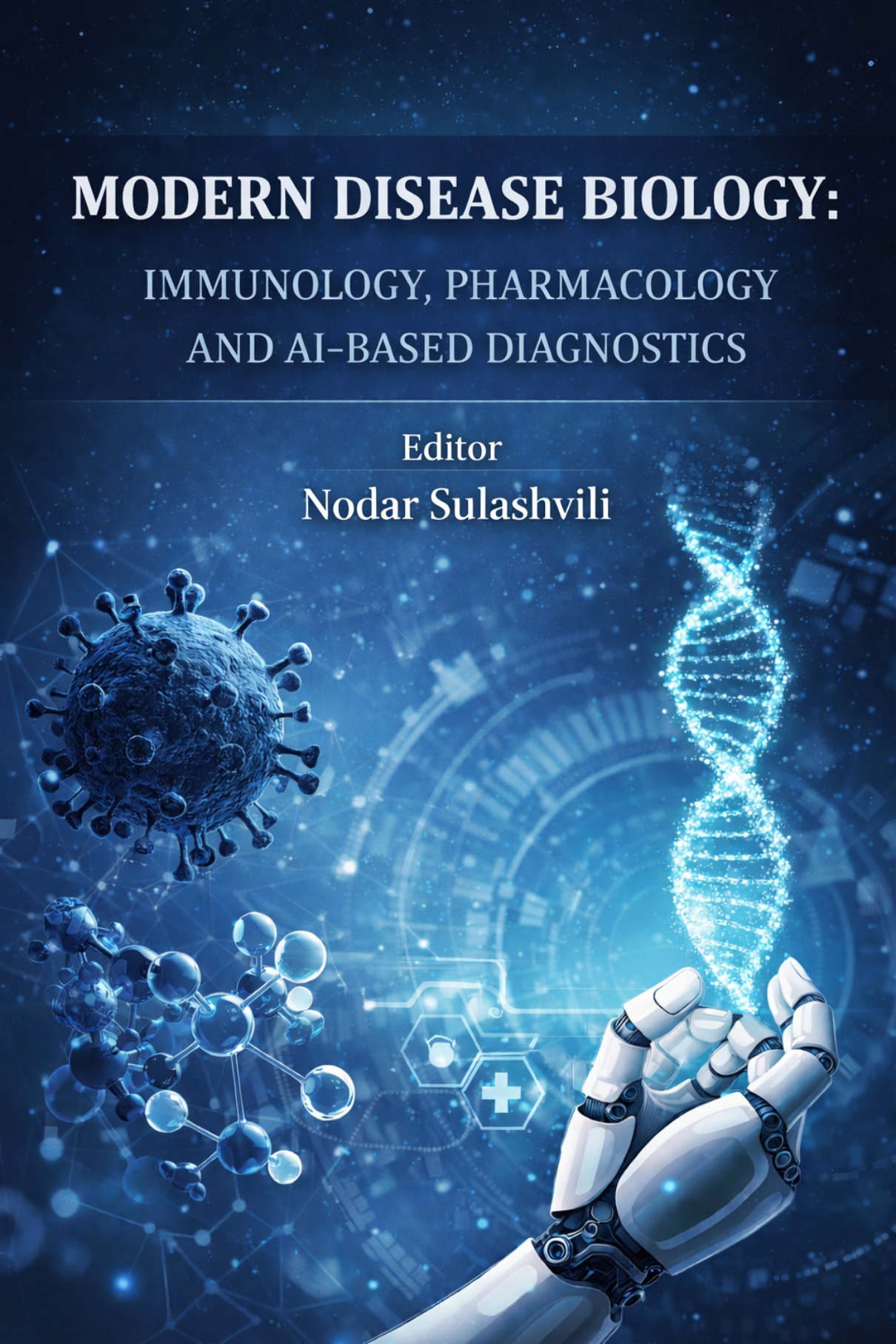
# MODERN DISEASE BIOLOGY:

## IMMUNOLOGY, PHARMACOLOGY AND AI-BASED DIAGNOSTICS

Editor

Nodar Sulashvili



# MODERN DISEASE BIOLOGY: IMMUNOLOGY, PHARMACOLOGY AND AI-BASED DIAGNOSTICS-2026

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# MODERN DISEASE BIOLOGY: IMMUNOLOGY, PHARMACOLOGY AND AI-BASED DIAGNOSTICS

**EDITOR**
Nodar Sulashvili

**AUTHORS**
Prof. Dr. R. SRINIVASAN
Prof. Dr. Arli Aditya PARIKESIT
Asst. Prof. Dr. V. RAKSHANA
R.THELSHATH
Snehamayee MAHAPATRA
V. HARIHARAN
Sarukesh.K
G. Thoufiq ALI
A. VIVEK
K. DIVYA
S. JAYASHREE
L. ABINAYA
T. Blessy FLARANCE
Theshia Veronica Kusuma Yun MEY
Michael Branson LEE
Angelo Christiano Aouad TOMODOK
Nathaniel Emmanuel MULIANA
Dhea PRISKILA
Muammar SADRAWI

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## PREFACE

This volume presents a multidisciplinary exploration of contemporary biomedical challenges, uniting advances in pharmacology, infectious disease control, neurodegeneration, and veterinary medicine. Each chapter offers a focused investigation into a distinct area of health science, reflecting the evolving landscape of research and innovation.

The opening chapter, Advances in Antidepressant Pharmacology, delves into the mechanistic underpinnings of depression and highlights emerging therapeutic strategies. It underscores the shift toward precision medicine and novel targets that may redefine how we approach mental health treatment.

In the second chapter, the efficacy of two feline calicivirus vaccines—Leucofeligen™ FeLV/RCP and Purevax™ RCPFeLV—is evaluated following a single injection. This comparative study contributes valuable insights into veterinary immunology and the optimization of feline disease prevention.

The third chapter offers a comprehensive overview of cutaneous leishmaniasis, examining its epidemiology, immunopathogenesis, diagnostic tools, and therapeutic developments. It emphasizes the global health burden of this neglected tropical disease and the need for integrated control strategies.

Finally, the fourth chapter explores the detection of Alzheimer's disease through AI-driven analysis of epigenetic modifications in key genes. This innovative approach bridges computational biology and neurogenetics, offering promise for earlier and more accurate diagnosis of neurodegenerative disorders.

**Editorial Team**
**January 19, 2026**
**Türkiye**

# CHAPTER 1
# ADVANCES IN ANTIDEPRESSANT PHARMACOLOGY: MECHANISTIC INSIGHTS AND EMERGING THERAPEUTIC APPROACHES

[1]R.THELSHATH
[2]Snehamayee MAHAPATRA
[3]V.HARIHARAN
[4]Sarukesh.K

[1]Bharath Institute of Higher Education and Research, Tambaram, Chennai, ORCID ID: 0009-0001-2512-9922, thelshath.pharmacy@bharathuniv.ac.in
[2]Bharath Institute of Higher Education and Research, Tambaram, Chennai
[3]Bharath Institute of Higher Education and Research, Tambaram, Chennai
[4]Bharath Institute of Higher Education and Research, Tambaram, Chennai

## INTRODUCTION

Depression is a complex and multifaceted psychiatric disorder characterized by a persistent and pervasive sense of sadness, an anhedonia (loss of interest or pleasure) in previously enjoyed activities, debilitating fatigue, cognitive impairments such as difficulty concentrating and making decisions, as well as significant challenges in social interactions and functioning. According to the World Health Organization (WHO), more than 280 million individuals globally are affected by depression, underlining its status as a critical contributor to disability worldwide and a major component of the global burden of disease. Despite the availability of a range of antidepressant medications—including selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs)—a considerable number of patients do not experience sufficient therapeutic benefits. Research suggests that around 30% to 40% of individuals struggling with depression fail to achieve remission, indicating that traditional treatment approaches are not universally effective. Furthermore, it is important to note that most conventional treatments typically require a duration of 4 to 6 weeks to manifest their clinical effects, which can be challenging for patients seeking immediate relief from their symptoms. This underscores the need for ongoing research into alternative therapies and personalized treatment strategies to address the diverse manifestations of this debilitating disorder.

Traditionally, antidepressants have primarily focused on the monoaminergic system, which encompasses the modulation of key neurotransmitters: serotonin, norepinephrine, and dopamine. These neurotransmitters are vital players in orchestrating mood, emotion, and the intricate functions of the brain. However, recent research reveals that the pathophysiology of depression is far more intricate and multifaceted than previously understood. Emerging studies underscore the significant role of the glutamatergic system, which is crucial for synaptic plasticity—a fundamental process in learning and memory—as well as various cognitive functions. Dysregulation of glutamate signaling has been intricately linked to the onset of depressive symptoms, suggesting that glutamate modulators could pave the way for innovative treatment options.

Moreover, alterations in GABAergic signaling, which governs inhibitory neurotransmission, have been implicated in the development of anxiety and mood disorders, pointing to another promising target for therapeutic intervention. This expanding understanding highlights the need for a more comprehensive approach to depression treatment, one that considers the complex interplay of various neurotransmitter systems.

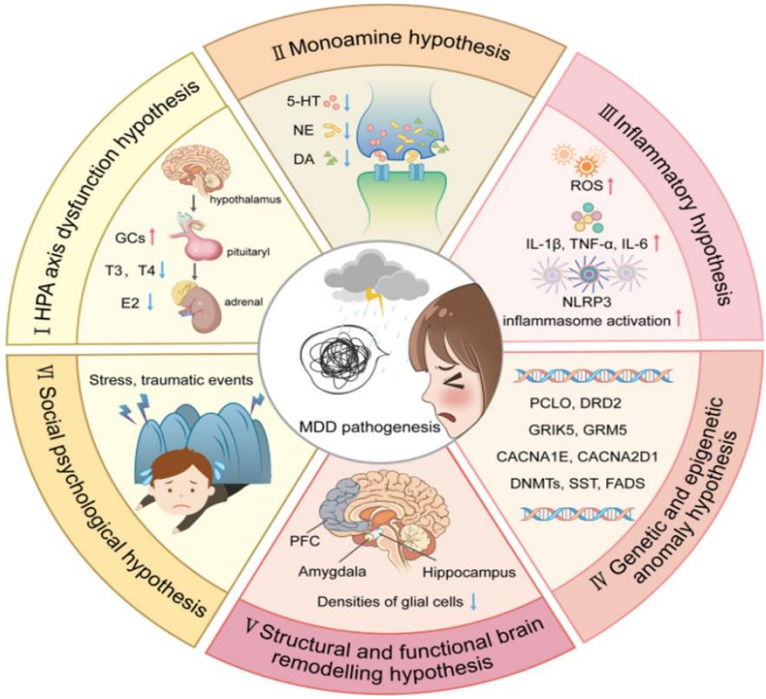


**Figure 1**. Major depressive disorder pathogenesis

The hypothalamic-pituitary-adrenal (HPA) axis serves as a crucial cornerstone of the body's intricate stress response system, and it plays a significant role in the development and manifestation of depression. Prolonged exposure to chronic stress can lead to a state of HPA axis dysregulation, marked by persistently elevated cortisol levels. This hormonal imbalance has far-reaching consequences, negatively impacting overall brain function and contributing to a spectrum of depressive symptoms, including fatigue, hopelessness, and cognitive impairments.

In addition to the hormonal disruptions, neuroinflammation has emerged as a pivotal player in the onset and progression of depression. This phenomenon is characterized by heightened levels of inflammatory cytokines, signaling an inflammatory response that can further exacerbate mood disturbances. The growing recognition of neuroinflammation in depression underscores the potential for anti-inflammatory strategies to serve as innovative and effective treatments, offering hope for those battling this challenging mental health condition.

Impaired neuroplasticity, which refers to the brain's remarkable capacity to adapt and reorganize itself in response to experiences, plays a crucial role in the pathology of depression. This phenomenon can significantly affect an individual's cognitive and emotional resilience. Prolonged exposure to stress is especially detrimental, as it can severely hinder neurogenesis the process by which new neurons are formed primarily within the hippocampus. This brain region is essential for regulating mood, memory, and cognitive functions, thereby underscoring its vital role in maintaining emotional well-being. Research indicates that chronic stress not only reduces the production of new neurons but also impacts synaptic plasticity the ability of synapses to strengthen or weaken over time, facilitating learning and memory. As a result, the intricate connectivity among key brain regions is disrupted, leading to impaired information processing and emotional regulation. These findings collectively highlight the urgent need for innovative treatment strategies that go beyond traditional monoamine modulation, which primarily focuses on altering neurotransmitter levels in the brain. Future therapeutic approaches should aim to address the deeper, underlying mechanisms of depression, including promoting neuroplasticity and neurogenesis. By targeting these foundational processes, we can develop more effective and holistic interventions that not only alleviate symptoms but also foster long-term recovery and resilience against the recurrence of depressive episodes. This paradigm shift in treatment philosophy could pave the way for breakthroughs that fundamentally change the way we understand and manage depression.

Recent advancements in the field of pharmacology have resulted in the innovative development of rapid-acting antidepressants, notably ketamine and its enantiomer, esketamine.

Unlike traditional antidepressants that typically require several weeks for noticeable effects, these compounds have shown the ability to alleviate symptoms of depression within a matter of hours. Esketamine, specifically, is administered as a nasal spray and has received FDA approval for treatment-resistant depression. This highlights its significant role in acute interventions for individuals who have not responded to conventional therapies.

