

INVESTIGATING AND BIOCHEMICAL BASIS OF NEURODEGENERATIVE DISORDERS

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Submitted towards the Requirements for the Award of Degree of

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Submitted By

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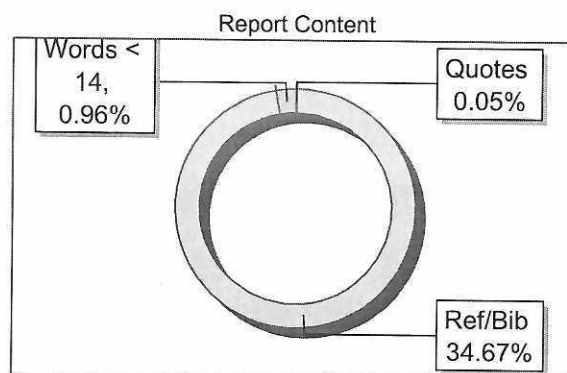
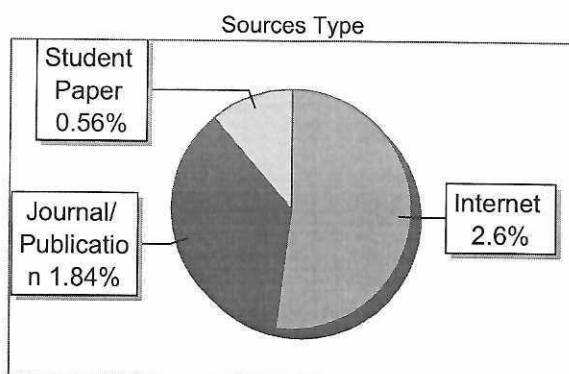
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ABSTRACT

Parkinson's disease (PD) and Alzheimer's disease (AD) are progressive neurodegenerative disorders characterized by neuronal dysfunction, neuroinflammation, oxidative stress, and impaired cellular homeostasis. MicroRNAs (miRNAs), which regulate gene expression at the post-transcriptional level, have emerged as important contributors to the pathogenesis of these disorders. The present study investigated miRNA-mediated regulatory mechanisms in PD and AD using peripheral blood mononuclear cells (PBMCs) as a minimally invasive and clinically relevant model system. PBMCs were isolated using Ficoll-Paque density gradient centrifugation, and total RNA was extracted using the TRIzol method. RNA quality and integrity were confirmed through agarose gel electrophoresis and spectrophotometric analysis. High-throughput miRNA sequencing was performed, followed by differential expression analysis, target gene prediction, functional enrichment, pathway analysis, network construction, and biomarker evaluation. The results revealed distinct miRNA expression profiles in PD and AD compared with healthy controls, indicating disease-specific molecular signatures. Several dysregulated miRNAs were associated with key pathological processes, including neuroinflammation, oxidative stress, apoptosis, synaptic dysfunction, and mitochondrial impairment. Functional enrichment analysis identified significant involvement of neurological disease pathways, particularly SMAD2/3, PI3K-Akt, MAPK/ERK, and Neuregulin signalling pathways. Target gene analysis demonstrated extensive post-transcriptional regulation, especially in PD, suggesting complex molecular rewiring associated with disease progression. Network analysis identified hub miRNAs that may serve as central regulators of multiple disease-related pathways. Receiver Operating Characteristic (ROC) curve analysis demonstrated excellent diagnostic performance of the identified miRNA signatures, with upregulated miRNAs showing an area under the curve (AUC) of approximately 0.99 and downregulated miRNAs exhibiting an AUC of approximately 0.88. Furthermore, ten potential miRNA biomarkers were identified with strong associations to neurodegenerative processes and significant translational potential. The present study demonstrates that miRNA dysregulation plays a critical role in the molecular pathogenesis of PD and AD. The identified miRNA signatures provide valuable insights into disease mechanisms and hold promise as non-invasive biomarkers and potential therapeutic targets for neurodegenerative disorders.

Keywords: Parkinson's disease, Alzheimer's disease, microRNA, PBMCs, neurodegeneration, biomarkers, neuroinflammation, pathway analysis.

INTRODUCTION

Neurodegenerative diseases constitute a major and growing global health burden, characterized by progressive loss of neuronal structure and function, ultimately leading to cognitive and motor impairments (S. Wang *et al.*, 2024). Among these disorders, Parkinson’s disease (PD) and Alzheimer’s disease (AD) are the most prevalent, affecting millions of individuals worldwide and posing significant challenges to healthcare systems. Parkinson’s disease is primarily associated with the degeneration of dopaminergic neurons in the substantia nigra, resulting in motor symptoms such as tremors, rigidity, and bradykinesia (Z. D. Zhou *et al.*, 2023). In contrast, Alzheimer’s disease is characterized by progressive cognitive decline, memory loss, and behavioral changes, largely attributed to the accumulation of amyloid-beta plaques and neurofibrillary tangles (Tenchov *et al.*, 2024).

Despite extensive research, the precise molecular mechanisms underlying these diseases remain incompletely understood due to their multifactorial and heterogeneous nature, involving complex interactions between genetic, epigenetic, and environmental factors. In recent years, advances in molecular biology and high-throughput sequencing technologies have significantly enhanced our ability to investigate the underlying mechanisms of neurodegenerative diseases. Among these advances, the study of non-coding RNAs, particularly microRNAs (miRNAs), has gained considerable attention (Nemeth *et al.*, 2024). MicroRNAs are short, endogenous RNA molecules that regulate gene expression at the post-transcriptional level by targeting messenger RNAs for degradation or translational repression (He & Hannon, 2004). In the context of Parkinson’s disease, miRNAs have been shown to influence key biological processes such as neuronal survival, oxidative stress response, mitochondrial function, and protein aggregation. Importantly, miRNAs do not function independently; rather, they participate in intricate regulatory networks that control multiple genes and pathways simultaneously (K. Liu & Cai, 2025). Therefore, understanding the system-level involvement of miRNAs in Parkinson’s disease is crucial for uncovering the complex regulatory mechanisms that drive disease progression.

Parallel to the study of miRNAs, epigenetic modifications have emerged as critical contributors to the pathogenesis of Alzheimer's disease. Epigenetics encompasses reversible and heritable changes in gene expression that occur without alterations in the DNA sequence, including DNA methylation, histone modifications, and chromatin remodeling (Lacal & Ventura, 2018). These modifications play a vital role in regulating neuronal gene expression and maintaining brain homeostasis. In Alzheimer's disease, aberrant epigenetic patterns have been associated with dysregulation of genes involved in synaptic function, inflammation, and amyloid processing (Tiwari *et al.*, 2025). Unlike genetic mutations, epigenetic changes are dynamic and can be influenced by environmental and lifestyle factors, making them promising targets for therapeutic intervention. The integration of epigenetic data within the framework of the human interactome representing the complete set of molecular interactions in a biological system provides a comprehensive approach to understanding how these modifications impact cellular networks and contribute to disease pathology (Chen & Tyagi, 2020). The concept of the human interactome has become increasingly important in systems biology, particularly in the study of complex diseases such as Alzheimer's. By analyzing protein-protein interaction networks and gene regulatory circuits, researchers can identify key hubs and pathways that are disrupted in disease conditions (Akbarzadeh *et al.*, 2024).

Computational approaches enable the integration of diverse datasets, including genomic, transcriptomic, and epigenomic information, to construct detailed interaction networks (Morabito *et al.*, 2025). In Alzheimer's disease, such analyses can reveal how epigenetic alterations influence network topology and lead to the dysregulation of critical biological processes (X. Gao *et al.*, 2022). This systems-level perspective allows for the identification of potential biomarkers and therapeutic targets that may not be apparent through traditional reductionist approaches. Another important dimension in neurodegenerative disease research is transcriptomic analysis RNA sequencing (RNA-Seq), which provides a comprehensive snapshot of gene expression across the genome (Lake *et al.*, 2021). Comparative analysis of RNA-Seq data from different tissue types, particularly brain and peripheral blood samples, has emerged as a powerful strategy for identifying disease-associated molecular signatures in Parkinson's disease (Jin *et al.*, 2021). While brain tissue offers direct insights into the affected organ, its accessibility is limited, especially in living patients. Blood-based transcriptomic profiling, on the other hand, provides a minimally invasive alternative for monitoring disease progression and

identifying potential biomarkers (Mohamed *et al.*, 2025). By comparing gene expression patterns between brain and blood samples, it is possible to identify overlapping and distinct molecular changes, thereby enhancing our understanding of systemic and tissue-specific aspects of Parkinson's disease.

Despite these significant advancements, several critical research gaps remain in the current understanding of neurodegenerative diseases. Although numerous studies have identified individual miRNAs associated with Parkinson's disease, there is a lack of comprehensive system-level analyses that integrate miRNA regulation with gene expression and protein interaction networks (Shaheen *et al.*, 2024). Most existing studies focus on isolated molecular components, failing to capture the complexity of regulatory interactions that drive disease progression. This limitation underscores the need for integrative approaches that consider the collective behavior of miRNAs within the broader molecular network (Fan *et al.*, 2025). While epigenetic alterations in Alzheimer's disease have been widely reported, there is insufficient integration of epigenetic data with the human interactome to fully understand how these modifications influence network dynamics. Current research often examines epigenetic changes in isolation, without considering their impact on protein-protein interactions and signalling pathways.

As a result, the systems-level consequences of epigenetic dysregulation in Alzheimer's disease remain poorly understood (Yücel & Gladyshev, 2026). Addressing this gap requires the application of computational models that can integrate epigenetic information with interactome data to identify key regulatory nodes and pathways (Agamah *et al.*, 2022). Although RNA-Seq studies have provided valuable insights into gene expression changes in Parkinson's disease, comparative analyses between brain and blood samples are still limited. Many studies focus on a single tissue type, which restricts the ability to identify robust and clinically relevant biomarkers. Furthermore, there is a lack of integrative analyses that combine transcriptomic data with miRNA and epigenetic profiles (C. Xu *et al.*, 2017).

Such integration is essential for understanding the multilayered regulation of gene expression and for identifying consistent molecular signatures across different biological compartments. Finally, while neurodegenerative diseases are a major focus of contemporary research, there is a need for more comprehensive and integrative frameworks that combine multiple omics approaches with advanced computational

methods. The complexity of these diseases necessitates a shift from traditional single-layer analyses to systems biology approaches that can capture the interplay between various molecular factors (Fischer *et al.*, 2025). Bridging this gap will not only enhance our understanding of disease mechanisms but also facilitate the development of more effective diagnostic and therapeutic strategies.

In light of these gaps, the present study aims to adopt an integrative and systems-level approach to investigate neurodegenerative diseases. Specifically, it focuses on the system-level involvement of microRNAs in Parkinson's disease, the epigenetic landscape of Alzheimer's disease within the human interactome, and the comparative analysis of RNA-Seq data from brain and blood samples in Parkinson's disease. By combining these approaches, the study seeks to provide a comprehensive understanding of the molecular mechanisms underlying neurodegeneration and to identify potential biomarkers and therapeutic targets. This integrative framework is expected to contribute significantly to the advancement of neurodegenerative disease research and to address the existing limitations in the field.

