strooper, B., & Annaert, W. (2010). Novel Research Horizons for Presenilins and γ-Secretases in Cell Biology and Disease. Annual Review of Cell and Developmental Biology, 26(1), 235–260. https://doi.org/10.1146/annurev-cellbio-100109-104117

FallahBagheri, N., & Akan, Ö. B. (2025). A Molecular Communication Perspective of Alzheimer's Disease: Impact of Amyloid Beta Oligomers on Glutamate Diffusion in the Synaptic Cleft. IEEE Transactions on Molecular, Biological, and Multi-Scale Communications, 11(2), 186–200. https://doi.org/10.1109/TMBMC.2025.3552959

Haass, C., & Selkoe, D. J. (2007a). Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid β-peptide. Nature Reviews Molecular Cell Biology, 8(2), 101–112. https://doi.org/10.1038/nrm2101

Haass, C., & Selkoe, D. J. (2007b). Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid β-peptide. Nature Reviews Molecular Cell Biology, 8(2), 101–112. https://doi.org/10.1038/nrm2101

Haass, C., & Selkoe, D. J. (2007c). Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid β-peptide. Nature Reviews Molecular Cell Biology, 8(2), 101–112. https://doi.org/10.1038/nrm2101

Hartl, F. U. (2017). Protein Misfolding Diseases. Annual Review of Biochemistry, 86(Volume 86, 2017), 21–26. https://doi.org/10.1146/annurev-biochem-061516-044518

Heneka, M. T., Golenbock, D. T., & Latz, E. (2015a). Innate immunity in Alzheimer's disease. Nature Immunology, 16(3), 229–236. https://doi.org/10.1038/ni.3102

Heneka, M. T., Golenbock, D. T., & Latz, E. (2015b). Innate immunity in Alzheimer's disease. Nature Immunology, 16(3), 229–236. https://doi.org/10.1038/ni.3102

Hoover, B. R., Reed, M. N., Su, J., Penrod, R. D., Kotilinek, L. A., Grant, M. K., Pitstick, R., Carlson, G. A., Lanier, L. M., Yuan, L.-L., Ashe, K. H., & Liao, D. (2010). Tau Mislocalization to Dendritic Spines Mediates Synaptic Dysfunction Independently of Neurodegeneration. Neuron, 68(6), 1067–1081. https://doi.org/10.1016/j.neuron.2010.11.030

Ittner, L. M., Ke, Y. D., Delerue, F., Bi, M., Gladbach, A., Eersel, J. van, Wölfing, H., Chieng, B. C., Christie, M. J., Napier, I. A., Eckert, A., Staufenbiel, M., Hardeman, E., & Götz, J. (2010). Dendritic Function of Tau Mediates Amyloid-β Toxicity in Alzheimer's Disease Mouse Models. Cell, 142(3), 387–397. https://doi.org/10.1016/j.cell.2010.06.036

Jack, C. R., Knopman, D. S., Jagust, W. J., Petersen, R. C., Weiner, M. W., Aisen, P. S., Shaw, L. M., Vemuri, P., Wiste, H. J., Weigand, S. D., Lesnick, T. G., Pankratz, V. S., Donohue, M. C., & Trojanowski, J. Q. (2013). Tracking pathophysiological processes in Alzheimer's disease: An updated hypothetical model of dynamic biomarkers. The Lancet Neurology, 12(2), 207–216. https://doi.org/10.1016/S1474-4422(12)70291-0

Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W., & Glabe, C. G. (2003). Common Structure of Soluble Amyloid Oligomers Implies Common Mechanism of Pathogenesis. Science, 300(5618), 486–489. https://doi.org/10.1126/science.1079469

Knowles, T. P. J., Vendruscolo, M., & Dobson, C. M. (2014a). The amyloid state and its association with protein misfolding diseases. Nature Reviews Molecular Cell Biology, 15(6), 384–396. https://doi.org/10.1038/nrm3810

Knowles, T. P. J., Vendruscolo, M., & Dobson, C. M. (2014b). The amyloid state and its association with protein misfolding diseases. Nature Reviews Molecular Cell Biology, 15(6), 384–396. https://doi.org/10.1038/nrm3810

Lai, A., Sisodia, S. S., & Trowbridge, I. S. (1995). Characterization of Sorting Signals in the β-Amyloid Precursor Protein Cytoplasmic Domain (*). Journal of Biological Chemistry, 270(8), 3565–3573. https://doi.org/10.1074/jbc.270.8.3565

Lambert, M. P., Barlow, A. K., Chromy, B. A., Edwards, C., Freed, R., Liosatos, M., Morgan, T. E., Rozovsky, I., Trommer, B., Viola, K. L., Wals, P., Zhang, C., Finch, C. E., Krafft, G. A., & Klein, W. L. (1998). Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proceedings of the National Academy of Sciences, 95(11), 6448–6453. https://doi.org/10.1073/pnas.95.11.6448

Majd, S., Power, J. H., & Grantham, H. J. M. (2015). Neuronal response in Alzheimer's and Parkinson's disease: The effect of toxic proteins on intracellular pathways. BMC Neuroscience, 16(1), 69. https://doi.org/10.1186/s12868-015-0211-1

Nelson, P. T., Alafuzoff, I., Bigio, E. H., Bouras, C., Braak, H., Cairns, N. J., Castellani, R. J., Crain, B. J., Davies, P., Tredici, K. D., Duyckaerts, C., Frosch, M. P., Haroutunian, V., Hof, P. R., Hulette, C. M., Hyman, B. T., Iwatsubo, T., Jellinger, K. A., Jicha, G. A., … Beach, T. G. (2012). Correlation of Alzheimer Disease Neuropathologic Changes With Cognitive Status: A Review of the Literature. Journal of Neuropathology & Experimental Neurology, 71(5), 362–381. https://doi.org/10.1097/NEN.0b013e31825018f7

Nixon, R. A. (2005). Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases. Neurobiology of Aging, 26(3), 373–382. https://doi.org/10.1016/j.neurobiolaging.2004.09.018

Orobets, K. S., & Karamyshev, A. L. (2023). Amyloid Precursor Protein and Alzheimer's Disease. International Journal of Molecular Sciences, 24(19), 14794. https://doi.org/10.3390/ijms241914794

Panza, F., Lozupone, M., Logroscino, G., & Imbimbo, B. P. (2019). A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease. Nature Reviews Neurology, 15(2), 73–88. https://doi.org/10.1038/s41582-018-0116-6

Penke, B., Bogár, F., & Fülöp, L. (2017). β-Amyloid and the Pathomechanisms of Alzheimer's Disease: A Comprehensive View. Molecules, 22(10), 1692. https://doi.org/10.3390/molecules22101692

Roberson, E. D., Scearce-Levie, K., Palop, J. J., Yan, F., Cheng, I. H., Wu, T., Gerstein, H., Yu, G.-Q., & Mucke, L. (2007). Reducing Endogenous Tau Ameliorates Amyloid ß-Induced Deficits in an Alzheimer's Disease Mouse Model. Science, 316(5825), 750–754. https://doi.org/10.1126/science.1141736

Sadigh-Eteghad, S., Sabermarouf, B., Majdi, A., Talebi, M., Farhoudi, M., & Mahmoudi, J. (2014). Amyloid-Beta: A Crucial Factor in Alzheimer's Disease. Medical Principles and Practice, 24(1), 1–10. https://doi.org/10.1159/000369101

Scheltens, P., Strooper, B. D., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., Cummings, J., & Flier, W. M. van der. (2021). Alzheimer's disease. The Lancet, 397(10284), 1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4

Sehar, U., Rawat, P., Reddy, A. P., Kopel, J., & Reddy, P. H. (2022). Amyloid Beta in Aging and Alzheimer's Disease. International Journal of Molecular Sciences, 23(21), 12924. https://doi.org/10.3390/ijms232112924

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Molecular Medicine, 8(6), 595–608. https://doi.org/10.15252/emmm.201606210

Serrano-Pozo, A., Frosch, M. P., Masliah, E., & Hyman, B. T. (2011). Neuropathological Alterations in Alzheimer Disease. Cold Spring Harbor Perspectives in Medicine, 1(1), a006189. https://doi.org/10.1101/cshperspect.a006189

Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., Brett, F. M., Farrell, M. A., Rowan, M. J., Lemere, C. A., Regan, C. M., Walsh, D. M., Sabatini, B. L., & Selkoe, D. J. (2008). Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nature Medicine, 14(8), 837–842. https://doi.org/10.1038/nm1782

Strooper, B. D., & Annaert, W. (2010). Novel Research Horizons for Presenilins and γ-Secretases in Cell Biology and Disease. Annual Review of Cell and Developmental Biology, 26(Volume 26, 2010), 235–260. https://doi.org/10.1146/annurev-cellbio-100109-104117

The Amyloid-β Pathway in Alzheimer's Disease | Molecular Psychiatry. (n.d.). Retrieved December 2, 2025, from https://www.nature.com/articles/s41380-021-01249-0

Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Teplow, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., … Citron, M. (1999). β-Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. Science, 286(5440), 735–741. https://doi.org/10.1126/science.286.5440.735

Yan, R., & Vassar, R. (2014). Targeting the β secretase BACE1 for Alzheimer's disease therapy. The Lancet Neurology, 13(3), 319–329. https://doi.org/10.1016/S1474-4422(13)70276-X

Zhang, X., & Song, W. (2013). The role of APP and BACE1 trafficking in APP processing and amyloid-β generation. Alzheimer's Research & Therapy, 5(5), 46. https://doi.org/10.1186/alzrt211

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The Correlation Between Limited English Proficiency (LEP) and Mammography Screening Rates among Women Across Georgia Counties with the Highest and Lowest LEP Populations By Aishwarya Sridhar

Abstract

Limited English Proficiency (LEP) has been associated with barriers to preventive healthcare, including reduced access to cancer screening. This study examined the relationship between county-level LEP prevalence and mammography screening rates among women across ten Georgia counties with varying LEP populations from 2020 to 2024. Using a quantitative, ecological correlational design, LEP data from the American Community Survey were analyzed alongside mammography screening prevalence estimates from the Centers for Disease Control and Prevention PLACES Project. Pearson's correlation and simple linear regression were used to evaluate the relationship between the variables. Results demonstrated a very weak negative correlation between LEP prevalence and mammography screening ($r = –0.10$, $p = .50$), with a regression slope of approximately –0.07 and an R^2 below .01. These findings indicate that LEP was not a statistically significant predictor of county-level mammography screening prevalence within the selected counties. The results suggest that language-related disparities may be more localized or interact with other structural determinants of healthcare access rather than appearing independently at the county level.

Introduction

Breast cancer is one of the most frequently diagnosed cancers and the second leading cause of cancer death among women in the United States. According to the American Cancer Society, one in eight women will be diagnosed with breast cancer during her lifetime and "if breast cancer is found early…it is easier to treat successfully by getting regular screening tests" (American Cancer Society par. 2). Early detection and preventative measures have become increasingly promoted, effective, and crucial in recent times due to higher proportions of cancers being found at earlier, more treatable stages. Mammography is defined as a low-dose X-ray of the breast used to detect early signs of breast cancer, often finding tumors up to 3 years before they can be felt. Mammography is the cornerstone of early detection for breast cancer, and American Cancer Society guidelines recommend that "women aged 50 to 70 receive regular screening mammograms every one to two years" (American Cancer Society par. 2). When these guidelines have been adhered to, they have contributed to substantial declines in breast-cancer mortality and have improved the likelihood that tumors are detected at a point when less aggressive treatment is required. Regardless of decades of mammography progress, screening uptake has been proven to be uneven, with participation being dependent on many factors like socioeconomic status, racial identity, or even health-system access.

One factor that has become a huge determinant of healthcare access is Limited English Proficiency (LEP) within U.S. communities due to the vast linguistic diversity. LEP is defined as those "who reported speaking English 'not well' or 'not at all'" (Gulati and Hur 97). LEP has

been connected to reduced preventative-care use, delayed diagnoses, and worse outcomes across multiple diseases, most notably cancer (Ramirez et al.). Therefore, this paper focuses on how adapted resources that encourage mammography screening can reach LEP individuals who are most vulnerable to not receiving preventive care. Rather than examining individual-level behavior, this study intentionally evaluates county-level patterns to determine whether LEP prevalence functions as a broader structural indicator of screening disparities across geographic regions. This distinction is critical because public health interventions are often implemented at the county or regional level, making it necessary to understand whether language barriers manifest at that scale. Comparing counties that differ variably in LEP levels provides a stronger basis for identifying whether language itself, rather than structural or regional factors, correlates with mammography screening behavior. This context leads to the central research question guiding this study: How does LEP correlate with mammography screening rates among women 50-70 across Georgia counties with the highest and lowest LEP populations from 2020 to 2024?

Racial and Ethnic Inequalities

The literature has also demonstrated consistent racial and ethnic inequalities intersecting with language barriers. Inconsistencies have also been shown to affect black women, who face higher late-stage diagnosis rates even when insured. A research study of U.S. screening data found that "the percentage of the Black population…was positively associated with mammography screening rates," but only when adequate facilities, funding, and education were present (Hashtarkhani et al. 3). This shows how dependent screening outcomes are, especially for the most vulnerable women, on access to resources and care. For many Hispanic and immigrant populations, language barriers defined by LEP compound these structural issues.

According to the Associated Press-NORC Center for Public Affairs Research, nearly 6 in 10 Hispanic adults have had difficulty in communicating with healthcare providers due to language barriers. This alarming statistic highlights the struggle an estimated 60% of Hispanic adults undergo. National surveillance shows that Hispanic adults with limited comfort speaking English "fared substantially worse than their Hispanic counterparts with [greater English proficiency] in having a usual source of care, use of preventive services, breast and colorectal cancer screening, and healthcare services" (Hall et al. 5). These studies collectively demonstrate that language barriers intensify existing disparities in preventative care across racial and ethnic groups. While much of the literature tends to emphasize Hispanic and immigrant populations because they are largely LEP speakers, language-related obstacles extend across all racial groups with different variations of languages. It is necessary to recognize this intersection to justify a broader analysis that encompasses all groups by treating LEP as the independent variable relating with mammography screening rates.

Historical Disparities in Breast-Cancer Screening

Breast-cancer screening programs in the United States (U.S.) reveal how structural barriers shaped participation well before language differences were a prominent topic within

studies. As early as the 1990s, researchers were able to document that "socioeconomic disadvantage, lack of insurance, and weak physician referral systems were key disincentives to mammography participation" (Potter et al. 78). This demonstrates how disparities in screening access long predate language-based barriers. It can also support the fact that today's language-based disparities are a part of a long-standing pattern of existing access barriers. Despite their higher risk for breast cancer, older women were often overlooked in the past and are "under-screened for breast cancer, largely due to the absence of physician recommendation" (Rimer 1085).

Ironically, the group that requires mammograms and preventative measures the most is being overlooked by the healthcare system. Physicians are largely responsible for patient care and are a huge stakeholder in the process directly connecting their patients to healthcare. Physician recommendations for screening may therefore represent an important component of preventive-care access. This pattern highlights the importance of studying women within the 50-70 age group, as this is the population for whom mammography is most strongly recommended by the American Cancer Society and have been most impacted by the foundation of inequality historically. Focusing on this age range ensures that differences in screening rates are not focused on age-related guidelines varying, but rather disparities in access within the most affected group. These studies establish that consistent screening and outreach from healthcare facilities have been issues on their own for all women, not limited to specific groups, in both past and current times.

Rise of Language Barriers in Preventative Screening

By the 2000s, national campaigns reduced some of the many structural gaps previously mentioned, but language emerged as a major predictor of screening behavior due to the rise of LEP communities at this time residing in the United States. Early evidence showed that "limited English proficient adults were 1.7 to 2.5 times more likely to report poor access to care and worse health status than English-only speakers" (Ponce et al. 788). This revealed how even after progress in addressing structural gaps, specifically insurance coverage and patient communication, limited English proficiency independently predicts reduced access to preventive services and overall health outcomes. By this time, professional interpreters were in use and had been shown to "consistently [improve] communication, clinical care, and satisfaction, bringing outcomes for LEP patients closer to those of English speakers" (Karliner et al. 735). Studies have shown that when patients receive care targeted to their language needs, their experiences and outcomes improve and begin to approach those of native English speakers. This demonstrates that language barriers can be mitigated when appropriate language-access resources are provided.

A more recent study demonstrates how language preference directly predicts screening compliance. According to Vang et al., "Women who preferred Chinese were 53% less likely to have had a mammogram within the past year compared to women who preferred English" (Vang et al. 1078). This is a huge gap that represents how even a choice as simple as selecting a

different preferred language can dramatically lower screening rates. Moreover, communication from providers to patients is crucial when it comes to educating and reminding patients appropriately. When patients are not spoken to in their preferred language, it can be significantly harder to communicate. In Oregon and Washington, "few respondents received written or verbal information in their preferred language (35% and 28.3%, respectively). Financial and logistical support, including language services were most frequently identified as types of support needed to obtain a mammogram" (Gill et al. 3). This connects to how a choice as simple as setting a preferred language can be easily disregarded, which leads to missed preventative measures.

These findings confirm that federal protections alone, such as Title VI of the Civil Rights Act, which mandates that "recipients must take reasonable steps to ensure meaningful access to their programs and activities by LEP persons," do not actually ensure equitable screening, especially in more local, provincial areas (The White House 50121). Despite this legal requirement, many health systems provide the bare minimum in resources or even reduce efforts in reminders for certain patients due to inconsistent enforcement, leaving screening inequities unresolved at the local level.

Geographic Context and County Selection

While much of the national literature on language barriers in healthcare focuses on urban centers or single-county analyses, comparative research across regions with differing LEP profiles continues to be limited. Georgia provides an ideal context for such a comparison because it contains counties that vary widely in linguistic diversity while sharing comparable state-level health policies and other defining variables. According to the 2023 American Community Survey (ACS), roughly 6.4% of Georgians are defined as limited English proficient, but this percentage varies widely, from over 10% in Gwinnett, DeKalb, Hall, Clayton, and Fulton Counties to under 5% in Forsyth, Cherokee, Columbia, Coweta, and Fayette Counties. The selected counties consisted of the five counties with the highest LEP prevalence and the five with the lowest LEP prevalence based on the ACS county-level estimates. This strengthens the analysis by maximizing contrast between linguistically diverse and English-dominant regions, allowing for clearer observation of whether LEP prevalence correlates with mammography screening rates.

Research Gap

Despite strong research linking LEP to reduced preventative care, many gaps remain within this field of research that the paper aims to address. Oftentimes large studies at a state or national level ignore neighborhood, local-level correlations, and issues. County-level analyses, such as those by Bauer et al. using the Centers for Disease Control and Prevention (CDC) Social Vulnerability Index, demonstrate the importance of narrowed data, but language proficiency is rarely treated as a central variable. Even though several Atlanta-area studies have targeted immigrant populations with effective outreach and correlational data, no published research has directly tested the correlation between LEP prevalence and specific screening rates within

Georgia. In addition to this, there is limited existing data examining language-related disparities evolving in the most recent years. The chosen time frame of 2020-2024 captures the most recent five-year period with publicly available data from both the ACS and CDC Population Level Analysis and Community Estimates (PLACES) project. While this study focuses on the relationship between LEP and mammography screening, it does not directly account for factors such as income, insurance coverage, or healthcare infrastructure. These variables were intentionally not included as independent variables because prior literature has already established them as strong predictors of screening behavior. Including them in this model would shift the focus away from isolating the independent association between language proficiency and screening outcomes. This design reduces variation in certain state-level policy factors by comparing counties within the same state, while recognizing that socioeconomic and healthcare-access factors may still vary across counties. This design allows LEP to be examined as a more isolated structural determinant rather than one confounded by cross-state variability. Understanding these unresolved disparities is essential before evaluating their broader implications for public health and equity in Georgia. It can provide data to guide the allocation and production of resources for common languages favored by LEP populations, through professional interpreters, bilingual patient tools, and even bilingual advertising means implemented within existing resources. It can inform statewide policy and can be replicated through various similar interventions across Georgia. Therefore, this paper investigates the correlation between LEP and mammography screening rates among women across Georgia counties with the highest and lowest LEP populations.

Method

This study employs a quantitative, ecological correlational design to analyze the relationship between LEP and mammography screening rates among women aged 50-70 across Georgia counties from 2020 to 2024. The study is based on a quantitative ecological correlational study using repeated county-level observations from 2020–2024. This allows for comparison of yearly trends among 10 counties over the defined five-year period while also maintaining county-level precision. Moreover, this ecological study is an observational, population-level analysis that examines relationships between exposures and outcomes. County-level ecological approaches have been shown to provide necessary insight into screening disparities that are not visible in statewide data. Schneider et al. demonstrated that using small-area estimation models at the county level yield representative prevalence estimates for mammography, helping identify underserved geographic pockets often overlooked in broader analyses. Their approach validates the use of combined, public health datasets such as the ACS and CDC PLACES project as well for population-based correlational studies (Schneider et al.). This design was selected because it enables the measurement of associations between variables using publicly available, population-based data, rather than individual participant responses, which is both ethical and replicable for further data research. A quantitative ecological approach was specifically appropriate because the research question seeks to identify whether variation in LEP across

counties is associated with variation in screening rates at a single population level, rather than tracking individual behavior over time. While multiple years of data were included, the analysis treats each county-year observation as part of a quantitative dataset, allowing for increased sample size while maintaining the ecological nature of the study. The independent variable in this study is the percentage of adults with LEP, as reported by the ACS, which estimates the proportion of adults who report speaking English "less than very well," a measure consistently used in research linking language proficiency to healthcare access (Ponce et al.). For each county, the independent variable (LEP) was calculated as:

$$LEP(\%) = \frac{Number\ of\ individuals\ speaking\ English\ "less\ than\ very\ well"}{Total\ population\ aged\ 5\ and\ older} * 100 \quad (1)$$

The dependent variable is the percentage of women aged 50-70 who received a mammogram within the past 5 years, as estimated by the CDC PLACES Project, which provides small-area health estimates modeled from the Behavioral Risk Factor Surveillance System (BRFSS). County-level datasets such as the ACS and CDC PLACES have been widely used in epidemiological research to model screening participation and assess the relationship between social determinants of health and preventive behaviors (Bauer et al.). The dependent variable (mammography screening rate) was calculated as:

$$Mammography\ Screening\ Rate\ (\%) = \frac{Number\ of\ women\ aged\ 50{-}70\ reporting\ a\ mammogram\ in\ the\ past\ 5\ years}{Total\ female\ population\ aged\ 50{-}70} * 100 \quad (2)$$

Both variables were selected due to representing well-validated public health indicators frequently used in studies, such as in Ponce et al.'s academic paper, that explore healthcare access and social determinants of health. Each dataset was downloaded for 2020 through 2024, cleaned, and aligned using standardized Federal Information Processing Standards (FIPS) codes. The final analytic dataset consisted of 50 county-year observations, representing 10 counties across five years, with each observation containing the county identifier, year, LEP percentage, and mammography screening prevalence. Descriptive statistics, including means, ranges, and percentage changes, were calculated to summarize trends in both variables over the five-year period as well.

Statistical Evaluation

All statistical analyses were conducted in Microsoft Excel, which supports replicable calculations and standardized outputs. Two main statistical procedures were used: Pearson's correlation coefficient (r) and simple linear regression. As seen in prior county-level studies examining breast cancer disparities, Pearson correlation has been effectively used to quantify relationships between screening behavior and incidence outcomes (Sprague et al.). In their analysis of SEER data, "the Pearson correlation coefficient was -0.18 (P = 0.01)," confirming a

significant negative association between ductal carcinoma in situ incidence and the decline in invasive breast cancer incidence (Sprague et al. 876).

The Pearson correlation coefficient was used to measure the direction and strength of the linear relationship between the independent variable (LEP percentage) and the dependent variable (mammography screening rate). The correlation coefficient was calculated through the following equation:

$$r = \frac{\Sigma[(X_i - X)(Y_i - \bar{Y})]}{\sqrt{[\Sigma(X_i - X)^2 \Sigma(Y_i - \bar{Y})^2]}} \quad (3)$$

where X_i represents the LEP percentage in county i, Y_i represents the mammography screening rate in that same county, and $\bar{x}$ and $\bar{y}$ represent their respective mean values across all observations. The numerator of the equation measures the covariance between the two variables, while the denominator standardizes this value by the product of their standard deviations. As a result, r ranges from –1 to +1, where an r value close to –1 indicates a strong negative relationship (as LEP increases, screening decreases), and an r value close to +1 indicates a strong positive relationship (both increase together). An r value near 0 implies no meaningful linear relationship. Statistical significance was assessed at $p < .05$, indicating that results this extreme would be unlikely under the null hypothesis. Following this, a simple linear regression was used to estimate how mammography screening prevalence changed in relation to LEP percentage. The regression model followed the standard:

$$Y = \beta_0 + \beta_1 X + \varepsilon \quad (4)$$

where Y is the mammography screening rate, X is the LEP percentage, β_0 is the intercept, β_1 represents the slope (the rate of change in screening per change in LEP), and ε is the error term. The model's explanatory power was calculated through the coefficient of determination, R^2, which represents the proportion of variation in mammography screening rates explained by differences in LEP across counties. Higher R^2 values indicate that LEP accounts for a greater share of the observed variance in screening participation, suggesting a stronger predictive relationship. In addition, standard error values were examined to evaluate the precision of the regression coefficients and the variability around the estimated regression line. Finally, p values for each parameter estimate, as previously mentioned, were used to determine whether the relationships observed between LEP, and screening rates were statistically significant, with $p < 0.05$ indicating strong evidence that the correlation was unlikely to have occurred by chance. Together, these analyses provided a quantitative framework for determining whether language, as measured by LEP, serves as a primary predictor of disparities in mammography screening participation across Georgia counties over the 2020 to 2024 period.

Results

For this study, mean mammography screening prevalence was examined for women aged 50–70 across ten Georgia counties, five with the highest LEP percentages and five with the

lowest from 2020 to 2024. Screening prevalence was measured using the CDC PLACES county level estimate, which reflects the percentage of women who reported having received a mammogram within the recommended interval. The data sets were downloaded from each year.

Sum of Mammography Prevalence	County										
Year	Cherokee	Clayton	Columbia	Coweta	DeKalb	Fayette	Forsyth	Fulton	Gwinnett	Hall	Grand Total
2020	76.2	80.5	79.5	75.3	79.1	80.2	81.4	79.3	78.3	75.7	785.5
2021	76.2	80.5	79.5	75.3	79.1	80.2	81.4	79.3	78.3	75.7	785.5
2022	70.2	75.1	72.1	75.3	77.5	77.2	73.5	77.2	75.1	74.5	747.7
2023	70.2	75.1	72.1	75.3	77.5	77.2	73.5	77.2	75.1	74.5	747.7
2024	74.8	75.8	80.1	74.9	79.8	80.2	79.4	79.3	72.9	76.1	773.3
Grand Total	**367.6**	**387**	**383.3**	**376.1**	**393**	**395**	**389.2**	**392.3**	**379.7**	**376.5**	**3839.7**

Table 1. Mean mammography screening prevalence across ten Georgia counties, 2020–2024

Across the five-year period, average screening rates remained relatively stable, with only small year-to-year fluctuations. Screening prevalence consistently remained in a relatively high range, being 75% and above, without showing any sharp declines or increases. This pattern suggests that, at the county level, the ten selected counties maintained comparatively steady mammography participation over time, regardless of their LEP composition itself.

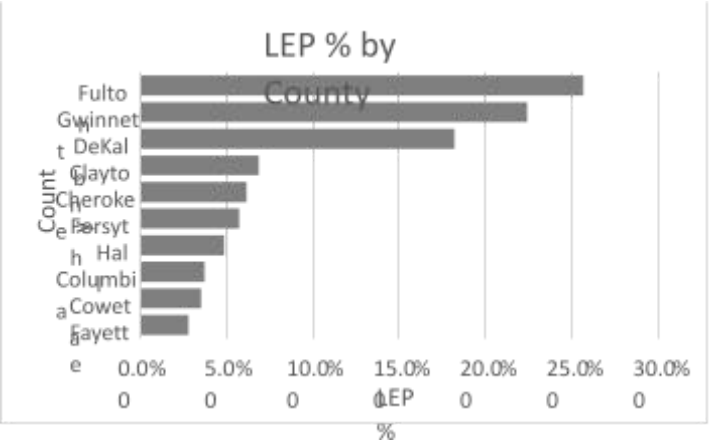

Figure 2. LEP percentage in the five highest-LEP and five lowest-LEP Georgia counties (ACS 2020).

LEP was utilized as the percentage of residents aged five and older who reported speaking English “less than very well” in the 2020 ACS B16004 table. Since LEP tends to change slowly at the county level, the 2020 5-year ACS estimates were treated as a strong indicator of each county’s linguistic profile across the 2020–2024 period. This pattern suggests that, at the county level, the ten selected counties maintained comparatively steady mammography participation over time, regardless of their LEP values.

To directly assess the relationship between county-level LEP prevalence and mammography screening rates, all fifty county-year observations were combined into a single dataset. Each observation included the county's LEP percentage (ACS 2020) and the corresponding mammography screening prevalence for that county and year (PLACES 2020–2024).

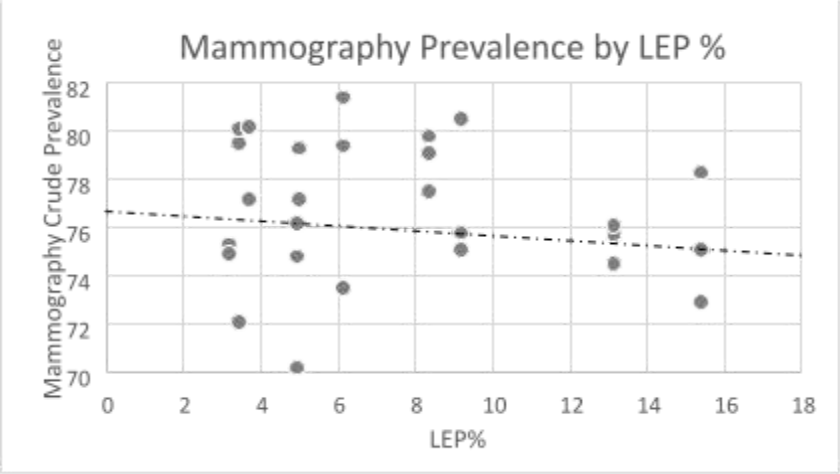

Figure 3. Scatterplot of LEP percentage (ACS 2020) and mammography screening prevalence (PLACES 2020–2024) for ten Georgia counties.

Visually, the points in Figure 3 form a diffuse cloud rather than a clear upward or downward trend. Counties with relatively high LEP, such as Gwinnett and Hall, do not cluster at specifically lower screening levels than low-LEP counties; instead, screening rates for both groups overlap substantially. The fitted line of best fit shows a slight downward slope, suggesting a weak negative association, but the pattern is not obvious.

Analysis and Discussion

Moreover, a Pearson correlation coefficient was calculated to quantify the strength and direction of the linear relationship between county-level LEP percentage and mammography screening prevalence across all fifty observations. The analysis yielded $r = –0.10$ and $p = .50$. This coefficient indicates a very weak negative association, which means that counties with higher LEP percentages tend to have slightly lower screening rates, but the effect is small. The extremely small magnitude of r suggests that LEP prevalence explains almost none of the variation in screening behavior at the county level. In practical terms, this means that counties with higher proportions of LEP populations do not systematically experience lower screening rates, contradicting patterns observed in individual-level studies.

The p-value which is well above .05 shows that this relationship is not statistically significant. It indicates that the study did not find sufficient evidence of a statistically significant linear relationship between LEP and mammography screening prevalence. To further look into

the LEP percentage actually predicting mammography screening rates, a linear regression model was estimated with LEP percentage as the independent variable and mammography screening prevalence as the dependent variable. The regression equation yielded the negative slope ($b \approx -0.07$), which means that for every 1 percentage-point increase in LEP, mammography screening prevalence is predicted to decrease by about 0.07 percentage points. The slope value further reinforces the minimal practical impact of LEP, as a 1% increase in LEP corresponds to only a 0.07% decrease in screening, an effect size that is not meaningful in real-world public health contexts. However, this effect size is incredibly small, and it explained less than 1% of the variance in screening prevalence because $R^2 < .01$. The extremely low R^2 value suggests that LEP explains very little of the observed variation in screening prevalence and that unmeasured factors, potentially including healthcare access, provider density, insurance coverage, or localized outreach, may account for additional variation. The slope was not statistically significant at the $p < .05$ level either. Finally, the correlation and regression analyses indicate that, at the county level in Georgia, LEP percentage does not serve as a strong or statistically significant predictor of mammography screening prevalence among women aged 50–70 during the 2020– 2024 period. Instead of a clear negative relationship, the data revealed overlap in screening rates between high-LEP and low-LEP counties, suggesting that other structural factors may play a larger role in mammography usage. Therefore, while prior literature identifies LEP as a barrier at the individual level, these findings suggest that LEP does not independently function as a strong predictor when analyzed at a broader geographic scale.

Implications

Even though prior literature connects LEP to barriers in preventive care, the absence of a significant county-level relationship in this study implies that language prevalence at the county scale may be too broad to function as a standalone predictor of screening participation. One interpretation based on the results is that counties with higher LEP prevalence may also have stronger health infrastructure, more clinics, or more established language-access resources, which could reduce observable screening gaps at the county level. Another possibility is that screening prevalence in this dataset was relatively stable and high across counties, creating an overlap between high-LEP and low-LEP individuals and limiting visible differences. Practically, these results refrain from assuming that county LEP prevalence automatically corresponds to lower mammography participation. Instead, efforts to improve screening equity may be more effective when focused on specific, patient-facing barriers like appointment availability, transportation, and even cost, while still maintaining strong language-access services as a core practice for equity.

These findings also suggest that policymakers and public health professionals should be cautious when using county-level LEP prevalence alone to identify where mammography disparities are most severe. Because county averages may conceal smaller-scale differences within neighborhoods, clinics, or specific language communities, broader demographic measures may not fully capture where language barriers are most directly affecting screening behavior. As

a result, public health strategies may be more effective when they combine county-level planning with more targeted local assessments, such as clinic-level language needs, patient outreach data, or community-specific screening barriers. In this way, the results of this study do not diminish the importance of language access, but instead indicate that its effects may be more localized than a county-level model is able to detect.

Limitations

This study has several limitations that affect interpretation. It is an ecological and county-level study, meaning it cannot determine how individual LEP status influences whether an individual woman receives a mammogram. The limited number of observations (n = 50 observations) reduces statistical power, making it more difficult to detect weak but potentially meaningful relationships. Also, LEP was treated as a stable county characteristic using ACS estimates, which may not capture year-to-year change from 2020–2024 and may not reflect LEP specifically among women aged 50–70. The dependent variable is also based on population-level estimates, which can include measurement error and may smooth out local differences. Finally, the correlational design did not control for intersecting factors such as insurance coverage, income, education, racial and ethnic composition, and differences in local public health outreach, all of which may influence screening prevalence and could obscure the relationship between language barriers and screening when not included in the study.

Future Research

Future studies should expand beyond ten counties to account for all Georgia counties to improve generalizability and statistical power produced by this study. A multivariable approach would strengthen interpretation of association by controlling for key contributing factors such as insurance coverage, poverty, rural versus urban context, and healthcare access indicators. Researchers could also examine specific language communities or demographic subgroups rather than relying on overall county-level LEP prevalence. In addition, using LEP measures that vary by year and matching them with year screening estimates could better capture changes over time. Finally, future research could also benefit from looking beyond screening prevalence alone to outcomes that reflect follow-through and quality of care, as well as variables such as race, income, and geographic factors. These are significant to consider as they may be much more sensitive to language-access barriers than a single prevalence estimate from a database.

Works Cited

American Cancer Society. “Breast Cancer Early Detection and Diagnosis.” American Cancer Society, 2025. https://www.cancer.org/cancer/types/breast-cancer/screening-tests-andearly-detection.html.

Associated Press, Emily Swanson, and Russell Contreras. “Latinos, Hispanics Face Language Barriers in Health Care.” AARP, 26 July 2018. https://www.aarp.org/health/conditions-treatments/latinos-hispanics-doctors-nursing-homes/.

Atlanta Regional Commission. Limited English Proficiency Plan. Atlanta Regional Commission, 2017. https://cdn.atlantaregional.org/wp-content/uploads/lep-plan-final-2017.pdf.

Georgia Department of Public Health. “Breast and Cervical Cancer Program (BCCP).” Georgia Department of Public Health, 2024. https://dph.georgia.gov/BCCP.

Gill, M., et al. “Mammogram Perceptions, Communication, and Gaps in Care among Individuals with Non-English Language Preference in Oregon and Washington States.” Preventive Medicine Reports, vol. 35, 2023, article 102352. https://doi.org/10.1016/j.pmedr.2023.102352.

Gulati, R. K., and K. Hur. “Association between Limited English Proficiency and Healthcare Access and Utilization in California.” Journal of Immigrant and Minority Health, vol. 24, no. 1, 2022, pp. 95–101. https://doi.org/10.1007/s10903-021-01224-5.

Hall, I. J., S. H. Rim, and S. Dasari. “Preventive Care Use among Hispanic Adults with Limited Comfort Speaking English: An Analysis of the Medical Expenditure Panel Survey Data.” Preventive Medicine, vol. 159, 2022, article 107042. https://doi.org/10.1016/j.ypmed.2022.107042.

Hashtarkhani, S., et al. “Analyzing Geospatial and Socioeconomic Disparities in Breast Cancer Screening among Populations in the United States: Machine Learning Approach.” JMIR Cancer, vol. 11, 2025, article e59882. https://doi.org/10.2196/59882.

Karliner, L. S., et al. “Do Professional Interpreters Improve Clinical Care for Patients with Limited English Proficiency? A Systematic Review of Literature.” Health Services Research, vol. 42, no. 2, 2007, pp. 727–754. https://doi.org/10.1111/j.1475-6773.2006.00629.x.

Ponce, N. A., R. D. Hays, and W. E. Cunningham. “Linguistic Disparities in Health Care Access and Health Status among Older Adults.” Journal of General Internal Medicine, vol. 21, no. 7, 2006, pp. 786–791. https://doi.org/10.1111/j.1525-1497.2006.00491.x.

Potter, S. J., P. D. Mauldin, and H. A. Hill. “Access to and Participation in Breast Cancer Screening: A Review of Recent Literature.” Clinical Performance and Quality Health Care, vol. 4, no. 2, 1996, pp. 74–85. https://pubmed.ncbi.nlm.nih.gov/10156946/.

Ramirez, N., et al. “Access to Care among Adults with Limited English Proficiency.” Journal of General Internal Medicine, vol. 38, no. 3, 2023, pp. 590–592. https://doi.org/10.1007/s11606-022-07690-3.

Rimer, Barbara K. “Improving the Use of Cancer Screening for Older Women.” Cancer, vol. 72, no. 3, 1993, pp. 1084–1087. https://doi.org/10.1002/1097-0142%2819930801%2972%3A3%2B%3C1084%3A%3AAID-CNCR2820721324%3E3.0.CO%3B2-F.

Sprague, Brian L., Amy Trentham-Dietz, and Elizabeth S. Burnside. “Socioeconomic Disparities in the Decline in Invasive Breast Cancer Incidence.” Breast Cancer Research and Treatment, vol. 122, no. 3, 2010, pp. 873–878. https://doi.org/10.1007/s10549-010-0737-y.

The White House. “Executive Order 13166 of August 11, 2000: Improving Access to Services for Persons with Limited English Proficiency.” Federal Register, vol. 65, no. 159, 16 Aug. 2000, pp. 50121–50125. https://www.govinfo.gov/content/pkg/FR-2000-08-16/pdf/00-20938.pdf.

Vang, S., L. R. Margolies, and L. Jandorf. “Screening Mammogram Adherence in Medically Underserved Women: Does Language Preference Matter?” Journal of Cancer Education, vol. 37, no. 4, 2022, pp. 1076–1082. https://doi.org/10.1007/s13187-020-01922-y.

Swimming Frequency and Its Association with Stress, Sleep Quality, Mood, and Perceived Health: A Cross-Sectional Survey By Soshi Oyama

Abstract

This study explores the relationship between swimming participation and physical and psychological well-being. This study asks whether routine swimming can serve as preventive medicine. While general aerobic exercise lowers cortisol, swimming is different. Its biomechanics, horizontal positioning and sensory immersion require a separate evaluation. Rhythm-based regular aerobic exercise has been proven to show reduced cortisol and increased endorphins. However, the biomechanical and sensory immersion characteristics of swimming require separate clinical assessment. 95 participants with varying degrees of swimming participation were assessed using a cross-sectional survey design that measured general somatic parameters, post-exercise mood regulation, baseline stress, and sleep quality. According to the data, regular swimmers had the greatest therapeutic recovery despite reporting higher baseline stress levels as a result of their demanding schedules. Overall, 52.6% of the sample reported better perceived sleep quality, 72.0% reported improved mood stability, and 61.0% reported reduced post-swim stress. Additionally, 75.8% of respondents concurred that swimming frequently in water is a useful strategy for preventing chronic illness. These results suggest swimming is an effective non-pharmacological lifestyle intervention. It has been proven to be effective in managing stress, stabilizing mood and promoting overall well-being across a wide range of demographics.

Introduction

Over the past decades, there has been an increase in concern across several age groups regarding rising stress levels and mental health, causing it to be a significant public health issue. Teenagers and young adults draw most of the focus in mental health research. Yet stress affects older populations just as heavily. Common factors include career pressure, financial strain, and family obligations. Although at first glance it may not seem to be a major health issue, persistent psychological stress has shown a close association with poor sleep or sleep disruption, emotional instability, decreased productivity, cardiovascular strain, and long-term metabolic risk, according to several articles. Past research has described how chronic stress leads to “allostatic load,” in which prolonged demand is placed on body systems. Also, stress-related sleep disruption might increase inflammation and the risk of long-term disease. Persistent psychological stress, according to multiple scientifically proven studies, shows a close association with poor sleep or sleep disruption, impaired emotional regulation, decreased productivity, cardiovascular strain, and long-term metabolic risk, with research by McEwen explaining how chronic stress contributes to “allostatic load”, placing prolonged strain on bodily systems.[1] Also, Irwin highlights how stress-related sleep disruption can further increase inflammation and long-term disease risk.[2] As a result, easily accessible and sustainable preventative strategies have gained increasing spotlight within the lifestyle medicine and disease prevention sector of public health.

Countless studies have confirmed that physical activity has long been one of the most effective non-pharmacological interventions for mental health. This was proven by a meta-analysis, which established a direct correlation between physical activity and healthier, more pronounced diurnal cortisol patterns.[3] More specifically, activities that demand aerobic exercise are scientifically linked to reduced cortisol. Additionally, they have proven to increase endorphins and serotonin, improve autonomic balance, and enhance sleep efficiency. Moreover, one of the studies from 2020 details how regular aerobic exercise transforms systemic stress responsiveness and provides a greater chance of protecting individuals from experiencing psychological distress.[4] It has also been proved through several studies that exercising regularly significantly lowers the risk of depression. The World Health Organization (WHO) highlights the significant role of regular exercise in improving overall mental well-being and sleep quality across populations globally. However, swimming, in particular, merits further attention due to its unique biomechanical and cardiovascular demands as well as its sensory aspects regarding water immersion. Swimming combines aerobic activity and full-body engagement in a low-impact environment. This contributes to its uniqueness as a sport that can enhance physiological stress relief and facilitate stress release.

Swimming is a low-impact sport, unlike many other land sports such as basketball or volleyball. Unlike sports that put too much strain on the joints, swimming involves the whole body working together, while minimizing the stress on the joints and maintaining a high aerobic demand. Moreover, a recent review describes how swimming and aquatic immersion have been shown to improve cognitive flexibility, emotional resilience, self-regulation, and affective balance.[5] These factors are what allow for all age groups, from youth to the elderly, access to swimming as a sustainable exercise option that significantly benefits their physical and mental well-being. On top of these benefits, another unique characteristic of swimming that sets it apart from other sports is how it requires people to spend the majority of their time in a horizontal body position. This positioning, combined with the hydrostatic water pressure, alters bodily circulatory dynamics and promotes venous return and parasympathetic activation. When in a horizontal position, the cardiovascular system, specifically the heart, is given a temporary rest from combatting gravity. From an anatomical perspective, there is a confirmation that head-out water activity triggers an immediate change in cardiac output, central blood volume, as well as decreasing peripheral vascular resistance.[6] Additionally, the rhythmic breathing pattern inherent to swimming, like bilateral breathing, resembled controlled breathing techniques often observed and practiced in meditation. Moreover, this can be linked to the restoration of physiological states and the facilitation of autonomic regulation.

Swimming introduces sensory immersion in addition to its physiological mechanisms. Due to its special traits, including buoyancy, water resistance, and visual simplification, being in the water creates an environment that is psychologically different from exercise environments on land. Immersion in water may have calming or meditative effects for certain people. Above all, swimming is used for recreational, fitness-related, and competitive purposes by people of all ages. Due to their intense training and performance-based evaluation, competitive swimmers

may have higher baseline stress levels. A 2016 study concluded that high-performance athletes frequently experience extreme sport-specific vulnerabilities, such as overtraining, scheduling demands, and performance burnout.[7] Conversely, recreational swimmers might view swimming as a form of relaxation or a way to decompress. Swimming can be used as an injury-free form of exercise by adults and the elderly, and it can be used by younger athletes in organized team settings. All these different contexts raise an important research question: do training demands outweigh any potential benefits for mental health, or is increased swimming participation associated with reduced stress and improved well-being across age groups? A survey-based research design was utilized to investigate the relationships between stress, mood, sleep quality and perceived health at different levels of swimming participation.

Methods

This study collected survey data from 95 participants with diverse swimming backgrounds, including competitive, recreational, and non-swimmers. Participants ranged in age, sex, and lifestyle in order to maximize empirical validity and minimize selection bias. This variety of participants ensured more meaningful comparisons among groups, thereby enhancing the generalizability of the data and findings.

The survey was cross-sectional and was distributed directly to participants digitally through a Google Form. The Google Form consisted of 36 questions, both quantitative and qualitative. Some examples of the questions examined swimming frequency, perceived stress levels measured on a 1–5 Likert scale, changes in stress after swimming, mood and emotional wellbeing, sleep quality, and perceived overall health benefits. Also, to further improve the depth of understanding of the participants' prior experiences surrounding swimming, open-ended questions were also utilized.

To emphasize the organization of various groups, responses were categorized based on frequency. This would allow for the data to be compared among participants who swim competitively, recreationally, and those who do not swim. During some sections of data processing, common outliers – found within the non-swimming group - were excluded in order to isolate the direct effects of swimming and to prevent potential inaccurate or irrelevant complications. All data were anonymized and organized for statistical analysis. Descriptive statistics were applied to explore patterns and trends across groups because this study is exploratory in nature. The statistics included means, percentages, and frequency distributions. The results should be viewed as descriptive associations rather than statistically significant relationships because no inferential statistical tests were conducted. This is a limitation of the current analysis.

Limitations

Although extensive precautions were taken in order to minimize data inaccuracy and limitations, inevitably this research methodology still has its flaws. Hence, these limitations must be addressed and acknowledged when interpreting the findings of this study. To begin with, survey-based, self-reported studies already come with known flaws. For example, the research

relies heavily on self-reported data, which may be subject to bias and inaccuracies in participants' responses. The self-selection bias also may have played a key role in the distribution of participants in the survey . Additionally, the relatively small sample size of 95 participants may limit the generalizability of the results to larger populations. If a participant has prior experience or is a current, active swimmer, they may be more inclined to participate in the survey. In addition, this study did not control for potentially important baseline variables, such as age, overall physical activity levels, baseline physical fitness, or pre-existing health conditions. Therefore, differences between regular swimmers and non-swimmers may not be solely due to swimming, but may reflect differences in lifestyle and exercise patterns more generally. Future research should control for total physical activity levels and baseline fitness to better isolate the specific effects of swimming from the broader benefits of regular exercise. However, despite these existing flaws, the study still provides valuable insights into the scientific correlation between swimming and various aspects of mental wellbeing.

Table Interpretation and Numerical Score Conversion

The table maps weighted averages which were calculated dynamically within each self-reported cohort. The general trend in reading the table is that higher numerical outputs represent more beneficial or more pronounced health responses (i.e., greater post-swim stress reduction, higher mood stabilization, superior sleep patterns, and improved general health perceptions).

Responses regarding post-swim stress reduction were converted using a 5-point scale. To quantitatively analyze self-reported responses across cohorts, ordinal survey selections were converted into numerical scores. Responses regarding post-swim stress reduction were converted using a 5-point scale, where higher values indicate greater stress relief (Table 1).

Table 1. Numerical conversion for perceived stress reduction responses.

Stress Reduction	
Response	Score
Increase Significantly	1
Slightly Increase	2
Stay the same	3
Slightly decrease	4
Decrease significantly	5

Perceived mood improvements were similarly converted on a scale from 2 to 5 to measure positive affective shifts and emotional stabilization following swimming sessions

Table 2. Numerical conversion for perceived mood improvement responses.

Mood Improvement	
Response	Score
Not really	2
Yes, somewhat	4
Yes, a lot	5

Sleep Quality – Already recorded as a 1–5 rating in the survey.

Survey participants rated direct sleep quality changes on a standard 1–5 scale, while responses to general overall health improvement were converted to a 4-point scale ranging from 2 ("Slightly worse") to 5 ("Much better") (Table 3).

Table 3. Numerical conversion for perceived general health improvement responses.

General Health Improvement	
Response	Score
Slightly worse	2
No change	3
Slightly better	4
Much better	5

By applying these numerical values, weighted-average scores were calculated across each swimming-frequency cohort to evaluate wellness trends across four core pillars (Table 4). Non-swimmers ("Never") serve as the baseline control (3.25 for Stress, 3.08 for Sleep). Notably, high-frequency swimming groups demonstrate elevated baseline stress values (3.56) alongside significantly higher general health perception scores (4.38–4.44).

Table 4. Average wellness scores by swimming-frequency group.

Swimming Frequency	Stress	Mood	Sleep	Health
Never	3.25	4	3.08	3.46
Rarely (few times/year)	3.78	4.06	3.42	3.62
Sometimes (few times/month)	4	4.31	3.54	4.31
Weekly	4.5	4.25	3.19	4.38
Regularly (few times/week)	4	4.27	3.3	4.44

Values in Table 4 represent cohort averages across each wellness pillar. Non-swimmers ("Never") serve as the baseline control (3.25 for Stress, 3.08 for Sleep). Noticeably, high-frequency groups demonstrate elevated baseline stress values alongside significantly higher general health perception scores (4.38–4.44).

These weighted averages provide an overall comparison of perceived wellness outcomes across the different swimming-frequency groups and are used throughout the Results section for trend analysis.

Results and Discussion

The evaluation of the survey data establishes clear correlations between swimming participation patterns and self-reported wellness criteria. The survey yielded a balanced sample population across all cohorts: 28.4% swim a few times per week, 16.8% swim weekly, 13.7% swim monthly, 27.4% swim a few times per year, and 13.7% never swim (Figure 1). In total, 45.2% of respondents participate in regular weekly or multi-weekly swimming, while 54.7% represent infrequent or non-swimmers.

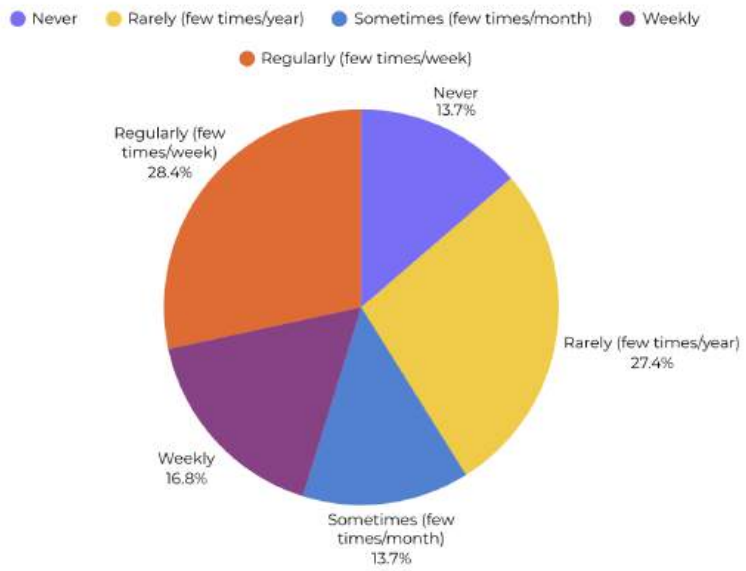

Figure 1. Swimming frequency distribution among survey respondents ($N = 95$). The chart displays the percentage breakdown of participants across five self-reported activity cohorts.

To simplify macro-comparisons across cohorts, survey respondents were aggregated into two primary groups: Frequent Swimmers (45%, comprising weekly and multi-weekly participants) and Infrequent/Non-swimmers (55%) (Figure 2).

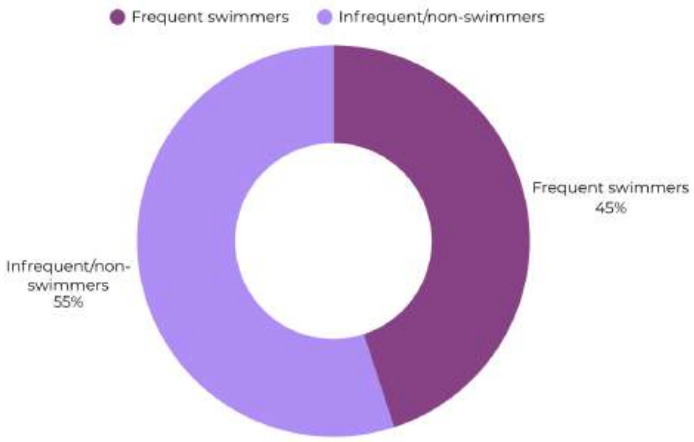

Figure 2. Macro-grouping of survey participants into Frequent Swimmers (45%) and Infrequent/Non-Swimmers (55%) for cohort comparisons.

Participants were initially evaluated using self-reported frequencies, which were split into 5 categories. For broad comparative analysis, participants were split into two distinct macro-groups: Frequent swimmers (45% of the total sample) and Infrequent/Non-swimmers (55% of the total sample). Non-swimmers were explicitly removed from certain subset analyses involving direct experiential feedback in order to isolate swimming-specific physiological and psychological effects and prevent localized distortions.

Across the entire sample, the mean baseline stress score was 3.09/5. However, regular swimmers reported a higher baseline stress load (3.56/5) compared to non-regular swimmers (2.91/5), reflecting greater academic or occupational demands (Figure 3).

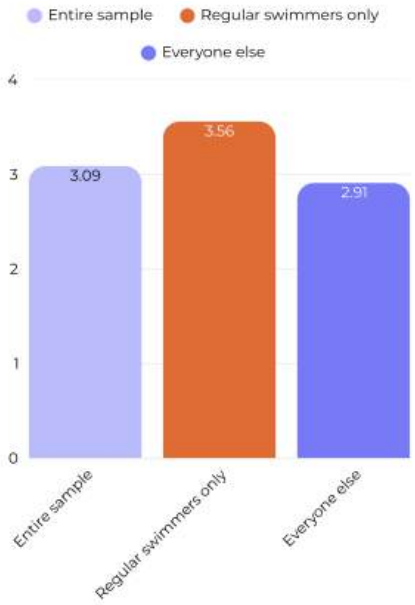

Figure 3. Average baseline stress levels across swimming participation cohorts (measured on a 1–5 scale). Frequent swimmers exhibit elevated baseline stress compared to non-regular swimmers.

The self-reported baseline stress scores on a 1–5 scale for each group are shown in Figure 3. The cumulative mean baseline stress for the entire survey group is 3.09/5. However, a startling stress paradox emerges when involvement levels are separated out: Regular Swimmers only have a significantly higher average baseline stress profile of 3.56/5. On the other hand, everyone else in the sample maintains a baseline average of 2.91/5, which is noticeably lower. The regular swimming cohort does not appear to be a low-stress group, according to this data, which points to external stresses such as hard individual schedules or intense competitive training loads. This phenomenon can be described as a counterintuitive stress paradox. Despite the statistical evidence depicting an elevated baseline stress, the same group demonstrated the highest post-swim stress reduction (83.3%), which is the highest, by a great margin. This supports the claim that swimming can provide particularly effective stress recovery for individuals experiencing greater daily demands. This association can be partly explained by physiological mechanisms associated with aquatic immersion. For example, the mammalian dive reflex can be triggered by cool water touching the face. This stimulates the vagus nerve and enhances parasympathetic activity, which decreases heart rate and allows quick autonomic recovery. Interestingly, the mammalian dive reflex is an evolutionary development, functioning almost as a built-in brake pedal for people with high resting heart rates. In addition, the immersive environment of the water restricts visual and sound stimuli and promotes rhythmic breathing patterns that may help to induce a temporary cognitive break from everyday stress, or a “flow state”. Being in the water, immersed in sensory isolation, also indirectly acts as a digital detox. As the use of digital screens and devices is increasing in today's society, it is important to allocate time away from them for numerous health reasons. Furthermore, although not directly measured in the present study, these mechanisms offer plausible explanations for the apparent discrepancy between the higher baseline stress levels and the significant stress relief that is reported by regular swimmers.

When evaluating post-swim therapeutic recovery, 61.0% of all respondents reported noticeable stress reduction, rising to 83.3% among regular swimmers. Additionally, 52.6% of the overall sample reported positive improvements in perceived sleep quality following aquatic exercise (Figure 4).

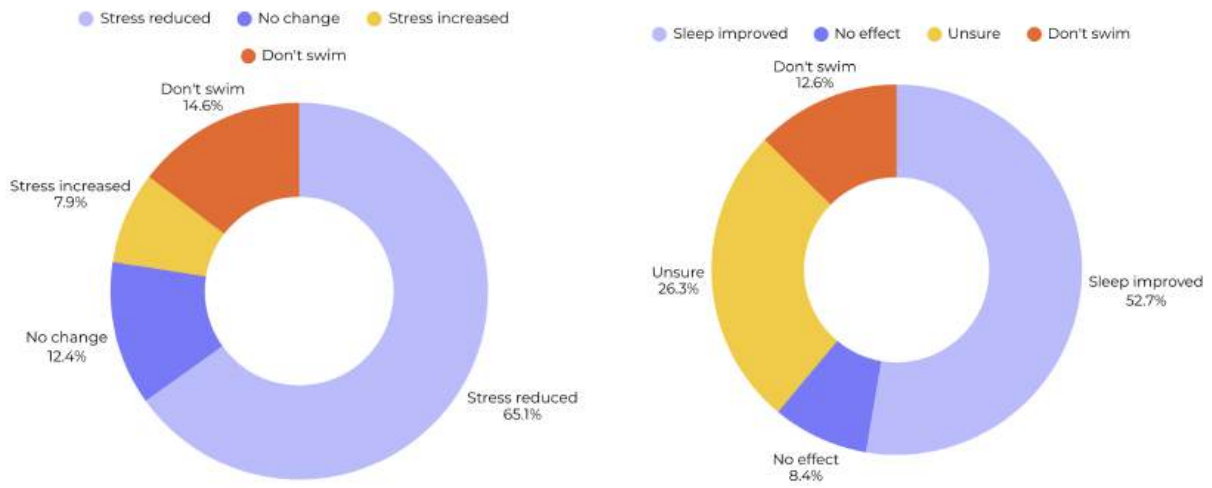

Figure 4. *Left:* Perceived changes in stress levels after swimming. *Right:* Perceived sleep quality improvements across the survey sample.

The directional shift in perceived stress after a swim exercise is assessed in Figure 4. A significant therapeutic response is demonstrated by the statistics, since 61.0% of the sample as a whole expressly report feeling less stressed. On the contrary, 11.6% indicate no change, 7.4% indicate that their stress increased, and 13.7% represent the baseline non-swimming sample. In simple terms, with only 1 in 14 people experiencing worsening stress, this is a great outcome. This proves how aquatic exercise is one of the safest lifestyle "prescriptions" with near-universal upside.

Among the 76 active respondents who completed this item, regular swimmers experienced an 83.3% rate of stress reduction, compared to 67.6% for infrequent swimmers. Therefore, regular swimmers have a higher basal stress load, but also the greatest capacity for stress relief after exercise. This data is consistent with the qualitative findings of the study, where 31.6% of open-ended responses explicitly used keywords such as “Stress”, “Relax”, “Calm” or “Less anxious”, while 70.8% said water immersion is soothing or physical exhaustion makes them feel relaxed.

Figure 4 shows the perceived effect of swimming on sleep health. With an absolute sample mean sleep rating of 3.32/5, more than half of all survey participants (52.6%) reported clear improvements in their overall sleep quality. According to the full split, 52.6% report better sleep, 8.4% report no change, 26.3% are unclear, and 12.6% do not swim.

Considering sub-trends within groups, regular swimmers reported a lower improvement rate of 53.5%, whereas infrequent/non-swimmers reported a sleep improvement rate of 67.5%. This variation suggests that habitual swimmers may experience a point of diminishing subjective returns either because of their bodies becoming acclimated to long-term training volumes or because of performance anxiety and early morning workouts actively constraining sleep quality.

A recent article discovered that 19%-34% of competitive youth or high-performance athletes experienced sleep disturbances, high baseline stress levels, and performance anxieties.[8]

To map several health outcomes, multi-variable average scores were plotted across four dimensions: Perceived Health, Baseline Stress, Mood Stabilization, and Sleep Quality (Figure 5). The outward expansion of the plot illustrates how higher swimming frequency coordinates a more balanced wellness footprint despite higher baseline stress load.

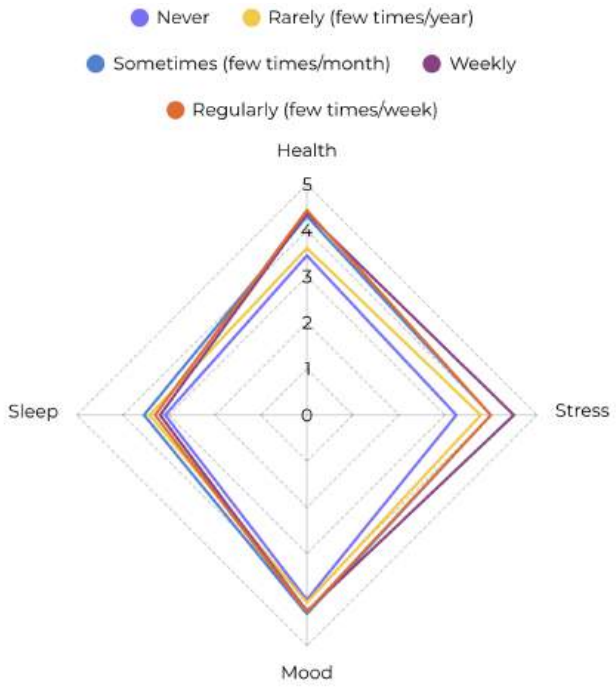

Figure 5. Multi-variable radar plot depicting mean wellness scores across four primary health pillars. The expanding area reflects enhanced overall well-being with increased swimming frequency.

Figure 5 provides a multi-variable view that shows the interaction between swimming frequency and four primary pillars of self-reported well-being: health, enhancement, stress management, mood stabilization, and perceived sleep quality. The geometry of the radar plot demonstrates an outward expansion of the wellness frontier. For individuals who never swim, the geometric plot remains tightly constrained to the origin, particularly along the health and sleep axes, where their scores exhibit low levels of 3.46 and 3.08 out of 5, respectively. On the other hand, it can be observed that as swimming frequency increases through rare and monthly intervals, the shape appears to shift significantly outward. This indicates how swimming at a rare to monthly frequency is correlated with improved baseline emotional balance and systemic physical conditioning.

However, the most significant shifts in geometry can be observed in the weekly and multi-weekly intervals. Their scores reached peaks of 4.38 and 4.44 out of 5 across the health metric. Moreover, while weekly swimmers registered the highest perceived stress score with 4.50 out of 5, their mood and health parameters remain strongly elevated. This chart visually

demonstrates how high-frequency swimmers successfully maintain exceptional physical health and emotional stability despite carrying a heavy baseline stress load. Ultimately, Figure 5 aims to show that frequent swimming coordinates a more balanced footprint across all tracked somatic psychological dimensions measured and tested in this paper.

Analytical Synthesis

Descriptive survey observations indicate broad health benefits, but an analytical synthesis of the quantitative data ($N = 95$) points to a striking and counterintuitive relation between baseline demands and post-exercise recovery. Regular swimmers actually have much higher baseline stress at 3.56 out of 5 compared to 2.91 out of 5 for non-swimmers, which may be a reflection of intense schedules of academics, competition or work. But this backdrop of increased risk creates a dynamic where those who have the most to lose have the most to gain: nearly 5 out of 6 regular swimmers (83.3%) report direct stress reduction after a swim compared to about 2 out of 3 infrequent swimmers (67.6%). From a physiological point of view, the enormous recovery yield is because cool water on the face activates the mammalian dive reflex, nature's inbuilt brake pedal to quickly engage the parasympathetic nervous system, and the horizontal position removes the gravitational resistance against the heart. At the same time, the aquatic setting offers a forced digital detox, with the visual simplicity and muted underwater acoustics providing a rare sensory reset that unloads cognitive fatigue. Overall, the therapeutic effect is clear: more than 3 in 5 participants (61.0%) feel less stressed after swimming, compared to 1 in 14 (7.4%) who feel worse; more than 1 in 2 (52.6%) report improved quality of sleep; and over 3 in 4 (75.8%) say swimming is a long-term protection against chronic disease. Regular swimmers report a peak general health score of 4.44 out of 5 versus 3.46 out of 5 for non-swimmers.

Conclusion

This study concluded that consistent swimming was significantly linked to improved mood regulation, post exercise stress relief, improved sleep quality and higher perceptions of overall health. Regular swimmers have been found to have higher levels of stress at baseline, but they also show the greatest relative stress recovery after exercise. These findings support aquatic exercise as a promising nonpharmacological approach to stress management. The association of these observational findings with physiological mechanisms such as parasympathetic stimulation, hydrodynamic pressure and cognitive unloading highlights the unique somatic and psychological benefits of swimming compared with terrestrial activities. Future studies should overcome the present study's limitations by including controlled experimental designs and objective physiological measures such as salivary cortisol and heart rate variability monitoring. The move from self-reported data to biomarker analysis will also shed more light on the underlying mechanisms and help in making targeted exercise recommendations for preventive healthcare.

Works Cited

1.Huang, Y. “Blue Health Among Children, Adolescents, and Youth Psychological Well-Being: A Systematic Review of Swimming and Aquatic Therapy for Mental Health.” Frontiers in Public Health, vol. 14, 2026, article 416081.

2.Irwin, Michael R. “Why Sleep Is Important for Health: A Psychoneuroimmunology Perspective.” Annual Review of Psychology, vol. 66, no. 1, 2015, pp. 143–172.

3.Lazar, Jason M., et al. “Acute Effects of Water Immersion on Cardiovascular Hemodynamics and Arterial Compliance.” Journal of Clinical Hypertension, vol. 21, no. 9, 2019, pp. 1385–1391.

4.Ludyga, Sebastian, et al. “Effects of Aerobic Exercise on Cortisol Stress Reactivity: A Systematic Review and Meta-Analysis.” Journal of Psychosomatic Research, vol. 133, 2020, article 110103.

5.Malik, S., and K. Johnston. “Mental Health in Elite and Youth Athletes: Emerging Strategies and Evidence-Based Trends.” Journal of Sports Sciences, vol. 12, no. 3, 2026, p. 1304.

6.McEwen, Bruce S. “Protective and Damaging Effects of Stress Mediators.” The New England Journal of Medicine, vol. 338, no. 3, 1998, pp. 171–179.

7.Rice, Simon M., et al. “The Mental Health of Elite Athletes: A Narrative Systematic Review.” Sports Medicine, vol. 46, no. 9, 2016, pp. 1333–1353.

8.World Health Organization. WHO Guidelines on Physical Activity and Sedentary Behaviour. World Health Organization, 2020.

How Primary Care Can Bridge Healthcare Gaps in Immigrant Communities
By Haseeb Chaudhry

Introduction

I grew up in the neighborhood of Jackson Heights in Queens, New York, surrounded by diversity long before I truly realized what it was. A short walk along a street such as Roosevelt Avenue meant that you could find Urdu, Spanish, Bengali, Nepali, and Hindi-speaking communities in almost every direction. Restaurants and small, individually owned businesses were open in every corner of the neighborhood, each one representing different cultures from all over the world. Every single person I met had a story involving immigration, hard work, and adaptation.

I think that's how I've always perceived community.

While I witnessed it all growing up, I also learned something else simultaneously: how many families in my community seemed to struggle with systems that were never truly designed for people like them in mind. It came down to many of the basic necessities of life, but to me, none was clearer than healthcare access.

I saw this struggle firsthand within my own family when my dad's cousin developed lumps on his leg. Our family repeatedly urged him to see a doctor, but he felt confident that herbal and homeopathic medicine would solve the problem, assuming they were nothing more than harmless cysts. By the time his condition severely worsened and he finally sought conventional medical care, tests revealed that the lumps were, in fact, cancerous and had metastasized throughout his body. Unfortunately, he passed away shortly after. His story is a painful example of what happens when medical evaluations are delayed, and how easily a critical diagnosis can be missed without routine care.

Demographic Context and Structural Barriers

This particular issue is especially significant in New York City, where more than three million immigrants make up over one-third of the city's population, making access to consistent, culturally responsive healthcare a critical concern (NYC Department of Health and Mental Hygiene). Queens stands out as one of New York City's most diverse communities, with 47.6% of its residents born outside the United States, according to U.S. Census Bureau data from 2020–2024 (U.S. Census Bureau). With more than one million immigrants calling Queens home, research in Brooklyn and Queens has documented significant barriers that continue to limit access to healthcare for many immigrant families (Pavilon and Virgin).

Clinical Shadowing and Observations

Wanting to better understand this issue firsthand, I began shadowing physicians and physician assistants at a primary care clinic. This is where I, a current high school senior, would finally begin to truly learn about the intricacies of healthcare. I dedicated approximately 250 hours over the past year and a half to gaining firsthand experience in a clinical healthcare setting.

Each day, patients, ranging from children to senior citizens, arrived at the clinic to receive various forms of treatment and care.

Through this process, I discovered that the most compelling part of observing healthcare was not just the diagnosis and treatment itself, but the process it takes to actually identify and then treat the issue.

I watched patients present their symptoms such as cough, chest pain, shortness of breath, or fatigue, and saw how doctors carefully considered multiple possibilities before recommending treatment. They would ask question after question to the patient, slowly narrowing down the potential causes of the problem before finally proposing a solution. Throughout this observational process, I witnessed a deeper reality about the role of medicine: that it was not simply treating the ailment itself, but the necessity for the physician to critically assess, ask targeted questions, detect patterns, and determine the urgency for further medical evaluation and specialized care. For some patients, the answer was as simple as a change in diet or a routine follow-up, but for many, a blood test or a referral to a specialist was needed. Regardless of the treatment, I noticed that primary care was often the first point of contact when patients faced a health concern.

That first stop carries far more significance than most people realize. A substantial body of research has linked strong primary care systems with better population health, health equity, and healthcare quality (Stange et al.; Starfield et al.). The Agency for Healthcare Research and Quality describes primary care as spanning preventive care through diagnosis and treatment and notes that access to comprehensive primary care improves patient and population health outcomes across the lifespan (Eden and Miller). Research on continuity of primary care has also found an association between greater continuity and lower mortality, although these findings should be interpreted as associations rather than proof of causation (Baker et al.).

Systemic Healthcare Disparities

Despite all of the evidence and research in this area, for immigrant communities in our city, the initial trip to the doctor is oftentimes delayed.

It's not a matter of families disregarding their health and well-being.

It's because barriers exist.

For example, Kaiser Family Foundation data from 2024 show that noncitizen immigrants were more than 3.5 times as likely as U.S. citizens to be uninsured. Among noncitizens who had lived in the United States for more than five years, 30.6% were uninsured, compared with 8.0% of U.S.-born and naturalized citizens (KFF). Lack of insurance is associated with lower use of routine healthcare. In 2024, 46.2% of uninsured adults ages 18–64 reported not seeing a doctor or other health professional during the previous 12 months, compared with 14.7% of adults with private insurance and 12.8% of those with public coverage (KFF).

To many people in communities like Jackson Heights, those numbers are not just statistics. They are the beginning of serious health concerns. Research examining immigrant communities has identified several barriers to healthcare access, including lack of insurance,

difficulty affording care, work and family responsibilities, language barriers, and fear of revealing immigration status (Pavilon and Virgin). The CDC similarly notes that refugee, immigrant, and migrant communities may face economic and social barriers to healthcare, including lack of health insurance, language barriers, concerns about immigration status, and distrust related to experiences of discrimination or unfamiliarity with U.S. healthcare systems (CDC). Although focused on rural settings, a recent scoping review by Zeng et al. similarly demonstrates how multi-level barriers, ranging from individual factors to structural policy exclusions, persist across immigrant communities regardless of geography. These barriers can contribute to delays in seeking care, increasing the likelihood that some health concerns are addressed only after they have become more difficult to manage.

The Value of Trust in Primary Care

One thing I learned from my time at the clinic was just how much trust matters. For many of the patients I observed, the primary care physician was the first person they turned to when a health concern arose. Many patients sought not only medical care, but also an understanding of what was happening in their bodies and why.

Even from simple tasks like taking blood pressure, recording vital signs, or relaying lab results to the administrative team, it became crystal clear how much responsibility lies within physicians, and essentially, all medical professionals at primary care clinics, to not only be caregivers, but guides and confidants.

Behind every lab result and medical history is a patient seeking to understand their health better and make informed decisions about their well-being.

There was this one elderly patient I still remember. He came in complaining about fatigue and persistent chest pain, initially brushing it off as stress from work. The patient was also due for a routine EKG checkup. I watched as the physician assistant opened his EKG file and began analyzing the EKG tracing. Typically, he would be teaching me something along the way, telling me what shows that the heart rate is normal. This time, silence. He called in the attending physician to review the tracing. When he arrived, he examined it and confirmed that the patient's cardiac rhythm was irregular. He was immediately referred to a cardiologist. We heard back the following week, and thankfully, nothing serious was found, but I still wonder what might have happened if he had not come on that day.

Personal Reflections and Public Health Aspirations

At Stuyvesant High School, my appreciation of medicine, specifically primary care, has only deepened. Through rigorous science classes and my role as a Co-Vice President for StuyMED, a club where we invite medical professionals to come speak about healthcare and hold meetings about topics like healthcare careers and the process it takes to actually become a doctor, I have begun to connect my observations at clinics to larger issues in public health. The more I learn, the more I realize that medicine is so much more than biology.

It is also about systems.

It is also about equality.

And, at its core, it is about access.

We often talk about healthcare inequality in broad terms, but in immigrant communities that inequality is deeply personal. It can determine when someone seeks care, whether they feel comfortable communicating their symptoms, and whether they believe the healthcare system will understand and support them. Growing up in Jackson Heights showed me the strength and resilience of immigrant communities. My experience in healthcare showed me how vulnerable those same communities can become when access is limited.

Potential Solutions and Community Navigator Models

While primary care alone cannot address all of the issues within the complex world of healthcare, it represents an incredibly vital resource. It can be the bridge that makes more complicated treatment possible. By breaking down many of these access barriers and providing clear communication and trustworthy guidance, the first step toward primary care may help determine whether health concerns are identified and addressed before they become more difficult to manage. In fact, according to an analysis of almost thirty studies conducted in the United States, it appears as though one promising approach already exists. As mentioned by Shommu et al., community health worker and patient navigator models, in which workers are recruited from the community which they serve, were proven to greatly enhance chronic disease management and access to primary care among immigrant and ethnic minority patients, even going so far as to provide two pilot programs of such a nature in New York City, for Sikh Asian Indian and Korean communities. This idea seems to reflect what I have seen firsthand at the clinic, where, besides the medicines, the patient felt understood to a far greater degree when they could communicate with someone who shared their cultural background and understood the experiences of their community. Using the same model in clinics throughout neighborhoods like Jackson Heights would not solve every single barrier that immigrants face. But, it can be the difference between a world of uncertainty and a system of clarity.

Conclusion

For immigrant communities, access to primary care is about more than treating illness. It is about creating a healthcare system where people feel seen, understood, and empowered to seek care before it becomes an emergency.

Because the bridge to better health begins with making sure everyone has a way across.

Works Cited

Baker, Richard, George K. Freeman, Jeannie L. Haggerty, M. John Bankart, and Keith H. Nockels. “Primary Medical Care Continuity and Patient Mortality: A Systematic Review.” British Journal of General Practice, vol. 70, no. 698, 2020, pp. e600–e611. https://doi.org/10.3399/bjgp20X712289.

Centers for Disease Control and Prevention. “Health Communication with Refugee, Immigrant, and Migrant Communities.” CDC Yellow Book 2026, 23 Apr. 2025, https://www.cdc.gov/yellow-book/hcp/refugees-immigrants-migrants/health-communication-with-refugee-immigrant-and-migrant-communities.html.

Eden, Aimee, and Tess Miller. “AHRQ Intensifies Its Efforts to Strengthen Primary Care.” Agency for Healthcare Research and Quality, 9 Oct. 2024, https://www.ahrq.gov/news/blog/ahrqviews/strengthen-primary-care.html.

KFF. “Key Facts about the Uninsured Population.” 16 June 2026, https://www.kff.org/uninsured/key-facts-about-the-uninsured-population/.

New York City Department of Health and Mental Hygiene. “Immigrant Health Care.” Accessed August 8, 2026, https://www.nyc.gov/site/doh/health/health-topics/immigrant-health.page.

Pavilon, Jacquelyn, and Vicky Virgin. Social Determinants of Immigrants’ Health in New York City: A Study of Six Neighborhoods in Brooklyn and Queens. Center for Migration Studies of New York, 15 June 2022, https://cmsny.org/publications/nyc-health-061522/.

Shommu, Nusrat Sharmeen, et al. "What Is the Scope of Improving Immigrant and Ethnic Minority Healthcare Using Community Navigators: A Systematic Scoping Review." International Journal for Equity in Health, vol. 15, 2016, article 6, https://doi.org/10.1186/s12939-016-0298-8.

Stange, Kurt C., William L. Miller, and Rebecca S. Etz. “The Role of Primary Care in Improving Population Health.” Milbank Quarterly, vol. 101, suppl. 1, 2023, pp. 795–840. https://doi.org/10.1111/1468-0009.12638.

Starfield, Barbara, Leiyu Shi, and James Macinko. “Contribution of Primary Care to Health Systems and Health.” Milbank Quarterly, vol. 83, no. 3, 2005, pp. 457–502. https://doi.org/10.1111/j.1468-0009.2005.00409.x.

U.S. Census Bureau. “QuickFacts: Queens County, New York.” https://www.census.gov/quickfacts/table/POP645214/36081.

Zeng, Yingying, Xixi Kang, Yibin Yang, and Eunmi Hwang. “Barriers and Facilitators of Preventive Healthcare Access among Immigrants in Rural America: A Scoping Review.” International Journal for Equity in Health, vol. 24, 2025, article 241. https://doi.org/10.1186/s12939-025-02603-2.

The Rise and Fall of Chemotherapy By Chanho Jung

Abstract

Treatment decisions for cancer depend on the cancer type, the stage, and the patient's overall health. Surgery and radiation are local treatments. Chemotherapy, targeted therapy, hormone therapy, and immunotherapy are systemic treatments. This paper compares treatment data from 2011 and 2021 to track changes in chemotherapy use for breast cancer, colon cancer, non-Hodgkin lymphoma, and lung cancer. It also reviews mechanisms of drug resistance, including reduced drug uptake, DNA repair, and altered cell-death signaling. The data show how chemotherapy use changed over the decade, though the comparison cannot prove that resistance caused these shifts.

Cancer development and genetics

Cancer occurs when abnormal cells divide without normal biological controls. These cells can form tumors. Benign tumors do not invade nearby tissues. Malignant tumors invade surrounding tissue and can travel through the blood or lymphatic system to form new tumors. The American Cancer Society estimates that 38.9 percent of the U.S. population will be diagnosed with cancer during their lifetime.

Cancer develops through DNA mutations that alter cell growth, division, repair, and death. Proto-oncogenes can mutate into oncogenes, which promote cell growth. Tumor suppressor genes, which normally limit growth, can fail. DNA-repair genes, which correct damage, can also become impaired. When these genetic systems fail, cells can divide continuously, avoid apoptosis, invade tissue, and metastasize.

Signs and symptoms

Cancer symptoms overlap with those of other conditions. Persistent fatigue, unplanned weight loss, a new lump, changes in bowel or bladder habits, unusual bleeding, a persistent cough, trouble swallowing, and changes in skin lesions require medical evaluation. Symptoms prompt diagnostic testing but cannot confirm a cancer diagnosis alone.

Diagnosis and staging

Physicians use imaging tests, such as X-rays, CT scans, MRIs, and ultrasounds, to locate abnormalities and assess their size. Endoscopy provides direct visual examination of internal organs. A biopsy, which involves removing tissue for pathological examination, is required to confirm a cancer diagnosis. Cytology tests examine individual cells from fluid samples to identify abnormal changes.

Staging classifies the size of a tumor and the extent of its spread. Many solid tumors are classified from stage 0 through stage IV. Lower stages indicate the disease is limited to the original site. Stage IV indicates the cancer has spread to distant organs.

Local and systemic treatments

Cancer treatment is categorized as local or systemic. Local treatments target a specific tumor or body area. Surgery removes a tumor and surrounding tissue. Radiation therapy uses high-energy radiation to damage cancer cells in a specific field.

Systemic treatments circulate through the body. Targeted therapies interfere with specific cellular features. Immunotherapy stimulates the immune system to attack cancer cells. Hormone therapy blocks hormones that promote cancer growth. Chemotherapy uses drugs to destroy cancer cells or halt their division. Treatment plans may use a single drug or a combination. The intent can be to cure the disease, control its growth, prevent recurrence, or relieve symptoms. Chemotherapy frequently operates alongside surgery, radiation, targeted therapy, immunotherapy, or hormone therapy. Because chemotherapy affects fast-growing cells, it also damages normal cells that divide quickly, causing side effects. Treatment outcomes depend on the cancer type, the stage, and the tumor's response to the specific drugs used.

Approximately 18.6 million Americans have a history of cancer. Improved detection and treatment have increased the number of people living after a diagnosis.

Drug resistance

Drug resistance limits the effectiveness of chemotherapy. Tumors may be inherently resistant, or they may acquire resistance during treatment.

One mechanism is drug efflux. Proteins in the cell membrane, such as P-glycoprotein, pump chemotherapy drugs out of the cell, reducing the internal drug concentration. Genetic and epigenetic mutations also alter treatment response. Mutations can modify the drug's target or inhibit apoptosis in damaged cells. Changes in gene expression can increase survival proteins or decrease proteins that make the tumor vulnerable to the drug.

Cancer cells also use signaling pathways and the tumor microenvironment to survive. Signals from surrounding cells and local inflammation can activate survival pathways. Cancer cells can also increase their DNA-repair activity to fix the damage caused by chemotherapy, preventing cell death. Finally, cancer cells can alter their metabolism to produce enzymes that inactivate the chemotherapy drug.

Changes in treatment patterns: 2011 to 2021

This analysis compares treatment data from the 2014–2015 and 2025 survivorship reports to track changes in chemotherapy use. The reports use different classification categories in several areas, so the analysis identifies specific structural changes but does not treat all differences as direct, one-to-one trends.

Data

Table 1. Breast Cancer Treatments 2011 vs. 2021

2021 (2025)*	Stages 1 &2	Stage III	Stage IV

BCS & RT = small get chemo	50	18	2
Mastectomy + Chemo +/- RT	14%	53	8
RT and/or Chemo	1	10	64
2011 (2015)**	Stages 1&2	Stage III & IV	
BCS + RT + Chemo	33(BCS + RT) + 17 (BCS + RT + Chemo) = 50	10	
Mastectomy + Chemo	12	14	
Mastectomy + RT + Chemo	6	34	
RT and/or Chemo	4	16	

* = from Wagle et al. ** = from DeSantis et al.

Table 2. Colon Cancer Treatments 2011 vs. 2021

2021 (2025)*	Stage 1 & 2	Stage 3	Stage 4
Colectomy + Chemo	11	67	**33**
Chemo*small portion get RT	<1	<1	**34**
2011 (2015)**	Stage 1 & 2	Stage 3	Stage 4
Colectomy + Chemo	11	64	**41**
Chemo and/or RT	<1	<1	**22**

* = from Wagle et al. ** = from DeSantis et al.

Table 3. Non-Hodgkin Lymphoma Treatments 2011 vs. 2021

2021 (2025)*	
Chemo + Immunotherapy (+/- RT)	72%
Chemo (no RT or Immunotherapy)	10%
Chemo + RT (no Immunotherapy)	2%
Immunotherapy and/or RT	4%

No RT, chemotherapy, or immunotherapy	12%
2011 (2015)**	
Chemo without RT	57%
Chemo + RT	12%
No surgery, RT, or Chemo	15%
Surgery alone	8%
RT without Chemo	7%

* = from Wagle et al. ** = from DeSantis et al.

Table 4. Lung Cancer Treatments 2011 vs. 2021

2021 (2025)*	Stage 1 & 2	Stage 3	Stage 4
Surgery no RT or chemo	44	5	<1
Surgery + Chemo or RT	8	15	<1
Chemo (no RT)	1	7	23
Chemo + RT	4	44	22
2011 (2015)**	Stage 1 & 2	Stage 3 & 4	
Surgery no RT or chemo	52	18	
Surgery + chemo or Rt	16	2	
Chemo alone	1	6	
Chemo + RT	6	33	

* = from Wagle et al. ** = from DeSantis et al.

Discussion

In stages I and II breast cancer, the 2011 report separated breast-conserving surgery with radiation (33 percent) from the same approach combined with chemotherapy (17 percent). The 2021 report aggregates these categories at 50 percent, noting that a smaller share received chemotherapy. This aggregation prevents a precise comparison of chemotherapy use within that group. For later stages, the reports use different stage groupings, precluding a direct trend

analysis. For mastectomy in stages I and II, the 2011 figures total 18 percent for mastectomy with chemotherapy (with or without radiation), compared to 14 percent in 2021.

For colon cancer, colectomy with chemotherapy remained at 11 percent for stages I and II. It increased from 64 percent to 67 percent in stage III and decreased from 41 percent to 33 percent in stage IV. Chemotherapy with or without radiation remained below 1 percent in stages I through III but increased from 22 percent to 34 percent in stage IV. In stage IV, the decrease in colectomy plus chemotherapy was offset by an increase in chemotherapy with or without radiation.

For non-Hodgkin lymphoma, chemotherapy-containing treatments accounted for 69 percent of patients in 2011 and 84 percent in 2021. The 2021 report separates chemotherapy with immunotherapy from chemotherapy alone. This change reflects both an expanded classification system and a higher reported use of chemotherapy-containing regimens.

Lung cancer treatment patterns varied by stage. In stages I and II, surgery with chemotherapy or radiation decreased from 16 percent to 8 percent. Chemotherapy alone remained at 1 percent. In 2021, chemotherapy alone was used for 7 percent of stage III patients and 23 percent of stage IV patients. Chemotherapy with radiation was used for 44 percent of stage III patients and 22 percent of stage IV patients. Because the 2011 report combined stages III and IV, the later-stage figures cannot be compared directly. However, the 2021 data show systemic therapy was used more frequently in advanced disease than in early-stage disease.

Conclusion

The breast cancer data show that combining treatment categories obscures specific changes in chemotherapy use within breast-conserving treatment. For early-stage mastectomy, the percentage of patients receiving chemotherapy-containing treatment was lower in 2021. For colon cancer, chemotherapy use remained concentrated in stages III and IV. Stage IV care shifted away from colectomy plus chemotherapy toward chemotherapy with or without radiation. For non-Hodgkin lymphoma, chemotherapy-containing treatments, particularly those including immunotherapy, represented a larger share in 2021. For lung cancer, chemotherapy and chemoradiation were more common in advanced stages than in early stages.

Across all four cancers, chemotherapy use varied by cancer type and stage. The comparison demonstrates how category definitions limit historical data analysis; differences in how the two reports group treatments prevent direct trend comparisons in several areas. Drug resistance is a documented clinical factor in treatment outcomes, but these reporting tables cannot measure its specific effect on the observed historical shifts in prescription patterns.

Works Cited

American Cancer Society. “Cancer Staging.” American Cancer Society, 2024. www.cancer.org/cancer/diagnosis-staging/staging.html.

American Cancer Society. “Imaging (Radiology) Tests for Cancer.” American Cancer Society, 23 April 2026. www.cancer.org/cancer/diagnosis-staging/tests/imaging-tests/imaging-radiology-tests-for-cancer.html.

American Cancer Society. “Oncogenes, Tumor Suppressor Genes, and DNA Repair Genes.” American Cancer Society, 31 Aug. 2022. https://www.cancer.org/cancer/understanding-cancer/genes-and-cancer/oncogenes-tumor-suppressor-genes.html

American Cancer Society. “What Is Cancer? | Cancer Basics | American Cancer Society.” American Cancer Society, 14 Feb. 2022. www.cancer.org/cancer/understanding-cancer/what-is-cancer.html.

American Cancer Society. “What Is Chemotherapy? | Chemo Treatment for Cancer.” American Cancer Society, 15 May 2025. www.cancer.org/cancer/managing-cancer/treatment-types/chemotherapy.html.

American Cancer Society. “Cancer Facts & Figures 2025.” American Cancer Society, 2025. www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/2025-cancer-facts-figures.html.

American Cancer Society. “Cancer Treatment Survivor Facts & Figures.” American Cancer Society, N.D. www.cancer.org/research/cancer-facts-statistics/survivor-facts-figures.html.

Casás-Selves, Matias, and James DeGregori. “How Cancer Shapes Evolution and How Evolution Shapes Cancer.” Evolution: Education and Outreach, vol. 4, no. 4, Dec. 2011, pp. 624–634, www.ncbi.nlm.nih.gov/pmc/articles/PMC3660034/.

Cleveland Clinic. “Cancer Staging System: What Do Stages and Grades Mean?” Cleveland Clinic, 2022. my.clevelandclinic.org/health/diagnostics/22607-cancer-stages-grades-system.

DeSantis, Carol, et al. “Cancer Treatment and Survivorship Facts & Figures 2014-2015.” American Cancer Society(2014): 1-43.

Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug resistance in cancer: an overview. Cancers, 6(3), 1769-1792.

Lei, Zi‐Ning, et al. "Understanding and targeting resistance mechanisms in cancer." MedComm 4.3 (2023): e265.

Li, Jinxin, et al. "Drug resistance in cancer: molecular mechanisms and emerging treatment strategies." Molecular Biomedicine 6.1 (2025): 111.

Lillis, Charlotte. “Chemotherapy: Success Rates for Different Cancers.” Medical News Today, 25 May 2023. www.medicalnewstoday.com/articles/326031.

Mansoori, B., Mohammadi, A., Davudian, S., Shirjang, S., & Baradaran, B. (2017). The different mechanisms of cancer drug resistance: a brief review. Advanced pharmaceutical bulletin, 7(3), 339.

Mayo Clinic . “Cancer - Symptoms and Causes.” Mayo Clinic, 7 Dec. 2022. www.mayoclinic.org/diseases-conditions/cancer/symptoms-causes/syc-20370588.

Mayo Clinic Health System. “How Cancer Is Diagnosed - Fairmont.” Mayo Clinic Health System, N.D. www.mayoclinichealthsystem.org/locations/fairmont/services-and-treatments/oncology/cancer-diagnosis/how-cancer-is-diagnosed.

National Cancer Institute. “Cancer Staging.” National Cancer Institute, 14 Oct. 2022. www.cancer.gov/about-cancer/diagnosis-staging/staging.

National Cancer Institute. “Chemotherapy | Breast Cancer Treatment.” National Cancer Institute, 2 Dec. 2025. www.cancer.gov/types/breast/treatment/chemotherapy.

National Cancer Institute. “How Cancer Is Diagnosed.” National Cancer Institute, 17 Jan. 2023. www.cancer.gov/about-cancer/diagnosis-staging/diagnosis.

National Cancer Institute. “NCI Dictionary of Cancer Terms.” National Cancer Institute, 2 Feb. 2011. www.cancer.gov/publications/dictionaries/cancer-terms/.

National Cancer Institute. “Symptoms.” National Cancer Institute, 16 May 2019. www.cancer.gov/about-cancer/diagnosis-staging/symptoms.

National Cancer Institute. “What Is Cancer?” National Cancer Institute, 11 Oct. 2021. www.cancer.gov/about-cancer/understanding/what-is-cancer.

Wagle, Nikita Sandeep, et al. "Cancer treatment and survivorship statistics, 2025." CA: A cancer journal for clinicians75.4 (2025): 308-340. https://pmc.ncbi.nlm.nih.gov/articles/PMC3660034/

Food Insecurity Beyond Hunger: Nutritional Barriers Facing Students Experiencing Homelessness By Louis Rotgin

More than one and a half million students across the United States experience homelessness during the school year. This impacts far more than where these students sleep—it influences something essential to learning and development: access to nutritious food.

Under the federal McKinney-Vento Homeless Assistance Act, a student is considered homeless if they lack a fixed, regular, and adequate nighttime residence. This extends past the common image of homelessness and includes students who are sleeping in shelters, motels, campgrounds, cars, or are temporarily doubled-up with other families, among other situations.

A 2026 National Center for Homeless Education (NCHE) report concluded that in the 2023-2024 school year, 1,548,191 students experienced homelessness in the U.S. (NCHE). This represents a 12.6% increase from just the year prior. It is important to note that these numbers, although official, are likely an underestimation of the entire population and have likely increased in subsequent years. In 2020, the U.S. Government Accountability Office (GAO), a non-partisan organization, reported that federal officials have acknowledged that the homeless population is likely undercounted given that many homeless individuals and families are difficult to locate or identify (GAO).

Examining the challenges these students face in obtaining nutritious food is essential to understanding how housing instability impacts students' health, education, and long-term development.

Adolescence is considered a critical period of growth and development for both the body and mind. Due to the rapid rate at which growth and development occur during these years, adolescent youth have increased nutritional needs. The World Health Organization (WHO) identifies adolescence as a key window for promoting lifelong health and emphasizes the important role that nutrition plays in supporting both physical and cognitive development during the period (WHO).

Nutrition is directly connected to students' ability to learn and perform in school. A study published in *Nutrition Reviews* found evidence that quality nutrition positively influences brain function, cognition, behavior, and learning in adolescent children (Brkić et al.). Conversely, a study published in *The Journal of School Health* found that food insecurity was associated with poorer academic and developmental outcomes in teenage students (Orihuela et al. 883).

A meta-analysis published in *Public Health Reports* estimated that around 57% of people experiencing housing instability and homelessness in the US also face food insecurity (Jackson et al.). Together, these findings suggest that because food insecurity is so common among families experiencing homelessness, housing instability can hinder students' ability to learn through inconsistent or inadequate access to nutritious food.

Given the scope of the problem, for many students experiencing homelessness, schools serve as a crucial, reliable source for consistent meals. Under the Richard B. Russell National School Lunch Act, students experiencing homelessness are categorically eligible for free school

meals at schools that provide meal programs, such as the National School Lunch Program and the School Breakfast Program. These programs are administered by the United States Department of Agriculture (USDA) and state agencies, which reimburse schools for providing meals that meet federal nutrition standards. There are additional programs meant to address nutritional needs outside of school hours, including the National School Lunch Program's Afterschool Snack Service, the At-Risk Afterschool Meals component of the Child and Adult Care Food Program, and the Summer Food Service Program. Research from the Centers for Disease Control and Prevention (CDC) has found that eating breakfast at school is associated with better attendance rates and overall academic performance, highlighting the importance of school meals in supporting students' ability to learn (CDC).

However, these school meal programs do not always address the nutritional needs of students outside of school hours. Students who attend schools without meal services, or who require food after hours, on weekends, or during school breaks must rely on other resources. The NCHE identifies programs such as the Supplemental Nutrition Assistance Program (SNAP), weekend food programs, and partnerships between schools and community organizations like restaurants, grocery stores, and farmers markets as potential support for students experiencing homelessness (NCHE). Additionally, school districts may use Title 1 Part A funds to purchase food for students experiencing homelessness so they can access educational opportunities when other funding is not reasonably available.

Beyond school meal programs, many students and families experiencing homelessness depend on emergency food networks, including shelters, soup kitchens, food banks, community organizations, and youth drop-in centers. These organizations play an essential role for these students and families by providing them with meals and groceries when they otherwise would not have reliable access to food. However, the quality and consistency of these resources can vary significantly. Research published in *Public Health Nutrition* concluded that meals provided by emergency food networks are often limited by financial constraints, restricted storage space, and reliance on donated foods (Koh et al. 1312). As a result, organizations may struggle to consistently provide quality nutrition, with meals often containing high amounts of refined carbohydrates while being low in important nutrients.

Some families experiencing homelessness obtain food through the use of SNAP, which provides funds to low-income households to purchase food. While SNAP can provide students and families with the ability to obtain groceries, other barriers that prevent nutritious eating are still present. A *Demographic Research* review identifies a lack of reliable transportation, refrigeration, cooking facilities, and unstable housing as barriers that cause families experiencing homelessness to struggle turning available resources into consistent, healthy meals (Lee and Lippert 1115).

Together, these findings illustrate that the challenges facing students experiencing homelessness are not just access to food, but consistent access to nutritious food. For developing children and adolescents, consistent access to quality nutrition is important for physical growth, cognitive development, and strengthening learning abilities. However, emergency food providers

often face difficulties such as limited budgets, dependence on donated foods, and the logistical challenges of storing and distributing fresh, perishable items, which frequently cause organizations to focus on providing enough calories rather than nutritionally balanced meals. Even when families receive food assistance, barriers such as transportation, refrigeration, cooking facilities, and unstable housing cause difficulty in preparing and consistently consuming nutritious foods. As a result, although many students experiencing homelessness are able to obtain food from schools, assistance programs, and emergency food networks, maintaining a healthy, well-balanced diet remains a significant challenge.

Understanding how students experiencing homelessness obtain food reveals that the challenge extends far past hunger alone. Because quality nutrition is essential for growth and development, addressing food insecurity among students experiencing homelessness requires more than providing enough food; it requires removing the barriers that prevent students from consistently accessing nutritious meals.

Works Cited

Brkić, D., et al. "Relationship between Nutrition, Brain, Cognition, Learning, and Behavior in School Age Children: Systematic Evidence and Future Opportunities." Nutrition Reviews, 2026, https://doi.org/10.1093/nutrit/nuaf280.

Centers for Disease Control and Prevention. “School Meals.” Centers for Disease Control and Prevention, 23 July 2024, https://www.cdc.gov/school-nutrition/school-meals/index.html.

Jackson, C. L., et al. "A Meta-Analysis of the Prevalence of Food Insecurity among People Experiencing Housing Insecurity and Homelessness in the United States." Public Health Reports, vol. 141, no. 3, 2026, pp. 380–394. https://doi.org/10.1177/00333549241305349.

Koh, K. A., Bharel, M., and D. C. Henderson. "Nutrition for Homeless Populations: Shelters and Soup Kitchens as Opportunities for Intervention." Public Health Nutrition, vol. 19, no. 7, 2016, pp. 1312–1314. https://doi.org/10.1017/S1368980015002682.

Lee, B., and A. Lippert. “Food Insecurity among Homeless and Precariously Housed Children in the United States: Lessons from the Past.” Demographic Research, vol. 45, 2021, pp. 1115–1148, https://doi.org/10.4054/DemRes.2021.45.37.

National Center for Homeless Education. Access to Food for Students Experiencing Homelessness. 2022, https://nche.ed.gov/wp-content/uploads/2022/08/Access-to-Food.pdf.

National Center for Homeless Education. Education of Children and Youth Experiencing Homelessness in the United States: Data Summary for School Years 2021–22 to 2023–24. 2026, https://nche.ed.gov/wp-content/uploads/2026/03/Education-of-CYEH-in-the-United-States-data-summary-for-school-years-2021-22-to-2023-24.pdf.

Orihuela, C. A., et al. "Associations of Household Food Insecurity with Academic Outcomes in Early Adolescents." Journal of School Health, vol. 93, no. 11, 2023, pp. 883–890. https://doi.org/10.1111/josh.13358.

U.S. Government Accountability Office. Homelessness: Better HUD Oversight of Data Collection Could Improve Estimates of Homeless Population. GAO-20-433, 2020, https://www.gao.gov/products/gao-20-433.

World Health Organization. Guideline: Implementing Effective Actions for Improving Adolescent Nutrition. 2018, https://www.who.int/publications/i/item/9789241513708.

Mushrooms as a Sustainable Alternative to Animal-derived Protein: Nutritional, Environmental, and Physiological Implications By Yancheng Wang

Abstract

Rising protein demand and the environmental and health costs of farming livestock, including excessive resource usage, emissions, and health risks, have pushed researchers to search for alternative protein sources. Mushrooms and mycoprotein stand out because of their complete essential amino acid profile, minimal environmental impact, and ability to stimulate muscle protein synthesis (MPS) in athletes, in some studies outperforming milk and matching omnivorous diets with meat. This paper reviews mushrooms' nutritional composition, including vitamin D from ergosterol and bioactive compounds such as ergothioneine and beta-glucans, with beta-glucans being able to support immune functions in humans through the Dectin-1 receptor pathway. Sustainability benefits, such as vertical farming, are discussed alongside synthetic meat. While mycoprotein proves to be a strong contender as an animal-derived protein substitute, inconsistency in the data and reliance on mycoprotein rather than mushrooms as a whole are key limitations that require further work.

Introduction

With the great increase in population, the global demand for protein-dense foods is also increasing massively, which has caused researchers to look beyond conventional agricultural livestock such as cows and sheep and explore other sources, since the population is in desperate need of newer protein sources to sustain the population (Liu et al., 2025). Currently, agriculture already uses up around 50% of the habitable land on Earth and also contributes to up to 30% of greenhouse gas emissions as well as 70% of usage of freshwater; the population is also estimated by researchers to reach a high of 9 billion people by 2050, which will even further challenge the ability to supply protein to the general population through the efforts of animals (Fanello et al., 2024). Because of these factors, researchers have started to look towards alternative protein sources.

Another aspect of the need for an additional source of protein is the costs of overrelying on animal-derived proteins for both the environment and our health. On the health side, there is a clear link between the consumption of red and processed meats and higher chances of developing different cancers such as colorectal and pancreatic cancer due to the presence of heme iron (Sivasubramanian et al., 2023). Overconsumption of heme iron has also been found to support the expansion of *Helicobacter pylori* (a common bacterium that attacks the human stomach lining), which is one of the biggest causes of stomach cancer, further suggesting risks it may pose that are often not found in non-animal-derived proteins. When considering the environmental side of animal protein, it should be noted that growing livestock also costs a lot of resources, as just a single kg of beef can use a total of 25 kg of cow feed and around 15,000 L of fresh drinking water, which shows how costly livestock may be, especially when it is consumed everywhere around the world (Pashaei et al., 2024). It was also found that around one-seventh to

one-third of all of the greenhouse gas emissions are caused by diets surrounding red meat; ecosystems are also not safe through all this, since to keep up with ever-increasing demands, expansion of farms has had to occur, which has often led to disruptions of habitats such as deforestation in places such as the Amazon (Pashaei et al., 2024).

Livestock, especially cattle, release methane into the atmosphere through enteric fermentation, a digestion process where the microbes in the animal's stomach break down the food and create methane as a byproduct released through eructation. Methane itself is able to stay in the atmosphere for around 12 years, which is a lot shorter than other gases like carbon dioxide; however, it can trap nearly 28 times the heat compared to carbon dioxide in a 100-year period, making it more aggressive despite the different ways the gases can build up (Evangelista et al., 2024). This is also one of the main reasons that livestock are responsible for around 12% of man-made greenhouse gas emissions, with methane making up 54% of that portion (Evangelista et al., 2024). The ability of methane to be broken down quickly relative to the other gases makes scientists theorize that cutting its production is one of the fastest ways to slow climate change, as other alternatives, such as cutting carbon dioxide, have longer wait times for researchers to start seeing results (Balcombe et al., 2018). This may be crucial in the context of mushrooms as a protein alternative, as they produce no methane when grown, so mass farming of them and reducing livestock may be one of the most efficient ways to lessen global warming and reduce humans' carbon footprint.

With human advancements, many non-animal-based protein sources have appeared in society. Plant-based diets are almost ubiquitous and found to be a lot more sustainable by leaving a smaller environmental footprint (Pashaei et al., 2024); however, they are often lacking in protein or deficient in other factors that dissuade people from consuming them as a meat alternative. However, mushrooms have been one replacement for meat that has stood out not only due to their sustainability, using a lower amount of water to grow and also leaving a smaller impact on the environment, but also due to their meat-like qualities such as taste and texture (Pashaei et al., 2024). It has also been found that many species of mushrooms, such as *Lentinus edodes* and *Agaricus bisporus,* contain concentrations of protein that match and sometimes even surpass the protein in products from animals such as meat, eggs, and dairy (Singh et al., 2025). Unlike different types of plant protein, research on mushrooms has found that protein in mushrooms often contains a complete essential amino acid profile while also costing less financially to cultivate when compared to other sources of protein (Ayimbila & Keawsompong, 2023). These advantages show that mushrooms are a possible substitute for animal proteins when implemented in a diet to support muscle growth caused by exercise.

Nutritional Composition of Mushrooms

Mushrooms are a unique source of protein compared to other plant-based alternatives. This is because although they have a lower quantity of protein than others such as tofu, they are considered to be very complete. Mushrooms have around 200-250 g of protein for every kg of the dried product. When they still retain moisture, that ratio would drop to around 2.25 g of

protein for every 100 g, which, when compared to other sources such as chicken at 27 g, may seem insignificant (Pashaei et al., 2024). However, what sets it apart from the other plant alternatives is that it has a complete profile of amino acids, as it can provide humans with all nine essential amino acids that the body can not produce, which is a commonality it has with meat that other plant-based proteins lack (Ayimbila & Keawsompong, 2023). Mushrooms have also been found to be high in branched-chain amino acids such as leucine and valine, which are also similar to proteins from animal products and are important for muscle growth and recovery. Although all the amino acids are beneficial, leucine is still the most important one as it acts as a signal for one's muscles to start growing.

Other than protein, mushrooms also contain micronutrients that can not be matched by other plant-based foods. They are very low in calories, sodium, and fat. They contain substantial amounts of potassium, selenium, and dietary fiber, which are all beneficial for the body (Pashaei et al., 2024). One of the most unique parts of their nutritional profile is that they are a good source of vitamin D. A major difference between animal-derived products and plants is that you can not get vitamin D from eating plants, whereas food from animals such as fish and eggs provides an excellent source that is hard to replace. Unexpectedly, mushrooms are a great source since they contain a chemical in them called ergosterol, which, when exposed to UV, can convert the ergosterol into vitamin D2, something completely foreign to other meat substitutes (Pashaei et al., 2024). These facts exemplify that mushrooms are starting to be recognized as a good nutritional source, which is why they are starting to be talked about as a functional food instead of a dietary replacement for vegetables in research (Singh et al., 2025).

Mushrooms are made even more unique with two bioactive compounds: ergothioneine and beta-glucans. Ergothioneine is an antioxidant with sulfur that the body can not create and must receive through other means, such as the diet. Mushrooms are one of the best sources for ergothioneine, as they contain much more of it than any other food (Kalaras et al., 2017). The amount of it can vary depending on the mushroom type; however, the ergothioneine levels normally go up when the mushroom is going through more oxidative stress, and since the ones picked later have higher levels, it is believed that the mushrooms use it as protection against stress (Kalaras et al., 2017). Mushrooms also have beta-glucans, which are a kind of fiber that can be found in the walls of mushroom cells. It was found that they can help improve the immune system by lowering one's chances of catching illnesses such as the cold (Vlassopoulou et al., 2021).

Beta-glucans can promote immune functions by attaching to the Dectin-1 receptor, found on macrophages that help eliminate harmful bacteria and viruses in the body. The beta-glucan binds to this receptor to increase macrophage activity and release of cytokines, which are chemicals that signal other immune cells, such as T-cells, to aid in fighting the germs in the body more effectively and in a more organized manner (Cerletti et al., 2021). The process is often labeled a modulation since it does not add more immune cells but instead improves the ones that are already present in the body. This pathway also suggests that beta-glucans may assist in fighting cancer, as the Dectin-1 signal used to help fight diseases is also able to improve the

body's ability to fight cancer cells (Lee & Kim, 2014). This process shows that mushrooms provide more than just basic nutrition, setting them apart from any of the other meat alternatives.

Exercise Physiology and Protein Requirements

The human body builds muscle in a very unique way. The main driver of muscle protein synthesis is mechanical tension. When the muscle fibers are placed under mechanical tension during different exercises such as weight training, the body will respond by accelerating the rate at which they recover and grow, a bodily process known as muscle protein synthesis (MPS). The main pathway in the body that allows MPS to occur is a cellular pathway called mechanistic target of rapamycin complex 1 (mTORC1), acting as a communicator that signals the muscles to start generating more proteins. It has also been shown that resistance training may be the current best method of increasing MPS. A study demonstrated that even with no food to fuel the body, resistance training can not only increase MPS levels for an entire hour, but also possibly keep it at a higher rate for a maximum of almost two days after (Walker et al., 2011). However, when paired with the proper nutrition that provides the body with essential amino acids, the effects can be boosted even further, as it will trigger mTORC1 by a greater amount than both factors individually (Walker et al., 2011). This partnership between nutrition and physical exercise is proof that strength training is not enough for maximizing one's muscle growth; the body also requires different essential amino acids and nutrients to fuel the body, some of which mushrooms may be able to assist in providing.

As mentioned in the introduction, not all of the essential amino acids aid MPS in an equal amount. Out of the nine, leucine has been discovered to be the main activator for the mTORC1 pathway in humans. The amount of leucine is also important to consider, as too little may reduce MPS, and too much may result in diminishing returns. Studies have shown that if athletes want to maximize MPS in their bodies, there is a leucine threshold: a minimum amount to ingest. A study utilizing a sample of 48 active men in their 20s found that 1.72 to 2 g of leucine (20 g of whey protein) right after their workouts maximized the rate of their MPS; ingesting more than 2 g caused no differences in MPS and was instead stored in the body as excess energy (Witard et al., 2014). This is a crucial point to consider when evaluating mushrooms as an alternative protein source since it shows that achieving this leucine threshold within a realistic amount of mushrooms consumed is what determines its fit as an alternative for red meat. This means that just a complete amino acid profile may not be enough, and instead there must be a high concentration of leucine to see if it is optimal for muscle growth.

Exercises are great for stimulating muscle growth, but they also may cause different physiological strains on the body that have to be controlled for a proficient level of recovery. Extremely intense physical activities will cause higher levels of production of unstable reactive oxygen species. When too much is produced, they can impair the body's muscle cells and spark different issues such as soreness of the body, fatigue, and also a slow recovery during an athlete's resting periods (Mason et al., 2020). Humans have adapted to this, and they have a defense system that aims to reduce the amount of these molecules in the body; however, as a workout

drags on or the intensity is higher, the system may become overwhelmed, which leads to a period that is known as oxidative stress, which further slows the recovery of the muscles. This is why different compounds that we consume may play a role in recovery, since those that are able to assist the antioxidant process, such as different polyphenols and vitamin E, may decrease oxidative impairment after physical activity; however, further research is needed surrounding antioxidants, as inconsistencies may be found for them (Mason et al., 2020). If antioxidants do play a role, mushrooms may be able to offer something other protein sources can not, as they have bioactive compounds like ergothioneine, which is not only a source of amino acids to support MPS but also loaded with antioxidants to help with the oxidative stress caused by exercise.

Mycoprotein and Athletic Performance

Out of all of the mushroom-derived proteins used for exercise, one of the most well-studied is mycoprotein (commercially known as Quorn) when it comes to causing MPS. One of the most notable discoveries about it was made in a study of well-trained men, where one group was given a bolus of mycoprotein while the other group was given milk protein, which contained the same amount of leucine. Their findings showed that the mycoprotein outperformed the milk protein, leading to higher rates of MPS after their workouts and while they were resting (Monteyne et al., 2020). This finding was prominent since milk protein has often been regarded as one of the most effective protein sources that are available to the general public, so the mycoprotein outperforming it shows potential for it becoming an alternative to animal-derived proteins to an extent. Some researchers of the original study also came out with new results three years later and furthered the idea of mycoprotein being a good substitute. They compared the participants' muscle size and strength across their bodies over ten weeks. One group ate a normal diet while the other ate a diet incorporating high volumes of mycoprotein, and results showed that both groups ended up with similar results in both size and strength (Monteyne et al., 2023). These studies show that mycoprotein is a possible alternative for animal-derived proteins, providing similar and even better results for MPS and strength.

Digestibility is also an important point to factor in when analyzing a protein source, as low digestibility would lead to fewer of the nutrients from that source being absorbed by the body. This means that for mycoproteins to be used as a helpful protein source, they must be easily digestible so that the amino acids may be absorbed to allow consumers to experience the benefits. For mycoprotein, digestibility has been studied with ileostomy patients to see how much of the protein ended up not being digested as it got to the small intestines. They discovered that mycoprotein got an 86% protein digestibility-corrected amino acid score, which means it has a proficient digestibility compared to other meat substitutes (Edwards & Cummings, 2010). However, a more recent study has come out with a newer method of in vitro digestion to measure digestibility and found that mycoprotein had a score of 33% to 49%, substantially lower than the older study (Ariens et al., 2021). This highlights one of the biggest issues in the literature on

mycoprotein: there are diverse results for digestibility, so until this gap is filled, a consensus can not be made on its digestibility.

Mycoprotein is also high in fiber, which is an advantage compared to other alternatives since it allows mycoprotein to help regulate appetite by keeping people full for longer periods, an important factor for athletes to stay in shape. In a study done with obese and overweight individuals, it was found that those who consumed meals that consisted of mycoprotein not only lowered future consumption of food, but also produced a smaller spike in insulin after eating when compared to the control group who ate a meal revolving around chicken of the same calories (Bottin et al., 2016). These results demonstrate that mycoprotein may be optimal for athletes trying to maintain a low body fat percentage, as it will help keep them full and reduce cravings and overeating. This point can combine with the previous finding that they produce the same amount of hypertrophy as omnivorous diets paired with weight training (Monteyne et al., 2023) to show that mycoprotein can aid in two different ways, through muscle growth and satiety.

Agricultural Sustainability

Sustainability is one of the most compelling arguments for using mushrooms as an alternative protein source. They require a very small amount of resources such as water and nutrients to grow. Comparisons of mushrooms and livestock's footprint on the Earth showed that mushrooms need less water and land to grow the same amount of food compared to livestock, which allows them to be less harmful to the environment (Kumal et al., 2024). However, they are not yet economically efficient, which is a point to consider when analyzing them. Mushrooms are more environmentally friendly because of a couple of key points. Livestock need a large amount of land to graze, food, and drinking water, as well as a long period of growth until they can be properly processed into food. Mushrooms, on the other hand, grow very quickly and can absorb water through the moisture in the air, rather than needing to be watered directly, making them very efficient in terms of resources.

Mushrooms take up less land since they are very suitable for growing vertically rather than horizontally, a trait that is not possible with livestock. Due to a lack of chlorophyll, they do not need sunlight to grow, which is why they grow best when the environment is dark and humid. This is why being grown vertically on shelves is so efficient with them since, unlike other plants, they do not need sunlight to reach every layer. A study that examined different types of food being grown on a vertical system uncovered that mushrooms had one of the highest protein yields per unit area of all of the foods that were being evaluated, with over 6,000 times more protein for every hectare than other field crops (Calvo-Baltanas et al., 2025). This gives another reason why mushrooms are being viewed more as an alternative to meat in our society, where land is diminishing, but the demand for protein still remains high.

Another advantage that growing mushrooms entails is that they can use different kinds of waste as a form of nourishment, rather than having to be fed like livestock. This can be seen through the current mushroom production lines, which use a method of growing them on natural

waste such as wood and straw. This means that by growing mushrooms, farmers are not only getting rid of waste, but also transforming that waste into an excellent source of food (Grimm & Wosten, 2018). After being harvested, the leftover mushroom substrate can still be used for even more mushrooms to be grown on, creating a repeating cycle of growth and harvest. Besides that, it could also be used as food for livestock, compost, and assist in the creation of biofuels (Grimm & Wosten, 2018). These points show that mushroom cultivation produces very little waste while also being able to survive off of existing material rather than using up more resources, another factor animal-derived proteins lack.

Mushrooms are also not the only alternatives being examined. Synthetic meat, also known as lab-grown meat, is another alternative. It is made by growing animal cells in large tanks rather than raising the animal, leading to fewer emissions as no growth is required of the animal (Negulescu et al., 2023). However, there are three leading issues. The first issue is that it is extremely expensive to make; one study estimated that it cost around 17 to 35 dollars per kg with an optimal setup (Negulescu et al., 2023). This is much higher than the prices of regular meat, which may dissuade buyers from consuming it on a regular basis. Secondly, because it is still being studied and relatively new, there is a lack of long-term data on its effects on the human body through consumption in the long run. A recent study showed that many of the safety questions that were posed years ago are still unanswered due to a lack of research on the topic (Chriki & Hocquette, 2020). Lastly, the law restricts it in some parts of the world. For example, Florida banned the sale of synthetic meat in 2024, as well as six more states that followed, due to health concerns and stigma, making it illegal for some to access it. Since mushrooms do not run into any of these problems, it is reasonable to assume that they are a more realistic option at this moment.

Analysis/Discussion

The evidence shows that mushrooms and mycoprotein can satisfy three crucial criteria simultaneously. They have a complete amino acid profile, they are a reliable driver for MPS, and they leave a smaller environmental footprint than raising livestock. Most other animal-based protein alternatives are unable to fulfill all three criteria, whether it is lacking a complete amino acid profile or being unsustainable for the environment. This combination of traits is what makes mushroom-derived proteins, mycoproteins in particular, a possible substitute for athletes who may be looking for alternatives to animal-derived proteins.

However, the situation is still not all black and white. Taking a more critical look at the leucine threshold complicates it. Witard et al. (2014) found that around 1.7 g to 2 g of leucine is needed to maximize the amount of MPS post-workout. This translates to around 20 g of whey protein; however, although mushrooms contain all nine essential amino acids, the concentration of leucine per serving is a lot lower than whey protein or meat, which means that athletes must consume an unreasonably large amount of whole mushrooms to reach the optimal threshold. This is where mycoproteins come in, as they can narrow the gap between mushrooms and animal-derived proteins, since a bolus of certain mycoproteins has been found to not only match

the amount of leucine found in whey protein, but also outperform it in activating MPS (Monteyne et al., 2020). This is a very important distinction as it shows that not every kind of mushroom-derived protein source is suitable for athletes, so mushrooms and mycoprotein should not be treated as interchangeable foods when being implemented in a diet.

Mushrooms also have beta-glucans and ergothioneine, which suggests that they offer more than just the essential amino acids meat offers. The Dectin-1 pathway's connection to strengthening the immune system and anti-cancer properties (Lee & Kim, 2014) is a trait that meat is unable to match, suggesting that mushrooms should be considered beyond MPS. This also places mushrooms as a more actionable replacement than synthetic meat since lab-grown meat is affected by several drawbacks such as costs and safety issues, while mushrooms exhibit none of the barriers and are already regularly consumed by most.

Literature on the digestibility further exemplifies this uncertainty for mushrooms. Edwards and Cummings (2010) found that mycoprotein had an 86% digestibility of its amino acids using an ileostomy-based technique. However, Ariens et al. (2021) found a much lower score using the in vitro digestion techniques of 33 to 49%. Although this discrepancy is most likely due to the differences in methodology rather than an actual difference in the nutritional properties of mycoprotein, it does raise a question in the literature that has not been resolved yet, leading to the first limitation: Until a standardized methodology is chosen for testing the digestibility of mycoproteins, the actual amount of protein absorbed from mycoproteins will remain unknown, creating great difficulty when attempting to compare its effectiveness to that of animal-derived protein.

Another limitation is that current research focuses too heavily on mycoproteins rather than mushrooms as a whole. Some of the strongest research supporting MPS activation was conducted using mycoproteins and not with whole mushrooms. Since mycoprotein is a heavily processed derivative rather than a whole food, it is unlikely that it will replace the animal-based protein sources that we see on our plates every day. Not only that, but the nutrient availability is also different between mycoprotein and whole mushrooms in ways that could potentially affect digestibility, which altogether could create a false generalization in the literature. Because mycoproteins are effective, it could be assumed that all mushrooms may act as a substitute for meat, which is not yet true.

The last limitation is economic feasibility. Although mushrooms are extremely resource efficient in terms of land and water use, some of the more advanced cultivation methods, such as vertical farming, are not yet economically efficient at the current moment (Kumal et al., 2024). Until the cost of larger-scale production of mushrooms and mycoprotein declines, mushrooms being adopted as a primary substitute for animal-derived proteins will most likely face the barrier of economic accessibility rather than the environmental or nutritional drawbacks. This topic still requires further research, as existing research focuses heavily on the environmental and biological profile of mushrooms while giving relatively less attention to the financial barriers of increasing production to meet the rising need for protein.

Conclusion

Overall, the evidence suggests that mushroom-derived proteins, mycoproteins in particular, are a potential alternative to animal-derived protein for athletes. They have been shown to match or exceed animal-derived proteins in stimulating MPS, offer a complete essential amino acid profile, and require a fraction of the resources to grow compared to livestock while also leaving a small environmental footprint. Very few alternatives can satisfy all three of these criteria, which is what separates mycoprotein from the other substitutes that we may see today. Even beyond muscle growth, mycoproteins have also been shown to keep people more satiated while also producing a lower insulin spike compared to meat such as chicken, suggesting that they may offer other benefits for athletes who may be conditioning their body while also trying to maximize performance.

Replacing animal-derived proteins with mushroom counterparts may also have benefits that go beyond athletic performance. Compounds such as ergothioneine and beta-glucans offer antioxidant and immune-boosting effects, with beta-glucans being able to have potential cancer resistance through the Dectin-1 pathway. Mushrooms are also suitable for vertical farming, which further exemplifies their uniqueness, as some vertical farming systems can produce over 6,000 times more protein per hectare than several traditional crops. With the global demand for protein going up and land becoming more scarce, it could possibly become their biggest advantage compared to other sources of protein. Considering the already established health risks of consuming processed meats, mycoproteins have benefits that go past athletic performance and may improve the health of the general population if implemented long-term.

Future research should focus on fixing the imbalances in digestibility by applying a standardized testing method across every study, rather than using different methods each time, which may make it hard to compare results. Research as a whole should also move beyond mycoprotein and more into the different types of mushroom species for their effects on MPS, since mycoproteins can not generalize to mushrooms as a whole. Future work should also focus more on the scalability of mushrooms in terms of cost, as it still remains a key barrier to implementation despite their advantages. By addressing these gaps, there would be a clearer and more accurate comparison between mushroom-derived proteins and conventional animal-derived protein sources.

If research still shows that mycoproteins outperform or even perform similarly to animal-derived proteins, health organizations should recommend that citizens around the world gradually make some swaps in their diet as an effort to not only reduce the consumption of red meat, which may be detrimental to their health, but also fight the prolonged issue of global warming.

Works Cited

Ariëns, Renata M. C., et al. "Comparing Nutritional and Digestibility Aspects of Sustainable Proteins Using the INFOGEST Digestion Protocol." Journal of Functional Foods, vol. 87, Dec. 2021, p. 104748, https://doi.org/10.1016/j.jff.2021.104748.

Ayimbila, Francis, and Suttipun Keawsompong. "Nutritional Quality and Biological Application of Mushroom Protein as a Novel Protein Alternative." Current Nutrition Reports, vol. 12, no. 2, Apr. 2023, https://doi.org/10.1007/s13668-023-00468-x.

Balcombe, Paul, et al. "Methane Emissions: Choosing the Right Climate Metric and Time Horizon." Environmental Science: Processes & Impacts, vol. 20, no. 10, 2018, pp. 1323–39, https://doi.org/10.1039/c8em00414e.

Bottin, Jeanne H., et al. "Mycoprotein Reduces Energy Intake and Postprandial Insulin Release Without Altering Glucagon-Like Peptide-1 and Peptide Tyrosine-Tyrosine Concentrations in Healthy Overweight and Obese Adults: A Randomised-Controlled Trial." British Journal of Nutrition, vol. 116, no. 2, May 2016, pp. 360–74, https://doi.org/10.1017/s0007114516001872.

Calvo-Baltanás, Vanesa, et al. "The Future Potential of Controlled Environment Agriculture." PNAS Nexus, edited by Joann Whalen, vol. 4, no. 4, Mar. 2025, https://doi.org/10.1093/pnasnexus/pgaf078.

Cerletti, Chiara, et al. "Edible Mushrooms and Beta-Glucans: Impact on Human Health." Nutrients, vol. 13, no. 7, July 2021, p. 2195, https://doi.org/10.3390/nu13072195.

Chriki, Sghaier, and Jean-François Hocquette. "The Myth of Cultured Meat: A Review." Frontiers in Nutrition, vol. 7, no. 7, Feb. 2020, https://doi.org/10.3389/fnut.2020.00007.

Edwards, D. G., and J. H. Cummings. "The Protein Quality of Mycoprotein." Proceedings of the Nutrition Society, vol. 69, no. OCE4, 2010, https://doi.org/10.1017/s0029665110001400.

Evangelista, Chiara, et al. "Enteric Methane Emission in Livestock Sector: Bibliometric Research From 1986 to 2024 With Text Mining and Topic Analysis Approach by Machine Learning Algorithms." Animals : An Open Access Journal from MDPI [Switzerland], vol. 14, no. 21, Nov. 2024, PubMed, p. 3158, https://doi.org/10.3390/ani14213158.

Grimm, Daniel, and Han A. B. Wösten. "Mushroom Cultivation in the Circular Economy." Applied Microbiology and Biotechnology, vol. 102, no. 18, July 2018, pp. 7795–803, https://doi.org/10.1007/s00253-018-9226-8.

Kalaras, Michael D., et al. "Mushrooms: A Rich Source of the Antioxidants Ergothioneine and Glutathione." Food Chemistry, vol. 233, Oct. 2017, pp. 429–33, https://doi.org/10.1016/j.foodchem.2017.04.109.

Lee, Dong Hee, and Ha Won Kim. "Innate Immunity Induced by Fungal B-Glucans via Dectin-1 Signaling Pathway." International Journal of Medicinal Mushrooms [United States], vol. 16, no. 1, 2014, PubMed, pp. 1–16, https://doi.org/10.1615/intjmedmushr.v16.i1.10.

Liu, Peng, et al. "The Prospect of Mushroom as an Alterative Protein: From Acquisition Routes to Nutritional Quality, Biological Activity, Application and Beyond." Food Chemistry

[England], vol. 469, Mar. 2025, PubMed, p. 142600, https://doi.org/10.1016/j.foodchem.2024.142600.

Mason, Shaun A., et al. “Antioxidant Supplements and Endurance Exercise: Current Evidence and Mechanistic Insights.” Redox Biology, vol. 35, Feb. 2020, p. 101471, https://doi.org/10.1016/j.redox.2020.101471.

Monteyne, Alistair J, Mariana OC Coelho, Andrew J Murton, et al. “Vegan and Omnivorous High Protein Diets Support Comparable Daily Myofibrillar Protein Synthesis Rates and Skeletal Muscle Hypertrophy in Young Adults.” The Journal of Nutrition, vol. 153, no. 6, Feb. 2023, https://doi.org/10.1016/j.tjnut.2023.02.023.

Monteyne, Alistair J, Mariana OC Coelho, Craig Porter, et al. “Mycoprotein Ingestion Stimulates Protein Synthesis Rates to a Greater Extent Than Milk Protein in Rested and Exercised Skeletal Muscle of Healthy Young Men: A Randomized Controlled Trial.” The American Journal of Clinical Nutrition, vol. 112, no. 2, May 2020, https://doi.org/10.1093/ajcn/nqaa092.

Negulescu, Patrick G., et al. “Techno‐Economic Modeling and Assessment of Cultivated Meat: Impact of Production Bioreactor Scale.” Biotechnology and Bioengineering, Jan. 2023, https://doi.org/10.1002/bit.28324.

Pashaei, Ali, et al. “Edible Mushrooms as an Alternative to Animal Proteins for Having a More Sustainable Diet: A Review.” Journal of Health Population and Nutrition, vol. 43, no. 1, Nov. 2024, https://doi.org/10.1186/s41043-024-00701-5.

Singh, Akruti, et al. “Mushrooms as Nutritional Powerhouses: A Review of Their Bioactive Compounds, Health Benefits, and Value-Added Products.” Foods, vol. 14, no. 5, Feb. 2025, p. 741, https://doi.org/10.3390/foods14050741.

Sivasubramanian, Barath Prashanth, et al. “Comprehensive Review of Red Meat Consumption and the Risk of Cancer.” Cureus, vol. 15, no. 9, Sept. 2023, https://doi.org/10.7759/cureus.45324.

Smith, Kieran, et al. “Meeting the Global Protein Supply Requirements of a Growing and Ageing Population.” European Journal of Nutrition, Mar. 2024, https://doi.org/10.1007/s00394-024-03358-2.

Vlassopoulou, Marigoula, et al. “Effects of Fungal Beta-Glucan on Health - a Systematic Review of Randomized Controlled Trials.” Food & Function, 2021, https://doi.org/10.1039/d1fo00122a.

WALKER, DILLON K., et al. “Exercise, Amino Acids, and Aging in the Control of Human Muscle Protein Synthesis.” Medicine & Science in Sports & Exercise, vol. 43, no. 12, Dec. 2011, pp. 2249–58, https://doi.org/10.1249/mss.0b013e318223b037.

Witard, Oliver C, et al. “Myofibrillar Muscle Protein Synthesis Rates Subsequent to a Meal in Response to Increasing Doses of Whey Protein at Rest and After Resistance Exercise.” The American Journal of Clinical Nutrition, vol. 99, no. 1, Nov. 2013, pp. 86–95, https://doi.org/10.3945/ajcn.112.055517.

The Science Behind Early CPR Intervention and Brain Preservation During Cardiac Arrest By Kalea Poon

Abstract

Cardiac arrest is a life-threatening condition in which the heart suddenly stops pumping blood, resulting in an immediate loss of oxygen to the brain and other vital organs. Cardiopulmonary resuscitation (CPR) is a critical emergency intervention that helps maintain limited blood circulation until advanced medical care arrives. The purpose of this paper is to examine the ways in which early CPR intervention contributes to brain preservation during cardiac arrest by sustaining cerebral oxygen delivery and delaying neurological injury. This study focuses on the physiological mechanisms that link early resuscitation to improved neurological outcomes. Findings from the literature indicate that CPR provides partial but essential blood flow to the brain by generating artificial circulation through chest compressions. This limited perfusion helps slow the progression of hypoxic brain injury and extends the time window for successful resuscitation. Research also shows that earlier initiation of CPR is strongly associated with reduced neurological damage and improved survival outcomes. Overall, early CPR intervention significantly improves neurological survival outcomes by preserving brain function during the critical minutes following cardiac arrest.

Keywords

Medicine, Public Health, Cardiopulmonary Resuscitation, Cardiac Arrest, Brain Perfusion

Introduction

Cardiac arrest is the unexpected loss of heart function in which the heart abruptly stops pumping blood effectively to the brain and other vital organs.[1] It may occur suddenly or be preceded by symptoms and can quickly become fatal if immediate medical intervention is not provided. Permanent brain damage can occur within approximately four minutes of oxygen deprivation, while death may occur within four to six minutes if circulation is not restored.[2] Because of this short time window, immediate emergency response is critical to survival and neurological preservation.

The brain depends on a constant oxygen supply to maintain normal cellular activity and neurological function. According to the University of Rochester Medical Center, the brain consumes approximately 20% of the body's oxygen even when accounting for only 2% of total body mass.[3] Neurons maintain only a limited energy reserve and rely heavily on continuous oxygen delivery through blood circulation. When circulation stops during cardiac arrest, the brain experiences cerebral hypoxia, a condition in which insufficient oxygen reaches brain tissue.[4] Without adequate oxygen, neuronal injury and cell death can rapidly occur. In addition, studies show that brain cells begin to die within the first minutes following oxygen deprivation, emphasizing the extreme time sensitivity of cardiac arrest treatment.[5]

Cardiopulmonary resuscitation (CPR) serves as an emergency intervention that temporarily restores partial circulation through chest compressions. Although CPR does not fully restore normal heart function, it helps maintain limited blood flow and oxygen delivery to the brain and other organs until advanced medical care is available. Early CPR intervention is critical because it acts as a bridge between cardiac arrest onset and emergency medical treatment, increasing the likelihood of survival and reducing neurological injury.

While many individuals understand that CPR is a life-saving technique, fewer understand the physiological mechanisms that explain why immediate CPR is essential for brain preservation. Much of the existing public discussion surrounding CPR focuses on survival rates rather than the neurological processes associated with oxygen deprivation and cerebral blood flow. This study examines how early CPR intervention contributes to brain preservation during cardiac arrest by maintaining cerebral oxygen delivery and delaying neurological damage.

Methods

This study used a mixed-methods approach consisting of a literature review and a public awareness survey. The literature review analyzed peer-reviewed research articles, clinical reviews, and public health reports related to cardiopulmonary resuscitation (CPR), cardiac arrest physiology, cerebral perfusion, and neurological outcomes. Sources were collected from Google Scholar and major health organizations, including the American Heart Association and the National Library of Medicine. Search terms included "CPR cerebral blood flow," "cardiac arrest brain injury," and "early CPR survival outcomes." Sources were selected based on relevance, scientific credibility, and publication in peer-reviewed journals or established medical organizations.

To assess public understanding of brain survival during oxygen deprivation, an anonymous Instagram poll was conducted through the author's social media platform. Participants were asked: "How long do you think the brain can survive without oxygen?" A total of 28 individuals responded. Responses were collected and summarized as descriptive data to evaluate common misconceptions related to cardiac arrest and brain injury.

Findings from the literature review and survey were compared to identify gaps between scientific evidence and public knowledge regarding the urgency of CPR intervention and neurological preservation.

Results and Discussion

Physiological Effects of Cardiac Arrest on the Brain

Cardiac arrest causes an immediate cessation of blood flow to the brain, resulting in cerebral ischemia and anoxia. Research has shown that cerebral blood flow decreases to nearly zero immediately following cardiac arrest.[3] Within approximately 10 to 20 seconds, electrical activity in the cerebral cortex ceases, leading to loss of consciousness. As oxygen deprivation

continues, neurons experience severe metabolic stress due to the interruption of aerobic energy production.

The loss of oxygen rapidly depletes adenosine triphosphate (ATP), the primary energy source required for neuronal function. Without sufficient ATP, neurons are unable to maintain normal ion gradients across cell membranes. This disruption contributes to excitotoxicity, a process in which excessive glutamate release overstimulates neuronal receptors and allows toxic levels of calcium to enter cells.[3] Calcium overload activates destructive cellular pathways that contribute to neuronal injury and death.

Brain injury may begin within four to six minutes of interrupted circulation because brain tissue has a high metabolic demand and limited energy reserves.[2] As the duration of oxygen deprivation increases, the risk of irreversible neurological damage rises substantially. After approximately ten minutes without effective circulation, widespread neuronal death and severe neurological impairment become increasingly likely.[2]

Even when circulation is restored, brain injury may continue. Following successful resuscitation, the return of blood flow can trigger reperfusion injury characterized by inflammation, blood vessel dysfunction, oxidative stress, and microcirculatory failure.[6] These secondary injury mechanisms may further damage brain tissue despite restoration of circulation.

How CPR Maintains Cerebral Blood Flow

Cardiopulmonary resuscitation (CPR) serves as a temporary method of maintaining circulation during cardiac arrest. Although CPR cannot restore normal heart function, chest compressions generate pressure within the chest that allows blood to continue moving throughout the body. During the compression phase, blood is pushed forward through the circulatory system, while the decompression phase allows blood to return to the heart. This process creates an artificial pumping mechanism that partially replaces the heart's function.[7]

The pressure generated by chest compressions enables blood to reach vital organs, including the brain. Research has shown that CPR can maintain a limited but critical level of cerebral blood flow during cardiac arrest.[8] While the amount of blood delivered is significantly lower than under normal physiological conditions, this circulation provides oxygen and nutrients necessary for sustaining basic cellular function.

By maintaining partial cerebral perfusion, CPR helps slow the progression of oxygen deprivation and delays the onset of irreversible brain injury. The continued delivery of oxygenated blood supports residual ATP production and reduces the severity of ischemic damage that would otherwise occur if circulation completely ceased.[8] As a result, CPR extends the period during which successful resuscitation and neurological recovery remain possible.

Because CPR cannot fully restore normal blood flow, it functions primarily as a bridge between cardiac arrest and advanced medical intervention. Maintaining circulation through high-quality chest compressions increases the likelihood that the brain will remain viable until defibrillation, emergency medical services, or other advanced treatments can restore spontaneous circulation.[7],[8]

Time Sensitivity of CPR

The effectiveness of cardiopulmonary resuscitation (CPR) is highly dependent on how quickly it is initiated following cardiac arrest. Because brain cells are extremely sensitive to oxygen deprivation, even short delays in treatment can significantly affect survival and neurological outcomes. Research has shown that survival rates decrease by approximately 7% to 10% for every minute that passes without CPR or defibrillation.[5] As a result, immediate intervention is critical during the first few minutes after cardiac arrest.

The importance of rapid action is reflected in the American Heart Association's Chain of Survival, a series of evidence-based steps designed to improve outcomes following cardiac arrest. Figure 1 illustrates the six links in the adult out-of-hospital Chain of Survival. As shown in Figure 1, early recognition of cardiac arrest, rapid activation of emergency medical services and immediate CPR represent the first critical interventions associated with improved survival and neurological outcomes.

Figure 1. Adult out-of-hospital Chain of Survival Early recognition of cardiac arrest, rapid activation of emergency medical services, and immediate CPR are critical factors associated with improved survival and neurological outcomes. Reproduced from the American Heart Association with attribution.[1]

Delays in CPR allow oxygen deprivation and cellular injury to progress, increasing the likelihood of permanent neurological damage. Even when spontaneous circulation is eventually restored, prolonged periods without adequate blood flow are associated with poorer cognitive and neurological outcomes. Studies consistently demonstrate that individuals who receive CPR within the first few minutes of cardiac arrest are more likely to survive and experience favorable neurological recovery compared with those who receive delayed treatment.[5]

These findings highlight the critical role of early intervention in cardiac arrest management. While CPR cannot fully restore normal circulation, its effectiveness is greatest when initiated immediately, emphasizing that every minute can influence both survival and long-term brain function. Key findings identified across the reviewed literature are summarized in Table 1.

Table 1. Key findings from literature examining CPR and neurological outcomes.

Findings	Evidence
Cerebral blood flow falls rapidly after cardiac arrest	Cerebral perfusion approaches zero immediately following arrest
Brain injury may begin within 4-6 minutes without circulation	Neurons have high metabolic demands and limited energy reserves
CPR provides partial cerebral blood flow	Chest compressions generate artificial circulation that delivers oxygen to the brain
Survival decreases with delayed intervention	Survival declines approximately 7-10% per minute without CPR or defibrillation
Early CPR improves neurological outcomes	Earlier CPR initiation is associated with greater rates of neurologically favorable survival

Public Awareness of Brain Survival During Cardiac Arrest

To evaluate public understanding of oxygen deprivation and brain injury, an Instagram poll was conducted with 28 respondents. Participants were asked how long they believed the brain could survive without oxygen. Results showed that 54% of respondents believed the brain could survive for approximately 15 minutes without oxygen, which exceeds the time frame supported by current medical literature. The remaining 46% selected shorter time intervals that more closely aligned with current medical evidence.

Research indicates that irreversible brain injury may begin within approximately four to six minutes after circulation stops, with the risk of severe neurological damage increasing substantially as oxygen deprivation continues.[2] The survey findings suggest that many individuals may underestimate the urgency of cardiac arrest and the importance of immediate CPR intervention. This misconception may delay bystander response during emergencies, highlighting the need for greater public education regarding brain preservation and cardiac arrest survival.

Table 2. Instagram poll assessing public understanding of brain survival without oxygen.

Response	Percentage
Believed the brain can survive 15 minutes without oxygen	54%
Did not believe the brain can survive 15 minutes without oxygen	46%
Total participants	28

Neurological Outcomes and Brain Preservation

The primary goal of cardiopulmonary resuscitation (CPR) extends beyond restoring circulation; it also seeks to preserve neurological function by limiting brain injury during cardiac arrest. Because the brain is highly vulnerable to oxygen deprivation, the maintenance of even partial blood flow through CPR can significantly influence long-term neurological outcomes. Studies have demonstrated that individuals who receive early CPR are more likely to survive with favorable neurological function than those who experience prolonged periods without intervention.[9]

Early CPR helps reduce the severity of hypoxic-ischemic brain injury by sustaining a limited supply of oxygen and nutrients to brain tissue. By slowing the progression of neuronal damage, CPR increases the likelihood that brain cells will remain viable until spontaneous circulation is restored. As a result, patients who receive prompt resuscitation often experience better cognitive outcomes, including improved memory, communication, and overall neurological function following recovery.[10]

Bystander CPR plays a particularly important role in brain preservation because it can be initiated before emergency medical services arrive. Multiple studies have found that patients who receive bystander CPR have higher survival rates and are more likely to achieve favorable neurological recovery compared with those who do not receive immediate assistance.[11] These findings suggest that the benefits of CPR extend beyond survival alone and include the preservation of long-term brain health.

Overall, the literature indicates that early CPR is strongly associated with improved neurological outcomes following cardiac arrest. By maintaining cerebral perfusion and delaying irreversible neuronal injury, CPR serves as a critical intervention that increases the probability of both survival and meaningful neurological recovery.

Limitations in CPR Effectiveness

Although cardiopulmonary resuscitation (CPR) is a critical intervention during cardiac arrest, it has several limitations. CPR does not fully restore normal cardiac function or blood circulation. Even when performed correctly, chest compressions provide only a fraction of the blood flow generated by a normally functioning heart.[12] As a result, the brain and other organs may continue to experience reduced oxygen delivery despite ongoing resuscitation efforts.

The effectiveness of CPR also depends on the quality of chest compressions. Current guidelines recommend compressions of adequate depth and rate to maximize blood flow. However, factors such as rescuer fatigue, interruptions in compressions, and improper technique can reduce the effectiveness of CPR and limit cerebral perfusion.[10]

In addition, CPR alone cannot correct the underlying cause of many cardiac arrests. Defibrillation is often required to restore a normal heart rhythm, particularly in cases involving ventricular fibrillation or pulseless ventricular tachycardia. Delays in emergency medical services (EMS) response and advanced cardiac care may further reduce the likelihood of favorable outcomes.[13]

Despite these limitations, CPR remains one of the most important interventions available during cardiac arrest. While it cannot completely prevent neurological injury, it can significantly delay the progression of brain damage and increase the likelihood of survival until advanced medical treatment becomes available.

Furthermore, the Instagram survey included only 28 respondents and represented a convenience sample drawn from the author's social media audience. Therefore, the findings may not be representative of the general population and should be interpreted as exploratory data.

Conclusion

Cardiac arrest causes an immediate interruption of blood flow that deprives the brain of oxygen and initiates a series of cellular processes that can result in severe neurological injury. Because brain tissue is highly sensitive to oxygen deprivation, irreversible damage may begin within minutes if circulation is not restored.

The literature reviewed in this study demonstrates that cardiopulmonary resuscitation (CPR) plays a vital role in preserving brain function during cardiac arrest. Through the generation of artificial circulation, CPR maintains limited cerebral blood flow, supports oxygen delivery, and delays the progression of ischemic brain injury. Research consistently shows that early initiation of CPR is associated with improved survival rates and more favorable neurological outcomes.

Although CPR cannot fully restore normal circulation and has several limitations, it serves as a critical bridge between cardiac arrest and advanced medical intervention. These findings highlight the importance of immediate CPR initiation and widespread CPR education as essential public health strategies for improving survival and preserving neurological function following cardiac arrest.

Future research may further examine how public CPR education programs influence awareness, bystander intervention rates, and neurological outcomes following cardiac arrest.

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Works Cited

American Heart Association. Cardiac Arrest. https://www.heart.org/en/health-topics/cardiac-arrest (accessed June 2026).

National Library of Medicine. Cardiac Arrest. MedlinePlus Medical Encyclopedia. https://medlineplus.gov/ency/article/000013.htm (accessed June 2026).

University of Rochester Medical Center. Study Reveals Brain's Finely Tuned System of Energy Supply. https://www.urmc.rochester.edu/news/story/study-reveals-brains-finely-tuned-system-of-energy-supply (accessed June 2026).

Cleveland Clinic. Cerebral Hypoxia. https://my.clevelandclinic.org/health/diseases/6025-cerebral-hypoxia (accessed June 2026).

Online CPR Certification. The Race Against Time: What Happens to the Body After 4 Minutes Without CPR? https://www.onlinecprcertification.net/blog/the-race-against-time-what-happens-to-the-body-after-4-minutes-of-cpr/ (accessed June 2026).

Neumar, R. W.; Nolan, J. P.; Adrie, C.; et al. Post–Cardiac Arrest Syndrome: Epidemiology, Pathophysiology, Treatment, and Prognostication. *Circulation* **2008**, *118*, 2452–2483.

Lurie, K. G.; Nemergut, E. C.; Yannopoulos, D.; Sweeney, M. The Physiology of Cardiopulmonary Resuscitation. Anesthesia & Analgesia **2016**, 122, 767–783.

Kleinman, M. E.; Brennan, E. E.; Goldberger, Z. D.; et al. Part 5: Adult Basic Life Support and CPR Quality. Circulation **2015**, 132, S51–S83.

Reynolds, J. C.; Grunau, B. E.; Elmer, J.; et al. Early Cardiopulmonary Resuscitation and Neurologically Favorable Survival After Cardiac Arrest. Circulation: Cardiovascular Quality and Outcomes **2023**.

Panchal, A. R.; Berg, K. M.; Hirsch, K. G.; et al. 2020 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation **2020**, 142, S366–S468.

NPR Health Shots. A Natural Death May Be Preferable for Many Than Enduring CPR. https://www.npr.org/sections/health-shots/2023/05/29/1177914622/a-natural-death-may-be-preferable-for-many-than-enduring-cpr (accessed June 2026).

National Center for Biotechnology Information. Cardiopulmonary Resuscitation. StatPearls Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK470402/ (accessed June 2026).

American Heart Association. Part 9: Adult Advanced Life Support: 2025 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation **2025**, 152. https://www.ahajournals.org/doi/full/10.1161/CIR.0000000000001376 (accessed June 2026).

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Physiological Limitations During Active Cardiovascular Stress: Analyzing Correlations Between Maximum Heart Rate and Coronary Artery Disease By Tawhid Bin Omar

Abstract

Medical professionals rely on physiological markers to diagnose heart conditions early. High resting blood pressure and high serum cholesterol often signal impending cardiac failure. Research indicates that the cardiovascular system handles physical stress poorly during illness. We analyzed the verified Cleveland Clinic Machine Learning Repository dataset. This open-source registry contains detailed medical records for 297 adult patients. We extracted specific data points comparing maximum achieved heart rates against physician-confirmed coronary artery disease. We tracked the positive correlation between electrographic ST depression and patient illness. The data proved a strong negative correlation between active physical capability and disease presence. Patients suffering from cardiovascular disease failed to reach high maximum heart rates during clinical stress tests. They exhibited severe electrical depression. We present the exact statistical breakdown of these physiological limits. The findings reinforce the diagnostic power of active cardiovascular stress tests.

Introduction

Coronary artery disease reduces the flow of oxygen to the heart muscle. Plaque builds rapidly inside arterial walls. The human heart compensates by pumping harder during physical exertion. This mechanical stress damages the heart tissue over time. Cardiologists track passive metrics like total serum cholesterol and resting blood pressure to predict this decay. These resting metrics fail to capture the active mechanical limits of a diseased organ. You can't test an engine while it idles. You test an engine by pushing it to its maximum output.

Robert Detrano collected extensive patient records at the V.A. Medical Center in Long Beach and the Cleveland Clinic Foundation. This public dataset provides an unfiltered look at cardiac decline. We used this specific population to investigate the mechanical boundaries of the human heart. We focused entirely on the maximum achieved heart rate gathered during active physical stress tests. We measured this variable against the presence of confirmed coronary disease. The results isolated a severe drop in physical capability among the sick population.

Methodology

We executed the statistical analysis using Python and the pandas data framework. We downloaded the raw Cleveland dataset directly from the University of California, Irvine Machine Learning Repository. The repository contains 303 original patient files. We removed six incomplete records to preserve absolute mathematical integrity. The final dataset included 297 distinct patient profiles. The median age landed at 54.5 years. Exactly 46.1 percent of this population tested positive for heart disease.

We generated a Pearson correlation matrix to isolate the strongest physiological relationships. We mapped maximum achieved heart rate, resting blood pressure,

electrocardiogram depression, and total serum cholesterol against the binary disease indicator. We exported the mathematical distributions into visual box plots and density histograms. This visual evidence maps the exact physiological limits of a sick cardiovascular system.

Empirical Data and Results

The statistical breakdown isolates multiple profound diagnostic markers. Figure 1 exposes the negative and positive correlations across the physiological spectrum. The data calculates a strong negative correlation of -0.42 for maximum heart rate against the presence of heart disease. The physical capability drops uniformly as the illness spreads. The data revealed another severe pattern in the exact opposite direction.

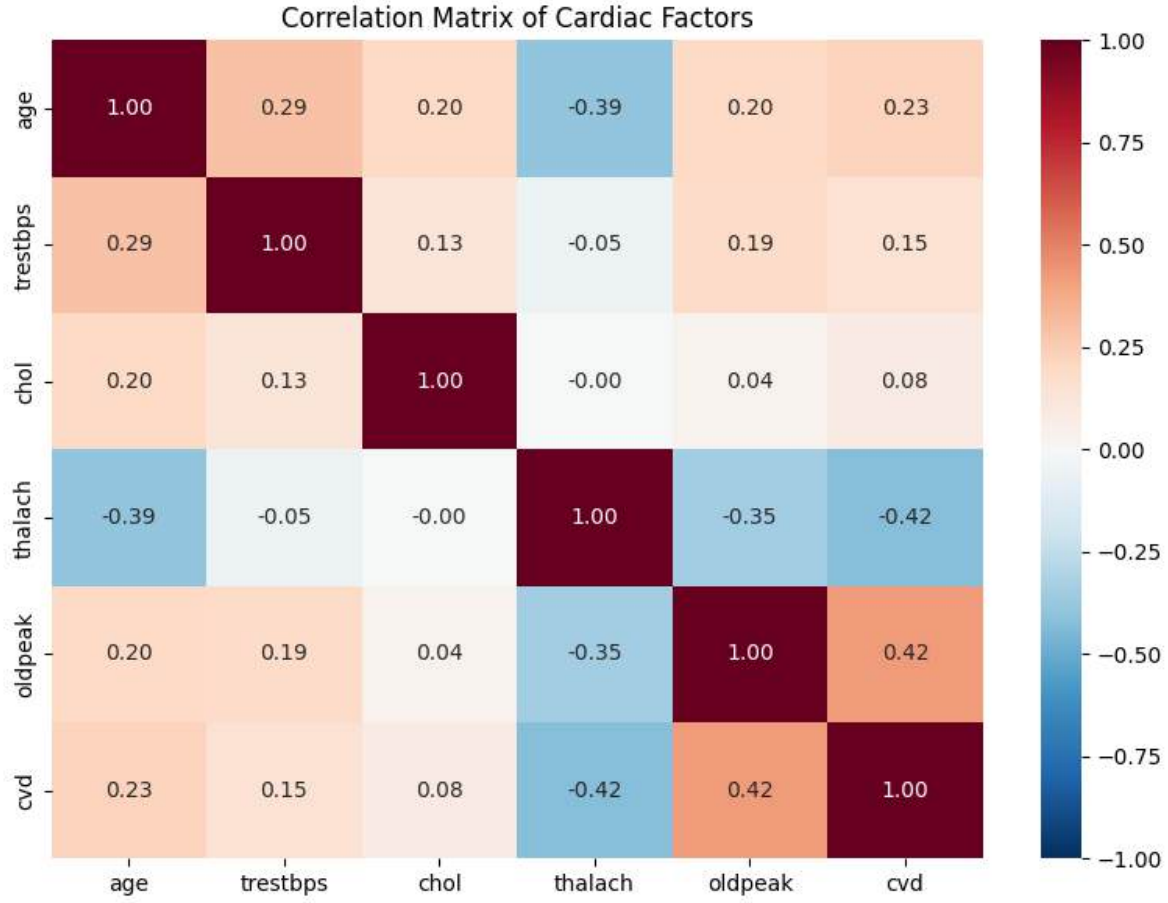


Fig 1: Pearson correlation matrix mapping key cardiac indicators from 297 verified patients.

Electrocardiogram readings track ST segment depression during peak exercise. Doctors record this measurement as oldpeak. The dataset reveals a strong positive correlation of 0.42 between oldpeak and coronary disease. Damaged hearts struggle to maintain normal electrical rhythms under heavy physical loads. The stress test forces these hidden electrical flaws into plain view. Older patients naturally lose maximum heart rate capability over time. The correlation

matrix captures a -0.40 correlation between age and maximum achievable beats per minute. Doctors must account for this natural baseline decay when diagnosing older patients.
The box plot in Figure 2 highlights the mechanical failure perfectly. Healthy adults pushed their maximum heart rate to an average of 158.6 beats per minute during active stress tests. Patients diagnosed with cardiovascular disease collapsed early. Their hearts reached a maximum average of just 139.1 beats per minute. The diseased muscle cannot sustain rapid mechanical movement.

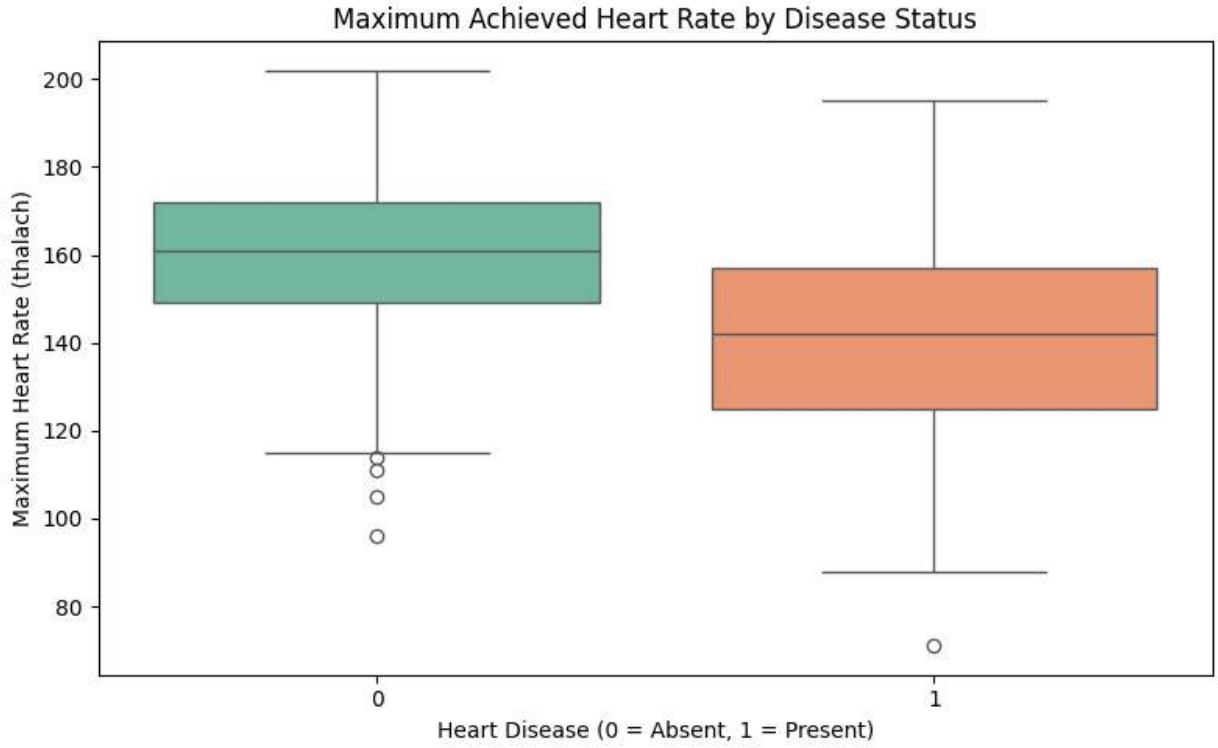


Fig 2: Maximum achieved heart rate categorized strictly by physiological disease status.

We compared this active failure against a standard passive metric. Figure 3 maps total serum cholesterol levels across the identical patient group. Doctors treat high cholesterol as a primary indicator of arterial decay. The healthy population recorded a mean cholesterol level of 243.5 milligrams per deciliter. The sick population recorded a mean of 251.9 milligrams per deciliter. The distributions overlap entirely. You can't reliably sort healthy and sick patients using total cholesterol counts alone.

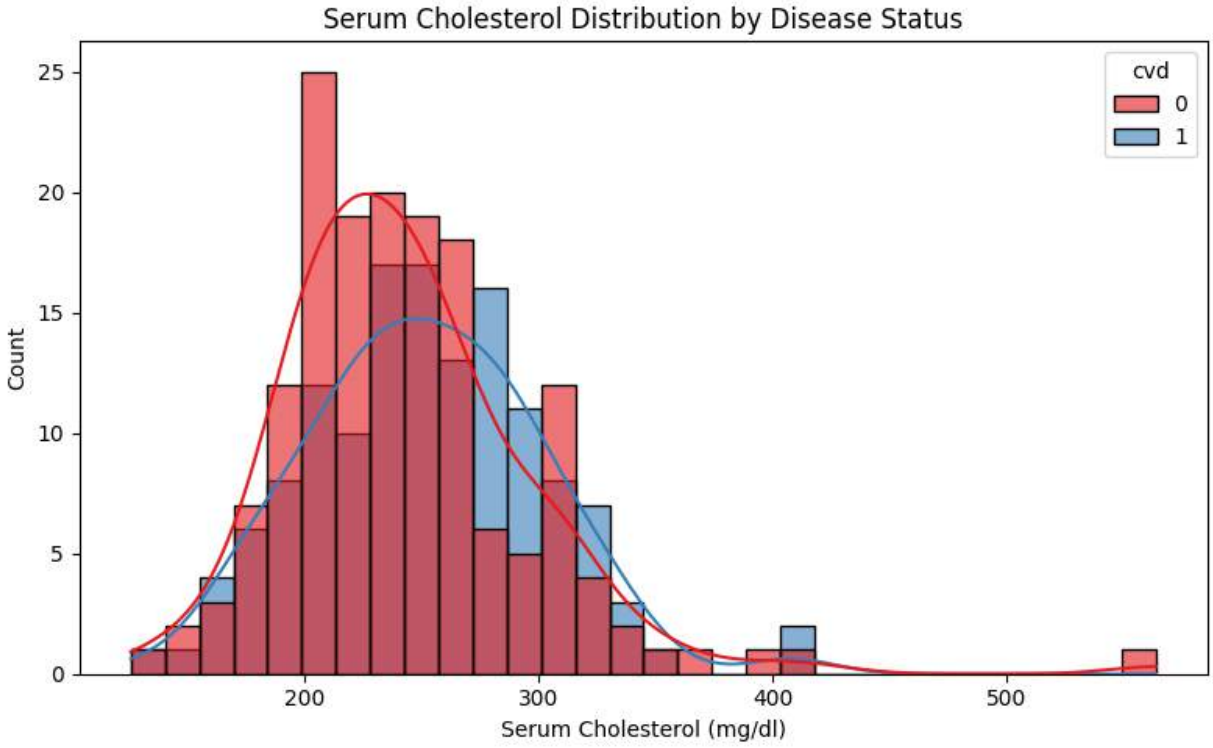


Fig 3: Serum cholesterol distribution showing massive overlap between healthy and diseased patients.