In addition to these developments, psychedelic-assisted therapies have gained momentum in clinical research as promising alternatives for treating depression. Among these, psilocybin the active compound found in certain species of mushrooms has garnered attention for its therapeutic potential. Clinical trials have demonstrated that psilocybin can lead to significant reductions in depressive symptoms and improvements in overall mood. Remarkably, many participants have experienced lasting therapeutic effects after just a few sessions, often requiring only a single administration to manifest substantial improvements in their mental health. This emerging field suggests a paradigm shift in how depression may be treated, emphasizing the need for further research into the long-term benefits and mechanisms of these rapid-acting and psychedelic treatments.

In recent years, significant advancements have been made in the development of neurosteroid-based medications for mood disorders, particularly postpartum depression. Brexanolone, administered through intravenous infusion, is one such treatment that binds to and modulates GABA-A receptors in the brain. This modulation results in a rapid improvement in depressive symptoms, often within hours, which is a remarkable contrast to traditional antidepressants that can take weeks to show effects.[20] Zuranolone, a novel oral treatment, similarly works on GABA-A receptors and represents a groundbreaking step in offering accessibility and convenience for patients. Both of these medications not only provide quick relief but also present a unique pharmacological approach, focusing on neurosteroid pathways that can fundamentally alter the neurochemical landscape associated with mood regulation. In addition to these innovative therapies, the rise of pharmacogenomics-guided personalized treatments is transforming the landscape of antidepressant therapy.

By leveraging an individual's genetic profile, healthcare providers can gain insights into how a patient's unique genetic makeup influences their response to specific medications. This personalized methodology allows for the identification of the most effective pharmacological options tailored to the individual, thereby optimizing treatment outcomes. As a result, healthcare providers can minimize the often frustrating and time-consuming trial-and-error process that accompanies the conventional prescribing of antidepressants. These advancements underscore the potential for more effective and personalized care in the treatment of mood disorders, enhancing the quality of life for many patients.

These innovative strategies collectively promise a swift onset of therapeutic action, marked by significantly reduced latency in symptom relief, heightened effectiveness characterized by improved response rates and remission outcomes, and greater tolerability designed to minimize adverse effects compared to traditional antidepressants. This heralds a transformative shift in the treatment landscape for mood disorders. In this chapter, we will delve into the evolution of antidepressant pharmacology, examining how recent advances in drug design and mechanism of action have led to groundbreaking discoveries. We will also explore promising pathways that encompass novel compounds, personalized treatment approaches, and integrative therapies, all aimed at creating a comprehensive and effective management strategy for depression. These emerging innovations not only expand our understanding of the neurobiological underpinnings of depression but also challenge long-held assumptions about treatment paradigms. By integrating insights from neuroscience, pharmacogenomics, and clinical practice, researchers are forging pathways toward more precise and individualized care. Such progress underscores the importance of interdisciplinary collaboration in addressing the complex and multifaceted nature of mood disorders. Ultimately, these advancements hold the potential to redefine therapeutic expectations and significantly improve long-term patient outcomes.

# 1. CLASSIFICATION OF ANTIDEPRESSANT DRUGS

## 1.1 Conventional Antidepressants

**Table 1.** Classification of Conventional Antidepressant Drugs

| Class | Examples | Mechanism of Action | Limitations |
|---|---|---|---|
| SSRIs | Fluoxetine, Escitalopram, Sertraline | Inhibit the reabsorption of serotonin | Onset of effect is delayed; may cause sexual dysfunction |
| SNRIs | Venlafaxine, Duloxetine | Inhibit the reabsorption of serotonin and norepinephrine | Can cause nausea and elevated blood pressure |
| TCAs | Amitriptyline, Imipramine | Block the reabsorption of serotonin and norepinephrine | Potentially high toxicity and anticholinergic side effects |
| MAOIs | Phenelzine, Selegiline | Inhibit the activity of monoamine oxidase enzymes | Require dietary restrictions and may interact with other medications |
| Atypical | Bupropion, Mirtazapine, Vortioxetine | Act on various targets | Limitations include specific side effects |

# 2. ADVANCES IN ANTIDEPRESSANT PHARMACOLOGY

## 2.1 NMDA Receptor Antagonists (Ketamine & Esketamine)

The mechanism of action primarily revolves around non-competitive antagonism of the N-methyl-D-aspartate (NMDA) receptors, a key process that leads to a substantial increase in the release of glutamate, the brain's critical excitatory neurotransmitter. This increase not only enhances synaptic plasticity the brain's ability to adapt and reorganize itself but also plays a crucial role in the rapid onset of antidepressant effects.

This innovative therapeutic strategy has demonstrated an extraordinary clinical impact, particularly for individuals grappling with treatment-resistant depression, a condition that often persists despite multiple treatment attempts. It offers renewed hope by targeting underlying mechanisms that conventional interventions often fail to address.

Esketamine, a derivative of ketamine, garnered significant attention in 2019 when it received FDA approval as a nasal spray formulation. This approval marked a groundbreaking advancement in psychiatric treatment, specifically designed to address refractory major depressive disorder (MDD). Esketamine offers renewed hope for patients who have run out of viable options, providing rapid relief from depressive symptoms within hours, rather than the weeks or months typically required for traditional antidepressants to take effect. By targeting the underlying neurobiological mechanisms of depression, esketamine represents a pivotal shift in the approach to treating severe mood disorders.

### 2.2 Psychedelic-Based Therapies (Psilocybin, LSD, DMT)

Concentrating on the role of serotonin 5-HT2A receptors is essential for effectively modulating neuroplasticity, which refers to the brain's dynamic ability to reorganize itself by forming new neural connections and strengthening existing ones. This neuroplastic process is crucial for adaptation, learning, and memory formation throughout an individual's life, as well as for recovery from brain injuries and mental health disorders.

Recent preliminary studies provide compelling evidence that even after administering a limited dosage of specific serotonergic compounds, such as atypical antipsychotics or novel antidepressants, patients experience enduring antidepressant effects that last long after the treatment has ended. These findings not only underscore the potential of 5-HT2A receptor modulation but also suggest that engaging this receptor may lead to innovative therapeutic approaches that offer long-lasting mental health benefits. This could significantly transform the landscape of depression and anxiety management, with implications for reducing relapse rates and improving overall patient well-being.

Nevertheless, the path toward broader therapeutic application is fraught with challenges. Key obstacles include overcoming regulatory hurdles that govern clinical trials and pharmaceutical approvals, as well as addressing significant concerns regarding potential psychotomimetic side effects. Sustained interdisciplinary collaboration will therefore be essential to ensure that these issues are navigated responsibly and effectively.

These side effects, which can include alterations in perception and cognition, may emerge during treatment and pose risks that need to be meticulously managed to ensure patient safety and treatment efficacy.

### 2.3 Neurosteroids (Brexanolone, Zuranolone)

Brexanolone: This revolutionary medication proudly holds the distinction of being the first FDA-approved treatment specifically for postpartum depression (PPD). It effectively acts as a positive allosteric modulator of the GABA-A receptor, significantly enhancing the impact of the neurotransmitter GABA. Administered intravenously over a precise 60-hour period, clinical trials unequivocally demonstrate its ability to rapidly alleviate depressive symptoms in postpartum women, providing much-needed relief within days.[37]

Zuranolone: This oral neuroactive steroid is currently under rigorous clinical investigation and is poised to deliver a faster onset of action than traditional antidepressants, which typically require weeks to take effect. By modulating GABA-A receptor activity in a manner akin to brexanolone, zuranolone is set to offer a swift response for individuals grappling with mood disorders, including postpartum depression. Preliminary studies affirm that it not only reduces symptoms rapidly but also boasts a favorable safety profile, making it a highly promising option for treating PPD.

### 2.4. Inflammatory Modulators

Emerging research has increasingly highlighted a significant connection between neuroinflammation and the pathology of depression. Studies indicate that neuroinflammatory processes, characterized by the activation of immune cells in the brain, may contribute to the onset and persistence of depressive symptoms by altering neurotransmitter systems such as serotonin, dopamine, and norepinephrine, as well as affecting critical neural circuits involved in mood regulation. Growing evidence also suggests that elevated levels of pro-inflammatory cytokines can disrupt neuroplasticity, further exacerbating mood-related dysfunction. Additionally, these inflammatory pathways may interact with stress-response systems, amplifying vulnerability to depressive episodes.

In response to these compelling findings, various anti-inflammatory agents are currently being investigated as potential adjunctive therapies alongside standard antidepressant treatment. Notably, tumor necrosis factor-alpha (TNF-α) blockers and minocycline are among the agents under exploration. TNF-α is a pro-inflammatory cytokine that has been implicated in the neurobiological mechanisms of depression. By blocking its action, researchers aim to reduce inflammation within the central nervous system, thereby alleviating depressive symptoms.

Minocycline, an antibiotic with anti-inflammatory properties, has also shown promise in preclinical studies for its potential to modulate neuroinflammation and promote neuroprotection. These agents aim not only to mitigate neuroinflammation but also to enhance the overall therapeutic outcomes for patients who experience inadequate relief from traditional antidepressants alone.

Currently, several clinical trials are being conducted to assess the efficacy, safety, side effects, and optimal dosages of these novel treatments when combined with conventional antidepressant modalities, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs). The goal of this research is to provide a more comprehensive treatment approach for individuals struggling with depression, ultimately leading to improved patient outcomes.

### 2.5. Pharmacogenomics & Personalized Antidepressant Therapy

Recent advancements in genomic sequencing technology have revolutionized the field of pharmacogenomics, enabling the identification of specific genetic variants that significantly impact drug metabolism. Notably, polymorphisms in genes such as CYP2C19 and CYP2D6 have been recognized for their critical roles in the biotransformation of various medications. For example, individuals with certain variants of the CYP2C19 gene may metabolize drugs like clopidogrel less effectively, which can diminish the drug's antiplatelet effects and increase the risk of cardiovascular events.

Similarly, variations in the CYP2D6 gene can affect the metabolism of a wide array of drugs, including antidepressants and opioids, leading to altered drug efficacy and heightened risk of adverse reactions. These genetic differences underscore the necessity for personalized approaches to medication management, ensuring that treatment regimens are tailored to the individual's unique genetic profile.[46]

Furthermore, the implementation of pharmacogenomic panels for personalized antidepressant selection represents a significant advancement in mental health care. By analyzing a patient's genetic makeup, healthcare providers can more accurately match individuals with antidepressants that align with their specific genetic variants, such as those affecting serotonin or norepinephrine pathways. This tailored approach not only aims to enhance therapeutic efficacy but also reduces the likelihood of negative side effects associated with trial-and-error prescribing. For instance, patients with certain gene profiles may have an increased risk of experiencing side effects from standard antidepressants, while others may require higher or lower doses to achieve optimal results.

## 3. COMPARATIVE EFFICACY OF NEW VS. CONVENTIONAL ANTIDEPRESSANTS

**Table 2.** Comparative Characteristics of New and Conventional Antidepressants

| Drug | Target | Onset of Action | Treatment-Resistant Depression Efficacy |
|---|---|---|---|
| SSRIs | SERT | 4–6 weeks | Low |
| Ketamine | NMDA | Within hours | High |
| Esketamine | NMDA | 24 hours | High |
| Psilocybin | 5-HT2A | 1–3 days | Moderate–High |
| Brexanolone | GABA-A | 24–48 hrs | High for PPD |

## 4. CHALLENGES AND LIMITATIONS

- **Safety Concerns:** Both ketamine and its derivative, esketamine, are associated with significant safety risks that warrant close scrutiny in clinical settings. These substances can induce dissociative experiences, which may manifest as feelings of detachment from reality, hallucinations, or altered perception of time and space. Additionally, there is a notable risk of misuse, particularly in individuals with a history of substance abuse disorders. Such concerns emphasize the critical importance of careful patient screening, informed consent, and monitoring protocols to safeguard against adverse effects and ensure patient safety during treatment.
- **Accessibility & Cost:** Advanced treatment options like ketamine and esketamine often come with exorbitant price tags, potentially ranging from several hundred to thousands of dollars per session, depending on the administration method and healthcare settings.
- Many patients encounter substantial barriers due to limited availability of these treatments in rural or underserved regions, exacerbating existing disparities in mental health care.This lack of access can prevent individuals experiencing severe depression or anxiety from receiving timely and effective interventions, thereby worsening their conditions and overall quality of life.[49,50]
- **Long-Term Outcomes:** Current research on the long-term effectiveness of psychedelics, such as psilocybin, and neurosteroids, including allopregnanolone, is still in its infancy, with a scarcity of comprehensive, longitudinal studies. Existing findings often lack consensus, leaving critical questions about the sustainability of their therapeutic benefits over extended periods unanswered. This gap in knowledge raises vital concerns about the durable impact of such treatments on mental health outcomes, necessitating further rigorous and well-structured research to explore the long-term effects and potential complications associated with their use.