REVIEW OF LITERATURE

Neurodegenerative disorders constitute a broad and complex group of diseases characterized by the progressive loss of neuronal structure, function, and ultimately viability (Gadhav *et al.*, 2024). These conditions primarily affect the central nervous system and are typically marked by a gradual decline in cognitive, motor, and behavioral functions. Unlike acute neurological injuries, neurodegenerative diseases develop insidiously and worsen over time, often leading to severe disability and death. The most prominent disorders within this category include Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, and amyotrophic lateral sclerosis (ALS), each of which presents with distinct clinical features but shares underlying biochemical and molecular mechanisms (Gadhav *et al.*, 2024). The increasing prevalence of these conditions, particularly in aging populations, has made them a major focus of biomedical research and public health concern. The global burden of neurodegenerative diseases has risen dramatically over recent decades, largely due to increased life expectancy and demographic shifts toward older populations. These disorders not only affect millions of individuals worldwide but also place a substantial emotional, social, and economic strain on families, caregivers, and healthcare systems (Moitra *et al.*, 2023). Alzheimer’s disease, for instance, is the leading cause of dementia and accounts for a significant proportion of cognitive decline in elderly individuals, while Parkinson’s disease is the second most common neurodegenerative disorder, primarily affecting motor function. Huntington’s disease, though less prevalent, is a genetically inherited condition with devastating neurological consequences, and ALS is known for its rapid progression and fatal outcome due to motor neuron degeneration (Andhale & Shrivastava, 2022). Despite differences in etiology and symptomatology, these diseases share common pathological hallmarks, including neuronal loss, abnormal protein accumulation, and disruption of cellular homeostasis.

2.1 Mechanistic Insights into Neurodegenerative Diseases

At the core of neurodegenerative processes lies a complex interplay of biochemical events that contribute to neuronal dysfunction and death (Sanghai &

Tranmer, 2023). One of the most significant features observed across many of these disorders is the accumulation of misfolded or aggregated proteins. These abnormal proteins interfere with normal cellular processes, impair synaptic communication, and trigger a cascade of toxic events within neurons (Rinauro *et al.*, 2024). For example, the aggregation of amyloid-beta peptides and tau proteins is central to Alzheimer's disease pathology, while alpha-synuclein accumulation plays a key role in Parkinson's disease. Similarly, mutant huntingtin protein is responsible for neuronal damage in Huntington's disease (Rinauro *et al.*, 2024). These proteinopathies highlight the importance of understanding protein folding, degradation pathways, and cellular quality control systems in maintaining neuronal integrity (Koszła & Sołek, 2024).

In addition to protein aggregation, oxidative stress is another critical biochemical factor implicated in neurodegeneration. Neurons are highly susceptible to oxidative damage due to their high metabolic activity and reliance on mitochondrial function for energy production (Wen *et al.*, 2025)). The excessive generation of reactive oxygen species (ROS), coupled with impaired antioxidant defense mechanisms, leads to damage of cellular components such as lipids, proteins, and DNA. This oxidative imbalance not only contributes directly to neuronal injury but also exacerbates other pathological processes, including inflammation and mitochondrial dysfunction (Wen *et al.*, 2025). Consequently, oxidative stress is considered both a cause and a consequence of neurodegenerative progression. Mitochondrial dysfunction further amplifies neuronal vulnerability in these disorders. As the primary source of cellular energy, mitochondria play a crucial role in maintaining neuronal survival and function. Impairments in mitochondrial activity result in decreased ATP production, disrupted calcium homeostasis, and increased production of reactive oxygen species. These alterations compromise neuronal metabolism and promote apoptotic pathways, ultimately leading to cell death (Wen *et al.*, 2025). Emerging evidence suggests that mitochondrial abnormalities may occur early in the disease process, indicating their potential role in the initiation as well as progression of neurodegenerative conditions (Wen *et al.*, 2025).

Another important aspect of neurodegenerative diseases is neuroinflammation, which involves the activation of the brain's immune cells, particularly microglia and astrocytes (Zhang *et al.*, 2023). While acute inflammatory responses can be protective, chronic neuroinflammation contributes to neuronal damage through the sustained release of pro-inflammatory cytokines, chemokines, and other toxic mediators. This prolonged

inflammatory state creates a hostile microenvironment within the brain, further accelerating neurodegenerative processes. The interaction between inflammation, oxidative stress, and protein aggregation forms a vicious cycle that perpetuates neuronal injury and disease progression (Dash *et al.*, 2025).

Genetic and epigenetic factors also play a significant role in the development and progression of neurodegenerative disorders (Migliore & Coppedè, 2009). Certain forms of these diseases are inherited and linked to specific genetic mutations, such as those affecting amyloid precursor protein in Alzheimer's disease or the huntingtin gene in Huntington's disease (Orobets & Karamyshev, 2023). However, the majority of cases are sporadic and involve a combination of genetic susceptibility and environmental influences. Epigenetic modifications, including DNA methylation and histone modification, regulate gene expression without altering the DNA sequence and have been increasingly recognized as important contributors to disease mechanisms (Mohana Devi *et al.*, 2021). These changes can influence key pathways involved in neuronal survival, inflammation, and protein metabolism. Furthermore, disruptions in neurotransmitter systems are commonly observed in neurodegenerative diseases and contribute significantly to clinical symptoms ((Teleanu *et al.*, 2022)). For instance, the loss of cholinergic neurons in Alzheimer's disease leads to cognitive impairment, while degeneration of dopaminergic neurons in Parkinson's disease results in motor dysfunction. Similarly, glutamate-mediated excitotoxicity has been implicated in neuronal death across several disorders. Understanding these biochemical alterations is essential for developing targeted therapeutic strategies aimed at restoring neurotransmitter balance and improving neuronal function.

In recent years, significant advancements have been made in identifying biomarkers and developing diagnostic tools for neurodegenerative diseases (Hansson, 2021). Biochemical markers in cerebrospinal fluid and blood, along with advanced neuroimaging techniques, have improved early detection and disease monitoring. Despite these advances, effective treatments that can halt or reverse disease progression remain limited (Selvarajah *et al.*, 2023). Most current therapies are symptomatic and do not address the underlying biochemical causes of neuronal degeneration.

This highlights the urgent need for a deeper understanding of the molecular and biochemical basis of these disorders to facilitate the development of disease-modifying

interventions. This chapter aims to provide a comprehensive review of the existing literature on the biochemical mechanisms underlying neurodegenerative disorders. It explores key molecular pathways, including protein aggregation, oxidative stress, mitochondrial dysfunction, and neuroinflammation, as well as the role of genetic and environmental factors.

By synthesizing current research findings, this review seeks to identify gaps in knowledge and highlight potential directions for future studies. A thorough understanding of these complex biochemical processes is essential for advancing diagnostic approaches, improving therapeutic strategies, and ultimately reducing the global burden of neurodegenerative diseases.

2.2 Overview of Neurodegenerative Disorders

Neurodegenerative disorders constitute a diverse group of chronic, progressive conditions that primarily affect the structure and function of neurons in the central and peripheral nervous systems (Chand & Subramanian, 2024). These disorders are marked by the gradual and irreversible loss of neuronal cells, which leads to a decline in neurological function over time (Gadhav *et al.*, 2024). Unlike acute neurological injuries, such as stroke or trauma, neurodegenerative diseases typically develop insidiously and worsen steadily, often over years or decades. A central pathological hallmark shared by many of these conditions is the abnormal accumulation of misfolded or aggregated proteins within neurons or in the extracellular space (Gadhav *et al.*, 2024). These protein aggregates disrupt essential cellular processes, including protein degradation pathways, mitochondrial function, axonal transport, and synaptic communication. As a result, neuronal dysfunction progresses to cell death, ultimately leading to widespread impairment of brain and nervous system functions ((Moujalled *et al.*, 2021)). The impact of these diseases extends beyond the biological level, affecting cognitive abilities, motor skills, emotional regulation, and overall quality of life, often placing a significant burden on patients, caregivers, and healthcare systems. The neurodegenerative diseases emphasize both their progressive nature and their association with specific pathological mechanisms (Gadhav *et al.*, 2024). Neuronal loss is not random but tends to follow distinct patterns depending on the disease, often targeting particular types of neurons or neural circuits (Gorman, 2008). For example, some disorders predominantly affect cortical neurons involved in higher cognitive functions,

while others target subcortical or spinal motor neurons responsible for movement. In addition to neuronal death, other pathological features such as neuroinflammation, oxidative stress, and impaired cellular repair mechanisms contribute to disease progression. Importantly, many neurodegenerative diseases exhibit selective vulnerability, meaning that certain neuronal populations are more susceptible to degeneration than others (Fu *et al.*, 2018). This selectivity helps explain why different disorders present with distinct clinical symptoms, even though they may share common underlying mechanisms such as protein misfolding and aggregation.

Classification of neurodegenerative disorders is essential for understanding their heterogeneity and guiding diagnosis, research, and treatment strategies (Perezcano & Pérez-Coria, 2026). One widely used classification approach is based on clinical manifestations, which broadly divides these conditions into cognitive disorders and motor disorders (Reed *et al.*, 2019). Cognitive neurodegenerative diseases primarily affect mental processes such as memory, attention, language, and executive function (Hugo & Ganguli, 2014). Patients with these conditions often experience progressive memory loss, confusion, impaired judgment, and changes in personality or behaviour (Tenchov *et al.*, 2024). In contrast, motor neurodegenerative disorders predominantly impair movement, leading to symptoms such as tremors, muscle rigidity, bradykinesia (slowness of movement), loss of coordination, and muscle weakness (Tanner & Ostrem, 2024). Although this distinction is useful, it is important to note that many neurodegenerative diseases exhibit overlapping features. For instance, a disorder that initially presents with motor symptoms may later involve cognitive decline, and vice versa, reflecting the spread of pathology to multiple brain regions over time. Another important basis for classification is the specific brain regions or neural systems that are most affected by the degenerative process. Different neurodegenerative diseases target distinct anatomical areas, and this regional specificity plays a critical role in determining the clinical presentation (Winalai & Bicout, 2026). For example, degeneration of the hippocampus and cerebral cortex is closely associated with memory impairment and cognitive decline, as these regions are crucial for learning and information processing. On the other hand, disorders that primarily affect the basal ganglia and related motor circuits result in movement abnormalities, since these structures are essential for the regulation and coordination of voluntary movements (Mattis & Rabinovitz, 2014). Similarly, degeneration of the cerebellum can lead to problems with balance and

coordination, while involvement of the spinal cord and motor neurons may cause muscle weakness and paralysis (Irani, 2021). Understanding which brain regions are affected not only aids in diagnosis but also provides insight into disease mechanisms and progression, as well as potential targets for therapeutic intervention.

A third major classification framework focuses on the type of protein aggregates that accumulate in the brain, which has become increasingly important with advances in molecular neuropathology. Many neurodegenerative diseases are now categorized as “proteinopathies,” reflecting the central role of abnormal protein folding and aggregation in their pathogenesis. For instance, some disorders are characterized by the accumulation of tau protein, which forms neurofibrillary tangles within neurons and disrupts microtubule stability and intracellular transport. Others are associated with extracellular deposits of amyloid-beta peptides, which form plaques that interfere with neuronal communication and trigger inflammatory responses (Jankovska *et al.*, 2020). Another group of diseases involves the aggregation of alpha-synuclein, a protein that forms intracellular inclusions known as Lewy bodies, contributing to synaptic dysfunction and neuronal death. Each type of protein aggregate is associated with distinct biochemical properties, patterns of spread, and clinical features, allowing for more precise classification and diagnosis (Kraus *et al.*, 2019). Moreover, the identification of these protein signatures has opened new avenues for the development of targeted therapies aimed at preventing protein misfolding, enhancing clearance mechanisms, or blocking the toxic effects of aggregates. In addition to these primary classification criteria, neurodegenerative diseases can also be distinguished based on genetic versus sporadic origins, age of onset, and rate of progression. Some disorders are strongly linked to inherited genetic mutations, while others arise sporadically due to a combination of environmental and genetic risk factors. Early-onset forms of certain diseases may progress more rapidly or exhibit different pathological features compared to late-onset forms (Hickman *et al.*, 2025). Furthermore, advances in neuroimaging, biomarker research, and molecular diagnostics have enabled more refined classification systems that integrate clinical, anatomical, and biochemical data. These multidimensional approaches are increasingly important for early detection, accurate diagnosis, and personalized treatment strategies.