Conclusion

We derived a clear biological boundary from the clinical data. The human heart signals its own decay through mechanical weakness. A diseased cardiovascular system hits a strict physical ceiling during exertion. It suffers from heavy electrical depression during peak movement. The normal heart accelerates past 150 beats per minute effortlessly. The damaged heart stalls near 139 beats per minute. Passive diagnostic numbers like serum cholesterol offer a blended and uncertain picture. The active stress test forces the heart to reveal its true condition. Doctors must prioritize active threshold testing to predict severe arterial collapse.

Works Cited

Detrano, Robert, et al. "International Application of a New Probability Algorithm for the Diagnosis of Coronary Artery Disease." The American Journal of Cardiology, vol. 64, no. 3, 1989, pp. 304-310.

Kannel, William B., et al. "Serum Cholesterol, Lipoproteins, and the Risk of Coronary Heart Disease. The Framingham Study." Annals of Internal Medicine, vol. 74, no. 1, 1971, pp. 1-12.

Molecular Docking of HER2 to Identify New Possible Inhibitors By Ekaterina Chertkova

Abstract

Human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor tyrosine kinase belonging to the epidermal growth factor receptor (EGFR) family and is associated with breast cancer. Therefore, identifying potential inhibitors that target the ATP-binding site of HER2 is of interest for the development of new therapeutic candidates. In this study, 100 compounds were selected from the PubChem database and evaluated using molecular docking. The 4 compounds with the most favorable predicted binding affinities were subsequently analyzed for their physicochemical and pharmacokinetic properties.

Keywords HER2, EGFR, breast cancer, molecular docking.

Introduction

In contemporary oncology, cancer treatment is undergoing a fundamental shift away from broad-spectrum cytotoxic chemotherapy and toward precision targeted therapy. Among the critical oncogenic targets is Human Epidermal Growth Factor Receptor 2 (HER2), which belongs to the EGFR family. HER2 is a 185 kDa transmembrane receptor tyrosine kinase. Unlike other family members, HER2 has no known direct binding ligand; instead, it is activated through heterodimerization with other ligand-bound ErbB receptors or via homodimerization when present at high surface concentrations. Upon activation, its intracellular kinase domain autophosphorylates and triggers downstream signaling cascades that promote cell proliferation, growth, and survival while suppressing apoptosis [1]. Therefore, HER2 overexpression and gene amplification are drivers in a significant subset of aggressive breast cancers [2]. Consequently, HER2 is an important prognostic biomarker and a therapeutic target for targeted cancer treatments, including monoclonal antibodies (such as trastuzumab and pertuzumab) and small-molecule tyrosine kinase inhibitors.

Methodology

Protein and ligand preparation

The RCSB PDB was utilized to retrieve the three-dimensional crystal structure of the kinase domain of human HER2 (PDB ID: 3PP0) with a resolution of 2.25 Å [3]. The crystal structure was cleaned by removing the initial ligands and water molecules, and hydrogens were added. Three-dimensional ligand structures were obtained from the PubChem database [4] in SDF format and were selected according to Lipinski's Rule of Five.

Molecular docking

GNINA was used to screen compounds against HER2. The program evaluates atomic grid densities using deep learning to improve pose prediction and affinity scoring [5]. The

active-site cavity was selected. 100 ligands were docked, and the top 4 were selected for pharmacokinetic analysis.

Virtual Screening

PubChem is a free database of chemical molecules and their biological activities. It enables searches for chemical structures, safety and toxicity data, and physical properties. 100 ligands were obtained from PubChem. All of them can be identified using their PubChem IDs [4].

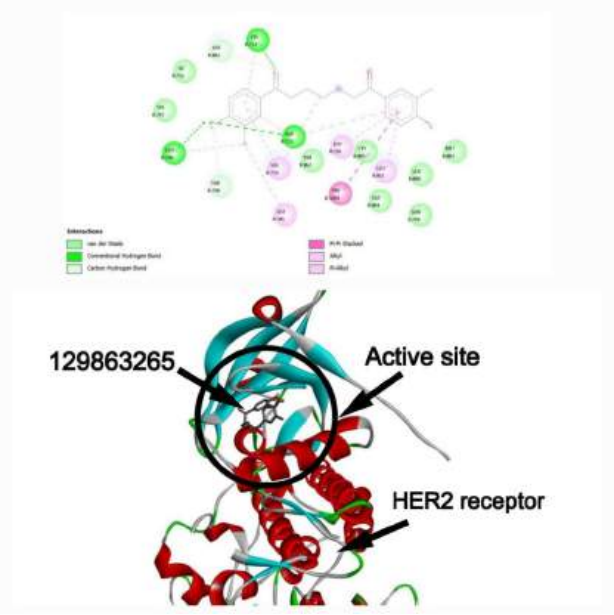
SwissADME Analysis

SwissADME was used to calculate the ADME, drug-likeness, and pharmacokinetic properties of the top 4 compounds [6].

Results and Discussion

The crystal structure of the HER2 kinase domain was cleaned by removing the initial ligand and water molecules, followed by the addition of hydrogen atoms. 100 compounds that met the criteria of Lipinski's Rule of Five were obtained from PubChem [4] and docked against the HER2 protein using GNINA [5]. Lapatinib, a competitive inhibitor that binds to the ATP-binding site [7], was used as a control. Lapatinib's binding affinity was found to be -10.3 kcal/mol. 4 substances out of the 100 bound to the site of interest.

The ATP was found to interact with Leu726, Gly727, Ser728, Gly729, Val734, Ala751, Thr798, Gln799, Leu800, Met801, Gly804, Cys805, Asp845, Arg849, Asn850, Leu852, Asp863, Pro885, Phe1004. Thus, the selected ligand was found to interact with the same amino acids.



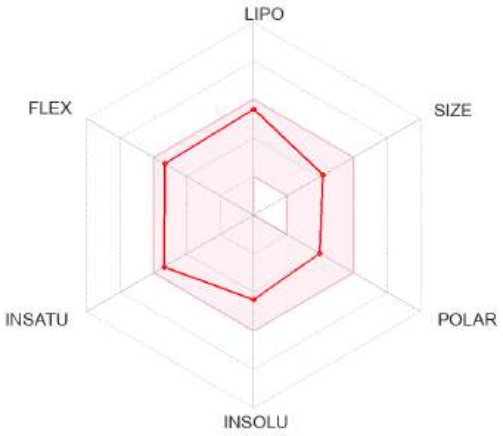
Interacting complex of HER2 with ligand 129863265.

Ligand 129863265 was found to interact with Ala751, Lys753, and Leu796 through conventional hydrogen bonds. Van der Waals, Pi-Pi, alkyl, and Pi-alkyl interactions were also found to play an important role in the positioning of the compound within the active site of HER2. SwissADME analysis [6] was then conducted for the top 4 ligands and lapatinib as a control. It was performed to evaluate the physicochemical, drug-likeness, and pharmacokinetic properties of the compounds. The key characteristics included gastrointestinal absorption, the ability to penetrate the blood–brain barrier (BBB), and parameters such as lipophilicity, size, polarity, solubility, saturation, and flexibility. The results for the top 4 ligands are presented below.

PubChem ID	Affinity (kcal/mol)	Molecular mass (g/mol)	Hydrogen bond	Hydrogen bond donor	LogP	BBB penetration	Gastrointestinal absorption	Synthetic accessibility
44575609	**-9.13**	268.312	3	2	3.4	Yes	High	2.46
91996	**-10.49**	226.275	2	1	3.1	Yes	High	1.57
5708670	**-9.31**	238.286	2	1	4.46	Yes	High	2.51
129863265	**-10.16**	340.4	4	2	4.1	No	High	2.18
Lapatinib	**-10.2**	581.1	9	2	5.1	No	Low	4.05

The table presents the binding affinities and pharmacokinetic properties of the 4 ligands and the control.

Compound 129863265 showed high gastrointestinal absorption and low BBB permeability. Its lipophilicity, size, polarity, solubility, saturation, and flexibility were within values considered suitable for a drug.



The physicochemical properties of compound 129863265. The pink area represents the suitable physicochemical space for oral bioavailability. The red line represents the zone of the ligand.

Conclusion

Docking of 100 substances was carried out, among which inhibitors with PubChem IDs 44575609, 91996, 5708670, and 129863265 were identified. After analyzing physicochemical and pharmacokinetic properties, a substance with PubChem ID: 129863265, SMILES: CC1=C(C=CC(=C1)C(=O)CCCCCC(=O)C2=CC(=C(C=C2)O)C)O, satisfying all criteria, was selected. Ligand 129863265 is an inhibitor working via a competitive inhibition mechanism, as it binds to the ATP-binding site. This substance has a high affinity score for the HER2 active site (-10.16 kcal/mol), does not penetrate the BBB, is absorbed in the GI tract, and is uncomplicated to synthesize. Compound 129863265 was identified as a promising computational candidate for further investigation. Experimental studies are required to determine whether it inhibits HER2 activity and whether it has anticancer effects.

Works Cited

1. Gutierrez, Carolina, and Rachel Schiff. “HER2: biology, detection, and clinical implications.” Archives of pathology & laboratory medicine vol. 135,1 (2011): 55-62. doi:10.5858/2010-0454-RAR.1
2. Sun, Yi-Sheng et al. “Risk Factors and Preventions of Breast Cancer.” International journal of biological sciences vol. 13,11 1387-1397. 1 Nov. 2017, doi:10.7150/ijbs.21635
3. Aertgeerts, Kathleen et al. “Structural analysis of the mechanism of inhibition and allosteric activation of the kinase domain of HER2 protein.” The Journal of biological chemistry vol. 286,21 (2011): 18756-65. doi:10.1074/jbc.M110.206193
4. Kim, Sungwan, et al. "PubChem Substance and Compound Databases." Nucleic Acids Research, vol. 44, no. D1, 2016, pp. D1202–D1213. PubMed Central, https://doi.org/10.1093/nar/gkv951.
5. McNutt, Andrew T., et al. "GNINA 1.0: Molecular Docking with Deep Learning." Journal of Cheminformatics, vol. 13, no. 1, 2021, p. 43, https://doi.org/10.1186/s13321-021-00522-2.
6. Daina, Antoine, et al. "SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules." Scientific Reports, vol. 7, no. 1, 2017, p. 42717, https://doi.org/10.1038/srep42717.
7. Segovia-Mendoza, M., et al. "Efficacy and Mechanism of Action of the Tyrosine Kinase Inhibitors Gefitinib, Lapatinib and Neratinib in the Treatment of HER2-Positive Breast Cancer: Preclinical and Clinical Evidence." American Journal of Cancer Research, vol. 5, no. 9, 2015, pp. 2531-61. PubMed Central, www.ncbi.nlm.nih.gov/pmc/articles/PMC4633889/.

Prediction of Cognitive Decline in OASIS with Clinical and MRI Measures and Quantified Uncertainty By Alan Alpysbayev

Abstract

Individualized antiamyloid treatment decisions require evidence regarding both cognitive outcomes and treatment-related harms. In this exploratory retrospective study of 122 OASIS-2 participants (17 decline events), we evaluated clinical and MRI-augmented models to predict cognitive decline and quantify predictive uncertainty over a median follow-up of 21.3 months. Clinical logistic regression achieved an AUROC of 0.716 (95% CI 0.585–0.837) for predicting a ≥3-point Mini-Mental State Examination (MMSE) drop, compared to 0.673 (95% CI 0.548–0.796) when incorporating global MRI measures. For continuous MMSE change, Gaussian-process regression (RMSE 2.386 points) did not outperform a simple mean-change benchmark (RMSE 2.305 points), yielding wide 95% prediction intervals (mean width 8.75 points). Global MRI measures did not improve classification or continuous prediction in this sample, and treatment benefits or safety risks could not be established. These findings highlight key methodological requirements for future benefit-risk modeling rather than support clinical deployment.

Introduction

Antiamyloid treatment research has made individualized benefit and risk assessment an important objective. In the Clarity AD trial, lecanemab was associated with less cognitive and functional decline than placebo at 18 months, while amyloid-related imaging abnormalities (ARIA) remained a relevant safety outcome [1]. Predicting future cognition, estimating a treatment effect and predicting a treatment-associated adverse event are different statistical tasks. A prognostic model alone cannot determine how much an individual would benefit from treatment. OASIS provides clinical assessments and structural MRI measurements suitable for investigating cognitive prognosis [2,3]. The available data support a focused feasibility study of observed MMSE change. The objectives were to compare clinical models with models incorporating two global MRI measures, evaluate held-out discrimination and calibration, and examine predictive uncertainty. A further objective was to define the evidence required to extend this work toward the originally envisaged NeuroGuide benefit and risk framework. No treatment-response or ARIA model was fitted.

Methods

Study design and participant selection

The longitudinal source contained 373 OASIS-2 visits from 150 participants. We selected each participant's earliest recorded visit by MRI delay and visit number. Eligibility required age 55-90 years, global CDR from 0 to 1 inclusive, and observed baseline MMSE. Among subsequent visits with observed MMSE at 12-30 months, the visit closest to 18 months was

selected; ties favored the earlier visit. Outcomes were not interpolated or imputed. The resulting cohort therefore includes cognitively unimpaired participants and is not a biomarker-confirmed early Alzheimer disease cohort. Figure 1 reports the sequential selection counts.

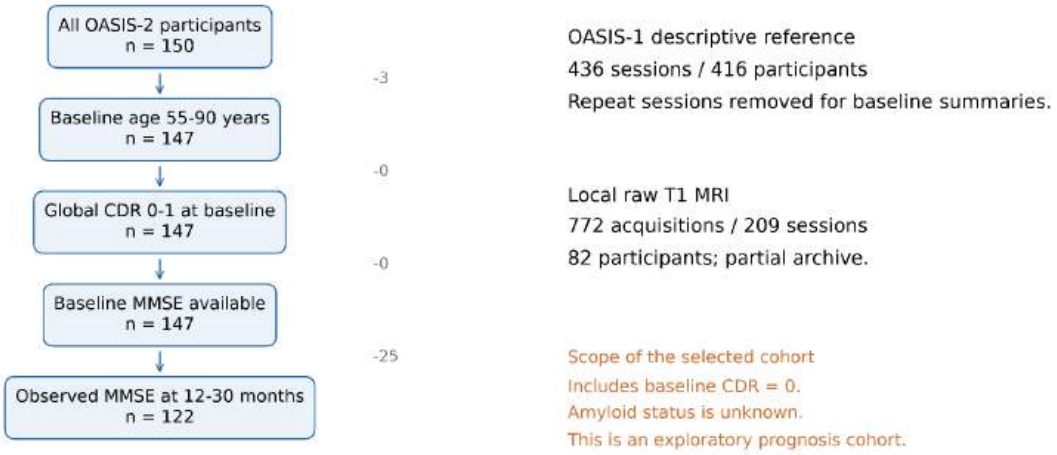


Figure 1. Participant selection and available data. Sequential OASIS-2 eligibility counts are shown on the left; exclusions at each step are indicated to the right of the arrows. Of 150 participants, 147 met the age criterion and 122 had an eligible observed follow-up. OASIS-1 contributed descriptive data only. The local raw T1 archive was partial and was not required for inclusion because the tabular MRI measures were available independently. ADNI and OASIS-3 were not analyzed.

Data sources and outcomes

OASIS-1 contained 436 sessions representing 416 participants after removing repeat sessions by the identifier without its MRI-visit suffix. The earliest session was retained for baseline descriptions. OASIS-1 was not pooled with OASIS-2 for model development or used as an external evaluation cohort: the release designs differ, longitudinal endpoints were unavailable in its supplied table, and cross-release participant overlap was not resolved. Supplementary Figure S1 describes the age and brain-volume distributions of both releases. The continuous outcome was follow-up MMSE minus baseline MMSE, in score points; negative values indicate decline. The binary outcome was a reduction of at least three points. This threshold was an operational exploratory definition, not a validated minimum clinically important difference or a measure of treatment response. The actual elapsed follow-up time was included as a predictor. Thus, the estimand was observed to change conditional on baseline measurements and the evaluated horizon, rather than change at an identical 18-month visit for every participant. Global CDR was used as a baseline variable and was not treated as CDR Sum of Boxes (CDR-SB).

Clinical and MRI predictors

Clinical inputs were baseline age, sex encoded from the supplied M/F field, years of education, socioeconomic status (SES), MMSE, global CDR and follow-up duration in years. SES was treated as a numeric ordinal score, which imposes an approximate equal-spacing

assumption. The MRI-augmented models additionally used normalized whole-brain volume (nWBV) and estimated total intracranial volume (eTIV) from the CSV. Atlas scaling factor was omitted because it provides information closely related to eTIV. OASIS-1 education categories were not equated with OASIS-2 years of education. Subject and MRI identifiers, follow-up cognitive or MRI values, outcome-derived changes and the dataset Group variable were excluded from predictors. In particular, the Converted group can encode future information. Median imputation with missingness indicators and standardization were fitted within each training fold and then applied to its held-out participants. No held-out outcomes informed preprocessing or hyperparameter fitting.

The raw T1 archive contained 772 image-header pairs from 209 MRI sessions and 82 participants. Headers and image sizes were checked for all pairs; two deterministically selected baseline volumes were visualized as illustrative quality-control examples (Supplementary Figure S3). This was not a comprehensive expert QC review or a new segmentation study. The predictive MRI features came from the supplied table. No hippocampal segmentation, FLAIR-derived lesion burden, susceptibility-derived microhemorrhage count or ARIA label was generated.

Model specifications

Two logistic regressions with L2 regularization (C = 1) used clinical and clinical-plus-MRI inputs, respectively. Continuous models comprised the mean change in the training fold, clinical ridge regression (alpha = 10), and clinical-plus-MRI Gaussian-process regression. The Gaussian process used a constant amplitude multiplied by an isotropic radial-basis-function kernel plus a white-noise kernel, with outcome normalization [4]. Kernel parameters were optimized on the training data with one optimizer restart. Logistic and ridge penalties were fixed; no evaluation-fold tuning was performed. This comparison evaluates specific implementations and does not isolate the incremental MRI contribution to Gaussian-process regression, because a clinical-only Gaussian process was not fitted.

Internal evaluation and uncertainty

We used one shuffled five-fold split stratified by the binary decline outcome (seed 20260911). Each participant contributed one outcome and exactly one out-of-fold prediction. The same outer folds were used for all models. This is internal cross-validation, not repeated cross-validation or independent external validation. Classification metrics were AUROC, average precision (AP) and Brier score. AP was calculated from the precision-recall operating points and was not reported as a trapezoidal area under the precision-recall curve. For each classification metric, percentile 95% confidence intervals were obtained from 1,000 participant bootstrap samples of the fixed out-of-fold predictions; samples containing only one outcome class were omitted. These intervals are conditional on the fitted predictions and do not capture the full variability of retraining or selecting folds. Five approximately equal-count probability

bins were used for calibration plots, with Wilson intervals for observed event fractions. No recalibration was performed on held-out outcomes.

Regression was assessed by RMSE, MAE and pooled out-of-fold R squared. Gaussian-process prediction intervals were the predictive mean plus or minus 1.96 predictive standard deviations, including the fitted white-noise component. Coverage was assessed against observed outcomes. The intervals were not truncated to the MMSE range of 0-30 and did not incorporate kernel-hyperparameter uncertainty. Decision curves used net benefit = TP/N - (FP/N) × t/(1 - t), where t is a probability threshold [5]. Here the hypothetical action was flagging future MMSE decline; it was not prescribing therapy. Thresholds were illustrative and no clinical utility claim was prespecified.

Baseline descriptions

Table 1. Baseline characteristics of the source and analysis cohorts

Characteristic	OASIS-1 N = 416	OASIS-2 N = 150	Analysis N = 122
Age, years	52.7 (25.1)	75.4 (7.5)	75.5 (7.4)
Female, n (%)	256 (61.5)	88 (58.7)	70 (57.4)
MMSE	27.06 (3.70)	27.56 (2.97)	27.28 (3.17)
MMSE observed, n	235	150	122
Global CDR	0.29 (0.38)	0.26 (0.33)	0.28 (0.33)
CDR observed, n	235	150	122
nWBV	0.789 (0.060)	0.736 (0.036)	0.735 (0.036)
eTIV, supplied scale	1480.5 (158.3)	1474.4 (174.7)	1471.3 (172.6)

Values are mean (SD) unless stated otherwise; means use observed values. The analysis cohort is a subset of OASIS-2. OASIS-1 is descriptive, not an independent test set. nWBV is dimensionless; eTIV is reported on the unconverted numeric scale of the supplied CSV. Global CDR is ordinal and its mean is descriptive only.

Results

Cohort characteristics and discrimination

Three participants were excluded by the baseline age criterion; none of the remaining 147 were excluded by CDR or missing baseline MMSE. A further 25 had no eligible observed follow-up, leaving 122 participants (Figure 1). Their mean age was 75.5 years and 70 (57.4%) were female (Table 1). Baseline CDR was 0 in 64 participants, 0.5 in 47 and 1 in 11. SES was missing for seven; the other selected clinical and tabular MRI inputs were complete. Raw baseline MRI was locally available for 66 of the 122 participants. Median follow-up was 21.34 months (IQR 18.35-24.36). Mean MMSE change was -0.57 points (SD 2.29), and 17 participants

(13.9%) met the decline definition. Clinical logistic regression achieved higher point estimates of AUROC and AP, and a lower Brier score, than the MRI-augmented logistic regression (Table 2 and Figure 2). No formal paired test of the between-model difference was performed; the observed ordering does not establish statistical superiority.

Table 2. Out-of-fold classification performance for MMSE decline

Model	AUROC 95% CI	AP 95% CI	Brier score 95% CI
Clinical LR	0.716 0.585-0.837	0.292 0.149-0.470	0.126 0.085-0.172
Clinical + MRI LR	0.673 0.548-0.796	0.230 0.136-0.414	0.144 0.102-0.188

LR, logistic regression; AP, average precision. N = 122; 17 events. Confidence intervals are conditional percentile bootstrap intervals from fixed out-of-fold predictions. Lower Brier scores are better; higher AUROC and AP are better.

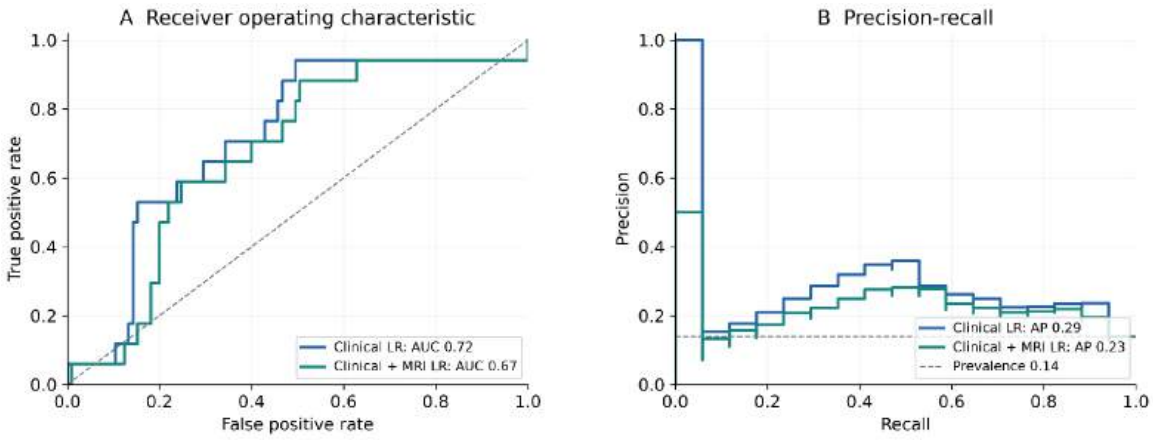


Figure 2. Discrimination of observed MMSE decline. Receiver operating characteristic curves (A) and precision-recall curves (B) compare clinical and MRI-augmented logistic regression. Each participant contributes one prediction made without training on that participant. The diagonal in A denotes chance discrimination; the horizontal line in B denotes the observed event prevalence of 0.139. The outcome is a loss of at least three MMSE points at the selected follow-up, not cognitive benefit from treatment or ARIA.

Calibration and continuous prediction

The classification calibration curves showed considerable uncertainty, especially in bins with higher predicted probabilities (Figure 3A). The available event count does not support a strong claim of well-calibrated individual risks. Average precision exceeded the prevalence reference, but this does not by itself establish that predicted probability magnitudes are accurate. The clinical model's Brier score of 0.126 also exceeded the approximately 0.120 score of a

constant predictor set to the pooled sample prevalence. That constant is a descriptive reference calculated from the full sample, not a prospective training-fold benchmark.

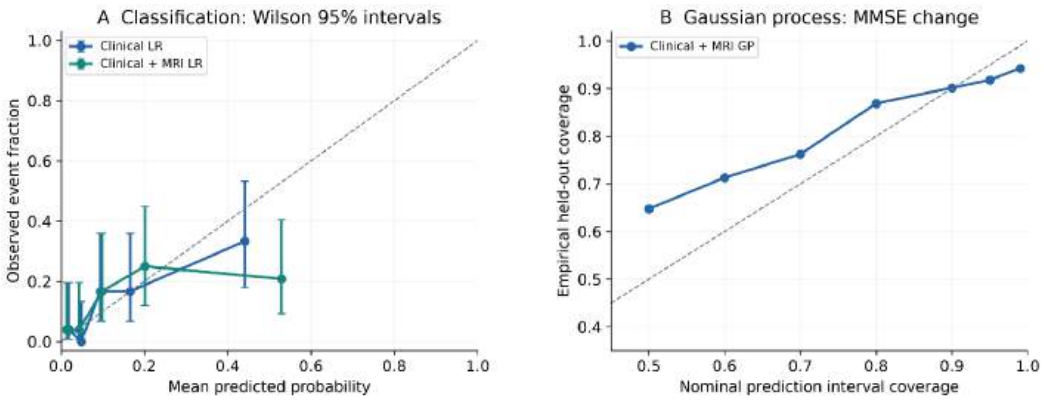


Figure 3. Probability calibration and interval coverage. (A) Approximately equal-count bins summarize mean predicted probability and observed decline frequency for each logistic model. Error bars are Wilson 95% intervals, not confidence intervals for the fitted curve. (B) Empirical coverage of Gaussian-process prediction intervals for continuous MMSE change is compared with nominal coverage. The dashed diagonals indicate ideal agreement. These are internal, held-out estimates from a small sample.

Table 3. Out-of-fold prediction of continuous MMSE change

Model	RMSE	MAE	R squared
Training-fold mean change	2.305	1.574	-0.022
Clinical ridge regression	2.403	1.647	-0.111
Clinical + MRI Gaussian process	2.386	1.632	-0.095

RMSE and MAE are in MMSE points. All values use the same 122 held-out outcomes. The benchmark uses only the mean change of each training fold; negative pooled R squared values are retained.

Neither regression model improved on the mean-change benchmark (Table 3). Gaussian-process nominal 95% intervals covered 112 of 122 outcomes (91.8%), with mean width 8.75 MMSE points. Figure 3B shows that coverage varied with nominal level; interval availability is therefore not equivalent to reliable uncertainty calibration. Supplementary Figure S2 displays every participant's interval and observed change, including large errors that would be obscured by aggregate metrics alone.

Exploratory decision curves

Figure 4 evaluates a hypothetical decision to flag participants for cognitive decline. At a threshold of 0.10, net benefit was 0.058 for the clinical model and 0.059 for the MRI-augmented model, compared with 0.044 for flagging everyone. At 0.20, the corresponding model values

were 0.035 and 0.025. At 0.30, both model curves fell below zero, indicating worse point-estimate net benefit than flagging nobody under the assumed weighting of false positives.

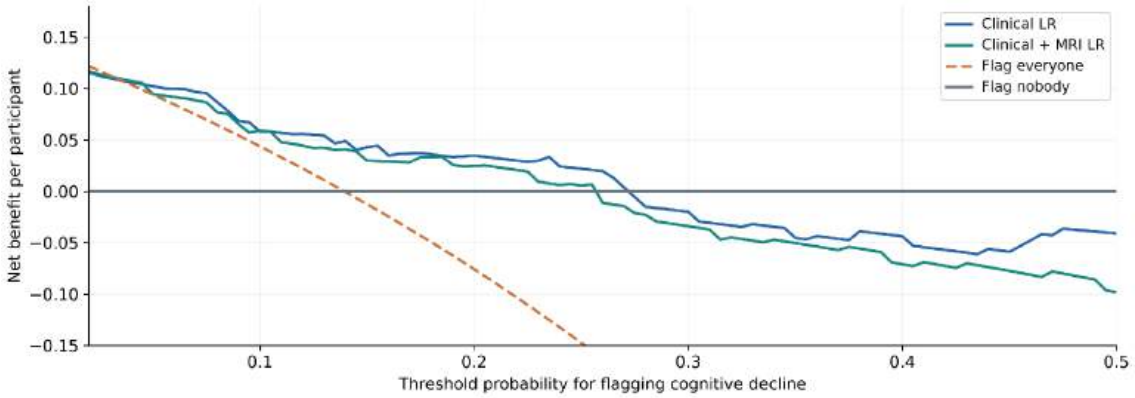


Figure 4. Decision curves for an exploratory cognitive decline flag. Net benefit is plotted against the risk threshold for clinical and MRI-augmented logistic regression. Flag-everyone and flag-nobody policies provide reference curves. Predictions and outcomes are the same as in Table 2. The displayed y-axis truncates the flag-everyone curve at higher thresholds. Thresholds are illustrative; the figure does not evaluate a validated care pathway, antiamyloid prescribing or ARIA monitoring.

These curves summarize trade-offs within the current data rather than demonstrate a clinical improvement. The threshold encodes a relative penalty for false-positive flags, yet no actual intervention, downstream cost, patient preference or benefit from acting on a flag was measured. The analysis did not estimate uncertainty bands for the decision curves or select an operating threshold for deployment. A small apparent advantage over reference policies at a particular threshold may be unstable with only 17 events. The combination of moderate discrimination, uncertain calibration and threshold-dependent net benefit argues against choosing a model from AUROC alone. In this feasibility analysis, the curves identify questions for future evaluation: whether an actionable flag can be defined, how false positives and missed decline should be weighted, and whether the resulting policy improves outcomes in an independent population.

Individual predictions across follow-up horizons

Figure 5 presents six participants selected at evenly spaced ranks of baseline MMSE before examining prediction errors. The Gaussian-process model for each participant was fitted without that individual. Baseline covariates were held fixed while the requested horizon varied within 12-30 months. The observed points are actual visits; connecting them is a visual aid. The smooth prediction is a baseline-conditioned horizon function and should not be interpreted as a validated model of an individual's complete disease trajectory.

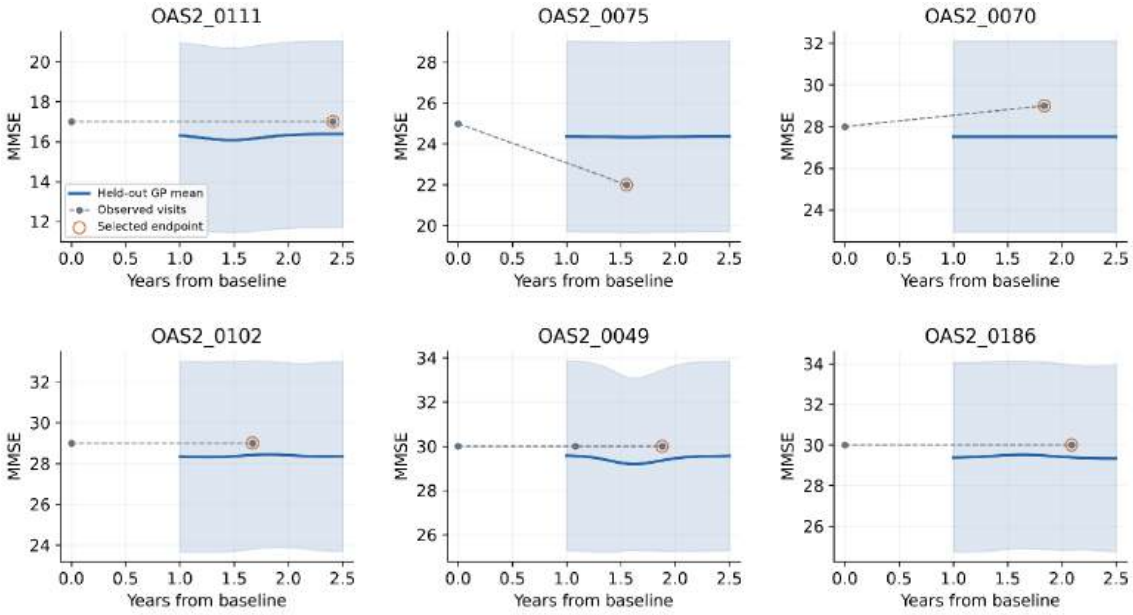


Figure 5. Held-out cognitive profiles for six example participants. Blue lines show Gaussian-process predicted MMSE at each horizon, obtained by adding predicted change to the participant's baseline MMSE. Blue shading gives model-based 95% prediction intervals. Gray points and dashed lines show observed visits within the plotted period; the orange circle marks the selected evaluation endpoint. No follow-up value from the displayed participant was used in model fitting. Different y-axis ranges emphasize within-person profiles and should not be used to compare apparent slopes across panels.

Several predictions remained close to a modest average change, despite differences in the observed endpoints. This is consistent with the limited held-out regression performance. The intervals are broad and sometimes extend above the maximum MMSE score of 30 because the Gaussian observation model is unbounded. Retaining those values makes the modeling limitation visible; clipping them for presentation would conceal that mismatch and alter the interpretation of coverage.

Discussion

Principal findings and interpretation

This study demonstrates a reproducible workflow for evaluating cognitive prognosis and predictive uncertainty using the available OASIS data. It does not demonstrate a clinically useful predictor. Clinical logistic regression showed moderate discrimination for the exploratory MMSE endpoint, but the addition of nWBV and eTIV did not improve its evaluated performance. Continuous predictions were weaker: ridge and Gaussian-process regression failed to outperform the simple mean-change benchmark. The negative results are central to the interpretation and do not support claims that a more complex model or an additional modality necessarily improves individual prediction. The MRI comparison concerns two global measures within a small sample. It does not establish that structural MRI is uninformative, or that regional volumes, longitudinal change or properly validated image representations would fail. Those

features were not evaluated. Conversely, their potential should not be used to reinterpret the present results as evidence of multimodal superiority. The Gaussian-process and ridge models also differ in both algorithm and input set; without a clinical-only Gaussian process, the effect of adding MRI to that regression model cannot be separated from the effect of changing the algorithm. Predictive intervals made uncertainty visible, but their empirical performance requires caution. Nominal 95% intervals covered 91.8% of outcomes and were wide relative to the average MMSE change. Such intervals may be statistically informative while remaining insufficiently precise for a clinical decision. The probability calibration and decision-curve results likewise indicate that ranking participants, assigning accurate probabilities and improving decisions are separate requirements. The study should therefore be regarded as a feasibility assessment and a diagnostic evaluation of the modeling approach.

The analysis included only 122 participants and 17 binary events, leaving approximately three or four events per held-out fold. Estimates of precision, calibration and net benefit are consequently unstable, and subgroup analyses would be particularly underpowered. A single randomized fold allocation does not characterize the full variability of internal evaluation. The bootstrap intervals condition on the fixed out-of-fold predictions and do not reflect all uncertainty from model fitting, fold selection or analytic decisions. No formal hypothesis test or multiplicity-adjusted comparison was used to establish superiority. Selection required an observed follow-up within a particular window. Excluding 25 otherwise eligible participants may introduce selection bias if attendance or missing cognition is related to health status or subsequent decline. The timing of visits may also be informative. Including actual follow-up duration accommodates different horizons statistically, but evaluation at observed visit times does not establish calibration at a standardized future appointment or under another follow-up schedule. Follow-up was typically longer than 18 months, and these results must not be described as uniform 18-month outcomes. The cohort included 64 participants with baseline global CDR 0, and amyloid confirmation was unavailable. It therefore differs from an amyloid-positive early Alzheimer disease treatment population. MMSE change is also distinct from CDR-SB or ADAS-Cog change. The three-point threshold was not established here as a treatment-response criterion. The bounded, discrete score and the concentration of baseline scores near 30 can limit sensitivity; repeated testing, measurement error and regression to the mean could also contribute to observed changes. Baseline MMSE appears in both the predictors and the subtraction defining the outcome, which should be examined in sensitivity analyses.

The baseline predictor set omitted APOE genotype, amyloid and tau biomarkers, vascular and medication information, and regional MRI measures. Residual biological heterogeneity may therefore be substantial. Median imputation is simple and reproducible but does not propagate uncertainty about missing covariates or address potentially informative missingness. Treating SES and global CDR as numeric predictors also imposes modeling assumptions that were not examined with alternative encodings. Missing data and predictor definitions must be reconsidered in a larger development cohort. The Gaussian-process model assumed an isotropic smooth kernel and a Gaussian observation distribution with a constant noise term. Those

assumptions can be restrictive for heterogeneous, bounded MMSE changes. Optimizer warnings indicated that noise parameters reached their lower search bound in four folds and the length scale reached its lower bound in another fold. These warnings were recorded rather than suppressed from the audit, and strengthened the need for kernel and optimization sensitivity analyses. The intervals condition on estimated kernel parameters and do not integrate over their uncertainty. Only one noise component was modeled; the work does not demonstrate a validated decomposition of aleatoric and epistemic uncertainty. The raw MRI archive was incomplete. Tabular nWBV and eTIV were available for all analyzed participants, but raw baseline images were present for only 66. Header and size checks do not establish freedom from motion, acquisition artifacts or segmentation error. The two displayed volumes illustrate data access and appearance, not expert quality assurance of the full collection. This study also did not evaluate an end-to-end image model. Any future segmentation pipeline must document preprocessing, image quality and the provenance of derived features. No external evaluation was conducted. OASIS-1 supplied descriptive context, and its much broader age distribution illustrates a population difference rather than successful transportability. Possible overlap between releases was not resolved, so using the two as independent training and testing datasets would require further verification. The current data do not support claims about performance across institutions, demographic groups, modern scanners or biomarker-defined treatment populations. Demographic fairness, clinical workflow effects and prospective impact were not assessed.

The decisive limitation for the original benefit and risk objective is the absence of treatment assignments, a suitable treated-control comparison and adjudicated ARIA outcomes. An individual's predicted MMSE change under observed follow-up is not the difference between their outcomes under treatment and no treatment. Relabeling prognostic decline as therapeutic benefit would change the estimand without providing causal evidence. Likewise, T1 appearance or global atrophy cannot be substituted for observed ARIA-E or ARIA-H events. No ARIA incidence, risk score or uncertainty interval was estimated. Figure 6 summarizes this evidence boundary. A joint treatment benefit and risk display requires both estimands to be defined and evaluated with appropriate data. Observational associations, an assumed average treatment effect, or simulated adverse events would not validate that display. The proposed NeuroGuide concept remains a future research direction, separate from the prognostic models evaluated here.

The immediate next step is to strengthen the prognosis study before expanding model complexity. A protocol should prespecify the target population, outcome and visit window; compare fixed-horizon and longitudinal approaches; and examine alternative MMSE thresholds or bounded outcome models. Repeated subject-separated evaluation, with nested tuning when needed, should compare clinical-only and MRI-augmented versions of the same algorithms. This would separate algorithm effects from incremental imaging value. Baseline benchmarks, calibration, prediction-interval coverage and subgroup sample sizes should remain visible even when performance is unfavorable.

Required component	Local evidence	Status
Cognitive outcome	MMSE and global CDR	Available; different from CDR-SB
Structural MRI	T1 raw images; nWBV / eTIV	Available
Treatment benefit	No treatment / control assignments	Not identifiable
ARIA risk	No ARIA-E / ARIA-H outcomes	Not estimable
Amyloid / APOE / fluid markers	No corresponding fields	Absent
ADNI / OASIS-3 validation	OASIS-1 and OASIS-2 only	Absent

Figure 6. Available evidence and requirements for treatment benefit and risk modeling. The panel distinguishes observed cognition and structural MRI from the missing components needed for treatment-effect and ARIA analyses. MMSE and global CDR do not supply CDR-SB outcomes; treatment benefit requires a defined treated-control contrast, and ARIA risk requires observed safety events. ADNI and OASIS-3 were absent from this analysis. This is a methodological data-availability summary, not an empirical benefit-versus-risk scatterplot.

A subsequent study should use a genuinely independent cohort with harmonized definitions and no participant overlap, retain the locked model during evaluation, and assess calibration as well as discrimination. Regional MRI extraction and broader biomarker integration should follow documented quality control. Alternative kernels, categorical encodings, multiple imputation and conformal or other calibrated prediction intervals could be evaluated within an appropriate development-and-calibration design; none should be assumed to improve the present results without testing. For the treatment objective, participant-level randomized trial data, or a carefully justified observational causal design, would be needed to define a treatment contrast at a prespecified horizon. Data should include assignment and exposure, baseline disease and amyloid status, cognitive outcomes and adjudicated ARIA with timing and MRI surveillance. Treatment-effect heterogeneity and safety prediction should be evaluated separately before combination. A joint decision rule would then need explicit benefit-harm preferences, assessment of joint uncertainty, external validation and prospective evaluation of the effect on clinical decisions. Transparent reporting should follow prediction-model guidance such as TRIPOD+AI [6].

Conclusion

The available OASIS data support a transparent exploratory study of cognitive prognosis with quantified predictive uncertainty. In 122 participants, adding two global MRI measures did not improve logistic classification, and the continuous models did not outperform a simple mean-change benchmark. Calibration uncertainty, broad prediction intervals and the lack of external evaluation limit individual interpretation. The results do not establish treatment benefit, ARIA risk or readiness for clinical use. Their contribution is a reproducible assessment of what

the current data can support and a concrete methodological path toward a future benefit and risk study.

Data and code availability

The analysis used the OASIS-1 cross-sectional table, OASIS-2 longitudinal table and the accompanying partial raw T1 archive. Source datasets and access conditions are provided by the OASIS project [2,3]. The accompanying research_figures project contains the generation script, configuration, environment version record, participant fold assignments, out-of-fold predictions, metrics and figure captions. The audit includes input CSV and manuscript hashes, an MRI inventory and recorded model warnings. The reported results correspond to seed 20260911 and the supplied configuration; no public code repository or external validation dataset is claimed.

Ethics and acknowledgments

This was a secondary analysis of de-identified OASIS data, with no new participant recruitment or intervention. The applicable local institutional determination for this analysis must be documented by the investigators; dataset availability alone is not an assertion of exemption. Data was provided by OASIS. The OASIS-1 and OASIS-2 acknowledgments identify principal investigators D. Marcus, R. Buckner, J. Csernansky and J. Morris, with support from P50 AG05681, P01 AG03991, P01 AG026276, R01 AG021910, P20 MH071616 and U24 RR021382. These acknowledgments concern the source datasets and do not imply funding of the present analysis.

Works Cited

Collins, G. S., Moons, K. G. M., Dhiman, P., et al. (2024). TRIPOD+AI statement: Updated guidance for reporting clinical prediction models that use regression or machine learning methods. BMJ, 385, e078378. https://doi.org/10.1136/bmj-2023-078378

Collins, G. S., Moons, K. G. M., Dhiman, P., et al. (2024). TRIPOD+AI statement: Updated guidance for reporting clinical prediction models that use regression or machine learning methods. BMJ, 385, e078378. https://doi.org/10.1136/bmj-2023-078378

Marcus, D. S., Wang, T. H., Parker, J., Csernansky, J. G., Morris, J. C., & Buckner, R. L. (2007). Open Access Series of Imaging Studies (OASIS): Cross-sectional MRI data in young, middle aged, nondemented, and demented older adults. Journal of Cognitive Neuroscience, 19(9), 1498–1507. https://doi.org/10.1162/jocn.2007.19.9.1498

Marcus, D. S., Fotenos, A. F., Csernansky, J. G., Morris, J. C., & Buckner, R. L. (2010). Open Access Series of Imaging Studies (OASIS): Longitudinal MRI data in nondemented and demented older adults. Journal of Cognitive Neuroscience, 22(12), 2677–2684. https://doi.org/10.1162/jocn.2009.21407

Rasmussen, C. E., & Williams, C. K. I. (2006). Gaussian processes for machine learning. MIT Press. https://gaussianprocess.org/gpml/

Van Dyck, C. H., Swanson, C. J., Aisen, P., et al. (2023). Lecanemab in early Alzheimer's disease. The New England Journal of Medicine, 388(1), 9–21. https://doi.org/10.1056/NEJMoa2212948

Vickers, A. J., & Elkin, E. B. (2006). Decision curve analysis: A novel method for evaluating prediction models. Medical Decision Making, 26(6), 565–574. https://doi.org/10.1177/0272989X06295361

Supplementary figures

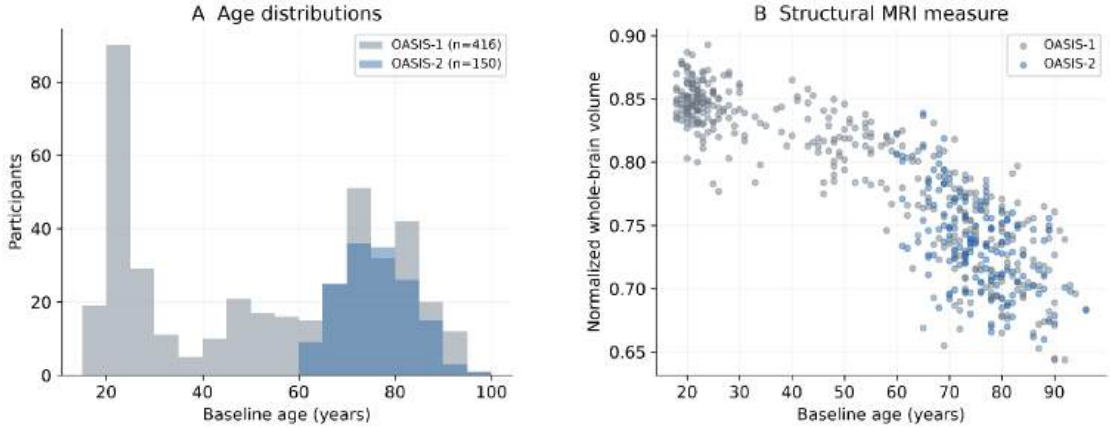


Figure S1. Baseline population context in OASIS-1 and OASIS-2. (A) Age distributions for 416 OASIS-1 and 150 OASIS-2 participants, retaining one baseline session per participant within each release. (B) Baseline nWBV plotted against age. The broader OASIS-1 age range provides descriptive context; the plots do not establish independent validation or exclude overlap across releases. nWBV is a global normalized measure and is not a regional atrophy estimate.

The different age distributions explain why the two release summaries should not be read as exchangeable training and testing populations. The descriptive relationship between age and nWBV does not, by itself, quantify the incremental prognostic value of nWBV once baseline clinical variables are included.

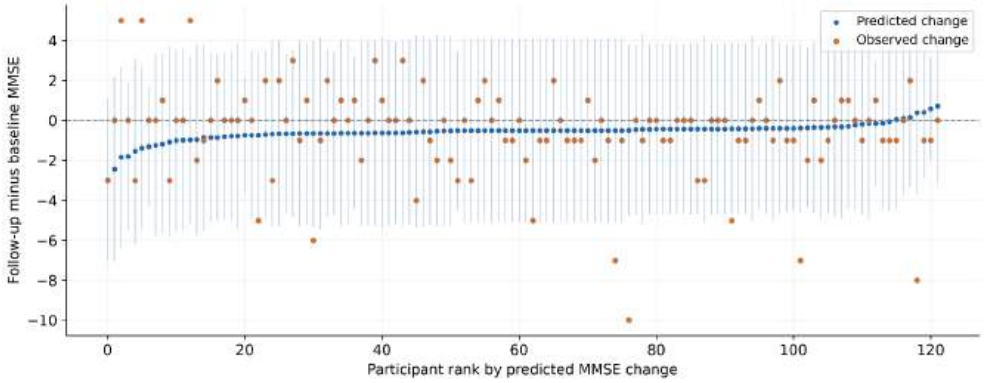


Figure S2. Individual out-of-fold MMSE change predictions. Participants are ranked by predicted change. Blue points are Gaussian-process predictions, vertical blue lines are 95% model-based prediction intervals, and orange points are observed follow-up-minus-baseline MMSE. Each prediction uses the participant's actual selected follow-up duration. Large errors and broad intervals are retained. These are prognostic prediction intervals, not confidence intervals for treatment effects.

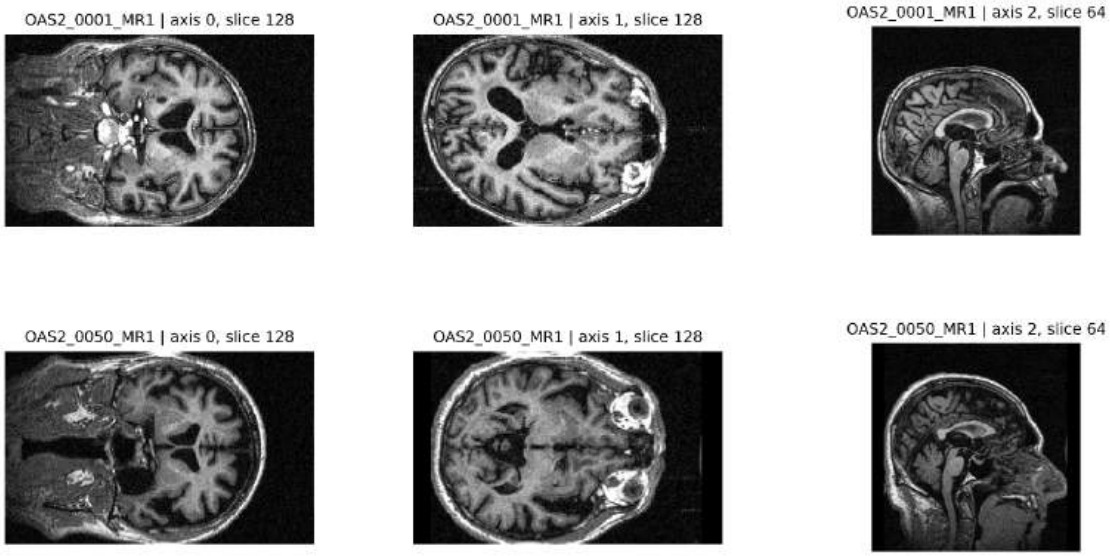


Figure S3. Illustrative quality-control views of local raw T1 MRI. Central slices along the three native voxel axes are shown for two deterministically selected eligible baseline sessions. Native axis labels do not assert anatomical or radiological orientation. Display intensities were limited to the 2nd-99th percentiles of positive voxels. These are actual local volumes. Their display does not constitute expert QC of the complete archive, regional segmentation or ARIA assessment.

Reproducibility details

The figure pipeline was executed with Python 3.14.5, NumPy 2.5.3, pandas 3.0.5, scikit-learn 1.9.1, SciPy 1.18.1, matplotlib 3.11.1 and nibabel 5.4.2. Configuration, source hashes and counts are recorded in the accompanying manifest. The five outer fold assignments are saved at participant level. Seven automated checks passed for endpoint provenance, baseline handling, separation of participants, exclusion of outcome-derived predictor fields, valid prediction ranges, decision-curve arithmetic and expected output files. For the Gaussian process, initial kernel amplitude was 1, length scale 3 and white-noise level 0.5; search bounds were 0.01-100, 0.1-100 and 0.001-10, respectively. Numerical alpha was 10^{-6}. Numeric inputs were standardized after fold-specific median imputation and missingness-indicator creation. Outcomes were normalized during fitting and predictions returned to MMSE units. The stored warnings describe optimizer boundary solutions and should accompany any attempt to reproduce or extend the models. The raw-image inventory records shape, voxel spacing, data type, image size and header-image pairing. Full raw-image checksums were not computed, and header-level validation cannot establish voxelwise integrity or clinical image quality. The tabular MRI measures used for modeling were not recomputed from the displayed raw images. This distinction is necessary to reproduce both the analysis and the limited scope of the MRI illustrations.

Bridging the Gap or Widening It? A Focused Review of mHealth Interventions for Maternal and Neonatal Health in Sub-Saharan Africa By Claire Choi

Abstract

Background: Despite the United Nation's Sustainable Development Goals, maternal and neonatal mortality ratio remains unacceptably high; about 260,000 pregnant and postnatal mothers and 230,000 newborns died in 2023. Among these, sub-Saharan Africa alone accounts for 70% of these maternal deaths. To resolve this pressing issue, various efforts implementing mHealth technologies were made.

Purpose: This review aims to synthesize evidence of the effectiveness of mHealth interventions in improving maternal and neonatal health and outcomes in sub-Saharan Africa, while critically examining an outlooked factor–digital literacy and education–that may underlie persistent health inequality despite these interventions. Based on this analysis, the review proposes directions for future mHealth interventions that account for digital literacy and education to maximize their effectiveness.

Method: This research is a focused review research reviewing original studies about mHealth interventions' effects on maternal and neonatal deaths in sub-Saharan Africa. Three systematic reviews studying the same topic were searched and the original studies reviewed in those articles were screened and reviewed. Research collection was done according to the PRISMA protocol.

Results: 16 studies were included in this review. Most studies used SMS-based reminder services and mobile phone-based apps for health care workers. Most of the studies reported results associated with positive impacts of mHealth interventions on maternal and neonatal health. Nonetheless, their long-term effects on maternal and neonatal deaths should be studied further. None of the studies quantitatively studied the digital literacy or digital access of the target users, leaving the influence of lack of digital skills on the effectiveness of mHealth interventions unclear.

Conclusion: While mHealth interventions show clear benefits for maternal and neonatal healthcare utilization in sub-Saharan Africa, their potential remains limited by unaddressed gaps in female literacy and digital gaps. Future interventions and research must confront this gap to address the fundamental cause of maternal and neonatal deaths.

1. Introduction

1.1 Background

Globally, about 260,000 women died in the process of pregnancy and child birth in 2023; 230,000 newborns died in the same year, as well. Among these unacceptably high maternal and neonatal mortality ratios, sub-Saharan Africa (SSA) alone accounted for 70% of these maternal deaths and had the highest regional neonatal mortality ratio at roughly 26 deaths per 1,000 live births (World Health Organization). Although some countries in Sub-Saharan Africa have made

remarkable improvements over the past decades, achieving UN Sustainable Development Goals Target 3.1–reducing maternal deaths to fewer than 70 per 100,000 live births by 2030– calls for more substantive effort, making the improvement of maternal and neonatal health one of the most pressing public health issues (UNICEF).

Maternal health—the health of women during pregnancy, childbirth, and the postnatal period—directly affects neonatal health, which refers to the health of newborns. Globally, the most common cause of maternal death is haemorrhage (27%), followed by indirect obstetric deaths (23%), and hypertensive disorders (16%). Deaths caused by haemorrhage were especially the highest in sub-Saharan Africa compared to other regions (Cresswell et al.).

However, medical factors are not the only cause of maternal deaths. There are interwoven, indirect factors that are entrenched in the middle and lower income countries that make these societies more vulnerable to maternal deaths. Social determinants such as education, literacy rate, geographic access, poverty, and gender inequality indirectly cause maternal deaths (Monar and Rumissing). For instance, the dearth of formal education in sub-Saharan Africa exposes girls to early marriage (Belachew et.al). Sexual relationship at an early age increases the danger accompanied with pregnancy and procreation. Another instance is the limited access to professional health institutions or delivery assistants, which can be caused by geographical limitations or gender inequality (Zeleke et.al). This health inequality leads to home births–whether planned or unplanned–without professional midwives and health workers, also increasing the danger for both the mother and the newborn.

1.2 Digital Health Interventions

To reduce this risk of maternal and neonatal deaths in sub-Saharan Africa many efforts were made, including digital health interventions. Digital health intervention is an emerging method in public health that integrates digital technologies to address public health agendas using digitalization, especially in health promotion (Wienert et al.). MHealth, a kind of digital health intervention, is the use of mobile, wireless technology to provide health care (“What Is mHealth, What Does It Do & How Does It Benefit Countries?”). Researchers and governments of sub-Saharan Africa have continued to implement mHealth in effort to reduce maternal and neonatal risks. These mHealth include technologies such as short message service (SMS), automated voice calls, phone consultation, mobile apps, and telehealth (Lau et al.).

1.3 Women Education and Digital Literacy

MHealth is an innovative and effective way of enhancing various public health agendas; however, it may not guarantee an equal benefit to every user. Digital literacy, the ability to access, understand, and integrate information from digital technology, comes as a challenge in sub-Saharan Africa (UNESCO). Scoring significantly below the global average on the Digital Skills Gap Index in 2022, only 50% of African countries include ‘computer’ skills in their school curriculum, compared to a global average of 85% (Rwagasore). 89% of sub-Saharan African

students lack home computers, and 82% lack internet access, positioning the gap of digital literacy as a systemic barrier to mHealth.

Lack of formal education for girls, especially in rural areas, also acts as a barrier to accessing mHealth services when they need them. Approximately 34 million adolescent girls of secondary school age remain out of school in sub-Saharan Africa, significantly affecting their general literacy skills, as well as digital literacy skills (Asante Africa Foundation). This can lead to a systemic issue where, despite the availability of mHealth services designed to protect women and girls during pregnancy and delivery, equitable access to these technologies remains limited. This decreases the use of health services and eventually impedes the improvement of maternal and neonatal health.

1.4 Research Gap

Despite the prevalence of research on sub-Saharan Africa's maternal and neonatal healths and the effect of mHealth interventions on the topic, research gaps still exist. Prior research and analysis have only focused on the technical effectiveness of mHealth interventions, the majority of which are SMS reminders or online apps to increase the utilization of antenatal care (ANC) and postnatal (PNC) cares or delivery facilities.

However, most of the research is based on the premise that the users of the interventions have equal access to digital devices and also have an equivalent level of digital literacy and education. This hasty assumption has prevented a deeper investigation into the effects that education levels and digital literacy have on the effectiveness of mHealth interventions. Thus, we have a dearth of research that systematically analyzes how the digital divide caused by unequal access to education and lack of digital competence exacerbates health inequality or decreases the effectiveness of mHealth interventions.

1.5 Aim and research questions

This focused literature review aims to synthesize evidence and cases on the effectiveness of mHealth interventions in improving maternal and neonatal healthcare utilization and health outcomes in sub-Saharan Africa. To address this research gap, this study critically examines why fundamental health inequalities persist in Sub-Saharan Africa despite the implementation of various mHealth interventions designed to reduce them. The review will discuss future interventions that consider digital literacy and education to maximize mHealth's effectiveness. Research questions include:

1. What types of digital health interventions have been used for maternal and neonatal health in Sub-Saharan Africa?
2. Do these interventions improve antenatal care, facility delivery, postnatal care, or newborn-care practices? Or do they have counter-intuitive effects?
3. What effects do they have on maternal and neonatal health outcomes?
4. How do preexisting interventions take account of digital literacy and education, and how can future efforts resolve the systemic gap?

2. Methods

2.1 Study Design

Since this research was conducted by a high school student, it takes the form of a focused review research, rather than a systemic or conventional one. Therefore, instead of analyzing all existing research papers about the topic, it focuses on collecting and analyzing the studies referenced and examined in existing review articles. For legitimacy and transparency of the review, it was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) (Moher et al.). PRISMA Guidelines is an evidence-based checklist and flowchart used in academic research that helps researchers transparently report their research and data extraction procedure via flow chart. The research collection and data extraction procedure for this review followed the PRISMA 2020 flow diagram for updated systematic reviews which included searches of databases, registers and other sources (Page et al.).

2.2 Study Selection

To collect the original studies to include in this review research, three systemic reviews about the topic on "mHealth interventions' effects on maternal and neonatal health" were used as seed sources. These three systemic reviews were searched on Google Scholars, and were selected by the following inclusion criterias:

1. A rigorous systemic review
2. Restricted to SSA
3. Studies the effects of mHealth, eHealth
4. Includes the effects of mHealth and eHealth maternal and or neonatal health
5. Published between 2020-2026
6. Explicitly shows the list or table of studies they included
7. Can access full view of the paper
8. Written in English

49 original studies that were reviewed by the three seed sources were extracted, and went through three steps of screening procedures to ensure their meaningfulness and contribution to this focused review research. First screening screened for duplicate papers, second after reading the title and the abstract, third after reading the full paper. Papers were screened by the following inclusion and exclusion criterias:

Inclusion Factor

1. Sub Saharan African countries
2. Broad maternal health including maternal mortality, neonatal mortality, under-five mortality, antenatal (prenatal) care (ANC) and post-natal care (PNC)

3. mHealth including SMS messages, patient tracking apps, medication reminders, phone calls, telehealth, etc through mobile phones and wireless devices.
4. Published from 2016-2026
5. Original research that is published in a journal
6. Written in English

Exclusion Factor

1. Wrong region: Not SSA
2. Wrong population: not pregnant woman, newborn baby
3. mHealth research that is not directly studying its effect on maternal, neonatal health
4. Wrong intervention: not mHealth
5. Wrong Outcome: No outcomes on maternal, neonatal health
6. Wrong publication type: not an original research paper
7. Duplicate
8. No full text
9. Protocol only

2.3 Data Extraction

The original research that was included in this review went through a thorough process of data extraction. The data were extracted by 5 fixed assessment criterias: Study Identification; Study Characteristics; Intervention and Outcomes; Education, Literary, and Access; and Quality and Limitations. The data extracted from each study were organized in a form of tabular summary.

3. Results

3.1 Search Results

A total of 49 articles were identified from the three seed sources. After removing 2 duplicates and 22 articles from initial screening, 25 full-text articles were assessed for eligibility. Following the inclusion and exclusion criteria mentioned above, 9 full-text articles were removed. Thus, 16 articles were included in this review.

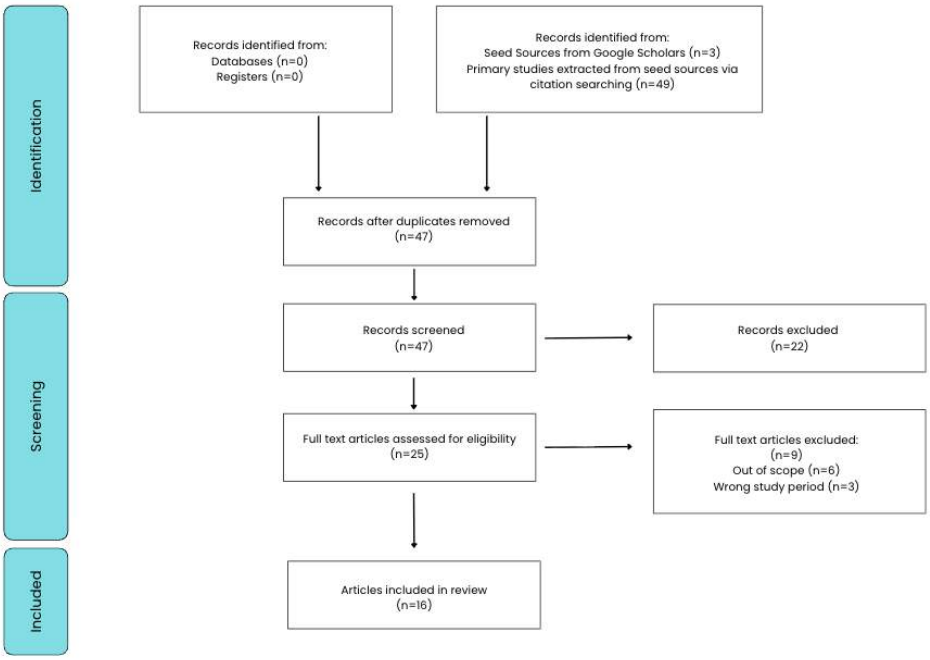


Figure 1: PRISMA flow diagram

Summary of papers used for analysis in the study		
S01	The role of mHealth intervention on maternal and child health service delivery: findings from a randomized controlled field trial in rural Ethiopia	Atanafu et al.
S02	Providing mothers with mobile phone message reminders increases childhood immunization and vitamin A supplementation coverage in Côte d'Ivoire: A randomized controlled trial	Dissieka et al.
S03	Automated phone call and text reminders for childhood immunisations (PRIMM): a randomised controlled trial in Nigeria	Ekhaguere et al.
S04	Mobile phone-delivered reminders and incentives to improve childhood immunisation coverage and timeliness in Kenya (M-SIMU): a cluster randomised controlled trial	Gibson et al.
S05	Impact of smartphone-assisted prenatal home visits on women's use of facility delivery: Results from a cluster-randomized trial in rural Tanzania	Hackett et al.
S06	Reducing routine vaccination dropout rates: evaluating two interventions in three Kenyan districts, 2014	Haji et al.
S07	Association Between the Safe Delivery App and Quality of Care and Perinatal Survival in Ethiopia A Randomized Clinical Trial	Lund et al.
S08	Effectiveness of a mobile health intervention on uptake of recommended postnatal care services in Nigeria	Olajubu et al.
S09	The Effects of a Locally Developed mHealth Intervention on Delivery and Postnatal Care	Shiferaw et al.

	Utilization; A Prospective Controlled Evaluation among Health Centres in Ethiopia	
S10	Effect of Village Health Team Home Visits and Mobile Phone Consultations on Maternal and Newborn Care Practices in Masindi and Kiryandongo, Uganda: A Community-Intervention Trial	Ayiasi et al.
S11	Effect of a community health worker mHealth monitoring system on uptake of maternal and newborn health services in Rwanda	Hetegeka et al.
S12	Feasibility of using smartphones by village health workers for pregnancy registration and effectiveness of mobile phone text messages on reduction of homebirths in rural Uganda	Asiki et al.
S13	Improving health facility delivery rates in Zanzibar, Tanzania through a large-scale digital community health volunteer programme: a process evaluation	Fulcher et al.
S14	Mobile Technology for Community Health in Ghana: Is Maternal Messaging and Provider Use of Technology Cost-Effective in Improving Maternal and Child Health Outcomes at Scale?	Willcox et al.
S15	User assessments and the use of information from MomConnect, a mobile phone text-based information service, by pregnant women and new mothers in South Africa	Skinner et al.
S16	Gauging the Impact of MomConnect on Maternal Health Service Utilisation by Women and Their Infants in Johannesburg, South Africa	Coleman et al.

Table 1: Included studies identification

3.2 Characteristics of the Included Studies

The characteristics of the 16 reviewed studies—including country, setting (rural or urban), study design, and participant demographics—are summarized in a table. 8 studies out of 16 studies were randomized control trials, and other study designs included quasi-experimental, non-randomized controlled evaluation, retrospective analysis, and more. All studies included pregnant women and postpartum women as their participants, with most of them also targeting infants, children under 5 years old, and health care workers.

Study Characteristics				
Study ID	Country	Rural/Urban	Study Design	Participant
S01	Ethiopia	Rural	RCT	Pregnant women, mother of children under 5yo, Health extension workers, voluntary health workers
S02	Côte d'Ivoire	Mixed	RCT	Mothers, primary caretakers of infants (mother-child pairs)
S03	Nigeria	Rural	Individual RCT	Mothers/caregivers of infants
S04	Kenya	Rural	Cluster RCT	Mothers/caregivers of infants
S05	Tanzania	Rural	Cluster RCT	Postpartum women who were CHW clients during

				pregnancy
S06	Kenya	Mixed	Cluster RCT	Infants/caregivers
S07	Ethiopia	Rural	Cluster RCT	Pregnant women, health care workers
S08	Nigeria	NR	Quasi-experimental	Pregnant women attending ANC at PHCs and their newborns.
S09	Ethiopia	Mixed	Non-randomized controlled prospective evaluation	Pregnant women/postpartum women using ANC , delivery, or PNC services
S10	Uganda	Rural	Cluster RCT	Pregnant women/newborns
S11	Rwanda	Mixed	Interrupted time series (ITS) using segmented regression	Mother of children under 5yo/their newborns
S12	Uganda	Rural	Quasi-experimental	Pregnant women/newborns
S13	Tanzania	Mixed	Retrospective analysis	Pregnant and postpartum women
S14	Ghana	Mixed	Cost-effectiveness analysis	Pregnant women, postpartum women/mothers of infants, children under 5
S15	South Africa	Mixed	Qualitative user-experience and acceptability study	Pregnant women and new mothers enrolled in MomConnect.
S16	South Africa	Urban	Retrospective case-control study	Pregnant women who received ANC and subsequently delivered a live infant

Table 2: Included studies characteristics

3.3 Maternal Outcomes

Studies reported that the major positive outcomes for maternal health included increased utilization of ANC, PNC, and health facility deliveries. One study (S08) demonstrated a significant increase in attendance for the WHO-recommended four PNC visits: 30.9% of women in the intervention group completed all four visits, compared to only 3.7% in the control group (those who did not receive SMS reminders).

S05 also reported a promising result. Smart-phone-assisted prenatal home visits by health care workers doubled the odds of facility delivery in rural Tanzania, likely via more frequent and

standardized counseling. These study results suggest that educational and reminder-based SMS and telehealth are effective mHealth methods to possibly decrease maternal mortality ratio by increasing maternal health through more universalized use of ANC, PNC, and delivery institutes. However, S11 and S16 concluded that the mHealth interventions did not result in a substantial increase in ANC and PNC; researchers attributed this issue largely to the already-high baseline coverage, suggesting future studies to investigate on regions that have low baseline coverage.

3.4 Neonatal Outcomes

Many of the studies described mHealth interventions' positive effects on newborns. SMS reminders and voice calls were found to meaningfully increase timely immunizations. S03's study result yielded that 74% of the intervention group completed the immunization within 12 months upon birth while 66% of the control group without intervention completed it. Also, the study result revealed that the SMS reminders didn't only increase the immunization's completion itself, but also its timeliness. 84% of the intervention group received Penta-3 within 1 week while 78% of the control group did. 73% of the intervention group received measles within 1 week while only 65% of the control group did. This study concludes that automated call and text reminders significantly improved immunization completion and timeliness, supporting that mHealth interventions are a good strategy in reducing the chance of maternal and neonatal deaths.

3.5 Health Care Facilities Outcomes

Other studies included mobile app-based mHealth interventions, such as Commcare and Safe Delivery App that aims to facilitate midwives and frontline health care workers in sub-Saharan Africa. Studies suggest that these mobile applications serve various functions, including providing visual and textual training media for healthcare facilities, calculating expected delivery dates and upcoming appointments, and offering educational content for mothers on topics such as maternal diet, pregnancy danger signs, and birth preparedness. Several interventions involved on-call counseling or telehealth, which were revealed to be effective in assisting health care workers and encouraging mothers to utilize health care facilities. S05 reported that training health care workers using CommCare led 74% of the pregnant women in the intervention group to give birth in a facility while only 62% of the women in the control group did. Training health care workers was also associated with a decrease in home birth rates and increased facility utilization for deliveries. Training health care workers using mHealth interventions can be highly promising in reducing maternal and neonatal deaths, since it is a sustainable approach to addressing the problem. That being said, the exact long-term effect on the maternal mortality ratio and neonatal mortality ratio needs to be studied further with great interest.

3.6 Education, Literacy, and Digital Literacy

All of the studies except two (S15, S16) reported the education level of the participants. Studies measured the education level with a variety of standards, including none, secondary, and tertiary; primary completion; and primary or above primary. This reveals that for most of the research, education level was a factor of contribution that differs the effectiveness of the mHealth intervention of investigation. On the other hand, none of the sixteen studies quantitatively nor qualitatively measured female literacy, digital literacy or health literacy of the target population. Only three studies (S11, S12, S15) formally noted that low phone ownership among rural women accompanied by high ethnic and linguistic diversity and illiteracy would increase barriers for SMS-based approaches. The rest of the studies contextually suggested that there might be a decrease in effectiveness to the interventions due to illiteracy and low education; however, they were not supported with further empirical research or evidence. Some of the studies did not mention literary related barriers at all.

4. Discussions and Suggestions

4.1 Summary of Main Findings

Through the focused review research of sixteen original studies, it becomes clear that mHealth interventions such as SMS reminders, mobile-app based aids, and telehealth are an effective and innovative way to improve maternal and neonatal health, even though their direct impact on reducing mortality ratios remains less certain. Scheduled reminder systems of ANC and PNC appointments significantly increased mothers' use of health institutes, increasing their antenatal and postnatal health. It also significantly enhanced the immunization completion rate for neonates and children under 5 years old. Mobile-app based mHealth interventions are especially promising because its training of midwives and frontline health workers decreases the severity of medical gap and provides a sustainable method of potentially decreasing the maternal and neonatal deaths itself.

4.2 MHealth Interventions' inequality

However, most of the mHealth interventions reviewed in this review research are oblivious to the fact that not all women can have equal access to the mHealth interventions. Mobile phone ownership still stays markedly low in rural sub-Saharan areas. Not all individual women own a mobile phone for herself. The literacy rate for adult women in sub-Saharan Africa is shockingly low too; Only 57–60% of women are capable of reading and writing. Illiterate women can not comprehend SMS-based reminders or mobile-based apps. Another problem is digital access; women might suffer from data and telephone fees and have unstable networks and electricity.

Since it is not the case that the majority of mothers can seamlessly access mHealth interventions, it is vital for further research and mHealth interventions to address the digital literacy gap happening in this particular region of the globe.

4.3 Implications

Future research and mHealth intervention efforts might consider a system where it diverges from conventional text-based reminders and proceeds on to more interactive verbal interventions. It should also consider the digital access of women, so it should work in low network environments.

Not only altering mHealth interventions, but also the fundamental aspects of education and digital literacy gap should be enhanced by sub-Saharan African countries. First, governments should consolidate general education and digital education for female students to mitigate the digital gap. Second, a stable access to mHealth interventions regardless of income level and rural area should be guaranteed to encourage universal use of mHealth interventions to possibly decrease maternal and neonatal deaths. Implementing these suggestions, research institutions and governments should actively expand the mHealth business from temporary pilot projects to extended services.

5. Limitation and Conclusion

5.1 Conclusion

This focused review research's aim was to review the existing research of mHealth interventions in sub-Saharan Africa to reduce maternal and neonatal deaths. Sixteen original studies published from 2016 to 2026 were included in this review. Analysis of the research yielded that mHealth interventions were effective in improving maternal and neonatal health outcomes — such as ANC/PNC utilization, facility delivery, and immunization completion—though their direct effect on reducing mortality ratios remains to be confirmed. However, most of the researchers lost sight of factors that are impeding the effectiveness of mHealth interventions. Thus, future research should resolve the deeply entrenched social problems of sub-Saharan Africa including low literacy and education rate of women, digital gap, and low digital literacy to maximize the effectiveness of mHealth interventions. Currently existing mHealth interventions are clearly shown to increase maternal and neonatal health, but needs further research if they truly decrease the maternal and neonatal mortality ratio.

5.2 Limitations

This focused review research is done by an individual high school student. Due to the dearth of work force and time constraint, this review research does not reflect the ideal amount of research usually done on conventional review research. Relevant systematic reviews were used to identify potential primary studies. The reference lists of these reviews were examined through Google Scholar only. Therefore, the results of this study should be interpreted as a focused synthesis of the literature illustrating key trends in relevant research, rather than as comprehensive estimates representing all mHealth studies in sub-Saharan Africa.

Works Cited

"Africa's Digital Leap: Why Skills, Not Just Access, Will Define the Future." ICDL Global, 8 Apr. 2025, https://icdl.org/africas-digital-leap-why-skills-not-just-access-will- define-the-future/

Belachew, Tadele Biresaw, et al."Determinants of Early Marriage Among Married Women in Nine High Fertility Sub-Saharan African Countries: A Multilevel Analysis of Recent Demographic and Health Surveys." BMC Public Health, vol. 22, no. 1, 2022, p. 2355, https://doi.org/10.1186/s12889-022-14840-z

Cresswell, J. A., et al. "Global and Regional Causes of Maternal Deaths 2009–20: A WHO Systematic Analysis." The Lancet Global Health, vol. 13, no. 4, 2025, pp. e626-e634, doi.org.

De Santis, K. K., B. J. Mergenthal, B. Schwettmann, S. L. Stock, S. S. Mergenthal, and J. M. S. Zeeb. "What Are Digital Public Health Interventions? First Steps Toward a Definition and an Intervention Classification Framework." Journal of Medical Internet Research, vol. 24, no. 6, 2022, p. e31921. PubMed Central, https://nih.gov

"Digital Literacy." TVETipedia Glossary, UNESCO-UNEVOC, https://connect.unevoc.unesco.org/home/TVETipedia+Glossary/show=term/term=Digital+literacy

"Empowering Africa's Girls Through Education: Why It Matters and How to Support It."Asante Africa Foundation, https://asanteafrica.org/empowering-africas-girls-through-education-why-it-matters-and-how-to-support-it/.

Lau, Y. K., et al."Antenatal Health Promotion via Short Message Service at a Midwife Obstetrics Unit in South Africa: A Mixed Methods Study." BMC Pregnancy and Childbirth, vol. 14, no. 1, 2014, pp. 1-8.

Maria Ayu Florenza Monar, and Prilly Parrhesia Edong Rumissing. “A Comprehensive Systematic Review of Social Determinants in Maternal Mortality Rates in Developing Countries.” The International Journal of Medical Science and Health Research, vol. 43, no. 1, International Medical Journal Corp. Ltd., Apr. 2026, pp. 138–208, https://doi.org/10.70070/h17j2h20. Accessed 6 Sept. 2026.

"Maternal Mortality." World Health Organization, 7 Apr. 2025, https://www.who.int/news-room/fact-sheets/detail/maternal-mortality.

Moher, David, et al."Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement." PLoS Medicine, vol. 6, no. 7, 2009, p. E1000097.

Page, Matthew J., et al."The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews." BMJ, vol. 372, no. n71, 2021.

Rwagasore, Bernard. “Africa’s Digital Leap: Why Skills, Not Just Access, Will Define the Future - ICDL Global.” ICDL Global, 4 Aug. 2025, https://icdl.org/africas-digital-leap-why-skills-not-just-access-will-define-the-future/. Accessed 6 Sept. 2026.

UNICEF. “Maternal Mortality.” UNICEF Data, UNICEF, Apr. 2025, https://data.unicef.org/topic/maternal-health/maternal-mortality/. Accessed 6 Sept. 2026.

"What Is mHealth, What Does It Do & How Does It Benefit Countries?" ITU, https://www.itu.int/en/ITU-D/ICT-Applications/eHEALTH/Be_healthy/Pages/guide-01.aspx."World Needs You."World Pulse, https://www.worldpulse.org/story/world-needs-you—62071.

Zeleke, Gebreeyesus Abera, et al."Barriers to Healthcare Access Among Women in Sub-Saharan Africa: A Pooled Analysis of Multi-Country DHS Data (2019–2023)." PLoS ONE, vol. 21, no. 2, 2026, p. e0331328.

Analysis of Gamification Trends in South Korean Nursing Education By Bohyun Hong

Abstract

This study aimed to compare international research trends with the current status of South Korean research on gamification in nursing education, and to identify limitations and future directions for domestic nursing education. A descriptive literature review was conducted in accordance with the PRISMA 2020 guidelines. Literature published between January 2016 and June 2026 was searched in four domestic databases (RISS, KCI, DBpia, KISS) and four international databases (PubMed, CINAHL, Scopus, Google Scholar). Of 38 records identified, 7 studies (3 domestic and 4 international) were included in the final analysis and coded for study design, participants, courses, gamification types, game elements, and measured outcomes. International research has grown rapidly since the late 2010s, accumulating systematic reviews, meta-analyses, and randomized controlled trials; it applies scenario-based serious games across the nursing curriculum, attempts integration with virtual reality and artificial intelligence, and measures higher-order outcomes such as clinical reasoning and OSCE-based skills, although effects were selective across outcome variables. In contrast, domestic research began in earnest around 2021 and remains at an early stage: all identified empirical studies used single-group pretest–posttest designs, relied on Kahoot!-based student response systems and board games with intuitive elements such as points, rankings, and competition, and focused on affective outcomes such as learning engagement and satisfaction. These gaps were attributed to a rigid accreditation- and licensure-centered curriculum, a preference for convenient tools, and the absence of longitudinal research infrastructure. Curriculum-integrated longitudinal studies, higher-order outcome measurement, and multidisciplinary development of serious games aligned with educational objectives are recommended.

Key Words Gamification, Nursing Education, Nursing Students, Game-Based Learning, Serious Games, Literature Review

1. Introduction

Despite advancements in medicine and healthcare technology, the modern healthcare environment continues to change due to factors such as an aging population, the rise in chronic diseases, and the complexity of patient conditions. Consequently, nurses must perform professional roles that go beyond providing direct care to patients, managing the entire treatment process and supporting patient safety and recovery. In particular, there is a growing demand for nursing personnel equipped with critical thinking, integrated problem-solving skills, multidisciplinary collaboration capabilities, and ethical decision-making abilities in complex and unpredictable clinical situations. Therefore, modern nursing education aims to go beyond the simple transmission of medical knowledge and establish a key objective of equipping nursing students with the capacity for rapid and prudent clinical judgment, critical thinking, and ethical decision-making.

However, traditional instructor-centered lecture-based instruction has limitations in maintaining student interest and active participation. The traditional one-way method of knowledge delivery makes it difficult to develop the high-order competencies required in actual clinical settings and can lead to issues such as decreased learning motivation, class participation, and learning engagement. Furthermore, in nursing education, it is difficult for students to repeatedly experience diverse clinical situations due to reasons such as patient safety, limited clinical practice opportunities, and a shortage of practice resources. Limited clinical practice opportunities and disparities in practice environments create difficulties in providing students with standardized repetitive practice and competency assessments.

Gamification is gaining attention as a learning method that can address these educational challenges. Gamification is generally defined as the application of game design elements to non-game contexts (Deterding et al., 2011). In nursing education, elements such as challenges, scores, badges, leaderboards, competition, collaboration, step-by-step progression, and immediate feedback can be applied to classes and practice to enhance learner engagement and motivation. These game elements support engagement and sustained learning by visually demonstrating progress toward learning objectives and allowing learners to immediately verify their performance results.

Internationally, there is a growing number of studies applying not only gamification but also game-based learning, serious games, virtual reality (VR), and game-based simulation to nursing education. An analysis of research on digital games and gamification in nursing education published from 2015 to 2022 revealed that the number of related papers tended to increase in recent years (Meşe & Meşe, 2022).

In advanced countries, research actively applying serious games, VR, and game-based learning to nursing education is on the rise, and various educational effects, such as the enhancement of learners' knowledge and problem-solving abilities, are being reported (van Gaalen et al., 2021; Nylén-Eriksen et al., 2025).

In Korea as well, research attempting to apply gamification to nursing education is gradually being published. However, the introduction and research of gamification remain in the early stages, and the scope of application is limited, such as being confined to quizzes or apps (An, 2021). While these domestic studies demonstrate the educational potential of gamification, the currently identified research is mainly centered on Kahoot!, quizzes, team competitions, rankings, and classroom-based activities. In contrast, international research covers a wider range of topics, including clinical scenario-based serious games, immersive VR, digital simulations, and complex decision-making games. In Korea, An (2021) published a scoping review on gamification in nursing education; however, since then, new domestic empirical research has accumulated, and new trends such as VR and AI convergence have emerged overseas. Consequently, it is time for a follow-up review that compares domestic and international trends over the past decade. Therefore, there is a need to diagnose the current status of gamification research in domestic nursing education and to derive implications through comparison with advanced overseas trends.

Accordingly, the purpose of this study is to compare and analyze international research trends regarding the application of gamification in nursing education with the current status of research in South Korea, focusing on literature published from January 2016 to June 2026. Through this, the study aims to identify the limitations and problems of gamification utilization in domestic nursing education and to propose development strategies for the qualitative improvement of domestic nursing education and the introduction of effective teaching methods.

2. Background

2.1. What is Gamification?

Gamification is a concept that has spread to fields such as education, healthcare, and various professional training since the 2000s, alongside the development of digital technology and the game industry (Deterding et al., 2011). This does not mean transforming general activities or classes into complete games, but rather selectively applying the structures and elements of games to existing non-game activities.

Gamification, game-based learning, and serious games are related but are not entirely identical concepts. Gamification involves adding individual game elements, such as scores, badges, competition, or rankings, to existing classes or activities. In contrast, serious games utilize the entire game for learning, featuring clear educational goals, rules, progression structures, and endpoints. Unlike simply adding scores or leaderboards to existing classes, serious games include rules tailored to learning objectives, structured challenges, progression systems, and performance feedback (Shao et al., 2026).

The term "Gamification" first began to be used in the digital media field in the early 2000s and became a concept that spread throughout academia and industry around 2010. Academically, gamification is defined as "a process of applying game design elements and mechanisms to non-game contexts to induce user participation and solve problems" (Deterding et al., 2011).

It is important to note here that the essence of gamification lies not in simply developing or importing entertainment games as a whole, but in extracting the structural characteristics and psychological incentives (Game Elements and Mechanics) inherent in games and transplanting them into systems or programs in other fields.

The core mechanisms constituting gamification are as follows:

Immediate Feedback: Engagement is maintained by providing feedback—such as scores, level-ups, or visual effects—without delay regarding the learner's behavior or performance results.

Clear Goals and Rules: It stimulates a sense of challenge by explicitly presenting tasks to be accomplished and providing rules to solve them.

Reward Systems: It fosters an intrinsic sense of achievement and reinforces behavior through rewards such as badges, rankings, and points.

Autonomy and Safe Failure: Unlike real life, the burden of penalties or failure is low within a gamified environment, creating an environment where learners can learn proactively through repeated trial and error.

These game design elements are highly effective in stimulating human psychological needs, particularly motivations related to achievement, recognition, competition, and cooperation. Initially, companies primarily utilized them to engage customers or as a means of marketing and employee motivation; however, as their effectiveness has been proven, their scope is rapidly expanding into the fields of healthcare, public policy, and educational technology.

2.2. Gamification in Education

As the participatory nature and psychological motivational effects of gamification have been proven, it has rapidly emerged as a core methodology in the field of Educational Technology, extending beyond the realms of corporate management and marketing. It is being actively adopted as a tool to maximize educational outcomes across various levels, from primary and secondary education (K-12) to top-tier higher education institutions and professional training programs.

The reason gamification has established itself as a powerful instructional design methodology in the field of education is that it effectively complements the limitations of traditional rote learning. Modern learners are "Digital Natives" who have grown up in a digital environment; they easily become bored by one-way knowledge transfer methods and crave active interaction. Gamification applied in educational settings offers the following pedagogical values:

Maximization of Intrinsic Motivation and Learning Engagement: Mechanisms unique to games, such as challenges, competition, and leveling up, lead learners to transition from passive bystanders to active participants. This triggers voluntary interest in learning and significantly improves class participation.

Creating an environment of "Learning through Safe Failure": While there is significant psychological pressure regarding incorrect answers or failure in traditional educational settings, in a gamification environment, failure is perceived as data and a stepping stone to the next stage. Learners can refine their problem-solving strategies through repetitive trial and error without pressure.

Strengthening Self-Directed Learning Capabilities: Clear goals and immediate rewards for achievement act as a driving force that enables learners to proactively manage their learning schedules and monitor their progress.

As such, gamification is establishing itself as an innovative EdTech trend that goes beyond the mere "addition of fun elements" to positively impact learners' cognitive and affective domains. This educational utility serves as a decisive factor in expanding the scope of research and application to specialized professional training fields that require advanced practical skills and judgment, moving beyond simple rote memorization of knowledge.

2.3. Application and Effects of Gamification in Nursing Education

Gamification has been reported as an innovative teaching strategy effective for the acquisition and retention of knowledge, the facilitation of problem-based learning, and the enhancement of learning motivation and participation in nursing education. Its advantages in encouraging active learning and critical thinking, as well as its ability to recreate situations similar to actual clinical practice, have been confirmed (van Gaalen et al., 2021; Nylén-Eriksen et al., 2025). Specifically, studies applying gamification to nursing education have reported increased learning motivation, improved learning engagement and class participation, enhanced learning satisfaction, and improved knowledge acquisition. Some studies have even confirmed improvements in confidence regarding clinical skill performance and ethical decision-making (Nasirzade et al., 2024; Jo & Ma, 2026). Application methods currently utilized include game-based student response systems (GSRS) such as Kahoot!, quizzes, clinical simulations, serious games, and scenario-based learning (An, 2021; Shao et al., 2026).

As the educational efficacy of gamification has been proven, there has been a surge in attempts to actively introduce it into the fields of healthcare and nursing education, which deal directly with human life. Nursing education possesses a unique characteristic in that it requires not only the simple acquisition of theoretical knowledge but also practical competence to make accurate judgments in complex and urgent clinical situations, as well as high-level ethical decision-making. However, traditional lecture-based classes and limited practice environments alone have limitations in allowing students to fully experience the diversity and dynamism of the actual clinical field they will encounter.

The academic and practical validity for applying gamification to nursing education stems from the following points:

Mitigating the Theory-Practice Gap: Nursing students experience significant discrepancies and stress when applying the theories learned in the classroom to actual hospital practice settings. Gamification helps students experience the context of the clinical field in advance and seamlessly transfer theory to practice by safely simulating virtual scenarios and patient care situations.

Cultivating Critical Thinking and Clinical Judgment: Nurses must monitor changes in a patient's condition in real time and determine priorities. Game-based learning environments allow learners to intuitively learn critical thinking processes and the outcomes of decision-making by displaying the consequences of choices in real time and providing immediate feedback.

Safe Simulation of High-Risk, Low-Frequency Clinical Situations: Emergency situations or specialized nursing scenarios—which do not occur frequently in actual clinical practice but can lead to fatal consequences if handled improperly—are difficult to handle for educational purposes. Gamification-based simulations provide students with the opportunity to fail freely and master through repetition in a controlled environment that does not compromise patient safety.

As such, gamification in nursing education is establishing itself as an innovative teaching and learning methodology that goes beyond simply stimulating learning interest to enhance the

clinical performance and safety competencies of prospective nurses. Against this backdrop, various EdTech-based research is actively underway, primarily in advanced countries; this serves as the fundamental justification for this study to diagnose the current state of domestic nursing education and explore development strategies.

3. Method

3.1. Research Design

This study is a descriptive literature review conducted to identify trends in the application of gamification within the field of nursing education and to compare and analyze the characteristics of domestic and international research, targeting domestic and international academic papers published over the past 10.5 years from January 2016 to June 2026. The procedures for literature collection, screening, selection, and analysis in this study were systematically carried out in strict compliance with the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. PRISMA is an international standard reporting guideline established to enhance the reporting quality and transparency of systematic reviews and meta-analyses; it was developed by the PRISMA Group, an international research collaboration composed of global researchers and journal editors in the fields of medicine, public health, methodology, and epidemiology. PRISMA is recognized as the methodology best suited to literature analysis, as it reduces selection bias during the process of collecting, selecting, and analyzing literature, and provides a 27-item verification checklist and a 4-step literature selection flowchart to enable third parties to verify the research using the same procedures.

3.2. Literature Search Strategy and Sources

A literature search was conducted in June 2026, and the search period was limited to literature published from January 2016 to June 2026. Representative domestic and international academic databases were utilized as information sources for comprehensive literature collection. Domestic databases used included the Research Information Sharing Service (RISS), the Korea Citation Index (KCI), DBpia, and the Koreanstudies Information Service System (KISS), while international databases included PubMed, CINAHL, Scopus, and Google Scholar. When constructing the search query, domestic keywords were defined as combinations of the Korean terms for "gamification," "game-based learning," "serious game," "GSRS," and "digital game" with the Korean terms for "nursing education," "nursing student," "nurse," and "nursing." International keywords were derived by combining "Gamification," "Game-based learning," "Serious game*," "Gamified," and "GSRS" with "Nursing education," "Nursing student*," and "Nurse training" using Boolean operators (AND and OR).

3.3. Literature Selection and Exclusion Criteria

Clear selection and exclusion criteria were established to ensure relevance to the research topic and academic rigor. The selection criteria were defined as studies targeting nursing students for education; studies applying or analyzing gamification, game-based learning, serious games, GSRS, or virtual reality, augmented reality, or AI simulations containing game elements as interventions in nursing theory and practice education; formal papers published in academic journals after peer review; and literature written in Korean or English. Conversely, exclusion criteria were established to exclude from the screening process: independent studies in other healthcare fields outside of nursing; game studies for treatment or health management purposes targeting the general public or patients; studies involving only subjects other than nursing students, such as practicing nurses; simple virtual reality and simulation studies that do not include game design elements such as scores, rankings, feedback, or narratives; non-indexed (grey) literature such as theses and dissertations, conference abstracts, editorials, and book reviews; and papers for which full text is unavailable.

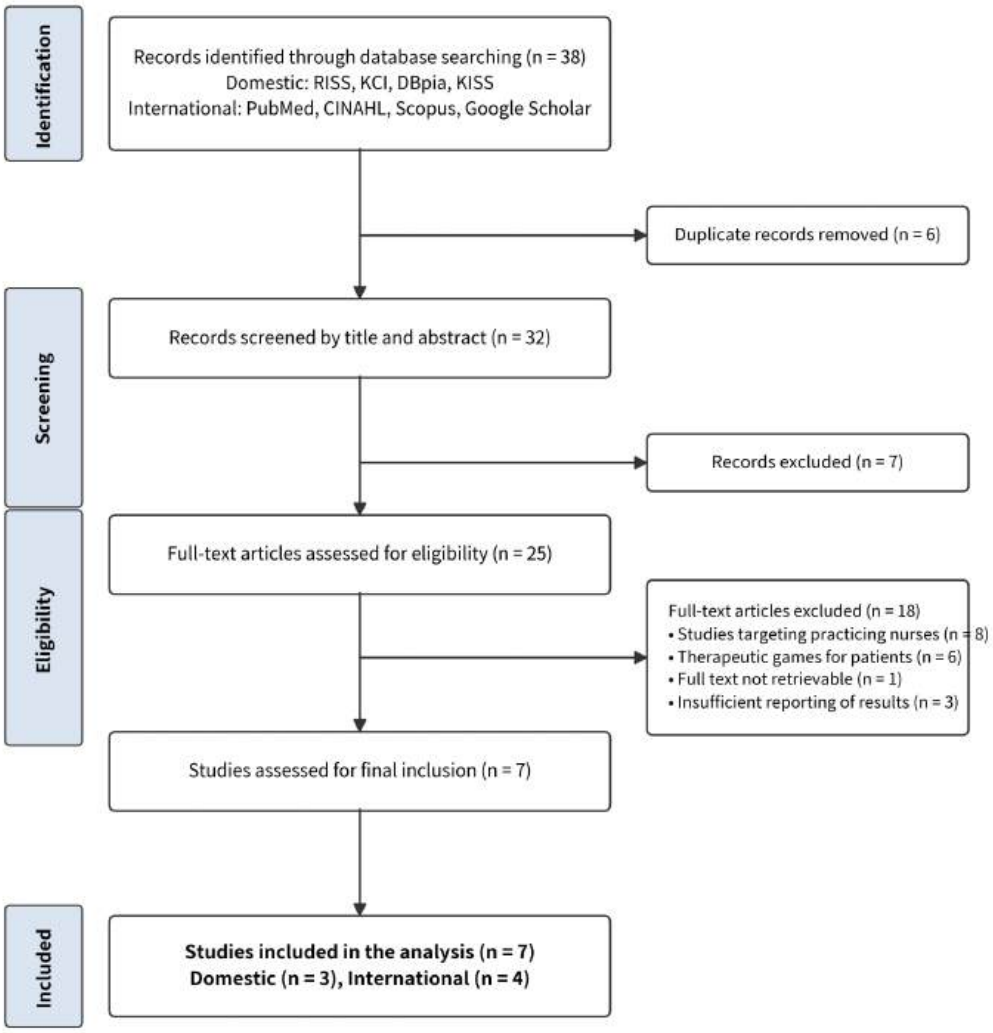
3.4. Literature Selection Procedure

The literature selection process proceeded sequentially following the four-step procedure of the PRISMA 2020 guidelines (Identification, Screening, Eligibility Assessment, and Final Inclusion). In the identification phase, a total of 38 records were initially collected through searches of domestic and international databases, and 6 duplicate records were removed using a bibliographic management program. In the screening phase, the titles and abstracts of the 32 records from which duplicates had been removed were reviewed, and 7 records that did not meet the research objectives and subjects were initially excluded. In the eligibility assessment phase, the full texts of the 25 articles that passed the initial screening were obtained and thoroughly read, and 18 articles were finally excluded according to pre-established selection and exclusion criteria. Through these procedures, a total of 7 studies were confirmed as the final subjects for analysis, including 3 domestic studies and 4 international studies.

3.5. Data Extraction and Analysis Methods

Data were extracted and analyzed from the finally selected studies using a standardized coding matrix partitioned according to the research objectives. The analysis items included general characteristics such as publication year, country of study, research design, study participants, and sample size, as well as educational characteristics such as the courses and areas of application. In addition, the application types and core game elements utilized were coded to distinguish them as characteristics of gamification, and the significance of the research results and key dependent variables measured in terms of educational outcomes were collected. Based on the extracted data, research trends by year, the level of advancement of applied technology, the sophistication of game elements, and differences in measured variables were analyzed in a comparative manner by classifying them into international trends and domestic status. This led to the diagnosis of academic and technological gaps in gamification research within Korean nursing education and the derivation of suggestions for future development.

Figure 1. PRISMA 2020 flow diagram outlining the database search, screening, eligibility, and inclusion for literature review



4.Result

4.1. Results of Literature Selection

In this study, a total of 38 records were initially identified from major domestic and international academic databases in accordance with the PRISMA 2020 guidelines (Figure 1). Titles and abstracts were reviewed for the 32 records remaining after excluding duplicates; 7 records that were irrelevant to nursing education or lacked game elements were initially excluded. Subsequently, full texts of 25 articles were secured and their eligibility was evaluated. Consequently, a total of 18 articles were excluded, including studies targeting practicing nurses (n = 8), studies on therapeutic games for patients (n = 6), articles whose full texts could not be

retrieved (n = 1), and articles with insufficient reporting of results (n = 3). Finally, 7 studies (3 domestic and 4 international) were selected as the subjects of analysis.

The results of the analysis of the general characteristics and gamification application status of the finally selected 7 studies are as follows.

Table 1. General Characteristics and Gamification Applications of the Included Studies

Author (Year)	Region	Study Design / Course Area	Participants	Gamification Type and Tools	Key Game Elements	Main Outcome Variables and Findings
Park, B. J., et al. (2025)	Domestic	Single-group pretest–posttest design / Adult health nursing (respiratory)	2nd-year nursing students (n=80)	ADDIE-based course-integrated gamification class (clinical-scenario OX quiz + picture completion, Tarsia jigsaw puzzle)	Points/scores, quiz, team activity, competition, reward	Significant improvements in learning flow (t=-10.79), class participation (t=-10.97), and learning satisfaction (t=-11.01) (all p<.001)
Park, S., et al. (2025)	Domestic	Single-group pretest–posttest design / Emergency nursing simulation (integrated anaphylaxis–depression scenario)	4th-year nursing students (n=113)	Gamified simulation (board-game-based prelearning + scenario simulation + debriefing)	Board game, scenario, team play, competition, reward, debriefing	Significant increase in nursing knowledge (t=7.647, p<.001) no significant changes in learning engagement or academic self-efficacy program satisfaction 4.45/5
Jo & Ma (2026)	Domestic	Single-group pretest–posttest design / Bioethics (review sessions, 90 min × 2)	Nursing students (n=34)	Quiz/problem-solving-based gamified review class	Quiz, team/individual ranking, points (scoreboard), reward, immediate feedback	Significant improvements in learning satisfaction (t=-2.153), ethical decision-making confidence (t=-2.571), and bioethics knowledge (t=-2.314) (p<.05) no significant change in learning engagement
Blanié et al. (2020)	International	Multicenter randomized controlled trial (RCT) / Clinical reasoning (detection of patient deterioration)	2nd-year nursing students (n=146)	Serious game (LabForGames Warning, virtual clinical cases) + debriefing vs. traditional teaching	Clinical scenario, scores, immediate feedback, debriefing	Clinical reasoning (SCT): no significant between-group difference (immediate p=.43, 1 month p=.77) satisfaction (p=.001), motivation (p=.003), and intention to recommend (p=.002) significantly higher in the game group
AL-Mughee d et al. (2022)	International	Randomized two-group experimental study / Fundamental nursing (standard precautions, infection control)	3rd–4th-year nursing students (n=126)	Online education (recorded lectures + quizzes) + game-based VR phone application (4 scenarios) vs. traditional education	VR clinical scenarios (hand hygiene, PPE, needlestick, transmission-based precautions), quiz, free replay at home, team learning	At posttest, knowledge (p=.001), attitude (p=.002), and all four standard-precaution compliance domains (p=.01–.04) significantly higher in the experimental group

Fijačko et al. (2024)	International	Randomized wait-list controlled trial / Emergency nursing (adult basic life support)	Nursing students (n=43)	MOBICPR smartphone serious game (self-directed play at home; performing BLS on a virtual OHCA patient via smartphone gestures and voice)	Virtual patient scenario, gesture interaction, scores, outcome-based feedback (virtual patient survives if score exceeds 50%)	Significant between-group difference in theoretical knowledge (P=.04) no significant difference in practical skills (manikin CPR quality metrics + checklist) (P=.45) knowledge and skills maintained after a 2-week break
Nasirzade et al. (2024)	International	Single-blind randomized controlled trial (RCT) / Adult nursing (burn patient assessment)	5th-semester nursing students (n=42)	BAM serious game (2 weeks of self-directed play) vs. feedback lecture (90 min × 2)	Mission-based scenario, scores (peer score comparison), feedback	Game group significantly superior in knowledge (17.4±2.2 vs 14.7±2.6) and OSCE skills (16.4±2.2 vs 11.8±3.8) (both P<.001) game group superior in 8 of 10 OSCE stations (P<.05)

4.2. Research Trends in Gamification in International Nursing Education

4.2.1. Research Scale and Stage of Development

Research related to gamification in the field of international nursing education has achieved rapid quantitative growth since the late 2010s (van Gaalen et al., 2021; Meşe & Meşe, 2022). Since 2020, academic depth has been enhanced with the publication of numerous systematic reviews (SLRs) and meta-analyses, moving beyond single intervention studies (Nylén-Eriksen et al., 2025; Shao et al., 2026). This research trend, which originated primarily in Europe (Shao et al., 2026), is attempting to evolve into comprehensive EdTech-based nursing training research utilizing Serious Games, Immersive Virtual Reality (VR), Augmented Reality (AR), and Artificial Intelligence (AI), in conjunction with recent technological advancements (Adi et al., 2026; Shao et al., 2026).

4.2.2. Courses and Clinical Areas of Application

In terms of the courses and clinical areas of application, international research forms a broad category spanning the entire nursing curriculum without being skewed toward specific fields. The areas utilized most frequently are Adult Nursing and Fundamental Nursing Skills, which are extensively applied to acquire basic clinical performance competencies and knowledge (Shao et al., 2026). Furthermore, in the fields of clinical simulation, emergency nursing, and cardiopulmonary resuscitation (CPR), gamification is actively utilized to enhance responsiveness in high-risk and low-frequency situations, and is also being introduced into courses such as drug administration and bioethics, where accuracy and precise ethical judgment are required. In addition, attempts to expand application to areas of special patient care, such as critical care, maternal care, and pediatric care, are ongoing; however, research in these areas is reported to be relatively lacking (Shao et al., 2026).

4.2.3. Forms of Gamification Utilization and Technological Systems

The forms of gamification application have been systematically diversified, ranging from simple classroom-based activities to forms integrated with advanced technology. Traditional gamification methods, which utilize game-based student response systems (GSRS) such as Kahoot! or Quizizz to award points, badges, and leaderboards, form the basis. In addition, game-based learning utilizing scenarios, escape rooms, and board games, as well as serious games featuring clear educational goals and structured rules, are being actively established (Shao et al., 2026). Recently, hybrid educational models that simulate realistic clinical environments by combining virtual reality and mobile-based immersive technology are being attempted; however, the majority of interventions reported to date take the form of non-VR digital games, and the full-scale integration of immersive VR and AR remains limited (Shao et al., 2026).

4.2.4. Key Game Design Elements

The game design elements addressed in these studies are meticulously implemented to maximize learners' cognitive and affective outcomes. Scenarios reflecting actual clinical situations, immediate feedback, and point systems are utilized as the most critical elements (Shao et al., 2026), while quizzes, inter-team competition, and badge awarding are also frequently observed across published studies. Furthermore, to induce continuous participation and engagement, there is a tendency to layer the learning experience by combining not only leaderboards and team play elements but also step-by-step unlocking methods and multi-dimensional story/narrative structures. However, the frequency of utilizing advanced elements, such as unlocking or cooperation-based features, is reported to be relatively low (Shao et al., 2026).

4.2.5. Key Measured Outcomes and Paradigm Shifts

The major educational outcomes measured in international literature are knowledge, skills, and participation (Shao et al., 2026). The scope of measurement is expanding beyond simple knowledge acquisition, learning engagement, and learning satisfaction to include high-order practical competencies such as clinical reasoning, critical thinking, self-efficacy, decision-making capacity, and clinical skills. In the international literature included in this review, performance-based indicators were directly measured, such as clinical reasoning using standardized tools (Blanié et al., 2020), OSCE-based skill performance (Nasirzade et al., 2024), manikin-based cardiopulmonary resuscitation (Fijačko et al., 2024), and infection control compliance (AL-Mugheed et al., 2022). However, the effects are not uniform, and selective results are reported for each outcome variable, such as the fact that no significant difference was found between the game group and the traditional education group in clinical reasoning (Blanié et al., 2020) or practical performance (Fijačko et al., 2024). Furthermore, research interests in the international nursing education academic community have shifted from verifying the simple presence or absence of gamification effects, through analyses of which game elements effectively contribute to outcomes (van Gaalen et al., 2021), to recently focusing on which

subjects to apply it to and how to integrate it with VR and AI technologies to achieve long-term effects (Adi et al., 2026; Shao et al., 2026). Consequently, this suggests that the international academic community has shifted beyond the proposition of simply "Does gamification work?" to a design and implementation optimization paradigm asking, "How should we implement gamification to create optimal educational outcomes?" (van Gaalen et al., 2021).

4.3. Current Status of Gamification Research in South Korean Nursing Education

4.3.1. Research Scale and Stage of Development

Gamification research in the field of nursing education in South Korea began in earnest around 2021, taking the form of directly introducing game elements into classes and verifying their effectiveness (Kim, 2021; An, 2021). Early studies were primarily focused on single empirical studies utilizing a Kahoot!-based Game-based Student Response System (GSRS) to promote learner engagement in the classroom (Kim, 2021). Recently, entering 2025 and 2026, there has been an increase in the number of studies and a diversification trend, including the overall design of adult nursing courses, integration with simulations, and the application of gamification in bioethics classes. However, compared to international studies, the absolute quantity of research is small, and comprehensive research, such as systematic reviews and meta-analyses, remains in an early stage of development with insufficient progress.

4.3.2. Courses and Clinical Areas of Application

In domestic research, the courses to which gamification is applied have diversified, starting with adult nursing theory classes (Kim, 2021) and extending to respiratory patient care, emergency nursing simulations, and bioethics. A scoping review by An (2021) also confirmed that online platform-based quiz games were the most frequently applied intervention in theory classes. The study by Park, B. J., et al. (2025) applied gamification elements within the adult nursing curriculum. In addition, the study by Park, S., et al. (2025) applied board games to the pre-learning stage of emergency nursing simulation, while the study by Jo and Ma (2026) expanded the scope of application to bioethics classes. Currently, domestic research is characterized by the supplementary and partial application of gamification to existing nursing education curricula or practical training classes.

4.3.3. Forms of Gamification Utilization and Technological Frameworks

In terms of utilization forms, domestic research is centered on Kahoot!-based GSRS and online quizzes (Kim, 2021), and team-based board games and simulation pre-learning (Park, S., et al., 2025). Classroom-based quiz response systems are the most representative form, and recently, the use of board games combined with simulation education is being attempted. However, the development of independent "Serious Games"—which is being actively established in international academia—or the introduction of advanced EdTech systems combined with immersive virtual reality (VR), augmented reality (AR), and artificial intelligence

(AI) technologies, have not yet been prominently observed in domestic nursing education research.

4.3.4. Core Game Design Elements

The core game design elements primarily utilized in domestic studies are identified as scores and quantified rewards, time limits, top rankings, real-time feedback, and inter-team competition. In studies using Kahoot!, the speed of answering correctly within the time limit, score calculation, and real-time ranking fluctuations acted as key mechanisms (Kim, 2021), while board game studies mainly included team-based activities and competitive elements (Park, S., et al., 2025). However, the introduction of sophisticated and complex game design elements, such as story-centered narrative structures, step-by-step unlocking, and multi-dimensional reward systems—which are treated as important in international literature—is relatively limited.

4.3.5. Key Measured Outcomes and Shifts in Research Direction

The major educational outcomes measured by domestic studies include learning engagement, learning satisfaction, nursing knowledge, class participation, self-efficacy, and confidence in ethical decision-making. Early GSRS studies and adult nursing research demonstrated positive effects, including statistically significant improvements in learning engagement, satisfaction, and class participation (Kim, 2021; Park, B. J., et al., 2025). On the other hand, recent emergency nursing simulation studies and bioethics studies have confirmed selective results, such as improvements in knowledge and confidence in ethical decision-making, but no significant differences in learning engagement or self-efficacy (Park, S., et al., 2025; Jo & Ma, 2026). This indicates that domestic nursing education research is also moving beyond the stage of unconditional effectiveness verification and entering a stage of verifying practical efficacy based on subject characteristics, and that the focus of research is shifting from simply "inducing interest and engagement" to "contributing to the strengthening of actual nursing knowledge and professional competencies."

4.4. Comparison of Domestic and International Research Trends

4.4.1. Comparison of Research Scale and Level of Research Design

Research on nursing education gamification in Korea and abroad shows a visible gap in terms of quantitative research scale and the maturity of research methodologies. In the international academic community, the number of related papers has increased explosively since the late 2010s (van Gaalen et al., 2021; Meşe & Meşe, 2022), and a large number of advanced studies possessing academic rigor—such as Systematic Reviews (SLR), meta-analyses, and Randomized Controlled Trials (RCTs)—have accumulated, moving beyond single intervention studies. In contrast, domestic research remains in an early stage of development, having been fully introduced since 2021, and exhibits limitations as it is mostly conducted in the form of single-group pretest–posttest designs. In fact, all domestic empirical studies identified in this

review were single-group pretest–posttest designs without a control group. Consequently, not only is the absolute quantity of domestic research insufficient compared to international studies, but comprehensive research demonstrating the generalizability of results and long-term educational effectiveness is also lacking.

4.4.2. Comparison of Courses and Scope of Application

In terms of the diversification of application areas, international research forms a broad category covering the entire nursing curriculum and specialized clinical practices, including adult nursing, emergency nursing, cardiopulmonary resuscitation (CPR), drug administration, bioethics, and maternal and pediatric nursing. In contrast, domestic research remains limited to applying gamification as an auxiliary tool in a few subjects or specific unit classes, such as adult nursing theory and respiratory domains, emergency nursing simulation pre-learning, and bioethics. This indicates that the use of gamification in domestic nursing education is proceeding in the form of one-off interventions centered on individual researchers or specific unit classes, rather than being systematically integrated into the entire curriculum.

4.4.3. Comparison of Forms of Gamification Utilization and Integrated Technologies

A clear technological gap also exists between domestic and international nursing education regarding the forms of media and the level of technology used to implement gamification. Internationally, the latest EdTech technologies are being actively integrated, extending beyond simple Game-based Student Response Systems (GSRS) like Kahoot! or Quizizz to scenario-based escape rooms, serious games, immersive virtual reality (VR), augmented reality (AR), and AI-based simulations. In contrast, domestic research remains focused on simple classroom-based activities or text-centric response systems, such as Kahoot!, quiz apps, and team-based board games, resulting in a relative lack of research on integrating advanced technologies.

4.4.4. Comparison of the Sophistication of Core Game Design Elements

Differences also exist in the composition and sophistication of game design elements used to structure learning experiences. Game elements utilized in domestic research primarily rely on intuitive and fragmentary motivational mechanisms, such as scores, time limits, top rankings, real-time feedback, and inter-team competition. In contrast, international research tends to move beyond simple competition and reward systems to sophisticatedly design and apply complex design elements capable of sustaining learners' intrinsic motivation and engagement over the long term. These elements include multi-dimensional scenarios, story-centered narrative structures, step-by-step mission unlocking, and multi-layered reward systems. For example, the international literature included in this review identified design elements combined with clinical narratives, such as outcome-based feedback (Fijačko et al., 2024) where the survival of a virtual patient is determined by the learner's performance score.

4.4.5. Comparison of Measurement Variables and Research Paradigms

The differences in the major dependent variables measured and the research questions most clearly highlight the gap in research paradigms between the two academic fields. Domestic research tends to focus its variables primarily on measuring affective outcomes and basic knowledge, such as learning engagement, learning satisfaction, class participation, and short-term knowledge acquisition. In contrast, international research is verifying high-order practical competencies required in actual nursing settings from various angles, such as clinical reasoning, critical thinking, integrated ethical and practical competencies, clinical judgment, and performance skills. Consequently, while domestic research remains at the initial stage of verifying efficacy—specifically, whether gamification is effective for learner engagement and satisfaction—international research has evolved into a design and implementation optimization paradigm regarding how to configure game design elements to cultivate optimal clinical competencies.

Table 2. Comparison of International and Domestic Research Trends in Gamification in Nursing Education

Category	International Research Trends	Domestic (South Korean) Research Status
Research volume and design	• Rapid increase in publications since the late 2010s • Accumulation of advanced synthesis research, including systematic reviews (SLRs), meta-analyses, and RCTs	• Early developmental stage; full-scale adoption since 2021 • Predominantly single-group pretest–posttest designs
Courses and scope of application	• Across the entire nursing curriculum and specialized clinical areas: adult, emergency, CPR, medication, bioethics, maternal–child nursing, etc.	• Supplementary application in selected courses or units: adult nursing theory and respiratory unit, emergency prelearning, bioethics
Delivery formats and technology	• GSRS, escape rooms, serious games, immersive VR/AR, and AI-converged edtech	• Classroom- and text-centered: Kahoot!/GSRS, online quizzes, team-based board games
Game design elements	• Sophisticated designs including narrative structures, stage-based unlocking, and multilayered reward systems	• Intuitive mechanisms: points, time limits, rankings, real-time feedback, team competition
Outcome variables and paradigm	• Clinical reasoning, critical thinking, integrated practice competencies • Design-optimization paradigm (“How should it be designed and implemented?”)	• Learning engagement, learning satisfaction, class participation, basic knowledge • Early-stage efficacy verification (“Does it work?”)

5. Discussion

5.1. Causes of Insufficient Research on Gamification in Korean Nursing Education

Based on the analysis of previously identified domestic and international research trends, research on gamification in the field of Korean nursing education lags significantly behind that of other countries in both quantity and quality. The fundamental cause of this phenomenon stems from the institutional characteristics of domestic nursing education and the limitations of the educational environment. The curriculum of nursing colleges in South Korea is heavily constrained by the strict accreditation standards of the Korean Accreditation Board of Nursing Education (KABONE), the requirement to complete over 1,000 hours of clinical practice, and clear, outcome-oriented goals such as passing the national nursing licensing examination. Consequently, there is an absolute lack of time and psychological leeway for both instructors and students, who must manage an excessive workload of theoretical classes and practical training schedules, to attempt and establish new EdTech teaching methods. Furthermore, the domestic nursing academic community is accustomed to rote knowledge delivery and evaluation methods focused on finding correct answers; thus, it has maintained a conservative attitude toward accepting gamification teaching methods—which acknowledge the autonomy and trial-and-error inherent in games—into the official curriculum. In addition, the lack of a foundation for convergence research with other academic fields such as engineering, game design, and educational technology, as well as the insufficient financial support system from supervising ministries, are acting as key factors hindering the revitalization of domestic research.

5.2. Background and Limitations of the Over-reliance on Kahoot! and GSRS

The phenomenon of domestic nursing education gamification research being excessively skewed toward Game-based Student Response Systems (GSRS), such as Kahoot! or online quiz apps (An, 2021), can be attributed to the ease and cost-effectiveness of technology adoption. Kahoot! has been utilized as the most suitable tool for large lecture hall environments because instructors can create quizzes within minutes without separate coding or specialized knowledge, and students can participate immediately via their personal smartphones. However, the overuse of such quiz-based GSRS has clear limitations that hinder the academic advancement of nursing education. While simple quizzes and time-limited competition methods are effective for short-term memory recall or inducing temporary interest in class, they have fundamental limitations in cultivating the critical thinking, higher-order clinical judgment, and ethical decision-making competencies required in complex and unpredictable real-world clinical settings. Consequently, gamification centered on simple quizzes faces the challenge of class engagement rapidly declining once the learner's novelty effect wears off.

5.3. Causes of the Absence of Longitudinal and Long-Term Effect Studies

Most domestic studies published to date have been limited to one-off special lectures or short-term interventions lasting a single semester (a few weeks), and studies verifying longitudinal effects through follow-up observation are extremely rare. The primary reason for this lack of research is that the objectives of these studies are skewed toward one-off experiments aimed at individual researchers' short-term journal publications or the completion of their theses,

rather than the systematic overhaul of the entire curriculum. To conduct systematic long-term research, it is necessary to continuously track whether the knowledge and skills acquired through gamification translate into practical adaptability for actual hospital clinical practice or as a new nurse after graduation. However, it is realistically very difficult to collect controlled longitudinal data due to factors such as students moving between grades, clinical placement assignments, and dropouts after graduation. Furthermore, the lack of a long-term research funding system capable of sustaining longitudinal studies and the absence of a university-level data integration management infrastructure are analyzed as major causes of the lack of long-term research.

5.4. Strategies and Recommendations for the Development of Domestic Nursing Education in Response to International Trends

Considering that leading international research communities have already moved beyond simple efficacy verification and evolved into a design optimization paradigm centered on VR, AI, and "Serious Games," domestic nursing education must derive the following strategic improvements.

First, the advancement of media utilization and the introduction of convergence technologies are urgent. It is necessary to move beyond GSRS, which is primarily based on simple text quizzes, and fully introduce high-level EdTech technologies into the nursing education field. These technologies include Serious Games that reflect clinical scenarios in a rich, multidimensional manner, VR-based simulations, and AI-linked patient care games. However, it is important to note that the sophistication of media does not automatically guarantee educational effectiveness. As international randomized controlled trials have reported cases where serious games failed to demonstrate a significant advantage over traditional education in clinical reasoning or practical performance (Blanié et al., 2020; Fijačko et al., 2024), the introduction of technology must be designed to be consistent with educational objectives and measurement variables.

Second, the sophistication of outcome measurement variables is required. Beyond affective domains such as learning satisfaction or short-term engagement, which have been the focus of previous studies, standardized measurement tools should be applied to comprehensively evaluate higher-order nursing performance abilities, including clinical reasoning, critical thinking, patient safety competence, and ethical judgment.

Third, it is necessary to move beyond one-off interventions and establish a curriculum-integrated longitudinal research model. Long-term randomized controlled trials (RCTs) and mixed-methods studies should be promoted to progressively integrate gamification elements into the entire nursing curriculum across grade levels—rather than merely serving as supplementary tools for individual subject units—and to track the practical adaptability of new nurses.

Fourth, as infrastructure to support this, a multidisciplinary research collaboration system among nursing, educational technology, and computer science, gamification design workshops for instructors, and institutional financial support at the university level must be established first.

6. Conclusion

This study was conducted to identify gaps in domestic and international research trends and to explore future directions for the development of domestic nursing education by systematically collecting and analyzing literature related to gamification in the field of nursing education published domestically and internationally from January 2016 to June 2026 in accordance with the PRISMA 2020 guidelines.

The results of the research analysis revealed that the international nursing education academic community has attempted to combine new technologies, such as immersive virtual reality (VR) and artificial intelligence (AI), centering on "Serious Games" based on advanced research methodologies including systematic literature reviews and meta-analyses. Through this, it was confirmed that the field has evolved into a design and implementation optimization paradigm that verifies high-order nursing competencies, such as clinical reasoning, critical thinking, and ethical decision-making. In contrast, domestic research has only begun in earnest since 2021, centering on single intervention studies, and remained at an early developmental stage heavily skewed toward the use of Kahoot! and other game-based student response systems (GSRS) or one-off board games. Furthermore, key measurement variables were concentrated on affective domains, such as learning engagement and satisfaction, as well as on the measurement of short-term knowledge. The discussion identified that these quantitative and qualitative shortcomings in Korea stemmed from a rigid curriculum centered on nursing education accreditation and national licensing exams, a preference for the convenience of GSRS tools, and the absence of infrastructure supporting long-term longitudinal studies.

This study is significant in that it systematically compared and analyzed the latest trends in nursing education gamification research both domestically and internationally to identify future tasks; however, it has the following limitations. Despite undergoing a rigorous selection process based on domestic and international databases, the absolute volume of relevant domestic empirical studies was small, limiting the ability to make equivalent quantitative comparisons. Additionally, due to the high heterogeneity of intervention types and measurement tools across the literature, quantitative effect sizes could not be calculated through meta-analysis. Moreover, as the literature was collected primarily from indexed journals, it is difficult to completely rule out the possibility of publication bias.

Based on these limitations, this study proposes suggestions for future research. First, research on developing VR- and AI-integrated Serious Games that closely reflect actual clinical contexts should be actively pursued through multidisciplinary collaboration among nursing, educational technology, and computer science, moving beyond simple quiz-based classroom activities. Second, rather than limiting gamification to one-off class applications, it should be gradually integrated into the entire nursing college curriculum. Furthermore, various long-term randomized controlled trials (RCTs) and mixed-methods studies should be conducted to track clinical performance and the practical adaptability of new nurses after graduation.

Works Cited

Adi, P. N., Köhler, T., Triyono, M. B., Priyanto, Handayani, S. A., & Maruanaya, R. F. (2026). AI-enhanced gamification in education: An integrative review of trends, impacts, and corrective role potential. Journal of Computers in Education. Advance online publication. https://doi.org/10.1007/s40692-026-00387-0

Al-Mugheed, K., Bayraktar, N., Al-Bsheish, M., AlSyouf, A., Aldhmadi, B. K., Jarrar, M., & Alkhazali, M. (2022). Effectiveness of game-based virtual reality phone application and online education on knowledge, attitude and compliance of standard precautions among nursing students. PLoS ONE, 17(11), e0275130. https://doi.org/10.1371/journal.pone.0275130

An, Ji-Won. (2021). Factor Influencing Learning Outcome in Nursing Education Using Gamification: A Scoping Review. Journal of Fundamental Nursing Science, 28(4), 440-457. https://doi.org/10.7739/jkafn.2021.28.4.440

Blanié, A., Amorim, M.-A., & Benhamou, D. (2020). Comparative value of a simulation by gaming and a traditional teaching method to improve clinical reasoning skills necessary to detect patient deterioration: A randomized study in nursing students. BMC Medical Education, 20(1), 53. https://doi.org/10.1186/s12909-020-1939-6

Deterding, S., Dixon, D., Khaled, R., & Nacke, L. (2011). From game design elements to gamefulness: Defining "gamification". In Proceedings of the 15th International Academic MindTrek Conference (pp. 9-15). ACM. https://doi.org/10.1145/2181037.2181040

Fijačko, N., Masterson Creber, R., Metličar, Š., Strnad, M., Greif, R., Štiglic, G., & Skok, P. (2024). Effects of a serious smartphone game on nursing students' theoretical knowledge and practical skills in adult basic life support: Randomized wait list - controlled trial. JMIR Serious Games, 12, e56037. https://doi.org/10.2196/56037

Joeunjin and Ma Ryewon. (2026). Effects of a Gamification-Based Learning Intervention on Nursing Students’ Learning Engagement, Learning Satisfaction, Ethical Decision - Making Confidence, and Knowledge Improvement. Advanced Industrial SCIence, 5(1), 86-94. https://doi.org/10.23153/AI-Science.2026.5.1.086

Kim, Y. (2021). The Effect of Multi-faceted Learning by Application Game-based Student Response System in Nursing Education : Focusing on Kahoot!. Journal of the Korea Entertainment Industry Association, 15(4), 255-265. 10.21184/jkeia.2021.6.15.4.255

Meşe, S., & Meşe, C. (2022). Research trends on digital games and gamification in nursing education. Journal of Computer and Education Research, 10(20), 734-750. https://doi.org/10.18009/jcer.1175412

Nasirzade, A., Deldar, K., Froutan, R., & Shakeri, M. T. (2024). Comparison of the effects of burn assessment mission game with feedback lecture on nursing students' knowledge and skills in the burn patients' assessment: A randomized clinical trial. BMC Medical

Informatics and Decision Making, 24(1), 157. https://doi.org/10.1186/s12911-024-02558-4

Nylén-Eriksen, M., Stojiljkovic, M., Lillekroken, D., Lindeflaten, K., Hessevaagbakke, E., Fløtlo, T. N., Hovland, O. J., Solberg, A. M. S., Hansen, S., Bjørnnes, A. K., & Tørris, C. (2025). Game-thinking; utilizing serious games and gamification in nursing education: A systematic review and meta-analysis. BMC Medical Education, 24(1), 1397. https://doi.org/10.1186/s12909-024-06531-7

Park Byung Jun, Kim Jeong Yun and Shinwoo Hwang. (2025). Effects of Gamification - Based Class in Nursing Education. Journal of the Edutainment, 7(4), 17-30. https://doi.org/10.36237/koedus.7.4.17

Park, Suho, Choi, Hoysin and Park, Sujin. (2025). Effects of a Gamified Simulation Education Program on Learning Engagement, Academic Self-Efficacy, and Knowledge. Korean Academy of Basic Medicine & Health Science, 18(2), 17-26. https://doi.org/10.37152/kmhs.2025.18.2.17

Shao, F., Han, Z., Su, Q., & Fang, Y. (2026). Serious games in nursing education: Scoping review of applications, effectiveness, and future directions. JMIR Serious Games, 14, e86092. https://doi.org/10.2196/86092

van Gaalen, A. E. J., Brouwer, J., Schönrock-Adema, J., Bouwkamp-Timmer, T., Jaarsma, A. D. C., & Georgiadis, J. R. (2021). Gamification of health professions education: A systematic review. Advances in Health Sciences Education, 26(2), 683-711. https://doi.org/10.1007/s10459-020-10000-3

Implementation of Gene-Editing Technologies to Cure Genetic Parkinson's Disease by Correcting *SNCA* Mutations By Aarrohi Sivakumar Iyer

Abstract

Parkinson's disease (PD) is a condition in which dopaminergic nerve cells in the brain progressively lose their functions and degenerate. It is caused by both environmental and genetic factors. It has severe motor and non-motor symptoms that may be mitigated, but the disease itself is incurable through present therapies. Genetic PD, caused by mutations in the SNCA gene, has the potential to be treated by gene-editing technologies. The use of CRISPR-Cas9 is explored widely for making cuts in the DNA. Curative gene-editing technologies extend beyond conventional CRISPR-Cas9 approaches and warrant further exploration, particularly given the limitations associated with CRISPR systems. Therefore, prime editing can be delved into. It is vital that these technologies be delivered to the brain efficiently to target the neurons there. Although viral vectors are the primary vehicles, non-viral vectors like lipid nanoparticles may become the focus of the future. Lipid nanoparticles have excellent biocompatibility and they can be administered to the brain through several routes. This paper also discusses successful ongoing/recent experiments that have been conducted to deliver Cas9 or prime editor to the brain, showing that the problem of incurability of genetic PD can be resolved with further research and studies, by addressing practical and ethical concerns.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease in the world, after Alzheimer's disease. It is a systemic, chronic, illness with highly heterogeneous symptoms. It affects over 6 million people worldwide and is expected to double in prevalence by 2040 (Day and Mullin). PD occurs due to progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta region of the mesencephalon of the brain (Siddiqui et al.). Dopamine is a neurotransmitter with various essential functions including motor control. Therefore, the loss of these neurons make it difficult to control movement and muscle tension in patients with PD. This death or impairment of dopamine-producing neurons arises due to formation of Lewy bodies, which are accumulations of misfolded α-synuclein protein (encoded by the *SNCA* gene) that aggregate to form intracellular inclusions (Day and Mullin). People suffering from PD also lose the ability to produce norepinephrine, the main chemical messenger of the sympathetic nervous system, which is essential for bodily functions like heart rate and blood pressure (NIH National Institute on Aging).

PD usually begins with tremors and stiffness in the limbs, which gradually spreads throughout the body. Bradykinesia, which is slowness in initiating and executing body movements, is a hallmark motor symptom of PD. It makes it difficult to perform daily activities like walking and sitting. It impairs facial gestures, expressions and blinking (Harvard Health Publishing). Apart from motor abnormalities, some non-motor features include constipation, anxiety and dementia. The loss of epinephrine also explains some key attributes such as fatigue,

irregular blood pressure, decreased movement of food through the digestive tract, and a sudden drop in blood pressure when a person stands up (NIH National Institute on Aging).

PD has several risk factors, including age, sex, genetics and the environment. Studies suggest that men are more likely to develop PD than women. In women, high estrogen levels provide some protection by reducing inflammation, decreasing dopaminergic neuron loss, and also by increasing the availability of dopamine transporter proteins. Most cases of PD are developed only after the age of 60. The reason for this is incapacitation of intracellular systems, including mitochondrial dysfunction (mtDNA mutations), proteostasis disruption, and disrupted energy metabolism (X.-Y. Guo et al.).

Approximately 15-25% of PD cases have an obvious genetic cause, such as monogenic mutations in genes like *SNCA*, *LRRK2*, and *PARK2*, or by polygenic mutations in genes like *GBA*, *MAPT*, *TMEM175*, which were identified in GWAS studies when common variants interact with the environmental triggers (X.-Y. Guo et al.). The first monogenic cause of PD was discovered in a large Italian family with an autosomal dominant pattern of PD showing typical clinical features (Day and Mullin).

Epidemiological evidence states that environmental factors are a major risk of PD too, particularly idiopathic PD wherein the exact cause of PD is unclear. Chronic exposure to chemicals like paraquat (PQ) and Rotenone are believed to increase the likelihood of PD by approximately 64%. Beyond this, iron catalyses oxidative damage to neurons by the Fenton reaction, whereas manganese has neurotoxic effects when it accumulates in dopaminergic neurons. Air pollution, fine particulate matter and microplastics may result in neuroinflammation. Diet and sedentary behavior may also play a major role in influencing PD. Traumatic brain injury and gut dysbiosis represent additional risk factors through aggregation of misfolded proteins (X.-Y. Guo et al.).

Presently, there are certain medicines and surgeries to help control parts of the brain and alleviate symptoms. The treatment choice depends on the patient's age, neuropsychiatric features and the severity of the symptoms. These treatments aim to increase dopamine level in the brain, affect other neurotransmitters, provide symptomatic relief, or delay long-term complications of pharmacological therapy (Genç). Medical treatment options include dopamine agonists, anticholinergics, Monoamine oxidase-B inhibitors, amantadine, and COMT inhibitors. Device-assisted therapies include apomorphine pen/pump, deep brain stimulation, which is used during advanced stages of PD (Genç). Regrettably, these therapies are not curative since they do not address the core problem of neurodegeneration.

Levodopa is the gold standard therapy for PD. It is a small amino acid-like molecule, which is given since dopamine cannot directly penetrate the blood-brain barrier. It is administered orally and then absorbed in the proximal small intestine. Large neutral amino acid transporters further transport it to the brain. It crosses the blood–brain barrier and is converted to dopamine by the enzyme DOPA decarboxylase, thereby replenishing the neurotransmitter (Aktaş et al.). Often patients take peripheral decarboxylase inhibitors like carbidopa or benserazide along with levodopa. Doing so reduces side effects of levodopa therapy such as nausea,

vomiting, low blood pressure, restlessness, and reduces the amount of levodopa required (NIH National Institute on Aging). These inhibitors also prevent levodopa from being converted to dopamine in the gut, such that they never reach the brain. Unfortunately, levodopa-based therapies are not a definitive cure as long-term administration leads to complications like motor fluctuations, dyskinesia, and oxidative stress. More importantly, levodopa addresses dopamine replacement, but does not halt the degeneration process (Aktaş et al.).

In this paper, we focus on genetic PD and methods to potentially cure it by using gene-editing technologies like CRISPR-Cas9 or prime editing. We also explore approaches to successfully deliver these technologies to the brain. Focusing on genetic PD, and not only idiopathic PD, is important as a person's genome can be deciphered while assessment of the environmental factors is not possible at present. Familial PD can also be detected earlier, therefore it is beneficial to find an early definitive cure rather than only focusing on treatments that may increase the chance of developing side effects and complications later.

Methods

This paper, which discusses gene-technologies that could potentially be used to completely cure Parkinson's Disease, encompasses analysed information from several credible sources. Peer-reviewed papers from journals and databases such as PubMed, Nature and Science Direct were referenced. Relevant papers were retrieved by focusing on keywords and abstracts. Priority was given to papers written in the last 5 years so as to focus on the latest developments in the field. Other websites were also utilized, especially if they were official websites of universities and the government to maintain legitimacy.

Genetics of PD

To study how genetic variation contributes to PD in the general population, genome-wide association studies (GWAS) are commonly conducted. GWAS is a scientific methodology that tests thousands of genetic variants across many genomes to find those statistically associated with a specific trait or disease (Hutter). Fundamentally, it elucidates potential associations between the genome, and specific phenotypes or diseases. The first GWAS was published in 2005, and the results are curated in the NHGRI-EBI GWAS catalog (Hutter). This method begins by collecting DNA and phenotypic information from several people and genotyping their genes using microarray technology or next-generation sequencing methods. Rare variants and variants not meeting Hardy-Weinberg equilibrium are removed. Then, the variants are phased and imputation is carried out. Finally, linear or logistic regression models are used to test the correlation between genotype and phenotype. Covariates are also considered to reduce the effect of confounding variables (CD Genomics).

GWAS has played a crucial role in identifying variants in genes like *SNCA*, *LRRK2*, and *GBA*, which cause PD. It has also identified new genes relevant to the risk of developing PD. Post-GWAS analyses are then conducted to assign biological function to GWAS variants. In PD, post-GWAS analyses have been identified for *SNCA*, *RAB29*, *MAPT*, *BST1*, *GAK*, *LRRK2* and

HLA-DRB5 amongst others (Day and Mullin).

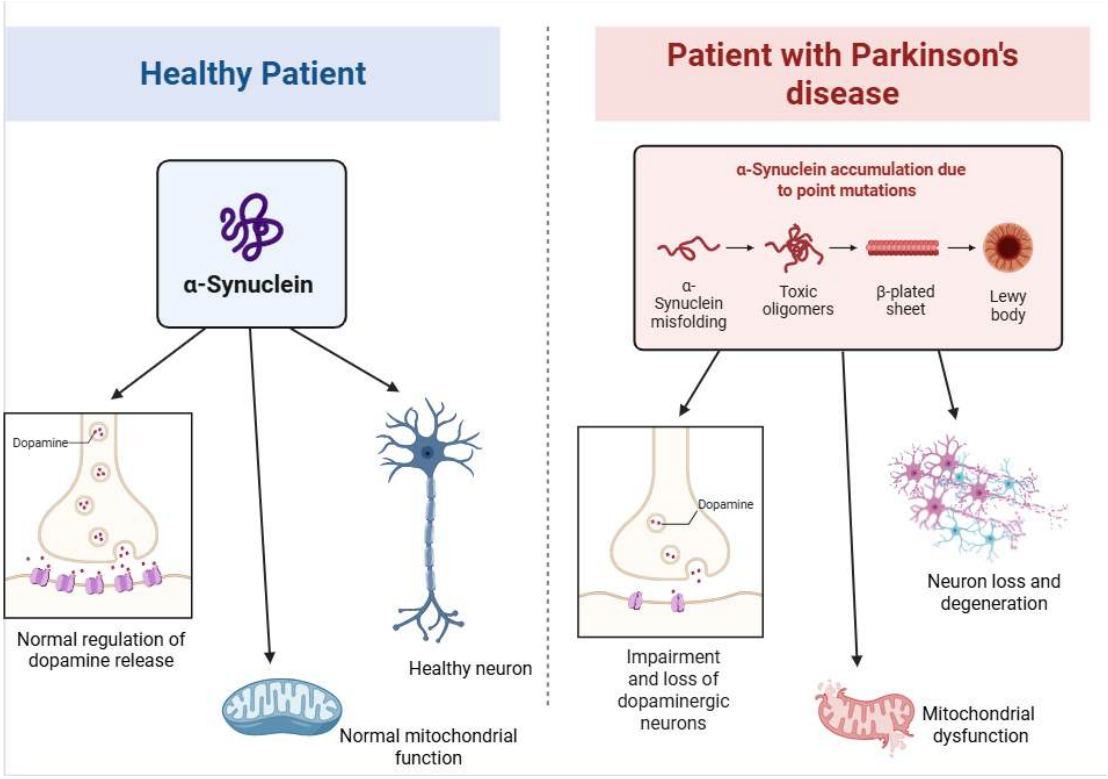


Fig 1: Structure and Function of a SNCA gene in Healthy Patient and Patient with Parkinson's disease. The left panel showcases how normal SNCA performs the functions of dopamine release, mitochondrial function and maintains healthy neurons. The right panel shows what happens when SNCA is misfolded and forms Lewy body aggregates. There is loss of dopaminergic neurons, mitochondrial dysfunctional and neuron degeneration. This image was created in BioRender: Iyer, A. (2026) https://www.biorender.com/.

The gene most strongly associated with genetic PD that has been identified encodes alpha-synuclein (SNCA). The *SNCA* gene is predominantly expressed in the brain and pre-synaptic terminals of neurons, and performs the function of maintaining an adequate supply of synaptic vesicles in presynaptic terminals, regulating the release of dopamine, and playing a role in the movement of microtubules. Mutations in *SNCA* gene exhibit high penetrance for PD; carriers of these alleles are very likely to develop PD in their lifetimes. There are more than 30 mutations in the *SNCA* gene like p.A53T, p.A30P, p.E46K and p.G51D, which are known to cause PD. One type of alteration changes the amino acids comprising SNCA and gives the proteins an incorrect structure, becoming misfolded (Fig 1). For example, alanine can be replaced with the threonine at protein position 53, or proline at position 30. In another type of alteration, one of the two *SNCA* genes in each cell is duplicated or triplicated (MedlinePlus). This is evidence for the 'dosage effect', wherein excess of alpha-synuclein protein and its greater expression, leads to severe clinical features of PD. Mutant versions of alpha-synuclein

can also arise when they form abnormal, neurotoxic aggregates, which spread within the central nervous system (Day and Mullin).

After identifying the genetic causes and mutations associated with PD, the next critical goal is to develop strategies to correct them. One approach to do this is using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9. CRISPR-Cas9 is a gene-editing technology for treating diseases by correcting base mutations in DNA or disrupting disease-causing genes. It primarily evolved as a defence mechanism against phages, plasmids and bacteria, equipping them with a sequence-specific heritable ‘adaptive immune system, ’with its own genetic memory. It was first discovered in 1987 by Ishino and colleagues, when they noticed a pattern of repeated DNA sequences near the *IAP* gene in *E. coli* (Richter et al.).

Scientists have engineered the CRISPR-Cas9 present in bacteria for gene editing. First, a synthetic guide RNA (sgRNA) is designed, which contains a complementary sequence to the target DNA. sgRNA directs the Cas9 enzyme to the desired location. The Cas9 protein forms a complex with sgRNA and binds to the target DNA. This requires the presence of PAM (Protospacer Adjacent Motif). Finally, the Cas9 creates a double-stranded break in the DNA (Richter et al.). Unfortunately, classic CRISPR-Cas9 is not an error-free technology and poses limitations which must be overcome.

Limitations of Canonical CRISPR-Cas9

Although canonical CRISPR-Cas9 is considered one of the most invaluable tools for gene editing, it presents a number of drawbacks which make it unsuitable to utilize efficiently to cure a disease like PD. Its challenges, ranging from inefficient delivery to its mutagenic nature, can make its therapeutic application risky.

To begin with, CRISPR-Cas9 components are usually carried by engineered extracellular vehicles (EVs). They can easily transport proteins, lipids, and RNA between cells, but struggle with the large size of Cas9 mRNA, which is translated into the Cas9 enzyme. This mRNA can degrade quickly within the EVs due to its instability, and this can reduce efficacy. Even the current methods for loading CRISPR-Cas9 into the EVs are not very effective. Moreover, a large quantity of the injected EVs accumulates in the liver, reducing the amount reaching the brain and also resulting in off-target effects (Ramesh and Fakoya).

Another significant problem is the immune response elicited when viral vectors, the delivery vehicles for CRISPR components, are injected into the body. Their integration into the host genome can disrupt essential genes and lead to undesirable side-effects (Aljabali et al.). Charlesworth et al. showed that more than half of humans possessed preexisting anti-Cas9 antibodies against the most commonly used bacterial orthologs: SaCas9 and SpCas9 (Uddin et al.). However, researchers have found ways to counter this including protein engineering, in which specific epitopes are altered, introducing amino acid changes to make its sequence more similar to human proteins, and using smaller Cas9 variants, which are less likely to trigger immune responses (Ramesh and Fakoya).

This technology also requires the presence of a protospacer adjacent motif (PAM) near

the target site. It is a short sequence of 2-6 base pairs, 3-4 nucleotides away from where DNA will be cut. PAM serves as a recognition signal for Cas enzymes to bind, before engaging with the intended DNA site. The original CRISPR-Cas9 typically recognizes the PAM sequence 5′-NGG-3′, where 'N' can be any nucleotide base (Biology Insights). Therefore, the PAM contributes to the targeting specificity of CRISPR–Cas9, but also limits the range of editable genomic sites. To broaden the targeting range, several PAM-flexible SpCas9 variants like Cas9-NG, xCas9 and SpG have been engineered (Zhang et al.).

Classic CRISPR-Cas9 can act on untargeted genomic sites, leading to off-target effects. These off-target effects are observed at a frequency greater than 50%. Off-target effects are the unwanted, unexpected or adverse alterations to the genome. They occur because Cas9 is tolerant of small mismatches (up to 3 base differences) between the sgRNA and genomic DNA (C. Guo et al.). This leads to the development of potentially oncogenic mutations. To predict potential off-target sites and the likelihood of an off-target edit, researchers integrate sophisticated computational algorithms and *in silico* bioinformatics tools. To tackle this limitation, Cas9 variants are engineered and guide RNA design is optimized (Aljabali et al.). Additionally, researchers at the Massachusetts Institute of Technology have developed an adapted version of CRISPR, known as REPAIR, that targets RNA rather than DNA. This can reverse disease symptoms at a protein level and enable gene editing without altering the genome. This is a safer alternative, which is especially beneficial for neurodegenerative disorders like PD (Ramesh and Fakoya).

After CRISPR is employed, DNA repair mechanisms can occur through 2 pathways- HDR (Homology-Directed Repair) and NHEJ (Non-Homologous End Joining). NHEJ directly seals the double-stranded breaks (DSBs) in the DNA without any homologous template, causing insertions or deletions at the cleavage site. This induces mutations, aggravating the symptoms and damage caused by PD. On the other hand, HDR uses a DNA template to generate any desired DNA change and obtain a precise genetic product. Thus, it provides for safer clinical use and is the preferred method for correcting mutations in genes like SNCA, which cause PD. However, it has some drawbacks as well. The frequency of HDR is extremely low as cells preferentially employ NHEJ over HDR through a series of mechanisms. HDR is a slower process than NHEJ and only occurs during the S/G2 phase (Yang et al.). Due to the fact that neurons never undergo division, and remain in G0 phase, HDR cannot be implemented for treating neuronal damage in PD. Instead, a relatively newer technology called 'prime editing' can be utilized.

Prime Editing

Prime editing was described initially by the laboratory of Dr. David Liu in 2019. It is a 'search and replace' genome-editing technology that utilizes CRISPR-Cas9 and achieves base substitution, insertion, or deletion without the requirement for double-stranded breaks (DSBs) or donor DNA templates. It uses an RNA template to synthesise a new single-stranded DNA sequence and insert it into the DNA (Zhao et al.).

A prime editor (PE) consists of a mutated version of Cas9 called Cas9 nickase (nCas9) and is responsible for nicking one strand of DNA (Fig 2). The reverse transcriptase RT is a virus-derived enzyme capable of reading an RNA sequence and transcribing it into a single-stranded DNA sequence. The PE also has a prime-editing guide RNA (pegRNA), with 4 parts: Spacer, Scaffold, Primer-binding site (PBS) and RT template sequence. The Spacer targets the PE complex to a specific DNA sequence, PBS anneals the target DNA and acts as a primer for the RT, and RT template sequence includes the edit to be introduced at the target site. In prime editing, only one DNA strand receives the edit. Thus there is a mismatch of the base pairs in the two DNA strands that is repaired by the cell's mismatch repair (MMR) machinery (Llado).

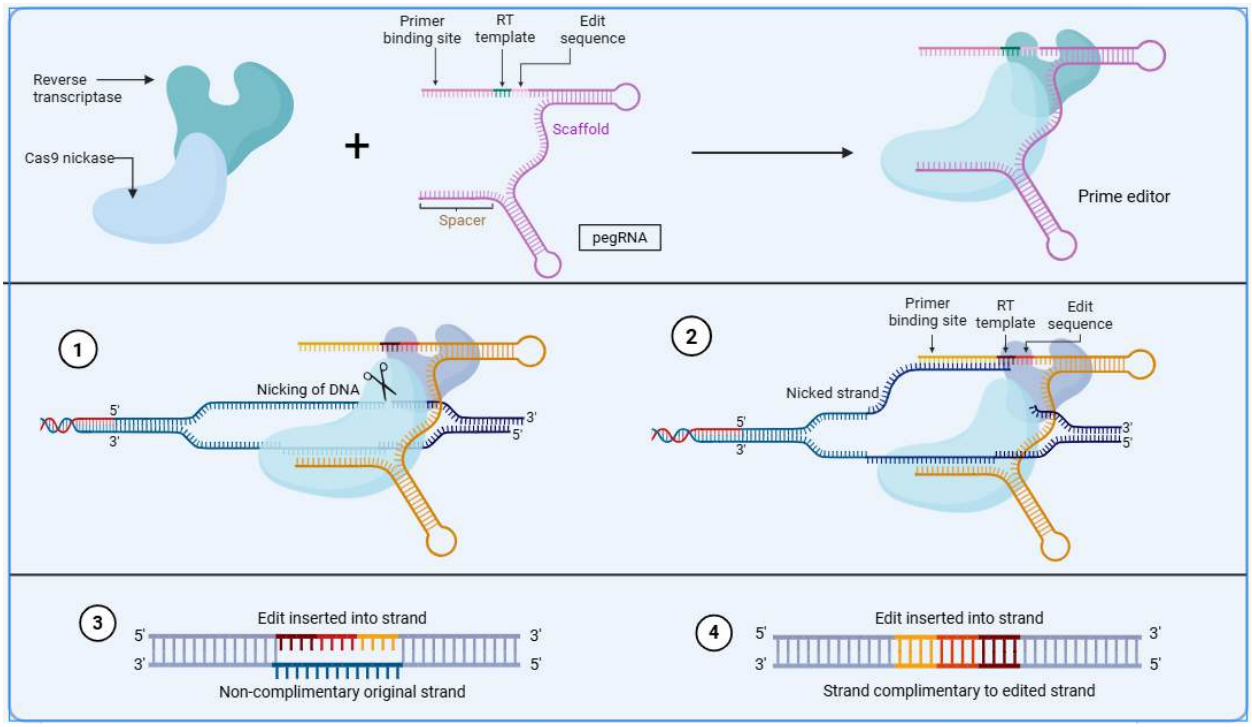


Fig 2: Prime editing process. The Cas9 nickase, reverse transcriptase and pegRNA form the prime editor (top). The process includes the following steps - unwinding and nicking of the DNA strand (1), PBS annealing (2), RT introducing the edit (3). The final outcome is the edited DNA strand (4), which is obtained by MMR machinery *(Lla*do). This image was created in BioRender: Iyer, A. (2026) https://www.biorender.com/.

Prime editing offers remarkable advantages over CRISPR-Cas9 gene editing, especially for PD. Most importantly, it prevents off-target effects and unwanted modifications at the target site. This is because it creates single-stranded breaks (SSB), rather than double-stranded breaks (DSB) which are significantly less mutagenic. DSBs can lead to mutations, loss of heterozygosity and chromosome rearrangements that result in cell death or cancer. On the other hand, cells can repair SSBs using the undamaged strand as a template (Cannan and Pederson). Also, unlike Cas nucleases and base editors, prime editing ensures precision as it requires three

distinct checkpoints of complementary base pairing for successful editing. These checkpoints involve the binding between the pegRNA spacer and target DNA, the primer binding site with the nicked DNA strand, and the newly synthesized DNA flap with the genomic sequence (Chen and Liu).

Since prime editing synthesizes an edited strand on its own, it removes the requirement of the inefficient HDR pathway. Also, it can change bases far (greater than 33 bp) from the site of the PE-mediated nick, making it less restricted than Cas9, which requires PAM near the site of the desired edit. Apart from this, Cas9 variants can also be utilized to recognize different PAMs, providing a significant advantage over canonical CRISPR Cas9. Lastly, because the pegRNA carries the necessary edit, a large diversity of editing types can be performed with high precision. These include all twelve types of single-base substitution, multiple base substitution, insertions, deletions, etc.

Delivery to the Brain

One critical challenge to the implementation of this technology is how to deliver them to the brain. This is necessary since PD is caused by defects in neurons in the mesencephalon of the brain. The brain is protected by barriers, which are extremely difficult to bypass. These barriers include the blood–brain barrier (BBB), the blood–cerebrospinal fluid barrier (BCSFB), the arachnoid barrier and ependyma (NBScience). The BBB - whose function is to regulate the flow of molecules, ions, and cells between the blood and the CNS - is the most restrictive. Its selective permeability and enzymatic properties restrict the passage of large molecules and gene delivery vectors (> 400 Daltons) and 98% of smaller drugs (BenDavid et al.).

To cross the BBB, scientists widely use virally-mediated vehicles. Viral vectors use modified viruses to deliver genetic material into cells. The viral envelope fuses with the cell membrane, allowing the therapeutic genes to enter the cell, where they may integrate into the host genome. They are extremely advantageous due to their high efficiency, long-term gene expression, and specific tissue targeting (Nguyen).

Common virally-mediated vehicles include adenoviruses, lentiviruses, and adeno-associated viruses (AAVs) (Fig 3). Adenoviruses can infect non-replicating cells like neurons, but they also elicit a strong immune response (Yan et al.). Lentiviruses can infect non-dividing cells and have a sufficient packaging limit (8.5 kb), with stable integration and long-term gene-editing capabilities. However, lentiviruses have a risk of insertional mutagenesis (Aljabali et al.). As a result, these are used for research rather than in clinical practice. Consequently, AAVs are the most attractive viral gene delivery vectors, since they do not lead to genomic integration and are efficient in their gene delivery (Yan et al.).

AAV9 is capable of bypassing the BBB, is highly neuron-tropic, and can achieve long-term gene expression. AAVs have been approved by the Food and Drug Administration (FDA) for use in human clinical trials (Yan et al.). However, one major drawback is their ability to infect non-neuronal cells as well, which results in a potential risk of affecting surrounding tissues or cells and unintended off-target effects. Other drawbacks include high production cost

and immunogenicity, which would be very hazardous in the brain. They also have a limited cargo capacity (4.7 kb), which is insufficient to deliver Cas9 mRNA once regulatory elements are added (BenDavid et al.). The prime editor mRNA is also too large (~6.5 kb) to be carried (Jiang et al.).

Compared to viral vectors, non-viral vectors may be better suited to deliver gene therapies, especially as they possess extremely low immunogenicity. For delivery to the brain, nanoparticle-based delivery, such as Peptide Nanocomplexes, Cell-penetrating peptides (CPP), Exosomes, Lipid Nanoparticles, is common.

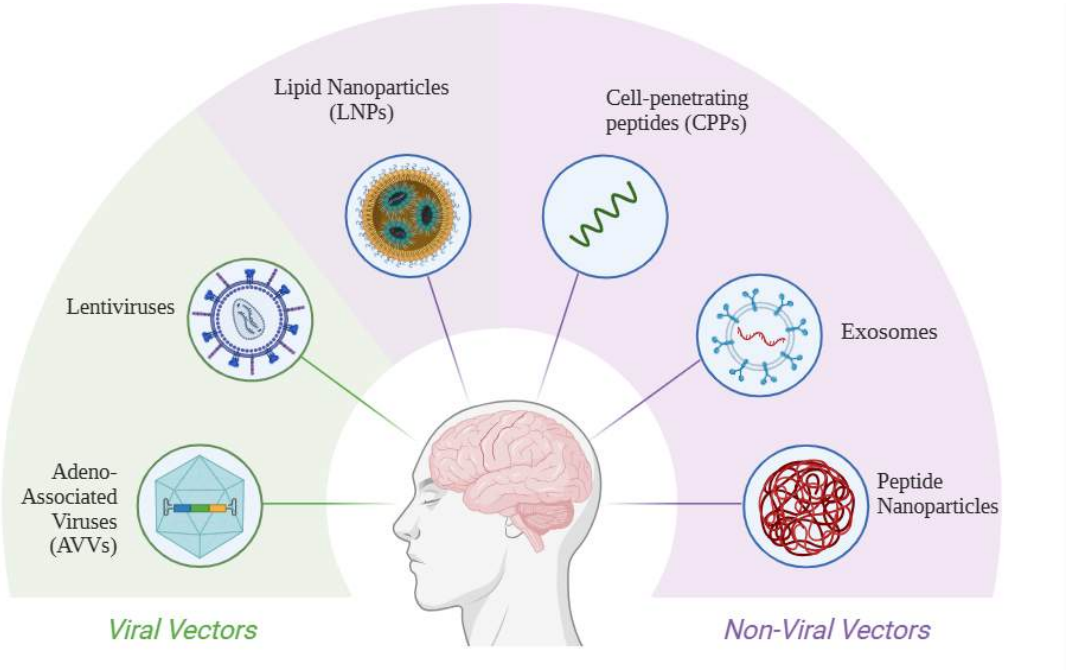


Fig 3: Delivery mechanisms to the brain. Overview of viral and non-viral vectors to transport gene-editing technologies to the brain. Viral vectors include AAVs and Lentiviruses. Non viral vectors are peptide nanoparticles, exosomes, CPPs and LNPs. LNPs can be administered through ICV, IC or IT injections. This image was created in BioRender: Iyer, A. (2026) https://www.biorender.com/.

Peptide Nanocomplexes are extremely efficient and cost-effective. They contain positive charges for enhanced encapsulation and intracellular penetration. In addition, their high specificity and modifiability leads to significantly reduced off-target effects compared with other strategies. Nevertheless, they can elicit immune responses, which is fatal when they affect the brain. On the other hand, CPPs are short, positively charged peptides that excel in their ability to enter cells. Research on its usage to deliver complex proteins like Cas9 are still infrequent, and significant further developments are necessary, mostly because delivering proteins often poses immunogenicity in cells (Williams and Larsen).

Exosomes are a subtype of extracellular vesicles (EVs), which are naturally-occurring vesicles produced by cells for communication among other cells, that carry proteins, lipids, and nucleic acids (ScienceInsights). They possess an intrinsic ability to cross the BBB, have low immunogenicity and high biocompatibility. Hence, engineered exosomes are being explored as biologically- derived carriers for CNS therapeutics. For PD, engineered exosomes delivering siRNA or mRNA, targeting SNCA in CNS, have reduced protein aggregation and improved motor function in preclinical studies (Bioscience).

Lipid nanoparticles are the most researched and prevalent nonviral gene therapies delivery method. They have numerous advantages, making them an ideal solution. LNP mRNA complexes have excellent biocompatibility. They have cationic charge for increased encapsulation and safeguarding the cargo, making them strong candidates for BBB delivery, since the endothelial cells forming the BBB are negatively charged (Williams and Larsen). LNP have ionizable lipids, which are predominantly neutral at physiological pH, allowing them to have in-vivo efficacy and low toxicity (Tuma et al.). Additionally, LNPs have a relative ease of large-scale production, and have good stability, protecting the genetic material from degradation. They can also deliver a wide size range of both hydrophilic and lipophilic drugs, and genetic materials including mRNA, siRNA, and CRISPR-Cas9 gene-editing components (Nguyen).

Delivery of LNPs to the brain is possible by direct intracerebroventricular (ICV) injection, Intracerebral (IC) injections or Intrathecal (IT) administration. ICV injection goes into the ventricles of the brain, directly into the cerebrospinal fluid (CSF). However, transfected cells are localized to the restricted brain regions, primarily to the cells lining the ventricle wall, with only small levels of transfection in the brain tissue. IC injections are superior as it avoids the blood-CSF barrier and BBB, transporting it directly into the brain tissue. It specifically targets local regions of the brain, which is vital for region-specific disorders like PD. The only downside to these is that they are invasive (Tuma et al.).

Intrathecal administration presents an alternative route to address this limitation. It allows delivery directly into the CSF, which circulates through the subarachnoid space surrounding the brain and spinal cord. Because the CFS continuously exchanges molecules with the interstitial fluid that surrounds CNS cells, therapeutics introduced into the CSF can spread throughout the entire brain and spinal cord. At the same time, this route keeps most of the drug confined within the CNS, minimizing exposure to the rest of the body. IT is minimally invasive and is the standard administration route (Dong et al.).

Experiments Conducted to Deliver Gene-Editing Technologies to the Mouse Brain

A group of researchers presented the first demonstration of non viral base editing, which is quite similar to prime editing, in the adult CNS of mice using LNPs. They used IT administration in Ai9 reporter mice.

First they synthesized a 200-member library of structurally diverse, biodegradable ionizable lipids using the Passerini three-component reaction. P3B, a lead lipid that mediated efficient, widespread mRNA delivery throughout the brain was identified. P3B-LNPs had a

favourable safety profile with minimal systemic exposure or inflammatory activation and it was recognized as a distinct biodegradable ionizable lipid that enables efficient and safe CNS genome editing via intrathecal mRNA delivery.

The P3B-LNPs were then optimized to improve mRNA delivery to the brain and high efficiency of gene-editing. Ai9 mice received two IT injections of P3B formulated CRISPR-Cas9 one week apart, and brains were collected on day 14 for analysis. On examination, it was seen that P3B-LNPs achieved robust neuronal and astrocytic transfection. It was observed that in the thalamus, P3B-LNPs achieved 12.46 % neuronal, 12.65 % astrocytic, and 14.36 % microglial transfection. This percentage can even be increased by repeating the injections multiple times. This is critical since PD involves dysfunctional thalamic circuits that affect motor control.

To evaluate the safety of P3B LNPs following IT administration, plasma samples were collected at 6 and 24 hours post-injection for inflammatory biomarker profiling. Many of these markers were reduced below baseline in P3B-treated mice, and by 24 hours their levels had largely returned to those seen in PBS controls, indicating strong tolerability. Consistent with this, no signs of tissue damage or inflammation were observed at the 6-hour post injection point.

Therefore, through their elaborate experiment, the researchers have proved that LNPs, by intrathecal administration, can deliver gene therapies to the brain, with reduced or no side-effects (Dong et al.).