- **Regulatory Barriers:** The approval processes for psychedelics and other innovative therapeutic agents involve rigorous clinical trials and extensive regulatory scrutiny, which can span several years or even decades before reaching the market. These stringent regulations, while essential for ensuring safety and efficacy, can significantly delay the timely availability of potentially transformative treatments. As a result, many patients may find themselves without access to these promising alternatives, underscoring the need for a reevaluation of regulatory frameworks to facilitate a more efficient pathway for innovation in mental health therapeutics. [52,53]

## 5. FUTURE PERSPECTIVES

- **Integration of AI and Computational Pharmacology for Drug Discovery:** Utilizing cutting-edge machine learning algorithms alongside sophisticated bioinformatics tools to enhance the drug discovery pipeline. This involves automating the identification of novel drug compounds through predictive modeling techniques, which assess chemical libraries for potential efficacy. Additionally, AI-driven simulations can predict pharmacokinetic properties and potential drug-drug interactions, significantly reducing the time and cost typically associated with the optimization phase. Consequently, this approach accelerates the development of new therapeutics, allowing researchers to focus resources on the most promising candidates.
- **Development of Multi-Target Antidepressants:** Focusing on the design of advanced antidepressant therapies that integrate multiple pharmacological mechanisms. These therapies aim to combine serotonin and norepinephrine reuptake inhibition with glutamatergic modulation—particularly through NMDA receptor antagonism—to maximize therapeutic effects. Furthermore, these novel compounds are tailored to enhance neurotrophic signaling pathways, specifically the upregulation of brain-derived neurotrophic factor (BDNF), which is crucial for neurogenesis and synaptic plasticity. This multifaceted approach is particularly beneficial for patients suffering from treatment-resistant depression, offering hope for improved clinical outcomes.

- **Advancement in Brain Stimulation-Assisted Pharmacotherapy:** Broadening the scope of non-invasive brain stimulation techniques, such as Transcranial Magnetic Stimulation (TMS) and transcranial Direct Current Stimulation (tDCS), which are being increasingly integrated into pharmacological treatment plans. These techniques provide a method to modulate neuronal activity and enhance neuroplasticity, thereby augmenting the therapeutic effects of psychotropic medications. By using these innovative stimulation tools, clinicians can improve patient responses to treatment while minimizing common side effects, resulting in a more holistic approach to managing mental health disorders.
- **Wider Use of Digital Biomarkers for Personalized Treatment Prediction:** Harnessing the capabilities of digital health technologies such as wearable sensors, mobile health applications, and advanced data analytics to capture real-time physiological and behavioral data. This comprehensive monitoring facilitates the creation of personalized treatment regimens that are dynamically adjusted based on individual patient responses and progress. By analyzing trends and patterns in health metrics, healthcare providers can devise targeted interventions, ensuring that treatment plans are not only customized but also responsive to the changing needs of each patient, ultimately leading to enhanced therapeutic outcomes. [59,60]

## CONCLUSION

The landscape of antidepressant pharmacology is evolving beyond traditional monoaminergic agents, such as SSRIs and tricyclic antidepressants. Emerging treatments like ketamine and its stereoisomer, esketamine, are revolutionizing depression management with rapid-acting effects, providing relief within hours for some treatment-resistant patients. Additionally, psychedelics like psilocybin and neurosteroids like allopregnanolone are gaining attention for their potential to promote neurogenesis and address depression from a neurobiological viewpoint. Pharmacogenomic-guided strategies further enhance this evolution by customizing interventions based on individual genetic profiles, optimizing drug selection, and minimizing side effects.

However, addressing crucial concerns regarding safety, accessibility, and cost-effectiveness remains vital for achieving widespread clinical acceptance of these innovative therapies. Ensuring equitable access and conducting thorough studies on long-term safety will be essential for realizing the full potential of these advancements in fighting depression.

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# CHAPTER 2
# VACCINES EFFICACY AGAINST INFECTION WITH CIRCULATING FELINE CALICIVIRUS AFTER ONE SINGLE INJECTION: COMPARISON OF LEUCOFELIGEN™ FELV/RCP AND PUREVAX™ RCPFELV VACCINES

[1]G. Thoufiq ALI
[2]A. VIVEK
[3]K. DIVYA
[4]S. JAYASHREE
[5]L. ABINAYA
[6]T. Blessy FLARANCE
[7] Asst. Prof. Dr. V. RAKSHANA
[8]Prof. Dr. R. SRINIVASAN

[1]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[2]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[3]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[4]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[5]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[6]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[7]Bharath institute of Higher Education and Research Institute, Faculty of Pharmacy, Chennai, rakshanamoorthypharmd@gmail.com, ORCID ID: 0009-0009-3173-9071
[8]Bharath İnstitute Of Higher Education and Research Institute, Faculty of Pharmacy, Chennai

## INTRODUCTION

Feline calicivirus (FCV) is a highly contagious virus that primarily causes respiratory illness in cats. However, it is also linked to other health issues such as polyarthritis, gingivostomatitis, and systemic vasculitis. FCV is part of the *Vesi virus* genus within the *Caliciviridae* family. Its small, nonenveloped structure makes it resilient in the environment and easily transmissible through fomites like pet owners and veterinary staff. The virus contains a single-stranded RNA genome, which has a high mutation rate—greater than that of feline herpesvirus (FHV-1). This contributes to variations in antigenicity and virulence among different FCV strains.

The gene responsible for encoding the capsomer protein, a key structural component of the virus, has variable regions that distinguish one FCV strain from another. These regions contain immunologically important epitopes, which leads to significant antigenic diversity across strains. While most vaccines provide cross-protection against various FCV strains due to antigenic overlap, they may not offer equal effectiveness against all field strains. Additionally, genetic variations can influence the disease manifestations caused by the virus, though these differences are not directly linked to antigenic variability. This genetic diversity poses challenges for vaccine design and development efforts.

## 1. TRANSMISSION AND PATHOGENESIS

Feline calicivirus (FCV) is excreted through secretions from the nose, conjunctiva, and oropharynx. The virus is most effectively transmitted via direct contact between cats or through fomites, while aerosol transmission is less significant, as sneezed droplets travel less than 4 feet. A primary source of infection is asymptomatic carrier cats that continuously shed the virus. Unlike feline herpesvirus (FHV-1), FCV shedding is not influenced by stress. FCV is prevalent in healthy cats, with up to 24% showing evidence of the virus depending on the test used. Carrier cats may shed the virus for months, years, or even for their entire lives. However, one study found that 50% of infected cats stopped shedding within 75 days. A long-term study of FCV shedding in naturally infected colonies identified three patterns: consistent shedding, intermittent shedding, or no shedding. Re-infection after recovery is possible.

FCV primarily infects the epithelia of the upper respiratory tract, oral cavity, and conjunctiva. Unlike FHV-1, it does not cause corneal infections or ulcerations. Oral ulceration is the most typical lesion associated with FCV, often beginning as vesicular lesions in the mouth, particularly on the tongue's edges. As the epithelial tissue in these areas necroses, it leads to inflamed, ulcerated lesions. FCV can also infect the alveolar epithelia of the lower respiratory tract, with some strains showing a strong affinity for lung tissue, resulting in severe interstitial pneumonia

The virus may cause a temporary viremia, spreading throughout the body. This systemic dissemination rarely leads to clinical symptoms, but on occasion, FCV can affect other systems. Acute synovitis may cause lameness, although the exact mechanism is unclear; viral antigens have been detected in joint macrophages.

In rare cases, FCV can lead to a virulent systemic form (VS-FCV), sometimes causing outbreaks in cat populations. This severe syndrome is characterized by widespread vasculitis, multiorgan failure, and epithelial necrosis, affecting the skin and mucous membranes. Ulcerations may appear on the ears, face, and paws, and this condition has been observed even in vaccinated cats. Unfortunately, the mortality rate for VS-FCV is very high.

The pathogenesis of this severe clinical form appears to stem from viral mutations that result in hypervirulence, though the specific mutations remain unidentified. In reported outbreaks, the virulent strains seem to have emerged spontaneously through mutations in caliciviruses already circulating within the affected group. Each outbreak has involved genetically unique isolates, with VS-FCV strains not belonging to a single clade. Instead, these mutant viruses arise from diverse lineages intermixed with other field strains of FCV. Additionally, the emergence of these variants seems to be influenced by both host and environmental factors. While no common mutation has been identified, at least one study has observed point mutations creating an extra glycosylation site in the capsomer protein of certain hemorrhagic isolates.

Interestingly, most VS-FCV outbreaks have been reported in shelter or rescue environments. It is theorized that FCV infections may be endemic in such populations.

Under these conditions, the immunity of the population could select for rapidly replicating virus strains that achieve high titers in a short period. When introduced into a susceptible group, these "hot" variants can lead to widespread systemic disease.

Host factors are also believed to play a role in VS-FCV cases. Immunopathological mechanisms might contribute to the development of disease. Localized cytokine modulation observed in lesions may be linked to vasculitis and the increased vascular permeability associated with this condition.

## 2. TREATMENT

The treatment for feline calicivirus (FCV) infection focuses on symptomatic and supportive care. For anorectic cats, fluid therapy and nutritional support, such as esophagostomy or gastrostomy tube feeding, are essential. Cats with breathing difficulties may require oxygen therapy, while suspected bacterial infections are managed with broad-spectrum antibiotics. Although recombinant feline interferon has shown antiviral activity in vitro, its effectiveness in vivo remains uncertain, and no specific antiviral drugs for FCV currently exist.

Recent advancements highlight the potential of virus-specific compounds, like phosphorodiamidate morpholino oligomer (PMO), which inhibit viral protein translation by targeting viral RNA. Research indicates PMO is safe and effective in reducing disease progression, virus shedding, and mortality, raising hopes for the future development of a commercial medication.

For virulent systemic feline calicivirus (VS-FCV), intensive care is vital, alongside corticosteroids to address the immunopathologic response. Additionally, oral interferon-alpha has been employed in some cases, though its impact on survival is unclear.

## 3. PREVENTION

Vaccination is a key measure to protect cats from feline calicivirus (FCV). There are vaccines which help to prevent and control feline calcivirus :

- Leucofeligen FeLV/ RCP
- Purevax RCP FeLV
- Comparison of Leucofeligen FeLV/ RCP and PUREVAX

### 3.1 Leucofeligen FeLV/RCP

Leucofeligen FeLV/RCP is a vaccine designed to protect cats from eight weeks old against several diseases. These diseases include feline calicivirosis, which is a flu-like illness with mouth inflammation caused by a calicivirus, and feline viral rhinotracheitis, another flu-like illness caused by a herpesvirus. The vaccine also protects against feline panleucopenia, a severe illness characterized by bloody diarrhea and a decrease in white blood cells, caused by a parvovirus. Additionally, it offers protection against feline leukemia, a disease that affects the immune system and leads to symptoms such as loss of appetite, weight loss, poor fur condition, fever, pale gums, and diarrhea, caused by a retrovirus.

The primary benefit of the vaccine is its ability to reduce the signs of these illnesses. Moreover, it prevents both feline panleucopenia and persistent viremia for feline leukemia, which involves the presence of the feline leukemia virus in the blood. The vaccine includes live, attenuated (weakened) feline viruses to ensure they do not cause the diseases: these include the feline calicivirus (strain F9), viral rhinotracheitis virus (strain F2), and feline panleucopenia virus (strain LR 72). Additionally, it contains a protein from the feline leukemia virus (FeLV).

#### *How is Leycofeligen FeLV/ RCP Used*

Leucofeligen FeLV/RCP is administered to kittens as two subcutaneous injections. The first dose is given when the kitten is around eight weeks old, followed by the second dose three or four weeks later. A booster vaccination is required one year after the initial vaccination.

Protection against panleucopenia and leukaemia begins three weeks after the second injection. Protection against calicivirosis starts three weeks after the first vaccine injection, and protection against viral rhinotracheitis begins four weeks after the second injection.

The protection against leukaemia lasts for three years after a booster vaccination, whereas protection against calicivirosis, panleucopenia, and viral rhinotracheitis lasts for one year.

Therefore, older cats need revaccination with Leucofeligen FeLV/RCP every three years. In the intervening years, annual revaccination with Feligen RCP is necessary to protect against calicivirosis, rhinotracheitis, and panleucopenia.

Leucofeligen FeLV/RCP is supplied as two vials: one containing a white powder pellet and the other containing a liquid. Before administration, the contents of the two vials are mixed together to form a suspension for injection.

### *How does Leucofeligen Works?*

Leucofeligen FeLV/RCP is a vaccine that helps the immune system learn how to defend itself against diseases. It contains small amounts of three weakened viruses and a protein from the outer layer of the FeLV virus called 'envelope p45 protein'. This FeLV protein is not extracted from viruses but is produced using recombinant DNA technology in a bacterium.

When a cat receives the vaccine, the immune system recognizes the weakened viruses and FeLV proteins as foreign invaders and produces antibodies against them. In the future, if the cat is exposed to the disease-causing forms of these viruses, its immune system will quickly produce antibodies to fight them, providing protection against the diseases.

The vaccine also contains aluminium hydroxide and Quillaja saponaria extract, which act as adjuvants to strengthen the immune response.

## 3.2 Purevax®

PUREVAX® vaccines are a series of nonadjuvanted feline vaccines created by Boehringer Ingelheim. These vaccines aim to protect cats from various infectious diseases without the use of adjuvants, which can sometimes lead to injection site reactions and chronic inflammation.