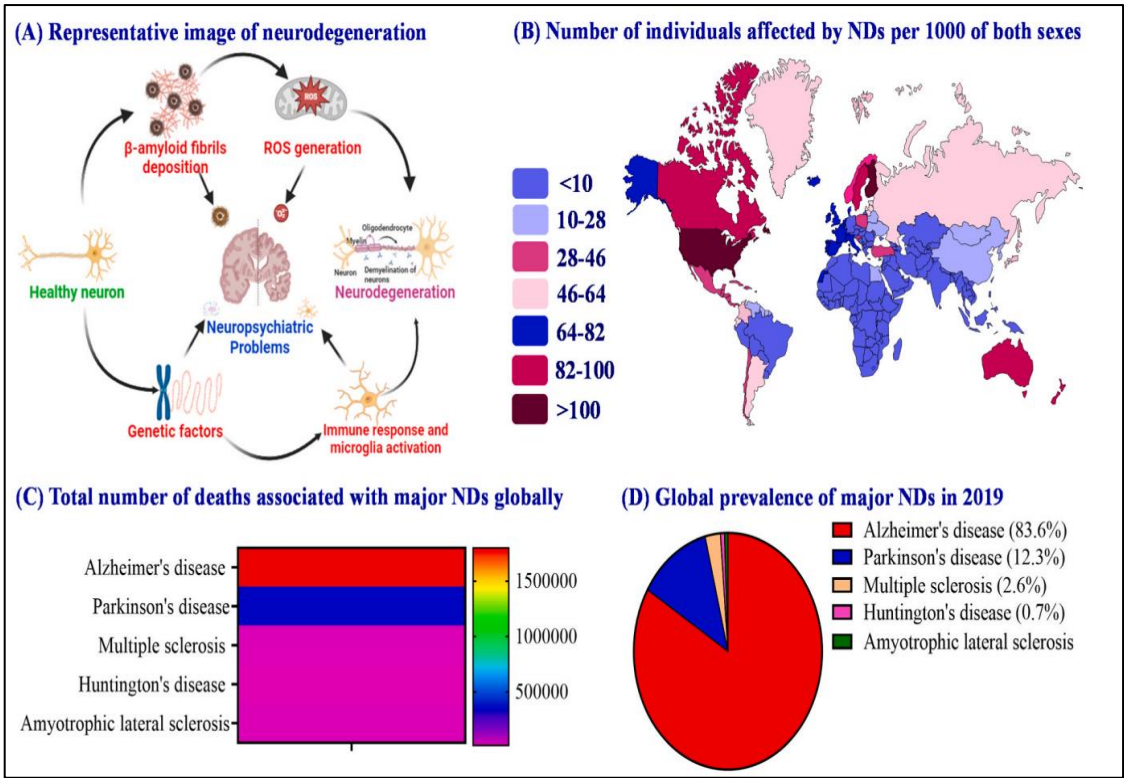


Figure 2.1: Statistical overview of neurodegenerative diseases (NDs) at both biological and global levels.

Figure 2.1 provides a comprehensive visual and statistical overview of neurodegenerative diseases (NDs) at both biological and global levels. Panel (A) illustrates a general representation of neurodegeneration, typically depicting progressive neuronal loss, synaptic dysfunction, and brain atrophy. This visual emphasizes that NDs are characterized by gradual deterioration of neurons and their connections, which ultimately impairs communication within the nervous system. Panel (B) presents a global map showing the incidence of NDs across different countries, expressed as the number of affected individuals per 100,000 population. The color gradient in the map reflects disease burden, with darker shades indicating higher prevalence rates. This type of geographic distribution highlights disparities between regions, often showing higher reported prevalence in aging populations and underdiagnosis in low-resource settings. Panel (C) illustrates the global mortality burden caused by major neurodegenerative diseases, providing a comparative breakdown of deaths attributed to different conditions. Panel (D) presents statistical data on the worldwide prevalence of major NDs as of 2019, offering insight into how widespread these disorders are and reinforcing their significance as a global health concern.

2.2.1 Epidemiology and Global Burden

The epidemiology of neurodegenerative disorders reflects a rapidly growing public health challenge, largely driven by demographic transitions, particularly the global increase in life expectancy (Wang *et al.*, 2024). Since advancing age is the most significant risk factor for most neurodegenerative diseases, the expansion of elderly populations worldwide has led to a marked rise in both incidence (new cases) and prevalence (total cases). These disorders are relatively uncommon in younger individuals but become increasingly prevalent after the age of 60, with risk rising exponentially in later decades of life (Fatima *et al.*, 2022). As healthcare systems improve and mortality from infectious diseases declines, more individuals survive into older age, thereby increasing the population at risk for neurodegenerative conditions. Among all neurodegenerative diseases, Alzheimer's disease is the most prevalent, accounting for the majority of dementia cases globally (Tahami Monfared *et al.*, 2022). It is estimated that tens of millions of people worldwide are living with Alzheimer's disease, and this number is expected to grow substantially in the coming decades. The disease is characterized by progressive cognitive decline, particularly in memory and executive function, and its prevalence doubles approximately every five years after the age of 65 (Monteiro *et al.*, 2023). Following Alzheimer's disease, Parkinson's disease is the second most common neurodegenerative disorder, primarily affecting motor function through symptoms such as tremors, rigidity, and bradykinesia. While less prevalent than Alzheimer's disease, Parkinson's disease still affects millions globally and is also strongly associated with aging, though it can occur earlier in some individuals.

The global burden of neurodegenerative diseases is not limited to prevalence alone but also includes significant impacts on mortality, disability, and quality of life (Wang *et al.*, 2024). These disorders are among the leading causes of disability-adjusted life years (DALYs), a measure that combines years of life lost due to premature death with years lived with disability (Ferrari *et al.*, 2024). Unlike many acute illnesses, neurodegenerative diseases often involve prolonged periods of progressive disability, requiring long-term medical care, rehabilitation, and social support. Patients may experience gradual loss of independence, necessitating assistance with daily activities such as eating, dressing, and mobility. This creates substantial emotional, physical, and financial strain not only on patients but also on caregivers and families. From a geographic perspective, the burden of neurodegenerative diseases is shifting increasingly

toward low- and middle-income countries (Kumar & Mirza, 2025). Historically, these disorders were more commonly reported in high-income nations due to longer life expectancies and better diagnostic capabilities. However, developing regions are now experiencing rapid population aging, often without corresponding improvements in healthcare infrastructure. As a result, a significant rise in the number of affected individuals is expected in countries across Asia, Africa, and Latin America. Limited access to early diagnosis, specialized care, and long-term management exacerbates the impact of these diseases in such settings (Ebi *et al.*, 2021). Furthermore, cultural factors, stigma, and lack of awareness may lead to underreporting and delayed treatment.

In addition to aging, several other epidemiological factors influence the distribution of neurodegenerative diseases. Genetic predisposition plays a role in certain cases, particularly in early-onset forms, but most cases are sporadic and influenced by a complex interplay of environmental and lifestyle factors (Motsinger-Reif *et al.*, 2024). These may include exposure to toxins, dietary habits, physical inactivity, cardiovascular health, and educational level (Sule *et al.*, 2025). For example, conditions such as hypertension, diabetes, and obesity have been linked to an increased risk of cognitive decline and dementia. Moreover, gender differences have been observed, with Alzheimer's disease being more common in women, possibly due to longer life expectancy and hormonal influences, while Parkinson's disease is slightly more prevalent in men. The economic burden associated with neurodegenerative diseases is immense and continues to rise (Feigin *et al.*, 2020). Costs include direct medical expenses such as hospitalization, medications, and long-term care facilities, as well as indirect costs related to lost productivity and informal caregiving. In many cases, family members provide unpaid care, which can lead to reduced workforce participation and financial hardship. As the number of affected individuals increases, healthcare systems worldwide face mounting pressure to allocate resources for diagnosis, treatment, and supportive services. This underscores the urgent need for effective public health strategies, including early detection, risk reduction, and the development of disease-modifying therapies.

2.3 Molecular and Biochemical Basis of Neurodegeneration

The molecular and biochemical basis of neurodegeneration centres on a complex interplay of cellular dysfunctions that ultimately lead to progressive neuronal damage and death (Sanghai & Tranmer, 2023). Although different neurodegenerative diseases

exhibit distinct clinical and pathological features, many share common underlying mechanisms at the molecular level. One of the most prominent features is the misfolding, aggregation, and accumulation of specific proteins within neurons or in the extracellular environment (Soto & Pritzkow, 2018). Under normal conditions, proteins are synthesized, folded into precise three-dimensional structures, and degraded when no longer needed. However, in neurodegenerative disorders, this tightly regulated process becomes disrupted, resulting in the formation of toxic protein aggregates that interfere with cellular homeostasis.

A key concept in this context is protein misfolding and aggregation. Proteins such as tau, amyloid-beta, and alpha-synuclein can adopt abnormal conformations that promote their aggregation into insoluble fibrils or inclusions (Louros *et al.*, 2023). These aggregates impair neuronal function by disrupting intracellular transport systems, damaging cellular membranes, and interfering with synaptic communication. In many cases, they also overwhelm the cell's protein quality control systems, including the ubiquitin-proteasome system and autophagy-lysosomal pathways, which are responsible for degrading damaged or misfolded proteins (Pohl & Dikic, 2019). As these clearance mechanisms fail, toxic proteins accumulate further, creating a vicious cycle that accelerates neuronal injury. Another critical biochemical mechanism is oxidative stress, which arises from an imbalance between the production of reactive oxygen species (ROS) and the cell's ability to neutralize them through antioxidant defenses (Garcia-Llorens *et al.*, 2025). Neurons are particularly vulnerable to oxidative damage due to their high metabolic activity and reliance on mitochondrial function for energy production. Excess ROS can damage lipids, proteins, and DNA, leading to impaired cellular function and activation of cell death pathways. Mitochondrial dysfunction plays a central role in this process, as damaged mitochondria not only produce more ROS but also fail to generate sufficient ATP, thereby compromising neuronal survival.

Neuroinflammation is also a significant contributor to neurodegeneration. The activation of microglia, the resident immune cells of the central nervous system, is a common feature in many neurodegenerative diseases (Gao *et al.*, 2023). While acute inflammation can be protective, chronic activation of microglia leads to the sustained release of pro-inflammatory cytokines, chemokines, and reactive species that exacerbate neuronal damage. This prolonged inflammatory state creates a toxic environment that further promotes protein aggregation and neuronal loss, highlighting the interplay

between immune responses and neurodegenerative processes (Zhang *et al.*, 2023). Disruption of cellular homeostasis extends to abnormalities in calcium signalling and excitotoxicity. Neurons rely on tightly controlled calcium levels for processes such as neurotransmitter release and synaptic plasticity. However, excessive calcium influx often due to overstimulation of glutamate receptors can trigger a cascade of harmful events, including activation of degradative enzymes, mitochondrial damage, and eventual cell death. This phenomenon, known as excitotoxicity, is particularly relevant in conditions where glutamate regulation is impaired. Another important biochemical aspect is the impairment of axonal transport and synaptic function. Neurons depend on efficient transport systems to move organelles, proteins, and signalling molecules along axons. Protein aggregates and cytoskeletal abnormalities can disrupt these transport pathways, leading to synaptic dysfunction and loss of connectivity between neurons (Cason & Holzbaur, 2022). Since synaptic loss is closely correlated with cognitive and motor deficits, this mechanism plays a crucial role in the clinical manifestations of neurodegenerative diseases.