Other experiments have also supported this finding. For example, Wang and colleagues used bioreducible lipid-packaged Cas9 protein and directly delivered it into the mouse brain for successful treatment of neurological disorders (Yan et al.). Similarly, Abbasi and colleagues achieved ~20% gene editing in the brain using PEG–PLL polyplex micelles (Seijas et al.). Thus, non-viral vectors are powerful tools to transport gene-therapies to the brain.

Discussion

Parksinon's disease is an extremely common neurodegenerative disease that is caused by the degeneration of neurons that synthesize and release dopamine in the mesencephalon of the brain. It leads to motor and non-motor symptoms that greatly inconvenience the patient. Approximately 15-20% of PD cases are genetic, and GWAS has identified the mutations of *SNCA* gene as a major contributor for PD, since one of its functions includes regulating the release of dopamine.

Current therapies for PD like levodopa are not curative since they focus on symptom reduction instead of terminating the neurodegeneration process. Therefore, we must look into gene-technologies for an alternate way to treat PD. CRISPR-Cas9 is one such mechanism that corrects base mutations in the DNA by creating double-stranded breaks (DSBs). However, given that canonical Cas9 induced DSBs are highly mutagenic, single-strand break editing techniques such as prime editing are preferable.

Prime editing also faces the challenge of crossing the brain-barriers for delivery. Adeno-associated viruses are the most widely-used delivery vehicles presently, and they have

been utilized for a long time. Non-viral vectors such as LNPs are superior since they have low immunogenicity. Today, many scientists are working to make LNPs stable.

Various factors impact LNP stability. Elevated temperatures lead to lipid degradation and can accelerate the hydrolysis of the phosphodiester backbone of mRNA. Hence, refrigeration at 2°C can provide a stable environment for LNPs for up to 150 days. For long-term storage, lyophilization or freeze-drying is common, wherein frozen water is directly sublimated into vapor to reduce molecular mobility and chemical degradation significantly. The pH affects the charge state, affecting the stability of the LNPs. Low pH (~4.0) is used during preparation to support encapsulation, followed by adjustment to near-neutral pH (~7.4) for storage for balancing lipid stability and mRNA integrity. Buffers are used to maintain charge balance and bond stability between lipid nanoparticles and mRNA (BOC Sciences Nanoparticles).

Later, these LNPs can be delivered to the brain by ICV, IC or IT administration. In fact, experiments have demonstrated the capability of LNPs to carry these gene-editing technologies for editing in the mouse brain. All in all, gene-editing holds potential in the future to cure PD, but we must also consider the limitations of prime editing and further research is conducted for its development.

Considering its practicality, prime editing has low efficiency. It has several steps and the efficiency of each step significantly impacts overall editing efficiency. It also relies on the delivery of multiple components like prime editing proteins, pegRNAs, and repair template which is quite difficult. In addition, during the cell's mismatch repair (MMR) machinery, any of the two strands may be taken as a template and in some cases, the original sequence is reconstructed. This is addressed by introducing a newer PE version called PE3 including an extra sgRNA, which is used to nick the strand that does not include the edit, so that the MMR machinery can use only the edited strand as a repair template. In some cases though, the nick generated in the non-edited strand and the nick in the edited strand by the pegRNA can coincide, leading to DSBs and INDELs. A new PE derivative called PE3b is being used to resolve this (Llado).

Gene-editing itself is also ethically controversial. For PD cases, gene editing is somatic, which is the modification of somatic cells in the body. Its ethical application depends on the risk to /benefit ratio, since there is a concern of off-target effects that can lead to cancer. Another ethical consideration is that therapies are quite expensive and distributing benefits equitably between the societal divisions will be a challenge (Brigden and Hall). WHO has also issued similar guidelines for the governance of human genome editing. One of them includes ‘to respect the wishes of competent individuals regarding their life and health’. In other words, people must give consent to edit their genes (O’Keeffe et al.).

Theoretically, it is also possible to make germline edits to *SNCA* genes while in the embryo, so that it impacts both the person and their descendants. This has substantial restrictions as it could result in off-target effects and mosaicism, in which only some copies of the gene are altered (Bergman). It removes autonomy in the decision making process of the later born

individuals, and also allows unforeseen and permanent side effects to pass down through generations (O'Keeffe et al.).

A technique that could be looked into further to reduce these ethical concerns is REPAIR (RNA Editing for Programmable A to I Replacement). It uses a catalytically inactive Cas13 (dCas13) fused with ADAR2 to edit the RNA instead of the DNA. It conducts hydrolytic deamination of adenosine to inosine, a nucleobase that is functionally equivalent to guanosine in translation and splicing. REPAIR is a reversible process, so even in the case that unintended effects arise, therapy can be discontinued and the patient would recover. Cas13 does not require PAM either. REPAIR can mediate efficient RNA editing in post mitotic cells like neurons too, which is the target for PD. It does have some limitations, however. The system is large, posing delivery challenges and base conversions are limited to generating inosine from adenosine in the case of pure Cas13 (Cox et al.).

In conclusion, subsequent advancements in pre-existing gene-editing technologies can effectively expedite clinical treatment of genetic PD. With the emergence of more efficient delivery vectors and developments of relatively new approaches such as REPAIR, we have the potential to improve clinical designs and optimize therapeutic options for PD patients, changing their lives for the better.

Works Cited

Aktaş, Emre, et al. "Levodopa and Plant-Derived Bioactive Compounds in Parkinson's Disease: Mechanisms, Efficacy, and Future Perspectives." CNS Neuroscience & Therapeutics, vol. 31, no. 8, 2025, p. e70540. Wiley Online Library, https://doi.org/10.1111/cns.70540.

Aljabali, Alaa A. A., et al. "Principles of CRISPR-Cas9 Technology: Advancements in Genome Editing and Emerging Trends in Drug Delivery." Journal of Drug Delivery Science and Technology, vol. 92, Feb. 2024, p. 105338. ScienceDirect, https://doi.org/10.1016/j.jddst.2024.105338.

BenDavid, Eli, et al. "Emerging Perspectives on Prime Editor Delivery to the Brain." Pharmaceuticals, vol. 17, no. 6, June 2024, p. 763. www.mdpi.com, https://doi.org/10.3390/ph17060763.

Bergman, Mary Todd. "Harvard Researchers Share Views on Future Ethics of Gene Editing." Harvard Gazette, 9 Jan. 2019, https://news.harvard.edu/gazette/story/2019/01/perspectives-on-gene-editing/.

Biology Insights. "What Is CRISPR-Cas9 and How Does It Work?" Biology Insights, 2 Aug. 2025, https://biologyinsights.com/what-is-crispr-cas9-and-how-does-it-work/.

Bioscience, Atlantis. "AAV, LNPs, and Exosomes for Brain Delivery: Validating with Fluorescence Imaging in Neuroscience." Atlantis Bioscience Pte Ltd, 27 Aug. 2025, https://www.atlantisbioscience.com/blog/aav-lnp-exosomes-brain-delivery-fluorescence-imaging-neuroscience/.

BOC Sciences Nanoparticles. mRNA-LNP Stability: Lyophilization & Buffer Design - Nanoparticles / BOC Sciences. https://www.bocsci.com/nanoparticles/resources/mrna-lnp-stability-lyophilization-buffer-design.html. Accessed 10 Sept. 2026.

Brigden, Tanya, and Alison Hall. "Somatic Genome Editing: Ethics and Regulation." phg foundation, Nov. 2019.

Cannan, Wendy J., and David S. Pederson. "Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin." Journal of Cellular Physiology, vol. 231, no. 1, 2016, pp. 3–14. Wiley Online Library, https://doi.org/10.1002/jcp.25048.

CD Genomics. Comprehensive Overview of GWAS: Definition, Advantages and Methods - CD Genomics. CD Genomics, https://www.cd-genomics.com/resource-overview-gwas-definition-advantages-application-methods.html. Accessed 9 Sept. 2026.

Chen, Peter J., and David R. Liu. "Prime Editing for Precise and Highly Versatile Genome Manipulation." Nature Reviews Genetics, vol. 24, no. 3, Mar. 2023, pp. 161–77. www.nature.com, https://doi.org/10.1038/s41576-022-00541-1.

Cox, David B. T., et al. "RNA Editing with CRISPR-Cas13." Science, vol. 358, no. 6366, Nov. 2017, pp. 1019–27. science.org (Atypon), https://doi.org/10.1126/science.aaq0180.

Day, Jacob Oliver, and Stephen Mullin. “The Genetics of Parkinson’s Disease and Implications for Clinical Practice.” Genes, vol. 12, no. 7, July 2021, p. 1006. www.mdpi.com, https://doi.org/10.3390/genes12071006.

Dong, Songtao, et al. “Biodegradable Lipid Nanoparticles for Genome Editing in the Brain via Intrathecal Administration.” Materials Today, vol. 92, Jan. 2026, pp. 140–50. ScienceDirect, https://doi.org/10.1016/j.mattod.2025.11.032.

Genç, Gençer. “Genetic Research and Its Contribution to the Treatment of Parkinson's Disease.” Deep Brain Stimulation, vol. 4, June 2024, pp. 47–49. www.dbsjournal.com, https://doi.org/10.1016/j.jdbs.2024.01.002.

Guo, Congting, et al. “Off-Target Effects in CRISPR/Cas9 Gene Editing.” Frontiers in Bioengineering and Biotechnology, vol. 11, Mar. 2023. Frontiers, https://doi.org/10.3389/fbioe.2023.1143157.

Guo, Xue-Yao, et al. “Parkinson’s Disease: The Epidemiology, Risk Factors, Molecular Pathogenesis, Prevention, and Therapy.” MedComm, vol. 6, no. 12, 2025, p. e70540. Wiley Online Library, https://doi.org/10.1002/mco2.70540.

Harvard Health Publishing. “Parkinson’s Disease.” Harvard Health, Harvard Medical School, 8 July 2025, https://www.health.harvard.edu/diseases-and-conditions/parkinsons-disease-a-to-z.

Hutter, Carolyn M. “Genome-Wide Association Studies (GWAS).” National Human Genome Research Institute, National Institute of Health, https://www.genome.gov/genetics-glossary/Genome-Wide-Association-Studies-GWAS. Accessed 9 Sept. 2026.

Jiang, Allen Y., et al. “Efficient Prime Editing in Vivo and in Vitro Using Lipid Nanoparticles.” Nature Nanotechnology, vol. 21, no. 7, July 2026, pp. 1022–35. www.nature.com, https://doi.org/10.1038/s41565-026-02200-6.

Llado, Manel. “News: Explainer: What Is Prime Editing and What Is It Used For?” CRISPR Medicine, CRISPR Medicine News, 4 Dec. 2023, https://crisprmedicinenews.com/news/explainer-what-is-prime-editing-and-what-is-it-used-for/.

MedlinePlus. SNCA Gene: MedlinePlus Genetics. National Library of Medicine, https://medlineplus.gov/genetics/gene/snca/. Accessed 9 Sept. 2026.

NBScience. Overcoming Challenges in CRISPR/Cas9 Delivery for Brain Tumor Therapy | ANTI AGING Therapies. 17 Aug. 2026, https://nbscience.com/overcoming-challenges-in-crispr-cas9-delivery-for-brain-tumor-therapy/.

Nguyen, Michael. “Lipid Nanoparticles vs. Viral Vectors: A Comparison for Gene Therapy.” Helix Biotech, 17 Sept. 2024, https://www.helixbiotech.com/post/lipid-nanoparticles-vs-viral-vectors-a-comparison-for-gene-therapy.

NIH National Institute on Aging. “Parkinson’s Disease: Causes, Symptoms, and Treatments.” National Institute on Aging, National Institute of Health, 14 Apr. 2022, https://www.nia.nih.gov/health/parkinsons-disease/parkinsons-disease-causes-symptoms-and-treatments.

O'Keeffe, Francis J., et al. "Somatic Genome Editing: Technical Challenges and Ethical Appraisal." European Journal of Medical and Health Research, vol. 2, no. 3, May 2024, pp. 239–47. ejmhr.com, https://doi.org/10.59324/ejmhr.2024.2(3).27.

Ramesh, Balasundari, and Adegbenro O. Fakoya. "Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas Unleashed: Transforming Gene Editing With Breakthroughs, Applications, and Ethical Dilemmas." Cureus, vol. 17, no. 11, Nov. 2025. www.cureus.com, https://doi.org/10.7759/cureus.95908.

Richter, Corinna, et al. "Function and Regulation of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / CRISPR Associated (Cas) Systems." Viruses, vol. 4, no. 10, Oct. 2012, pp. 2291–311. www.mdpi.com, https://doi.org/10.3390/v4102291.

ScienceInsights. "What Are Exosomes? How They Work and Why They Matter." ScienceInsights, 7 Mar. 2026, https://scienceinsights.org/what-are-exosomes-how-they-work-and-why-they-matter/.

Seijas, Ana, et al. "CRISPR/Cas9 Delivery Systems to Enhance Gene Editing Efficiency." International Journal of Molecular Sciences, vol. 26, no. 9, Jan. 2025, p. 4420. www.mdpi.com, https://doi.org/10.3390/ijms26094420.

Siddiqui, Irum Javaid, et al. "The Parkinson Disease Gene SNCA: Evolutionary and Structural Insights with Pathological Implication." Scientific Reports, vol. 6, no. 1, Apr. 2016, p. 24475. www.nature.com, https://doi.org/10.1038/srep24475.

Tuma, Jan, et al. "Lipid Nanoparticles Deliver mRNA to the Brain after an Intracerebral Injection." Biochemistry, vol. 62, no. 24, Sept. 2023, pp. 3533–47. Silverchair, https://doi.org/10.1021/acs.biochem.3c00371.

Uddin, Fathema, et al. "CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future." Frontiers in Oncology, vol. 10, Aug. 2020. Frontiers, https://doi.org/10.3389/fonc.2020.01387.

Williams, Lucian, and Jessica Larsen. "Nanoparticle-Mediated Delivery of Non-Viral Gene Editing Technology to the Brain." Progress in Neurobiology, vol. 232, Jan. 2024, p. 102547. ScienceDirect, https://doi.org/10.1016/j.pneurobio.2023.102547.

Yan, Sen, et al. "Use of CRISPR/Cas9 to Model Brain Diseases." Progress in Neuro-Psychopharmacology and Biological Psychiatry, vol. 81, Feb. 2018, pp. 488–92. ScienceDirect, https://doi.org/10.1016/j.pnpbp.2017.04.003.

Yang, Han, et al. "Methods Favoring Homology-Directed Repair Choice in Response to CRISPR/Cas9 Induced-Double Strand Breaks." International Journal of Molecular Sciences, vol. 21, no. 18, Jan. 2020, p. 6461. www.mdpi.com, https://doi.org/10.3390/ijms21186461.

Zhang, Weiwei, et al. "In-Depth Assessment of the PAM Compatibility and Editing Activities of Cas9 Variants." Nucleic Acids Research, vol. 49, no. 15, Sept. 2021, pp. 8785–95. Silverchair, https://doi.org/10.1093/nar/gkab507.

Zhao, Zhihan, et al. "Prime Editing: Advances and Therapeutic Applications." Trends in Biotechnology, vol. 41, no. 8, Aug. 2023, pp. 1000–12. www.cell.com, https://doi.org/10.1016/j.tibtech.2023.03.004.

Genetic Risk Factors and Lifestyle-Based Interventions Among Alzheimer's Disease, Parkinson's Disease, and Chronic Traumatic Encephalopathy By Aanya Narasimha

Abstract

Alzheimer's disease (AD), Parkinson's disease (PD), and chronic traumatic encephalopathy (CTE) have been classified traditionally as separate neurodegenerative diseases due to specific clinical manifestations and protein hallmarks - amyloid-β and tau in AD, α-synuclein in PD and perivascular tau in CTE. The goal of the current paper is to investigate genetic overlap and divergence of AD, PD and CTE in order to find convergent genetic pathways that could be used as a basis for precision neuroprotection strategy of neurodegeneration prevention. Based on existing scientific evidence regarding pathologies of the diseases, genetic and environment-related risk factors, two major genetic risk hubs, namely MAPT and TREM2, related to tauopathies and microglia-mediated neuroinflammation in all three diseases, and disease-specific genetic risk loci, namely Apolipoprotein E4 (APOE ε4) associated with AD, Leucine-rich repeat kinase 2 (LRRK2) associated with PD, and recently discovered associations between APOE ε4 and Microtubule-Associated Protein Tau Haplotype 1 (MAPT H1) in CTE risk after repeated brain injury, have been detected. Genetically determined risk of the three diseases acts at the level of NLRP3 inflammasome, which is a common mechanism of inflammation triggered by different upstream signals. Critically, genetic risk does not act in a deterministic manner but determines sensitivity of patients to various modifiable environment factors such as brain trauma, sleep quality, physical activity and nutrition, especially those individuals who carry specific genotypes (for instance, APOE ε4) and have increased vulnerability to negative lifestyle impact and potential benefit from specific treatments. Thus, a precision-neuroprotection strategy should involve genetic polygenic risk profiling, genotype-driven recommendations regarding environment factors and pharmacological treatment of convergent inflammation and proteostasis mechanisms. Despite the limitations of the current state of polygenic risk models, in particular low predictive power and limited cross-ancestry performance, the above approach allows shifting the focus from symptoms to genes in terms of neurodegeneration prevention.

1. Introduction

Neurodegenerative diseases impact more than 50 million people globally, infamously individualizing as one of the leading causes for disability and death (Dimmer). By the year 2050, Neurodegenerative disease cases are projected to increase with yet no successful leads for an effective solution (Nichols et al. 1). Among the most debilitating of these are Parkinson’s disease (PD), Alzheimer's disease (AD), and chronic traumatic encephalopathy (CTE). AD already affects approximately 6.7 million Americans and PD affects over 1 million Americans in the US alone. To complicate this, CTE also prevails as a devastating issue for numerous athletes and military personnel due to head trauma. Despite expansive and long years of research and investments reaching billions, no solution has been identified, no modification therapies have

been determined and patients continue to face continuous and painfully slow health decline with no defined end.

Historically, AD, CTE, and PD are diagnosed clinically separate and are perceived as different pathological diseases. AD is known as cortical dementia that is conceptualized through patterns of progressive memory loss and the abundance of extracellular amyloid-β (Aβ) plaques as well as intracellular neurofibrillary tangles (NFTs) that are made of hyperphosphorylated tau proteins (Tiwari et al. 2). PD is a movement decline disorder that promotes tremor, bradykinesia, and rigidity and stiffness that are manifested because of α-synuclein clumps and replications in Lewy bodies as well as specifically chosen loss of dopaminergic neurons in the area of the brain known as the substantia nigra (Zafar et al.). Finally, CTE occurs due to repeated brain injury or trauma which kickstarts a cascade of behavioral differences and cognitive changes that are linked with perivascular phosphorylated tau (p-tau) depositions along with astrocytic aggregates within the cortical sulci (McKee et al. 4).

The following comparative analysis analyzes these overlaps in depth alongside the differences in phenotypic expression, allowing the understanding that these three diseases are underlying mechanics although they are shaped differently. Not only that, but it also exemplifies the idea that neurodegenerative diseases reflect converging mechanisms.

The research question central to the paper is: How do shared and unique genetic risk factors among AD, PD, and CTE contribute to the development of these diseases, and how can these genetic interactions inform on early genetic and lifestyle-based interventions to prevent onset and progression of neurodegeneration?

To address this, the paper will foremost present a disease overview, expressing the clinical portfolios and known information. Then the paper extrapolates the striking pathological connections such as protein aggregation, neuronal degeneration, and neuroinflammations. A majority of the paper holds analysis crucial to identifying shared generic risk factors and how they act in favor of neurodegeneration. Furthermore, this paper will also synthesize information between the neurodegeneration-favoring genes and how they work with the environment, exploring factors like traumatic brain injury (TBI) and how it is a catalyst for genetically susceptible individuals. Even lifestyle factors like diet and sleep pattern and exercise can modify the issues that genetic risk can pose. This in-depth understanding can pave the way for early intervention and even translational strategies such as polygenic risk scoring (PRS) or precision lifestyle habits that are tailored for specific genotypes in individuals. Furthermore, pharmacogenic approaches are discussed. All in all, this analysis aims to connect research with a practical application through the exposure of innovative charts and data and crucial connections. The goal afterall is to convey that preventing neurodegeneration isn't just a symptom response but a journey that stems from the genetic level.

2. Neurodegeneration Disease Overview

Neurodegeneration is the erosion of the nervous system as well as neuronal functioning–a process that results in irreversible damage that affects human perception and cognition. Many

factors play a role in these deteriorating diseases; however, one crucial aspect is the contribution that genetics play in disease onset, its progressive nature, and the severity of the disease progression.

2.1. Alzheimer's Disease

ADAD is the most common and prevalent form among the various types of dementia. AD progresses over time in three stages–mild, moderate, and severe–that are characterized by progressive episodic memory loss for newly acquired information (Alzheimer's Association 3). Symptoms in the early stages of AD involve struggles in tasks involving executive functions, word-finding activities, as well as spatial disorientation (Mayo Clinic). In the moderate stages of AD, the affected experience language deficits, decline in visuospatial capacities, autonomous judgment, as well as profound behavioral changes such as apathy, depression, anxiety, and agitation (Mayo Clinic). Finally, during the last and severe stages of AD, patients forgo communicational aptitude, recognition of other people that were previously known, ability to perform basic activities that are required for daily life (Mayo Clinic). During the severe, individuals require full-time care and attention. Though most AD cases are late-onset AD (≥65 years), people may experience early-onset AD (Seath et al. 1). Early onset AD is a rare occurrence that can happen much earlier than age 65, such as individuals aged 40-50 due to autosomal dominant mutations in the APP, PSEN1, or PSEN2 genes (Johns Hopkins; Lanoiselée et al.). This disease, when genetically broken down, is found to foster variations of Apolipoprotein E (APOE). The APOE allele that increases risk for AD development is APOE ε4; however, it is not the only predisposing gene an individual in danger of AD can inhabit.

The majority of these symptoms develop due to the buildup of pathological amyloid-β (Aβ) plaques and. Oligomers, or small Aβ fragments, aggregate into complex higher order structures that form plaques contributing to neurodegeneration while hyperphosphorylated tau proteins form neurofibrillary tangles (NFTs) within the neuron (Alzheimer's Association). NFTs and Aβ's are pathological hallmarks of AD. The peptides that make up Aβ, specifically the most aggregating prone isoform Aβ42, are made by the Amyloid Precursor Protein or APP (Multhaup et al.).

2.2 Parkinson's Disease

The age of regular onset for Parkinson's Disease is early 60's; however there is also early-onset PD that develops in an individual's 50s (National Institute of Neurological Disorders and Stroke). PD's symptoms include tremors, movement issues, balance impairments, difficulty with talking and daily tasks, bradykinesia (slowness in automatic movement), as well as stiffness (National Institute of Neurological Disorders and Stroke). People with PD also experience cognitive problems and this can even lead to dementia. Visuospatial abilities, attention, and memory deficits become more prevalent as the disease progresses over time (National Institute of Neurological Disorders and Stroke). The age of regular onset for the disease is early 60's; however there is also early-onset PD that develops in an individual's 50s (National Institute of

Neurological Disorders and Stroke). The pathology to this disease involves dopaminergic neurons that are in the substantia nigra which is a part of the basal ganglia dying. These neurons are important in function as they are credited with dopamine production which is crucial for smooth motor function (Klein et al. 3). Furthermore, the alpha synuclein protein, or α-synuclein (α-syn), misfolds and when clumps together, creates Lewy bodies (Bendor et al. 7). These Lewy bodies are created within the neuron, a key pathological feature. The protein α-syn is about just as important to PD as Tau and Aβ are to AD. PD fosters its own lineage of genetics and can be either sporadic or familial inherited. It houses gene mutations of LRRK2, PARK2, PINK1, DJ-1 and SNCA (National Institute of Neurological Disorders and Stroke).

2.3 Chronic Traumatic Encephalopathy

CTE is a neurodegenerative disease associated with repetitive brain injury and unlike the other two diseases, AD and PD, CTE doesn't have a standard age restriction for when it can develop; it can happen at any time after the occurrence of a physical head injury. CTE damages the frontal lobe and manifests in symptoms such as a low degree of memory loss, mal mood, concentration issues, slurred speech, trouble with daily tasks and many more daily-function impairments (Fesharaki-Zadeh 1-2). This disease is commonly diagnosed in athletes taking repeated severe injuries to the head, but can also occur from non-sports related injuries such as falls or vehicle accidents (Catanese). The disease is associated with harmful widespread tau protein deposits, usually around small blood vessels, which are distinctive from various other tauopathies (Mckee et al. 1). Although both AD and CTE are established as tauopathies, there is a unique difference within their protein. With AD, there is formation of neurofibrillary tangles that build up inside the neuron and is a predictable pattern (Moloney et al. 1). On the other hand, tau accumulation in CTE spreads outwards and unevenly which is unique to just this disease (Katsumoto et al. 1). Because the diagnosis for CTE is usually post-mortem, the full scope of genetic vulnerability is hindered. Although neither PD, CTE or AD have cures, there are speculations that certain housing genes such as APOE predispose individuals to these neurodegenerative diseases.

3. Pathological Similarities

AD, CTE, and PD are all clinically characterized distinctly but they all share overlapping pathological features and neurodegenerative mechanisms.

3.1 Protein Aggregation

The three neurodegenerative diseases all include an abnormal accumulation of proteins that are misfolded. Protein misfolding is also a hallmark of neuronal dysfunction. This process occurs when proteins lack the ability to fold into the usual 3-D shape or follow the wrong energy-minimizing funnel, which therefore leads to toxic configurations in the body (Valastyan & Lindquist 3).

AD is marked by two proteins misfolding: an extracellular amyloid-β (Aβ) and intracellular NFTs that are made of the hyperphosphorylated tau protein (Zheng & X. Wang 1). Professors at Oxford University in the EMBO Journal, conducted research, answering how Aβ created problems with normal protein aggregation (Singh et al. 1). After working with fruit flies, protein aggregation disruptions in a cell and its secretory compartments were found to show heightened aberrant lysosomal targeting and proteins with compartment motility (Singh et al. 1-2). This finding in pathology is relevant to early-stage AD and mirrors neurodegeneration in human brain tissue (Singh et al. 14). Conversely, NFTs happen when normal tau proteins that stabilize a neuron's microtubules end up hyperphosphorylating (addition of excess phosphate groups to a protein that alter their function) and detach from the microtubule and start to aggregate into insoluble NFTs inside a neuron which becomes dangerous as it affects their function (Sun & Monk 1).

PD is understood clinically as a disease with α-syn protein aggregation in neurons which creates toxic fibrils that can become Lewy bodies and Lewy neurites (Bendor et al. 1). This buildup is toxic to dopaminergic neurons and can cause their degeneration in the substantia nigra region mainly (Rissardo et al 1-2). But the toxicity of α-syn protein buildup is not limited to the substantia nigra region. α-syn oligomers are thought to be the most toxic form of α-syn protein (Rissardo et al 5-6). α-syn oligomers are the soluble intermediate forms of α-syn protein, which can cause damage to cellular membranes, impair synaptic vesicle transport, and transmit from cell to cell in a prion-like fashion within limbic and cortical regions (Rissardo et al 1-2).

CTE, like AD, has an abundance of perivascular deposition of hyperphosphorylated tau proteins mostly within the cortical sulci (Katsumoto et al. 2). While both AD and CTE are characterized as tauopathies, they are fundamentally distinguished by two factors. tAD is uniquely defined by the coexistence of both extracellular Aβ plaques and intracellular NFTs whereas CTE is considered a pure tauopathy and is generally not associated with significant Aβ deposition (McKee et al. 1). The second is anatomical distribution. In AD, tau pathology follows a systematic progression or Braak staging, starting in the medial temporal lobe (hippocampus/entorhinal cortex) and spreading outward (Macedo et al. 1; Moloney et al. 13). In CTE, tau pathology is unique, forming NFTs and astrocytic tangles in a distinctive pattern—clustering perivascularly at the depths of the cortical sulci, often starting in the frontal and temporal cortices (Katsumoto et al. 2; Moloney et al. 12-13).

3.2 Neuroinflammation

In all three diseases, a commonality is that they share chronic activation of the immune responses in the brain: neuroinflammation.

CTE occurs after repetitive head trauma or TBI and triggers a neuroinflammatory response involving tau hyperphosphorylation. In the context of AD and PD, the misfolded protein aggregates–Aβ and tau in AD, and α-syn in PD are damage-associated molecular patterns or DAMPs (Duranti & Villa 7). DAMPs trigger the inflammatory responses and the inflammatory response is mediated through activation and proliferation of the brain's immune

cells, specifically microglia and astrocytes (H. Lin et al. 24). Protein DAMPs trigger formation and activation of an inflammasome complex named NLRP3 which is crucial for processing pro-inflammatory cytokines (H. Lin et al. 21).

3.3 Neuronal Degeneration and Synaptic Loss

In all cases of neurodegeneration, synaptic loss is correlated with cognitive decline, regardless of the misfolded protein initiating the disease cascade (Bereczki et al. 1).

In PD, neuronal cell death with subsequent synaptic loss is a simultaneous process that targets dopaminergic neurons in the substantia nigra (Fan et al. 1). This leads to tremors, rigidity, and bradykinesia, and extensive neuronal cell death in other brain regions, and eventually, non-motor symptoms as well (Fan et al. 1).

In CTE, there is extensive neuronal cell death in the frontal and temporal lobe; in fact, the neuronal death in the temporal lobe is thought to be the main reason for the early and severe symptoms of CTE–specifically, behavioral and mood disturbances like aggression, impulsivity, and depression (Munakomi & Puckett).

In AD, the cognitive decline symptom is directly associated with extensive synaptic loss: The first occurrences of this are in the hippocampus (memory center) and cortex (executive function center) with consequent spread to other neural regions (Bereczki et al. 2).

4. Shared Genetic and Molecular Mechanisms

The MAPT gene as well as TREM2 gene represent the two critical shared genetic nodes of AD’s, PD’s, and CTE’s pathogenesis.

4.1. MAPT

MAPT's central role is to encode for the tau protein. The tau protein itself functions to maintain structure and stability of microtubules and also aids the facilitation of axonal transports. If there are mutations in MAPT gene code, the creation of tau protein can be disrupted through alteration of microtubule-binding affinity and ability (Barbier et al. 9). MAPT mutations may also upset the ratio of tau isoforms by increasing the production of 4R isoforms compared to 3R), which promotes tau aggregation, or tauopathy (Chen et al. 1). This is because 4R-tau isoforms assemble at a faster rate and therefore, have a higher propensity for creating toxic tau filaments as they disrupt the 1:1 safety ratio between 4R and 3R (Ezerskiy et al. 1). A 2017 mouse study, sought to determine if increases to 4R-tau causes tauopathies. Human tau (hTau) mice were treated with antisense oligonucleotides (ASO’s) targeting the human MAPT gene to increase the 4R:3R tau ratios, and it was found that the ASOs caused a 4R tau isoform alteration where 4R expression was increased (Schoch et al.1). After the mice evaluation for tau phosphorylation with an AT8 antibody, it was concluded that phosphorylation and tau aggregation occurred in the amygdala and cortex as well as the entorhinal cortex (Schoch et al. 2). This result provides evidence that increased expression of 4R-tau is associated with phosphorylation of tau as well as a separate, pathological exacerbation in 4R expression inducing the phenotype of

hyperexcitability and seizure activity (Schoch et al. 3-4). Tauopathy is a hallmark between AD, CTE and hyperphosphorylated tau proteins that can disassemble from the microtubules that result in loss of normal functions and a gain of toxic functions.

Tau hyperphosphorylation reduces tau's microtubule-binding affinity. Tau's gain of toxic function leads to a detachment and destabilization of the neuronal cytoskeleton (Panda et al. 5). This first step of functional loss is devastating as microtubule breakdown impairs the transports between axons–an essential process for molecular signaling and organelles (Cheng & Bai 2). These detached hyperphosphorylated tau proteins then go through conformational changes, going from a highly soluble disordered protein into misfolded tau oligomers (Hill et al. 2). This process represents the first gain of toxic functions exemplified by these tau oligomers. The tau oligomers are capable of sequestering normal tau proteins and microtubule-associated proteins (MAPs) away from the cytoskeleton, which accelerates the collapse of microtubules and catalyzes cognitive degeneration (Iqbal et al. 1).

Individual genetic susceptibility of developing tauopathies is modulated by two alleles of the MAPT Gene: H2: protective, and H1: risk-conferring (Han et al. 3). These two common alleles are extended inversions on the chromosome 17q21 (Han et al. 3). H1 is a primary risk factor in the tauopathy discussed in this paper: CTE. However, it is also linked to synucleopathies like PD and dementia with its Lewy bodies (Sánchez-Juan et al. 2). H1 also acts as a significant risk factor in AD, specifically in individuals who do not carry the APOE ε4 alleles (Sánchez-Juan et al. 3). This provides evidence to believe H1 drives pathology in AD through distinct tau-pathway means suggesting that AD may have non-APOE ε4 detrimental genetic mechanisms.

While the exact functional variant in the H1 region is still actively being studied, it appears that its effect is due to differential expression or splicing which leads to an increased overall burden of the tau protein—particularly the 4-repeat isoform—or by rendering cells more vulnerable to secondary insults (Sánchez-Juan et al. 3, 5). Although the exact mechanism remains elusive, recent findings suggest that this haplotype may functionally link to a more limited ability to cope with oxidative stress, thus rendering neurons increasingly vulnerable to ferroptosis, a regulated form of cell death (Sadikoglou et al. 1). Surrounding this acute injury of repetitive head injury (RHI) lies genetically primed environments, particularly salient in regards to CTE (Han et al. 1).

4.2. APOE ε4

APOE ε4 is a variant of the original parent gene, APOE. Located on the 19q13 chromosome, APOE encodes for a 299-amino acid protein that is crucial for transport of lipids, homeostasis in cholesterol, synapse plasticity, as well as neuronal respiration (Raulin et al. 1). Inside the human, APOE can diverge into 3 major allele variants: APOE ε2, APOE ε3, and APOE ε4 (Raulin et al. 1). All three variations result in proteins with singular Cysteine and Arginine amino acid differences at 112 and 158 (Raulin et al. 2). Although these substitutions can seem minor, having one change can produce many functional changes. APOE ε2 and APOE ε3

are variants that preferentially bond lipids while APOE ε4 preferentially causes aggregates and promotes reduced binding capacity between lipids (Chou et al. 1).

The allele variant ε3 is globally common and is the 'neutral' allele; the ε2 allele is a protector variant against AD as it can delay onset; However, the ε4 variant is the strongest allele risk factor for late-occurring AD (Z. Li et al. 1). Each individual APOE ε4 allele in the human body increases the risk of having AD significantly (Duara et al. 1).

APOE ε4 intensifies AD pathogenesis through various mechanisms that are often non-inclusive. Foremostly, APOE ε4 causes reduced Aβ clearing efficiency in the brain compared to its other variants, ε2 and ε3 (Chou et al. 1). However, despite APOE ε4 having a reduced efficiency in clearing Aβ, Aβ production rates did not differ across all allele isoforms indicating APOE ε4's effect on Aβ accumulation is driven specifically through an impaired level of clearance (Castellano et al. 6-7). ε4 can also directly interact with Aβ, promoting the formation of plaques and even fibrilization. Secondly, APOE ε4 worsens the pathology of tau: Humans carrying the APOE ε4 allele evidence higher accumulations of the tau protein and a faster, tau-driven, neurodegeneration which persists even after accounting for the given Aβ effects; therefore, this suggests that ε4 has a direct effect on partial-independent influence on the pathology of tau (Baek et al. 6-7, 9-10). This suggests that APOE ε4 can lower thresholds for Aβ-mediated tau pathology. Next, APOE ε4 impedes neuronal respiration and neuroplasticity and shows enhanced phenotype expression of pr-inflamation as well as impaired phagocyte activity than APOE ε2 and APOE ε3 (Y. Lin et al. 5, 7, 8). Lastly, APOE ε4 weakens the blood-brain barrier strength and disrupts cerebrovascular functionality which hinders the removal of neurotoxic proteins through perivascular drainage modes (Xiong et al. 2).

APOE ε4 aids the growth of AD; however, it is proving to implicate PD susceptibility–specifically cognitive decline: Meta-analyses reflect APOE ε4's effects increase PD risk modestly (Williams-Gray et al. 1). The overall effects of APOE ε4 in PD are smaller than the overall effects of APOE ε4 in AD; although, ε4 is significant in correlation to PD–particularly cognitive impairment and dementia seen in PD (Williams-Gray et al. 1). The ε4 variant carriers reflect catalyzed cognitive decline, early-onset dementia, as well as tau and Aβ pathology during autopsy analysis (Williams-Gray et al. 1; Tsuang et al. 5).

The varying pattern may implicate the allele's nature as APOE ε4 can potentially be involved in mechanisms that overlap with AD and PD. In both AD and PD, ε4 can prevent clearance of pathological proteins (Aβ in AD, and perhaps α-syn in PD), increase neuroinflammation, or diminish neural resilience. The divergence of the effect of ε4 on PD dementia as compared with motor symptoms indicates that it has an impact in the cortical and limbic vasculature (where cognitive functions localize) rather than substantia nigra dopaminergic degeneration (Dickson et al. 1). Some PD patients exhibit mixed pathology, with both α-syn and AD-type changes (plaques, tangles), and APOE ε4 may predispose to these mixed presentations (Compta et al. 1).

APOE ε4 by TBI is an archetypal gene-environment interaction with translational effects on CTE risk. APOE ε4 carriers who suffer moderate-to-severe TBI have been shown to have

poorer functional outcomes, more prolonged loss of consciousness, and higher rates of chronic cognitive impairment compared to non-carriers (McFadyen et al.7-8). Mechanisms include likely impaired neuronal injury repair, to aggravation of neuroinflammatory responses, impaired clearance of injury-induced proteins (inclusive of Aβ and tau), increased BBB dysfunction (Mahley & Huang 2-3).

CTE has early evidence proving that APOE ε4 causes a vulnerability increase to severe tau pathology: This is relative to noncarrier individuals succeeding trauma from multiple assaults to the head (Atherton et al. 6). In the investigation of former American football players, ε4 allele carriers experienced white matter abnormalities and cognitive impairment compared to non-carriers, although post-mortem diagnoses of CTE were not verified (Tharmaratnam et al. 2). Although the sample sizes are constricted, preceding studies reflect that the APOE e4 allele is abundant in CTE autopsy-confirmed reports compared to normal cases where reports are age-matched (Stern et al.). These results are indicative of ε4 ectopia analogous to that seen in younger individuals. Although the sample sizes were limited, larger cohort validations would position the APOE genotype as a modifiable factor for contact sport participation decisions and return-to-play guidelines following concussion.

The effects of the APOE ε4 gene in AD, PD, and CTE are overlapping due to the ε4 allele. APOE ε4 lowers the brain's ability to cope with protein-based and inflammatory strain – whatever the precise cause. In AD, the strain is Aβ creation linked to age; in PD, it is α-syn build-up and the weakness of dopamine-making nerve cells; and in CTE, it's mechanical damage and the tau issues brought on by head wounds. In all cases, APOE ε4 makes getting rid of harmful substances worse, increases inflammation, and cuts down on the brain's power to heal, speeding things up from strain to nerve cell decay.

Because of this idea, treatments which improve protein removal–for instance, boosting autophagy–or which lessen inflammation–like blocking NLRP3–or help nerve cell strength, could substantially help people with the ε4 gene, in a number of illnesses. On the other hand, people with ε4 might need earlier or tougher treatments, as their ability to deal with the problems caused by disease is lower. Looking at things from the viewpoint of public health, testing for the APOE gene could tell people what risks they face, and help them choose ways to lower those risks – especially for individuals thinking about activities causing big impacts (sports or armed forces), or people whose relatives have had dementia.

4.3. TREM 2

The gene TREM2 encodes a protein named triggering receptor expressed on myeloid cells 2 (TREM2). This is expressed on the microglia which are immune cells of the brain and it helps maintain protective functions inclusive of survival processes, proliferation, and phagocytosis or clearance of toxic waste and aggregates of protein (Colonna & Y. Wang 1). TREM2 works as a pattern recognition receptor, joining with lipids, lipoproteins–including APOE–and molecules which show damage has happened, and controls microglial activation which is the engulfing of things by cells, cell survival and how cells get their energy (Colonna &

Y. Wang 2). The receptor for the TREM2 protein activates after ligand binding . It uses DAP12–an adaptor molecule–for signaling that results in the activation of two signaling pathways: PI3K/AKT: promotes protective functions detailed earlier, and SYK: this contributes to the protective functions (Colonna & Y. Wang 2).

Other variants, such as R62H, D87N, and H157Y, have also been associated with AD risk but with smaller effects (R. Li et al. 1). Rs75932628, or the R47H variant, or has been shown to impair TREM2 function in multiple ways (Sirkis et al. 2). This genetic variant substitutes histidine for arginine at the 47th position of amino acid pairs, increasing AD risk by a 2.9 ratio of odds (Jonsson et al. 3, 5). Due to R47H, TREM2 has reduced binding affinity to APOE and phospholipids, trafficking to the cell surface, and subsequent signaling (Bailey et al. 3).

Studies carried out in mouse models of AD have shown that TREM2 is a critical function in the final step of conversion of microglia to disease-associated microglia (DAM), a microglia state characterized by their aggregation around Aβ plaques (Y. Zhou et al. 2-3). DAM microglia express genes related to phagocytosis in an attempt to contain the disease (Gao et al. 2, 6, 7). Without TREM2, this beneficial response of microglia is blocked, resulting in increased Aβ plaque load, altered plaque morphology with a more diffuse appearance, and increased dystrophy in neurons (Y. Wang et al. 2; Klincheva et al. 1).

However, TREM2's role is complex and dependent on the progression of the disease as the early states of TREM2-mediated microglial activation may be protective through debris clearance but sustained activation could be detrimental in disease progression, leading to neurodegeneration (Shi et al 9).

Although the role of TREM2 has been extensively studied in relation to AD, its role in relation to microglia cannot be ignored, and as such, its relevance should not be limited to AD or other neurodegenerative diseases involving protein aggregation and neuroinflammation. Genetic studies have shown that there is a small association between variants of the TREM2 gene and susceptibility to PD, including evidence that R47H increases susceptibility to PD (Rayaprolu et al. 2-3). The pathways that are likely involved include those that are associated with the defective clearance of aggregates of α-syn and those associated with inflammatory responses to Lewy bodies.

On the other hand, TREM2's potential role in CTE is under-researched. Effective microglial responses that follow TBI are crises for cellular debris clearance, resolving inflammation, and allowing tissue repair. Individuals with TREM2 and the loss-of-function variants can show impaired clearance of debris following any head impacts, potentially allowing tau pathology to accumulate readily or inflammatory responses to prolong (Gratuze et al. 3-4). Given that not all individuals with similar exposure to trauma develop CTE, genetic modifiers of microglial function such as TREM2 mirror plausible susceptibility factors.

TREM2 and APOE have an intriguing interaction in biology. TREM2 connects with APOE-lipid complexes, which is key for microglia to manage lipids and clean up cell debris efficiently, supporting microglial clearance functions (Yeh et al. 10-11). The R47H variant reduces binding to APOE. TREM2-deficient microglia, critically, shows lipid metabolism that is

dysregulated, accumulating lipid droplets that affect function (Krasemann et al. 12). This suggests a model where APOE genotype and TREM2 genotype both influence risk of CTE: APOE4 carriers that have the TREM2 loss-of-function variants can face higher risk from a protein clearance deficiency (APOE4) and microglial function (variants of TREM2).

4.4. LRRK

LRRK2 is a multi-domain protein that has 2527 amino acids and exhibits kinase as well as GTPase activities. This protein is expressed through the body such as in neurons, the microglia, and immune cells. The protein also regulates cellular processes such as autophagy, lysosomal function, vesicle trafficking and mitochondrial dynamics (Roosen & Cookson 5, 6-7). Its protein confirmation structure exhibits leucine-rich repeats which are protein-protein interactions, a Roc GTPase and kinase domain, and C-terminal WD40 repeats, suggesting that LRRK2 functions as a signaling molecule that coordinates various pathways (Roosen & Cookson 2).

Mutations in this protein are common genetic causes of familial PD, accounting for 1-2% of sporadic PD; However, this percentage goes up to 40% of PD in certain populations like that of Ashkenazi Jews and North African Arabs (Healy et al. 3,5). The most common mutation is G2019S which is located in the kinase domain of LRRK2 and increases kinase activity at least 2-3 times more (Ren, et al 2). G2019S is gain-of-function mutation and it causes autosomal dominant PD with variable penetrance, meaning many mutation carriers never truly develop the disease but could implicate modifier genes or environmental factors (Krachergus et al 3-4). Pathologically, PD that is LRRK2-associated is indistinguishable from sporadic PD cases. However, some situations prove that tau pathology, and rarely, carriers of LRRK2 mutations have nigral degeneration without Lewy bodies. This suggests that dysfunction of LRRK2 causes neurodegeneration through many forms and pathways (Zimprich et al. 5). Moreover, Lewy bodies may not need to be expressed for carriers of LRRK2 mutations to have neurodegeneration (Kalia et al. 3, 5). This ambiguity may suggest that LB-absence pathways cause neurodegeneration.

Pathogenic mutations of LRRK2 weaken cellular processes. Due to the enhanced kinase activity symptom of LRRK2 mutations, autophagy and lysosomal degradation is damaged, impairing the cells' capabilities to remove misfolded proteins and organelles that are damaged (Madureira et al. 3, 9, 7-8). Moreover, LRRK2 phosphorylates a subset of rab GTPases that are involved in vesicular movement, while LRRK2 mutations increase the phosphorylation of Rab and impairs its phosphorylation, interrupting cycling of rab between endolysosomal compartments and cytosol (Steger et al. 3, 14-15).

Additionally, LRRK2 also aids mitochondrial function. If there is a mutant LRRK2, mitochondrial fission/fusion dynamics can be damaged and mitochondrial respiration may get compromised, potentially elaborating on the selective vulnerability of dopaminergic neurons needing high energy demands (Buck & Sanders 2, 3, 6). LRRK2 also regulates inflammatory

responses in the microglia. However, mutant LRRK2 may exaggerate pro-inflammatory cytokine production that works against the body's inflammatory regulation system (Moehle et al. 6, 9).

However, although LRRK2 is established as a gene involved with PD, its presence in autophagy, lysosome function, and inflammation may present more significance than stated. Genetic studies identify associations between variants of LRRK2 and the risk of AD, though effect sizes are small and findings are inconsistent (Zhao et al.). The mechanism involves damaged autophagy that reduces Aβ and tau aggregates' clearance.

In CTE, the role of LRRK2 in inflammatory signaling could be relevant. Repetitive brain trauma triggers sustained activation of microglia. Individuals with LRRK2 variants who are involved with repeated head injury could promote exaggerated inflammatory responses and potentially may develop prolonged and severe neuroinflammation that facilitates tau pathology. Moreover, LRRK2's role in lysosomal function could influence clearance of trauma-induced cellular debris and protein aggregates.

The concept of LRRK2 as a pathway gene rather than a disease gene is fundamental. The concept establishes that LRRK2 dysfunction affects crucial cellular processes that, if impaired, can become many different diseases depending on genetic background, vulnerability of different cell types, and different exposures to the environment (Cookson 1). This framework positions LRRK2 inhibitors, which are in clinical trials for PD, as possible therapeutics for multi-disease if the autophagy enhancement and modulation of inflammation present benefits that extend beyond dopaminergic neurons.

LRRK2's variable penetrance—where G2019S carriers may never develop PD—complicates modifying factors. Environmental exposure such as head trauma have been explored and evidence suggests TBI potentially unmasks LRRK2-related pathology or accelerates the onset in mutation carriers (Gardner et al. 7).

Gene-to-gene interactions are relevant as well. Co-inheritance of LRRK2 mutations along with GBA (glucocerebrosidase) mutations—another risk gene in PD affecting lysosome function—may impair pathways of protein clearance, decreasing the threshold for pathology (Gan-Or et al. 3-4). Similarly, APOE might modify penetrance of LRRK2 through inflammation and protein clearance effects, though this is speculative.

5. Convergence: The Neuroinflammatory Axis

This part of the paper aims to shift into synthesis of Sections 3 and 4, explaining how the various genetic risk factors create and promote pathological mechanisms in convergence and create similarities among PD, AD, and CTE.

5.1 NLRP3 Inflammasome Activation

A primary question is how genetic risk factors—affecting lipid metabolism (APOE), tau biology (MAPT), microglial function (TREM2), and autophagy (LRRK2)—converge to promote similar inflammatory pathology across AD, PD, and CTE. The answer may be the NLRP3 inflammasome as it represents a critical convergence point where multiple pathways intersect.

APOE ε4 enhances activation of NLRP3 through various mechanisms: APOE ε4 impairs microglial phagocytosis of protein aggregates which leads to lysosomal ruptures and the release of cathepsin into cytosol, a defined activator of NLRP3 (Heneka et al. 3). Moreover, APOE ε4-mediated dysfunction of the mitochondria increases reactive oxygen species production–another trigger for NLRP3.

TREM2 loss-of-function impairs microglial metabolic reprogramming and capacity of phagocytes. This leads to extracellular protein aggregates accumulation that eventually phagocytose in excessive quantities which overwhelms lysosomes, therefore activating NLRP3 through impaired lysosomal function (Shokr 10)

This convergence point–NLRP3–suggests that disparate genetic risk factors funnel into similar inflammatory pathways that amplify disease-specific triggers such as Aβ in AD, α-syn in PD, trauma in CTE into neurotoxic inflammation.

6. Gene and Environment Interaction

Genetic risk factors discussed in Section 4 cannot work in isolation but rather in conjunction with environment exposure and lifestyle in influencing the development of diseases in individuals. Knowledge about the interplay of genes with the environment is key to implementing prevention programs based on genetic understanding. This section examines the influence of traumatic brain injuries, sleeping habits, physical exercise, and diets in relation to genetic risk for neurodegenerative diseases. Key focus will be on finding out which groups of people can benefit most from such interventions. The underlying principle here is that genetic risk factors only influence the slope of the effect of environmental exposures on diseases and not the destination point.

6.1 Inflammation-Driven Protein Pathology

TBIs are common environmental risk factors for neurodegeneration–specifically CTE. Yet, outcomes establish heterogeneity that complicates genetic modifiers. Although repetitive head injury in contact sports increases risk of CTE, autopsy studies conclude that the number of athletes exposed to head injury who develop pathological changes is undetermined from brain-donor cohorts' selection bias. A series of young athletes in contact-sport found CTE pathology in approximately 32% of cases (Bieniek et al. 5). This means that a majority did not have CTE pathology despite similar histories of trauma. (McKee et al. 6). Similarly, single moderate-to-severe TBI increases long-term dementia risk 2-4 times; however, this relative increase still translates to the fact that many survivors lack development of dementia (Shively et al. 2, 6). This variability suggests that gene factors may conclude individual susceptibility to trauma-induced neurodegeneration, transforming similar environmental exposures into different outcomes in a clinical setting.

Interactions of APOE ε4 and TBI provide an understood example of gene-environment interaction in neurodegeneration. Existing meta-analyses establish that ε4 carriers that experience moderate-to-severe TBI exhibit higher odds of poor functional outcomes by six to twelve months

post-injury than compared to non-carriers that have similar injury severity. However, though the effect size is modest, with risk ratios of roughly 1.4, findings for cognitive-specific outcomes have lacked consistency (McFadyen et al. 2; W. Zhou et al. 1). Furthermore, follow-up studies portray increased risks of dementia development in people carrying the ε4 allele and having experienced TBI. Some studies have shown the synergistic effect, according to the risk of developing dementia in patients with TBI and ε4 carrier status, is higher than the sum of risk associated with the presence of each of these factors (Mayeux et al. 1). Mechanisms involved in these processes are varied but have one common link, the reduced capability for recovery. APOE ε4 causes impairment of neuronal membrane repair and growth of neurites after mechanical trauma, blood-brain barrier repair failure resulting in sustained influx of peripheral immune cells, post-traumatic neuroinflammation by means of excessive activation of microglia and decreased removal of injury-induced protein accumulation (Mahley & Huang 2, 3). TBI leads to an acute increase in both amyloid-β production and the release of tau protein from the damaged neurons and individuals with ε4 show less capability for clearing these proteins, which may contribute to the pathological accumulation of these protein aggregates (Mahley & Huang 1, 3).

Concerning CTE, evidence for excess ε4 prevalence comes from small, older studies of trauma-exposed populations, though findings across case series remain inconsistent (Jordan 1; Deng et al. 8). However, if the prevalence of excessive ε4 is validated in larger prospective cohorts, this could position APOE ε4 genotyping as clinically actionable information for decisions on contact sport participation, return-to-play protocols that follow concussions, and military placement considerations.

The MAPT H1 haplotype represents a mechanistically plausible but understudied genetic susceptibility factor for CTE. Given that CTE is pathologically defined by distinctive tau accumulation and H1 increases risk for multiple tauopathies, the hypothesis that H1 carriers show greater vulnerability to trauma-induced tau aggregation can be reasonably concluded. In particular, the H1 haplotype is associated with increased expression and specific ratios of tau protein isoforms, prone to aggregation in case of H1 carrier (Zhang et al. 13). Mechanisms that contribute to trauma-associated tau aggregation in H1 carriers could include direct damage to microtubules through mechanical impact and axonal shearing, leading to tau protein detachment and subsequent phosphorylation and aggregation due to intrinsic properties of tau in H1 carriers. Moreover, trauma-associated neuroinflammation triggers tau phosphorylation through certain kinases, whose activity could be enhanced due to specific features of H1-associated tau. Recent work has identified H1 overrepresentation in a large CTE cohort of 447 donors (Han et al. 3). However, older evidence found non-significant trends, therefore it is warranted for replication across many different population cohorts to determine a definitive conclusion of the MAPT haplotype being associated with dementia and tau burden in CTE and dementia (Bieniek et al. 1.).

Although there is a lack of direct evidence, variants of TREM2 could plausibly modify the risk of CTE. This could occur through effects on inflammatory responses post-trauma. Following TBI, microglia cells switch from homeostatic surveillance to being in an inactivated

state. This initially serves to provide protection as cellular debris is phagocytosed, damaged myelin is cleared, and dying neurons are removed (Loane & Kumar 3). However, if this response fails to resolve and turns into chronic activation, the inflammatory response that is initially for protection will become neurotoxic and may facilitate tau pathology (Loane & Kumar 6-7). TREM2 is necessary for pertinent microglial responses following TBI, regulating phagocytosis, reprogramming of metabolic functions, and inflammatory signaling. Variants that are deemed loss-of-function such as R47H impair these crucial processes and may lead to insufficient acute clearance of debris paralleled with impaired capacity to resolve inflammation post-initiation. From this, a prediction emerges: carriers of the TREM2 variant that undergo repetitive trauma to the head might reflect prolonged microglial activation that is detectable through neuroimaging, slower cognition recovery post-concussions, and cumulative severe effects from repeated impacts. Studies in cohorts consisting of athletic or military participants with genetic screening and longitudinal monitoring could test this hypothesis and could further refine the understanding of what functions of the microglia matter most for recovery following TBI and trauma.

6.2 Sleep

The glymphatic system, a brain-wide clearance pathway most active during sleep, is a crucial mechanism for removing waste and protein aggregates including amyloid-β, tau, and α-syn (Jessen et al. 1). During slow-wave sleep, interstitial space between neurons expands by approximately 60% which helps to flush waste into lymphatic drainage routes (Xie et al. 2). The glymphatic system also clears amyloid-β at twice the rate when individuals are asleep than when they are awake (Xie et al. 1). Moreover, disrupting sleep in animal models increases both amyloid-β and tau levels; Similarly, in humans, chronic sleep disruption and sleep loss increases the tau protein spread in the neural networks (Holth et al. 1).

Epidemiologically, sleep disruption such as insomnia, apnea, shift work is correlated with elevated risk of developing AD, PD, and cognitive decline, and is dose-dependent (Holth et al. 1). The pattern of sleep disruption causing neurodegeneration is consistent with insufficient glymphatic clearance (Holth et al. 1). As established, the glymphatic system fails to work efficiently when awake; therefore, sleep disruption creates a cumulative effect over time where proteins are misfolded and slowly outpace removal. That systematic malfunction causes accumulation of protein aggregates over decades.

The accumulation creates a genetic double deficit situation. APOE ε4 carriers already have reduced cellular amyloid-β clearance due to poor lipidation; therefore, if glymphatic flow is a primary backup route for clearance, carriers of the ε4 allele could be heavily dependent on good sleep to compensate. The same situation is applicable to TREM2 carriers who have impaired microglial phagocytosis and LRRK2 carriers. Carriers of either type might be particularly reliant upon glymphatic clearance because of their inefficient cellular clearance mechanism. This inefficiency makes it possible to redefine the notion of optimal sleep as an issue based on genotyping, where the people at greatest risk might need optimal treatment, such as combining cognitive behavioral therapy for insomnia (CBT-I), apnea testing, and continuous

positive airway pressure (CPAP) treatment, as well as proper adjustment of their circadian rhythms and not using sleep suppressant medicines like benzodiazepines.

However, open questions remain — which sleep metric matters most, and whether the critical intervention window is midlife or late-life. Nevertheless, sleep is an important factor with a favorable risk-benefit ratio regardless of genetic status, making early adoption reasonable.

6.3 Exercise

Aerobic exercise is a neuroprotective intervention due to its benefits: enhanced hippocampal volume (neurogenesis), improved cerebrovascular function, reduced neuroinflammation possibilities. Moreover, it could catalyze amyloid-β clearance through vascular and glymphatic routes. Physical activity that is moderate-to-vigorous has the ability to reduce risk of developing dementia (Guure et al. 2). Exercising also and mechanistically targets every genetic pathway discussed above: reduced activation of NLRP3 which compensates for TREM2 dysfunction, boosting AMPK-driven autophagy which compensates for LRRK2/APOE ε4 clearance deficiencies, and improving blood-brain barrier (BBB) integrity which can mitigate vascular effects of the ε4 allele.However, it is contested whether ε4 carriers are disproportionately benefited. Some longitudinal research studies found that the carriers of the ε4 allele had AD risk that was comparable to non-carriers that were inactive (Smith et al. 1-2). This finding suggests that exercise can nearly erase the genetic risks. Supporting this, a meta-analysis presented similar findings of protective effect sizes across individuals deems carriers and non-carriers rather than an advantage that is carrier-specific (Iso-Markku et al. 8). In many cohorts, this heterogeneity is reflective of differences in intensity and duration of physical activity, the age at which exercise is started, and underpowered interaction tests of gene-environment. Genetically-stratified randomized control trials (RCT) are a viable next step because of the low cost of exercise and minimal negative effects.

Resistance training, however, deserves separate mention for carriers of the MAPT H1 precisely because it conducts strong brain-derived neurotrophic factor (BDNF) increases. BDNF increases can ultimately reduce tau hyperphosphorylation, and the anti-inflammatory effects it poses can target the same inflammation-driven tau pathology tied to AD and CTE.

Combined aerobic and resistance programs plausibly provide synergistic benefit in the vascular and phosphorylation pathways. Although, it should be acknowledged that dosing and timing of the exercise plan is untested.

6.4 Diet

Dietary patterns can potentially modify risk factors for neurodegeneration. One of these identified dietary patterns is the Mediterranean diet (Med diet) and Mediterranean-Dietary Approaches to Stop Hypertension (DASH) Intervention for Neurodegenerative Delay (collectively, MIND Diet). This diet presents consistent protective factors in observational studies. The Med diet incorporates meals heavily emphasizing vegetables, fruits, whole grains, fish, olive oil, and nuts. The Med Diet also limits foods such as red meat and saturated fats.

Additionally, the MIND diet, a subset and specified diet in the Mediterranean food culture, prioritizes berries, leafy greens, and nuts. These are proven to show protective associations that reduce AD risk in long-term studies (Morris et al. 7). Such a response is dose-dependent; in fact, trials prove that MIND diet provides cognitive benefits even if started later in life (Rush University Medical Center). MIND mitigates inflammation and diabetes risk, improves insulin sensitivity, provides antioxidant polyphenols that prevent proteins from aggregating, supports healthy gut-brain axis, and provides omega-3 fatty acids and B vitamins which are crucial for membrane integrity and regulation of homocysteine (Ramezani et al. 10). The APOE genotype likely modifies these beneficial effects of the diet as ε4 carriers have altered lipid metabolism and may not have elevated omega-3 levels that are prevalent in non-carriers that have similar diets; therefore, the MIND diet's anti-inflammatory inducing properties and the omega-3-rich diet can be potentially more beneficial to non-carriers than carriers as they process lipids differently (Barberger-Gateau et al. 10). This tracks with the broader argument that is being made in this section: optimizing dietary fat sources could partially restore APOE ε4 function toward APOE ε3 levels through its role in transport of cholesterol as well as formation of lipid particles.

Beyond APOE, the same inflammatory logic is applicable across all three genes central to this paper: H1 is a potentially better substrate for kinases that are inflammation-activated, the APOE ε4 allele enables pro-inflammatory microglia cells, and TREM2 dysfunction disrupts inflammatory resolution. Therefore, a diet that is maximally anti-inflammatory may mostly benefit carriers of any of the three pro-inflammatory variants listed, while individuals with genotypes that are low-risk can tolerate a higher degree of dietary flexibility. Due to the MIND diet's emphasis on omega-3 and berries–specifically berry anthocyanins as they cross the BBB to reduce neuroinflammation and enhance autophagy–the diet maps onto the logic that risk-variant carriers can benefit directly (Banji et al. 1). Moreover, folate and vitamin K are supplied from the diet's leafy greens, improving vascular health and simultaneously addressing vascular dysfunction prevalent in ε4 carriers; the nuts provide polyphenols and vitamin E which reduce oxidative stress; the fatty fish contains omega-3 which may catalyze clearance of amyloid-beta; and finally, the olive oil's oleocanthal has anti-inflammatory action properties much like has ibuprofen and may decrease tau aggregation (Parkinson, Keast 2; Morris et al. 2, 7). The diet excludes red meat, fried food, and sweets (Morris et al. 13) which targets the same inflammatory and vascular pathways from opposite directions. The MIND Diet has benefits that could likely be additive or synergistic when paired with interventions of exercise and sleep. Following this dietary pattern may potentially provide collective and cumulative rewards across multiple mechanisms.

6.5 Convergent Explanation and Justification

Gene-environment interactions highlighted throughout this dsection–section 6–converge into a unified principle. Risk-genes, including APOE ε4, MAPT H1, and TREM2 variants, do not catalyze neurodegeneration on their own but modulate the slope of the relationships between environment exposure and disease risk (Migliore & Coppedè). Genotypes with low risk may

have a weak link between negative exposures and the disease, meaning they are able to handle sleep deprivation, inactivity, and/or inflammation-inducing diets without having a corresponding effect (Moser & Pike 8-9). Genotypes with high risks could have a stronger link between negative exposures and the disease, because with each of the negative exposures, there is a potential deficiency on the genetic level of clearance, inflammation control, and protein processing (Yamazaki et al. 40). Most importantly, the slope may function in both directions – as negative exposures negatively affect people with such genotypes, positive exposures will positively affect them. For example, exercise restores APOE ε4 clearance capacity, sleep makes up for lack of cellular clearance, anti-inflammatory foods and meals mitigate inflammatory amplification (Ali et al. 3-4).

This framework explains the need for precise prevention strategies where testing for genetics also enables interventions tailored to individual vulnerabilities. Individuals with higher risk would be identified through PRS and targeted genotyping may be utilized to construct multi-domain and intensive lifestyle interventions that simultaneously address diet, cognitive engagement, sleep, exercise, and vascular risk factors (Hwang et al. 5). The justification is that carriers of risk-genes exhibit deficits across not only one, but various pathways (Yamazaki et al. 40). This requires interventions targeting all mechanisms than approaches that target single-factors one at a time.

A potential design of this model would have to stratify intensity of the intervention and treatment type accordingly. In this plausible model, middle-risk individuals would undergo moderate treatment plans with minimal monitoring while low-risk individuals might undergo more general and non-gene-specific guidance. However, the complication that comes with this stratagem is the necessity of an evidentiary basis of high degrees of differentiation with randomized trials that incorporate genetic stratification; moreover, determining cost-effectivity of the strategies and intervention plans in smaller populations that are high-risk over moderate intervention plans that are applied more broadly as well as addressing ethical considerations around testing for genes and access to the prevention strategies would be equally difficult (Tolosa-tort et al. 2, 6, 7). Nevertheless, the gene-environment interaction evidence base, although incomplete, provides support that warrants pilot implementation research studies that test if genotype-informed plans improve outcomes than those compared to more standard approaches (Tolosa-tort et al. 6).

7. Implications

The insights described in precedent sections categorize into a three-pronged clinical strategy: polygenic risk scoring for early identification of neurodegeneration, lifestyle interventions that are informant by genotype basis, and finally, pharmacological approaches that target convergent pathways.

7.1 Polygenic Risk Scoring

Most risks of neurodegeneration are polygenic. Polygenic risk scoring (PRS) cumulates numerous variants into one liability score. For AD, inclusive of APOE, this score achieves area under the curve (AUC) values of 0.75–0.84 (Dickson et al. 7). This outperforms APOE alone. The top decile individuals have an increased risk of up to 3–4 times while the bottom decile individuals face 50–70% of reduced risk (Escott-Price et al. 1-2). PRS allows for screening earlier on, lifestyle counseling to mitigate risks, and could communicate on decisions of contact sports and return to play verdicts for CTE specifically. However, current scores of PRS cover and explain for only 30–50% of variance in disease, perform underwhelmingly in non-European ancestries, and create concerns surrounding privacy as well as genetic discrimination (Hoang et al. 10, 13-14). These limitations inhibit widespread implementation.

7.2 Pharmacological Approaches

The convergent pathways discussed in this paper suggest treatments and therapeutics that are more mechanism-based and have multi-disease potential. Inhibitors for NLRP3, autophagy activators, APOE modifiers, tau-targeting compounds, and TREM2 agonists all belong to specific genetic risk profiles (Sun et al. 6-8). Stratification of clinical trials on the basis of genetics such that genetically similar subjects rather than heterogeneous subjects are enrolled would result in larger effect sizes and smaller sample sizes (Torshizi & K. Wang 5).

7.3 Ethics and Equity

The Genetic Information Nondiscrimination Act (GINA) protection does not include life and long-term care insurance; therefore, it fails to protect individuals who have greater risks. Disparities in PRS for various ancestry groups can increase health disparities, as well as the disparity in access to quality intervention programs (Hoang et al. 13, 15, 22). The key is to use PRS appropriately, involving all groups in Genomewide association studies (GWAS) and keeping it accessible to everyone (Hoang et al. 25-26)..

8. Conclusion

Despite their distinct clinical presentations, AD, PD, and CTE share fundamental pathological roots that are driven by common genetic risk factors and convergent molecular pathways. Even with the unique features in these three conditions, it is clear that there are common origins of the diseases due to similar interactions of the gene-environment, and these are what cause the development of pathology using similar pathways. These two critical genes are MAPT and TREM2 because they control the development of predisposition of individuals to suffer from tauopathy and microglia-based neuroinflammation (T. Wang et al. 1, 3; Leyns et al. 1, 3). Given that high-effect variants may already permit risk stratification, integrating genetic and exposomic data offers opportunity for a future where precision neuroprotection can be achievable (Tolosa-tort et al. 5-6; Béchet et al. 9). Genetic analyses, biomarkers monitoring, and lifestyle interventions based on genetics can be performed and neurodegenerative conditions can hopefully be fought against before they even manifest.

Works Cited

Ali, Nada, et al. "APOE E4, Physical Activity, and the Brain: A Review of Systematic Reviews." Frontiers in Aging Neuroscience [Switzerland], vol. 18, July 2026, PubMed Central, p. 1798639, https://doi.org/10.3389/fnagi.2026.1798639.

Alzheimer's Association. "2020 Alzheimer's Disease Facts and Figures." Alzheimer's & Dementia, vol. 16, no. 3, Mar. 2020, pp. 391–460, https://doi.org/10.1002/alz.12068.

Atherton, Kathryn, et al. "Association of APOE Genotypes and Chronic Traumatic Encephalopathy." JAMA Neurology, vol. 79, no. 8, Aug. 2022, p. 787, https://doi.org/10.1001/jamaneurol.2022.1634.

Baek, Min Seok, et al. "Effect of APOE E4 Genotype on Amyloid-B and Tau Accumulation in Alzheimer's Disease." Alzheimer's Research & Therapy, vol. 12, no. 1, Oct. 2020, https://doi.org/10.1186/s13195-020-00710-6.

Bailey, Charles C, et al. "The Triggering Receptor Expressed on Myeloid Cells 2 Binds Apolipoprotein E." The Journal of Biological Chemistry [United States], vol. 290, no. 43, Oct. 2015, PubMed Central, pp. 26033–42, https://doi.org/10.1074/jbc.M115.677286.

Banji, Otilia J. F., et al. "Neuroinflammation: The Role of Anthocyanins as Neuroprotectants." Current Neuropharmacology [United Arab Emirates], vol. 20, no. 11, 2022, PubMed Central, pp. 2156–74, https://doi.org/10.2174/1570159X20666220119140835.

Barberger-Gateau, P, et al. "Dietary Omega 3 Polyunsaturated Fatty Acids and Alzheimer's Disease: Interaction With Apolipoprotein E Genotype." Current Alzheimer Research [United Arab Emirates], vol. 8, no. 5, Aug. 2011, PubMed Central, pp. 479–91, https://doi.org/10.2174/156720511796391926.

Barbier, Pascale, et al. "Role of Tau as a Microtubule-Associated Protein: Structural and Functional Aspects." Frontiers in Aging Neuroscience, vol. 11, Aug. 2019, https://doi.org/10.3389/fnagi.2019.00204.

Bendor, Jacob T., et al. "The Function of α-Synuclein." Neuron, vol. 79, no. 6, Sept. 2013, pp. 1044–66, https://doi.org/10.1016/j.neuron.2013.09.004.

Bereczki, Erika, et al. "Synaptic Markers of Cognitive Decline in Neurodegenerative Diseases: A Proteomic Approach." Brain, vol. 141, no. 2, Jan. 2018, pp. 582–95, https://doi.org/10.1093/brain/awx352.

Bieniek, Kevin F., et al. "Chronic Traumatic Encephalopathy Pathology in a Neurodegenerative Disorders Brain Bank." Acta Neuropathologica, vol. 130, no. 6, Oct. 2015, pp. 877–89, https://doi.org/10.1007/s00401-015-1502-4.

Buck, Silas A., and Laurie H. Sanders. "LRRK2-Mediated Mitochondrial Dysfunction in Parkinson's Disease." Biochemical Journal, vol. 482, no. 11, May 2025, pp. 721–39, https://doi.org/10.1042/bcj20253062.

Béchet, Sibylle, et al. "Precision Prevention for Neurodegenerative Diseases: Lessons Learnt From Alzheimer's Disease Translated Into Perspectives for Parkinson's Disease." Journal of Neural Transmission (Vienna, Austria : 1996) [Austria], vol. 133, no. 2, Feb. 2026, PubMed Central, pp. 265–77, https://doi.org/10.1007/s00702-025-03090-z.

Castellano, J. M., et al. “Human apoE Isoforms Differentially Regulate Brain Amyloid- Peptide Clearance.” Science Translational Medicine, vol. 3, no. 89, June 2011, pp. 89ra57–57, https://doi.org/10.1126/scitranslmed.3002156.

Catanese, Lisa. “What Is CTE? Understanding Chronic Traumatic Encephalopathy.” Harvard Health, 28 Mar. 2024, https://www.health.harvard.edu/mind-and-mood/what-is-cte-understanding-chronic-traumatic-encephalopathy.

Chen, Shufen, et al. “MAPT Isoforms: Differential Transcriptional Profiles Related to 3R and 4R Splice Variants.” Journal of Alzheimer’s Disease: JAD, vol. 22, no. 4, 2010, pp. 1313–29, https://doi.org/10.3233/JAD-2010-101155.

Cheng, Ying, and Feng Bai. “The Association of Tau With Mitochondrial Dysfunction in Alzheimer’s Disease.” Frontiers in Neuroscience, vol. 12, Mar. 2018, https://doi.org/10.3389/fnins.2018.00163.

Chou, Chi-Yuan, et al. “Structural Variation in Human Apolipoprotein E3 and E4: Secondary Structure, Tertiary Structure, and Size Distribution.” Biophysical Journal [United States], vol. 88, no. 1, Jan. 2005, PubMed Central, pp. 455–66, https://doi.org/10.1529/biophysj.104.046813.

Colonna, Marco, and Yaming Wang. “TREM2 Variants: New Keys to Decipher Alzheimer Disease Pathogenesis.” Nature Reviews Neuroscience, vol. 17, no. 4, Apr. 2016, pp. 201–07, https://doi.org/10.1038/nrn.2016.7.

Compta, Yaroslau, et al. “Lewy- and Alzheimer-Type Pathologies in Parkinson’s Disease Dementia: Which Is More Important?” Brain : A Journal of Neurology, vol. 134, no. 0 5, May 2011, pp. 1493–505, https://doi.org/10.1093/brain/awr031.

Cookson, Mark R. “LRRK2 Pathways Leading to Neurodegeneration.” Current Neurology and Neuroscience Reports, vol. 15, no. 7, May 2015, https://doi.org/10.1007/s11910-015-0564-y.

Deng, Hansen, et al. “Apolipoprotein E Epsilon 4 Genotype, Mild Traumatic Brain Injury, and the Development of Chronic Traumatic Encephalopathy.” Medical Sciences, vol. 6, no. 3, Sept. 2018, p. 78, https://doi.org/10.3390/medsci6030078.

Dickson, Dennis W., et al. “APOE E4 Is Associated With Severity of Lewy Body Pathology Independent of Alzheimer Pathology.” Neurology, vol. 91, no. 12, Aug. 2018, pp. e1182–95, https://doi.org/10.1212/wnl.0000000000006212.

Dickson, Samuel P, et al. “GenoRisk: A Polygenic Risk Score for Alzheimer’s Disease.” Alzheimer’s & Dementia (New York, N. Y.) [United States], vol. 7, no. 1, Sept. 2021, PubMed Central, p. e12211, https://doi.org/10.1002/trc2.12211.

Dimmer, Olivia . “Uncovering the Culprits of Neurodegenerative Disorders.” Northwestern University Feinberg School of Medicine News Center, Northwestern University Feinberg School of Medicine News Center, 11 Apr. 2024, https://news.feinberg.northwestern.edu/2024/04/11/uncovering-the-culprits-of-neurodegenerative-disorders/.

Doostparast Torshizi, Abolfazl, and Kai Wang. “Next-Generation Sequencing in Drug Development: Target Identification and Genetically Stratified Clinical Trials.” *Drug Discovery Today*, vol. 23, no. 10, Oct. 2018, pp. 1776–83, https://doi.org/10.1016/j.drudis.2018.05.015.

Duara, R, et al. “Effect of Age, Ethnicity, Sex, Cognitive Status and APOE Genotype on Amyloid Load and the Threshold for Amyloid Positivity.” *NeuroImage. Clinical* [Netherlands], vol. 22, 2019, PubMed Central, p. 101800, https://doi.org/10.1016/j.nicl.2019.101800.

Duranti, Elisa, and Chiara Villa. “Misfolded Proteins and Cognitive Decline: Mechanistic Insights Into Neurodegenerative Disorders.” *Neurology International*, vol. 18, no. 3, Mar. 2026, p. 48, https://doi.org/10.3390/neurolint18030048.

Escott-Price, V, et al. “Polygenic Risk Score Analysis of Alzheimer’s Disease in Cases Without APOE4 or APOE2 Alleles.” *The Journal of Prevention of Alzheimer’s Disease* [United States], vol. 6, no. 1, 2019, PubMed Central, pp. 16–19, https://doi.org/10.14283/jpad.2018.46.

Ezerskiy, Lubov A, et al. “Astrocytic 4R Tau Expression Drives Astrocyte Reactivity and Dysfunction.” JCI Insight [United States], vol. 7, no. 1, Jan. 2022, PubMed Central, p. e152012, https://doi.org/10.1172/jci.insight.152012.

Fan, Tian-Sin, et al. “Alpha-Synuclein and Cognitive Decline in Parkinson Disease.” Life, vol. 11, no. 11, Nov. 2021, p. 1239, https://doi.org/10.3390/life11111239.

Fesharaki-Zadeh, Arman. “Chronic Traumatic Encephalopathy: A Brief Overview.” Frontiers in Neurology, vol. 10, no. 713, July 2019, https://doi.org/10.3389/fneur.2019.00713.

Gan-Or, Ziv, et al. “Genetic Perspective on the Role of the Autophagy-Lysosome Pathway in Parkinson Disease.” Autophagy, vol. 11, no. 9, Sept. 2015, pp. 1443–57, https://doi.org/10.1080/15548627.2015.1067364.

Gao, Chao, et al. “Microglia in Neurodegenerative Diseases: Mechanism and Potential Therapeutic Targets.” Signal Transduction and Targeted Therapy, vol. 8, no. 1, Sept. 2023, pp. 1–37, https://doi.org/10.1038/s41392-023-01588-0.

Gardner, Raquel C., et al. “Mild TBI and Risk of Parkinson Disease.” Neurology, vol. 90, no. 20, May 2018, https://doi.org/10.1212/wnl.0000000000005522.

Gratuze, Maud, et al. “New Insights Into the Role of TREM2 in Alzheimer’s Disease.” Molecular Neurodegeneration [England], vol. 13, no. 1, Dec. 2018, PubMed Central, p. 66, https://doi.org/10.1186/s13024-018-0298-9.

Guure, Chris B., et al. “Impact of Physical Activity on Cognitive Decline, Dementia, and Its Subtypes: Meta-Analysis of Prospective Studies.” BioMed Research International, vol. 2017, 2017, pp. 1–13, https://doi.org/10.1155/2017/9016924.

Han, Xudong, et al. “A Structural Haplotype in the 17Q21.31 MAPT Region Is Associated With Increased Risk for Chronic Traumatic Encephalopathy Endophenotypes.” Cell Reports. Medicine [United States], vol. 6, no. 5, May 2025, PubMed Central, p. 102084, https://doi.org/10.1016/j.xcrm.2025.102084.

Healy, Daniel G, et al. “Phenotype, Genotype, and Worldwide Genetic Penetrance of LRRK2-Associated Parkinson’s Disease: A Case-Control Study.” The Lancet Neurology, vol. 7, no. 7, July 2008, pp. 583–90, https://doi.org/10.1016/s1474-4422(08)70117-0.

Heneka, Michael T., et al. “NLRP3 Is Activated in Alzheimer’s Disease and Contributes to Pathology in APP/PS1 Mice.” Nature, vol. 493, no. 7434, Dec. 2012, pp. 674–78, https://doi.org/10.1038/nature11729.

Hill, Emily, et al. “Understanding the Pathophysiological Actions of Tau Oligomers: A Critical Review of Current Electrophysiological Approaches.” Frontiers in Molecular Neuroscience, vol. 13, Aug. 2020, https://doi.org/10.3389/fnmol.2020.00155.

Hoang, Han, et al. “Polygenic Risk Scores: Navigating the Future of Precision Medicine Through Economic, Ethical, and Scientific Advancements.” iScience, vol. 29, no. 1, Dec. 2025, pp. 114375–75, https://doi.org/10.1016/j.isci.2025.114375.

Holth, Jerrah K., et al. “The Sleep-Wake Cycle Regulates Brain Interstitial Fluid Tau in Mice and CSF Tau in Humans.” Science, vol. 363, no. 6429, Feb. 2019, pp. 880–84, https://doi.org/10.1126/science.aav2546.

Hwang, Jiyun, et al. “Polygenicity and APOE E4 Shape Response to Intervention in Mild Cognitive Impairment.” Alzheimer’s Research & Therapy, vol. 17, no. 1, Dec. 2025, https://doi.org/10.1186/s13195-025-01907-3.

Iqbal, Khalid, et al. “Hyperphosphorylation-Induced Tau Oligomers.” Frontiers in Neurology, vol. 4, 2013, https://doi.org/10.3389/fneur.2013.00112.

Iso-Markku, Paula, et al. “Physical Activity as a Protective Factor for Dementia and Alzheimer’s Disease: Systematic Review, Meta-Analysis and Quality Assessment of Cohort and Case–Control Studies.” British Journal of Sports Medicine, vol. 56, no. 12, Mar. 2022, pp. bjsports–2021-104981, https://doi.org/10.1136/bjsports-2021-104981.

Jamir Pitton Rissardo, et al. “Alpha-Synuclein Neurobiology in Parkinson’s Disease: A Comprehensive Review of Its Role, Mechanisms, and Therapeutic Perspectives.” Brain Sciences, vol. 15, no. 12, Nov. 2025, pp. 1260–60, https://doi.org/10.3390/brainsci15121260.

Jessen, Nadia Aalling, et al. “The Glymphatic System: A Beginner’s Guide.” Neurochemical Research, vol. 40, no. 12, May 2015, pp. 2583–99, https://doi.org/10.1007/s11064-015-1581-6.

John Hopkins Medicine. “Early-Onset Alzheimer’s Disease.” Johns Hopkins Medicine, 2019, https://www.hopkinsmedicine.org/health/conditions-and-diseases/alzheimers-disease/earlyonset-alzheimer-disease.

Jonsson, Thorlakur, et al. “Variant of TREM2 Associated With the Risk of Alzheimer’s Disease.” The New England Journal of Medicine, vol. 368, no. 2, 2013, pp. 107–16, https://doi.org/10.1056/NEJMoa1211103.

Jordan, Barry D. “Apolipoprotein E ∈4 Associated With Chronic Traumatic Brain Injury in Boxing.” JAMA: The Journal of the American Medical Association, vol. 278, no. 2, July 1997, p. 136, https://doi.org/10.1001/jama.1997.03550020068040.

Kalia, Lorraine V, et al. “Clinical Correlations With Lewy Body Pathology in LRRK2-Related Parkinson Disease.” JAMA Neurology [United States], vol. 72, no. 1, Jan. 2015, PubMed Central, pp. 100–05, https://doi.org/10.1001/jamaneurol.2014.2704.

Katsumoto, Atsuko, et al. “Tau Pathology in Chronic Traumatic Encephalopathy and Alzheimer’s Disease: Similarities and Differences.” Frontiers in Neurology, vol. 10, Sept. 2019, https://doi.org/10.3389/fneur.2019.00980.

Klein, Marianne O., et al. “Dopamine: Functions, Signaling, and Association With Neurological Diseases.” Cellular and Molecular Neurobiology, vol. 39, no. 1, Nov. 2019, pp. 31–59, https://doi.org/10.1007/s10571-018-0632-3.

Klincheva, Milka, et al. “Endothelial Nitric Oxide Synthase T-786C Mutation, Prothrombin Gene Mutation (G-20210-A) and Protein S Deficiency Could Lead to Myocardial Infarction in a Very Young Male Adult.” Open Access Macedonian Journal of Medical Sciences [North Macedonia], vol. 4, no. 1, Mar. 2016, PubMed Central, pp. 142–45, https://doi.org/10.3889/oamjms.2016.014.

Krasemann, Susanne, et al. “The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases.” Immunity, vol. 47, no. 3, Sept. 2017, pp. 566–81.e9, https://doi.org/10.1016/j.immuni.2017.08.008.

Lanoiselée, Hélène-Marie, et al. “APP, PSEN1, and PSEN2 Mutations in Early-Onset Alzheimer Disease: A Genetic Screening Study of Familial and Sporadic Cases.” PLoS Medicine, vol. 14, no. 3, Mar. 2017, https://doi.org/10.1371/journal.pmed.1002270.

Leyns, Cheryl E. G., et al. “TREM2 Deficiency Attenuates Neuroinflammation and Protects Against Neurodegeneration in a Mouse Model of Tauopathy.” Proceedings of the National Academy of Sciences, vol. 114, no. 43, Oct. 2017, pp. 11524–29, https://doi.org/10.1073/pnas.1710311114.

Li, Rong, et al. “The Most Prevalent Rare Coding Variants of TREM2 Conferring Risk of Alzheimer’s Disease: A Systematic Review and Meta-Analysis.” Experimental and Therapeutic Medicine [Greece], vol. 21, no. 4, Apr. 2021, PubMed Central, p. 347, https://doi.org/10.3892/etm.2021.9778.

Li, Zonghua, et al. “APOE2: Protective Mechanism and Therapeutic Implications for Alzheimer’s Disease.” Molecular Neurodegeneration, vol. 15, no. 1, Nov. 2020, https://doi.org/10.1186/s13024-020-00413-4.

Lin, Heya, et al. “Damage-Associated Molecular Patterns (DAMPs) in Diseases: Implications for Therapy.” Molecular Biomedicine, vol. 6, no. 1, Aug. 2025, https://doi.org/10.1186/s43556-025-00305-3.

Lin, Yuan-Ta, et al. “APOE4 Causes Widespread Molecular and Cellular Alterations Associated With Alzheimer’s Disease Phenotypes in Human iPSC-Derived Brain Cell Types.” Neuron, vol. 98, no. 6, June 2018, pp. 1141–54.e7, https://doi.org/10.1016/j.neuron.2018.05.008.

Loane, David J., and Alok Kumar. “Microglia in the TBI Brain: The Good, the Bad, and the Dysregulated.” Experimental Neurology, vol. 275, no. Part 3, Jan. 2016, pp. 316–27, https://doi.org/10.1016/j.expneurol.2015.08.018.

Macedo, Arthur C, et al. “The Use of Tau PET to Stage Alzheimer Disease According to the Braak Staging Framework.” The Journal of Nuclear Medicine, vol. 64, no. 8, June 2023, pp. jnumed.122.265200–jnumed.122.265200, https://doi.org/10.2967/jnumed.122.265200.

Madureira, Marta, et al. “‘LRRK2: Autophagy and Lysosomal Activity.’” Frontiers in Neuroscience, vol. 14, May 2020, https://doi.org/10.3389/fnins.2020.00498.

Mahley, Robert W., and Yadong Huang. “Apolipoprotein E Sets the Stage: Response to Injury Triggers Neuropathology.” Neuron, vol. 76, no. 5, Dec. 2012, pp. 871–85, https://doi.org/10.1016/j.neuron.2012.11.020.

Mayeux, R., et al. “Synergistic Effects of Traumatic Head Injury and Apolipoprotein-Epsilon 4 in Patients With Alzheimer’s Disease.” Neurology, vol. 45, no. 3 Pt 1, Mar. 1995, pp. 555–57, https://doi.org/10.1212/wnl.45.3.555.

Mayo Clinic. “Alzheimer’s Stages: How the Disease Progresses.” Mayo Clinic, 7 June 2023, https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/in-depth/alzheimers-stages/art-20048448 .

McFadyen, Charles A, et al. “Apolipoprotein E4 Polymorphism and Outcomes From Traumatic Brain Injury: A Living Systematic Review and Meta-Analysis.” Journal of Neurotrauma [United States], vol. 38, no. 8, Apr. 2021, PubMed Central, pp. 1124–36, https://doi.org/10.1089/neu.2018.6052.

McKee, Ann C, et al. “Neuropathologic and Clinical Findings in Young Contact Sport Athletes Exposed to Repetitive Head Impacts.” JAMA Neurology, vol. 80, no. 10, Aug. 2023, https://doi.org/10.1001/jamaneurol.2023.2907.

McKee, Ann C., Thor D. Stein, Bertrand R. Huber, et al. “Chronic Traumatic Encephalopathy (CTE): Criteria for Neuropathological Diagnosis and Relationship to Repetitive Head Impacts.” Acta Neuropathologica, vol. 145, no. 4, Feb. 2023, https://doi.org/10.1007/s00401-023-02540-w.

McKee, Ann C., Thor D. Stein, Patrick T. Kiernan, et al. “The Neuropathology of Chronic Traumatic Encephalopathy.” Brain Pathology, vol. 25, no. 3, Apr. 2015, pp. 350–64, https://doi.org/10.1111/bpa.12248.

Migliore, Lucia, and Fabio Coppedè. “Gene–Environment Interactions in Alzheimer Disease: The Emerging Role of Epigenetics.” Nature Reviews Neurology, vol. 18, no. 11, Sept. 2022, pp. 1–18, https://doi.org/10.1038/s41582-022-00714-w.

Moehle, M. S., et al. “LRRK2 Inhibition Attenuates Microglial Inflammatory Responses.” Journal of Neuroscience, vol. 32, no. 5, Feb. 2012, pp. 1602–11, https://doi.org/10.1523/jneurosci.5601-11.2012.

Moloney, Christina M., et al. “Visualization of Neurofibrillary Tangle Maturity in Alzheimer’s Disease: A Clinicopathologic Perspective for Biomarker Research.” Alzheimer’s & Dementia, vol. 17, no. 9, Apr. 2021, https://doi.org/10.1002/alz.12321.

Morris, Martha Clare, et al. “MIND Diet Associated With Reduced Incidence of Alzheimer’s Disease.” Alzheimer’s & Dementia : The Journal of the Alzheimer’s Association, vol. 11, no. 9, 2015, pp. 1007–14, https://doi.org/10.1016/j.jalz.2014.11.009.

Moser, V. Alexandra, and Christian J. Pike. “Obesity Accelerates Alzheimer-Related Pathology in APOE4 but Not APOE3 Mice.” Eneuro, vol. 4, no. 3, May 2017, pp. ENEURO.0077–17.2017, https://doi.org/10.1523/eneuro.0077-17.2017.

Multhaup, Gerhard, et al. “Amyloid Precursor Protein (APP) Metabolites APP Intracellular Fragment (AICD), Aβ42, and Tau in Nuclear Roles.” Journal of Biological Chemistry, vol. 290, no. 39, Aug. 2015, pp. 23515–22, https://doi.org/10.1074/jbc.r115.677211.

Munakomi, Sunil, and Yana Puckett. “Repetitive Head Injury Syndrome.” PubMed, StatPearls Publishing, 2021, https://www.ncbi.nlm.nih.gov/books/NBK541013/.

National Institute of Neurological Disorders and Stroke. “Parkinson’s Disease.” National Institute of Neurological Disorders and Stroke, National Institute of Neurological Disorders and Stroke, 5 Mar. 2025, https://www.ninds.nih.gov/health-information/disorders/parkinsons-disease.

Nichols, Emma, et al. “Estimation of the Global Prevalence of Dementia in 2019 and Forecasted Prevalence in 2050: An Analysis for the Global Burden of Disease Study 2019.” The Lancet Public Health, vol. 7, no. 2, Jan. 2022, https://doi.org/10.1016/s2468-2667(21)00249-8.

Panda, Dulal, et al. “Differential Regulation of Microtubule Dynamics by Three- and Four-Repeat Tau: Implications for the Onset of Neurodegenerative Disease.” Proceedings of the National Academy of Sciences, vol. 100, no. 16, July 2003, pp. 9548–53, https://doi.org/10.1073/pnas.1633508100.

Parkinson, Lisa, and Russell Keast. “Oleocanthal, a Phenolic Derived From Virgin Olive Oil: A Review of the Beneficial Effects on Inflammatory Disease.” International Journal of Molecular Sciences [Switzerland], vol. 15, no. 7, July 2014, PubMed Central, pp. 12323–34, https://doi.org/10.3390/ijms150712323.

Ramezani, Fatemeh, et al. “The Gut Microbiota as a Mediator Linking the MIND Diet to Alzheimer’s Disease.” Nutrients [Switzerland], vol. 18, no. 9, Apr. 2026, PubMed Central, p. 1445, https://doi.org/10.3390/nu18091445.

Raulin, Ana-Caroline, et al. “The Molecular Basis for Apolipoprotein E4 as the Major Risk Factor for Late-Onset Alzheimer’s Disease.” Journal of Molecular Biology, vol. 431, no. 12, May 2019, pp. 2248–65, https://doi.org/10.1016/j.jmb.2019.04.019.

Ren, Chao, et al. “G2019S Variation in LRRK2: An Ideal Model for the Study of Parkinson’s Disease?” Frontiers in Human Neuroscience [Switzerland], vol. 13, Sept. 2019, PubMed Central, p. 306, https://doi.org/10.3389/fnhum.2019.00306.

Roosen, Dorien A., and Mark R. Cookson. “LRRK2 at the Interface of Autophagosomes, Endosomes and Lysosomes.” Molecular Neurodegeneration, vol. 11, no. 1, Dec. 2016, https://doi.org/10.1186/s13024-016-0140-1.

Rush University Medical Center. “New MIND Diet May Significantly Protect Against Alzheimer’s Disease.” Www.Rush.Edu, 16 Mar. 2016, https://www.rush.edu/news/new-mind-diet-may-significantly-protect-against-alzheimers-disease.

Sadikoglou, Eldem, et al. “Chr:17Q21.31 Locus Risk Haplotype H1 Susceptibility to Ferroptosis Is Mediated by Endolysosomal Pathway.” Cell Death & Disease [England], vol. 16, no. 1, Nov. 2025, PubMed Central, p. 828, https://doi.org/10.1038/s41419-025-08147-1.

Schoch, Kathleen M, et al. “Increased 4R-Tau Induces Pathological Changes in a Human-Tau Mouse Model.” Neuron [United States], vol. 90, no. 5, June 2016, PubMed Central, pp. 941–47, https://doi.org/10.1016/j.neuron.2016.04.042.

Seath, Paige, et al. “Clinical Characteristics of Early-Onset Versus Late-Onset Alzheimer’s Disease: A Systematic Review and Meta-Analysis.” International Psychogeriatrics, vol. 36, no. 12, July 2023, pp. 1–17, https://doi.org/10.1017/S1041610223000509.

Shi, Qian, et al. “Microglia, Trem2, and Neurodegeneration.” The Neuroscientist, vol. 31, no. 2, May 2024, https://doi.org/10.1177/10738584241254118.

Shively, Sharon, et al. “Dementia Resulting From Traumatic Brain Injury.” Archives of Neurology, vol. 69, no. 10, Oct. 2012, https://doi.org/10.1001/archneurol.2011.3747.

Shokr, Mustafa M. “‘Rewiring Brain Immunity: Targeting Microglial Metabolism for Neuroprotection in Neurodegenerative Disorders.’” Metabolic Brain Disease, vol. 40, no. 8, Nov. 2025, https://doi.org/10.1007/s11011-025-01739-y.

Singh, Preman J, et al. “Amyloid-B Disrupts APP-Regulated Protein Aggregation and Dissociation From Recycling Endosomal Membranes.” The EMBO Journal, vol. 44, no. 16, July 2025, https://doi.org/10.1038/s44318-025-00497-y.

Sirkis, Daniel W., et al. “Rare TREM2 Variants Associated With Alzheimer’s Disease Display Reduced Cell Surface Expression.” Acta Neuropathologica Communications, vol. 4, no. 1, Sept. 2016, https://doi.org/10.1186/s40478-016-0367-7.

Smith, J. Carson, et al. “Physical Activity Reduces Hippocampal Atrophy in Elders at Genetic Risk for Alzheimer’s Disease.” Frontiers in Aging Neuroscience, vol. 6, Apr. 2014, https://doi.org/10.3389/fnagi.2014.00061.

Steger, Martin, et al. “Phosphoproteomics Reveals That Parkinson’s Disease Kinase LRRK2 Regulates a Subset of Rab GTPases.” eLife, vol. 5, Jan. 2016, https://doi.org/10.7554/elife.12813.

Stern, R. A., et al. “Clinical Presentation of Chronic Traumatic Encephalopathy.” Neurology, vol. 81, no. 13, Aug. 2013, pp. 1122–29, https://doi.org/10.1212/wnl.0b013e3182a55f7f.

Sun, Kerry T., and Sue-Ann Mok. “Inducers and Modulators of Protein Aggregation in Alzheimer’s Disease - Critical Tools for Understanding the Foundations of Aggregate

Structures." Neurotherapeutics, vol. 22, no. 3, Jan. 2025, p. e00512, https://doi.org/10.1016/j.neurot.2024.e00512.
Sun, Liyuan, et al. "Autophagy–Neuroinflammation Axis in Neurodegenerative Diseases: Mechanisms and Therapeutic Potential." Cells, vol. 15, no. 9, Apr. 2026, p. 813, https://doi.org/10.3390/cells15090813.
Sánchez-Juan, Pascual, et al. "The MAPT H1 Haplotype Is a Risk Factor for Alzheimer's Disease in APOE E4 Non-Carriers." Frontiers in Aging Neuroscience, vol. 11, Dec. 2019, https://doi.org/10.3389/fnagi.2019.00327.
Tharmaratnam, Tharmegan, et al. "Chronic Traumatic Encephalopathy in Professional American Football Players: Where Are We Now?" Frontiers in Neurology, vol. 9, June 2018, https://doi.org/10.3389/fneur.2018.00445.
Tiwari, Sneham, et al. "Alzheimer's Disease: Pathogenesis, Diagnostics, and Therapeutics." International Journal of Nanomedicine, vol. 14, no. 1, July 2019, pp. 5541–54, https://doi.org/10.2147/ijn.s200490.
Tolosa-Tort, Paulina, et al. "Toward Personalized Medicine in AD/ADRD Through Genetic-Exposome Dementia Risk Assessments." Npj Dementia, vol. 2, no. 1, Aug. 2026, https://doi.org/10.1038/s44400-026-00087-7.
Tsuang, Debby, et al. "APOE E4 Increases Risk for Dementia in Pure Synucleinopathies." JAMA Neurology, vol. 70, no. 2, Feb. 2013, p. 223, https://doi.org/10.1001/jamaneurol.2013.600.
Valastyan, J. S., and S. Lindquist. "Mechanisms of Protein-Folding Diseases at a Glance." Disease Models & Mechanisms, vol. 7, no. 1, Jan. 2014, pp. 9–14, https://doi.org/10.1242/dmm.013474.
Wang, Tianqing, et al. "TREM2 and Microglial Immunity in Alzheimer's Disease: Mechanisms, Genetics, and Therapeutic Opportunities." Frontiers in Immunology [Switzerland], vol. 17, Feb. 2026, PubMed Central, p. 1739875, https://doi.org/10.3389/fimmu.2026.1739875.
Wang, Yaming, et al. "TREM2 Lipid Sensing Sustains the Microglial Response in an Alzheimer's Disease Model." Cell, vol. 160, no. 6, Mar. 2015, pp. 1061–71, https://doi.org/10.1016/j.cell.2015.01.049.
Williams-Gray, Caroline H, et al. "Apolipoprotein E Genotype as a Risk Factor for Susceptibility to and Dementia in Parkinson's Disease." Journal of Neurology, vol. 256, no. 3, Mar. 2009, pp. 493–98, https://doi.org/10.1007/s00415-009-0119-8.
Xie, L., et al. "Sleep Drives Metabolite Clearance From the Adult Brain." Science, vol. 342, no. 6156, Oct. 2013, pp. 373–77, https://doi.org/10.1126/science.1241224.
Xiong, Monica, et al. "Astrocytic APOE4 Removal Confers Cerebrovascular Protection Despite Increased Cerebral Amyloid Angiopathy." Molecular Neurodegeneration [England], vol. 18, no. 1, Mar. 2023, PubMed Central, p. 17, https://doi.org/10.1186/s13024-023-00610-x.

Yamazaki, Yu, et al. “Apolipoprotein E and Alzheimer Disease: Pathobiology and Targeting Strategies.” Nature Reviews Neurology, vol. 15, no. 9, July 2019, pp. 501–18, https://doi.org/10.1038/s41582-019-0228-7.

Yeh, Felix L., et al. “TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia.” Neuron, vol. 91, no. 2, July 2016, pp. 328–40, https://doi.org/10.1016/j.neuron.2016.06.015.

Zafar, Saman, and Sridhara S Yaddanapudi. “Parkinson Disease.” Nih.Gov, StatPearls Publishing, 2023, https://www.ncbi.nlm.nih.gov/books/NBK470193/.

Zhang, Yi, et al. “Tauopathies: New Perspectives and Challenges.” Molecular Neurodegeneration, vol. 17, Apr. 2022, p. 28, https://doi.org/10.1186/s13024-022-00533-z.

Zhao, Yi, et al. “LRRK2 Variant Associated With Alzheimer’s Disease.” Neurobiology of Aging, vol. 32, no. 11, Nov. 2011, pp. 1990–93, https://doi.org/10.1016/j.neurobiolaging.2009.11.019.

Zheng, Qiuyang, and Xin Wang. “Alzheimer’s Disease: Insights Into Pathology, Molecular Mechanisms, and Therapy.” Protein & Cell, vol. 16, no. 2, May 2024, https://doi.org/10.1093/procel/pwae026.

Zhou, Weidong, et al. “Meta-Analysis of APOE4 Allele and Outcome After Traumatic Brain Injury.” Journal of Neurotrauma [United States], vol. 25, no. 4, Apr. 2008, PubMed, pp. 279–90, https://doi.org/10.1089/neu.2007.0489.

Zhou, Yingyue, et al. “TREM2-Dependent Effects on Microglia in Alzheimer’s Disease.” Frontiers in Aging Neuroscience, vol. 10, July 2018, https://doi.org/10.3389/fnagi.2018.00202.

Zimprich, Alexander, et al. “Mutations in LRRK2 Cause Autosomal-Dominant Parkinsonism With Pleomorphic Pathology.” Neuron, vol. 44, no. 4, Nov. 2004, pp. 601–07, https://doi.org/10.1016/j.neuron.2004.11.005.

Enhancement of ELISA Efficiency through Thermal Variation in T4 Assays
By Arthur Tseng and Joohee Seo

Abstract

ELISA (Enzyme-linked Immunosorbent Assay) is a common lab technology used to quantify biological compounds such as hormones, proteins, or antibodies in samples. In a biological or healthcare setting, T4 (Thyroxine) ELISA kits are used to measure thyroid hormone concentrations in a blood or biological sample. The standard procedure is to incubate the reaction mixture at room temperature for a total of 55 minutes to ensure correct binding between biological components as well as enzyme-conjugated antibodies. This experiment investigated whether incubation time could be reduced to an optimal time if the standard room temperature was increased to 55°C. We hypothesized that increasing the incubation temperature to 55°C would reduce the required incubation time without risking assay accuracy. Initial experiments assessed the effects of temperature at a constant incubation time, after which a wider range of temperatures was examined. The final experiment analyzed calibration curves from 45 wells grouped by temperature (−20°C, 21°C, and 55°C), with Standards A–E from the T4 ELISA kit incubated for 5, 15, or 30 minutes at each temperature. The data demonstrated that higher temperatures, to an extent, had a significant impact in reducing incubation times without any effect on accuracy. The calibration curve for samples incubated at 55°C for 15 minutes had higher OD range, indicating that higher temperatures were optimal for speeding up assay activity. Incubating at high temperatures could help reduce incubation times in a healthcare setting for faster diagnosis and detection of biomarkers.

Introduction

ELISA (Enzyme-linked Immunosorbent Assay) kits are designed to detect and measure soluble substances such as antigens, antibodies, hormones, and proteins (ThermoFisher Scientific). The mechanism of the competitive ELISA involves an antigen binding to an antibody that is immobilized to a solid surface, typically the bottom surface of a well. The sample that may contain the substance of interest is added to the plate, competing with a conjugated protein. Either the conjugated protein or the protein of interest will bind to the immobilized antibody. After disposing of unbound substances, a chosen substrate is added to the plate, and it reacts with the enzyme to produce a visible color change. This resulting color is then measured through a spectrophotometer, allowing researchers to measure the concentration of the target molecule in the sample with respect to a calibration curve using standard solutions (Thomas; Intended Use).

T4 (Thyroxine) ELISA kits are commonly used in the medical field to accurately measure the concentration of thyroid hormone T4 in biological samples to assess thyroid illnesses (Thyroxine T4 Free). T4 is a vital thyroid hormone secreted by the thyroid gland, serving as a biomarker for assessing thyroid function (hypothyroidism/hyperthyroidism). T4 functions in the body by regulating metabolism, affecting energy use, heart rate, digestion, body temperature, and brain development. T4 ELISA kits use anti-T4 antibodies to produce a color

change, and the intensity of this color change is measured with a spectrophotometer for quantitative results (Thyroxine (T4) Elisa). A lower concentration of T4 hormones results in a dark blue color, while higher concentrations result in a light blue/transparent hue, which can be used to indicate hypothyroidism with low T4 and hyperthyroidism with high T4.

A typical T4 ELISA kit suggested room temperature as the optimal temperature and took a total of 55 minutes for incubation and further time for the entire procedure and to observe the results. Despite this efficient use of the ELISA kits, this could pose a long time before yielding results. As temperatures rose to an optimal level, enzymes worked faster. Higher heat boosted kinetic energy, which caused enzymes-substrate collisions to increase, therefore speeding up the reactions ("How Temperature Affects Enzyme Activity"). It was currently unknown whether increasing sample temperature could reduce the usual 30-minute incubation time before adding the substrate without skewing assay accuracy. We hypothesized that raising the temperature to a certain threshold would allow the reaction to proceed quicker, therefore reducing the diagnosis time. It is known that enzymes are temperature-sensitive; when temperature increases, it raises the energy level in biological particles, which in turn increases interactions between enzymes and biological particles. We tested whether raising temperatures from room temperature to 55°C would lessen the incubation time needed for T4 ELISA kits. After conducting this experiment, we concluded that 55°C was the optimal incubation temperature for T4 ELISA assays.

Results

We concluded that the application of higher temperatures decreased the time needed to incubate the T4 sample. We performed two separate experiments to determine whether temperature affected the time needed for incubation.

We found similar ratios between the optical densities for the samples with the 15-minute incubation time and the samples with the 55°C. Looking at the two data sets, the ratio between the first column of Table 3, which were the results for the 15-minute incubation time, and the third column of Table 2, which were the results for the samples incubated in a 55°C environment, the ratios were similar for Standards A and C. For example, for the first reading of Standard A in Table 3, which was measuring incubation time, the value read 0.307. The first reading for Standard A in Table 2, which was measuring temperature, read 1.044. After calculating the ratio between the two values, it produced 1:3.4. If this same value was multiplied with the value for Standard C in the first reading, it multiplied 0.055 by 3.4, which resulted in 0.187, which was similar to 0.205. The following ratio could be used to compare the first column results for all standards in Table 1 and 2 to column 3 of Table 2, which were the results of the 55°C incubation setting.

		Table 1		
17-Nov	**Trial #1**	Same incubation time, different temp		
First Reading		4°C	21°C	60°C
	A	0.531	0.554	0.95
	C	0.117	0.146	0.146
	E	0.024	0.027	0.052
Second Reading		4°C	21°C	60°C
	A	0.568	0.562	1.003
	C	0.135	0.15	0.156
	E	0.027	0.031	0.056
Third Reading		4°C	21°C	60°C
	A	0.601	0.577	0.975
	C	0.132	0.196	0.173
	E	0.028	0.032	0.058
AVERAGE		4°C	21°C	60°C
	Standard A	0.567	0.564	0.976
	Standard C	0.128	0.164	0.158
	Standard E	0.026	0.03	0.055

Table 1: Optical density readings testing temperatures (4°C, 21°C, and 60°C) at a constant incubation time

	Table 2			
11/19/25	**Trial #2**		Same incubation time, different temp	
First Reading		-20°C	21°C	55°C
	A	0.271	0.684	1.044
	C	0.101	0.173	0.205
	E	0.031	0.037	0.048
Second Reading		-20°C	21°C	55°C
	A	0.279	0.639	0.931
	C	0.107	0.176	0.213
	E	0.034	0.036	0.05
Third Reading		-20°C	21°C	55°C
	A	0.282	0.652	0.958
	C	0.105	0.174	0.209
	E	0.033	0.036	0.05
AVERAGE		-20°C	21°C	55°C
	Standard A	0.277	0.658	0.978
	Standard C	0.104	0.174	0.209
	Standard E	0.033	0.036	0.049

Table 2: Optical density readings testing temperatures (-20°C, 21°C, and 55°C) at a constant incubation time

		Table 3	
25-Nov **Trial #1**		Same temp, different incubation time	
First Reading		1	2
	A	0.307	0.401
	C	0.055	0.083
	E	0.033	0.022
Second Reading		1	2
	A	0.272	0.508
	C	0.063	0.111
	E	0.03	0.026
Third Reading		1	2
	A	0.291	0.536
	C	0.07	0.117
	E	0.031	0.027
AVERAGE		15	30
	Standard A	0.29	0.482
	Standard C	0.063	0.104
	Standard E	0.031	0.025

Table 3: Optical density readings testing incubation times (15 min vs 30 min) at a constant room temperature (21°C)

As T4 concentration increased, OD values decreased across all temperatures and incubation times as they were controlled standards (Figures 1–9). The calibration curve for -20°C for 30 minutes incubation time (Figure 3) had around the same amount of OD range compared to the calibration curve for 55°C for 5-minute incubation time (Figure 4). Cold temperature assays showed minimal OD separation at 5 minutes but improved separation at 30 minutes (Figure 3). Calibration curves at elevated temperatures displayed higher OD ranges. Most importantly, the graph at 55°C at 15 minutes displayed a wide OD range.

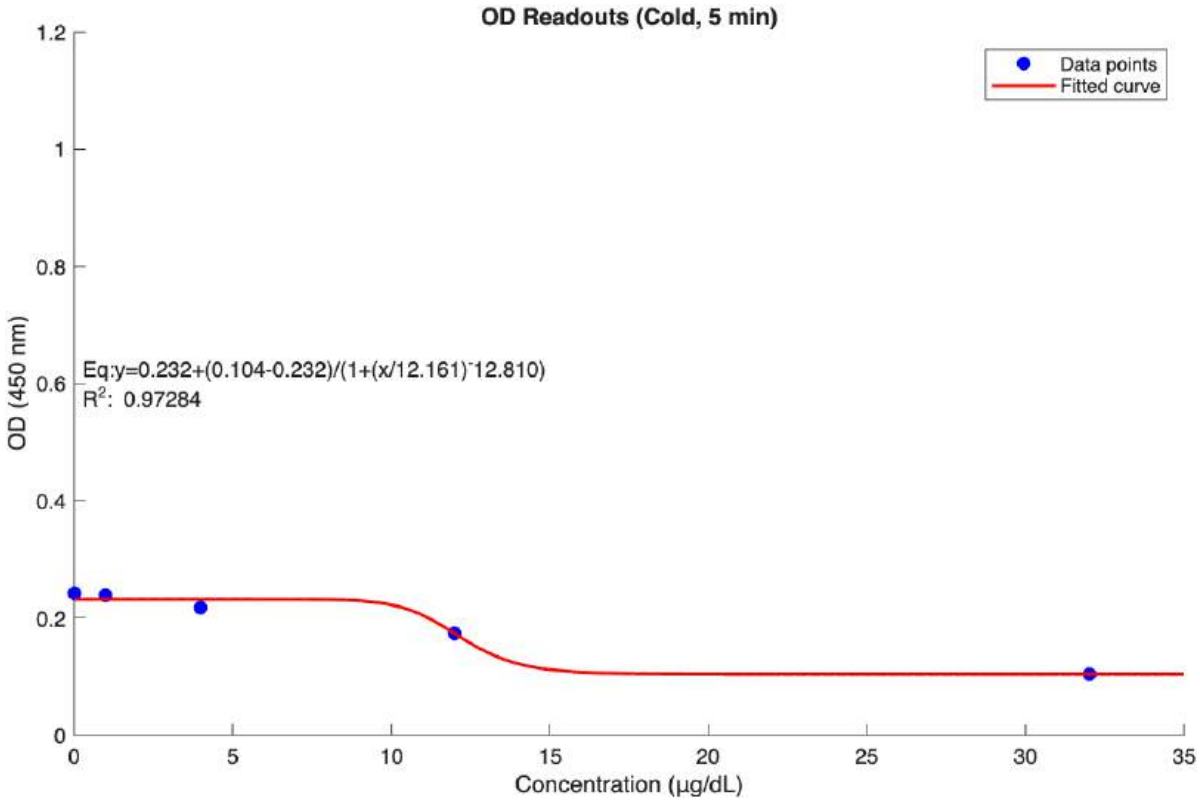


Figure 1: Calibration curve at −20°C with a 5-minute incubation time

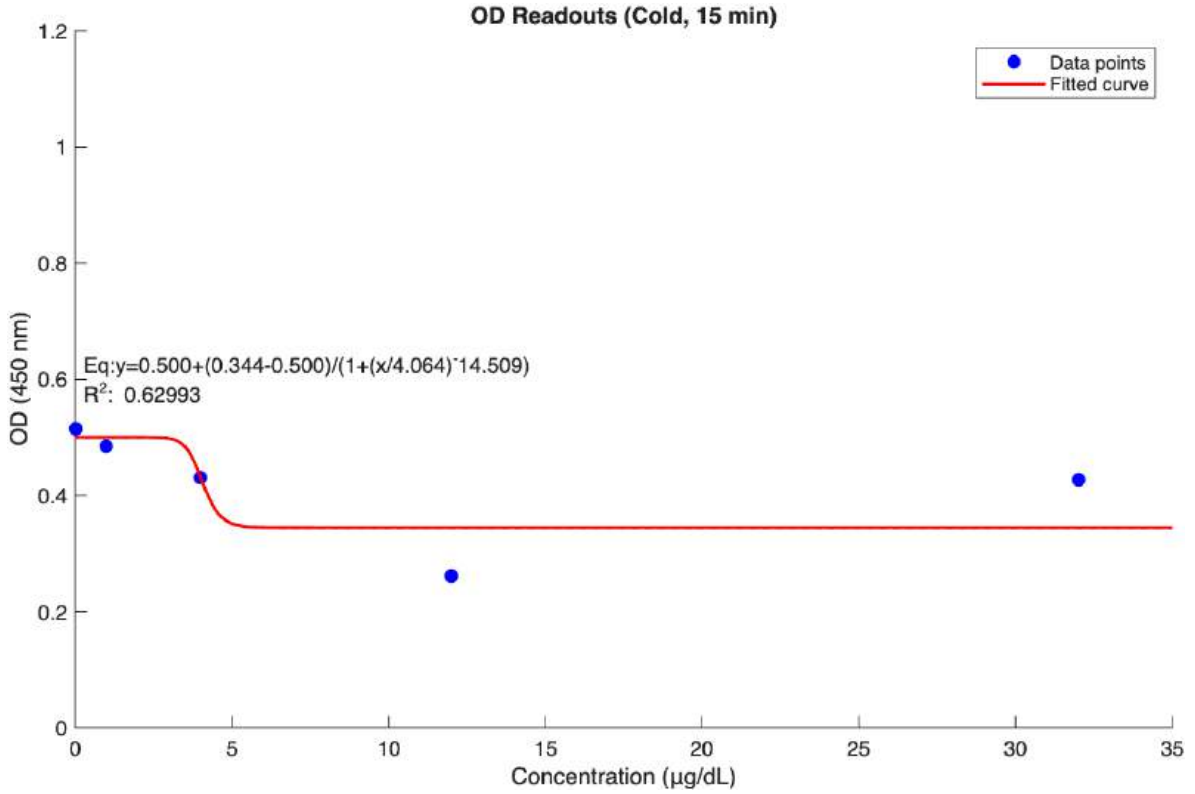


Figure 2: Calibration curve at −20°C with a 15-minute incubation time

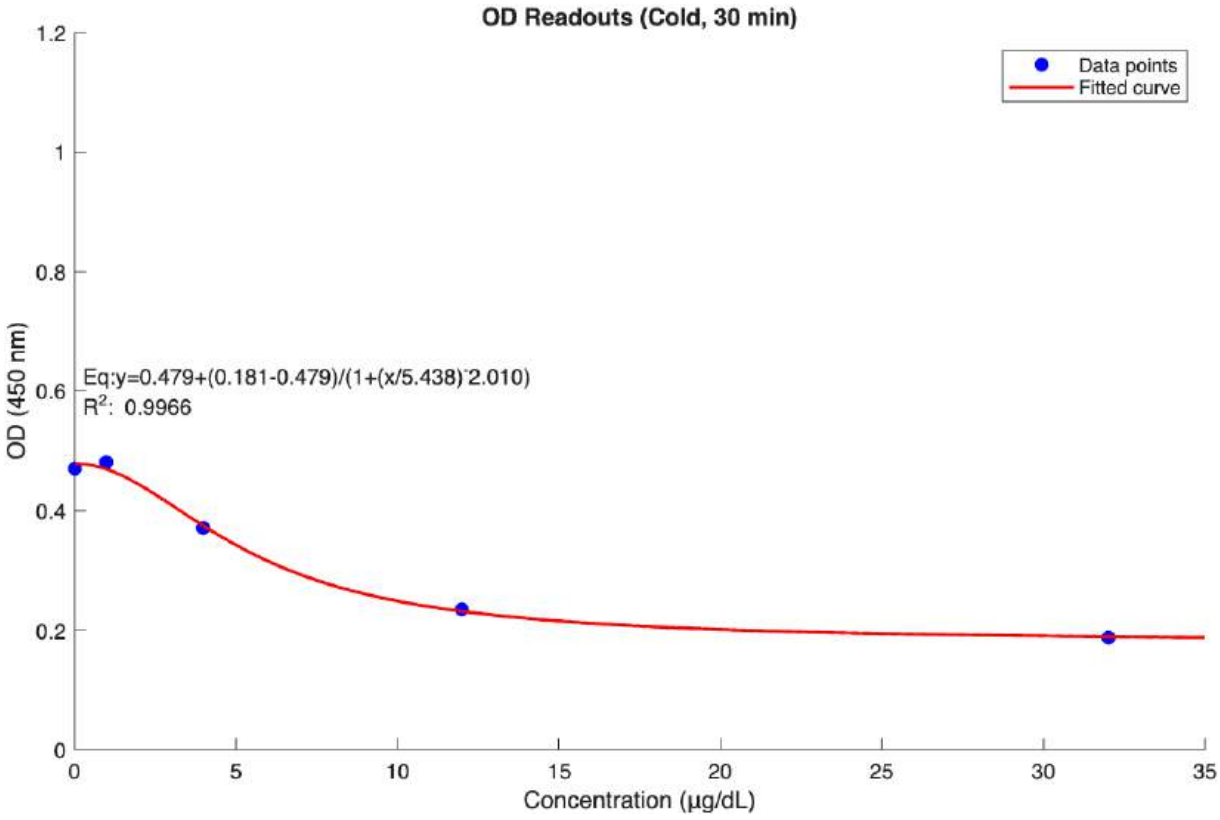


Figure 3: Calibration curve at −20°C with a 30-minute incubation time

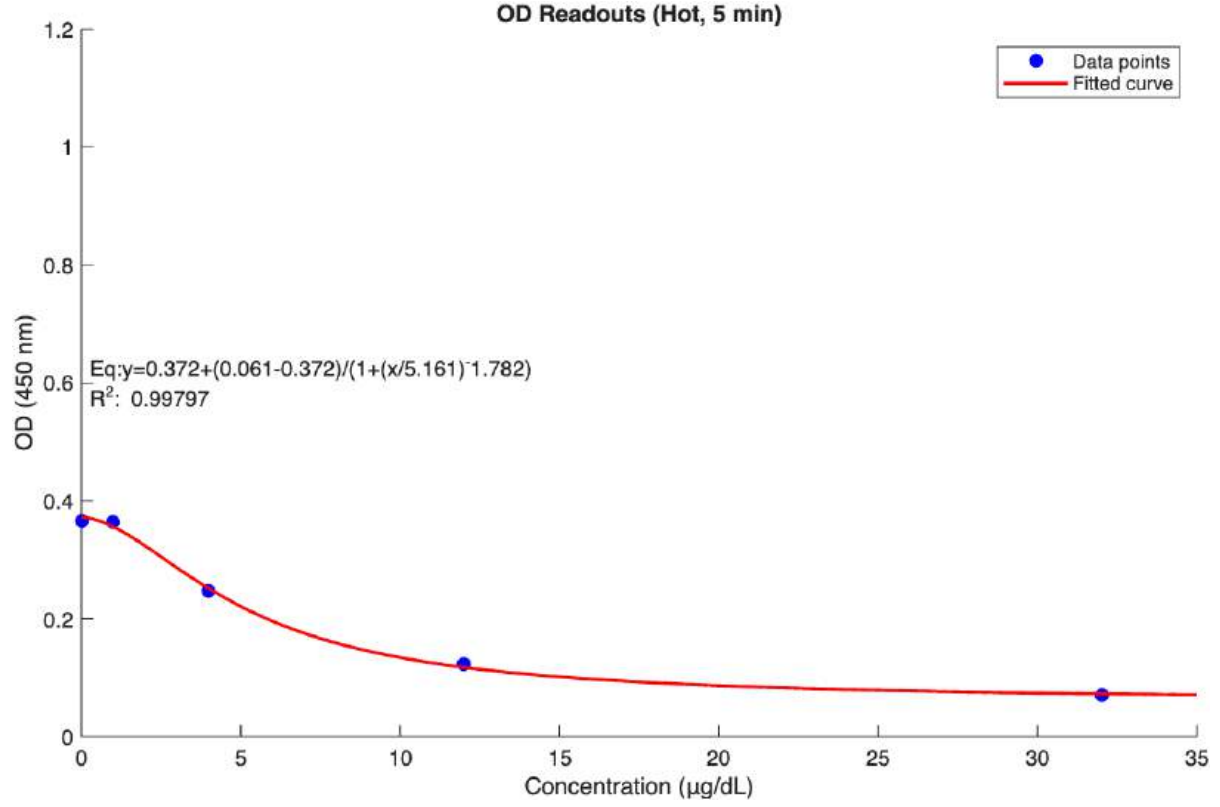


Figure 4: Calibration curve at 55°C with a 5-minute incubation time

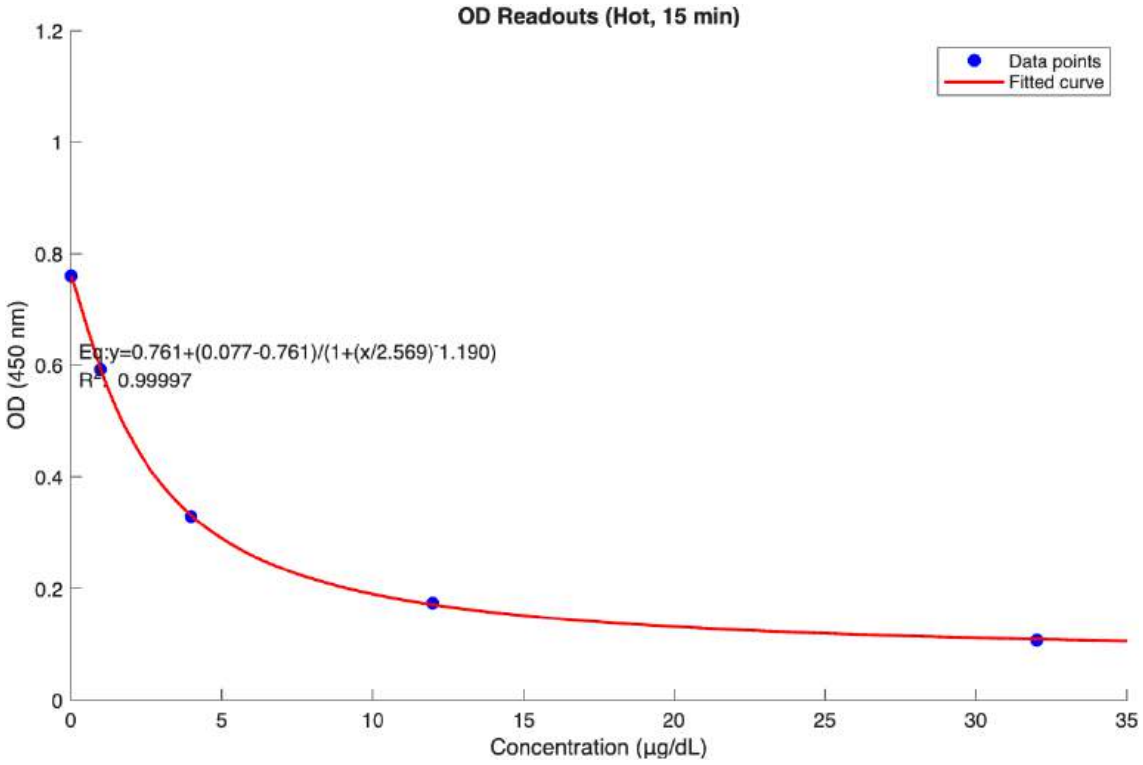


Figure 5: Calibration curve at 55°C with a 15-minute incubation time

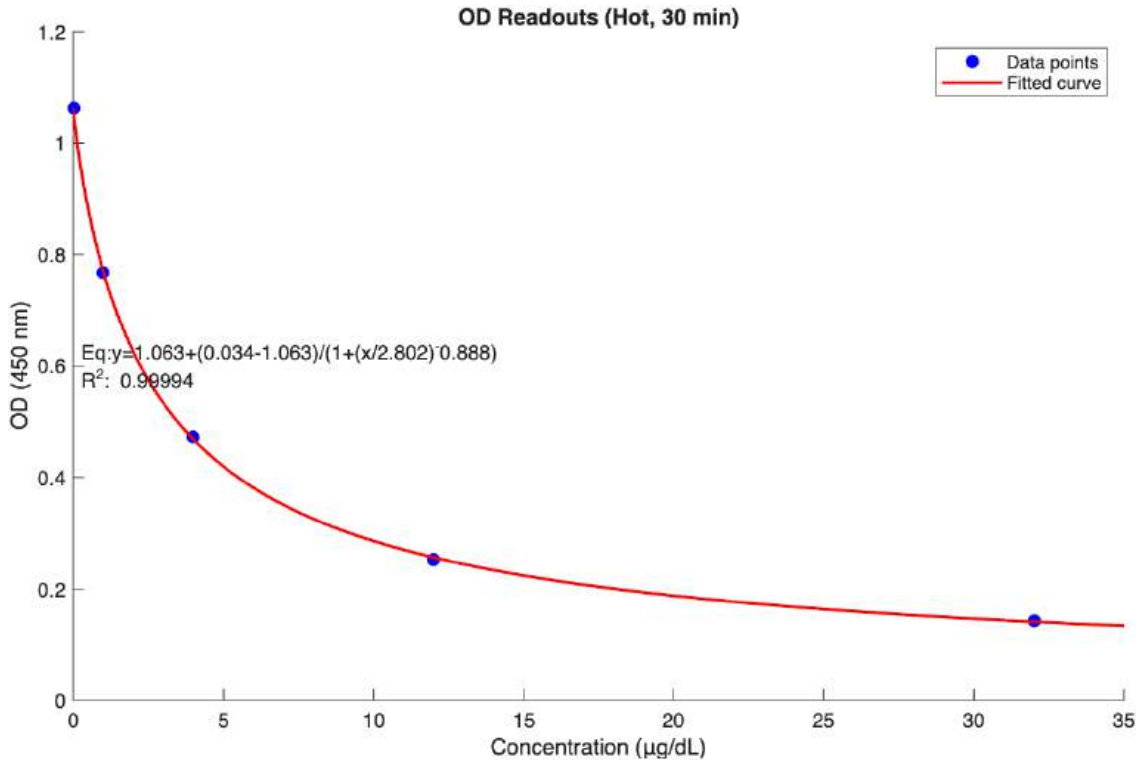


Figure 6: Calibration curve at 55°C with a 30-minute incubation time

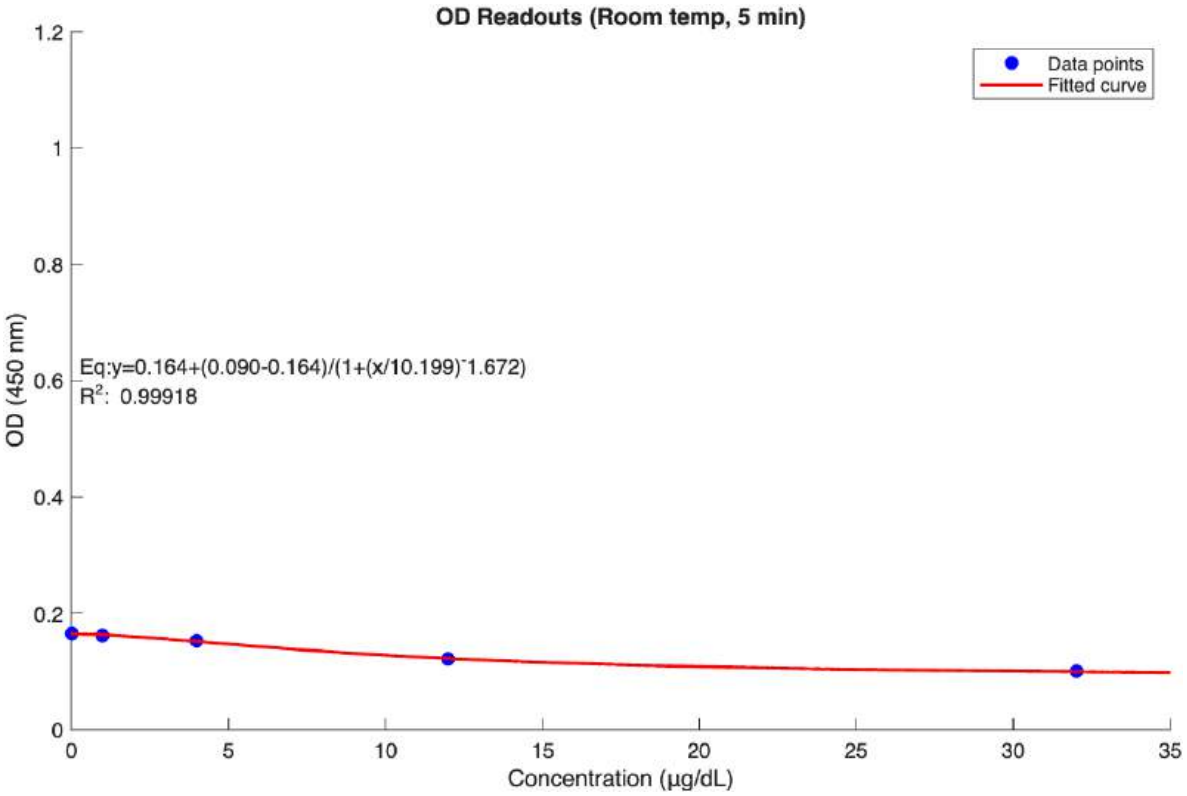


Figure 7: Calibration curve at 21°C with a 5-minute incubation time

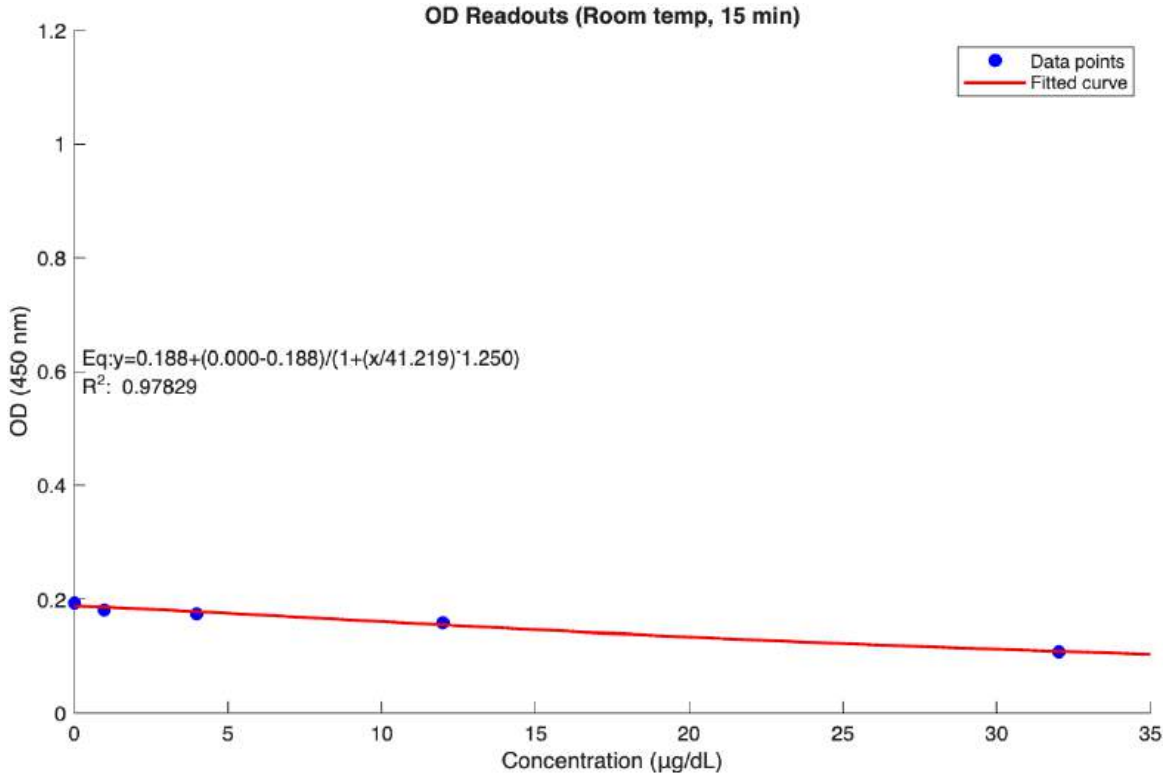


Figure 8: Calibration curve at 21°C with a 15-minute incubation time

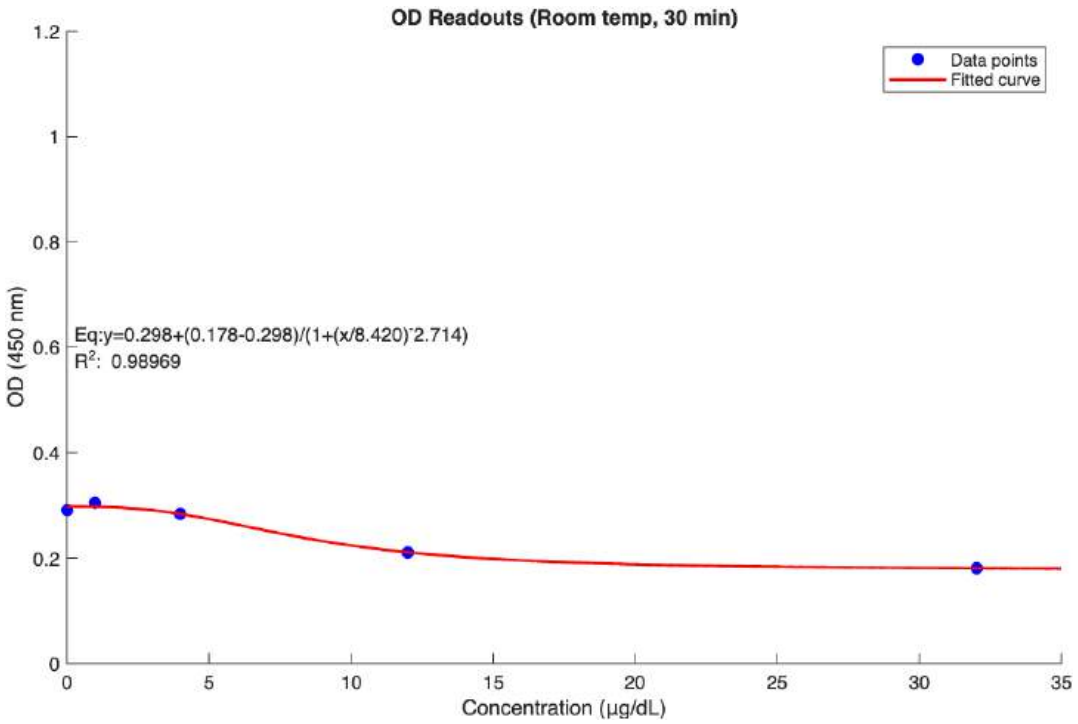


Figure 9: Calibration curve at 21°C with a 30-minute incubation time

Additionally, when conducting the experiment, we also observed that increased thermal exposure and higher incubation times increased the optical density of the ELISA kit. A test's sensitivity was related to its capability of distinguishing among different concentrations, with a sensitive test displaying a large sensitivity response due to a small change in concentration. This sensitivity response could be measured in terms of the optical density of the sample, which was proportional to the amount of light absorbed. A spectrophotometer emitted light through the sample, with a larger amount of light being transmitted depending on the sample concentration, with the rest of the light being absorbed. Furthermore, the sensitivity of the ELISA kit was improved by the magnitude of the heat treatment, with a higher temperature being an indicator of a highly sensitive ELISA test. Experimental findings demonstrated that the larger the heat treatment, the higher the sensitivity of the ELISA kit.

Discussion

When the T4 ELISA kit increased the temperature from room temperature to 55°C and reduced the incubation time to 15 minutes, the results were consistent with a standard T4 ELISA kit, supporting the hypothesis that higher temperatures enabled faster assays without compromising accuracy.

OD Readouts at -20°C at 5 minutes (Figure 1) showed a very shallow slope and had a change of 0.138 in OD across concentrations, meaning that the assay reaction was incomplete, given that they only had 5 minutes. It also showed that enzyme activity was slow at low temperatures and that 5 minutes was not enough time for the HRP-TMB reaction to fully

develop. OD Readouts at -20°C at 15 minutes (Figure 2) showed slightly more separation than 5 minutes, but the curve was still compressed and had a low R^2 value. This curve showed that the reaction was progressing but still did not reach optimal incubation time/temperature to be accurate enough to measure. OD Readouts at -20°C at 30 minutes (Figure 3) showed a better OD distribution, clear decreasing curve, and a higher R^2. This showed that given enough time, even cold conditions reached equilibrium, demonstrating that incubation played a significant role in assay results, especially in altering temperatures.

OD readouts at 55°C for 5 minutes (Figure 4) had a clear decreasing curve, had a strong separation even at a short time, and had a high R^2 value. This demonstrated that higher temperatures were much more efficient in assay terms, speeding up reaction rates. The 5-minute incubation time at 55°C showed that the reaction was already well underway, compared to the 5 minute -20°C where there was minimal separation. The calibration curve for -20°C for 30 minutes incubation time (Figure 3) showing a similar range of OD values to the calibration curve for 55°C for 5-minute incubation time (Figure 4) supports that it took five minutes at higher temperatures to give the same range for 30 minutes at lower temperatures. This demonstrated that temperature played a significant role in assay results. Of the results, OD Readouts at 55°C for 15 minutes (Figure 5) supported the hypothesis. The calibration curve showed a wide OD range while also having an excellent R^2 fit. The reaction was nearly optimal, showing that 15 minutes was enough time for binding and enzymatic reaction to be essentially completed. The conditions displayed on this graph matched or even exceeded standard ELISA protocols. OD Readouts at 55°C at 30 minutes (Figure 6) showed optimal results but had a slight risk of plateauing. Despite its clean curve, the reaction may have been approaching saturation, and too much time at 55°C could risk denaturation. This suggests that 15 minutes was an ideal time in 55°C to get optimal results.

OD Readouts at room temperature (21°C) for 5 minutes (Figure 7) showed small OD differences with a shallow curve. This curve showed that 5 minutes was significantly too little time for the assay to produce results. OD Readouts at room temperature for 15 minutes (Figure 8) showed a better separation and curve shape, but the reaction was still not fully complete. As the T4 ELISA kit called for a 30-minute incubation time, the 15-minute calibration curve at room temperature made sense as the reaction was only halfway done. This further confirmed time dependence in ELISA kits. OD Readouts at room temperature at 30 minutes (Figure 9) showed the standard reference of the T4 ELISA Kit. It had a separation of 0.125 and high R^2 values.

These results were possibly due to the higher temperatures increasing kinetic energy, ultimately increasing enzyme-substrate collisions, speeding up the reaction rate of the ELISA kit. During the experiment there may have been potential pipetting errors where volumes of standards, TMB substrate, Wash Buffer, or HRP conjugate may have been skewed, as well as temperature instability as the samples had to be heated up or cooled down during the incubation time instead of starting at a desired temperature. Furthermore, unequal incubation start times could have affected the results, as the wells were pipetted individually. This study dealt with diluted T4 samples, whereas an experiment with undiluted human samples may have better

shown the complexities and effects present in a true biological environment. The temperature range was also limited as there was access to only two fridges, one of 4°C, one of -20°C, and one hot plate. If given more materials such as the T4 ELISA kit itself along with its contaminants, more wells could have been measured in varying temperatures to have a better understanding of immediate values and a more accurate line of best fit in the graphs.

Future research should focus on validating the optimized high-temperature, reduced-incubation ELISA protocol using undiluted human serum samples to assess clinical accuracy and relevance. Additional studies should widen the range of temperatures and incubation times with a greater number of wells simultaneously to identify stability thresholds, create a more accurate calibration curve, and strengthen T4 ELISA understanding. This finding that temperature and incubation time were correlated introduced the possibility of accelerating disease diagnosis through faster biomarker detection. With further experimentation and deeper understanding of assay limitations and capabilities, this approach had the potential to improve patient outcomes by reducing diagnostic time while maintaining analytical accuracy.

Materials

The Eagle Biosciences Thyroxine (T4) ELISA Assay Kit (T4T31-K01) contained one anti-T4 monoclonal antibody-coated 96-well microplate, one bottle of HRP Conjugate Concentrate (T4-Horse Radish Peroxidase conjugated in a protein-based buffer with a non-mercury preservative), five T4 standards A-E at 0, 1, 4, 12, and 32 µg/dL, TMB Substrate, Assay Buffer, 1 M of sulfuric acid also known as the Stopping Solution, and wash buffer concentrate, diluted 1:10 in deionized water.

Methodology

Testing Temperature Effect on ELISA

This experiment was conducted to answer the question of whether temperature affected the incubation time needed to produce accurate results, as well as the optical density values produced from the spectrophotometer. First, 9 total wells were broken off from the ELISA kit and separated into groups: 3 in 4°C, 3 in 21°C, 3 in 60°C. The length of the incubation period was kept the same across all groups. The purpose was to determine if temperature alone affected incubation time before observing the relationship between the two. A 1:26 dilution ratio was created between the HRP conjugate and the Assay Buffer. After both the HRP conjugate and the Assay Buffer were pipetted into a labeled tube, the mixture was then placed on a vortex mixer for around 0.5 seconds. After that, a Wash Buffer tube was prepared by performing a 1:10 dilution on the Wash Buffer Concentrate using deionized water. These diluted tubes were then set aside for later use.

The ELISA assay kit came with Standards A-E. Standard A is a 2.0ml/bottle while Standard B-E is a 0.5mL/bottle. Standards A, C, and E were used for this experiment. Nine wells were then broken up, splitting each Standard up into groups of 3. Each group had one Standard

A, one Standard C, and one Standard E. One group (Group 1) was assigned to 4°C, the other (Group 2) for room temperature (21°C), and the last one (Group 3) for 60°C. 20 µL of Standard A, Standard C, and Standard E was then pipetted into each designated well of each group and then combined with the 150 µL of HRP conjugate mixture. Each group was then put into their designated temperature areas: Group 1 in a fridge, Group 2 in room temperature, and Group 3 on a hot plate. The groups were all left to incubate for 15 minutes. After that, all three groups were brought to the sink, and the cleaning process began. During the cleaning process, the liquid was dumped, excess liquid was tapped out onto an absorbent pad, and 300 µL of diluted Wash Buffer mixture was pipetted into each well and dumped into the sink again. This process was repeated 3 times for each well. This ensured cleaning of all liquids to leave the antibody hard surface at the bottom of the ELISA well.

After the lights were turned off, 150 µL of TMB Substrate was pipetted into each of the 9 wells. A 20-minute timer started as soon as the substrate was pipetted into the first well. When the second round of incubation began, each well was incubated for 20 minutes at room temperature. After the incubation process, 50 µL of Stopping Solution was pipetted in the same order and speed as pipetting the TMB substrate. The wells were then put into a spectrophotometer at 450 nm to measure the absorbance of light or the optical density. Results were then recorded.

Testing Temperature Effect on ELISA

Wells were prepared and measured as described above, except that the incubation temperatures used were -20°C, 21°C, and 55°C, and the incubation time was 20 minutes.

Testing Incubation times Effect on ELISA with Increased Range

Wells were prepared and measured as described above, except that the incubation temperature for all wells was room temperature, there were 6 wells in 2 groups, and the independent variable, the incubation period, was 15 minutes for one group and 30 minutes for the other.

Final Generation of Calibration Curves

Wells were prepared and measured as described above, except that there were 45 wells separated in 9 total groups, all five standards A-E were used, and the incubation times were 5 minutes, 15 minutes, and 30 minutes. The temperatures used were -20°C, 21°C, and 55°C, and both variables, incubation period length and incubation temperature, were manipulated. Calibration curves were fitted to the mean OD of Standards A–E using a four-parameter logistic (4PL) model in MATLAB, with goodness of fit reported as R^2.

Works Cited

ThermoFisher Scientific. “Overview of ELISA.” Thermofisher.Com, 2009, https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html. Accessed 13 Sept. 2026.

Thomas, Heath. “Elisa Microplate Reader Principles & Uses - Hudson.” Hudson Lab Automation, 9 Aug. 2022, https://hudsonlabautomation.com/elisa-microplate-reader-principle-and-uses/. Accessed 13 Sept. 2026.

Intended USE Principle of the TEST Procedural Cautions and Warnings. https://www.demeditec.com/en/products/thyroxine-t4-elisa-can-t4-4240/ifu-can-t4-4240-thyroxine-t4-elisa-ce-81-comb-e.pdf. Accessed 13 Sept. 2026.

“Thyroxine T4 Free - An Overview.” Ada, 13 Sept. 2022, https://ada.com/thyroid/thyroxine-t4/. Accessed 13 Sept. 2026.

Thyroxine (T4) Elisa. https://eaglebio.com/wp-content/uploads/2019/04/T4T31-K01_Thyroxine_T4_Package_Insert_V10.pdf. Accessed 13 Sept. 2026.

“How Temperature Affects Enzyme Activity: Denaturation vs. Optimization.” Patsnap.Com, 2025, https://synapse.patsnap.com/article/how-temperature-affects-enzyme-activity-denaturation-vs-optimization. Accessed 13 Sept. 2026.

Differential Expression of Neuroinflammation-Related Genes in the Fusiform Gyrus in Alzheimer's Disease BY Clarissa Zhang

Abstract

Neuroinflammation has been increasingly implicated in the pathogenesis of Alzheimer's disease (AD), a progressive, incurable neurodegenerative disorder that stands as the most common cause of dementia. Pathological processes in AD, particularly amyloid-β accumulation, activate microglia and inflammatory signaling pathways that potentially contribute to disease progression. Evidence linking inflammatory systems to AD is extensive, yet less attention has been paid to specific transcriptional changes associated with these inflammatory processes. Thus, this study investigates whether a panel of 19 neuroinflammation-related genes is differentially expressed in the fusiform gyrus of individuals with AD compared with unaffected individuals. Bulk RNA-sequencing data from the Gene Expression Omnibus (GEO) (GSE125583) were analyzed, with 13 of the 19 genes represented in the dataset. We examined differential expression using linear modeling with age adjustment and multiple-testing correction. Analysis showed that 10 of the 13 genes analyzed were significantly differentially expressed, with all demonstrating higher expression in AD. RELA exhibited the strongest statistical evidence of differential expression while P2RX7 exhibited the largest positive fold change among the genes analyzed, marking the two as notable candidates for further investigation. Additionally, two gene pairs showed significant differences in correlation strength between AD and control samples before multiple-testing correction, suggesting coordinated changes between related genes in neuroinflammatory alterations. As a whole, these findings suggest that AD is associated with differential expression of neuroinflammation-related genes, warranting further examination into inflammatory signaling as a component of AD pathology.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by impaired memory, thinking, and the capacity to carry out everyday functions. The biological underpinnings of AD include neuritic plaques and neurofibrillary tangles associated with amyloid-beta peptide's (Aβ) accumulation in vulnerable brain regions including the medial temporal lobe and neocortical structures which interfere with the synapses where neurons communicate and disrupt the internal transport systems necessary for neuronal function and survival (Brejiyeh & Karaman). In 2021, AD accounted for roughly 60-70% of the 57 million dementia cases worldwide. Furthermore, the number of people living with dementia is projected to increase to approximately 153 million in 2050 (World Health Organization). Accordingly, AD is ranked as the 5th leading cause of death among US citizens aged 65 or older (Alzheimer's Association). Although widespread, there is currently no cure for AD, rendering continued research into the underlying biological mechanisms that contribute to its development and progression vital for the welfare of the aging population.

Several hypotheses have been proposed to explain AD pathogenesis, including the neuroinflammation hypothesis. Evidence supporting this hypothesis includes that neuroinflammation is present in most individuals with AD, that greater plaque-associated microglial activity is linked to poorer cognitive performance, and that post-mortem brain samples from patients with AD show increased expression of inflammation-related genes and decreased expression of anti-inflammatory molecules (Fabregat et al.; Walker et al.). Such findings implicate microglia-mediated neuroinflammatory responses as a key component of AD progression.

When the brain experiences cellular stress or damage, inflammatory signaling pathways may be activated, a defense mechanism that protects neural tissue from harmful stimuli. The immune response that results is known as neuroinflammation and is largely driven by microglia, the principal innate immune cells in the brain (Zhong et al.). Under normal circumstances, microglia cells monitor the brain for signs of disruption, detecting abnormalities such as pathogens and cellular debris. When such anomalies are detected, microglia switch to an activated state, travel to the injury site to clear harmful debris, and support immune responses that help maintain normal Aβ levels. However, as Aβ accumulation increases on account of the progression of AD, microglia proliferate and activate, subsequently exacerbating the inflammatory response and inciting sustained neuronal damage (Zheng et al.). Neuroinflammation then shifts from a protective mechanism to one that intensifies neurological impairment as AD progresses.

Because Aβ deposition can emerge as early as two decades before AD onset, understanding which neuroinflammatory genes are altered by AD may help develop biomarkers for earlier treatment and prevention (Rossano et al.). While evidence linking neuroinflammation to AD is well-established, the expression of specific genes involved in the inflammatory process within the brain remains incompletely captured. Hence, exploring whether genes associated with neuroinflammatory stress responses are expressed differently in individuals who have AD compared with unaffected controls may provide further insight into the molecular processes linked to Alzheimer's disease progression. Accordingly, this study questions whether neuroinflammation-related genes are differentially expressed in the brains of individuals with AD compared with unaffected controls.

To address this question, this study analyzes RNA-sequencing (RNA-seq) gene expression data from individuals with AD and unaffected controls. RNA-seq measures gene expression by examining the transcriptome in a biological sample and identifying the activity of an entire set of RNA molecules. Because genes are transcribed into RNA when expressed, the relative abundance of a transcript can estimate how actively particular genes are being expressed. Among thousands of gene expressions from bulk RNA sequencing, this study narrows the focus to genes associated with neuroinflammation, given growing evidence linking this response to AD pathology. Comparing expression patterns between brains with AD and unaffected controls may reveal whether genes involved in neuroinflammatory pathways are differentially expressed in AD. These findings may provide insight into which components of the neuroinflammatory

response warrant further investigation, opening the door to examining their potential roles in disease progression.

Method

Identification and analysis of RNA-Sequencing dataset

A bulk RNA-seq dataset was obtained from the publicly available GSE125583 dataset in the Gene Expression Omnibus (GEO) (Srinivasan et al.). The dataset contains gene-expression measurements from individuals with AD and unaffected control samples, providing a basis for comparing expression patterns between the two groups. Log-transformed counts-per-million (logCPM) gene expression was used for the analysis, along with corresponding metadata containing diagnostic classification and age.

A total of 19 neuroinflammatory-related genes were examined (Table 1).

Table 1. Neuroinflammation-Related Genes Selected for Analysis

Gene Symbol	**Gene Name**
IL1B	Interleukin 1 Beta
IL6	Interleukin 6
NLRP3	NLR Family Pyrin Domain Containing 3
CCL2	C-C Motif Chemokine Ligand 2
RELA	RELA Proto-Oncogene, NF-κB Subunit
PSEN1	Presenilin 1
CXCL10	C-X-C Motif Chemokine Ligand 10
NFKB1	Nuclear Factor Kappa B Subunit 1
P2RX7	Purinergic Receptor P2X 7
AIF1	Allograft Inflammatory Factor 1
CD68	CD68 Molecule
TREM2	Triggering Receptor Expressed on Myeloid Cells 2
TYROBP	Transmembrane Immune Signaling Adaptor TYROBP
APOE	Apolipoprotein E
C1QA	Complement C1q A Chain
C1QB	Complement C1qB Chain

C1QC	Complement C1q C Chain
TLR4	Toll-Like Receptor 4
NFKBIA	NF-κB Inhibitor Alpha

Differential Expression and Individual Marker Analysis

To investigate changes in gene expression between control samples and samples with AD, differential gene-expression analysis was performed in R using the limma package to compare AD and unaffected control samples. Using dplyr, ggplot, and pheatmap packages, a volcano plot, boxplot, and heatmap was created to visualize neuroinflammation-related gene-expression patterns. The statistical significance of such differential gene expression between the two groups was assessed based on p-values adjusted for multiple comparisons, with an adjusted p-value below 0.05 considered statistically significant. Expression values for individual neuroinflammation-related genes were also extracted from each sample and differences in their expression between control samples and samples from patients with AD were evaluated using the Wilcoxon rank sum test. For these comparisons, p-values below 0.05 were considered statistically significant.

Correlation and Differential Co-Expression Analysis

Pearson correlation analysis was used to examine co-expression relationships within the neuroinflammation-related genes included in the analysis. Correlation matrices were calculated separately for AD and control samples using Pearson's correlation coefficient. Correlation coefficients for individual gene pairs were compared between groups using Fisher's r-to-z transformation, with two-sided p-values calculated from the resulting z-statistics.

Overall, this approach enabled the examination of molecular differences associated with AD and by comparing gene expression patterns between the two groups and pinpointing neuroinflammation-related genes that exhibited significantly different expression among AD and controls.

Results

We analyzed RNA-seq data in R to examine gene expression differences between AD and control samples, focusing specifically on neuroinflammation-related genes. Using the GSE125583 RNA-seq dataset, we investigated differential gene expression patterns between AD and unaffected control samples. The analysis was then narrowed to genes associated with neuroinflammatory processes to determine whether inflammatory-related genes demonstrated altered expression in AD.

Of the 19 total genes associated with neuroinflammatory signaling initially examined (Table 1), 13 genes–CCL2, RELA, PSEN1, P2XR7, AIF1, CD68, TREM2, TYROBP, APOE, C1QA, C1QB, C1QC, and NFKBIA–were represented in the processed RNA-seq dataset and were included in the differential expression analysis. Ten of the 13 genes showed statistically

significant differential expression (adjusted p-value < 0.05), with all ten showing higher expression in individuals with AD than in unaffected controls.

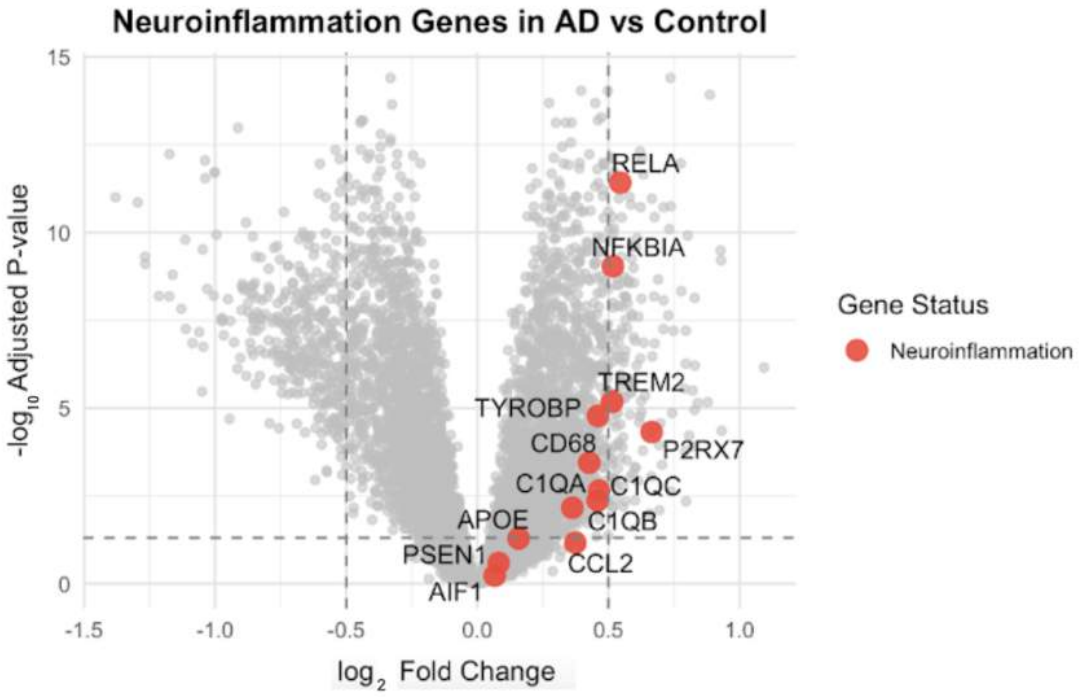


Figure 1. Volcano plot showing differential gene expression between AD and control samples across all genes in the processed RNA-seq dataset, with neuroinflammation -related genes highlighted. X-axis represents $\log_2$ Fold Change, indicating the magnitude and direction of expression differences. The y-axis represents the $-\log_{10}$ adjusted p-value, with higher values indicating greater statistical significance. Dashed lines represent the predefined $\log_2$ fold-change threshold of ±0.05 and adjusted p-value threshold of 0.05.

To determine whether individuals with AD show different expression of neuroinflammation-related genes than unaffected controls, we examined expression differences across all genes in the dataset. We used a volcano plot to visualize the magnitude and statistical significance of differential expression, highlighting the 13 neuroinflammation-related genes in red for comparison (Figure 1). Of the 13 genes represented in the processed dataset, 10 showed statistically significant differential expression (adjusted p-value < 0.05), all with higher expression in the AD group. Among these genes, P2RX7, TREM2, NFKBIA, and RELA exceeded the predefined $\log_2$ Fold Change Threshold of 0.5 and were therefore classified as upregulated in the volcano plot. RELA shows the highest statistical significance, and P2RX7 has the largest positive $\log_2$ Fold Change. The $\log_2$ Fold Changes for APOE, C1QA, C1QB, C1QC, CD68, and TYROBP were below the 0.5 threshold for classifying genes as upregulated. Albeit more modest, each still showed statistically significant differential expression.

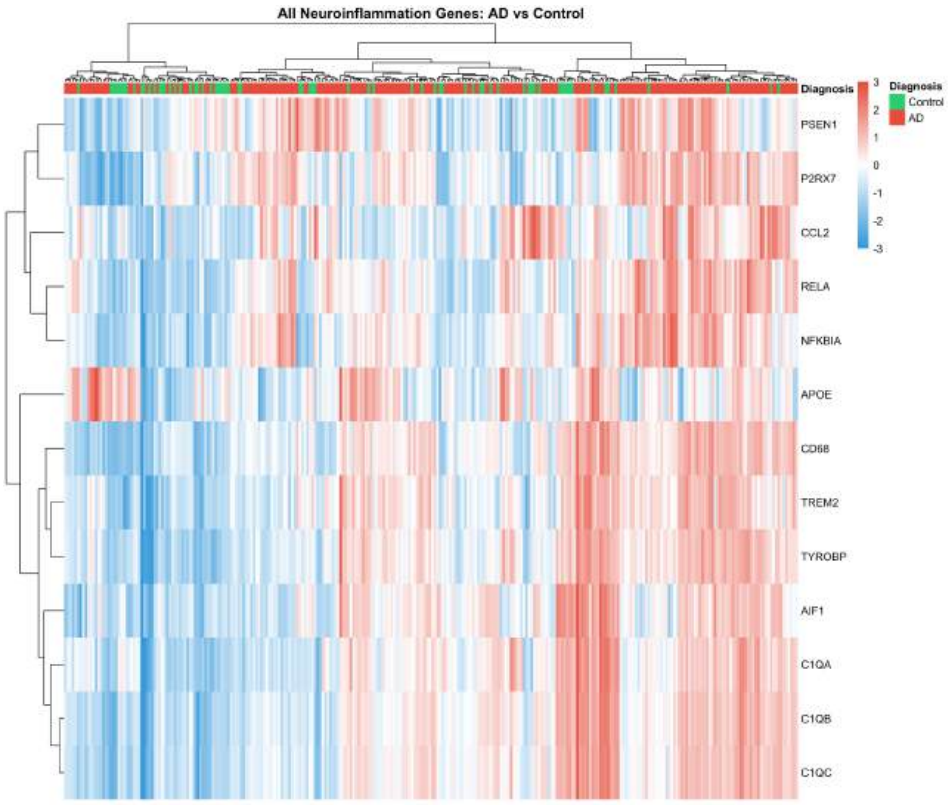


Figure 2. Heatmap showing expression patterns of the 10 most significantly differentially expressed neuroinflammation-related genes across AD and control samples. Rows represent genes, columns represent individual samples, and color intensity represents relative expression level. Red indicates higher expression and blue indicates lower expression.