### *Key Features of PUREVAX® Vaccines*

- **Nonadjuvanted:** These vaccines do not contain adjuvants. Adjuvants are substances added to vaccines to enhance the immune response but can sometimes cause injection site reactions, chronic inflammation, and even sarcoma formation in cats.
- **Safety: PUREVAX®** vaccines are specifically designed for cats and kittens, ensuring a high level of safety. They are considered the safest rabies vaccination on the market for cats.
- **Innovative Technology:** These vaccines use recombinant canarypox-vectored technology, which stimulates both humoral and cell-mediated immune responses without the risk of reversion to virulence.
- **Comprehensive Protection:** The PUREVAX® family includes vaccines for feline rabies, feline leukemia virus (FeLV), feline rhinotracheitis, calicivirus, panleukopenia, and chlamydia psittaci.
- **Convenience:** PUREVAX® Feline Rabies vaccines are available in both 1-year and 3-year duration of immunity preparations, providing flexibility for pet owners and veterinarians.
- **Half-mL Doses:** PUREVAX® offers the first and only half-mL feline rabies vaccine, which may help further enhance compliance with vaccination site recommendations.

### *Types of PUREVAX® Vaccines*

- PUREVAX® Feline Rabies: Available in 1-year and 3-year duration of immunity.
- PUREVAX® Recombinant FeLV: Protects against feline leukemia virus.
- PUREVAX® Feline 3 (RCP): Protects against feline rhinotracheitis, calicivirus, and panleukopenia.
- PUREVAX® Feline 4 (RCCP): Protects against feline rhinotracheitis, calicivirus, panleukopenia, and chlamydia psittaci.
- PUREVAX® Feline 3/Rabies (RCP + rRabies-1YR): Combination vaccine for feline rhinotracheitis, calicivirus, panleukopenia, and rabies.

- PUREVAX® Feline 4/Rabies (RCCP + rRabies-1YR): Combination vaccine for feline rhinotracheitis, calicivirus, panleukopenia, chlamydia psittaci, and rabies.

***Advantages of PUREVAX® Vaccines***

- Nonadjuvanted: Reduces the risk of injection site reactions and chronic inflammation.
- Safety: High level of safety for cats and kittens.
- Innovative Technology: Uses recombinant canarypox-vectored technology.
- Comprehensive Protection: Protects against multiple feline diseases.
- Convenience: Available in both 1-year and 3-year duration of immunity.
- Half-mL Doses: First and only half-mL feline rabies vaccine.

### 3.3 Comparison of Leucofeligen FeLV/ RCP and PUREVAX®

When it comes to protecting cats from diseases, two vaccines stand out: Leucofeligen and Purevax. These two vaccines have their different way of providing therapeutic efficacy.While both vaccines have their strengths and weaknesses, there are key differences that set them apart. This comparison will explore the composition, efficacy, safety, and convenience of each vaccine.

***Composition***

***Leucofeligen***

Leucofeligen is a powerful, multivalent vaccine that safeguards cats against four major diseases:

- Feline leukemia virus (FeLV)
- Feline calicivirus (FCV)
- Feline viral rhinotracheitis (FVR)
- Feline panleucopenia (FPV)

This vaccine combines live, attenuated viruses with a recombinant FeLV protein, providing comprehensive protection.

### *PUREVAX®*

Purevax, on the other hand, is a non-adjuvanted, recombinant vaccine that protects cats against:

- Feline leukemia virus (FeLV)
- Feline calicivirus (FCV)
- Feline viral rhinotracheitis (FVR)
- Feline panleucopenia (FPV)
- Rabies (in select formulations)

Purevax contains recombinant proteins and viral antigens, eliminating the need for live or attenuated viruses.

### *Efficacy Comparison*

Both Leucofeligen and Purevax have consistently demonstrated hig efficacy in safeguarding cats against targeted diseases.

### *Leucofeligen's Efficacy Profile*

Studies have confirmed that Leucofeligen:

- Induces robust antibody responses: Generating high titers against FeLV, FCV, FVR, and FPV
- Provides extended protection: Offering long-term immunity against FeLV and FPV for at least 3 years
- Offers broad cross-protection: Shielding against diverse strains of FCV and FVR

### *Purevax's Efficacy Profile*

Similarly, Purevax has demonstrated:

- High antibody titers: Against FeLV, FCV, FVR, and FPV
- Long-term immunity: Protecting against FeLV and FPV for at least 3 years
- Effective cross-protection: Guarding against various strains of FCV and FVR

Both vaccines have shown remarkable efficacy in protecting cats against these serious diseases. Consult with your veterinarian to determine the best vaccine for your feline companion.

### *Safety Comparison*

Both Leucofeligen and Purevax have a favorable safety profile, but some differences are notable:

### *Leucofeligen's Safety Profile*

While generally safe, Leucofeligen has been linked to:

- Mild adverse reactions: Including transient fever, vomiting, and diarrhea
- Rare but serious adverse events: Such as anaphylaxis and autoimmune disorders, which are uncommon but require immediate attention.

### *Purevax's Safety Profile*

In comparison, Purevax has been associated with:

- Fewer and milder reactions: With reduced reports of fever, vomiting, and diarrhea compared to Leucofeligen
- Lower anaphylaxis risk: Due to its non-adjuvanted formulation, which minimizes the risk of severe allergic reactions

### *Convenience Comparison*

Both Leucofeligen and Purevax are administered via injection, but their vaccination schedules differ:

Leucofeligen's Vaccination Schedule:

Leucofeligen requires:

- Initial vaccinations: Two injections, given 3-4 weeks apart
- Annual boosters: To maintain protection against targeted diseases

Purevax's Vaccination Schedule:

In comparison, Purevax requires:

- Initial vaccinations: Two injections, given 3-4 weeks apart

Booster shots:

- Every 3 years: For FeLV and FPV

- Annually: For FCV, FVR, and rabies (if included)

## CONCLUSION

In conclusion, the substantial genetic and antigenic diversity of FCV underscores the complexity of controlling the virus and highlights the need for continued molecular characterization of circulating strains. A deeper understanding of the variable genomic regions and their relationship to virulence and immune recognition will play a critical role in refining current vaccines and guiding future vaccine development.

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# CHAPTER 3
# CUTANEOUS LEISHMANIASIS: CURRENT UNDERSTANDING OF EPIDEMIOLOGY, IMMUNOPATHOGENESIS, DIAGNOSTICS, AND THERAPEUTIC ADVANCES

[1]Asst. Prof. Dr. V. RAKSHANA
[2]L. ABINAYA
[3]K. DIVYA

[1]Bharath institute of Higher Education and Research Institute, Faculty of Pharmacy, Chennai, rakshanamoorthypharmd@gmail.com, ORCID ID: 0009-0009-3173-9071
[2]Faculty Of Pharmacy, Bharath Institute Of Higher Education And Research, Selaiyur, Chennai
[3]Faculty Of Pharmacy, Bharath Institute Of Higher Education And Research, Selaiyur, Chennai

## INTRODUCTION

Cutaneous Leishmaniasis (CL) is a neglected tropical disease caused by protozoan parasites of the Leishmania genus. It is transmitted to humans through the bite of infected female sandflies, primarily from the Phlebotomus and Lutzomyia genera. Among the various forms of leishmaniasis, the cutaneous type is the most common, affecting the skin and resulting in ulcerative lesions. Though rarely life-threatening, it can lead to significant scarring and social stigma, especially in endemic regions.

The disease occurs predominantly in tropical and subtropical areas, including parts of the Middle East, North Africa, Central and South America, and the Indian subcontinent. Environmental changes such as deforestation, urbanization, and climate change have influenced the spread and distribution of the sandfly vector, contributing to the emergence and re-emergence of CL in certain regions.

After a sandfly bite, the parasite enters the human body in its promastigote form and is taken up by macrophages, where it transforms into the amastigote form and multiplies. The clinical presentation typically involves the appearance of one or more sores or ulcers on exposed skin, often beginning as papules or nodules. Over time, these lesions may ulcerate, forming a central crater with raised edges. The severity, number, and appearance of lesions can vary depending on the Leishmania species involved and the immune status of the individual.

Diagnosis is primarily clinical, supported by laboratory tests such as microscopy, culture, PCR, and serological methods. Treatment options vary and may include topical therapies, systemic drugs like pentavalent antimonials, amphotericin B, or oral medications such as miltefosine. Preventive strategies include vector control (e.g., insecticide-treated nets, repellents), health education, and in some areas, reservoir control.

### 1. Direct Microscopy, Histopathology, and Culture

#### *Direct Microscopy*

Purpose: To detect Leishmania parasites (amastigotes) directly from skin lesions. Procedure:

A skin smear or scraping is taken from the edge of the ulcer. The sample is stained with Giemsa stain.

Viewed under a microscope for amastigotes (also called Leishman-Donovan (LD) bodies). Advantages:

Simple, fast, and inexpensive. Limitations:

Requires skilled personnel.

Lower sensitivity, especially in chronic or old lesions.

### *Histopathology*

Purpose: To examine tissue structure and identify Leishmania within tissue sections. Procedure:

A biopsy is taken from the lesion.

Tissue is fixed, sectioned, and stained (usually with Hematoxylin & Eosin or Giemsa). Microscopic examination is done to look for:

Inflammatory infiltrates. Amastigotes within macrophages. Advantages:

Helps rule out other skin diseases (e.g., fungal infections, tuberculosis, cancer). Limitations:

Moderate sensitivity.

More time-consuming than direct smear.

### *Culture*

Purpose: To grow and identify the Leishmania parasite.Procedure:

Samples from lesions are inoculated into special culture media (e.g., NNN medium or Schneider's medium). Parasites grow in promastigote form.

Incubation at 22–26°C for up to 2–4 weeks. Advantages:

Confirms live parasite presence.

Can be used for species identification. Limitations: Slow process.

Requires proper lab conditions and is prone to contamination.

Additionally, culture success can vary depending on parasite load and sample quality. It may also fail in chronic cases where viable parasites are scarce. Despite these challenges, culture remains a valuable tool when combined with other diagnostic methods.

### *Leishmania Skin Test (Montenegro Test)*

What is it?

The Leishmania Skin Test is a delayed-type hypersensitivity (DTH) test used to detect past or current exposure to Leishmania parasites. It measures the cell-mediated immune response.

Principle:

In individuals previously exposed to Leishmania, their immune system reacts to a Leishmania antigen injected into the skin.

If immune memory exists, a local skin reaction (induration) develops within 48–72 hours. Procedure:

- Intradermal injection of 0.1 mL of killed Leishmania antigen into the forearm.
- Wait 48–72 hours.
- Measure the diameter of induration (hardened swelling) at the injection site.

  ≥5 mm induration = Positive result

  Interpretation:

  Result Induration Interpretation Positive ≥5 mm Past or current infection

  Negative <5 mm No exposure or immunosuppressed

  Slide 1:

  Title: Leishmania Skin Test (Montenegro Test)

  Subtitle: A Diagnostic Tool for Cutaneous Leishmaniasis

  (Add a background image of a healthcare worker doing a skin test)

  Slide 2: Principle

  Show a diagram of how the antigen is injected into the skin.

  Add labels like “Killed Leishmania antigen”, “Skin reaction” (Use arrows to show immune cells reacting)

  Slide 3: Procedure Picture sequence:

- Antigen injection into forearm
- Time lapse (48–72 hours)
- Measuring induration using a ruler

  Slide 4: Positive vs Negative Reaction Side-by-side photos:

  Positive test: Swollen, red area Negative test: Barely visible mark

Slide 5: Limitations

Not useful in very early stages.

May be false-negative in immunocompromised people (e.g., HIV).

## 2. Treatment of Cutaneous Leishmaniasis (CL)

The treatment of Cutaneous Leishmaniasis (CL) depends on various factors such as the Leishmania species involved, the number and location of lesions, the risk of mucosal involvement, and the immune status of the patient. The primary goals of treatment are to eliminate the parasite, speed up healing, reduce the risk of complications, and minimize scarring.

For mild or uncomplicated cases, especially when the lesion is small, not located on the face, and not caused by species associated with mucosal spread, local treatment may be sufficient. Local therapies include cryotherapy (freezing the lesion with liquid nitrogen), heat therapy (thermotherapy), topical antimonial creams, or intralesional injections of antimonial drugs like sodium stibogluconate. These methods are effective in controlling localized infections and have fewer systemic side effects.

In moderate to severe cases, or when lesions are multiple, large, on sensitive areas like the face, or associated with species like Leishmania braziliensis that may cause mucocutaneous leishmaniasis, systemic treatment is required. The most commonly used drugs are pentavalent antimonials (such as sodium stibogluconate or meglumine antimoniate), which are usually given by injection for 20–30 days. In resistant or relapsing cases, liposomal amphotericin B is highly effective, especially in immunocompromised patients. Miltefosine, an oral drug, has become a valuable option due to its ease of use and effectiveness against several species.

Other treatments like azole antifungal drugs (ketoconazole, fluconazole) are sometimes used, though their effectiveness is generally lower. It is also important to identify the exact species of Leishmania, since the choice and success of treatment can vary based on this. Not all cases of CL need drug treatment, as some lesions may heal spontaneously. Additionally, factors such as the patient's immune status and the extent of the lesions play an important role in guiding therapy.