Genetic and epigenetic factors also contribute significantly to the molecular basis of neurodegeneration (Yuan *et al.*, 2022). Mutations in specific genes can increase the production or aggregation propensity of pathological proteins, impair degradation pathways, or alter cellular stress responses (Tsoi *et al.*, 2023). In addition, epigenetic changes such as DNA methylation and histone modification can influence gene expression patterns in ways that promote disease progression. These molecular alterations may interact with environmental factors, such as toxins or lifestyle influences, further increasing susceptibility to neurodegeneration. Finally, programmed cell death pathways, including apoptosis and other forms of regulated cell death, are activated in response to the cumulative cellular damage (Qian *et al.*, 2024). As neurons lose their ability to maintain homeostasis, signalling pathways that trigger cell death become increasingly active, leading to the progressive loss of neuronal populations. Unlike some other cell types, neurons have limited regenerative capacity, making their loss largely irreversible.

2.3.1 Protein Misfolding and Aggregation

Protein misfolding and aggregation represent one of the central pathological mechanisms underlying a wide range of neurodegenerative disorders (Louros *et al.*,

2023). Under normal physiological conditions, proteins are synthesized as linear chains of amino acids that must fold into precise three-dimensional conformations to become functionally active. This folding process is highly regulated by cellular quality control systems, including molecular chaperones that assist in proper folding and proteolytic pathways that degrade damaged or misfolded proteins (Louros *et al.*, 2023). However, when this tightly controlled system fails, proteins may adopt abnormal conformations that expose hydrophobic regions, making them prone to aggregation. These misfolded proteins can accumulate within neurons or in the extracellular space, forming insoluble structures that disrupt cellular function and ultimately contribute to neuronal death (Soto & Pritzkow, 2018).

A hallmark feature of many neurodegenerative diseases is the presence of disease-specific protein aggregates. In Alzheimer's disease, two major types of abnormal protein deposits are observed: extracellular amyloid-beta plaques and intracellular tau neurofibrillary tangles (Gulisano *et al.*, 2018). Amyloid-beta is derived from the cleavage of a larger precursor protein and tends to aggregate into oligomers and fibrils that accumulate outside neurons. These aggregates interfere with synaptic communication, disrupt membrane integrity, and activate inflammatory responses. Meanwhile, tau protein, which normally stabilizes microtubules within neurons, becomes hyperphosphorylated and detaches from microtubules, forming twisted filamentous structures known as neurofibrillary tangles (Alonso *et al.*, 2018). This disrupts intracellular transport systems and contributes to neuronal dysfunction. In Parkinson's disease, the key pathological feature is the aggregation of alpha-synuclein, a presynaptic protein involved in neurotransmitter release (Calabresi *et al.*, 2023). Misfolded alpha-synuclein accumulates into intracellular inclusions called Lewy bodies, which are toxic to neurons, particularly in regions controlling movement such as the substantia nigra. These aggregates impair mitochondrial function, disrupt vesicular trafficking, and promote oxidative stress, leading to progressive degeneration of dopaminergic neurons (Lurette *et al.*, 2023). Similarly, in Huntington's disease, the presence of a mutant form of the huntingtin protein caused by an expanded CAG repeat in the gene leads to the formation of intracellular aggregates (Tabrizi *et al.*, 2020). These aggregates interfere with transcriptional regulation, protein degradation pathways, and neuronal signalling, ultimately causing selective neuronal loss, especially in the striatum. The process of protein aggregation typically follows a multistep pathway beginning with the formation

of misfolded monomers, which then assemble into soluble oligomers and eventually into insoluble fibrils and large aggregates (Ye *et al.*, 2022). Interestingly, growing evidence suggests that the smaller, soluble oligomeric forms of these proteins may be more toxic than the larger aggregates. These oligomers can interact with cellular membranes, form pore-like structures, and disrupt ion homeostasis, particularly calcium balance, leading to cellular dysfunction. Over time, the accumulation of both soluble and insoluble aggregates overwhelms the cell's capacity to maintain homeostasis (Waigi *et al.*, 2023).

One of the critical consequences of protein aggregation is the disruption of cellular proteostasis, or protein homeostasis. The cell relies on two major systems to manage protein quality: the ubiquitin-proteasome system and the autophagy-lysosomal pathway (Cohen-Kaplan *et al.*, 2016). The former degrades short-lived or misfolded proteins, while the latter is responsible for clearing larger protein aggregates and damaged organelles. In neurodegenerative diseases, these systems often become impaired, either due to genetic mutations, aging, or the toxic effects of accumulated proteins themselves. As a result, the clearance of misfolded proteins is reduced, leading to their progressive accumulation and further exacerbation of cellular stress (Hommen *et al.*, 2022). Protein aggregates also interfere with several essential cellular functions. They can disrupt cytoskeletal integrity, impair axonal transport, and hinder synaptic function (Verma *et al.*, 2024). Neurons, with their long axons and high metabolic demands, are particularly vulnerable to such disruptions (Beirowski, 2022). For instance, tau aggregation destabilizes microtubules, which are essential for transporting nutrients and organelles along axons (Cario & Berger, 2023). Similarly, alpha-synuclein aggregates can impair synaptic vesicle recycling, reducing neurotransmitter release and affecting neuronal communication (Gao *et al.*, 2023). These disruptions contribute directly to the cognitive and motor deficits observed in neurodegenerative diseases. Another important aspect of protein misfolding is its potential to propagate in a prion-like manner. Misfolded proteins can act as templates that induce the misfolding of normal proteins, thereby spreading pathology from one cell to another (Louros *et al.*, 2023). This mechanism helps explain the characteristic progression of neurodegenerative diseases, where pathology appears to spread through interconnected brain regions over time. For example, tau pathology in Alzheimer's disease and alpha-synuclein pathology in Parkinson's disease have been shown to follow predictable patterns of spread, correlating with disease progression and symptom severity. In addition to their direct toxic effects, protein

aggregates can trigger secondary pathological processes such as oxidative stress and neuroinflammation (Grune, 2020). The presence of aggregates activates microglia and astrocytes, leading to the release of pro-inflammatory cytokines and reactive oxygen species. This creates a harmful environment that further damages neurons and promotes additional protein misfolding. Thus, protein aggregation is both a cause and a consequence of broader cellular dysfunction, forming a self-reinforcing cycle of neurodegeneration (Wu *et al.*, 2024). Genetic factors also play a significant role in protein misfolding and aggregation (Louros *et al.*, 2023). Mutations in genes encoding specific proteins can increase their tendency to misfold or aggregate. For example, mutations affecting amyloid precursor protein or tau can enhance amyloid-beta production or tau phosphorylation, respectively. In Huntington's disease, the expanded polyglutamine tract in the huntingtin protein directly promotes aggregation. Even in sporadic cases, age-related changes in protein folding capacity and degradation efficiency can predispose individuals to aggregation. Environmental factors such as toxins, oxidative stress, and metabolic disturbances may further exacerbate these processes.

2.3.2 Oxidative stress

Oxidative stress is a fundamental biochemical mechanism contributing to the pathogenesis of many neurodegenerative disorders (Liu *et al.*, 2025). It arises when there is an imbalance between the production of reactive oxygen species (ROS) and the ability of the cell's antioxidant defense systems to neutralize and eliminate these reactive molecules. ROS are highly reactive derivatives of oxygen, including free radicals such as superoxide anion and hydroxyl radicals, as well as non-radical species like hydrogen peroxide (Sies & Jones, 2020). Under normal physiological conditions, ROS are generated as byproducts of cellular metabolism particularly during mitochondrial oxidative phosphorylation and play important roles in cell signalling and homeostasis (Palma *et al.*, 2024). However, when their production exceeds the cell's capacity to detoxify them, ROS accumulate and begin to damage essential cellular components, ultimately leading to neuronal dysfunction and death.

One of the primary sources of ROS in neurons is the mitochondria, which are responsible for producing ATP through the electron transport chain (Nolfi-Donagan *et al.*, 2020). During this process, a small percentage of electrons can leak and react with oxygen to form superoxide radicals. In healthy cells, antioxidant systems such as

superoxide dismutase, catalase, and glutathione peroxidase rapidly convert these reactive species into less harmful molecules (Ighodaro & Akinloye, 2018). However, in neurodegenerative conditions, mitochondrial dysfunction often leads to increased electron leakage and excessive ROS generation (Wen *et al.*, 2025). Damaged mitochondria not only produce more ROS but also become less efficient at generating energy, creating a dual burden of oxidative damage and energy deficiency that severely compromises neuronal survival. In addition to increased ROS production, oxidative stress is exacerbated by impaired antioxidant defense systems. Neurons rely on both enzymatic and non-enzymatic antioxidants to maintain redox balance (Lee *et al.*, 2020). Enzymatic defenses include molecules like superoxide dismutase and catalase, while non-enzymatic defenses include glutathione, vitamin E, and vitamin C. In many neurodegenerative diseases, these protective systems are either overwhelmed or functionally impaired (Gadhav *et al.*, 2024). For example, reduced levels of glutathione a major intracellular antioxidant have been observed in affected brain regions, limiting the cell's ability to neutralize ROS. Furthermore, genetic mutations affecting antioxidant enzymes can further weaken the defense system, increasing susceptibility to oxidative damage.

The accumulation of ROS leads to widespread damage of cellular macromolecules, including lipids, proteins, and DNA (Juan *et al.*, 2021). Lipid peroxidation is one of the most significant consequences of oxidative stress, particularly in neurons, which have membranes rich in polyunsaturated fatty acids (Milatovic & Aschner, 2011). ROS attack these lipids, initiating chain reactions that compromise membrane integrity, fluidity, and function. This can disrupt ion gradients, impair receptor activity, and ultimately lead to cell death. Protein oxidation is another critical effect, where ROS modify amino acid side chains, alter protein structure, and impair enzymatic activity. Oxidized proteins may lose their function or become prone to aggregation, further contributing to cellular dysfunction and linking oxidative stress to protein misfolding mechanisms seen in neurodegenerative diseases (Grune, 2020). DNA damage caused by oxidative stress is equally detrimental. ROS can induce strand breaks, base modifications, and cross-linking in both nuclear and mitochondrial DNA. Mitochondrial DNA is particularly vulnerable due to its proximity to the electron transport chain and lack of protective histones. Damage to mitochondrial DNA impairs the synthesis of essential components of the respiratory chain, further exacerbating mitochondrial

dysfunction and ROS production. In the nucleus, DNA damage can disrupt gene expression and activate cell death pathways, including apoptosis. Although cells possess repair mechanisms to correct oxidative DNA damage, these systems may become less efficient with age or overwhelmed under conditions of chronic oxidative stress. Neurons are especially vulnerable to oxidative stress for several reasons. First, they have exceptionally high metabolic demands and rely heavily on oxidative phosphorylation for energy production, making them a major site of ROS generation (Wen *et al.*, 2025). Second, neuronal membranes are rich in lipids that are highly susceptible to peroxidation. Third, neurons have relatively limited regenerative capacity compared to other cell types, meaning that once damaged, they cannot be easily replaced. Additionally, neurons have lower levels of certain antioxidant enzymes compared to other tissues, further increasing their susceptibility to oxidative injury (Wen *et al.*, 2025). This combination of high ROS production, vulnerable cellular structures, and limited repair capacity makes oxidative stress a particularly potent driver of neurodegeneration. Oxidative stress also interacts closely with other pathological processes, amplifying neuronal damage. For instance, it can promote protein misfolding and aggregation by oxidizing proteins and impairing degradation pathways. It also plays a key role in neuroinflammation by activating microglia and astrocytes, which in turn release additional ROS and pro-inflammatory cytokines, creating a self-perpetuating cycle of damage. Moreover, oxidative stress contributes to excitotoxicity by affecting glutamate transport and receptor function, leading to excessive calcium influx and further mitochondrial damage.