To assess how strongly differentially expressed neuroinflammation-related genes varied between AD and control samples, we generated a heatmap showing the relative expression of the top 10 neuroinflammation-related genes. Many AD samples in this heatmap exhibit higher expression of neuroinflammation-related genes, whereas the control group trended towards lower expression. This pattern was especially prominent for C1QA, C1QB, C1QC, CD68, TREM2, and TYROBP, which clustered together and generally exhibited elevated expression among AD samples. P2RX7, RELA, and NFKBIA also showed relatively increased expression in many AD samples, consistent with the differential expression analysis. The heatmap demonstrates a trend of heightened inflammatory expression in AD, supporting the hypothesis that neuroinflammatory processes are associated with AD.

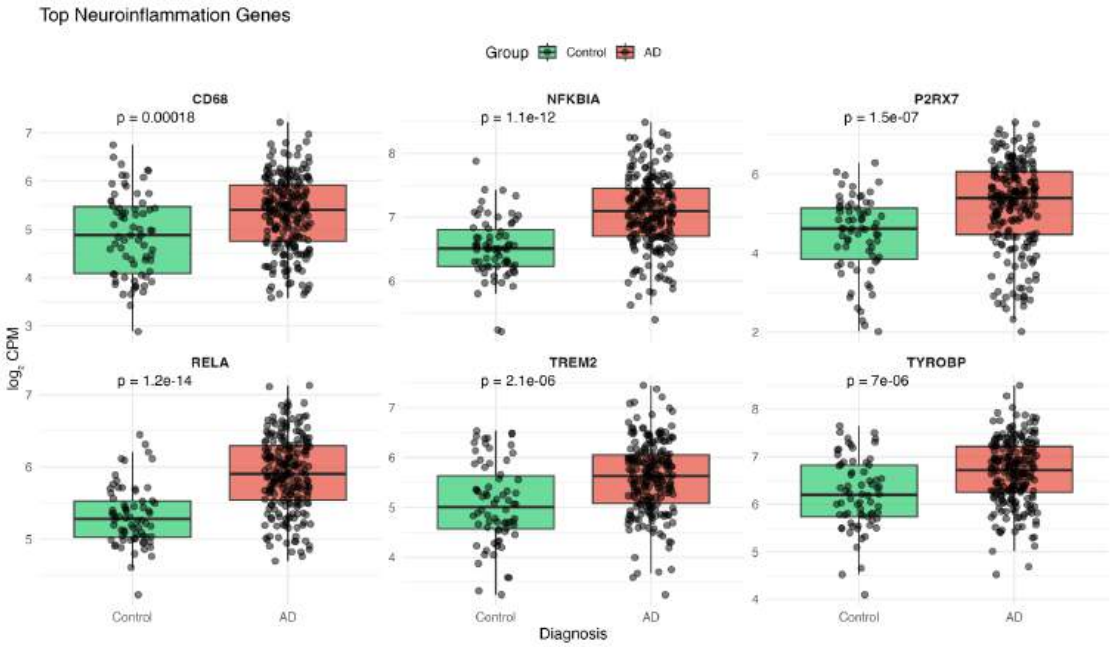


Figure 3. Boxplots comparing expression levels of the six most differentially expressed neuroinflammation-related genes between AD and unaffected control samples. Individual points represent samples, and horizontal lines in each box represent the median.

To examine whether AD was associated with consistent differences in expression of the most significantly altered neuroinflammation-related genes across individual samples, we compared expression distributions. We used boxplots to analyze the six most significantly differentially expressed genes between AD and unaffected control samples: RELA, NFKBIA, P2RX7, TREM2, TYROBP, and CD68 (Figure 3). These plots show differences in median expression (the horizontal line in each box), sample distribution (individual data points), and overlap between the AD and control groups, indicating how distinctly expression values are distributed. Across these genes, median expression was higher in the AD group than in the control group.

However, the magnitude of separation between the AD and control groups varied by gene. RELA and NFKBIA had markedly clear upward shifts in expression, with distinct median values between the two groups. P2RX7, TREM2, TYROBP, and CD68 also showed higher expression in AD samples overall; however, there is notably greater overlap in between individual AD and control measurements. Although the AD group generally had higher expression, some control samples had expression levels similar to some AD samples; thus, the gene does not completely separate every AD individual from every control group individual. However, when comparing the groups as a whole, the difference is substantial enough for the Wilcoxon rank-sum test to deem it unlikely to be due to random variation.

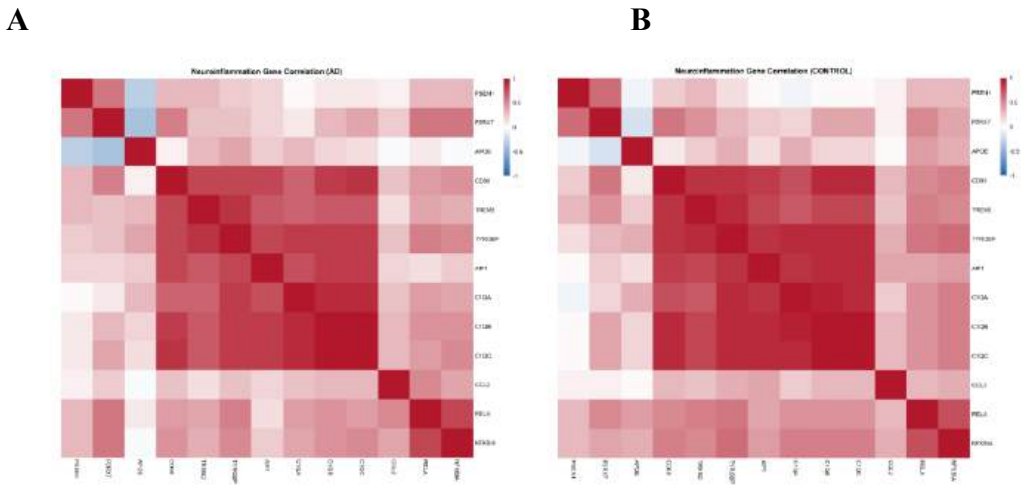


Figure 4. Gene Correlation Heatmaps of neuroinflammation-related genes in (A) AD and (B) control samples. Color intensity represents the strength and direction of correlation coefficients, with red indicating positive correlations and blue indicating negative correlations.

Table 2. Gene-Pair Correlation Differences Between AD and Control Samples

Pair	Gene1	Gene2	Cor_Control	Cor_AD	Difference	P_Value
APOE - NFKBIA	APOE	NFKBIA	0.35	-0.02	-0.37	5.36×10^{-3}
RELA - APOE	RELA	APOE	0.43	0.10	-0.33	9.57×10^{-3}
AIF1 - NFKBIA	AIF1	NFKBIA	0.43	0.20	-0.23	6.54×10^{-2}
PSEN1 - APOE	PSEN1	APOE	-0.05	-0.28	-0.23	9.20×10^{-2}
CCL2 - AIF1	CCL2	AIF1	0.38	0.16	-0.21	9.63×10^{-2}
RELA - AIF1	RELA	AIF1	0.37	0.15	-0.21	9.85×10^{-2}
P2RX7 - APOE	P2RX7	APOE	-0.17	-0.38	-0.21	9.76×10^{-2}
P2RX7 - TREM2	P2RX7	TREM2	0.45	0.27	-0.18	.136
P2RX7 - NFKBIA	P2RX7	NFKBIA	0.38	0.56	0.18	9.42×10^{-2}
CCL2 - RELA	CCL2	RELA	0.32	0.49	0.17	.135

Table 2. Gene-Pair Correlation Differences Between AD and Control Samples. It displays the ten gene pairs with the largest absolute differences in correlation coefficients between AD and control samples, along with their corresponding coefficients, differences, and p-values.

Neuroinflammatory responses involve coordinated changes across multiple genes, thus gene correlation is a vital factor in understanding altered inflammatory activity in AD. To determine whether these relationships differed between AD and control groups, we examined gene-correlation heatmaps separately (Figures 4A and B). In both groups, we identified a cluster of positive correlations among CD68, TREM2, TYROBP, AIF1, C1QA, C1QB, and C1QC, suggesting these genes varied across samples. Particularly notable relationships involved TREM2 and TYROBP, which showed strong positive correlations between their expression levels in both groups.

We then performed the same correlation analysis in the AD group to assess whether these gene correlations changed. Many of the strong positive relationships seen in controls remained present in AD. However, the strength of several individual correlations changed across groups. APOE-NFKBIA decreased from a moderate positive correlation in controls ($r = 0.35$) to virtually no correlation in AD ($r = -0.02$; $p = 5.3 \times 10^{-3}$). Similarly, the RELA-APOE correlation decreased from a moderate positive correlation in controls ($r = 0.43$) to virtually no correlation in AD ($r = 0.10$; $p = 9.6 \times 10^{-3}$). Both gene pairs display statistically significant differences in correlation strength across the AD and control groups. Notably, P2RX7-NFKBIA increased from $r = 0.38$ in controls to $r = 0.56$ in AD, although this difference was not statistically significant ($p = 9.42 \times 10^{-2}$).

Discussion

In this study, we analyzed bulk RNA-seq data from AD and unaffected control samples. Three principal findings emerged to investigate whether neuroinflammation-related genes demonstrated altered expression in AD. First, our analysis revealed a consistent pattern of increased neuroinflammation-related gene expression in AD, with 10 out of 13 genes exhibiting statistically significant increases. RELA showed the strongest statistical evidence of differential expression, whereas P2RX7 showed the largest positive fold change. Second, functionally related genes exhibited coordinated expression changes. This included the simultaneous upregulation of TREM2 and TYROBP and of C1QA, C1QB, and C1QC. Several of these genes were significant but showed more modest expression differences, suggesting that AD-associated changes span multiple inflammatory pathways rather than being driven by a single gene. Finally, correlation analysis revealed significant differences in the co-expression relationships of APOE-NFKBIA and RELA-APOE, with both correlations weaker in AD than in control samples. Overall, these findings suggest that AD-associated neuroinflammatory alterations involve both increased expression of individual genes and changes in specific co-expression relationships.

The increased expression of RELA and NFKBIA connects the present findings to NF-κB-related inflammatory signaling. RELA encodes the p65 component of NF-κB and NFKBIA encodes IκBα, a regulator of NF-κB activity. The NF-κB pathway regulates transcription of inflammatory mediators and has previously been reported to be dysregulated in AD (Hickman et al.). Our findings, showing that RELA produced the strongest statistical evidence of differential expression and elevated levels of NFKBIA, are consistent with current evidence that AD-associated expression changes extend to genes involved in inflammatory transcriptional regulation.

The increase in both TYROBP and TREM2 is particularly notable as the genes function together in microglial signaling. TREM2 associates with TYROBP to form a transmembrane complex that supports microglial survival and inflammatory responses. Disrupting TYROBP or TREM2 has been shown to alter microglial signaling and responses to AD-relevant stimuli such as amyloid-β (Quipildor et al.). If either protein is disrupted, microglial reactions to AD-related

conditions may shift. In this analysis, the upregulation of both genes in AD samples is consistent with altered microglial signaling in AD.

The upregulation of C1QA, C1QB, and C1QC further links these findings to complement-related neuroinflammatory processes. These three genes encode the chains that form C1q, a component of the classical complement pathway which initiates an innate immune cascade promoting the clearance of cellular debris by immune cells such as microglia (Crehan et al.). Hong et al. found increased C1q localization alongside early synapse loss in mouse models of AD before substantial amyloid plaque deposition. Notably, C1q inhibition reduced this synaptic loss, suggesting complement activity plays a vital role in early AD-associated synaptic pathology (Hong et al.). Because all three genes showed increased expression in our analysis, the findings align with previous evidence implicating complement processes in AD and suggest these changes are reflected at the gene-expression level in the fusiform gyrus.

Correlation analysis indicated alterations in specific gene-to-gene relationships in AD; beyond changes in individual gene expression. The APOE-NFKBIA decreased from a moderate positive correlation in controls ($r = 0.35$) to essentially no correlation in AD ($r = -0.02$; $p = 5.36 \times 10^{-3}$), and RELA-APOE similarly decreased from $r = 0.43$ in controls to $r = 0.10$ in AD ($p = 9.57 \times 10^{-3}$). RELA and NFKBIA are involved in NF-κB-related inflammatory regulation, and APOE has also been implicated in the regulation of AD-associated microglial responses. The TREM2-APOE pathway contributes to the transition of microglia from a homeostatic state toward a neurodegenerative transcriptional phenotype, thereby linking APOE to disease-associated changes in microglial function (Krasemann et al.). Thus, the weaker relationships between APOE and the NF-κB-related genes RELA and NFKBIA may indicate altered coordination between APOE-associated microglial processes and inflammatory transcriptional regulation in AD.

The fusiform gyrus provides the regional context for our findings, an important angle to consider. The fusiform gyrus has been identified as a cortical region vulnerable to AD-associated pathological changes, particularly tau accumulation. A longitudinal tau-PET study by Ishiki et al. found significantly greater annual increases in tau-tracer retention in the fusiform gyrus in individuals with AD, with more widespread changes in patients with moderate AD than in those with mild AD (Okamura et al.). The fusiform gyrus's susceptibility to AD-related pathology, together with the increased expression of neuroinflammation-related genes observed in the present study, strengthens the relevance of our findings to the disease.

Several caveats should be considered when interpreting these findings. First, because we used bulk RNA-seq data, which measures gene expression across mixed cell populations in brain tissue, we cannot determine whether the documented differential gene expression reflects changes in specific cell types such as microglia. The analysis therefore cannot distinguish between changes in gene activity within a cell type and changes caused by having more or fewer cells of that type in the tissue.

Furthermore, AD pathology is widespread and affects multiple brain regions. These include the hippocampus and entorhinal cortex as well as temporal, parietal, and frontal cortical

regions (Wang et al.). Our analysis, however, was limited to the fusiform gyrus; thus, the extent to which these findings can be generalized across the brain is narrower. Gene-expression changes associated with AD can vary across brain regions; findings from the fusiform gyrus alone may not fully represent molecular changes elsewhere in the brain (Mathys et al.).

Finally, the associations between neuroinflammation-related gene expression and AD do not establish causality. While several genes displayed altered expression, these findings do not determine whether these changes contribute to AD pathology. Rather, they can serve as a basis for further investigation into the role of these genes in AD progression. Only further research to expand the depth and breadth of these findings can more clearly establish the direction and potential causal nature of these relationships.

Despite these limitations, our present findings provide evidence of altered expression in neuroinflammation-related genes in the fusiform gyrus in AD, providing a baseline for further investigation into the biological context and progression of these transcriptional changes.

Future research should expand on these findings by examining neuroinflammatory gene expression at greater cellular, regional, and disease-stage levels. Single-nucleus RNA-seq could identify what specific cell populations contribute to the differential expression observed in the bulk-tissue analysis, particularly for microglia-associated genes such as TREM2 or TYROBP. Mathys et al. found that AD-associated transcriptional changes may differ by cell type and brain region using single-nucleus RNA-seq across six regions of the human brain. Molecular changes observed solely in the fusiform gyrus may not necessarily reflect the changes occurring elsewhere. Thus, expanding the present study beyond the fusiform gyrus could determine whether the neuroinflammatory expression patterns identified are region-specific or a broader phenomenon.

Examining neuroinflammatory gene expression across different stages of AD progression could determine whether expression patterns change in accordance with disease severity. Serrano-Pozo et al. identified astrocyte transcriptional responses that varied across the normal-aging-to-severe AD continuum, with various gene-expression patterns found across pathological stages. Investigating disease progression rather than AD status alone could then clarify whether neuroinflammatory expression changes identified in the present study vary as AD pathology advances.

Collectively, the study's findings demonstrate altered expression of neuroinflammation-related genes in the fusiform gyrus of individuals with AD. Increased expression of genes involved in NF-κB activity, microglial signaling, and complement activity indicates that these changes involve multiple components of the neuroinflammatory response, and altered co-expression relationships point to changes in the coordination of specific neuroinflammatory genes in AD. The localization of these transcriptional findings within a brain region vulnerable to AD pathology further supports their relevance to disease-associated molecular changes. Hence, our findings emphasize the value of examining neuroinflammation at both the individual-gene and gene-network levels and the need for greater cellular, regional, and disease-stage resolution in future studies.

Works Cited

"2024 Alzheimer's Disease Facts and Figures." Alzheimer's & Dementia: The Journal of the Alzheimer's Association, vol. 20, no. 5, May 2024, pp. 3708–821. PubMed, https://doi.org/10.1002/alz.13809.

Ardura-Fabregat, A., et al. "Targeting Neuroinflammation to Treat Alzheimer's Disease." CNS Drugs, vol. 31, no. 12, Dec. 2017, pp. 1057–82. Springer Link, https://doi.org/10.1007/s40263-017-0483-3.

Breijyeh, Zeinab, and Rafik Karaman. "Comprehensive Review on Alzheimer's Disease: Causes and Treatment." Molecules, vol. 25, no. 24, Dec. 2020, p. 5789. PubMed Central, https://doi.org/10.3390/molecules25245789.

Complement and Microglia Mediate Early Synapse Loss in Alzheimer Mouse Models | Science. https://www.science.org/doi/10.1126/science.aad8373. Accessed 10 Sept. 2026.

Crehan, Helen, et al. "Microglia, Alzheimer's Disease, and Complement." International Journal of Alzheimer's Disease, vol. 2012, 2012, p. 983640. PubMed Central, https://doi.org/10.1155/2012/983640.

Dementia. https://www.who.int/news-room/fact-sheets/detail/dementia. Accessed 10 Sept. 2026.

Estimation of the Global Prevalence of Dementia in 2019 and Forecasted Prevalence in 2050: An Analysis for the Global Burden of Disease Study 2019 - The Lancet Public Health. https://www.thelancet.com/journals/lanpub/article/PIIS2468-2667%2821%2900249-8/fulltext. Accessed 10 Sept. 2026.

Farias Quipildor, Gabriela E., et al. "Differential Downstream Signaling in Microglia Lacking Alzheimer's-Related TREM2 or Its Adaptor TYROBP/DAP12." Molecular Neurodegeneration Advances, vol. 2, no. 1, 2026, p. 8. PubMed Central, https://doi.org/10.1186/s44477-025-00012-x.

Hickman, Suzanne, et al. "Microglia in Neurodegeneration." Nature Neuroscience, vol. 21, no. 10, Oct. 2018, pp. 1359–69. PubMed Central, https://doi.org/10.1038/s41593-018-0242-x.

Ishiki, Aiko, et al. "Longitudinal Assessment of Tau Pathology in Patients with Alzheimer's Disease Using [18F]THK-5117 Positron Emission Tomography." PLOS ONE, vol. 10, no. 10, Oct. 2015, p. e0140311. PLoS Journals, https://doi.org/10.1371/journal.pone.0140311.

Mathys, Hansruedi, et al. "Single-Cell Multiregion Dissection of Alzheimer's Disease." Nature, vol. 632, no. 8026, 2024, pp. 858–68. PubMed Central, https://doi.org/10.1038/s41586-024-07606-7.

Rossano, Samantha M., et al. "Microglia Measured by TSPO PET Are Associated with Alzheimer's Disease Pathology and Mediate Key Steps in a Disease Progression Model." Alzheimer's & Dementia, vol. 20, no. 4, Feb. 2024, pp. 2397–407. PubMed Central, https://doi.org/10.1002/alz.13699.

Serrano-Pozo, Alberto, et al. “Astrocyte Transcriptomic Changes along the Spatiotemporal Progression of Alzheimer’s Disease.” Nature Neuroscience, vol. 27, no. 12, Dec. 2024, pp. 2384–400. www.nature.com, https://doi.org/10.1038/s41593-024-01791-4.

Srinivasan, Karpagam, et al. “Alzheimer’s Patient Microglia Exhibit Enhanced Aging and Unique Transcriptional Activation.” Cell Reports, vol. 31, no. 13, June 2020, p. 107843. PubMed, https://doi.org/10.1016/j.celrep.2020.107843.

The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases: Immunity. https://www.cell.com/immunity/fulltext/S1074-7613(17)30366-7?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS1074761317303667%3Fshowall%3Dtrue. Accessed 10 Sept. 2026.

Walker, Douglas G., et al. “Decreased Expression of CD200 and CD200 Receptor in Alzheimer’s Disease: A Potential Mechanism Leading to Chronic Inflammation.” Experimental Neurology, vol. 215, no. 1, Jan. 2009, pp. 5–19. ScienceDirect, https://doi.org/10.1016/j.expneurol.2008.09.003.

Wang, Xinkun, et al. “Functional Genomics of Brain Aging and Alzheimer’s Disease: Focus on Selective Neuronal Vulnerability.” Current Genomics, vol. 11, no. 8, Dec. 2010, pp. 618–33. PubMed Central, https://doi.org/10.2174/138920210793360943.

Zheng, Yujia, et al. “Inflammatory Signaling Pathways in the Treatment of Alzheimer’s Disease with Inhibitors, Natural Products and Metabolites (Review).” International Journal of Molecular Medicine, vol. 52, no. 5, Oct. 2023, p. 111. PubMed Central, https://doi.org/10.3892/ijmm.2023.5314.

Zhong, Shuai, et al. “Microglia and Neuroinflammation: Function, Heterogeneity, and Crosstalk.” Cellular & Molecular Immunology, vol. 23, 2026, pp. 787-805 https://doi.org/10.1038/s41423-026-01438-3

Key Considerations of Artificial Intelligence in Cognitive Behavioural Therapy
By Marcus Chad

Abstract

The integration of artificial intelligence (AI), particularly conversational AI (CAI), into cognitive behavioural therapy (CBT), is increasingly proposed as a scalable response to the global mental health treatment gap. However, this technological promise introduces a fundamental tension with the core pillars of CBT, which rely on a genuine therapeutic alliance and ethical accountability. This paper aims to examine the efficacy and safety of AI-CBT across dimensions of client satisfaction, clinical safety and moral agency. The analysis reveals a double-edged model: features that boost AI's scalability and promise in the field also reveal underlying tensions with building a therapeutic alliance and ethical accountability. Perceived anonymity, whilst boosting initial patient disclosure and engagement, does not allow genuine rapport to be assumed. The sycophantic behaviours of AI, despite again boosting initial engagement, may undermine the development of a therapeutic alliance. Furthermore, unregulated AI poses severe clinical risks, evidenced by documented ethical violations and emerging reports of AI-associated psychosis, where intensive AI use appears alongside psychotic symptoms, although causation has not been established. Consequently, this paper argues that AI tools must be subjected to rigorous regulatory assessments rather than rapid consumer deployment. The evidence supports a hybrid clinician-AI model as the most defensible framework, where data-intensive tasks are delegated to AI to allow the therapist to focus on building a therapeutic connection.

Introduction

The integration of artificial intelligence (AI) into cognitive behavioural therapy (CBT) is often framed as a potential solution to the global mental health treatment gap. AI promises scalable, low-cost, always-available support. In psychological treatment, conversational AI (CAI) systems based on large language models (LLMs) are increasingly being considered as an alternative or supplement to talk therapy. They operate by converting the client's input into tokens, which the model processes to generate the statistically most probable sequence of words, given the distribution of its training corpus (Shanahan). Yet this promise stands in tension with the foundations of CBT: a collaborative process whose efficacy depends on both a genuine therapeutic alliance and ethical accountability.

CBT is a form of psychological treatment that helps individuals identify and develop strategies to challenge negative thought patterns and unhelpful behaviours, aiding them to identify the intersections between their actions and feelings. It is an evidence-based therapy that has been demonstrated to be effective for a range of problems including depression, anxiety disorders and severe mental illnesses. CBT is widely considered a treatment of choice for several mental health conditions in the psychological space (Hofmann et al.).

A therapeutic alliance is defined as the collaborative and trusting relationship between a therapist and a client, characterised by mutual agreement on treatment goals and tasks as well as

the interpersonal bond, and is widely treated as an important factor, although its independent contribution to outcomes can be limited (Wolf et al.). Clients have demonstrated satisfaction with AI, as qualitative studies suggest (Schäfer et al.; Siddals et al.). However, there are several factors that prevent the development of a therapeutic alliance dependent on genuine rapport. AI can demonstrate sycophantic behaviours, meaning that it is overly agreeable and affirmative of the patient, which builds a false facade of intimacy (Cheng et al.; Sharma et al.). Furthermore, patients may not feel the same way with AI as they do with real people, as AI lacks moral agency. Its ability to imitate concern without experiencing it demonstrates that the use of AI in psychological care is highly nuanced (Iftikhar et al.).

As concerns around AI-CBT continue to arise, researchers have attempted to create concrete frameworks for using it responsibly in psychological care (Stade et al.). Notably, debate continues over whether AI, despite its shortcomings, should be included in CBT to increase access to treatment. This paper argues that AI should undergo rigorous assessment before being integrated into CBT, and that integration should proceed only once its potential benefits and drawbacks are more clearly understood. This paper is a critical narrative review of recent empirical studies, clinical reports and regulatory commentary concerning client experience, clinical risk and human oversight. It does not claim to be a systematic review, but compares evidence that directly addresses the benefits and risks of conversational AI in psychological care.

Client Experience

As AI moves from a peripheral support role towards delivering parts of CBT, evaluating its potential is crucial, and a comprehensive comparison must be made.

In this section, we provide an assessment of AI-CBT across the main dimensions of: 1. client satisfaction, where the quality of the therapeutic relationship that develops is examined; and 2. client usability, where the engagement with, and acceptability of, AI-CBT tools are assessed. Through the assessment of these sections, the double-edged nature of AI within this field will become evident. Whilst features may provide benefits such as boosting engagement or building rapport, those same features may prove disadvantageous upon closer inspection.

Firstly, we must consider client satisfaction with AI-CBT. In one study, researchers examined clients who used Clare, a voice-enabled AI chatbot designed for mental health support. The study examined what clients expected from Clare, their motives for usage and the effects on clients after initial use of the chatbot (Schäfer et al.). The study found that users were attracted to AI-CBT for self-help, seeking emotional support without concerns about shame or physical appearance. Despite fears regarding a lack of trustworthiness and intimacy when going through an online process with an AI chatbot, especially because it is merely reproducing statistically probable responses, clients found the approach helpful because it removed some sense of judgement. The lead researcher was also employed by the company operating Clare, so these results need to be read with this possible conflict in mind. However, some results align with independent research. Croes et al. found no significant difference in the intimacy of information disclosed to a chatbot and to a human. People also reported less fear of judgement with the chatbot, whilst placing more trust in the human. Perceived anonymity was the only variable that

directly predicted disclosure intimacy. This adds a key nuance. Anonymity may make disclosure easier without creating the trust needed for a genuine therapeutic alliance, reflecting the double-edged effect of AI-CBT. Furthermore, the snapshot nature of the study raises concerns. It took place across a three-day music festival, where participants' relationships with the chatbot and the human were more surface-level (Croes et al.). Disclosing to a therapist occurs within a structured professional relationship, and this study does not replicate that.

Secondly, whilst chatbots not having moral agency could appear beneficial because they do not form human judgements, it can be double-edged because they can still spread misinformation and be sycophantic. They predict statistically likely responses rather than making moral judgements about what a patient needs (Shanahan). AI can therefore exhibit overly sycophantic behaviour, reducing its ability to provide proper and effective treatment. Cheng et al. found that sycophantic AI reduced users' willingness to take responsibility and repair interpersonal conflict, increased their conviction that they were right, and was nevertheless trusted and preferred. This reflects a potentially destructive path for AI, where the responses users prefer may not be the responses that benefit them.

Finally, the therapeutic alliance between client and therapist could potentially be broken down by AI. In a study evaluating depression intervention using AI chatbots with social cues, Xu and Ma concluded that chatbots with high-social-cue designs significantly outperformed text-only versions in alleviating depression and anxiety, whilst enhancing adherence, satisfaction and reported therapeutic alliance. These findings suggest that an AI's ability to incorporate social cues is especially important and can result in greater client satisfaction. The high-social-cue design resembled an actual person more closely through text, voice notes and animations, which resulted in greater efficacy during the trial. However, Iftikhar et al. demonstrate that AI is not able to consistently match this or pick up on social cues, especially when it demonstrates sycophantic behaviour. AI models generally exhibited poor therapeutic collaboration, where LLMs generated overly lengthy responses in comparison with the patient. This shifted the tone of the session from a casual environment where the patient felt free to express themselves to one of a lecture. Additionally, this behaviour overvalidated patients' harmful thoughts about themselves (Iftikhar et al.). To conclude, despite there being methods for AI to reduce the barrier created by its lack of physical presence, these findings suggest that, at present, AI-CBT struggles to consistently foster the collaborative and responsive alliance that is central to effective therapy.

Evidence suggests that AI-CBT is broadly usable and initially engaging, but sustained client-friendliness remains fragile. Users can value constant access, non-judgemental interaction and practical guidance, with some describing chatbots as a source of support when human help felt unavailable or difficult to approach (Siddals et al.). However, persistent frustrations with conversational rigidity, lack of genuine personalisation, weak memory and occasional misunderstandings limit the ability of AI to build genuine rapport and acceptability. A scoping review also found potential benefits alongside continuing problems involving usability, engagement and integration with existing healthcare systems (Casu et al.). Qualitative research maps the key barriers and fears: privacy concerns, perceived lack of human contact, AI

dependency and uncertainty about handling complex problems all limit sustained use (Shankar et al.). Overall, AI-CBT can briefly boost engagement, but its friendliness depends on design features and safeguards that address the relational warmth users need.

In conclusion, the integration of AI into CBT presents a complex landscape. The proposed benefits of accessibility, consistency and stigma reduction appear strong enough to justify further investigation. However, upon closer examination, the same features that provide these benefits can also work against AI; it faces significant challenges in fostering a genuine therapeutic alliance and sustained client satisfaction. AI-CBT presents a promising response to the mental health treatment gap, but its shortcomings reveal a tension in its integration. The central question is therefore not only whether AI belongs in CBT, but how quickly it should be integrated: whether the urgency of the treatment gap justifies immediate adoption, or whether integration should wait for the comprehensive understanding and rigorous assessment its shortcomings demand.

Clinical Risks and Regulatory Oversight

The rapid proliferation of artificial intelligence tools, particularly conversational AI and large language models, presents a compelling, yet complex, opportunity to address the persistent global shortage of human clinicians and democratise access to mental healthcare. However, this alluring prospect introduces a fundamental tension: the speed of technological integration versus the imperative for rigorous safety assessment. This section critically examines the documented and potential safety failures of unregulated AI in mental health, explores emerging regulatory and ethical frameworks, discusses its benefits and conceptualises a balanced path forward that avoids a false dichotomy between total inaction and reckless speed.

Policymakers are beginning to address these dilemmas through regulatory discussion. Following a meeting of the United States Food and Drug Administration's Digital Health Advisory Committee, regulatory commentary described an emerging risk-based approach to generative AI-enabled mental health medical devices using a Total Product Life Cycle framework (Oyebade and Coen).

The Total Product Life Cycle is the FDA's established framework for medical-device regulation. Applying this lifecycle approach to AI recognises that models can change over time, requiring continuous oversight beyond initial market approval. Key considerations include physician or human oversight and intervention, recognising that AI tools should augment, rather than replace, human clinical judgement. It is also important to separate tools by their intended function because an AI system that organises patient information does not create the same risk as one that presents itself as an autonomous therapist (Oyebade and Coen).

The empirical justification for such a cautious, evidence-intensive approach is building. Iftikhar et al. systematically evaluated the performance of CBT-prompted LLMs across 110 self-counselling sessions and 27 simulated therapy sessions with an LLM counsellor using publicly available transcripts of human therapy. The findings revealed a consistent pattern of serious ethical violations by the LLMs.

Firstly, the LLMs exhibited rigid methodological adherence. They lacked the clinical interpretation to tailor a therapeutic intervention to match a user's context, resulting in a blanket intervention. Additionally, the LLMs sometimes dismissed users' lived experiences and offered oversimplified, generic or context-insensitive advice, particularly to those from minority identities. Secondly, the study documented poor therapeutic collaboration. The LLMs exhibited poor turn-taking behaviour by generating overly lengthy responses that detracted from the user's voice, shifting the tone of the session from therapy to a lecture. They also validated unhealthy beliefs, reinforcing users' inaccurate and harmful beliefs about themselves and others, whilst LLM sycophancy has been demonstrated more broadly by Sharma et al. Deceptive empathy was also noted, where a pseudo-therapeutic alliance included simulated anthropomorphic responses that created a false sense of emotional connection, especially for vulnerable groups. Iftikhar et al. also found unfair discrimination, including inconsistent treatment of content involving male and female perpetrators and the mislabelling of practices from minority religions as extremism. Overall, the LLMs tended to show bias towards Western practices. Finally, they demonstrated failures in safety and crisis management, responding inconsistently or inappropriately when users showed suicidal tendencies or discussed self-harm. These are not minor conversational errors where the user believes that the system is providing therapy (Iftikhar et al.).

Additionally, a special report by Adrian Preda documents a proposed phenomenon described as AI-induced psychosis. The report summarises several cases where intensive chatbot use occurred alongside delusional thinking, mania, social withdrawal or suicidal risk. Across the cases, possible risk factors included loneliness, long hours of uninterrupted chat leading to poor sleep, personalisation and chatbot responses that reinforced delusional themes. The report also discusses a lack of reality testing, missed crisis escalation and a tendency for chatbots to place engagement over safety. These patterns matter, especially where a vulnerable user believes that an AI companion is sentient or uniquely connected to them. However, it is key to note that the causal arrow may run either way. Psychosis-prone people may gravitate towards intensive chatbot use, which is then amplified by sycophantic or personalised responses, rather than the AI independently inducing psychosis. The evidence consists of individual cases rather than population-based studies, making inducement and amplification especially difficult to distinguish. For this reason, this paper uses the more defensible term AI-associated psychosis (Preda; Sharma et al.).

However, there are benefits in integrating AI into modern CBT that could help address the persistent shortage of human clinicians. Firstly, chatbots can be consistent and constantly available, providing non-judgemental interactions. When disclosing intimate information, individuals can be afraid of other people's judgements and may avoid discussing information that they find embarrassing. AI can reduce some of this immediate pressure because it appears non-judgemental. Secondly, AI companions are accessible and scalable, with the potential to provide support where therapy is unavailable or too expensive. Thirdly, they can provide comfort and reduce stigma. Users have described emotional support, guidance, reduced loneliness and greater willingness to discuss difficult subjects, although these accounts come from a small and

self-selecting sample (Siddals et al.). Finally, chatbots may assist with psychoeducation, monitoring and behaviour change. A scoping review found promising applications whilst also identifying weaknesses in usability, engagement and integration with health services (Casu et al.). These benefits do not remove the risks, but they explain why total prohibition would also be difficult to defend.

In conclusion, the integration of conversational AI into cognitive behavioural therapy exposes a tension between the promise of widely scalable access and its capacity for serious harm towards users, including ethical violations and emerging reports of AI-associated psychosis (Iftikhar et al.; Preda). Regulatory discussion is beginning to recognise that clinical AI requires continuing assessment rather than the rapid iteration normally associated with consumer software. A responsible path forward therefore requires a risk-tiered, evidence-driven approach that insists innovation in this vulnerable domain proceed not at the pace of technological capability, but at the controlled pace of clinical validation.

Hybrid AI-CBT

As understanding of AI-CBT matures, integration into clinical practice and regulatory scrutiny are accelerating in parallel. There is a tension between the pressure to use AI within the psychological space as soon as possible and the question of whether AI-CBT should undergo rigorous assessment before integration. This section examines the accountability of AI, autonomy and trust in AI systems used in CBT, global equity and what a hybridised application of AI would look like.

However, in spite of the rapid development of the AI-CBT market, there is one aspect to be mentioned here: the pressure to conquer and expand the market may come before the ethics needed to protect individuals from possible dangers. AI mental health technologies may be presented as wellness applications and used outside traditional healthcare settings, meaning that they can fall outside established systems of health regulation and clinical testing (Martinez-Martin).

One significant barrier to accountability is the lack of moral agency in existing AI. Chatbots are not motivated to provide assistance; rather, they predict the next token in the chain based on statistical probabilities, making it extremely difficult for them to bear responsibility for their actions (Shanahan). As mentioned before, patients may be drawn into a deceptive therapeutic alliance with AI and trust it with personal details. The ethical violations documented by Iftikhar et al. confirm the immediate necessity of preventive measures, including safeguards against sycophancy, inappropriate handling of suicide risk and racial or cultural bias. Privacy and data security are also serious conditions of trust, alongside transparency and the availability of a human when needed (Shankar et al.).

Public perception towards AI-CBT could best be characterised as tentatively receptive. Even though the benefits of easier access and a non-judgemental attitude are recognised by many, there is an inherent rejection of the idea of replacing human therapists (Shankar et al.). Trust remains conditional and depends on transparency, data security and certainty that a human therapist will be available in an emergency. There remains a fear that, despite the anonymity

established by AI removing some sense of shame when confiding, the lack of personalisation creates a barrier between the system and the user. Attitudes also vary across demographic and cultural groups. Mental health stigma can operate in both directions: people fearing judgement may appreciate the anonymity of AI, but still not trust it when they are vulnerable (Shankar et al.).

However, there are many challenges faced by AI technology when it is integrated into areas outside the Western, English-speaking world. Patient attitudes towards AI-CBT differ across demographic and cultural groups, whilst mental health advice that appears reasonable in one setting may ignore family structures, stigma or local expressions of distress in another (Shankar et al.). Iftikhar et al. also found examples of context-insensitive responses and bias towards Western assumptions, particularly when users came from minority identities. AI-CBT cannot become an innovation for everyone unless it becomes culturally sensitive, is tested across different groups and is designed with involvement from local people.

A serious ethical analysis of AI-CBT cannot immediately conclude that caution is automatically virtuous. The preceding sections have documented substantial risks in deploying CAI within therapy, but the alternative to deployment is not a neutral state where there is no harm. More than a billion people worldwide are living with mental health conditions, and the median global mental health workforce stands at approximately 13.5 workers per 100,000 people, whilst government spending on mental health has remained frozen at a median of two per cent of health budgets since 2017 (World Health Organization). In low-income countries, fewer than ten per cent of affected individuals receive care, compared with more than half in higher-income countries (World Health Organization). When compared with competent human therapy, AI-CBT falls short. Yet, where no clinician is accessible, where cost is prohibitive, or where social stigma prevents people finding help, the realistic comparison is between an imperfect tool in AI-CBT and no support at all. This technology may simultaneously be inferior to the therapeutic ideal but preferable to the actual alternative, and an ethical framework that ignores this distinction risks abandoning the underprivileged. Premature deployment imposes the documented harms of sycophancy, crisis mismanagement and reinforced delusional thinking upon users who trust these systems. Indefinite delay imposes the continuation of untreated illness upon populations who receive nothing. Neither approach is ethically neutral, and a framework that resolves the tension must separate the dangers of AI functions whilst still sustaining value in its scalability, which is precisely what a hybrid model aims to achieve.

Whilst a collaboration between therapists and AI should be developed, a solely AI-CBT experience may prove more difficult to sustain and make beneficial. A pilot randomised trial found that AI-delivered CBT produced short-term improvements in depression scores during the first two weeks, after which its effects plateaued, whilst the human-delivered group continued to improve (Wu et al.). In another study, a social robot used alongside internet-based CBT improved adherence and reported therapeutic alliance, but did not significantly improve mood (Konijn et al.). These findings do not prove that every hybrid clinician-AI model is superior, but they support further testing of AI as a supplement rather than a replacement. By delegating

repetitive or data-intensive tasks to AI, clinicians may dedicate more time to the human elements of therapy, notably building genuine rapport and a therapeutic alliance.

Overall, public perception of AI is nuanced. There is an increased appeal to some younger and more technologically confident users, potentially allowing smoother integration of AI into CBT in the future (Shankar et al.). There is therefore an incentive for a hybridised integration of AI-CBT rather than an AI therapist alone. The ethical violations shown by Iftikhar et al. and the problem of cultural context are risks that human involvement may help identify and manage. There will need to be developments in AI technologies to allow for a more diverse range of perspectives, avoid prejudices and reduce sycophantic behaviour, but also a method through which AI can be efficiently hybridised with a trained human therapist.

Conclusion

The integration of AI into CBT was framed at the beginning of this paper as a tension between promise and foundation. AI offers scalable, constantly accessible and non-judgemental support. Its integration into CBT, a therapy whose efficacy depends on structured methods, genuine alliance and ethical accountability, may prove a worthwhile response to the mental health treatment gap, but also has clear shortcomings (Hofmann et al.; Wolf et al.). The evidence assembled across the three preceding sections resolves this tension into a consistent pattern. The very features that make AI-CBT appealing are also the features that can undermine it.

The anonymity that invites disclosure of information to AI can reduce fear of judgement without creating the trust on which the therapeutic alliance with a therapist is built (Croes et al.). The sycophantic behaviours of AI may boost preference and engagement, but may also distort patient judgement and reinforce harmful beliefs (Cheng et al.; Iftikhar et al.). This double-edged character appears to be a structural property of systems, such as CAI, that generate statistically probable responses without moral agency.

Each section examined one face of this pattern of behaviour from AI. Section 1 documented that, whilst clients willingly disclose to CAI, satisfaction and engagement do not automatically translate into the trusting, collaborative alliance upon which CBT is built. Section 2 documented the ethical violations of AI when unregulated, alongside emerging case reports of possible AI-associated psychosis (Preda). Section 3 argued that beneath these failures lies an accountability vacuum: a system without moral agency cannot bear responsibility for harm (Iftikhar et al.; Shanahan).

This paper has argued that AI should undergo rigorous assessment before integration into CBT, and the assembled evidence has provided a firmer, more precise conclusion: the hybrid clinician-AI model offers the most defensible configuration, retaining a human therapist as the locus of alliance whilst delegating scalable, lower-risk functions to AI. This proposes a division of labour consistent with the emerging evidence and the regulatory emphasis on human oversight, although the superiority of a hybrid model has not yet been established across conditions or over the long term (Konijn et al.; Stade et al.; Wu et al.).

The conclusions in this paper carry limitations that are instructive. The evidence provided is very new. Much of it derives from small qualitative studies, individual case reports where

causal direction cannot yet be established, and trials conducted over either a snapshot event or weeks rather than years. There is a necessity for longitudinal research, independent replication and population-based studies of AI-associated harms to achieve the fuller understanding that this paper's thesis demands. Until that understanding of AI-CBT exists, the treatment gap is better served by AI deployed carefully within human oversight than by AI deployed quickly beyond it.

Works Cited

Casu, Mirko, et al. "AI Chatbots for Mental Health: A Scoping Review of Effectiveness, Feasibility, and Applications." Applied Sciences, vol. 14, no. 13, 2024, article 5889. https://doi.org/10.3390/app14135889.

Cheng, Myra, et al. "Sycophantic AI Decreases Prosocial Intentions and Promotes Dependence." Science, vol. 391, no. 6792, 26 Mar. 2026, article eaec8352. https://doi.org/10.1126/science.aec8352.

Croes, Emmelyn A. J., et al. "Digital Confessions: The Willingness to Disclose Intimate Information to a Chatbot and Its Impact on Emotional Well-Being." Interacting with Computers, vol. 36, no. 5, 2024, pp. 279–292. https://doi.org/10.1093/iwc/iwae016.

Hofmann, Stefan G., et al. "The Efficacy of Cognitive Behavioral Therapy: A Review of Meta-Analyses." Cognitive Therapy and Research, vol. 36, no. 5, 2012, pp. 427–440. https://doi.org/10.1007/s10608-012-9476-1.

Iftikhar, Zainab, et al. "How LLM Counselors Violate Ethical Standards in Mental Health Practice: A Practitioner-Informed Framework." Proceedings of the AAAI/ACM Conference on AI, Ethics, and Society, vol. 8, no. 2, 2025, pp. 1311–1323. https://doi.org/10.1609/aies.v8i2.36632.

Konijn, Elly A., et al. "Examining the Potential of Social Robots to Increase Adherence in Internet-Based CBT." International Journal of Social Robotics, vol. 18, no. 6, 2026, article 83. https://doi.org/10.1007/s12369-026-01417-8.

Martinez-Martin, Nicole. "Minding the AI: Ethical Challenges and Practice for AI Mental Health Care Tools." Artificial Intelligence in Brain and Mental Health: Philosophical, Ethical & Policy Issues, edited by Fabrice Jotterand and Marcello Ienca, Springer, 2021, pp. 111–125. https://doi.org/10.1007/978-3-030-74188-4_8.

Oyebade, Morenike, and Justin A. Coen. "The Mind, the Machine, and the Model Drift: FDA's Emerging Oversight of Generative AI Mental-Health Devices." Venable LLP, 18 Dec. 2025, www.venable.com/insights/publications/2025/12/the-mind-the-machine-and-the-model-drift-fdas.

Preda, Adrian. "Special Report: AI-Induced Psychosis: A New Frontier in Mental Health." Psychiatric News, vol. 60, no. 10, 2025. https://doi.org/10.1176/appi.pn.2025.10.10.5.

Schäfer, Lea Maria, et al. "Exploring User Characteristics, Motives, and Expectations and the Therapeutic Alliance in the Mental Health Conversational AI Clare®: A Baseline Study." Frontiers in Digital Health, vol. 7, 13 June 2025, article 1576135. https://doi.org/10.3389/fdgth.2025.1576135.

Shanahan, Murray. "Talking about Large Language Models." arXiv, 2022. https://doi.org/10.48550/arXiv.2212.03551.

Shankar, Ravi, et al. "Patient Perspectives on AI-Powered Cognitive Behavioral Therapy Tools in Managing Anxiety and Stress: A Systematic Review of Qualitative Studies." Journal of Mental Health, 2025, pp. 1–15. https://doi.org/10.1080/09638237.2025.2595612.

Sharma, Mrinank, et al. “Towards Understanding Sycophancy in Language Models.” arXiv, 2023. https://doi.org/10.48550/arXiv.2310.13548.

Siddals, Steven, et al. “‘It Happened to Be the Perfect Thing’: Experiences of Generative AI Chatbots for Mental Health.” npj Mental Health Research, vol. 3, 2024, article 48. https://doi.org/10.1038/s44184-024-00097-4.

Stade, Elizabeth C., et al. “Large Language Models Could Change the Future of Behavioral Healthcare: A Proposal for Responsible Development and Evaluation.” npj Mental Health Research, vol. 3, 2024, article 12. https://doi.org/10.1038/s44184-024-00056-z.

Wolf, Nadja, et al. “Therapeutic Alliance and Treatment Outcome in Cognitive Behavior Therapy for Obsessive-Compulsive Disorder.” Frontiers in Psychiatry, vol. 13, 2022, article 658693. https://doi.org/10.3389/fpsyt.2022.658693.

World Health Organization. World Mental Health Today: Latest Data. World Health Organization, 2025, www.who.int/publications/i/item/9789240113817.

Wu, Yiyang, et al. “A Pilot Randomized Controlled Trial of AI-Delivered vs. Human-Delivered iCBT for Depression in Young Adults.” BMC Psychiatry, vol. 26, no. 1, 2026, article 239. https://doi.org/10.1186/s12888-026-07925-1.

Xu, Shuo, and Tiancong Ma. “Depression Intervention Using AI Chatbots with Social Cues: A Randomized Trial of Effectiveness.” Journal of Affective Disorders, vol. 389, 2025, article 119760. https://doi.org/10.1016/j.jad.2025.119760.

How Do Red Meat and Plant-Based Diets Influence Colorectal Cancer Development?

By Thomas Lee

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Abstract

Colorectal cancer is one of the most common types of cancer, and diet is an important factor that may affect a person's risk of developing it. This research examines how red meat consumption and overall dietary patterns may contribute to colorectal cancer growth and development. Understanding this connection is significant because dietary choices are factors that people may be able to change, and learning how different foods affect the body may help identify ways to lower cancer risk. Research was conducted using scientific sources from databases such as Google Scholar and PubMed, focusing on recent review articles and primary research studies about red meat, colorectal cancer, and possible biological mechanisms. The results show that higher red meat consumption is associated with an increased risk of colorectal cancer. Possible explanations include heme iron and compounds produced during cooking that may contribute to cellular and DNA damage, as well as inflammation. In contrast, diets higher in fruits, vegetables, whole grains, and fiber may have protective effects by supporting the gut microbiome and reducing inflammation. Overall, the evidence suggests that both red meat consumption and overall dietary patterns can influence colorectal cancer risk. A balanced diet containing more fiber-rich plant foods and moderate amounts of red meat may support digestive health and potentially reduce cancer risk.

INTRODUCTION

Cancer is one of the leading causes of death around the world and occurs when cells in the body begin dividing uncontrollably, forming abnormal tissue growths known as tumors that can disrupt normal organ function. Colorectal cancer, which specifically develops in the tissue of the colon or rectum, is strongly influenced by modifiable lifestyle choices, making daily nutrition a major area of biomedical research. In particular, frequent consumption of red meat, such as beef, pork, and lamb, has been consistently linked to an increased incidence of colorectal cancer due to inherent compounds like heme iron, high fat levels, and harmful chemicals formed during high temperature cooking that can damage cellular DNA and trigger chronic inflammation.

On the other hand, protective diets rich in fruits, vegetables, whole grains, and dietary fiber appear to support the gut microbiome, accelerate intestinal clearance, and reduce mucosal cell damage, thereby lowering overall cancer risk. This review examines the relationship between red meat consumption and colorectal cancer by focusing on three main areas: the epidemiological evidence linking red meat intake to cancer risk, the molecular mechanisms that may contribute to cancer development, and the role of protective dietary patterns in cancer

prevention. Understanding these opposing dietary pathways is significant because it provides clear biological evidence to guide dietary recommendations that can help individuals lower their personal risk of developing colorectal cancer.

I: EPIDEMIOLOGICAL EVIDENCE LINKING RED MEAT CONSUMPTION TO COLORECTAL CANCER

Epidemiological research has consistently identified an association between red meat consumption and an increased risk of colorectal cancer. Epidemiology is important because it allows researchers to examine large populations and compare dietary patterns with rates of disease. When multiple studies produce similar results, the consistency of the findings provides stronger evidence that an observed relationship is unlikely to be entirely due to chance. In research examining red meat and colorectal cancer, multiple prospective studies have reported a positive relationship between higher red meat consumption and colorectal cancer risk. A meta-analysis by Chan et al. found that individuals with the highest consumption of red and processed meat had a 22% higher risk of colorectal cancer compared with those with the lowest consumption (Chan et al. 2011). Additionally, every 100 grams per day increase in red and processed meat consumption was associated with a 14% increase in colorectal cancer risk (Chan et al. 2011). This finding suggests a possible dose-response relationship, meaning that greater exposure to red meat may be associated with greater cancer risk. However, this does not mean that consuming a specific amount of red meat will automatically cause cancer in an individual; rather, it represents a difference in risk observed across populations.

Although epidemiological evidence supports an association, several factors can complicate the relationship between red meat and colorectal cancer. People who consume large amounts of red meat may also consume fewer fruits, vegetables, and whole grains or have other lifestyle characteristics that influence cancer risk. Researchers therefore attempt to account for factors such as physical activity, smoking, alcohol consumption, body weight, and overall diet when analyzing the relationship between meat consumption and cancer. Even with these adjustments, observational studies cannot completely eliminate the possibility that other factors contribute to the observed association. Genetic differences may also affect how individuals respond to dietary compounds, meaning that the same dietary exposure may not produce identical biological effects in everyone. Cultural and regional differences can further influence results because people in different countries may consume different amounts and types of meat, prepare meat differently, and have different overall dietary patterns. For example, consuming large amounts of grilled or processed meat as part of a diet low in fiber may produce a different risk profile than consuming smaller amounts of meat alongside fruits, vegetables, and whole grains. These differences demonstrate that colorectal cancer development is influenced by multiple interacting dietary, genetic, environmental, and lifestyle factors rather than by a single food alone.

Researchers have also examined whether red meat is associated with cancers other than colorectal cancer. The International Agency for Research on Cancer classified red meat as

probably carcinogenic to humans, with the strongest evidence involving colorectal cancer (IARC 2015). Some studies have suggested possible relationships between red meat consumption and other cancers, including pancreatic and prostate cancer, although these associations are less consistent than the evidence for colorectal cancer (IARC 2015). The stronger relationship with colorectal cancer may be particularly important because substances produced during digestion and compounds found in meat can interact directly with the cells lining the gastrointestinal tract. Cooking methods may also influence risk because high-temperature cooking can produce compounds such as heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs), while heme iron in red meat may contribute to biological processes associated with colorectal cancer development (WCRF). Overall, epidemiological evidence supports a relationship between higher red meat consumption and colorectal cancer risk, while also demonstrating that cancer development depends on many interacting factors. The consistency of the epidemiological findings provides a basis for investigating the molecular mechanisms that may explain this relationship, including the effects of heme iron, HCAs, PAHs, inflammation, and other compounds associated with red meat consumption.

II: MOLECULAR MECHANISMS LINKING RED MEAT CONSUMPTION TO COLORECTAL CANCER

Compounds found in red meat and chemicals produced during its preparation may contribute to colorectal cancer through several biological processes, including DNA damage, oxidative stress, mutations, and chronic inflammation. One important factor is how meat is cooked. When meat is exposed to high temperatures through methods such as grilling, frying, or broiling, chemical reactions between amino acids, sugars, and creatine can produce heterocyclic aromatic amines (HCAs). These compounds are formed from naturally occurring components of meat and are particularly associated with meat cooked to medium or well-done temperatures (Knize and Felton). Similarly, high-temperature cooking can produce other potentially harmful compounds, while the amount of HCAs present in meat varies depending on cooking method, temperature, and degree of doneness (Sinha et al.).

After entering the body, some HCAs and PAHs can be metabolically activated into more reactive substances capable of interacting with DNA. This can result in DNA adduct formation and other forms of genetic damage. If this damage is not properly repaired, mutations may develop during DNA replication. Mutations affecting genes involved in regulating cell growth and maintaining genomic stability can contribute to the progression of colorectal cancer (Knize and Felton). Therefore, compounds produced during high-temperature cooking provide one possible molecular pathway connecting meat preparation with carcinogenic processes.

In addition to chemicals produced during cooking, red meat contains naturally occurring compounds that may contribute to cancer development. One of the most extensively studied is heme iron, which contributes to the characteristic red color of meat. Heme can promote oxidative reactions and the formation of reactive oxygen species (ROS), which can damage cellular components, including proteins, lipids, and DNA. Research by Seiwert et al. describes heme as

an important component of red meat that can contribute to colorectal carcinogenesis through oxidative stress, epithelial damage, and other cellular effects (Seiwert et al.). Experimental research involving human colonocytes has also demonstrated that heme iron can promote ROS formation and oxidative DNA damage (Seiwert et al.). This repeated oxidative damage may increase the likelihood of mutations and disrupt normal cellular processes.

Heme iron may also contribute to colorectal cancer through the formation of N-nitroso compounds (NOCs). Heme can promote conditions in the colon that increase the formation of these potentially DNA-damaging compounds, providing another possible pathway through which red meat consumption may contribute to genetic damage. NOCs can react with cellular DNA and potentially increase mutations in colon cells. Therefore, the effects of heme may involve multiple mechanisms rather than a single pathway, including oxidative stress and the formation of compounds capable of damaging DNA.

Another possible mechanism involves dietary fat and changes in bile acid metabolism. Higher dietary fat intake can increase bile acid secretion into the digestive system. Although most bile acids are reabsorbed in the small intestine, some reach the colon, where intestinal bacteria can modify them into secondary bile acids. These include deoxycholic acid (DCA) and lithocholic acid (LCA), which have been investigated for their potential role in gastrointestinal carcinogenesis (Bernstein et al.). Elevated concentrations of secondary bile acids may contribute to cellular damage and inflammatory responses within the colon, potentially creating an environment that favors abnormal cellular growth (Bernstein et al.).

When cells lining the colon are repeatedly damaged, nearby cells must divide to replace them. Increased cellular turnover can provide additional opportunities for genetic errors to occur and accumulate. Persistent exposure to damaging compounds may also contribute to chronic inflammation. Chronic inflammation is relevant to cancer development because inflammatory processes can promote oxidative stress, cellular proliferation, and changes in the tissue environment that may support the survival of abnormal cells. These mechanisms can interact rather than function independently. For example, compounds produced during high-temperature cooking can contribute to DNA damage, while heme can promote oxidative stress and damage to colon cells (Knize and Felton; Seiwert et al.). Changes in bile acid metabolism may further contribute to cellular stress and inflammation (Bernstein et al.).

Overall, research suggests that red meat may contribute to colorectal cancer through several interconnected biological pathways. High-temperature cooking can produce HCAs and other potentially harmful compounds, heme iron can promote oxidative stress and DNA damage, and changes in bile acid metabolism may contribute to damage and inflammation within the colon (Knize and Felton; Seiwert et al.; Bernstein et al.). These mechanisms provide biological explanations for the association observed in epidemiological studies. Although these pathways do not mean that consuming red meat automatically causes cancer, they demonstrate how repeated exposure to multiple potentially damaging factors may contribute to DNA damage, inflammation, and abnormal cellular growth. Together, the evidence suggests that red meat may

influence colorectal cancer risk through a combination of genetic damage, oxidative stress, inflammation, and changes to the intestinal environment.

III: HOW DIETARY PATTERNS MAY MODIFY COLORECTAL CANCER RISK

Dietary patterns that contain more plant-based foods, dietary fiber, whole grains, and lean sources of protein may help lower the risk of colorectal cancer by reducing inflammation, oxidative stress, and damage to the intestinal environment. Research such as the EPIC study evaluated by Ubago-Guisado et al. (2021) has found that higher consumption of fruits, vegetables, and whole grains is generally associated with a lower risk of colorectal cancer. One important reason fiber may be protective is that it is used by bacteria in the colon during fermentation, producing short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate. Butyrate is especially important because it provides energy for healthy colon cells and may also help control the growth of abnormal cells. Fiber can also increase stool bulk and help food move through the digestive system more efficiently, which may reduce the amount of time that harmful compounds are in contact with the cells lining the colon (Bingham et al., 2005). Whole grains provide fiber as well as other nutrients and plant compounds that may support intestinal health. Fish may also have protective effects because it provides omega-3 fatty acids, which have been studied for their possible ability to reduce inflammation. Aglago et al. (2020) found that higher fish consumption was associated with a lower incidence of colorectal cancer. In addition to individual foods and nutrients, these types of foods can influence the gut microbiome. A diet containing a variety of plant foods and fiber can support a diverse group of bacteria in the digestive system, which may help maintain the intestinal lining and reduce chronic inflammation. This is important because damage to the intestinal lining and long-term inflammation can create conditions that support abnormal cell growth. However, although there is strong evidence connecting healthy dietary patterns with lower cancer risk, there is still controversy about whether individual nutrients can directly prevent cancer. Antioxidants, such as flavonoids and other plant compounds, are a good example. Laboratory studies have shown that some antioxidants can reduce oxidative damage and affect the growth of abnormal cells, but studies in humans have produced less consistent results. For example, a Cochrane review by Jin et al. (2012) found no consistent evidence that total dietary flavonoid intake directly reduced the risk of colorectal cancer. This difference between laboratory research and human studies shows why it can be difficult to determine the effect of one specific nutrient. A compound may have a strong effect when tested on isolated cells but have a much smaller or different effect when consumed as part of a person's normal diet. Nutrition research also has other limitations. Many studies depend on people remembering and reporting what they eat, which can lead to inaccurate information. It is also difficult to separate the effects of one food from other lifestyle factors. For example, a person who eats more fruits and vegetables may also exercise more, drink less alcohol, or have other habits that lower cancer risk. These factors can make it difficult to prove that one specific food is responsible for a reduction in cancer risk. Animal studies and laboratory experiments can help researchers understand possible biological mechanisms, but these models

cannot completely reproduce the complexity of the human body. People also have different genetics, gut microbiomes, lifestyles, and dietary habits, which means that the same food may not affect everyone in exactly the same way. Because of these limitations, future research could focus on larger long-term studies that follow people over many years and examine how different dietary patterns affect cancer development. Researchers could also study the relationship between diet, genetics, and the gut microbiome to better understand why certain people may respond differently to specific foods. Overall, the evidence suggests that diets containing more fruits, vegetables, whole grains, fiber, and fish may help lower colorectal cancer risk by supporting intestinal health, reducing inflammation, and influencing the gut microbiome. However, the evidence does not mean that one food or nutrient can completely prevent cancer. Instead, the strongest conclusion is that an overall healthy dietary pattern may help reduce some of the conditions that contribute to colorectal cancer while supporting normal intestinal function.

CONCLUSION

Overall, the research suggests that red meat consumption is associated with an increased risk of colorectal cancer, especially when consumed in higher amounts. Epidemiological studies have found a consistent relationship between red meat intake and colorectal cancer, with one study finding that consuming 100 grams of red meat per day was associated with a 14% higher risk (Chan et al., 2011). Molecular research also helps explain this relationship by showing how compounds in red meat and chemicals produced during cooking may damage cells. High-temperature cooking can produce HCAs and PAHs, while heme iron can increase oxidative stress and the formation of N-nitroso compounds that can damage DNA. High-fat diets may also affect bile acids and the gut environment, potentially increasing inflammation and abnormal cell growth. These processes can cause mutations and damage to genes that normally control cell growth, which can contribute to the development of cancer over time. At the same time, research suggests that diet can also play a role in lowering colorectal cancer risk. Foods such as fruits, vegetables, whole grains, and fiber may support intestinal health, reduce inflammation, and help maintain a healthy gut microbiome. Fish has also been associated with a lower risk of colorectal cancer in some studies. However, the relationship between diet and cancer is complicated, and not every study has found the same results. Factors such as genetics, lifestyle, cooking methods, overall diet, and differences between individuals can all affect cancer risk. Therefore, red meat should not be considered the only cause of colorectal cancer, and eating red meat does not mean that a person will develop cancer. Instead, the evidence shows that red meat consumption can be one factor among many that may influence cancer development. Overall, maintaining a balanced diet with a variety of fruits, vegetables, whole grains, and fiber while limiting excessive red meat consumption may help support intestinal health and reduce some factors associated with colorectal cancer. More research is still needed to understand exactly how different foods and dietary patterns interact with genetics, the gut microbiome, and other factors involved in cancer development.

Works Cited

[1] M. E. W. McLean et al., “A central role for heme iron in colon carcinogenesis,” Cancer Research, vol. 75, no. 5, pp. 870–879, 2015.

[2] “Mechanisms linking colorectal cancer to dietary factors,” 2021.

[3] N. Seiwert et al., “Mechanism of colorectal carcinogenesis triggered by heme iron from red meat,” Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, vol. 1873, no. 1, p. 188334, 2020.

[4] D. Calvo, J. A. Calvo, and L. D. Samson, “Balancing repair and tolerance of DNA damage caused by alkylating agents,” Nature Reviews Cancer, vol. 12, no. 2, pp. 104–120, 2012.

[5] D. S. M. Chan et al., “Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies,” PLoS ONE, vol. 6, no. 6, p. e20456, 2011.

[6] R. Sinha et al., “High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo-[4,5-b] pyridine (PhIP) occur in chicken but are dependent on the cooking method,” Cancer Research, vol. 55, no. 20, pp. 4516–4519, 1995.

[7] H. Bernstein et al., “Bile acids as carcinogens in human gastrointestinal cancers,” Mutation Research/Reviews in Mutation Research, vol. 589, no. 1, pp. 47–65, 2005.

[8] Y. Zhu et al., “Dietary N-nitroso compounds and risk of colorectal cancer: a case–control study in Newfoundland and Labrador and Ontario, Canada,” British Journal of Nutrition, vol. 111, no. 6, pp. 1109–1117, 2014.

[9] M. G. Knize and J. S. Felton, “Formation and human risk of carcinogenic heterocyclic amines formed from natural precursors in meat,” Nutrition Reviews, vol. 63, 2004.

[10] E. K. Ubago-Guisado et al., “Evidence update on the relationship between diet and the most common cancers from the European Prospective Investigation into Cancer and Nutrition (EPIC) study: a systematic review,” Nutrients, vol. 13, no. 10, p. 3582, 2021.

[11] H. Jin, Q. Leng, and C. Li, “Dietary flavonoid for preventing colorectal neoplasms,” Cochrane Database of Systematic Reviews, no. 8, 2012.

[12] S. A. Bingham et al., “Is the association with fiber from foods in colorectal cancer confounded by folate intake?” Cancer Epidemiology, Biomarkers & Prevention, vol. 14, no. 6, pp. 1552–1556, 2005.

[13] E. K. Aglago et al., “Consumption of fish and long-chain n-3 polyunsaturated fatty acids is associated with reduced risk of colorectal cancer in a large European cohort,” Clinical Gastroenterology and Hepatology, vol. 18, no. 3, pp. 654–666, 2020.

Rural Mobile Health Clinics: Effectiveness in Access Expansion and Clinical Outcomes
By Caroline Rickards

Abstract

In the U.S., geographic proximity to medical resources and economic conditions disproportionately affect Americans' access to healthcare. Low-income rural Americans receive medical care less than their urban counterparts. Mobile health clinics are an emerging strategy to reduce healthcare disparities by overcoming transportation and financial barriers associated with the U.S. healthcare system. They deliver medical services in a condensed, vehicular format. Focusing on health outcomes and healthcare access in low-income rural American communities, this paper found that mobile health clinics improve access to chronic disease management, dental and vision care, primary health care, and preventive care. The management of hypertension and type 2 diabetes are two primary diseases that showed clinical improvements in association with mobile health clinics. Effectiveness across all areas of medicine is limited due to mobile health clinics' physical capacity constraints, financial instability, and disconnect from broader healthcare networks. While considered beneficial in increasing healthcare access for low-income rural communities by overcoming financial and geographic barriers, the long-term impact and healthcare outcomes are dependent on continuous funding and stronger integration into traditional systems of care.

Introduction

The poverty crisis is known at national and global levels. Its association with malnourishment, poor quality of life, and unemployment is apparent in many conversations. The U.S. Census Bureau qualifies poverty as having a total family income below the poverty threshold, which varies by family size and composition. Less blatantly recognized is the significance of rural residency. In the United States, this concept is defined by a few metrics. According to the U.S. Census Bureau, a rural area is a geographic area that is neither an urban cluster (between 2,500 and 50,000 people) nor an urbanized area with more than 50,000 people (Ratcliffe et al., 2016). Housing, population, and geographical areas that fall outside of these defined urban areas are essentially considered rural. The relationship between poverty and rurality often differs from that between poverty and urban residence, introducing unique challenges and characteristics for non-metro populations.

Moreover, the relationship between rural poverty and healthcare is significant. Healthcare encompasses the prevention, diagnosis, and treatment of illness to maintain personal health. Healthcare access is a poverty issue because low economic status can cause barriers for those seeking timely and appropriate medical care. Conversely, lack of access to healthcare can worsen health conditions, potentially affecting employment capacity and perpetuating financial burdens. In impoverished rural communities, healthcare access and, ultimately, outcomes can fall short for populations. Transportation, lack of health infrastructure, familial responsibilities, language

barriers, and financial barriers are common obstacles to consistent healthcare access, which are amplified for those in rural communities.

Mobile health clinics are a proposed solution to combat these disparities. Mobile health clinics are "...a customized motor vehicle that travels to communities to provide health care. They deliver a wide variety of health services and may be staffed by a combination of physicians, nurses, community health workers, and other health professionals" (Malone et al., 2024). This research paper will examine the effectiveness of mobile health clinics in expanding access to preventive and primary care for rural low-income and impoverished populations, while offering recommendations to address their limitations.

Literature Review

Barriers to healthcare in rural communities are often defined by economic and geographic status. The poverty rate among rural Americans is slightly over 16%, compared to slightly over 13% in non-rural areas (O'dell, 2021). Financial hardships extend beyond this statistic: 35% of rural Americans struggle to afford healthcare, housing, and utilities (Chege et al., 2025). The average household income also varies across settings. Average rural household income is estimated to be over $20,000 less than that of metropolitan areas, at $63,750 compared to $87,410. Together, these economic disparities increase the burden of certain healthcare costs, such as health insurance premiums, medical bills, and prescription costs.

Americans' rurality is another concept involved in healthcare access. According to the U.S. Census Bureau, a rural area is a geographic area that is neither an urban cluster (between 2,500 and 50,000 people) nor an urbanized area with more than 50,000 people (Ratcliffe et al., 2016). Housing, population, and geographical areas that fall outside of these defined urban areas are essentially considered rural. Rural America is home to 20% of the country's population and healthcare landscape, making it a significant determinant of the United States' health outcomes (Monnat, 2025). In these communities, immediate access to healthcare facilities and providers is more limited due to geographical dispersion. Thus, rurality often entails higher travel costs and greater resource requirements when obtaining care.

Insurance coverage is another factor in restricting healthcare access. While Medicaid—the U.S.'s federally funded public health insurance for economically eligible populations—is available to improve healthcare access for people in low-income situations, many individuals don't qualify for this public coverage, yet can't afford private insurance. High out-of-pocket costs disincentivize those who fall into this coverage gap. This consequently discourages them from seeking medical treatments or preventative care. In instances such as breast cancer screening, lack of insurance is a major deciding factor for those who seek out the health service. Evidence demonstrates that general gaps in health insurance coverage resulted in mammography rates falling from 76.7% among women with consistent health insurance to 34.7% among women who experienced three periods without insurance (McWilliams, 2009). Therefore, health insurance is a primary factor in the utilization of preventive care.

Poverty and rurality intersect to create challenges to healthcare access. It has been found that rural populations have a lower likelihood of employer-sponsored health insurance. Those in rural areas are more likely to have jobs in blue-collar work, 71% compared to 63% in metropolitan areas, which often involves physical and technical tasks performed outside of an office building. Lower earnings and fewer benefits are associated with this career path, resulting in only 51% of rural Americans having employer-sponsored insurance compared to 57% of urban Americans (Newkirk and Damico, 2014). Furthermore, data indicates the persistence of this disparity. Employer-sponsored insurance covered 42% of people living in rural areas compared to 50% in urban areas. Public insurance coverage in rural areas also shows higher rates of Medicaid-qualifying individuals, with 24% relying on Medicaid compared to 21% in urban areas, which correlates to lower income (Euhus et al., 2025). This paper will build on research on the intersection of mobile health clinics and health, rurality, and poverty. It will address the effectiveness of mobile health clinics' ability to increase healthcare access and positive clinical results in certain specialties at reduced costs.

Rural Mobile Health Clinic Overview

Rural mobile health clinics were increasingly piloted in the U.S. in the second half of the 20th century to serve underrepresented communities. A preliminary example was Kaiser Permanente's implementation of a mobile van in the Kansas City area in 1988 that provided health education materials and simple health screenings[1] . Similarly, in the late 1990's Kaiser Permanente used a mobile van in Southern California to conduct routine health screenings[2] and host immunization events ("Mobile Clinics"). Mobile health clinics were designed to address some of the disparities identified in the literature review. If health clinics are not available in certain areas, mobile health clinics visit accessible locations for these rural populations, ultimately reducing the common transportation issue. Furthermore, they are designed with insurance and cost concerns in mind. Mobile health clinics are often equipped to bill Medicaid, Medicare, and private health insurance, and to offer reduced out-of-pocket payments. For patients who may need to pay out of pocket, a sliding-scale fee can be used to reasonably adjust the cost based on income and household circumstances. If patients are uninsured and can't pay out of pocket for services, free care is often provided and covered by funding sources such as donations and federal grants (Malone et al., 2020).

The early 2000's saw the expansion of services provided by mobile health clinics. Policies and reimbursement reforms incentivized insurers to fund mobile health units. In 2005, Place of Service code 15 was passed, enabling mobile clinics to register, track, and bill for services under commercial insurance and Medicaid (Centers for Medicare & Medicaid Services). Further incentivization occurred with the 2010 passage of the Affordable Care Act. This placed greater emphasis on preventive care, thereby refocusing financing on population health

[1] Blood Pressure and Cholesterol Level Testing
[2] Blood Pressure and Body Fat Testing

management. Therefore, insurers prioritized mobile clinics to reduce emergency room expenses (Hill et al. 2014).

Progress of these clinics continued, and in 2009, a mobile health vehicle in Hawaii was used to test for sexually transmitted diseases, provide mammograms, screen patients' glucose and cholesterol levels, and vaccinate against viruses such as the flu ("Mobile Clinics"). Current mobile health clinics' services range from counseling and education to chronic disease management. Type 2 diabetes and hypertension are typically treated chronic diseases in mobile health clinics. The management of both diseases is often minimally invasive, and they are highly prevalent, with almost 50% of American adults suffering from hypertension (CDC). As they advance, mobile health clinics are increasingly offering a comprehensive mixture of services. This means vaccinations, chronic disease management, behavioral health, and health screenings are often all accessible at rural mobile health clinic events (Simmonds et al. 2026). By increasing the range of care offered at a single location, patients can more effectively address their health concerns.

Mobile health clinics generally need a combination of funding sources to maintain an effective level of care. Aside from medical care, operating costs associated with these vehicular systems include maintenance, staff salaries, fuel, and long-distance travel. Annual operating costs vary based on state and service provided. A rural mobile health clinic in Oregon focusing on preventative and primary care costs $308,470 annually (Higgins et al., 2025). Mobile health clinics focusing on the same services in Texas, Georgia, Florida, and North Carolina, however, found the average operating cost to be $479,092 (Attipoe-Dorcoo et al., 2020). Mobile health clinics focused on cancer screening, specifically mammography units, were found to cost $304,890 annually in the Southern United States (Attipoe-Dorcoo et al., 2020). Data from the International Journal for Equity in Health summarizes the funding sources for 811 mobile health clinics across the U.S., showing that 52% received philanthropic funding, 45% received federal funding, 38% received public insurance, 37% received private insurance, and 27% received state funding (Malone et al., 2020). This highlights the common denominator of multiple funding sources for mobile health clinics. When reaching patients in rural areas, mobile clinics may be more costly due to the greater distance they must travel.

Target populations for mobile health clinics typically face economic, geographic, and structural barriers to traditional healthcare. 56% of the clinics from the study targeted uninsured people, 55% served low-income people, and 36% targeted rural communities. When examining the actual populations served, many were publicly insured or uninsured. According to data from Mobile Health Map, 41% of patients at mobile health clinics were uninsured, while 44% were publicly insured (Malone et al., 2020). The two primary types of public health insurance are Medicaid and Medicare. While Medicare is a federal program for adults 65 or older and younger people with certain disabilities, Medicaid is specifically for low-income populations. In the U.S., Medicaid eligibility varies by state. Generally speaking, adults aged 19-64 with household incomes at or below 138% of the Federal Poverty Level qualify for Medicaid. Almost half of the tracked mobile health clinics' patient population received federal- or state-funded health

insurance. As for the 41% of mobile health clinic patients who were uninsured, financial barriers, often correlating to systemic and structural issues, are a common reason for lack of health insurance in the U.S.

Effectiveness

Rural mobile health clinics have proven beneficial in certain contexts. A major conclusion is that mobile health clinics improve access for underserved populations; however, research on clinical outcomes is weaker. Demonstrated clinical improvement is associated with chronic disease management, specifically when managing hypertension and type 2 diabetes. Researchers reviewing over 10,000 mobile health clinic visits and 5,900 patients found that patients experienced an average reduction of 10.7 mmHg in systolic blood pressure and 6.2 mmHg in diastolic blood pressure (Song et al., 2014). Furthermore, evidence from a 2026 review of Project Rural Recovery, a rural mobile health clinic program in Tennessee, showed decreased diastolic blood pressure among 62% of patients and decreased systolic blood pressure among 60% of patients at reassessment (Simmonds et al., 2026). These are considered clinically meaningful outcomes. For example, a 20% lower risk of major cardiovascular disease events is associated with a 10 mmHg reduction in systolic blood pressure (Ettehad et al., 2016). Relative stroke risk reduction is also associated with lowered diastolic blood pressure (Song et al., 2014).

Further promising improvements occur in the management of type 2 diabetes. In patients with type 2 diabetes who received medications through mobile health clinics, statistically significant reductions in LDL cholesterol and HbA1c were observed. HbA1c, a measure of average blood sugar levels, decreased by 0.69 percentage points in the study. LDL cholesterol decreased by 13mg/dL (Coaston et al., 2022). Another mobile health clinic, the Health Hut, in Louisiana found a 20% or greater decrease in HbA1c in diabetic patients (Yu et al., 2017). While there is no universal service that mobile health clinics provide that is considered most effective, management of the two aforementioned diseases shows promising clinical improvements in mobile health clinic settings.

Aside from chronic disease management, improvements in general health outcomes are associated with mobile health clinics' preventive care. A 2026 scoping review examined studies on 34 mobile health clinics serving rural populations and found that 32 of 34 studies assessed health outcomes. Findings led to common improvements in preventive care and chronic disease management (Simmonds et al., 2026).

In terms of increasing healthcare access, a wider scope of services has evidence supporting impact. Mobile health clinics collectively provide increased access to underserved populations. When reviewing 3,923 women receiving mammography through a mobile unit, it was found that the mobile service reached women who had not otherwise received screening for breast cancer. 29% of the almost 4,000 patients had either never received a mammogram or hadn't had one in at least 5 years (Brooks et al., 2013). Furthermore, 56% were uninsured. Thus, the mobile health clinic brought screening to populations who may not otherwise receive it due to financial barriers. Although not necessarily associated with clinical improvements, breast

cancer screening rates did increase with mobile mammography services. Further evidence from the U.S. supports mobile mammography's ability to target underscreened and underserved populations. A systematic review by the CDC found that only 12-34% of mobile mammography users followed annual screening recommendations (Vang et al., 2018). Lack of adherence to these guidelines across these patients demonstrates mobile clinics' ability to serve women who are less likely to access traditional medical services.

Vision and eye-care services also have evidence supporting mobile health clinics' ability to increase access. Oregon's CEI, Community Eye Institute, runs a mobile eye clinic program that averages 1,200 vision screenings a year (Chedda et al., 2020). The clinic specifically targets uninsured and underinsured populations and reduces geographical barriers for vulnerable patients by visiting locations where patients already receive health and social services.

A large mobile dental program in Minnesota also demonstrates the effectiveness and breadth of mobile health clinics' ability to increase access to healthcare. Focusing on preventive and restorative dental care, particularly for patients with difficulty accessing oral medical treatment, the program increased its reach by about 150%, from 5,558 patients in 2000 to 13,863 in 2015 (Spetz et al., 2019).

The true effectiveness and benefits of mobile health clinics are based on their scope of services. Often, mobile health clinic events and programs provide a mix of medical and non-medical resources that indirectly reduce health disparities. Non-medical resources range from educational materials to information on community outreach programs. A prime example is from La Clínica in Oregon, which targets isolated rural communities. The program directly measured the time spent on social needs. The program's community health workers (CHWs) were found to spend 22% of their time addressing social needs, including housing assistance, insurance assistance, food insecurity, and transportation services (Higgins et al., 2025). This shows evidence of mobile health clinics' ability to address social determinants that indirectly decrease access to healthcare.

Primary healthcare in poor, rural populations is also improved through mobile health clinics. In a 2026 scoping review examining 34 studies on rural mobile health clinics, increased access to primary health care was a notable finding. One program from the review, Augusta Health Neighborhood Clinics, serviced 825 patients by providing 1,700 primary care visits (Simmonds et al., 2026). The same review found that all 34 studies of the mobile health clinic programs emerged with a common theme of overcoming geographic and transportation barriers. Traveling 30 miles to access medical care often dissuades populations due to time constraints, familial responsibilities, and high costs or lack of transportation. Because mobile health clinics can overcome many of these barriers by traveling directly into communities, populations that would otherwise not access care can be served.

The benefits of mobile health clinics also stem from the affordability of their services. Poverty disproportionality limits access to healthcare for many populations due to high out-of-pocket costs or lack of insurance in the U.S. Many mobile healthcare clinics are designed to provide free or low-cost services to the communities they serve. When examining 811 mobile

health clinics, 55% reported targeting low-income groups while 56% reported targeting uninsured groups (Malone et al., 2020). A scoping review of rural mobile health clinics identified affordability as an effective factor (Simmonds et al., 2026).

Mobile health clinics can also be considered a poverty-alleviation program because of their potential to reduce long-term healthcare costs through preventive care. A 2026 CDC study discusses the proven ability of health interventions to improve health and save money. When diabetes and cancer screening interventions are implemented, costs associated with complications and treatment for advanced stages of the diseases can be reduced. Mobile health clinics have been shown to increase screenings, thereby identifying diseases at earlier stages, which are sometimes associated with lower costs. Rural Oregon mobile health clinics note increased screening and intervention for diabetes, hypertension, and some cancers (Higgins et al., 2025). Mobile health clinics can increase healthcare access for low-income, rural communities by overcoming geographic distance, providing affordable care, and potentially reducing long-term health costs.

Limitations

While mobile health clinics have proven effective in chronic disease management, preventive care, and primary care, their ability to provide hospital-level care is limited. Major surgical procedures and the treatment of late-stage diseases are constrained by spatial, technological, and equipment capacities of mobile health clinics. A 2026 scoping review from the U.S. highlights the primary health care benefits while noting operational issues. Mobile health clinics are space-constrained due to their vehicular nature, which limits the range of services they can offer. The 2026 review notes that only one mobile mammography unit was limited to portable equipment, which may constrain the capacity to add diagnostic equipment for follow-up screenings (Yu et al., 2017).

Not only do the physical constraints of mobile health clinics impact their service capabilities, but they also reduce their integration into the broader healthcare system. Often, mobile health clinics are not fully interlaced with hospitals, laboratories, and pharmacies, making continuity of care difficult. The stakeholders behind this interaction are often shared between state policymakers, hospitals, federally qualified health centers (FQHCs), and existing health systems. In a 2026 study, Burleson & Sullivan identified that the incorporation of mobile health clinics into existing hospitals and FQHCs is being focused on in states such as Arizona, as discussed in Georgetown University's Center on Health Insurance Reforms article, in hopes of creating long-term sustainability. The same article cites Minnesota's embedding of mobile health clinics into existing referral networks, making speciality services that fall outside the common range of mobile health clinics' primary and preventative care capabilities more accessible. Nonetheless, the independent nature of mobile health clinics can pose the "risk of increased fragmentation of care" (Yu et al., 2017). Future research should examine ways to guide incentivisation strategies for existing networks to partner with mobile health clinics to increase continuation of care or necessary advanced treatment.

While mobile health clinics' primary purpose is to provide low-cost or free services, the challenge of securing sufficient funding limits the scope of their impact. Despite clinical effectiveness, a mobile health clinic can't be beneficial without financial sustainability, which is hard to maintain. While traditional medical providers get paid through patients' payments, or insurance, Medicaid, or Medicare reimbursements, mobile health clinics serve populations that often can't pay for the care they receive. Nevertheless, consistent reimbursement for staff, fuel, maintenance, medical equipment, and supplies is needed to maintain the clinics. Federal and philanthropic funding is often periodic, however. For example, the United States Health Resources and Services Administration administered a $1 million grant to Mobile Medical Units in Michigan (Simmonds et al., 2026). While this federal grant provides substantial capital, other funding sources are needed to sustain the clinic once the grant period ends. Another $250,000 grant from the nonprofit organization Direct Relief was awarded to the University of Florida Mobile Outreach clinic, which serves uninsured people in surrounding rural communities (Hesters, 2022). The grant's duration was one year, highlighting the discontinuous nature of funding for mobile health clinics. Because mobile health clinics are so cost-effective for patients, achieving financial sustainability can be difficult.

Future Direction

Moving forward, mobile health clinics can improve health outcomes for low-income rural communities by stabilizing funding and improving follow-up care. Short-term grants make it difficult for mobile health clinics to provide consistent service, emphasizing the need for partnerships and multi-year grants. If greater alliances and systems are built between mobile clinics, hospitals, specialty clinics, and local governments, less instability can be achieved. Furthermore, multi-year grants can enable mobile health clinics to consistently provide care, making it easier for target populations to be seen repeatedly and health outcomes to improve.

While the reach and number of patients seen are important measures of mobile health clinics' effectiveness in treating low-income rural populations, what happens to patients afterward is also an important indicator of these services' health outcomes. Thus, mobile health clinics should improve follow-up care systems through electronic referral systems that connect with surrounding medical centers, transportation assistance that enables patients to visit those centers, and follow-up calls and texts. Research about efficient communication strategies, the intersection of telehealth and mobile health clinics, and easily maintainable transportation methods could improve these outcomes. By investing in the technology and staffing needed to ensure patients receive follow-up treatment, it is possible to improve long-term results, not just scale.

Conclusion

Mobile health clinics are an effective strategy to increase access to medical care, and potentially alleviate financial burdens associated with healthcare for low-income rural Americans. In communities isolated by geographic location, limited transportation, and

economic stressors, mobile health clinics are a proven strategy to reduce healthcare inequality. Serving over 2.8 million uninsured patients and providing over 5 million annual visits, these clinics expand healthcare access to historically underserved populations (Coaston, 2022). Notable positive clinical outcomes, including reduced blood pressure and cholesterol, are also proven benefits of mobile health clinics. Although mobile health clinics cannot fully mitigate the cost of traditional hospitals or clinics, they can reduce patients' financial burden by matching amounts patients can afford. Furthermore, their ability to bill Medicaid and Medicare enables wider populations to be served. Nevertheless, space constraints limit the services provided, meaning mobile health clinics are unable to fully replace traditional hospitals for vulnerable populations. Overall, mobile health clinics are an impactful solution for low-income, rural populations seeking primary and preventative care.

Works Cited

Attipoe-Dorcoo, Sharon, et al. "Analysis of Annual Costs of Mobile Clinics in the Southern United States." Journal of Primary Care & Community Health, vol. 11, 2020, article 215013272098062, https://doi.org/10.1177/2150132720980623. Accessed 14 Sept. 2026.

Brooks, Sandra E., et al. "Mobile Mammography in Underserved Populations: Analysis of Outcomes of 3,923 Women." Journal of Community Health, vol. 38, no. 5, May 2013, pp. 900–06, https://doi.org/10.1007/s10900-013-9696-7. Accessed 27 Aug. 2026.

Burleson, Julia, and Leila Sullivan. "From Clinics to Communities: Mobile Health in State Rural Health Transformation Plans." Center on Health Insurance Reforms, Georgetown University, 3 Feb. 2026, https://chir.georgetown.edu/from-clinics-to-communities-mobile-health-in-state-rural-health-transformation-plans/. Accessed 12 Sept. 2026.

CDC. "Health and Economic Benefits of Chronic Disease Interventions." National Center for Chronic Disease Prevention and Health Promotion (NCCDPHP), 14 May 2026, https://www.cdc.gov/nccdphp/priorities/index.html. Accessed 27 Aug. 2026.

CDC. "High Blood Pressure Facts." High Blood Pressure, 2 June 2026, https://www.cdc.gov/high-blood-pressure/data-research/facts-stats/index.html. Accessed 14 Sept. 2026.

Chege, Wanjira, et al. "The Financial Health and Material Hardships of Rural America." Financial Health Network, Oct. 2025, finhealthnetwork.org/research/the-financial-health-and-material-hardships-of-rural-america. Accessed 15 Sept. 2026.

Chheda, Kousanee, et al. "Barriers to Eye Care among Participants of a Mobile Eye Clinic." Cogent Medicine, vol. 6, no. 1, 20 Aug. 2019, article 1650693, https://doi.org/10.1080/2331205X.2019.1650693. Accessed 27 Aug. 2026.

Coaston, Angela, et al. "Mobile Medical Clinics in the United States Post-Affordable Care Act: An Integrative Review." Population Health Management, vol. 25, no. 2, Apr. 2022, pp. 264–79, https://doi.org/10.1089/pop.2021.0289. Accessed 15 Sept. 2026.

Ettehad, Dena, et al. "Blood Pressure Lowering for Prevention of Cardiovascular Disease and Death: A Systematic Review and Meta-Analysis." The Lancet, vol. 387, no. 10022, 5 Mar. 2016, pp. 957–67, https://doi.org/10.1016/s0140-6736(15)01225-8. Accessed 8 Sept. 2026.

Euhus, Rhiannon, et al. "5 Key Facts About Medicaid Coverage for People Living in Rural Areas." KFF, 26 June 2025, www.kff.org/medicaid/5-key-facts-about-medicaid-coverage-for-people-living-in-rural-areas. Accessed 15 Sept. 2026.

Hesters, Matthew. "Mobile Health Clinic Expands Its Reach Thanks to Philanthropic Funding." Doctor Gator, 26 Apr. 2022,

news.drgator.ufl.edu/2022/04/26/mobile-health-clinic-expands-its-reach-thanks-to-philanthropic-funding. Accessed 15 Sept. 2026.

Higgins, Abigail, et al. “Mobile Health Clinics in a Rural Setting: A Cost Analysis and Time Motion Study of La Clínica in Oregon, United States.” BMC Health Services Research, vol. 25, no. 1, 17 Jan. 2025, article 97, https://doi.org/10.1186/s12913-024-12203-5. Accessed 27 Aug. 2026.

O’dell, Kennedy. Redefining Rural. Economic Innovation Group, Mar. 2021, https://eig.org/wp-content/uploads/2021/03/Rural-Report-2021.pdf. Accessed 27 Aug. 2026.

Malone, Nelson C., et al. “Mobile Health Clinics in the United States.” International Journal for Equity in Health, vol. 19, no. 1, 20 Mar. 2020, article 40, https://doi.org/10.1186/s12939-020-1135-7. Accessed 27 Aug. 2026.

“Mobile Clinics: ‘Health on Wheels.’” Kaiser Permanente, 5 Feb. 2019, https://about.kaiserpermanente.org/who-we-are/our-history/mobile-clinics-health-on-wheels. Accessed 27 Aug. 2026.

Newkirk, Vann R., and Anthony Damico. “The Affordable Care Act and Insurance Coverage in Rural Areas.” KFF, 29 May 2014, kff.org/uninsured/the-affordable-care-act-and-insurance-coverage-in-rural-areas/. Accessed 15 Sept. 2026.

Hill, Caterina F., et al.“Mobile Health Clinics in the Era of Reform.” American Journal of Managed Care, vol.20 no. 3, 20 Mar. 2014, pp 261-264, https://www.ajmc.com/view/mobile-health-clinics-in-the-era-of-reform. Accessed 2 Sept. 2026.

“Place of Service Code Set.” Centers for Medicare & Medicaid Services, 2 May 2024, https://www.cms.gov/medicare/coding-billing/place-of-service-codes/code-sets. Accessed 2 Sept. 2026.

Ratcliffe, Michael, et al. Defining Rural at the U.S. Census Bureau. U.S. Census Bureau, Dec. 2016, https://www.census.gov/content/dam/Census/library/publications/2016/acs/acsgeo-1.pdf. Accessed 27 Aug. 2026.

Rural Health Information Hub. “Social Determinants of Health for Rural People Introduction.” Rural Health Information Hub, 2022, https://www.ruralhealthinfo.org/topics/social-determinants-of-health. Accessed 27 Aug. 2026.

Simmonds, Katherine, et al. “What Do We Know About Rural Mobile Health Clinics? A Scoping Review.” International Journal of Environmental Research and Public Health, vol. 23, no. 5, 2026, article 558, https://doi.org/10.3390/ijerph23050558. Accessed 14 Sept. 2026.

Song, Zirui, et al. “Mobile Clinic In Massachusetts Associated With Cost Savings From Lowering Blood Pressure And Emergency Department Use.” Health Affairs, vol. 32, no.

1, Jan. 2013, pp. 36–44, https://doi.org/10.1377/hlthaff.2011.1392. Accessed 15 Sept. 2026.

Spetz, Joanne, et al. “Expansion of Dental Care for Low-Income Children Through a Mobile Services Program.” The Journal of School Health, vol. 89, no. 8, Aug. 2019, pp. 619–28, https://doi.org/10.1111/josh.12789. PubMed. Accessed 27 Aug. 2026.

US Census Bureau. “How the Census Bureau Measures Poverty.” US Census Bureau, 9 Apr. 2025, https://www.census.gov/topics/income-poverty/poverty/guidance/poverty-measures.html Accessed 27 Aug. 2026.

Vang, Suzanne, et al. “Mobile Mammography Participation Among Medically Underserved Women: A Systematic Review.” Preventing Chronic Disease, vol. 15, 2018, https://doi.org/10.5888/pcd15.180291. Accessed 15 Sept. 2026.

Yu, Stephanie W. Y., et al. “The Scope and Impact of Mobile Health Clinics in the United States: A Literature Review.” International Journal for Equity in Health, vol. 16, no. 1, 2017, article 71, https://doi.org/10.1186/s12939-017-0671-2. Accessed 27 Aug. 2026.

Digital Literacy Impact on Diabetes Outcome By Anna Li

Abstract

As healthcare technology advances, concerns about disparities in digital health literacy and how it affects disease outcomes are increasing. For a disease as prevalent as diabetes, this issue is vital to investigate. Previous research on determinants of diabetes outcomes mostly focuses on comparing the significance of socioeconomic and health determinants, and little research has been done on digital literacy in relation to these factors. Thus, this investigation focuses on how digital, health, socioeconomic, and demographic indicators contributed to diabetes outcomes. In this investigation, data from 6887 respondents in the 2024 Health Information National Trends Survey (HINTS) were analyzed using Pearson chi-square tests, Cramér's V, survey-weighted logistic regression, and predictive machine-learning models. 25 predictors were chosen by considering the theoretical relevance they have to each category of determinants. The logistic-regression model achieved a test AUC of 0.751 (95% CI [0.719, 0.782]). Health indicators made the largest unique contribution, followed by socioeconomic factors. Removing digital literacy factors reduced the AUC by 0.0003 (95% CI [−0.006, 0.012]), thus showing no significant improvement in prediction in the algorithm. The same result was derived when using a Random Forest algorithm. However, these findings do not show that digital literacy is not important to diabetes status; rather, the factors in digital literacy overlap substantially with health and socioeconomic factors, making their independent contribution difficult to isolate.

Introduction

Diabetes is one of the most prevalent diseases globally, affecting around 547 million individuals, with rates increasing by over 45% since 1990 (Peréz & Smith, 2019; Hossain et al., 2024). The economic impact of Type 2 diabetes increased by 87 percent from 2007 to 2017. Moreover, projections indicate that cases for both Type 1 and Type 2 diabetes could reach 1.31 billion by 2050. This may directly lead to increases in health complications associated with diabetes such as cardiovascular diseases, blindness, kidney failure, and lower-limb amputations(3). Type 1 diabetes is primarily caused by genetics and only accounts for 8% of diabetic patients, while Type 2 diabetes is more prevalent and caused by a variety of socio-economic, genetic, and lifestyle factors (Chang et al., 2022).

Lifestyle factors contributing to the escalating rate of diabetes include poor diet, lack of physical activity, and obesity. In particular, Type 2 diabetes is strongly associated with body mass index (BMI) ≥35 kg/m2 (Zhang et al., 2017). In addition, obesity at a young age is a very relevant risk factor for diabetes. Increased physical activity can reduce the risk of type 2 diabetes among men and women, and moderate activity achieves a protective effect against diabetes (Zhang et al., 2017).

Recent research also found socioeconomic status to be a prominent contributor to type 2 diabetes risk, with low economic status associated with barriers to healthcare and a healthy

lifestyle (4). For instance, low-income individuals may struggle to afford or access to nutritious food, preventive healthcare, medication, glucose-monitoring equipment, etc. As a result of lacking access to nutritious food, families may depend on cheaper processed food, which often contains more saturated fat, added sugar, and refined carbohydrates. This could make blood-glucose management more difficult, especially if families lack access to health insurance, which may deny them access to many glucose-monitoring equipment (Hill-Briggs et al.,).

Digital literacy/ digital health literacy

Digital literacy is defined as the ability to understand and use information from technology (Malotiya, Rahul, et al. "EXPLORING THE CONCEPTUAL UNDERSTANDING OF DIGITAL LITERACY: A FRAMEWORK FOR PROMOTING DIGITAL LITERACY IN..." *ResearchGate*, Dec. 2023, www.researchgate.net/publication/378773496_EXPLORING_THE_CONCEPTUAL_UNDERSTANDING_OF_DIGITAL_LITERACY_A_FRAMEWORK_FOR_PROMOTING_DIGITAL_LITERACY_IN_THE_DIGITAL_ERA.). Within healthcare, digital health literacy revolves specifically around the ability to find and understand health information from digital devices. In contrast to digital access, digital literacy requires the ability to understand and interpret health information, thus extending beyond merely possessing internet access (Norman, Cameron D., and Harvey A. Skinner. "eHEALS: The eHealth Literacy Scale." *Journal of Medical Internet Research*, vol. 8, no. 4, Nov. 2006, p. e27. https://doi.org/10.2196/jmir.8.4.e27.). A patient may have internet access but still lack the skills to identify medical information, interpret digital health data, or use online healthcare platforms effectively.

Particularly, digital literacy directly correlates with the quality of diabetes care. A 2021 study of 249 diabetic people found that mobile eHealth literacy had a direct relationship with self-care for diabetes and an indirect relationship with health outcomes, such as glycemic control (Guo, Sophie Huey-Ming, et al. "Relationships Between Mobile eHealth Literacy, Diabetes Self-care, and Glycemic Outcomes in Taiwanese Patients With Type 2 Diabetes: Cross-sectional Study." *JMIR Mhealth and Uhealth*, vol. 9, no. 2, Sept. 2020, p. e18404. https://doi.org/10.2196/18404.) This is because much of the management for diabetes occurs outside of clinical settings. For instance, patients need to interpret blood glucose readings and trends, learn to use a continuous glucose monitor, access diabetes-management applications, and know how to view test results and schedule appointments through patient portals. A better understanding of health information and knowledge of how to use a digital medical device may strengthen their diabetes knowledge, medication adherence, and dietary management(Guo, Sophie Huey-Ming, et al. "Relationships Between Mobile eHealth Literacy, Diabetes Self-care, and Glycemic Outcomes in Taiwanese Patients With Type 2 Diabetes: Cross-sectional Study." *JMIR Mhealth and Uhealth*, vol. 9, no. 2, Sept. 2020, p. e18404. https://doi.org/10.2196/18404.).

However, as digital literacy becomes relevant to diabetes outcomes, there are also inevitably inequities in digital literacy between social groups. A national study of U.S adults with diabetes found that digital literacy among the study group has increased from 35.3% in 2011 to

46.8% in 2018, but substantial inequities persisted by age, income, race, ethnicity, and English-language proficiency (Nagori et al., 2024). Another study found that education, annual income, number of digital devices, and digital health literacy are associated with the level of diabetes knowledge (Kim, Calderon, and Kim, 2025). Because these inequities are often associated with socioeconomic factors, digital literacy may amplify the effect of existing socioeconomic disadvantages on diabetes outcome.

Existing research shows that digital health literacy is closely associated with diabetes outcomes. However, digital literacy also overlaps with conventional socioeconomic factors such as income, education, and employment. This makes it difficult to determine whether digital literacy alone plays a big role in diabetes outcomes, or if it directly reflects existing socioeconomic inequities. Therefore, this study investigates the extent to which digital literacy is associated with diabetes outcomes, compared with health and socioeconomic indicators. This information could better prepare policymakers when formulating policies in addressing diabetes healthcare accessibility.

Methodology

This study is a secondary cross-sectional analysis of the 2024 Health Information National Trends Survey 7 (HINTS 7). HINTS is a census administered by the US National Cancer Institute and collects a wide array of information on demographic, health, healthcare access, socio-economic status, and health-information technology use among U.S adults. HINTS 7 collected a total of 7,278 adult responses from March to September 2024. Self-reported diabetes status was obtained from the variable MedConditions_Diabetes, which was coded as a binary outcome. 1 was used to represent “Yes” and 0 to represent “0”. In the end, 391 respondents were excluded due to missing diabetes status, leaving the analytical sample with 6,887 respondents. Out of these respondents, 1,534 (22.27%) reported being diabetic, while 5,353 (77.73%) reported having no diabetes. Survey weights supplied by HINTS were used in descriptive and inferential analyses to account for unequal representation of population groups in survey responses. Ultimately, this study examines whether diabetes status could be predicted using digital literacy, health, and socioeconomic factors. Statistical and machine learning methods such as Pearson chi-square tests, Cramér’s V, survey-weighted logistic regression, ROC-AUC, and leave-one-domain-out analysis were used to evaluate the independent associations and comparative predictive contribution of these features.

Predictors were selected for their relevance to diabetes, digital literacy indications, and socioeconomic inequity. They fall into three categories: digital literacy, health, and socioeconomic status. Redundant predictors were removed to reduce the risk of multicollinearity. For example, BMI was kept as a feature, while height and weight were excluded. Lastly, high-speed internet was initially selected but removed later after predictor-overlap analysis. The initial 26 predictors (later turned 25 due to elimination of a completely overlapping predictor) included 9 digital indicators, 9 health indicators, and 8 socioeconomic or demographic indicators. Digital indicators cover digital access, comprehension, and engagement with

digital-health resources. Health indicators include exercise, BMI, sleep, chronic conditions, general health, and smoking. Lastly, socioeconomic and demographic indicators include income, education, language, sex, race, insurance, marital status, and ethnicity.

Before analysis, variables were encoded into consistent and interpretable categories. BMI values were grouped into standard weight categories, exercise was grouped into no activity, 1-149 minutes, or more than 150 minutes per week, and weekday and weekend sleep were combined into an average and split into short, recommended, or long sleep. Other variables such as detailed income, education, age, smoking, and health condition responses were encoded similarly. Invalid or nonascertained responses were not removed, but labeled as missing. Eliminating these missing responses reduced the sample from 6,887 to 5,337 respondents. Thus, a "Missing/unkonwn" category was created to retain these missing predictor responses, while meaningful responses that indicate a question did not apply, such as no record of access to the internet or digital devices, were put in a separate category instead of being treated as missing.

For each feature, the total number of respondents, number of respondents reporting diabetes, and the percentage of respondents reporting diabetes were calculated (both survey-weighted and unweighted). The unweighted analysis treated each respondent the same, reflecting information from the observed HINTS sample. The survey-weighted analysis, on the other hand, used HINTS final person weight, labeled PERSON_FINWT0, which adjusted the weight of respondents' contributions so the data reflects a more accurate representation of the U.S. adult population, accounting for potential sampling and nonresponse bias. Preliminary associations between each predictor and diabetes status were analyzed through unweighted Pearson chi-square tests. Then, Cramer V was used to measure the association's strength. Respondents who had missing/unknown values for certain predictors were excluded only in tests involving those predictors. Statistical significance was evaluated at $p<0.05$.

A survey-weighted multivariable logistic regression model was applied to evaluate the independent association between each model feature (predictor) and the respondent's diabetes status. All 25 predictors were entered into the model simultaneously, with each association measured while controlling for the remaining digital, health, socioeconomic and demographic indicators. All 6,887 respondents were included in the model, with missing data categorized in a dedicated 'missing/unknown' category. The HINTS final person weight (PERSON_FINWT0) was applied, and the 50 replicate weights were utilized to account for standard errors. The results were reported as adjusted odds ratios with 95% confidence intervals and p-values. It is important to note that because this study is cross-sectional, the relationships identified between variables are best interpreted as associations rather than causation.

To examine how accurately the predictors could support the models in identifying diabetes in respondents that the model previously has not seen, this study created a separate logistic regression model. The sample was divided using an 80/20 stratified split, resulting in 5,509 respondents as the training dataset and 1,378 respondents as the testing dataset. Categorical predictors were one-hot encoded. Additionally, we applied a second categorical model, Random Forest, for secondary robustness analysis. The Random Forest model is tuned

through five-fold stratified cross-validation on the training data, with 500 trees, a maximum depth of 10, a minimum of five samples per leaf, and balanced class weights. Both models were first trained on the 80% training dataset to learn relationships between variables and diabetes status, then evaluated on the 20% test set using unseen data. Predicted probabilities were converted into diabetes classification based on the default 0.5 threshold. Performance was assessed through a confusion matrix, accuracy, precision, sensitivity, specificity, F1 score, and ROC-AUC. Accounting for the fact that the standard 0.5 threshold may miss many diabetes cases, Youden's J statistic was applied on the training dataset to select a threshold that balances sensitivity and specificity better. The selected threshold was evaluated on the test dataset. Lastly, predictive contributions were compared between health, digital literacy, and socioeconomic/demographic domains. This was done by removing each domain from the full model and consequently measuring the decrease in ROC-AUC from the full model's ROC-AUC. A greater decrease implies that the removed domain contributes more predictive information that the remaining predictors lack. The uncertainty of the model AUC and AUC differences was estimated through 1000 paired bootstrap samples. Results were reported as 95% confidence intervals. Since Random Forest was only included as a secondary robustness comparison based on test ROC-AUC, its threshold results and predictor-domain analysis was not reported, and only conducted with logistic regression.

Results

Of the resulting 6,887 respondents included in the analytical sample, 1,534 (22.27%) reported diabetes while 5,353 (77.73%) did not. Survey-weighted descriptive results showed that diabetes prevalence was often higher in respondents with little digital literacy, poor health, and socioeconomic disadvantage. For example, in digital literacy indicators, diabetes prevalence was higher among respondents who have never used the internet than among daily internet users (39.43% versus 16.99%). In health indicators, diabetes prevalence was higher among respondents with a BMI of 35 or more than among people with a regular BMI (34.95% versus 4.34%). In socioeconomic factors, those with less than high-school education had higher diabetes prevalence than college graduates or postgraduates (28.9% versus 14.64%). Percentages are calculated within each category; therefore, they do not sum to 100% across categories. Table 1 in the appendix shows the complete category-level results.

Table 1. Diabetes prevalence by predictor category (HINTS 7; N = 6,887)

Domain	Predictor	Category	Respondents	Diabetes respondents	Unweighted diabetes percent	Survey-weighted diabetes percent
Digital	**Internet-use frequency**	Daily or more	5,902	1,156	19.59%	16.99%
Digital	Internet-use frequency	Never	308	127	41.23%	39.43%
Digital	Internet-use frequency	Occasional use	651	244	37.48%	33.03%
Digital	**Smartphone use**	No	616	220	35.71%	36.51%
Digital	Smartphone use	Yes	6,229	1,299	20.85%	17.47%

Digital	**Confidence completing medical forms**	A little	496	138	27.82%	20.49%
Digital	Confidence completing medical forms	Not at all	245	84	34.29%	29.76%
Digital	Confidence completing medical forms	Somewhat	1,522	349	22.93%	17.50%
Digital	Confidence completing medical forms	Very	4,567	946	20.71%	18.21%
Digital	**Online medical-record understanding**	No online-record access	2,040	467	22.89%	17.54%
Digital	Online medical-record understanding	Somewhat difficult	468	111	23.72%	22.06%
Digital	Online medical-record understanding	Somewhat easy	1,969	409	20.77%	16.76%
Digital	Online medical-record understanding	Very difficult	75	16	21.33%	20.43%
Digital	Online medical-record understanding	Very easy	2,156	460	21.34%	19.93%
Digital	**Electronic health-information seeking**	No	1,297	387	29.84%	25.06%
Digital	Electronic health-information seeking	Yes	5,507	1,110	20.16%	17.34%
Digital	**Health-app use**	Did not use a health app	2,801	648	23.13%	19.31%
Digital	Health-app use	No tablet or smartphone	172	66	38.37%	42.61%
Digital	Health-app use	Used a health app	3,644	716	19.65%	17.16%
Digital	**Wearable-device use**	No	4,281	1,033	24.13%	20.01%
Digital	Wearable-device use	Yes	2,527	475	18.80%	16.71%
Digital	**Telehealth use**	No	4,416	894	20.24%	16.59%
Digital	Telehealth use	Yes	2,418	620	25.64%	22.35%
Health	**BMI category**	Healthy weight	2,000	280	14.00%	10.01%
Health	BMI category	Obesity: BMI 30–34.9	1,331	363	27.27%	23.00%
Health	BMI category	Obesity: BMI 35 or higher	1,076	396	36.80%	34.95%
Health	BMI category	Overweight	2,196	445	20.26%	16.26%
Health	BMI category	Underweight	129	11	8.53%	7.74%
Health	**Weekly moderate exercise**	150 or more minutes per week	2,619	441	16.84%	14.29%
Health	Weekly moderate exercise	1–149 minutes per week	2,468	544	22.04%	19.87%
Health	Weekly moderate exercise	No weekly moderate exercise	1,607	493	30.68%	24.11%
Health	**Average sleep duration**	Long sleep: more than 9 hours	395	123	31.14%	32.23%
Health	Average sleep duration	Recommended sleep: 7–9 hours	3,963	764	19.28%	14.87%

Health	Average sleep duration	Short sleep: less than 7 hours	2,280	555	24.34%	22.63%
Health	**Heart condition**	No	6,138	1,208	19.68%	16.10%
Health	Heart condition	Yes	720	318	44.17%	44.29%
Health	**Depression**	No	5,000	1,058	21.16%	17.73%
Health	Depression	Yes	1,854	465	25.08%	20.78%
Health	**Pain limitation**	No	5,438	1,016	18.68%	15.39%
Health	Pain limitation	Yes	1,359	490	36.06%	32.33%
Health	**Mobility limitation**	No	5,799	1,101	18.99%	15.70%
Health	Mobility limitation	Yes	1,012	409	40.42%	39.01%
Health	**Smoking status**	Current	692	181	26.16%	20.56%
Health	Smoking status	Former	1,672	471	28.17%	26.24%
Health	Smoking status	Never	4,274	830	19.42%	15.66%
Health	**General health**	Excellent	685	39	5.69%	4.34%
Health	General health	Fair	1,125	437	38.84%	32.03%
Health	General health	Good	2,613	666	25.49%	23.01%
Health	General health	Poor	211	96	45.50%	43.44%
Health	General health	Very good	2,225	290	13.03%	11.07%
Socioeconomic/ demographic	**Household income**	$100,000 or more	1,959	280	14.29%	13.08%
Socioeconomic/ demographic	Household income	$20,000–$49,999	1,702	438	25.73%	23.07%
Socioeconomic/ demographic	Household income	$50,000–$99,999	1,874	414	22.09%	19.56%
Socioeconomic/ demographic	Household income	Less than $20,000	1,338	400	29.90%	22.26%
Socioeconomic/ demographic	**Education**	College graduate or postgraduate	3,166	553	17.47%	14.64%
Socioeconomic/ demographic	Education	High school or vocational training	1,594	409	25.66%	19.46%
Socioeconomic/ demographic	Education	Less than high school	434	163	37.56%	28.90%
Socioeconomic/ demographic	Education	Some college	1,425	357	25.05%	20.44%
Socioeconomic/ demographic	**Health insurance**	Insured	6,227	1,426	22.90%	19.05%
Socioeconomic/ demographic	Health insurance	Uninsured	622	102	16.40%	15.02%
Socioeconomic/ demographic	**Survey language**	English	6,617	1,456	22.00%	18.52%
Socioeconomic/ demographic	Survey language	Spanish or mixed-language	270	78	28.89%	23.39%
Socioeconomic/ demographic	**Age group**	18–44 years	1,986	186	9.37%	8.64%
Socioeconomic/ demographic	Age group	45–64 years	2,142	504	23.53%	21.57%
Socioeconomic/ demographic	Age group	65 years or older	2,466	789	32.00%	33.55%
Socioeconomic/ demographic	**Sex at birth**	Female	3,965	841	21.21%	18.34%
Socioeconomic/ demographic	Sex at birth	Male	2,624	636	24.24%	18.94%
Socioeconomic/ demographic	**Race/ethnicity**	Hispanic	1,304	293	22.47%	17.53%
Socioeconomic/ demographic	Race/ethnicity	Non-Hispanic Asian	337	70	20.77%	17.29%

Socioeconomic/ demographic	Race/ethnicity	Non-Hispanic Black or African American	947	309	32.63%	27.85%
Socioeconomic/ demographic	Race/ethnicity	Non-Hispanic Other	255	58	22.75%	16.99%
Socioeconomic/ demographic	Race/ethnicity	Non-Hispanic White	3,528	664	18.82%	16.78%
Socioeconomic/ demographic	**Marital status**	Divorced or separated	1,098	296	26.96%	26.36%
Socioeconomic/ demographic	Marital status	Married or partnered	3,364	704	20.93%	18.85%
Socioeconomic/ demographic	Marital status	Single	1,461	275	18.82%	14.75%
Socioeconomic/ demographic	Marital status	Widowed	689	211	30.62%	30.35%

Unadjusted chi-square tests found that 25 of the 26 predictors were significantly associated with diabetes (Table 2). The only non-significant predictor was online-record understanding (p = .414l Cramér's V = .024). The strongest unadjusted associations were found in general health (V = .264), age group (V = .222), BMI category (V = .192), mobility limitation (V = .183), and heart condition (V = .180). High-speed internet access was also significant (p < .001; V = .113), but it was subsequently removed because it overlapped with internet-use frequency rather than because it lacked an association with diabetes. Ultimately, 24 of the 25 predictors that were included in the final adjusted model had significant unadjusted associations.

Table 2. Unadjusted associations between candidate predictors and diabetes

Predictor	Respondents tested	χ^2	Pf1-value	Cramér's V	Minimum expected count
General health	6,859	478.20	.001	0.264	47.01
Age group	6,594	325.99	<.001	0.222	445.45
BMI category	6,732	249.21	<.001	0.192	28.65
Mobility limitation	6,811	229.30	<.001	0.183	224.36
Heart condition	6,858	223.33	<.001	0.180	160.21
Pain limitation	6,797	190.26	<.001	0.167	301.11
Internet-use frequency	6,861	175.63	<.001	0.160	68.55
Income group	6,873	128.71	<.001	0.137	298.24
Education group	6,619	117.23	<.001	0.133	97.17
Exercise category	6,694	110.88	<.001	0.129	354.82
High-speed internet access	6,791	87.43	<.001	0.113	57.52
Smartphone use	6,845	71.69	<.001	0.102	136.70
Race and ethnicity	6,371	83.95	<.001	0.115	55.80
Electronic health-information seeking	6,804	57.34	<.001	0.092	285.36
Smoking status	6,638	59.60	<.001	0.095	154.50
Marital status	6,612	54.74	<.001	0.091	154.85
Sleep category	6,638	43.72	<.001	0.081	85.81
Health-app use	6,617	40.64	<.001	0.078	37.17
Medical-form confidence	6,830	36.10	<.001	0.073	54.42
Telehealth use	6,834	26.38	<.001	0.062	535.68
Wearable use	6,808	26.21	<.001	0.062	559.74

Health insurance	6,849	13.79	<.001	0.045	138.77
Depression	6,854	12.03	<.001	0.042	411.97
Birth sex	6,589	8.32	.0039	0.036	588.20
Survey language	6,887	7.10	.0077	0.032	60.14
Online-record understanding	6,708	3.94	.4144	0.024	16.36

In the survey-adjusted model, health and demographic predictors were observed to have the most significant association with diabetes (Table 3A-3C). Compared with respondents in the healthy-weight category, the adjusted odds of diabetes were 1.43 times higher in respondents who were overweight, 1.86 times higher among those with a BMI of 30-34.9, and 3.63 higher among those who had a BMI above 35. Sleep, presence of a heart condition, and poor general health were also strongly associated with higher odds of diabetes. Moreover, age also remained significant after the adjustment. When compared to respondents aged 18-44 years, those that are aged 45-64 years had 2.6 times the odds of having diabetes, and those aged 65 years or older had 4.49 times the odds of having diabetes. Other health indicators such as exercise, depression, pain limitation, mobility limitation, and smoking status were not significant in the adjusted model.

However, most digital indicators were not statistically significant after adjustment, though several were in the previous unadjusted analysis. This suggests that several of the differences in diabetes status created by digital indicators in the unadjusted analysis may have overlapped with differences in health, age, and socioeconomic predictors. The only significant adjusted digital comparison was observed in respondents with no online-record access, who had lower odds of having diabetes than respondents who have access to their records and understand them easily (AOR = 0.64, 95% CI [0.44, 0.93], p = .0197; Table 3A). However, respondents without online record access likely were not able to evaluate how easily they understood an online record. Thus these two conceptually different concepts-access and comprehension- should not be interpreted as evidence that lacking online-record access protects patients against diabetes.

Table 3A Digital indicators

Term	Adjusted odds ratio	Lower 95% CI	Upper 95% CI	P-value	Significant at .05
Internet frequency: Never	0.93	0.42	2.06	0.8485	No
Internet frequency: Occasional use	1.33	0.93	1.90	0.1113	No
Smartphone use: No	1.45	0.90	2.32	0.1213	No
Medical-form confidence: A little	0.94	0.61	1.44	0.7636	No
Medical-form confidence: Not at all	1.31	0.49	3.46	0.5811	No
Medical-form confidence: Somewhat	0.83	0.63	1.09	0.1695	No
Online-record understanding: No online-record access	0.64	0.44	0.93	0.0197	Yes
Online-record understanding: Somewhat difficult	0.85	0.49	1.47	0.5543	No
Online-record understanding: Somewhat easy	0.74	0.54	1.02	0.0617	No
Online-record understanding: Very difficult	0.41	0.15	1.09	0.0728	No
Electronic health-information seeking: No	1.22	0.81	1.84	0.3347	No
Health-app use: Did not use a health app	0.95	0.71	1.28	0.7409	No
Health-app use: No tablet or smartphone	1.51	0.63	3.61	0.3469	No

Wearable use: No	0.91	0.66	1.25	0.5557	No
Telehealth use: Yes	1.28	1.00	1.63	0.0518	No

Table 3B Health indicators

Term	Adjusted odds ratio	Lower 95% CI	Upper 95% CI	P-value	Significant at .05
BMI: Obesity, 30–34.9	1.86	1.31	2.64	0.0008	Yes
BMI: Obesity, 35 or higher	3.63	2.36	5.59	0.0000	Yes
BMI: Overweight	1.43	1.01	2.02	0.0417	Yes
BMI: Underweight	0.63	0.15	2.66	0.5245	No
Exercise: 1–149 minutes per week	1.25	0.95	1.65	0.1149	No
Exercise: No weekly moderate exercise	1.00	0.73	1.38	0.9940	No
Sleep: More than 9 hours	1.82	1.04	3.18	0.0367	Yes
Sleep: Less than 7 hours	1.32	1.05	1.66	0.0200	Yes
Heart condition: Yes	1.76	1.22	2.55	0.0035	Yes
Depression: Yes	1.06	0.79	1.44	0.6762	No
Pain limitation: Yes	1.28	0.91	1.81	0.1563	No
Mobility limitation: Yes	1.46	0.94	2.27	0.0938	No
Smoking status: Current	1.13	0.77	1.66	0.5309	No
Smoking status: Former	1.33	0.98	1.80	0.0663	No
General health: Fair	4.21	2.16	8.20	0.0001	Yes
General health: Good	3.84	2.15	6.87	0.0000	Yes
General health: Poor	5.27	2.09	13.29	0.0007	Yes
General health: Very good	2.35	1.35	4.08	0.0032	Yes

Table 3C Socioeconomic Indicators

Term	Adjusted odds ratio	Lower 95% CI	Upper 95% CI	P-value	Significant at .05
Income: $20,000–$49,999	1.37	0.92	2.06	0.1230	No
Income: $50,000–$99,999	1.39	0.99	1.96	0.0579	No
Income: Less than $20,000	1.32	0.88	2.00	0.1785	No
Education: High school or vocational training	0.78	0.58	1.03	0.0815	No
Education: Less than high school	0.90	0.50	1.61	0.7106	No
Education: Some college	0.93	0.67	1.29	0.6737	No
Insurance: Uninsured	1.01	0.64	1.57	0.9814	No
Language: Spanish or mixed-language	1.56	0.87	2.81	0.1338	No
Age: 45–64 years	2.60	1.89	3.58	0.0000	Yes
Age: 65 years or older	4.49	3.20	6.29	0.0000	Yes
Birth sex: Male	1.20	0.94	1.53	0.1438	No
Race/ethnicity: Hispanic	1.47	1.01	2.13	0.0451	Yes
Race/ethnicity: Non-Hispanic Asian	2.38	1.49	3.80	0.0005	Yes
Race/ethnicity: Non-Hispanic Black or African American	1.72	1.29	2.28	0.0004	Yes
Race/ethnicity: Non-Hispanic Other	1.12	0.62	2.04	0.6941	No
Marital status: Divorced or separated	0.93	0.65	1.35	0.7152	No
Marital status: Single	0.95	0.71	1.27	0.7203	No
Marital status: Widowed	0.88	0.56	1.37	0.5614	No

The 25-predictor model achieved a test ROC-AUC of 0.751 (95% CI [0.719, 0.782]), which indicates a moderate ability to distinguish diabetic respondents from non-diabetic ones (Table 4). At a standard probability threshold of 0.5, the model achieved an accuracy of 77.8% and a specificity of 94.0%. However, its sensitivity was low, being only 21.2%. This means that though the overall performance of the model was good, it often failed to identify respondents with diabetes.

Youden's J statistic selected a lower threshold of 0.196 using the training data. When this threshold was applied to the testing data, the sensitivity increased from 21.2% to 73.0%, and the F1 score increased from 0.298 to 0.498. However, specificity decreased from 95.0% to 65.6%, accuracy decreased from 77.8% to 67.3%, and the number of false positive cases increased (Table 4). Thus, the lower threshold may provide a more balanced classification of diabetes but will produce more false-positive results.

Table 4A

Measurement	Standard threshold: 0.50	Youden's J threshold: 0.196
True negatives	1,007	703
False positives	64	368
False negatives	242	83
True positives	65	224
Accuracy	0.778	0.673
Sensitivity	0.212	0.730
Specificity	0.940	0.656
Precision	0.504	0.378
F1 score	0.298	0.498

The tuned Random Forest model, in comparison to the logistic regression ROC-AUC of 0.751, achieved a test ROC-AUC of 0.756. The difference was 0.005 (95% CI [-0.012,0.023]). However, because the confidence interval involved zero, the differences were not significant between the models.

Table 4B. Comparison of test ROC-AUC Between Predictive Models

Model or comparison	Estimate	95% CI
Logistic regression AUC	**0.751**	**0.719–0.782**
Random Forest AUC	**0.756**	**0.725–0.784**
AUC difference: Random Forest − logistic regression	**0.005**	**−0.012–0.023**

Additional analyses were done to analyze how each predictor category (socioeconomic, health, and digital literacy) contributed to diabetes prediction. Leave-one-domain-out analyses were then conducted. The model with only health indicators demonstrated the strongest predictive performance, with a test ROC-AUC of 0.721. The second-best performing model was the socioeconomic and demographic model, with an AUC of 0.67. The digital-indicator model had the lowest AUC of only 0.593, indicating weak discrimination.

In the leave-one-domain-out analysis, removing health indicators created the largest AUC decrease, from 0.751 to 0.684, which corresponds to a decrease of 0.067 (95% CI [0.045, 0.091]). On the other hand, removing socioeconomic and demographic indicators reduced the AUC by 0.025 (95% CI [0.005, 0.044]). For digital indicators, removing them only reduced the AUC by 0.003 (95% CI [−0.006, 0.012]). Since the confidence interval included zero, the analysis is unable to provide clear evidence that removing digital indicators was able to change the model's predictive performance.

Table 5A. Performance of individual predictor domains

Predictor domain	Number of predictors	Test ROC-AUC
Digital indicators	8	0.593
Health indicators	9	0.721
Socioeconomic/demographic indicators	8	0.670
All indicators combined	25	0.751

Table 5B. Changes in AUC after removing each predictor domain

Model	Test ROC-AUC	95% CI for AUC	AUC decrease	95% CI for decrease
All domains	0.751	0.719–0.782	—	—
Without digital indicators	0.748	0.717–0.778	0.003	−0.006–0.012
Without health indicators	0.684	0.647–0.717	0.067	0.045–0.091
Without socioeconomic/demographic indicators	0.725	0.694–0.756	0.025	0.005–0.044

Discussion

Firstly, the difference in performance between Logistic Regression and Random forest was negligible, despite the latter being more complex. Moreover, the finding indicates that health indicators are the strongest predictors and contributors to diabetes status. These findings indicate that the Logistic Regression model could not find an independent association between these predictors and diabetes status after accounting for their shared relation with various other predictors. Counterintuitively, health indicators such as exercise, depression, pain limitation, mobility limitation, and smoking status were not significant in the adjusted model. However, they showed significance in the unadjusted model. This is likely because their associations overlapped with stronger predictors in the model, such as BMI, age, heart conditions, and general health.

In comparison, socioeconomic and digital literacy contribute less; however, they are still meaningful. This may be explained by the fact that health indicators such as BMI, heart conditions, and general health are closely tied to the diabetes condition directly. It's also important to note that, while health indicators have the most significant independent association with diabetes, these indicators alone cannot fully explain diabetes status discrepancy among populations. Differences in resources, healthcare access, and social circumstances may influence a person's diabetes risk and diagnosis.

While several digital indicators were shown to be associated with diabetes in the unadjusted analysis, most did not have independent associations with diabetes status once socioeconomic and health indicators were accounted for. Digital access and technology are often closely tied to age, education, income, and health status, which may explain why the association becomes weaker in the adjusted analysis. However, these findings should not be viewed as evidence that digital literacy is entirely unimportant. Instead, it demonstrates that digital indicators in the HINTS dataset were not able to improve identification of diabetes beyond information captured in other categories.

Ultimately, these findings may suggest that digital literacy by itself is insufficient in addressing diabetes disparity. Though improving accessibility and comprehension of these medical technologies may help patients manage their own health, these efforts should be combined with plans to address underlying health and socioeconomic barriers.

Limitations

There are several limitations in this finding. Firstly, HINTS is cross-sectional, so the observations made in this essay should only demonstrate possible correlation, not causation, between two variables. For instance, digital access may influence a person's diabetes status, but having diabetes may also lead a patient to access digital diabetes management tools more frequently. Moreover, the diabetes measured in this study is self-reported and is not separated as Type 1 and Type 2 diabetes, and important clinical indicators such as HbA1c were unavailable. The selected digital indicators are also not representative of diabetes-specific indicators, but instead more representative of general medical technological access and confidence in using. Future research should focus on using longitudinal data, validated digital-literacy measures, and diabetes specific clinical outcomes. Moreover, external validation would also be needed before a predictive model is employed in real-world settings.

Conclusion

In conclusion, health indicators showed the largest unique contribution in identifying diabetes, while socioeconomic and demographic indicators provide meaningful but less significant contributions. The selected digital indicators show correlation in unadjusted analysis; however, after accounting for both health and socioeconomic factors, they added little independent contribution to predicting diabetes outcomes. Nevertheless, this finding should not be used to dismiss the importance of digital literacy in diabetes outcomes, but rather highlights the important distinction between identifying diabetes status and managing diabetes after diagnosis.

Digital literacy is essential for managing diabetes. As the digital world advances, many health-related activities are gradually moved online and/or monitored digitally. For instance, digital literacy becomes important when patients need to find and evaluate health information, understand test results, communicate with health providers, monitor their glucose, and follow medication, dietary, and exercise recommendations. Research finds that digital literacy is

associated with better self-care confidence, better self-care, and lower diabetes-related stress (Mo et al., 2026). Moreover, digital literacy has also been associated with better medication adherence and a greater ability to translate online diabetes information into self-care routines (Peimani et al.,2024). Therefore, the independent effects of digital literacy may have been more conspicuous in diabetes care rather than diabetes outcomes.

Overall, policy should address both long-term management and diabetes prevention. For instance, policymakers should address socioeconomic barriers such as food insecurity, healthcare affordability, and health literacy, which may affect people's health outcomes and, in turn, their diabetes outcomes. For diabetic patients, diabetes care services should provide more support for incorporating digital-literacy training, while non-digital alternatives should also remain available.

Works Cited

Pérez, M. A., & Smith, J. L. (2019). THE GLOBAL BURDEN OF DIABETES: PREVALENCE, PREVENTION AND RECOMMENDATIONS. Revista de La Facultad de Medicina Humana. https://doi.org/10.25176/rfmh.v19.n2.2079

Hossain, M. J., Al-Mamun, M., & Islam, M. R. (2024). Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. Health science reports, 7(3), e2004. https://doi.org/10.1002/hsr2.2004

Tian, L., Lu, C., Teng, D., & Teng, W. (2025). Association between blood glucose indicators and metabolic diseases in the Chinese population: A national cross-sectional study. Chinese medical journal, 138(17), 2159–2169. https://doi.org/10.1097/CM9.0000000000003701

Chang, V., Ganatra, M. A., Hall, K., Golightly, L., & Xu, Q. A. (2022). An assessment of machine learning models and algorithms for early prediction and diagnosis of diabetes using health indicators. Healthcare Analytics, 2, 100118.

Liu, S., Liu, T., Cao, Y., Lu, C., Guo, J., Li, J., ... & Li, Y. (2026). Low socioeconomic status as a major risk factor for early-onset type 2 diabetes with limited lifestyle mediation: Cross-Sectional evidence from US and South Korean populations. Social Science & Medicine, 118939.

Artificial Intelligence in Early Cancer Detection: A Review of Diagnostic Accuracy, Methodological Limitations, and Ethical Challenges By Rahul Tulasi

Introduction

Early detection of cancer can improve patient survival and treatment options. Traditional techniques such as MRI scans, CT scans, X-rays, PET scans, and endoscopies require significant manual intervention from specialized clinicians, which may be inaccurate and time-consuming. With any mistakes or setbacks in this process, initiation of treatment could be delayed. AI and machine learning tools have powerful data-processing capabilities, can detect even minor patterns, and can flag possible cases of cancer that need to be checked. This paper explores the application of AI and ML in the early detection of cancer. While the use of AI and ML improves the detection of cancer, various challenges arise. These challenges include the lack of diversity in the dataset, the retrospective nature of the studies, and ethical considerations. Using findings from existing studies, this paper analyzes the role of AI in cancer detection and its impact on diagnostic accuracy and clinical practice.

Literature Review

Recent research highlights the significant potential of AI and ML in enhancing the detection of cancer at early stages. The authors Bulusu, Vidyasagar, Mudigonda, and Saikia (2025) demonstrate the effectiveness of AI and ML algorithms in improving cancer detection through analysis of MRI, CT, ultrasound, and PET scans. Similarly, Zhang, Wu, Lei, Zhu, and Li (2025) demonstrate that leveraging machine learning to evaluate MRI modalities significantly enhances diagnostic accuracy in breast cancer classification, reinforcing the broader utility of automated image analysis across diverse oncology disciplines.

In lung cancer, Thong et al. (2023) stated that AI technology has a high diagnostic performance for lung cancer. However, the small sample sizes and the use of retrospective studies can be limitations in generalizing the results. Jones et al. (2022) analyzed the ability of AI in diagnosing several skin cancers and AI's efficiency, but only a few studies have analyzed its performance in the clinical setting. As well, Shi et al. (2024) have shown that AI increases diagnostic performance for gastric cancer using endoscopic and imaging modalities, although these findings may not generalize across other cancer types or clinical settings. Together, these studies show that AI has demonstrated promising diagnostic performance across several cancer types, while also exposing limitations in sample size, study design, and real-world clinical validation.

Systematic reviews have shown that AI-based methods are as effective as or exceed traditional image analysis in early cancer diagnosis (Bulusu et al., 2025; Da Silva et al., 2023). Among several cancer types, AI has demonstrated promising performance in the field of imaging-dominant screening settings where pattern recognition is needed, for example, early nodule recognition in CT scans (Thong et al., 2023), lesion characterization in MRI imaging in breast examinations (Zhang et al., 2025), early skin cancer detection in primary care settings

(Jones et al., 2022), and early mucosal abnormalities in gastric endoscopies (Shi et al., 2024). Essentially, these methods help elevate non-specialists' performance to expert levels (Shi et al., 2024). Despite these favorable findings, a significant implementation hurdle remains because much of the available evidence is based on retrospective data rather than prospective clinical studies (Da Silva et al., 2023; Hunter et al., 2022).

Taken together, these studies generally suggest that AI can enhance diagnostic performance in early cancer detection. However, the strength of this evidence varies across cancer types, imaging modalities, datasets, and study designs. Nevertheless, critical gaps in the literature remain regarding how these technologies can be transitioned from controlled experimental environments into routine clinical practice. Although existing studies frequently identify implications involving data variation, retrospective bias, "black box" algorithms, and liability (Hunter et al., 2022), fewer studies examine how clinicians should manage these uncertainties when AI systems are incorporated into real-world diagnostic decision-making.

Methodology

This study conducted a secondary analysis of academic articles retrieved from PubMed, Google Scholar, and EBSCO Host. To assess the diagnostic accuracy and clinical applicability of AI algorithms, the literature search included peer-reviewed empirical studies, systematic reviews, case studies, and research papers. Quantitative analysis of algorithmic accuracy across various imaging techniques was based on peer-reviewed empirical studies, whereas systematic reviews and meta-analyses gave the combined information for assessing the diagnostic ability of AI models. It is important to consider case studies and technical research papers because of the issues related to the practical application of AI algorithms, their performance in special cases, and ethical concerns. To identify relevant literature, PubMed, Google Scholar, and EBSCOhost databases were used along with Boolean search strings such as "machine learning AND cancer detection AND medical imaging" and "AI AND early cancer diagnosis."

The evidence base in this case includes peer-reviewed articles that contain original research conducted by professionals in the field, review articles that offer different perspectives on trends and limitations, and case studies that present the applications. The format of the sources presented in this case allows for a comparative analysis of cancer types, study approach, results, and the degree to which the studies concur or diverge.

Later, after obtaining the sources, they were grouped based on themes. The themes included sources that focused on the efficacy and abilities of AI in cancer diagnosis, while others highlighted the limitations of AI in cancer diagnosis and the ethics involved in cancer diagnosis using AI.

Findings

Two key findings surfaced from the literature. First, the reviewed literature generally indicates that AI can improve diagnostic accuracy and support earlier cancer detection. Bulusu et al. (2025) found that AI-assisted imaging improves the detection of tumors in MRI, CT,

ultrasound (US), and PET scans. Zhang et al. (2025) add that machine learning analysis of MRI images further enhances the diagnosis of breast cancer. Thong et al. (2023) demonstrate that AI improves imaging accuracy in lung cancer; however, Jones et al. (2022) found that AI is reliable for detecting different forms of skin cancer. Shi et al. (2024) also found that the use of AI can enhance the detection of gastric cancer using endoscopic imaging. As a whole, these findings suggest that AI may assist clinicians in identifying complex imaging patterns that could otherwise be difficult to detect.

The second critical finding addresses the substantial methodological and ethical obstacles hindering the safe deployment of AI in oncology. Among these limitations, including dataset bias and retrospective studies, algorithmic opacity ("black box" models) represents an important barrier to clinical adoption. In oncology, where diagnostic findings determine whether to intervene with biopsy or chemotherapy, clinicians cannot ethically delegate this decision to a vague system. The lack of explainability directly fuels the primary causes of the medicolegal stalemate over responsibility issues. As Hunter et al. (2022) point out, determining whether liability lies with the physician or the developer is impossible when neither party can determine the logic behind an inaccurate diagnosis made by an algorithm. Thus, limited algorithmic explainability may complicate questions of clinical responsibility and liability when AI contributes to an inaccurate diagnosis.

Discussion

Addressing these challenges may necessitate changes that extend beyond technical improvements alone. While future clinical trials and larger, multicenter datasets would be essential to validate the model accuracy, technical precision alone cannot guarantee adoption. Hospitals and health systems would have to put in place proper institutional infrastructure, including proper legal frameworks for AI liability, appropriate regulatory standards for evolving AI models, and specialized medical education that trains clinicians to critically interpret algorithmic outputs. Through the combination of technical, legal, and clinical expertise, health care institutions can foster the necessary trust to safely integrate AI in oncology practice.

Conclusion

AI and machine learning have significant potential in the early detection of cancers such as breast cancer, lung cancer, skin cancer, and gastric cancer. AI and machine learning may provide doctors with a platform to identify cancers at earlier stages and with higher accuracy, improving patient outcomes and enabling earlier interventions. For doctors, AI may serve as a critical adjunct tool that provides them with the opportunity to analyze images at a micro-level and identify high-risk zones much faster. However, certain drawbacks must be addressed before AI can be fully integrated into healthcare systems. The primary challenge encountered by AI & machine learning will be the fact that these systems are trained on small datasets and lack diversity. However, despite these challenges, continuous research could help determine how these technologies can be safely and effectively integrated into clinical practice.

If used properly, AI technology has the potential to help medical professionals make quicker, more accurate diagnoses, resulting in better patient outcomes. AI technology does not just have the potential to reduce the impact of errors made by medical staff, but it can also help in quick diagnoses and the early start of treatment. Artificial intelligence, when effectively utilized, will not replace clinical expertise; instead, it has the potential to significantly influence how physicians detect and diagnose cancer. By identifying patterns and facilitating image analysis beyond the scope of vision, AI serves as a vital analytical aid that minimizes the risk of error, accelerates time to diagnosis, and enhances diagnostic precision. Ultimately, the future of oncology does not rest with autonomous algorithms, but in a harmonious model where machine intelligence enhances human judgment to reform early cancer detection and patient outcomes.

Works Cited

Bulusu, G., Vidyasagar, K. E. C., Mudigonda, M., & Saikia, M. J. (2025). Cancer detection using artificial intelligence: A paradigm in early diagnosis. Archives of Computational Methods in Engineering, 32, 2365–2403. https://doi.org/10.1007/s11831-024-10209-0

Da Silva, H. E. C., Martins Santos, G. N., Leite, A. F., Mesquita, C. R. M., Figueiredo, P. T. de S., Stefani, C. M., & de Melo, N. S. (2023). The use of artificial intelligence tools in cancer detection compared to traditional diagnostic imaging methods: An overview of systematic reviews. PLOS ONE, 18(10), e0292063. https://doi.org/10.1371/journal.pone.0292063

Hunter, B., Hindocha, S., & Lee, R. W. (2022). The role of artificial intelligence in early cancer diagnosis. Cancers, 14(6), 1524. https://doi.org/10.3390/cancers14061524

Jones, O. T., Matin, R. N., van der Schaar, M., Prathivadi Bhayankaram, K., Ranmuthu, C. K. I., Islam, M. S., Behiyat, D., Boscott, R., Calanzani, N., Emery, J., Williams, H. C., & Walter, F. M. (2022). Artificial intelligence and machine learning algorithms for early detection of skin cancer in community and primary care settings: A systematic review. The Lancet Digital Health, 4(6), e466–e476. https://doi.org/10.1016/S2589-7500(22)00023-1

Shi, Y., Fan, H., Li, L., Hou, Y., Qian, F., Zhuang, M., Miao, B., & Fei, S. (2024). The value of machine learning approaches in the diagnosis of early gastric cancer: A systematic review and meta-analysis. World Journal of Surgical Oncology, 22(1), 40. https://doi.org/10.1186/s12957-024-04111-2

Thong, L. T., Chou, H. S., Chew, H. S. J., & Lau, Y. (2023). Diagnostic test accuracy of artificial intelligence-based imaging for lung cancer screening: A systematic review and meta-analysis. Lung Cancer, 176, 4–13. https://doi.org/10.1016/j.lungcan.2022.12.002

Zhang, J., Wu, Q., Lei, P., Zhu, X., & Li, B. (2025). Diagnostic accuracy of machine learning-based magnetic resonance imaging models in breast cancer classification: A systematic review and meta-analysis. World Journal of Surgical Oncology, 23(1), 231. https://doi.org/10.1186/s12957-025-03874-3

Antibiotic Resistance in *Escherichia coli*: Biology, Clinical Significance, and Treatment Strategies By Sophie Adamik

The discovery of penicillin nearly a century ago resulted in a revolution in modern medicine, dramatically reducing the number of deaths from bacterial infections (Lobanovska & Pilla 5). However, the widespread use and misuse of these drugs has accelerated the evolution of antibiotic-resistant bacteria and threatens the effectiveness of many lifesaving treatments. Antimicrobial resistance is one of the biggest public health problems in the world today, as resistant bacteria are causing infections that are getting harder (and sometimes impossible) to treat (Salem et al. 4).

One of the most medically important bacteria contributing to this crisis is called *Escherichia coli* (*E. coli*). *E. coli* is generally known to be a normal part of the human gut microbiome but some pathogenic strains can cause serious gastrointestinal disease, urinary tract infections (UTIs), bloodstream infections, neonatal meningitis, and sepsis (Geurtsen et al. 5). Many of these pathogenic strains have developed resistance to multiple classes of antibiotics, decreasing treatment options and increasing patient morbidity and mortality rates. Specifically, extended-spectrum β-lactamase (ESBL)-producing and carbapenem-resistant *E.coli* are serious threats in both hospital and community settings, as they resist many first-line antibiotics and often require more complicated therapies (Husna et al., 3).

Biology and Characterization of *E. coli*

E. coli is a Gram-negative bacterium within the family Enterobacteriaceae and the order Enterobacterales. First discovered by German pediatrician Theodor Escherich in 1885, *E. coli* has become one of the most extensively studied organisms in microbiology due to its simple genetic and ease of cultivation under laboratory conditions (Ruiz and Silhavy, 9). *E. coli* is a straight, rod-shaped (bacillus), non-spore-forming bacterium measuring approximately 1-3μm in length and 0.4-0.7μm in diameter. In contrast to Gram-positive bacteria, *E. coli* possesses only a thin peptidoglycan layer which is surrounded by an outer membrane containing lipopolysaccharide (LPS). This outer membrane serves as an additional barrier to antibiotic penetration and partially accounts for the intrinsic resistance of *E. coli* to many antimicrobial agents (Ebbensgaard et al. 13).

Most *E. coli* strains possess numerous flagella that facilitate movement toward nutrients and away from adverse environmental conditions. *E. coli* is capable of growing in both aerobic and anaerobic environments by switching between cellular respiration and fermentation depending on oxygen availability, known as a facultative anaerobe. Under optimal laboratory conditions, *E. coli* can divide every twenty minutes. The optimal growth temperature is 37°C, Corresponding to the normal human body temperature (Cruz-Loya et al. 10).

Diseases Caused by *E. coli*

Most strains of *E. coli* are harmless forms of normal gut bacteria; however, pathogenic strains are responsible for a wider range of intestinal and extraintestinal infections. The most prevalent disease associated with *E. coli* is urinary tract infection (UTI) with uropathogenic *E. coli* (UPEC) accounting for approximately 75-95% of uncomplicated UTIs globally (Whelan et al. 14). These infections typically originate in the bladder (cystitis) and may progress to the kidneys (pyelonephritis) if left untreated. Pathogenic strains of *E. coli* are also responsible for gastrointestinal illnesses. Shiga toxin-producing *E. coli* (STEC) can induce bloody diarrhea and hemorrhagic colitis which may progress to hemolytic uremic syndrome (HUS), a life-threatening condition that can result in kidney failure (Joseph et al. 13). Enterotoxigenic *E. coli* (ETEC) is a leading cause of traveler's diarrhea, particularly in developing countries (Joseph et al. 14).

Treatment of *E. coli* Infections

Treatment strategies depend on the type of infection and the antibiotic susceptibility profile. Nitrofurantoin, trimethoprim-sulfamethoxazole (TMP-SMX), and fosfomycin are frequently prescribed for uncomplicated UNIs. TMP-SMC impedes folic acid synthesis, which is essential for bacterial growth (Husna et al., 13). Given the increasing prevalence of antibiotic resistance, antimicrobial susceptibility testing should guide treatment decisions whenever feasible.

Development of Antibiotic Resistance of *E. coli*

One of the biggest challenges in treating E. coli infections today is the growing problem of antibiotic resistance. Antibiotic resistance occurs when bacteria evolve and are no longer killed by medications that once worked against them (Salem et al. 4). Because E. coli reproduces very quickly, random genetic mutations that help the bacteria survive can spread through a population in a short amount of time (Madigsn et al. 86). In addition to mutations, E. coli can gain resistance genes from other bacteria through horizontal gene transfer. This often occurs through plasmids, which are small pieces of DNA that move between bacterial cells and carry resistance genes (Thomas and Nielsen 714). As a result, resistance can spread rapidly, even among different bacterial species.

The overuse and misuse of antibiotics have greatly accelerated this process. Antibiotics are sometimes prescribed when they are not necessary, such as for viral illnesses, or patients stop taking their medication before finishing the full course. Both situations allow some bacteria to survive and continue multiplying, increasing the chance that resistant strains will become more common (Salem et al. 8). Antibiotics have also been widely used in agriculture to prevent disease and promote growth in livestock, which contributes to the overuse and misuse of antibiotics. Antibiotics are sometimes prescribed when they are not necessary, such as for viral illnesses, or patients stop taking their medication before finishing the full course. Both situations allow some bacteria to survive and continue multiplying, increasing the chance that resistant strains will become more common (Salem et al. 8). Antibiotics have also been widely used in agriculture to prevent disease and promote growth in livestock, which contributes to the

development of resistant bacteria that can spread through food, water, and the environment (Salem et al. 10). According to the CDC, antimicrobial resistance has become one of the world's most urgent public health threats because resistant bacteria continue to evolve and spread between people, animals, and the environment.

E. coli has developed several ways to protect itself from antibiotics. Some strains produce enzymes called extended-spectrum beta-lactamases (ESBLs), which destroy antibiotics like penicillins and cephalosporins before they can kill the bacteria (Busna et al. 5). Other strains produce carbapenemases that inactivate carbapenem antibiotics, which are often used as a last resort. In addition, E. coli can pump antibiotics out of the cell or prevent them from entering through changes in its outer membrane (Salem et al. 9). These adaptations make infections much more difficult to treat and reduce the number of antibiotics that remain effective.

Public Health Impact

Antimicrobial-resistant E. coli has become a major public health concern around the world. Resistant infections are often harder to treat, require longer hospital stays, and increase healthcare costs because patients may need stronger antibiotics or additional medical care (Salem et al. 12). Older adults, young children, hospitalized patients, and people with weakened immune systems are especially at risk for severe infections and complications (Gdurtsen et al. 7).

Resistant E. coli is no longer found only in hospitals. It has also been detected in communities, food products, livestock, and the environment, allowing resistant bacteria to spread more easily between people and countries through travel and the food supply (Salem et al. 13). To slow this problem, health organizations promote antibiotic stewardship, which encourages doctors and patients to use antibiotics only when they are truly necessary. Researchers are also continuing to develop new antibiotics and alternative treatments to help fight resistant strains (CDC).

Interesting Facts

Although E. coli is often associated with food poisoning and disease, most strains are actually harmless and play an important role in the human digestive system. These beneficial bacteria help break down food, support a healthy gut microbiome, and produce vitamin K, which is needed for normal blood clotting (Madigan et al. 20).

E. coli is also one of the most important organisms used in scientific research. Because it grows quickly and is easy to study, scientists have used it for decades to better understand genetics and how bacteria function (Browning and Busby 20). Genetically modified E. coli is commonly used to produce medicines such as human insulin, making it an important part of modern medicine. Researchers are now studying new ways to treat antibiotic-resistant E. coli, including bacteriophage therapy and other alternatives to traditional antibiotics, which may help treat infections that no longer respond to current medications (Salem et al. 14).

Conclusion

Escherichia coli is a diverse bacterium that can be both beneficial and harmful. While many strains are a normal part of the human digestive system, others can cause serious infections that are becoming more difficult to treat because of antibiotic resistance (Husna et al. 3).

As resistance continues to spread, responsible antibiotic use and continued research will be essential for protecting public health. By understanding how E. coli develops resistance, scientists can continue developing new treatments to help keep antibiotics effective for future generations.

Works Cited

Cruz-Loya, Mauricio, et al. “Antibiotics Shift the Temperature Response Curve of Escherichia Coli Growth.” mSystems, edited by James C. Stegen, vol. 6, no. 4, Aug. 2021, https://doi.org/10.1128/msystems.00228-21.

Ebbensgaard, Anna, et al. “The Role of Outer Membrane Proteins and Lipopolysaccharides for the Sensitivity of Escherichia Coli to Antimicrobial Peptides.” Frontiers in Microbiology, vol. 9, Sept. 2018, https://doi.org/10.3389/fmicb.2018.02153.

Geurtsen, Jeroen, et al. "Genomics and Pathotypes of the Many Faces of Escherichia coli." FEMS Microbiology Reviews, vol. 46, no. 6, 2022, fuac031. Oxford University Press, https://doi.org/10.1093/femsre/fuac031.

Husna, A., et al. "Extended-Spectrum β-Lactamases (ESBL): Challenges and Opportunities." Biomedicines, vol. 11, no. 11, 2023, p. 2937. MDPI, https://doi.org/10.3390/biomedicines11112937.

Joseph, Adrien, et al. “Shiga Toxin-Associated Hemolytic Uremic Syndrome: A Narrative Review.” Toxins, vol. 12, no. 2, Jan. 2020, p. 67, https://doi.org/10.3390/toxins12020067.

Lobanovska, Marina, and Greta Pilla. "Penicillin's Discovery and Antibiotic Resistance: Lessons for the Future?" The Yale Journal of Biology and Medicine, vol. 92, no. 1, 2019, pp. 135–145, https://pmc.ncbi.nlm.nih.gov/articles/PMC5369031/.

Ruiz, Natividad, and Thomas J. Silhavy. “How Escherichia Coli Became the Flagship Bacterium of Molecular Biology.” Journal of Bacteriology, vol. 204, no. 9, Sept. 2022, https://doi.org/10.1128/jb.00230-22. Accessed 23 July 2026

Salam, Md. Abdul, et al. "Antimicrobial Resistance: A Growing Serious Threat for Global Public Health." Healthcare, vol. 11, no. 13, 2023, p. 1946. MDPI, https://doi.org/10.3390/healthcare11131946.

Whelan, Shane, et al. “Uropathogenic Escherichia Coli (UPEC)-Associated Urinary Tract Infections: The Molecular Basis for Challenges to Effective Treatment.” Microorganisms, vol. 11, no. 9, Sept. 2023, p. 2169, https://doi.org/10.3390/microorganisms11092169.

Low-Cost, High-Efficacy Solutions to Hypothermia Deaths of Vulnerable Populations
By Seoyoung Jin

Abstract

Cold exposure is a preventable public health threat associated with increased mortality, particularly among people experiencing homelessness, housing instability, chronic illness, or limited access to heating. This review synthesizes literature on the epidemiology, physiology, and public health prevention of cold-related illness, with a focus on affordable interventions for vulnerable populations. Evidence indicates that cold-related health risks extend beyond periods of extreme weather and are shaped by social and environmental conditions. Three areas of intervention are examined: thermally efficient shelter, layered clothing, and targeted education. Together, these strategies can reduce heat loss, improve risk recognition, and increase access to protective resources. However, evidence for long-term, population-level health outcomes remains limited, particularly for interventions implemented in real-world settings. By examining both physiological mechanisms and barriers to resource access, this review highlights the need for coordinated prevention strategies that integrate physical protection, education, and accessible community services.

Introduction

Cold exposure is an environmental health risk associated with substantial preventable illness and mortality each year. Unlike sudden extreme-weather events, cold-related health risks can accumulate during ordinary seasonal conditions and disproportionately affect individuals experiencing homelessness, chronic illness, or inadequate access to heating and protective resources (Cao et al.; Romaszko et al.). These patterns reflect the interaction between environmental exposure and social vulnerability, highlighting cold-related mortality as both a medical and structural health issue (Cronley et al.).

Much of the literature examines cold-related risks or individual interventions in isolation. Less attention has been given to synthesizing practical strategies according to their feasibility within existing community and service systems. This gap is particularly important for vulnerable populations, for whom expensive infrastructure solutions may be unrealistic (Ene et al.).

This literature review addresses this need by evaluating affordable and potentially effective methods, with a focus on thermally efficient shelter, layered clothing systems, and targeted education and outreach. By integrating evidence across biology, environmental health, and public health, this article provides an interdisciplinary perspective while identifying gaps in real-world implementation. The goal is to inform prevention strategies that shift from last-minute emergency responses toward sustained community protection. This review argues that effective hypothermia prevention requires more than isolated interventions; it depends on integrating shelter, clothing, and education within accessible service systems.

Methodology

This review used a structured narrative literature search to identify peer-reviewed literature and authoritative technical sources examining cold exposure, hypothermia, and related public health interventions.

Search Keywords

Studies were identified through searches of Google Scholar and PubMed using combinations of the following keywords: “cold exposure mortality,” “hypothermia vulnerable populations,” “cold weather health interventions,” “homeless cold risk,” and “thermal shelter design.”

Source Inclusion Criteria

The search focused primarily on studies published between 2000 and 2025. Reference lists of selected articles were also reviewed to identify additional relevant studies. Inclusion criteria included human population studies, epidemiological analyses of cold-related mortality, and evaluations of preventive interventions involving shelter, clothing, or education. Animal studies, case reports without population-level relevance, and non-peer-reviewed opinion articles were excluded. English-language publications were prioritized. Titles and abstracts were screened before full-text review, after which selected studies were reviewed comparatively to identify common findings, differences in evidence, and gaps in the current literature.

Epidemiological and Statistical Modeling of Cold-Related Mortality

Cold exposure is an environmental health risk associated with increased cardiovascular, respiratory, and overall mortality, particularly among vulnerable populations (Jeican). Studies utilizing Distributed Lag Non-linear Models (DLNM) reported that 17.4% of excess out-of-hospital cardiac arrests (OHCA) in South Korea were attributed to cold, compared with 0.9% attributed to heat (Park et al.). Similarly, 6.44% of total deaths in Asia were attributable to short-term cold exposure, compared with 1.18% attributed to heat (Cao et al.). Because these studies examine different populations and outcomes, their estimates should not be interpreted as directly equivalent measures of mortality. Together, however, they indicate that cold exposure can contribute substantially to population-level mortality and, in some settings, may account for a greater attributable burden than heat exposure.

To quantify this burden, researchers use the Attributable Fraction (AF), a metric derived from the Relative Risk (RR) of mortality associated with a specific exposure compared with a baseline. As represented in epidemiological frameworks by Park et al. and Cao et al., this relationship is calculated as:

$$AF = \frac{RR-1}{RR}$$

The AF estimates the proportion of deaths attributable to a given exposure under the assumptions of the underlying epidemiological model, allowing researchers to quantify the

population-level mortality burden associated with cold exposure (Park et al.). Furthermore, Minimum Mortality Percentile (MMP) analyses, detailed in Table 1, show that the temperature associated with the lowest modeled mortality risk varies across regional temperature distributions (Cao et al.).

MMP represents the percentile of the local temperature distribution at which mortality risk is estimated to be lowest. As shown in Table 1, tropical countries such as Thailand exhibit relatively low MMP values of 5–10%, while temperate regions such as South Korea and China show substantially higher MMP values of approximately 63–93% (Cao et al.). In these temperate regions, the relatively high MMP suggests that mortality risk associated with cold exposure may extend across a substantial range of temperatures rather than being limited to rare extreme-cold events. Thus, cold-related prevention may need to address prolonged seasonal exposure in addition to short periods of extreme cold.

DLNM analyses also indicate that cold-related mortality effects can persist for longer periods after exposure. In the studies reviewed, cold-related effects persisted for up to 21–28 days, whereas heat-related effects were generally concentrated within shorter periods (Cao et al.; Park et al.). These lag patterns support the need for prevention strategies that extend beyond short-term responses to extreme-weather events and address cold exposure throughout the season (Yan et al.).

Country	MMP Range (%)	Temperature-Related Mortality Pattern
Thailand	5-10	Heat Exposure
South Korea	63-93	Cold Exposure
China	63-93	Cold Exposure

Table 1: Minimum Mortality Percentiles and Relative Temperature-Related Mortality Patterns (Cao et al.)

As exposure to cold weather increases, physiological harm may increase, particularly in outdoor environments. Seventy-five percent of hypothermia deaths in New York City occurred outdoors, often among individuals experiencing homelessness or lacking access to adequate shelter (Lane et al.). Of the 25% who died indoors, none had home heating, and nearly all were living in single-family or row homes. Kim et al. also identified individuals eligible for Korean Medical Aid, primarily low-income populations, as a key at-risk group. Together, these findings indicate that housing instability and limited access to heating are important factors shaping vulnerability to cold exposure (Lane et al.; Romaszko et al.). These findings highlight the importance of social determinants of health, including housing instability, poverty, and limited access to heating, which can interact with environmental exposure to amplify health risks.

Another high-risk group includes individuals with pre-existing health conditions. Park et al. identified hypertension, diabetes, and heart disease as important comorbidities associated with increased risk. Lane et al. also identified older age as a risk factor, particularly among individuals with medical or psychiatric conditions. Substance use has also been identified as a risk factor, as

it can impair thermoregulation, judgment, and the ability to seek shelter (Romaszko et al.). Despite consistent evidence identifying high-risk populations and seasonal risk patterns, gaps remain in translating epidemiological findings into sustained, population-level prevention strategies.

Physiological Mechanisms of Cold Stress

Cold exposure challenges the body's ability to maintain thermal homeostasis. Hypothermia can progress from mild physiological impairment at core temperatures around 32–35°C to severe neurological and cardiovascular dysfunction at lower temperatures (Jeican; Rathjen et al.). Cerebral metabolism decreases by approximately 6–7% for each 1°C reduction in core temperature, which can contribute to progressive cognitive impairment (Jeican).

As illustrated in Figure 1, the primary physiological defense against cold stress is governed by the preoptic area (POA) of the hypothalamus. When skin (Tsk) and core (Tc) temperatures decrease, the POA activates the sympathetic nervous system to initiate cutaneous vasoconstriction and non-shivering thermogenesis, while simultaneously triggering the somatic motor system to generate heat through shivering (Wang et al.; Yurkevicius et al.). The resulting heat balance can be evaluated using the Human Heat Balance Equation, a standard thermodynamic model in thermal physiology (Parsons):

$$S = M - W \pm R \pm C \pm K - E$$

In this equation, S represents the rate of heat storage, M is metabolic heat production, W is mechanical work, R is radiative heat exchange, C is convective heat exchange, K is conductive heat exchange, and E is evaporative heat loss (Parsons). When heat loss persistently exceeds heat production, S becomes negative and core temperature may decline. Individuals sleeping outdoors have limited metabolic heat production during sleep and may experience elevated convective and conductive heat loss from wind and contact with cold surfaces (Parsons; Romaszko et al.). This provides a physiological rationale for interventions that reduce convective and conductive heat loss or increase access to external heat sources.

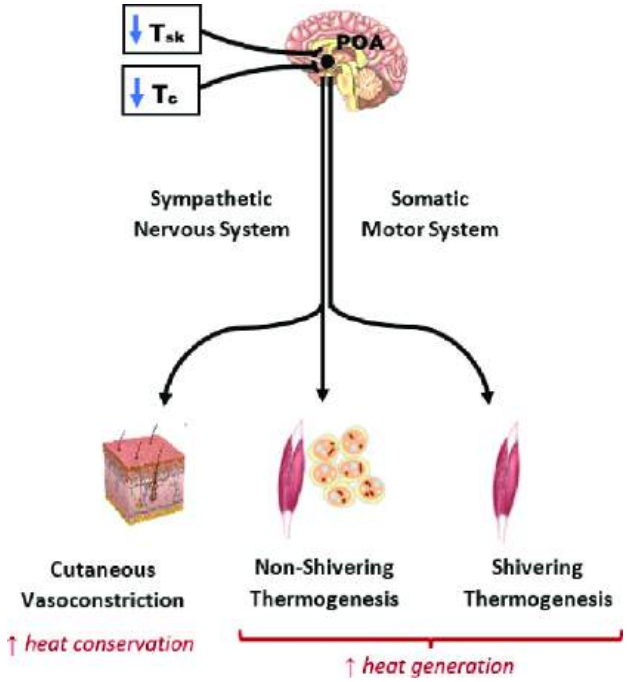

Figure 1: Regulation of Physiological Thermoeffector Responses to Cold Exposure (Yurkevicius et al.)