## CONCLUSION

Cutaneous Leishmaniasis (CL) remains a major public health concern in many parts of the world, especially in tropical and subtropical regions. It is the most common form of leishmaniasis, caused by protozoan parasites of the genus Leishmania, and transmitted through the bite of infected female sandflies. Although it is not typically life-threatening, it can lead to disfiguring skin lesions, permanent scarring, and significant social and psychological consequences for affected individuals.

One of the main challenges in managing CL is its wide range of clinical presentations, which can vary depending on the species involved and the immune response of the host. The disease can present as a single, localized ulcer or multiple chronic, non-healing lesions. Diagnosis relies on several methods including direct microscopy, histopathology, culture, and more advanced techniques like PCR. Each diagnostic method has its own advantages and limitations in terms of sensitivity, specificity, and availability in resource-limited settings.

Treatment options for CL have improved significantly over the years, but they still require careful consideration. While some cases may heal spontaneously, others require targeted treatment to prevent complications. Local treatments such as cryotherapy, thermotherapy, and intralesional antimonials are effective for smaller or non- complicated lesions. In more severe cases, or where there is a risk of mucosal spread, systemic treatment is essential. Drugs like pentavalent antimonials, liposomal amphotericin B, and oral miltefosine are commonly used, though each comes with its own potential side effects and limitations. Species identification plays an important role in selecting the appropriate therapy.

In addition to treatment, prevention and control strategies are crucial. Public health measures such as sandfly control, use of insecticide-treated nets, protective clothing, and health education campaigns are necessary to reduce transmission. Surveillance and early detection programs can also help identify outbreaks and initiate timely interventions. Raising awareness among at-risk populations and healthcare providers is equally important. In conclusion, Cutaneous Leishmaniasis represents more than just a dermatological disease it is a complex condition influenced by environmental, biological, and social.

Although not fatal, its consequences can be long-lasting and deeply affect a person's quality of life. A comprehensive approach involving early diagnosis, effective treatment, prevention, and education is needed to combat the disease. Continued research, global collaboration, and investment in healthcare infrastructure are essential to reduce the burden of CL and improve outcomes for affected individuals worldwide.

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# CHAPTER 4
# EMERGING TRENDS AND METHODOLOGIES IN DIABETES MELLITUS RESEARCH

[1]Asst. Prof. Dr. V. RAKSHANA
[2]K. DIVYA
[3]S. JAYASHREE
[4] L. ABINAYA
[5]T.Blessy FLARANCE
[6]A.VIVEK
[7]G.Thoufiq ALI
[8] Prof. Dr. R. SRINIVASAN

[1]Bharath institute of Higher Education and Research Institute, Faculty of Pharmacy, Chennai, rakshanamoorthypharmd@gmail.com, ORCID ID: 0009-0009-3173-9071
[2]Faculty Of Pharmacy, Bharath Institute Of Higher Education And Research, Selaiyur, Chennai
[3]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[4]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[5]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[6]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[7]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai
[8]Bharath institute of higher Education and Research Institute, Faculty of Pharmacy, Chennai

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels (hyperglycemia), resulting from defects in insulin secretion, insulin action, or both. Insulin is a hormone produced by the pancreas that plays a crucial role in regulating blood glucose levels by facilitating the uptake of glucose into cells for energy production. When insulin production is insufficient or when cells become resistant to its effects, glucose accumulates in the bloodstream, leading to the development of diabetes.

There are several types of diabetes mellitus:

***Type 1 Diabetes Mellitus (T1DM):*** An autoimmune condition where the immune system attacks and destroys insulin-producing beta cells in the pancreas, leading to absolute insulin deficiency. It is typically diagnosed in childhood or adolescence.

***Type 2 Diabetes Mellitus (T2DM):*** Characterized by insulin resistance, where the body's cells do not respond effectively to insulin, and eventual pancreatic beta-cell dysfunction. It is more common in adults and is often associated with lifestyle factors such as obesity, physical inactivity, and poor diet.

***Gestational Diabetes Mellitus (GDM):*** Occurs during pregnancy and is characterized by high blood glucose levels. While it often resolves after childbirth, women who have had GDM are at increased risk of developing T2DM later in life.

***Other Specific Types:*** Includes monogenic diabetes and diabetes resulting from other causes such as diseases of the exocrine pancreas or drug-induced diabetes.

The global prevalence of diabetes has been increasing steadily. According to the International Diabetes Federation, approximately 537 million adults aged 20-79 years had diabetes in 2021, and this number is projected to rise significantly in the coming years . This upward trend is attributed to factors such as aging populations, urbanization, sedentary lifestyles, and dietary changes.

Uncontrolled diabetes can lead to serious complications affecting various organs and systems, including the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), heart, and blood vessels.

Therefore, early diagnosis and effective management are crucial to prevent or delay the onset of these complications. Management of diabetes involves a combination of lifestyle modifications, such as adopting a healthy diet and engaging in regular physical activity, along with pharmacological interventions when necessary. For individuals with T1DM, lifelong insulin therapy is essential. For those with T2DM, treatment may include oral medications, insulin, or other injectable agents, depending on the severity of the condition.

Ongoing research continues to explore new treatments and technologies to improve the quality of life for individuals with diabetes and to find potential cures. Public health initiatives focusing on prevention, early detection, and education are vital in addressing the growing diabetes epidemic worldwide.

## 1. METHODOLOGY IN DIABETES MELLITUS RESEARCH

### *Study Design*

Observational Studies: These studies observe and analyze outcomes without intervention, providing insights into disease progression and real-world treatment effects. Randomized Controlled Trials (RCTs): Considered the gold standard, RCTs randomly assign participants to treatment or control groups to assess the efficacy and safety of interventions. Longitudinal Studies: Track participants over time to observe the development and progression of diabetes and its complications.

### *Data Collection Methods*

Clinical Assessments: Regular monitoring of blood glucose levels, HbA1c, insulin sensitivity, and other metabolic parameters.

Patient Surveys and Questionnaires: Gather information on lifestyle factors, medication adherence, and quality of life.

Biomarker Analysis: Utilization of blood and urine samples to identify genetic, proteomic, and metabolomic markers associated with diabetes.

Technological Tools: Incorporation of continuous glucose monitors (CGMs) and mobile health applications to collect real-time data.

### *Statistical Analysis*

Descriptive Statistics: Summarize demographic and clinical characteristics of study populations.

Inferential Statistics: Apply tests such as t-tests, chi-square tests, and regression analyses to determine associations and differences between groups.

Power Analysis: Conducted during study planning to ensure sufficient sample size for detecting significant effects.

### *Ethical Consideration*

Informed Consent: Ensuring participants are fully aware of the study's purpose, procedures, and potential risks.

Ethics Committee Approval: Obtaining approval from institutional review boards to uphold participant rights and safety.

Data Confidentiality: Implementing measures to protect personal and medical information.

### *Emerging Methodologies*

Mixed-Methods Research: Combines quantitative and qualitative approaches to provide a comprehensive understanding of diabetes management and patient experiences.

Adaptive Clinical Trials: Allow modifications to the trial design based on interim results, enhancing flexibility and efficiency in testing interventions.

N-of-1 Trials: Single-patient studies that assess individual responses to treatments, contributing to personalized medicine approaches.

### *Different types of Diabetes*

There are several types of diabetes mellitus, each with different causes and characteristics. Here's a summary of the main types:

### *Type 1 Diabetes mellitus (T1DM)*

Cause: Autoimmune destruction of insulin-producing beta cells in the pancreas. Onset: Usually in childhood or adolescence, but can occur at any age.

Treatment: Requires lifelong insulin therapy.

Characteristics: Rapid onset of symptoms; prone to diabetic ketoacidosis.

***Type 2 Diabetes Mellitus (T2DM)***

Cause: Insulin resistance and eventual beta-cell dysfunction.

Onset: Common in adults over 40, but increasingly seen in younger people due to obesity. Treatment: Lifestyle changes, oral medications, insulin in later stages.

Characteristics: Often asymptomatic in early stages; linked to obesity and sedentary lifestyle.

***Gestational Diabetes Mellitus ( GDM)***

Cause: Hormonal changes during pregnancy causing insulin resistance. Onset: During the second or third trimester of pregnancy.

Treatment: Diet, exercise, insulin if needed.

Characteristics: Usually resolves after delivery but increases risk of T2DM later.

***Maturity – Oneset Diabetes of the Young (MODY)***

Cause: Genetic mutation affecting insulin production. Onset: Typically before age 25.

Treatment: Often managed with oral medications; sometimes insulin. Characteristics: Inherited in an autosomal dominant pattern.

***Secondary Diabetes***

Cause: Resulting from other medical conditions (e.g., pancreatitis, Cushing's syndrome) or use of medications like steroids.

Onset: Depends on underlying cause.

Treatment: Focuses on managing both the underlying condition and blood glucose. Characteristics: Often resolves if the primary cause is treated.

***Latent Autoimmune Diabetes in adults ( LADA)***

Cause: Slow-progressing autoimmune destruction of pancreatic beta cells. Onset: Adulthood (typically >30 years).

Treatment: Initially may respond to oral meds, but eventually requires insulin. Characteristics: Shares features of both Type 1 and Type 2 diabetes.

The genetic role in diabetes mellitus is significant, particularly in determining a person's susceptibility to both type 1 and type 2 diabetes. Here's an overview:

## 2. GENETIC ROLE IN DIABETES MELLITUS

### 2.1 Type 1 Diabetes Mellitus

Type 1 diabetes is an autoimmune disease where the body's immune system destroys insulin-producing beta cells in the pancreas. Genetics play a strong role, though environmental factors also contribute.

Key genetic factors:

HLA genes (Human Leukocyte Antigen): Variants like HLA-DR3 and HLA-DR4 are strongly associated with increased risk.

INS gene: Encodes insulin; certain variants increase autoimmune attack likelihood. PTPN22, IL2RA, and CTLA4: Other genes involved in immune regulation and autoimmunity. Family risk:

If a first-degree relative (like a parent or sibling) has type 1 diabetes, the risk increases significantly compared to the general population.

### 2.2 Type 2 Diabetes Mellitus

Type 2 diabetes has a stronger genetic component than type 1. It results from a combination of genetic predisposition and lifestyle factors like diet and physical inactivity.

Key genetic factors:

TCF7L2 gene: One of the strongest genetic links to type 2 diabetes. FTO gene: Associated with obesity, which is a major risk factor.Other genes influence insulin resistance, beta-cell function, fat metabolism, and glucose transport (e.g., PPARG, KCNJ11, SLC30A8).

Family risk:

If one parent has type 2 diabetes, the risk is about 40%; if both have it, the risk may exceed 70%

### 2.3 Monogenic Diabetes

This form results from a single gene mutation and includes:

MODY (Maturity-Onset Diabetes of the Young): Caused by mutations in genes like HNF1A, HNF4A, and GCK.

Neonatal Diabetes: Appears in the first 6 months of life and may result from mutations in genes such as KCNJ11 or INS.

These types are rare but highlight how powerful a single genetic mutation can be in causing diabetes.

## CONCLUSION

Genetics significantly influence the risk of developing diabetes. In type 1, they predispose the immune system to attack insulin-producing cells. In type 2, they affect metabolism and insulin function. While you can't change your genes, understanding genetic risk can help guide early screening, preventive measures, and personalized treatment.

Moreover, genetic insights can help identify individuals who may benefit from more intensive lifestyle interventions. They also support the development of targeted therapies aimed at specific molecular pathways. Ultimately, integrating genetic information into clinical care enhances the precision and effectiveness of diabetes management.

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# CHAPTER 5
# DETECTION OF ALZHEIMER'S DISEASE THROUGH AI-DRIVEN AND METHYLATION DIFFERENCE REGION ANALYSIS OF SIGNIFICANT EPIGENETIC MODIFICATIONS IN APP, PSEN1, PSEN2, APOE, MAPT, AND TREM2 GENES

[1]Theshia Veronica Kusuma Yun MEY
[2]Michael Branson LEE
[3]Angelo Christiano Aouad TOMODOK
[4]Nathaniel Emmanuel MULIANA
[5]Dhea PRISKILA
[6]Muammar SADRAWI
[7]Prof. Dr. Arli Aditya PARIKESIT

[1]Indonesia International Institute of Life Sciences, School of Life Sciences, Department of Biomedicine, theshia.mey@student.i3l.ac.id, ORCID ID: 0009-0008-8716-5110
[2]Indonesia International Institute of Life Sciences, School of Life Sciences, Department of Biomedicine, michael.lee@student.i3l.ac.id, ORCID ID: 0009-0006-1350-5982
[3]Indonesia International Institute of Life Sciences, School of Life Sciences, Department of Biomedicine, angelo.tomodok@student.i3l.ac.id, ORCID ID: 0009-0007-9156-3763
[4]Indonesia International Institute of Life Sciences, School of Life Sciences, Department of Biomedicine, nathaniel.muliana@student.i3l.ac.id, ORCID ID: 0009-0008-5999-1191
[5]Indonesia International Institute of Life Sciences, School of Life Sciences, Department of Biomedicine, dhea.priskila@student.i3l.ac.id, ORCID ID: 0009-0005-4259-5123
[6]Indonesia International Institute of Life Sciences, School of Life Sciences, Department of Biotechnology, muammar.sadrawi@i3l.ac.id, ORCID ID: 0000-0002-2818-242X
[7]Indonesia International Institute of Life Sciences, School of Life Sciences, Department of Biotechnology, arli.parikesit@i3l.ac.id, ORCID ID: 0000-0001-8716-3926

## INTRODUCTION

Alzheimer's Disease (AD) is a progressive neurological disorder that primarily affects memory, thinking, and behavior (Kamatham et al., 2024). In AD, there are several factors that influence the susceptibility towards this disease. This was evident as the risk for AD was said to increase with age, family records of this disease, unhealthy lifestyle and dietary habits, head trauma and even genetics (Breijyek & Karaman, 2020). Genetics is the most crucial factor that plays a role in the development of AD as genetic factors were known to be involved in at least 70% AD cases (Silva et al., 2019). The inheritance of AD involved two categories of genes, deterministic genes and risk genes ; Deterministic genes are genes that follow the pattern of Mendelian inheritance and can confidently guarantee the development of the disease inherited in most cases (Harden et al., 2022). In the case of AD, mutations in APP and PSEN1/PSEN2 guarantee a 77% pathogenicity of early-onset familial AD ( Lanoiselée et al., 2017). Additionally, risk genes are genes that can increase the susceptibility of the disease without absolute guarantee of the disease development, this category is generally impacted by other factors such as, lifestyle, environment and even other genes. Thus, polymorphisms in APOE such as APOE ε4 were said to increase the susceptibility of late-onset AD cases (Polsinelli et al., 2023).