Another important aspect of oxidative stress in neurodegeneration is its role in aging, which is the greatest risk factor for most neurodegenerative diseases. Over time, the efficiency of mitochondrial function declines, antioxidant defenses weaken, and cumulative oxidative damage increases (Xu *et al.*, 2025). This age-related decline in cellular resilience makes neurons more susceptible to stressors and accelerates the onset and progression of neurodegenerative conditions. Environmental factors such as exposure to toxins, pollutants, and radiation can further increase oxidative stress, highlighting the interplay between intrinsic and extrinsic factors in disease development.

2.3.3 Mitochondrial Dysfunction

Mitochondrial dysfunction is a central and unifying feature in the pathogenesis of many neurodegenerative disorders, reflecting the critical role that mitochondria play in

maintaining neuronal health and survival (Clemente-Suárez *et al.*, 2023). Mitochondria are often described as the “powerhouses” of the cell because they generate adenosine triphosphate (ATP) through oxidative phosphorylation, a process that supplies the energy required for nearly all cellular activities. In neurons, which have exceptionally high energy demands due to continuous synaptic activity, ion transport, and axonal signalling, efficient mitochondrial function is especially vital (Clemente-Suárez *et al.*, 2023). Beyond energy production, mitochondria are also involved in regulating calcium homeostasis, generating reactive oxygen species (ROS), and controlling apoptotic (programmed cell death) pathways. When mitochondrial function becomes impaired, these essential processes are disrupted, leading to a cascade of biochemical events that ultimately contribute to neuronal degeneration ((Kathiresan *et al.*, 2025)). One of the most significant consequences of mitochondrial dysfunction is reduced ATP production. The electron transport chain (ETC), located in the inner mitochondrial membrane, is responsible for generating ATP by transferring electrons through a series of protein complexes. In neurodegenerative diseases, defects in these complexes whether due to genetic mutations, oxidative damage, or age-related decline can impair electron flow and reduce ATP synthesis (Wen *et al.*, 2025). As a result, neurons experience an energy deficit that compromises their ability to maintain ion gradients, support synaptic transmission, and carry out essential metabolic processes. This energy failure is particularly detrimental in neurons because they rely almost exclusively on oxidative phosphorylation rather than glycolysis for energy production. Even slight reductions in ATP levels can disrupt neuronal function and make cells more susceptible to injury and death.

In addition to reduced energy production, dysfunctional mitochondria are a major source of increased ROS generation. Under normal conditions, mitochondria produce small amounts of ROS as byproducts of electron transport, which are neutralized by antioxidant systems ((Palma *et al.*, 2024)). However, when the ETC is impaired, electrons may leak prematurely and react with oxygen to form excessive ROS. This overproduction of ROS contributes to oxidative stress, damaging mitochondrial membranes, proteins, and DNA. Importantly, this creates a vicious cycle: damaged mitochondria produce more ROS, which in turn causes further mitochondrial damage (Palma *et al.*, 2024). This self-amplifying process accelerates neuronal injury and is a key link between mitochondrial dysfunction and other pathological mechanisms, such as oxidative stress and protein misfolding. Another critical aspect of mitochondrial dysfunction is the disruption of

calcium homeostasis. Mitochondria play a crucial role in buffering intracellular calcium levels by taking up and releasing calcium ions as needed. This function is essential for regulating processes such as neurotransmitter release, synaptic plasticity, and enzyme activity. However, when mitochondria become dysfunctional, their ability to regulate calcium is compromised. Excessive calcium accumulation within mitochondria can trigger the opening of the mitochondrial permeability transition pore, leading to loss of membrane potential, swelling of the organelle, and eventual release of pro-apoptotic factors such as cytochrome c. This initiates cell death pathways, contributing to the progressive loss of neurons observed in neurodegenerative diseases (Palma *et al.*, 2024). At the same time, elevated cytosolic calcium levels can activate harmful enzymes, including proteases and nucleases, further exacerbating cellular damage.

Mitochondrial DNA (mtDNA) mutations also play a significant role in the development and progression of neurodegenerative disorders (Mei *et al.*, 2025). Unlike nuclear DNA, mtDNA is located within the mitochondria and encodes essential components of the electron transport chain. It is particularly vulnerable to damage due to its proximity to ROS production sites and its lack of protective histones. Mutations in mtDNA can impair the synthesis of ETC proteins, leading to defective energy production and increased ROS generation (Wen *et al.*, 2025). Over time, the accumulation of mtDNA mutations can result in a population of dysfunctional mitochondria within neurons, further compromising cellular function. Both inherited and acquired mtDNA mutations have been implicated in various neurodegenerative conditions, highlighting the importance of mitochondrial genetics in disease pathology (Mei *et al.*, 2025). In addition to these core mechanisms, mitochondrial dysfunction affects several other aspects of neuronal health. For example, mitochondria are essential for axonal transport, as they must be distributed along the long neuronal processes to supply energy where it is needed most. Impaired mitochondrial movement along microtubules can lead to localized energy deficits at synapses, contributing to synaptic failure and loss of connectivity between neurons. Furthermore, mitochondria are involved in the regulation of apoptosis through the release of factors that activate caspases, the enzymes responsible for executing cell death (Wang & Youle, 2009). In neurodegenerative diseases, dysregulation of these pathways can lead to inappropriate activation of apoptosis, resulting in excessive neuronal loss. Mitochondrial dysfunction is also closely linked with other pathogenic processes, forming an interconnected network of cellular damage. For instance, oxidative

stress can damage mitochondrial components, while dysfunctional mitochondria generate more ROS, reinforcing oxidative injury (Chen *et al.*, 2024). Similarly, protein aggregates such as those seen in various neurodegenerative diseases can impair mitochondrial function by interfering with mitochondrial dynamics, including fission and fusion processes that are essential for maintaining mitochondrial integrity (Tsoi *et al.*, 2023). Neuroinflammation further exacerbates mitochondrial dysfunction by exposing neurons to inflammatory mediators that disrupt mitochondrial activity. This complex interplay highlights the central role of mitochondria as both a target and a driver of neurodegenerative processes. Age-related changes further amplify mitochondrial dysfunction. As individuals age, mitochondrial efficiency naturally declines, and the accumulation of oxidative damage increases. The capacity for mitochondrial repair and biogenesis (the formation of new mitochondria) also diminishes over time (Jia *et al.*, 2025). These changes reduce the resilience of neurons to stress and increase their vulnerability to degeneration. Environmental factors, such as exposure to toxins, pesticides, and heavy metals, can also impair mitochondrial function, providing an additional link between external risk factors and neurodegenerative disease development.

2.3.4 Neuroinflammation

Neuroinflammation refers to the immune response that occurs within the central nervous system (CNS) and is increasingly recognized as a central mechanism in the development and progression of neurodegenerative disorders (Kölliker-Frers *et al.*, 2021). Unlike peripheral inflammation, which involves a wide range of immune cells, neuroinflammation is primarily mediated by resident CNS cells, particularly microglia and astrocytes. Under normal conditions, these cells play protective and supportive roles: microglia act as immune sentinels that detect and remove pathogens or cellular debris, while astrocytes maintain neuronal homeostasis, regulate neurotransmitter levels, and support the blood-brain barrier (Garland *et al.*, 2022). However, in neurodegenerative diseases, these cells become chronically activated, leading to a sustained inflammatory response that contributes significantly to neuronal injury and disease progression. Microglial activation is one of the earliest and most prominent features of neuroinflammation. When triggered by factors such as protein aggregates, damaged neurons, or oxidative stress, microglia shift from a resting state to an activated phenotype (Gao *et al.*, 2023). In the short term, this activation can be beneficial, as microglia help

clear toxic proteins and dead cells through phagocytosis. However, chronic activation leads to a detrimental state in which microglia continuously release pro-inflammatory mediators, including cytokines (such as interleukin-1 β , tumor necrosis factor- α , and interleukin-6), chemokines, and reactive oxygen and nitrogen species. These substances, while intended to combat damage, can instead create a toxic microenvironment that exacerbates neuronal dysfunction and death (Kim *et al.*, 2024). Over time, the persistent release of these inflammatory factors contributes to a cycle of ongoing damage and immune activation.

Astrocytes also play a crucial role in neuroinflammation (Huang *et al.*, 2025). In response to injury or disease, astrocytes undergo a process known as reactive astrogliosis, characterized by changes in morphology, gene expression, and function. Reactive astrocytes can release inflammatory cytokines and contribute to the overall inflammatory milieu (Patani *et al.*, 2023). Additionally, they may lose some of their normal supportive functions, such as regulating extracellular glutamate levels and maintaining ion balance. This dysfunction can lead to excitotoxicity, where excessive glutamate overstimulates neurons and causes calcium overload, further contributing to neuronal damage. Astrocytes also influence the integrity of the blood-brain barrier, and their dysfunction can increase its permeability, allowing peripheral immune cells and inflammatory molecules to enter the brain and amplify the inflammatory response (Sobral *et al.*, 2025). One of the key consequences of chronic neuroinflammation is the sustained release of pro-inflammatory cytokines. These signalling molecules are essential for coordinating immune responses, but when produced in excess, they can have harmful effects on neurons (Starchenko & Lauffenburger, 2018). Pro-inflammatory cytokines can alter synaptic function, impair neurotransmission, and disrupt neural networks. They may also activate intracellular signalling pathways that lead to apoptosis or other forms of programmed cell death (Starchenko & Lauffenburger, 2018). Furthermore, cytokines can enhance the production and aggregation of misfolded proteins, linking neuroinflammation with other pathological processes such as protein aggregation and oxidative stress. This interconnectedness highlights how neuroinflammation not only contributes to neuronal damage but also amplifies other mechanisms of neurodegeneration. Neuroinflammation has a profound impact on synaptic function and plasticity, which are essential for learning, memory, and overall brain function (Kölliker-Frers *et al.*, 2021). Chronic inflammation can lead to synaptic loss, reduced synaptic

strength, and impaired communication between neurons. Microglia, in particular, play a role in synaptic pruning a normal developmental process where unnecessary synapses are eliminated. However, in disease states, this process may become dysregulated, leading to excessive removal of synapses and contributing to cognitive decline. The disruption of synaptic networks is closely associated with the clinical symptoms of many neurodegenerative diseases, including memory impairment and motor dysfunction (Li *et al.*, 2026).