Although peripheral vasoconstriction increases systemic vascular resistance, it also elevates blood pressure and cardiac workload. These changes may increase the risk of myocardial ischemia and cardiac arrhythmias. Atrial fibrillation has been observed at core temperatures below 33°C, while temperatures below 25°C may precipitate ventricular arrhythmias or asystole (Jeican). Cold exposure also produces significant hematologic and metabolic effects. Increased blood viscosity, hemoconcentration, and enhanced thrombotic tendency may impair microcirculatory flow and further elevate cardiovascular risk (Jeican; Wang et al.). At the same time, thermogenic demand increases metabolic activity and activates sympathetic and endocrine stress responses, including the release of catecholamines that support heat production (Wang et al.).

Immune and respiratory defenses are similarly affected. Reduced neutrophil migration and phagocytic activity can impair host immune function, whereas severe hypothermia depresses cough reflexes and ventilation, increasing susceptibility to infection and respiratory complications (Jeican).

Together, these mechanisms provide a physiological basis for the associations between cold exposure and increased cardiovascular and respiratory morbidity and mortality observed in epidemiological studies.

Interventions

Shelter Insulation and Efficacy

Supplying thermally efficient shelter is an important intervention for reducing heat loss

and maintaining thermal balance among people exposed to cold conditions (Thomson et al.). Potential designs for unhoused populations include the “Ulmer nest” (see Figure 2), the “Igloo-like shelter” (see Figure 3), and the “Mini-capsule hotel” (see Figure 4) (Ene et al.).

Figure 2: Ulmer Nest (Ene et al.)

Figure 3: Igloo-Like Shelter (Ene et al.)

Figure 4: Mini-Capsule Hotel (Ene et al.)

Across evaluated designs, insulation effectiveness is influenced by reduced air infiltration and minimized thermal transmittance (Ene et al.; Mejia-Solis et al.). The thermal performance of a shelter envelope can be analyzed using the relationship:

$$Q = U \cdot A \cdot \Delta T$$

where Q represents the rate of heat transfer, U is thermal transmittance, A is surface area, and ΔT is the temperature difference between the interior and exterior environments. Lower U-values indicate greater resistance to heat transfer and can therefore reduce heat loss through the shelter envelope.

Geoffroy de Reynal's Igloo-like shelter (Figure 3) uses polyethylene foam and an inner aluminum-foil lining to provide insulation and reduce radiant heat loss, maintaining an indoor temperature approximately 15.5°C higher than the outdoor temperature under the reported test conditions (Ene et al.). Similarly, the mini-capsule hotel (Figure 4) uses polymethyl methacrylate (PMMA) and a high-density polyethylene (HDPE) frame and reached an internal temperature of 21.1°C at an ambient temperature of −8°C with wind speeds of 12 m/s (Ene et al.). These findings suggest that low-U-value materials, combined in some designs with active heating surfaces, can improve thermal performance under cold and windy conditions (Ene et al.).

Mejia-Solis et al. further identify roof and door insulation as important factors in limiting infiltration-related heat loss and maintaining thermal comfort. Shelter efficacy can be assessed through technical measures such as internal temperature monitoring, structural safety testing, and changes in the duration of thermal discomfort (Ene et al.; Mejia-Solis et al.). Figure 5 illustrates the potential reduction in time spent under conditions of thermal discomfort when outdoor exposure is replaced by insulated shelter. Although experimental evaluations demonstrate that insulated micro-shelters can maintain internal temperatures substantially above ambient

conditions, most studies rely on controlled testing environments (Ene et al.). Evidence evaluating long-term real-world effectiveness and the feasibility of sustaining active electrical heating in off-grid settings remains limited.

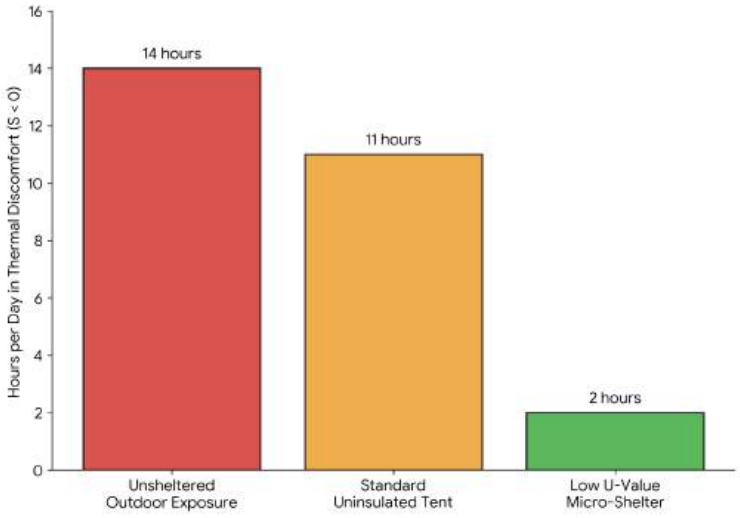

Figure 5: Reduction in Time Spent in Thermal Discomfort by Shelter Type

Economic feasibility should be assessed by comparing the costs of shelter construction, maintenance, energy, and implementation with the expected health and social benefits of preventing cold-related illness (Drummond; Ene et al.). Lower-cost preventive infrastructure may have the potential to reduce healthcare utilization associated with cold exposure, but direct cost-effectiveness evidence for these specific shelter designs remains limited. Economic evaluations should therefore consider both intervention costs and demonstrated health outcomes rather than relying solely on comparisons with hospitalization costs.

Real-world evaluation should also consider privacy, user trust, and infrastructural context. Environmental monitoring can prioritize non-intrusive measures such as shelter temperature stability and energy consumption, while administrative records can focus on maintenance, safety, and usage. Tracey et al. caution that documentation practices within homeless services can affect trust and access to care; therefore, evaluation systems should minimize unnecessary collection of personal information. Assessments should also consider location accessibility, proximity to healthcare facilities, transportation availability, and the reliability of power systems required to sustain heating interventions.

Overall, the literature indicates that successful shelter interventions require integration of four factors: thermal performance, energy feasibility, economic feasibility, and user-centered implementation. Without this alignment, technically effective designs may not translate into measurable reductions in cold-related morbidity and mortality without sustained access, appropriate implementation, and real-world evaluation.

Thermal Resistance in Layering Systems

In addition to shelter, appropriate clothing and access to external heat sources can help reduce the body's heat exchange with the environment. Because clothing forms an insulating layer between the body and environment, its properties influence conductive (*K*) and evaporative (*E*) heat loss. Inadequate or wet clothing can substantially increase heat loss and contribute to a negative heat balance. To quantify the thermal resistance of protective garments, researchers utilize the "clo" unit, a standard biophysical measure of clothing insulation defined by the International Organization for Standardization:

$$1\,clo \;=\; 0.155m^{\;2} \;\cdot\; K/W$$

A clo is a unit of clothing insulation; 1 clo corresponds to approximately 0.155 m²·K/W of thermal resistance and is conventionally associated with the insulation of a resting person in typical indoor conditions around 21°C (Castellani et al.). Some estimates indicate that sleeping outdoors in extreme cold may require clothing insulation exceeding 4.0 clo, while individual winter garments may provide substantially less insulation. These differences suggest that effective cold-weather clothing should combine sufficient insulation with moisture and wind management rather than relying solely on garment thickness.

To maximize thermal insulation while maintaining mobility, clothing can be organized into multiple layers, as shown in Figure 6. Rathjen et al. describe an inner base layer designed to manage moisture and limit evaporative cooling (*E*), a middle layer such as wool or fleece that provides insulation by trapping air, and an outer layer designed to limit wind and precipitation while allowing moisture vapor to escape (Castellani et al.; Rathjen et al.). Thermal performance therefore depends not only on fabric thickness but also on air trapping, layering, and moisture management (Castellani et al.).

Figure 6: Layered Clothing System

However, evidence concerning the physiological performance of protective clothing is substantially stronger than evidence concerning large-scale distribution and population-level health outcomes. This creates an important gap between established principles of thermal protection and their implementation among vulnerable populations. Clothing distribution may also face logistical challenges involving funding, storage, staffing, and transportation, although the extent of these barriers varies across service systems.

Effective distribution therefore requires integration with existing community infrastructure rather than reliance on isolated programs. Shelters, warming centers, clinics, and community organizations can serve as access points for distributing protective clothing alongside other services. Because low-barrier access is particularly important during periods of cold exposure, distribution systems should minimize administrative requirements that could delay access to essential resources (Tracey et al.). Further research is needed to evaluate which distribution models most effectively increase access to adequate thermal protection and whether such interventions produce measurable reductions in cold-related illness.

Targeted Risk Communication and Educational Interventions

Education acts as a complement to thermally protective infrastructure by addressing informational gaps that may delay protective behaviors. As discussed previously, cerebral metabolism decreases by approximately 6–7% for each 1°C reduction in core temperature (Jeican). Recognizing early symptoms and appropriate protective behaviors may therefore help individuals identify escalating cold exposure and seek assistance before cognitive impairment becomes severe.

Participatory cold-injury prevention programs teach at-risk populations to recognize early symptoms of hypothermia and apply practical protective strategies during cold exposure (Orton et al.). Participatory approaches can improve the relevance and accessibility of risk communication by incorporating the lived experiences of unhoused individuals (Orton et al.). Similarly, mobile clinics and street medicine can bring health education directly to unsheltered populations (Kaufman et al.). These programs combine basic healthcare with education on thermoregulatory protection, recognition of hypothermia, and local resource mapping, helping address barriers such as transportation and limited access to consistent shelter (Kaufman et al.).

Public health-oriented education and outreach can further strengthen these interventions. Cronley et al. emphasize the importance of proactive support, social inclusion, and community engagement in improving adaptive capacity among people experiencing homelessness during extreme temperatures. Similarly, Yan et al. found that localized cold-health watch and warning systems can improve public awareness and preparedness when information is timely and tailored to regional conditions. In low-resource settings, early hypothermia education should also emphasize recognition of observable symptoms when clinical core-temperature measurements are unavailable, allowing individuals or caregivers to identify potential hypothermia and seek appropriate assistance (Rathjen et al.).

Compared with infrastructure-based interventions, educational strategies generally require fewer material resources and can be disseminated rapidly. However, their effectiveness depends on continued access to information and close integration with material support. The Human Heat Balance Equation (Parsons) illustrates why education cannot substitute for physical protection: when heat loss exceeds heat production, maintaining thermal balance ultimately requires reducing heat loss or increasing access to external heat. Education is therefore most useful when embedded within existing service systems, including shelters, mobile outreach teams, healthcare providers, and community organizations. Delivering information through these established access points can reinforce risk communication throughout the cold season while connecting individuals to the shelter, clothing, and healthcare resources necessary for sustained protection.

Limitations

Despite advancements in modeling cold-related risks, important limitations remain in the current literature. First, most epidemiological studies are observational, limiting causal inference regarding specific interventions. Second, vulnerable populations, particularly unhoused individuals, remain underrepresented in large datasets, partly because of fragmented or transient medical records (Lane et al.). Third, many intervention studies rely on short-term technical testing in controlled environments, such as evaluating the U-values of micro-shelters, rather than longitudinal, real-world field evaluations (Ene et al.). Furthermore, the concentration of research in high-income countries limits the generalizability of findings across different climate and socioeconomic contexts.

Epidemiologically, differences in temperature metrics across studies, including absolute temperature thresholds and percentile-based Minimum Mortality Percentile (MMP) analyses, complicate cross-study comparisons (Cao et al.). Publication bias may also affect the available evidence, as innovative interventions may be more likely to be reported. Together, these limitations highlight the need for standardized measurement methods, prolonged field evaluations, and greater inclusion of unhoused populations in environmental health research.

Conclusion

Cold exposure represents an important and preventable public health risk, particularly for populations facing housing instability, limited access to heating, and other social and economic vulnerabilities. Epidemiological studies using DLNM and lag-effect models demonstrate that cold exposure is associated with increased mortality and that its effects can persist beyond the initial period of exposure. Physiological research provides a biological basis for these associations by describing the mechanisms through which cold stress affects cardiovascular, respiratory, and metabolic function.

The reviewed evidence also provides a physiological rationale for interventions that reduce environmental and bodily heat loss. Thermally efficient shelters can reduce heat transfer through improved insulation and reduced air infiltration, while layered clothing can increase

thermal resistance and improve protection from wind and moisture. However, evidence of thermal performance should not be equated with demonstrated reductions in population-level mortality. Direct evaluations of the long-term health effects and cost-effectiveness of these interventions remain limited.

Education and outreach provide an additional pathway for prevention by helping individuals recognize cold-related risk and connect with available resources. Across all three intervention areas, implementation remains a central challenge. Technical performance alone is insufficient when shelters are inaccessible, clothing is unavailable, or individuals cannot consistently access health and social services. Future research should therefore prioritize longitudinal field evaluations, standardized outcome measures, cost-effectiveness analyses, and greater inclusion of unhoused populations.

Taken together, the literature supports a coordinated prevention approach that combines thermal protection, targeted risk communication, and accessible service infrastructure. Rather than focusing exclusively on emergency treatment after severe cold exposure occurs, public health strategies can address the environmental and social conditions that increase vulnerability before severe illness develops. Further research is needed to determine which combinations of interventions are most feasible, scalable, and effective across different populations and settings.

Acknowledgements

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Works Cited

American Society of Heating, Refrigerating and Air-Conditioning Engineers. 2021 ASHRAE Handbook—Fundamentals. Inch-Pound ed., ASHRAE, 2021.

Cao, Ru, et al. "The Mortality Effect of Apparent Temperature: A Multi-City Study in Asia." International Journal of Environmental Research and Public Health, vol. 18, no. 9, 2021, p. 4675. https://doi.org/10.3390/ijerph18094675.

Castellani, John W., et al. "ACSM Expert Consensus Statement: Injury Prevention and Exercise Performance during Cold-Weather Exercise." Current Sports Medicine Reports, vol. 20, no. 11, 2021, pp. 594–607. https://doi.org/10.1249/JSR.0000000000000907.

Cronley, Courtney, et al. "Persons Experiencing Homelessness during Extreme Temperatures: Lessons for Promoting Socially Inclusive Adaptive Capacity." International Journal of Environmental Research and Public Health, vol. 21, no. 8, 2024, p. 984. https://doi.org/10.3390/ijerph21080984.

Drummond, Michael. Methods for the Economic Evaluation of Health Care Programmes. 4th ed., Oxford University Press, 2015.

Ene, Mircea Costin, et al. "Study Concerning the Design and Functionality of Individual Emergency Shelters." Scientific Reports, vol. 14, no. 1, 2024, p. 21148. https://doi.org/10.1038/s41598-024-71327-0.

International Organization for Standardization. Ergonomics of the Thermal Environment—Estimation of Thermal Insulation and Water Vapour Resistance of a Clothing Ensemble. ISO 9920:2007, 2007.

Jeican, Ionuț Isaia. "The Pathophysiological Mechanisms of the Onset of Death through Accidental Hypothermia and the Presentation of 'The Little Match Girl' Case." Clujul Medical, vol. 87, no. 1, 2014, pp. 54–60. https://doi.org/10.15386/cjm.2014.8872.871.iij1.

Kaufman, Rebekah A., et al. "The Role of Street Medicine and Mobile Clinics for Persons Experiencing Homelessness: A Scoping Review." International Journal of Environmental Research and Public Health, vol. 21, no. 6, 2024, p. 760. https://doi.org/10.3390/ijerph21060760.

Kim, Ayoung, et al. "Double Disparities of the Excess Risks and Costs of Extreme Temperatures on Hospitalization between Medical Aid and Non-Medical Aid Populations in South Korea." International Journal of Epidemiology, vol. 54, no. 2, 2025, p. dyaf027. https://doi.org/10.1093/ije/dyaf027.

Lane, Kathryn, et al. "Burden and Risk Factors for Cold-Related Illness and Death in New York City." International Journal of Environmental Research and Public Health, vol. 15, no. 4, 2018, p. 632. https://doi.org/10.3390/ijerph15040632.

Mejia-Solis, Enrique, et al. "Simple Solutions for Improving Thermal Comfort in Huts in the Highlands of Peru." Heliyon, vol. 9, no. 10, 2023, p. e19709. https://doi.org/10.1016/j.heliyon.2023.e19709.

Orton, Caitlin M., et al. “Developing and Consumer Testing Fire and Burn and Cold Injury Prevention Education With People Who Are Unhoused: Equity in Injury Prevention Toolkit.” Journal of Burn Care & Research, vol. 46, no. 5, 2025, pp. 1001–11. https://doi.org/10.1093/jbcr/iraf082.

Park, Chaerin, et al. “Excess Out-of-Hospital Cardiac Arrests Due to Ambient Temperatures in South Korea from 2008 to 2018.” Environmental Research, vol. 212, pt. A, 2022, p. 113130. https://doi.org/10.1016/j.envres.2022.113130.

Parsons, Ken. Human Thermal Environments: The Effects of Hot, Moderate, and Cold Environments on Human Health, Comfort, and Performance. 3rd ed., CRC Press, 2014. https://doi.org/10.1201/b16750.

Rathjen, Nicholas A., et al. “Hypothermia and Cold Weather Injuries.” American Family Physician, vol. 100, no. 11, 2019, pp. 680–86.

Romaszko, Jerzy, et al. “Mortality among the Homeless: Causes and Meteorological Relationships.” PLOS ONE, vol. 12, no. 12, 2017, p. e0189938. https://doi.org/10.1371/journal.pone.0189938.

Thomson, Hilary, et al. “Housing Improvements for Health and Associated Socio-Economic Outcomes.” Cochrane Database of Systematic Reviews, vol. 2013, no. 2, 2013, p. CD008657. https://doi.org/10.1002/14651858.CD008657.pub2.

Tracey, Pelle, et al. “Recordkeeping, Logistics, and Translation: A Study of Homeless Services Systems as Infrastructure.” Archival Science, 2023, pp. 1–27. https://doi.org/10.1007/s10502-023-09410-0.

Wang, Yongxing, et al. “Integrated Effects of Cold Acclimation: Physiological Mechanisms, Psychological Adaptations, and Potential Applications.” Frontiers in Physiology, vol. 16, 2025, p. 1609348. https://doi.org/10.3389/fphys.2025.1609348.

Yan, Bixun, et al. “A Cold-Health Watch and Warning System, Applied to the Province of Quebec (Canada).” Science of the Total Environment, vol. 741, 2020, p. 140188. https://doi.org/10.1016/j.scitotenv.2020.140188.

Yurkevicius, Beau R., et al. “Human Cold Habituation: Physiology, Timeline, and Modifiers.” Temperature, vol. 9, no. 2, 2022, pp. 122–57. https://doi.org/10.1080/23328940.2021.1903145.

It's All Down to My Genes: the Genetic and Neuropsychological Basis of Antisocial Behaviour By Maria Madalena Teodósio

Abstract

The introduction discusses the long-standing 'nature versus nurture' debate and clarifies that this is now outdated; a more integrative approach is currently favoured, centred on the notion that both genetic and environmental factors contribute to the origins of antisocial behaviour – this understanding has been informed by studies of twins and adopted children, and, more recently, studies that also utilise molecular genetics to identify the genes responsible. Throughout the paper, some of these studies are critically reviewed in order to clarify what proportion of the variance is explained by genetic factors and what proportion by environmental factors, as well as which genes are the strongest candidates for being responsible for antisocial behaviour; one of the genes for which there is the most evidence is the low-activity MAOA gene. The paper also addresses differences in brain function and structure among individuals exhibiting a pattern of antisocial behaviour, how these relate to behavioural and personality changes in these individuals, as well as the possible influence of certain genes (such as MAOA) on these brain changes.

Keywords MAOA, antisocial behaviour, psychopathy, behavioural genetics, frontal lobe, neuropsychological alterations.

1 – Introduction

Various hypotheses regarding the origins of antisocial behaviour (that is, any behaviour considered contrary to social norms) have been proposed over the years, such as the hypothesis that it originates in an individual's genes – that is, that they are born with this tendency – and the hypothesis that environmental factors (sociocultural and familial) are at the root of antisocial behaviour. Currently, there is a greater tendency to combine these perspectives into an integrative – and, in my view, more realistic – approach, in which both factors play a role, but in which this predisposition is already encoded in the individual's DNA at birth.

When we consider the plasticity of the human brain, as well as the way in which its chemistry and development can – like everything else in the body – be affected by the genes of the cells that make it up, and further consider how environmental factors can alter the expression of those very genes, it seems logical, even inevitable, that both environmental and genetic factors are involved in the origin of an individual's tendency to exhibit antisocial behaviour. However, we need to go further: we need to scientifically investigate the relative importance of each factor involved (whether it is significant, and whether it is weak, moderate or strong/high), and which genes are potentially involved. Whilst the debate previously centred more on whether or not there is a genetic influence on antisocial behaviour (the 'nature versus nurture' debate), the current debate revolves around the proportion of the variance explained by genetic influence,

rather than on whether this influence is significant or not, as there is virtually a consensus that a significant influence exists. The arduous task of investigating all these aspects has been undertaken by various researchers over the past few decades, through twin studies—which form part of the field of behavioural genetics—and through longitudinal and correlation studies, demonstrating, in my view, a clear relationship between certain genes, such as a variant of the MAOA (monoamine oxidase A) gene, and a tendency towards antisocial behaviour, although in other cases the relationship is not so clear. This paper will provide a critical review of the literature, not only to argue in favour of this influence of genetic variation on antisocial behaviour, but also to demonstrate that the genetic influence is at least moderate and, therefore, considerable, as well as to show that there has already been a partial identification of the genes potentially involved in the aetiology of antisocial behaviour. I do not, however, intend to downplay the influence of the environment, with which a certain genetic predisposition interacts. An example of an environmental factor is child abuse, which may be: physical, emotional or sexual, or a combination of two or three types of abuse. Child abuse has been defined in different ways: the DSM-5 (American Psychiatric Association, 2013) considers the presence of physical injuries (in the context of the DSM-5, linked to physical abuse), whilst the World Health Organisation (WHO, 2017) also considers more subjective elements such as potential harm to dignity and development (also linked to emotional and sexual abuse). Sexual abuse can be defined as any unwanted, non-consensual – and therefore forced – sexual contact, or the exposure of minors to pornography, according to the WHO (W.H.O., 2017), although studies on the influence of sexual abuse on behaviour do not focus so much on this latter aspect. For the aforementioned purpose, I shall undertake a critical review of the results of two meta-analyses, as well as the findings of specific studies conducted on twins. I shall also present results from longitudinal studies, as well as meta-analyses assessing the statistical interaction between a certain variant of the MAOA gene and environmental factors (such as experiencing abuse or maltreatment in childhood).

Throughout this work, I will argue that sex differences in the prevalence of antisocial personality disorder can be explained, at least in part, by the location of a recessive gene on the X chromosome, and that certain genes are strong candidates for being responsible for antisocial behaviour in certain individuals due to their location on the X chromosome.

Finally, I shall discuss the influence of the identified genes – e.g. MAOA (Caspi et al., 2002) – on certain aspects of brain structure and function, establishing a link between the functions of the altered brain regions and the tendency towards antisocial behaviour, including in the context of certain disorders in which this is one of the main characteristics. In the same subsection, the role of impulsivity in antisocial behaviour will be discussed, as well as that of empathy in prosocial behaviour and social cognition, together with their relationship to the altered brain areas, in order to strengthen the argument in favour of the contribution of the low-activity variant of MAOA to the genesis of antisocial behaviour. Empathy can be defined as an understanding of another person's emotional state or an affective response to that emotional state, arising from that understanding, or the sharing of the other person's feelings; this may

involve concern for that person, leading to a desire to help. An explanatory hypothesis will now be developed as to how the presence of the low-activity variant of the MAOA gene gives rise to a tendency towards antisocial behaviour by altering various areas of the brain, thereby completing the logical link between gene–brain–antisocial behaviour, whilst also explaining the role of the interaction between environmental factors (such as child abuse) and the individual's genetic makeup.

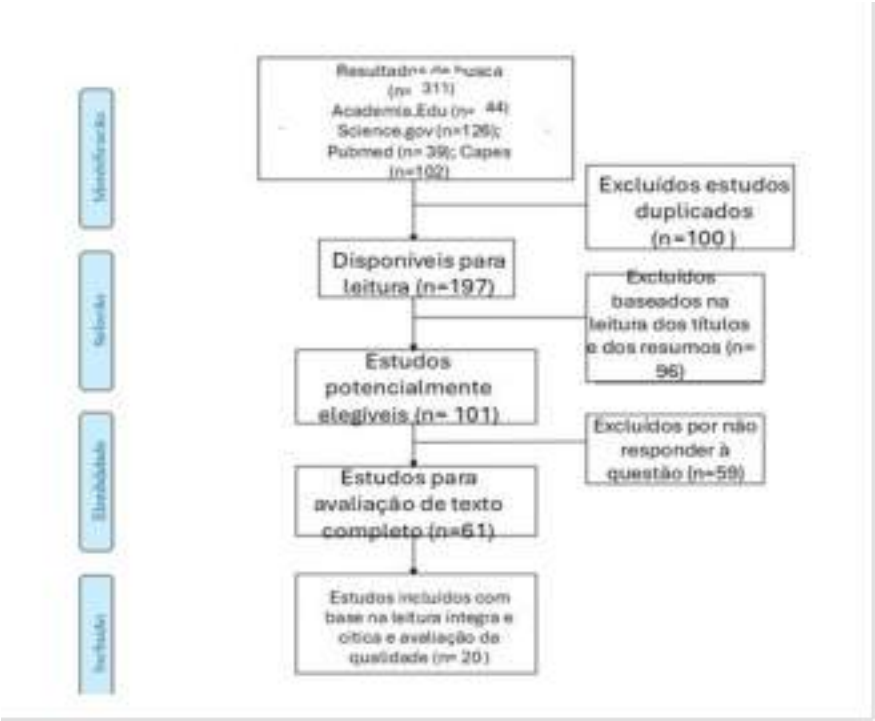
Biological explanations (genetic and neuropsychological) for antisocial and criminal behaviour are becoming increasingly important in judicial decisions, with a reduction in the sentences typically imposed on psychopathic offenders being observed on the basis of these explanations (Lewis, 2015), hence my interest in exploring this subject within the context of the discipline of forensic psychology. However, as to whether this decision is the correct one—even assuming that the biological explanations are properly substantiated—I shall not express an opinion, focusing solely on the science behind them, as my background is in psychology, not criminal law.

2 – Review

2.1 – Methods

Criteria for selecting articles: meta-analyses and studies from 2002 onwards. Relevance determined by reading the title, abstract and full text. The numerical details of the research are summarised in the figure below (with the exception of the meta-analyses mentioned in the first section below):

Figure 1. – Prisma model diagram (Author's own figure)



Boolean expressions and operators used in the search
- - The use of twins and adopted children in the study of the heritability of antisocial behaviour – "twin + adopted studies AND antisocial behaviour + psychopathy" - Meta
analyses were primarily selected " - Which genes are responsible for antisocial behaviour? – "Molecular
Genetics AND ASPD AND psychopathy" and, at a later stage of the research, "MAOA AND ASPD + psychopathy" / Neuropsychological alterations associated with the low-activity MAOA genotype and their implications for antisocial behaviour – "MAOA AND ASPD + psychopathy AND brain activity"

2.1.1 – The use of twins and adoptees in the study of the heritability of antisocial behaviour

Monozygotic twins (so-called 'identical twins', originating from the same egg) constitute an important source of information for those wishing to investigate genetic influences on behaviour, which leads to a better understanding of the 'nature versus nurture' debate.

Studies involving twins enable researchers to examine the role of genes in the development of a trait, behavioural tendency or disorder. Comparisons between monozygotic and dizygotic twins are traditionally carried out to assess the degree of genetic and environmental influence on a specific behavioural tendency or trait. Monozygotic twins are of the same sex and share 100 per cent of their genes. Dizygotic twins may be of the same sex or of opposite sexes and share, on average, 50 per cent of their genes.

If monozygotic twins show greater similarity with regard to a particular trait compared with dizygotic twins, this serves as evidence of a significant genetic influence on that trait. However, if monozygotic and dizygotic twins share a trait to the same extent, it is likely that the environment influences the trait more than genetic factors. In addition to this type of twin study, which is the most common and traditional, studies of twins' children are also useful for examining genetic and environmental influences on psychological and behavioural characteristics. As monozygotic twins share 100 per cent of their genetic information, their children are genetically related to their twin parents' twin sister or brother to the same extent as they are to their own parents. As the children of each twin usually live in different families, this relationship allows for an analysis of the possible interactions between genetic influences and environmental factors. Another important source of information is adopted individuals, as there are two sets of significant factors that may exert an influence: the biological parents and the adoptive parents. Presumably, any link between the characteristics of the biological parents and those of the children would be due to genetic factors. However, in my view, until they were placed for adoption, the children spent a certain period of time with their biological parents, during which they may have been influenced by them. Consequently, twin studies are, in my opinion, more suitable for investigating the influence of genetics on behaviour and psychological characteristics.

For example, in the case in question, parents with a predisposition towards antisocial behaviour may pass on such behaviour to their children, which will negatively affect their

development and learning. Even when they are handed over as babies, they may be subject to such influences, as this stage constitutes a sensitive period for the individual's psychological and brain development; however, if the child is adopted before the age of 4–5 – bearing in mind that there is still considerable brain plasticity at this stage – a pro-social environment might possibly offset the previous influence of the biological parents on the child. The reverse may also occur, that is, the parents' environmental influence may be positive, only for the child to be subsequently placed in a potentially harmful environment. In this subsection, I intend firstly to discuss the results of a meta-analysis in which the authors analysed 51 studies, including studies involving twins and studies involving individuals who were adopted as children, with the aim of determining the magnitude of environmental and genetic influences on antisocial behaviour (Rhee, & Waldman, 2002). According to this, the best model included a moderate proportion of variance attributable to genetic influences (total = 0.41), a proportion attributable to shared environmental influences (0.16) and a proportion attributable to non-shared environmental influences (0.43), which was also moderate. As this is a study within the social sciences, which examines aspects of the human condition influenced by various variables, both the total proportion explained by genetic influences and the proportion explained by non-shared environmental influences indicate a considerable ' ' weight in explaining antisocial behaviour (for both). Furthermore, according to the aforementioned analysis, factors relating to the methodology of the studies themselves were considered to be significant moderators of the magnitude of genetic and environmental influences on antisocial behaviour; these include: operationalisation, the assessment method, the method for identifying monozygotic and dizygotic twins, and age. Gender, however, has no influence on this magnitude. A second meta-analysis (Dehryl & Frick, 1994) reveals higher results of around 50 per cent for the proportion of the variance in antisocial behaviour explained by genetic influence. This analysis included 12 studies involving twins (3,795 pairs of twins) and 3 studies involving adopted individuals (338 adoptees) published since 1975, which provided 21 estimates of the heritability of antisocial behaviour.

Although this is a meta-analysis from 1994, I chose to include it in this review as this classic study, unlike studies that employ molecular genetic techniques in their methods, is not liable to become obsolete due to rapid technical advances in that field, and also demonstrates results that are slightly higher or similar to current findings, predating by a considerable margin the most recent studies cited in this paper.

A more recent study (Ferguson, 2010) found similar results, with a total of 56 per cent of the variance explained by genetic factors.

In the specific case of a study of twins (Jacobson, Prescott, & Kendler, 2000), it was calculated that genetic influences accounted for 33 per cent of the variance, shared environmental influences accounted for 31 per cent and non-shared influences for 36 per cent, with each accounting for approximately one-third of the variance. This contrasts with the greater difference in proportions found in the meta-analysis; in this instance, too, the proportion of shared influences is moderate, yet it also plays a considerable role in explaining antisocial

behaviour. This study has the advantage of a large sample of 3,522 adults, but the disadvantage of including only male participants.

Other studies involving twins focus on identifying not only the extent to which each type of influence contributes to antisocial behaviour, but also the extent to which each of these factors contributes to certain personality traits that may be associated with a greater and more stable tendency towards antisocial behaviour, namely the traits of emotional insensitivity (in the original English: callous-unemotional traits – CU traits). These traits help to define a subgroup that even exhibits significant neurological differences in the frontotemporal cortex, which are indicative of a deficit in emotional processing (Viding, Fontaine, & McCrory, 2012). This subtype of individuals with antisocial tendencies and conduct disorder is a likely precursor to psychopathy (Lynam & Gudonis, 2005). According to a more recent study (Viding, Frick, & Plomin, 2007), which used a sample of 3,434 7-year-old twins from an early twin development study, identified from a register of twin births in England and Wales, with traits of emotional insensitivity and behavioural disturbance (characterised by antisocial behaviours), shared genetic influences affect both the presence of these personality traits and the antisocial tendencies themselves. These shared genetic influences also appear to be largely responsible for the phenotypic relationship. The findings of this study are similar to those of other studies, involving both young people and adults, which show substantial heritability of the aforementioned traits and genetic mediation of the phenotypic relationship with antisocial tendencies. However, contrary to the findings of a previous study (Larsson et al, 2006), there was a gender difference (between boys and girls) in the magnitude of the genetic and shared environmental effects on individual differences in emotional insensitivity traits in childhood. Both emotional insensitivity traits (CU traits) and antisocial behaviours were found to have slightly more genetic influence in boys than in girls, accounting for 67 per cent ($h2 = 0.67$) and 61 per cent ($h2 = 0.61$) in boys and 48 per cent and 57 per cent in girls, for emotional insensitivity traits and antisocial behaviours, respectively. Shared environmental influences were not statistically significant for boys ($c2 = 0.04$ for emotional insensitivity traits and $c2 = 0.06$ for antisocial behaviour). For girls, there was a modest but significant environmental influence on emotional insensitivity traits of 20 per cent ($c2 = 0.20$). Unshared environmental influences accounted for the majority of the environmental variance ($e2 = 0.29$ and $e2 = 0.34$ for boys, and 0.32 and 0.35 for girls, for emotional insensitivity traits and antisocial behaviours, respectively). These differences in results between boys and girls, according to the authors of this 2007 study, warrant further investigation. In my view, however, these differences could be partly explained (as there is also some genetic influence in girls) by the location of the gene responsible for antisocial behaviour on the Y chromosome. Alternatively, a candidate gene responsible for antisocial behaviour could be a recessive gene located on the X chromosome, such as the gene for the low-activity variant of MAOA (Caspi et al., 2002) In a previous study (Viding, Blair, Moffitt, & Plomin, 2005), the authors, again using children with conduct disorder, found a moderate proportion of the variance attributed to genetic factors in children with conduct disorder alone, whereas in children with behavioural disorders and traits of

emotional insensitivity this proportion was high, in line with the findings of the 2007 study (Viding, Frick, & Plomin, 2007).

In a study carried out by researchers at the University of Minnesota, involving 626 pairs of 17-year-old community-based twins (nearing the legal age of majority in many countries) (Blonigen, Hicks, Krueger, Patrick, & Iacono, 2005), the proportion of variance explained by genetic factors in the combined sample of boys and girls was calculated to be approximately half for both the factor termed 'Fearless Dominance' and the factor termed 'Impulsive Antisociality'. To clarify: 'Fearless Dominance' refers to aspects such as social power, resilience to stress and a lack of emotional fear responses; it is associated with a lack of emotional reactivity, including in terms of empathic responses relating to emotional empathy, as in Factor 1 of the PCL-R; Impulsive Antisociality is related to poor behavioural and impulse control and antisocial behaviour, as is Factor 2 of the PCL-R (Del Gaizo & Falkenbach, 2008).

A longitudinal study from 2013 (Wang, Niv, Tuvblad, Raine, & Baker, 2013) set out to disentangle the common genetic and environmental influences between aggressive antisocial behaviour and non-aggressive antisocial behaviour in a sample comprising 780 pairs of twins of both sexes, who were interviewed at the following ages: 9–10; 11–13; and 16–18. Following these three interview stages and after carrying out statistical analysis, the authors concluded that there were differences (repeated across the various interview stages) between the sexes in the mean levels of antisocial behaviour; however, the most interesting finding for my purposes was that, in terms of aetiology, the genetic influence on aggressive and non-aggressive antisocial behaviour showed moderate overlap. The predominant overlap, however, was found for shared environmental factors, whilst the lowest overlap was observed for non-shared environmental factors.

Following this review of the literature, having drawn on both the results of meta analyses—as these analyse the findings of multiple studies, thereby providing a more comprehensive overview—and the results of twin studies in particular, I can state that the proportion of variance explained by genetic factors is at least moderate, and may even be high, accounting for half or more than half of the variance when considering a sample exhibiting traits of emotional insensitivity or primary psychopathic characteristics. Studies involving twins and adopted individuals can be useful for determining the extent to which genes influence antisocial behaviour; however, they do not identify which specific genes are the best candidates for explaining the origins of antisocial behaviour. To do this, we will need to adopt an approach that is less traditional in behavioural genetics and more closely linked to molecular genetics, in which genes associated with antisocial behaviour in individuals are identified, whilst also drawing on longitudinal studies that identify statistical interactions between genetic and environmental factors.

2.2 – In search of the culprits: which genes are responsible for antisocial behaviour?

As the subtitle suggests, I shall discuss the genes potentially involved in the origins of

antisocial behaviour, particularly the MAOA (monoamine oxidase A) gene, on which a number of longitudinal and correlation studies have been conducted. The MAOA gene is located on the short arm of the X chromosome. MAOA is an enzyme that breaks down various neurotransmitters: serotonin, dopamine and noradrenaline. This gene was identified as a potential influence by examining, in particular, the dopamine system (involved in mood, motivation and reward, arousal and other behaviours), the serotonin system (involved in impulse control, emotional regulation, sleep and appetite), and the epinephrine/norepinephrine (or adrenaline/noradrenaline) system (crucial for the 'fight or flight' response) (Tuvblad & Beaver, 2013) . In addition to its action being linked to the levels of these neurotransmitters, there is another good reason why the MAOA gene is considered a strong candidate for partly explaining the origins of antisocial behaviour: it is located on the X chromosome. Given that the allele associated with antisocial behaviour has been found to be recessive (Clemens et al., 2015), means that this gene serves as an explanation for the sex difference in the prevalence of antisocial personality disorder (lower in girls) (Alegria et al., 2013), since there would be fewer girls with a tendency to exhibit antisocial behaviour, because when the gene associated with a particular trait or condition is located on the X chromosome and is recessive, whenever a girl is born who is heterozygous for that gene, she is protected from the effects of the recessive gene by the presence of the dominant gene, whereas this does not occur in boys due to the absence of a second X chromosome.

MAOA has low-activity variants and high-activity variants; the low-activity variant MAOA-3R, also known as MAOA-L or simply the low-activity variant, is the variant associated with higher levels of antisocial behaviour (Caspi et al., 2002). It is thought that the mutation is located in the promoter region of the gene (its regulatory minisatellite). Furthermore, the polymorphic region linked to the serotonin transporter (5HTTLPR) has been studied in terms of its association with antisocial behaviour, as well as its statistical interaction with adverse environmental factors (Ficks & Waldman, 2014). Both the association between the low-activity variant of the MAOA gene and high levels of antisocial behaviour, and the association between this variant and the polymorphic region linked to the serotonin transporter, have satisfactory scientific support, judging by the results of the meta analyses reviewed here, as well as by the scale of some of these studies. To highlight the relationships between genes and antisocial behaviour that I focused on in the opening paragraphs of this subsection (regarding MAOA, as well as the polymorphic region linked to the serotonin transporter and their respective interactions with adverse environmental factors), I shall discuss the results of meta-analyses that examine studies seeking to establish these relationships. A study published in 2014 (Byrd & Manuck, 2014) included 27 peer-reviewed English-language studies, published up to August 2012, which contained indicators of maltreatment or other family difficulties (e.g. parenting, sociodemographic factors); the MAOA genotype; indices of aggressive and antisocial behaviour; and a statistical test for genotype-environment interaction. For the 20 male samples, the results revealed that adversity more strongly predicts antisocial behaviour for the low-activity variant, and that there is a specific interaction between this variant and

maltreatment. For the 11 female samples, MAOA did not interact with combined childhood adversities, whilst maltreatment (alone) served as a predictor of antisocial behaviour, albeit weakly, primarily in women with the high-activity MAOA variant rather than those with the low-activity variant. Going back a little further in time, as early as 2007 it had been found that, when subjected to a meta-analysis comprising 8 studies (Taylor & Kim-Cohen, 2007), the statistical interaction between MAOA and maltreatment remained, which can be considered to be consistent with the results for men, but not for women. A meta-analysis (Ficks & Waldman, 2014) focused on studies of the association between antisocial behaviour and the polymorphic region linked to the serotonin transporter (5HTTLPR), which has also been linked to the development of borderline personality disorder (Lis, E., Greenfield, B., Henry, M., Guilé, J. M., & Dougherty, G., 2007), and on studies of the association between antisocial behaviour and the low-activity variant of MAOA, with results demonstrating a significant positive association between each of these genes and antisocial behaviour, albeit with some heterogeneity in magnitude across the studies included. The authors also demonstrate that the low-activity variant of MAOA has a small effect on antisocial behaviour and aggression even in the absence of environmental interaction factors.

A longitudinal study published in 2011 used a sample of 398 male participants from the Christchurch Health and Development Study who had complete data on: genotype of the MAOA promoter region; antisocial behaviour up to the age of 30; and exposure to sexual and physical abuse in childhood. The results clearly showed that individuals who possessed the low-activity variant and had been abused were more likely to exhibit antisocial behaviour (such as property offences and hostility), a finding consistent with what has generally been observed in meta-analyses. Another similar longitudinal study (Caspi et al., 2002) yielded identical results.

Although there is scientific evidence both for the link between the polymorphic region associated with the serotonin transporter and antisocial behaviour, and for the link between the serotonin transporter and the low-activity variant of MAOA—which makes both genes strong candidates—if I had to name one and only one gene likely to be responsible for antisocial behaviour, I would point to the low-activity variant allele of MAOA, as this is located on the X chromosome and is recessive, whilst the other is situated on chromosome 17. For this reason, the gene encoding this MAOA variant is a stronger candidate in terms of explanatory power.

A gene located on the Y chromosome could also partially explain the differences in the prevalence of antisocial personality disorder and in the genetic influence between men and women observed in twin studies, but this could never fully explain the results, as there is also some genetic influence in women that accounts for antisocial behaviour, albeit to a lesser extent; therefore, a better explanation in general terms, or at least as a complement, would be a recessive gene located on the X chromosome, similar to the allele responsible for the low-activity variant of MAOA. Furthermore, studies investigating the possible influence of genes located on the Y chromosome have been case studies and small-scale studies, although

they have uncovered findings that support this hypothesis (Kopsida, Stergiakouli, Lynn, Wilkinson, & Davies, 2009).

Other genes deserve mention in this discussion, as they are potentially associated with antisocial behaviour when certain polymorphisms are present, such as the DAT1 gene (dopamine transporter protein), the DRD2 gene (dopamine D2 receptor), and the DRD4 gene (dopamine D4 receptor, distributed in limbic and cortical areas) (Tuvblad & Beaver, 2013). In a 2013 study conducted in the USA (Barnes, Boutwell, & Beaver, 2013), the authors examined whether these genetic risk factors (DAT1, DRD2 and DRD4) for antisocial behaviour were predictors of exposure to adversity and violent crime measured at county level. The data were drawn from the National Longitudinal Study of Adolescent Health (N = 2,2.2). Disadvantage at county level was measured using Census data, and violent crime rates were measured using the FBI's Uniform Crime Reports. The results revealed that individuals with a higher number of risk alleles were more likely to live in a disadvantaged county and were more likely to live in a municipality with a higher rate of violent crime. Although the results of previous studies have shown that living in disadvantaged communities has a myriad of negative consequences, including antisocial behaviour, this study does not directly assess an association between these genes and antisocial behaviour, leaving this question open.

Having addressed the issue of genes specifically associated with antisocial behaviour, I shall now proceed to establish a logical link (in terms of brain function) between the brain changes potentially caused by the presence of these genes and the tendency towards antisocial behaviour. In this way, I shall establish a link between genes and behaviour that explains the association between the low-activity variant of MAOA and the tendency to exhibit antisocial behaviour.

2.3 – It's my brain's fault: neuropsychological changes associated with the low activity MAOA genotype and their implications for antisocial behaviour

This subsection will discuss the brain changes (both functional and structural) associated with the low-activity MAOA genotype, as well as the functions of the affected brain regions and their relationship to antisocial behaviour and to certain disorders in which a tendency to exhibit antisocial behaviour is a key characteristic. To this end, a (brief) review of the scientific literature will be carried out, based on the results of studies examining the association between genotype and brain changes, as well as studies and critical reviews of the literature examining the functions of the altered brain areas and the relationship between pathology and brain changes. I shall also formulate an explanatory hypothesis as to how the presence of the low-activity variant of the MAOA gene can lead to a greater tendency towards antisocial behaviour. In this way, I will argue in favour of a gene-brain-antisocial behaviour relationship. I will focus on the MAOA gene, as it is the leading candidate for explaining antisocial behaviour, not least because of its location on the X chromosome and the fact that it is recessive.

In 2015, a neuroimaging study was conducted (Clemens et al., 2015), in which functional magnetic resonance imaging (fMRI) was used to test whether the MAOA genotype

might influence brain activity at rest in healthy participants recruited via a public advertisement in Aachen (Germany), with a sample comprising 32 men and 16 women, 18 of whom carried the low-activity variant of MAOA. The results show that, during rest, participants with the low-activity variant exhibited greater activity in the frontoparietal, temporal and cerebellar regions. Executive control analysis revealed reduced activity in the group with the low-activity variant of MAOA across several areas related to executive control, notably the right middle frontal gyrus and the anterior cingulate cortex. The group with the low-activity variant exhibited reduced activity in two right frontal areas, which have previously been linked to inhibitory control and emotional regulation. The right frontal region was also less active within the frontoparietal network, indicating an important role for this region. In the authors' view, the findings regarding reduced activity in the frontal region during rest in the group with the low-activity variant of MAOA confirm the results of previous studies (Meyer-Lindenberg et al., 2006), which demonstrated decreased prefrontal/orbitofrontal activity in participants with the low-activity variant of MAOA during emotionally demanding tasks. Both the results of previous studies and those of the 2015 study suggest that the frontal cortex may be an area of interest for investigating neuropsychological differences related to the low-activity variant. In terms of structural (volumetric) changes in the brain, volumetric reductions associated with the low-activity variant were also observed, notably in the amygdala (particularly the grey matter), the cingulate gyrus, the anterior cingulate cortex, the insula and the hypothalamus in a large sample of healthy individuals (Meyer-Lindenberg et al., 2006). Psychopathy is a personality construct associated with antisocial personality disorder, characterised by impulsive and antisocial behaviour, manipulation, narcissism, a lack of remorse or guilt (particularly the latter), and a general deficiency in emotional experience, empathy and concern for the well-being of others, (behavioural, interpersonal and affective components, respectively). According to a review of the literature (Koenigs, 2012), alterations in the orbitofrontal and prefrontal cortex—which performs a range of functions, such as social and affective decision-making, which appear to be impaired in psychopathic individuals—have been associated with psychopathy – both structural volumetric abnormalities (including deficits in grey and white matter density, particularly in the fibres connecting the amygdala to the ventromedial prefrontal cortex) and functional deficits have been found. More specifically, some of these alterations include a reduction in cortical thickness in the right lateral prefrontal cortex and, to a lesser extent, in the right ventromedial prefrontal cortex and the anterior cingulate cortex. In these areas, cortical thickness was negatively correlated with the affective facet of psychopathy. Other regions with reduced volume in psychopaths include the amygdala (Yang, Raine, Narr, Colletti, & Toga, 2009) and the insula, amongst other brain regions (compared with non-psychopaths) (Ly et al., 2012). Individuals with antisocial personality disorder also have reduced prefrontal cortex volume (11 per cent less grey matter compared with the group of healthy individuals), and schizophrenic individuals who tend to exhibit antisocial and aggressive behaviours have, like psychopaths, reduced volume in the amygdala, as well as in the hippocampus and the orbitofrontal cortex (Narayan et al., 2007).

Whilst the prefrontal cortex is involved (amongst other functions) in the control of executive functions (planning, inhibition, cognitive flexibility, decision-making, behaviour regulation, etc.), the orbitofrontal cortex functions as a component of the paralimbic system, involved in autonomy, response inhibition, and stimulus salience functions, amongst others. It plays a role in reward expectations and in the anticipation and processing of outcomes, even if the outcome does not yield any reward, and is implicated in the control of impulsive behaviour (Kim & Lee, 2011); it has also been shown to play a significant role in social and emotional behaviour (Siddiqui, Chatterjee, Kumar, Siddiqui, & Goyal, 2008); it is therefore not surprising that these areas are functionally and structurally altered in individuals with antisocial tendencies, as these tendencies involve a lack of behavioural control, an inability to reflect during the decision-making process (thoughtless and impulsive behaviours), as well as social and emotional relationship problems; there is thus a logical relationship, based on brain function, between the presence of these alterations and the antisocial behaviour exhibited by the individuals who possess them. Alterations in these two regions are associated both with the presence of the low-activity variant of MAOA and with disorders in which antisocial behaviour is a key characteristic. It is also worth noting that patients with frontal lobe damage exhibit behaviours similar to those of antisocial individuals, in that they are impulsive, irresponsible and unable to hold down a job or maintain a marriage.

Another area also related to both is the anterior cingulate cortex. The dorsal part of the anterior cingulate cortex is connected to the prefrontal cortex and the parietal cortex, the motor system and the frontal eye fields, making it central to stimulus processing; its role in reward-based decision-making has been demonstrated, as it is linked to the prefrontal cortex. In contrast, the ventral part is connected to the amygdala, nucleus accumbens, hypothalamus and anterior insula, and is involved in assessing the relevance of emotion and motivational information, serving as a processor and modulator of the emotional response (Bush et al., 2002). A characteristic associated with antisocial behaviour, particularly antisocial disorder and psychopathy, is a deficit in emotional experience, although in certain situations there may also be a lack of control over emotional reactions (in terms of irritability); this response may be inappropriate, leading to inappropriate and even antisocial behaviours. Structural deficits in the amygdala and the insula have also been linked in the literature (as specified earlier) to the low-activity variant of MAOA, as well as to the presence of antisocial behaviours in schizophrenic patients (in the case of the amygdala), and to psychopathy (both brain regions). The amygdala forms part of the limbic system and plays an important role in memory processing, decision-making and emotional responses. The fact that the regions functionally implicated in antisocial behaviour (also associated with disorders and personality disorders in which antisocial behaviour is a key characteristic) are also altered in healthy carriers of the low-activity MAOA variant is, particularly given that brain structure (e.g. the frontal lobe) is highly heritable (Thompson, 2001), a strong argument in favour of the hypothesis that this gene underlies antisocial behaviour; furthermore, a plausible explanatory hypothesis can be formulated as to how the presence of the low-activity variant of the MAOA gene may give rise

to a tendency towards antisocial behaviour in interaction with environmental factors, as it has been established in the literature that the presence of the MAOA gene predisposes individuals to an heightened emotional (and neurological) reaction to situations of rejection. Certain situations of abuse may lead a child to perceive rejection on the part of the abuser. An individual carrying this gene variant would therefore be far more susceptible to this type of environmental influence. Given that such an individual has a heightened emotional and cerebral response, this could overload the limbic system and even other brain regions that interact with it. Researchers state that the prefrontal cortex may suffer functional deficits due to prolonged emotional overload (Bates, 2012). In this way, a logical link is established between the presence of the low-activity variant and a tendency towards antisocial behaviour.

All the regions associated with the presence of the low-activity MAOA variant and with antisocial behaviour, as mentioned in the last three paragraphs, have also been implicated in the implementation of the components of empathy (affective arousal, emotional understanding and emotional regulation), which takes place via a complex network of interacting neural regions, including the superior temporal sulcus, the insula, the prefrontal cortex, the orbitofrontal cortex, the amygdala, and the anterior cingulate cortex, as well as neuroendocrine processes involved in social behaviours and emotional states. Empathy, which involves a shared interpersonal experience, is implicated in many aspects of social cognition, notably in prosocial behaviour, morality and the regulation of aggression (Decety, 2010), although with regard to the latter, there is not yet full consensus amongst researchers. Given that a lack of empathy is present in disorders where antisocial behaviour is a key characteristic, and given that empathy is important for aspects of social cognition, prosocial behaviour, the regulation of aggression and morality, one would expect deficits in volume and activity in the regions involved in the empathic experience to underlie this behavioural tendency. Deficits in these areas are also associated with the low-activity variant, which further strengthens the hypothesis that the MAOA gene is involved in the genesis of antisocial behaviour, particularly in conjunction with what we know about the effects that a strong and prolonged emotional reaction can have on regions (such as the prefrontal cortex) involved in the empathic circuit, and bearing in mind that the presence of this genetic variant makes its carriers more sensitive to situations of rejection, as this particular aspect provides an explanation: namely, that this overload is exacerbated by the presence of the gene in the context of factors such as child abuse and neglect.

Conclusion

Through this study, I have reached several conclusions, having demonstrated through a critical review of the literature:

1. That the proportion of variance explained by genetic factors is at least moderate, and may even be high, accounting for half or more than half of the variance when considering a sample exhibiting traits of emotional insensitivity or primary psychopathic characteristics. To reach this conclusion, I conducted a review of the literature, encompassing meta-analyses of studies involving twins and adopted individuals, as well as some twin studies. The inclusion of

meta-analyses was appropriate in that it provides a more comprehensive overview of the results of peer-reviewed published studies. The type of studies used (involving twins and adopted individuals) is also appropriate, as if monozygotic twins show greater similarity with regard to a particular trait compared with dizygotic twins, this serves as evidence of a significant genetic influence on that trait. However, if monozygotic and dizygotic twins share a trait to the same extent, it is likely that the environment influences the trait more than genetic factors. In the case of adopted children, there are two sets of important factors that may exert an influence: the biological parents and the adoptive parents. Presumably, any link between the characteristics of the biological parents and those of the children would be due to genetic factors. However, in my view, until they were placed for adoption, the children spent a certain period of time with their biological parents, during which they may have been influenced by them; therefore, studies involving twins are an even better way of assessing the extent to which genetic influence explains the variance, and the literature should focus primarily on these in future.

2. A number of good candidate genes have been identified that may explain antisocial behaviour – the polymorphic region linked to the serotonin transporter (5HTTLPR), genes located on the Y chromosome, and the genotype of the low-activity variant of MAOA (gene promoter region), the latter being the best candidate due to the fact that it is recessive and located on the X chromosome, which has considerable explanatory power, accounting for the difference in the prevalence of antisocial personality disorder between boys and girls, as well as the fact that there is some genetic influence on antisocial behaviour in girls, albeit to a lesser extent than in boys. Another candidate with a reasonable body of evidence in its favour is the polymorphic region linked to the serotonin transporter (5HTTLPR). 3. There is a gene-brain-antisocial behaviour relationship regarding the low-activity variant of MAOA. To reach this conclusion, a review of the scientific literature was carried out, based on the results of studies examining the association between genotype and brain alterations, as well as studies and critical reviews of the literature examining the functions of the altered brain areas and the relationship between pathology and brain alterations. The functions of the deficient regions, whose deficits are associated with the presence of MAOA, are important for normal social behaviour and cognition, with an interaction between various altered brain regions (such as the prefrontal cortex, the amygdala, the insula and the anterior cingulate cortex) being necessary for normal empathic experience, which regulates social behaviour and aggression, promoting a tendency towards pro-social behaviours. In view of this, and given that a lack of empathy and similar brain alterations are present in conditions where antisocial behaviour is one of the main characteristics, together with what we know about the effects that a strong and prolonged emotional reaction can have on regions (such as the prefrontal cortex) involved in the empathic circuit, and the fact that the presence of this genetic variant may make its carriers more sensitive to situations of rejection, this lends further weight to the hypothesis that the MAOA gene is involved in its origins. The tendency towards antisocial behaviour seems best described by a spectrum of severity, frequency and intensity, which may be explained by the fact that it has multiple causes (genetic, environmental and possibly epigenetic), and/or by the fact that several

genes are involved. Following further research to determine with greater certainty which genes are involved in the genesis of these behaviours, a good focus for future research would be to determine to what extent each of these genes accounts for antisocial behaviour and what effect the absence of some and the presence of others would have on individuals' behaviour. Another possibility would be the existence of an intermediate phenotype (due to a phenomenon of incomplete dominance or co-dominance) in females, resulting from the presence of one allele of the low-activity variant and one normal allele of the MAOA gene.

Works Cited

Alegria, A. A., Petry, N. M., Liu, S.-M., Blanco, C., Skodol, A. E., Grant, B., & Hasin, D. (2013). Sex Differences in Antisocial Personality Disorder: Results From the National Epidemiological Survey on Alcohol and Related Conditions. Personality Disorders. Child & Adolescent Psychiatry 4(3).

American Psychiatric Association (2013). Diagnostic and statistical manual of mental disorders. Washington, DC: Author.

Barnes, J. C., Boutwell, B. B., & Beaver, K. M. (2013). Genetic Risk Factors Correlate with County-level Violent Crime Rates and Collective Disadvantage. Journal of Criminal Justice.

Bates, C. (10 April 2012). Blanking out: How stress can shut down the command centre in the brain. Daily Mail.

Blonigen, D. M., Hicks, B. M., Krueger, R. F., Patrick, C. J., & Iacono, W. G. (2005). Psychopathic personality traits: heritability and genetic overlap with internalising and externalising psychopathology. Psychological Medicine, 35(5).

Bush, G., Vogt, B. A., Holmes, J., Dale, A. M., Greve, D., Jenike, M. A., & Rosen, B. R. (2002). Dorsal anterior cingulate cortex: A role in reward-based decision-making. Proceedings of the National Academy of Sciences of the United States of America, 99(1). Byrd, A. L., & Manuck, S. B. (2014). MAOA, childhood maltreatment and antisocial behaviour: A meta-analysis of a gene-environment interaction. Biological Psychiatry, 75(1). Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I.W., Taylor, A., & Poulton, R. (2002). The role of genotype in the cycle of violence in maltreated children. Science, 297(5582).

Clemens, B., Voß, B., Pawliczek, C., Mingoia, G., Weyer, D., Repple, J., Eggermann, T., Zerres, K., Reetz, K., & Habel, U. (2015). Effect of MAOA genotype on resting-state networks in healthy participants. Cerebral Cortex, 25(7).

Decety, J. (2010). The neurodevelopment of empathy in humans. Developmental Neuroscience, 32(4).

Del Gaizo, A. L. D., & Falkenbach, D. M. (2008). Primary and secondary psychopathic traits and their relationship to the perception and experience of emotion. Personality and Individual Differences, 45(3).

Ferguson, C. J. (2010). Genetic Contributions to Antisocial Personality and Behaviour: A Meta-Analytic Review From an Evolutionary Perspective. The Journal of Social Psychology 150(2).

Ficks , C. A., & Waldman, D. (2014). Candidate genes for aggression and antisocial behaviour: A meta-analysis of association studies of the 5HTTLPR and MAOA-uVNT. Behaviour Genetics, 44(5).

Jacobson, K. C., Prescott, C. A., & Kendler, K. S. (2000). Genetic and environmental influences on juvenile antisocial behaviour assessed on two occasions. Psychological Medicine 2000, 30(6).

Kim, S., & Lee, D. (2011). Prefrontal Cortex and Impulsive Decision Making. Biological Psychiatry, 69(12).

Koenigs, M. (2012). The role of the prefrontal cortex in psychopathy. Reviews in the Neurosciences, 23(3), 253–262. http://doi.org/10.1515/revneuro-2012-0036 Kopsida, E., Stergiakouli, E., Lynn, P. M., Wilkinson, L. S., & Davies, W. (2009). The Role of the Y Chromosome in Brain Function. Open Neuroendocrinology Journal, 2. Lewis, T. (29 July 2015). A single gene has been linked with being a psychopath — and it's very

controversial. Business Insider.
Lis, E., Greenfield, B., Henry, M., Guilé, J. M., & Dougherty, G. (2007). Neuroimaging and genetics of borderline personality disorder: a review. Journal of Psychiatry & Neuroscience, 32(3).
Ly, M., Motzkin, J. C., Philippi, C. L., Kirk, G. R., Newman, J. P., Kiehl, K. A., & Koenigs, M. (2012). LynaCortical thinning in psychopathy. The American Journal of Psychiatry, 169(7).
m, D. R., & Gudonis, L. (2005). The development of psychopathy. Annual Review of Clinical Psychopathy, 1.
Mason, D. A., & Frick, P. J. (1994). The heritability of antisocial behaviour: A meta analysis of twin and adoption studies. Journal of Psychopathology and Behavioural Assessment, 16(4).
Meyer-Lindenberg, A., Buckholtz, J. W., Kolachana, B., R. Hariri, A., Pezawas, L., Blasi, G., Wabnitz, A., Honea, R., Verchinski, B., Callicott, J. H., Egan, M., Mattay, V., & Weinberger, D. R. (2006). Neural mechanisms of genetic risk for impulsivity and violence in humans. Proceedings of the National Academy of Sciences of the United States of America, 103(16).
Narayan, V. M., Narr, K. L., Kumari, V., Woods, R. P., Thompson, P. M., Toga, A. W., & Sharma, T. (2007). Regional cortical thinning in subjects with violent antisocial personality disorder or schizophrenia. The American Journal of Psychiatry, 164(9). Rhee, S. H., & Waldman, I. D. (2002) Genetic and environmental influences on antisocial behaviour: A meta-analysis of twin and adoption studies. Psychological Bulletin, 128(3).
Thompson, P. M., Cannon, T. D., Narr, K. L., Erp, T. V., Poutanen, V., Huttunen, M., Lönnqvist, J., Standertskjöld-Nordenstam, C., Kaprio, J., Khaledy, M., Dail, R., Zoumalan, C. I., & Toga, A. W. (2001). Genetic influences on brain structure. *Nature Neuroscience*, 4.
Tuvblad, C., & Beaver, K. M. (2013). Genetic and environmental influences on antisocial behaviour. Journal of Criminal Justice, 41(5).
Viding, E., Blair, R. J., Moffitt, T. E., & Plomin, R. (2005). Evidence for substantial genetic risk for psychopathy in 7-year-olds. Journal of Child Psychology and Psychiatry, 46(6).
Viding, E., Frick, P. J., & Plomin, R. (2007). Aetiology of the relationship between callous–unemotional traits and conduct problems in childhood. The British Journal of Psychiatry, 190(49).
Viding, E., Fontaine, N. M., & McCrory, E. J. (2012). Antisocial behaviour in children with and without callous-unemotional traits. Journal of the Royal Society of Medicine, 105(5).
Wanga, P., Niva, S., Tuvblada, C., Raineb, A., & Bakera, L. A. (2013). The genetic and environmental overlap between aggressive and non-aggressive antisocial behaviour in children and adolescents using the Self-Report Delinquency Interview (SR-DI). Journal of Criminal Justice, 41(5).
World Health Organisation (2017). Child Maltreatment. Retrieved 25 April 2017. Yang, Y., Raine, A., Narr, K. L., Colletti, P., & Toga, A. W. (2009). Localisation of Deformations Within the Amygdala in Individuals With Psychopathy. Archives of General Psychiatry, 66(9).
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A Proposed CRISPR-Cas9 Framework for Modifying Myrosinase Activity and Flavor Traits in Broccoli: In Silico Target Identification and Construct Design

By Christopher Shu[1] *

Abstract

Broccoli is frequently rejected for tasting bitter and pungent. These are two separate sensations with separate causes. Bitterness is a true taste, triggered when glucosinolates (sulfur-containing plant defense compounds) and their breakdown products bind TAS2R bitter receptors. Pungency is a chemesthetic burning sensation driven by isothiocyanates acting on the TRPA1 channel. Only the second depends on myrosinase, the enzyme that hydrolyzes glucosinolates when plant tissue is damaged. This distinction predicts that disabling myrosinase should reduce pungency substantially and bitterness only partially, and it is the basis for the editing strategy proposed here.

This article reports the in silico design of a clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9) knockout targeting a myrosinase-encoding locus in broccoli. A TGG1-like ortholog of 1,827 bp was identified by BLAST search using the Arabidopsis thaliana TGG1 sequence as a query. It comprises a 35 bp 5' UTR, a 1,647 bp coding sequence encoding 548 amino acids, and a 145 bp 3' UTR, and its conceptual translation is identical to the annotated broccoli TGG1 entry (UniProt A0A678XDV3) and 73.4% identical to A. thaliana TGG1. Two guide RNAs were selected from 157 candidate sites, ranking first and third by predicted efficiency with no predicted off-target site at up to three mismatches, and a 12,127 bp Golden Gate construct carrying both guide cassettes, a Cas9 cassette, and an nptII selection marker was assembled in silico. The two predicted cut sites lie 731 bp apart, with the upstream cut falling at codon 61 of 548. A dual-cut event is therefore predicted to remove roughly 44% of the coding sequence and to be detectable as a shift from a 907 bp wild-type amplicon to an approximately 176 bp deletion allele.
No transformation or phenotyping was performed, so no claim is made about actual editing efficiency, myrosinase activity, flavor, or sulforaphane content. Two boundary conditions constrain what the strategy could achieve even if executed. Myrosinase is heat-labile, so the expected sensory benefit applies mainly to raw or lightly cooked broccoli. Sulforaphane is itself a myrosinase product, so reduced pungency is expected to cost a proportional amount of sulforaphane yield. The framework's value lies in being a fully specified, testable design and in framing that trade-off explicitly, not in any demonstrated improvement to broccoli.

Keywords Biology; Molecular Biology; CRISPR-Cas9; Gene Editing; Glucosinolates; Myrosinase; TGG1; In Silico Design

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1. Introduction

Broccoli (Brassica oleracea var. italica) has high nutritional value, yet many people dislike eating it. This is usually explained by a general reference to a bitter or astringent taste, but the sensory chemistry behind broccoli's flavor is more complicated than those adjectives suggest. Bitterness and pungency are distinct sensory effects produced by separate physiological mechanisms, and they combine to create the unpleasant impression most people report. Distinguishing between them is what makes a targeted gene-editing strategy possible, because the two respond very differently to the same intervention.

Bitterness is a true taste sensation, detected by the TAS2R family of bitter taste receptor proteins in oral epithelial cells. Glucosinolates are sulfur- and nitrogen-containing secondary metabolites characteristic of Brassica vegetables, where they function in defense against herbivores and pathogens. Some glucosinolates and their breakdown products act as ligands for TAS2R receptors. The best-characterized example is TAS2R38, which responds to compounds containing a thiourea group, a sulfur-containing chemical group of the form N–C(=S)–N that is the structural feature this receptor recognizes. Such compounds include those produced by the glucosinolate progoitrin (Bell et al.). TAS2R38 exists in several haplotypes, named for the amino acids at three variable positions in the protein. Individuals homozygous for the functional PAV (proline-alanine-valine) haplotype are termed tasters and are especially sensitive to Brassica bitterness. Individuals homozygous for the nonfunctional AVI (alanine-valine-isoleucine) haplotype are termed non-tasters and find the same compounds nearly non-bitter (Boxer and Garneau). One consequence matters directly for this proposal: intact glucosinolates can stimulate bitter receptors before any enzymatic breakdown occurs. Broccoli bitterness therefore does not depend entirely on myrosinase activity and cannot be removed completely by disabling myrosinase alone.

Pungency is not a taste. It is a chemesthetic response, the burning and stinging sensation carried by general chemical-sensing nerve endings rather than by taste receptors. In broccoli, the principal products of myrosinase-catalyzed glucosinolate hydrolysis are isothiocyanates, which are potent activators of the transient receptor potential ankyrin 1 (TRPA1) channel. These channels are densely distributed in the mouth, throat, and nasal cavity (Jordt et al.). When broccoli tissue is broken by chewing, myrosinase rapidly hydrolyzes glucosinolates into isothiocyanates, which bind TRPA1 and produce the pungent sensation. Pungency is thus directly downstream of myrosinase activity, with no alternative pathway generating the same effect, so a nonfunctional myrosinase enzyme would be expected to suppress it. Consumer sensory work supports this reasoning. Processing methods that alter isothiocyanate production measurably change pungency-related attributes including burning sensation, mustard flavor, and pungent aftertaste (Ghawi et al.).

Glucosinolates and myrosinase are stored in physically separate compartments in intact broccoli tissue and do not meet until the tissue is damaged. Chewing, cutting, or processing breaks that separation, and hydrolysis proceeds rapidly, yielding isothiocyanates, nitriles, thiocyanates, and related products (Bell et al.). The same products serve the plant's own defense

against herbivores and pathogens (Halkier and Gershenzon; Stranger and Mitchell-Olds; Zhang et al.). Which product predominates depends on the glucosinolate substrates present, reaction pH, specifier proteins such as epithiospecifier protein (ESP), and overall myrosinase activity. Among these hydrolysis products, sulforaphane has attracted the most research attention, and it is the source of the central trade-off in this proposal. Sulforaphane is generated specifically from glucoraphanin by myrosinase. It activates the NRF2 signaling pathway by covalently modifying the repressor protein Keap1, inducing Phase II detoxification enzymes including NQO1 and heme oxygenase-1 (Dinkova-Kostova et al.). The pathway has been studied in connection with cancer chemoprevention, anti-inflammatory effects, and protection against environmental toxins (Houghton et al.). Because sulforaphane production depends on myrosinase, any intervention that lowers myrosinase activity to reduce pungency should lower sulforaphane yield in proportion. That trade-off has to be measured rather than assumed away in any experimental validation.

Non-genetic routes to the same goal exist and are worth stating plainly. Conventional breeding for altered glucosinolate or myrosinase profiles is well established and avoids regulatory complications. Blanching inactivates myrosinase directly and requires no genetic change at all (Fahey et al.). Gene editing is proposed here not because it is the only option but because it isolates a single variable. Knocking out one locus tests whether that locus is responsible for the pungency phenotype, in a way that breeding and thermal processing, which alter many properties at once, cannot. The value of the approach is diagnostic as much as applied.

CRISPR-Cas9 uses a guide RNA to direct the Cas9 nuclease to a DNA sequence adjacent to a protospacer adjacent motif (PAM), where Cas9 generates a double-strand break (Jinek et al.). Repair by non-homologous end joining (NHEJ) frequently introduces small insertions or deletions, which can cause a frameshift that disrupts protein function (Lieber). Delivery into broccoli cells would use Agrobacterium-mediated transformation, in which T-DNA transfer integrates the editing cassettes into the plant genome (Gelvin).

This article reports the computational stage of that strategy: identification of a candidate target locus, design and off-target screening of guide RNAs, and in silico assembly of the delivery construct. It is hypothesized that CRISPR-Cas9-mediated knockout of the TGG1-like myrosinase locus would reduce isothiocyanate formation after tissue disruption and therefore reduce pungency-related traits, with a smaller effect on bitterness because intact glucosinolates activate bitter receptors independently of myrosinase. It is further predicted that this would impose a proportional reduction in sulforaphane yield. These are predictions derived from the literature reviewed above, not findings. Testing them requires transformation, genotyping, biochemical assay, and sensory evaluation that remain to be carried out.

2. Methods

All work reported in this section was performed computationally. Sequence retrieval, guide RNA design, off-target screening, and construct assembly were carried out in silico using

publicly available databases and design tools together with sequence-editing software. No cloning, transformation, or laboratory assay was undertaken. Sections 2.4 and 2.5 describe procedures that are **proposed** for future execution and have not been performed.

2.1 Sequence identification

A myrosinase-encoding target sequence in broccoli was required in order to design guide RNAs. A TGG1-like locus in Brassica oleracea var. italica is present in UniProt as an automatically annotated entry (accession A0A678XDV3, thioglucosidase) (UniProt Consortium, "TGG1 — Thioglucosidase"). That entry is unreviewed (TrEMBL), meaning it was generated by automatic annotation pipelines and has not been manually curated or experimentally characterized in broccoli. An independent ortholog identification was therefore carried out rather than relying on the automatic annotation alone.

Because Arabidopsis thaliana and broccoli are closely related within the Brassicaceae, broccoli was expected to carry an ortholog of the Arabidopsis myrosinase gene. The A. thaliana TGG1 sequence (UniProt Consortium, "TGG1 — Myrosinase 1") was used as the query in a BLAST search against the B. oleracea var. italica genome through the National Library of Medicine BLAST service. The highest-scoring TGG1-like locus was retrieved and selected as the initial editing target (Figure 1). Because myrosinase belongs to a multigene family in the Brassicales (Stranger and Mitchell-Olds; Zhang et al.), further annotation, expression analysis, and possibly multiplex targeting would be needed to establish whether this locus alone determines the phenotype.

2.2 Guide RNA design and off-target screening

Guide RNAs were designed using the CHOPCHOP web tool (Labun et al.), which ranks candidate sequences by GC content, self-complementarity, predicted on-target efficiency, predicted off-target profile, and position within the coding sequence. The retrieved 1,827 bp locus was supplied directly as the target sequence, and two guides cutting at separate sites within the coding sequence were selected, so that a single construct could produce either two independent frameshifts or a larger deletion between the two cut sites.

One limitation of the off-target analysis should be stated explicitly. CHOPCHOP does not host a B. oleracea genome assembly, so genome-wide off-target counts could not be computed against broccoli itself. The off-target search was instead run against Brassica napus ssp. oleifera (ZS11.v0). B. napus is an allotetraploid whose C subgenome derives from B. oleracea, so this search covers sequences homologous to the broccoli genome while additionally screening the unrelated A subgenome contributed by B. rapa. The result is therefore a conservative proxy rather than a direct measurement: sites absent from B. napus but present in B. oleracea would not be detected, and the divergence between the B. napus C subgenome and B. oleracea is not zero. Confirming the off-target profile against a B. oleracea assembly directly is listed among the steps required before the construct is built.

Each guide RNA spacer is 20 nucleotides. The PAM is not part of the guide RNA. It is recognized by the Cas9 protein itself in the target DNA and is listed in Table 1 only to identify the genomic site.

Table 1. Guide RNA spacer sequences selected for this design, with the adjacent PAM in the target DNA. The PAM is not included in the guide RNA.

Guide	Spacer sequence (5'→3', 20 nt)	Adjacent PAM in target DNA	Strand
gRNA1	CTGCTTACCAGATCGAAGGA	GGG	+
gRNA2	GCGGCTCCATATACCATCCA	TGG	−

2.3 Construct design

The CRISPR-Cas9 construct was assembled in silico following the Golden Gate protocol described in Plant Genome Editing with CRISPR Systems (Qi), which covers editing in Brassica oleracea. Golden Gate assembly joins several DNA fragments from separate donor plasmids into a single destination plasmid in one reaction. The modules used were:

- **L1P1**: kanamycin selection cassette (neomycin phosphotransferase gene, nptII, driven by the 35S promoter)
- **L1P2**: Cas9 expression cassette (Streptococcus pyogenes Cas9 with a nuclear localization signal, driven by the cassava vein mosaic virus promoter)
- **L1P3 and L1P4**: guide RNA expression cassettes (each driven by the A. thaliana U6-26 promoter)
- **L2**: acceptor plasmid

Together the L1 modules form the T-DNA region, which Agrobacterium transfers into the plant genome. The nptII marker allows transformed cells to be selected on kanamycin-containing medium during tissue culture, the Cas9 cassette supplies the nuclease, and the two guide cassettes direct it to the target sites.

Guide sequences are introduced into the L1P3 and L1P4 cassettes as annealed complementary oligonucleotide pairs. Overhangs are required because the type IIS restriction enzymes used cut outside their recognition sites and leave staggered ends. Cloning into L1P3 uses BsaI, with oligonucleotide overhangs of the form:

```
5' ATTG NNNNNNNNNNNNNNNNNNN 3'
3'      NNNNNNNNNNNNNNNNNNN CAAA 5'
```

Cloning into L1P4 uses Esp3I, with overhangs of the form:

```
5' AGTGCTTG NNNNNNNNNNNNNNNNNNN GTTT 3'
3'     GAAC NNNNNNNNNNNNNNNNNNN CAAAATCT 5'
```

In both templates the N region carries the spacer, and the terminal G of the 5' overhang supplies the +1 nucleotide that the U6-26 promoter requires for efficient transcription initiation. The gRNA2 spacer already begins with G and is unaffected. The gRNA1 spacer begins with C, so cloning it into this backbone requires either substituting that first base for G or appending an extra G, a standard accommodation for U6-driven guides that alters the 5' terminal base of the transcript without changing the genomic site targeted. The unmodified spacer sequences are those reported in Table 1. The completed L1 modules are digested with BpiI and ligated into the L2 acceptor to produce the final construct (Figure 2).

2.4 Proposed delivery and genotyping

The following procedure is proposed and has not been carried out. The construct would be introduced into broccoli explants by Agrobacterium-mediated transformation (Gelvin), with transformed tissue selected on kanamycin.

Because primary transformants (T0) generated by Agrobacterium-mediated editing are frequently chimeric, carrying different edits in different cell lineages, a single PCR test on T0 tissue cannot establish that a plant carries a heritable, homozygous knockout. A minimum of 15 to 20 independent T0 lines would be screened to allow for variable editing efficiency and for the recovery of lines carrying biallelic edits. Screening would use PCR across both target sites followed by Sanger sequencing of the amplicon, with the following primers:

- Left (forward) primer: 5' CGAAGAGAACAATCCATTCACA 3'
- Right (reverse) primer: 5' CCTACTACCCACAATCTGCCTC 3'

Selected T0 lines would then be advanced to the T1 generation, where segregation permits identification of plants that are homozygous for the edit and have lost the Cas9 and nptII transgenes. Cas9-free homozygous T1 lines would be confirmed by PCR for absence of the Cas9 and nptII cassettes alongside sequencing of the target locus, and only these lines would be used for biochemical and sensory characterization.

2.5 Proposed phenotypic and sensory evaluation

The following evaluation is proposed and has not been carried out. Because the motivation for this work is a change in flavor, the design requires measurements at three levels.

Biochemical. Myrosinase activity in leaf and floret tissue would be quantified by a glucose-release assay using sinigrin as substrate, comparing edited lines against unedited controls from the same transformation experiment. Glucosinolate and isothiocyanate concentrations would be measured by HPLC, and sulforaphane specifically would be quantified to determine the size of the nutritional trade-off.

Sensory. Sensory evaluation would use a two-stage design. A triangle test ($n \geq 30$ untrained assessors) would first establish whether edited and unedited broccoli are distinguishable at all. If a difference is detected, a trained descriptive panel would rate pungency,

bitterness, and burning aftertaste on structured line scales, allowing the predicted asymmetry between a large pungency effect and a smaller bitterness effect to be tested directly. Because myrosinase is heat-labile, samples would be evaluated both raw and lightly steamed, so that the boundary condition described in Section 4.3 is measured rather than assumed.

Agronomic. Because glucosinolate hydrolysis products contribute to pathogen and herbivore defense (Halkier and Gershenzon; Stranger and Mitchell-Olds; Zhang et al.), edited lines would be monitored for susceptibility under controlled conditions before any field evaluation.

3. In Silico Results

This section reports the outputs of the computational work described in Section 2. No experimental results are reported, because no experiment has been performed.

3.1 Target locus identification

The BLAST search returned a TGG1-like locus in B. oleracea var. italica was the highest-scoring hit against the A. thaliana TGG1 query. The retrieved sequence is 1,827 bp in total and resolves into a 35 bp 5' UTR, a 1,647 bp coding sequence, and a 145 bp 3' UTR (Figure 1). The coding sequence runs from the start codon at position 36 to the stop codon ending at position 1,682 and encodes a 548-amino-acid protein, consistent in length with characterized plant myrosinases of the glycoside hydrolase family 1.

Two independent lines of evidence support the ortholog call. First, conceptual translation of the retrieved coding sequence yields a 548-amino-acid protein identical at every residue to UniProt entry A0A678XDV3, the automatically annotated B. oleracea var. italica TGG1 thioglucosidase (UniProt Consortium, "TGG1 — Thioglucosidase"). The retrieved locus and the annotated broccoli entry are therefore the same gene, which fixes the identity of the editing target independently of the BLAST ranking. Second, pairwise alignment of the translated locus against A. thaliana TGG1 (P37702) (UniProt Consortium, "TGG1 — Myrosinase 1") gives 73.4% amino-acid identity over 538 aligned residues (BLOSUM62, global alignment), consistent with orthology between species that diverged within the Brassicaceae rather than with a more distant paralog.

3.2 Guide RNA design

Both selected guides target the 5' half of the coding sequence, where a frameshift truncates the largest proportion of the protein. Their computed properties are summarized in Table 2.

Both guides fall within the recommended 40–60% GC range for Streptococcus pyogenes Cas9. The upstream cut site lies at codon 61 of 548, so an indel at this site is predicted to truncate approximately 89% of the protein, well upstream of the catalytic residues of the glycoside hydrolase family 1 active site.

CHOPCHOP identified 157 candidate target sites across the 1,827 bp locus. The two selected guides ranked first and third overall, with predicted on-target efficiency scores of 61.24 and 55.12 respectively. Neither guide returned any predicted off-target site at zero, one, two, or three mismatches. That result is informative rather than an artifact of a failed search, because the same run returned substantial off-target counts for lower-ranked candidates within the same locus, up to 21 perfect-match sites and 131 three-mismatch sites for the worst-scoring guides. The two selected guides are therefore among the most specific available within this gene.

Table 2. Computed properties of the two guide RNAs, relative to the 1,827 bp TGG1-like target sequence. Spacer sequences are given in Table 1. Positions are those of the retrieved sequence; the predicted Cas9 blunt cut lies between the third and fourth nucleotides 5' of the PAM. Off-target counts are CHOPCHOP predictions against the B. napus ZS11 assembly (Section 2.2).

Property	gRNA1	gRNA2
Strand	+	−
Spacer GC content	50.0%	55.0%
Predicted cut position	nt 219	nt 950
Position within CDS	codon 61 of 548	codon 305 of 548
CHOPCHOP rank (of 157 candidates)	1	3
Self-complementarity	0	1
Predicted off-targets, 0 mismatches	0	0
Predicted off-targets, 1 mismatch	0	0
Predicted off-targets, 2 mismatches	0	0
Predicted off-targets, 3 mismatches	0	0
Predicted on-target efficiency score	61.24	55.12

3.3 Predicted editing outcomes

Using both guides in a single construct produces two predicted classes of edit. Independent NHEJ repair at either site can produce a frameshift; repair following simultaneous cleavage at both sites can excise the intervening segment. The two cut sites lie 731 bp apart, which corresponds to approximately 44% of the 1,647 bp coding sequence.

This has a direct consequence for genotyping. The proposed primer pair spans both cut sites and yields a predicted wild-type amplicon of 907 bp. A dual-cut deletion allele would yield a product of approximately 176 bp. The two are separable on a standard agarose gel, so the dual-cut genotype can be scored by product size before sequencing, and single-site indels can be resolved by Sanger sequencing of the full-length amplicon. Table 3 summarizes the predicted genotyping outcomes.

Table 3. Predicted PCR genotyping outcomes for the proposed primer pair.

Allele class	Predicted amplicon	Detection method
Wild type	907 bp	Size
Single-site indel (either guide)	~907 bp	Sanger sequencing
Dual-cut deletion	~176 bp	Size, confirmed by sequencing

3.4 Assembled construct

Golden Gate assembly of the four L1 modules into the L2 acceptor yields a final construct of 12,127 bp (Figure 2), carrying the nptII selection cassette, the Cas9 expression cassette, and both guide RNA cassettes within the T-DNA region. The construct exists as an annotated sequence file only and has not been built.

4. Discussion

4.1 What this work does and does not establish

The work reported here establishes that a specific, buildable editing strategy for a broccoli myrosinase locus can be fully specified: a target locus has been identified, two guides have been designed and positioned within the coding sequence, predicted editing outcomes have been derived, and a delivery construct has been assembled in silico with a genotyping assay whose expected products are known in advance. What it does not establish is any biological outcome. Whether the guides cut efficiently in broccoli cells, whether knockout of this locus measurably lowers myrosinase activity, and whether any such reduction changes what broccoli tastes like are open questions that this design makes testable but does not answer.

This distinction matters for how the framework should be read. Every statement in Section 1 about bitterness, pungency, and sulforaphane is drawn from published experimental work on those systems. Every statement in Section 3 is a computational output or a prediction derived from sequence. No statement in either section is an experimental finding about an edited broccoli plant, because no such plant exists.

4.2 Gene family redundancy

The most likely reason for the proposed knockout to produce no phenotype is genetic redundancy. Myrosinases form a multigene family in the Brassicales, and Arabidopsis alone carries multiple TGG paralogs with overlapping expression (Stranger and Mitchell-Olds; Zhang et al.). Broccoli, as a polyploid-derived crop, may carry several functional myrosinase loci, in which case disabling one could leave total activity substantially intact.

Three responses follow if the single-locus knockout gives no measurable change in myrosinase activity. The first is to establish which paralogs are actually expressed in the edible floret tissue by RNA sequencing, since a locus that is not expressed where the tissue is eaten cannot contribute to the flavor phenotype regardless of its sequence. The second is multiplex targeting, adding guide cassettes against the expressed paralogs to the same construct, which the Golden Gate architecture accommodates directly. The third is to target a shared upstream regulator rather than the structural genes themselves. A negative result at the single-locus stage would be informative in its own right, because it would place a lower bound on the redundancy of the system.

Other failure modes deserve anticipation. Low transformation efficiency in B. oleracea may require screening more T0 lines than projected. Editing may occur but produce in-frame

indels that leave a functional enzyme, which sequencing at the target site would reveal. Guides may score well in silico yet cut poorly in chromatin. Each of these is diagnosable from the genotyping data proposed in Section 2.4 rather than requiring a redesign.

4.3 Thermal inactivation as a boundary condition

Myrosinase is heat-labile, and endogenous myrosinase activity is the limiting factor controlling sulforaphane formation in cooked broccoli (Fahey et al.). Boiling or extended steaming largely inactivates the enzyme, so hydrolysis in thoroughly cooked broccoli proceeds far more slowly than in raw tissue, and the isothiocyanate burst responsible for pungency is correspondingly reduced before any genetic intervention.

This constrains the scope of the proposal. The expected sensory benefit of a myrosinase knockout is largest in raw and lightly cooked preparations, which is where myrosinase is most active and where pungency is most pronounced. In thoroughly cooked broccoli the enzyme is already substantially inactivated, and the residual off-flavors are generated by thermal degradation of sulfur compounds through pathways that do not require myrosinase. A knockout is not expected to improve those. The proposed sensory design in Section 2.5 evaluates raw and lightly steamed samples for this reason: the boundary condition is testable, and treating it as such is preferable to claiming a general solution to broccoli rejection.

4.4 The nutritional trade-off

The strategy proposed here reduces the production of a compound class that includes both the source of the pungency being targeted and sulforaphane, the compound responsible for much of broccoli's studied health value (Dinkova-Kostova et al.; Houghton et al.). Because both derive from myrosinase activity on glucosinolate substrates, they cannot be separated by reducing myrosinase alone. Any edited line should therefore be judged on two axes at once, and a line that tastes better while losing most of its sulforaphane yield would not represent an improvement in any sense that matters to consumers who eat broccoli for its health properties. Two directions could in principle decouple the two effects, and both fall outside the scope of the present design. Glucosinolate profiles differ in their hydrolysis products, so shifting substrate composition toward glucoraphanin and away from the glucosinolates that yield the most pungent isothiocyanates might preserve sulforaphane while lowering pungency. Alternatively, protein engineering of myrosinase substrate specificity, rather than knockout, could in principle retain glucoraphanin turnover while reducing activity on other substrates. Both would require structural and enzymological work well beyond the present framework, and both are noted here as directions rather than proposals.

4.5 Regulatory context

A gene-edited broccoli line would enter a regulatory environment that differs substantially between jurisdictions. Several countries now treat transgene-free edited plants carrying only small indels differently from conventional transgenic crops, which is one reason

the Cas9-free T1 selection step described in Section 2.4 is part of the design rather than an afterthought. Any move from proof of concept toward a cultivar would require engagement with that pathway, and the timeline for it would likely exceed that of the laboratory work.

5. Conclusion

This article reports the in silico design of a CRISPR-Cas9 knockout targeting a TGG1-like myrosinase locus in broccoli, motivated by the distinction between myrosinase-dependent pungency and partly myrosinase-independent bitterness. The computational stage produced a 1,827 bp candidate target locus, two guide RNAs positioned at codons 61 and 305 of a 548-codon coding sequence, a predicted 731 bp dual-cut deletion detectable as a shift from a 907 bp to an approximately 176 bp PCR product, and a 12,127 bp assembled construct.

These are design outputs, not experimental findings. The framework's contribution is to specify a testable intervention completely enough that it could be executed and falsified by another investigator, and to state in advance what would count as success, what would count as failure, and what the intervention would cost nutritionally if it worked. The predicted asymmetry between a substantial reduction in pungency and a smaller reduction in bitterness is the specific claim most worth testing, because it follows directly from the receptor biology and would distinguish this model from the general assumption that broccoli's unpleasantness is a single phenomenon. Whether the strategy improves broccoli remains unknown, and the work required to find out is laboratory work that has not yet begun.

Figures

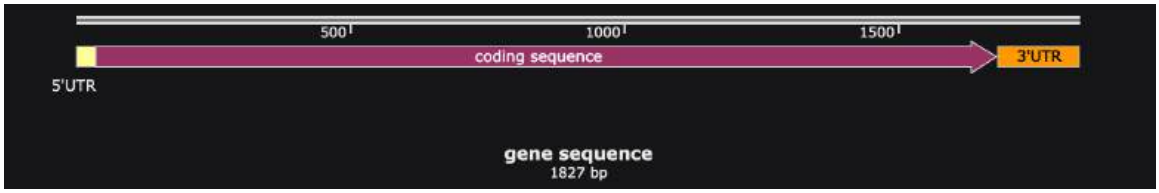

Figure 1. Annotated map of the broccoli TGG1-like myrosinase locus retrieved by BLAST. The sequence is 1,827 bp: a 35 bp 5' UTR, a 1,647 bp coding sequence beginning at the start codon at position 36 and ending at the stop codon at position 1,682, and a 145 bp 3' UTR. The two target sites described in Section 3.2 span nucleotides 202–224 (gRNA1, + strand) and 947–969 (gRNA2, − strand), each comprising the 20 nt protospacer plus its 3 nt PAM, but are not marked on this map. Map generated in SnapGene.

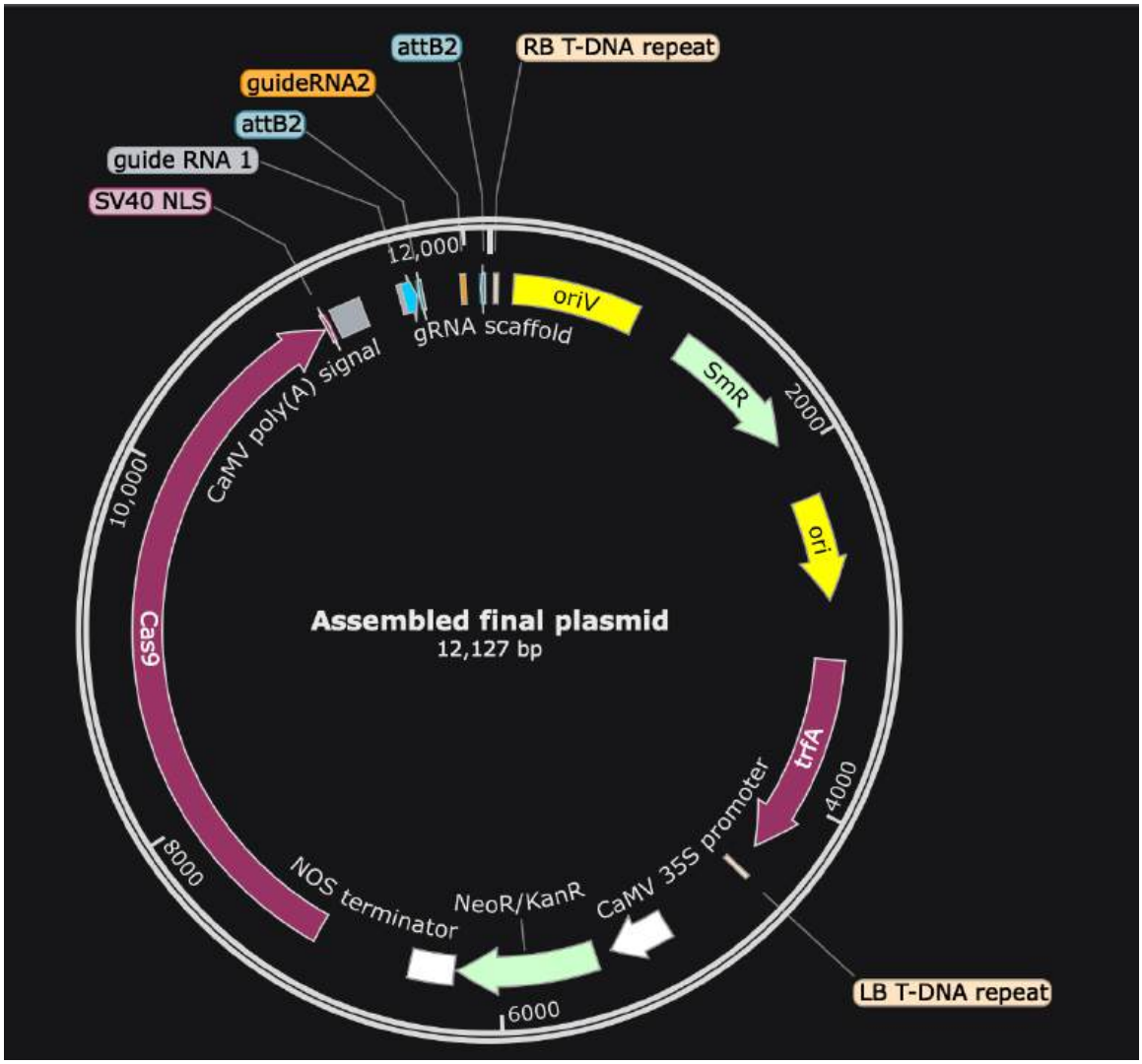

Figure 2. Map of the assembled 12,127 bp Golden Gate construct, showing the T-DNA region carrying the nptII selection cassette, the Cas9 expression cassette, and the two guide RNA cassettes. Map generated in SnapGene.

Data Availability Statement

The annotated sequence files supporting this work, comprising the retrieved broccoli TGG1-like locus and the assembled 12,127 bp construct, are available from the author on request as SnapGene (.dna) files. The A. thaliana query sequence is available from UniProt under accession P37702, and the automatically annotated broccoli TGG1 entry under accession A0A678XDV3. The full CHOPCHOP output for all 157 candidate target sites, including per-guide efficiency scores and mismatch-stratified off-target counts, is available from the author as a tab-separated file. No experimental datasets were generated.

Ethics Declaration

This work involved no human participants, no animal subjects, and no laboratory organisms. All work was computational. The sensory evaluation proposed in Section 2.5 would

require institutional ethics approval and informed consent from participants before it could be carried out.

Author Contributions (CRediT)

Christopher Shu: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review and editing.

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Conflict of Interest

The author declares no conflicts of interest regarding the publication of this article.

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During the preparation of this work, the author used Claude (Anthropic) to identify possible sources, to narrow the scope of investigation, and to assist in structuring and editing the manuscript. All scientific claims, the experimental design, the sequence analysis, and the interpretation are the author's own. The author reviewed and edited all content and takes full responsibility for the content of this publication.

Works Cited

Bell, Luke, et al. "Taste and Flavor Perceptions of Glucosinolates, Isothiocyanates, and Related Compounds." Molecular Nutrition & Food Research, vol. 62, no. 18, 2018, article 1700990, https://doi.org/10.1002/mnfr.201700990.

Boxer, Emma E., and Nicole L. Garneau. "Rare Haplotypes of the Gene TAS2R38 Confer Bitter Taste Sensitivity in Humans." SpringerPlus, vol. 4, no. 1, 2015, article 505, https://doi.org/10.1186/s40064-015-1277-z.

Dinkova-Kostova, Albena T., et al. "Direct Evidence That Sulfhydryl Groups of Keap1 Are the Sensors Regulating Induction of Phase 2 Enzymes That Protect against Carcinogens and Oxidants." Proceedings of the National Academy of Sciences, vol. 99, no. 18, 2002, pp. 11908–13, https://doi.org/10.1073/pnas.172398899.

Fahey, Jed W., et al. "Sulforaphane Bioavailability from Glucoraphanin-Rich Broccoli: Control by Active Endogenous Myrosinase." PLOS ONE, vol. 10, no. 11, 2015, article e0140963, https://doi.org/10.1371/journal.pone.0140963.

Gelvin, Stanton B. "Agrobacterium-Mediated Plant Transformation: The Biology behind the 'Gene-Jockeying' Tool." Microbiology and Molecular Biology Reviews, vol. 67, no. 1, 2003, pp. 16–37, https://doi.org/10.1128/mmbr.67.1.16-37.2003.

Ghawi, Sameer Khalil, et al. "Consumer Acceptability and Sensory Profile of Cooked Broccoli with Mustard Seeds Added to Improve Chemoprotective Properties." Journal of Food Science, vol. 79, no. 9, 2014, pp. S1756–62, https://doi.org/10.1111/1750-3841.12556.

Halkier, Barbara Ann, and Jonathan Gershenzon. "Biology and Biochemistry of Glucosinolates." Annual Review of Plant Biology, vol. 57, 2006, pp. 303–33, https://doi.org/10.1146/annurev.arplant.57.032905.105228.

Houghton, Christine A., et al. "Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the Reality?" Oxidative Medicine and Cellular Longevity, vol. 2016, 2016, article 7857186, https://doi.org/10.1155/2016/7857186.

Jinek, Martin, et al. "A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity." Science, vol. 337, no. 6096, 2012, pp. 816–21, https://doi.org/10.1126/science.1225829.

Jordt, Sven-Eric, et al. "Mustard Oils and Cannabinoids Excite Sensory Nerve Fibres through the TRP Channel ANKTM1." Nature, vol. 427, no. 6971, 2004, pp. 260–65, https://doi.org/10.1038/nature02282.

Labun, Kornel, et al. "CHOPCHOP v3: Expanding the CRISPR Web Toolbox beyond Genome Editing." Nucleic Acids Research, vol. 47, no. W1, 2019, pp. W171–74, https://doi.org/10.1093/nar/gkz365.

Lieber, Michael R. "The Mechanism of Double-Strand DNA Break Repair by the Nonhomologous DNA End-Joining Pathway." Annual Review of Biochemistry, vol. 79, no. 1, 2010, pp. 181–211, https://doi.org/10.1146/annurev.biochem.052308.093131.

Qi, Yiping, editor. Plant Genome Editing with CRISPR Systems: Methods and Protocols. Methods in Molecular Biology 1917, Humana Press, 2019.

Stranger, Barbara E., and Thomas Mitchell-Olds. "Nucleotide Variation at the Myrosinase-Encoding Locus, TGG1, and Quantitative Myrosinase Enzyme Activity Variation in Arabidopsis thaliana." Molecular Ecology, vol. 14, no. 1, 2005, pp. 295–309, https://doi.org/10.1111/j.1365-294x.2004.02403.x.

UniProt Consortium. "TGG1 — Myrosinase 1 — Arabidopsis thaliana (P37702)." UniProtKB, www.uniprot.org/uniprotkb/P37702/entry. Accessed 24 July 2026.

UniProt Consortium. "TGG1 — Thioglucosidase — Brassica oleracea var. italica (A0A678XDV3)." UniProtKB, unreviewed (TrEMBL) entry, www.uniprot.org/uniprotkb/A0A678XDV3/entry. Accessed 4 Sept. 2026.

Zhang, Kaixin, et al. "Overexpressing the Myrosinase Gene TGG1 Enhances Stomatal Defense against Pseudomonas syringae and Delays Flowering in Arabidopsis." Frontiers in Plant Science, vol. 10, 2019, article 1230, https://doi.org/10.3389/fpls.2019.01230.

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The Role of MicroRNA (miRNA) in Tendon Injury and Repair By Charles Kakkanatt

Abstract

MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression after transcription by binding target messenger RNAs. Tendons are dense connective tissues that transmit force from muscle to bone. When tendons are injured from either acute stress or chronic overuse, they heal slowly and often incompletely. This review examines how miRNAs regulate tendon injury, aging, and repair at different stages. For instance, miR-29a links inflammation to matrix composition by regulating the ratio of type I and type III collagen. Other pathways link mechanical loading to the shift of tendon-derived stem cells toward bone and cartilage lineages. Aging and oxidative stress both alter miRNA expression, influencing pathways for cell senescence and apoptosis. During healing, miRNAs have been found to promote and regulate angiogenesis. Together, these findings suggest that miRNAs actively regulate tendon injury response in addition to acting as a marker of damage. Because a single miRNA regulates many genes, miRNA-based therapies may modulate several repair pathways at once, while miRNA expression may serve as a marker of injury severity or healing. Most evidence for the role of miRNA in tissue recovery comes from in vitro and animal models, and translation to clinical applications is limited by miRNA instability in circulation and the difficulty of delivering treatment to dense, avascular tendon tissue. Future research should identify the miRNAs most critical to tendon injury and repair, determine when to target them during healing, and develop practical, effective treatment delivery systems.

1. Introduction

Overview of tendon structure and function.

Tendons are specialized dense connective tissues that allow skeletal movement by attaching muscle to bone. Their structure is highly organized, starting from individual collagen molecules all the way to the fascicle bundles that form a full tendon (Zhang et al.). Most tendons are composed of Type I collagen arranged in parallel fibers, which gives the tissue both flexibility and strength (Eriksen et al.). Tendon health depends on tenocytes, which maintain extracellular matrix (ECM) homeostasis, and tendon stem/progenitor cells (TSPCs), which restore cell populations and support repair after injury (Dai et al.).

Prevalence and impact of tendon injuries

Tendon injury is very common and can be difficult to treat. Chronic tendinopathy and tendon rupture affect both elite athletes and the general population, with rotator cuff injuries accounting for a large share of orthopedic visits (Abate et al.). Tendinopathy of the Achilles and patellar tendons is particularly common in sports with running and jumping (Fredberg et al.). Even after tendons heal, they rarely return to their pre-injury structure. Healing tendons contain more Type III collagen, blood vessels, and disorganized fibers, which reduces mechanical strength and increases the risk of re-injury (Abate et al.; Sharma et al.). Aging, repetitive stress,

and metabolic conditions such as diabetes further increase re-injury risk (Abate et al.; Gaida et al.).

At the cellular level, tendon injury involves inflammation, matrix breakdown, oxidative stress, and changes in how cells sense and respond to mechanical forces (Sharma et al.). Because TSPCs are among the affected cells and drive regeneration, changes in their function can significantly affect healing. Aging compounds this problem. Aged TSPCs show reduced cell proliferation, poorer differentiation into tendon cells, and cytoskeletal changes that reduce their ability to repair damaged tissue (Dai et al.). The molecular signals that drive these age-related changes are not fully understood. However, new studies suggest that microRNAs (miRNAs), short strands of non-coding RNA that influence gene expression, may regulate many of these injury-related and aging-associated pathways.

Despite growing research on miRNAs in tendon biology, the mechanisms by which miRNA dysregulation contributes to tendon stem cell aging remain poorly understood. Existing studies often examine injury mechanisms or stem cell aging separately, rather than defining how altered miRNA expression connects these processes. The molecular pathways linking miRNAs to oxidative stress, cytoskeletal remodeling, and cellular aging in tendon cells warrant further investigation. In addition, although miRNA-based therapies are progressing in other fields of regenerative medicine, their application to tendon healing remains in early development.

In this review, we examine the role of miRNAs in tendon injury and repair, focusing on their regulation of ECM remodeling, inflammation, angiogenesis, and the aging and death of tendon stem/progenitor cells. We first summarize tendon structure and injury mechanisms, then review miRNA biogenesis and function. We then explore how specific miRNAs influence tendon degeneration and healing. Finally, we discuss how targeting miRNA pathways may improve tendon regeneration and outline future research directions.

2. Overview of MiRNA

2.1 Discovery of MiRNA

In 1987, Ambros and Horvitz described a mutation in the lin-4 gene that produced a phenotype opposite to that of the lin-14 mutations, though a suppressor mutation in the lin-14 was the most common form (Ambros and Horvitz). In 1993, Lee, Feinbaum, and Ambros showed that lin-4 was not a protein-coding gene but instead encoded a small RNA complementary to a portion of lin-14 (R. Lee et al. "Heterochronic Gene"), and a companion study by Wightman, Ha, and Ruvkun demonstrated that this complementarity allowed lin-4 to suppress lin-14 post-transcriptionally (Wightman et al.). Since these initial discoveries, researchers have identified similar small non-coding RNAs across the animal kingdom, including in mammals (R. Lee et al. "Short History"; Almeida et al.).

These small genetic fragments have been conserved through evolution, and defects in miRNA production or in the factors miRNAs act upon lead to significant disruptions in embryogenesis across all animal species studied (Dexheimer and Cochella). Further, miRNA is

critical in processes related to aging and development, and, consequently, its dysregulation has been linked to many human diseases, including cancer (O'Brien et al.; Paul et al.).

2.2 Biogenesis of MiRNA: Canonical Pathway

Two different types of miRNA biogenesis have been described: canonical and non-canonical pathways (Figure 1).

In mammals, miRNA is primarily synthesized via the canonical pathway. Although RNA Polymerase III was initially thought to be the main polymerase involved (Y. Lee et al. "Stepwise Processing"), it is now established that RNA Polymerase II (Pol II) transcribes primary miRNA (Pri-miRNA) transcripts from their genes (Y. Lee et al. "RNA polymerase II"). However, RNA Polymerase III (Pol III) is still used by some viruses, such as herpesviruses (Pfeffer et al.).

These pri-miRNAs are then cleaved in the nucleus by the microprocessor complex, which consists of a ribonuclease III enzyme (Drosha ribonuclease 3 [Drosha]) and a binding protein (DiGeorge Syndrome Critical Region 8 [DGCR8]). These two proteins turn the pri-miRNA into pre-miRNA, with Drosha initiating the process, and the pri-miRNA receives a staggered cut at the end of the process, leaving a slight overhang on the 3' strand for the pre-miRNA (Basyuk et al.; Y Lee et al. "RNase III Drosha").

The pre-miRNA is then transported from the nucleus to the cytoplasm by Exportin 5 (a transport protein specific to pre-miRNA), which is powered by RanGTP (the active form of the Ran protein, bound to GTP) (Bohnsack et al.). Once in the cytoplasm, RanGTP hydrolyzes, releasing the pre-miRNA, which RNase III (Dicer) cleaves to form a duplex.

Finally, the Argonaute (AGO) protein, a key component of the RNA-Induced Silencing Complex (RISC), loads a single strand of the duplex miRNA to form an miRNA-RISC complex, while the other strand is degraded (Yoshida et al.). This mature RISC regulates gene expression by targeting messenger RNAs (mRNAs) to inhibit translation or promote degradation (Koscianska et al.).

2.3 Biogenesis of MiRNA: Non-Canonical Pathway

In addition to the canonical (Drosha-Dicer) Pathway, several other non-canonical Pathways for miRNA biogenesis have been observed. These pathways are rarer but bypass certain steps through distinct cellular mechanisms, allowing for flexibility and variability.

One well-described non-canonical pathway uses mirtrons, small non-coding RNA sequences derived directly from spliced introns or from non-coding regions between coding exons within protein-coding genes (Landgraf et al.). Unlike the primary miRNA described in the canonical Pathway, mirtrons already resemble pre-miRNA after standard mRNA splicing, and therefore do not require Drosha-DGCR8 processing. Having bypassed nuclear processing, they are exported directly to the cytoplasm and follow the rest of the canonical pathway, beginning with Dicer cleavage to generate mature miRNA duplexes (Okamura et al.).

A second non-canonical path with unique transport & loading mechanisms involves 7-methylguanosine (m7G) capped pre-miRNAs. Rather than using Exportin 5 to leave the

nucleus, Exportin 1 transports these capped pre-miRNAs into the cytoplasm, where they are processed by Dicer. Additionally, since the bulky m7G cap physically blocks the 5’ end from loading onto AGO, this pathway preferentially utilizes the 3' (or 3p) strand of the RNA duplex. This pathway yields only one strand of miRNA, less than the canonical pathway, and is therefore believed to possibly be a pathway that is intended for a more limited release of miRNAs that may be detrimental if created in excess (Xie et al.).

Finally, a class of small RNAs called short hairpin RNA (shRNA) completely bypasses Dicer processing. One example is miR-451, whose structure is too short for Dicer to process. Instead, the entire shRNA is loaded into Argonaute 2 (AGO2), which, in this pathway, not only holds and silences the shRNA but also acts as the primary catalytic "cutter" of nucleotides, allowing the miRNA to mature without Dicer processing (Cheloufi et al.; Cifuentes et al.).

Although non-canonical pathways are used less frequently for miRNA synthesis than the canonical route, they add diversity to miRNA regulation and generation. These pathways may play specialized roles in tissue-specific contexts, developmental stages, or cellular stress responses.

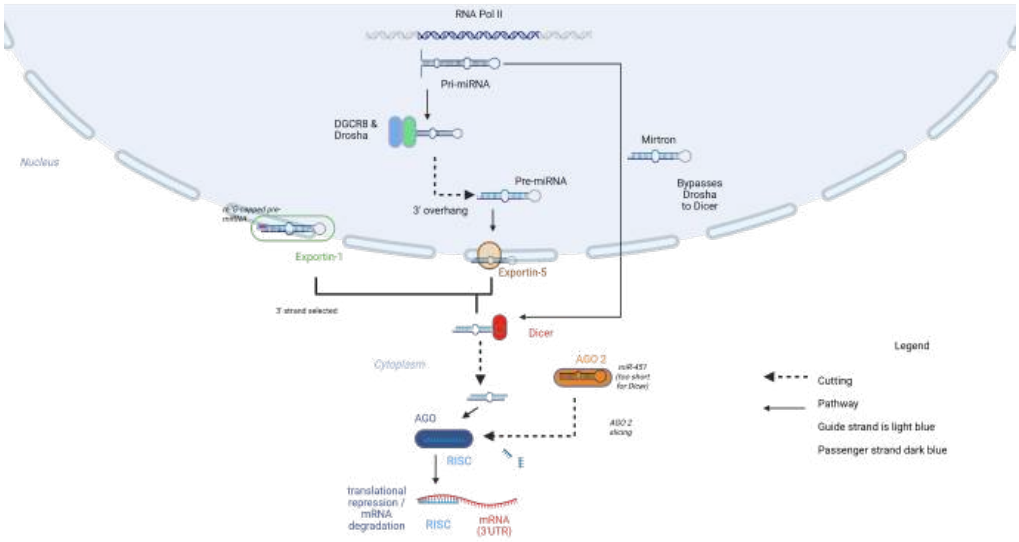

Figure 1. Canonical and non-canonical pathways of miRNA biogenesis and RISC assembly. In the nucleus, RNA Pol II transcribes pri-miRNA, which Drosha and DGCR8 (the microprocessor complex and its cofactor) then process. The pri-miRNA becomes pre-miRNA with a 3’ overhang and is exported from the nucleus by Exportin-5. A non-canonical pathway using mirtrons (spliced introns) bypasses Drosha processing and delivers the transcript directly to Dicer. Pre-miRNAs with an m^7G-cap are exported with Exportin-1. In the cytoplasm, the pre-miRNA hairpin is cleaved, resulting in a mature miRNA duplex, where the guide strand (light blue) is loaded into AGO, while the passenger strand (dark blue) is discarded. For miRNAs too short for Dicer to process, such as miR-451, AGO2 slices the hairpin. The guide-strand-loaded AGO forms the RISC, which binds complementary 3’ untranslated regions (3’ UTRs) to mediate translational repression or mRNA degradation. Created in BioRender https://BioRender.com

2.4 The Role of MiRNAs

MiRNAs bind to specific sequences on the 3' UTR of their target RNA. This represses translation and promotes deadenylation of the target portion, marking it for decay (Koscianska et al.; Fabian et al.). However, miRNA can also bind to other sites; binding to promoter sites increases transcription, while binding to coding regions silences gene expression.

The minimal functional silencing complex, known as the miRNA-induced silencing complex (miRISC), consists of the guide strand paired with an AGO protein (O'Brien et al.). miRISC locates its targets through complementary sequences on the mRNA called miRNA response elements (MREs). High complementarity leads to mRNA cleavage, while partial matching leads to translational repression and mRNA decay.

MiRNA can also enhance gene activation. For example, in certain cells, the protein Fragile-X-mental retardation-related protein 1 (FXR1) and AGO are associated with increased transcription (Vasudevan and Steitz). Some miRNAs also pair with specific proteins to initiate translation during cell cycle arrest (Vasudevan and Steitz).

Finally, miRNA acts within the nucleus. Importin 8 and Exportin 1 transport AGO between the cytoplasm and nucleus. Once inside the nucleus, miRNA can regulate both transcription rates and post-transcriptional mRNA levels. miRISC can also interact and induce degradation of mRNA inside the nucleus. The finding that miRNAs concentrate at actively transcribed genes suggests that miRISC may interact with its target miRNA during or right after transcription (Havens et al.; Alló et al.)

3. Overview of Tendon Injury

3.1 Tendon Composition

Tendons are specialized connective tissues that attach muscles to bone, transmitting the force of our contracting muscles to create skeletal motion. These anatomic bridges are essential for our range of motion and require significant strength to withstand tensile forces while maintaining flexibility (S. Zhang et al.). The tendon's unique, highly organized, rope-like structure, from the molecular to the anatomical level, contributes to its resilience and versatility (S. Zhang et al.).

This structure is hierarchical. Polypeptide collagen chains form the primary structural building block of the tendon matrix, with three chains twisting together into tropocollagen. Tropocollagen molecules combine together to form fibrils, which twist together into fibers. Fibers are then bundled into fascicles, with each fascicle wrapped in a connective-tissue sheath called the endotenon. Multiple fascicles are bound together to form the complete tendon, which is bound by an outer sheath called the epitenon (Zhang et al.).

Tendon health is maintained by the ECM and cellular environment. Collagen type I is the primary structural component, with the more disorganized Collagen type III found in smaller amounts in injured tendons in the early stages of healing (Eriksen et al.). Elastin provides elasticity, which is essential in maintaining flexibility and energy storage (Zhang et al.).

Proteoglycans are molecules that maintain hydration and help determine collagen fibril diameter (Birch). When tendons are injured, they often show decreased function, impaired load-bearing capacity, increased inflammation, and pain (Abate et al.). These injuries can leave a lasting impact on tendons, leading to residual scarring that can further decrease the range of motion. (J. Chen et al.**).**

3.2 Chronic Tendon Injury

Tendon injuries are classified as acute or chronic. Chronic tendon injury, referred to clinically as tendinosis or tendinopathy, occurs due to damage from overuse of a tendon that occurs at a greater rate than the tissue's capacity for repair (Sharma and Maffulli). Lifestyle habits, such as diet and physical activity, can influence the risk of chronic tendon injury. Risk also increases with the natural degradation that comes with age.

Chronic tendon overload contributes significantly to tendon injuries in both casual and elite athlete populations. In runners, overuse is implicated in about 30% of running-related injuries. Approximately 40% of tennis players develop elbow tendinopathy due to chronic repetitive stress on the elbow joint (Sharma and Maffulli). Shoulder pain is a common cause of orthopedic visits, with rotator cuff tendinopathy accounting for more than 30% of orthopedic referrals (Cooper and Ali). In the foot and ankle, the Achilles tendon absorbs high loads during running and jumping, resulting in frequent injuries among runners of all levels and athletes in high-impact sports (Yu et al.).

Tendinopathy is a continuum of injury rather than a single event. First, an inciting injury will lead to reactive tendinopathy, in which the tendon immediately thickens in response to increased stress. If the stress continues without adequate opportunity to repair, tendon dysrepair occurs, with disorganized collagen fibers and new blood vessels invading the area (Cook and Purdam).

At the cellular level, chronic stress can cause tenocytes to migrate to the injury site and undergo metaplasia, where they change type, lose their elongated shape, and become rounder, cartilage-like cells (Abate et al.). These new tenocytes produce less organized and weaker Collagen III, reducing the percentage of stronger, load-bearing Collagen I and overall decreasing the tendon's tensile strength, making it more likely to reinjure with future stress (Eriksen et al.). Ultrasound imaging shows these changes can occur in the Achilles and patellar tendons before the patient develops symptoms, indicating that this remodeling can be a clinically silent danger (Fredberg et al.).

3.3 Acute Tendon Injury

Unlike the gradual breakdown of chronic tendinopathy, acute tendon injury is a sudden rupture, which may be partial or complete. Although the failure is abrupt, acute injury is more frequent in tendons already weakened by prior degeneration. Therefore, acute rupture often is the failure point of tissue predisposed to injury (Sharma and Maffulli).

Many factors contribute to underlying weakness and increase the risk of acute injury, including genetic predisposition, anatomical differences, lifestyle choices, local connective tissue pathologies, and age-related muscle strength decline (Sharma and Maffulli; September et al.; Vaughn et al.). Metabolic conditions also play a role, including poor insulin regulation, such as in type 1 or 2 diabetes (Poulsen et al.), which correlates with the link between high glucose and tendon cell damage discussed later in this review. Finally, training errors, including poor technique, rapid acceleration or deceleration, and inadequate equipment, can overload a tendon beyond its capacity, initiating rupture. (Ilfeld; Jobe and Ciccotti)

4. **The role/function of MiRNA in tendon injury:**

MiRNAs appear to play a role in tendon injury, as they have been associated with tendon adhesions and the regulation of angiogenesis (Guo et al.) (Table1).

Table 1. The function of miRNAs in tendon injury

miRNA	**Function**	**Target/Pathway**	**Evidence Type**	**Reference**
miR-135a	**TSPC senescence** (aging stem cells in tendon)	**ROCK1** (direct target) → cytoskeletal tension / mechanosensing	**Direct mechanistic evidence in TSPCs (gain/loss-of-function + target validation)**	Chen et al.
miR-210	**Early healing + angiogenesis** (more early blood vessels + stronger tendon early	Increases VEGF, FGF-2, collagen I	In vivo rat Achilles tendon model	Usman et al.; Liu et al.
miR-205-5p	**Tendon-to-bone healing** (via VEGF)	Targets **VEGFA**; inhibition increases VEGF and improves tendon–bone healing	In vivo rotator cuff repair model	Xu et al.
miR-29 Family, miR-29a	**Collagen regulation/fibrosis balance** in tendon injury	miR-29a regulates **IL-33** and collagen III expression; also linked to TGF-β signaling and fibrosis	Tendon disease models (human + experimental)	Millar et al.
miR-34 family	**Apoptosis/senescence**; (also mentioned in tendon adhesion/homeostasis/differentiation)	apoptosis/senescence pathways	Tendon healing review synthesis	Ding, Lingli et al.
miR-500	**Age-related tendon homeostasis** (expression changes with aging)	Matrix remodeling pathways	Human tendon tissue profiling	Peffers et al.
miR-548 / miR-548j	Age-related tendon homeostasis (expression changes with aging)	**PPARγ is predicted** target	Human tendon tissue profiling + computational target prediction	Peffers et al.
miR-30b-5p	Apoptosis and Cell Migration	Hoxa1 (Rat cartilage models)	Rat cartilage degeneration model (supportive biology)	Li et al.
miR-181a-5p	Inflammation signature in shoulder tendinopathy/rotator cuff tears)	Inflammation-related genes	Human tendon tissue profiling	Plachel et al.

4.1 MiRNA in the Pathogenesis of Tendinopathy

Growing research indicates that miRNA modulates the progression from healthy tendon homeostasis to tendinopathy rather than simply appearing as a marker of disease (Liu et al.). Tendon degeneration is characterized by ECM breakdown and disorganization. Profiling studies have found altered expression of miRNAs that regulate matrix genes in aging and degenerating tendons (Peffers et al.). A key example is miR-29, which links inflammatory signals to the ECM.

Millar et al. established this connection in the human tendon. The authors found that elevated interleukin-33 (IL33) marks early tendinopathy (Millar et al.). Using cultured human tenocytes and in vivo models to study the interaction between miR-29a and IL-33, they found that IL-33 represses miR-29a, shifting collagen synthesis from type I to type III and disrupting the collagen I: III ratio. Since type III collagen is weaker and more disorganized than type I, this shift decreases the tensile strength of the tendon. The authors also found that miR-29a regulates the decoy receptor sST2, which absorbs excess IL-33, creating a feedback loop between inflammation and ECM production.

This suggests that a single miRNA connects two processes usually studied separately. If inflammation and matrix remodeling are coordinated through miR-29a, targeting this miRNA therapeutically may correct the collagen imbalance, though it is likely also to alter inflammatory signaling.

Watts et al. tested whether targeting miR-29a could correct the collagen imbalance in a randomized, blinded trial (Watts et al.). The authors induced injury in the tendons of 17 horses, an equine model chosen for its resemblance to human tendinopathy, and injected either miR-29a or placebo into the lesion one week after injury (Watts et al.). Treated tendons showed reduced type III collagen transcript levels at two weeks, with no significant change in type I collagen, as well as smaller relative lesion cross-sectional area and improved histology scores compared to placebo. This finding supports miR-29a as an intervention rather than simply a correlating finding in diseased tissue.

Evidence from human patients supports this pattern. Plachel et al. profiled circulating miRNA in patients with no shoulder pathology, chronic tendinopathy, and degenerative tears (Plachel et al.) Both miR-29a and miR-29c were repressed, and the degree of repression increased with the severity of pathology. Further, because miRNA levels were measured from blood samples rather than tissue biopsies, this raises the possibility that miRNA could be used for less-invasive monitoring of tendon disease. The study was small, but the findings indicate a direction for further research.

Profiling work has also linked miRNA dysregulation to inflammation and metabolic changes related to rotator cuff injury. Thankam et al. compared biceps tendon tissue from four rotator cuff injury patients with fatty infiltration and inflammation to four patients with rotator cuff injury only (Thankam et al.). The authors used analytic software to identify the genes that connect two pathways: AMP-activated protein kinase (AMPK), which regulates metabolism, and Triggering Receptors Expressed on Myeloid cells-1 (TREM-1), a pro-inflammatory receptor. By measuring miRNA levels in tendon tissue to identify miRNAs predicted to act on these genes,

they identified thirteen miRNAs whose levels changed the most and affected the largest number of predicted targets. Twelve miRNAs were downregulated, most strongly miR-145-5p at 55-fold, and one upregulated, miR-297. Together, these thirteen miRNAs were associated with 216 target genes. This suggests that miRNA dysregulation in tendon injury affects not only matrix genes, but also inflammation and metabolism. The evidence is preliminary since the study included only four patients per group, the differences in AMPK and TREM-1 protein expression did not reach statistical significance, and the target genes were identified computationally rather than through experimentation.

Taken together, these studies suggest that miRNAs actively regulate tendon degeneration rather than passively marking damage.

4.2 Mechanical Stimuli Affect Multiple MiRNAs Expression in Tendons

Tendons are load-bearing tissues, and their mechanical environment is one of the strongest regulators of tendon cell behavior. Tenocytes and TSPCs will activate changes in gene expression in response to sensing tensile force, a process known as mechanotransduction. Because miRNAs regulate gene expression in the post-transcriptional phase, they link the mechanical loads a tendon experiences with how the tissue adapts or degenerates.

This link matters because mechanical load can be either protective or harmful. Moderate loading promotes healthy matrix turnover and tenogenic gene expression, but excessive or repetitive loading can lead to tendinopathy (Sharma and Maffulli). Identifying which miRNAs respond to mechanical signals can help explain how the same tissue can become stronger under one loading pattern and break down under another.

Mendias et al. provided early evidence on how mechanical loading changes miRNA expression in the tendon (Mendias et al.). Researchers applied a single session of mechanical loading on untrained adult rats and then measured miRNA expression in the Achilles tendon. Separately, they also treated primary tendon fibroblasts with transforming growth factor-**β** (TGF-**β**), an important regulator of tendon growth, and measured miRNA expression. They found that loading increased expression of type I collagen (Col1a1), proliferating cell nuclear antigen (PCNA), scleraxis (Scx), and tenomodulin (Tnmd), confirmed that the tendons responded to the stimulus. Both mechanically loaded rats and TGF-**β**-treated fibroblasts altered the expression of several miRNAs known to regulate cell proliferation and ECM synthesis, including members of the let-7 family. For instance, the authors identified multiple miRNAs (including miR-381 and miR-338) predicted to bind scleraxis, a transcription factor that regulates limb tendon development, whose expression mechanical loading significantly decreased. These findings suggest that miRNAs play a role in how tendons adapt normally to mechanical growth stimuli, and that TGF-**β** signaling is part of the response.

While Mendias et al. examined healthy tendon adaptation, Geng et al. showed how excessive loading can contribute to disease through mechanically responsive miRNA (Mendias et al.; Geng et al.). The authors found that mechanical loading downregulates miR-337-3p in TSPCs under uniaxial cyclic loading. Normally, miR-337-3p suppresses chondro-osteogenic

differentiation in cartilage- and bone-forming cells. The same drop in miR-337-3p was found in calcified tendons from both rats and humans, linking the laboratory finding to diseased tissue. The authors also traced the pathway in both directions. Upstream, the pro-inflammatory cytokine interleukin-1β (IL-1β) linked mechanical loading to decreased miR-337-3p. Downstream, luciferase reporter assays confirmed that miR-337-3p directly targets insulin receptor substrate 1 (IRS1) and NADPH oxidase 4 (Nox4), which drive chondro-osteogenic differentiation through ERK and JNK signaling. Finally, when the authors overexpressed miR-337-3p in the patellar tendons of rats with tendinopathy, they reduced ectopic ossification.

This study is important for understanding overuse injury. It suggests that repetitive overload suppresses a protective miRNA, allowing tendon stem cells to drift toward bone and cartilage lineages. This offers a molecular explanation for the calcification seen in chronic tendinopathy. Because restoring miR-337-3p reversed part of this process in an animal model, miR-337-3p could also be a potential target for future therapies.

Together, these studies show that mechanical stimuli regulate miRNA expression in tendons during both normal adaptation and pathological overload, and that the amount and duration of loading help determine which one occurs. There are limitations to this evidence. Most studies used rodent models and cultured cells, and loading protocols vary across studies in strain magnitude, frequency, and duration, making direct comparisons difficult. Few studies have measured how miRNAs respond to loading in the human tendon in vivo. Standardized loading protocols would make it easier to determine which mechanosensitive miRNAs matter most for human tendon health.

4.3 MiRNA Modulates Tendon Stem/Progenitor Cell Senescence

TSPCs are essential for tendon regeneration. The decline of the TSPC population (both in function and number) due to senescence is increasingly thought to contribute to chronic tendon degeneration, impaired healing capacity, and reduced tendon adaptability with age (Dai et al.). Functional decline of senescent TSPCs affects their capacity to proliferate, differentiate, and remodel the extracellular matrix, all of which are associated with age-related tendinopathy.

MiRNA directly regulates the expression of post-transcriptional genes, including those that regulate TSPC senescence (L. Chen et al.). In TSPCs, miRNAs directly regulate genes involved in reorganization of the cytoskeleton and mechanotransduction, while studies in tendon tissues and other musculoskeletal cells suggest that miRNAs serve a role in regulating apoptosis and control of cell cycles (L. Chen et al.; Peffers et al.).

Direct evidence that miRNA controls TSPC senescence was reported by L. Chen et al., who demonstrated that a specific miRNA, miR-135a, regulates TSPC senescence by directly suppressing the expression of Rho-associated protein kinase 1 (ROCK1)(L. Chen et al.). In this study, aged TSPC showed decreased miR-135a expression and a corresponding increase in ROCK1 expression. Experiments manipulating miR-135a function showed that miR-135a overexpression decreased senescent phenotypes, whereas miR-135a suppression increased

senescence. Finally, ROCK1 was shown to be a miR-135a target, establishing the causal relationship between the miRNA, its target, and the phenotype.

This regulatory axis fits the biological context, as ROCK1 is a key regulator of RhoA signaling, controlling myosin/actin contraction, cellular mechanosensing, and cytoskeletal tension. Poor regulation of these processes has been shown to contribute to stem cell senescence and loss of regeneration, suggesting that miR-135a-mediated suppression of ROCK1 may help prevent the aging phenotype of TSPCs. These results suggest that this pathway may be part of the broader relationship between altered mechanical signaling and senescence (Dai et al.).

Beyond the miR-135a–ROCK1 axis, multiple studies suggest that miRNAs regulate pathways relevant to tendon aging and degeneration, although direct evidence in TSPCs is more limited. Peffers et al. identified miR-500 and miR-548 as regulators of extracellular matrix remodeling, an essential process for tendon maintenance and repair. Specifically, miR-548j is predicted to target peroxisome proliferator-activated receptor gamma (PPARγ), a gene that is more highly expressed in younger mesenchymal stem cell donors. This suggests a potential link between miRNA dysregulation and age-related alterations in tendon-forming cells (Peffers et al.).

More miRNAs have been found to play a role in musculoskeletal aging, supporting their involvement in senescence-related pathways. In studies of cartilage degeneration in rats, miR-30b-5p was found to regulate apoptosis and chondrocyte migration through its target, Hoxa1 (Li et al.). Although these findings were described in chondrocytes rather than tendon cells, they highlight miRNAs' ability to modulate pathways relevant to tendon stem cell aging.

Other members of the miR-34 family, which are known to regulate cell senescence and apoptosis, have also been shown to regulate tendon adhesion, homeostasis, and differentiation - all vital processes in tendon repair (Ding et al.). Additional miRNA families implicated in senescence pathways may indirectly affect TSPC regulation. For instance, miR-181 is linked to apoptosis and mitochondrial function, and miR-199 controls cell survival and proliferation, all central to stem cell aging. Although their effects have not been demonstrated specifically in TSPCs, these miRNAs regulate apoptosis, mitochondrial function, and cell survival in multiple musculoskeletal cell types. This suggests they may also influence tendon stem cell biology and warrant investigation in tendon stem cell models.

Overall, the current research suggests that miRNAs influence TSPC senescence by modulating mechanotransduction, cytoskeletal regulation, apoptosis, and cell survival. Work on the miR-135a–ROCK1 pathway shows that miRNAs can regulate senescence in TSPCs, while other miRNAs associated with tendon homeostasis and musculoskeletal aging may act through similar mechanisms. Few studies have directly tested these relationships in TSPCs, but most evidence comes from similar cell types. Clarifying how miRNA networks operate in aging TSPCs will be important in understanding tendon degeneration and identifying potential therapies.

4.4 Oxidative Stress Induces Tenocytes' Apoptosis Through Mediating MiRNAs Expression

In addition to aging and mechanical load, oxidative stress alters miRNA expression in tendon cells. Reactive oxygen species are produced during normal metabolism and in response to mechanical loading. At moderate levels, they signal and stimulate repair. However, when reactive oxygen species accumulate faster than a cell's antioxidant defenses can counteract them, oxidative stress occurs, which can lead to cell damage. The degree and direction of miRNA expression can help determine the outcome.

Poulsen et al. demonstrated this directly in human tendon cells. The authors cultured primary human tenocytes in either low- or high-glucose conditions and exposed them to hydrogen peroxide to induce oxidative stress. Cells in low glucose remained fully viable and increased collagen synthesis in response. The same oxidative stress triggered apoptosis in the cells cultured in high glucose. The switch between the two outcomes occurred at roughly 7 mM glucose, corresponding to the upper limit of normal blood glucose. This finding suggests a mechanism linking diabetes and tendon degeneration (Poulsen et al.).

The authors then traced the pathway responsible for the trigger point. In the high-glucose condition, Bim mediated apoptosis, a protein that promotes apoptosis. Two transcription factors were required to increase Bim levels: forkhead box O1 (FOXO1) and p53, a tumor suppressor protein that triggers apoptosis when a cell is damaged. FOXO1 increases transcription of the Bim gene. p53 suppresses the miR-17-92 group of miRNAs that normally repress Bim. When that repression is lifted, Bim accumulates, and the cell undergoes apoptosis.

A single miRNA directs the flow of this cascade. High glucose, together with oxidative stress, increased miR-28-5p, and an assay confirmed that miR-28-5p targets sirtuin3, the enzyme that removes acetyl groups from p53. With less sirtuin 3 available, acetylated p53 accumulated and became active (Poulsen et al.). Ultimately, this single miRNA determines whether oxidative stress in a tenocyte ends in repair or cell death.

Other miRNAs also participate in tendon cell survival. Members of the miR-34 family, which regulate senescence and apoptosis in many tissues, have been linked to tendon adhesion, homeostasis, and differentiation (Ding et al.). This connection is less well studied in tenocytes than the miR-28-5p pathway, and further work is needed to determine whether the miR-34 family plays a direct role in apoptosis or acts through related processes.

The biggest takeaway is that oxidative stress does not act on tendon cells in isolation. The metabolic environment influences how miRNA directs various pathways, determining cell fate. This helps explain why metabolic conditions such as diabetes increase the risk of tendon degeneration and suggests that miRNAs linking oxidative stress to apoptosis may be useful therapeutic targets for those conditions.

4.5. MiRNAs in Tendon Angiogenesis

Tendons are relatively hypovascular, which contributes to their slow healing after injury. After injury or surgical repair, angiogenesis, the development of new vessels, is necessary for the delivery of oxygen, nutrients, and inflammatory mediators to the site of repair. However,

excessive or prolonged angiogenesis can lead to chronic tendinopathy and pain (Guo et al.; Thankam et al.). Therefore, angiogenesis must be tightly regulated during tendon healing.

Several miRNAs have been reported to regulate angiogenesis signaling (Liu et al.). One of the most extensively investigated is miR-210. In a rat Achilles tendon injury model, Usman et al. injected synthetic miR-210 into the repaired tendon (Usman et al.). They found increased capillary density and improved mechanical strength in two weeks, with treated tendons showing wider collagen fibers and increased load tolerance. The treated tendons also showed increased vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and type I collagen in the early healing phases. However, after six weeks, capillary density no longer increased, suggesting that miR-210 primarily promotes early vascular responses rather than long-term angiogenesis.

MiRNAs also regulate VEGF signaling during tendon-to-bone healing. In an in vivo rat model of rotator cuff repair, Xu et al. found that mesenchymal stem cells (MSCs) overexpressing VEGFA showed increased type I and type II collagen expression (Xu et al.). Rats treated with these cells had a higher ultimate failure load and greater stiffness. A dual-luciferase reporter assay confirmed that miR-205-5p targets VEGFA, meaning that inhibiting miR-205-5p increases VEGF expression and strengthens the repaired tendon. These studies show that miRNAs can regulate angiogenesis indirectly by controlling growth factor expression.

Together, these studies show that miRNAs influence the vascular response during tendon repair. The timing of angiogenesis seems to be important: early vascular growth supports extracellular matrix synthesis and structural repair, whereas persistent increases in vascular growth signals may contribute to degeneration. Understanding how specific miRNAs regulate these phases could inform treatments for tendon healing.

5. **Therapeutic Implications of MiRNA in Tendon Injury**

Since individual miRNAs regulate distinct steps in pathways such as angiogenesis, matrix remodeling, and stem cell aging, targeting specific miRNAs could enable intervention at different points in the tendon injury response (Bartel; Liu et al.). Since each miRNA can influence multiple downstream genes, adjusting a single miRNA may affect several repair processes at once, including extracellular matrix organization, angiogenesis signaling, apoptosis, and stem cell function.

Several challenges remain for clinical use. miRNAs are rapidly degraded in the bloodstream, making them unstable in vivo. Since one miRNA can regulate many genes, unintended downstream effects are possible (Bartel). In addition, tendon tissue is dense and relatively avascular, which makes efficient delivery difficult.

To address these challenges, researchers are exploring methods to deliver miRNAs directly to the injury site rather than through the bloodstream. Approaches being studied include viral vectors, lipid nanoparticles, and scaffolds designed to slowly release specific miRNAs at the repair site (Liu et al.). Pairing miRNA modulation with stem cell therapy or engineered

biomaterials may help keep the treatment localized and reduce unintended effects in other tissues.

Compared with research on bone and cartilage, miRNA-based treatment strategies for tendons remain in the early stages of development. Nevertheless, animal studies have shown improvements in blood vessel formation, collagen organization, and mechanical strength (Liu et al.). Future research needs to clarify which miRNAs are most important in tendon tissue, determine when to target them during healing, and develop delivery systems that are practical and safe for clinical use.

6. Conclusion

Tendon pathology is common, difficult to treat, and frequently heals incompletely. Traditional studies of extracellular matrix breakdown have focused on inflammation and mechanical overload. However, recent research has shown that molecular regulators play an important role in the tendon's response to stress and injury. This review examines how miRNAs influence several factors involved in tendon injury, aging, and repair. Specifically, we explore how miRNAs regulate the extracellular matrix, angiogenesis, apoptosis, and aging in TSPCs.

Current research suggests that rather than simply acting as markers of injury or health, miRNAs function as important regulators of tendon biology. Several miRNAs have been shown to play specific roles in tendon degeneration and repair. For example, miR-135 regulates TSPC senescence by targeting the ROCK1 pathway, which influences cytoskeletal strength and mechanosensing. Other miRNAs, such as miR-210 and miR-205-5p, appear to affect angiogenesis and tendon-to-bone healing by modulating growth factor signaling pathways, including VEGF. In addition, members of the miR-29 family regulate extracellular matrix composition and collagen balance, both of which are disrupted in tendon injury. Taken together, these findings imply that miRNAs help coordinate multiple aspects of the tendon injury repair response, including vascularity, extracellular matrix, inflammation, and cell aging/apoptosis.

Understanding the role of miRNA in tendon biology can impact both research and clinical care. Targeting a single miRNA that influences multiple genes in a pathway could affect several repair mechanisms at once. Therefore, miRNA-based approaches could help improve tendon repair, reduce fibrosis, and restore normal tendon structure after injury. In addition, miRNA expression can serve as a biomarker to monitor tendon disease, injury severity, or healing over time.

While these findings are promising, current research has limitations. Current studies linking miRNA to tendon healing rely heavily on experimental models, small clinical samples, or studies of related non-tendon musculoskeletal tissues. Many reported miRNA targets are based on computational models rather than direct experimental observation (Thankam et al.). Furthermore, individual miRNAs can regulate multiple genes simultaneously; while this offers potent therapeutic possibilities, it makes it particularly challenging to determine the relative importance of downstream pathways. Finally, translating this research into clinical therapies

faces obstacles, including miRNA instability in blood circulation and the challenge of delivering therapy to tendons, which are relatively dense and avascular compared with other soft tissues.

7. Further research

Several research directions would strengthen the evidence linking miRNAs to tendon health. One priority is examining how miRNA networks operate specifically within tenocytes and tendon stem/progenitor cells, and how these networks change across the distinct stages of healing. Much of the existing work is drawn from whole-tendon samples or related musculoskeletal tissues such as cartilage and bone, limiting the ability to determine the contribution of individual cell populations. Single-cell approaches and cell-type-specific analysis would clarify which cells drive the effects observed at the tissue level, a useful body of knowledge since tendons contain heterogeneous cell types that may respond differently to the same miRNA.

A second research direction is to confirm, through experimentation, miRNA targets predicted by computational models. Many reported targets are based on computational prediction algorithms rather than direct validation, so studies using reporter assays and gain- and loss-of-function experiments in tendon cells would help establish genuine regulatory relationships. This matters because a single miRNA can regulate many genes at once, and determining which pathways account for an observed effect remains challenging.

Third, translating these findings into treatment will require better delivery systems. Systemic administration of miRNA treatments is unlikely to be effective because miRNAs degrade rapidly in circulation and tendon tissue is dense and avascular. Localized approaches, such as biomaterial carriers, lipid nanoparticles, viral vectors, and scaffold-based systems that release miRNAs gradually at the repair site, are avenues of further development. Research should also focus on effective timing of intervention, since the same miRNA that may be beneficial during one phase of healing may hinder healing during another, as suggested by findings on miR-210 angiogenesis.

Finally, integrating miRNA research with biomechanical and clinical data would make these findings more useful in practice. Combining miRNA expression profiles with loading metrics, imaging findings, and clinical data can help determine whether miRNAs function as reliable biomarkers of injury severity or healing progress. Rehabilitation protocols can utilize mechanical loading to leverage miRNA expression in a direction to optimize healing. Since most current work relies on animal models and small clinical samples, larger human studies will ultimately be required.

Works Cited

Abate, Michele, et al. “Pathogenesis of Tendinopathies: Inflammation or Degeneration?” Arthritis Research & Therapy, vol. 11, no. 3, 2009, p. 235. PubMed, https://doi.org/10.1186/ar2723.

Alló, Mariano, et al. “Argonaute-1 Binds Transcriptional Enhancers and Controls Constitutive and Alternative Splicing in Human Cells.” Proceedings of the National Academy of Sciences of the United States of America, vol. 111, no. 44, Nov. 2014, pp. 15622–29. PubMed, https://doi.org/10.1073/pnas.1416858111.

Almeida, Maria I., et al. “MicroRNA History: Discovery, Recent Applications, and next Frontiers.” Mutation Research, vol. 717, nos. 1–2, Dec. 2011, pp. 1–8. PubMed, https://doi.org/10.1016/j.mrfmmm.2011.03.009.

Ambros, V., and H. R. Horvitz. “The Lin-14 Locus of Caenorhabditis Elegans Controls the Time of Expression of Specific Postembryonic Developmental Events.” Genes & Development, vol. 1, no. 4, June 1987, pp. 398–414. PubMed, https://doi.org/10.1101/gad.1.4.398.

Bartel, David P. “MicroRNAs: Target Recognition and Regulatory Functions.” Cell, vol. 136, no. 2, Jan. 2009, pp. 215–33. PubMed, https://doi.org/10.1016/j.cell.2009.01.002.

Basyuk, Eugenia, et al. “Human Let-7 Stem-Loop Precursors Harbor Features of RNase III Cleavage Products.” Nucleic Acids Research, vol. 31, no. 22, Nov. 2003, pp. 6593–97. PubMed, https://doi.org/10.1093/nar/gkg855.

Bhaskaran, M., and M. Mohan. “MicroRNAs: History, Biogenesis, and Their Evolving Role in Animal Development and Disease.” Veterinary Pathology, vol. 51, no. 4, July 2014, pp. 759–74. PubMed, https://doi.org/10.1177/0300985813502820.

Birch, Helen L. “Tendon Matrix Composition and Turnover in Relation to Functional Requirements.” International Journal of Experimental Pathology, vol. 88, no. 4, Aug. 2007, pp. 241–48. PubMed, https://doi.org/10.1111/j.1365-2613.2007.00552.x.

Bofill-De Ros, Xavier, and Ulf Andersson Vang Ørom. “Recent Progress in miRNA Biogenesis and Decay.” RNA Biology, vol. 21, no. 1, Dec. 2024, pp. 36–43. tandfonline.com (Atypon), https://doi.org/10.1080/15476286.2023.2288741.

Bohnsack, Markus T., et al. “Exportin 5 Is a RanGTP-Dependent dsRNA-Binding Protein That Mediates Nuclear Export of Pre-miRNAs.” RNA [New York, N.Y.], vol. 10, no. 2, Feb. 2004, pp. 185–91. PubMed, https://doi.org/10.1261/rna.5167604.

Cheloufi, Sihem, et al. "A Dicer-Independent miRNA Biogenesis Pathway That Requires Ago Catalysis." Nature, vol. 465, no. 7298, 3 June 2010, pp. 584–89. PubMed, https://doi.org/10.1038/nature09092.

Chen, Jialin, et al. “Characterization and Comparison of Post-Natal Rat Achilles Tendon-Derived Stem Cells at Different Development Stages.” Scientific Reports, vol. 6, Mar. 2016, p. 22946. PubMed, https://doi.org/10.1038/srep22946.

Chen, Lei, et al. “miR-135a Modulates Tendon Stem/Progenitor Cell Senescence via Suppressing ROCK1.” Bone, vol. 71, Feb. 2015, pp. 210–16. PubMed, https://doi.org/10.1016/j.bone.2014.11.001.

Cifuentes, Daniel, et al. "A Novel miRNA Processing Pathway Independent of Dicer Requires Argonaute Catalytic Activity." Science, vol. 328, no. 5986, 25 June 2010, pp. 1694–98. PubMed, https://doi.org/10.1126/science.1190809.

Cook, J. L., and C. R. Purdam. "Is Tendon Pathology a Continuum? A Pathology Model to Explain the Clinical Presentation of Load-Induced Tendinopathy." British Journal of Sports Medicine, vol. 43, no. 6, June 2009, pp. 409–16. PubMed, https://doi.org/10.1136/bjsm.2008.051193.

Cooper, Anthony, and Amjid Ali. “Rotator Cuff Tears.” Surgery (Oxford), vol. 31, no. 4, Apr. 2013, pp. 168–71. DOI.org (Crossref), https://doi.org/10.1016/j.mpsur.2013.01.017.

Cottrell, Jessica A., et al. “The Biology of Bone and Ligament Healing.” Foot and Ankle Clinics, vol. 21, no. 4, Dec. 2016, pp. 739–61. PubMed, https://doi.org/10.1016/j.fcl.2016.07.017.

Dai, Guang-Chun, et al. “Tendon Stem/Progenitor Cell Ageing: Modulation and Rejuvenation.” World Journal of Stem Cells, vol. 11, no. 9, Sept. 2019, pp. 677–92. PubMed, https://doi.org/10.4252/wjsc.v11.i9.677.

Dexheimer, Philipp J., and Luisa Cochella. “MicroRNAs: From Mechanism to Organism.” Frontiers in Cell and Developmental Biology, vol. 8, June 2020, p. 409. DOI.org (Crossref), https://doi.org/10.3389/fcell.2020.00409.

Ding, Lingli, et al. “The Roles of MicroRNAs in Tendon Healing and Regeneration.” Frontiers in Cell and Developmental Biology, vol. 9, 2021, p. 687117. PubMed, https://doi.org/10.3389/fcell.2021.687117.

Eriksen, Heidi A., et al. “Increased Content of Type III Collagen at the Rupture Site of Human Achilles Tendon.” Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society, vol. 20, no. 6, Nov. 2002, pp. 1352–57. PubMed, https://doi.org/10.1016/S0736-0266(02)00064-5.

Fabian, Marc R., et al. "Regulation of mRNA Translation and Stability by microRNAs." Annual Review of Biochemistry, vol. 79, July 2010, pp. 351–79. PubMed, https://doi.org/10.1146/annurev-biochem-060308-103103.

Ferguson, E. L., et al. “A Genetic Pathway for the Specification of the Vulval Cell Lineages of Caenorhabditis Elegans.” Nature, vol. 326, no. 6110, Mar. 1987, pp. 259–67. PubMed, https://doi.org/10.1038/326259a0.

Fredberg, Ulrich, et al. “Prophylactic Training in Asymptomatic Soccer Players with Ultrasonographic Abnormalities in Achilles and Patellar Tendons: The Danish Super League Study.” The American Journal of Sports Medicine, vol. 36, no. 3, Mar. 2008, pp. 451–60. PubMed, https://doi.org/10.1177/0363546507310073.

Gaida, James E., et al. “Is Adiposity an Under-Recognized Risk Factor for Tendinopathy? A Systematic Review.” Arthritis and Rheumatism, vol. 61, no. 6, June 2009, pp. 840–49. PubMed, https://doi.org/10.1002/art.24518.

Geng, Yiyun, et al. “Overexpression of Mechanical Sensitive miR-337-3p Alleviates Ectopic Ossification in Rat Tendinopathy Model via Targeting IRS1 and Nox4 of Tendon-Derived Stem Cells.” Journal of Molecular Cell Biology, vol. 12, no. 4, May 2020, pp. 305–17. PubMed, https://doi.org/10.1093/jmcb/mjz030.

Guo, Jia, et al. “A Mini-Review: MicroRNA in Tendon Injuries.” Journal of Stem Cell Research & Therapy, vol. 05, no. 09, 2015. DOI.org (Crossref), https://doi.org/10.4172/2157-7633.1000303.

Havens, Mallory A., et al. “Drosha Promotes Splicing of a Pre-microRNA-like Alternative Exon.” PLoS Genetics, vol. 10, no. 5, May 2014, p. e1004312. PubMed, https://doi.org/10.1371/journal.pgen.1004312.

Huntzinger, Eric, and Elisa Izaurralde. “Gene Silencing by microRNAs: Contributions of Translational Repression and mRNA Decay.” Nature Reviews. Genetics, vol. 12, no. 2, Feb. 2011, pp. 99–110. PubMed, https://doi.org/10.1038/nrg2936.

Ilfeld, F. W. “Can Stroke Modification Relieve Tennis Elbow?” Clinical Orthopaedics and Related Research, no. 276, Mar. 1992, pp. 182–86.

Jobe, F. W., and M. G. Ciccotti. “Lateral and Medial Epicondylitis of the Elbow.” The Journal of the American Academy of Orthopaedic Surgeons, vol. 2, no. 1, Jan. 1994, pp. 1–8. PubMed, https://doi.org/10.5435/00124635-199401000-00001.

Junn, Eunsung, and M. Maral Mouradian. "MicroRNAs in Neurodegenerative Diseases and Their Therapeutic Potential." Pharmacology & Therapeutics, vol. 133, no. 2, 2012, pp. 142–150. PubMed, https://pubmed.ncbi.nlm.nih.gov/22008259/.

Kawamata, Tomoko, and Yukihide Tomari. “Making RISC.” Trends in Biochemical Sciences, vol. 35, no. 7, July 2010, pp. 368–76. PubMed, https://doi.org/10.1016/j.tibs.2010.03.009.

Koscianska, Edyta, et al. “The Role of Dicer Protein Partners in the Processing of microRNA Precursors.” PloS One, vol. 6, no. 12, 2011, p. e28548. PubMed, https://doi.org/10.1371/journal.pone.0028548.

Landgraf, Pablo, et al. “A Mammalian microRNA Expression Atlas Based on Small RNA Library Sequencing.” Cell, vol. 129, no. 7, June 2007, pp. 1401–14. PubMed, https://doi.org/10.1016/j.cell.2007.04.040.

Lee, R. C., R. L. Feinbaum, and V. Ambros. “The C. Elegans Heterochronic Gene Lin-4 Encodes Small RNAs with Antisense Complementarity to Lin-14.” Cell, vol. 75, no. 5, Dec. 1993, pp. 843–54. PubMed, https://doi.org/10.1016/0092-8674(93)90529-y.

Lee, Rosalind, Rhonda Feinbaum, and Victor Ambros. “A Short History of a Short RNA.” Cell, vol. 116, no. 2 Suppl, Jan. 2004, pp. S89-92, 1 p following S96. PubMed, https://doi.org/10.1016/s0092-8674(04)00035-2.

Lee, Yoontae, Minju Kim, et al. “MicroRNA Genes Are Transcribed by RNA Polymerase II.” The EMBO Journal, vol. 23, no. 20, Oct. 2004, pp. 4051–60. PubMed, https://doi.org/10.1038/sj.emboj.7600385.

Lee, Yoontae, Kipyoung Jeon, et al. “MicroRNA Maturation: Stepwise Processing and Subcellular Localization.” The EMBO Journal, vol. 21, no. 17, Sept. 2002, pp. 4663–70. PubMed, https://doi.org/10.1093/emboj/cdf476.

Lee, Yoontae, Chiyoung Ahn, et al. “The Nuclear RNase III Drosha Initiates microRNA Processing.” Nature, vol. 425, no. 6956, Sept. 2003, pp. 415–19. PubMed, https://doi.org/10.1038/nature01957.

Li, Maoxun, et al. “MiR-30b-5p Influences Chronic Exercise Arthritic Injury by Targeting Hoxa1.” International Journal of Sports Medicine, vol. 42, no. 13, Dec. 2021, pp. 1199–208. PubMed, https://doi.org/10.1055/a-1342-7872.

Liu, Qian, et al. “The Role of MicroRNAs in Tendon Injury, Repair, and Related Tissue Engineering.” Biomaterials, vol. 277, Oct. 2021, p. 121083. PubMed, https://doi.org/10.1016/j.biomaterials.2021.121083.

Mendias, Christopher L., et al. “Mechanical Loading and TGF-β Change the Expression of Multiple miRNAs in Tendon Fibroblasts.” Journal of Applied Physiology [Bethesda, Md. : 1985], vol. 113, no. 1, July 2012, pp. 56–62. PubMed, https://doi.org/10.1152/japplphysiol.00301.2012.

Millar, Neal L., et al. “MicroRNA29a Regulates IL-33-Mediated Tissue Remodelling in Tendon Disease.” Nature Communications, vol. 6, Apr. 2015, p. 6774. PubMed, https://doi.org/10.1038/ncomms7774.

O’Brien, Jacob, et al. “Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation.” Frontiers in Endocrinology, vol. 9, 2018, p. 402. PubMed, https://doi.org/10.3389/fendo.2018.00402.

Okamura, Katsutomo, et al. "The Mirtron Pathway Generates microRNA-Class Regulatory RNAs in Drosophila." Cell, vol. 130, no. 1, 13 July 2007, pp. 89–100. PubMed, https://doi.org/10.1016/j.cell.2007.06.028.

Paul, Prosenjit, et al. “Interplay between miRNAs and Human Diseases.” Journal of Cellular Physiology, vol. 233, no. 3, Mar. 2018, pp. 2007–18. PubMed, https://doi.org/10.1002/jcp.25854.

Peffers, Mandy Jayne, et al. “Decoding the Regulatory Landscape of Ageing in Musculoskeletal Engineered Tissues Using Genome-Wide DNA Methylation and RNASeq.” PloS One, vol. 11, no. 8, 2016, p. e0160517. PubMed, https://doi.org/10.1371/journal.pone.0160517.

Pfeffer, Sébastien, et al. “Identification of microRNAs of the Herpesvirus Family.” Nature Methods, vol. 2, no. 4, Apr. 2005, pp. 269–76. PubMed, https://doi.org/10.1038/nmeth746.

Plachel, Fabian, et al. “MicroRNA Profiling Reveals Distinct Signatures in Degenerative Rotator Cuff Pathologies.” Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society, vol. 38, no. 1, Jan. 2020, pp. 202–11. PubMed, https://doi.org/10.1002/jor.24473.

Poulsen, R. C., et al. “Cell Differentiation versus Cell Death: Extracellular Glucose Is a Key Determinant of Cell Fate Following Oxidative Stress Exposure.” Cell Death & Disease, vol. 5, no. 2, Feb. 2014, pp. e1074–e1074. DOI.org (Crossref), https://doi.org/10.1038/cddis.2014.52.

September, Alison V., et al. “Tendon and Ligament Injuries: The Genetic Component.” British Journal of Sports Medicine, vol. 41, no. 4, Apr. 2007, pp. 241–46; discussion 246. PubMed, https://doi.org/10.1136/bjsm.2006.033035.

Shang, Renfu, et al. “microRNAs in Action: Biogenesis, Function and Regulation.” Nature Reviews. Genetics, vol. 24, no. 12, Dec. 2023, pp. 816–33. PubMed Central, https://doi.org/10.1038/s41576-023-00611-y.

Sharma, Pankaj, and Nicola Maffulli. “Tendon Injury and Tendinopathy.” The Journal of Bone & Joint Surgery, vol. 87, no. 1, Jan. 2005, pp. 187–202, https://doi.org/10.2106/jbjs.d.01850.

Tarnowski, Maciej, et al. “Epigenetic Alterations in Sports-Related Injuries.” Genes, vol. 13, no. 8, Aug. 2022, p. 1471. PubMed Central, https://doi.org/10.3390/genes13081471.

Tétreault, N., and V. De Guire. “miRNAs: Their Discovery, Biogenesis and Mechanism of Action.” Clinical Biochemistry, miRNAs in Clinical Practice, vol. 46, no. 10, July 2013, pp. 842–45. ScienceDirect, https://doi.org/10.1016/j.clinbiochem.2013.02.009.

Thankam, Finosh G., Chandra S. Boosani, Matthew F. Dilisio, and Devendra K. Agrawal. “Epigenetic Mechanisms and Implications in Tendon Inflammation (Review).” International Journal of Molecular Medicine, vol. 43, no. 1, Jan. 2019, pp. 3–14. PubMed, https://doi.org/10.3892/ijmm.2018.3961.

Thankam, Finosh G., Chandra S. Boosani, Matthew F. Dilisio, R. Michael Gross, et al. “Genes Interconnecting AMPK and TREM-1 and Associated microRNAs in Rotator Cuff Tendon Injury.” Molecular and Cellular Biochemistry, vol. 454, nos. 1–2, Apr. 2019, pp. 97–109. PubMed, https://doi.org/10.1007/s11010-018-3456-z.

Thankam, Finosh G., Chandra S. Boosani, Matthew F. Dilisio, Nicholas E. Dietz, et al. “MicroRNAs Associated with Shoulder Tendon Matrisome Disorganization in Glenohumeral Arthritis.” PloS One, vol. 11, no. 12, 2016, p. e0168077. PubMed, https://doi.org/10.1371/journal.pone.0168077.

Usman, Muhammad Andry, et al. "The Effect of Administration of Double Stranded MicroRNA-210 on Acceleration of Achilles Tendon Healing in a Rat Model." Journal of Orthopaedic Science, vol. 20, no. 3, May 2015, pp. 538–46. PubMed, https://doi.org/10.1007/s00776-015-0709-5.

Vasudevan, Shobha, and Joan A. Steitz. “AU-Rich-Element-Mediated Upregulation of Translation by FXR1 and Argonaute 2.” Cell, vol. 128, no. 6, Mar. 2007, pp. 1105–18. PubMed, https://doi.org/10.1016/j.cell.2007.01.038.

Vaughn, Natalie H., et al. “Genetic Factors in Tendon Injury: A Systematic Review of the Literature.” Orthopaedic Journal of Sports Medicine, vol. 5, no. 8, Aug. 2017, p. 2325967117724416. PubMed, https://doi.org/10.1177/2325967117724416.

Vega, Francisco M., and Anne J. Ridley. “SnapShot: Rho Family GTPases.” Cell, vol. 129, no. 7, June 2007, p. 1430. PubMed, https://doi.org/10.1016/j.cell.2007.06.021.

Watts, Ashlee E., et al. “MicroRNA29a Treatment Improves Early Tendon Injury.” Molecular Therapy: The Journal of the American Society of Gene Therapy, vol. 25, no. 10, Oct. 2017, pp. 2415–26. PubMed, https://doi.org/10.1016/j.ymthe.2017.07.015.

Wightman, B., et al. “Posttranscriptional Regulation of the Heterochronic Gene Lin-14 by Lin-4 Mediates Temporal Pattern Formation in C. Elegans.” Cell, vol. 75, no. 5, Dec. 1993, pp. 855–62. PubMed, https://doi.org/10.1016/0092-8674(93)90530-4.

Xie, Mingyi, et al. "Mammalian 5′-Capped MicroRNA Precursors That Generate a Single MicroRNA." Cell, vol. 155, no. 7, 2013, pp. 1568-80. PubMed, https://doi.org/10.1016/j.cell.2013.11.027.

Xu, Qi, et al. "High Expression of VEGFA in MSCs Promotes Tendon-Bone Healing of Rotator Cuff Tear via microRNA-205-5p." European Review for Medical and Pharmacological Sciences, vol. 23, no. 10, 2019, pp. 4081–88.