Epigenetic changes have played an important role in the development and progression of Alzheimer's Disease, affecting several key genes in the pathology (Behl et al., 2024). These modifications, which alter the gene expression without changing the DNA sequence can help uncover the complex mechanisms underlying Alzheimer's disease and are able to be used for early detection and intervention (Alkhammash & Alotaibi, 2025). APP gene or the Amyloid Precursor Protein gene can be considered as the central of Alzheimer's disease pathology, as it encodes for the Amyloid Precursor Protein (APP) (Hampel et al, 2021). While the function of the protein itself is unknown, scientists hypothesize that it may bind to other proteins on the surface of cells or help cells attach to one another which helps direct nerve cell movement (Orobets & Karamyshev, 2023). PSEN1 and PSEN2 genes encode the components of the gamma-secretase complex, which is involved in APP processing (Valdes et al., 2025).

Some studies suggest that PSEN1 mutations influence the methylation of other AD-related genes such as MAPT while PSEN2 expression is downregulated in specific brain regions of AD patients due to epigenetic modifications (De Plano et al., 2024; Xiao et al., 2020). The APOE gene, particularly the ε4 allele, is the strongest genetic risk factor for late-onset AD. Studies stated that the ε4 variant introduces an extra CpG site due to specific single nucleotide polymorphisms (SNPs) (Giallongo et al., 2022). This can increase methylation, potentially silencing or altering APOE expression which affects the amyloid-beta (Aβ) clearance, lipid transport, and neuronal health (Lozupone et al., 2023). The MAPT gene encodes the tau protein production and can be influenced by epigenetic modifications. Silencing epigenetic marks (e.g., methylation) may reduce tau levels, potentially protecting against abnormal tau aggregation while activating marks (e.g., histone acetylation) could increase tau production, raising the risk of aggregation and neurodegeneration (Yu et al., 2019). TREM2 gene encodes the protein found on microglia. Protein helps microglia clear out harmful debris, like amyloid plaques, which are sticky protein clumps linked to Alzheimer's disease (Lue et al., 2015). If the TREM2 gene is methylated, it might be silenced, leading to less TREM2 protein. This could weaken the microglia's ability to clean up plaques, worsening disease progression.

With the progress of research, validation of significant known methylation and genetic targets were widely studied in order to understand gene expression with respect towards methylation rates that may have a critical impact towards the downstream pathway signalling related to Alzheimer disease.This was evident as highly significant gene targets such as, APP, PSEN1, PSEN2, APOE, MAPT, TREM2 are often further researched to observe the significance and relevance of this gene with respect towards new gene targets (Neuner et al., 2020; Vega et al., 2022). Furthermore with the progress of technological advancements, AI (Artificial Intelligence) has contributed greatly towards gene-based diagnostics of Alzheimer's biomarkers (Winchester et al. 2023). Whereas, these AI models relied on deep learning workflows such as multi-stage deep learning, support vector machine learning, generalized machine learning, and random forest. They were used to analyze a variety of genomic and methylation data.

This approach allowed for the identification of specific genomic and epigenetic factors that influence the pathogenesis of Alzheimer's disease (Jo et al., 2025; Bahado-Singh et al., 2022).Thus, in order to further AI development and detection of Alzheimer's disease, AI-driven analysis and DMR analysis will be used concurrently in this study to understand key methylation factors significantly expressed in the target genes APP, PSEN1, PSEN2, APOE, MAPT, TREM2.

## 1. METHODS

### *Data Collection for DMR Analysis and KEGG Pathway Analysis*

The dataset used for DMR analysis was collected from the Gene Expression Omnibus (GEO) with the accession code of GSE244352. This data contained methylation data of genomic DNA taken from the peripheral blood samples of 12 clinically diagnosed Alzheimer's patients and 12 subjects with normal cognition. In order to extract the genomic data, Maxwell RSC Instrument (Promega, USA) with Maxwell RSC Buffy Coat DNA Kit (Promega, USA) was used and further methylation profiling was performed with the use of Illumina HiSeq 2500. This dataset is referenced from the study conducted in "Identification of diagnostic DNA methylation markers in the blood of Japanese Alzheimer's disease patients using methylation capture sequencing" (Mitsumori et al.,2025).

### *Data Collection for AI-Driven Analysis of RNA-seq Data*

Additionally, datasets such as GSE48350 and GSE11882 were also collected fromthe Gene Expression Omnibus. Both of these datasets contained microarray gene expression profiles on four different brain regions, such as the hippocampus (HC), entorhinal cortex (EC), superior frontal gyrus (SG), and postcentral gyrus (PCG). Postmortem brain tissue was collected from the ADRC brain banks. The total RNA was then extracted from frozen, unfixed tissue using TRIzol reagent (Invitrogen, Carlsbad, CA) and purified using quick spin columns (Qiagen, Valencia, CA). A UV spectrophotometer and Agilent BioAnalyzer were used to measure RNA quality for sharp peaks and 28S/18S RNA.

### *AI/ML Implementation*

To elucidate the role of epigenetic modifications in AD, multilayer perceptron models will be employed with publicly available transcriptomic datasets GSE48350 and GSE11882 from GEO. These models will be utilized to detect the status of a patient's well-being of developing AD based on the selected gene's expression. Furthermore, the analysis will incorporate relevant factors such as sex, age, and Braak stage (stage 0 - II were classified as Normal, stage III-VI were considered indicative of AD pathology) to comprehensively assess their impact on the AD-related genes.

### *Data Extraction and Preprocessing*

The datasets were extracted using GEOparse for fast and efficient loading in the Google Colab environment. Then, the expression data and metadata contained in the dataset were defined and extracted. After that, some columns inside the metadata were dropped as they were unnecessary to be used in the development of the machine learning model. Finally, both of these separate datasets that were already preprocessed and cleaned were combined into one using the concat feature.

### *Feature Construction and Integration*

The set of targets of AD related genes selected were processed further. Each of the genes were specified with associated Affymetrix probe IDs and the average expression value across probes was also calculated to obtain gene-level expression. These genes were chosen and combined with standardized demographic features. To guarantee compatibility with the model, the brain region variable was one-hot encoded. StandardScaler was used to standardize all numerical features, and the resulting matrix was split into training and testing sets (80:20 ratio) with the train_test_split function.

### *Multilayer Perceptron (MLP) Model Construction and Evaluation*

First, the target was defined, which holds a series of 0s and 1s, identified as normal and AD, respectively, corresponding to the disease status of each sample in the same order as the rows of the features in the dataset.

Then, the data was split into x_train, y_train, x_test, and y_test sets. The targets were then converted into Numpy arrays for compatibility and to optimize performance for training a Keras neural network. Lastly, the model was designed using the Tensorflow/Keras machine learning pipeline, configured and trained up to 200 training iterations for a binary classification task, such as differentiating 'Normal' from 'AD' based on earlier data preprocessing steps.

Afterwards, the held-out test set was then used to assess the trained MLP model's performance. The trained model was applied to the scaled test features to generate predictions, and the probabilities results were converted into binary class labels (0 for Normal, 1 for AD) using a threshold of 0.5. Additionally, the classification_report and confusion_matrix functions in scikit-learn were used to compute the evaluation metrics, including accuracy, precision, recall, and F1-score.

## 1.1 Differential Methylated Regions (DMR) Analysis

In this research DMR analysis on GSE244352 was performed in order to find significant methylated regions associated with the targets APP, PSEN1, PSEN2, APOE, MAPT, TREM2. This was done by cleaning and manipulation, visualization, genomic annotation, followed by KEGG enrichment and pathway analysis of the given DMR data. This process would enable the selection of significantly methylated regions and annotating these regions with respect towards the human chromosome database, thus understanding the targeted genes affected by methylation through the specific chromosome location/regions. Lastly, these genes will be enriched and the KEGG pathway will be performed. This DMR analysis will be conducted by the use of.

### *Data Cleaning, Filtering, and Manipulation*

From the given dataset, raw DMR data will be loaded by the use of library(data.table). This allows for rapid loading of the dataset containing data on chromosome, start sequence, end sequence, p-value, q-value and methylation difference data. Thus allowing for further data manipulation. After this was done, cleaning, filtering, and transformation were carried out using library(dplyr).

This allowed for the screening of data considered significant by selecting data that met the thresholds of q-value < 0.05 and methylation difference > 15.Therefore, deleting null data and enabling the collection of significantly methylated regions of the targeted genes in the chromosomes, which allow for visualization and further gene annotation.

### *Methylation Profiling Analysis with Manhattan Plot and Volcano Plot*

After preprocessing of the data, visualization of the significant data was done by the use of library(ggplot) in order to illustrate the significance of the selected DMRs. This was done by the use of Manhattan plot to visualize the significance of each DMRs across the chromosome and Volcano plot in order to visualize the of each DMRs across the methylation differences(%). In both data, significant DMRs are annotated with the color red and non-significant DMRs are annotated with the color grey.

### *Genomic Annotation*

Aside from visualization, annotating the DMR data is crucial in order to understand the upstream and downstream genes affected by the methylated regions. This was done by manipulating the data structure of the DMR coordinates by arranging these data as GRanges objects by the use of a library(GenomicRanges).

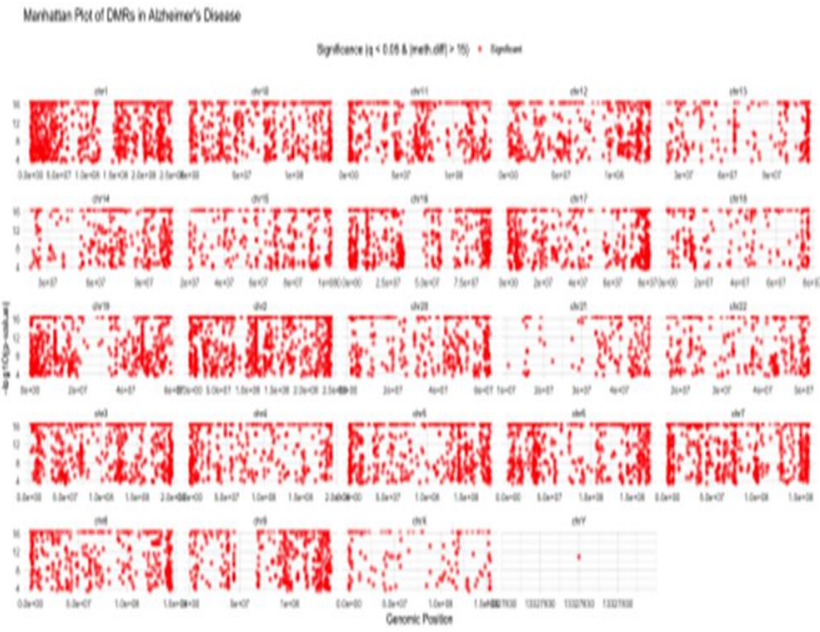
Following this, library(TxDb.Hsapiens.UCSC.hg38.knownGene) was loaded to provide a reference for human genome annotation database to map DMR based on the hg38 assembly. Furthermore, annotation is carried out by the library(ChIPseeker) as this library allows for the annotation of GRanges objects with library and library (TxDb.Hsapiens.UCSC.hg38.knownGene), thus associating the coordinates of the DMR with genomic features such as gene id, annotation (promoter, intron, exon etc), gene symbol and distance to transcription start sites (tss) which is crucial for pathway analysis. In order to allow for smooth annotation, library(rtracklayer) to ensure the compatibility of genomic ranges format during annotation.

### *KEGG Pathway Enrichment & Visualization*

In order to conduct the KEGG pathway enrichment, the library(KEGGREST) extracted KEGG pathway data as well as genesets for the annotated gene of interest (Kanehisa et al, 2025; Kaneshisa & Goto, 2000; Kanehisa, 2019). Thus, allowing for pathway visualization. After this was conducted, KEGG pathway enrichment was conducted with library(clusterProfiler), which conducted functional enrichment analysis, allowing for the identification of major pathways and subsets of related pathways affecting the pathogenesis of Alzheimer disease. With this identification, visualization of major and related pathways was conducted with a library(pathview) which visualizes the KEGG plot with reference to the methylation difference(%) on the Alzheimer disease pathway of the KEGG Pathway ID.