An important shift in scientific understanding is the recognition that neuroinflammation is not merely a secondary response to neuronal damage but can act as a primary driver of neurodegeneration (Zhang *et al.*, 2023). Initially, it was believed that inflammation occurred only as a reaction to protein aggregates or dying neurons. However, accumulating evidence suggests that inflammatory processes can precede and even initiate neuronal damage (Zhang *et al.*, 2023). For example, chronic activation of microglia may occur in response to subtle changes in the brain environment, such as aging or metabolic disturbances, setting the stage for subsequent neurodegenerative changes. This perspective has significant implications for therapeutic strategies, as it highlights the potential of targeting inflammatory pathways to slow or prevent disease progression. The relationship between neuroinflammation and aging is particularly important. Aging is associated with a gradual increase in baseline inflammatory activity in the brain, a phenomenon sometimes referred to as “inflammaging” (Soraci *et al.*, 2024). This low-grade, chronic inflammation primes microglia and astrocytes to respond more aggressively to stimuli, increasing the likelihood of a sustained inflammatory response. As a result, older individuals are more susceptible to the harmful effects of neuroinflammation, which partly explains why neurodegenerative diseases are more common in the elderly population (Soraci *et al.*, 2024). Additionally, age-related declines in the ability to resolve inflammation further contribute to its persistence and detrimental effects. Neuroinflammation also interacts closely with other pathological processes involved in neurodegeneration (W. Zhang *et al.*, 2023). For instance, oxidative stress can activate microglia, while inflammatory mediators can increase the production of reactive oxygen species, creating a feedback loop that amplifies cellular damage. Similarly, protein aggregates can trigger inflammatory responses, and inflammation can promote further protein misfolding and aggregation (Louros *et al.*, 2023). Mitochondrial dysfunction, another key feature of neurodegeneration, can both result from and

contribute to inflammatory processes. These interactions form a complex network of self-reinforcing mechanisms that drive disease progression.

2.4 Genetic and Epigenetic Factors

2.4.1 Genetic Mutations

Genetic mutations play a crucial role in the development and progression of many neurodegenerative disorders, particularly in familial or early-onset forms of disease (Ardalan, 2023). These mutations typically affect genes involved in protein production, folding, trafficking, and degradation, thereby increasing the likelihood of abnormal protein accumulation and neuronal toxicity. While most neurodegenerative diseases are sporadic and influenced by a combination of genetic and environmental factors, the study of inherited mutations has provided critical insights into disease mechanisms. In many cases, these mutations alter the structure or processing of specific proteins, making them more prone to misfolding and aggregation processes that are central to neurodegeneration. In Alzheimer's disease, several key genes have been identified that are associated with early-onset familial forms of the disease (Andrade-Guerrero *et al.*, 2023). The APP (amyloid precursor protein) gene encodes a protein that, when cleaved, produces amyloid-beta peptides. Mutations in APP can increase the production of amyloid-beta or favor the formation of longer, more aggregation-prone forms of the peptide (Li *et al.*, 2019). These peptides accumulate extracellularly to form amyloid plaques, one of the pathological hallmarks of the disease. Similarly, mutations in PSEN1 (presenilin 1) and PSEN2 (presenilin 2) which encode components of the gamma-secretase enzyme complex responsible for APP cleavage can alter enzymatic activity, leading to increased generation of toxic amyloid-beta species (Hernandez-Sapiens *et al.*, 2022). These genetic alterations accelerate plaque formation and are strongly linked to early and aggressive disease progression.

In Parkinson's disease, mutations in genes such as SNCA and LRRK2 are among the most well-studied genetic contributors. The SNCA gene encodes alpha-synuclein, a protein involved in synaptic function and neurotransmitter release (Butler *et al.*, 2017). Mutations or gene duplications in SNCA can lead to overproduction or structural changes in alpha-synuclein, increasing its tendency to misfold and aggregate into Lewy bodies

within neurons. These aggregates disrupt normal cellular processes, including mitochondrial function and vesicle trafficking (Choi *et al.*, 2025).

The LRRK2 (leucine-rich repeat kinase 2) gene encodes a protein involved in multiple cellular pathways, including vesicular transport, cytoskeletal dynamics, and autophagy (Chen, 2018). Mutations in LRRK2 can enhance its kinase activity, leading to abnormal phosphorylation of target proteins and contributing to neuronal toxicity. Unlike some other genetic forms, LRRK2 mutations are also found in late-onset cases, making them particularly important in understanding both familial and sporadic Parkinson's disease. In Huntington's disease, the genetic basis is well-defined and involves a mutation in the HTT gene, which encodes the huntingtin protein (Anjum *et al.*, 2026). This mutation is caused by an abnormal expansion of CAG trinucleotide repeats within the gene, resulting in an elongated polyglutamine tract in the protein. The mutated huntingtin protein becomes prone to misfolding and aggregation, forming intracellular inclusions that interfere with essential cellular processes such as transcription, protein degradation, and mitochondrial function (Shaha & Godad, 2026). Huntington's disease is inherited in an autosomal dominant manner, meaning that a single copy of the mutated gene is sufficient to cause the disorder. The number of CAG repeats is directly related to disease severity and age of onset, with larger expansions leading to earlier and more severe symptoms (Shaha & Godad, 2026).

A common theme across these genetic mutations is their impact on protein homeostasis, often referred to as proteostasis (Xie & Jarosz, 2018). Mutations may increase the production of toxic proteins, alter their structure, or impair their clearance, all of which contribute to their accumulation within neurons. This accumulation disrupts cellular functions, including synaptic signalling, intracellular transport, and energy metabolism (Singh *et al.*, 2025). Furthermore, these mutations can impair the function of cellular quality control systems such as the ubiquitin-proteasome system and autophagy pathways, reducing the cell's ability to remove damaged or misfolded proteins. As a result, a toxic environment develops within neurons, leading to progressive dysfunction and eventual cell death. It is also important to note that genetic mutations often interact with other pathogenic mechanisms, such as oxidative stress, mitochondrial dysfunction, and neuroinflammation. For example, mutated proteins may impair mitochondrial function, leading to increased production of reactive oxygen species, which in turn can further damage proteins and DNA (Chen *et al.*, 2024). Similarly, protein aggregates

formed as a result of genetic mutations can activate microglia, triggering chronic inflammation that exacerbates neuronal damage. These interconnected processes highlight the complexity of neurodegenerative diseases and the central role of genetic factors in initiating and driving these pathological cascades.

2.4.2 Epigenetic Modifications

Epigenetic modifications refer to heritable and reversible changes in gene expression that occur without altering the underlying DNA sequence (Mohana Devi *et al.*, 2021). These changes regulate when, where, and how genes are expressed, allowing cells with the same genetic material to perform different functions. In the context of neurodegenerative disorders, epigenetic mechanisms have emerged as critical regulators of disease onset and progression (Berson *et al.*, 2018). Unlike fixed genetic mutations, epigenetic changes are dynamic and can be influenced by aging, environmental exposures, lifestyle factors, and disease states. This makes them particularly important in explaining why some individuals develop neurodegenerative diseases while others with similar genetic backgrounds do not. The major epigenetic mechanisms include DNA methylation, histone modifications, and regulation by non-coding RNAs, all of which play significant roles in neuronal function and survival (Ijomone *et al.*, 2020). DNA methylation is one of the most extensively studied epigenetic modifications (Smith *et al.*, 2025). It involves the addition of a methyl group to cytosine bases in DNA, typically at CpG sites, leading to repression of gene transcription. In neurons, proper DNA methylation patterns are essential for maintaining normal gene expression involved in synaptic function, plasticity, and memory formation (Feng *et al.*, 2010). However, in neurodegenerative diseases, abnormal methylation patterns can disrupt these processes. For example, hypermethylation of promoter regions may silence genes that are important for neuronal protection, repair, or antioxidant defense, while hypomethylation may lead to overexpression of genes that promote inflammation or protein aggregation.

Age-related changes in DNA methylation, often referred to as “epigenetic drift,” further contribute to the vulnerability of neurons, as the precision of gene regulation declines over time (K. Wang *et al.*, 2022). These alterations can accumulate gradually, influencing disease susceptibility and progression. Histone modifications represent another crucial layer of epigenetic regulation. DNA in the nucleus is wrapped around histone proteins, forming a structure known as chromatin. The accessibility of DNA for

transcription depends on how tightly it is packed within this structure. Chemical modifications of histones such as acetylation, methylation, phosphorylation, and ubiquitination can either loosen or tighten chromatin, thereby activating or repressing gene expression (R. Liu *et al.*, 2023).

For instance, histone acetylation generally promotes gene transcription by relaxing chromatin structure, while histone deacetylation leads to gene repression. In neurodegenerative diseases, dysregulation of histone-modifying enzymes can result in inappropriate activation or silencing of genes critical for neuronal health. Reduced histone acetylation has been associated with impaired learning and memory, as well as decreased expression of neuroprotective genes. Conversely, abnormal histone methylation patterns can alter the expression of genes involved in inflammation, apoptosis, and protein homeostasis, further contributing to neuronal dysfunction (Liu *et al.*, 2023).

Non-coding RNAs (ncRNAs) have emerged as powerful regulators of gene expression at the post-transcriptional level (Nemeth *et al.*, 2024). Unlike messenger RNA, which codes for proteins, non-coding RNAs function primarily to regulate gene expression through various mechanisms. Among them, microRNAs (miRNAs) are particularly important in the nervous system (Cao *et al.*, 2016). These small RNA molecules bind to complementary sequences on target mRNAs, leading to their degradation or inhibition of translation. In neurodegenerative diseases, dysregulation of specific miRNAs can disrupt the balance of protein production, leading to either deficiency of essential proteins or accumulation of toxic ones (Cao *et al.*, 2016). For example, certain miRNAs may regulate the expression of proteins involved in synaptic function, inflammation, or protein aggregation (Song, 2020). Alterations in miRNA expression can therefore contribute to multiple aspects of disease pathology. Other types of non-coding RNAs, such as long non-coding RNAs (lncRNAs), also play roles in chromatin remodeling and transcriptional regulation, further adding complexity to epigenetic control (Mattick *et al.*, 2023). Epigenetic modifications are closely linked with other molecular mechanisms involved in neurodegeneration. For instance, oxidative stress can alter DNA methylation patterns and histone modifications, while inflammation can influence the expression of non-coding RNAs (García-Giménez *et al.*, 2025).

Similarly, protein aggregates such as those found in neurodegenerative diseases can interfere with epigenetic regulatory machinery, leading to widespread changes in gene expression. These interactions create a complex network in which epigenetic changes both influence and are influenced by other pathological processes. Importantly, because epigenetic modifications are reversible, they provide a potential link between environmental factors and genetic susceptibility, helping to explain how external influences such as diet, toxins, stress, and physical activity can affect disease risk.

Another important aspect of epigenetics in neurodegeneration is its role in aging, which is the strongest risk factor for most neurodegenerative diseases (Lardenoije *et al.*, 2015). As individuals age, epigenetic regulation becomes less precise, leading to increased variability in gene expression. This can result in reduced expression of genes involved in cellular repair and increased expression of genes that promote inflammation and cell death. These age-related epigenetic changes may prime neurons for degeneration, making them more susceptible to additional stressors (Avila, 2021).

Furthermore, cumulative exposure to environmental factors over a lifetime can modify the epigenome, contributing to the gradual development of disease. The study of epigenetic modifications has important implications for diagnosis and therapy. Epigenetic markers, such as specific DNA methylation patterns or circulating microRNAs, have the potential to serve as biomarkers for early detection and disease monitoring (Kim *et al.*, 2018). Additionally, therapeutic strategies targeting epigenetic mechanisms are being actively explored. For example, drugs that inhibit histone deacetylases (HDAC inhibitors) aim to restore normal gene expression by increasing histone acetylation. Similarly, approaches that modulate microRNA activity or correct abnormal DNA methylation patterns are under investigation. These strategies offer the possibility of modifying disease progression rather than simply treating symptoms.