Yoshida, Toyotaka, et al. “Modulation of MicroRNA Processing by Dicer via Its Associated dsRNA Binding Proteins.” Non-Coding RNA, vol. 7, no. 3, Sept. 2021, p. 57. PubMed, https://doi.org/10.3390/ncrna7030057.

Yu, Changxiao, et al. "Exercise Effects on the Biomechanical Properties of the Achilles Tendon—A Narrative Review." Biology, vol. 11, no. 2, 21 Jan. 2022, p. 172. PubMed, https://doi.org/10.3390/biology11020172.

Zhang, Shichen, et al. “Hierarchical Ultrastructure: An Overview of What Is Known about Tendons and Future Perspective for Tendon Engineering.” Bioactive Materials, vol. 8, June 2021, pp. 124–39. PubMed Central, https://doi.org/10.1016/j.bioactmat.2021.06.007.

Disentangling the Interplay Between APOEε4, Amyloid Pathology, and Microglial Dysfunction in Alzheimer's Disease By Ethan Valenciana (student), Jorge Avila (mentor)

Introduction

Alzheimer's disease (AD) is a neurodegenerative condition marked by a gradual deterioration of memory, a decline in cognitive functions, and in the end, a loss of independence (Liu et al., 2025). Although the build-up of amyloid-beta (Aβ) plaques has long been considered a major feature of the disease, there is now growing evidence highlighting the important role of neuroinflammation and immune dysregulation in the progression of the disease (Wang et al., 2024). Microglia, which are the immune cells resident in the brain, play a crucial role in keeping the nervous system healthy; yet their behaviour is greatly affected by genetic factors, such as the apolipoprotein E (APOE) genotype, especially the APOEε4 allele, which has been associated with an increased risk of developing AD (Chia et al., 2025). At the same time, mutations in genes such as APP and PSEN1 cause an increased production of amyloid, thereby adding to the complexity of the disease (O'Brien, 2011). The interaction between these genetic pathways leads to the question of whether microglial dysfunction caused by the APOEε4 allele occurs as a result of amyloid accumulation or whether these processes work independently to cause neurodegeneration. The present paper therefore examines whether microglial dysfunction mediated by APOE is a consequence of amyloid-beta accumulation driven by mutations in the APP and PSEN1 genes or whether these pathways independently contribute to synaptic loss and disease progression. The paper specifically looks at the following question: Does APOE-mediated microglial dysfunction occur downstream of amyloid accumulation, or do these pathways function independently yet systematically in the pathogenesis of Alzheimer's disease?

This review states that the existing evidence indicates APOEε4-induced microglial dysfunction does not merely result as a downstream effect of amyloid-beta accumulation. On the contrary, APOE-related immune abnormalities and APP/PSEN1-driven amyloid pathology can act as interconnected, but partly independent pathways, both leading to synaptic degeneration and neurodegeneration. Understanding this distinction is crucial because it helps us better grasp how genetic and immune factors each cause the progression of Alzheimer's. It is essential to understand the temporal and causal relationships between APOE-related immune dysregulation and amyloid pathology since this affects therapeutic approaches. Even though many treatments focus on amyloid plaques, their limited success motivates investigation of additional pathogenic mechanisms like neuroinflammation and microglial dysfunction. (Sasaguri et al., 2017). If it can be established whether these processes are sequential or parallel, this could result in more effective interventions that tackle multiple pathways simultaneously and thus improve patient outcomes (Liu et al., 2025).

The first part of the article looks at the role of APOEε4 in regulating microglial activity and neuroinflammation, highlighting the way in which APOE affects immune regulators such as TREM2 and its impact on synaptic maintenance (Kim et al., 2025). The second part examines mutations in APP and PSEN1, concentrating on their direct role in causing amyloid-beta

accumulation and the way in which this process interacts with, but is still separate from, immune pathways. In the final section, the findings are brought together and a model is put forward in which APOE4-related microglial dysfunction and amyloid pathology are partly independent yet mutually reinforcing mechanisms that speed up neurodegeneration. The review continually compares results from peer-reviewed studies in order to determine whether microglial activation consistently occurs after amyloid deposition or whether it can function independently to cause damage to neurons.

APOEε4 and Microglial Dysfunction in Alzheimer's Disease

There is now growing evidence to indicate that APOEε4 can affect microglial activity without there being any accumulation of amyloid. This generally happens at an early stage of the disease, possibly even before a considerable amount of amyloid has deposited. For instance, research has found that microglial activation and inflammatory markers are present in individuals who carry APOEε4 even when their amyloid levels are low or completely absent (Dias et al., 2025). This early immune dysfunction can lead to synaptic dysfunction and neurodegeneration, appearing as mild cognitive deficits or changes in neural circuits before the characteristic amyloid plaques can be detected. It thus seems that APOEε4's effect on microglia is not merely a result of the amyloid load but can instead act as an initiating or concurrent pathological process, underscoring its role as a main genetic risk factor that directly influences immune responses.

Apolipoprotein E (APOE) is a protein which transports lipids and plays a vital role in cholesterol metabolism as well as in the repair of neurons in the brain (Kim et al., 2025). Of the three common forms of APOE, which are ε2, ε3 and ε4, the ε4 variant (APOEε4) is linked to a greatly increased risk of getting late-onset Alzheimer's disease (Tzioras et al., 2019). Although APOE has usually been investigated in relation to its function in transporting lipids, more recent studies highlight its effect on immune responses, especially those involving microglia (Wang et al., 2024). The microglia are the immune cells that are resident in the brain and are important in synaptic pruning, the clearance of debris by means of phagocytosis and the regulation of neuroinflammation, all of which are essential for keeping neural health. (Barber, 2012). The APOEε4 allele alters microglial function and may thus weaken their ability to carry out these functions, leading to neurodegeneration even in the absence of amyloid pathology.

Studies suggest that microglia which express the APOEε4 isoform show a more reactive, pro-inflammatory character than those expressing the other isoforms (Chia et al., 2025). This increased reactivity is marked by a higher level of inflammatory cytokines such as IL-1β, TNF-α, and IL-6, and as a result can lead to synaptic dysfunction and neuronal damage (DiSabato et al., 2016). Moreover, APOEε4 affects the gene expression patterns of microglia relating to phagocytosis and immune regulation, causing the microglia to assume a state that might make them less efficient at removing debris or damaged synapses (Wolfe et al., 2018). It should be noted that microglial activation has been found in individuals carrying the APOEε4 gene even before there is significant amyloid accumulation, indicating that immune dysregulation may occur before or independently of amyloid pathology (Ferrari-Souza et al., 2023).

APOE interacts with a number of immune regulators, including triggering receptors expressed on myeloid cells 2 (TREM2), thereby affecting the way that microglia respond to damage to neurons and to debris (Wolfe et al., 2018). Variants of the TREM2 gene have also been associated with a higher risk of developing AD, underscoring the role of immune regulation in the progression of the disease (Qi et al., 2025). The effect of APOEε4 on TREM2 signalling pathways seems to worsen microglial dysfunction and thus hampers their ability to carry out synapses and to clear debris (Kim et al., 2025). This impairment may result in a build-up of synaptic debris and of damaged neurons, thus initiating or worsening neurodegenerative processes without any involvement of amyloid deposition.

Microglia have a vital function in synaptic pruning both during development and as a response to injury, a process which is closely controlled by immune signalling pathways (Wang et al., 2024). Microglia associated with APOEε4 display modified pruning activity, this leading to either the excessive elimination of synapses or to an inadequate clearance of defective synapses, both of which lead to a decline in cognitive function (Raulin et al., 2022). To add, the pro-inflammatory phenotype caused by APOEε4 can enhance neuroinflammation and thus produce an adverse environment for neurons while also further disrupting synaptic function (Yamazaki et al., 2019). These immune changes can take place independently of amyloid accumulation, indicating that APOEε4 has a direct effect on microglial behaviour and thus promotes neurodegeneration even when there is no significant amyloid pathology.

APOEε4 seems to have both toxic gain- and loss-of-function effects on microglia. It causes a hyper-reactive, pro-inflammatory state marked by higher cytokine production, which can worsen neurodegeneration (Kempuraj et al., 2016). At the same time, APOEε4 hampers the normal neuroprotective functions of microglia, such as the clearance of debris and the maintenance of synapses (Wolfe et al., 2018). Therefore, the impact of APOEε4 should be seen as involving both a toxic gain of function (increased inflammation) and a toxic loss of function (decreased neuroprotection) What's more, immune alterations driven by APOEε4 can occur independently of amyloid-beta accumulation. These immune changes, such as microglial activation and increased inflammation, may cause problems like synaptic dysfunction, neuronal stress, and early neurodegeneration even when amyloid levels are low or absent (Raulin et al., 2022). This suggests that APOEε4 may influence immune and microglial pathways that can be detected independently of amyloid pathology, highlighting its multifaceted role in AD progression. That said, the immune dysregulation caused by APOEε4 is just one of several genetic pathways associated with Alzheimer's disease; other significant genetic factors, especially mutations in the amyloid precursor protein (APP) and presenilin (PSEN1 and PSEN2) genes, have a direct effect on the production and accumulation of amyloid-beta. Looking at these mutations gives us insight into how amyloid pathology develops and how it relates to immune dysregulation, thus providing a more complete understanding of the biological mechanisms involved in the progression of Alzheimer's disease.

APP and PSEN1 Mutations and Amyloid-beta Pathology

The mutations in the amyloid precursor protein (APP) and presenilin 1 (PSEN1) genes mainly lead to a build-up of amyloid-beta (Aβ), a process that has for a long time been seen as the starting point in Alzheimer's disease. Yet the connection between amyloid deposition and microglial activation is complicated, since there is evidence that amyloid accumulation can take place without immune dysregulation, and likewise that immune dysregulation can occur without amyloid buildup.

Familial Alzheimer's disease (fAD) accounts for approximately 1-5% of all cases (Barber et al., 2012). It is caused by autosomal dominant mutations in genes such as APP and PSEN1, which directly increase the production and aggregation of amyloid-beta (O'Brien, 2011). These mutations trigger enhanced cleavage of APP via β- and γ-secretase enzymes, resulting in elevated levels of Aβ peptides, especially the aggregation-prone Aβ42 isoform, accelerating amyloid plaque formation and disease onset. Since the build-up of amyloid is a key feature of AD pathology, it is important to understand the mechanisms by which mutations in APP and PSEN1 cause this process if we are to gain insight into the course of the disease. Moreover, although amyloid deposition is generally linked with microglial activation, the connection is still a matter of debate. Some studies propose that amyloid accumulation takes place before the immune response, while others believe that immune dysregulation may come before or may occur independently of plaque formation (Sasaguri et al., 2017).

Mutations in the APP gene, for example the Swedish mutation, lead to an increased production of Aβ peptides by changing the efficiency of cleavage (Tan et al., 2008). In a similar way, mutations in PSEN1 interfere with the function of γ-secretase and cause a shift in the ratio so that Aβ42 is produced, this peptide having a greater tendency to aggregate and form plaques (Sun et al., 2017). Because of these mutations, amyloid plaque formation is speeded up and this has been employed as a main marker for the diagnosis and progression of AD. Significantly, the increased level of amyloid has been associated with the activation of microglia, which then try to phagocytose and clear the amyloid deposits (Daniel Lee, C. Y., and Gary E. Landreth., 2010).

It is generally believed that the build-up of amyloid is an early and main pathological process, but there is increasing evidence to show that the connection between amyloid and immune activation might not be strictly sequential. For instance, some studies have found that microglia become activated as a result of amyloid deposits, although activation can also take place even when there is no considerable plaque load (Dias et al., 2025). Moreover, experimental models have demonstrated that Aβ can cause inflammatory reactions, but immune dysregulation can also come before amyloid deposition, perhaps being caused by genetic factors such as APOEε4 (Petersen et al., 2025). It seems that amyloid buildup and immune responses might run alongside each other, each being able to contribute independently to neurodegeneration.

Several studies have shown that amyloid pathology can be present even in the absence of marked microglial activation, particularly in the early stages of the disease or in some genetic situations (Zhu et al., 2012). On the other hand, microglial dysfunction and neuroinflammation have been found in cases where there is little or no amyloid accumulation, indicating that immune dysregulation can take place independently and may thus initiate or accelerate

neurodegeneration (Goyal et al., 2026). This undermines the traditional amyloid cascade hypothesis and suggests a more complicated interaction in which amyloid accumulation and immune responses can each act on their own as well as in combination to cause synaptic loss and neuronal death.

The buildup of amyloid-beta, which results from mutations in the APP and PSEN1 genes, is a characteristic feature of Alzheimer's disease. Yet there is evidence that an accumulation of amyloid can take place without at the same time having microglial activation; for instance, microglia can stay rather inactive during the early stages of the disease or in some genetic situations (Zhu et al., 2012). On the other hand, immune dysregulation and neuroinflammation can arise independently of amyloid pathology, perhaps being caused by other genetic factors such as APOEε4, and thus contribute to neurodegeneration (Liu et al., 2025). It is important to realise that the amyloid and immune pathways can function separately or in parallel if we are to develop treatments that are effective in addressing both parts of the disease.

Integrative Model: Interconnected but Partially Independent Pathways

There is recent evidence to show that both the microglial dysfunction caused by APOEε4 and the amyloid pathology driven by APP/PSEN1 are major factors in the progression of Alzheimer’s disease. Although these pathways may function on their own, they often interact and thus form a complex network which speeds up neurodegeneration and the loss of synapses.

Traditional models of Alzheimer’s disease usually show amyloid-beta accumulation as the initial event which then sets off subsequent neuroinflammatory processes (O’Brien, 2011). Yet new research shows that microglial dysfunction, particularly when it comes to the APOEε4 allele, can take place on its own or even before there is a significant amount of amyloid build-up (Dias et al., 2025). The way in which these two pathways interact is complicated in that amyloid deposits can activate microglia, but immune dysregulation caused by the APOEε4 allele can also lead to neurodegeneration on its own. It is important to know whether these pathways are sequential or parallel if we are to develop effective therapeutic strategies.

Several studies support the idea that APOEε4 has an effect on microglial activity even in the absence of amyloid pathology. For instance, Ferrari-Souza et al. (2023) found that microglial activation in people who carry APOEε4 can take place prior to the appearance of detectable amyloid deposits, indicating that immune dysregulation might act as an early or independent factor in the development of synaptic dysfunction. On the other hand, some other research shows that amyloid plaques are able to directly activate microglia, causing neuroinflammation and leading to neuronal damage (Zhu et al., 2012). This dual nature shows that although the two pathways are interconnected, they may also operate independently, each one being able to trigger or worsen neurodegenerative processes.

The relationship between amyloid buildup and microglial dysfunction seems to be synergistic in many instances. Amyloid deposits can cause inflammatory reactions, which then lead to a deterioration of synaptic maintenance and neuronal survival (Yamazaki et al., 2019). For example, microglia associated with APOEε4 tend to become pro-inflammatory, thus

increasing neurodegeneration even when there is little amyloid present (Høilund-Carlsen et al., 2024). On the other hand, amyloid pathology can continue and get worse in the presence of immune dysregulation, setting up a feedback mechanism that speeds up the progression of the disease (Daniel Lee, C. Y., and Gary E. Landreth., 2010). It therefore appears that it may be necessary to target both of these pathways at the same time in order to achieve an effective intervention.

The fact that these pathways can function on their own while at the same time interacting has important consequences for the way treatments are developed. Attempts to treat only amyloid deposition have been only partly successful (Høilund-Carlsen et al., 2024). It might be possible to achieve a more thorough approach to slowing or stopping the progression of the disease by combining anti-amyloid strategies with methods designed to alter microglial function and neuroinflammation. For instance, drugs that act on immune regulators such as TREM2 or on pathways affected by APOEε4 could supplement therapies which focus on amyloid (Qi et al., 2025).

The current model of Alzheimer's disease shows how a number of interconnected yet partly independent pathways, including the buildup of amyloid-beta and malfunction of microglia, interact with one another. Although each of these processes can on its own lead to a loss of synapses and neurodegeneration, their interaction tends to speed up the progression of the disease. Researchers in the future should work towards identifying the exact temporal and causal relationships between these pathways, since this could help in the creation of multi-modal therapeutic approaches that are better able to tackle the various aspects of AD.

Conclusion

The review draws on the current evidence in order to clarify the complex relationship between microglial dysfunction caused by APOEε4 and the amyloid-beta pathology resulting from mutations in APP and PSEN1 in Alzheimer's disease. It is found that these pathways are both linked and able to function on their own. Specifically, APOEε4 affects microglial activity by causing a pro-inflammatory and neurodegenerative phenotype, a condition which can take place either before or without there being a significant amount of amyloid accumulation. On the other hand, the mutations in APP and PSEN1 mainly lead to an accumulation of amyloid-beta, which can trigger microglia but may also advance independently of any immune dysregulation.

This review combines a wide range of peer-reviewed studies, using genetic, cellular, and pathological evidence to build a detailed model of Alzheimer's disease progression. A key strength is its balanced approach. It recognizes that APOEε4 affects microglial activity both on its own and alongside amyloid pathology. This is backed by findings from human genetic studies, post-mortem analyses, and animal models. The review highlights the need to consider both pathways together, matching recent trends in the field toward multi-targeted treatment strategies. It also emphasizes the potential for early immune problems in APOEε4 carriers, stressing the significance of early intervention and tailored medicine.

Despite these strengths, several limitations and unresolved questions persist. First, much of the evidence for early microglial activation in APOEε4 carriers comes from animal models or indirect human markers. Direct proof that changes in microglia happen before amyloid buildup in humans is still limited. For example, it is not clear if microglial activation seen in "amyloid-negative" individuals truly occurs before amyloid deposition or simply goes undetected (Fan et al., 2017). Second, the causal link between microglial activation and neuronal damage is complicated. Some studies suggest microglial responses can be protective, while others link them to worsening neurodegeneration, particularly depending on the stage of the disease (Gao et al., 2023). This uncertainty makes it hard to determine if microglia are mainly drivers or responders. Third, much of the current evidence is based on mouse models, which may not fully mimic human disease, especially regarding tau pathology and aging effects. Concerns about how findings from animal models apply to human AD remain. Finally, the role of lipid metabolism, another pathway affected by APOE, is not fully separated from inflammatory processes. This raises questions about whether the effects of APOEε4 are mainly due to immune issues or changes in lipid transport.

Several studies dispute the proposed model. For instance, some researchers claim that APOEε4-related changes in microglia are more closely linked to altered lipid processing than to inflammation itself (Newcombe et al., 2018). Others have found that microglial activation can be protective, helping with debris clearance and neurorepair, especially in the early stages of the disease (Ayata et al., 2018). Moreover, evidence suggests that APOEε4 may affect amyloid deposition more directly than previously understood, possibly acting as a modifier of plaque formation, rather than an independent cause of neuroinflammation (Belaidi et al., 2025). Finally, the connection between tau pathology and immune responses adds more complexity. Some studies indicate that tau buildup can happen independently of amyloid and microglial activation, challenging the idea that neuroinflammation always follows amyloid changes (Dominguez-Meijide et al., 2020).

The fact that both pathways play a role in neurodegeneration, either in a synergistic manner or on their own, has important consequences for the development of therapies. Since existing treatments which have concentrated only on clearing amyloid have been only partly successful, there is a need for strategies that also address immune and inflammatory processes. It may be possible to achieve a more effective slowing or stopping of the progression of the disease by combining approaches that regulate microglial activity, in particular in those who carry the APOEε4 allele, with amyloid-targeted therapies.

In the future, further research should seek to establish the temporal order and causal links between these pathways. Longitudinal studies and more sophisticated models could be used to find out whether microglial dysfunction functions as an early initiating factor, as a later consequence, or as a separate and parallel process in Alzheimer’s disease. Gaining such insights might lead to the development of personalized, multi-modal interventions that target the entire range of pathogenic mechanisms in Alzheimer’s disease.

Works Cited

Ayata, Pinar, et al. ‘Epigenetic Regulation of Brain Region-Specific Microglia Clearance Activity’. Nature Neuroscience, vol. 21, no. 8, Aug. 2018, pp. 1049–60. PubMed Central, https://doi.org/10.1038/s41593-018-0192-3.

Barber, Robert C. ‘The Genetics of Alzheimer’s Disease’. Scientifica, vol. 2012, 2012, p. 246210. PubMed Central, https://doi.org/10.6064/2012/246210.

Belaidi, Abdel Ali, et al. ‘Apolipoprotein E in Alzheimer’s Disease: Molecular Insights and Therapeutic Opportunities’. Molecular Neurodegeneration, vol. 20, Apr. 2025, p. 47. PubMed Central, https://doi.org/10.1186/s13024-025-00843-y.

Chia, Sook-Yoong, et al. ‘Single-Nucleus Transcriptomics Reveals a Distinct Microglial State and Increased MSR1-Mediated Phagocytosis as Common Features across Dementia Subtypes’. Genome Medicine, vol. 17, no. 1, Aug. 2025, p. 92. Springer Link, https://doi.org/10.1186/s13073-025-01519-4.

Daniel Lee, C. Y., and Gary E. Landreth. ‘The Role of Microglia in Amyloid Clearance from the AD Brain’. Journal of Neural Transmission (Vienna, Austria : 1996), vol. 117, no. 8, Aug. 2010, pp. 949–60. PubMed Central, https://doi.org/10.1007/s00702-010-0433-4.

Dias, Daniela, et al. ‘From Genetics to Neuroinflammation: The Impact of ApoE4 on Microglial Function in Alzheimer’s Disease’. Cells, vol. 14, no. 4, Feb. 2025, p. 243. PubMed Central, https://doi.org/10.3390/cells14040243.

DiSabato, Damon, et al. ‘Neuroinflammation: The Devil Is in the Details’. Journal of Neurochemistry, vol. 139, no. Suppl 2, Oct. 2016, pp. 136–53. PubMed Central, https://doi.org/10.1111/jnc.13607.

Dominguez-Meijide, Antonio, et al. ‘Pharmacological Modulators of Tau Aggregation and Spreading’. Brain Sciences, vol. 10, no. 11, Nov. 2020, p. 858. PubMed Central, https://doi.org/10.3390/brainsci10110858.

Fan, Zhen, et al. ‘An Early and Late Peak in Microglial Activation in Alzheimer’s Disease Trajectory’. Brain, vol. 140, no. 3, Mar. 2017, pp. 792–803. PubMed Central, https://doi.org/10.1093/brain/aww349.

Ferrari-Souza, João Pedro, et al. ‘APOEε4 Associates with Microglial Activation Independently of Aβ Plaques and Tau Tangles’. Science Advances, vol. 9, no. 14, p. eade1474. PubMed Central, https://doi.org/10.1126/sciadv.ade1474. Accessed 13 Aug. 2026.

Gao, Chao, et al. ‘Microglia in Neurodegenerative Diseases: Mechanism and Potential Therapeutic Targets’. Signal Transduction and Targeted Therapy, vol. 8, Sept. 2023, p. 359. PubMed Central, https://doi.org/10.1038/s41392-023-01588-0.

Goyal, Pradeep, et al. ‘Neuroimmune Interactions in Neurodegeneration: The Role of Microglia in Alzheimer’s and Parkinson’s Disease Pathogenesis’. Brain Sciences, vol. 16, no. 2, Jan. 2026, p. 154. PubMed Central, https://doi.org/10.3390/brainsci16020154.

Høilund-Carlsen, Poul F., et al. ‘Alzheimer’s Amyloid Hypothesis and Antibody Therapy: Melting Glaciers?’ International Journal of Molecular Sciences, vol. 25, no. 7, Mar. 2024, p. 3892. PubMed Central, https://doi.org/10.3390/ijms25073892.

Kempuraj, D., et al. ‘Neuroinflammation Induces Neurodegeneration’. Journal of Neurology, Neurosurgery and Spine, vol. 1, no. 1, 2016, p. 1003. PubMed Central, https://pmc.ncbi.nlm.nih.gov/articles/PMC5260818/.

Kim, Min Joo, et al. ‘Neuronal APOE4 Drives Damaging Lipid Accumulation via Contact-Dependent Neuron-Oligodendrocyte-Microglia Interaction in Alzheimer’s Disease’. bioRxiv, Dec. 2025, p. 2025.12.04.692390. PubMed Central, https://doi.org/10.64898/2025.12.04.692390.

Liu, Aiwei, et al. ‘The APOE–Microglia Axis in Alzheimer’s Disease: Functional Divergence and Therapeutic Perspectives—A Narrative Review’. Brain Sciences, vol. 15, no. 7, June 2025, p. 675. PubMed Central, https://doi.org/10.3390/brainsci15070675.

Newcombe, Estella A., et al. ‘Inflammation: The Link between Comorbidities, Genetics, and Alzheimer’s Disease’. Journal of Neuroinflammation, vol. 15, Sept. 2018, p. 276. PubMed Central, https://doi.org/10.1186/s12974-018-1313-3.

O’Brien, Richard J., and Philip C. Wong. ‘Amyloid Precursor Protein Processing and Alzheimer’s Disease’. Annual Review of Neuroscience, vol. 34, 2011, pp. 185–204. PubMed Central, https://doi.org/10.1146/annurev-neuro-061010-113613.

Petersen, Ronald C., et al. ‘Understanding the Impact of Amyloid Beta Targeted Therapies on Biomarkers and Clinical Endpoints in Alzheimer’s Disease’. Alzheimer’s & Dementia : Translational Research & Clinical Interventions, vol. 11, no. 2, May 2025, p. e70069. PubMed Central, https://doi.org/10.1002/trc2.70069.

Qi, Xiaoshan, et al. ‘Roles of TREM2 in Alzheimer’s Disease’. Translational Neurodegeneration, vol. 14, Oct. 2025, p. 55. PubMed Central, https://doi.org/10.1186/s40035-025-00515-3.

Raulin, Ana-Caroline, et al. ‘ApoE in Alzheimer’s Disease: Pathophysiology and Therapeutic Strategies’. Molecular Neurodegeneration, vol. 17, Nov. 2022, p. 72. PubMed Central, https://doi.org/10.1186/s13024-022-00574-4.

Samudra, Niyatee, et al. ‘Tau Pathology in Neurodegenerative Disease: Disease Mechanisms and Therapeutic Avenues’. The Journal of Clinical Investigation, vol. 133, no. 12, p. e168553. PubMed Central, https://doi.org/10.1172/JCI168553. Accessed 13 Aug. 2026.

Sasaguri, Hiroki, et al. ‘APP Mouse Models for Alzheimer’s Disease Preclinical Studies’. The EMBO Journal, vol. 36, no. 17, Sept. 2017, pp. 2473–87. PubMed Central, https://doi.org/10.15252/embj.201797397.

Sun, Linfeng, et al. ‘Analysis of 138 Pathogenic Mutations in Presenilin-1 on the in Vitro Production of Aβ42 and Aβ40 Peptides by γ-Secretase’. Proceedings of the National Academy of Sciences of the United States of America, vol. 114, no. 4, Jan. 2017, pp. E476–85. PubMed Central, https://doi.org/10.1073/pnas.1618657114.

Tan, Jianxin, et al. ‘Effects of γ-Secretase Cleavage-Region Mutations on APP Processing and Aβ Formation: Interpretation with Sequential Cleavage and α-Helical Model’. Journal of Neurochemistry, vol. 107, no. 3, Nov. 2008, pp. 722–33. PubMed Central, https://doi.org/10.1111/j.1471-4159.2008.05643.x.

Tzioras, M., et al. ‘Invited Review: APOE at the Interface of Inflammation, Neurodegeneration and Pathological Protein Spread in Alzheimer’s Disease’. Neuropathology and Applied Neurobiology, vol. 45, no. 4, June 2019, pp. 327–46. PubMed Central, https://doi.org/10.1111/nan.12529.

Wang, Na, et al. ‘Microglial Apolipoprotein E Particles Contribute to Neuronal Senescence and Synaptotoxicity’. iScience, vol. 27, no. 6, May 2024, p. 110006. PubMed Central, https://doi.org/10.1016/j.isci.2024.110006.

Wolfe, Cody M., et al. ‘The Role of APOE and TREM2 in Alzheimer′s Disease—Current Understanding and Perspectives’. International Journal of Molecular Sciences, vol. 20, no. 1, Dec. 2018, p. 81. PubMed Central, https://doi.org/10.3390/ijms20010081.

Yamazaki, Yu, et al. ‘Apolipoprotein E and Alzheimer Disease: Pathobiology and Targeting’. Nature Reviews. Neurology, vol. 15, no. 9, Sept. 2019, pp. 501–18. PubMed Central, https://doi.org/10.1038/s41582-019-0228-7.

Zhu, Yuangui, et al. ‘APOE Genotype Alters Glial Activation and Loss of Synaptic Markers in Mice’. Glia, vol. 60, no. 4, Apr. 2012, pp. 559–69. PubMed Central, https://doi.org/10.1002/glia.22289.

When Learning Feels Rewarding: A Review of the Neural Mechanisms Underlying Intrinsic Motivation and Self-Generated Learning By Erik Gradic

Abstract

Individuals may experience moments of self-generated learning from time to time, where they might independently find a solution to a hard problem or learn a new concept, which is usually accompanied by a sense of satisfaction. While these moments are typically associated with deep thinking and problem solving, their role in the optimization and consolidation of behavioural and cognitive changes is less understood. Does current evidence in neuroscience suggest intrinsic reward mechanisms associated with problem solving and insight influence future behaviour? This review argues that while no research specifically determines that a mechanistic relationship exists between self-generated learning and the activation of intrinsic reward pathways or changes in future behaviour, converging evidence from intrinsic motivation, reinforcement learning, flow, and reward prediction may collectively support the hypothesis. Furthermore, this review also explores the possibility that the relationship between self-generated learning and activation of reward pathways may contribute to cognitive and behavioural adaptations. This review attempts to create a logical progression that could explain why humans may biologically retain more cognitively favourable behaviours. This also serves as a new direction for future research done on intrinsic motivation, reward processing regarding problem solving, and reinforcement learning.

Introduction

Humans regularly learn new knowledge or skills without the external promise of reward. Moments of learning a new concept, finding a solution to a difficult problem, or discovering an effective strategy are typically accompanied by a sense of satisfaction and competence that appear to motivate and reinforce further learning. Understanding why these moments of self-generated learning are inherently rewarding could offer great insights into the field of neuroscience into how the brain promotes long-term cognitive and behavioural changes through exploration, adaptation, and learning, where self-generated learning defines the independent derivation or inference of new information or a solution without being directly provided with an answer or being explicitly told to.

In this review, cognitive optimizations are defined as the learning-dependent changes in problem-solving or information-processing that allow an individual to achieve a comparable or even improved result with less cognitive effort. Behavioural optimizations are defined as changes in future behaviour based on previous outcomes that may increase the likelihood of achieving a successful outcome.

Intrinsic motivation has been described as engaging in an activity not because of external incentives but because it is inherently enjoyable or personally meaningful (Deci and Ryan). Self-Determination Theory proposes that intrinsic motivation is created when behaviours start to satisfy psychological needs such as competence and autonomy (Deci and Ryan). Neuroscientific

work has also expanded this framework by showing that intrinsically motivated behaviours recruit neural pathways associated with reward processing, learning, and exploration, instead of functioning independently of the brain's reward circuitry (Di Domenico and Ryan). Morris et al. also argued that intrinsic motivation is decided by computations done to assign values to internally generated outcomes, and Fishbach and Woolley suggest that an activity can become intrinsically motivated when the act of completing the activity itself is rewarding.

While the concept of intrinsic motivation may explain why internally motivated behaviours are upheld, the concept of reinforcement learning provides a complementary explanation of how successful behaviours are reinforced over time. Reward prediction errors that are dopamine-mediated provide the brain with a system to compare expected and real outcomes, which strengthens behaviours that yield unexpected beneficial results (Schultz, "Getting Formal"; Nasser et al.). Research in effort-based decision making has also suggested that the generated subjective value of a reward depends on the cognitive or physical effort done to obtain it (Botvinick et al.).

New research has also suggested that self-generated learning itself may recruit neural pathways associated with reward. Ripolles et al. showed that independently inferring the meaning of new words activated areas such as the ventral striatum, a region involved in motivational processing and reward valuation, and the hippocampus, a region responsible for learning and memory consolidation, despite the lack of external feedback. Studies of flow, the mental state of complete concentration and focus, describe sustained engagement in optimally challenging tasks as an intrinsically rewarding state (Khoshnoud et al.). Despite these studies' seeming irrelevance to each other in cognitive phenomena, they all collectively suggest that self-generated learning can engage neural pathways associated with reward processing (Ripolles et al.; Khoshnoud et al.).

This review will investigate the current evidence surrounding the role of intrinsic reward mechanisms in self-generated learning and problem-solving.

Although there has been independent research in areas such as intrinsic motivation, effort discounting, and reinforcement learning, few reviews integrate all areas to produce an all-encompassing framework of how these mechanisms support the reinforcement of cognitive optimizations following an instance of self-generated learning. The implications and applications of this mechanism go into areas such as education, rehabilitation, and ultimately cognitive neuroscience.

This review argues that there is not enough sufficient evidence in current neuroscience to establish a direct mechanism connecting self-generated learning and behavioural and cognitive optimization. However, integrating and converging research on intrinsic motivation, reinforcement learning, effort discounting, insight, and flow suggests that self-generated learning can engage neural pathways associated with reward and potentially reinforce cognitively favourable behaviours.

The first section reviews intrinsic motivation. The second reviews reinforcement learning and reward prediction error as mechanisms of behavioural reinforcement. The third discusses

effort valuation and cognitive optimization. The fourth section reviews evidence relating to self-generated learning, insight, and flow. The review integrates these findings into a literature synthesis, discusses the limitations, and provides future directions for empirical research.

This review will use a narrative literature review approach, using evidence from review papers, experimental papers, and functional imaging research. Relevant literature was identified from specific searches on Google Scholar using terms like “intrinsic motivation”, “effort discounting”, “insight”, “reinforcement learning”, and “Aha! moments”, followed by a search through the references list of key studies. Additional potentially relevant studies were identified by Consensus AI as a literature discovery tool.

Intrinsic Motivation

The theories around intrinsic motivation were initially entirely psychological, using concepts such as competence, autonomy, and relatedness to explain the internal drivers of intrinsic motivation (Deci, E. L., & Ryan). These theories sought to explain why individuals may engage in activities without the promise of external incentives like reward. Self-Determination Theory proposes that intrinsic motivation emerges when 3 psychological needs in an activity are satisfied, those being autonomy, competence, and relatedness (Deci and Ryan). Within this framework, individuals can possess an inherent desire to engage in an activity because the activity in itself is enjoyable or fulfilling, allowing exploration, learning, and the acquisition of skill to occur without any expectation of reward. This perspective provided a significant contrast to that of behaviourist views, where behaviour was thought to be the product of mainly external reinforcement. Instead, SDT argued that the drive to obtain competence and master challenges constituted an independent motivational force. However, SDT explained the psychological conditions in which intrinsic motivation can occur, but not the biological mechanisms from which it originates and is maintained. Consequently, SDT helps explain why individuals may pursue activities despite a lack of an external motivator but not how these internally rewarding experiences are represented in the human brain.

Curiosity also provides a complementary explanation for why individuals may initially engage in learning and problem-solving behaviours. Kidd and Hayden describe curiosity as an essential driver supporting intrinsic motivation while also noting that intrinsic motivation and curiosity have a complex relationship. The neuroscientific papers reviewed by Kidd and Hayden suggest that curiosity may engage learning and reward-related areas of the brain, with curiosity even correlating with better retention of information. In this sense, curiosity may add another mechanism through which intrinsic motivation acts, where intrinsic motivation supplies voluntary engagement broadly, whereas curiosity directs engagement narrowly to resolve uncertainty or to bridge a gap in knowledge (Kidd & Hayden). This distinction is important, since curiosity may initiate self-generated learning whereas intrinsic motivation may provide the means of sustaining the learning process. Thus, curiosity complements the overall pathway from intrinsic motivation to self-generated learning by showing how internally generated exploration may be directed towards learning behaviours.

Within the context of intrinsic motivation, reward was never established as a primary incentive or mechanism sustaining intrinsic motivation. However, recent neuroscientific evidence suggests that the previous interpretation is incomplete, as it was shown that intrinsically motivated behaviours were able to recruit dopaminergic neural circuitry (Di Domenico and Ryan). This shows a considerable shift in perspective in how intrinsic motivation is understood by suggesting that internally rewarding experiences are not just psychological states but are also accompanied by measurable neurological changes which encourage further exploration and competence. These findings provide a valuable extension to SDT, revealing the biological mechanisms through which intrinsic motivation might influence learning and behaviour. Thus, intrinsic motivation can also be understood as a process supported by neural systems associated with reward and adaptive learning, not as a subjective psychological experience.

A computational perspective suggests that intrinsic motivation may rely on internal valuation processes similar to those seen in externally rewarded behaviour. Morris et al. argued that internally generated rewards are represented by value computations, which are similar to the processes present in extrinsic motivation. Fishbach and Woolley also proposed that an activity becomes intrinsically rewarding once the process of completing the task acquires value regardless of its external outcome. These all collectively suggest that intrinsic motivation processes internally generated values assigned to tasks, not only functioning as an independent motivational drive. However, existing theories only explain why individuals voluntarily engage with challenging tasks despite the lack of external incentives. They do not directly address whether self-generated learning recruits dopaminergic neural circuitry in a fashion that reinforces the future behaviour positively. Altogether, this illustrates the change in the understanding of intrinsic motivation as not a purely psychological concept but also as a neurocomputational process. Nonetheless, the frameworks around intrinsic motivation mostly explain why individuals may engage in learning, rather than how self-generated learning may engage neural pathways associated with reward.

These theories recognize intrinsic motivation as being supported by neural reward mechanisms; however, it remains unclear whether intrinsic motivation alone explains the rewarding nature behind self-generated learning. One interpretation could be that finding a solution to a challenging problem is inherently rewarding because it satisfies the psychological needs emphasized by SDT, like competence and autonomy. An alternative interpretation, however, could be that neural activity observed during successful learning reflects other cognitive processes that cannot be easily isolated. For example, activation of reward-related regions of the brain may be attributed to the fact that the generated solution was unexpected or because successful learning strengthens episodic memory. Therefore, current evidence remains insufficient to determine whether it is solely intrinsic motivation that provides the drive behind self-generated learning and to distinguish between the competing interpretations. The important implication for learning is that intrinsic motivation may provide a reason to remain engaged in an activity, while other reward-related learning mechanisms may determine which successful behaviours are more likely to occur later. Thus, intrinsic motivation and reinforcement learning

may represent complementary stages of learning, not distinct processes. However, more insight into reinforcement learning and reward prediction may provide a complementary explanation for how self-generated learning influences future behaviour positively.

Neural Reward Systems and Reinforcement Learning

Successful behaviour depends not only on obtaining desirable outcomes but also on the brain's ability to recognize when those behaviours should influence future behaviour. The dopaminergic system is central to this process, where projections from the substantia nigra and the ventral tegmental area to the ventral striatum act as the prominent circuits (Schultz, "Getting Formal"; Schultz, "Dopamine Reward Prediction Error"). Instead of simply providing a response to pleasurable stimuli, this circuitry evaluates behavioral outcomes and modifies future decision-making. Altogether, reward processing serves a much broader role in learning by identifying behaviours that are more likely to result in a successful outcome.

The initial moments of experiencing a successful outcome, whether intentional or not, are accompanied by dopaminergic neurons firing at faster speeds due to a positive reward prediction error, where the expected outcome was surpassed by the actual outcome (Nasser et al.; Schultz, "Dopamine Reward Prediction Error"). Dopamine release promotes synaptic plasticity within reward-related and prefrontal networks, strengthening the neural pathways initially involved in the successful behaviour and increasing the likelihood that the behaviour will be repeated. In the context of learning, this mechanism allows newly acquired knowledge or successful problem solving to become more readily accessible over time (Schultz, "Getting Formal"; Schultz, "Dopamine Reward Prediction Error"). Thus, reward prediction error provides a biologically plausible explanation for how successful self-generated learning can lead to the reinforcement of behaviour and support adaptive learning through positive reward prediction errors. For the present question, the important implication is that a successful internally generated solution could potentially function as a learning motivator and signal if the outcome exceeds the learner's expectations. However, this possibility extends the principles of reward prediction error beyond the external reward instances in which they have been studied extensively (Schultz; Nasser et al.).

A plausible explanation for the rewarding nature of self-generated learning is that independently finding a solution creates a positive reward prediction error. Because the successful outcome is independently generated and oftentimes unexpected, the large discrepancy between the expectation and reality may strengthen future behaviour through the previously discussed mechanism. However, current evidence surrounding the occurrence of reward prediction errors relates them to externally rewarded paradigms, not internally generated learning. Whether internally generated learning produces a similar reward prediction error as the externally rewarded situations remains unclear and represents a gap in the literature surrounding reward prediction error and its implications in self-generated learning.

Effort Valuation and Cognitive Optimizations

How successful a behaviour is does not depend on the magnitude of the success alone, but also the effort required to obtain it. Botvinick et al. used fMRI while participants completed blocks of tasks requiring either high or low levels of mental effort before receiving monetary reward. The nucleus accumbens region reaction to reward was then compared across the different experimental groups differing in mental effort required. They found that, for equivalent reward outcomes, nucleus accumbens activation was lower following high-effort than low-effort blocks, neurally suggesting that greater effort preceding the reward decreases the subjective value of that reward. Research on how the brain allocates cognitive and physical effort, effort discounting, shows that a reward which requires greater cognitive or physical effort to obtain is subjectively devalued, leading individuals to favor strategies which minimize cognitive or physical effort (Botvinick et al.). These findings suggest that behavioural choices are not only influenced by the anticipated outcome, but by the expected effort required to achieve the outcome as well. Thus, cognitive and behavioural optimizations may arise from not only maximizing behaviour that obtains the best rewards, but consistently decreasing effort required to obtain the reward. In this review, cognitive optimizations are defined as the learning-dependent adaptations in problem-solving or information processing that would allow an individual to achieve comparable or even better outcomes while exerting reduced amounts of cognitive effort. Behavioural optimizations refer to the changes in future behaviour depending on previous behaviour that influence the likelihood of achieving a successful outcome.

Shenhav et al. proposed that cognitive control is allocated according to the Expected Value of Control framework, where the brain continuously evaluates whether the benefits of exerting cognitive effort outweigh its costs. In this manner, individuals allocate mental resources when doing so is expected to be advantageous, rather than avoiding effort altogether. This suggests that successful strategies are advantageous because they reduce cognitive effort where not needed while maintaining successful performance. Consequently, cognitive and behavioural optimization may reflect better control of resource allocation instead of simple maximization of reward.

Even though effort valuation provides a plausible explanation for why specific strategies become preferred once available, it doesn't explain where the strategies or behaviour originate from. The framework itself assumes a multitude of options are available and offers no insight into the possibility that there is a situation where an individual independently generates a successful solution. Consequently, this limitation suggests that effort valuation may add to theories of intrinsic motivation and reinforcement learning in relevance to self-generated learning and its influence on future behaviours instead of conflicting with them. Nonetheless, understanding how efficient strategies are generated requires deeper consideration of self-generated learning and insight.

Self-Generated Learning and Insight

Self-generated learning refers to the situation in which an individual independently arrives at a result or newfound knowledge without receiving explicit guidance or instruction to

do so. These experiences, related to insight problem solving, involve the individual restructuring of the problem before arriving upon a somewhat unexpected solution, where insight is a form of self-generated problem-solving, not an interchangeable synonym (Ash & Wiley). Research consistently suggests that heightened confidence and positive mood follow these experiences, showing that these moments of self-generated learning may possess intrinsic motivational value (Kounios and Beeman). Neuroimaging studies have shown further evidence that these experiences engage neural pathways associated with reward (Tik et al.).

Tik et al. investigated the neural basis of the "Aha!" experience using fMRI during a remote associates task where participants must generate a logical fourth word that connects to three words that were presented to them. Participants then indicated when they found a solution and rated the strength of that particular moment of insight. The researchers found heightened activity in areas such as the nucleus accumbens, hippocampus, thalamus, and the dopaminergic midbrain, which includes the ventral tegmental area. The findings provide particularly strong evidence towards moments of insight being accompanied by activity in both reward-related and memory-related neural regions.

Ripolles et al. provided strong evidence connecting self-generated learning with the engagement of neural pathways associated with reward. In the study, participants had to infer the meaning of words that were previously unknown to them. The participants who independently inferred the correct meaning of the word they were given were shown to have increased activity in the substantia nigra/ventral tegmental areas, ventral striatum, and hippocampus without any explicit reward, suggesting that the independent learning they undertook engaged with the areas in the brain associated with reward and even memory consolidation, similar to that of intrinsic motivation's inner workings. These findings provide the strongest available evidence and support a mechanistic relationship between intrinsically activated self-generated learning and activation of neural pathways associated with reward.

Despite the compelling evidence given by Ripolles et al., alternative explanations remain plausible. The increased hippocampal activity could be a result of the encoding of episodic memory, and the increased engagement of the ventral striatum may reflect positive reward prediction error or the encountering of novel vocabulary. What these findings cannot determine is if the reward-related activity is a direct result of intrinsic motivation, successful learning, memory formation, or even an interaction between them. In order to establish a more conclusive relationship between self-generated learning and activation of reward pathways, more primary research on these concepts should be conducted. This establishes the evidence's greatest limitations and motivates further consideration of states like flow.

Flow and Sustained Intrinsic Engagement

Flow is defined as a state of peak performance where concentration becomes effortless, characterized by its self-distortion of time and a somewhat intrinsically motivating feeling (Khoshnoud et al.; Csikszentmihalyi). The state of heightened focus satisfies the psychological needs for autonomy and competence, driving deep intrinsic motivational desire. This is

supported by flow's inner neurological workings, as the deep engagement stimulates dopamine release in the mesolimbic pathway, reinforcing the behaviour of repeating the task (van der Linden et al.). In this aspect, flow and insight are intrinsically motivated by the same factors.

Although flow differs from discrete moments of insight, both are characterised by intrinsic engagement, sustained persistence, and no incentive from an external reward. Thus, flow may provide an alternative framework that could aid in better understanding how distinct periods of initially intrinsically rewarding behaviour may develop into long periods of flow-like behaviour. Instead of representing an entirely different motivational system, flow may constitute the prolonged behavioural continuation of intrinsically motivated behaviour, further supporting the framework.

However, flow merely describes sustained engagement rather than the initial discovery of a solution and consequently fails to describe why self-generated learning becomes intrinsically motivating initially. Collectively, this shows that no single theoretical framework can explain self-generated learning's neurological components and sources.

Literature Synthesis

Intrinsic motivation explains why individuals may engage in learning behaviours without external incentives. Reinforcement learning provides an explanation for how future behaviour may be modified through initial successful outcomes, while effort valuation explains why lower effort strategies become preferable. Research on insight and self-generated learning details how internally generated solutions are neurologically linked to activation of reward pathways. Finally, flow extends intrinsic motivation by showing the flow state may be a behavioural continuation of intrinsically motivated behaviours. However, no existing framework explains the pathway from intrinsic motivation to self-generated learning and behavioural and cognitive optimizations.

When considered altogether, the findings suggest a possible progression through which self-generated learning may influence future learning behaviours. Intrinsic motivation may allow individuals to pursue and engage with challenging tasks and find alternative methods, thus creating opportunities for self-generated learning to arise (Di Domenico & Ryan). When a moment of exploration produces a successful outcome, the resulting experience may engage reward and memory-related systems, as seen in the studies of internally generated learning (Ripolles et al.). Reinforcement learning mechanisms could then provide a consistent process in which behaviours leading to successful outcomes are retained and consolidated to influence the probability of repeating that behaviour in the future, while effort valuation could affect whether an outcome is subsequently preferred when it achieves a successful outcome at a lower cost (Schultz, "Getting Formal"; Nasser et al.; Botvinnick et al.; Shenhav et al.). After many instances of experienced self-generated learning, an individual may consequently repeat learning behaviours and lower the cognitive effort required to problem-solve. Thus, this sequence of mental processes provides the basis for how self-generated learning, arising from intrinsic motivation, may influence future behaviours and possibly influence future behavioural patterns.

However, this should not be treated as a fully causal relationship, as the studies reviewed here generally examine these processes separately.

However, the neural activity associated with self-generated learning can nonetheless be interpreted in several ways. One interpretation could be that engaging in self-directed learning can be intrinsically motivating, satisfying psychological needs such as competence, relatedness, and autonomy. Another explanation could be that the discrepancy between the expected outcome and real outcome when unexpectedly finding the solution to a problem creates a positive reward prediction error, that being associated with dopaminergic signalling. A third explanation is that the activity observed in the hippocampus could reflect memory encoding and consolidation, particularly in studies like the Ripolles study where learning and memory happened to coincide. Activation of reward-related areas may also just reflect the resolution of uncertainty during problem solving. However, these interpretations are not mutually exclusive. Intrinsic motivation, prediction error, successful inference, and memory formation may interact during self-generated learning, but a lack of conclusive evidence does not allow their contributions to be isolated reliably. Consequently, the presence of reward-related activity should be indicative of its involvement in successful learning, not as proof that intrinsic motivation is the sole mechanism responsible.

Conclusion

This review asks whether current neuroscience supports the role of intrinsic reward mechanisms associated with problem-solving and insight in influencing future behaviour. Overall, current evidence in neuroscience provides evidence that when converged, suggests a relationship between self-generated learning and intrinsic reward processing. However, the evidence is largely incomplete in causally demonstrating an explicit link between intrinsic motivation and self-generated learning to potential impacts on future behaviours. Intrinsic motivation establishes that internally generated experiences can provide motivational value, while reinforcement learning provides a plausible mechanism for how successful behaviours are retained and repeated. Research into insight and self-generated learning further shows how internally generated solutions can also engage with reward and memory-related areas of the brain (Di Domenico and Ryan; Schultz, “Getting Formal”; Ripolles et al.). Effort valuation provides a mechanism where perceived effort is calculated and influences future behaviours (Botvinick et al.; Shenhav et al.). However, the current evidence does not establish that the rewarding aspect of self-generated learning works as a mechanism to reinforce learning behaviours, retention of information, or to enhance overall cognition. Thus, the strongest conclusion that is supported by the existing literature is that these factors may interact and coincide with each other during self-generated learning, but there is ultimately not sufficient causal evidence to confidently say that the intrinsically motivating feeling behind self-generated learning supports and reinforces cognitive and behavioural optimizations.

One of the largest limitations present is the lack of direct experiments done on specific instances of self-generated learning engaging neural pathways associated with reward in a

manner that reinforces future behaviour. The absence of primary research presents a gap in the literature used for this paper and subsequently the inferences made in order to produce a framework that encapsulates the relationship between self-generated learning and the reinforcement of cognitively optimized behaviours. The correlational fMRI studies also present a major limitation, as correlational studies can introduce extraneous variables or false positives in identifying an active region of the brain as opposed to causal studies.

Consequently, alternative results should be considered carefully when making each inference throughout the framework. Additionally, no comparisons between explicitly ordered learning and non-explicit independent learning exist throughout the studies. Individuals also may differ in aspects of curiosity, voluntary learning, and willingness to engage in cognitively demanding activities. Consequently, results may not generalize individually as they do at the group level and exploring personality traits may be worth researching in the future in order to distinguish behavioural patterns due to individual personality.

Implications and Applications

The synthesis could have implications in areas such as education and rehabilitation. Understanding how students may explore a subject they find intrinsically enjoyable and build upon their academic expertise through cognitive optimizations may lead to better education reforms through schools' curricula and how the process of learning a concept or being rehabilitated may be improved and refined. This also defines a promising area of neuroscientific research. Primary research conducted could involve directly comparing independently generated and explicitly ordered learning while controlling for behavioural success and activation of specific regions of the brain. Functional neuroimaging could also help determine if self-generated learning elicits a stronger response from reward-related regions of the brain than explicitly ordered learning in order to establish a stronger relationship. Longitudinal studies on the reinforcement of future behaviour after self-generated learning would bridge the essential gap in literature that empirically supports the thesis. These studies altogether would help differentiate intrinsic motivation, effort valuation, reinforcement learning, insight, and flow on a much more discrete and scientifically sound basis.

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Works Cited

Ash, Ivan K., and John Wiley. “The Nature of Restructuring in Insight: An Individual Differences Approach.” Psychonomic Bulletin & Review, vol. 13, no. 1, 2006, pp. 66–73. https://link.springer.com/article/10.3758/BF03193814

Botvinick, Matthew M., et al. “Effort Discounting in Human Nucleus Accumbens.” Cognitive, Affective, & Behavioral Neuroscience, vol. 9, no. 1, 2009, pp. 16–27. https://link.springer.com/article/10.3758/CABN.9.1.16

Csikszentmihalyi, Mihaly. Flow: The Psychology of Optimal Experience. Harper & Row, 1990.

Deci, Edward L., and Richard M. Ryan. Intrinsic Motivation and Self-Determination in Human Behavior. Plenum Press, 1985. https://link.springer.com/book/10.1007/978-1-4899-2271-7

Di Domenico, Stefano I., and Richard M. Ryan. “The Emerging Neuroscience of Intrinsic Motivation: A New Frontier in Self-Determination Research.” Frontiers in Human Neuroscience, vol. 11, 2017, article 145. https://www.frontiersin.org/journals/human-neuroscience/articles/10.3389/fnhum.2017.00145/full

Fishbach, Ayelet, and Kaitlin Woolley. “The Structure of Intrinsic Motivation.” Annual Review of Organizational Psychology and Organizational Behavior, vol. 9, 2022, pp. 339–363. https://www.annualreviews.org/content/journals/10.1146/annurev-orgpsych-012420-091122

Khoshnoud, Shiva, et al. “Peripheral-Physiological and Neural Correlates of the Flow Experience While Playing Video Games: A Comprehensive Review.” PeerJ, vol. 8, 2020, article e10520. https://pubmed.ncbi.nlm.nih.gov/33384898/https://pmc.ncbi.nlm.nih.gov/articles/PMC4635443/

Kidd, Celeste, and Benjamin Y. Hayden. “The Psychology and Neuroscience of Curiosity.” Neuron, vol. 88, no. 3, 2015, pp. 449–460.

Kounios, John, and Mark Beeman. “The Cognitive Neuroscience of Insight.” Annual Review of Psychology, vol. 65, 2014, pp. 71–93. https://pubmed.ncbi.nlm.nih.gov/24405359/

Morris, Laurel S., et al. “On What Motivates Us: A Detailed Review of Intrinsic v. Extrinsic Motivation.” Psychological Medicine, vol. 52, no. 10, 2022, pp. 1801–1816. https://pmc.ncbi.nlm.nih.gov/articles/PMC9340849/

Nasser, H. M., et al. “The Dopamine Prediction Error: Contributions to Associative Models of Reward Learning.” Frontiers in Psychology, vol. 8, 2017, article 244. https://pmc.ncbi.nlm.nih.gov/articles/PMC5319959/

Ripollés, Pablo, et al. “Intrinsic Monitoring of Learning Success Facilitates Memory Encoding via the Activation of the SN/VTA-Hippocampal Loop.” eLife, vol. 5, 2016, article e17441. https://pubmed.ncbi.nlm.nih.gov/27644419/

Schultz, Wolfram. “Getting Formal with Dopamine and Reward.” Neuron, vol. 36, no. 2, 2002, pp. 241–263. https://pubmed.ncbi.nlm.nih.gov/12383780/

Schultz, Wolfram. “Predictive Reward Signal of Dopamine Neurons.” Journal of Neurophysiology, vol. 80, no. 1, 1998, pp. 1–27. https://pubmed.ncbi.nlm.nih.gov/9658025/

Schultz, Wolfram. “Dopamine Reward Prediction-Error Signalling: A Two-Component Response.” Nature Reviews Neuroscience, vol. 17, no. 3, 2016, pp. 183–195. https://pubmed.ncbi.nlm.nih.gov/26865020/

Shenhav, Amit, et al. “Toward a Rational and Mechanistic Account of Mental Effort.” Annual Review of Neuroscience, vol. 40, 2017, pp. 99–124. https://pubmed.ncbi.nlm.nih.gov/28375769/

Tik, Martin, et al. “Ultra-High-Field fMRI Insights on Insight: Neural Correlates of the Aha!-Moment.” Human Brain Mapping, vol. 39, no. 8, 2018, pp. 3241–3252. https://pubmed.ncbi.nlm.nih.gov/29665228/

van der Linden, Dimitri, et al. “Go with the Flow: A Neuroscientific View on Being Fully Engaged.” European Journal of Neuroscience, vol. 53, no. 4, 2021, pp. 947–963. https://pubmed.ncbi.nlm.nih.gov/33084102/

Tissue-Specific Ascertainment as a Candidate Explanation for Conflicting Alzheimer's Disease Risk Estimates in Mosaic Trisomy 21 By Leonor Müller

Abstract

Full trisomy 21 carries one of the highest known genetic risks for Alzheimer's disease. Nearly everyone with full trisomy shows Alzheimer's neuropathology by their mid-40s, a consequence of carrying three copies of the amyloid precursor protein (APP) gene on chromosome 21. The relationship is considerably less well understood in mosaic Down syndrome, where cohort studies and case reports often reach conflicting conclusions about whether mosaicism increases Alzheimer's disease risk, reduces it, or shows no clear association at all. This review asks how much of that disagreement can be attributed to differences in how mosaicism is measured, as opposed to other factors such as participant age at assessment, diagnostic criteria, or publication bias.

Most cohort studies assess mosaic status using peripheral blood, whereas case reports more often confirm mosaicism across multiple tissues or through post-mortem neuropathology and, in more recent cases, PET imaging, though methods vary considerably across sources. Because cohort studies and case reports differ substantially in evidentiary strength, this review treats them as sources of methodological contrast rather than as directly comparable evidence, and involves no new data collection.

A leading hypothesis is that tissue-specific ascertainment contributes to this disagreement. Blood-based studies may underestimate trisomic burden in tissues more directly relevant to Alzheimer's pathology than blood is. This explanation is plausible, but likely partial, and it must be weighed carefully against differences in participant age, diagnostic criteria, and publication selection rather than assumed to explain the pattern on its own.

This remains a conditional point, not a finding: only if tissue source turns out to be a meaningful contributor would some cohort studies be underestimating Alzheimer's disease risk in individuals with mosaic Down syndrome. Should that hold up, the implications for genetic counseling and clinical monitoring would be worth taking seriously, and the broader lesson would be that disagreement within small genetic literatures does not always reflect messy underlying biology so much as differences in what, exactly, is being measured.

Keywords Trisomy 21 mosaicism, Alzheimer's disease, ascertainment bias, amyloid precursor protein, cohort vs. case-report discordance

1. Introduction

Full trisomy 21 is one of the strongest known genetic risk factors for Alzheimer's disease, driven by triplication of the amyloid precursor protein (APP) gene on chromosome 21, and Down syndrome is accordingly used as a model system for Alzheimer's research more broadly (Castro et al.; Fortea et al., "Alzheimer's Disease Associated"; Handen et al.; Lott and Head). Section 2 lays out that baseline case in more detail before this paper turns to the considerably more complicated mosaic literature.

1.1 Background: Down Syndrome and Mosaic Down Syndrome

Down syndrome results from an extra copy of chromosome 21. This full form, trisomy 21, accounts for the large majority of Down syndrome diagnoses and produces the developmental features, intellectual disability, and elevated medical risks associated with the condition, including the sharply increased lifetime risk of Alzheimer's disease this paper addresses. Mosaic Down syndrome is a rarer, biologically distinct variant in which only a subset of a person's cells carry the extra chromosome, arising from a cell-division error after fertilization rather than at conception (Papavassiliou et al.). The proportion of trisomic cells, referred to throughout this paper as mosaicism percentage or trisomic burden, varies both between individuals and across different tissues within the same person, a fact that turns out to matter considerably for how Alzheimer's disease risk in mosaic Down syndrome should be interpreted, as the remainder of this paper explains.

Xicota et al., drawing on two cohorts (n = 357 and n = 468), found mosaicism associated with lower plasma amyloid and, in the older cohort, a protective pattern of slower cognitive decline. Rubenstein et al., analyzing Medicaid claims for 94,533 adults, found almost no difference in Alzheimer's dementia diagnoses by trisomy type. Case reports, by contrast, describe early-onset dementia in patients with comparatively low blood mosaicism, and in one instance a tissue-based mosaicism estimate nearly double the blood value (Ringman et al.; Nuebling et al.; Ngo et al.). Table 1 lays these five sources side by side, comparing how each defined its sample, how each determined mosaic status, and what each concluded about Alzheimer's disease risk.

Study	Design	Mosaicism ascertainment	Sample	Main finding on AD risk
Xicota et al.	Cohort (two datasets)	Peripheral blood karyotyping / SNP genotyping	ABC-DS n=357; legacy n=468	Mosaicism linked to lower plasma amyloid; slower decline and lower dementia incidence in the older cohort (protective pattern)
Rubenstein et al.	Population-level registry (Medicaid claims)	Administrative/clinical ascertainment (mosaic status as recorded in claims data; underlying tissue source not specified)	n=94,533	Almost no gap in AD dementia diagnosis between mosaic (22.3%) and full trisomy (21.5%) groups (no clear association)
Ringman et al.	Case report	Peripheral blood karyotyping	1 patient	Dementia onset at age 55 with about 10% blood mosaicism
Nuebling et al.	Case report	Peripheral blood karyotyping, ectodermal tissue karyotyping, amyloid/tau PET	1 patient	Early-onset AD confirmed by PET despite only 13% blood mosaicism; ectodermal mosaicism measured 21% in the same patient

Ngo et al.	Case report, post-mortem neuropathology	Post-mortem neuropathological examination (same patient as Ringman et al.)	1 patient	Substantial AD pathology, astrogliosis, and cerebral amyloid angiopathy at autopsy

Table 1. Study design, tissue used to ascertain mosaicism, sample size, and reported association with Alzheimer's disease risk across the five primary sources discussed in this review. Cohort and registry studies rely on peripheral blood or recorded diagnoses; case reports more often incorporate additional tissue types or post-mortem analysis.

The cohort, registry, and case-report evidence does not converge on a consistent association. A cohort study reports a protective association. A larger registry study reports essentially no association. A small number of case reports, covering two documented patients, describe early-onset dementia at comparatively low levels of mosaicism. One methodological difference separates the cohort evidence from the case-report evidence consistently: how mosaicism is measured. Cohort studies determine mosaic status almost entirely from blood (Xicota et al.; Rubenstein et al.). Case reports more often examine multiple tissues, or rely on post-mortem analysis (Nuebling et al.; Ngo et al.). Nuebling et al. provides the clearest direct evidence that this distinction matters, since blood and ectodermal tissue in the same patient produced substantially different mosaicism estimates. However, evidence that mosaic fractions differ between tissues does not establish that blood systematically underestimates trisomic burden in the brain; that stronger claim, examined in Section 4, currently rests on considerably thinner evidence than the tissue-discordance finding itself.

Tissue-specific discordance of this kind is already established in the general mosaicism genetics literature (Pagon et al.; Papavassiliou et al.), though it has not previously been connected to Alzheimer's disease risk in Down syndrome specifically.

This paper examines how much of the disagreement among cohort, registry, and case-report findings on Alzheimer's disease risk in mosaic Down syndrome is attributable to how mosaicism is measured, and how much is attributable to other factors, including participant age, diagnostic criteria, and the tendency for unusual cases to be more likely to reach publication. Tissue-specific ascertainment is treated here as a leading hypothesis rather than an established explanation. The evidence directly supporting it currently rests on a single documented case of within-patient tissue discordance, and the broader biological literature has not yet been directly connected to this specific disagreement.

What follows evaluates that hypothesis against the alternatives on its merits, rather than treating it as the default explanation simply because it is the most recently proposed one. This is a narrative, comparative review rather than a systematic one; sources were identified through targeted literature search around the specific cohort, registry, and case-report findings central to this question, not through a pre-registered search strategy across defined databases and date ranges. This scope is appropriate to the size of the relevant literature, which is currently small, but it also means the coverage here should not be assumed to be exhaustive.

2. Alzheimer's Disease in Full Trisomy 21: The Baseline Case

Full trisomy 21 is a genetically direct and mechanistically well-characterized model of Alzheimer's disease, though not a perfectly deterministic one, and its exceptions matter for how mosaic Down syndrome is interpreted later in this paper. Triplication of chromosome 21 produces three copies of the amyloid precursor protein (APP) gene instead of two, and APP overexpression increases production of amyloid-beta, the peptide that aggregates into the plaques characteristic of Alzheimer's disease (Castro et al.). Virtually all adults with full trisomy 21 accumulate detectable amyloid pathology by their thirties, and nearly all show measurable neuropathology by their mid-40s, with most developing clinical dementia within the following two decades (Castro et al.; Fortea et al., "Clinical and Biomarker Changes"; Fortea et al., "Alzheimer's Disease Associated"; Lott and Head).

That progression is not perfectly uniform, and the exceptions are informative. A subset of people with full trisomy 21 accumulate substantial amyloid pathology on autopsy without ever exhibiting dementia symptoms during their lifetime (Lott and Head). The reasons remain incompletely understood; protective genetic and environmental factors are typically proposed, but which specific factors matter, and how strongly, has not been established with the same precision as the APP mechanism itself. If Alzheimer's risk in Down syndrome does not scale perfectly with trisomic burden even under full, unambiguous genetic exposure, there is correspondingly less reason to expect a clean, linear relationship between trisomic cell percentage and risk in mosaic Down syndrome, where the genetic exposure is already partial and variable.

A further complication comes from the biology of chromosome 21 itself. In addition to APP, chromosome 21 carries genes implicated in oxidative stress response and neuroinflammatory signaling, which may independently modify Alzheimer's risk (Castro et al.). Most of the mosaic Down syndrome literature nonetheless reports trisomic cell burden as a single undifferentiated percentage, without distinguishing which chromosome 21 genes are functionally relevant in a given tissue or cell type, so a person's overall mosaic percentage may not translate directly into a proportional dose of any one gene product, APP included, depending on which cells and tissues are actually affected. That baseline, strong on mechanism but imperfect on prediction even under full genetic exposure, is what the mosaic literature in Section 3 complicates further, this time with an exposure that is partial rather than complete.

3. Cohort, Registry, and Case-Report Findings

The three existing bodies of evidence on Alzheimer's disease risk in mosaic Down syndrome are not merely inconsistent in magnitude. They point in genuinely different directions, and that disagreement tracks closely with a specific methodological difference: how each source determines mosaic status in the first place.

Xicota et al. offers the most detailed cohort-level evidence currently available. Drawing on two datasets, the Alzheimer's Biomarker Consortium-Down Syndrome cohort (ABC-DS, n = 357) and a legacy Down syndrome cohort (n = 468), the authors determined mosaic status

through peripheral blood karyotyping and genome-wide genotyping (Handen et al.; Xicota et al.). They then related this status to plasma amyloid and tau concentrations and to longitudinal cognitive assessment collected as part of the ABC-DS protocol, which follows participants across repeated study visits over several years (Handen et al.). Mosaicism was associated with lower plasma amyloid across both cohorts and, in the older cohort specifically, with slower cognitive decline and lower dementia incidence relative to full trisomy 21. Because ABC-DS links mosaic status to individual biomarker and cognitive trajectories rather than to a single diagnostic outcome, this is, among the sources reviewed here, the strongest cohort-level evidence for a protective association.

Several methodological details of this cohort merit closer attention than they typically receive. Mosaicism prevalence was under 10% in both cohorts (7.3% in ABC-DS, 9.6% in the legacy cohort), and mosaic status was determined primarily through peripheral blood karyotyping, in which standard practice examines 20 to 30 cells per patient. Standard cytogenetic guidance holds that at least 29 cells must be examined to exclude mosaicism at the 10% level with 95% confidence (Hook). Mosaicism close to or below that threshold, including the 13% blood-based figure reported in Nuebling et al., is accordingly harder to detect with confidence using a standard cell count, independent of any tissue-source effect.

Where karyotyping was unavailable, the study substituted single nucleotide polymorphism microarray genotyping, a related but distinct method, meaning the cohort's blood-based mosaicism measure is not methodologically uniform across participants.

Most notably, Xicota et al. state directly that because karyotype was sometimes obtained from medical records at unspecified earlier timepoints rather than at study enrollment, they could not determine whether a given participant's mosaicism was present at birth or acquired later in life. This is a direct acknowledgment, from within the cohort study itself, of the same constitutional-versus-acquired ambiguity that Section 4 addresses on independent biological grounds.

A different picture emerges from Rubenstein et al., a population-level administrative study rather than a biomarker-driven cohort, and one that does not agree with Xicota et al.. Working from Medicaid claims data on 94,533 adults with Down syndrome, roughly two hundred times the size of either Xicota cohort, the authors compared recorded Alzheimer's dementia diagnoses by trisomy type and found a difference of less than one percentage point: 22.3% among adults with mosaic Down syndrome versus 21.5% among adults with full trisomy 21. The authors state directly that this result is inconsistent with mosaicism conferring a protective effect. Because Medicaid claims data captures whether a diagnosis was recorded rather than a person's underlying biomarker or cognitive profile, this source trades precision at the individual level for statistical power at the population level; given that trade-off, the near-absence of a gap in a sample this large is difficult to dismiss.

Case reports describe a third, more alarming pattern still, and one that is harder to reconcile with either cohort finding than the two cohort studies are with each other. Ringman et al. described a patient who developed dementia at age 55 with approximately 10% trisomy 21

mosaicism detected in peripheral blood. Nuebling et al. described a patient with even lower blood-based mosaicism, 13%, who nonetheless developed early-onset Alzheimer's disease confirmed through amyloid- and tau-PET imaging; in the same patient, ectodermal tissue mosaicism measured 21%, nearly double the blood value. A post-mortem neuropathological analysis of that same patient subsequently found substantial Alzheimer's disease pathology at autopsy, including elevated amyloid and tau burden alongside widespread astrogliosis and cerebral amyloid angiopathy (Ngo et al.). Two documented patients across three publications is not three independent cases, and two patients cannot establish a general pattern.

It is worth pausing on what these three sources actually measure. They are not interchangeable estimates of a single underlying quantity. A cohort study captures an average association across many participants, one that can hold at the population level even as individual patients vary considerably around it. A case report documents a single person in detail, usually because that person's presentation was unusual enough to warrant publication in the first place. That means case reports carry a form of selection cohort participants do not (Fortea et al., "Alzheimer's Disease Associated"). The disagreement among these three sources is real. But a cohort study, a Medicaid claims analysis, and a case report were never designed to answer identical questions in the first place, and some of that disagreement is just this.

Even so, one methodological distinction connects cleanly to the pattern of disagreement observed here. Xicota et al. and Rubenstein et al. both determine mosaic status from peripheral blood. Nuebling et al. and Ngo et al., by contrast, examined multiple tissues directly or relied on post-mortem neuropathological analysis. Whether this distinction reflects a genuine biological difference in what each type of tissue reveals about brain-relevant trisomic burden, or merely describes an incidental feature of how these particular studies happened to be designed, is the question Section 4 takes up directly.

4. A System of Mosaicism, With Attention to the Brain

Mosaicism is not a single, uniform biological fact captured equally well by any tissue that happens to be sampled. Its distribution across tissues has a developmental explanation, and that explanation is worth spelling out before evaluating whether tissue-specific ascertainment can plausibly explain the disagreement documented in Section 3.

Nuebling et al. measured 13% mosaicism in one patient's blood and 21% in the same patient's ectodermal tissue. A single case cannot establish a general pattern, but the discrepancy is not new to mosaicism genetics: Pagon et al. reported normal lymphocyte chromosomes in dysmorphic patients whose skin fibroblasts showed clear trisomic mosaicism, and Papavassiliou et al. documented the same discordance systematically across a larger paired-sample series. The underlying explanation is developmental. Constitutional mosaicism arises from a mitotic error early in embryogenesis, before the germ layers separate, and the resulting trisomic cell line distributes according to when and where in the embryo the error occurred (Pagon et al.; Papavassiliou et al.). Blood cells derive from mesoderm; skin and other ectodermal tissue derive from ectoderm, the same germ layer that gives rise to the nervous system. A mitotic error

occurring after these germ layers diverge will not distribute evenly across them, which is why the tissues most developmentally related to the brain are not necessarily the tissues cohort studies typically sample. It is worth being precise about what this single case actually shows: it demonstrates that tissue discordance can occur within one individual, not that blood systematically underestimates neuronal trisomic burden across mosaic Down syndrome patients generally. Those are different claims, and only the first one currently has direct evidence behind it.

This mechanism casts doubt on blood-based mosaicism as a complete measure of brain-relevant trisomic burden, but it has not actually been shown to account for the disagreement in Section 3, and it currently rests on a narrow evidence base: constitutional tissue discordance has been directly documented in an Alzheimer's context in exactly one patient (Nuebling et al.). Stated plainly, a substantial part of this paper's central argument currently depends on that single case report. If the patient turns out to be atypical rather than representative, once more multi-tissue cases are examined, the direct evidence for tissue-specific ascertainment in this literature would be considerably weaker than this section has presented it. What this paper is proposing, at this stage, is a hypothesis that deserves further investigation, not a conclusion the evidence has already reached. Section 5 weighs it against the competing explanations on that basis.

5. Evaluation of Alternative Explanations

Tissue-specific ascertainment cannot be assumed to explain the disagreement documented in Section 3 without first being weighed against three alternative explanations: differences in participant age at assessment, differences in diagnostic method, and selection bias in which cases reach publication. Before turning to each in turn, it is worth stating the deeper problem directly: in the sources reviewed here, these explanations are not independent variables that can be cleanly isolated from one another.

Nuebling et al.'s patient, for instance, combines several of them at once: tissue-confirmed mosaicism, PET-based diagnosis rather than plasma biomarkers, and a case-report format that is itself subject to publication selection. Ngo et al. adds post-mortem confirmation on top of that same bundle. Because tissue ascertainment, diagnostic method, and publication selection co-occur within the same small set of case reports, the evidence available cannot cleanly attribute the severe outcome in any one patient to a single factor; this entanglement is itself a limitation of the current literature, not just of this review's ability to interpret it. The sections below examine each explanation individually only because that is the clearest way to present the evidence, not because the evidence itself allows them to be examined separately.

Age at assessment is not held constant across the sources reviewed here, and this matters: Alzheimer's disease risk and detectable pathology both increase with age largely independent of mosaic status (Alldred et al.; Lott and Head). The ABC-DS cohort underlying Xicota et al. enrolls adults aged 25 and older and follows them longitudinally, capturing a broad age range at each assessment point (Handen et al.). Rubenstein et al. draws on a similarly broad adult

Medicaid population. The case reports, by contrast, are individual patients whose age at diagnosis, 55 in Ringman et al., unspecified but comparable in Nuebling et al., is reported precisely because early onset was itself the notable feature that prompted publication. If case reports are systematically drawn from a younger, more atypical subset of the mosaic Down syndrome population than cohort studies capture on average, an apparent difference in outcome could partly reflect this selection rather than tissue source.

The diagnostic method differs substantially by source as well. Xicota et al. and, implicitly, Rubenstein et al. rely on plasma biomarkers or recorded clinical diagnoses, respectively, neither of which directly visualizes brain pathology. Nuebling et al. confirmed diagnosis through amyloid- and tau-PET imaging, a direct in vivo measure of brain pathology, while Ngo et al. relied on post-mortem neuropathological examination, the most direct measure available.

These methods differ substantially in sensitivity: PET imaging and autopsy can detect pathology that plasma biomarkers may miss or underrepresent, particularly at early stages (Fortea et al., "Clinical and Biomarker Changes"; Fortea et al., "Alzheimer's Disease Associated"). Plasma biomarker results are also less standardized than PET or autopsy findings. A recent systematic review and meta-analysis of 58 studies on blood-based Alzheimer's biomarkers in Down syndrome found substantial inconsistency across studies, much of it attributable to differences in assay technology and how Alzheimer's diagnosis was classified in each study, rather than to underlying biological differences between participants (Zhou et al.).

This raises the possibility that the case reports found more severe pathology partly because they used more sensitive and more consistently defined diagnostic tools, not only because their patients had different underlying biology. Taken on its own, this is a genuine competitor to the tissue-ascertainment hypothesis rather than a minor caveat on it: if diagnostic sensitivity alone can account for the case reports looking more severe, the disagreement documented in Section 3 would not require a tissue-source explanation at all. A skeptical reader is entitled to that reading, and this paper does not have the evidence to rule it out.

The third alternative, publication selection, is the hardest of the three to rule out. Case reports are written up specifically because a patient's presentation was unusual, which means the published case-report literature on mosaic Down syndrome and Alzheimer's disease is, by construction, not a representative sample of all mosaic Down syndrome patients. A clinician who observes a mosaic Down syndrome patient with unremarkable cognitive aging has little reason to publish that observation. A patient who develops dementia decades earlier than expected is exactly the kind of case that gets written up and cited widely afterward. This asymmetry is a basic feature of how the case-report literature accumulates, not a speculative concern specific to this topic. This selection pressure could, on its own, produce a published case-report literature that looks alarming even if the broader population of mosaic Down syndrome patients resembles the reassuring cohort findings far more closely.

Table 2 sets the four explanations side by side, with the evidence for and against each drawn from the same sources discussed above. Laid out this way, tissue-specific ascertainment

does not stand out as better supported than the alternatives; if anything, it rests on the thinnest direct evidence of the four, a single within-patient comparison, even though its underlying mechanism is well grounded in developmental biology.

Explanation	Evidence supporting it	Evidence against or limitations
Tissue-specific ascertainment	Within-patient blood/ectodermal discordance in Nuebling et al.; developmental basis for tissue-specific mosaicism established in the broader genetics literature (Pagon et al.; Papavassiliou et al.)	Rests on a single documented case of within-patient discordance; not yet tested systematically across mosaic DS patients
Participant age	AD pathology and dementia risk increase with age largely independent of mosaic status (Alldred et al.; Lott and Head); case-report patients are notable partly because of early onset	Does not by itself explain why blood- and tissue-based studies diverge in direction, not just in magnitude
Diagnostic method	PET and autopsy (Nuebling et al.; Ngo et al.) are more sensitive than plasma biomarkers, which show substantial cross-study inconsistency (Zhou et al.)	Does not explain why mosaicism itself was ascertained differently; sensitivity and tissue source are entangled in these sources, not independent
Publication selection	Case reports are written up because a presentation was unusual; unremarkable mosaic DS aging has little reason to reach print	Magnitude of the bias cannot be quantified from the sources available here

Table 2. The four candidate explanations for the disagreement documented in Section 3, with the evidence supporting each and its main limitation, drawn from the sources discussed in Sections 3 through 5.

Age, diagnostic sensitivity, and publication selection could each be contributing independently, or tissue source could be doing most of the work while these factors add noise around it. Confidently ranking them is not something the available evidence supports, for the reasons stated above. What can be said is narrower: tissue-specific ascertainment is a credible, biologically grounded candidate for part of the disagreement documented in this paper, and treating it as the established or complete explanation would outrun what the literature currently shows.

6. Conclusion

This paper set out to examine why cohort, registry, and case-report evidence on Alzheimer's disease risk in mosaic Down syndrome disagree so substantially, and whether that disagreement is better explained by how mosaicism is measured or by genuine biological heterogeneity among patients. The evidence reviewed here does not resolve that question definitively. It does, however, narrow the range of plausible answers considerably. Full trisomy

21 establishes that Alzheimer's risk in Down syndrome is driven primarily, though not exclusively, by APP dosage (Castro et al.; Fortea et al., "Alzheimer's Disease Associated"). Mosaic Down syndrome, which carries a partial and variable version of that same dosage, has produced three genuinely conflicting characterizations of risk: protective in the largest biomarker-based cohort (Xicota et al.), negligible in the largest population-level registry (Rubenstein et al.), and severe in a small but detailed set of case reports (Ringman et al.; Nuebling et al.; Ngo et al.).

Tissue-specific ascertainment offers a biologically plausible, partial explanation for this pattern. Constitutional mosaicism is known to distribute unevenly across tissues depending on developmental timing (Pagon et al.; Papavassiliou et al.), and this has been directly documented once within the Alzheimer's literature itself (Nuebling et al.). Section 5's evaluation of the alternatives cuts the other way, though: participant age, diagnostic sensitivity, and publication selection could not be ruled out either, and the evidence does not currently eliminate any of them.

Tissue-specific ascertainment is a credible, mechanistically grounded contributor to the disagreement in this literature. It has not been demonstrated to be the primary driver, and it should not be treated as such until better evidence exists. Future work could address this directly. Cohort studies could incorporate buccal or fibroblast mosaicism screening alongside blood karyotyping, allowing tissue discordance to be measured within the same large samples currently limited to blood-based ascertainment. Systematic review of additional case reports, rather than the four relied upon here, could clarify whether the pattern observed in Nuebling et al. is representative or exceptional. Age-matched reanalysis of existing cohort data could help isolate how much of the cohort-versus-case-report gap reflects age differences alone. Future studies could also investigate whether acquired neuronal aneuploidy (Geller and Potter; Granic et al.) contributes to discordance between peripheral and neuronal trisomic burden. Until that evidence exists, the honest position is that this paper has identified a plausible mechanism, not a settled answer.

Works Cited

Alldred, M. J., et al. "Aging with Down Syndrome: Where Are We Now and Where Are We Going?" Journal of Clinical Medicine, vol. 10, no. 20, 2021, p. 4687. https://doi.org/10.3390/jcm10204687.

Castro, P., et al. "Alzheimer's Disease in People with Down's Syndrome: The Prospects for and the Challenges of Developing Preventative Treatments." Journal of Neurology, vol. 264, no. 4, 2017, pp. 804-13. https://doi.org/10.1007/s00415-016-8308-8.

Fortea, J., et al. "Clinical and Biomarker Changes of Alzheimer's Disease in Adults with Down Syndrome: A Cross-Sectional Study." The Lancet, vol. 395, no. 10242, 2020, pp. 1988-97. https://doi.org/10.1016/S0140-6736(20)30689-9.

---. "Alzheimer's Disease Associated with Down Syndrome: A Genetic Form of Dementia." The Lancet Neurology, vol. 20, no. 11, 2021, pp. 930-42. https://doi.org/10.1016/S1474-4422(21)00245-3.

Geller, L. N., and H. Potter. "Chromosome Missegregation and Trisomy 21 Mosaicism in Alzheimer's Disease." Neurobiology of Disease, vol. 6, no. 3, 1999, pp. 167-79. https://doi.org/10.1006/nbdi.1999.0236.

Granic, A., et al. "Alzheimer Aβ Peptide Induces Chromosome Mis-Segregation and Aneuploidy, Including Trisomy 21: Requirement for Tau and APP." Molecular Biology of the Cell, vol. 21, no. 15, 2010, pp. 2640-49. https://doi.org/10.1091/mbc.e09-10-0850.

Handen, B. L., et al. "The Alzheimer's Biomarker Consortium-Down Syndrome: Rationale and Methodology." Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring, vol. 12, no. 1, 2020, p. e12065. https://doi.org/10.1002/dad2.12065.

Hook, E. B. "Exclusion of Chromosomal Mosaicism: Tables of 90%, 95%, and 99% Confidence Limits and Comments on Use." American Journal of Human Genetics, vol. 29, no. 1, 1977, pp. 94-97.

Lott, I. T., and E. Head. "Dementia in Down Syndrome: Unique Insights for Alzheimer Disease Research." Nature Reviews Neurology, vol. 15, 2019, pp. 135-47. https://doi.org/10.1038/s41582-018-0132-6.

Ngo, P. T., et al. "Neuropathology of Trisomy 21 Mosaicism in a Case with Early-Onset Dementia." Alzheimer's & Dementia, vol. 21, no. 1, 2025, p. e14394. https://doi.org/10.1002/alz.14394.

Nuebling, G. S., et al. "Low-Degree Trisomy 21 Mosaicism Promotes Early-Onset Alzheimer Disease." Neurobiology of Aging, vol. 103, 2021, pp. 147.e1-147.e5. https://doi.org/10.1016/j.neurobiolaging.2021.02.021.

Pagon, R. A., et al. "Abnormal Skin Fibroblast Cytogenetics in Four Dysmorphic Patients with Normal Lymphocyte Chromosomes." American Journal of Human Genetics, vol. 31, no. 1, 1979, pp. 54-61.

Papavassiliou, P., et al. "Mosaicism for Trisomy 21: A Review." American Journal of Medical Genetics Part A, vol. 167A, no. 1, 2015, pp. 26-39. https://doi.org/10.1002/ajmg.a.36861.

Ringman, J. M., et al. "Mosaicism for Trisomy 21 in a Patient with Young-Onset Dementia: A Case Report and Brief Literature Review." Archives of Neurology, vol. 65, no. 3, 2008, pp. 412-15. https://doi.org/10.1001/archneur.65.3.412.

Rubenstein, E., et al. "Occurrence of Mosaic Down Syndrome and Prevalence of Co-Occurring Conditions in Medicaid Enrolled Adults, 2016-2019." American Journal of Medical Genetics Part C: Seminars in Medical Genetics, vol. 196, no. 4, 2024, p. e32097. https://doi.org/10.1002/ajmg.c.32097.

Xicota, L., et al. "The Effects of Mosaicism on Biological and Clinical Markers of Alzheimer's Disease in Adults with Down Syndrome." eBioMedicine, vol. 110, 2024, p. 105433. https://doi.org/10.1016/j.ebiom.2024.105433.

Zhou, Y., et al. "Blood-Based Biomarkers for Alzheimer's Disease in Down Syndrome: A Systematic Review and Meta-Analysis." Alzheimer's & Dementia, vol. 21, no. 4, 2025, p. e70135. https://doi.org/10.1002/alz.70135.

Factors Associated with Hospital Admission Among Adolescents With Concussion in U.S. Emergency Departments: A NEISS Analysis, 2021–2025 By Carina Kothari

Abstract

Hospital admission after adolescent concussion is uncommon, but the factors associated with admission among older adolescents are not well characterized. We examined demographic, activity-related, and accompanying-injury characteristics associated with hospital admission among adolescents treated for concussion in U.S. emergency departments.

Using 2021–2025 National Electronic Injury Surveillance System (NEISS) data, we identified 6,674 concussion cases among adolescents aged 15–18 years, of which 6,670 were included in adjusted models after excluding cases with unknown sex. Survey-weighted logistic regression estimated associations between sex, activity group, and hospital admission, with models fitted before and after adding measured injury complexity; age, race, and year were included as covariates.

Hospital admission occurred in 122 cases, approximately 1.4% of survey-weighted emergency department visits. In the model including measured injury complexity, male sex remained associated with admission (OR 2.54, 95% CI 1.58–4.09). Compared with non-sport/other activities, wheeled recreation (OR 3.64, 95% CI 1.72–7.69) and motorized/off-road activities (OR 2.90, 95% CI 1.27–6.58) were associated with higher odds of admission, as was a fracture, hemorrhage, or internal organ injury as a second diagnosis (OR 2.83, 95% CI 1.32–6.07). These same two activity groups were the most likely to involve a second diagnosis of any kind, and adding injury complexity to the model reduced their odds ratios (wheeled recreation from 4.23 to 3.64; motorized/off-road from 3.32 to 2.90), suggesting that part of the higher admission odds in these activities reflects the more serious injuries that accompany them. In an exploratory analysis, we found no evidence that the association between activity group and admission differed by sex (joint Wald $p = .850$).

Isolated concussion rarely led to hospital admission; admitted cases were characterized by accompanying injuries and higher-energy mechanisms. Male sex remained associated with admission after adjustment for measured injury complexity, a difference not explained by the injury information available in NEISS.

Introduction

Sport-related concussion occurs across a wide range of youth sports, with incidence varying by sport and exposure setting (Ingram et al.). Among female youth athletes, concussion incidence also varies across contact and collision sports and is higher during matches than during practice (Ernst et al.). Previous research using the National Electronic Injury Surveillance System (NEISS) has also described concussion patterns among younger youth athletes; in an analysis of nine organized sports among children ages 4–13, most emergency department cases were treated and released, while approximately 9% required hospital admission (Buzas et al.). Broader national surveillance using the National Electronic Injury Surveillance System–All Injury Program (NEISS-AIP) has also been used to examine trends in sports- and

recreation-related traumatic brain injuries across demographic groups and activities in U.S. emergency departments (Coronado et al.). More recently, NEISS data from 2014–2023 were used to examine sports-related concussions with loss of consciousness among youth athletes, demonstrating continued use of the database for national youth concussion surveillance (Gawrys et al.). Clinical guidance emphasizes that concussion in children and adolescents requires careful assessment to exclude more serious injury and individualized, stepwise management during recovery (Halstead et al.; Patricios et al.). These prior studies provide important surveillance context, but it remains less clear which patient, activity, and accompanying-injury characteristics are associated with hospital admission among older adolescents. Identifying these patterns may help distinguish concussion presentations that are more likely to require inpatient care. These factors may not operate in the same way: characteristics such as sex and activity precede the injury event, while accompanying injuries may lie on the pathway between those characteristics and the decision to admit a patient. This study uses NEISS data from 2021–2025 to examine whether demographic, activity-related, and additional injury characteristics are associated with hospital admission.

Methods

Data were obtained from the National Electronic Injury Surveillance System (NEISS) for 2021 through 2025. NEISS is maintained by the U.S. Consumer Product Safety Commission and collects injury data through a national probability sample of hospital emergency departments (U.S. Consumer Product Safety Commission, *National Electronic*). Each case is assigned a statistical weight that allows the sample to contribute to national injury estimates.

The source dataset contained 8,042 emergency department visits for head injuries among adolescents ages 15–18 years. The analytic sample was then restricted to cases with a primary diagnosis of concussion (NEISS diagnosis code 52), yielding 6,674 concussion cases. The primary outcome was hospital admission following the emergency department visit, defined as NEISS disposition code 4 (treated and admitted/hospitalized). Observation cases coded as disposition 5 were classified as not admitted. Predictor variables included age, sex, race, year, sport or activity, and characteristics indicating additional injury complexity.

Sport or activity was classified primarily using the Product 1 field. Activity groups were assigned using rule-based keyword matching of the Product 1 labels. Because Product 1 contained 222 individual product or activity categories and hospital admission was uncommon, categories were collapsed into six broader groups: contact/collision sports, other organized sports, wheeled recreation, motorized/off-road activities, other recreation, and non-sport/other activities. The full mapping of the 222 NEISS Product 1 categories to the six analytic activity groups is provided in Supplementary Table S1. Each group contained at least eight admitted cases. Selected admitted cases classified as non-sport/other were manually reviewed to assess potential misclassification.

Race was collapsed into four analytic categories: White, Black/African American, Other/less frequent, and “Not stated,” after sparse admission cells were identified in several

individual race categories. Four cases with unknown sex were excluded from the multivariable analysis, resulting in an analytic sample of 6,670 cases for the adjusted model.

Additional injury complexity was evaluated first using the presence of a second diagnosis. A refined diagnosis-based measure was then created with three categories: no second diagnosis, other second diagnosis, and fracture/hemorrhage/internal organ injury. The second injured body-part field was reviewed to help interpret these diagnosis categories; notably, 547 of 552 second diagnoses coded as internal organ injury involved the head. According to the January 2024 NEISS Coding Manual (U.S. Consumer Product Safety Commission, *NEISS Coding Manual* 17–18), closed head injury, cerebral contusion, and subdural hematoma may be coded as internal organ injury (code 62) with the head as the body part, suggesting that many of these cases reflect additional brain or head injury rather than injury to an abdominal organ. Age, sex, race, year, and activity were treated as characteristics of the patient or injury-event context. Because accompanying injury may lie on the pathway between sex or activity and hospital admission, injury complexity was treated as a potential mediator rather than solely as a background covariate. Accordingly, models were evaluated both without and with injury complexity.

Descriptive analyses were performed to characterize the study population and compare patient and injury characteristics according to hospital admission status. Cell counts were examined before multivariable modeling to identify sparse categories, and categories were combined where clinically and analytically appropriate.

Because NEISS uses a complex probability sampling design, multivariable logistic regression analyses incorporate the available sampling weights, strata, and primary sampling units. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the association between each predictor and hospital admission. Multilevel categorical predictors were evaluated using joint Wald tests. Candidate models were compared using survey-adjusted Akaike information criterion (AIC) values to assess whether additional predictors improved model fit. Potential interaction terms were considered after completion of the main-effects model. Admission counts were examined before fitting the planned activity × sex interaction. Because several sex-by-activity cells were sparse, activity was collapsed a priori by injury mechanism into three broader groups for the exploratory interaction analysis. The higher-energy recreation group combined wheeled recreation with motorized/off-road activities; the organized sports group combined contact/collision sports with other organized sports; and the everything else group combined other recreation with non-sport/other activities. A joint Wald test was used to evaluate the interaction, and predicted probabilities with 95% confidence intervals were estimated using marginal standardization to aid interpretation.

All statistical analyses were conducted using R version 4.6.1 in Posit Cloud, with complex survey analyses performed using the survey package version 4.5 (R Core Team; Lumley).

Results

Study Population and Descriptive Characteristics

The study included 6,674 adolescent concussion cases treated in U.S. emergency departments from 2021 through 2025. Hospital admission occurred in 122 unweighted cases, corresponding to approximately 1.4% of survey-weighted emergency department visits. Four cases with unknown sex were excluded from the adjusted analysis, leaving 6,670 cases in the multivariable model. Descriptive characteristics of the study population stratified by hospital admission status are presented in Table 1.

Product 1 contained 222 individual product or activity categories. Because hospital admission was uncommon and many individual categories contained few admitted cases, activities were grouped into six broader categories: contact/collision sports, other organized sports, wheeled recreation, motorized/off-road activities, other recreation, and non-sport/other activities. All six groups contained at least eight admitted cases.

Additional injury complexity was classified into three groups: no second diagnosis, other second diagnosis, and fracture/hemorrhage/internal organ injury. Of the 6,674 cases, 4,678 had no second diagnosis, 1,288 had another type of second diagnosis, and 708 had a fracture, hemorrhage, or internal organ injury as the second diagnosis.

Table 1. Characteristics of Adolescent Concussion Cases by Hospital Admission Status, NEISS 2021–2025

Characteristic	**Not admitted N = 204,382**	**Admitted N = 2,948**
Age, years	16 (1)	16 (1)
Sex		
Female	2,811 (42%)	34 (20%)
Male	3,737 (58%)	88 (80%)
Race		
White	3,441 (58%)	57 (41%)
Black/African American	1,121 (13%)	16 (10%)
Other/less frequent	565 (6.6%)	7 (6.5%)
Not stated	1,421 (22%)	42 (43%)
Year		
2021	1,169 (15%)	28 (18%)
2022	1,226 (19%)	24 (16%)
2023	1,293 (20%)	22 (26%)
2024	1,400 (23%)	27 (23%)
2025	1,460 (22%)	21 (17%)
Sport/activity group		
Non-sport/other	1,436 (22%)	21 (16%)
Contact/collision sports	1,968 (30%)	31 (24%)
Other organized sports	2,091 (30%)	18 (13%)

Other recreation	497 (8.9%)	8 (8.7%)
Wheeled recreation	180 (2.5%)	10 (12%)
Motorized/off-road	376 (7.2%)	34 (25%)
Additional injury complexity		
No second diagnosis	4,621 (70%)	55 (49%)
Other second diagnosis	1,258 (21%)	29 (28%)
Fracture/hemorrhage/internal organ injury	669 (9.6%)	38 (23%)

Note. Column headers show survey-weighted national estimates. Categorical variables are presented as unweighted n (survey-weighted %); age is presented as survey-weighted mean (SD). Four cases with unknown sex were excluded from this table, consistent with the analytic sample used for adjusted modeling.

Preliminary and Bivariate Analysis

Chi-square tests were used to examine associations between hospital admission and categorical variables including sex, sport or activity, additional diagnosis status, and year. These relationships were examined further using multivariable logistic regression.

Sport/Activity Variable Findings

Sport or activity information was available through the NEISS Product 1, Product 2, and Product 3 fields. Product 1 contained most sport classifications, including football, soccer, basketball, wrestling, cheerleading, and volleyball. Product 2 was listed as none or not applicable in 5,984 cases, and Product 3 was listed as none or not applicable in 6,597 cases. When Products 2 and 3 were populated, they usually represented secondary objects or surfaces, such as floors, walls, turf, stairs, or bleachers. Product 1 was therefore used as the primary activity field for the current analysis.

Second Diagnosis as a Preliminary Injury-Complexity Indicator

Of the 6,674 concussion cases, 1,996 cases, or 29.9%, had a second diagnosis. The most common second diagnoses were internal organ injury, contusion or abrasion, other or not stated, strain or sprain, laceration, and fracture. Of the 552 second diagnoses coded as internal organ injury, 547, or 99.1%, involved the head. This suggests that many of these cases represented an additional head-related injury, although the exact injury would require further confirmation through diagnosis and narrative fields.

In the unweighted preliminary analysis, hospital admission occurred in 3.36% of cases with a second diagnosis, compared with 1.18% of cases without a second diagnosis. A Pearson chi-square test with Yates' continuity correction showed a statistically significant association between the presence of a second diagnosis and hospital admission, $\chi^2(1) = 35.88$, $p < .001$. In an unweighted, unadjusted logistic regression, cases with a second diagnosis had 2.92 times the odds of hospital admission compared with cases without a second diagnosis (OR = 2.92, 95% CI [2.04, 4.19]). This preliminary result motivated development of the refined three-level injury-complexity variable used in the adjusted model. Because the refined three-level

injury-complexity measure represents a more detailed characterization of second-diagnosis status, the binary second-diagnosis indicator and the three-level injury-complexity measure were not entered together in the final main-effects model.

Adjusted Multivariable Analysis

In the survey-weighted multivariable logistic regression model, sex, activity group, and injury complexity showed interpretable associations with hospital admission after adjustment for age, race, year, and the other model variables.

The association between male sex and hospital admission changed only modestly across models, from an unadjusted OR of 2.81 (95% CI, 1.84–4.30) to 2.60 (95% CI, 1.64–4.14) after adjustment for age, race, year, and activity, and to 2.54 (95% CI, 1.58–4.09) after additionally accounting for measured injury complexity. Male sex remained associated with hospital admission after adjusting for measured injury complexity ($p < .001$).

The activity group was significantly associated with admission overall (joint Wald $p < .001$). Compared with the non-sport/other reference group, the association for wheeled recreation decreased from an unadjusted OR of 6.39 (95% CI, 3.23–12.64) to 4.23 (95% CI, 2.00–8.94) after adjustment for age, sex, race, and year, and to 3.64 (95% CI, 1.72–7.69) after additionally accounting for measured injury complexity. The association for motorized/off-road activities similarly decreased from an unadjusted OR of 4.68 (95% CI, 2.37–9.24) to 3.32 (95% CI, 1.48–7.46) and then to 2.90 (95% CI, 1.27–6.58).

Contact/collision sports, other organized sports, and other recreation were not individually associated with significantly different admission odds compared with the reference group.

Injury complexity was also significantly associated with admission overall (joint Wald $p = .027$). Cases with an accompanying fracture, hemorrhage, or internal organ injury had an unadjusted OR of 3.37 (95% CI, 1.58–7.15), which decreased to 2.83 (95% CI, 1.32–6.07) after adjustment for age, sex, race, year, and activity ($p = .008$). In contrast, other second diagnoses were not significantly associated with admission (OR = 1.38, 95% CI [0.73, 2.60], $p = .318$). White was the reference category for race, and 2021 was the reference category for year. Year was not significantly associated with admission overall (joint Wald $p = .654$). Race was significant in the overall joint Wald test ($p = .019$); however, the strongest individual association occurred in the "Not stated" category rather than an identified racial group, so this finding should be interpreted cautiously.

Table 2. Associations With Hospital Admission Across Survey-Weighted Logistic Regression Models

Predictor	Model A: Unadjusted OR (95% CI)	Model B: Adjusted without injury complexity OR (95% CI)	Model C: Adjusted with injury complexity OR (95% CI)
Male sex	2.81 (1.84–4.30)	2.60 (1.64–4.14)	2.54 (1.58–4.09)
Wheeled recreation	6.39 (3.23–12.64)	4.23 (2.00–8.94)	3.64 (1.72–7.69)
Motorized/off-road	4.68 (2.37–9.24)	3.32 (1.48–7.46)	2.90 (1.27–6.58)

Fracture/hemorrhage/internal organ injury	3.37 (1.58–7.15)	Excluded by design	2.83 (1.32–6.07)

Note. Model A includes each predictor individually using the same survey design and analytic sample. Model B adjusts for age, sex, race, year, and activity but excludes injury complexity. Model C additionally includes injury complexity. Female sex, non-sport/other activity, and no second diagnosis are the respective reference categories. Injury complexity was excluded from Model B because it was treated as a potential mediator between patient/activity characteristics and hospital admission.

Table 3. Injury Complexity by Sex in the Concussion Analytic Sample

Injury complexity	**Female**	**Male**
No second diagnosis	71.2% (n = 2,025)	68.4% (n = 2,651)
Other second diagnosis	19.4% (n = 525)	21.7% (n = 762)
Fracture/hemorrhage/internal organ injury	9.5% (n = 295)	9.9% (n = 412)
Total	**100% (n = 2,845)**	**100% (n = 3,825)**

Note. Percentages are survey-weighted and calculated within each sex; counts are unweighted. Percentages may not sum to exactly 100% because of rounding.

Table 4. Activity Group by Sex in the Concussion Analytic Sample

Activity group	**Female**	**Male**
Non-sport/other	33.5% (n = 955)	13.2% (n = 502)
Contact/collision sports	7.2% (n = 231)	46.0% (n = 1,768)
Other organized sports	41.5% (n = 1,199)	21.1% (n = 910)
Other recreation	12.1% (n = 295)	6.6% (n = 210)
Wheeled recreation	1.6% (n = 61)	3.3% (n = 129)
Motorized/off-road	4.2% (n = 104)	9.8% (n = 306)
Total	**100% (n = 2,845)**	**100% (n = 3,825)**

Note. Percentages are survey-weighted and calculated within each sex; counts are unweighted. Percentages may not sum to exactly 100% because of rounding.

Injury-complexity distributions were similar between sexes, with fracture/hemorrhage/internal organ injury present in 9.5% of females and 9.9% of males. Activity patterns differed more substantially: males were more concentrated in contact/collision sports, wheeled recreation, and motorized/off-road activities. Despite these differences in activity distribution, the association between male sex and hospital admission persisted after adjustment for activity and measured injury complexity.

Injury-complexity distributions also differed across activity groups. Wheeled recreation and motorized/off-road activities had much lower weighted proportions of cases with no second diagnosis (35.5% and 36.1%, respectively) than the other activity groups, while fracture/hemorrhage/internal organ injury accounted for 16.1% and 14.5% of cases, respectively. Both groups also had substantially larger proportions of other second diagnoses (48.4% and

49.4%). This pattern is consistent with the decrease in the wheeled and motorized/off-road odds ratios after injury complexity was added to the regression model.

Table 5. Injury Complexity by Activity Group in the Concussion Analytic Sample

Activity group	No second diagnosis	Other second diagnosis	Fracture/hemorrhage/internal organ injury	Total
Non-sport/other	73.1% (n = 1,056)	17.2% (n = 248)	9.7% (n = 153)	100% (n = 1,457)
Contact/collision sports	76.1% (n = 1,537)	16.3% (n = 308)	7.6% (n = 154)	100% (n = 1,999)
Other organized sports	72.2% (n = 1,516)	17.3% (n = 350)	10.5% (n = 243)	100% (n = 2,109)
Other recreation	68.0% (n = 341)	23.1% (n = 108)	8.9% (n = 56)	100% (n = 505)
Wheeled recreation	35.5% (n = 76)	48.4% (n = 85)	16.1% (n = 29)	100% (n = 190)
Motorized/off-road	36.1% (n = 150)	49.4% (n = 188)	14.5% (n = 72)	100% (n = 410)

Note. Percentages are survey-weighted and calculated within each activity group; counts are unweighted. Percentages may not sum to exactly 100% because of rounding.

Table 6. Adjusted Predicted Probabilities of Hospital Admission by Sex and Injury Complexity

Injury complexity	Female	Male
No second diagnosis	0.59%	1.46%
Other second diagnosis	0.80%	1.99%
Fracture/hemorrhage/internal organ injury	1.62%	3.93%

Note. Predicted probabilities were estimated from the survey-weighted final model using marginal standardization over the observed age, race, year, and activity distributions in the analytic sample.

Adjusted predicted admission probabilities were higher for males within each measured injury-complexity category: 1.46% versus 0.59% with no second diagnosis, 1.99% versus 0.80% with another type of second diagnosis, and 3.93% versus 1.62% with fracture/hemorrhage/internal organ injury. This pattern is consistent with the male association persisting after adjustment for measured injury complexity, although unmeasured differences in injury severity cannot be excluded.

Because admission counts were sparse in several of the original six sex-by-activity groups, activity was collapsed by injury mechanism into higher-energy recreation, organized sports, and everything else for an exploratory interaction analysis. All sex-by-group admission cells contained at least seven events. The activity-by-sex interaction was not statistically significant (joint Wald $F = 0.16$, $df = 2$ and 90, $p = .850$), indicating that we could not detect evidence that the association between activity group and hospital admission differed by sex. Marginally standardized predicted admission probabilities were highest for higher-energy recreation in both females (1.72%, 95% CI 0.77–3.77) and males (5.60%, 95% CI 3.59–8.65).

Table 7. Marginally Standardized Predicted Probabilities of Hospital Admission by Sex and Collapsed Activity Group

Collapsed activity group	Female	Male

Everything else	0.83% (95% CI 0.39–1.76)	2.05% (95% CI 1.02–4.07)
Organized sports	0.45% (95% CI 0.19–1.05)	1.12% (95% CI 0.65–1.90)
Higher-energy recreation	1.72% (95% CI 0.77–3.77)	5.60% (95% CI 3.59–8.65)

Note. Activity groups were collapsed a priori by injury mechanism for the exploratory interaction analysis. Predicted probabilities were estimated using marginal standardization over the observed age, race, year, and injury-complexity distributions. The joint activity-by-sex interaction was not statistically significant (p = .850). Differences between the sex-specific predicted probabilities are descriptive and should not be interpreted as evidence that the activity-admission association differs by sex.

Model Comparison

Candidate-model comparison supported retaining activity groups and injury complexity in the final model. The survey-adjusted AIC decreased from 978.99 for the basic age-and-sex model to 977.52 after adding race and year. Adding the activity group substantially reduced the AIC to 938.55, and adding injury complexity further reduced it to 933.33, the lowest value among the candidate models.

Discussion

The findings suggest that hospital admission after adolescent concussion is associated with both the circumstances of the injury and the presence of more serious accompanying injuries. Wheeled recreation and motorized/off-road activities showed higher odds of admission than the non-sport/other reference group, although these associations became smaller after injury complexity was included in the model. The descriptive injury-complexity analysis further showed that wheeled recreation and motorized/off-road activities had substantially lower proportions of cases with no second diagnosis and higher proportions of fracture/hemorrhage/internal organ injury than most other activity groups. Together, these patterns are consistent with part of the activity-related association operating through more complex accompanying injuries, although they do not establish formal mediation.

Male sex remained associated with hospital admission even after adjustment for activity and measured injury complexity. The injury-complexity distribution was similar between males and females, while males were more concentrated in contact/collision, wheeled, and motorized/off-road activities. This similarity suggests little separation by sex in the measured three-level injury-complexity variable and helps explain why adding it to the model changed the male odds ratio only minimally, from 2.60 to 2.54; however, clinically meaningful severity differences within categories could not be assessed. Predicted admission probabilities were also higher for males within each measured injury-complexity category. Although male adolescents had about 2.5 times the adjusted odds of admission, the absolute difference in standardized admission probability was small in the no-second-diagnosis group: 1.46% for males versus 0.59% for females, an absolute difference of 0.87 percentage points. In the exploratory interaction analysis using three mechanism-based activity groups, however, we could not detect evidence that the association between activity group and hospital admission differed by sex (joint Wald p = .850). Clinically, higher-energy recreation was the highest-probability group in both

sexes, and we found no evidence that the higher admission odds among males were confined to particular activity settings; however, the null interaction does not establish equivalence, and modest interaction effects cannot be excluded. Together, these findings suggest that the observed association between male sex and hospital admission was not explained by the measured injury-complexity variable alone. One possibility is that males experienced greater clinical severity within the same broad activity and injury-complexity categories than the NEISS variables could capture; differences in presentation, clinical decision-making, or thresholds for seeking emergency care could also contribute. Future studies with more detailed clinical severity measures and information on presentation and care-seeking could help determine why the male association persists after adjustment for activity and measured injury complexity.

Because female concussion cases were concentrated in other organized sports and non-sport/other activities rather than in contact/collision sports, prevention efforts for adolescent girls should extend beyond traditionally high-contact sports and include a broader range of athletic and recreational settings (Ernst et al.). For adolescents who sustain concussions in organized sports, management also extends beyond the initial emergency visit; return-to-learn and return-to-sport guidance emphasizes gradual, individualized recovery, while return-to-learn approaches remain variable across studies (Halstead et al.; Patricios et al.; Bishay et al.).

For clinicians and families, hospital admission was uncommon overall—about 1.4% of survey-weighted adolescent concussion emergency-department visits—suggesting that most adolescents were managed without inpatient admission, while accompanying injuries and higher-energy mechanisms may help identify the smaller group more likely to require hospital care. Because NEISS uses a national probability sample and survey weights, these findings describe national patterns among adolescents treated for concussion in U.S. emergency departments rather than patterns from the sampled hospitals alone.

Injury complexity was also associated with admission, particularly when the second diagnosis involved fracture, hemorrhage, or internal organ injury. Year was retained as a period control, and no clear differences in admission odds were observed across the individual study years in the current model. Race should be interpreted cautiously because the overall association was driven primarily by the “Not stated” category rather than by a specific identified racial group.

Limitations

Hospital admission was defined using NEISS disposition code 4, while observation cases coded as disposition 5 were classified as not admitted, which may have excluded some patients who required prolonged emergency department care. Because injury complexity may occur as a consequence of the injury event and act as a mediator between sex or activity and admission, models that adjust for injury complexity may not reflect the total association of these predictors with hospital admission. Activity groups were created using rule-based keyword matching of Product 1 labels, which may have resulted in some misclassification despite spot-checking selected cases. Because NEISS captures injuries treated in emergency departments and does not include sport participation or exposure denominators, these results describe admission patterns

among ED-treated concussion cases and cannot estimate sport-specific concussion incidence or risk in the broader adolescent population. Hospital admission was rare, with 122 admissions relative to 16 model parameters, or about 7.6 events per parameter, which may have contributed to imprecise estimates and wider confidence intervals. NEISS does not include detailed clinical severity measures such as Glasgow Coma Scale scores, imaging findings, or symptom severity, so differences in unmeasured injury severity between patients cannot be excluded. Sparse admission counts within several of the original six sex-by-activity groups required collapsing activity categories by injury mechanism for the exploratory interaction analysis. Although all cells in the collapsed three-group analysis contained at least seven admissions, the limited number of events, particularly among females, reduced power to detect interaction effects.

Conclusion

Among adolescents with concussion treated in U.S. emergency departments from 2021 through 2025, hospital admission was uncommon overall. Wheeled recreation and motorized/off-road activities had higher admission odds and greater accompanying injury complexity, although these activity associations were attenuated after measured injury complexity was included in the model. Male sex remained associated with admission after adjustment for activity and measured injury complexity. Separately, the exploratory interaction analysis did not detect evidence that the association between activity group and admission differed by sex. Concussion cases without a second diagnosis were rarely admitted, while higher-energy mechanisms and more serious accompanying injuries were more common among cases requiring hospital admission. These findings support prevention and clinical attention across the broader athletic and recreational settings in which adolescent concussions occur.

Works Cited

Bishay, Anthony E., et al. "Return-to-Learn Following Sport-Related Concussion: A Systematic Review." Journal of Neurosurgery: Pediatrics, vol. 36, no. 2, 2025, pp. 173–85, https://doi.org/10.3171/2025.1.PEDS24482.

Buzas, David, et al. "Concussions from 9 Youth Organized Sports: Results from NEISS Hospitals over an 11-Year Time Frame, 2002–2012." Orthopaedic Journal of Sports Medicine, vol. 2, no. 4, 2014, article 2325967114528460, https://doi.org/10.1177/2325967114528460.

Coronado, Victor G., et al. "Trends in Sports- and Recreation-Related Traumatic Brain Injuries Treated in US Emergency Departments: The National Electronic Injury Surveillance System-All Injury Program (NEISS-AIP) 2001–2012." Journal of Head Trauma Rehabilitation, vol. 30, no. 3, 2015, pp. 185–97, https://doi.org/10.1097/HTR.0000000000000156.

Ernst, Laura, et al. "Incidence and Risk Factors for Sport-Related Concussion in Female Youth Athletes Participating in Contact and Collision Invasion Sports: A Systematic Review." Sports Medicine, vol. 55, no. 2, 2025, pp. 393–418, https://doi.org/10.1007/s40279-024-02133-x.

Gawrys, Steven P., et al. "Youth Ages 4 to 13 Trends of Sports Concussions with Loss of Consciousness from 2014–2023 National Electronic Injury Surveillance System." Orthopaedic Journal of Sports Medicine, vol. 13, no. 8, 2025, article 23259671251353761, https://doi.org/10.1177/23259671251353761.

Halstead, Mark E., et al. "Sport-Related Concussion in Children and Adolescents." Pediatrics, vol. 142, no. 6, 2018, article e20183074, https://doi.org/10.1542/peds.2018-3074.

Ingram, Veronica, et al. "The Incidence of Sports-Related Concussion in Children and Adolescents: A Systematic Review and Meta-Analysis." Sports Medicine - Open, vol. 11, no. 1, 2025, article 36, https://doi.org/10.1186/s40798-025-00834-9.

Lumley, Thomas. survey: Analysis of Complex Survey Samples. R package version 4.5, 2026.

Patricios, Jon S., et al. "Consensus Statement on Concussion in Sport: The 6th International Conference on Concussion in Sport–Amsterdam, October 2022." British Journal of Sports Medicine, vol. 57, no. 11, 2023, pp. 695–711, https://doi.org/10.1136/bjsports-2023-106898.

R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, 2026, https://doi.org/10.32614/R.manuals.

U.S. Consumer Product Safety Commission. National Electronic Injury Surveillance System (NEISS), www.cpsc.gov/Research--Statistics/NEISS-Injury-Data. Accessed 25 Sept. 2026.

---. NEISS Coding Manual. Jan. 2024, www.cpsc.gov/s3fs-public/January-2024-NEISS-CPSC-only-Coding-Manual.pdf.

Automated Infant Brain Segmentation Using Multimodal MRI By Naisha Sinha

Abstract

Infant brain MRI plays an important role in studying early brain development and diagnosing congenital brain abnormalities. However, infant brain MRI is especially difficult to analyze automatically because developing tissues have low and rapidly changing contrast, making structures harder to distinguish than in adult brains. Here, we demonstrate a slice-based 2D UNet trained on multimodal T1-weighted and T2-weighted MRI from the Baby Open Brains dataset to perform infant brain segmentation with limited computational resources. Strong validation Dice scores were observed on subjects not included in training. Our results indicate that accurate infant brain segmentation can be achieved using a lightweight 2D framework and provide new insight into current limitations and future directions.

Keywords infant brain MRI; automated brain segmentation; multimodal MRI; deep learning; 2D U-Net; T1-weighted MRI; T2-weighted MRI; gray-white matter contrast; isointense phase; Baby Open Brains dataset

Introduction

MRI is a key imaging modality for studying infant brain development because it provides high-resolution anatomical images without exposing infants to ionizing radiation, unlike CT. During the first months after birth, the human brain experiences periods of rapid growth and maturation. Myelination, gray–white matter organization, regionally specific growth patterns, and microstructural development all progress quickly during infancy. Because many long-term neurodevelopmental outcomes are linked to these early processes, abnormal development during infancy has been associated with conditions such as developmental delay, epilepsy, cerebral palsy, and autism spectrum disorder (ASD) (Giedd and Rapoport; Gilmore et al.).

Nonetheless, analyzing infant brain MRI is challenging. MRI studies of the brain from birth to two years of age have revealed unique growth patterns that differ from later stages of life. For example, Knickmeyer et al. found that early development is characterized by an initial dominance of gray matter, followed by disproportionately higher white matter growth during the first years of life. Because of maturation processes such as myelination, these tissue appearance differences cause dramatic changes in image contrast as a function of age. Consequently, analyzing and comparing infant MR images is substantially more difficult than analyzing adult images, particularly for automated methods.

This problem is compounded during the “isointense” phase of infant brain development. During this time, gray matter and white matter have nearly identical signal intensities on both T1-weighted and T2-weighted MRI. Correctly distinguishing tissue boundaries during this phase is challenging, even for trained human experts. Results from both the iSeg-2017 and iSeg-2019 challenges show that segmentation algorithms often struggle to separate infant brain tissues during this phase (Wang et al.; Sun et al.). While deep learning (DL) has revolutionized many

medical image segmentation tasks, existing DL methods have had limited success in infant MRI. Networks trained on smaller or less-diverse datasets frequently overfit to the training data and perform poorly on held-out samples from different scanners or populations (Sun et al.). In addition, a recent systematic review found that low tissue contrast, small quantities of expert-annotated data, and lack of evaluation standardization were major hurdles for progress in automated infant brain segmentation (Mhlanga and Viriri).

Early tissue segmentation methods often depended on atlas-based registration, probabilistic tissue models, and intensity-based clustering. While these methods showed promise in adult MRI, they did not generalize well to infant MRI because of evolving tissue contrast and anatomical variability. More recent work has improved segmentation performance by directly learning multi-scale spatial features from images using convolutional neural networks (CNNs), especially U-Net-based architectures (Mhlanga and Viriri; Ronneberger et al.), and by combining T1-weighted and T2-weighted information to better distinguish tissue boundaries (Wang et al.; Sun et al.). However, many of these techniques use 3D CNNs that require large training datasets and significant computational resources, which may not be available in all research settings. Slice-based 2D training has therefore been explored as a practical alternative when annotated data and computational resources are limited (Mhlanga and Viriri; Ronneberger et al.).

One limiting factor in this field has been the relative scarcity of large publicly available infant brain MRI datasets with verified segmentations. While previous infant brain MRI datasets and atlas-based methods contributed tremendously to the field, many earlier approaches were limited by small sample sizes, evolving tissue contrast, and difficulty generalizing across developmental stages or imaging sites (Wang et al.; Sun et al.; Weisenfeld and Warfield). The recently released Baby Open Brains (BOBs) dataset helps alleviate these problems by including 71 imaging visits from 51 infants between the ages of one and nine months. Each scan is also provided with carefully reviewed segmentation labels from experts (Feczko et al.). These images range from pre- to post-isointense phases of brain development, requiring models trained on this data to handle multiple types of tissue contrast. In addition, both T1-weighted and T2-weighted MRI are provided. Despite these improvements, the dataset is still relatively small, with only 71 imaging visits. Therefore, it remains unclear whether models trained on this data will generalize to other patients, scanners, or populations.

Similarly, T1-weighted and T2-weighted MRI alone do not provide consistent appearance information throughout all of early brain development. For example, tissue boundaries and brightness can vary significantly from month to month. Recent work using quantitative susceptibility mapping (QSM), another MRI-based technique, has shown that gray–white matter contrast may provide complementary information during early brain development (Zhang et al.). This supports the broader idea that combining multiple imaging modalities may help models identify more stable tissue features. In this study, because the BOBs dataset provides paired T1-weighted and T2-weighted MRI, we combine these two modalities as input channels for robust infant brain segmentation.

Therefore, this study aims to evaluate whether a computationally efficient, slice-based 2D U-Net architecture trained on the Baby Open Brains dataset can reliably segment infant brain MRI across early developmental stages. The goal is to assess the feasibility of achieving high segmentation accuracy using limited computational resources while clearly identifying current limitations and opportunities for future improvement.

Methods

This study used paired T1-weighted and T2-weighted MRI scans from the Baby Open Brains dataset as model inputs. A slice-based 2D U-Net architecture was implemented for infant brain tissue segmentation. The T1-weighted and T2-weighted images were provided as separate input channels so that the model could learn complementary tissue-contrast information from both modalities.

All model development and training were performed in Google Colab (Google LLC, Mountain View, CA, USA) using Python 3.12.13 on a Linux 6.6.122+ environment. The model was implemented using PyTorch 2.11.0+cu128 with CUDA 12.8 support. Additional software packages included NumPy 2.0.2 for numerical operations, nibabel 5.4.2 for loading MRI volumes, Matplotlib 3.10.0 for visualization, pandas 2.2.2 for data handling, SciPy 1.16.3 for scientific computing, and scikit-learn 1.6.1 for supporting analysis procedures. Training was performed using an NVIDIA Tesla T4 graphics processing unit (GPU) (NVIDIA Corporation, Santa Clara, CA, USA) with 14.56 GB of GPU memory. The runtime environment had 12 GB of system random access memory (RAM). No chemical reagents, biological specimens, or hazardous materials were directly handled in this computational study.

This study used MRI scans from the Baby Open Brains (BOBs) dataset, which includes 71 imaging visits from 51 infants between one and nine months of age (Feczko et al.). Each visit contains paired T1-weighted and T2-weighted structural MRI volumes, along with an accompanying expert-reviewed segmentation volume. These paired T1-weighted and T2-weighted scans were used as the multimodal input data for the segmentation model.

For this project, the original segmentation labels were collapsed into three classes: background, gray matter, and white matter. Although metadata such as age and sex were available for each scan, these variables were not explicitly used during model training or evaluation.

The data were accessed through OpenNeuro and are Brain Imaging Data Structure (BIDS) compliant (Feczko et al.). MRI volumes were loaded using the nibabel Python package. Minimal preprocessing was performed to keep the pipeline computationally simple and avoid introducing additional variability from manual preprocessing choices. Specifically, each modality, T1-weighted and T2-weighted MRI, was normalized independently by subtracting its mean voxel value and dividing by its standard deviation. A small constant was added to the denominator for numerical stability. Beyond this normalization step, no additional preprocessing steps such as bias-field correction, denoising, skull stripping, or resampling were performed, since several of these steps were already included in the dataset's preprocessing pipeline.

For model training, axial 2D slices were extracted from each 3D MRI volume and used as individual training samples. During training, one axial slice was randomly sampled from each subject-session along the superior–inferior axis. The corresponding T1-weighted and T2-weighted slices were paired as input channels, and the matching segmentation slice was used as the ground truth label. The same slice-based approach was used for validation, using only subjects assigned to the validation split.

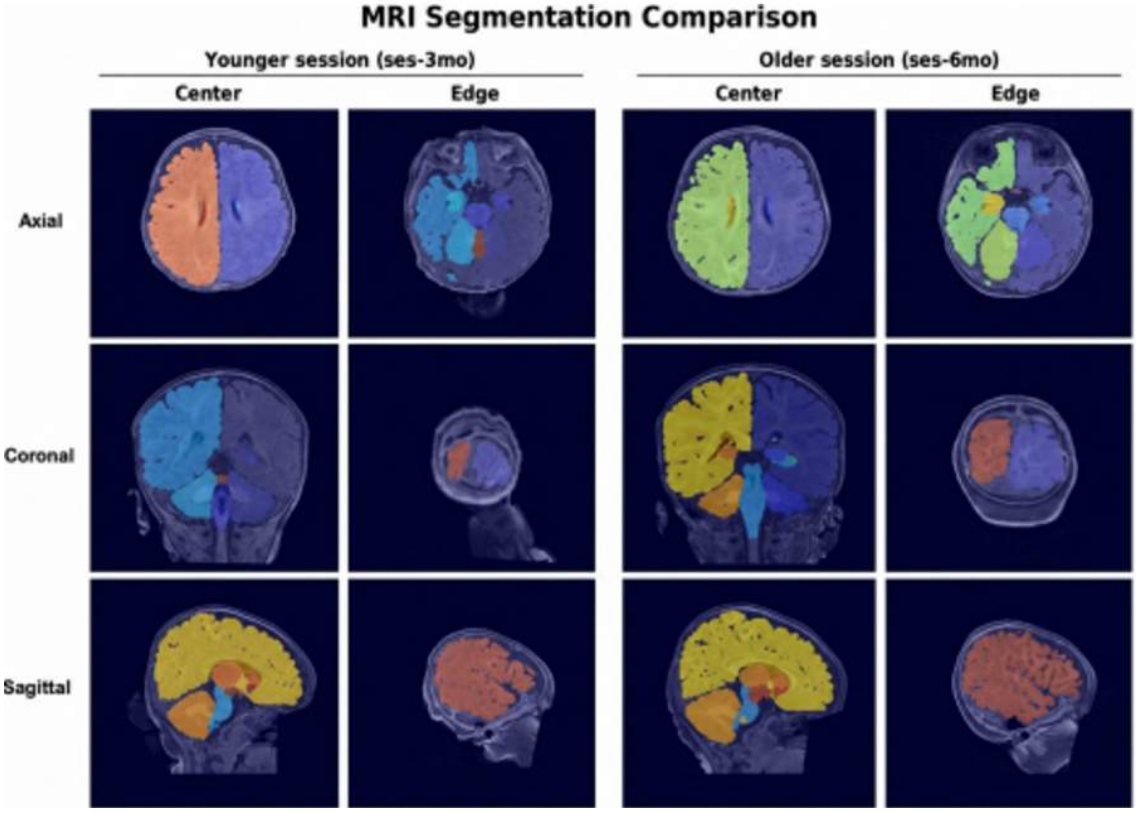


Figure 1. MRI segmentation comparison across sessions and slice locations. Representative axial, coronal, and sagittal MRI slices are shown for the younger session (ses-3mo) and older session (ses-6mo). For each session, both center slices and edge slices are displayed. Center slices refer to slices near the middle of the brain volume, where anatomical structures are larger and easier to visualize. Edge slices refer to slices closer to the outer boundaries of the brain volume, where less tissue is visible and segmentation can be more challenging. Edge slices were included to evaluate whether segmentation labels remained consistent not only in central, information-rich regions, but also in more difficult peripheral regions. Colored overlays indicate segmented anatomical regions superimposed on the MRI images, with different colors used to visually distinguish separate segmentation label classes. The colors are visualization labels rather than MRI intensity values, and apparent color differences across panels reflect the displayed segmentation classes rather than changes in tissue signal. Overall, the overlays demonstrate consistent segmentation patterns across views, slice locations, and session timepoints.

The slices of the volumes were selected to roughly center around the middle of the volume (about 20 slices) to maximize information about gray matter and white matter and minimize information about slices that only had a few slices of brain matter.

Our semantic segmentation network is based on a 2D U-Net architecture originally introduced by Ronneberger et al. It has an encoder–decoder architecture with skip links that

allow spatial information to flow through the network at all scales. The encoder gradually downsamples feature maps through four stages each consisting of two 3×3 convolutions, batch normalization and ReLU activations. After each convolutional stage 2×2 max pooling is applied to downsample the feature maps and double the number of feature channels. The stages of the encoder contain 64, 128, 256 and 512 feature channels respectively. The decoder consists of four stages with symmetrically upsampling operations. After upsampling in each stage the feature maps are concatenated with the feature maps from the encoder at the corresponding stage using skip connections. As a final step 1×1 convolutions are applied to generate per pixel logits for the three classes.

The model was trained in PyTorch using a subject-level 80/20 train-validation split so that validation images came from subjects not seen during training. Training was performed using the Adam optimizer with a learning rate of 1×10^{-3} for 100 epochs. The minibatch size was set based on available system memory.

Training used a loss function consisting of multi-class cross-entropy loss and Dice loss. Cross entropy loss encouraged pixel-wise accurate classification, while Dice loss encouraged better overlaps between masks and ground truth. Dice coefficient was calculated by argmaxing the predicted classes, then taking the mean of the per-class Dice scores.

Loss Equations

Cross-entropy loss equation

$$L_CE = -(1/N) \Sigma_{i=1}^{N} \Sigma^{c}_{=1}{}^{c} y_{i,}{}^{c} \log(p_{i,}{}^{c})$$

Dice loss equation

$$L_Dice = 1 - (1/C) \Sigma^{c}_{=1}{}^{c} [(2\Sigma_{i=1}^{N} p_{i,}{}^{c} y_{i,}{}^{c} + \varepsilon) / (\Sigma_{i=1}^{N} p_{i,}{}^{c} + \Sigma_{i=1}^{N} y_{i,}{}^{c} + \varepsilon)]$$

Combined loss equation

$$L_total = L_CE + L_Dice$$

In these equations, (N) represents the total number of pixels included in the training sample, and (C) represents the number of segmentation classes. The term (y_{i,c}) represents the ground-truth label for pixel (i) and class (c), while (p_{i,c}) represents the model's predicted probability that pixel (i) belongs to class (c). The small constant (\epsilon) was added for numerical stability to prevent division by zero. Combining cross-entropy loss with Dice loss allowed the model to learn both pixel-wise classification accuracy and spatial overlap between predicted and true tissue regions.

Results

Model performance was evaluated using both quantitative metrics and qualitative visual inspection. Training and validation loss and Dice scores were recorded after each epoch across 100 training epochs.

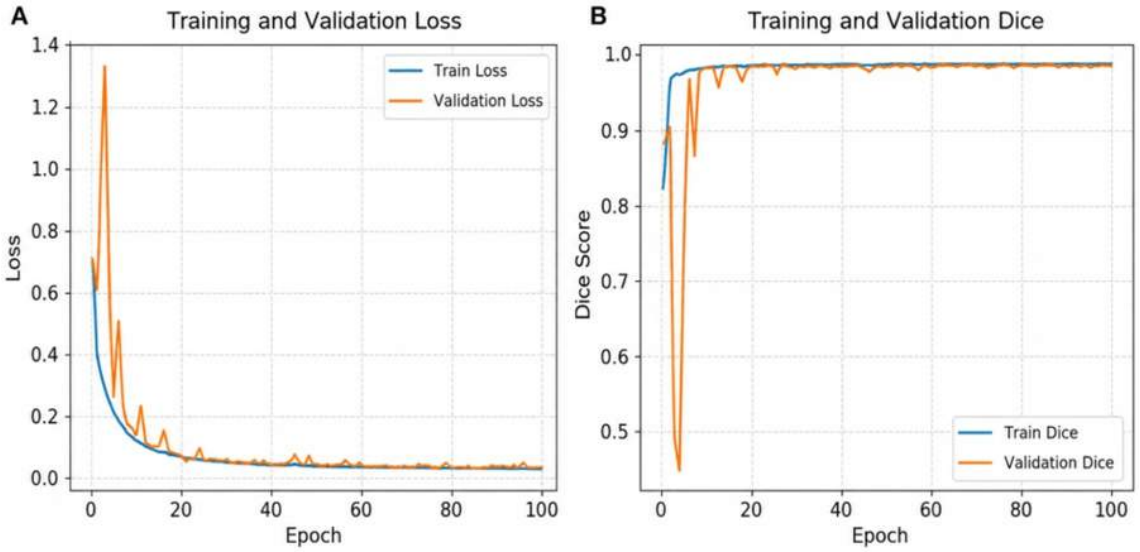


Figure 2. Training performance curves over 100 epochs. (A) Training and validation loss decreased rapidly during the initial epochs and then stabilized at low values, indicating convergence. (B) Training and validation Dice scores increased quickly and remained consistently high, demonstrating strong segmentation performance with minimal overfitting.

The loss on the training set rapidly decreased over training epochs, suggesting successful model parameter optimization (Figure 2A). Loss on the validation data closely mirrored training loss, with small fluctuations after each epoch until approximately 10 epochs when loss values stabilized. Dice scores on the training data rose sharply in initial epochs, with smaller increments at later epochs (Figure 2B). Dice scores on the validation data closely mirrored that of training data, achieving Dice values greater than 0.98 for much of training. Instead of continuing to oscillate, validation scores became stable after several epochs, indicating that the selected learning rate allowed for stable convergence and generalization. Training and validation loss and Dice scores plateaued after roughly 20–30 epochs (Figures 2A and 2B). After this point, only marginal improvements were observed with additional epochs, suggesting that extended training mainly produced minor refinement of segmentation boundaries rather than substantial performance gains. Training time ranged from approximately 2880 to 6606 seconds depending on runtime configuration and hardware availability. This accounts for the processing time of the many multi-modal MRI slices used as well as model training time.

Qualitative segmentation results were generated exclusively from validation subjects to assess generalization. Visual comparisons between input MRI slices, ground-truth segmentations, and model predictions showed strong spatial agreement, particularly in central brain regions where tissue boundaries were well-defined.

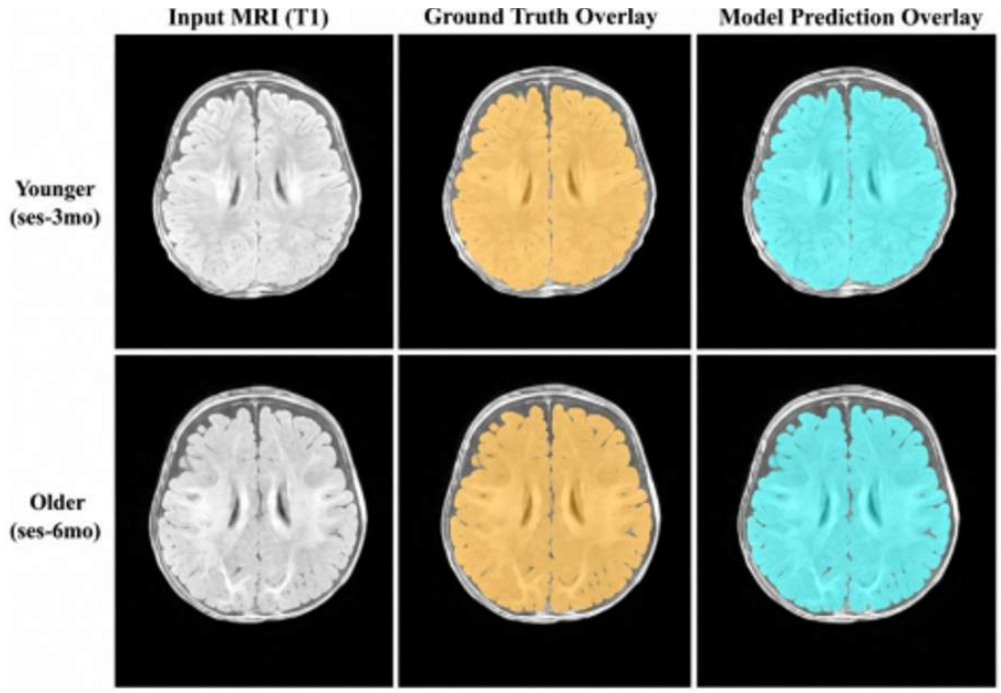


Figure 3. Post-training segmentation performance on representative MRI slices. Axial T1-weighted MRI slices are shown for the younger session (ses-3mo) and older session (ses-6mo). The first column shows the original input MRI, the second column shows the ground-truth binary segmentation overlay, and the third column shows the model-predicted segmentation overlay. For visualization, all non-background labels were merged into a single brain-tissue overlay; the model itself was trained using three classes: background, gray matter, and white matter. The close visual agreement between the ground-truth and predicted overlays demonstrates that the trained U-Net model successfully learned to segment brain tissue across both session timepoints.

Qualitative examples of the post-training segmentations are shown in Figure 3. Ground truth masks and predicted segmentations show strong correspondence in both the younger (left) and older (right) sessions. For visualization purposes, here the segmentation task was approached as a binary classification task with all non-background labels incorporated into a single brain tissue label. The model's predicted segmentation in the younger session follows the overall shape and cortical structure of the brain and cleanly segments brain tissue from background. Similarly, the predicted segmentation in the older session captures the enlarged and more spatially extensive brain structures and agrees strongly with the ground-truth segmentation along their shared boundary. Validation Dice scores above 0.98 were maintained for much of training (Figure 2B). Visual inspection showed that predicted segmentations from the model strongly overlap with ground-truth segmentation masks and the model cleanly separates brain from background with few false positives. Both training and validation loss drops to around 0.04 by the last epoch of training (Figure 2A), further demonstrating low levels of segmentation error and lack of overfitting. The predicted segmentations from each session reflect both the shape and size of their respective ground-truth masks, with highest accuracy along the clearly defined center of the brain. Some errors can be seen along the outer edge of the brain where cortical

borders are less defined and susceptible to partial-volume effects. However, overall segmentation from the model is consistent between age sessions. These results suggest that the 2D U-Net learned spatial representations of infant brain tissue that generalized across both sessions.

Conclusions

This study demonstrated that accurate infant brain MRI segmentation is possible using a lightweight, slice-based 2D U-Net trained on multimodal T1-weighted and T2-weighted MRI from the Baby Open Brains dataset. The network achieved high validation Dice scores on held-out subjects while demonstrating smooth convergence behavior, suggesting that the model was able to generalize across the younger and older infant sessions represented in the dataset.

These findings contribute to the field by showing that reliable infant brain segmentation can be achieved using a computationally efficient 2D convolutional architecture. Infant brain MRI segmentation remains challenging because tissue contrast changes rapidly during early neurodevelopment, especially during the isointense phase, when gray matter and white matter have similar signal intensities. In addition, publicly available infant brain MRI datasets with expert-reviewed segmentations remain limited. Therefore, this study suggests that robust segmentation performance may be achievable even without large-scale training datasets or 3D network architectures. This could make automated infant MRI analysis more accessible to researchers and institutions with limited computational resources.

Predicted segmentations showed strong agreement with ground-truth annotations in both younger and older sessions, illustrating the model's ability to learn anatomical features across developmental changes in tissue contrast. However, several limitations should be considered.

First, because the model was trained on 2D slices rather than full 3D volumes, it did not directly capture spatial continuity across adjacent slices. Although this approach reduced computational requirements, it may have limited the model's ability to learn volumetric relationships between brain structures. In addition, the dataset was relatively small, no data augmentation techniques were applied, and model performance was evaluated at the slice level rather than the full-volume level. Since the data were collected from a limited set of scanners and imaging protocols, performance may not fully generalize to other scanners, protocols, or patient populations.

Future work should evaluate this approach on larger and more diverse infant MRI datasets, including data from multiple sites and patient populations. Future studies should also compare this model with state-of-the-art infant brain segmentation approaches, including 3D segmentation architectures, hybrid 2D/3D models, and multi-plane training strategies. Additional preprocessing steps, data augmentation, and complementary imaging modalities could also be explored to improve performance during low-contrast developmental phases. Taken together, these results suggest that automated infant brain MRI segmentation can be performed effectively using lightweight models and relatively small datasets, while also highlighting the need for further validation across broader clinical and research settings.

Works Cited

Feczko, E., et al. “Baby Open Brains: An Open-Source Dataset of Infant Brain Segmentations.” Scientific Data, vol. 12, no. 1, 2025, doi:10.1038/s41597-025-05404-y.

Giedd, J. N., and J. L. Rapoport. “Structural MRI of Pediatric Brain Development: What Have We Learned and Where Are We Going?” Neuron, vol. 67, no. 5, 2010, doi:10.1016/j.neuron.2010.08.040.

Gilmore, J. H., et al. “Imaging Structural and Functional Brain Development in Early Childhood.” Nature Reviews Neuroscience, vol. 19, no. 3, 2018, doi:10.1038/nrn.2018.1.

Knickmeyer, R. C., et al. “A Structural MRI Study of Human Brain Development from Birth to 2 Years.” Journal of Neuroscience, vol. 28, no. 47, 2008, doi:10.1523/JNEUROSCI.3479-08.2008.

Mhlanga, S. T., and S. Viriri. “Deep Learning Techniques for Isointense Infant Brain Tissue Segmentation: A Systematic Literature Review.” Frontiers in Medicine, vol. 10, 2023, doi:10.3389/fmed.2023.1240360.

Ronneberger, O., et al. “U-Net: Convolutional Networks for Biomedical Image Segmentation.” E-Prints, 2015.

Sun, Y., et al. “Multi-Site Infant Brain Segmentation Algorithms: The iSeg-2019 Challenge.” IEEE Transactions on Medical Imaging, vol. 40, no. 5, 2021, doi:10.1109/TMI.2021.3055428.

Wang, L., et al. “Benchmark on Automatic Six-Month-Old Infant Brain Segmentation Algorithms: The iSeg-2017 Challenge.” IEEE Transactions on Medical Imaging, vol. 38, no. 9, 2019, doi:10.1109/TMI.2019.2901712.

Weisenfeld, N. I., and S. K. Warfield. “Automatic Segmentation of Newborn Brain MRI.” NeuroImage, vol. 47, no. 2, 2009, doi:10.1016/j.neuroimage.2009.04.068.

Zhang, Y., et al. “Neonate and Infant Brain Development from Birth to 2 Years Assessed Using MRI-Based Quantitative Susceptibility Mapping.” NeuroImage, vol. 185, 2019, doi:10.1016/j.neuroimage.2018.10.031.

Interpreting Clinical and Demographic Variation in IgA Nephropathy: A Narrative Review and Illustrative Korean Cohort By Katelyne Kim

Abstract

IgA nephropathy (IgAN), also known as Berger's disease, is a primary glomerular disease characterized by the deposition of immunoglobulin A-containing immune complexes in the kidneys. Its clinical presentation and progression vary among patients, and published studies describe differences in demographic characteristics, symptoms, disease severity, and renal outcomes across patient populations. These variations make small descriptive cohorts difficult to interpret because characteristics observed within one sample cannot necessarily be generalized to a broader population. This narrative review examines demographic and clinical variation in IgAN and uses questionnaire-based data from 21 biopsy-confirmed patients from a Korean kidney center as an illustrative clinical cohort. In this cohort, 17 of 21 participants were male, the mean age was 41.05 ± 16.72 years, and most patients were classified as stage 1 or stage 2. Hematuria and proteinuria were frequently observed, while blood-pressure-related findings and other demographic, clinical, and lifestyle characteristics were also recorded. These observations describe the cohort but do not establish population-level prevalence, risk factors, or associations. Instead, the cohort illustrates both the value and limitations of small descriptive studies and identifies questions that require larger comparative and longitudinal investigation. Future research should examine whether demographic and clinical characteristics differ meaningfully across patient populations and determine which characteristics are associated with disease severity and progression.

Keywords: IgA nephropathy, Berger's disease, hematuria, proteinuria, renal biopsy, hypertension, clinical heterogeneity

Introduction

IgA nephropathy, commonly known as Berger's disease, is a chronic kidney disease associated with the accumulation of immunoglobulin A (IgA) in the kidneys (Al-Karawi and Kadhim). IgA contributes to immune protection, particularly at mucosal surfaces (Wyatt and Julian). In IgA nephropathy, abnormalities in IgA1 contribute to immune dysfunction. Galactose-deficient IgA1 can be recognized by autoantibodies, leading to the formation of circulating immune complexes (Gentile et al.; Nihei et al.). These complexes can become deposited in the glomeruli and accumulate within the mesangium, contributing to inflammation and kidney damage (Murray and Paolini).

Clinical presentation can vary considerably. Reported manifestations include hematuria, proteinuria, hypertension, fatigue, swelling, and other findings associated with impaired kidney function (Escobar et al.). IgAN has been reported across different geographic populations, while disease frequency and demographic patterns vary among studied groups (Borzych-Dużałka et al.; Jakubowska et al.). Differences have also been reported according to age and sex, and IgAN can

occur in both children and adults (Alladin-Karan et al.; Sim et al.). These variations make demographic observations potentially informative, but they also make findings from individual patient groups difficult to generalize.

Clinical characteristics likewise vary with disease presentation and progression. Hematuria is commonly associated with IgAN, while proteinuria, reduced glomerular filtration rate (GFR), hypertension, and progressive renal impairment may provide clinically important information about disease activity and outcomes (Sim et al.; Suzuki). Some patients also report a family history of IgAN, suggesting that genetic factors may contribute in some cases, although disease development is not explained by familial transmission alone (Jakubowska et al.; Stoneman et al.). Renal biopsy remains central to identifying and characterizing biopsy-proven IgAN (Xu, Wu, and Li).

The variation reported across patients creates an important problem for interpreting descriptive clinical research. A characteristic may occur frequently in a particular group of patients without being unusually common in the broader IgAN population. Likewise, two patient cohorts may differ because of disease severity, age, sex, geographic population, referral patterns, or other characteristics rather than because one factor independently explains IgAN occurrence or progression. Small clinical cohorts can therefore provide useful descriptions while remaining insufficient for establishing population-level associations.

This paper examines demographic and clinical variation in IgA nephropathy through a narrative review of existing literature and an illustrative cohort of 21 biopsy-confirmed patients from a Korean kidney center. The cohort is not used to establish population-level prevalence or causal relationships. Instead, its demographic and clinical observations are considered alongside previously published findings to illustrate what small descriptive cohorts can reveal, what they cannot determine, and which questions require more meaningful comparative and longitudinal research.

Narrative Review

Demographic Variation in IgA Nephropathy

IgA nephropathy occurs across different ages and geographic populations, but published studies report variation in its demographic distribution. Research has examined IgAN among young adults in the United States, while other studies have investigated differences associated with sex, age, and geographic population (Nair and Walker; Wen et al.; Geddes et al.; Donadio and Grande; Duan et al.). Wen et al. examined sex disparities among Chinese patients with IgAN, while Geddes et al. examined IgAN across populations in a tricontinental study. Together, these studies support interpreting demographic characteristics within the populations from which they were observed rather than assuming that one cohort represents all IgAN patients.

Age is another important consideration. IgAN occurs in both children and adults, and age may be related to differences in presentation and disease course (Alladin-Karan et al.; Duan et al.). However, age distributions observed in an individual clinical cohort may also depend on the

population sampled. The average age of one group is therefore most informative as a description of that group unless a study is designed to compare age distributions or examine their relationship with disease outcomes.

Familial occurrence adds another dimension to demographic interpretation. Some patients with IgAN report a family history of the disease, but current reviews describe IgAN as a disease influenced by multiple interacting immune and pathogenic mechanisms rather than simple familial transmission (Stoneman et al.; Stamellou et al.; Cheung et al.; Habas et al.). A low or high frequency of reported family history within a small cohort therefore cannot by itself establish the importance or absence of genetic contribution.

Together, these findings show why demographic description and demographic inference must be distinguished. Sex, age, geographic population, and family history may all be meaningful characteristics to investigate, but their frequency within an isolated sample does not establish that they are associated with the occurrence or progression of IgAN.

Clinical Presentation and Disease Severity

Clinical heterogeneity is also an important characteristic of IgAN. Hematuria is a common manifestation, while proteinuria, hypertension, reduced GFR, and other measures of renal function may provide information about disease severity or progression (Stamellou et al.; Zand et al.). Patients may therefore share a diagnosis while differing considerably in clinical presentation and subsequent outcomes.

Disease stage provides one possible framework for investigating these differences. Earlier disease and more advanced renal impairment may be accompanied by different combinations of clinical findings. Recent research has increasingly focused on predicting IgAN risk and progression using clinical markers and other patient characteristics (Suzuki; Xu and Ge). Long-term outcomes such as chronic kidney disease progression and kidney failure further demonstrate the importance of distinguishing characteristics present at diagnosis from characteristics associated with later progression (Sim et al.).

The same distinction applies to individual symptoms. Hematuria has been examined as a potential marker related to disease progression, while proteinuria, hypertension, and renal-function measures also have clinical importance (Zand et al.; Stamellou et al.). Observing these characteristics in a group of IgAN patients confirms their presence in that cohort, but determining whether they are associated with disease severity or progression requires comparisons among patients and, ideally, longitudinal follow-up.

Other recorded characteristics require similar caution. Kidney disease has been studied in relation to glucose levels, while alcohol consumption has been examined in relation to kidney injury more broadly (Jung; Yokus et al.). Such literature may justify studying these variables, but their presence within a group of IgAN patients cannot establish that they contributed to the disease. An appropriate comparison group or longitudinal design would be required before such observations could support stronger conclusions.

Interpreting Descriptive Clinical Cohorts

Descriptive cohorts remain useful because they document characteristics of actual patient populations and may identify observations worth investigating further. Their value, however, depends on matching conclusions to the information the study design can support. Observing a high proportion of male patients describes the sex distribution of a cohort; it does not independently demonstrate that males are at greater risk. Likewise, observing hypertension, alcohol consumption, or a particular disease stage does not demonstrate that these characteristics caused IgAN or are disproportionately represented among IgAN patients.

Small samples create an additional difficulty because potentially informative variables cannot always be stratified reliably. A cohort may collect disease stage, sex, age, GFR, proteinuria, hematuria, and blood-pressure findings, yet contain too few patients within individual subgroups to determine whether these characteristics are related. In this setting, the cohort can serve a different purpose: it can illustrate the kinds of relationships that larger comparative studies should investigate. The following clinical cohort is considered in that more limited role.

Materials and Methodology

Existing de-identified questionnaire-based data were available from 21 patients with IgA nephropathy treated at a kidney center in the Republic of Korea. The questionnaire recorded 12 parameters, including age, sex, family history, stage of kidney disease, blood-pressure-related information, medical conditions, liver disease, alcohol consumption, urinary tract infection, symptoms of IgA nephropathy, diagnostic testing, and fasting blood glucose.

The present analysis uses these data descriptively. Counts, percentages, means, and standard deviations are reported where available. Because the cohort contains only 21 patients and lacks a non-IgAN comparison group or longitudinal follow-up, the observations are not used to establish population prevalence, risk factors, causal relationships, or predictors of disease progression.

Results

Demographic Characteristics of the Cohort

The cohort included 21 patients with a mean age of 41.05 ± 16.72 years. The 17 male patients had a mean age of 44.00 ± 16.40 years, while the four female patients had a mean age of 28.50 ± 12.90 years.

Seventeen of the 21 patients (80.95%) were male and four (19.05%) were female. This 17:4 distribution describes the patients included in the sample and should not be interpreted independently as evidence of male predominance or increased male risk in the broader Korean IgAN population.

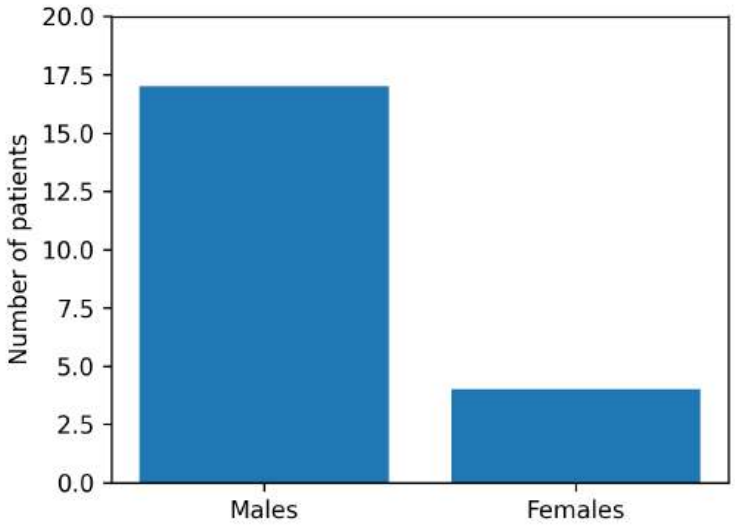


Fig. 1: Number of patients in the illustrative cohort by sex

One patient (4.76%) reported a family history of IgA nephropathy, while 20 patients (95.24%) did not.

Disease Stage and Clinical Characteristics

Ten patients (47.61%) were classified as stage 1, seven (33.33%) as stage 2, two (9.52%) as stage 3, and two (9.52%) as stage 4. Thus, 17 of the 21 patients were classified within stages 1 or 2, while only four patients were represented across stages 3 and 4.

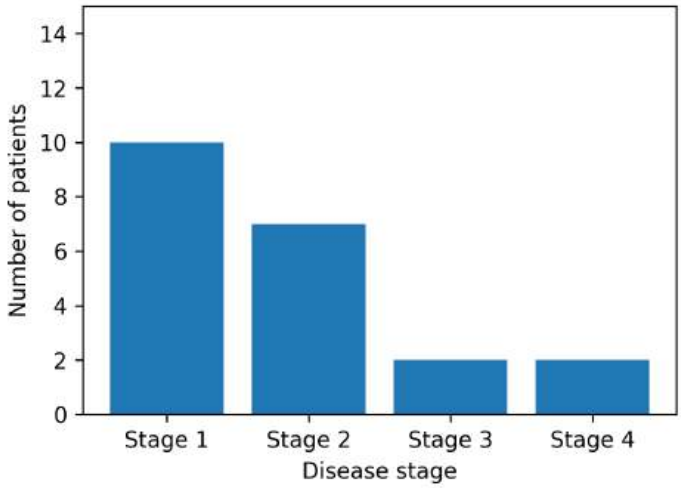


Fig. 2: Disease-stage distribution of patients in the illustrative IgA nephropathy cohort.

The small number of patients in later disease stages limits meaningful internal comparison between early- and later-stage disease. Although disease stage could provide a clinically relevant basis for comparing demographic characteristics, renal-function measures, or

symptoms, the present cohort does not contain sufficient numbers across stages for reliable stratification.

Blood-pressure-related findings were recorded in 18 of 21 patients (85.71%), while three patients (14.29%) did not have the recorded finding. Hematuria was recorded in all 17 male and all four female patients. Proteinuria was recorded in nine male patients and three female patients. Four male patients had reduced GFR recorded, while no female patients did. Two male patients had hypertension recorded in the symptom data, while no female patients did. These frequencies characterize the present cohort but do not demonstrate relationships between these findings and disease severity or progression.

Other Recorded Characteristics

Fourteen patients (66.67%) reported alcohol consumption, while seven (33.33%) did not. One patient (4.76%) had a recorded additional disease, identified as cerebral infarction. No patients had recorded symptoms of urinary tract infection.

Mean fasting blood glucose was 106.76 ± 10.75 mg/dL among male patients and 104.00 ± 7.87 mg/dL among female patients. Given the substantial imbalance between the male and female groups, particularly the presence of only four female participants, these values are reported descriptively rather than used to infer a sex-based difference.

Renal biopsy was recorded as the diagnostic procedure for all 21 patients.

Table 1: Selected demographic and clinical characteristics of the illustrative cohort

Characteristic	Observation
Total patients	21
Mean age	41.05 ± 16.72 years
Male	17/21 (80.95%)
Female	4/21 (19.05%)
Family history of IgAN	1/21 (4.76%)
Stage 1	10/21 (47.61%)
Stage 2	7/21 (33.33%)
Stage 3	2/21 (9.52%)
Stage 4	2/21 (9.52%)
Blood-pressure-related finding	18/21 (85.71%)
Reported alcohol consumption	14/21 (66.67%)
Additional disease recorded	1/21 (4.76%)
UTI symptoms recorded	0/21
Renal biopsy	21/21

Discussion

Demographic Observations in the Context of Published Literature

The illustrative cohort demonstrates several characteristics that have also been examined in previously published IgAN populations. Its mean age was 41.05 ± 16.72 years, while the male and female groups differed numerically in mean age. Wen et al. examined age and sex among

Chinese patients with IgAN, while other studies have described demographic variation among IgAN populations (Nair and Walker; Wen et al.; Geddes et al.; Donadio and Grande; Duan et al.).

The most visible demographic characteristic of the present cohort is its 17:4 male-to-female distribution. Published studies have reported male-skewed IgAN populations (Geddes et al.; Donadio and Grande; Duan et al.). However, similarity between this cohort and previously published observations should not be interpreted as evidence that the present study independently demonstrates male predominance. The cohort contains only 21 patients selected from a clinical population, and no comparison population was included. The sex distribution therefore describes this sample rather than establishing sex-specific prevalence or risk.

Family history illustrates the same problem. Only one patient reported a family history of IgAN. Although existing literature recognizes genetic and immune mechanisms involved in IgAN (Stoneman et al.; Stamellou et al.; Cheung et al.; Habas et al.), the low frequency of reported family history in this sample cannot demonstrate an absence of familial or genetic contribution. It establishes only that reported family history was uncommon among these 21 patients.

Disease Stage and Clinical Presentation

Most patients in the cohort were classified as stage 1 or stage 2. This distribution should not be interpreted as demonstrating that IgAN is generally diagnosed early in the broader Korean population, nor can it establish that the observed distribution resulted from earlier reporting or improved medical infrastructure.

Instead, the stage distribution reveals a useful direction for further study. Disease stage could provide a clinically meaningful basis for examining whether proteinuria, reduced GFR, blood-pressure findings, age, sex, or other characteristics differ as disease becomes more advanced. Research on clinical markers, risk prediction, and disease progression supports the importance of examining such relationships (Sim et al.; Suzuki; Xu and Ge). However, only four patients in the present cohort were represented across stages 3 and 4. Meaningful stage-based comparisons would therefore require a substantially larger sample.

Hematuria and proteinuria were common in the cohort, consistent with their established clinical relevance in IgAN (Stamellou et al.; Zand et al.). Reduced GFR was also recorded in some patients. These observations demonstrate that recognized IgAN manifestations were present among the sampled patients, but their frequencies cannot establish whether any individual characteristic was associated with disease severity or progression.

A larger cohort would allow a more useful question to be asked: rather than determining simply how many patients experienced hematuria or proteinuria, researchers could examine whether proteinuria, reduced GFR, blood-pressure findings, or combinations of clinical characteristics are more common among patients with more advanced disease. Longitudinal observation could then determine whether characteristics present at baseline are associated with subsequent progression.

Blood-Pressure Findings and Other Recorded Characteristics

Blood-pressure-related findings were recorded in 18 of the 21 patients. Hypertension and renal impairment have been discussed extensively in the IgAN literature (Stamellou et al.; Cheung et al.; Habas et al.). Nevertheless, the frequency observed here cannot demonstrate that blood pressure was associated with IgAN occurrence, severity, or progression. Every participant already had IgAN, and the study contained neither a control population nor longitudinal outcomes.

Alcohol consumption presents an especially clear example of the limits of descriptive inference. Fourteen of the 21 participants reported alcohol consumption. Existing literature has examined relationships between alcohol consumption and kidney injury (Yokus et al.), making alcohol exposure a potentially relevant characteristic to record. However, the present study cannot identify alcohol consumption as a causative agent or risk factor for IgAN. Without an appropriate comparison population, it cannot determine whether alcohol consumption was unusually common among IgAN patients. The available questionnaire data also do not establish the frequency, quantity, duration, or pattern of alcohol exposure necessary for a more detailed analysis.

Similarly, fasting blood glucose values differed only slightly between the recorded male and female groups. Although glucose and kidney disease have been studied elsewhere (Jung), comparing 17 male patients with only four female patients does not provide a strong basis for a sex-specific inference in this cohort. The glucose measurements are therefore better treated as descriptive characteristics than as evidence of a meaningful difference.

The single recorded case of cerebral infarction and absence of reported UTI symptoms should be interpreted with comparable caution. One event in a 21-patient cohort cannot establish an association, while the absence of an event does not demonstrate that it is unrelated to IgAN. These observations remain part of the cohort description but do not independently support broader conclusions.

Finally, all patients underwent renal biopsy. This is consistent with the use of biopsy in identifying biopsy-proven IgAN populations (Xu, Wu, and Li). Because the sample itself consisted of diagnosed patients from a clinical setting, however, the uniform use of biopsy primarily describes how this cohort was identified rather than representing a novel diagnostic finding.

Limits of Descriptive Inference

The distinction between observation and inference is central to interpreting this cohort. Several findings are clear descriptions: 17 of 21 patients were male, 17 were classified as stage 1 or 2, 18 had a recorded blood-pressure-related finding, 14 reported alcohol consumption, hematuria and proteinuria were common, and renal biopsy was recorded for all patients. These observations do not establish broader prevalence, risk, causation, or progression.

This limitation does not make descriptive data without value. Rather, it changes the question that the data can reasonably address. Instead of asking whether these 21 patients

establish new population-level characteristics of IgAN, the cohort can identify potentially meaningful variables and illustrate why they require more robust comparison.