## 2. RESULTS AND DISCUSSION

### *Manhattan Plot of DMRs in Alzheimer Disease*



***Volcano Plot of DMRs in Alzheimer Disease***

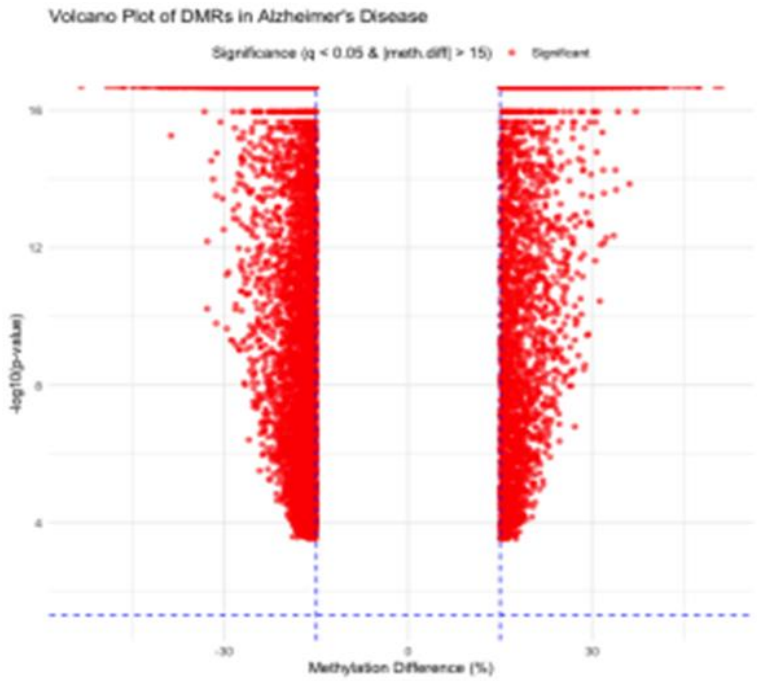



**Figure 1.** Combined Volcano and Manhattan Plots of differentially methylated regions (DMRs) across the genome with significance threshold of q value < 0.05 and methylation differential value > 15.

## 2.1 Manhattan Plot

Figure 1 also shows a Manhattan plot that gives an example of how most of the DNA methylation changes (called DMRs) are found on chromosomes 14 and 17. The tall peaks in the plot go above the cut-off line ($q < 0.05$), showing strong signals in areas where two important Alzheimer's genes are found: PSEN1 and MAPT. Furthermore, chromosomes 13,18, and 20 also display noticeable clusters of red dots, but lower statistical strength, indicating even though while DMRs are present, they less associated with Alzheimer pathology, one possible explanation is that these regions involves genes that have an indirect roles (e.g.immune response, synaptic function, and etc), rather than the primary amyloid/tau pathways (Feng et al., 2024).

Moreover, researchers have also studied DNA methylation across the whole genome and found that certain gene areas might be linked to Alzheimer's specifically to their pathology. For example, Monti et al. (2020) found that the PSEN1 gene had much lower methylation in Alzheimer's brains, which was linked to higher gene activity. Mori et al. (2024) also found methylation changes in the MAPT gene, suggesting this gene may not be working properly in the disease. Additionally, Gao et al. (2022) found methylation changes in both PSEN1 and MAPT genes in the front part of the brain (called the frontal cortex) in Alzheimer's patients, showing that these genes are likely involved in changes to gene regulation in the disease.

### 2.2 Volcano Plot

Many differentially methylated regions (DMRs) in Alzheimer's disease had high significance p-values and significant methylation differences, with several above 30% change, either rising or decreasing, as seen in the volcano plot in Figure 1. The majority of DMRs satisfy the $q < 0.05$ and methylation difference > 15% criterion, indicating a significant and pervasive epigenetic change between the control and Alzheimer's samples. To ensure that less than 5% of significant results are probable false positives, the false discovery rate is then controlled using a q-value threshold of <0.05 (Maksimovic et al., 2016). To identify methylation variants that are biologically significant, a cutoff of greater than 15% is frequently employed; this eliminates small variations that have no effect on gene activity (Chowdhury et al., 2016). Large-scale epigenome wide association studies (EWAS), which have often found strong DNA methylation signatures linked to Alzheimer's pathology across several brain areas, are consistent with the findings. More than 334 cortical DMPs and 220 cross-cortex CpGs have been reported by meta-analyses, glial-driven epigenetic changes in neurodegeneration are demonstrated by a significant percentage of these cells originating in non-neuronal cells, including astrocytes and microglia (Shireby et al., 2022). These findings highlight the critical role of epigenetic regulation in Alzheimer's disease and suggest that specific methylation changes may serve as potential biomarkers for early diagnosis.

**Table 1.** DMR identification of Alzheimer Related Genes, PSEN1 and MAPT that are significantly methylated

| Chr | Start | End strand | P-value Qvalue | Meth.diff |
|---|---|---|---|---|
| Chr14 | 73113602 | 73113602 | +0.000000e+00 0.000000e+00 | -30.08647 |
| Chr14 | 73198335 | 73198335 | +0.000000e+00 0.000000e+00 | 19.11400 |
| Chr17 | 45889839 | 45889839 | +8.121932e-10 1.471765e-07 | -24.09307 |

**Table 2.** Gene Annotations Associated with PSEN1 and MAPT

| Chr | Annotation | Gene_id Gene_symbol | Dictance_ to_tss |
|---|---|---|---|
| Chr14 | Promoter (2-3kb) | 5663 PSEN1 | -2816 |
| Chr14 | Promoter (1-2kb) | 5663 PSEN1 | 1823 |
| Chr17 | Intron (ESNT00000634876.2/100128977, intron 1 of 6) | 4137 MAPT | -4688 |

DNA Methylation functions as an epigenetic process which controls gene expression through alterations in gene activity without modifying DNA sequences (Kiselev et al., 2021). Gene expression becomes abnormal when disruptions occur in the methylation patterns that regulate gene expression leading to neurodegenerative disease (Rasmi et al., 2022). Tables 1 and 2 show significant differences in DNA methylation with several genomic loci of PSEN1 and MAPT. Notably, the site on chromosome 14 at position 73113602 marked as hypomethylation, with a methylation difference of -30.09% (P = 0, Q = 0). On the other hand, another site on the same chromosome at position 73198335 marked as hypermethylation, showing a +19.11% increase in methylation (P = 0, Q = 0). Both regions were annotated to the promoter region of the PSEN1 gene which was located at 2.8 kb upstream and 1.8 kb downstream of the transcription start site (TSS), respectively.

The process of methylation in promoter regions controls gene expression through silencing when hypermethylated and activation when hypomethylated (Wang et al., 2020). The opposing methylation patterns indicate complex regulatory mechanisms that could be affected by cell type and disease stage or multiple unidentified factors (Dor & Cedar, 2018).

Meanwhile, the site at 17 position 45898939 showed significant hypomethylation with -24.09% (P = 8.12 x 10-10, Q = 1.47 x 10-7) within the intronic region of the MAPT gene which is 4.7 kb upstream of the TSS. The MAPT gene produces tau protein, which has a role to stabilize microtubules in neurons. However, during Alzheimer's disease, the tau protein becomes hyperphosphorylated, and losing its ability to bind microtubules and forming the neurofibrillary tangles, this is a characteristic of Alzheimer's disease (Strang et al., 2019; Rawat et al., 2022). In addition, changes in MAPT intronic methylation patterns may impact tau transcript levels as well as tau isoform expression, both of which have been connected to tauopathy progression. The biological function of the many tau isoforms generated by MAPT alternative splicing varies because some of them are more prone to aggregation. The alterations between various tau isoforms are produced by changes in intronic methylation patterns, which could contribute to the advancement of the disease (Creekmore et al., 2024). These findings suggest that intronic methylation changes in the MAPT gene may play a direct role in modulating tau pathology in Alzheimer's disease. Targeting these epigenetic modifications could therefore offer a promising avenue for therapeutic intervention aimed at preventing or reducing tau aggregation.

**Table 3.** First Batch of KEGG pathway enrichment results of respective PSEN1 and MAPT with reference towards KEGG pathway ID of hsa05010 and hsa05022

| ID | Description | GeneRatio | BgRatio | RichFactor | FoldEnrichment |
|---|---|---|---|---|---|
| hsa05010 | Alzheimer disease | 2/2 | 391/9396 | 0.005115090 | 24.03069 |
| hsa05022 | Alzheimer disease | 2/2 | 483/9396 | 0.004140787 | 19.45342 |

**Table 4.** Second Batch of KEGG pathway enrichment results of respective PSEN1 and MAPT with reference towards KEGG pathway ID of hsa05010 and hsa05022

| ID | Z-Score | P-value | P.adjust | Q-value GeneID | Count |
|---|---|---|---|---|---|
| hsa05010 | 6.787215 | 0.001727434 | 0.01054907 | 0.004164108 4137/5663 | 2 |
| hsa05022 | 6.075423 | 0.002637269 | 0.01054907 | 0.004164108 4137/5663 | 2 |

Using KEGG pathway enrichment analysis, Table 3 and 4 is showing the biological significance of the differentially methylated genes for PSEN1 and MAPT. The results showed both genes which correspond to KEGG IDs hsa05010 and hsa05022, were significantly linked to the Alzheimer's pathway.. The GeneRatio for both pathways is 2/2, meaning that both genes were found in each pathway. This suggests a pathway overlap with the input genes, highlighting their potential role in Alzheimer's related pathways. Furthermore, the BgRatio (Background Ratio) offers context for how frequently certain pathways occur within the gene set (Olgun et al., 2019). The BgRatio for hsa05010 is 391/9396, while for hsa05022, it is 438/9396, meaning that these pathways are connected with 391 and 483 genes, respectively, from the total of 9396 reference genes.

The rich factor values were statistically significant, with values of 0.0051 (hsa05010) and 0.0041 (hsa05022). The ratio of input genes that map a particular pathway to the total number of genes known to be in that pathway is known as the RichFactor (Hiransuchalert et al., 2024). In short, it is a measure of how dense the representation of the input genes is within a pathway. In addition, the fold enrichment values for hsa05010 and hsa05022 are 24.03 and 19.45, respectively. Fold Enrichment is calculated by comparing the GeneRatio to the BgRatio (Wu et al., 2021). In this case, both pathways are more than 19 to 24 times enriched, indicating that these genes are not just present but highly concentrated in Alzheimer's pathways. To validate the biological relevance of the observed enrichment, several statistical parameters were used: Z-score, P-value, adjusted P-value (P.adjust), and Q-value. The Z-score measures how far the observed enrichment deviates from a random distribution.

In this analysis, the Z-scores are 6.78 (hsa05010) and 6.07 (hsa05022). These are considered statistically significant, the score above 6 represents very strong evidence, as the Z-scores above 2 are generally considered statistically significant (Andrade, 2021). The P-Values quantifies the probability of observing such enrichment under the assumption of null hypothesis . The P-values of 0.00127 and 0.00263 for the two pathways indicate that there is a 0.127% and 0.263% chance of obtaining such results by random chance, providing strong evidence of the null hypothesis (Chen et al., 2023). The adjusted P-value addresses the problem of multiple testing. Since many pathways are tested, the likelihood of false positives increases (Chicco & Agapito, 2022). Here, P.adjust values of 0.0105 for both pathways suggest that the results remain statistically significant. The Q-value on the other hand, estimates the False Discovery Rate (FDR) is 0.0041 for both pathways (Lai, 2017). This indicates that only 0.41% of the pathways found significant might actually be false positives. Q-value below 0.05 is generally accepted as high confidence (Li et al., 2021).

As seen in Figure 2. Detailed KEGG pathway illustration of hsa05010 was shown above, whereas molecular pathways and cellular effects of various mutated genes due to methylation involved in Alzheimer disease were shown in this picture. In relation to the results obtained by DMR analysis, mutation in Presenillin 1 (PSEN1) was shown to be involved in several parts of the pathways as seen in the red colored writing above. Whereas, mutations in PSEN1 was observed to affect the synthesis of γ-secretase complex, this was validated by the fact that mutations in PSEN1 functioned as a catalytic subunit in the γ-secretase which cleaved the APP (Amyloid precursor protein), thus generating amyloid beta plaques variants such as, aggregation prone (Aβ42), a classic hallmark of Alzheimer's disease (Do et al., 2023). In addition, mutations in PSEN1 was also known to affect the calcium signalling pathway, promoting stress in the endoplasmic reticulum (ER), this was supported by the fact that these mutations in PSEN1 affected calcium exchange pump in the ER by binding towards the sarco/endoplasmic reticulum calcium-ATPase (SERCA) ; Furthermore, mutations in PSEN1 may also even act as a leakage channel (Deaton & Johnson, 2021).