2.5 Neurotransmitter Imbalance

2.5.1 Cholinergic System Dysfunction

Neurotransmitter imbalance is a fundamental feature of neurodegenerative diseases, and among these, dysfunction of the cholinergic system has been extensively studied in Alzheimer's disease (Sharma *et al.*, 2024). The cholinergic hypothesis, first proposed in the late 20th century, remains one of the most influential frameworks for

understanding cognitive decline in Alzheimer's disease. It suggests that degeneration of cholinergic neurons and the resulting reduction in acetylcholine levels are primary contributors to memory impairment and cognitive dysfunction. Early postmortem studies by researchers such as (Whitehouse *et al.*, 1982) demonstrated a significant loss of cholinergic neurons in the basal forebrain, particularly in the nucleus basalis of Meynert, correlating strongly with the severity of dementia. Subsequent biochemical studies confirmed that acetylcholine levels and the activity of choline acetyltransferase (the enzyme responsible for acetylcholine synthesis) are markedly reduced in the brains of Alzheimer's patients (Stanciu *et al.*, 2019). For example, Hampel (2018) reported a profound decrease in choline acetyltransferase activity in cortical regions associated with memory and cognition (Hampel *et al.*, 2018). This reduction directly impairs synaptic transmission in cholinergic pathways connecting the basal forebrain to the hippocampus and cortex, which are essential for learning and memory processes. Functional imaging studies, including positron emission tomography (PET), have further supported these findings by demonstrating reduced cholinergic activity in living patients, particularly in early stages of the disease (Anand *et al.*, 2009). In addition to reduced synthesis, increased breakdown of acetylcholine also contributes to cholinergic dysfunction. Studies have shown elevated activity of acetylcholinesterase in certain brain regions, which accelerates the degradation of acetylcholine in the synaptic cleft. This has been supported by pharmacological research demonstrating that inhibition of acetylcholinesterase can temporarily improve cognitive function. Clinical trials involving drugs such as donepezil, rivastigmine, and galantamine have shown modest but significant improvements in memory, attention, and daily functioning in patients with mild to moderate Alzheimer's disease (Li *et al.*, 2019). These findings provide strong indirect evidence supporting the central role of acetylcholine deficiency in cognitive decline.

Receptor-level changes have also been extensively investigated. Both nicotinic and muscarinic acetylcholine receptors are altered in Alzheimer's disease. Studies have shown a reduction in nicotinic receptor density, particularly the $\alpha 4 \beta 2$ subtype, which is important for cognitive processing. At the same time, muscarinic receptors may show altered signalling efficiency even when their numbers are relatively preserved. Research by Wu *et al.* (2021) suggests that dysfunction of these receptors contributes to impaired synaptic plasticity, a key mechanism underlying learning and memory (Q.-L. Wu *et al.*,

2022). These receptor abnormalities further compound the effects of reduced acetylcholine availability. Importantly, cholinergic dysfunction does not occur in isolation but interacts closely with other pathological processes. Experimental studies have demonstrated that amyloid-beta peptides can directly impair cholinergic neurotransmission by reducing acetylcholine release and damaging cholinergic neurons. For instance, research by Satyabrata and Quirion (2004) showed that amyloid-beta exposure decreases cholinergic synaptic activity in animal models (Kar & Quirion, 2004). Similarly, tau pathology disrupts axonal transport, affecting the delivery of essential components required for acetylcholine synthesis and release. This interaction between protein aggregation and neurotransmitter dysfunction suggests a synergistic mechanism driving disease progression.

More recent studies have expanded the cholinergic hypothesis to include its role in modulating inflammation and neuroprotection. Acetylcholine has been shown to regulate inflammatory responses through the “cholinergic anti-inflammatory pathway.” Research indicates that reduced cholinergic signalling may lead to increased neuroinflammation, further contributing to neuronal damage. For example, Pavlov and Tracey (2017) highlighted the role of acetylcholine in suppressing pro-inflammatory cytokine release, suggesting that its deficiency may exacerbate inflammatory processes in Alzheimer’s disease (Pavlov & Tracey, 2017). Longitudinal and clinical studies also support the relationship between cholinergic decline and cognitive impairment. Patients with greater loss of cholinergic markers tend to exhibit more severe cognitive deficits and faster disease progression. Moreover, imaging studies using cholinergic tracers have shown that reductions in cholinergic activity can be detected even before significant clinical symptoms appear, indicating that cholinergic dysfunction may be an early event in the disease process.

2.5.2 Dopaminergic System Degeneration

Dopaminergic system degeneration is a central pathological feature of Parkinson’s disease and plays a critical role in the development of its characteristic motor and non-motor symptoms (Z. D. Zhou *et al.*, 2023). The dopaminergic system refers to neurons that produce and release dopamine, a neurotransmitter essential for regulating movement, motivation, reward, and certain cognitive functions. In Parkinson’s disease, there is a progressive and selective loss of dopaminergic neurons, particularly in a region

of the midbrain known as the substantia nigra, specifically its pars compacta. These neurons project to the striatum (part of the basal ganglia), forming the nigrostriatal pathway, which is crucial for the coordination and smooth execution of voluntary movements (Florio *et al.*, 2018). Dopamine acts as a modulatory neurotransmitter within the basal ganglia circuitry, helping to balance two major pathways: the direct pathway, which facilitates movement, and the indirect pathway, which inhibits movement. In a healthy brain, dopamine stimulates the direct pathway and suppresses the indirect pathway, thereby promoting smooth and controlled motor activity. However, in Parkinson's disease, the loss of dopaminergic neurons disrupts this balance (Ramesh & Arachchige, 2023). Reduced dopamine levels lead to decreased activation of the direct pathway and increased activity of the indirect pathway, resulting in excessive inhibition of movement. This imbalance manifests clinically as the hallmark motor symptoms of Parkinson's disease, including bradykinesia (slowness of movement), resting tremor, muscle rigidity, and postural instability.

A key aspect of dopaminergic degeneration is that symptoms typically appear only after a substantial loss approximately 70-80% of dopaminergic neurons in the substantia nigra (Ramesh & Arachchige, 2023). This delayed onset highlights the brain's ability to compensate for neuronal loss in early stages, but once this threshold is crossed, motor deficits become evident. For example, a patient may initially notice subtle symptoms such as reduced arm swing while walking or slight stiffness, which gradually progress to more pronounced tremors and difficulty initiating movement (Hess & Hallett, 2017). A classic example is a resting tremor in one hand, often described as a "pill-rolling" motion, which becomes more noticeable when the limb is at rest and diminishes during voluntary movement. At the cellular level, dopaminergic neuron degeneration is closely associated with the accumulation of misfolded alpha-synuclein protein, which forms intracellular inclusions known as Lewy bodies (Calabresi *et al.*, 2023). These aggregates disrupt normal cellular functions, including mitochondrial activity, protein degradation pathways, and synaptic vesicle trafficking.

For instance, impaired vesicle transport can reduce the release of dopamine at synaptic terminals, further exacerbating neurotransmitter deficiency. Additionally, mitochondrial dysfunction in these neurons leads to increased oxidative stress, making them more vulnerable to degeneration. Dopaminergic neurons are particularly susceptible because dopamine metabolism itself can generate reactive oxygen species,

adding to the oxidative burden (Delcambre *et al.*, 2016). The consequences of dopaminergic degeneration extend beyond motor symptoms. Dopamine also plays a role in cognitive and emotional processes, which explains the presence of non-motor symptoms in Parkinson's disease. Patients may experience depression, apathy, anxiety, sleep disturbances, and cognitive impairment. For example, reduced dopamine levels in the mesolimbic pathway can affect motivation and reward processing, leading to decreased interest in daily activities. Similarly, involvement of other brain regions may contribute to executive dysfunction and memory problems in later stages of the disease. Therapeutically, the central role of dopamine deficiency has led to the development of treatments aimed at restoring dopaminergic signalling. The most common treatment is levodopa (L-DOPA), a precursor of dopamine that can cross the blood-brain barrier and be converted into dopamine within the brain (Riederer *et al.*, 2025). For example, a patient treated with levodopa may show significant improvement in motor symptoms, such as reduced rigidity and improved movement speed. However, long-term use can lead to complications such as motor fluctuations and dyskinesias (involuntary movements). Other treatments include dopamine agonists, which directly stimulate dopamine receptors, and inhibitors of enzymes that break down dopamine, thereby prolonging its action (Riederer *et al.*, 2025).

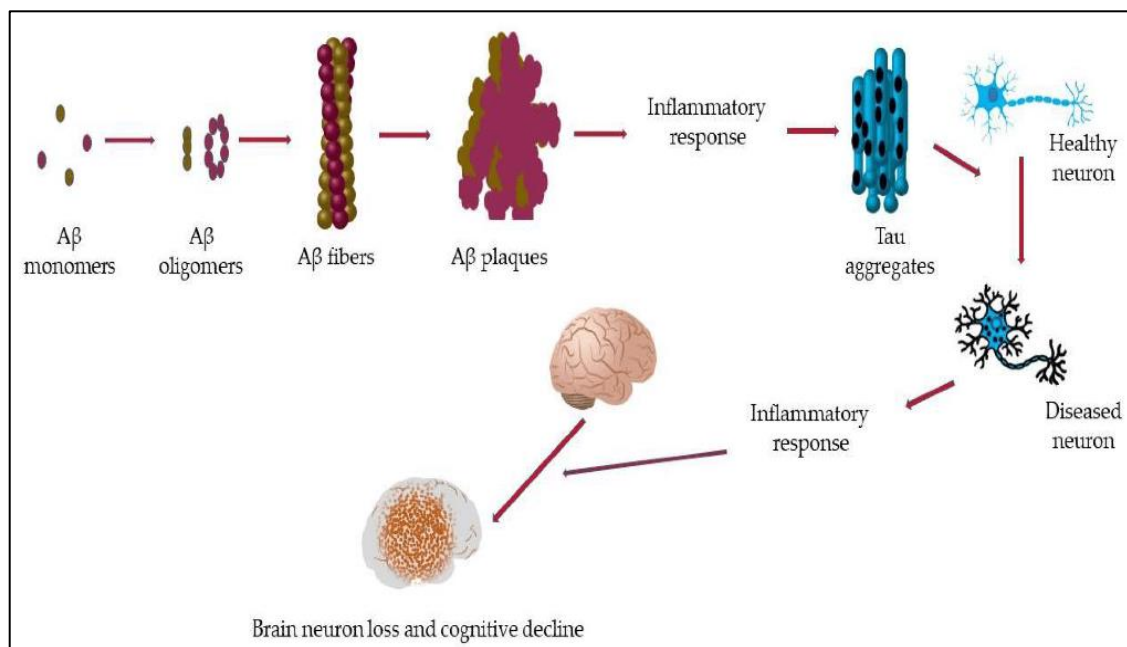


Figure 2.2: Illustration of the molecular and cellular cascade underlying neurodegeneration, particularly in Alzheimer's disease.