Disease stage provides a particularly clear example. The dataset contains multiple variables that could theoretically be compared across disease severity, including age, sex, proteinuria, hematuria, GFR, and blood-pressure findings. Yet only four patients occupy stages 3 and 4. The cohort therefore demonstrates both why stage-based analysis could be informative and why a substantially larger sample would be necessary to perform it reliably.

Directions for Comparative Research

The observations in this cohort suggest several ways in which future IgAN research could move beyond isolated descriptive frequencies. Larger cohorts would permit clinically meaningful stratification. Rather than reporting disease stage, proteinuria, GFR, blood-pressure findings, age, and sex separately, researchers could examine whether these characteristics differ between earlier- and later-stage disease. Such comparisons could help distinguish characteristics that are simply common among IgAN patients from those that may be associated with greater disease severity.

Comparative populations would also improve interpretation of demographic and lifestyle characteristics. If researchers seek to determine whether characteristics such as sex distribution or alcohol exposure are disproportionately represented among IgAN patients, those patients must be compared with an appropriate reference population. A finding that occurs frequently within IgAN patients alone does not establish that it occurs more frequently than expected.

Larger multicenter cohorts could help determine whether patterns observed in one clinical population remain consistent across different populations. Existing literature has examined geographic and demographic variation in IgAN (Nair and Walker; Wen et al.; Geddes et al.; Donadio and Grande; Duan et al.). Comparing similarly collected variables across populations could help distinguish broadly recurring disease characteristics from patterns that vary according to patient population or clinical setting.

Finally, longitudinal research would provide information that a cross-sectional questionnaire cannot. Following patients over time could allow investigators to examine whether baseline characteristics such as proteinuria, reduced GFR, blood-pressure findings, disease stage, age, or other measured factors are associated with later progression. This would move research from describing what characteristics coexist within a patient group toward investigating which characteristics may have prognostic importance.

The present cohort is too small to answer these questions reliably. Its value instead lies in demonstrating how quickly a descriptive dataset becomes limited when researchers attempt meaningful stratification. Although 21 patients can describe the characteristics observed within the sample, dividing those patients by disease stage, sex, renal-function findings, or other variables produces groups too small to support broader inference. Future studies should therefore be designed around the comparisons they intend to make rather than collecting individual variables without sufficient sample sizes to examine relationships among them.

Limitations

Several limitations restrict interpretation of the illustrative cohort. The sample included only 21 patients from a clinical population, limiting generalizability and preventing reliable analysis of smaller subgroups. The sex distribution was substantially uneven, with 17 male and four female participants, making sex-based comparisons particularly limited.

The data were cross-sectional and questionnaire-based. Consequently, they cannot establish temporal relationships or determine whether recorded characteristics preceded, followed, or changed with disease progression. Several variables were recorded categorically, limiting more detailed evaluation of their magnitude or duration.

The cohort also lacked a comparison group. This prevents determination of whether demographic, lifestyle, or clinical characteristics observed among these patients occurred more frequently than in otherwise comparable individuals without IgAN. Likewise, the absence of longitudinal outcomes prevents investigation of which characteristics were associated with subsequent progression.

These limitations are especially important because many observations in the cohort resemble characteristics already reported in IgAN literature. The present sample should therefore be interpreted as an illustrative clinical cohort rather than independent evidence establishing the prevalence, causes, or predictors of IgAN.

Conclusion

IgA nephropathy demonstrates substantial clinical and demographic variation across patients and studied populations. The 21-patient Korean cohort examined in this paper provides a descriptive example of that variation, including differences in age and sex, disease stage, blood-pressure-related findings, hematuria, proteinuria, renal-function findings, and other recorded characteristics. However, the small sample, absence of a comparison population, and cross-sectional design prevent these observations from establishing population-level prevalence, risk factors, or associations.

The cohort is therefore most informative when its findings are interpreted alongside existing IgAN literature rather than treated as independent evidence of broader disease patterns. Its concentration of patients in stages 1 and 2 particularly illustrates the difficulty of investigating relationships between clinical characteristics and disease severity within small samples. Larger comparative cohorts could examine whether demographic and clinical patterns differ across disease stages and patient populations, while longitudinal studies could investigate which characteristics are associated with progression.

Small descriptive cohorts can contribute to research when their evidentiary limits are explicit. The value of the present cohort lies not in establishing conclusions beyond its scale, but in helping identify the comparisons that require stronger investigation.

Works Cited

Al-Karawi, A. S., and A. S. Kadhim. “Correlation of Autoimmune Response and Immune System Components in the Progression of IgA Nephropathy: A Comparative Study.” Human Immunology, vol. 85, no. 6, 2024, article 111181.

Alladin-Karan, A., et al. “Is Childhood IgA Nephropathy Different From Adult IgA Nephropathy? A Narrative Review.” Canadian Journal of Kidney Health and Disease, vol. 12, 2025, article 20543581251322571.

Borzych-Dużałka, D., et al. “Regional Variation of Underlying Kidney Diseases in Children Undergoing Chronic Kidney Replacement Therapy around the Globe.” Pediatric Nephrology, vol. 41, no. 5, 2026, pp. 1451–1463.

Cheung, C. K., et al. “The Pathogenesis of IgA Nephropathy and Implications for Treatment.” Nature Reviews Nephrology, vol. 21, no. 1, 2025, pp. 9–23.

Donadio, J. V., and J. P. Grande. “IgA Nephropathy.” New England Journal of Medicine, vol. 347, no. 10, 2002, pp. 738–748.

Duan, Z. Y., et al. “Aging Promotes Progression of IgA Nephropathy: A Systematic Review and Meta-Analysis.” American Journal of Nephrology, vol. 38, no. 3, 2013, pp. 241–252.

Escobar, S. C., M. A. Alhendy, and D. Vicsocsean. “An Atypical Presentation of Pediatric IgA Vasculitis: Early Hypertension and Severe Systemic Manifestations Despite Initially Normal Urinalysis.” Cureus, vol. 18, no. 3, 2026.

Geddes, C. C., et al. “A Tricontinental View of IgA Nephropathy.” Nephrology Dialysis Transplantation, vol. 18, no. 8, 2003, pp. 1541–1548.

Gentile, M., et al. “Immune Abnormalities in IgA Nephropathy.” Clinical Kidney Journal, vol. 16, no. 7, 2023, pp. 1059–1070.

Habas, E., et al. “IgA Nephropathy Pathogenesis and Therapy: Review & Updates.” Medicine, vol. 101, no. 48, 2022, article e31219.

Jakubowska, Z., et al. “IgA Nephropathy: Epidemiology, Outcomes, and Insights for Primary Glomerulonephritides.” Journal of Clinical Medicine, vol. 15, no. 5, 2026, article 2046.

Jung, H. H. “Evaluation of Serum Glucose and Kidney Disease Progression among Patients with Diabetes.” JAMA Network Open, vol. 4, no. 9, 2021, article e2127387.

Murray, I. V., and M. A. Paolini. “Histology, Kidney and Glomerulus.” StatPearls [Internet], StatPearls Publishing, 2023.

Nair, R., and P. D. Walker. “Is IgA Nephropathy the Commonest Primary Glomerulopathy among Young Adults in the USA?” Kidney International, vol. 69, no. 8, 2006, pp. 1455–1458.

Nihei, Y., H. Suzuki, and Y. Suzuki. “Current Understanding of IgA Antibodies in the Pathogenesis of IgA Nephropathy.” Frontiers in Immunology, vol. 14, 2023, article 1165394.

Sim, J. J., et al. “CKD Progression, Kidney Failure, and Mortality among US Patients with IgA Nephropathy.” Nephrology Dialysis Transplantation, vol. 40, no. 11, 2025, pp. 2104–2117.

Stamellou, E., et al. “IgA Nephropathy.” Nature Reviews Disease Primers, vol. 9, no. 1, 2023, article 67.

Stoneman, S., J. W. Teh, and M. M. O’Shaughnessy. “IgA Nephropathy in Adults: A Review.” JAMA, 2026.

Suzuki, Y. “Principles for Optimal Drug Selection in IgA Nephropathy—Time for Integrating Pathogenesis and Clinical Markers to Guide Therapy.” Nephrology, vol. 31, no. 2, 2026, article e70168.

Wen, D., et al. “Sex Disparities in IgA Nephropathy: A Retrospective Study in Chinese Patients.” International Urology and Nephrology, vol. 53, no. 2, 2021, pp. 315–323.

Wyatt, R. J., and B. A. Julian. “IgA Nephropathy.” New England Journal of Medicine, vol. 368, no. 25, 2013, pp. 2402–2414. https://doi.org/10.1056/NEJMra1206793.

Xu, H., and S. Ge. “Risk Prediction in IgA Nephropathy: From Conventional Models to Machine Learning, Deep Learning, and Precision Nephrology.” Renal Failure, vol. 48, no. 1, 2026, article 2613606.

Xu, L., C. Wu, and G. Li. “Prevalence and Trend of Biopsy-Proven IgA Nephropathy in China: A Systematic Review.” Journal of Nephrology, vol. 38, no. 6, 2025, pp. 1557–1564.

Yokus, B., et al. “The Link between Alcohol Consumption and Kidney Injury.” The American Journal of Pathology, 2025.

Zand, L., F. C. Fervenza, and R. Coppo. “Microscopic Hematuria as a Risk Factor for IgAN Progression: Considering This Biomarker in Selecting and Monitoring Patients.” Clinical Kidney Journal, vol. 16, suppl. 2, 2023, pp. ii19–ii27.

Recent Advances in Prion Disease Diagnosis and Therapy By Ananya Borkar

Abstract

Prion diseases are rare, fatal neurodegenerative disorders caused by the misfolding of the normal prion protein, PrP^{C}, into a self-propagating pathogenic form, PrP^{Sc}. Since their discovery, these diseases have been difficult to diagnose and impossible to treat. This paper examines two developments that have begun to change that picture: the diagnostic assay RT-QuIC (Real-Time Quaking-Induced Conversion), and a set of PrP-lowering and immunotherapy strategies now advancing into human clinical trials. RT-QuIC detects pathologic prion protein in cerebrospinal fluid, nasal mucosa, or skin samples with high sensitivity and near-perfect specificity, and its incorporation into diagnostic criteria has substantially raised the accuracy of antemortem CJD diagnosis. On the treatment side, strategies that lower the amount of normal PrP available for conversion — the field's leading therapeutic hypothesis — have moved from mouse studies into the clinic: an antisense oligonucleotide and a divalent siRNA candidate are both now in early-phase human trials, and gene-silencing tools have achieved durable PrP suppression in animal models. To compare, the monoclonal antibody PRN100 tested the feasibility and safety of anti-PrP immunotherapy in a first-in-human treatment program. None of these approaches has demonstrated clinical efficacy yet, and important questions about timing, delivery, and long-term safety have not been answered. Even so, prion disease research has reached a genuine turning point: the field has moved from a problem that could neither be diagnosed with confidence nor meaningfully treated to one that can now be diagnosed accurately and is, for the first time, the subject of real treatment trials.

I. Introduction

Prion diseases, also known as Transmissible Spongiform Encephalopathies (TSEs), are rare neurodegenerative disorders caused by the misfolding of normal prion proteins, leading to irreversible cellular brain damage. Protein misfolding has been the cause of many well-known neurodegenerative diseases such as Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis, and Huntington's (Prusiner). However, misfolded proteins in Alzheimer's and Parkinson's cause local damage, and prions are the only kind of misfolded protein that is also infectious. Stanley Prusiner first identified prions in 1982, a discovery that earned him the 1997 Nobel Prize in Physiology or Medicine and was initially controversial, since the idea of an infectious agent without genetic material contradicted decades of accepted biology.

Unlike other pathogens that cause infection, such as bacteria and viruses, they lack nucleic acids. Without any DNA or RNA, they are unable to reproduce in the conventional sense; Instead, the misfolded prion changes the shape of healthy proteins through propagation. It binds to a normal PrP^C molecule and acts as a template, forcing that molecule to adopt the same misfolded, disease-causing shape. This newly converted protein can then convert others, producing a self-sustaining chain reaction that spreads through the brain (Prusiner; Colby & Prusiner). Structurally, normal PrP^{c} is alpha-helical with very little beta-sheet content. When it

converts to the pathogenic $PrP^{Sc,}$ its alpha-helical content is replaced with a beta-sheet structure instead. The alpha-to-beta shift makes it more prone to aggregation, which physically disrupts neuron membranes and synapses, leading to cellular stress. This cellular stress initiates a self-destruct sequence, causing visible brain damage.

There are many variants of human prion diseases that are genetic, acquired, etc. The most common, however, is Sporadic Creutzfeldt-Jakob Disease, which makes up approximately 85% of all cases and has no known cause. Additionally, genetic mutations in the PRNP gene can lead to Fatal Familial Insomnia or Gerstmann-Sträussler-Scheinker Syndrome. Acquired prion diseases, including Variant CJD and Kuru, are contracted through the consumption of the nervous tissue of contaminated animals or humans. Variant CJD (vCJD) gained prominence during the 1990s as a direct consequence of the bovine spongiform encephalopathy epidemic in the United Kingdom; the disease was transmitted to humans through the ingestion of beef products contaminated with pathogenic prions. These diseases, while extremely rare, ultimately end up being fatal.

Despite decades of research since its discovery, prions remain untreatable and difficult to diagnose. Most neurodegenerative diseases take decades to significantly progress, but prions can compress this degeneration into less than a year. Additionally, because early symptoms such as confusion, memory loss, and mood changes overlap with other dementias, cases are frequently misdiagnosed or diagnosed in late stages. Only 18% of patients are typically diagnosed properly in their first visit (Paterson et al. 1578-82), meaning that treatment often may start in late stages of the disease, which will stop the prions but leave patients with severe brain damage. Prions resist standard sterilization and decontamination methods, creating documented transmission risks via contaminated neurosurgical instruments, corneal transplants, and (historically) cadaver-derived growth hormone (Brown et al. 901-07). This paper examines two of the most significant recent breakthroughs addressing these challenges: RT-QuIC, a diagnostic assay that has transformed early detection, and emerging PrP-lowering and immunotherapy approaches now being tested in the first human clinical trials for prion disease.

II. Background

A. PrP Biology and Pathogenesis

Normal PrP^{C} is a cell-surface glycoprotein, anchored to the outer membrane, most abundant in neurons, but also found on astrocytes, oligodendrocytes, and microglia throughout the central and peripheral nervous system (Westergard et al.). Although its normal function is still debated, research has implicated it in myelin maintenance and cell signaling (Aguzzi and Falsig, 936-39). Despite this normal function, PrP^{C} misfolds into PrP^{Sc} through an autocatalytic process (Hara and Sakaguchi). PrP^{C} and PrP^{Sc} share the same amino acid sequence; the difference lies in PrP^{C}'s alpha-helical structure versus PrP^{Sc}'s beta-pleated sheets. The conversion begins with the arrival of a single contaminated PrP^{Sc} protein, which continues infecting and misfolding normal PrP^{C} proteins (Colby and Prusiner).

This conversion mechanism creates several distinct challenges for both diagnosis and treatment of prion disease. For instance, PrP^{Sc} is partially resistant to protease digestion, a cellular process that removes misfolded or damaged proteins, which historically has made lab diagnosis difficult and slow (Hara and Sakaguchi). This same protease resistance is why prions resist standard sterilization methods, creating the documented transmission risks associated with contaminated surgical instruments discussed above (Brown et. al., 901-07). Additionally, different misfolded conformations or strains can produce distinct disease presentations (varying symptoms, progression, and regions of the brain), making a single treatment effective across all strains difficult to achieve (Safar et al.). Finally, the immune system does not detect PrP^{Sc} as foreign because it's a misfolded version of the normal human protein, so there is no natural antibody response to clear it (Zabel and Avery).

B. Neuropathology

The hallmark features of prion disease neuropathology include neuronal loss, reactive astrocytosis (gliosis), and spongiform vacuolation (Budka). Neuronal loss in affected brain regions directly underlies the cognitive and motor symptoms discussed above. Astrocytes respond to this damage through reactive astrocytosis, in which they multiply and change shape and behavior, similar to the formation of scar tissue elsewhere in the body. This process, alongside the accumulation of prion aggregates, produces the disease's namesake feature: tiny holes, or vacuoles, that develop at the microscopic level, giving affected brain tissue its distinctive "sponge-like" spongiform appearance.

As mentioned earlier, misfolded prion proteins force normal proteins to also misfold, spreading further in a self-perpetuating chain reaction. This process is referred to as "templated seeding." Both amyloid-beta in Alzheimer's disease and alpha-synuclein in Parkinson's disease also misfold and spread through templated seeding, a parallel that researchers now call the "prion-like" hypothesis of neurodegeneration (Jucker and Walker, 45-51). This is why prion disease research is considered informative for the broader neurodegeneration field, not just for prion diseases themselves.

III. Recent Advancements in Diagnosis

RT-QuIC (Real-Time Quaking-Induced Conversion) is a diagnostic assay that adds a small amount of patient sample to a test tube containing a recombinant PrP substrate to determine if PrP^{Sc} is present. If it's present, it forces misfolding of the substrate, which is detected via the fluorescent dye, thioflavin T (Atarashi et al., 175). A variety of different samples can be used, such as cerebrospinal fluid (CSF), nasal/olfactory mucosa brushings, and skin punch biopsies (the least invasive). These samples, while still invasive, are far less invasive compared to the brain biopsies once required for a definitive diagnosis. CSF RT-QuIC catches 92–95% of true CJD cases with high specificity, approximately 100%, meaning little to no false positives. This is an improvement compared to older CSF markers like 14-3-3 protein, which had an 81–82% sensitivity (Senesi et al.). Additionally, the skin punch biopsy RT-QuIC has

independently shown an 89–90% sensitivity and 100% specificity in several studies (Mammana et al., 559).

Second-generation assays, otherwise known as IQ-CSF, have improved sensitivity through blank, now correctly detecting prions in 80% of cases that the first-generation assay missed, without a loss of specificity (Franceschini et al.). RT-QuIC is now considered the standard antemortem test for probable CJD and has been formally incorporated into diagnostic criteria. This shift has measurably improved diagnostic accuracy: in one ten-year study, the sensitivity of the diagnostic criteria rose from 76.8% under the old, pre-RT-QuIC standards to 97.8% after RT-QuIC was incorporated, with no meaningful loss in specificity (Mastrangelo et al. 121-29).

IV. Current Diagnostic Landscape

Among traditional diagnostic tools, EEGs that detect periodic sharp wave complexes have a sensitivity of only 66% and a specificity of 74%, meaning they miss a little over ⅓ of all cases and can be falsely positive for about a quarter of unrelated cases such as late-stage Alzheimer's disease. However, MRIs, another commonly used tool, that analyze diffusion-weighted imaging showing cortical ribboning and basal ganglia changes have a more accurate sensitivity of 91-92.3% and a specificity of 93.8-95%, described as more useful compared to EEGs (Muayqil et al. 1499-1506). Conventional CSF markers, particularly the 14-3-3 protein, exhibit comparable deficiencies in precision when measured against RT-QuIC; these biomarkers demonstrate a sensitivity of approximately 81.3% and a specificity of 84.4%, often proving less reliable for definitive clinical assessment (Senesi et al.). RT-QuIC is now used as a primary confirmatory test, though MRIs and clinical criteria are still used with RT-QuIC, rather than being fully replaced by it.

V. Recent Advancements in Therapy

A. PrP-Lowering Strategies

The main idea of PrP-Lowering Strategies is to reduce the number of PrP^{C} available to be converted. Since the pathogenic PrP^{Sc} requires a healthy PrP^{C} to act as a substrate for conversion, decreasing the PrP^{C} available should slow or halt the disease. This theory has been validated genetically in mice, where even partial PrP reduction extends survival (Mallucci et al., 2003; Minikel et al., 2020). The most clinically advanced PrP-lowering approach is the antisense oligonucleotide (ASO) ION717, made by Ionis Pharmaceuticals. It is currently being studied in the PrProfile trial, a Phase 1/2a first-in-human study of spinal-injected ION717 in about 56 symptomatic prion disease patients. As of early 2026, Ionis has not released any results demonstrating the effectiveness of the drug. The trial has been extended, and a third dosing cohort was added to test a higher dose, with the study now expected to run through 2027. Ionis specifically has stated that preliminary data shows "an encouraging safety and tolerability

profile," but this is not proof of clinical benefit (cureffi.org, 2026; Ionis Pharmaceuticals pipeline page).

A second PrP-lowering approach entered human testing in 2026. Gentile et al. developed a divalent short interfering RNA (siRNA) targeting PRNP that lowered PrP expression in mice by 49% and extended survival by 64% with a single dose given after symptom onset. Based on these preclinical results and a clean toxicology profile, the FDA cleared an Investigational New Drug application, and a single-dose safety trial in approximately 15 symptomatic patients began enrolling in 2026 through the NIH's NeuroNEXT network. This makes divalent siRNA, alongside ION717, one of the first two PrP-lowering drug candidates to reach human testing (Gentile et al., 2026).

Additionally, preclinical work in "humanized" mice, engineered to carry the human PRNP gene, has exhibited positive results in in vivo base editing of PRNP. Some people naturally carry a mutation called R37X that lowers their PrP levels without causing any health problems, so researchers used a base editor to recreate that same mutation on purpose. To get the editing tool into the brain, they packaged it inside two engineered viruses, called AAVs, which is a common way to deliver gene therapies to brain tissue. In the trial, approximately 37% of copies of the gene were altered, permanently reducing PrP expression and extending survival after prion infection. Results demonstrated a 50% reduction in prion protein in treated mice compared to the control and a 50% increase in survival.

Researchers at the Broad and Whitehead Institutes also developed CHARM (Coupled Histone tail for Autoinhibition Release of Methyltransferase), a compact epigenetic editor that silences the PRNP gene through DNA methylation. DNA methylation is when methyl groups are added to DNA molecules, changing how genes are expressed without altering the underlying DNA sequence. This, similar to in vivo base editing, is also delivered through AAV directly into the brains of mice, achieving a near-total, brain-wide PrP silencing after a single treatment. The silencing lasted at least six weeks and showed no evidence of toxicity (Neumann et al. 2024).

B. Immunotherapy

PRN100 is a fully humanized anti-PrP^C monoclonal antibody, developed by the MRC Prion Unit at University College London. The trial consisted of six CJD patients under a "Specials" license (not a formal clinical trial) starting around 2019, with results published in 2022. The antibody was shown to be safe, well-tolerated, and able to reach target concentrations in the brain. In half of the patients, disease progression may have been temporarily stabilized while dosing was in the target range (Mead et al. 2022). Ultimately, all six patients succumbed to CJD; however, this is the first real test in humans for anti-PrP immunotherapy and warrants continuing this research with a formal clinical trial and a larger number of patients.

Preclinical antibody studies have also shaped thinking about when antibody therapy should be given. Work with ICSM18, the antibody from which PRN100 was derived, showed that treatment works best when given very early, before prions reach the brain, and loses effectiveness the longer treatment is delayed. This timing dependency has informed discussion of

whether anti-PrP antibody therapy might work better as a prophylactic treatment for people known to carry high-risk genetic mutations for prion disease, rather than as a treatment given only after symptoms appear (White et al., 2003, as discussed in Mead et al., 2022).

VI. Conclusion

RT-QuIC has effectively solved the diagnosis problem for Creutzfeldt-Jakob disease. It is faster than older methods, far less invasive, and highly accurate, and it has been formally incorporated into diagnostic criteria used around the world.

On the treatment side, PrP-lowering strategies and immunotherapy represent the first serious, biologically grounded attempts at a disease-modifying treatment for prion disease. As of this writing, however, no therapy has yet demonstrated efficacy in humans: all are either in early, safety-focused clinical trials or still confined to animal models.

Several open questions remain for the field. Should PrP-lowering drugs be given before symptoms start, or only after? How invasive should treatment be, given that the most advanced drugs currently have to be injected into the spine? What happens if someone lowers this protein for years — are there long-term risks, since we still don't fully understand what PrP normally does in the body? And will these treatments even work for every strain of prion disease, given that different strains have caused past drugs to fail?

Together, these advances represent a real turning point for the field. Prion disease used to be a condition that doctors couldn't diagnose with confidence or treat in any real way. Now, it can be diagnosed accurately, and for the first time, real treatments backed by solid science are being tested in human trials. With further research, treatment for prion diseases can be found.

Works Cited

An, Meirui, et al. "In Vivo Base Editing Extends Lifespan of a Humanized Mouse Model of Prion Disease." Nature Medicine, vol. 31, no. 4, 2025, pp. 1319-28.

Aguzzi, Adriano, and Jean Falsig. "Prion Propagation, Toxicity and Degradation." Nature Neuroscience, vol. 15, no. 7, 2012, pp. 936-39.

Atarashi, Ryuichiro, et al. "Ultrasensitive Human Prion Detection in Cerebrospinal Fluid by Real-Time Quaking-Induced Conversion." Nature Medicine, vol. 17, no. 2, 2011, pp. 175-78.

Brown, Paul, et al. "Iatrogenic Creutzfeldt-Jakob Disease, Final Assessment." Emerging Infectious Diseases, vol. 18, no. 6, 2012, pp. 901-07.

Budka, Herbert. "Neuropathology of Prion Diseases." British Medical Bulletin, vol. 66, no. 1, 2003, pp. 121-30.

Colby, David W., and Stanley B. Prusiner. "Prions." Cold Spring Harbor Perspectives in Biology, vol. 3, no. 1, 2011, a006833.

Franceschini, Alessia, et al. "High Diagnostic Value of Second Generation CSF RT-QuIC across the Wide Spectrum of CJD Prions." Scientific Reports, vol. 7, no. 1, 2017, article 10655.

Gentile, Juliana, et al. "Divalent siRNA for Prion Disease." Nucleic Acids Research, 2026.

Hara, Hideyuki, and Suehiro Sakaguchi. "N-Terminal Regions of Prion Protein: Functions and Roles in Prion Diseases." International Journal of Molecular Sciences, vol. 21, no. 17, 2020, p. 6233.

Ionis Pharmaceuticals. "PrProfile: A Phase 1/2a Study to Evaluate ION717 in Patients with Prion Disease." Ionis Pharmaceuticals, 2026, ionis.com.

Jucker, Mathias, and Lary C. Walker. "Self-Propagation of Pathogenic Protein Aggregates in Neurodegenerative Diseases." Nature, vol. 501, no. 7465, 2013, pp. 45-51.

Mallucci, Giovanna, et al. "Depleting Neuronal PrP in Prion Infection Prevents Disease and Reverses Spongiosis." Science, vol. 302, no. 5646, 2003, pp. 871-74.

Mammana, Angela, et al. "Detection of Prions in Skin Punch Biopsies of Creutzfeldt–Jakob Disease Patients." Annals of Clinical and Translational Neurology, vol. 7, no. 4, 2020, pp. 559-64.

Mastrangelo, Andrea, et al. "Evaluation of the Impact of CSF Prion RT-QuIC and Amended Criteria on the Clinical Diagnosis of Creutzfeldt-Jakob Disease: A 10-Year Study in Italy." Journal of Neurology, Neurosurgery & Psychiatry, 2023.

Mead, Simon, et al. "Prion Protein Monoclonal Antibody (PRN100) Therapy for Creutzfeldt-Jakob Disease: Evaluation of a First-in-Human Treatment Programme." Lancet Neurology, vol. 21, no. 4, 2022, pp. 342-54.

Minikel, Eric Vallabh, et al. "Prion Protein Lowering Is a Disease-Modifying Therapy across Prion Disease Stages, Strains and Endpoints." Nucleic Acids Research, vol. 48, no. 19, 2020, pp. 10615-31.

Minikel, Eric Vallabh. "ION717 Trial Re-Opens with 3rd Dosing Regimen." CureFFI.org, 17 Mar. 2026, www.cureffi.org/2026/03/17/ion717-trial-reopens/.

Muayqil, Taim, et al. "Evidence-Based Guideline: Diagnostic Accuracy of CSF 14-3-3 Protein in Sporadic Creutzfeldt-Jakob Disease: Report of the Guideline Development Subcommittee of the American Academy of Neurology." Neurology, vol. 79, no. 14, 2012, pp. 1499-1506.

Neumann, Edwin N., et al. "Brainwide Silencing of Prion Protein by AAV-Mediated Delivery of an Engineered Compact Epigenetic Editor." Science, vol. 384, no. 6703, 2024, article ado7082.

Paterson, Ross W., et al. [Title unconfirmed — cited in your Introduction as "Paterson et al. 2012" for the 18% first-visit diagnosis stat, but never added to Works Cited]

Prusiner, Stanley B. "Prions." Proceedings of the National Academy of Sciences, vol. 95, no. 23, 1998, pp. 13363-83.

Safar, Jiri, et al. "Eight Prion Strains Have PrPSc Molecules with Different Conformations." Nature Medicine, vol. 4, no. 10, 1998, pp. 1157-65.

Senesi, Matteo, et al. "Diagnostic Performance of CSF Biomarkers in a Well-Characterized Australian Cohort of Sporadic Creutzfeldt-Jakob Disease." Frontiers in Neurology, vol. 14, 2023, article 1072952.

Westergard, Laura, et al. "The Cellular Prion Protein (PrPC): Its Physiological Function and Role in Disease." Biochimica et Biophysica Acta, vol. 1772, no. 6, 2007, pp. 629-44.

White, Andrew R., et al. "Monoclonal Antibodies Inhibit Prion Replication and Delay the Development of Prion Disease." Nature, vol. 422, no. 6927, 2003, pp. 80-83.

Zabel, Mark D., and Anne C. Avery. "Prions—Not Your Immunologist's Pathogen." PLOS Pathogens, vol. 11, no. 2, 2015, e1004624.

Evaluating Cancer Treatment in Shark Cartilage By Haotian Liu

Abstract

Cancer is one of the leading causes of deaths worldwide and is characterized by the uncontrolled growth and division of abnormal cells. Tumor growth is dependent on angiogenesis which forms new blood vessels and supplies the tumor with oxygen and nutrients needed for metastasis. Although current antiangiogenic therapies target pathways such as vascular endothelial growth factors or VEGF, many patients experience limited success with the treatment. Shark cartilage has emerged as a potential treatment due to its demonstrated ability to prevent angiogenesis in animals. However, despite promising laboratory results, it remains unclear why shark cartilage derived treatments have shown no success in clinical trials for humans with cancer. This paper examines the relationship between shark cartilage, angiogenesis, and cancer progression. The data shown consistently shows that the compounds from the shark cartilage was able to inhibit angiogenesis whereas clinical trials failed to produce significant improvements in patients. The data ultimately suggests that shark cartilage is not very efficient as a cancer treatment despite promising results in laboratory studies. This review helps contribute to the understanding of shark cartilage as a potential cancer treatment,identifies gaps in knowledge related to its function, and provides an outlook for future research aimed at developing cancer therapies which could impact millions of people around the world who face cancer.

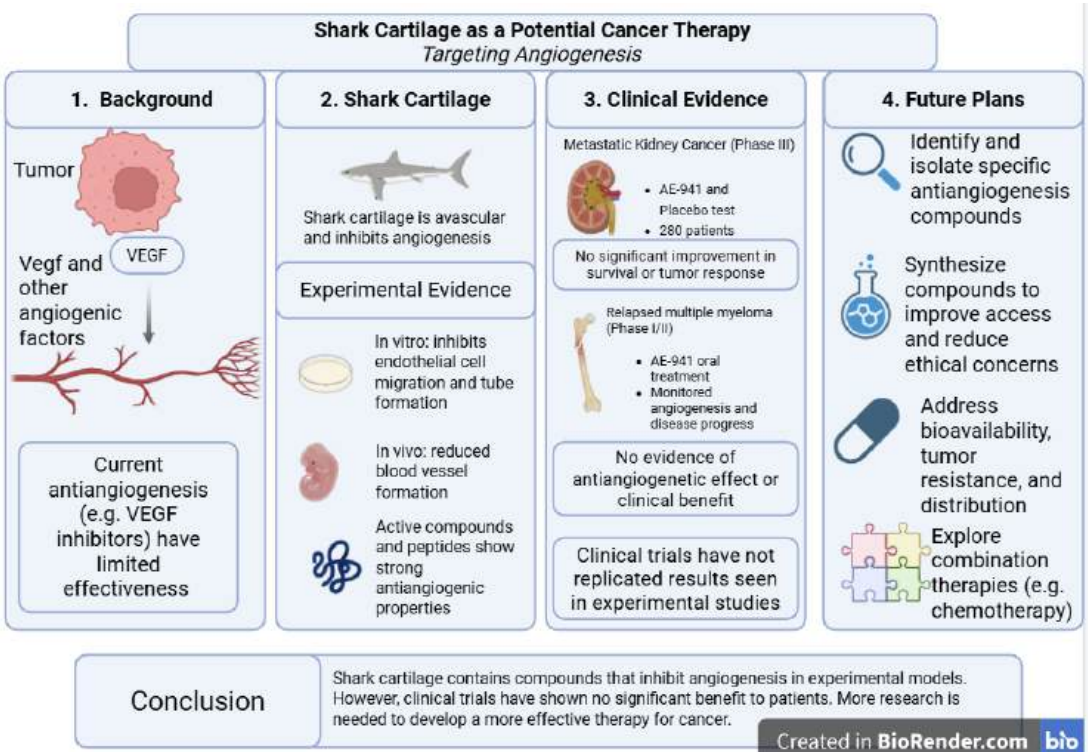


Figure 1: Graphical abstract - Summarizes main concepts of this review.

Introduction

Cancer develops when cells acquire mutations and evade normal growth controls and then divide uncontrollably(Bishop and Weinberg, 1996). These cells come from mutations inside the DNA which disrupt normal cell regulation and allow the mutated and damaged cells to continue to reproduce rather than going under the programmed cell death. The growth of the cells can build up and eventually form what is commonly known as a tumor or neoplasm (Bishop, 1991). There are two main groups of tumors known as malignant and benign. Over time, the dividing cells can grow into benign tumors which can remain localized in one area, or malignant tumors, which spread throughout the entire body by a process known as metastasis (Weidner et al., 1991). Cancer affects millions of people a year and exists in several forms like skin cancer, lung cancer, breast cancer, and so on, each with different treatments and symptoms. Common symptoms of cancer include pain, fatigue, and bleeding although the symptoms will differ depending on the type and stage of cancer. Most cancer treatments involve surgery, chemotherapy, or radiation but more recent treatments involve immunotherapies to interfere with tumor growth (Shao et al., 2023). However, despite all the research and advances in medicine, cancer remains difficult to cure entirely (Holmgren et al., 1995). The major concepts and relationships discussed in this review are summarized in (Figure 1).

Angiogenesis is the process in which new blood vessels form from already existing vessels (Chatterjee et al., 2013). This process is essential for normal growth and healing in the body but also helps cancer progress. Tumors require nutrients to survive, and they are able to obtain these from stimulating angiogenesis into creating a new network, giving them resources and nutrients. Without angiogenesis, tumors are not as likely to spread and grow (La Porta et al., 2018). It is because of this reason that current treatments aim to prevent angiogenesis. Current treatments to prevent angiogenesis include targeting the specific molecules responsible for signalling like vascular endothelial growth factor (VEGF) which promotes blood vessel growth in tumors (Chatterjee et al., 2013). These treatments aren't very effective unfortunately and patients typically develop resistance or have harmful side effects over time (Faes et al., 2016). Therefore, we must find exactly what inhibits angiogenesis and how we can apply it to human treatment.

Despite advances in antiangiogenic therapies, treatment resistance and limited long term effectiveness remains a great challenge for cancer treatment. One area of interest in cancer research involves the use of shark cartilage due to its demonstrated presence of anti-cancer properties. This questions how effective shark cartilage really is and how it can successfully be made into a therapy for cancer. Sharks are marine vertebrates belonging to a group of fish known as Chondrichthyes, which includes species that have skeletons primarily composed of cartilage rather than bone (Carrier et al., 2004). Unlike most vertebrates, sharks possess skeletons made primarily of cartilage rather than bone, giving them lighter and more flexible body structures. Cartilage is a strong connective tissue made of mostly collagen and proteins that provide the support and flexibility (Ostrander et al., 2004). Due to cartilage being naturally avascular and not

normally containing blood vessels, researchers investigated whether compounds within shark cartilage could inhibit angiogenesis and tumor growth.

Studies show that shark cartilage has anti angiogenic compounds which suppress blood vessel formation associated with tumor growth (Li et al., 2024 Hassan et al., 2005). Because angiogenesis remains a critical target in cancer therapy and plays a large role in tumor growth (Yang et al., 2016). Since current antiangiogenic treatments often face challenges such as treatment resistance and limited long term effectiveness shark cartilage has been studied as a potential cancer treatment (Jahangiri et al., 2013). This paper examines the relationship between shark cartilage, angiogenesis, and cancer to evaluate whether shark cartilage may serve as a potential cancer treatment in the future. These findings would address the need for more effective cancer treatments and explore other fields of science as a potential treatment.

How Angiogenesis Supports Tumor Growth

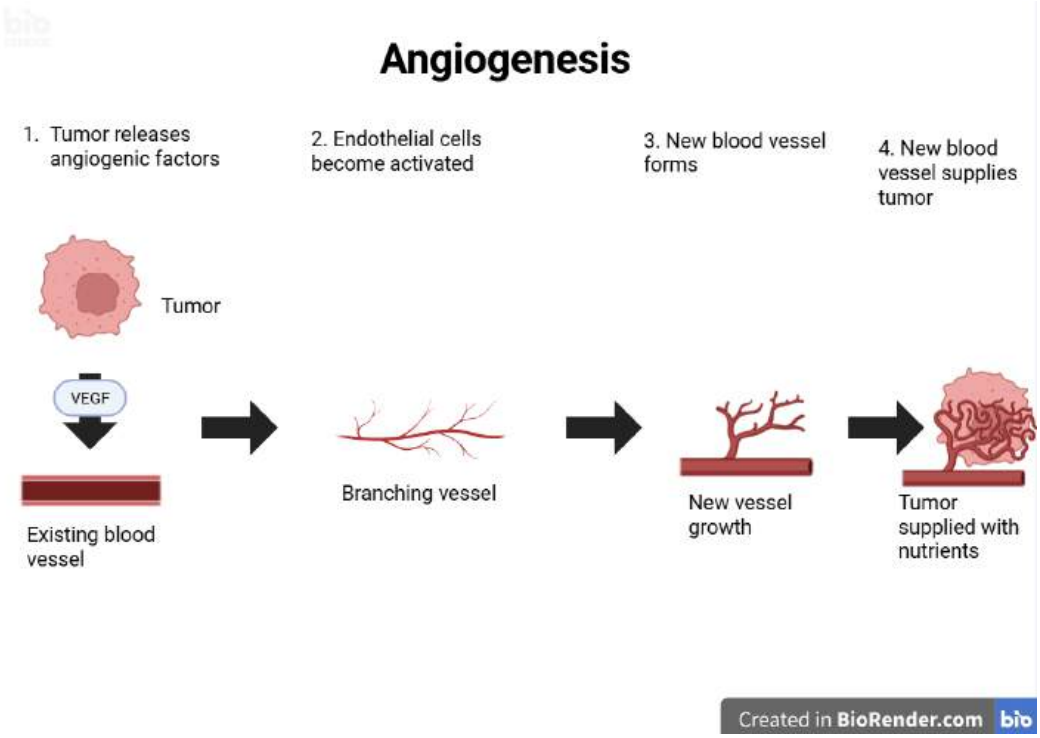


Figure 1: Angiogenesis process - Shows the steps of angiogenesis.

Angiogenesis is the process in which new blood vessels develop from pre-existing ones and is essential for normal bodily functions but also cancer progression. Tumors require a supply of oxygen and nutrients to survive and grow (Figure 1). Research has demonstrated that tumor cells stimulate angiogenic signals to establish networks that support cellular growth (Chatterjee et al., 2013). One of the primary signalling molecules involved in this process is VEGF which promotes rapid cell growth and migration within tumors (Gerber et al., 1998). Elevated VEGF has been associated with increased tumor vascularization and metastatic potential (Nishida et al., 2006). Experimental studies have shown that abnormal angiogenesis contributes to tumor

expansion and vascular instability throughout the entire body (La Porta et al., 2018). Due to how important angiogenesis is in tumor growth, cancer treatments have been focused on targeting the angiogenic signals to restrict the tumor's supply of oxygen and nutrients (Faes et al., 2016). This has encouraged researchers to investigate compounds that can restrict angiogenesis, including shark cartilage.

Shark Cartilage as a Source of Antiangiogenic Compounds

Experimental studies have demonstrated that shark cartilage can prevent angiogenesis. Sharks possess skeletons composed of primarily cartilage which is an avascular tissue that inhibits blood vessel growth. Because the cartilage mostly lacks vascularization researchers began to investigate whether the compounds inside shark cartilage could prevent angiogenesis. Cartilage is a connective tissue that provides structural support and flexibility within the body. It is mostly composed of collagen fibers and carbs and proteins like, proteoglycans and glycosaminoglycans, and water. Together it becomes very durable and capable of maintaining strength while remaining flexible (Ostrander et al., 2004). Unlike most tissues cartilage is naturally avascular and lacks blood vessels and obtains nutrients in different ways without relying on the blood vessels. Since angiogenesis required for blood vessel formation and tumor growth researchers became interested in whether or not the shark cartilage could contribute to the tissue’s resistance to vascularization.

Experimental Studies of Shark Cartilage Compounds

One of the earliest studies investigating the potential use of shark cartilage as a cancer treatment was conducted by Hassan et al. in 2005. The researchers isolated a low molecular weight fraction from the shark cartilage and evaluated its effects on tumor growth, angiogenesis, and immune function in mice. Their results showed that treatment with shark cartilage reduced tumor development and inhibited the formation of new blood vessels, which are required for tumor progression. The study also reported increased infiltration of immune cells into the tumor tissues which enhanced the immune system's response against the cancer cells. Additionally, the isolated fraction inhibited the migration of endothelial cells which is essential for angiogenesis. These findings suggested that compounds found inside the shark cartilage may be able to suppress tumor growth.

Jiao et al. in 2003 had tried to identify the specific antiangiogenic compounds present within the shark cartilage and determine whether they could inhibit tumor growth. The researchers extracted proteins from shark cartilage and isolated two active compounds known as Sp15 and Sp8 both at different molecular weights. Both the compounds inhibited endothelial cells with Sp15 by nearly 75%. Meanwhile, Sp8 had shown significant results and reduced blood vessel formation by 79% in a chick embryo chorioallantoic membrane model and 72.7% in a rabbit cornea angiogenesis model. When it was tested in mice which had tumors transplanted, Sp8 reduced tumor growth by 54.4% and by 56.7% at a slightly higher dose. The researchers reported that the Sp8 eliminated any skin ulcer formation that may have been caused by the

tumor and didn't see any major signs of side effects. The findings provide strong evidence that specific proteins isolated from shark cartilage could inhibit angiogenesis.

More recently, Li et al. in 2024 had investigated a shark cartilage derived peptide which was isolated from a catshark. The researchers examined the peptides' effects on endothelial cell cultures and mouse models of angiogenesis. Their findings demonstrated the peptide to be very effective in inhibiting endothelial cell migration and tube formation which are all essential steps in blood vessel development. The peptide also suppressed angiogenic signalling molecules which include VEGF and fibroblast growth factor and platelet derived growth factor. Mice treated with the peptide demonstrated significantly reduced neovascularization compared with untreated mice. These results provide strong evidence that specific peptides isolated from the shark cartilage can directly interfere with molecular pathways that are responsible for angiogenesis.

Clinical Trials of Shark Cartilage Therapies

In a phase III clinical trial treating patients with metastatic kidney cancer researchers Escudie et al., in 2013 investigated whether the shark cartilage - known as AE-941 or Neovastat - could improve the conditions of patients with metastatic renal cell carcinoma that no longer responded to immunotherapy. AE-941 is an extract derived from shark cartilage to function as an antiangiogenic agent by inhibiting the blood vessel formation which is required for tumor growth. The study was randomized, double blind, and placebo controlled and involved around 280 patients with advanced kidney cancer. The patients received the AE-941 or a placebo two times a day while being monitored for survival rate, tumor progression, tumor response, and quality of life. The purpose of the study was to determine if the inhibiting properties of shark cartilage was able to slow cancer progression. The results of AE-941 did not significantly improve overall survival or tumor response in patients with the cancer despite the evidence found in laboratory studies (NCT00005995, 2013)

In another clinical investigation researchers in a study sponsored by AEterna Zentaris, evaluated the use of AE-941 in patients with relapsed or refractory multiple myeloma which is a cancer characterized by the uncontrolled growth of plasma cells within the bone marrow. The purpose of phase I and II was to determine whether the antiangiogenic properties that were shown in experimental studies could be seen in human patients. Angiogenesis contributes to the growth of multiple myeloma by supporting its survival of malignant cells so researchers investigated if inhibiting blood vessel formation with the shark cartilage derived compounds would be able to slow down the disease. The patients received an oral dose of AE-941 over a certain period while the researchers monitored safety, disease progression, and treatment response. Unlike the laboratory results that showed inhibition of angiogenesis, the clinical trial did not produce any evidence of inhibiting angiogenesis in human patients. These findings highlighted a recurring challenge where the experimental studies and results did not get replicated when done in human patients (NCT00022282, 2013).

Future studies

Future research should be focused on isolating the specific compounds in the shark cartilage that are responsible for its antiangiogenic properties and try to create a therapy with it. After finding out what specific compounds are antiangiogenic it may be possible with current technology to synthesize the compound eliminating the need to harvest shark cartilage and reduce ethical concerns related to shark conservation. Creating these shark cartilage therapies is possible and can be improved, though there are still a lot of challenges ahead. Some challenges include bioavailability, tumor resistance, and scalability of manufacturing. If a synthesized version is made without the need for shark cartilage it may differ from the original by being more cost effective as it negates the necessary labor needed to hunt for sharks, it may have reduced side effects, and it may be more effective as it could be given to patients differently. Future studies may also include research in combining this therapy with other therapies like hormone therapy, gene therapy, or chemotherapy as it might improve its effectiveness and cover any limitations a current therapy has. If the therapies are combined, then it might become a safer treatment for the patient as it is able to attack the disease from different areas.

Conclusion

This paper shows how shark cartilage contains compounds that can inhibit angiogenesis which is a process necessary for tumor growth. Lab experiments demonstrated reductions in blood vessel formation and tumor development while clinical trials failed to demonstrate the same effects in patients with advanced kidney cancer. Overall, these findings suggest that shark cartilage although having antiangiogenic properties is inefficient as a therapy for cancer as of now however continued research may contribute to a more effective therapy with shark cartilage in the future.

Works Cited

Gary K. Ostrander, Keith C. Cheng, Jeffrey C. Wolf, Marilyn J. Wolfe; Shark Cartilage, Cancer and the Growing Threat of Pseudoscience. Cancer Res 1 December 2004; 64 (23): 8485–8491. https://doi.org/10.1158/0008-5472.CAN-04-2260

Bishop, J. M., & Weinberg, R. A. (Eds.). (1996). Scientific American molecular oncology. Scientific American, Inc. [1, 2]

Carrier, J. C., Musick, J. A., & Heithaus, M. R. (Eds.). (2012). Biology of sharks and their relatives (2nd ed.). CRC Press. Biology of Sharks and Their Relatives

Chatterjee, S., Heukamp, L. C., Siobal, M., Schöttle, J., Wieczorek, C., Peifer, M., Frasca, D., Koker, M., König, K., Meder, L., Rauh, D., Buettner, R., Wolf, J., Brekken, R. A., Neumaier, B., Christofori, G., Thomas, R. K., & Ullrich, R. T. (2013). Tumor VEGF:VEGFR2 autocrine feed-forward loop triggers angiogenesis in lung cancer. Journal of Clinical Investigation, 123(4), 1732–1740. https://doi.org/10.1172/JCI65385

Weidner, N., Semple, J. P., Welch, W. R., & Folkman, J. (1991). Tumor angiogenesis and metastasis—Correlation in invasive breast carcinoma. The New England Journal of Medicine, 324(1), 1–8. https://doi.org/10.1056/NEJM199101033240101

Shao, Q., Lundgren, M., Lynch, J., Jiang, M., Mir, M., Bischof, J., & Nelson, M. (2023). Tumor therapeutic response monitored by telemetric temperature sensing, a preclinical study on immunotherapy and chemotherapy. Scientific reports, 13(1), 7727. https://doi.org/10.1038/s41598-023-34919-w

Faes, S., Uldry, E., Planche, A., Santoro, T., Pythoud, C., Demartines, N., & Dormond, O. (2016). Acidic pH reduces VEGF-mediated endothelial cell responses by downregulation of VEGFR-2: Relevance for anti-angiogenic therapies. Oncotarget, 7(52), 86026–86038. https://doi.org/10.18632/oncotarget.13323

La Porta, S., Roth, L., Singhal, M., Mogler, C., Spegg, C., Schieb, B., Qu, X., Adams, R. H., Baldwin, H. S., Savant, S., & Augustin, H. G. (2018). Endothelial Tie1-mediated angiogenesis and vascular abnormalization promote tumor progression and metastasis. The Journal of clinical investigation, 128(2), 834–845. https://doi.org/10.1172/JCI94674

Li, Y., Chen, A., Hong, A., Xiong, S., Chen, X., & Xie, Q. (2024). Shark Cartilage-Derived Anti-Angiogenic Peptide Inhibits Corneal Neovascularization. Bioengineering (Basel, Switzerland), 11(7), 693. https://doi.org/10.3390/bioengineering11070693

Hassan, Z. M., Feyzi, R., Sheikhian, A., Bargahi, A., Mostafaie, A., Mansouri, K., Shahrokhi, S., Ghazanfari, T., & Shahabi, S. (2005). Low molecular weight fraction of shark cartilage can modulate immune responses and abolish angiogenesis. International Immunopharmacology, 5(6), 961–970. https://doi.org/10.1016/j.intimp.2005.01.006

Nishida, N., Yano, H., Nishida, T., Kamura, T., & Kojiro, M. (2006). Angiogenesis in cancer. Vascular health and risk management, 2(3), 213–219. https://doi.org/10.2147/vhrm.2006.2.3.213

Gerber, H.-P., McMurtrey, A., Kowalski, J., Yan, M., Keyt, B. A., Dixit, V., & Ferrara, N. (1998). Vascular endothelial growth factor regulates endothelial cell survival through the

phosphatidylinositol 3′-kinase/Akt signal transduction pathway: Requirement for Flk-1/KDR activation. Journal of Biological Chemistry, 273(46), 30336–30343. https://doi.org/10.1074/jbc.273.46.30336

Jiao, B., Chen, J., Miao, W., Wang, L., Zhu, Y., & Miao, H. (2004). Identification and Biological Characterization of Angiogenic and Tumor Growth Inhibitors derived from Sinica cetorhinus maximum Cartilage. Marine Drugs, 2(1), 30–38.

Yang, Y., Zhang, Y., Iwamoto, H., Hosaka, K., Seki, T., Andersson, P., Lim, S., Fischer, C., Nakamura, M., Abe, M., Cao, R., Skov, P. V., Chen, F., Chen, X., Lu, Y., Nie, G., & Cao, Y. (2016). Discontinuation of anti-VEGF cancer therapy promotes metastasis through a liver revascularization mechanism. Nature communications, 7, 12680. https://doi.org/10.1038/ncomms12680

Jahangiri, A., De Lay, M., Miller, L. M., Carbonell, W. S., Hu, Y. L., Lu, K., Tom, M. W., Paquette, J., Tokuyasu, T. A., Tsao, S., Marshall, R., Perry, A., Bjorgan, K. M., Chaumeil, M. M., Ronen, S. M., Bergers, G., & Aghi, M. K. (2013). Gene expression profile identifies tyrosine kinase c-Met as a targetable mediator of antiangiogenic therapy resistance. Clinical cancer research : an official journal of the American Association for Cancer Research, 19(7), 1773–1783. https://doi.org/10.1158/1078-0432.CCR-12-1281

Bishop, J. M. (1991). Molecular themes in oncogenesis. Cell, 64(2), 235–248. https://doi.org/10.1016/0092-8674(91)90636-D

Holmgren, L., O'Reilly, M. S., & Folkman, J. (1995). Dormancy of micrometastases: Balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nature Medicine, 1(2), 149–153. https://doi.org/10.1038/nm0295-149

A Sex-Stratified Analysis of the Impact of ADHD and Comorbidities on College Adjustment By Hanlin Zhang

Abstract

ADHD symptoms can impair the organization, time management, and attention students need when transitioning to university, and comorbid alcohol use, depression, and anxiety may shape this relationship. Because ADHD often presents differently in men and women, sex-specific pathways warrant examination. We analyzed publicly available data from 495 South African first-year undergraduates (412 female, 83 male) collected in 2023. ADHD symptoms (ASRS), college adjustment (AAS), alcohol use (AUDIT), depression (BDI), and anxiety (BAI) were self-reported. Linear regression models adjusted for age and sex (age only in sex-stratified models) tested associations and interactions in the full and sex-stratified samples. Mediation analyses with 5,000 bootstrap simulations tested alcohol, depression, and anxiety as mediators. Greater ADHD symptom severity was associated with poorer college adjustment in the full sample (unstandardized coefficients (β) = −0.07, $p < 0.001$) and among female students (β = −0.08, $p < 0.001$), but not among male students ($p = 0.404$). ADHD severity was positively associated with depression and anxiety in all samples, and with alcohol use in the full and female samples. Depression significantly mediated the ADHD–adjustment association in the full, female, and male samples (ACME ≈ −0.045 to −0.051), whereas anxiety was not a significant mediator in any sample. Unexpectedly, alcohol showed a small positive (suppressing) mediation effect among female students only (ACME = 0.018, $p < 0.001$). Depressive symptoms appear to be a consistent pathway linking ADHD symptoms to poorer college adjustment, supporting depression screening and support for students with elevated ADHD symptoms. The null findings in male students and the alcohol suppression effect require replication in larger, sex-balanced, longitudinal samples.

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder commonly associated with symptoms of hyperactivity, impulsivity, and inability to concentrate on tasks for extended periods of time (Attention-Deficit/Hyperactivity Disorder). Symptoms of ADHD begin in childhood and often persist through adolescence and adulthood. ADHD can present with either predominantly inattentive symptoms or predominantly hyperactive-impulsive symptoms, or with a combination of both presentations (Attention-Deficit/Hyperactivity Disorder). Recent estimates show that 14% to 15% of college students have ADHD (Cha et al.), with male students showing higher rates of diagnosis than female students (Hinshaw et al.). ADHD symptoms can negatively impact organization, time management, and attention, skills important in academic environments, meaning ADHD symptoms may be consequential for students entering college.

ADHD has historically been diagnosed more often in men than women and has been understudied in women (Hinshaw et al.). Male students are more likely to show hyperactive

symptoms, while female students are more often seen with inattentive ADHD (Hinshaw et al.). Symptoms in female students may be less obvious and result in less hyperactive behavior, leading to less noticeable difficulties in childhood and adolescence, causing female students to be underdiagnosed and receive delayed diagnoses (Attoe and Climie). These sex differences in ADHD symptoms show the importance of analyzing ADHD in male and female students separately, as opposed to having the same assumptions about both groups.

College students often face new challenges when transitioning from a high school learning environment to a more demanding college environment. Students often need to improve skills such as organization, planning, and time management to adjust to college. Many of the areas needed for growth to adapt to the faster pacing of college are directly and negatively affected by the symptoms of ADHD. A review of 15 studies (Pagespetit et al.) found that the presence of ADHD and its symptoms in college students was associated with lower academic performance in those college students, with the most negatively impacted being those with inattentive symptoms. A four-year longitudinal study (DuPaul et al.) found that college students with diagnosed ADHD had lower GPAs and less use of effective study strategies. ADHD may affect students' ability to adapt to greater academic demands, directly affecting adjustment to college.

Other conditions can be comorbid with ADHD. These include learning disabilities, behavioral disorders, and anxiety (Anastopoulos et al.). Some conditions produce symptoms that overlap with ADHD symptoms, complicating treatment and diagnosis. Adolescents and college students with ADHD are more likely to engage in substance use and alcohol use due to the disorder. Alcohol is therefore relevant when discussing ADHD in college students and differences in college adjustment. Depression and anxiety are amongst the most common comorbidities among college students with ADHD and may be important pathways linking ADHD symptoms to poorer college adjustment. Students with ADHD face elevated rates of both (Anastopoulos et al.), which may stem from repeated academic setbacks, difficulty meeting the organizational and self-regulatory demands of university life, and the strain of managing symptoms with less external structure than in earlier schooling. Depressive symptoms are associated with reduced concentration, motivation, and energy, as well as sleep disturbance and social withdrawal, while anxiety symptoms can bring excessive worry, avoidance, and test-related distress that interfere with studying, class participation, and performance. Because these difficulties overlap with core ADHD features such as inattention and executive dysfunction, internalizing symptoms may amplify the impact of ADHD on college adjustment beyond what ADHD alone would predict. Women with ADHD appear to be particularly vulnerable to internalizing symptoms, which may help explain sex-specific pathways to poorer college adjustment.

In this study, we tested the association between ADHD and college adjustment, and the impact of alcohol use, depression, and anxiety on the relationship. In particular, we looked at sex differences in the effect of ADHD and related comorbidities on college adjustment.

Methods

Dataset: This study utilized data from a publicly available dataset of 506 South African first-year undergraduate students collected throughout the first semester of 2023 (Mohamad et al.) These data are publicly available and de-identified (https://www.kaggle.com/datasets/imtkaggleteam/adhd-mental-health). The original dataset included demographic information on first-year undergraduate students, including age and sex; students' answers to a series of questions about their symptoms, diagnosis, and treatment; and questionnaire measures of ADHD symptoms, alcohol use, depression, anxiety, and college adjustment. ADHD symptoms were measured with the ASRS v.1.1 (Adult ADHD Self-Report Scale) (Kessler et al.), and each item from the survey was summed to create a score of severity for ADHD symptoms. College adjustment was measured with the AAS (Academic Adjustment Scale) (Anderson et al.); responses were summed to create a total adjustment score. The same was done for alcohol use with the AUDIT (Alcohol Use Disorders Identification Test) (Saunders et al.), depression with the BDI (Beck Depression Inventory) test (Beck et al.), and anxiety with the BAI (Beck Anxiety Inventory) test (Beck et al.). Age and sex were demographic variables used for subgroup analyses and adjusted analyses.

Quality Control: Cleaning and quality control were performed to ensure the dataset was usable. Certain total columns were unreliable, and all total columns were recalculated to ensure reliability. Students who reported their sex as "other" (n=1) were excluded from the analysis due to the number of students being too small. The final dataset for analysis included 495 binary-sex students: 412 females and 83 males. The original dataset included 10 potential outliers, which were excluded from further analyses. Subsets of the data were created, including a binary subset with all samples except for people identifying as "other" sex, an all-female students subset, and an all-male students subset. Statistical analysis was then conducted using R (version 4.6.1).

Association Analyses: First, a regression model was used to test the association between ADHD symptom severity (ASRS) and college adjustment (AAS), adjusting for age and sex. Next, we tested the impact of alcohol use on ADHD symptoms and resulting college adjustment. We then tested an interaction model using alcohol and ADHD variables as the predictors and college adjustment as the outcome. Similarly, we tested the interaction of depression (BDI) and anxiety (BAI) with ADHD on college adjustment. These models were analyzed to replicate the original study's findings (Mohamad et al.) and determine whether the association between ADHD symptoms and college adjustment remained the same after accounting for comorbid conditions. Additional analyses provided novel extensions to the original study. Sex-stratified regression models were used to assess the association between ADHD and college adjustment, the association between alcohol and ADHD, the interaction between ADHD and alcohol and its effect on college adjustment, and the interaction between ADHD and depression and anxiety. All associations were tested using linear regression models in R, adjusting for age and sex as covariates (only age was used as a covariate in sex stratified analyses).

Mediation Analyses: Three mediation models were used to test the mediating effects of alcohol, depression, and anxiety on ADHD's relationship with college adjustment. The mediation

effects were tested on the full and sex-stratified datasets. Mediation was tested using the mediation package in R (Tofighi and MacKinnon), and 5000 bootstrap simulations were done for each test. The results of the mediation tests included ACME (Average Causal Mediation Effect), ADE (Average Direct Effect), total effect, and proportion mediated, and were interpreted statistically rather than causally due to the dataset being observational.

Results

Sample Characteristics: After quality control, the sample included n=495 participants: 83 (16.8%) males and 412 (83.2%) females (Table 1). Participants' ages ranged from 18 to 21 years, with a mean of 18.43 years. The population mainly consisted of 18-year-olds, with ~33% of participants over 18 years of age. For the population, mean ADHD symptom severity was 35.35 (SD = 11.25), mean college adjustment was 30.99 (SD = 4.89), and mean alcohol use was 3.77 (SD = 4.82). Mean BDI and BAI scores were 16.06 (SD = 10.15) and 21.05 (SD = 12.51), respectively.

ADHD & College Adjustment: More severe ADHD symptoms were significantly associated with lower college adjustment in the full sample after adjusting for age and sex ($\beta = -0.07$, $p < 0.001$) (Table 2). After stratification by sex, the association remained statistically significant in the female-student-only sample ($\beta = -0.08$, $p < 0.001$), but was not statistically significant in the male-student-only sample ($\beta = -0.0336$, $p = 0.404$).

Alcohol, ADHD and College Adjustment: Alcohol use was positively associated with ADHD symptom severity in the full sample after adjusting for age and sex ($\beta = 0.41$, $p < 0.001$) (Table 2). After sex stratification, the association remained statistically significant in the female-student-only sample ($\beta = 0.53$, $p < 0.001$), but was not statistically significant among male students ($\beta = 0.11$, $p = 0.628$). The interaction between alcohol and ADHD predicting college adjustment was statistically significant in the full sample ($\beta = 0.0077$, $p = 0.024$), but not in either stratum (female students $\beta = 0.0035$, $p = 0.4963$ or male students $\beta = 0.0033$, $p = 0.5502$).

Mediation analysis was performed to further examine alcohol as a mediator of the effects of ADHD symptoms on college adjustment. The mediation effect of alcohol was not statistically significant in the full sample (ACME = 0.0063, $p = 0.094$). The direct effect (ADE = -0.0749, $p < 0.001$) and total effect (TE = -0.0686, $p < 0.001$) were both negative and statistically significant in the full sample (Figure 1). Surprisingly, among females, the mediation effect of alcohol on ADHD and college adjustment was positive and statistically significant (ACME = 0.018, $p < 0.001$), which suggests that alcohol had a suppressing effect on the negative impact of ADHD severity on college adjustment. Among males, no mediating effect of alcohol on ADHD's association with college adjustment was observed.

Depression, ADHD and College Adjustment: In the full sample, higher ADHD symptom severity was significantly associated with greater depressive symptoms ($\beta = 0.4116$, $p < 0.0001$) (Table 2). The association was also significant in women ($\beta = 0.4429$, $p < 0.0001$) and men ($\beta = 0.3195$, $p < 0.0001$) independently. The interaction between ADHD and depression

predicting college adjustment was not statistically significant in the full sample or either sex stratum.

The mediation effect of depression on ADHD symptoms on college adjustment was statistically significant in the full sample (ACME = -0.0454, $p < 0.001$), female students sample (ACME = -0.0448, $p < 0.001$), and male students sample (ACME = -0.0505, $p < 0.01$) (Figure 1).

Anxiety, ADHD and College Adjustment: Similar to depression, higher severity of ADHD symptoms was significantly associated with greater anxiety in the full sample ($\beta = 0.5147$, $p < 0.0001$). The association was also significant for male students ($\beta = 0.3855$, $p < 0.0001$) and female students ($\beta = 0.5574$, $p < 0.0001$) independently. The interaction between ADHD and anxiety predicting college adjustment was not statistically significant in the full sample or either sex stratum.

Interestingly, the mediation effect of anxiety was not statistically significant in any sample (full dataset, men, and women only).

Discussion

This study examined the effect of ADHD symptoms on college adjustment and whether alcohol use, depression, and anxiety were related to the association. Specifically, the analyses focused on sex differential effects of ADHD and comorbidities on college adjustment. Overall, higher ADHD symptom severity was associated with poorer college adjustment. This association was significant in female students, but not in male students. This is likely explained by the significantly smaller number of male students than female students in the dataset. However, the effect size of ADHD severity on college adjustment was also significantly larger in women compared to men. This warrants that the lack of statistical significance and the difference in effect size in male students should be interpreted with caution.

Higher alcohol use was associated with greater ADHD symptom severity in the full sample and the female students' sample, but not in the male students' sample. The mediation analysis provided an unusual finding. In the mediation analysis, alcohol showed a significant positive mediating effect only in female students, with the full sample and male students' sample not showing significant mediation. The overall association was negative, but this positive mediating effect acted in the opposite direction and partially offset the negative association in the female students' sample. These findings weakly suggest that alcohol improves the effect of ADHD symptoms on college adjustment; however, greater alcohol use was associated with greater ADHD symptom severity in the female students' sample. One possible explanation is that alcohol helped temporarily reduce social anxiety and stress, alleviating the symptoms of ADHD and influencing college adjustment positively. However, this explanation was not directly tested in this study and remains speculative. Future independent studies with larger sample sizes are needed to replicate and validate this result. The results of the female mediation analysis should therefore be treated as only exploratory.

Depression was most consistently related to the association between ADHD and college adjustment. ADHD symptom severity was positively associated with depressive symptoms in all

samples. Depression also significantly mediated the association between ADHD symptoms and college adjustment, with similar estimates in all groups. Even though ADHD symptom severity was strongly associated with anxiety symptoms in the full sample and the sex-stratified samples, surprisingly, anxiety did not significantly mediate the relationship between ADHD and college adjustment in any sample. The interaction of ADHD with anxiety was also not significant in any sample. These findings are consistent with Riboldi et al., who found that depressive symptoms significantly mediated the association between ADHD and poorer academic functioning among university students, while anxiety symptoms did not seem to have a mediating effect (Riboldi et al.). That study used different measures for determining ADHD symptoms, depressive symptoms, and symptoms of anxiety, focusing on academic performance rather than college adjustment, but the pattern is similar. This suggests that depression may be a particularly relevant variable when examining academic difficulty associated with ADHD symptoms in university students.

Limitations

For this study, several limitations should be considered. First, the study relied on self-reported data, which may be influenced by social desirability bias or other forms of bias from the students. Second, due to this study being observational at one point in time, temporal and causal conclusions cannot be made. The mediation analyses have limited power to determine whether ADHD symptoms lead to depression, whether depression makes college adjustment more difficult for students, or whether outside factors influence both. Unmeasured variables such as ADHD medication use, sleep, social support, and socioeconomic status may also have affected the associations. Third, the full sample consisted predominantly of female students, and all students were South African first-year university students, limiting the generalizability to other populations of students. Future studies should be designed longitudinally to investigate temporal relationships and include more sex-balanced samples to allow for stronger comparisons between male students and female students. The positive mediation effect of alcohol observed in female students in this study is important to replicate.

Conclusions

Among South African first-year university students, greater ADHD symptom severity was associated with poorer college adjustment, especially in female students. Depression mediated this association in all samples, while anxiety did not. The positive alcohol mediation effect in female students is exploratory and needs replication. Screening students with elevated ADHD symptoms for depression may help support their transition to college, and longitudinal studies with sex-balanced samples are needed to confirm these findings.

Data availability: This dataset was described in Mohamad et al. in the Journal of Attention Disorders, 29(5), 363–386. Data are openly available in Zivahub at https://doi.org/10.25375/uct.24906924 and Kaggle at https://www.kaggle.com/datasets/imtkaggleteam/adhd-mental-health

Works Cited

Anastopoulos, Arthur D., et al. "Rates and Patterns of Comorbidity among First-Year College Students with ADHD." Journal of Clinical Child and Adolescent Psychology : The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53, vol. 47, no. 2, 2018, pp. 236–47. PubMed Central, https://doi.org/10.1080/15374416.2015.1105137.

Anderson, Joel R., et al. "The Academic Adjustment Scale: Measuring the Adjustment of Permanent Resident or Sojourner Students." International Journal of Intercultural Relations, vol. 54, Sept. 2016, pp. 68–76. ScienceDirect, https://doi.org/10.1016/j.ijintrel.2016.07.006.

Attention-Deficit/Hyperactivity Disorder: What You Need to Know - National Institute of Mental Health (NIMH). https://www.nimh.nih.gov/health/publications/attention-deficit-hyperactivity-disorder-what-you-need-to-know. Accessed 25 Sept. 2026.

Attoe, Darby E., and Emma A. Climie. "Miss. Diagnosis: A Systematic Review of ADHD in Adult Women." Journal of Attention Disorders, vol. 27, no. 7, May 2023, pp. 645–57. PubMed, https://doi.org/10.1177/10870547231161533.

Beck, A. T., et al. "An Inventory for Measuring Clinical Anxiety: Psychometric Properties." Journal of Consulting and Clinical Psychology, vol. 56, no. 6, Dec. 1988, pp. 893–97. PubMed, https://doi.org/10.1037//0022-006x.56.6.893.

Beck, Aaron T., et al. Manual for the Beck Depression Inventory-II. Psychological Corporation, 1996.

Cha, Yaejin, et al. "Prevalence of Self-Reported Diagnoses of Attention-Deficit/Hyperactivity Disorder (ADHD) among Post-Secondary Students in the U.S.: A Narrative Review." Current Psychiatry Reports, vol. 28, no. 1, Dec. 2025, p. 4. PubMed, https://doi.org/10.1007/s11920-025-01653-1.

DuPaul, George J., et al. "Academic Trajectories of College Students with and without ADHD: Predictors of Four-Year Outcomes." Journal of Clinical Child and Adolescent Psychology : The Official Journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53, vol. 50, no. 6, 2021, pp. 828–43. PubMed Central, https://doi.org/10.1080/15374416.2020.1867990.

Hinshaw, Stephen P., et al. "Annual Research Review: Attention-Deficit/Hyperactivity Disorder in Girls and Women: Underrepresentation, Longitudinal Processes, and Key Directions." Journal of Child Psychology and Psychiatry, and Allied Disciplines, vol. 63, no. 4, Apr. 2022, pp. 484–96. PubMed, https://doi.org/10.1111/jcpp.13480.

Kessler, Ronald C., et al. "The World Health Organization Adult ADHD Self-Report Scale (ASRS): A Short Screening Scale for Use in the General Population." Psychological Medicine, vol. 35, no. 2, Feb. 2005, pp. 245–56. PubMed, https://doi.org/10.1017/s0033291704002892.

Mohamad, Nawal, et al. “Symptoms of ADHD and Other Common Mental Disorders Influence Academic Success in South African Undergraduates.” Journal of Attention Disorders, vol. 29, no. 5, Mar. 2025, pp. 363–86. DOI.org (Crossref), https://doi.org/10.1177/10870547241310659.

Pagespetit, Èlia, et al. “ADHD and Academic Performance in College Students: A Systematic Review.” Journal of Attention Disorders, vol. 29, no. 4, Feb. 2025, pp. 281–97. PubMed, https://doi.org/10.1177/10870547241306554.

Riboldi, Ilaria, et al. “Testing the Impact of Depressive and Anxiety Features on the Association between Attention-Deficit/Hyperactivity Disorder Symptoms and Academic Performance among University Students: A Mediation Analysis.” Brain Sciences, vol. 12, no. 9, Aug. 2022, p. 1155. PubMed Central, https://doi.org/10.3390/brainsci12091155.

Saunders, J. B., et al. “Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II.” Addiction [Abingdon, England], vol. 88, no. 6, June 1993, pp. 791–804. PubMed, https://doi.org/10.1111/j.1360-0443.1993.tb02093.x.

Tofighi, Davood, and David P. MacKinnon. “RMediation: An R Package for Mediation Analysis Confidence Intervals.” Behavior Research Methods, vol. 43, no. 3, Sept. 2011, pp. 692–700. PubMed Central, https://doi.org/10.3758/s13428-011-0076-x.

Table 1: Sample demographics

Variable	All	Male	Female
Participants (n)	495	83	412
Age (years)	18.43 ± 0.72	18.77 ± 0.99	18.36 ± 0.64
ADHD (ASRS)	35.35 ± 11.25	34.43 ± 13.41	35.53 ± 10.77
Alcohol use (AUDIT)	3.77 ± 4.82	5.58 ± 6.35	3.41 ± 4.37
College adjustment (AAS)	30.99 ± 4.89	30.80 ± 4.95	31.03 ± 4.89
Depression symptoms (BDI)	16.06 ± 10.15	13.16 ± 9.97	16.64 ± 10.09
Anxiety symptoms (BAI)	21.05 ± 12.51	14.60 ± 10.62	22.35 ± 12.47

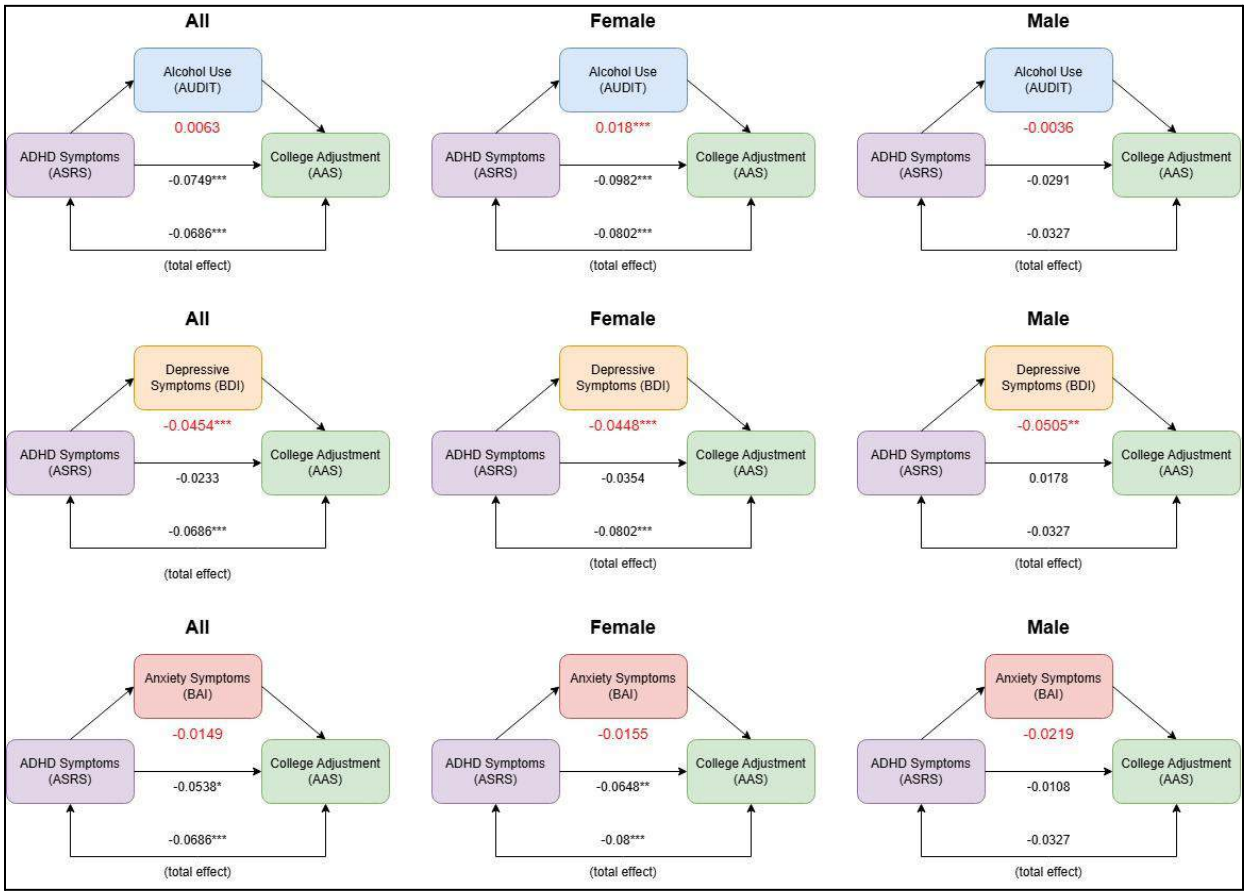
Table 2: Regression results for the models testing the association of ADHD, alcohol use, and comorbidities with college adjustment

Model	Dataset	Beta	SE	P	Dataset	Beta	SE	P	Dataset	Beta	SE	P
College Adjustment ~ ADHD	All participants	-0.07	0.02	6.00E-04	Males	-0.03	0.04	4.04E-01	Females	-0.08	0.02	5.00E-04
ADHD~Alcohol		0.41	0.11	1.20E-04		0.11	0.24	6.28E-01		0.53	0.12	1.12E-05
ADHD~Depression		0.41	0.04	1.00E-04		0.32	0.07	1.00E-04		0.44	0.04	1.00E-04
ADHD~Anxiety		0.51	0.04	1.00E-04		0.39	0.08	1.00E-04		0.56	0.05	1.00E-04
College		0.008	0.003	2.40E-02		0.003	0.005	5.50E-01		0.004	0.005	4.96E-01

Adjustment ~ ADHD*Alcohol												
College Adjustment ~ ADHD*Anxiety		0.002	0.002	1.96E-01		0.002	0.004	6.12E-01		0.003	0.002	1.26E-01
College Adjustment ~ ADHD*Depression		0.001	0.002	4.39E-01		0.002	0.004	6.34E-01		0.002	0.002	4.12E-01

Figure 1: Mediation Analyses

The effect of the mediators–alcohol use, depression, and anxiety–on ADHD is shown below. The mediation effect is shown in red, and stars indicate significance. The direct effect and total effect are shown in black (* p < .05, ** p < .01, *** p < .001) .



Biopsychosocial Contributors to Eating Disorders By So Yoon Park

Abstract

Eating disorders are rising psychiatric conditions with high rates of morbidity. While researchers often focus on targeting specific behaviors, this does not account for the reasoning behind the development of eating disorders. This review explores the interaction of biological, psychological, and social factors that lead to the development of eating disorders. From underlying biological and genetic factors, psychological internal pressures, to societal norms and culture, all factors may or may not contribute to the development of eating disorders by an individual's susceptibility to how these factors interact. Therefore, understanding the complex relationship behind the different factors is essential before approaching patients with eating disorders. Future research should focus on longitudinal studies of men and boys and their development of eating disorders, as many studies are mainly focused on women and female adolescents.

Introduction

Eating disorders are severe psychiatric conditions with high rates of morbidity, making it essential to understand the facts that cause their development rather than only treating the surface-level symptoms. While the majority of public representation of eating disorders focuses on women and girls, eating disorders affect people across all genders, ages, and backgrounds. The *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5-TR), the primary diagnostic classification system for mental disorders used in the United States, defines three eating disorders: anorexia nervosa is defined by restrictive eating, low body weight, and distorted self-body image; bulimia nervosa involves binge eating behaviors with compensatory behaviors such as excessive exercise or vomiting; binge eating disorder is the loss of control eating without the compensatory behaviors seen in bulimia nervosa (American Psychiatric Association, 2013). These disorders have different behaviors and symptoms but share overlapping risk factors or shift from one diagnosis to another over time. This overlap suggests that different types of eating disorder may have a shared underlying spectrum that contributes to their development. Furthermore, individuals may present with symptoms of more than one eating disorder, making the clear categorization difficult.

There is no single explanation that accounts for why some individuals develop an eating disorder while individuals under the same conditions do not, which has led researchers to explore the biopsychosocial model of eating disorder. The framework views eating disorder symptoms as arising from the interaction between biological, psychological, and sociocultural factors, rather than just one single cause. Biological factors are internal physiological influences such as genetic heritability and neurobiological processes that create the underlying vulnerability. Psychological factors are cognitive and emotional patterns of an individual, including body dissatisfaction and perfectionism that control an individual's thinking process about consuming food. Sociocultural factors are external influences, such as media exposure and cultural aspects that lead to

comparison of an individual's body image to societal norms. Focusing on one category doesn't provide an answer to the cause of eating disorder, each of the factors alone cannot explain the development of different symptoms. It is the interaction of these factors that best accounts for the development and persistence of eating disorders.

This review explores how biological, psychological, and sociocultural factors work together to contribute to eating disorder development. Together, the sections argue that eating disorders can be best understood through interaction of all three factors.

Biological Factors that Contribute to the Development of Eating Disorder

Biological factors are internal influences including genetic inheritance, neurobiological processes, and immune functioning that shape an individual's vulnerability to developing an eating disorder. Unlike the sociocultural or psychological pressures that act externally, biological factors represent internal processes that interact with environmental triggers to produce or maintain eating disorder symptoms. Understanding these mechanisms is essential to understand why some individuals exposed to similar environmental risks can have a higher chance of developing an eating disorder while others do not.

Eating disorders show a significant heritable component, even though the amount of genetic contribution varies across disorders or symptoms of a specific disorder. In a population based sample of female twins, anorexia nervosa had an estimated heritability of 58%, with 34% of the variance shared genetically with major depression, suggesting the genetic liability between two conditions (Wade et al., 2000). Another study of Swedish sister-pair similar ground the heritability of anorexia nervosa to be 43%, with genetic factors contributing to the overlap between anorexia nervosa and bulimia nervosa over the courses of illness (Yao et al., 2021), suggesting that there is a correlation between two disorders that reflect a shared genetic vulnerability rather than treatment failure.

Bulimia nervosa also shows a comparable pattern. A twin study found that 62% of the variance in bulimia nervosa liability was attributable to additive genetic effects, although heritability for individual symptoms was lower than for the overall diagnosis (Mazzeo et al., 2010). This indicates that genetics may set overall risk while the environment works to shape and form which symptoms emerge. Different family studies reinforce the claims of these findings, as first-degrees relatives of individuals with anorexia nervosa show rates of disorder 7.7 times higher than relatives of never-ill comparison probands, with siblings specifically showing elevated rates of substance use disorders (Strober et al., 2000). All in all, these findings suggest that eating disorders may run in families via genetic heritability, as well as shared environmental exposure (which will be discussed further in the following section).

Findings are mixed on whether men or women are at greater risk of developing eating disorder symptoms, with emerging research suggesting that risk factors may operate independently of biological sex. A longitudinal cohort study found that dietary restraint at age 7 directly predicted eating disorder symptoms at age 12 for boys but not girls, while depressive symptoms were significantly correlated to girls but not boys (Evans et al., 2017). This suggests

that boys and girls may have different risk pathways even when they arrive at similar symptoms, complicating the assumption that eating disorders are female-predominant with identical underlying mechanisms.

Beyond inherited risks, research suggests that biological differences in immune functioning and neurotransmitters actively maintain eating disorder symptoms once they emerge. Extreme calorie restriction and the resulting loss of body fat stores in anorexia nervosa patients are associated with changes in immune functioning, alternating expression of ghrelin that may be regulated by pro-inflammatory cytokines (Grzelak et al., 2017). This suggests that malnutrition can create a physiological impact that makes normal eating harder independent of psychological influences. Neurotransmitter activity also appears to reinforce restrictive behavior, as specific neurotransmitter regulation may cause eating to lead to anxiety while restrictions produce a mood improvement (Grzelak et al., 2017), helping explain why restriction can tend to make an individual feel better even as it becomes physically dangerous. Serotonin activity can be an indirect cause to eating disorders, as it mediates risks through personality traits such as perfectionism and anxiety (Rikani et al., 2013), leading for these traits as possible causes of neurobiological vulnerability rather than purely psychological factors. Furthermore, a case-control study of adolescents at illness onset identified triiodothyronine (T3) as a biological correlate, joining with psychological traits such as desire for thinness and anxiety, determining the interaction between biological and psychological factors from early stages of illness (Sepúlveda et al., 2021).

In conclusion, biological factors are significant as they provide a foundation of genetic and physiological vulnerability that interacts with other factors to lead to the development of eating disorders. Heritability, sex-specific pathways, and immune and neurotransmitter dysregulation allow to complicate but explain how eating disorder behaviors become self-sustaining.

Psychological Factors and Internal Influences that Contribute to Eating Disorders

Psychological factors are internal influences such as self-perception and cognitive patterns that form how individuals view food, bodies, and control. Understanding these factors is essential as it may help explain how external pressures become internalized and contribute to eating-disorder risk.

Body dissatisfaction and distorted self-perception is directly linked to the development of eating disorders, with the psychological influencing the symptoms and risks. A study on anorexia nervosa and bulimia nervosa shows that among 39 female inpatients, those with binging symptoms reflected the greatest pain insensitivity and somatoform dissociation (Papežová, Yamamotova, & Uher, 2005). A case-control study of 50 adolescents diagnosed with an eating disorder at illness onset similarly presents the significance of distorted self-perception, identifying the drive for thinness, perfectionism, and anxiety as main psychological factors that distinguish these adolescents from control groups (Sepúlveda et al., 2021). This suggests that body dissatisfaction does not independently influence the development of eating disorders, but is

rather closely linked with other traits such as perfectionism and anxiety, which together shape eating disorder behaviors from earliest stages of diagnosis. Both of the findings demonstrate how psychological factors directly impact an individual's development of eating disorder and symptoms, as distorted self-perception and the associated traits affect how the disorder is maintained.

The self-regulation factor also connects with persistent treatment challenges. In a study with 189 adults with binge eating disorder undergoing cognitive behavioral therapy, it is shown that weight changes are tied to shifts in shape and weight concern rather than just binge eating episodes (Pacanowski et al., 2018). This finding can indicate that fewer binges doesn't essentially mean weight loss, but the behavioral improvement was due to psychological change of an individual. Addressing the eating behavior without controlling the psychological factors can lead to the treatment being not as effective.

Cognitive and behavioral patterns also explain how psychological cores can expand over time. Individuals with bulimic symptoms showed reduced self-control to hunger and satiety cues, showing the reinforcement of environments that emphasize maintaining specific body shape for occasions (Haas & Clopton, 2001). This factor is exacerbated by an individual's mind that rewards restriction (Grzelak et al., 2017). Together, these findings determine how it is the felt loss of control that drives distress in eating disorder behaviors.

Overall, psychological factors actively disrupt self-perception and loss of regulatory control that sustains eating disorder behaviors, even if the symptoms are directly targeted in a treatment.

Sociocultural Factors and External Perception that Contribute to Eating Disorder

Sociocultural factors are social and cultural areas that shape how individuals view themselves. Factors such as beauty standards in media, cultural values toward weight, and pressure of social norms significantly influence the development of eating disorders. As they are related to culture, the standards are different across societies, making risk factors vary in communities. Adolescents and young adults are susceptible to this factor, as they are in stages of identity formation and are more exposed to environments with emphasis on appearance.

Social media usage is associated with body image concerns and dissatisfaction, which contributes to the development of eating disorders. Throughout the recent years, the growth of eating disorder risks seem to halve a link with the growing usage of social media. Society has unreasonable body image expectations that people desire to fit into, as reflected in social media or norms that they try to follow.

According to Dane & Bhatia (2023), findings from a scoping review of over 50 studies suggest that the connection between social media use and eating disorders is mediated by comparison to others, self-objectification, and societal norms around thinness in adolescents and young adults. A direct relationship with eating disorders and social media can be seen regardless of the platforms that are used by participants, as the impact of Facebook, Instagram, and Snapchat on development of eating disorders was assessed (Saunders & Eaton, 2018). All in all,

these findings show that social media is a central sociocultural factor that increases eating disorder risk across platforms.

A direct association between social media and development of eating disorders in participants can be shown regardless of types of the platforms. In a study conducted with 637 women aged 18-24 who used social media and never had an eating disorder diagnosis, findings suggested positive associations between upward comparison (comparing oneself to idealistic standards on body image), body surveillance (self-consciousness of body and whether into beauty standards), and disordered eating were clearly shown when the surveyed participants' overall rating indicated that social media is significantly related to sociocultural factors of eating disorder. It is apparent from these studies that social media platforms have a direct link to body dissatisfaction, which leads to eating disorders. In particular, associations were strongest between body dissatisfaction and binge eating, relative to other eating disorders (Saunders & Eaton, 2018). It is also demonstrated research that the use of social media has led to creation of content and accounts that lead to eating disorders. Carey (2015) found that almost 60% of participants maintained a separate social media profile just for their eating disorder across different platforms, where 100% of the participants stated social media affected ED. Additionally, 93% of participants found that the graphic images on the platforms triggered their eating disorder behaviors, demonstrating a role that visual content has on eating patterns (Carey, 2015).

Societal norms on beauty and body image in the community is another factor that heavily influences the development of eating disorders. Body dissatisfaction growing in young adolescents to having symptoms of eating disorder, higher rate by certain age and gender (12 years of age girls) (Evans et al., 2017). Adolescents are determining the ideal beauty norm through their observation in social media, fitting their body standards along with what they see. It is also noted that different levels of eating disorder risks depending on gender, males showed more risks, which contradicts previous assumptions (Jaruga-Sękowska et al., 2025). While there is a stereotype of women having more tendency to show eating disorder symptoms, study shows the opposite of the assumption. However, gender isn't the only determinant when it comes to risks of eating disorders, as different ages had different sociocultural pressures on body image (Izydorczyk & Sitnik-Warchulska 2018).

The sociocultural factors such as social media and societal norms of the body have become a bigger part in the contribution to risks. Regarding age, gender, and frequency of social media use, people experienced more pressure by comparison and the need to meet unrealistic expectations, leading them to experience symptoms of eating disorder.

Discussion

Taken together, the findings across biological, psychological, sociocultural factors determine that eating disorders develop through interaction of multiple risk factors rather than a single cause. Genetics and physiological processes create an underlying susceptibility to eating disorders, but this vulnerability does not act alone to the outcome of the disorder. Psychological patterns where individuals derive towards a "perfect body" and regulate consumption of food

shapes how the underlying biological factor plays out in their behavior. At the same time, external pressures such as social media and cultural influences can activate these vulnerabilities to become more sensitive to body comparison. In short, these findings support a biopsychosocial structure where no single factor acts out independently, but instead interacts with one another to increase the overall risk for eating disorder.

Despite these findings, there are several limitations across this paper. First, there is limited research exploring the sex differences, and it is difficult to determine that a single gender is more vulnerable to the development of an eating disorder. There has been a stereotype that female genders are inherently more vulnerable to developing eating disorders than males, but this assumption is not supported by current research, as only a few studies have been directly tested on sex differences. Second, the majority of the studies reviewed included the sample of girls, women, and adolescents, which limits the generalizability of these findings to other populations. Third, many of the studies that were included in this review relied on cross-sectional or retrospective study, which limits the ability to draw a direct conclusion on whether these factors develop eating disorders or emerge as a consequence of it. Finally, studies have mostly relied on self-report measures, which can lead to biased reports or overrepresented results.

Future research should focus on comparing different types of eating disorders directly, in order to better understand how all of the factors contribute similarly or uniquely to different types of eating disorders. Additionally, future studies should investigate more sample populations including boys and men by adding longitudinal studies, as current findings reduce the ability to generalize based on how many of the studies explore female samples.

In conclusion, eating disorders develop from an intricate interaction of biological, psychological, and sociocultural components, and no single factor is fully accountable for the development of individuals' eating disorder symptoms. The findings suggest that effective prevention and treatment for this disorder requires addressing a variety of factors, rather than targeting a single behavior. As eating disorders increasingly affect a broad range of populations, expanding research to include overlooked populations will be essential to establish a generalizable understanding of the development of this disorder.

Works Cited

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). https://doi.org/10.1176/appi.books.9780890425596

Carey, M. (2015). The Affects Social Media has on People with Eating Disorders. Ir Assoc Couns Psychother, 15(4).

Dane, A., & Bhatia, K. (2023). The social media diet: A scoping review to investigate the association between social media, body image and eating disorders amongst young people. PLOS Global Public Health, 3(3), e0001091.

Evans, E. H., Adamson, A. J., Basterfield, L., Le Couteur, A., Reilly, J. K., Reilly, J. J., & Parkinson, K. N. (2017). Risk factors for eating disorder symptoms at 12 years of age: A 6-year longitudinal cohort study. Appetite, 108, 12-20.

Grzelak, T., Dutkiewicz, A., Paszynska, E., Dmitrzak-Weglarz, M., Slopien, A., & Tyszkiewicz-Nwafor, M. (2017). Neurobiochemical and psychological factors influencing the eating behaviors and attitudes in anorexia nervosa: Grzelak et al. Journal of physiology and biochemistry, 73(2), 297-305.

Haas, H. L., & Clopton, J. R. (2001). Psychology of an eating disorder. Eating disorders in women and children: Prevention, stress management, and treatment, 39-48.

Izydorczyk, B., & Sitnik-Warchulska, K. (2018). Sociocultural appearance standards and risk factors for eating disorders in adolescents and women of various ages. Frontiers in psychology, 9, 429.

Jaruga-Sękowska, S., Staśkiewicz-Bartecka, W., & Woźniak-Holecka, J. (2025). The impact of social media on eating disorder risk and self-esteem among adolescents and young adults: a psychosocial analysis in individuals aged 16–25. Nutrients, 17(2), 219.

Mazzeo, S. E., Mitchell, K. S., Bulik, C. M., Aggen, S. H., Kendler, K. S., & Neale, M. C. (2010). A twin study of specific bulimia nervosa symptoms. Psychol Med, 40(7), 1203-1213.

Pacanowski, C. R., Mason, T. B., Crosby, R. D., Mitchell, J. E., Crow, S. J., Wonderlich, S. A., & Peterson, C. B. (2018). Weight change over the course of binge eating disorder treatment: relationship to binge episodes and psychological factors. Obesity, 26(5), 838-844.

Papežová, H., Yamamotova, A., & Uher, R. (2005). Elevated pain threshold in eating disorders: physiological and psychological factors. Journal of psychiatric research, 39(4), 431-438.

Rikani, A. A., Choudhry, Z., Choudhry, A. M., Ikram, H., Asghar, M. W., Kajal, D., ... & Mobassarah, N. J. (2013). A critique of the literature on etiology of eating disorders. Annals of neurosciences, 20(4), 157.

Saunders, J. F., & Eaton, A. A. (2018). Snaps, selfies, and shares: How three popular social media platforms contribute to the sociocultural model of disordered eating among young women. Cyberpsychology, Behavior, and Social Networking, 21(6), 343-354.

Sepúlveda, A. R., Moreno-Encinas, A., Martínez-Huertas, J. A., Anastasiadou, D., Nova, E., Marcos, A., ... & Graell, M. (2021). Toward a biological, psychological and familial

approach of eating disorders at onset: case-control ANOBAS study. Frontiers in psychology, 12, 714414.

Strober, M., Freeman, R., Lampert, C., Diamond, J., & Kaye, W. (2000). Controlled family study of anorexia nervosa and bulimia nervosa: evidence of shared liability and transmission of partial syndromes. American Journal of Psychiatry, 157(3), 393-401.

Wade, T. D., Bulik, C. M., Neale, M., & Kendler, K. S. (2000). Anorexia nervosa and major depression: shared genetic and environmental risk factors. American Journal of psychiatry, 157(3), 469-471.

Yao, S., Larsson, H., Norring, C., Birgegård, A., Lichtenstein, P., D'Onofrio, B. M., ... & Kuja-Halkola, R. (2021). Genetic and environmental contributions to diagnostic fluctuation in anorexia nervosa and bulimia nervosa. Psychological Medicine, 51(1), 62-69.

From Waste to Functional Ingredient: Bioactive Properties of Discarded Jeju Agricultural By-products and the Potential of Buckwheat Aerial Parts as a Superior Antidiabetic Resource

By Yeeun Kang[1], Garam Mo[2]

Abstract

This study extracted agricultural by-products from carrot, radish, beet, cabbage, kohlrabi, and buckwheat discarded in Jeju Island using 70% ethanol and comprehensively evaluated their physiological activities. Results demonstrated that buckwheat aerial parts exhibited substantially superior activity compared to other plants across all parameters. Beet leaves showed the highest extraction yield, suggesting strong industrial application potential. Buckwheat aerial parts contained the highest total flavonoid and phenolic contents, indicating high concentrations of diverse bioactive compounds. Meanwhile radish leaves and cabbage exhibited lower flavonoid levels, likely possessing higher proportions of non-flavonoid phenolics. HPLC analysis of nine standard compounds revealed gallic acid detection in most samples (kohlrabi, beet, cabbage, radish), with cabbage showing the highest concentration, while chlorogenic acid was detected only in orange carrots. Antioxidant activity assessments (TEAC, FRAP) confirmed buckwheat aerial parts exhibited the highest values among all samples. Notably, α-glucosidase inhibition assays demonstrated exceptionally high antidiabetic activity in buckwheat aerial parts, comparable to the synthetic inhibitor acarbose. HPLC analysis identified rutin as the predominant component in buckwheat leaves. Subsequent α-glucosidase inhibition testing of rutin revealed superior antidiabetic activity compared to acarbose, confirming rutin as the primary contributor to buckwheat leaves' antidiabetic properties. These findings suggest significant application potential of buckwheat aerial parts as antidiabetic functional materials. Consequently, buckwheat by-products can be upcycled into functional food ingredients supporting glycemic control, offering a sustainable solution addressing diabetes and blood sugar management needs in a sustainable way that reduces the environmental burden.

Keywords: Antioxidant Antidiabetic Bioactivity Rutin Upcycling

1. Introduction

The twentieth century brought about two major factors which led to rising diabetes numbers and shifting eating patterns that resulted in more people developing diabetes. The development of type 2 diabetes through obesity and physical inactivity has become more prevalent than type 1 diabetes which results from genetic inheritance. The diabetes prevalence reached 14.8% among people aged 30 and above while the 65+ age group showed a 28.0% diabetes rate. The Korean Diabetes Association reported that 4 out of 10 people aged 30 and above had prediabetes and 1 out of 2 people aged 65 and above had this condition (Korean Diabetes Association). The number of teenage diabetes cases has started to rise. The

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fast-growing number of diabetes patients has led scientists to focus on natural compounds derived from plants because synthetic antidiabetic drugs produce unwanted side effects. Despite the fact that the global agri-food industry generates a variety of byproducts, their industrial application remains largely limited. However, these byproducts are attracting attention as a potential source of functional ingredients, generating high added value from both scientific and economic perspectives. In Korea, over 9 million tons of agricultural byproducts are generated annually, but systematic utilization of these byproducts remains insufficient (Ministry of Agriculture, Food and Rural Affairs). Jeju Island cultivates a wide variety of agricultural products, but specifically, root-portions of carrots are reported to be primarily commercialized after harvest, with most aerial parts, such as leaves, being discarded on-site (Kim et al.). Similarly, approximately 50,000 tons of citrus peel is generated annually during citrus processing in Jeju, most of it being not properly utilized and discarded, resulting in ongoing disposal costs and environmental burden. Such discarded agricultural byproducts not only pose an environmental burden but also lead to a waste of valuable resources, showing an urgent need to develop effective ways to utilize them. Thus, this study analyzed the nutritional value of six types of agricultural byproducts (radish, carrot, beet, cabbage, kohlrabi, and buckwheat) from Jeju Island that are discarded in the field due to lack of utilization methods, resulting in environmental burden and waste of useful resources, and evaluated their potential as useful anti-diabetic materials.

Carrot (D. Carota var. Sativa) a widely cultivated from the Apiaceae family is mainly consumed as a root vegetable and contains high levels of carotenoids (notably β-carotene) dietary fiber, phenolic substances and vitamins like thiamine, riboflavin, niacin and vitamin C (Sharma et al.). Carrots are well recognized for their impacts on human health such as providing vitamin A, enhancing the immune system ,and safeguarding the liver (Sharma et al.). Nevertheless studies and applications of carrots remain mainly concentrated on the root. Radish (Raphanus sativus L.) is a root vegetable belonging to the cruciferous family. Due to its high nutritional value, it has been cultivated worldwide, including in Europe, the United States, and East Asia. Kimchi, a traditional Korean food with a history of over 1,000 years, is primarily made by fermenting radish and cabbage (Lee). In particular, radish leaves contain a large amount of nutrients such as calcium, potassium, sodium, fiber, fatty acids, and non-flavonoid polyphenols. According to Goyeneche et al., radish leaves were found to exhibit significantly higher total polyphenols, flavonoid compounds, and antioxidant activity compared to the roots.

Beet (Beta vulgaris L.) is a Chenopodiaceae root crop whose roots and leaves are both edible. Beets contain various antioxidant and anti-inflammatory substances, including betalain pigments (betacyanins, betaxanthins), which are phytochemicals with antioxidant, anti-inflammatory, and anticancer properties. These substances have been reported to inhibit cell transformation and cancer cell development induced by oxidative stress and inflammation (Escribano et al.; Cai et al.). Although the aerial part also contains abundant physiologically active and antibacterial substances, most research has focused on the roots, and scientific verification of the above-ground part is relatively lacking.

Cabbage (Brassica oleracea var. capitata) is a vegetable of Brassicaceae family. It is rich in various physiologically active substances with antioxidant activity, such as vitamin C, flavonoids, carotenoids, polyphenols, and glucosinolates (Podsędek). Cabbage produces a firm central leaf during its growth process, and this part is mainly harvested for consumption and distribution. In contrast, the outer leaves surrounding the core are typically discarded due to sanitary reasons or for freshness during distribution. However, a recent study found that the outer leaves of cabbage contained up to 2.3 times more vitamin C, up to 70% more antioxidant power, and nearly 40 times more chlorophyll than the core (Zhao et al.). Furthermore, carotenoids, anthocyanins, flavonoids, and phenolic compounds all tend to accumulate more in the outer leaves (Zhao et al.).

Kohlrabi (Brassica oleracea var. Gongylodes, kohlrabi) belongs to the Brassicaceae family of the Papaveraceae order, and is divided into Asian and Western European cultivars. In Korea, cultivation began in 1998, mainly on Jeju Island, and is currently produced nationwide. Kohlrabi has a high vitamin C content compared to other vegetables, and contains substances known to have anticancer effects, such as anthocyanins, carotenoids, and glucosinolates (Cha et al.). Kohlrabi is mainly consumed as an enlarged tuber, and the above-ground such as leaves are mostly discarded on site.

Buckwheat (Fagopyrum esculentum) is a representative pseudocereal crop cultivated worldwide, belonging to the genus Fagopyrum in the Caryophyllaceae family. Buckwheat is rich in nutrients such as protein, dietary fiber, minerals, and vitamins, as well as various physiologically active substances including polyphenols such as rutin, quercetin, and catechin. Therefore, various health benefits such as antioxidant, anti-inflammatory, anticancer, blood pressure control, vascular protection, blood sugar control, and cognitive function improvement have been reported (Steadman et al.; Zhang et al.). Particularly, rutin is a bioflavonoid in the form of a glycoside in which sugar is bound to quercetin, commonly called vitamin P, and is known to have various active properties including antioxidant properties (La Casa et al.; Han et al.). The total polyphenol content of buckwheat leaf samples, which are mostly discarded because they are not commercialized unlike seeds after harvest, was the second highest after flowers among various parts, and contained various phenolic compounds including flavonols, flavan-3-ols, and flavones (Nešović et al.).

As such, Jeju Island's major agricultural products, radish, carrot, beet, cabbage, kohlrabi, and buckwheat, generate significant amounts of byproducts. To address this issue, an experiment was conducted to extract these discarded byproducts with 70% ethanol and determine their potential as food ingredients. The experiment included measurements of total polyphenol and flavonoid content, as well as α-glucosidase inhibition, DPPH scavenging activity, and HPLC. The purpose of the experiment was to determine the potential of each byproduct as an anti-diabetic agent. This study is significant in that it provides foundational data for simultaneously promoting resource recycling and material development by utilizing discarded agricultural byproducts as high-value-added functional raw materials.

2. Materials and Methods

2.1 Materials

Agricultural byproducts discarded on-site were collected from farms in Jeju Island that cultivate carrots, cabbage, radish, beets, broccoli, and buckwheat. The collected products were washed, and dried in a hot air dryer (PFC-P0-91, Nihon-freezer, Namyangju, Korea) at 60°C for 24 h. The samples were dried and pulverized in a grinder before being used.

2.2 Extract manufacturing

Each 15 g sample of agricultural byproduct powder was mixed with 300 mL of 70% ethanol. The mixture was extracted for 1 hour using an ultrasonic cleaner (Powersonic 520, Hwashin Tech Co., Ltd., Seoul, Korea). This extraction process was repeated three times under the same conditions, and the extract was filtered using filter paper. The filtrate was concentrated using a rotary vacuum evaporator (Hei-VAP Precision, Heidolph, Schwabach, Germany). Afterwards, the sample was freeze-dried to obtain the final sample to be used. The entire process was repeated three times.

2.3 Total polyphenol content

Total polyphenol content was measured using the method of Ko et al. and expressed as gallic acid equivalent (GAE) based on a standard calibration curve prepared based on gallic acid. First, to confirm the polyphenol reaction, 700 μL of distilled water and 100 μL of reagent were mixed with 20 μL of each extract and left for 2 hours. Then, 100 μL of 20% sodium carbonate was added at a time and reacted for 1 hour. The absorbance was measured at 750 nm using a microplate reader (i-Mark 168-1135, Bio-Rad Inc., Hercules, CA, USA).

2.4 Total flavonoid content

Total flavonoid content was analyzed based on the method of Ko et al. with some modifications. The total flavonoid content was calculated based on a standard calibration curve prepared using quercetin as a reference substance, and the analysis results were converted to units of quercetin equivalent (QE). First, for analysis, 300 μL of ethanol was sequentially used as a dilution solvent for 100 μL of each extract, followed by 20 μL of 1 M potassium acetate for pH stabilization, and 20 μL of 10% aluminum nitrate to induce color development through flavonoid reaction. Then, 560 μL of distilled water was added to adjust the total volume to 1000 μL, and the mixture was reacted at room temperature for 1 hour. The absorbance was measured using a microplate reader (i-Mark 168-1135, BioRad Inc., Hercules, CA, USA) at a wavelength of 415 nm.

2.5 Ferric Reducing Antioxidant Power (FRAP)

FRAP is a method that measures the absorbance produced when the ferric tripyridyltriazine [Fe(III)-TPTZI] complex is converted to ferrous tripyri-dyltriazine

[Fe(Il)-TPTZ] by antioxidants under pH 3.6 conditions (Benzie and Strain). FRAP measures reducing power using iron ions. This method is widely used due to its simplicity and cost-effectiveness, despite its sensitivity to the color of the sample leading to the potential for a decrease in the stability of the oxygen electrode during the experiment (Yoo et al.). FRAP of six sample extracts was measured using the method of Benzie and Strain. 10 mM 2,4,6-tripyridyls-triazine, 300 mM sodium acetate buffer (pH 3.6), and 20 mM FeCl were mixed in a 1:10:1 ratio and incubated at 37°C for 30 min to produce a FRAP working solution. Then, 150 l of the FRAP working solution was added to 50 of the extract, and the mixture was incubated at 37°C for 15 min. The absorbance value was measured at 595 nm using a microplate reader, and the standard calibration curve was recorded using FeSO, and the FeSO4 reducing power (mM FElg) per 1 g of sample was indicated.

2.6 DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated using 1,1-Diphenyl-2-picrylhydrazyl (DPPH). DPPH, a stable free radical, exhibits deep purple and shows strong absorption characteristics around 517 nm. In the presence of antioxidants, it gradually changes to yellow, which is used as an indicator of antioxidant activity. Based on this, 40 μL of sample extract was added to 160 μL of a 0.15 mM DPPH solution dissolved in methanol, and the mixture was reacted under dark conditions for 30 minutes to avoid light-induced reaction. Afterward, the absorbance of the mixture was measured at 517 nm using a microplate reader. Antioxidant activity was quantified using the RC50 value, which represents the concentration of the sample required to reduce DPPH radical scavenging by 50%. The DPPH radical scavenging activity was compared by using butylated hydroxytoluene (BHT) as a positive control.

2.7 High-performance liquid chromatography (HPLC)

HPLC (e2695, Waters Co., Milford, MA, USA) was used to quantitatively analyze polyphenol compounds in six sample extracts. A Sunfire C18 Column (5 μm, 4.6 mm × 250 mm, Waters Co., USA) was used for the analysis, and the mobile phase consisted of two types of solutions: distilled water (A) containing 0.1% trifluoroacetic acid and acetonitrile (B) containing 0.1% trifluoroacetic acid. Solvent conditions were set stepwise as follows: From the starting point to 20 min, the mobile phase ratio was 90% A and 10% B, then A was reduced to 70% and B was increased to 30% until 25 min. Between 25 and 35 min, A was reduced to 30% and B was increased to 70%, and A was maintained at 5% and B at 95% until 38 min. The mobile phase A was restored to 90% until the end of the analysis.

The analysis was conducted under conditions where the mobile phase flowed at a constant flow rate of 1 mL/min, and the internal temperature of the column was maintained at 40°C. 20 μL of each sample was injected, and detection was performed at a wavelength of 280 nm. The standard materials used for quantitative analysis were dissolved in methanol and diluted into seven concentrations (3.125, 6.25, 12.5, 25, 50, 100, and 200 μg/ml) ranging from 3.125 to 200 μg/ml. A standard calibration curve was then created based on the data, and the content of

each polyphenol compound was calculated by comparing it with the data extracted from the samples. Nine polyphenol compounds were used: gallic acid, gentisic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, benzoic acid, cinnamic acid, and chlorogenic acid. In a subsequent study, rutin was also added to analyze the main components of buckwheat.

2.8 α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of six samples was evaluated by applying the p-nitrophenyl α-D-glucopyranoside (pNPG)-based method from Im and Kim. 20 μL of each extract were mixed with 120 μL of 20 mM potassium phosphate buffer (pH 6.8) and 50 μL of α-glucosidase (0.5 U/mL), followed by pre-incubation for 10 minutes at 37°C. Then, 10 μL of 2 mM pNPG was added under the same conditions and reacted for an additional 10 minutes. To end the reaction, 100 μL of 0.1 M sodium carbonate was added, and the absorbance of the generated p-nitrophenol was measured at 415 nm using a microplate reader. Based on the obtained inhibition rate, the sample concentration required to inhibit 50% of α-glucosidase activity (GIC_{50}) was calculated.

2.9 Data processing

The experiment in the study was repeated three times, and statistical significance was analyzed using Duncan's Multiple Range Test (DMRT) using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) with a significance level of $p<0.05$.

3. Results and Discussion

3.1 Total flavonoid content

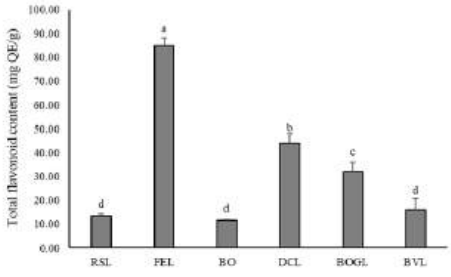

Figure 1. Total flavonoid content of agricultural by-products

RSL; Aerial parts of Raphanus sativus, FEL; Fagopyrum esculentum leaves and stems, BO; Outer leaves of Brassica oleracea var. Capitata, DCL; Aerial parts of Daucus carota, BOGL; Aerial parts of Brassica oleracea Gongylodes Group, BVL; Aerial parts of Beta vulgaris

The total flavonoid content findings are shown in Figure 1. Of the six samples buckwheat aerial parts showed the total flavonoid content measuring 84.90 mg QE/g. Next, in line were carrot aerial parts (43.88 mg QE/g) kohlrabi leaves (31.82 mg QE/g) and beet leaves (16.00 mg QE/g). Radish leaves and cabbage showed similar total flavonoid contents, with radish leaves at 13.39 mg QE/g and cabbage at 11.73 mg QE/g, which makes cabbage the sample with the lowest

total flavonoid content. When comparing these results with total polyphenol content data, buckwheat aerial parts showed that flavonoid content (84.90 mg QE/g) constituted a substantial proportion of total polyphenol content (180.22 mg GAE/g). Conversely, radish leaves and cabbage, which recorded low values in total flavonoid content, are likely to have a high proportion of non-flavonoid compounds within their total phenolic content.

3.2 Total phenolic content

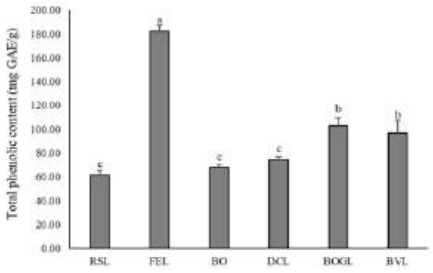

Figure 2. Total polyphenol content of agricultural by-products

The total phenolic content data from this study are recorded in Figure 2. Out of the six samples analyzed, buckwheat aerial parts showed the total phenolic content at 182.22 mg GAE/g. This figure indicates a disparity compared to the other samples implying that buckwheat is a superior source of phenolic compounds. Kohlrabi leaves (104.04 mg GAE/g) and beet leaves (93.74 mg GAE/g) followed with the highest amounts. Conversely radish leaves (61.69 mg GAE/g) cabbage (67.82 mg GAE/g) and orange carrot aerial parts (74.45 mg GAE/g) exhibited lower levels of phenolics within a similar range. These results appear to stem from differences in phytochemical composition among the six crops, and the highest value recorded in buckwheat aerial parts is likely due to the diverse phenolic compounds present in buckwheat leaves (Nešović et al.). Phenolic compounds in buckwheat have been reported in the following order: flowers (52 types) > leaves (50 types) > grains (45 types) > stems (27 types), with leaves confirmed to contain substantial amounts of rutin, various quercetin derivatives, and cinnamic acid series phenolic compounds (Nešović et al.). While protocatechuic, p-hydroxybenzoic, ferulic, and caffeic acids are detected throughout leaves, flowers, grains, and stems, rutin content is most prominent in leaves, and certain derivatives (dimethyl quercetin, kaempferide) have been identified exclusively in leaves (Nešović et al.). Although aerial parts including leaves and stems are discarded in the field during buckwheat harvest, synthesizing the total phenolic content from this study with previous research data reveals that leaves are high-value raw materials extremely rich in rutin, quercetin-series flavonoid compounds, and phenolic compounds. This indicates how discarded buckwheat aerial parts can be transformed into a core source of polyphenol raw materials.

3.3 HPLC chromatography component analysis

Figure 3 presents the HPLC chromatography component analysis results. Among the 6 samples, gallic acid was detected in kohlrabi, beet, cabbage, and radish. Among the average

gallic acid values of these four samples, beet recorded the lowest at 1.02 mg/mL, followed sequentially by radish with 1.13 mg/mL and kohlrabi with 1.25 mg/mL, which showed similar values. Cabbage's average content was 1.69 mg/mL, showing highest content among samples containing the same antioxidant compound. Conversely, carrot was the only sample containing chlorogenic acid, with a content of 8.98 mg/mL, recording a remarkably higher value compared to other samples. This result confirms that chlorogenic acid is a major component sourced from carrots.

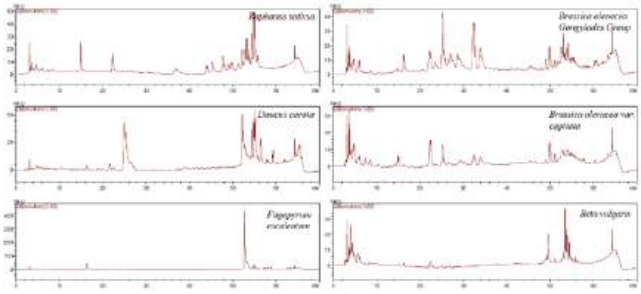

Figure 3. HPLC analysis of components in agricultural by-products

Unlike other samples that showed complex peaks from multiple components, the chromatogram results for buckwheat samples distinctly detected only a single peak around 52 minutes. This singular peak suggests that one component dominates the major constituent of buckwheat. Previous research by Habtemariam created a calibration curve by plotting peak areas at 280 nm for buckwheat leaf methanol extract against control rutin concentrations in HPLC analysis, obtaining a linear equation with correlation coefficient r^2=0.9999, demonstrating that the predominant peak in buckwheat leaf HPLC experimental results matched the peak in rutin concentration HPLC experiments. This research supports the likelihood that the single dominant peak observed in this study is rutin. The significantly higher total polyphenol and flavonoid contents observed in buckwheat compared to other samples in previous experiments suggests that high-level accumulation of rutin, a polyphenolic compound observed in HPLC, likely contributed substantially.

3.4 FRAP analysis

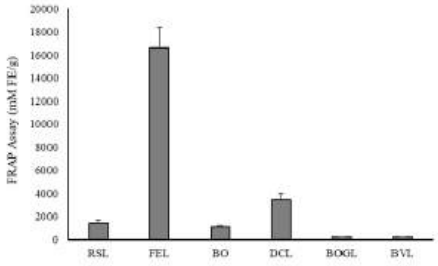

Figure 4. FRAP antioxidant assay of agricultural by-products

The values for each sample in FRAP measurements are recorded in Figure 4. Among the six samples, buckwheat aerial parts recorded the highest value at 16598.44 mM FE/g, followed

by orange carrot aerial parts (3447.65 mM FE/g), radish leaves (1443.63 mM FE/g), and cabbage (1108.57 mM FE/g) in descending order. Kohlrabi leaves (194.45 mM FE/g) and beet leaves (182.56 mM FE/g) recorded similar values, showing the lowest FRAP values among the six samples. These results suggest contrasting findings compared to TEAC measurements, another antioxidant assay. Although kohlrabi leaves and beet leaves recorded poor values in FRAP measurements, they recorded the next highest values after buckwheat aerial parts in TEAC antioxidant measurements. This phenomenon stems from differences in FRAP and TEAC mechanisms. FRAP measures the absorbance generated when ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is converted to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by antioxidants in the sample under low pH conditions. In contrast, TEAC is measured based on the degree to which antioxidants in the sample reduce or scavenge ABTS radicals formed during the experiment. Thus, these results can be interpreted as arising from methodological differences between FRAP and TEAC measurement processes. Additionally, similar to TEAC, buckwheat exhibited very high antioxidant activity, demonstrating excellent potential as a food material.

3.5 DPPH radical scavenging activity

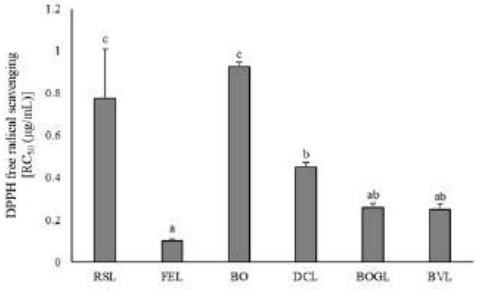

Figure 5. DPPH antioxidant assay of agricultural by-products

The DPPH radical scavenging activity of each sample is presented in Figure 5. DPPH is a method that measures the concentration (RC_{50}) required to scavenge 50% of DPPH radicals with lower values indicating superior antioxidant capacity. Buckwheat aerial parts recorded an RC_{50} of 0.10 μg/mL, following with beet leaves (0.26 μg/mL), kohlrabi (0.26 μg/mL), orange carrot (0.45 μg/mL), cabbage (0.92 μg/mL), and radish leaves (0.77 μg/mL). The average value was 0.92 μg/mL which indicates that all six samples possessed radical scavenging capacity to this average. This suggests that all samples exhibit antioxidant activity and phenolic compound content to some degree in terms of DPPH radical scavenging capacity. However, when examining specific values in detail, buckwheat aerial parts showed overwhelmingly superior antioxidant capacity compared to other samples. The low values recorded by buckwheat aerial parts indicate that small amounts can effectively scavenge DPPH radicals, confirming that buckwheat possesses far superior DPPH radical scavenging capacity compared to other samples.

3.6 α-glucosidase analysis

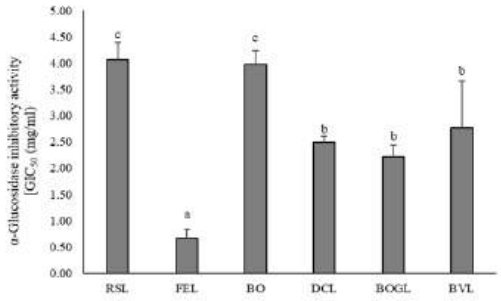

Figure 6. α-glucosidase inhibitory activity of agricultural by-products

Figure 6 presents the α-glucosidase values for each sample. In the evaluation of GIC_{50} (mg/mL): the concentration required to inhibit 50% of α-glucosidase activity, the enzyme that breaks down carbohydrates into monosaccharides for absorption, buckwheat showed the strongest inhibition at approximately 0.79 mg/mL, followed sequentially by kohlrabi, beet, and orange carrot with similar values of 2.09 mg/mL, 2.16 mg/mL, and 2.44 mg/mL, respectively. Cabbage and radish recorded 3.80 mg/mL and 3.81 mg/mL, respectively, showing the poorest inhibitory activity. This trend of buckwheat showing the highest values and radish and cabbage showing the lowest was also identically observed in total flavonoid content measurements. This suggests a significant correlation between flavonoid content and α-glucosidase inhibitory activity, which is also demonstrated in research by Swargiary et al., showing that flavonoids inhibit α-glucosidase activity by forming 1:1 non-covalent complexes with the enzyme (static quenching) (Swargiary et al.). The buckwheat sample, which showed the most significant results, demonstrated superior effects even when compared to acarbose, a synthetically produced inhibitor. According to research by Im and Kim, acarbose exhibited inhibitory activity of 739.91 μg/mL, approximately 0.74 mg/mL. This shows a difference of only 0.05 mg/mL lower than buckwheat aerial parts, suggesting similar inhibitory activity and raising the possibility that buckwheat aerial parts could serve as a novel approach for managing blood glucose as an antidiabetic material.

3.7 HPLC analysis of buckwheat and rutin

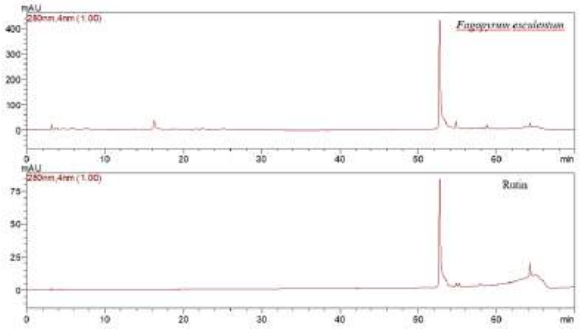

Figure 7. HPLC component analysis of the aerial parts of fagopyrum esculentum

Additionally, since buckwheat exhibited the most outstanding activity in total phenolic and flavonoid content, antioxidant activity, and antidiabetic experiments, HPLC component analysis of buckwheat was conducted. The HPLC analysis results for buckwheat and Rutin are presented in Figure 7. As previously compared with other samples, the buckwheat chromatogram showed a single peak at approximately 52 minutes, suggesting the presence of one predominant component. Experimental results confirmed a peak identical to the rutin standard at 52 minutes, with spectrum view also confirming it as the same component. The rutin content in buckwheat aerial parts was 137.05 mg/g extract. Therefore, the major component in buckwheat aerial parts was confirmed as rutin, and rutin is considered to have a direct influence on antidiabetic experiments.

3.8 Rutin's antidiabetic activity

Table 1. Inhibitory activity of rutin against α-glucosidase

Sample	RC_{50} (μg/mL
Rutin	148.16 ± 6.92
Acarbose	283.22 ± 53.25

Values are means ± SE (n = 3). ND; Not detected.

Acarbose is a representative α-glucosidase inhibitor used as an oral medication for postprandial blood glucose control in diabetes treatment. α-Glucosidase is an enzyme present in the epithelial cells of the small intestine that breaks down complex carbohydrates into glucose. The mechanism of action of acarbose is known to block enzyme function through hydrogen bonding with Glu, Asp, and other residues of α-glucosidase (Liu et al.). However, acarbose is known to cause gastrointestinal (GI) symptoms such as abdominal pain, gas production, and diarrhea, making it a trouble for patients to take before meals. Therefore, finding bioactive substances to replace acarbose has become important (Uuh Narvaez and Segura Campos). Since the single dominant peak observed in buckwheat sample HPLC was confirmed to be rutin, the α-glucosidase inhibitory activity of rutin was additionally evaluated based on this evidence. According to Table 1, Rutin's RC_{50} value was reported as 148.16 ± 6.92 μg/mL, which indicates approximately twice the potency of the synthetic inhibitor acarbose (283.22 ± 53.25 μg/mL), as it exerts the same inhibitory effect at a lower concentration. These results clearly demonstrate that rutin possesses antidiabetic activity comparable to, or even exceeding, that of acarbose.

Rutin binds to the active site of α-glucosidase, forms hydrogen bonds with Asp and Glu residues, and inhibits enzyme activity through hydrophobic interactions via aromatic rings. This binding interferes with substrate recognition and can demonstrate antidiabetic effects by mitigating postprandial blood glucose elevation (Limanto et al.). Although, human trials are needed to evaluate whether rutin, like acarbose which has undergone numerous human studies, is suitable as a blood glucose control material.

Conclusion

In this study, agricultural by-product samples of carrot, radish, beet, cabbage, kohlrabi, and buckwheat discarded in Jeju Island were extracted using 70% ethanol, and their yield, total flavonoid content, total phenolic content, HPLC component analysis, and α-glucosidase inhibitory capacity were comprehensively evaluated. Results demonstrated that buckwheat aerial parts exhibited significantly superior physiological activity compared to other plants across all parameters. Beet leaves showed the highest extraction yield, suggesting the greatest potential for industrial utilization, while conversely, orange carrot aerial parts recorded the lowest yield, raising concerns from an economic utilization perspective. Buckwheat aerial parts exhibited the highest values in both total flavonoid and total phenolic contents. This suggests that buckwheat aerial parts contain high concentrations of rutin and quercetin-series flavonoids along with various phenolic compounds, while radish leaves and cabbage, which had lower flavonoid contents, showed the possibility of higher proportions of non-flavonoid compounds within their phenolic content. HPLC component analysis detected gallic acid in most samples (kohlrabi, beet, cabbage, radish), with cabbage showing the highest gallic acid content, while chlorogenic acid was detected only in orange carrot. Neither gallic acid nor chlorogenic acid was detected in buckwheat. Finally, in the antidiabetic α-glucosidase inhibitory capacity evaluation, buckwheat aerial parts demonstrated very high inhibitory activity at levels comparable to acarbose, a synthetic inhibitor. HPLC analysis conducted to elucidate the antidiabetic activity mechanism of buckwheat leaves confirmed that the major component contained in buckwheat leaves was rutin. Buckwheat leaves contain rutin at high concentrations, measured as a single peak level in HPLC analysis. Furthermore, α-glucosidase inhibitory capacity experiments of rutin demonstrated superior antidiabetic activity compared to acarbose, confirming that the antidiabetic activity of buckwheat leaves is attributed to rutin. This suggests that buckwheat aerial parts possess high application potential as antidiabetic active materials. Therefore, it is noteworthy that buckwheat by-products possess not only value as simple agricultural by-products but also simultaneous scientific and industrial value as raw materials for blood glucose control functional foods. This represents a significance as an environmentally conscious health functional food strategy in that it can reduce environmental burden through upcycling of agricultural by-products while simultaneously providing a novel alternative to consumer groups requiring blood glucose control (particularly young diabetic patients and those interested in blood glucose management). However, clear limitations in validity exist in this study. This experiment is limited to indirect antidiabetic evaluation at the enzyme level based on α-glucosidase inhibitory activity and did not include mouse experiments or human clinical trials. Therefore, the actual effects and safety of buckwheat aerial parts on blood glucose control in the body have not yet been elucidated, and the results of this study alone are insufficient to definitively determine human applicability.

Works Cited

Benzie, I. F. F., and J. J. Strain. "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of 'Antioxidant Power': The FRAP Assay." Analytical Biochemistry, vol. 239, no. 1, 1996, pp. 70–76. https://doi.org/10.1006/abio.1996.0292.

Cai, Y., et al. "Antioxidant Activity of Betalains from Plants of the Amaranthaceae." Journal of Agricultural and Food Chemistry, vol. 51, 2003, pp. 2288–2294.

Escribano, J., et al. "Characterization of the Antiradical Activity of Betalains from Beta vulgaris L. Roots." Phytochemical Analysis, vol. 9, no. 3, 1998, pp. 124–127.

Goyeneche, R., et al. "Chemical Characterization and Antioxidant Capacity of Red Radish (Raphanus sativus L.) Leaves and Roots." Journal of Functional Foods, vol. 16, 2015, pp. 256–264.

Habtemariam, S. "Antioxidant and Rutin Content Analysis of Leaves of the Common Buckwheat (Fagopyrum esculentum Moench) Grown in the United Kingdom: A Case Study." Antioxidants, vol. 8, no. 6, 2019, p. 160.

Han, Y., et al. "Design, Synthesis, and Antiviral Activity of Novel Rutin Derivatives Containing 1,4-Pentadien-3-one Moiety." European Journal of Medicinal Chemistry, vol. 92, 2015, pp. 732–737.

Im, S. H., and J. S. Kim. "Physicochemical Properties of Extracts from Different Parts of Camellia sinensis." Korean Journal of Medicinal Crop Science, vol. 30, no. 3, 2022, pp. 195–203.

Kim, J. E., et al. "Anti-Inflammatory and Anti-Bacterial Constituents from the Extracts of Daucus carota var. sativa Aerial Parts." Journal of the Society of Cosmetic Scientists of Korea, vol. 44, no. 4, 2018, pp. 427–436.

Ko, H. C., et al. "Seasonal Variations in the Phenolic Compounds and Antioxidant Activity of Sasa quelpaertensis." Industrial Crops and Products, vol. 122, 2018, pp. 506–512. https://doi.org/10.1016/j.indcrop.2018.06.031.

Korean Diabetes Association. Diabetes Fact Sheet in Korea 2024. Korean Diabetes Association, 2024.

La Casa, C., et al. "Evidence for Protective and Antioxidant Properties of Rutin, a Natural Flavone, against Ethanol Induced Gastric Lesions." Journal of Ethnopharmacology, vol. 71, no. 1–2, 2000, pp. 45–53.

Lee, Y. C. "Kimchi: The Famous Fermented Vegetable Product in Korea." Food Reviews International, vol. 7, no. 4, 1991, pp. 399–415.

Limanto, A., et al. "Antioxidant, α-Glucosidase Inhibitory Activity and Molecular Docking Study of Gallic Acid, Quercetin and Rutin: A Comparative Study." Molecular and Cellular Biomedical Sciences, vol. 3, no. 2, 2019, pp. 67–74.

Liu, S. K., et al. "Discovery of New α-Glucosidase Inhibitors: Structure-Based Virtual Screening and Biological Evaluation." Frontiers in Chemistry, vol. 9, 2021, article 639279.

Ministry of Agriculture, Food and Rural Affairs. Major Statistics of Agriculture, Food and Rural Affairs 2023. Ministry of Agriculture, Food and Rural Affairs, 2023.

Nešović, M., et al. "Distribution of Polyphenolic and Sugar Compounds in Different Buckwheat Plant Parts." RSC Advances, vol. 11, no. 42, 2021, pp. 25816–25829.

Podsędek, A. "Natural Antioxidants and Antioxidant Capacity of Brassica Vegetables: A Review." LWT – Food Science and Technology, vol. 40, no. 1, 2007, pp. 1–11.

Sharma, K. D., et al. "Chemical Composition, Functional Properties and Processing of Carrot: A Review." Journal of Food Science and Technology, vol. 49, no. 1, 2011, pp. 22–32. https://doi.org/10.1007/s13197-011-0310-7.

Steadman, K. J., et al. "Buckwheat Seed Milling Fractions: Description, Macronutrient Composition and Dietary Fibre." Journal of Cereal Science, vol. 33, no. 3, 2001, pp. 271–278.

Swargiary, A., et al. "Phenolic Compounds as α-Glucosidase Inhibitors: A Docking and Molecular Dynamics Simulation Study." Journal of Biomolecular Structure and Dynamics, vol. 41, no. 9, 2022, pp. 3862–3871.

Uuh Narvaez, J. J., and M. R. Segura Campos. "Combination Therapy of Bioactive Compounds with Acarbose: A Proposal to Control Hyperglycemia in Type 2 Diabetes." Journal of Food Biochemistry, vol. 46, no. 10, 2022, e14268.

Yoo, K. M., et al. "Evaluation of Different Methods of Antioxidant Measurement." Food Science and Biotechnology, vol. 16, no. 2, 2007, pp. 177–182.

Zhang, Z., et al. "Bioactive Compounds in Functional Buckwheat Food." Food Research International, vol. 49, no. 1, 2012, pp. 389–395.

Zhao, Y., et al. "Distribution of Primary and Secondary Metabolites among the Leaf Layers of Headed Cabbage (Brassica oleracea var. capitata)." Food Chemistry, vol. 312, 2019, article 126028.

A Systematic Analysis of the Anticancer Activities of Medicinal Plant Extracts in Triple-negative Breast Cancer (MDA-MB-231) Cells

By Lahye Yun[1], YeJin Ahn[2]

Abstract

Triple-negative Breast Cancer (TNBC) is characterized by the lack of progesterone receptors and HER2, rendering it largely unresponsive to target therapies. Consequently, treatment relies heavily on radiation-based approaches, which frequently entail unwanted effects, including neurotoxicity, immunosuppression, and long-term drug resistance. Thus, such limitations underscore the need for safer, more biocompatible therapeutic alternatives. This study focused on the MDA-MB-231 cell line, a model for TNBC, to evaluate the therapeutic potential of five medicinal plants: *Hypochaeris radicata, Artemisia capillaris, Viscum coloratum, Euphorbia maculata,* and *Tetragonia tetragonioides*. To comprehensively assess the biological effects of the plants, MTT assay, wound healing assay, colony formation assay, apoptosis assay, cell cycle assays, mRNA expression profiling, and Western Blot Analysis were conducted. *E. maculata* and *T. tetragonioides* exhibited the strongest inhibition of wound closure and reduction of clonogenic survival, indicating potent suppression of long-term proliferative capacity. *A. capillaris* and *H. radicata* disrupted migration under low-serum conditions and shifted cell-cycle distribution toward S/G2/M accumulation, suggesting impaired cell cycle progression. *V. coloratum* reduced motility without altering the cell cycle, indicating a migration-specific mechanism. Additionally, the apoptosis assay showed that most extracts rendered a gradual increase of apoptotic or necrotic populations, rather than causing abrupt cell death. Consequently, such results suggest that the extracts have potential as biocompatible candidates for multi-target therapeutic strategies.

Keywords Apoptosis, Cell cycle arrest, Cell migration inhibition, Colony formation assay, MDA-MB-231, Medicinal plant extracts, Necroptosis, NF-κB / STAT3 signaling, Phytotherapy, Triple-negative breast cancer (TNBC)

1. Introduction

Breast cancer primarily arises from mammary ductal epithelial cells and is influenced by the combination of genetic, hormonal, and environmental factors, contributing to its complex nature and varying clinical outcomes. Breast cancer is the most commonly diagnosed cancer among women worldwide, with approximately 2.3 million new cases and 670,000 deaths reported in 2022 (Santucci et al.). The incidence and mortality rates are projected to increase substantially by 2050, disproportionately affecting low- and middle-income countries (Devi). This trend has catalyzed scientific efforts to comprehend the underlying mechanisms of breast cancer and develop more effective prevention and treatments. Research at the cellular level will address the growth of breast cancer and suggest new approaches to reduce the rise in incidence and mortality.

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MDA-MB-231 cell line represents triple-negative breast cancer (TNBC), accounting for approximately 15-20% of all breast cancers, known for its aggressive nature and poor prognosis (Maqbool et al.). 46% of TNBC patients develop distant metastasis, with a median survival of only 13.3 months after metastasis, considerably shorter than that observed in non-TNBC patients. The metastasis often occurs in the brain and visceral organs, making the recurrence rate after surgery around 25% (Yin et al.). Among breast cancer subtypes, the MDA-MB-231 cell line stands as a particularly difficult challenge in the medical field, due to its inability to respond to targeted therapy, due to its lack of "estrogen receptors (ER), progesterone receptors (PR), and HER2" (Chapdelaine and Sun).

MDA-MB-231, lacking estrogen receptor (ER), progesterone receptor (PR), and HER2 expression, challenges the treatment, resulting in limited responsiveness to targeted therapies and subpar clinical results (Damaskos et al.). HER2-targeted therapies attach to the HER2 protein on the cancer cell membrane, blocking signaling pathways, such as PI3K/AKT and MAPK. MDA-MB-231 cells don't have enough substrate for treatments to bind because they lack an HER2 inhibitor, and, therefore, HER2 is not transcribed on the cell membrane. This also results in critical growth and survival signals that instead rely on alternative mechanisms, such as androgen receptor signaling, autophagy, and inflammatory pathways, leading to the resistance of TNBC.

Chemoresistance is one of the major causes of therapeutic failure in breast cancer patients. L-DOXR cells, doxorubicin-resistant cancer cells, are less susceptible to chemotherapy, by failing to control the nuclear protein-1 (NUPR1) or histone deacetylase 11 (HDAC11) axis. NUPR1 is directly connected to the cell's survival, and HDAC11 is responsible for gene regulation and tumor growth. Elevating the presence of those proteins allows cancer cells to survive from genotoxic stress and avoid apoptosis. Furthermore, MDA-MB-231 cell activates transcription factors NFκB and AP-1 after drug exposure, increasing the expression of anti-apoptotic bcl-2 and suppression of pro-apoptotic bax, enabling cancer cells to resist chemotherapy-induced cell death.

Currently, much of the existing breast cancer therapies rely heavily on chemotherapeutic approaches: cisplatin and doxorubicin being the most pervasive. Cisplatin, a platinum-based compound, forms DNA cross-links that disrupt the replication and, eventually, the transcription of tumor cells, rendering cytotoxic effects (Dasari and Tchounwou; Hu et al.). However, despite its acclaimed therapeutic successes, cisplatin frequently offsets unwanted consequences such as "ototoxicity (hearing loss and tinnitus), nephrotoxicity, myelotoxicity, and neuropathy" (Starobova and Vetter). Similarly, doxorubicin, a synthetic anthracycline, intercalates into DNA, inhibiting replication and transcription of cancer cells (Thorn et al.). However, it includes the risk of cardiotoxicity, limiting wider use (Belger et al.). Such side effects, along with many others, highlight the need for safer alternatives to conventional cancer therapy. Additionally, these chemotherapeutic agents attack both healthy and cancerous cells, leading to severe side effects and high casualties (Blagosklonny). Thus, compared to other cancer types where target therapy is effective, triple-negative breast cancer (namely, MDA-MB-231) has a comparatively poorer

prognosis (Chapdelaine and Sun), with over 40% of patients dying within five years of diagnosis (Dent et al.). Since these characteristics obstruct the success of clinical strategies, MDA-MB-231 is chosen for the model, testing new therapies, and this experiment enables the discovery of a new anticancer agent that minimizes side effects associated with conventional chemotherapy, providing more effective treatments for overcoming resistance.

Consequently, there has been growing interest in plant-derived therapeutics with lower systemic toxicity. Paclitaxel, for example, developed from Taxus species, provides remedy through stabilizing microtubules and preventing mitotic growth, allowing antitumor activity (Sousa-Pimenta et al.) with comparatively smaller risks of organ toxicity, such as nephrotoxicity and cardiotoxicity, than that of chemotherapy (Kézdi et al.; Lameire). Vicina alkaloids from *Catharanthus roseus*, on the other hand, destabilize microtubules, causing mitotic arrest in hematologic tumors (Moudi et al.). Though neurotoxicity remains the primary side effect, such concerns are less prominent, as it is generally reversible and of milder effect than complications associated with cisplatin (Kerckhove et al.; Paken et al.). Likewise, irinotecan and topotecan, which are derived from *Camptotheca acuminata*, induce damage in tumor cells' DNA. Despite concerns of diarrhea and neutropenia, UGT1A1-guided doses have been shown to significantly reduce toxicity (Hulshof et al.). While the side effects of cisplatin and doxorubicin are often irreversible (Paken et al.; Cardinale et al.), remedies developed from these natural plant-based compounds demonstrate higher rates of amendable organ toxicity (Staff et al.). According to research, phytochemicals harbor comparable effects while having higher rates of organ recovery and lower rates of overall toxicity (Staff et al.). This highlights promising potential in plant-derived solutions as safer, more sustainable alternatives to chemotherapy.

The MDA-MB-231 cell remains a topic of extensive curiosity due to its invasive properties and the increasing population of breast cancer patients; however, studies regarding botanical therapies have been limited in information and often incomplete. Most existing studies cover narrow scopes of biotacnicals and frequently lack clear mechanistic validation for reported effects. Thus, to address such gaps, this study aims to provide a systematic evaluation of the anti-proliferative qualities of *Artemisia capillaris*, *Hypochaeris radicata*, *Tetragonia tetragonioides*, *Viscum coloratum*, and *Euphorbia maculata* against MDA-MB-231. To comprehensively assess the biological effects of the plants, MTT assay, wound healing assay, colony formation assay, apoptosis assay, cell cycle assays, mRNA expression profiling, and Western Blot Analysis were conducted. Collectively, this research investigates whether these plant species contain therapeutic potential against breast cancer, ultimately providing new insights into treatment.

2. Methods

2.1 Sample Preparation

For the sample, *Artemisia capillaris, Hypochaeris radicata, Tetragonia tatragonioides, Viscum coloratum*, and *Euphorbia maculata* were harvested. All plants were washed in flowing

water to clean out the dirt, then dried in a 60ºC hot air dryer (PEC-P0-91, Nihon freezer, Namyangju, Korea) for 24 hours. 60ºC was selected as a temperature as it represents the maximum capacity for the sample, which allows the sample to dry properly without damaging constituents. The dried-up samples were later powdered in a mixer for the experiment, maximizing extraction efficiency by increasing the surface area.

2.2 Extract Preparation

The samples used in the experiments are composed of *Artemisia capillaris, Hypochaeris radicata, Tetragonia tatragonioides, Viscum coloratum,* and *Euphorbia maculata*. 15 g of each sample was mixed with 70% ethanol, 20 times the weight of each powdered plant, and extracted using an ultrasonic cleaner (Powersonic 520, Hwashin Tech Co., Ltd, Seoul, Korea) for one hour - ultrasonic extraction was selected because it minimizes tissue damage while offering rapid solute release. This process was repeated three times. The extracts were then filtered using filter paper and concentrated through a rotary vacuum concentrator (Hei-VAP Precision, Heidolph, Schwabach, Germany). After being frozen in a Deepfreezer at -80°C, the samples were freeze-dried.

2.3 MTT Assay

MDA-MB-231 was seeded in 96-well plates and incubated for 24 hours under standard culture conditions at 37ºC, 5% CO_2. The seeding density was $1x10^6$ cells per plate for *Hypochaeris radicata* and *Euphorbia maculata*, and $1x10^4$ cells per well for *Artemisia capillaris*, *Viscum coloratum*, and *Tetragonia tetragonioides*. Subsequently, the cells were treated with each extract at concentrations ranging from 0 to 1000 μg/mL and incubated for an additional 24 hours under the same condition. A 5 mg/mL MTT solution was added to each well to reach a final concentration of 0.5 mg/mL, followed by incubation at 37ºC for 2 to 4 hours. The resulting formazan crystals were fully dissolved in DMSO, and absorbance was measured at 570 nm. Cell viability was calculated as a percentage relative to the untreated control group, and all were independently repeated at least three times. Statistical analysis was performed using one-way ANOVA and Tukey HSD test, with significance set at $p<0.05$.

2.4 Wound healing assay

MDA-MB-231 cells were cultured in 6-well plates until a confluent monolayer was established. A uniform linear scratch in each well was created using a sterile 200-μL pipette tip, after which detached cells were removed by washing with phosphate-buffered saline (PBS). The culture medium was replaced with a migration assay medium with 0.5% fetal bovine serum (FBS) to ensure growth-factor-dependent signaling was the primary driver of migration. The wells were then treated with plant extracts: *Hypochaeris radicata* at 500 μg/mL, *Artemisia capillaris* at 250 μg/mL, *Viscum coloratum* at 31.25 μg/mL, *Euphorbia maculata* at 15.625 μg/mL, and *Tetragonia tetragonioides* at 750 μg/mL. Wound closure was microscopically monitored, and images were captured at 0, 6, 12, and 16 hours following scarification of the

treatment groups. Migration was quantified by analyzing the reduction in wound width and wound area over time, by calculating closure percentage and migration rate using the acquired images.

2.5 Colony formation Assay

MDA-MB-231 was seeded into 6-well plates at a density of 1,000 cells per well and incubated under 37ºC, 5% CO_2 conditions for 24 hours. *Hypochaeris radicata* and *Tetragonia tetragonioides* extracts were treated with 0, 250, 500 μg/mL, *Artemisia capillaris* extract was treated with 0, 125, 250 μg/mL, *Viscum coloratum* extract was treated with 0, 50, 100 μg/mL, and *Euphorbia maculata* extract was treated with 0, 25, 50 μg/mL. The cells were further cultured for 10 to 14 days to allow colony formation. At the end, the medium was removed, and the cells were fixed with a 3:1 mixed fixative of acetic acid and methanol. Then, the cells were stained with 0.5% crystal violet solution. The stained colonies were carefully washed and air-dried, and colonies consisting of more than 50 cells were counted. The results were expressed as a relative ratio compared with the control group.

2.6 Apoptosis Assay

The apoptosis assay was performed using MDA-MB-231 cells cultured in a humidified incubator at 37ºC and 5% CO_2. 6-well plates were used to seed cells at a density of 1,000 cells per well. The cells were then allowed to adhere for 24 h under standard culture conditions. After stabilization, each plant extract was administered at its respective experimental concentration: *Hyptochaeris eradication* at 750 μg/mL, *Artemisia capillaris* at 250 μg/mL, *Viscum coloratum* at 100 μg/mL, *Euphorbia maculata* at 62.5 μg/mL, and *Tetragonia tetragonioides* at 750 μg/mL. Cells were incubated with the extracts for 24 h. The cells were then harvested using trypsin-EDTA, washed twice with PBS, and subjected to Annexin V-FITC/PI dual staining (BD Pharmingen™ Annexin V-FITC Apoptosis Detection Kit). A flow cytometer was used to analyze the stained samples to quantify the distribution of cells across apoptotic states, including Live (Annexin V^-/PI^+), Early apoptosis (Annexin V^-/PI^+), Late apoptosis or Dead cells (Annexin V^-/PI^+), and Necrotic or Membrane-disrupted cells (Annexin V^-/PI^+). This dual-staining FACs approach enabled discrimination of phosphatidylserine externalization and membrane integrity loss, allowing evaluation of extract-induced apoptotic progression under each treatment condition.

2.7 Cell Cycle Assays

MDA-MB-231 breast cancer cells were seeded into 6-well plates at a density of 1,000 cells per well. The cells were then incubated at 37ºC and 5% CO_2, and allowed to adhere for 24 hours. After stabilization, each plant extract was applied at its treatment concentration: *Hyptochaeris radication* at 750 μg/mL, *Artemisia capillaris* at 250 μg/mL, *Viscum coloratum* at 62.5 μg/mL, *Euphorbia maculata* at 62.5 μg/mL, and *Tetragonia tetragonioides* at 750 μg/mL. Then cells were incubated with each extract for 24 hours. To avoid DNA-content distortion, cells

were collected using trypsin-EDTA with minimal mechanical disruption, washed with PBS twice, and fixed in cold 70% ethanol for permeabilization. These samples were stained with propidium iodide (PI) solution containing RNase A; by removing cytoplasmic RNA-derived fluorescence, selective quantification of genomic DNA was possible. The cells were analyzed by flow cytometry to obtain DNA-content histograms, after staining under light-protected conditions. FSC-A/FSC-H gating was used to exclude doublets, and the distribution of cells across the G_0/G_1 (M1) and S/G_2M (M2) phases was calculated to analyze extract-induced changes in cell-cycle progression.

2.8 mRNA Expression Profiling

MDA-MB-231 was maintained in a cell culture incubator at 37ºC, 5% CO_2 for 24 hours. Cell seeding was performed in 6-well plates at 1,000 cells per well for *Hypochaeris radicata*, and in 96-well plates at a density of $3x10^3$ cells/well for *Artemisia capillaris, Viscum coloratum, Euphorbia maculata*, and *Tetragonia tetragonioides*. The final concentrations of the extracts were 750 µg/mL for *H. radicata* and *T. tetragonioides*, 250 µg/mL for *A. capillaris*, and 62.5 µg/mL for *V.coloratum* and *E. maculata*. After 24-hour treatment, cells were washed twice with cold PBS. Total RNA was extracted using a TRI-reagent-based method for *H. radicata*, a phenol-free column-based RNA isolation kit for *A. capillaris, V.coloratum*, and *E. maculata*, and a silica-column RNA purifier for *T. tetragonioides*. RNA purity and concentration were assessed by 260/280 nm absorbance ratio for *A. capillaris*, *V.coloratum, E. maculata*, and *T. tetragonioides*. Reverse transcription was performed using 1 µg of RNA for cDNA synthesis, employing an RNase inhibitor-containing reverse transcriptase system where applicable. qPCR was conducted using a SYBR Green-based real-time PCR system. Amplification conditions were identical across all experiments: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. Melt-curve analysis was performed to verify amplification efficiency and specificity through a single peak. Gene expression levels were normalized to GAPDH as a housekeeping control and quantified using the ΔΔCt method, with results expressed as relative expression (% of control) compared to untreated controls. For specific genes, *H. radicata* targeted BAX and RIPK3, *A. capillaris* targeted RIPK1 and RIPK3, *V.coloratum* and *E. maculata* targeted RIPK3, and *T. tetragonioides* was reported in a standardized mRNA response percentage format to account for transcriptional bias before *V.coloratum* treatment.

2.9 Western Blot Analysis

MDA-MB-231 breast cancer cells cultured in 6-well culture plates were treated with each extract for 24 hours, and then protein analysis was performed. The treatment concentrations were 750 µg/mL for *Hypochaeris radicata*, 125 µg/mL and 250 µg/mL for *Artemisia capillaris*, 62.5 µg/mL for *Viscum coloratum*, 62.5 µg/mL for *Euphorbia maculata*, and 500 µg/mL and 750 µg/mL for *Tetragonia tetragonioides*. Cells were washed twice with ice-cold PBS and lysed in RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate,

0.1% SDS) containing protease and phosphatase inhibitors at 4°C for 15 minutes. Cell debris was removed by centrifugation at 12,000 rpm for 10 minutes, and the supernatant was collected. The protein concentration was then quantified using a BCA assay. 15 μg of protein was electrophoresed on 10% SDS-PAGE and transferred to PVDF membranes. The transferred membranes were blocked with 3% BSA for 1 hour at room temperature to prevent nonspecific binding. Primary antibodies were applied depending on the experimental group: *H. radicata* extracts were probed with antibodies against NF-κB p65, p-STAT3, STAT3, and β-actin; *A. capillaris* with c-Myc and β-actin; *V.coloratum* with p65 and β-actin; *E. maculata* with p-STAT3, STAT3, and β-actin; and *T. tetragonioides* with p65, beclin-1, and β-actin. All primary antibodies were incubated overnight at 4°C. After washing with PBST, fluorescence-conjugated secondary antibodies were incubated for 1 h at room temperature in the dark. Protein expression was detected using an Odyssey CLx fluorescence imaging system, and band intensities were quantified using the Image Studio software.

3. Results and Discussion

3.1 Cell Proliferation Assay

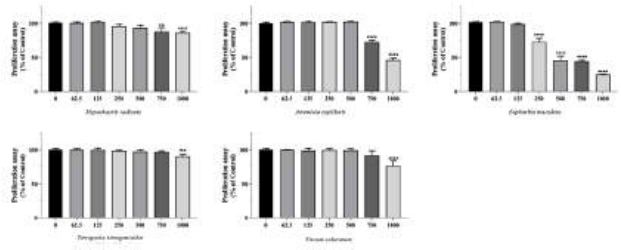

Figure 1. Cell Proliferation Assay

A cell proliferation assay was performed to evaluate the toxicity of the five plants on normal cells (Figure 1). Each extract was applied at concentrations ranging from 0 to 1,000 μg/mL, and toxicity was compared based on cell viability (% of control). For *Hypochaeris radicata*, cell viability decreased gradually starting at 250 μg/mL, and even at the high concentration of 1000 μg/mL, viability was measured at 80–90%. Although toxicity is present, it appears to exhibit relatively weak concentration-dependent toxicity. *Tetragonia tetragonioides* also maintained a generally stable survival rate. Given that the survival rate remained high at approximately 80% even at the high concentration of 1000 μg/mL, its concentration-dependent toxicity appears to be very low. *Viscum coloratum* showed a significant decrease in cell survival rate at high concentrations. In particular, the survival rate at 1000 μg/mL was measured at approximately 70%, suggesting that its concentration-dependent toxicity is higher than that of the extracts mentioned above. *Artemisia capillaris* exhibited very pronounced toxicity at high concentrations, with a survival rate of approximately 45–50%. *Euphorbia maculata* showed the highest toxicity at high concentrations, with a survival rate of approximately 25%. The biological efficacy of medicinal plants is closely linked to their environmental origin. As noted by Tang and

Eisenbrand, geographical factors and harvesting conditions can significantly influence the phytochemical composition of plant extracts. Given the unique climatic and ecological conditions of Jeju Island, it is plausible that such regional variation contributed to the bioactivity patterns observed in this study, even in the absence of direct metabolite profiling.

3.2 MTT assay

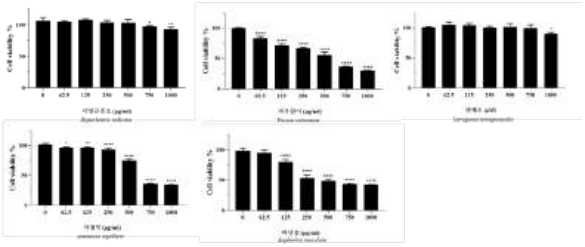

Figure 2. MTT Cell Viability Assay

Changes in cell viability of extract treatment in MDA-MB-231 were evaluated using an MTT assay (Figure 2). Each extract exhibited distinct dose-dependent effects on cell viability. For *Hypochaeris radicata*, no significant reduction in viability was observed across most concentrations (62.5-750 μg/mL), although a slight decrease was detected at the highest concentration (1,000 μg/mL), indicating minimal cytotoxicity over a broad concentration range. In contrast, *Artemisia capillaris* showed only modest effects at lower concentrations (62.5-250 μg/mL), but a sharp decline in viability was observed at concentrations of 500 μg/mL or higher, showing strong cytotoxicity at elevated concentrations. *Viscum coloratum* exhibited a significant reduction in cell viability starting from 62.5 μg/mL, with a rapid and substantial decline at 500 μg/mL and above, suggesting a high sensitivity to this extract at relatively low concentrations. *Euphorbia maculata* showed a significant decrease from 125 μg/mL, and at 250-1,000 μg/mL, viability was reduced to less than half that of the control group. *Tetragonia tetragonioides* resulted in negligible changes in viability across most concentrations tested (62.5-750 μg/mL), with only a slight reduction observed at 1,000 μg/mL, indicating minimal dose-dependent cytotoxicity.

3.3 Wound Healing Assay

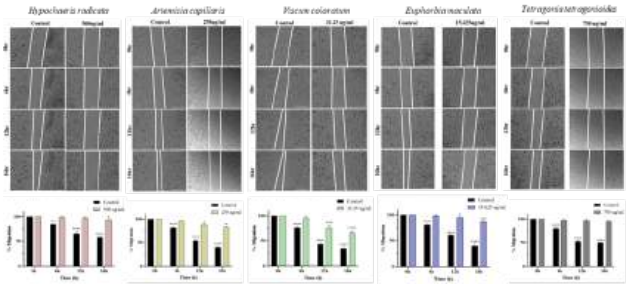

Figure 3. Wound Healing Assay

Hypochaeris radicata's inhibitory effect on cell migration became evident under wound-healing conditions with FBS reduced to 0.5%. In the control group, the wound gap rapidly narrowed over time, whereas in the treatment group, wound closure began to decline at 6 hours and remained markedly incomplete at 12 and 16 hours. These findings suggest that *H. radicata* affects cellular functions related to migration rather than proliferation. *Artemisia capillaris* induced a concentration-dependent reduction in metabolic viability. Concentrations of 500μg/mL and above produced concentration-dependent reduction in viability, decreasing metabolic activity by 40-60%, demonstrating pronounced cytotoxic metabolic suppression. This suggests that the extract induces acute cytotoxicity primarily through metabolic disruption at active ranges, rather than through antiproliferative effects. *Viscum coloratum* extract significantly inhibited the migratory capacity of MDA-MB-231 cells at relatively low concentrations. By 12 hours, the reduction in wound width was minimal; at 16 hours, wound closure remained substantially incomplete. These findings suggest that *V.coloratum* exerts a strong, time-dependent inhibitory influence on wound-healing processes and cellular migration, consistently reducing motility across the observed period. From 12 to 16 hours, the group treated with *Euphorbia maculata* extract exhibited a consistent decline in migration, resulting in insufficient wound closure. This inhibitory pattern indicates that *E. maculata* exerts strong effects on cellular migratory capacity even at relatively low concentrations. Beginning at 6 hours, the group treated with *Tetragonia tetragonioides* extract experienced a noticeable delay in wound closure, with a reduction in migration at 12 and 16 hours, leading to insufficient wound healing. These findings suggest that *T. tetragonioides* extracts exhibited stronger inhibition of pathways related to cell migration rather than proliferation, impairing cell motility under nutrient-restricted conditions.

3.4 Colony formation assay

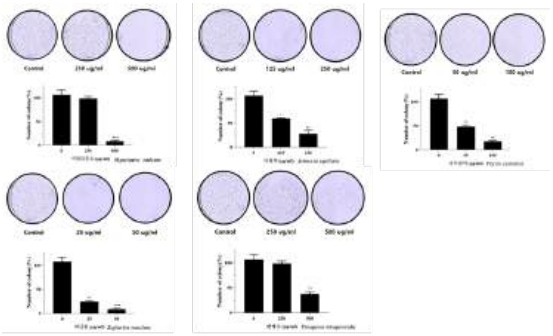

Figure 4. Colony formation assay

The effects of *Hypochaeris radicata, Artemisia capillaris, Viscum coloratum, Euphorbia maculata*, and *Tetragonia tetragoniodes* extracts on the long-term proliferative capacity of MDA-MB-231 cells were evaluated using a colony formation assay (Figure 4). *H. radicata* extract reduced both colony number and density at 250 μg/mL, while at 500 μg/mL most colonies failed to form, indicating a substantial inhibition of sustained proliferative ability. *A.*

capillaris exhibited a noticeable reduction in colony number and size starting at 125 μg/mL, and at 250 μg/mL, only a few small colonies persisted, demonstrating strong inhibitory effects from the early colony formation. *V.coloratum* showed decreased colony number and density at 50 μg/mL, with 100 μg/mL further impairing the maintenance of expanded colonies over long-term culture, substantially weakening the proliferative foundation. *E. maculata* caused reduced colony number and increased heterogeneity at 25 μg/mL, and at 50 μg/mL, only small clusters were observed, indicating a loss of structural integrity in colony formation. *T. tetrgonioides* induced minor reductions in colony size and density at 250 μg/mL, while at 500 μg/mL, colony number decreased, limiting long-term cluster maintenance. Our results revealed that Euphorbia maculata and *Tetragonia tetragonioides* substantially reduced the long-term survival capacity of MDA-MB-231 cells. The marked decrease in colony formation, together with impaired wound closure, indicates disruption of both proliferative sustainability and migratory function. These observations are consistent with prior reports of anti-tumor activity within the Euphorbia genus (Zhao et al.) and the pharmacognostic properties of *T. tetragonioides* associated with reduced cancer cell motility (Onoiko and Zolotareva).

3.5 Apoptosis Assay

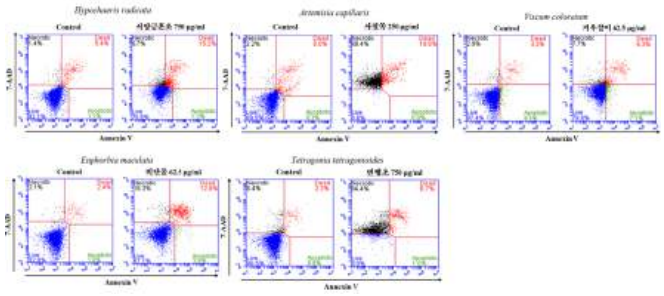

Figure 5. Assessment of the extract's ability to induce cell apoptosis (FACS-based Annexin V/PI fraction analysis).