Lastly, these mutations in PSEN1 may impair axonal transport, this is proven as these mutations was shown to induce defects in the axonal transport of organelles and vesicles through kinesin-1 phosphorylation, which was mediated by glycogen synthase kinase 3 (GSK-3) family (Guo et al., 2020). Consequently, disrupted axonal transport can contribute to neuronal dysfunction and degeneration, exacerbating the progression of Alzheimer's disease. Understanding the molecular mechanisms behind PSEN1 mutations may provide potential targets for therapeutic strategies aimed at preserving neuronal integrity.

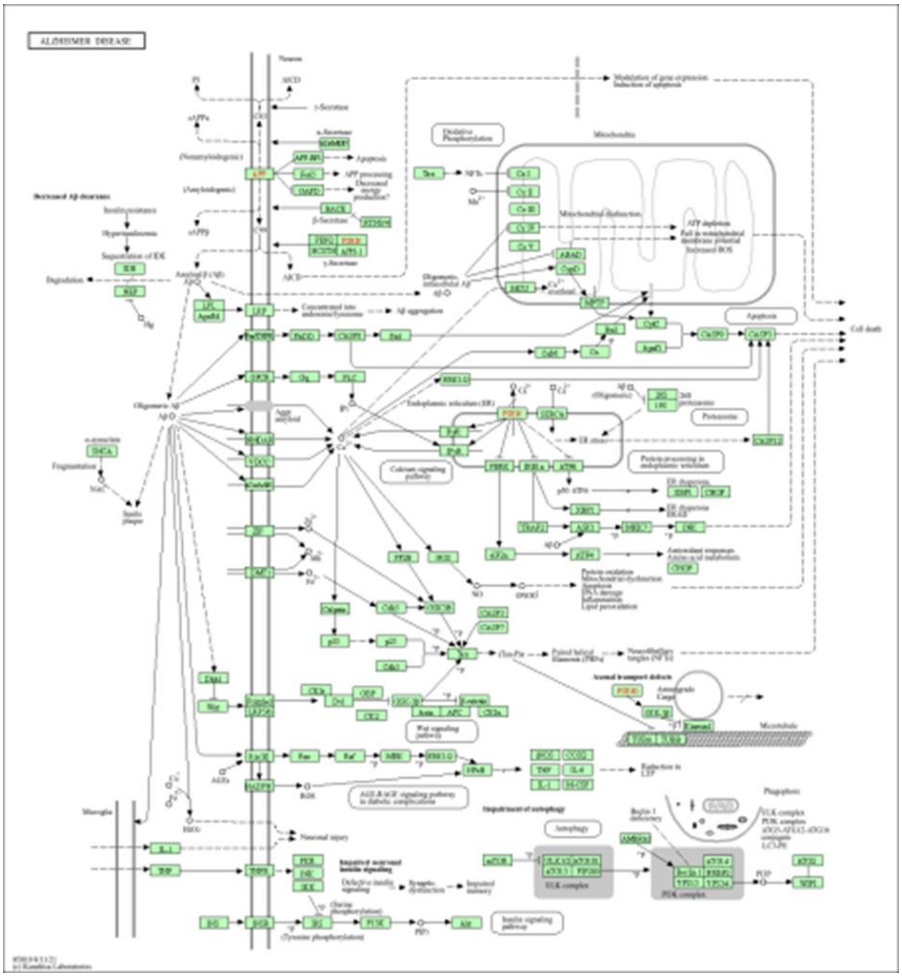


**Figure 2**. Alzheimer's Diseases Pathways of KEGG Pathway ID hsa05010

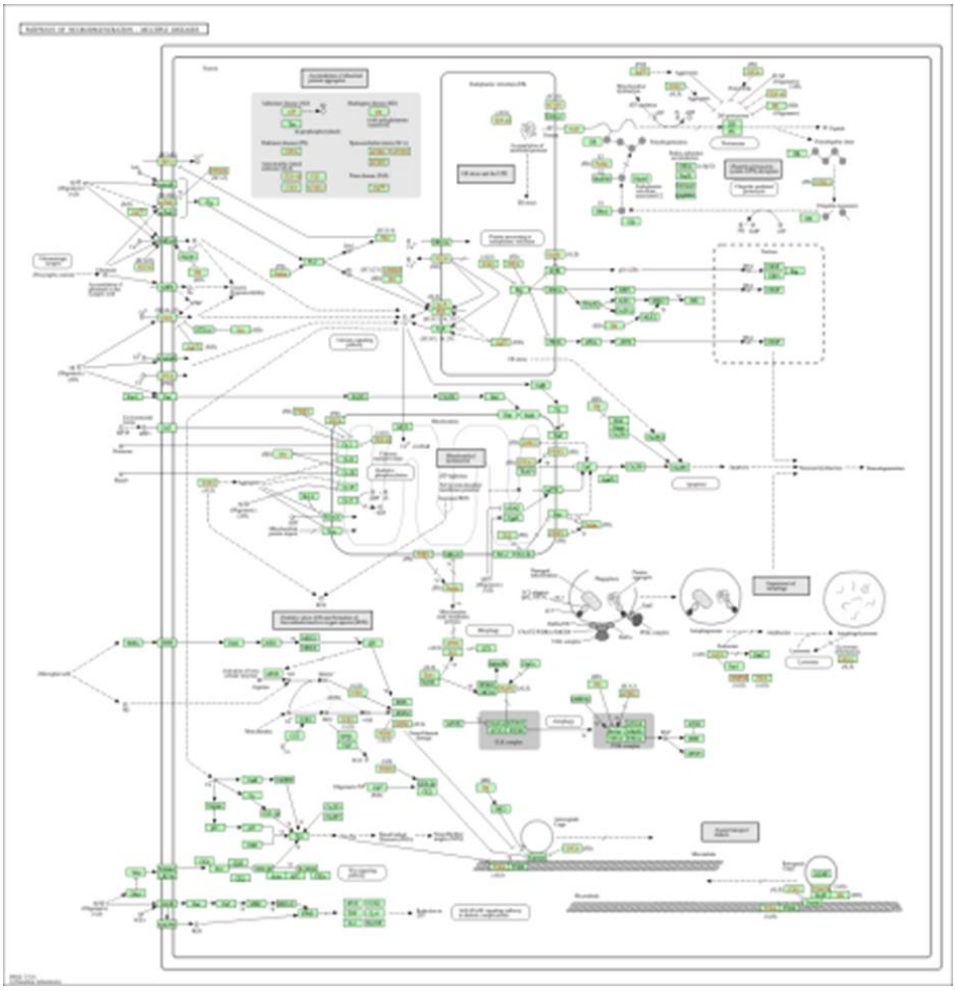


**Figure 3.** Alzheimer's Diseases Pathways of KEGG Pathway ID hsa05022

In Figure 3. Several critical KEGG pathways from hsa05022 influencing the pathology of several neuro-related diseases such as, Alzheimer disease, Parkinson disease, Amyotrophic lateral sclerosis, Huntington disease, Spinocerebellar ataxia (SCA) and Prion disease was shown above. Whereas, these pathways had validated the findings given in Figure 2., whereas mutations in PSEN1 was shown to affect calcium exchange in the endoplasmic reticulum through SERCA binding, disruption of axonal transport through GSK-3β mediated kinesin phosphorylation and amyloid beta plaque formation through APP cleavage.

Further analysis of the pathway has also shown the involvement of mutations in PSEN1 in the hyperphosphorylation of tau proteins, which was a known hall mark of Alzheimer's, this was evident by the fact that mutations in PSEN1 may induce PIK3K/Akt/GSK-3 mediated signalling of tau phosphorylation, thus inducing the formation of neurofibrillary tangles (Yang et al., 2023). However in both Figure 2. And Figure 3., the significance of mutations in MAPT, which is a significant pathway observed in DMR analysis, was not shown in the KEGG pathways analysis. This was due to the fact that mutations in MAPT had only been recently proven to affect the pathology of Alzheimer's whereas both MAPT mutation variants V337M and R406W produce tau filaments with Alzheimer fold, with V337M inducing triple tau filaments in Alzheimer's disease; Thus, increasing production of neurofibrillary tangles (Qi et al., 2024). Additionally other research had noted that inconsistencies were observed in the genetic association of MAPT mutations towards late-onset Alzheimer disease and incomplete assessment of MAPT mutation variants may also affect the lack of understanding in the role of MAPT mutations in the pathogenesis of Alzheimer's disease (Juan et al., 2019). Thus, further enrichment and research must be done in order to analyze the possible critical role of MAPT mutations in Alzheimer disease.

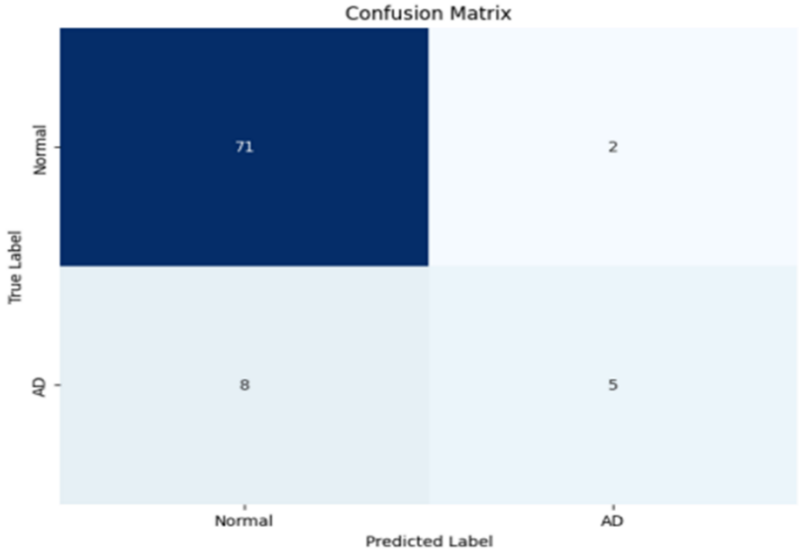



**Figure 4.** Machine learning Confusion Matrix

As shown in figure 4 it shows the confusion matrix that evaluates how well the machine learning model can distinguish between people with Alzheimer's Disease (AD) and people without Alzheimer Disease, which are normal patients. It can be visualised that the matrix in the group shows the true positives and true negative values. Based on the matrix, the model did a substantial job at identifying people who are normal, as shown it correctly predicted 71 out of 73 normal cases (which are called true negatives). Only 2 normal people were mistakenly predicted as having AD (which are called false positives), showing that the model has a high specificity, and also avoiding mistakes when it comes to healthy people.

Moreover, the model was able to find some people who had Alzheimer's. Out of 13 people with AD, it correctly detected 5 of them, being described as true positives. However, it didn't detect 8 people who had Alzhiemer's, and the model described it as normal (false negative). While this number is not significant, it still shows the model can recognize some signs of Alzheimer's.

One reason the model didn't perform well was because of the imbalanced information in the dataset in identifying the cases in Alzheimer's. In the data, there was an excess number of normal people when compared to Alzheimer's patients, which leads to the model learning far more about normal cases than Alzheimer's, and it can affect the decision-making and accuracy of the model itself (Albattah & Khan, 2025). Another reason that the model may predict wrong is due to the Braak stage assignment. While stages 0-2 is still too early to fulfill the clinical criteria for Alzheimer's dementia, they are still considered not normal. This factor may contribute to the positive instances mislabeled as negative, which could affect the model's performance (Rasmussen & Langerman, 2019).

## CONCLUSION

Overall, this study demonstrates the complex nature of AD by combining transcriptomic analysis based on artificial intelligence with epigenetic profiling to identify important molecular signatures and predictive patterns. The study also showed how differential methylation and gene expression patterns are important in the onset and progression of AD.

It specifically focused on genes that are strongly linked to the disease, including APP, PSEN1, APOE, MAPT, and TREM2.With the use of DMR analysis, both PSEN1 and MAPT genes have shown significant hyper and hypo-methylation patterns in Alzheimer's disease. Whereas further validation with the use of KEGG enrichment and pathway analysis have confirmed the role of these genes in molecular pathways involved in the Alzheimers such as, tau hyperphosphorylation, mitochondrial dysfunction, neuroinflammation and amyloid beta formation. There were several limitations encountered during this analysis whereas the results do not show the significance of other primary genes, thus indicating that these validated genes are less relevant. This is due to the small dataset size and lack of data variety. Thus, to solve this problem, future research needs to incorporate more datasets into the system in order to have a larger size and variance in the data. Therefore, decreasing biases towards certain genes.

In addition, the creation of a predictive model based on transcriptomic and clinical-demographic data was made possible by the complementary use of MLP. The model achieves moderate sensitivity for classifying AD and high specificity for detecting normal cases, despite certain limitations brought on by class imbalance. Although enhancements in sample balance and feature enrichment are required for clinical applicability, this implies its potential for early screening.

Finally, the integration of epigenetic knowledge with AI-driven prediction enhances the understanding of AD biology and lays the groundwork for the development of future, more complete tools. However, several limitations must be acknowledged. The class imbalance, where normal cases greatly outnumber AD cases affect the predictive model in identifying true AD cases. Additionally, although the model includes transcriptomic and clinical features, predictive accuracy could be increased by further enriching it with additional biomarkers or omics data. The findings have also not yet been verified on independent or external datasets which further limit the robustness of the findings. Moreover, the model absence of longitudinal data and real world clinical testing limits its applicability. Therefore, future studies should also concentrate on expanding the sample, verifying findings in different cohorts, incorporating longitudinal data, and examining real clinical settings.

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