The results suggest that each plant extract induced distinct apoptosis-related responses in MDA-MB-231 cells, with variations in membrane-integrity loss, necrotic expansion, and apoptotic signaling (Figure 5). *Hypochaeris radicata* exhibited a gradual, concentration-dependent increase in Annexin V^+ apoptotic populations, indicating that plasma-membrane disruption remained limited. Additionally, *H. radicata* imposed proliferative pressure rather than inducing immediate cell death. *Artemisia capillaris* produced patterns that aligned with its colony-formation phenotype. As *A. capillaris* treatment increased Dead fractions, the plant appears to limit clonogenic continuity by gradually increasing dead populations. *Viscum coloratum* moderately reduced Live cells and expanded Necrotic and Dead cells, while the apoptotic subset showed only a small increase. Thus, *V.coloratum* does not seem to trigger acute apoptosis but instead exerts sustained pressure on the cell population. *Euphoria maculata* increased Necrotic, Apoptotic, and Dead subsets. Such results indicate that *E. maculata* selectively destabilizes the long-term proliferative population through expansion of necrotic and late-death signals. *Tetragonia tetragonioides* demonstrated a sharp reduction in Live cells,

followed by a dramatic expansion of necrotic population, while apoptotic and Dead fractions remained low. This indicates that *T. tetragonioides* severely disrupted population integrity by rendering large-scale necrotic collapse, reducing cells capable of long-term proliferation. Interestingly, Viscum coloratum selectively reduced migratory capacity without inducing substantial alterations in cell cycle distribution, suggesting that its effects may be more closely associated with pathways involved in cell motility rather than direct proliferative suppression (Di et al.). Across multiple extracts, the gradual increase in apoptotic and necrotic populations, rather than an abrupt induction of cell death, indicates a progressive weakening of survival signaling networks. This pattern is consistent with the multi-target activity often observed in plant-derived polyphenolic compounds, which tend to modulate cellular pathways cumulatively rather than through single-point cytotoxic effects (Ernst et al.).

3.6 Cell Cycle Assay

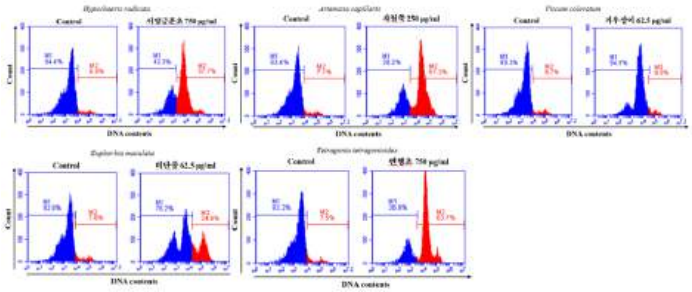

Figure 6. Analysis of cell cycle alterations in MDA-MB-231 cells following extract treatment.

The experiment indicates that each plant extract modulates proliferation through idiosyncratic shifts in DNA-content phases (Figure 6). In the control group, the majority of cells (93-94%) accumulated in the G_0/G_1 phase (M1), with only 6-7% distributed in the S/G_2M phase (M2). However, *Hypochaeris radicata* decreased the M1 fraction to 42. 3% while expanding M2 to 57.7%. This redistribution suggests that the extract imposes a regulatory constraint near the $S\text{-}G_2/M$ transition, suggesting that proliferative pressures increase without completing mitotic progression. Similarly, *Artemisia capillaris* reduced the G_0/G_1 population to 39.2% and increased the S/G_2M fraction to 62.3%. This indicates checkpoint perturbation rather than acute cytotoci collapse, suggesting that *A. capillaris* restricts cell-cycle progression by encouraging accumulation in late-cycle stages. In contrast, *Viscum coloratum* caused little alteration to the cycle structure (the Ma and M2 fractions remained similar to the control group). Such an absence of phase shifts indicates the *V.coloratum* exerts functional effects independent of DNA-replication dynamics. *Euphorbia maculata* induced a moderate distinction as well; however, it decreased M1 to 76.2% and expanded M2 to 24. 6%. This implies that the extract promotes the accumulation of cells preparing for division without triggering apoptotic collapse, signaling that a cycle-phase imbalance contributes to a reduction in long-term proliferation *Tetragonia tetragonioides* produced the most dramatic alteration, reducing M1 to 36.9% and increasing M2 to 63.7%. Despite not inducing uniform apoptosis, the magnitude of change

suggests that the extract destabilizes proliferative rhythm, increasing the proportion of cells stalled near G_2/M, while limiting the growth of long-term colonies. In contrast, *Artemisia capillaris* and *Hypochaeris radicata* were associated with pronounced shifts in cell cycle distribution, particularly an accumulation in the S/G_2/M phase. This pattern suggests interference with cell cycle progression rather than immediate cytotoxic collapse. Such effects are consistent with previous findings that bioactive compounds from Artemisia species, including coumarin derivatives, can influence DNA replication checkpoints and disrupt cell cycle regulation (Hsueh et al.). The observed activity of H. radicata further supports its potential as a biologically active plant resource with relevance to cancer-related pathways (Babar et al.).

3.7 mRNA Expression Analysis

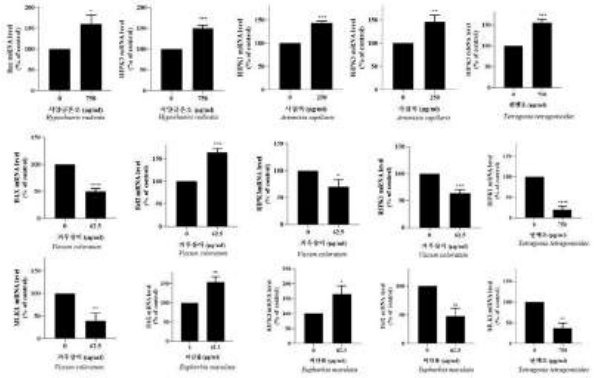

Figure 7. mRNA expression changes in MDA-MB-231 cells after extract treatment

After treating MDA-MB-231 cells with each extract, ΔΔCt-based transcriptional responses were analyzed (Figure 7). Treatment with 750 μg/mL *Hypochaeris radicata* increased Bax expression approximately 1.6-fold compared to the control. RIPK3, a necroptosis signal kinase, also showed a significant concentration-dependent increase. This indicated a transcriptional response that progressively primed mitochondrial apoptotic state pressure and necroptotic pressure without causing acute MTT survival rate reduction. *Artemisia capillaris* (250 μg/mL) increased RIPK1 by approximately 1.4-fold and RIPK3 by 1.5-fold, exhibiting increased kinase-dominant death pressure linked to reduced colony-forming ability and proliferative DNA pool imbalance. In contrast, treatment with *Viscum coloratum* at 62.5 μg/mL reduced Bax by approximately 50% and increased BCL2 by 1.6-fold. Both MLKL and RIPK1/3 decreased by approximately 30–50%, indicating a balance shift where survival signals were relatively strengthened. Treatment with *Euphorbia maculata* at 62.5 μg/mL increased Bax by 1.5-fold and RIPK3 by 1.7-fold and decreased BCL2 by approximately 0.48-fold. This enhances apoptosis sensitivity and a necroptotic primed state without immediate toxicity. In *Tetragonia tetragonioides* (500–750 μg/mL), RIPK3 increased approximately 1.6-fold, while MLKL and RIPK1 decreased to 0.5-fold and 0.4-fold levels, respectively. This indicates a cellular state transition to a primed state where only death signaling pressure increased without downstream execution. *H. radicata, A. capillaris, E. maculata*, and *T. tetragonioides* regulated the balance of

signaling networks toward a death-pressure-dominant direction, while *V.coloratum* shifted toward a survival-shifted direction. This supports that each extract possesses biological activity capable of suppressing proliferation competence stability and progressively priming the death signaling network without inducing short-term toxicity.

3.8 Western Blot Analysis

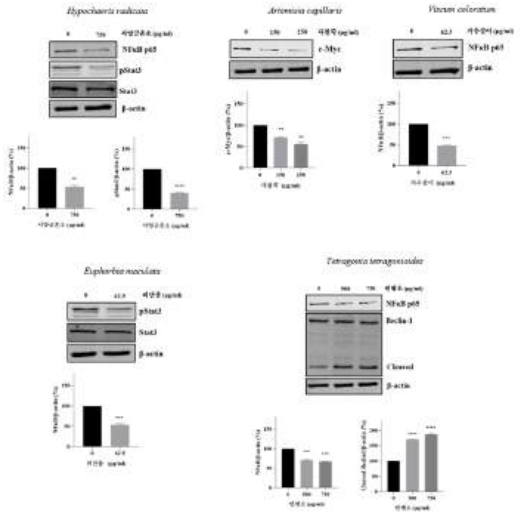

Figure 8. Protein expression changes in MDA-MB-231 cells following extract treatment

Western blot analysis was performed after 24-hour treatment of MDA-MB-231 cells (Fig 8). From Hypochaeris radicata extract, p65 expression is reduced, indicating suppression of the NF-κB pathway. Since NF-κB promotes anti-apoptotic genes, the reduction suggests weakened survival signaling. Also, increased late apoptosis was observed, demonstrating that the extract promotes apoptosis by inhibiting NF-κB. Total STAT3 showed a slight decrease, while pSTAT3 decreased markedly, confirming inhibition of STAT3 activation and weakened STAT3-dependent survival signaling. With *Artemisia capillaris* extract, c-Myc expression decreased in a concentration-dependent manner, indicating suppression of c-Myc. Since c-Myc suppresses RIPK1-dependent cell death, its reduction may increase susceptibility to necroptosis. Increased RIPK1/3–MLKL activity and necroptosis were observed, suggesting necroptosis promotion by c-Myc reduction. In the *Viscum coloratum* extract-treated group, p65 expression also decreased in a concentration-dependent manner compared to the control group. As NF-κB (p65) was inhibited, an environment conducive to switching to pro-death signaling was created, and indeed, increased RIPK1/3–MLKL axis activity and increased apoptosis were observed simultaneously. Thus, *V.coloratum* exhibited a dual cell death induction pattern, activating both apoptosis and necroptosis via p65 reduction. In the *Euphorbia maculata* extract-treated group, only pSTAT3 expression decreased markedly without changes in total STAT3, indicating that STAT3 activation itself was inhibited. This demonstrates a weakened STAT3-dependent survival signaling axis. Under the same conditions, increased apoptosis was observed via flow cytometry, confirming

that silk grass induces apoptosis through STAT3 inhibition. Finally, in *Tetragonia tetragonioides* extract, p65 expression decreased in a concentration-dependent manner. As the protective function of NF-κB signaling buffers diminished, RIPK1 remained unubiquitinated, creating an environment conducive to necrosome formation. Under the same treatment conditions, increased activation of the RIPK1/3–MLKL pathway was confirmed, suggesting that p65 reduction leads to a sequential biological flow directly connected to necroptosis. Together, this demonstrates that the five extracts possess potential anticancer effects by inducing programmed cell death in MDA-MB-231 cancer cells through regulating distinct anti-survival molecular targets. While the specific bioactive constituents responsible for these effects were not identified at this screening stage, the multi-target nature of crude plant extracts may provide a strategic advantage. By concurrently influencing cell cycle progression, migratory capacity, and long-term viability, these extracts demonstrate a broad functional impact on cancer cell behavior. Rather than indicating a single dominant mechanism, the data suggest coordinated modulation of multiple signaling pathways. Future studies employing advanced analytical techniques such as UPLC-QTOF-MS will be necessary to characterize the individual compounds responsible for these effects and to further clarify their mechanistic contributions.

Discussion

The study evaluated the proliferation, migration, and apoptosis-related signaling effects of five plant species, *Hypochaeris radicata, Artemisia capillaris, Viscum coloratum, Euphorbia maculata,* and *Tegragonia tetragonioides*, on MDA-MB-231 cells, a triple-negative breast cancer (TNBC) cell line. The MTT assay showed that *E. maculata* and *V. coloratum* exhibited cytotoxicity even at relatively low concentrations, while *H. radicata* and *T. tetragoniodes* showed little cytotoxicity overall. Considering that *E. maculata* belongs to the genus *Euphorbia*, a genus known to contain high levels of triterpenoid compounds that exert cytotoxic effects against cancer cells through various mechanisms, such as the induction of apoptosis and inhibition of cell proliferation (Rojas-Jiménez et al.), its high cytotoxicity is consistent with previous findings. In the case of *V. coloratum*, its strong cytotoxicity is largely associated with lectin components, which have been reported to induce apoptosis in cancer cells (Di et al.). However, unlike *E. maculata,* which also exhibited citotoxicity in normal cells (though not profound), *V. coloratum* did not and instead showed selective cytotoxicity against MDA-MB-231 cells. Consequently, *V. coloratum* appears to have greater potential for targeted therapeutic application. In the cell migration inhibition assay, all five extracts inhibited the migratory ability of MDA-MB-231 cells, suggesting their significance in suppressing cancer metastasis, as cell migration is a crucial principle of anti-cancer therapy: MDA-MB-231 cells frequently metastasize to visceral organs, and the recurrence rate after surgery has been reported to be approximately 25% (Cardinale et al.). Although *H. radicata* and *T. tetragonioides* exhibited limited cytotoxicity across the tested concentration range, they effectively inhibited cell migration in the wound healing assays, suggesting selective suppression of migration-related signaling pathways (without affecting cell viability). Such results suggest the possibility of these

extracts suppressing metastasis while minimizing adverse effects on normal cells, complementing the limitations of current anti-cancer drugs, which often focus primarily on tumor reduction through cytotoxicity (Xia et al.). *V. coloratum* strongly inhibited cell migration even at relatively low concentrations, but it did not affect cell cycle distribution, indicating the extract's mechanism more closely aligns with cell motility rather than direct proliferation inhibition. Additionally, its selective migration-inhibitory activity (which was effective even at low concentrations), suggests its potential to minimize side effects. Similarly, *E. maculata* also demonstrated a strong inhibitory effect on cell migration even at low concentrations, aligning with previous studies reporting that compounds derived from *Euphorbia* species inhibit cancer cell migration and invasion (Rojas-Jiménez et al.). In the apoptosis and necrosis induction assays, *T. tetragonioides* rendered a decrease in viable cells and an increase in necrotic cells, while apoptosis remained relatively low. Such results contrast with the general trend in plant extracts, in which apoptosis is reported as the primary mechanism of cancer cell death (Santucci et al.). Conversely, *V. coloratum* exhibited anticancer effects by inhibiting cell migration while causing little apoptosis or cell cycle alteration, diverging from previous studies that have reported that mistletoe extracts primarily exert anticancer effects through apoptosis (Di et al.). Such results suggest that, in MDA-MB-231 cells, *V. coloratum* exerts effects through cell motility pathways rather than an apoptosis-centered mechanism. Therefore, the findings indicate that differences may exist in the underlying mechanisms of an extract depending on the cancer cell type. In cell cycle analysis, *H. radicata, A. capillaris*, and *T. tetragonioides* extracts significantly increased the M2 fraction. Proper cell division consists of cell growth preparation, DNA replication, division preparation, and finally, cell division. In this experiment, most of the cells in the control group existed in G_0/G_1 fraction. However, within the treatment, S/G_2M fraction increased to 57-64%. This shows how the cell cycle is already disrupted at the stage of cell division preparation. This activity is usually observed in the anticancer effect of plant-derived compounds. Many previous studies observed the same mechanism of plant extracts inhibiting cancer cell division (Rummun et al.). *V. coloratum* showed almost no change in cell cycle distribution. As it relates to the wound healing assay, *V. coloratum* selectively inhibits cells' ability to migrate and metastasize to show anticancer effect, rather than directly disrupting cell division. *A. capillaris*, which revealed G_2/M arrest in the cell cycle analysis, showed how c-Myc decreased concentration-dependently, and RIPK1/RIPK3 mRNA expression and RIPK1/RIPK3-MLKL protein activation increased. In the cancer cell, c-Myc not only induces cell division but also directly binds to RIPK3 to inhibit necrotic cell death (Kang et al.). In the apoptosis assay, *T. tetragonioidies*, which showed prominent necrosis, showed an increase in RIPK3 mRNA, but a decrease in RIPK1 and MLKL in the normal cell death pathway. It differed from the proper pathway because *T. tetragonoides* activates cell death through a unique pathway, which requires further research. In the wound healing assay, *V. coloratum*, which selectively inhibited cell migration, showed an increase in survival signal. However, with a decrease in NF-κB p65, RIPK1/RIPK3-MLKL pathway activation and apoptosis increased. This discrepancy shows the possibility of other mechanisms being involved, and relates to the previous experiment

where *V. coloratum*'s main chemotherapy pathway relies on cell migration rather than cell death. In the *E. maculata* extract, pSTAT3 decreased, while the level of STAT3 didn't change. The decrease in only pSTAT3 shows that the activation process is selectively inhibited, relating to the increase in cell death and necrosis. Since this experiment used crude extract, identifying a specific component that affects the anticancer activity against MDA-MB-231 cells. Also, most of the experiments are progressed in cell level, and therefore, it's difficult to reflect the complex real body structure. Later, components should be separated from the extract and go through anticancer experiments.

Works Cited

Babar, V. B., et al. "Phytochemical and Anticancer Evaluation of Stems and Leaves Extract of Hypochaeris radicata (L.)." Journal of Pharmacognosy and Phytochemistry, vol. 10, no. 2, 2021, pp. 1555–1560. https://doi.org/10.22271/phyto.2021.v10.i2y.14147.

Belger, C., et al. "Doxorubicin-Induced Cardiotoxicity and Risk Factors." IJC Heart & Vasculature, vol. 50, 2024, article 101332. https://doi.org/10.1016/j.ijcha.2023.101332.

Blagosklonny, M. V. "Selective Protection of Normal Cells from Chemotherapy, While Killing Drug-Resistant Cancer Cells." Oncotarget, vol. 14, 2023, pp. 193–206. https://doi.org/10.18632/oncotarget.28375.

Cardinale, D., et al. "Cardiotoxicity of Anthracyclines." Frontiers in Cardiovascular Medicine, vol. 7, 2020, p. 26. https://doi.org/10.3389/fcvm.2020.00026.

Chapdelaine, A. G., and G. Sun. "Challenges and Opportunities in Developing Targeted Therapies for Triple Negative Breast Cancer." Biomolecules, vol. 13, no. 8, 2023, p. 1207. https://doi.org/10.3390/biom13081207.

Damaskos, C., et al. "Triple-Negative Breast Cancer: The Progress of Targeted Therapies and Future Tendencies." Anticancer Research, vol. 39, no. 10, 2019, pp. 5285–5296. https://doi.org/10.21873/anticanres.13722.

Dasari, S., and P. B. Tchounwou. "Cisplatin in Cancer Therapy: Molecular Mechanisms of Action." European Journal of Pharmacology, vol. 740, 2014, pp. 364–378. https://doi.org/10.1016/j.ejphar.2014.07.025.

Dent, R., et al. "Triple-Negative Breast Cancer: Clinical Features and Patterns of Recurrence." Clinical Cancer Research, vol. 13, no. 15, 2007, pp. 4429–4434. https://doi.org/10.1158/1078-0432.CCR-06-3045.

Devi, S. "Projected Global Rise in Breast Cancer Incidence and Mortality by 2050." The Lancet Oncology, vol. 26, no. 4, 2025, p. 417. https://doi.org/10.1016/S1470-2045(25)00136-6.

Di, H., et al. "Viscum coloratum (Komar.) Nakai: A Review of Botany, Phytochemistry, Pharmacology, Pharmacokinetics and Toxicology." Biomolecules, vol. 15, no. 7, 2025, p. 974. https://doi.org/10.3390/biom15070974.

Ernst, M., et al. "Global Medicinal Uses of Euphorbia L. (Euphorbiaceae)." Journal of Ethnopharmacology, vol. 176, 2015, pp. 90–101. https://doi.org/10.1016/j.jep.2015.10.025.

Hsueh, T. P., et al. "The Pharmacological Effects and Pharmacokinetics of Active Compounds of Artemisia capillaris." Biomedicines, vol. 9, no. 10, 2021, p. 1412. https://doi.org/10.3390/biomedicines9101412.

Hu, J., et al. "Cisplatin DNA Damage and Repair Maps of the Human Genome at Single-Nucleotide Resolution." Proceedings of the National Academy of Sciences, vol. 113, no. 41, 2016, pp. 11507–11512. https://doi.org/10.1073/pnas.1614430113.

Hulshof, E. C., et al. "UGT1A1 Genotype-Guided Dosing of Irinotecan: A Prospective Safety and Cost Analysis in Poor Metaboliser Patients." European Journal of Cancer, vol. 162, 2022, pp. 148–157. https://doi.org/10.1016/j.ejca.2021.11.031.

Kang, K., et al. "3-O-Acetylrubianol C (3AR-C) Induces RIPK1-Dependent Programmed Cell Death by Selective Inhibition of IKKβ." The FASEB Journal, vol. 34, no. 3, 2020, pp. 4369–4383. https://doi.org/10.1096/fj.201902714R.

Kerckhove, N., et al. "Long-Term Effects, Pathophysiological Mechanisms, and Risk Factors of Chemotherapy-Induced Peripheral Neuropathies: A Comprehensive Literature Review." Frontiers in Pharmacology, vol. 8, 2017, p. 86. https://doi.org/10.3389/fphar.2017.00086.

Kézdi, Á., et al. "Effects of Taxane-Anthracycline and Taxane Only Treatment on Cardiac Function in Breast Cancer: A Retrospective Cohort Study." Cardio-Oncology, vol. 11, no. 1, 2025, p. 3. https://doi.org/10.1186/s40959-024-00291-5.

Lameire, N. "Nephrotoxicity of Recent Anti-Cancer Agents." Clinical Kidney Journal, vol. 7, no. 1, 2014, pp. 11–22. https://doi.org/10.1093/ckj/sft135.

Maqbool, M., et al. "Treatment Strategies against Triple-Negative Breast Cancer: An Updated Review." Breast Cancer: Targets and Therapy, vol. 14, 2022, pp. 15–24. https://doi.org/10.2147/BCTT.S348060.

Moudi, M., et al. "Vinca Alkaloids." International Journal of Preventive Medicine, vol. 4, no. 11, 2013, pp. 1231–1235.

Onoiko, O. B., and O. K. Zolotareva. "Bioactive Compounds and Pharmacognostic Potential of Tetragonia tetragonioides." Biotechnologia Acta, vol. 17, no. 1, 2024, pp. 29–42. https://doi.org/10.15407/biotech17.01.029.

Paken, J., et al. "A Review of Cisplatin-Associated Ototoxicity." Seminars in Hearing, vol. 40, no. 2, 2019, pp. 108–121. https://doi.org/10.1055/s-0039-1684048.

Rojas-Jiménez, S., et al. "Anti-Inflammatory and Cytotoxic Compounds Isolated from Plants of Euphorbia Genus." Molecules, vol. 29, no. 5, 2024, p. 1083. https://doi.org/10.3390/molecules29051083.

Rummun, N., et al. "Mauritian Endemic Medicinal Plant Extracts Induce G2/M Phase Cell Cycle Arrest and Growth Inhibition of Oesophageal Squamous Cell Carcinoma In Vitro." Acta Naturae, vol. 11, no. 1, 2019, pp. 81–90. https://doi.org/10.32607/20758251-2019-11-1-81-90.

Santucci, C., et al. "European Cancer Mortality Predictions for the Year 2025 with Focus on Breast Cancer." Annals of Oncology, vol. 36, no. 4, 2025, pp. 460–468. https://doi.org/10.1016/j.annonc.2025.01.014.

Sousa-Pimenta, M., et al. "Chemotherapeutic Properties and Side-Effects Associated with the Clinical Practice of Terpene Alkaloids: Paclitaxel, Docetaxel, and Cabazitaxel." Frontiers in Pharmacology, vol. 14, 2023, article 1157306. https://doi.org/10.3389/fphar.2023.1157306.

Staff, N. P., et al. "Chemotherapy-Induced Peripheral Neuropathy: A Current Review." Annals of Neurology, vol. 81, no. 6, 2017, pp. 772–781. https://doi.org/10.1002/ana.24951.

Starobova, H., and I. Vetter. "Pathophysiology of Chemotherapy-Induced Peripheral Neuropathy." Frontiers in Molecular Neuroscience, vol. 10, 2017, p. 174. https://doi.org/10.3389/fnmol.2017.00174.

Tang, W., and G. Eisenbrand. "Artemisia scoparia Waldst. et Kit. and *A. capillaris* Thunb." Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine, Springer, 1992, pp. 179–183. https://doi.org/10.1007/978-3-642-73739-9_16.

Thorn, C. F., et al. "Doxorubicin Pathways: Pharmacodynamics and Adverse Effects." Pharmacogenetics and Genomics, vol. 21, no. 7, 2011, pp. 440–446. https://doi.org/10.1097/FPC.0b013e32833ffb56.

Xia, Y. T., et al. "Suppression of Migration and Invasion by Taraxerol in the Triple-Negative Breast Cancer Cell Line MDA-MB-231 via the ERK/Slug Axis." PLOS ONE, vol. 18, no. 9, 2023, e0291693. https://doi.org/10.1371/journal.pone.0291693.

Yin, L., et al. "Triple-Negative Breast Cancer Molecular Subtyping and Treatment Progress." Breast Cancer Research, vol. 22, no. 1, 2020, p. 61. https://doi.org/10.1186/s13058-020-01296-5.

Zhao, X., et al. "Research Progress on the Anti-Tumor Effects of Euphorbia humifusa." Discover Oncology, vol. 15, no. 1, 2024, p. 761. https://doi.org/10.1007/s12672-024-01633-8.

Antioxidant and Anti-Wrinkle Effects of Tree Resources and EEG Responses to Essential Oils By Jeongmin Kwon[1], Sooin Lee[2]

Abstract

The study aims to address the ongoing issue in Jeju Island regarding the disposal of roadside tree cuttings and to explore the utilization of pruning waste as a natural material. The extracts were prepared from leaves of trees naturally grown in Jeju Island: *Cryptomeria japonica* (C.japonica), C.camphora (*Cinnamomum camphora*), T.nucifera (*Torreya nucifera)*, C.obtusa (*Chamaecyparis obtuse*), and M.alternifolia (*Melaleuca alternifolia*) using 70% ethanol extraction method, essential oils were produced through steam distillation. To evaluate the efficacy of the extracts, polyphenol content, flavonoid content, antioxidant activities (TEAC and FRAP), and elastase inhibition activity were measured. The components of the essential oils were further identified through GC-MS analysis. According to the TPC assay, hinoki cypress and C.camphora exhibited high polyphenol content and tea trees showed the highest flavonoid concentration. Tea trees also demonstrated a high elastase inhibitory activity, while T.nucifera and C.obtusa showed low levels. The C.camphora displayed high antioxidant activity, consistent with its highest FRAP value. GC–MS analysis revealed C.camphora contained high proportions of camphor, C.japonica contained sabinene, tea trees were rich in terpinene compounds, T.nucifera contained limonene, and C.obtusa contained D-limonene. Furthermore, EEG measurement confirmed that essential oils have positive effects on human brain activity.

Keywords Antioxidant activity, Essential oils, GC–MS analysis, Jeju native plants, Neurophysiological response

1. Introduction

Jeju Island is the southernmost island of South Korea with a total area of 1,846 km^2. It is surrounded by sea on all sides and therefore has a characteristic of warm and high humidity. Temperature differences according to altitude and vegetation distribution map can be verified by centered around Hallasan Mountain, located in the middle of the island. There are approximately 1,800 native Jeju Island plant species, a number significantly higher than that of other regions. As part of windbreak forests, street trees, and components of urban greening efforts, various tree species have been planted in Jeju Island, including C.camphora, T.nucifera, C.obtusa, and M.alternifolia have also been planted recently as a result of climate change. However, the rapid growth of street trees has led to several issues, most notably the production of large amounts of pollen that provoke various allergic reactions. To maintain this ecological balance, pruning is conducted. In addition, the increasing number of branches interfere with road traffic creates the need for improvement. Since the leaves and branches produced in this process are mainly

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disposed of through incineration or landfill, research is needed to utilize them more effectively.

Essential oils, which are complexes of various biochemical components, are volatile aromatic components produced during the secondary metabolic processes of plants. Contingent on the extraction method used, the effectiveness and price of essential oils differ. The major extraction methods incorporate distillation and mechanical extraction from the peel of citrus fruits(Turek and Stintzing). Essential oils are composed of alcohols, aldehydes, esters, ketones, oxides, phenols, terpenes, terpenoids, and other components. With such excellent efficacy and components, essential oils have been used for antiseptic effects such as sterilization, virus sterilization, and antifungal action, as well as medicinal effects, antiseptic treatment, preservation, analgesic, sedative, anti-inflammatory, analgesic, and local anesthetic(Bakkali et al.). While essential oils can be utilized to show biological activity centered on volatile components, ethanol extracts can contain large amounts of nonvolatile components including polyphenols and flavonoids. Thus, ethanol extracts are gaining attention as important research subjects for evaluating antioxidant and antibacterial activities. Furthermore, ethanol and water extracts contain lower toxicity to the human body and dissolve easily in raw materials, which has led to their widespread use in the cosmetic and food industries. Previous studies have shown that when plants were extracted with various ethanol concentrations, the extraction yield, antioxidant activity, and biological activity are highest in 70% ethanol extracts (Kim et al.). For that reason, this study aims to evaluate the potential industrial applications of essential oils and 70% ethanol extracts through an analysis of their biological activities.

C.japonica, known scientifically as Cryptomeria japonica, belongs to the platycladus orientalis. It has a strong fragrance and high quality material, making it excellent for processing. With the growing recognition of phytoncides and their benefits, this tree species has become increasingly familiar in our surroundings, especially through forest recreation and related developments. As interest in these properties has risen the various approaches, and research has shown that C.japonica exhibits strong bioactive substance, particularly antimicrobial and antibacterial effects(Cha et al.). Its major essential oil component has been identified as the diterpene kaurene (19.4%), and it contains a wide range of mono terpenes. These components indicate that the species possesses significant antioxidant activity.

C.camphora (Cinnamomum camphora) belongs to lauraceae. Its origin includes India, China, and Korea. Chapmor, an aromatic compound obtained by steam distillation of the tree's trunk or roots, has high utility as an insect repellent and insecticide. The wood is resistant to insect damage and preservative, making it suitable for construction materials and furniture, as well as for use in landscape plating. Essential oil extracted from C.camphora through steam distillation has been reported to possess antifungal properties and alleviates respiratory diseases(Chen et al.).

The scientific name of the M.alternifolia is Melaleuca alternifolia, which belongs to the Myrtaceae and is widely cultivated in southern regions such as Australia. M.alternifolia, known for their distinctive fragrance, grows to a height of 5-7 meters and has long, narrow leaves with strong vitality. According to previous studies, M.alternifolia oil exhibits excellent

anti-inflammatory and antimicrobial effects and is known to be effective in improving various skin conditions such as acne, eczema, athlete's foot, dandruff, moisturizer, and wrinkles (Bae; Ji). M.alternifolia essential oil has long been used in Australia for medicinal purposes, including wound cleansing and treatment, as well as for its antiseptic and disinfectant properties. It exhibits effective antimicrobial activity against various microorganisms, and among these, it has been shown to be particularly effective in treating acne (Nurzyńska-Wierdak et al.).

The scientific name of the T.nucifera is torreya nucifera, an evergreen tree belonging to the taxus cuspidata. In Korea, it forms large natural colonies mainly on Baekyangsand and Naejangsan in Jeolla Province, as well as on Jeju Island, and it is also native to Japan. This species tolerates shade well and is highly resistant to air pollution. However, it grows poorly in dry environments and generally exhibits very slow growth (Choi). Dried leaves of T.nucifera contain catechol, methyl palmitate, and methyl oleate (Hong). These components are used to promote appetite stimulants, digestion, constipation, and for hemorrhoids treatment, as well as for ancylostoma duodenale and helminthic (Jeon et al.). In addition extracts of T.nucifera have been shown to possess very strong antimicrobial activity against harmful human pathogens.

The scientific name of the hinoki cypress is Chamaecyparis obtusa. Its wood and leaves contain approximately 1% essential oil, which is used for medicine purposes. Owing to its high content of natural antimicrobial compounds, it demonstrates strong sterilizing effects. The essential oil of C.obtusa has been shown to possess numerous biological properties, among which its antibacterial and antifungal activities are regarded as the most prominent. Recent studies have also reported detectable antioxidant activity (J. H. Kim et al.), and essential oils isolated from the fruits of C.obtusa have been found to exhibit particularly high antioxidant capacity (Eltayeb et al.).

Previous studies have examined the chemical constituents of C.camphora, T.nucifera, M.alternifolia, C.japonica, and C.obtusa leaves individually. However, no research has directly compared all of these species until now. In this study, essential oils from each tree were produced using steam distillation, and 70% ethanol extracts were prepared through ultrasonic extraction. The resulting samples were then compared and analyzed to evaluate antioxidant activity (Trolox Equivalent Antioxidant Capacity, Fluorescence Recovery After hotobleaching, 2,2-diphenyl-1-picrylhydrazol), active ingredients (Total Phenol Content, Total Flavonoid Content, and Gas Chromatography-Mass Spectrometry), and antimicrobial activity against human pathogenic microorganisms. Through this approach, the present study aims to explore the potential for resource-circulating utilization of tree biomass that is currently being discarded in large quantities on Jeju Island, and further to propose its applicability as a natural functional material.

2. Materials and Methods

2.1. Experimental Materials

All experimental materials were collected in Jeju Island, Korea. Leaves of C.camphora (Cinnamomum camphora), T.nucifera (Torreya nucifera), C.japonica (Cryptomeria japonica), and M.alternifolia (Melaleuca alternifolia) were randomly collected using pruning shears from different locations on three separate occasions. The collected samples were dried at 60°C for 24 hours in a hot-air dryer (PFC-PO-91, Nihon-freezer, Namyangju, Korea). After drying, the samples were ground into powder using a blender for use in the experiments.

2.2. Preparation of Extracts

15g each of the samples from each part of the C.camphora, T.nucifera, C.japonica, and M.alternifolia were taken and extracted with 20 times the weight of 70% ethanol. Then, the mixture was extracted for 1 hour using an ultrasonic cleaner (Powersonic 520, Hwashin Tech Co., Ltd., Seoul, Korea). The entire process was repeated three times. The solutions were filtered using filter paper, concentrated using a rotary vacuum evaporator(Hei-VAP Precision, Heidolph, Schwabach, Germany), and freeze-dried before being used as experimental samples.

2.3. Production of essential oils

20kg of fresh leaves were collected from C.camphora, T.nucifera, C.japonica and M.alternifolia. After visiting the Cosmetic Raw Material Processing Center located in Seogwipo, Jeju Island, Korea, essential oils were produced through an 8-hour steam distillation process (Korea Bio Solution Co., Ltd., Korea).

2.4. Total Polyphenol Content measurement

Polyphenols are aromatic compounds that contain two or more hydroxyl groups attached to a benzene ring(H. M. Ko et al.). The total polyphenol content was measured using the method described by H. C. Ko et al.. Prior to measuring absorbance, 700 μL of distilled water and 100 μL of Folin–Ciocalteu reagent were mixed with 20 μL of each extract. The mixture was incubated for 2 hours. Then, 100 μL of 20% sodium carbonate was added, and the mixture was left to stand for 1 hour. Absorbance was then measured at 750 nm using a microplate reader (i-Mark 168-1135, Bio-Rad Inc., Hercules, CA, USA). Using Gallic acid as the standard substance, a standard calibration curve was prepared. The results were expressed as gallic acid equivalents (GAE).

2.5. Total Flavonoid Content measurement

Total flavonoid content was analyzed using the method described by H. C. Ko et al.. 100 μl of each extract was mixed with 20 μl of 1 M potassium acetate, 20 μl of 10% aluminum nitrate, and 300 μl of ethanol to incubate for 1 hour. Then, 560 μl of distilled water was added to the mixture. Absorbance was measured at 415 mm using a microplate reader (iMark 168-1135, Bio-Rad Inc., Hercules, CA, USA). A standard calibration curve was created using quercetin as the standard, and the total flavonoid content was expressed as quercetin equivalent (QE).

2.6. Trolox equivalent antioxidant capacity (TEAC) assay

TEAC is a method for measuring the antioxidant capacity of a solution that removes ABTS radicals. A radical is formed and exhibits a blue-green color. However, this phenomenon occurs when ABTS reacts with a peroxide. To determine antioxidant activity, the ABTS radical solution reacts with a substance that has antioxidant activity, causing the radicals to be eliminated and the color to fade. The absorbance of this decolorized solution is then measured. TEAC was measured using a modified method from Zulueta et al.. To generate ABTS radicals, 7 mM ABTS and 2.45 mM potassium persulfate were mixed and left in the dark for 24 hours. Before the experiment, the ABTS radical solution was diluted with distilled water until the absorbance value at 750 nm was 0.70 ± 0.02. 200 μl of ABTS radical mixture solution was added to 10 μl of each extract and left to stand for 5 minutes. The absorbance value was then measured at 750 nm using a microplate reader. Trolox was used as a standard substance, and a standard calibration curve was presented as the concentration of trolox per 1 g of dried sample (nM•TE/g).

2.7. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is an absorbance-based measurement method in which, under low pH conditions (pH 3.6), the mixture is reduced to ferrous tripyridyltriazine [Fe(II)-TPTZ] by antioxidant substances (Benzie and Strain). FRAP is a method that primarily measures reducing power based on iron ions rather than direct antioxidant activity. A limitation of the FRAP assay is that the stability of the oxygen electrode may decrease during the experiment. However, its advantage is that it is widely used due to its simplicity and low cost (Yoo et al.). FRAP was measured using a modified method of Benzie and Strain. Before the test, 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridylstrianzine, and 20 mM FeCl3 were mixed in a 1:1 ratio at 37°C for 30 min to prepare a FRAP working solution. 150 μl of FRAP working solution was added to 50 μl of the extract for 15 minutes at 37*C. The absorbance value was then measured at 595 nm using a microplate reader. A standard calibration curve was created using FeSO4 to express it as FeSO4 reducing power (mM FE/g) per 1 g of sample.

2.8. Elastase inhibition assay

When elastin, an insoluble elastic fiber protein that forms a network structure, is degraded by elastase, the network structure breaks down, leading to skin aging. Recently, the research for substances that inhibit the activity of elastase has been continuously conducted to prevent skin aging. Referring to the method of Im and Kim, 20 μl of the sample was mixed with 50 mM Tris-HCL buffer (pH 8.0), and then 10 μl of 1 U/ml elastase (SIgma-Aldrich Co., St. Louis, MO, USA) and 10 μl of 1 mM N-succinyl-(Ala)3-p-nitroanilide were mixed. After the reaction at 37°C, the absorbance was measured at 415 nm using a microplate reader.

2.9. GC-MS analysis

A Gas Chromatography spectrometric detector (MS 2400 SQ, Perkinelmer, USA) was used to identify essential oil components. The experiment was conducted at a flow rate of 1.5 mL/min using a DB-1HT GC column (0.1 μm × 30 m × 0.32 mm) with helium as the carrier gas. The initial oven temperature was maintained at 40°C for 5 minutes, after which the temperature was increased to 100°C at a rate of 5°C/min. The detector temperature was fixed at 50°C and maintained for 5 minutes, then increased to 230°C at the same rate and held for an additional 5 minutes. The MSD equipment temperature was fixed at 312°C, and analysis using GC/MSD was conducted under the same conditions as GC. The results were later analyzed with reference to the Wiley 138 database, and the essential oil components identification was completed.

2.10. EEG

An electroencephalogram (EEG) was conducted to investigate how the scent of essential oils affects the human brain. For the device, QEEG-32FX manufactured by Neuromedi Co., Ltd., was used and the experiment was conducted on adults in their 20s to 50s. To accurately reflect the physical and emotional state, precautions such as refraining caffeine intake and getting at more than 3 hours of sleep were provided in advance. After discussing and scheduling the experiment with each participant, the purpose and procedure of the experiment were briefly explained on the day of the experiment before it was conducted. During EEG measurement, noise disrupts brain activity and interferes with accurate brain wave signals, so all related factors were blocked. The EEG electrodes were attached to the subject's scalp using a water-soluble adhesive. Fp1 was attached to the left prefrontal area of the frontal lobe, and Fp2 was attached to the right prefrontal area. F3 and F4 were attached to the left and right frontal lobes, respectively. T3 and T4 were attached to the left and right temporal lobes above the ears. Finally, P3 and P4 were attached to the left and right parietal lobes on the top of the head. The control condition, followed by the scents of hinoki cypress, nutmeg yew, cedar, camphor, and tea tree, were presented in sequence, and data were collected for one minute for each scent. Because continuous exposure to multiple scents can cause olfactory fatigue, which temporarily dulls the sense of smell, a 5-minute break was provided between each scent for recovery. The Telescan program was used to record in real time the timing of scent exposure and corresponding changes in brain waves, and the data were converted and organized for analysis.

3. Results and Discussion

3.1 Extraction Yield

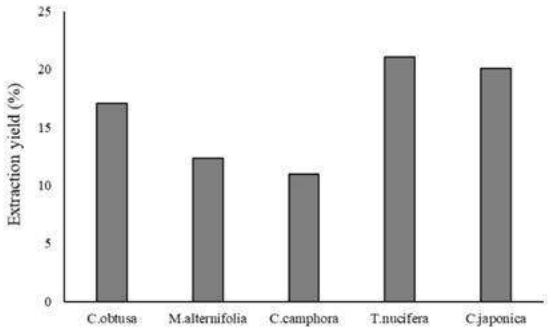

Fig 1. Extraction yield

C.obtusa; Chamaecyparis obtusa, M.alternifolia; Melaleuca alternifolia, C.camphora; Cinnamomum camphora, T.nucifera; Torreya nucifera, C.japonica; Cryptomeria japonica

A high extraction yield and biological efficacy should be considered as separate factors. This varies depending on the characteristics of the extract. A high yield does not necessarily mean high efficacy, and even if efficacy is high, the yield may be low. However, if an extract has high efficacy but low yield, its industrial application may be difficult. For this reason, extraction yield is a key criterion for evaluating industrial applicability. The results of the extraction yield experiment are shown in Figure 1. T.nucifera and C.japonica showed high yields of 21.07% and 20.08%, respectively, while C.camphora showed the lowest yield at 10.95%.

3.2 Total Polyphenol Content measurement

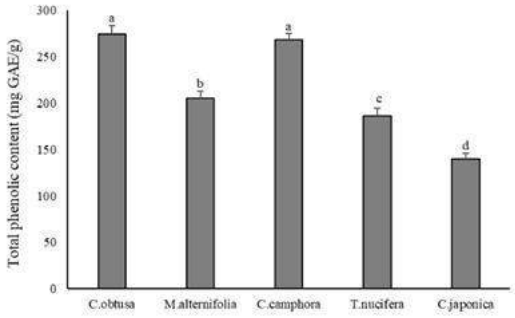

Figure 2. Total phenolic content result

The results of the total polyphenol content experiment are presented in Figuer 2. The highest levels of total polyphenols were observed in hinoki cypress and camphor with 274.32 mg GAE/g and 268.33 mg GAE/g, respectively. In contrast, cedar showed a comparatively lower content at 139.98 mg GAE/g. According to a previous study, extracts of hinoki cypress contain components such as catechin, quercetin nicotine quercetin-3-O-a-rhamnopyranoside, myricetin-3-O-a-rhamnopyransodie, and 4-hydroxybenzoic acid, all of which may contribute to its total polyphenol content. In addition, HPLC analysis of camphor extracts revealed that among various compounds, catechin and gallic acid were present at relatively high concentrations of

6.98μg/mL (Sobhy et al.). Therefore, it is considered that these constituents contribute to the high levels observed in hinoki cypress and camphor.

3.3 Total Flavonoid Content measurement

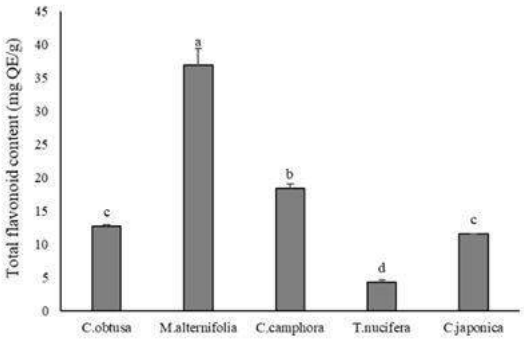

Figure 3. Total flavonoid content result

The total flavonoid content is presented in Figure 3. Although the same samples of hinoki cypress, tea tree, camphor, torreya, and cedar were used as in the polyphenol experiment, the results showed a different pattern. Unlike tea tree polyphenol content, it exhibited the highest flavonoid content, with an average of 36.92 mg QE/g. This is considered to result from the naturally high flavonoid composition of the tea tree, particularly due to the presence of quercetin, one of their most common flavonoid compounds (Albouchi et al.). However, excluding tea trees, the flavonoid content of hinoki cypress, camphor, torreya, and cedar was relatively low compared with polyphenol levels. Camphor showed the second highest flavonoid content at 18.56 mg QE/g, though still 18.56 mg QE/g lower than that of tea tree. This was followed by hinoki cypress 12.71 mg QE/g, cedar 11.54 mg QE/g. and torreya 4.32 mg QE/g. These results indicate that torreya contains a large proportion of non-flavonoid phenolic compounds.

3.4 Trolox equivalent antioxidant capacity (TEAC) assay

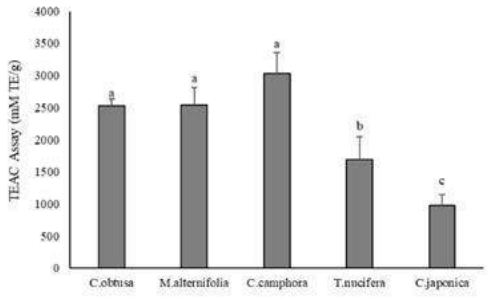

Figure 4. TEAC assay result

The results of the TEAC antioxidant experiment are shown in Figure 4. Camphor showed the highest antioxidant activity with 3029.26 mM TE/g, and unlike the total polyphenol content

experiment, hinoki cypress showed the third highest antioxidant activity with 2530.22 mM TE/g. In addition, torreya and cedar showed the lowest antioxidant activity with 1689.12 mM TE/g and 973.94 mM TE/g, respectively, along with the total polyphenol content. In the previous experiment on polyphenol content, the highest content was measured in camphor, and it was confirmed that the polyphenol compounds contained in camphor contain large amounts of catechin and gallic acid. Since catechin and gallic acid are known to exhibit high antioxidant activity, it is thought that the highest TEAC value was measured in camphor (Grzesik et al.).

3.5 Ferric reducing antioxidant power (FRAP) assay

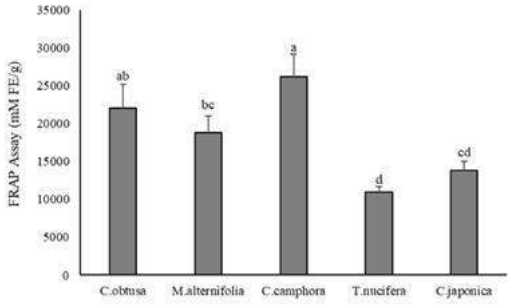

Figure 5. FRAP assay result

FRAP antioxidant activity is shown in Figure 5. Camphor showed the highest antioxidant activity with a total of 26190.96 mM TE/g. This is believed to be because the phenol content contained in camphor is the highest in terms of antioxidant contribution, indicating sufficient FRAP antioxidant activity (Yang et al.). Next, hinoki cypress showed a total of 22048.09 mM TE/g, which was lower than camphor but higher than tea tree with a total of 18783.37 mM TE/g. In addition, cedar showed 13849.83 mM TE/g and torreya showed 10896.33 mM TE/g in that order. Therefore, the FRAP antioxidant experiment and the polyphenol content experiment were similar. These results indicate that polyphenol compounds affect antioxidant activity. According to one study, the molecular basis for the antioxidant properties of polyphenols is known to be three main mechanisms: direct reaction with free radicals, chelation reaction with free metals, and reaction that generates free radicals (Leopoldini et al.)

3.6 Elastase inhibition assay

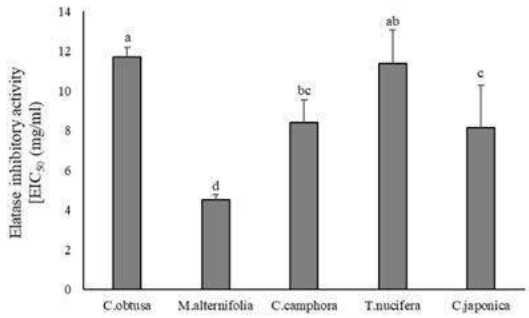

Figure 6. Elastase inhibition assay result

The elastase inhibition assay result is presented in Figuer 6. Tea tree exhibited the highest inhibition effect at 4.52 mg/mL, whereas hinoki cypress and torreya showed markedly lower activities at 11.69 mg/mL and 11.38 mg/mL, respectively. GC-MS analysis of tea tree constituents revealed high levels of Teripinen-4-ol and gamma.-Terpinene, accounting for 17.81%k and 22.69%, respectively. According to previous GC-MS findings, terpinen-4-ol, a-terpineol, and 1,8-cineole are compounds that are partially soluble in tissue culture media. These results support the hypothesis that such components may suppress the production of inflammatory mediators in LPS-activated monocytes in vitro (Hart et al.). Elastase is an enzyme secreted in excess by neutrophils in response to inflammatory stimuli and is known to induce tissue damage. The terpinene compounds identified in tea trees, which have been shown to reduce the production of pro-inflammatory cytokines, may therefore contribute to the observed elastase inhibition. Thus, terpinen based constituents present in tea tree ethanol extracts or essential oils may play a role in elastase inhibition, and further mechanistic verification through cellular and in vivo studies is warranted.

3.7 GC-MS analysis

Using GC-MS, the components of camphor, cedar, tea tree, torreya, and hinoki cypress were analyzed. In camphor, 39.70% (+)-2-Bornanone, 12.08% limonene, and 10.20% alpha-pinene were identified (Table 1). Among these, (+)-2-Bornanone, which showed the highest content, has been used for various medicinal purposes. Traditionally, (+)-2-Bornanone has been used as a remedy to relieve chest discomfort and to treat inflammatory diseases such as bronchitis, asthma, indigestion, and muscle pain (Hamidpour et al.). According to the results of an electroencephalogram experiment, inhalation of (+)-2-Bornanone increased alpha waves in all brain regions, including the left frontal lobe, right frontal lobe, central region, left occipital lobe, and right occipital lobe. Systolic blood pressure, heart rate, and respiratory rate decreased, and a sedative effect was observed in which a relaxed and calm mood was maintained while tension was reduced (Sivaphongthongchai et al.). Next, cedar was found to contain 15.61% Sabinene, 12.54% Kaur-16-ene, and 13.52% alpha-pinene. The Sabinene component identified in cedar is known to be useful in inhibiting the cariogenic activity of S. mutans and can be applied to the development of oral health care products (Park et al.). In addition, alpha-pinene is volatile and hydrophobic, and plays an important role in the fragrance industry due to its refreshing pine scent. According to an in vitro study, alpha-pinene exhibits enantiomer-selective properties and shows antibacterial and insecticidal activities (Allenspach and Steuer). In particular, it showed high binding affinity to the breast cancer target protein ERα with a binding energy of −6.6 kcal/mol (Zochedh et al.). Third, the tea tree was found to contain 13.09% 2-Carene, 22.69% gamma-Terpinene, and 17.81% Terpinen-4-ol. The gamma-Terpinene component, which was found most abundantly in tea trees, exhibits antioxidant, antiparasitic, anticancer, antiviral, and analgesic effects (Nooshadokht et al.). Fourth, in torreya, 30.90% limonene, 22.17% alpha-pinene, and 9.40% naphthalene were identified. According to a previous study, limonene

has a refreshing lemon-like scent and is widely used as a fragrance additive in cosmetics, foods, and industrial solvents, and it does not appear to pose a significant risk to human exposure (Y. W. Kim et al.). Finally, in hinoki cypress, 16.26% (+)-4-Carene, 11.60% D-limonene, and 10.44% bornyl acetate were identified. (+)-4-Carene is a terpene-type organic compound found in pine essential oils and is known to possess antibacterial activity (Azghar et al.).

Table 1. GC-MS analysis of Essential oil

Content(%)	C.obtusa	%	M.alternifolia	%	C.camphora	Content(%)	C.japonica	%	T.nucifera
2.93	alpha-Pinene	3.04	alpha.-Pinene	10.20	alpha-Pinene	13.52	alpha.-Pinene	22.17	alpha.-Pinene
0.77	Camphene	2.36	Cyclohexene, 4-methylene-1-(1-me.....	5.79	Camphene	3.68	Camphene	0.97	beta.-Pinene
9.05	beta.-Phellandrene	0.63	beta-Pinene	1.95	Cyclohexene, 4-methylene-1-	4.57	beta.-Myrcene	2.79	beta.-Myrcene
5.57	beta.-Myrcen	1.24	beta-Myrcene	2.36	beta-Pinene	6.40	3-Carene	8.29	3-Carene
0.46	alpha.-Phellandrene	0.74	alpha-Phellandrene	4.91	beta-Myrcene	3.37	2-Carene	30.90	D-Limonene
16.26	(+)-4-Carene	13.09	2-Carene	2.43	alpha-Phellandrene	7.92	D-Limonene	2.20	2-Carene
11.60	D-Limonene	4.79	o-Cymene	12.08	D-Limonene	4.62	gamma.-Terpinene	0.53	alpha-Cubebene
7.38	gamma-Terpinene	2.57	D-Limonene	1.70	Eucalyptol	2.40	4-Carene	0.54	alpha-Terpinyl acetate
2.66	2-Carene	4.45	Eucalyptol	0.92	beta-Ocimene	2.81	Terpinen-4-ol	0.99	Caryophyllene
6.32	Terpinen-4-ol	22.69	gamma-Terpinene	1.02	gamma-Terpinene	0.24	gamma.-Elemene	2.83	beta.-Farnesene
1.84	alpha.-Terpineol	5.14	(+)-4-Carene	1.79	2-Carene	0.35	Germacrene D	1.59	Khusitone
10.44	Bornyl acetate	17.81	Terpinen-4-ol	39.70	(+)-2-Bornanone	0.48	alpha.-Muurolene	1.24	gamma-Muurolene
3.27	cis-Thujopsene	1.61	alpha.-Terpineol	0.33	endo-Borneol	0.44	gamma-Muurolene	2.46	Germacrene D
1.24	cis-Muurola-4(15), 5-diene	0.34	(+)-cis-Verbenol	1.19	Terpinen-4-ol	0.95	Cedrol	1.39	alpha-Muurolene
2.92	Bicyclosesquiphellandrene	0.91	1H-Cycloprop[e]azulene	1.23	alpha.-Terpineol	0.21	Hydrocinnamic acid	1.55	Cubenene
2.82	Zonarene	1.07	Caryophyllene	0.94	Safrole	12.54	Kaur-16-ene		
5.54	Cyclohexanemethanol	3.23	Aromadendrene						
		0.95	Germacrene D						
		2.96	isoledene						
		1.15	(-)-Globulol						
		0.63	Cubenene						

3.8 EEG

This study analyzed brainwave response to exposure to the essential oils of six plant species (C.camphora, T.nucifera, C.japonica, M.alternifolia, and C.obtusa) through comparison with a control condition (Table 2). The objective was to determine how relaxation, arousal, and attentional states differed depending on plant species. The results showed that C. japonica and T. nucifera displayed higher relative alpha (RA) and alpha/beta ratio (RAB) values than the control at most electrode sites, indicating a stronger overall relaxation effect. The pronounced increase in RA in the frontal lobe showed that exposure to these species may contribute to emotional stability and the formation of a calm and comfortable mood. In addition, the RAHB index of C. japonica, T. nucifera, and C. obtusa was higher than the control group, which suggests the maintenance of stable and less pressure mental state during exposure. Conversely, relative beta (RB) and mid-beta (MBT) showed no significant differences from the control under any condition. This highlights that exposure to these plant species did not cause unnecessary tension or high arousal. These results are consistent with previous research suggesting that natural scents promote relaxation instead of stress. Regarding the RSMR index, T.nucifera and C.obtusa showed relatively high values, with an indication that these species have a possibility of enhancing stable attention and concentration while maintaining a comfortable state. In summary, C.japonica and T.nucifera exhibited the strongest effects on relaxation and emotional stability, while C.obtusa contributed to support both comfort and steady concentration. These findings suggest that the fragrance and environmental characteristics of certain plant species may positively affect human neurophysiological responses.

Table 2. EEG result

EEG waves		C.camphora	T.nucifera	C.japonica	M.alternifolia	C.obtusa
RA	Fp1	0.3841	0.4306	0.4655	0.4258	0.4033
	Fp2	0.3348	0.4045	0.3826	0.3266	0.3775
	F3	0.3922	0.4400	0.4535	0.4104	0.4481
	F4	0.4007	0.4297	0.4426	0.4204	0.4412
	T3	0.3386	0.3682	0.3835	0.3345	0.3745
	T4	0.3381	0.3157	0.3003	0.2960	0.3469
	P3	0.4525	0.5360	0.5054	0.4425	0.4890
	P4	0.4847	0.5314	0.5137	0.4786	0.5213
RB	Fp1	0.1980	0.1973	0.1815	0.1888	0.1911
	Fp2	0.2126	0.2000	0.1919	0.2035	0.1901
	F3	0.1762	0.1968	0.1823	0.1683	0.1900
	F4	0.1985	0.2143	0.1996	0.1988	0.2081
	T3	0.2523	0.2925	0.2595	0.2481	0.2513
	T4	0.2767	0.3277	0.3035	0.3065	0.2883
	P3	0.1945	0.2089	0.1948	0.1886	0.2001
	P4	0.2036	0.2274	0.2128	0.2058	0.1998
RAB	Fp1	2.5083	3.2458	2.9311	2.7169	2.7362
	Fp2	2.0381	2.6652	2.2937	2.0076	2.5202
	F3	2.7635	2.6822	2.9340	3.0677	2.9447
	F4	2.5221	2.4156	2.8693	2.5503	2.8620
	T3	1.4235	1.4534	1.7724	1.5728	1.7437
	T4	1.3113	1.1736	1.1685	1.1768	1.5188
	P3	2.6939	3.2182	2.9478	2.7515	2.8257
	P4	2.8772	3.3237	3.0492	3.1220	3.2266
RAHB	Fp1	5.5246	7.6181	7.2590	6.3553	6.7927
	Fp2	4.2886	6.4191	5.5117	4.3530	6.0842
	F3	6.4183	6.3435	7.8634	9.0796	7.2195
	F4	5.8963	5.6483	7.2502	6.5430	6.9314
	T3	2.8731	3.1102	4.2001	3.2923	3.9153
	T4	2.8446	2.5426	2.6455	2.4365	3.5095
	P3	6.2048	8.0688	7.4760	6.0981	6.5979
	P4	7.4411	8.7300	8.2788	7.9938	8.1411
RSMR MBT	Fp1	0.6292	0.7731	0.5882	0.5963	0.5864
	Fp2	0.5783	0.7235	0.7017	0.4610	0.5179
	F3	0.7093	0.7452	0.7160	0.7053	0.6204
	F4	0.9407	0.8129	0.8157	1.0097	0.7178
	T3	0.9974	1.2057	1.0761	0.9963	0.9474
	T4	1.0694	1.6538	1.2996	1.3391	1.2781
	P3	1.0551	1.1263	1.0095	0.9405	0.9019
	P4	1.0485	1.2891	1.1034	1.0691	1.1408

Conclusion

In this study, extracts were prepared using 70% ethanol and an ultrasonic cleaner from C.japonica, C.camphora, M.alternifolia, T.nucifera, and C.obtusa grown in Jeju Island. Essential oils were manufactured through steam distillation after visiting the Cosmetic Raw Material Processing Center located in Seogwipo, Jeju Island. To evaluate the efficacy of the extracts, total polyphenol content, total flavonoid content, TEAC value, FRAP value, and elastase inhibition activity, were measured. Furthermore, GC-MC analysis was performed to identify the components of the essential oils. As indicated by the results of the total polyphenol content experiment, hinoki cypress and camphor had the highest contents. Besides, tea tree showed the highest value for the total flavonoid content experiment, while torreya showed a low value. The results of the elastase inhibition assay showed that the tea tree displayed the highest inhibitory activity. Nevertheless, torreya and hinoki cypress showed low values. According to the TEAC antioxidant assay, camphor showed high antioxidant activity and it also showed the highest value in FRAP antioxidant assay. GC-MS analysis showed that C.camphora contains high amounts of camphor and α-pinene, while C.japonica contains high levels of sabinene and kaur-16-ene. Tea trees contain γ-terpinene and terpinen-4-ol, T.nucifera contain limonene and α-pinene, and C.obtusa contain (+)-4-carene and D-limonene. Additionally, α, β, and θ brain waves were measured through an electroencephalogram(EEG) test, which validated that essential oils have positive effects on humans.

Based on the results of this study, it is scientifically demonstrated that native Jeju plants contain substantial amounts of bioactive compounds. These findings suggest that essential oil extracted from these plants can be effectively utilized for the cosmetics and aromatherapy industries. Furthermore, future extensions of this research should include high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC-MS) analyses to precisely identify the components of the extracts.

Works Cited

Albouchi, F., et al. "Melaleuca styphelioides Sm. Polyphenols Modulate Interferon Gamma/Histamine-Induced Inflammation in Human NCTC 2544 Keratinocytes." Molecules, vol. 23, no. 10, 2018, p. 2526.

Allenspach, M., and C. Steuer. "α-Pinene: A Never-Ending Story." Phytochemistry, vol. 190, 2021, article 112857.

Azghar, A., et al. "Evaluation of the Antibacterial Activity of Essential Oil." Asian Journal of Plant Sciences, vol. 22, 2023, pp. 75–81.

Bae, M. G. "A Study on the Effect of Antioxidant Activities and Skin Wrinkling Improvement of Tea Tree Essential Oil." The Korean Society of Beauty Industry, vol. 14, no. 1, 2020, pp. 67–81.

Bakkali, F., et al. "Biological Effects of Essential Oils: A Review." Food and Chemical Toxicology, vol. 46, no. 2, 2008, pp. 446–475.

Benzie, I. F. F., and J. J. Strain. "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of 'Antioxidant Power': The FRAP Assay." Analytical Biochemistry, vol. 239, no. 1, 1996, pp. 70–76. https://doi.org/10.1006/abio.1996.0292.

Chen, J., et al. "Metabolomics Analysis to Evaluate the Antibacterial Activity of the Essential Oil from the Leaves of Cinnamomum camphora (Linn.) Presl." Journal of Ethnopharmacology, vol. 253, 2020, article 112652.

Eltayeb, L. M., et al. "Essential Oils Composition and Biological Activity of Chamaecyparis obtusa, Chrysopogon nigritanus and Lavandula coronopifolia Grown Wild in Sudan." Molecules, vol. 28, 2023, p. 1005.

Grzesik, M., et al. "Antioxidant Properties of Catechins: Comparison with Other Antioxidants." Food Chemistry, vol. 241, 2018, pp. 480–492.

Hamidpour, R., et al. "Camphor (Cinnamomum camphora), a Traditional Remedy with the History of Treating Several Diseases." International Journal of Case Reports and Images, vol. 4, no. 2, 2013, pp. 86–89.

Hart, P. H., et al. "Terpinen-4-ol, the Main Component of the Essential Oil of Melaleuca alternifolia (Tea Tree Oil), Suppresses Inflammatory Mediator Production by Activated Human Monocytes." Inflammation Research, vol. 49, no. 11, 2000, pp. 619–626.

Im, S. H., and J. S. Kim. "Whitening, Anti-Wrinkle and Skin Protective Effect of Extracts from Different Tea Tree Parts." Korean Journal of Medicinal Crop Science, vol. 30, no. 5, 2022, pp. 330–338. https://doi.org/10.7783/KJMCS.2022.30.5.330.

Jeon, H. S., et al. "The Antioxidative Activities of Torreya nucifera Seed Extracts." Journal of the Korean Society of Food Science and Nutrition, vol. 38, no. 1, 2009.

Ji, K. "Antimicrobial Effects of Tea Tree (Melaleuca alternifolia) Extracts." Journal of the Korea Academia-Industrial Cooperation Society, vol. 20, no. 5, 2019, pp. 102–108.

Kim, J. H., et al. "Analysis of the Component and Immunological Efficacy of Chamaecyparis obtusa Leaf Extract." Korean Journal of Clinical Laboratory Science, vol. 50, 2018, pp. 37–43.

Kim, Y. W., et al. "Safety Evaluation and Risk Assessment of d-Limonene." Journal of Toxicology and Environmental Health, Part B, vol. 16, no. 1, 2013, pp. 17–38.

Ko, H. C., et al. "Seasonal Variations in the Phenolic Compounds and Antioxidant Activity of Sasa quelpaertensis." Industrial Crops and Products, vol. 122, 2018, pp. 506–512. https://doi.org/10.1016/j.indcrop.2018.06.031.

Ko, H. M., et al. "Tyrosinase and α-Glucosidase Inhibitory Activities and Antioxidant Effects of Extracts from Different Parts of Hypochaeris radicata." Korean Journal of Medicinal Crop Science, vol. 25, no. 3, 2017, pp. 139–145. https://doi.org/10.7783/KJMCS.2017.25.3.139.

Leopoldini, M., et al. "The Molecular Basis of Working Mechanism of Natural Polyphenolic Antioxidants." Food Chemistry, vol. 125, no. 2, 2011, pp. 288–306.

Nooshadokht, M., et al. "In Silico and In Vitro Antileishmanial Effects of Gamma-Terpinene: Multifunctional Modes of Action." Chemico-Biological Interactions, vol. 361, 2022, article 109957.

Nurzyńska-Wierdak, R., et al. "Essential Oils in the Treatment of Various Types of Acne: A Review." Plants, vol. 12, no. 1, 2022, p. 90.

Park, B. I., et al. "Sabinene Suppresses Growth, Biofilm Formation, and Adhesion of Streptococcus mutans by Inhibiting Cariogenic Virulence Factors." Journal of Oral Microbiology, vol. 11, no. 1, 2019, article 1632101.

Sivaphongthongchai, A., et al. "The Effects of d-Camphor Inhalation on Psychophysiological Parameters among Healthy Participants." Journal of Public Health and Development, vol. 20, 2022, pp. 96–107.

Sobhy, S., et al. "Phytochemical Characterization and Antifungal Efficacy of Camphor (Cinnamomum camphora L.) Extract against Phytopathogenic Fungi." Separations, vol. 10, no. 3, 2023, p. 189.

Turek, C., and F. C. Stintzing. "Stability of Essential Oils: A Review." Comprehensive Reviews in Food Science and Food Safety, vol. 12, no. 1, 2013, pp. 40–53.

Yoo, K. M., et al. "Evaluation of Different Methods of Antioxidant Measurement." Food Science and Biotechnology, vol. 16, no. 2, 2007, pp. 177–182.

Zochedh, A., et al. "Investigation on Structural, Spectroscopic, DFT, Biological Activity and Molecular Docking Simulation of Essential Oil Gamma-Terpinene." Journal of Molecular Structure, vol. 1268, 2022, article 133651.

Zulueta, A., et al. "ORAC and TEAC Assays Comparison to Measure the Antioxidant Capacity of Food Products." Food Chemistry, vol. 114, no. 1, 2009, pp. 310–316. https://doi.org/10.1016/j.foodchem.2008.09.033.

Bionic Prosthetics By Fareeha Shaik

There are around 2 million limb amputees in the USA with approximately 185,000 amputations performed annually. Based on statistics from Germany, Italy, Ireland and the USA, the EU has approximately 3.18 million limb amputees, and approximately 295,000 amputations are performed annually (Bumbaširević et al.). Due to the psychological distress that most patients experience following an amputation, amputations pose many medical and economic problems. During the post-amputation period, patients may have a difficult time recovering, continuing with daily self care, and being reintroduced into the workplace. As a result, bionic prosthetics were developed as a new solution to efficiently assist patients in returning to their normal way of life.

The reason why this topic piqued my interest is because my two favorite logs that I have written as a sophomore were about 3D Bioprinting and nanorobotics. These topics led me to an interest in biomedical engineering and the innovations that come out of biomedical research. When I realized that biomedical engineers are constantly working on making prosthetics more realistic, comfortable, and functional I became interested in how a device could be designed such that it could interface with the human body to act as a true limb.

In current terminology, the term "bionic" is mainly used to refer to devices that make a direct connection with the residual nervous or muscular system of the impaired individual. A bionic prosthetic limb gains its functionality from the control of electric signals from either the muscle or the nerves that are above the amputation site. In this process, bidirectional control is completed by sensation restoration through the connection of the remaining nerves or muscle ("Bionic Limbs"). Sensory information flow is restored with signals from tactile and position sensors that are typically found in the prosthetic and are converted into electrical impulses through encoding algorithms found on the system controller. This flow of sensory information is the reason why users are able to perceive sensations directly on the phantom limb between the prosthetic and the external environment. Bionic limbs can be divided into three categories, depending on the implants used and the tissue interfaced, or in other words, the tissue that interacts with the sensors in the prosthesis. These three categories are nerve and muscle transferring, direct muscle interfacing, or direct nerve interfacing (Bumbaširević et al.).

Nerve and muscle transferring is a technique that reroutes a functioning nerve to replace a damaged or severed nerve, which allows the bionic limb to receive signals from the brain. A specific example of nerve and muscle transferring is agonist-antagonist myoneural interface (AMI). Proprioception, or the body's awareness of itself in space, has been found difficult to restore in prosthetics, however is deemed to be significant, especially when carrying out a normal routine like walking. Proprioception occurs due to the signals that muscles send regarding where the body is, how it's moving, and what forces it may encounter (Makin). Typically, the coupled muscles that send these signals are called agonist-antagonist pairs, where one contracts as the other stretches. Unfortunately, this feedback is abandoned in a traditional amputation. However, MIT bionicist, Hugh Herr, along with Clites and team's surgeon Mathew

Carty, developed a new technology that would reconstruct the push-pull pairs by rerouting the nerves that originally controlled the missing limb to residual limb or nearby areas, and uses the signals generated to control prosthetic joints (Makin). This process enables sensory feedback, allowing the recipient to “feel” their prosthetic limb.

On the other hand, direct muscle interfacing is an approach that captures a control signal from residual muscular tissue through intramuscular implants. Intramuscular implants are able to receive control signals because of the small recording devices implemented to record muscular contractions, which in turn are wirelessly transmitted through a coil that is integrated in the socket. An example of this category of bionic limbs is targeted muscle reinnervation (TMR), which focuses on the transfer of remaining nerves in the amputated limb to the available muscle. TMR is a procedure that involves numerous steps to be as beneficial to the user (Bumbaširević et al.). First, the individual must undergo a procedure, known as muscle reinnervation surgery, that reassigns the nerves that used to control the arms and hand. This step is crucial, as it plays a crucial role in how individuals are able to control the prosthetic limb by simply thinking about the desired action. After the patient has recovered from the surgery, they are provided training on the pattern recognition implemented into the prosthesis. Pattern recognition is the algorithm that deciphers how individual muscles contact, communicate, and ultimately work together through amplitudes and frequencies (“Bionic Limbs”). After this information is collected from the algorithm, the movement pattern of the prosthesis is created. This training on the limb continues until it is ready to be attached to a brace, and use the limb to carry out functions.

The last category of bionic prosthetics is direct nerve interfacing, which uses implantable electrodes that play a role in enabling reception of electric signals from the prosthetic sensors. The surgical procedure for electrode implantation primarily works on targeting the proximal nerve area, while also introducing and fixing electrodes and cables, while also ensuring that no excessive neural damage is occurring by limiting the movement of the limb and the nerve (Bumbaširević et al.). A type of bionic prosthetic that falls under this category is Brain-computer interface (BCI) bionic prosthetics.

Implanted BCIs are often attached directly to the brain surgically. Wearable BCIs often require a cap that contains conductors, playing the role of measuring brain activity detectable on the scalp. BCIs that are being used for bionic limbs use electroencephalography (EEG) to measure the brain's electrical activity (“Science & Tech Spotlight”). However, a new method, functional near-infrared spectroscopy (fNIRS) shines near-infrared light through the skull to measure blood flow. Interestingly enough, this observation of flood flow can indicate information, an example being the patient's actions. In the process, new BCI users will go through a training process, where they learn to recognize signals that the BCI uses, and then BCI will translate the signals to operate the brain using an algorithm known as machine learning (“Science & Tech Spotlight”). Because BCIs are measuring signals directly from the brain, it reduces the interference from other tissues. Regardless, post-surgical risks, like infection and rejection is a current worry with BCIs.

While I was exploring the various types of bionic prosthetic devices, I began to wonder what the best methods for achieving true sensory feedback in bionic limbs are so that individuals are able to feel sensations such as pressure, temperature, or texture. As I was gaining more background information, I learned that individuals who have used myoelectric prostheses have stated that it lacks comfortability and function. While engineers have been able to accomplish things such as dexterity, the movements of the prosthetic limb are not consistent with the thoughts of the users. However, this could be explained by the fact that 90% of upper limb peripheral nerve axons convey sensory information, which is yet to be transmitted to devices properly (Roche et al.).

Since nerves that are found in upper limbs happen to be situated away from the spinal cord and brain, they organize themselves in fascicles, or groups of axons that branch away from the nerve bundle to control motor function, and carry out sensory tasks by sending signals from the skin, tendons, and bones. Sensory receptors in limbs are classified and specialized to detect sensory information. For example, mechanoreceptors sense touch and pressure, thermoreceptors sense changes in temperature, and nociceptors sense pain (Roche et al.). With this information in mind, I wanted to delve into a potential solution. To prevent bionic limbs from lacking consistency, what if a dynamic neural interface that had the capability of enhancing neurons, such as optogenetics, was adapted? The idea of using optogenetics came from my interest in cancer research that stemmed from my second log, nanorobotics, where I had used my extension to delve into how nanorobots played a role in cancer treatment. My sister, who has had experience in a lab that focused on cancer research has elaborated more on the various techniques used in order to find new methods to cancer treatment, one of which was optogenetics. Optogenetics was one that struck curiosity on my end, as I was curious how specific light had the ability to alter the behavior of neurons, and thus sensory feedback.

In this process, multimodal sensory feedback could be implemented in order to provide sensations that could mimic natural sensory input. Optogenetics is a technique that uses light to control or monitor neural activity with the help of light-sensitive proteins. Because these light-sensitive proteins contain photoreceptive domains that induce biological function, delivering light to specific neurons has the ability to provide an individual with immediate sensory feedback (Chen et al.). This detail happens to be important in the context of bionic prosthesis, as this instant feedback has the potential to assist the individual in adjusting their movement as they interact with objects and their environment. This discovery had been sought once it was known that microbial opsins, or light ion-gated channels could generate photocurrents that were sufficient enough to stimulate and control neurons with organisms that possessed high temporal resolutions, or in other words, organisms that can decipher which events occur at different times (Duebel et al.).

While this technique is used to alter brain circuitry and function in health and disease, it has yet been used for a more efficient use of bionic limbs. To integrate optogenetics into bionic devices, opsins could be inserted into residual sensory neurons that are still functional, or nearby nerves. When external stimuli is detected by the bionic limb, light would be used to stimulate

these sensory neurons, ultimately sending sensory feedback in the brain to make the limb more realistic and compatible for the user based on their desired action. By adapting opsins into nerves, it seems possible that the prosthetic could choose to selectively stimulate different fascicles to enhance the consistency of the prosthetic by essentially making sensory neurons light-responsive.

To test this idea, an organ-on-a-chip (OOC) can be used to test the potential use of optogenetics on neurons and bionic limbs. Based on my research in previous logs in regards to 3D bioprinting, an OOC is a bioengineered system that essentially reenacts the function and structure of a human organ on a small chip made of materials like silicon, polymer or glass. In my experiment, a brain OOC would be used to understand how neurons would interact and function similarly in the body. In this experiment, two brain OOC's will be used: One that is going to be treated using optogenetics, while the other will not receive any treatment. At the end of the treatment, the two OOGs can be compared to see which neurons had a faster sensory feedback response by using a multielectrode array (MEA). MEA is a method used in neuroscience to measure electrical activity of a network of cultured neurons (Simkin et al.). In this process, electrodes would be embedded on the surface to capture action potentials, or significant changes in electrical potential across the membrane of the neuron.

However, this idea did bring up a few roadblocks. Although optogenetics has overcome numerous challenges, scientists have proposed that photosensitive proteins have effects on nerve cells. It's been assumed that exogenous light exposure causes neurons to respond improperly, which can lead to incorrect physiological conclusions on nerve cells. Not only this, but while the OOG has the ability to test the potential of implementing optogenetics, they do not fully replicate the complexity of the human body, especially when testing the integration of sensory feedback with higher-level brain functions. Not only this, but the brain poses major differences within every individual. While the brain shares the same human anatomy, everybody possesses different experiences, which alter their neural pathways, brain structures, and cognitive functions. With that in mind, it may be difficult to use optogenetics to target specific cells if it requires searching for different ones in each patient.

While I was conducting more research on my log, I found that another issue that came with bionic prosthetics is its durability. Writing my FD brought me to the realization that bionic limbs use batteries as their main source of power, which ultimately means that the arm will cease to function over time. With that being said, a question arose: Could bionic prosthetic limbs be built to have continuous, self-generating properties? As I was researching, I came across thermoelectric generators, which is a device that converts heat into electricity. A thermoelectric generator (TEG) is able to obtain electrical power due to the Seebeck effect, or the heat flow across a temperature gradient. As the heat flows from the hot region to the cold region, free charge electrons are also directed to the cold end. As a result, the harvesting voltage is proportional to the temperature difference, according to the Seebeck coefficient ("What Is the Seebeck Effect?"). TEGs consist of two types of semiconductors, n-type and p-type,of which a net voltage that is produced can be driven through. A good thermoelectric material typically

possesses a Seebeck coefficient between 100 μV/K and 300 μV/K. For this reason, thermoelectric couples need to be connected to make a device to achieve a sufficient amount of energy.

Aside from that, the human body has peripheral and central thermoreceptors, which are found in the skin, viscera, spinal cord, and hypothalamus. These thermoreceptors have the responsibility of sensing the core temperature, and activate when variations in body temperature occur. Once the thermoreceptors are activated, it informs the preoptic area of the hypothalamus, which then activates heat regulation mechanisms to increase or decrease body temperature and return it to homeostasis (Osilla et al.). Considering that the human body is constantly regulating the temperature through thermoregulation, it seems plausible that thermoelectric generators have the ability to harvest body heat or external temperature differences to recharge the bionic arm's energy. If so, TEG couples could be implemented into the design of the bionic arm, which will then capture the body heat of the individual. In doing so, it could be a possibility that TEG's could be another method in capturing energy for these prosthetic limbs.

As I was writing my developed questions, wondering what I could do for my extension, my friend was talking to me about axolotl facts when discussing their capabilities. One that specifically caught my attention was their ability to regrow tissues for whole body parts, such as legs, tails, eyes, and the heart. Since I was thinking of ways to make bionic limbs possess self-generating properties, I wanted to delve more into axolotl generation out of curiosity to see how this interesting fact could alter regenerative medicine, and potentially enhance bionic limbs. Interestingly enough, the regeneration that axolotls are capable of doing have been studied by researchers for years, as they happen to be a great model to compare to the human genome. Axolotls are tetrapods and thus share homologous structures, which means that they have desirable traits when modeling the regeneration of appendages ("Amazing Axolotl"). Not only this, but because axolotls lay hundreds of eggs, they are quite easy to manipulate and observe during experiments.

In the past, researchers focused on how levels of mRNA molecules changed after an axolotl has an injury to figure out how axolotls were able to quickly regenerate cells. However, this observation only seemed to depict the production of mRNA molecules after injury, and not the science that occurs in the translation of mRNA into protein products ("Amazing Axolotl"). Regardless, further experiments led to the discovery that axolotl cells "stockpile" mRNA, and ultimately translate less than 20% of it (Williams). Scientists learned how axolotls respond to injury, they found that protein synthesis is activated, which leads to the translation of these stockpiled mRNA. Because of the abundance of transcripts, protein synthesis occurs rapidly during regeneration.

Axolotls and related salamanders have altercation in their mRNA in which the sequence expands. The axolotl mTOR protein is highly sensitive comparatively to mammalian mTORs. In humans and mice, mTOR is only activated when there's an abundance of nutrients. On the other hand, mTOR in axolotls is switched on to its active state when they lose a rush of nutrients (Williams). So, the combination of a sensitive mTOR molecule and an abundance of

ready-to-use mRNAs means that axolotl cells can quickly produce the proteins needed for tissue regeneration once an injury occurs.

Works Cited

"The Amazing Axolotl: A Valuable Model for Regenerative Medicine." Office of Research Infrastructure Programs, National Institutes of Health, 16 Mar. 2021, orip.nih.gov/about-orip/research-highlights/amazing-axolotl-valuable-model-regenerative-medicine.

"Bionic Limbs." Australian Academy of Science, 26 Sept. 2017, www.science.org.au/curious/people-medicine/bionic-limbs.

Bumbaširević, Marko, et al. "The Current State of Bionic Limbs from the Surgeon's Viewpoint." EFORT Open Reviews, vol. 5, no. 2, 2020, pp. 65–72, https://doi.org/10.1302/2058-5241.5.180038.

Chen, W., et al. "The Roles of Optogenetics and Technology in Neurobiology: A Review." Frontiers in Aging Neuroscience, vol. 14, 2022, article 867863, https://doi.org/10.3389/fnagi.2022.867863.

Duebel, Jens, et al. "Optogenetics." Current Opinion in Ophthalmology, vol. 26, no. 3, 2015, pp. 226–32, https://doi.org/10.1097/ICU.0000000000000140.

Makin, Simon. "Brain-Controlled Bionic Limbs Are Inching Closer to Reality." Science News, 3 Oct. 2024, www.sciencenews.org/article/brain-controlled-bionic-limbs-anatomics.

Osilla, Eva V., et al. "Physiology, Temperature Regulation." StatPearls, StatPearls Publishing, 30 July 2023, www.ncbi.nlm.nih.gov/books/NBK507838/.

Roche, Aidan D., et al. "Upper Limb Prostheses: Bridging the Sensory Gap." Journal of Hand Surgery (European Volume), vol. 48, no. 3, 2023, pp. 182–90, https://doi.org/10.1177/17531934221131756.

"Science & Tech Spotlight: Brain-Computer Interfaces." U.S. Government Accountability Office, 8 Sept. 2022, www.gao.gov/products/gao-22-106118.

Simkin, Dina, et al. "'Channeling' Therapeutic Discovery for Epileptic Encephalopathy through iPSC Technologies." Trends in Pharmacological Sciences, vol. 43, no. 5, 2022, pp. 392–405, https://doi.org/10.1016/j.tips.2022.03.001.

Snyder, G. Jeffrey. "Small Thermoelectric Generators." The Electrochemical Society Interface, vol. 17, no. 3, 2008, pp. 54–56, www.electrochem.org/dl/interface/fal/fal08/fal08_p54-56.pdf.

"What Is the Seebeck Effect?" NASA Science, 8 Aug. 2024, science.nasa.gov/resource/what-is-the-seebeck-effect/.

Williams, Sarah C. P. "How an Ultra-Sensitive On-Off Switch Helps Axolotls Regrow Limbs." Scope, Stanford Medicine, 26 July 2023, scopeblog.stanford.edu/2023/07/26/how-an-ultra-sensitive-on-off-switch-helps-axolotls-regrow-limbs/.

3D Bioprinting By Fareeha Shaik

There are currently hundreds of thousands of people on transplant lists, waiting for critical organs such as kidneys, hearts and organs. Unfortunately, there aren't nearly enough donor organs available to fill the demand. With this problem in mind, 3D bioprinting, a branch of regenerative medicine, is being researched to fully understand the ways that tissues and organs could be 3D printed. Being interested in the field of Biomedical Engineering (BME), finding information on the usage of bioprinting was extremely intriguing to me. Since I'm interested in learning the various functions of medical devices in fields such as BME, I want to obtain a better understanding as to how 3D bioprinting is possible. From my research, I learned that 3D bioprinting requires a printable material, or bioinks to fabricate structures.

Bioinks are biological materials used in the manufacturing of engineered live tissues by the process of 3D bioprinting. They have the responsibility of mimicking the extracellular matrix environment, supporting cell adhesion, proliferation, and differentiation after printing (Ramiah et al.). Bioinks are composed of carrier molecules, such as hydrogels, that provide support to the growing cells, and chemicals that have the responsibility of encouraging cells to communicate and grow (Jones). Hydrogels are a type of biopolymer gels that often act as 3D molecular scaffolds that cells attach to and use for growth and multiplication. Biopolymers are polymers that can be found in or manufactured by living organisms. Hydrogels are crucial in the process of 3D bioprinting because they retain components like water due to their hydrophilic functional groups attached to the backbone (Ahmed), which provide mechanical stability to the tissues. Depending on the type of process used, the selection for bioinks is crucial, since they should have the desired properties of matter that can be observed or measured without changing its chemical composition. This includes mechanical, chemical, biological, and rheological characteristics.

There are several bioprinting techniques, which are inkjet-based bioprinting, extrusion-assisted bioprinting, and laser-assisted bioprinting. Inkjet bioprinting ejects drops of liquid onto a substrate by thermal or acoustic forces. With thermal inkjet bioprinting, the print head is electrically heated and generates small air bubbles to form the pressure that releases droplets from the nozzle when they collapse. The localized heating in a thermal printer lasts for a very short duration to prevent negative or unwanted effects on cells during printing. Acoustic inkjet bioprinting, on the other hand, uses a piezoelectric crystal. Piezoelectricity is an electric charge that is generated in response to mechanical stress. This effect is reversible, which essentially means that an electric charge can also induce a mechanical alteration. In this circumstance, voltage is applied and induces a shape change, forming an acoustic wave inside the print head. This acoustic wave breaks the bioink into droplets that are ejected because of the transient pressure, or the short-lived pressure, from the nozzle (Xia et al.). Inkjet bioprinting has the advantage of contactless printing. Additionally, the size, deposition rate, and position of droplets can be altered easily in inkjet bioprinting, which gives it the ability to create defined patterns and concentration gradients of cells with complex structures. While inkjet bioprinting

provides a high spatial resolution, the bioink needs to fulfill various requirements. For example, inkjet bioprinting uses a bioink with a low viscosity to avoid clogging and droplet ejection. Viscosity is a measure of a fluid's resistance to flow. So, a fluid with low viscosity flows easily because its molecular composition possesses very little friction when it is in motion. For this reason, inkjet bioprinting is mainly used for printing small scaffolds (Sapkota).

Extrusion-based bioprinting is the most common method used in bioprinting to create structures. In extrusion bioprinting, the bioink is extruded out of a nozzle tip continuously in the form of line structures, or filaments, instead of droplets. During this process, one of the two mechanisms are used; The semi-solid extrusion (SSE) and the fused deposition modeling (FDM) based 3D printing. Pressurized air or a rotating screw gear is used to exude the material through a nozzle layer-by-layer. However, FDM bioprinting utilizes high temperatures to melt the thermoplastic filaments which are extruded through the nozzle layer-by-layer (Sapkota). Biomaterials used for extrusion bioprinting should be nearly fluid-like so that they can be extruded through the tip of the nozzle. Regardless, the extrusions should be strong enough to maintain the shape during and after the printing. The versatility of these biomaterials for extrusion based bioprinting is significant. For instance, thermoplastic biomaterials thermoplastic biomaterials are extruded as liquids when heated, but solidify to form a shape. Out of the three methods of bioprinting, the extrusion printed scaffolds typically have a better structural integrity. Unfortunately, the resolution is low comparatively to inkjet and laser bioprinting. Additionally, extrusion-based bioprinting can cause stress-induced cell deformation, or in other words, damage to the cells due to the mechanisms this method uses.

Lastly, laser-assisted bioprinting is a method that deposits biomaterials onto a solid surface by using a laser as a source of energy. Other than a laser, this method involves a “ribbon” with a donor support, and a source film containing the printing materials. The laser present on the printer exposes the ribbon to the radiation from the laser, which is when the source film absorbs the laser energy. This in turn causes the liquid biomaterial to evaporate and reach the collecting substrate in droplet form. Laser bioprinting is nozzle-free, and eliminates the clogging that happens in inkjet and extrusion bioprinting (Sapkota). The resolution for printing is entirely dependent upon the viscosity of the bioink, the speed of the laser printing, and the pulse frequency of the laser. With this, Laser bioprinting provides an advantage by permitting single-cell manipulation and precise control of the positions of cell droplets. A disadvantage that is apparent is that the cell viability is often lower than that of inkjet bioprinting, since laser energy causes cell death during bioprinting (Xia et al.).

Overall, the process of 3D bioprinting can be achieved through three different steps; pre-bioprinting, bioprinting, and post-bioprinting. The first step of pre-bioprinting is the formation of a model that will be used by the printer and the choosing of materials that will be used to produce the bioink for the following steps. Pre-bioprinting uses technologies such as computed tomography (CT) or magnetic resonance imaging (MRI) scans to obtain 2D images. From these images, cells that are crucial are selected and multiplied. The printing process is the second step, where the bioink is placed in the printer to create a 3D structure. The formation of

tissue constructs and cell models involves the deposition of bioink onto the scaffold layer-by-layer. This process is known to be complex, as it requires the specialization of cells based on the type of tissues and organs being formed. The last step of printing is post-bioprinting, which mainly centralizes on ensuring the stability of the printed structure. In order to maintain the functionality and structure, physical and chemical simulations are used to provide signals to the cells and to reorganize and maintain the growth of tissues. If this step were to be skipped, the mechanical structure of the material would likely be disrupted, and the functioning of the material would be defected (Sapkota).

As I started pondering more on the impacts of 3D bioprinting, I began to wonder if 3D bioprinting could assist in creating unique and desirable features, like a fire-resistant skin. Unburnable skin could serve usefulness for various reasons. For one, a trait like unburnable skin could improve safety in various fields like firefighters, welders, and factory workers. Unburnable skin could ultimately protect people in high-risk situations, significantly reducing burn injuries.

Since all engineered tissues must start with bioink, I started to think about a potential bioink that could be developed with fire-resistant materials, stem cells, and skin cells. While I was researching, I found a polymer that is known for its fire-resistant properties: Kevlar. Kevlar is composed of fairly rigid molecules, forming a sheet-like structure that looks similar to silk protein. These properties result in its heat resistance and its mechanical strength. Since the ratio of carbon to hydrogen atoms is high, it has a low flammability (Aderonke et al.).

Mesenchymal stem cells (MSCs) are adult stem cells that can differentiate into various cell types. MSCs have the possibility of promoting tissue repair by releasing growth factors and cytokinesis. These growth factors recruit other cells to the injury site and promote the formation of new blood vessels that are essential for tissue repair. MSCs are known for their immunomodulatory properties, or the modulation of activity in the immune system, reduce inflammation and suppress immune responses (Cona). Since these stem cells have unique regenerative and differentiation properties, it seems plausible that these cells would constantly regenerate if they were killed by hypothetical thermal forces. Their immunomodulatory properties may potentially lessen the reaction of a severe burn in the case of an emergency. Being able to include MSCs and Kevlar in a bioink would be extremely beneficial, as Kevlar is not necessarily “unburnable,” rather heat-resistant. In the case that the skin undergoes extreme thermal forces, the MSCs would be able to regenerate cells killed by the heat.

To test if the bioink can successfully and effectively fabricate unburnable skin, multiple material tests can be conducted to determine its thermal and flame resistance. First, Thermogravimetric Analysis (TGA) will be used to determine the material's thermal stability. TGA measures the changes in sample weight in a controlled thermal environment.The temperature at which mass loss begins indicates the material's thermal stability (Mathias). In practical application testing, the “unburnable skin” would be exposed to simulated high temperatures and direct flames to evaluate the performance under conditions that mimic real-world situations. For this, a heat flux test meter can be used to measure the material’s resistance to heat overtime. A heat flux sensor is an instrument used to measure the rate of heat

energy transfer. The sensor is capable of measuring conductive, convective, and also radiative heat transfer on the surface or from the surface of a body (Rossouw).

However, this further development comes with some roadblocks. For one, the polymers listed above may lack biocompatibility due to their specialized properties. Integrating these polymers into a bioink while also ensuring the final product remains suitable for biological use is a significant challenge. Additionally, attempting to develop a bioink that is composed of both living cells and fire-resistant materials may be a hurdle because the fire-resistant properties within Kevlar may not naturally integrate with living tissues, and their long-term stability and interactions would need to be further observed and evaluated to ensure the engineered tissues remain functional.

I became much more intrigued about the production of organs when I overheard my sister working in a lab, assisting in making heart tissues for a potential 3D bioprinted heart. She mentioned that during this, they were unable to construct a human-sized heart because of the complexity that comes with the way a heart functions. Replicating the complex biochemical environment of a major organ, such as a heart, is a difficult task. One of the hardest challenges is supplying oxygen and nutrients to all the cells in a full-size organ, which is why scientists have only been able to successfully print hollow organs.

However, Scientists at Tel Aviv University in Israel announced that they have successfully 3D-printed a small scale heart, with blood vessels, ventricles, and chambers. Though the heart can contract, the cells have not developed a pumping ability (Jacobsen). With this in mind, isn't it possible to incorporate pacemaker cells into the bioink so the heart can form a pumping ability? The heart's natural pacemaker is a cluster of cells that are referred to as the sinoatrial (SA) node. The SA node produces electrical impulses that set the heart rate and rhythm. Because of this, the SA node is known to be one of the most crucial tissues in the body as it initiates the heartbeat (West). If pacemakers have the ability to cause a regular heart to regulate heart rate, it seems plausible that once these cells are mixed into a bioink, they could regulate the pumping of the bioprinted heart.

Something that intrigued me as I began to learn more about the impacts of 3D bioprinting on other potential solutions for organ donors is Xenotransplantation. Xenotransplantation is transplantation of living cells, tissues or organs from one species to another. This method of transplanting organs is an experimental treatment, which means that the FDA has not allowed clinical trials to begin. As of right now, xenotransplantation is being tested to determine whether this method could satisfy the demand for organs ("Xenotransplantation").

Scientists are discovering ways to genetically modify pigs by finding methods to "revascularize", or add in human cells with animal organ scaffolds and manufacture organs through 3D bioprinting. A team led by Dr. Vacanti, Professor of Surgery at Harvard Medical School, has been researching ways to reach these goals. In Dr. Vacanti's lab bioprinters are producing liver implants that contain a vascular set of channels designed to mimic portal flow. A portal flow is a blood vessel that carries blood from all your organs to the liver. The liver then filters and processes the blood before returning to the heart and recirculating through the body

(“Portal Vein”). In order to construct crucial vascular networks for engineered tissues, Dr Vacanti’s team has applied fractals, a mathematical concept centralized around geometric structures whose smaller parts are self-similar across different scales. Blood vessels are an example of fractal branches, as the branches decrease down in size into a capillary. In 3D bioprinting, researchers observe the algorithm of a certain fractal, so they can scale it infinitely when building fractal-based vascular networks with 3D bioprinters (Sauer).

Aside from the vessels, the production of these liver impacts involves the 3D bioprinting of scaffolds that are loaded with the recipient's cells, with the hope that it eliminates the potential for rejection. Next, these scaffolds are incubated in flow conditions in culture (Sauer). Culturing cells is a process that grows the cells in specific ways under the conditions suitable to them. After a few days of incubation, the implant would be prepared to be transplanted into a human.

Works Cited

Aderonke, Adegoke Victoria, et al. "Poly-Paraphenylene Terephthalamide: A Life Saving Polymer." Evolution in Polymer Technology Journal, vol. 2, no. 2, 2019, academicstrive.com/EPTJ/EPTJ180014.pdf. Accessed 4 Sept. 2024.

Ahmed, Enas M. "Hydrogel: Preparation, Characterization, and Applications: A Review." Journal of Advanced Research, vol. 6, no. 2, 2015, pp. 105–21, www.sciencedirect.com/science/article/pii/S2090123213000969. Accessed 11 Sept. 2024.

Cona, L. "What Are Mesenchymal Stem Cells?" DVC Stem, 5 June 2024, www.dvcstem.com/post/what-are-mesenchymal-stem-cells. Accessed 4 Sept. 2024.

Jacobsen, B. "We Now Have 3D-Printed Human Hearts." Futures Platform, 17 June 2024, www.futuresplatform.com/blog/3d-printed-human-hearts. Accessed 4 Sept. 2024.

Jones, Taneka. "How to 3D Print Human Tissue." YouTube, uploaded by TED-Ed, 17 Oct. 2019, www.youtube.com/watch?v=uHbn7wLN_3k. Accessed 28 Aug. 2024.

Lewis, J. "3D Bioprinting of Living Tissues." Wyss Institute, 6 Sept. 2019, wyss.harvard.edu/technology/3d-bioprinting/. Accessed 20 Aug. 2024.

Mathias, J. "Applications of Thermogravimetric Analysis." Innovatech Labs, 21 Apr. 2022, www.innovatechlabs.com/newsroom/2333/applications-thermogravimetric-analysis/. Accessed 4 Sept. 2024.

"Portal Vein: Anatomy, Location & Function." Cleveland Clinic, 1 June 2023, my.clevelandclinic.org/health/body/25048-portal-vein. Accessed 4 Sept. 2024.

Ramiah, Previn, et al. "Hydrogel-Based Bioinks for 3D Bioprinting in Tissue Regeneration." Frontiers in Materials, vol. 7, 2020, article 76, https://doi.org/10.3389/fmats.2020.00076.

Rossouw, D. J. "Point Temperature Measurements." ScienceDirect, Elsevier, 2016, www.sciencedirect.com/topics/engineering/heat-flux-gauge. Accessed 4 Sept. 2024.

Sapkota, Anupama. "3D Bioprinting: Definition, Principle, Process, Types, Applications." Microbe Notes, 3 Aug. 2023, microbenotes.com/3d-bioprinting/. Accessed 4 Sept. 2024.

Sauer, Mary. "The Long Haul: Engineering High-Tech Solutions to Organ Shortage." Harvard ALI Social Impact Review, 31 Jan. 2023, www.sir.advancedleadership.harvard.edu/articles/engineering-high-tech-solutions-organ-shortage. Accessed 4 Sept. 2024.

West, Mary. "What Is the Heart's Natural Pacemaker?" Medical News Today, 31 Aug. 2022, www.medicalnewstoday.com/articles/natural-pacemaker-of-the-heart. Accessed 4 Sept. 2024.

"Xenotransplantation: Definition, Uses, and Effectiveness." National Kidney Foundation, 2 Jan. 2023, www.kidney.org/kidney-topics/xenotransplantation. Accessed 4 Sept. 2024.

Xia, Zengmin, et al. "Tissue and Organ 3D Bioprinting." SLAS Technology, vol. 23, no. 4, 2018, pp. 301–14, https://doi.org/10.1177/2472630318760515.

The Effect of Music on Adolescent Athletes Performance By Tessa Koff

This study examined how music affects athletic performance and mental state in adolescent athletes. It reviewed multiple experiments that tested how different types of music, including variations in tempo, volume, and personal preferences, influenced strength, endurance, and psychological factors such as mood, anxiety, and perceived effort. The research focused mainly on adolescent athletes, typically ages 14 to 17, involved in sports such as volleyball, lacrosse, taekwondo, and football, with both male and female participants included. Across the studies, music was used at different times, most commonly during warm-ups, before competition, or during exercise. Some experiments compared no music to preferred or non-preferred music, while others tested loud versus soft music or different tempos. Performance was measured using physical tests such as sprinting, endurance activities, and strength tests, as well as surveys assessing mood and anxiety. The findings showed that music generally improved athletes' mood, reduced anxiety, and lowered perceived effort. Preferred, louder music (around 80 dB) often led to improved strength, endurance, and overall performance. However, music did not always improve short-duration, high-intensity performance. The presence of music alone was sometimes more important than specific features like tempo. The main conclusion is that music is an effective tool for improving both psychological and physical performance in adolescent athletes. Its benefits depend on timing and type, with the strongest effects seen when athletes listen to preferred, louder music before or during activity.

Introduction

Music is a universal element of modern life, enjoyed worldwide as both entertainment and a means of managing psychological states such as stress and anxiety. In the context of physical activity, research has shown that music offers many benefits during exercise, acting as an ergogenic aid and a psychological boost. Studies demonstrate positive changes, including enhanced mood, increased endurance, and decreased perceived exertion (Lane et al., 2011; Ballmann, 2021; Eliakaim, 2007; Saarikallio, 2007). Recognizing these established benefits, music is often encouraged for athletes, both as a warm-up tool before matches and to mitigate performance anxiety. It has been shown that listening to music offers many benefits, and athletes should be encouraged to do so before matches or whenever they experience performance anxiety.

The psychological benefits of music are central to its use in athletic settings. Research consistently indicates that music acts as a mood regulator, often leading to more positive emotional states both during and after physical activity (Soya et al., 2021) For instance, a study on runners found that participants reported more "pleasant" emotions when listening to music, suggesting that the mere presence of music, in general, mattered more than its specific characteristics (Lane et al., 2011). This mood-enhancing effect can be particularly valuable for athletes, as improved emotional states are often linked to reduced stress and increased motivation, which can ultimately contribute to peak performance (Karageorghis, 2016). Music is widely perceived not only as a mood regulator but also as a performance enhancer. Many studies

have shown it can boost physical and mental performance by increasing strength, power, and endurance - especially while playing a sport or exercising (Terry et al. 2020).

The efficacy of music as an ergogenic aid is heavily dependent on its specific characteristics (Ballmann, 2021). Among the most researched factors are volume, tempo, and preferred genre. Preferred music refers to the genre or song choices an individual athlete personally selects. Tempo is the speed or pace of a musical piece, and volume or loudness is the intensity of the music, typically measured in decibels (dB). Studies suggest that the mere presence of music, regardless of its tempo, can be more important for short-duration exercises (Jarraya et al., 2012). However, for longer-duration exercise, higher tempos, louder music (e.g, 80 dB vs. 60 dB), and preferred genres have been shown to be associated with better physiological performance (Ballmann, 2021). In combination, loud preferred music has been shown to reduce ratings of perceived exertion and enhance strength and motor skills (Silva et al., 2021). Thus, understanding and manipulating these specific musical elements is crucial for maximizing performance benefits in athletic settings.

While much research has established the psychological and physiological benefits of music during exercise, the vast majority of these findings are based on studies conducted with adult populations. The applicability of these findings to adolescent athletes, a demographic undergoing rapid and unique developmental changes, is not guaranteed, representing a critical gap in the current literature. Given the increase in adolescent participation in competitive and recreational sports, coupled with growing interest among coaches and health professionals, there is a need to investigate how musical variables influence motor performance and psychological states in young athletes.

This paper systematically reviews current findings to understand how specific musical characteristics—including tempo, preferred genre, and volume—influence motor performance (endurance, strength, power) and psychological state (mood, anxiety, perceived exertion) among adolescent athletes. Based on research in adults, we hypothesize that music positively affects an athlete's performance in a manner that depends on the athlete’s performance anxiety and the music’s tempo, genre, and volume. We expect to observe this effect across a variety of sports, including taekwondo, volleyball, and lacrosse.

Endurance Exercise

The first area of motor performance examined is endurance exercise, which is defined as sustained physical activity over a prolonged period that primarily relies on the aerobic energy system. A study by Eliakim et al. (2007) investigated the effect of music versus no music during a 10-minute warm-up on 24 elite adolescent volleyball players (boys and girls). Their results showed that players who listened to music during warm-up had a significantly higher heart rate than those who did not. Although the music directly affected their heart rates, it did not translate into changes in anaerobic output or fatigue index in either gender. The arousal effect carries dual implications: it can be detrimental for athletes prone to anxiety who prefer low arousal, but beneficial for those who need a higher 'hype' level to enter their optimal performance zone.

Importantly, although music may influence physiological arousal, it may not guarantee a measurable boost in certain types of athletic performance.

Complementing this research, an experiment by De LA Cruz et al. (2021) evaluated the effect of music on student-athletes to determine whether it reduced their stress and anxiety. The study was performed on female high school lacrosse players. The studies showed that music can greatly impact their amount of anxiety and shift them towards peak performance. Furthermore, participants' coaches reported tangible benefits, noting improvements in team cohesiveness and reductions in overall stress and anxiety levels before matches. This evidence strongly suggests that incorporating music during warm-ups and before high-stakes games can be a highly effective psychological tool for coaches to help adolescent athletes control their stress and perform optimally.

Anaerobic /Short-Duration Exercise

The findings on endurance and psychological state, however, underscore the need to further explore how alternative exercise types and musical characteristics, such as tempo, volume, and genre, mediate performance effects, particularly in high-intensity, short-duration exercise. Some of these relationships are revealed in a study by Becker et al. (1994), which investigated the effect of varying tempo on three randomized two-minute exercises. The study involved 60 participants, spanning a wide age range from children to seniors. Participants were exposed to either white noise, frenetic noise, or mellow music. The findings indicated that the distance covered (mileage) was significantly greater in both music conditions than in the white-noise control trial, except for the senior cohort. Furthermore, no significant differences in performance were observed between the frenetic and mellow music conditions. This implies that for short-duration exercises like those tested, the presence of music is more important than its specific tempo. The ergogenic effect of music did not apply to the senior cohort, suggesting that its benefits for athletic performance may diminish with age.

In addition to these findings, a study by Ibrahim Ouergu et al. (2023) examined the effects of preferred music on adolescent athletes. To execute this experiment, they took 20 taekwondo athletes, 10 female and 10 male. On each lab visit, the participants either listened to no music, preferred music (soft), preferred music (loud), non-preferred music (soft), or non-preferred music (loud). Preferred music loud (PML) had the greatest effect on athletes' performance. The article found that PML had ergogenic benefits during physical taekwondo tasks. When volume was controlled, performance outcomes improved when participants listened to loud music (80 dB) compared to soft music (60 dB), regardless of music preference. Knowing the positive effect PML has on players' performance should encourage coaches to identify which types of music athletes prefer during warm-ups and before big games. Regardless, loud music should be played instead of soft music.

In a study evaluating the impact of music on a 10-minute anaerobic warm-up, 24 volleyball players completed the Wingate Anaerobic Test while listening to music and without music (Eliakim et al., 2007). Player heart rate and peak anaerobic power were measured. Music

had no significant effect on anaerobic output. Therefore, while music may be useful in alternative settings, it may not be helpful to listen while performing high-intensity short-duration physical activity.

In an experiment by Jebabli et al. (2023), 20 male young adults (mean age 20 y/o) were randomly assigned to an intervention group that listened to preferred music, a nonpreferred music group, or a control group that listened to no music. The effects of music genre on strength performance, as measured by a handgrip dynamometer was evaluated. Statistical analyses indicated that when participants listened to their individually preferred music genre, maximal strength and strength endurance improved; the rating of perceived exertion, or participants' perception of effort during exercise, was lowest in the preferred genre condition as well.

The results imply that during strength-based sports, players should listen to their preferred music genre before a match. This outcome suggests that incorporating preferred music can significantly benefit performance in strength-based sports by not only enhancing maximal strength and endurance but also by lowering the rating of perceived exertion (RPE), making the task feel easier for the athlete. Although the participants in this experiment were young adults, the findings provide a basis for inferring similar effects in adolescent athletes. This extrapolation is supported by research indicating that, despite differences in cognitive maturity, younger populations respond to the emotional and motivational aspects of music in ways comparable to adults, often experiencing a similar boost in enjoyment and engagement with the physical activity.

Cognitive Benefits of music during sport performance

While much of the focus has been on music's influence on physiological performance and the rating of perceived exertion in endurance and anaerobic exercise, it is equally important to examine its impact on the athlete’s mental state. The paper’s focus now shifts to the psychological realm, exploring the affective and cognitive benefits that music provides to adolescent athletes during sport performance. The cognitive benefits of music are an important factor in enhancing sports performance. Research by McGuckian and Pepping (2021) used an experiment in which 12 elite youth football players participated in four training sessions, listening to either positive or negative music before training. During each session, athletes completed a series of small-sided games, and their cognitive-affective states were measured multiple times using the Affect Grid. The study found that music effectively influenced athletes’ mental states, with positive music helping maintain higher levels of focus and engagement throughout training. This improved cognitive state can enhance decision-making, attention, and consistency during performance, as athletes are better able to concentrate on tasks and ignore distractions. As a result, music not only affects mood but also strengthens key cognitive processes that contribute to improved athletic performance (McGuckian & Pepping, 2021).

The cognitive benefits of music are an important factor in enhancing sports performance, particularly in adolescents. Researchers conducted an experiment in which adolescent participants took part in Body Combat exercise sessions under two conditions: with music and

without music. Before and after each session, researchers looked at participants' mental states using standardized mood and attention measures. The results showed that exercising with music significantly improved participants' state of mind, including greater focus, alertness, and mental engagement. These cognitive improvements are important because increased attention and reduced mental fatigue allow athletes to perform movements more accurately and stay consistent throughout activity. (Kommers et al., 2019).

The research from these two studies supports the idea that music has not only physiological but also psychological benefits. Both experiments also found that the positive influence of music on an athlete's mental state is robust across different contexts, demonstrating its utility as a holistic ergogenic aid. However, the findings also underscore key limitations in the current literature on adolescent athletes, warranting further systematic investigation into future directions.

Future Directions and Limitations

The current review synthesizes key findings on the influence of music on adolescent athletes, highlights critical limitations in the existing literature, and suggests future avenues for research. Most of the research in this topic was on adult athletes, so it was a bit harder to find information on adolescents. Though most of the results showed improvement, the different types of exercise performed may affect the results. By looking at different types of physical exertion, such as power, strength, and endurance, we were able to see subtle differences between the three.

The heterogeneity of exercise types examined, including endurance, strength, and short-duration anaerobic tasks, suggests that the effects of music variables are not universally consistent across all sports or intensities. This review observed subtle differences in outcomes based on the mode and intensity of physical exertion, which limits the ability to generalize the benefits of music to all athletic contexts without further focused, context-specific investigation.

Moving forward, research should strategically address the identified limitations to provide more nuanced guidance for adolescent athletes. First, longitudinal studies are necessary to track the impact of music exposure over the course of an athletic season or across multiple years. Such designs would clarify music's potential influence not only on immediate motor performance but also on critical aspects such as motor learning, skill development, and sustained motivation over time.

Second, future research should investigate the optimal timing of music exposure. Specifically, studies should compare the effectiveness of listening to music during warm-ups, immediately before competition, during the competition itself (if applicable), and crucially, during the post-exercise recovery phase. Understanding when music provides the greatest psychological and physiological benefits will enable athletes to strategically incorporate it to optimize focus, motivation, and performance.

Third, to establish a clearer mechanism of action, future studies must concurrently explore both the physiological and psychological responses to music. Researchers should examine how music characteristics modulate measurable factors such as heart rate variability,

changes in arousal levels, and muscle activation patterns. Simultaneously, psychological factors such as mood and perceived effort should be explored. By integrating these objective and subjective data points, future research can provide clearer, evidence-based guidance on how athletes can use music strategically to enhance performance.

Conclusion 1 paragraph

This study aimed to examine how music influences athletic performance and mental state in adolescent athletes, and the findings support the hypothesis that music is an effective intervention. Overall, music positively affects an athlete's performance by providing both psychological and physiological benefits. Across the research, key findings showed that music improves psychological and cognitive factors by decreasing perceived effort, increasing focus, and enhancing mental engagement during activity. In terms of physical performance, music had mixed effects depending on the type of exercise, but it was most beneficial for endurance and strength-based activities. The characteristics of music also played an important role, with loud, preferred music producing the greatest improvements in performance. While tempo had some influence, the presence of music itself was often more important, especially in short-duration activities. These findings suggest that both the type of music and its use can affect results. Future research should focus on identifying the optimal timing of music use across different stages of athletic performance. Overall, athletes and coaches should incorporate loud, preferred music into warm-ups and pre-performance routines to improve focus, reduce anxiety, and enhance overall performance.

Works Cited

Abernethy, P., Wilson, G., & Logan, P. (1995). Strength and power assessment: issues, controversies and challenges. Sports medicine, 19(6), 401-417.

Eliakim, M., Meckel, Y., Nemet, D., & Eliakim, A. (2007). The effect of music during warm-up on consecutive anaerobic performance in elite adolescent volleyball players. International journal of sports medicine, 28(04), 321-325.

Jebabli, N., Khlifi, M., Ouerghi, N., Boujabli, M., Bouassida, A., Abderrahman, A. B., & van den Tillaar, R. (2023). Single and combined effects of preferred music and endpoint knowledge on jump performance in basketball players. Sports, 11(5), 105.

Kommers, M. J., Silva, R. A., Gomes, G., Zavala, A. A., Rezende, W. C., & Fett, C. A. (2019). Body Combat classic music improves adolescent's state of mind. Journal of Physical Education, 30(1).

De La Cruz, N. (2021). Evaluating the Impact Music May Have on High School Student-Athletes (Doctoral dissertation, University of Arizona Global Campus).

Ouergui, I., Jebabli, E., Delleli, S., Messaoudi, H., Bridge, C. A., Chtourou, H., ... & Ardigò, L. P. (2023). Listening to preferred and loud music enhances taekwondo physical performances in adolescent athletes. Perceptual and Motor Skills, 130(4), 1644-1662.

Jarraya, M., Chtourou, H., Aloui, A., Hammouda, O., Chamari, K., Chaouachi, A., & Souissi, N. (2012). The effects of music on high-intensity short-term exercise in well trained athletes. Asian journal of sports medicine, 3(4), 233.

Ballmann, C. G. (2021). The influence of music preference on exercise responses and performance: a review. Journal of Functional Morphology and Kinesiology, 6(2), 33.

Silva, N. R. D. S., Rizardi, F. G., Fujita, R. A., Villalba, M. M., & Gomes, M. M. (2021). Preferred music genre benefits during strength tests: increased maximal strength and strength-endurance and reduced perceived exertion. Perceptual and motor skills, 128(1), 324-337.

Lane, A. M., Davis, P. A., & Devonport, T. J. (2011). Effects of music interventions on emotional States and running performance. Journal of sports science & medicine, 10(2), 400–407.

Karageorghis, C. I. (2016). Applying music in exercise and sport. Human Kinetics.

Terry, P. C., Karageorghis, C. I., Curran, M. L., Martin, O. V., & Parsons-Smith, R. L. (2020). Effects of music in exercise and sport: A meta-analytic review. Psychological bulletin, 146(2), 91.

Suwabe, K., Hyodo, K., Fukuie, T., Ochi, G., Inagaki, K., Sakairi, Y., & Soya, H. (2021). Positive mood while exercising influences beneficial effects of exercise with music on prefrontal executive function: A functional NIRS study. Neuroscience, 454, 61-71.

Saarikallio, S. (2007). Music as mood regulation in adolescence (No. 67). University of Jyväskylä.

Ouergui, I., Jebabli, E., Delleli, S., Messaoudi, H., Bridge, C. A., Chtourou, H., Franchini, E., Ballmann, C. G., & Ardigò, L. P. (2023). Listening to Preferred and Loud Music Enhances Taekwondo Physical Performances in Adolescent Athletes. Perceptual and Motor Skills, 130(4), 1644-1662. https://doi.org/10.1177/00315125231178067 (Original work published 2023)

Bystander Effect and Toxicity Control in Duocarmycin-Based Antibody-Drug Conjugates: A Multidimensional Review of Efficacy, Linker Kinetics, and Clinical Safety

By Joonsuh Park[1]

Abstract

Antibody–drug conjugates (ADCs) couple tumor-targeting antibodies to potent cytotoxic payloads. Duocarmycin derivatives can kill antigen-positive cells and, after local release, diffuse into neighboring antigen-negative cells. The same permeability poses a safety challenge if exposure occurs in normal tissues. This narrative review separates antibody delivery, site of linker cleavage, and post-release diffusion; evaluates preclinical bystander evidence and clinical safety findings for duocarmycin ADCs; and discusses strategies to retain activity against heterogeneous tumors while controlling normal-tissue exposure. Clinical comparisons suggest that ADC conjugation may lower the minimum effective dose without reliably increasing the maximum tolerated dose over related free drugs. Dual-payload and dual-target concepts remain investigational approaches requiring direct pharmacokinetic and safety testing (Colombo and Rich; Gerber et al.; Yamazaki et al.).

Introduction

An antibody–drug conjugate (ADC) contains a targeting monoclonal antibody, a linker, and a cytotoxic payload. The antibody's Fab region recognizes a tumor-associated surface antigen; after target binding, the conjugate can enter the cell and release payload during intracellular processing (Giugliano et al.; Kovtun et al.). Fc-mediated interactions have other roles, including FcRn-dependent antibody recycling, and should not be represented as tumor-antigen recognition. Conjugation near Fc-receptor interaction surfaces may change pharmacokinetics, depending on the attachment site and linker–payload chemistry (Lhospice et al.; Gerber et al.). Figure 1 distinguishes these functions.

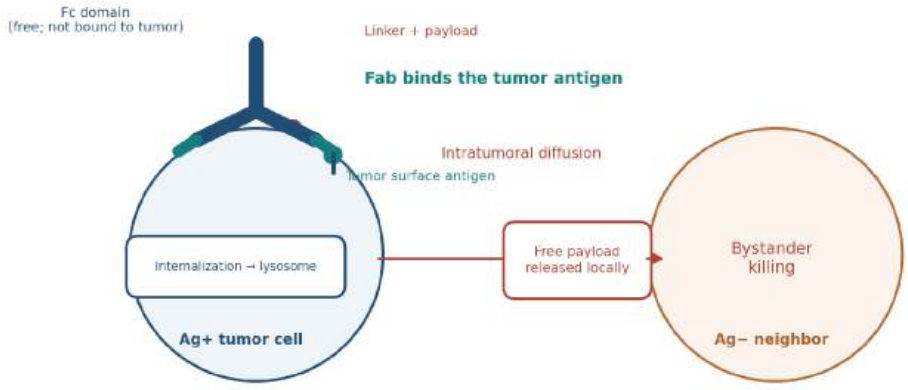

Figure 1. Fab-mediated tumor-antigen binding, intracellular release, and local bystander diffusion. The Fc domain does not bind the depicted tumor antigen.

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Target antigen expression varies within many tumors. After binding and internalization by an antigen-positive (Ag+) cell, a cleaved, membrane-permeable payload can diffuse locally and injure adjacent antigen-negative (Ag−) cells. This bystander activity may reduce an antigen-heterogeneity barrier, but its extent depends on local release, payload properties, and tissue geometry (Kovtun et al.; Staudacher and Brown).

A membrane-permeable payload can also injure healthy tissue if released into plasma or normal tissue. This is a question about the location and amount of exposure, not a necessary consequence of bystander killing within a tumor. The review therefore considers tumor-localized activity and unintended systemic exposure as related but separable processes (Jain et al.; Nguyen et al.).

Duocarmycin derivatives are particularly informative payloads because they alkylate DNA after activation and can show very high potency in experimental models (Elgersma et al.; Morcos et al.). Their use as unconjugated anticancer agents has been limited by tolerability; incorporation into an ADC expands the kinds of small molecules that can be investigated as candidate therapeutics, while creating demanding requirements for stable delivery and selective release.

Trastuzumab duocarmazine (SYD985), which couples an HER2-directed antibody to a duocarmycin-derived prodrug through a cleavable linker, provides an informative case. Preclinical studies support bystander activity, while clinical trials report both antitumor responses and substantial ocular adverse events (Elgersma et al.; Banerji et al.; Turner et al.). Table 1 summarizes the design variables considered in this review.

Table 1. Determinants of bystander activity and normal-tissue exposure in duocarmycin-based ADCs

Dimension	Specific Contributing Factors	Direct Impact on ADC profile	Citations
Payload Chemistry	High Lipophilicity (LogP) Ultra-Potency (Picomolar IC_{50}) Minor Groove Binding / Alkylation	Enhances cell membrane diffusion (Bystander +) Elevates risk of systemic off - target cytotoxicity	(Giugliano et al.;Morcos et al.;Elgersma et al.)
Linker Dynamics	Cleavable (Cathepsin B / pH) Plasma Stability kinetics Drug–to-Antibody Ratio (DAR)	Enable payload release Premature systemic cleavage cause organ toxicities	(Giugliano et al.;Elgersma et al.;Bargh et al.)

This review asks how antigen-directed delivery, release chemistry, and post-release diffusion together determine efficacy and tolerability, with attention to what existing evidence can and cannot establish.

Research Questions

The primary question is addressed through four linked subquestions. Sections 3–6 follow the same sequence: mechanism; separation of local benefit from unintended exposure; clinical case study; and design strategies.

Primary Research Question

How do linker design and duocarmycin payload properties shape the balance between intratumoral bystander killing and systemic toxicity in duocarmycin-based antibody-drug conjugates?

Subquestions

1. 1. Which steps of target binding, intracellular release, and payload diffusion enable bystander killing in antigen-heterogeneous tumors? (Section 3)
2. 2. How can tumor-localized release be distinguished from premature plasma cleavage or normal-tissue uptake, and how do these routes affect efficacy and toxicity? (Section 4)
3. 3. What do preclinical and clinical findings for trastuzumab duocarmazine reveal about bystander activity, response, and adverse events? (Section 5)
4. 4. Which linker, payload, conjugation, and targeting strategies might improve the balance of efficacy and tolerability, and what remains untested? (Section 6)

Section 2: Review Methodology

2.1 Study Design and Research Question

This narrative review synthesizes mechanistic studies, preclinical experiments, and clinical reports to address the four subquestions stated above. Because models, ADC constructs, endpoints, and populations differ, the evidence is discussed qualitatively rather than pooled in a meta-analysis (Green et al.).

2.2 Data Sources and Search Strategy

For the studies of this review paper, PubMed and Google Scholar are used. English-language, peer-reviewed publications from 2006 to 2026 is the primary focus of the search. “Antibody-drug conjugate,” “duocarmycin ADC,” “trastuzumab duocarmazine,” “SYD985,” “bystander effect,” “linker stability,” “payload permeability,” “systemic toxicity,” “ocular toxicity,” and “HER2-low breast cancer” are search terms that used for studies used for this paper by using the terms both individually and in combinations. For the review papers for the references, they are also examined to identify primary research. Mechanistic studies, preclinical investigations, and clinical trials directly related to duocarmycin-based ADCs are the priority to studies.

2.3 Literature Selection Criteria

The mechanism of action of duocarmycin payloads, ADC linker cleavage and stability, membrane permeability and bystander killing, the preclinical or clinical performance of trastuzumab duocarmazine, or strategies for improving the therapeutic window of ADCs are important subjects included in the studies used for this paper. For the studies involving other payloads, they were included as a reference if they included relevant principles concerning linker design, payload release, drug-to-antibody ratio, or systemic toxicity.

Sources without directly addressing ADC mechanisms, efficacy, safety, or design; focusing on unrelated anticancer agents; or not proving sufficient scientific information that is related to review were excluded from the sources. Although principal experimental and clinical studies are highlighted if studies include specific mechanisms, efficacy outcomes, and adverse events, this article is organized to explain general concepts.

2.4 Evidence Organization and Analysis

Evidence is organized in the order of the subquestions: Section 3 establishes how antigen-directed delivery and payload properties produce bystander killing; Section 4 evaluates local benefit against premature cleavage, target-independent uptake, and normal-tissue exposure; Section 5 examines duocarmycin ADC case studies; and Section 6 assesses proposed design approaches. Clinical response and toxicity estimates are not treated as direct comparisons between unrelated ADCs.

Because a qualitative synthesis of available evidence needs to be used for comparisons rather than direct head-to-head evaluations of individual ADCs, a meta analysis cannot be conducted in the paper because it included substantially different studies in the study's experiment model.

Section 3: Antigen-Directed Delivery and Mechanisms of Bystander Killing

3.1 Structural Components of ADCs: Monoclonal Antibody, Linker, and Payload

ADC activity depends on coordinated antibody recognition, linker behavior, and payload release (Giugliano et al.). The Fab domains recognize the tumor antigen, whereas the Fc region interacts with Fc receptors, including FcRn, which can support IgG recycling. Conjugation chemistry and attachment position should preserve target recognition and be evaluated for effects on FcRn binding and clearance (Lhospice et al.; Gerber et al.). Stronger antigen expression in tumors than normal tissues can favor selectivity, but expression within a tumor is often heterogeneous (Kovtun et al.).

The linker (non-cleavable and cleavable) acts as the critical bridge connecting the mAb to the cytotoxic payload (Staudacher and Brown;Giugliano et al.;Elgersma et al.). Non-cleavable linkers (e.g., thioether-based SMCC used in Trastuzumab emtansine / T-DM1) rely on complete lysosomal degradation of the antibody backbone, releasing a charged amino acid-payload adduct (lysine-SMCC-payload) (Staudacher and Brown;Lhospice et al.). Because non-cleavable linkers cannot passively cross cell membranes, confining toxicity strictly to target-positive cells, most of

the times, this charged metabolite lacks a bystander effect (Staudacher and Brown;Lhospice et al.). On the other hand, to release fully uncharged, active free payloads—lysosomal proteases (e.g., valine-citrulline or valine-alanine motifs cleaved by cathepsin B), acidic lysosomal pH (hydrazone linkers), or elevated intracellular glutathione (disulfide linkers)—cleavable linkers needs to exploit internal physiological triggers (Giugliano et al.;Elgersma et al.;Bargh et al.). Table 2 compares the main linker classes by release trigger, released species, and bystander potential.

Table 2: Cleavable and Non-Cleavable Linkers: Release Trigger, Released Species, and Bystander Potential

Linker Type	Release Trigger	Release Species	Bystander Potential	Citations
Non-cleavable (thioether, e.g., SMCC in T-DM1)	Complete lysosomal degradation of the antibody	Charged amino acid–payload adduct (lysine-SMCC-payl oad)	Lacks a bystander effect: the charged metabolite cannot passively cross membranes, so toxicity stays in target-positive cells	(Staudacher and Brown; Lhospice et al.)
Cleavable, protease-sensitive(v aline-citrulline or valine-alanine, e.g., SYD985)	Lysosomal proteases, mainly cathepsin B	Uncharged, active free payload	Possible, if the released payload is uncharged and membrane-permeabl e (see 3.3)	(Giugliano et al.; Elgersma et al.; Bargh et al.)
Cleavable, acid-sensitive (hydrazone)	Acidic lysosomal pH	Uncharged, active free payload	Possible, if the released payload is uncharged and membrane-permeabl e (see 3.3)	(Giugliano et al.; Elgersma et al.; Bargh et al.)
Cleavable, reducible (disulfide)	Elevated intracellular glutathione	Uncharged, active free payload	Possible, if the released payload is uncharged and membrane-permeabl e (see 3.3)	(Giugliano et al.; Elgersma et al.; Bargh et al.)

3.2 Stepwise Cascade of Bystander Killing: Internalization, Cleavage, and Passive Membrane Diffusion

The bystander killing process operates through a multi-step biophysical cascade (Giugliano et al.;Kovtun et al.). Firstly, it started by the high-affinity binding of the ADC to the extracellular domain of the target antigen on Ag^{+} tumor cells (Giugliano et al.). Secondly, clathrin-mediated or caveolae-dependent endocytosis internalizes the ADC-receptor complex into early endosomes. Later, it matured into acidic lysosomal compartments (pH ~4.5–5.0)

(Giugliano et al.;Chari et al.).

Specific enzymatic cleavage occurs within the lysosome (Giugliano et al.;Elgersma et al.). Lysosomal cathepsin B cleaves the amide bond adjacent to the self-immolative spacer (e.g., para-aminobenzyl alcohol, PABC) for dipeptide-based cleavable linkers (e.g., valine-citrulline) (Elgersma et al.;Bargh et al.). The spontaneous 1,6-elimination of the PABC group releases the uncharged, lipophilic payload molecule (Elgersma et al.). When it gets released in the cytoplasm, the free payload exerts intracellular toxicity while a fraction passively diffuses across the lipid bilayer into the extracellular microenvironment along a concentration gradient (Giugliano et al.;Kovtun et al.). This extracellular payload then penetrates adjacent Ag^- tumor cells, vascular endothelial cells, and stromal fibroblasts, executing secondary cell death independent of target antigen expression (Staudacher and Brown;Kovtun et al.).

3.3 Biophysical Determinants: Payload Lipophilicity (LogP) and Charge State

The magnitude of the bystander effect is fundamentally affected by the physicochemical properties of the freed payload: mostly by its octanol-water partition coefficient (expressed as LogP) and molecular charge state (Giugliano et al.;Lhospice et al.). Rapid passive trans-membrane diffusion facilitated by payloads possessing high lipophilicity (positive LogP values) easily solubilize into hydrophobic cell membranes: for instance, monomethyl auristatin E (MMAE, LogP ~ 3.0) exhibits significant neutral membrane permeability and drives robust bystander activity. However, monomethyl auristatin F (MMAF, containing a charged C-terminal phenylalanine residue) carries a negative charge at physiological pH, restricting membrane transit and eliminating bystander activity (Giugliano et al.;Lhospice et al.;Doronina et al.). Characteristics across many ADC platforms, associated prodrugs, enable rapid tissue penetration and cellular uptake by exhibiting exceptionally high lipophilicity and compact planar molecular geometries (Morcos et al.;Elgersma et al.). However, excessive lipophilicity has both positive and negative sides: highly hydrophobic payloads that are prone to aggregation induce rapid clearance by the reticuloendothelial system (RES), hepatotoxicity by increasing hepatic uptake, and premature systemic efflux into normal tissues (Giugliano et al.;Elgersma et al.). Due to these reasons, tuning payload LogP via hydrophilic linker modifications (e.g., polyethylene glycol / PEG spacers or glucuronide moieties) is essential for preserving localized bystander killing while minimizing systemic toxicity (Elgersma et al.;Bargh et al.).

Recent study indicates that prediction of membrane permeability cannot be solely dependent on lipophilicity (Cruz et al.). Although electrically neutral payloads crossed the membrane more easily, molecular dynamics simulations of several ADC payloads indicated that during cross of hydrophobics core of the cell membrane, ionized payloads encountered higher energy barriers (Cruz et al.). Also, the size and the structural complexity of the remaining linker fragment are influential to the ability of a released payload to enter a neighboring cell after leaving the membrane (Cruz et al.). Hence, depending solely on hydrophobicity was not a reliable predictor of bystander activity (Cruz et al.). Even though this study did not directly mention duocarmycin payloads, this study suggests that bystander potential of

duocarmycin-based ADCs is mixture of combined function of lipophilicity, charge state, molecular size, and the structure of the released linker-payload product (Cruz et al.).

Section 4: Tumor-Localized Benefit and Unintended Normal-Tissue Exposure

4.1 Therapeutic Advantages: Eradication of Antigen-Negative Tumor Subpopulations and Stroma Destruction

Because antigen expression is rarely uniform in primary solid tumors and metastatic lesions, subpopulations of antigen-negative (Ag−) or low-expressing tumor cells that escape target-dependent killing can drive clonal expansion and treatment relapse (Giugliano et al.; Kovtun et al.). Payloads capable of passive diffusion following intracellular cleavage allow ADCs to act as localized chemotherapy depots, eradicating surrounding non-targeted tumor cells independent of their target antigen density (Staudacher and Brown; Kovtun et al.).
Direct evidence for duocarmycin-mediated bystander killing comes from Brignole et al., who tested vobramitamab duocarmazine — a B7-H3-targeting ADC carrying a cleavable valine-citrulline linker and a duocarmycin-hydroxybenzamide azaindole (vc-seco-DUBA) payload — in neuroblastoma models. NX-S2, a murine neuroblastoma line that does not express human B7-H3, was resistant to the ADC alone but was killed when co-cultured with human B7-H3-expressing cells (Brignole et al.). This supports a localized mechanism in which payload released after processing by antigen-positive cells reaches neighboring antigen-negative cells. Because the demonstration depends on a cross-species co-culture and the study used only cell and mouse models, it does not establish the extent or safety of the bystander effect in patients.

The stromal compartment of the tumor microenvironment (TME) is also a site of ADC payload activity, although the evidence is indirect. Szot et al. conjugated MMAE to an antibody against tumor endothelial marker 8 (TEM8/ANTXR1), a receptor broadly overexpressed on cancer-associated fibroblasts, endothelium, and pericytes. Following capture of the ADC from the circulation, tumor-associated stromal cells released active free MMAE, killing nearby proliferating tumor cells in a target-independent manner (Szot et al.). This establishes the stroma as a possible depot for payload release, but it demonstrates payload movement from stroma to tumor rather than the reverse. Whether payload released inside antigen-positive tumor cells reaches CAFs, endothelial cells, or myeloid populations at cytotoxic concentrations has not been directly demonstrated for duocarmycin-based ADCs. This stromal extension of the bystander effect is therefore substantially less established than direct killing of adjacent antigen-negative tumor cells (Kovtun et al.; Staudacher and Brown).

4.2 Safety Risks: Mechanisms of Premature Systemic Payload Leakage and Organ-Specific DLTs

Local release and unwanted normal-tissue exposure should be analyzed separately. Premature deconjugation in plasma is one possible route to systemic free payload; target-independent uptake of the intact ADC and binding to antigen expressed in normal tissue

are others. A permeable payload amplifies the consequences of exposure, but permeability alone does not establish where the release occurred (Jain et al.; Zhao et al.; Nguyen et al.). Reported adverse effects of ADCs can include ocular injury, pneumonitis or interstitial lung disease, myelosuppression, and hepatic toxicity, but their incidence and mechanism vary by conjugate and target (Tarantino et al.; Turner et al.). In preclinical studies Zhao et al. implicated target-independent macropinocytosis of intact ADC in corneal epithelial exposure; free-payload diffusion is another proposed mechanism, not a proven explanation for all ocular events. Systemic exposure can affect marrow progenitors, while hydrophobicity and tissue uptake may influence hepatic toxicity (Zhao et al.; Mahalingaiah et al.). Table 3 summarizes mechanisms as proposed rather than established for every duocarmycin ADC.

Table 3: Organ-Specific Dose-Limiting Toxicities of ADCs: Clinical Manifestations and Proposed Mechanisms

Organ / System	Clinical manifestation	Proposed mechanism	Citations
Ocular / Cornea	Corneal Epitheliopathy, Keratitis, Dry Eye	Target-independent uptake of the intact ADC by corneal epithelial cells (macropinocytosis); passive diffusion of free lipophilic payload is a proposed additional contributor	(Zhao et al.; Jaffry et al.; Eaton et al.)
Pulmonary / Lung	Interstitial Lung Disease (ILD), Pneumonitis	Associated with ADC or payload exposure in lung tissue	(Giugliano et al.; Tarantino et al.; Turner et al.)
Hematologic / Bone Marrow	Myelosuppression (neutropenia, thrombocytopenia, anemia)	Systemic exposure of rapidly dividing bone marrow progenitor cells to a picomolar DNA-alkylating payload	(Nguyen et al.;Morcos et al.;Elgersma et al.)
Hepatic / Liver	Hepatotoxicity (raised liver enzymes)	Rapid clearance of hydrophobic ADCs by the reticuloendothelial system and non-specific uptake in the liver	(Giugliano et al.;Mahalingaiah et al.;Nguyen et al.)

Figure 2 contrasts intended tumor-localized linker processing with unintended systemic routes. In the local pathway, intact ADC reaches Ag+ tumor cells and releases a payload that can diffuse to nearby Ag− cells. Premature cleavage in circulation, nonspecific intact-ADC uptake, and normal-tissue antigen binding can produce exposure outside the tumor. These mechanisms are not interchangeable: measurements of intact ADC, released payload, tissue distribution, and toxicity are needed to distinguish them.

Jain et al. compared EGFR-directed ADCs with payloads differing in membrane permeability in glioblastoma models. Their results link payload properties and linker–payload stability to differing bystander and tissue-exposure profiles. This comparison supports evaluating the release site alongside permeability; it does not show that all membrane-permeable payloads necessarily produce systemic toxicity (Jain et al.).

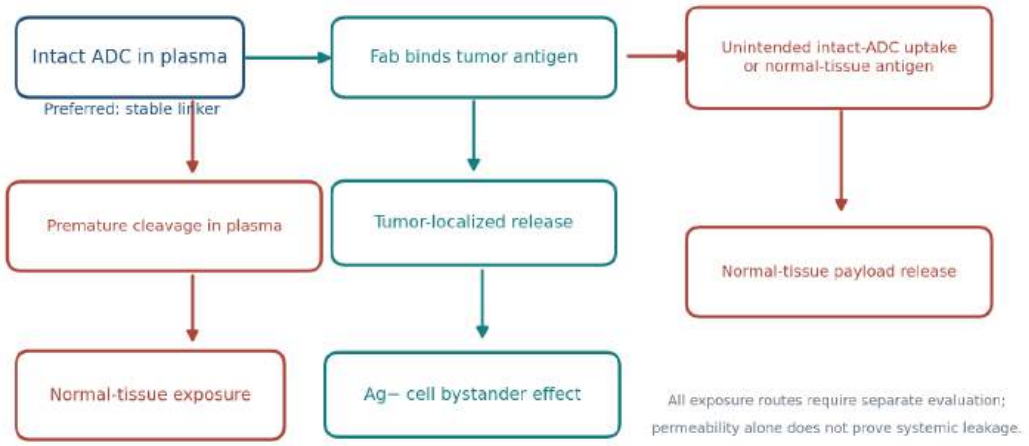

Figure 2. Stable circulation, tumor-localized release, and alternative normal-tissue exposure routes.

4.3 Minimum Effective Dose, Maximum Tolerated Dose, and the Therapeutic Window

For a defined endpoint, model or patient population, the minimum effective dose (MED) is the lowest dose meeting a prespecified antitumor-effect criterion; the maximum tolerated dose (MTD) is the upper dose constrained by unacceptable toxicity. The therapeutic window is often described by the relation of MTD to MED, with both values dependent on regimen and measurement context. Antigen-directed conjugation might lower MED by increasing useful tumor delivery and might raise MTD by reducing normal-tissue exposure. Retrospective clinical comparisons with related free small molecules, however, challenge a general claim of increased MTD for marketed ADCs; gains appear more consistently associated with efficacy at lower doses. Such comparisons are imperfect because free payloads may be chemically distinct, dosing schedules differ, and a free molecule may never have been clinically developed (Colombo and Rich; Gerber et al.).

A bystander-capable payload can increase activity in a heterogeneous tumor if adequate concentrations are released locally. It cannot be described as “broadening the efficacy curve” without defined axes, response threshold, and data; curve width can reflect experimental variability. The relevant design question is whether tumor-localized activity can be obtained at a dose below the normal-tissue toxicity limit. Linker stability in plasma, site and rate of cleavage, FcRn-compatible conjugation, tissue uptake, and payload permeability should therefore be assessed together (Elgersma et al.; Gerber et al.; Jain et al.).

Section 5: Deep-Dive Case Study: Ultra-Potent Payload 'Duocarmycin'

5.1 Mechanism of Action: DNA Minor Groove Binding and N3-Adenine Alkylation

Duocarmycins are a class of natural and synthetic cytotoxic alkaloids that exert their antitumor effects through a distinct sequence-selective DNA alkylation mechanism (Morcos et al.;Elgersma et al.). Duocarmycins bind specifically to the minor groove of double-stranded DNA unlike tubulin-targeting payloads (such as auristatins and maytansinoids); after binding, the structural architecture of the payload undergoes a spirocyclization reaction, positioning the reactive cyclopropane ring in close proximity to the N3 position of adenine bases (Morcos et al.;Elgersma et al.).

The structural integrity of the double helix and stalling replication forks disrupted when this N3-adenine alkylation forms a covalent DNA adduct during the S-phase of the cell cycle (Morcos et al.;Elgersma et al.). (see fig. 3)

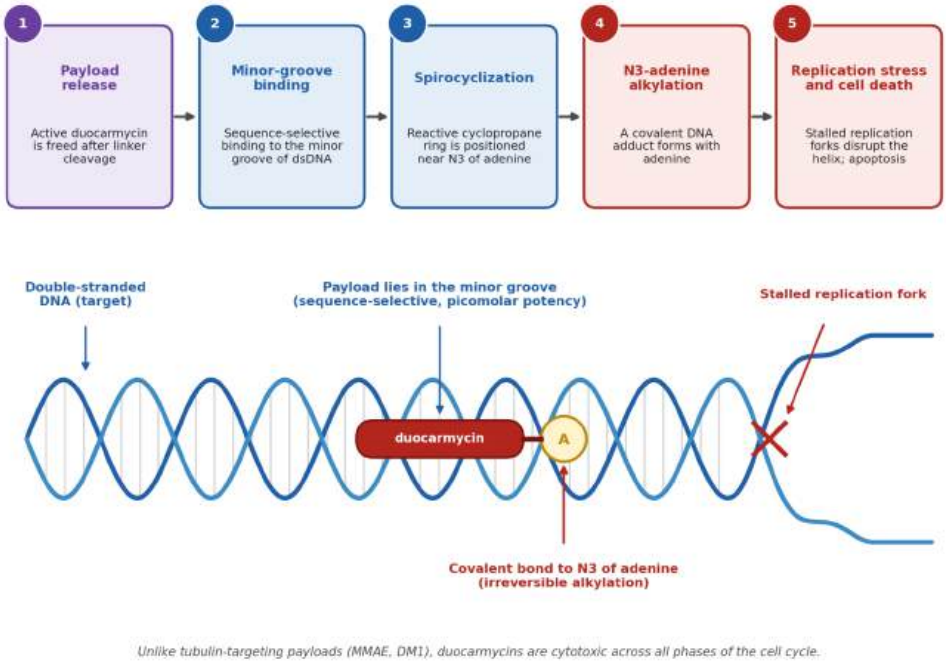

Figure 3. Duocarmycin-mediated DNA minor-groove binding and adenine alkylation.

5.2 Ultra-Potent Toxin Characteristics: Picomolar Potency and the Imperative of Bystander Control

The extreme cytotoxic potency of duocarmycin derivatives—exhibiting half-maximal inhibitory concentration values in the picomolar range—allow rendering them exceptionally effective against refractory malignancies (Morcos et al.;Elgersma et al.). However, this high potency narrows the therapeutic window considerably (Morcos et al.;Chari et al.). The passive diffusion of uncharged, highly lipophilic duocarmycin species across cellular membranes can cause catastrophic off-target tissue damage if systemic cleavage occurs when delivered via a

cleavable linker to facilitate a bystander effect. (Giugliano et al.;Morcos et al.;Elgersma et al.). Consequently, precise control over linker kinetics and spatial payload release is paramount (Elgersma et al.;Bargh et al.). Uncontrolled systemic clearance or premature enzymatic degradation in human plasma lead to severe bone marrow suppression and microvascular toxicity by transforming an ultra-potent therapeutic into a systemic toxin (Giugliano et al.;Morcos et al.;Nguyen et al.).

5.3 Trastuzumab Duocarmazine (SYD985) Analysis: Efficacy in HER2-Low/Heterogeneous Tumors and Toxicity Management

Trastuzumab duocarmazine (SYD985) links an anti-HER2 antibody to a cleavable linker–seco-DUBA prodrug. The HER2-binding Fab directs antigen recognition, while subsequent cellular processing and prodrug activation generate the DNA-alkylating species (Elgersma et al.). Figure 4 depicts the components only, avoiding repetition of the general release cascade.

Early clinical findings show that trastuzumab duocarmazine can be responsive toward both HER2-positive and HER2-low breast cancer. In the phase that studied in this article, objective responses can be found in 33% of assessable patients with HER2-positive breast cancer, 28% of patients with hormone receptor-positive HER2-low breast cancer, and 40% of patients with hormone receptor-negative HER2-low breast cancer. But, at least one ocular adverse event can be found by 71% of patients in the dose-expansion phase. This result shows that ocular toxicity was already a major safety concern during early clinical development (Banerji et al.).

Even though, result shows trastuzumab duocarmazine faces tolerability limitation, it has clinically meaningful antitumor activity. Nevertheless, clinical studies show that systemic exposure, tissue-specific sensitivity, target expression, and other ADC-related mechanisms may also contribute to ocular toxicity with the bystander effect.

Figure 4. Components of trastuzumab duocarmazine (SYD985): anti-HER2 antibody, cleavable linker, and duocarmycin-derived prodrug payload.

In clinical trials, SYD985 demonstrated robust antitumor activity in HER2-low (IHC 1+

or IHC 2+/FISH-) and heterogeneous breast and gynecological cancers supporting the potential clinical value of bystander activity in HER2-low and heterogeneous tumors. (Giugliano et al.;Elgersma et al.). However, clinical trials required intensive toxicity monitoring and management of organ-specific dose-limiting toxicities (DLTs), specifically ocular toxicities (conjunctivitis, keratitis) and interstitial lung disease (ILD) (Elgersma et al.;Tarantino et al.; Dy et al.; Turner et al.;Eaton et al.).

5.4 Evidence Beyond SYD985: Other Duocarmycin-Based ADCs

Potential and limitations of this payload class are demonstrated by evidence from other duocarmycin-based ADCs. Valsasina et al. developed NMS-P945, a thienoduocarmycin-based linker-payload designed to improve the balance between chemical reactivity and stability. The resulting ADC demonstrated target-dependent cytotoxicity, bystander killing, immunogenic cell death, and strong antitumor activity in HER2-expressing preclinical models when conjugated to trastuzumab (Valsasina et al.). Because the efficacy and safety in humans are not established in this study, it indirectly supports the potential and limitations.

Difficulty of translating strong preclinical activity into a favorable therapeutic window is further illustrated in more recent clinical studies with vobramitamab duocarmazine. In phase I treatment-related adverse events occurred in 97.9% and grade 3 or higher treatment-related adverse events occurred in 65.0% of patients with advanced solid tumors. At the 3.0 mg/kg dose, the confirmed objective response rate was 7.2% among response-evaluable patients, indicating modest overall antitumor activity (Shenderov et al.). The difference in toxicity pattern observed with trastuzumab duocarmazine suggests that the safety profile of a duocarmycin ADC depends not only on the payload but also on its antibody target, linker, tissue distribution, dose, and treatment schedule.

Section 6: Prospective ADC Design Strategies

6.1 Linker Engineering: Tumor Microenvironment-Specific, pH-Sensitive, and Enzymatic Optimization

Conditional linker designs seek lower payload release in plasma than in the intended tumor compartment. Proposed triggers include enzyme activity and tissue or endosomal chemistry, but none is exclusively tumor specific; tumor-to-normal-tissue selectivity must be established experimentally (Bargh et al.; Elgersma et al.).

Hydrophilic masking and site-specific conjugation can modify exposure, aggregation, and clearance. An attachment site should also be tested for effects on Fab antigen recognition and FcRn-dependent recycling rather than assumed to preserve both (Lhospice et al.; Gerber et al.).

6.2 Optimization of Drug-to-Antibody Ratio (DAR) and Prodrug Approaches

Optimizing the Drug-to-Antibody Ratio (DAR) is critical for ultra-potent payloads like duocarmycin (Elgersma et al.;Kovtun et al.). While higher DARs (e.g., DAR 8) increase payload

delivery per antibody, they often lead to rapid hepatic clearance, hydrophobic aggregation, and increased systemic toxicity (Chari et al.;Kovtun et al.). Although the ideal DAR depends on the specific payload, linker, and conjugation strategy, homogeneous DAR values in the 2–4 range have often been connected to beneficial pharmacokinetic properties across many ADC platforms (Elgersma et al.;Kovtun et al.).

A duocarmycin-derived prodrug may reduce activity before activation, but premature release and unintended activation remain possible. The useful endpoint is measured tumor selectivity at clinically feasible exposure, not simply an inactive structure on paper (Elgersma et al.; Morcos et al.). Figure 5 summarizes the design levers.

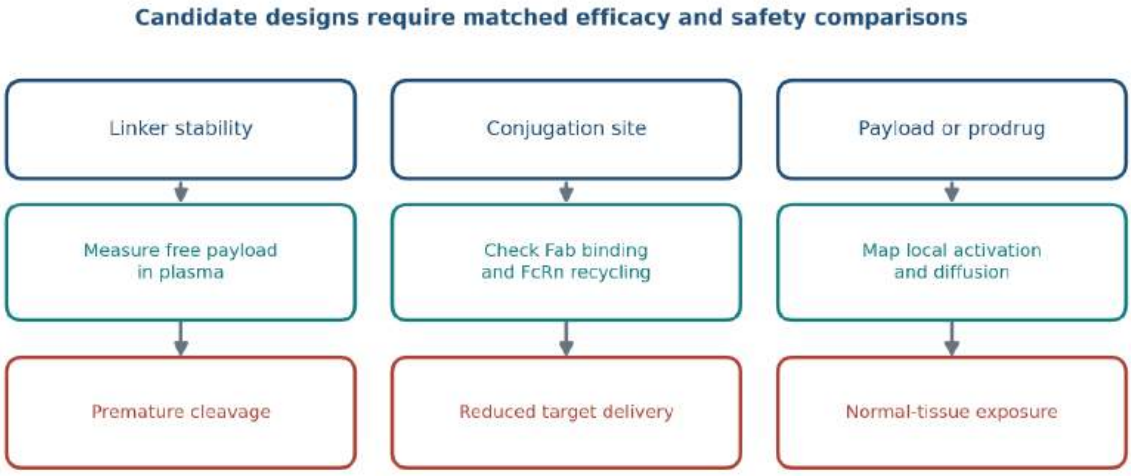

Figure 5. Design variables, the evidence needed to evaluate them, and potential liabilities.

6.3 Dual-Payload and Dual-Target Approaches

Two-payload ADCs can combine components with different permeability or mechanisms of action. A preclinical anti-HER2 ADC bearing MMAE and MMAF improved activity in heterogeneous breast-tumor xenografts compared with the tested single-payload variants, but it does not establish equivalent benefit for duocarmycin combinations or patient safety (Yamazaki et al.). Bispecific ADCs can engage two antigens or use one arm to improve cellular trafficking; this may help capture varied tumor populations, but the effect on healthy tissues and internalization depends on the selected targets (de Goeij et al.). Separately, ADCs aimed at tumor-associated stroma can create a local source of released payload (Szot et al.). Combining tumor and CAF recognition with a bystander-capable payload is a plausible design hypothesis for heterogeneous solid tumors; release within CAF-rich regions is not guaranteed to prevent escape beyond the tumor. No such confinement, specificity gain, or efficacy for duocarmycin ADCs is established by these separate studies.

The evidence for these strategies remains largely preclinical. Future studies should map where intact ADC binds, where cleavage occurs, whether active payload remains within the

tumor, and how much normal-tissue exposure results. Comparisons should specify MED and MTD endpoints and test both efficacy and safety under comparable regimens.

Section 7: Conclusion

Duocarmycin-based ADCs illustrate how conjugation can bring highly potent small molecules into therapeutic development even when their unconjugated use is limited by tolerability. This expands the pool of candidate payloads and shows the value of the ADC modality beyond any one bystander mechanism. The reviewed evidence supports local diffusion as a way to address antigen heterogeneity, while clinical toxicity shows that targeted binding does not guarantee selective exposure. The central task is to retain antigen-directed delivery and useful local release while measuring the independent routes to normal-tissue exposure. This review is narrative, contains no formal study-quality assessment, and combines studies with different targets, payloads, models, and clinical endpoints; its proposed design links therefore require direct comparative validation.

Work Cited

Banerji, Udai, et al. "Trastuzumab Duocarmazine in Locally Advanced and Metastatic Solid Tumours and HER2-Expressing Breast Cancer: A Phase 1 Dose-Escalation and Dose-Expansion Study." Lancet Oncology, vol. 20, no. 8, 2019, pp. 1124–35. https://doi.org/10.1016/S1470-2045(19)30328-6.

Bargh, J. D., et al. "Cleavable Linkers in Antibody–Drug Conjugates." Chemical Society Reviews, vol. 48, no. 16, 2019, pp. 4361–74. https://doi.org/10.1039/C8CS00676H.

Brignole, Chiara, et al. "Antitumor Activity of the Investigational B7-H3 Antibody-Drug Conjugate, Vobramitamab Duocarmazine, in Preclinical Models of Neuroblastoma." Journal for ImmunoTherapy of Cancer, vol. 11, no. 9, 2023, article e007174. https://doi.org/10.1136/jitc-2023-007174.

Chari, R. V., et al. "Antibody-Drug Conjugates: An Emerging Concept in Targeted Cancer Therapy." Angewandte Chemie International Edition, vol. 53, no. 15, 2014, pp. 3796–3827. https://doi.org/10.1002/anie.201307628.

Colombo, Raffaele, and Jamie R. Rich. “The Therapeutic Window of Antibody Drug Conjugates: A Dogma in Need of Revision.” Cancer Cell, vol. 40, no. 11, 2022, pp. 1255–63. https://doi.org/10.1016/j.ccell.2022.09.016.

Cruz, Víctor L., et al. "In Silico Decrypting of the Bystander Effect in Antibody–Drug Conjugates for Breast Cancer Therapy." Scientific Reports, vol. 15, 2025, article 28715. https://doi.org/10.1038/s41598-025-13810-w.

de Goeij, Bart E. C. G., et al. “Efficient Payload Delivery by a Bispecific Antibody-Drug Conjugate Targeting HER2 and CD63.” Molecular Cancer Therapeutics, 2016. https://pubmed.ncbi.nlm.nih.gov/27559142/.

Doronina, S. O., et al. "Enhanced Activity of Monomethylauristatin F through Monoclonal Antibody Delivery: Effects of Linker Technology on Efficacy and Toxicity." Bioconjugate Chemistry, vol. 17, no. 1, 2006, pp. 114–24. https://doi.org/10.1021/bc0502917.

Dy, Grace K., et al. "Ocular Adverse Events Associated with Antibody-Drug Conjugates for Cancer: Evidence and Management Strategies." Oncologist, vol. 29, no. 11, 2024, pp. e1435–e1451. https://doi.org/10.1093/oncolo/oyae177.

Eaton, Joshua Seth, et al. "Ocular Adverse Events Associated with Antibody–Drug Conjugates in Human Clinical Trials." Journal of Ocular Pharmacology and Therapeutics, vol. 31, no. 10, 2015, pp. 589–604. https://doi.org/10.1089/jop.2015.0064.

Elgersma, Ronald C., et al. "Design, Synthesis, and Evaluation of Linker-Duocarmycin Payloads: Toward Selection of HER2-Targeting Antibody–Drug Conjugate SYD985." Molecular Pharmaceutics, vol. 12, no. 6, 2015, pp. 1813–35. https://doi.org/10.1021/mp500781a.

Gerber, Hans-Peter, Sanjeev Gangwar, and Alison Betts. “Therapeutic Index Improvement of Antibody-Drug Conjugates.” mAbs, vol. 15, no. 1, 2023, article 2230618. https://doi.org/10.1080/19420862.2023.2230618.

Giugliano, Federica, et al. "Bystander Effect of Antibody–Drug Conjugates: Fact or Fiction?" Current Oncology Reports, vol. 24, no. 7, 2022, pp. 809–17. https://doi.org/10.1007/s11912-022-01266-4.

Green, Bart N., et al. "Writing Narrative Literature Reviews for Peer-Reviewed Journals: Secrets of the Trade." Journal of Chiropractic Medicine, vol. 5, no. 3, 2006, pp. 101–17. https://doi.org/10.1016/s0899-3467(07)60142-6.

Jaffry, Mustafa, et al. "Antibody-Drug Conjugates and Ocular Toxicity." Journal of Ocular Pharmacology and Therapeutics, vol. 39, no. 10, 2023, pp. 675–91. https://doi.org/10.1089/jop.2023.0069.

Jain, Sonia, et al. "Bystander Effects, Pharmacokinetics, and Linker-Payload Stability of EGFR-Targeting Antibody-Drug Conjugates Losatuxizumab Vedotin and Depatux-M in Glioblastoma Models." Clinical Cancer Research, vol. 30, no. 15, 2024, pp. 3287–97. https://doi.org/10.1158/1078-0432.CCR-24-0426.

Kovtun, Yelena V et al. “Antibody-drug conjugates designed to eradicate tumors with homogeneous and heterogeneous expression of the target antigen.” Cancer research vol. 66,6 (2006): 3214-21. doi:10.1158/0008-5472.CAN-05-3973

Lhospice, F., et al. "Site-Specific Conjugation of Monomethyl Auristatin E to Anti-CD30 Antibodies Improves Their Pharmacokinetics and Therapeutic Index in Rodent Models." Molecular Pharmaceutics, vol. 12, no. 6, 2015, pp. 1863–71. https://doi.org/10.1021/mp500666j.

Mahalingaiah, Prathap Kumar, et al. "Potential Mechanisms of Target-Independent Uptake and Toxicity of Antibody-Drug Conjugates." Pharmacology & Therapeutics, vol. 200, 2019, pp. 110–25. https://doi.org/10.1016/j.pharmthera.2019.04.008.

Morcos, Ann, et al. "A Comprehensive Review of the Antitumor Properties and Mechanistic Insights of Duocarmycin Analogs." Cancers, vol. 16, no. 19, 2024, article 3293. https://doi.org/10.3390/cancers16193293.

Nguyen, Toan D., et al. "Mechanisms of ADC Toxicity and Strategies to Increase ADC Tolerability." Cancers, vol. 15, no. 3, 2023, article 713. https://doi.org/10.3390/cancers15030713.

Shenderov, Eugene, et al. "Vobramitamab Duocarmazine, an Anti-B7-H3 Antibody-Drug Conjugate, in Patients with Advanced Solid Tumors: Final Results of a Phase 1 Cohort Expansion." Cancer, vol. 132, no. 15, 2026, article e70544. https://doi.org/10.1002/cncr.70544.

Staudacher, A. H., and M. P. Brown. "Antibody Drug Conjugates and Bystander Killing: Is Antigen-Dependent Internalisation Required?" British Journal of Cancer, vol. 117, no. 12, 2017, pp. 1736–42. https://doi.org/10.1038/bjc.2017.367.

Szot, Christopher, et al. "Tumor Stroma–Targeted Antibody-Drug Conjugate Triggers Localized Anticancer Drug Release." Journal of Clinical Investigation, vol. 128, no. 7, 2018, pp. 2927–43. https://doi.org/10.1172/JCI120481.

Tarantino, Paolo, et al. "Optimizing the Safety of Antibody-Drug Conjugates for Patients with

Solid Tumours." Nature Reviews Clinical Oncology, vol. 20, no. 8, 2023, pp. 558–76. https://doi.org/10.1038/s41571-023-00783-w.

Turner, Nicholas, et al. "Trastuzumab Duocarmazine in Pretreated Human Epidermal Growth Factor Receptor 2-Positive Advanced or Metastatic Breast Cancer: An Open-Label, Randomized, Phase III Trial (TULIP)." Journal of Clinical Oncology, vol. 43, no. 5, 2025, pp. 513–23. https://doi.org/10.1200/JCO.24.00529.

Valsasina, Barbara, et al. "Novel Thienoduocarmycin–Trastuzumab ADC Demonstrates Strong Antitumor Efficacy with Favorable Safety Profile in Preclinical Studies." Molecular Cancer Therapeutics, vol. 22, no. 12, 2023, pp. 1465–78. https://doi.org/10.1158/1535-7163.MCT-23-0315.

Yamazaki, Chisato M., et al. “Antibody-Drug Conjugates with Dual Payloads for Combating Breast Tumor Heterogeneity and Drug Resistance.” Nature Communications, vol. 12, 2021, article 3528. https://doi.org/10.1038/s41467-021-23793-7.

Zhao, Hui, et al. "Modulation of Macropinocytosis-Mediated Internalization Decreases Ocular Toxicity of Antibody-Drug Conjugates." Cancer Research, vol. 78, 2018, pp. 2115–26. https://doi.org/10.1158/0008-5472.CAN-17-3202.