Figure 2.2 illustrates the molecular and cellular cascade underlying neurodegeneration, particularly in Alzheimer's disease, emphasizing the progressive transition from protein misfolding to widespread neuronal dysfunction and cognitive impairment. The process begins with the aggregation of amyloid-beta ($A\beta$) peptides, which are derived from the proteolytic cleavage of amyloid precursor protein (APP) via β - and γ -secretase activity (Haass *et al.*, 2012). Under pathological conditions, $A\beta$ monomers undergo conformational changes that promote misfolding and self-association, leading to the formation of soluble oligomers. These oligomeric species are structurally heterogeneous and are considered highly neurotoxic due to their ability to disrupt synaptic function and alter membrane integrity. As aggregation progresses, $A\beta$ oligomers further assemble into protofibrils and fibrillar structures through β -sheet stabilization (Haass *et al.*, 2012). These fibrils subsequently accumulate extracellularly to form dense-core amyloid plaques. The deposition of $A\beta$ plaques is not merely a passive event but actively contributes to neurotoxicity by triggering a series of downstream pathological mechanisms. One of the key consequences is the activation of the brain's innate immune system, particularly microglia and astrocytes, leading to a sustained neuroinflammatory response. Activated glial cells release pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS), which exacerbate neuronal stress and damage (Figure 2.2) (Haass *et al.*, 2012). Concomitant with amyloid pathology is the abnormal modification of tau protein, a microtubule-associated protein responsible for stabilizing the neuronal cytoskeleton. In response to $A\beta$ -induced toxicity and inflammatory signalling, tau undergoes hyperphosphorylation, which reduces its affinity for microtubules. This leads to microtubule destabilization, impaired axonal transport, and the formation of intracellular neurofibrillary tangles composed of aggregated tau. These tau aggregates propagate in a prion-like manner across connected neuronal networks, further amplifying neurodegeneration.

The combined effects of extracellular $A\beta$ deposition and intracellular tau pathology result in profound synaptic dysfunction, which is an early and critical event in disease progression (H. Zhang *et al.*, 2021). Synaptic loss correlates strongly with cognitive decline, as it disrupts neural circuitry involved in memory, learning, and executive function. Additionally, mitochondrial dysfunction, calcium dysregulation, and oxidative stress further compromise neuronal viability. As the number of damaged neurons increases, a self-perpetuating cycle of neurodegeneration is established,

characterized by escalating protein aggregation, inflammation, and cellular dysfunction. Ultimately, this cascade leads to widespread neuronal death, cortical and hippocampal atrophy, and progressive impairment of brain function (Dash *et al.*, 2025). Clinically, these pathological changes manifest as cognitive decline, memory loss, and behavioral disturbances. Thus, Figure 2 represents a multifactorial and interconnected model of neurodegeneration, where amyloid-beta aggregation acts as an initiating factor that triggers downstream tau pathology, neuroinflammation, and neuronal loss, culminating in the clinical symptoms of Alzheimer's disease.

2.5.3 Glutamate Excitotoxicity

Excess glutamate induced neurotoxicity, commonly referred to as excitotoxicity, is a well-established mechanism contributing to neuronal injury in many neurodegenerative disorders (Wu *et al.*, 2025). Glutamate is the primary excitatory neurotransmitter in the central nervous system and is essential for normal processes such as learning, memory, and synaptic plasticity. However, when present in excessive amounts in the synaptic cleft, glutamate becomes toxic to neurons. This imbalance can result from increased release, impaired reuptake by astrocytes, or dysfunction of glutamate transporters (K. Gao & Cheung-Hoi Yu, 2025). A large body of experimental and clinical research has demonstrated that chronic glutamate excitotoxicity plays a significant role in diseases such as Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis. One of the primary consequences of excess glutamate is the overactivation of NMDA (N-methyl-D-aspartate) receptors, which are a subtype of ionotropic glutamate receptors located on neuronal membranes (Neves *et al.*, 2023). Under normal conditions, NMDA receptors play a crucial role in synaptic plasticity and memory formation by allowing controlled calcium influx.

However, studies such as those by Aizenman *et al.*, 2020, who first described excitotoxicity, and demonstrated that prolonged or excessive activation of NMDA receptors leads to neuronal injury (Aizenman *et al.*, 2020). When glutamate levels remain elevated, NMDA receptors become persistently activated, causing continuous opening of their ion channels. This results in an abnormal influx of calcium ions (Ca^{2+}) into the neuron, which is the central event in excitotoxic damage. The increase in intracellular calcium is a critical step linking NMDA receptor overactivation to neuronal death. Calcium is normally tightly regulated within neurons because it acts as a signalling

molecule controlling various cellular processes. However, excessive calcium entry overwhelms the cell's buffering capacity. Research by Sattler and Tymianski (2001) showed that elevated intracellular calcium activates a range of calcium-dependent enzymes, including proteases, phospholipases, and endonucleases (Szydlowska & Tymianski, 2010). These enzymes begin to degrade essential cellular components such as cytoskeletal proteins, membrane lipids, and nucleic acids. Additionally, high calcium levels disrupt mitochondrial function by impairing the electron transport chain and triggering the release of pro-apoptotic factors (S. H. Lee *et al.*, 2023). This mitochondrial dysfunction further increases the production of reactive oxygen species (ROS), amplifying oxidative stress and cellular damage. Another important aspect of glutamate excitotoxicity is its effect on neuronal survival pathways. Studies have shown that excessive NMDA receptor activation can alter gene expression and activate signalling cascades that promote apoptosis (programmed cell death). For instance, research by Hardingham and Bading (2010) demonstrated that synaptic NMDA receptor activity can be protective, whereas excessive activation of extrasynaptic NMDA receptors triggers cell death pathways (Hardingham & Bading, 2010). This distinction highlights that not all NMDA receptor activity is harmful, but rather that dysregulated and prolonged activation shifts the balance toward neurotoxicity.

Glutamate excitotoxicity is particularly relevant in Amyotrophic Lateral Sclerosis, where impaired glutamate clearance due to dysfunction of astrocytic transporters (especially EAAT2) leads to elevated extracellular glutamate levels. Previous studies found reduced expression of glutamate transporters in ALS patients (Rothstein *et al.*, 1995), supporting the idea that impaired reuptake contributes to neuronal damage.

Similarly, in Alzheimer's disease, amyloid-beta has been shown to disrupt glutamate regulation and enhance NMDA receptor activity, thereby promoting excitotoxicity. Research by Hynd *et al.* (2004) and others has linked this mechanism to synaptic loss and cognitive decline (Y. Chen *et al.*, 2019). In Parkinson's disease, alterations in glutamatergic signalling within the basal ganglia contribute to motor dysfunction and neuronal stress. Experimental evidence from animal models further supports the role of excitotoxicity in neurodegeneration. For example, administration of glutamate analogs such as kainic acid or NMDA in laboratory animals induces neuronal death patterns similar to those observed in human neurodegenerative diseases (Zheng *et*

et al., 2011). These models have been widely used to study the molecular pathways involved and to test potential neuroprotective therapies. From a therapeutic perspective, targeting glutamate excitotoxicity has led to the development of drugs that modulate NMDA receptor activity. One example is memantine, an NMDA receptor antagonist used in moderate to severe Alzheimer's disease.

Clinical studies have shown that memantine can reduce excessive calcium influx without completely blocking normal receptor function, thereby providing neuroprotection while preserving physiological signalling. This supports the concept that controlled modulation, rather than complete inhibition, of glutamatergic activity is essential for maintaining neuronal health (Magdaleno Roman & Chapa González, 2024).

2.6 Role of Environmental and Lifestyle Factors

2.6.1 Environmental Toxins

Environmental toxins are increasingly recognized as important contributors to the development and progression of neurodegenerative diseases (Kulcsárová *et al.*, 2025). While genetic and age-related factors play a major role, exposure to harmful substances in the environment can significantly increase the risk of neuronal damage by triggering molecular pathways such as oxidative stress, mitochondrial dysfunction, neuroinflammation, and protein aggregation. These toxins may enter the body through inhalation, ingestion, or skin contact and can accumulate over time, particularly affecting the nervous system due to its high metabolic activity and sensitivity (Kulcsárová *et al.*, 2025). Long-term or repeated exposure even at low levels can disrupt normal neuronal function and increase susceptibility to disorders such as Parkinson's disease and Alzheimer's disease. One of the most extensively studied groups of environmental toxins is pesticides. Epidemiological and experimental studies have consistently linked pesticide exposure to an increased risk of neurodegeneration, particularly Parkinson's disease. Compounds such as paraquat and rotenone are known to induce oxidative stress and mitochondrial dysfunction in neurons (Tanner *et al.*, 2011). For example, rotenone directly inhibits complex I of the mitochondrial electron transport chain, leading to reduced ATP production and increased generation of reactive oxygen species (ROS). Animal studies have shown that chronic exposure to these pesticides can reproduce key features of Parkinson's disease, including the selective degeneration of dopaminergic

neurons in the substantia nigra. Similarly, paraquat has been shown to promote oxidative damage and may enhance the aggregation of alpha-synuclein, a protein implicated in Parkinson's pathology (Tanner *et al.*, 2011). Occupational exposure among agricultural workers has been associated with a higher incidence of Parkinson's disease, further supporting this link.

Heavy metals are another major category of environmental neurotoxins. Metals such as lead, mercury, arsenic, and manganese can accumulate in the brain and interfere with normal neuronal processes (Althomali *et al.*, 2024). Lead exposure, especially during early development, has long-term effects on cognitive function and may increase the risk of neurodegenerative diseases later in life (Althomali *et al.*, 2024). Mercury, particularly in its organic form (methylmercury), is highly neurotoxic and can disrupt neuronal membranes, impair neurotransmitter systems, and induce oxidative stress. Manganese exposure, often seen in industrial settings such as mining or welding, is associated with a Parkinson-like syndrome known as manganism, characterized by motor deficits similar to those seen in Parkinson's disease. These metals can also interfere with protein folding and promote aggregation, linking them to broader neurodegenerative mechanisms. Industrial chemicals, including solvents, air pollutants, and synthetic compounds, have also been implicated in neurodegeneration (Althomali *et al.*, 2024).

Exposure to substances such as polychlorinated biphenyls (PCBs), volatile organic compounds, and particulate matter in air pollution can lead to chronic inflammation and oxidative stress in the brain. Studies have shown that long-term exposure to air pollution is associated with an increased risk of cognitive decline and dementia. These pollutants can cross the blood-brain barrier or enter the brain via the olfactory system, triggering inflammatory responses and neuronal injury. In urban environments, where exposure to such pollutants is higher, there is growing concern about their contribution to the rising prevalence of neurodegenerative diseases. The mechanisms by which environmental toxins contribute to neurodegeneration are multifactorial and interconnected (Kulcsárová *et al.*, 2025).

Many toxins induce oxidative stress by increasing the production of ROS, which damage cellular components such as lipids, proteins, and DNA. Others impair mitochondrial function, leading to energy deficits and further ROS generation. Neuroinflammation is another common pathway, as toxins can activate microglia and

astrocytes, resulting in the release of pro-inflammatory cytokines that exacerbate neuronal damage. Additionally, some toxins interfere with neurotransmitter systems or promote the misfolding and aggregation of proteins, thereby linking environmental exposure to the core pathological features of neurodegenerative diseases (Kulcsárová *et al.*, 2025). It is also important to consider that environmental toxin exposure often interacts with genetic susceptibility and lifestyle factors. Individuals with certain genetic backgrounds may be more vulnerable to the harmful effects of toxins, while factors such as diet, physical activity, and overall health can influence the body's ability to detoxify and repair damage. For example, inadequate antioxidant intake may reduce the ability to counteract oxidative stress induced by toxins, increasing the risk of neuronal injury (Kulcsárová *et al.*, 2025).

2.6.2 Diet and Nutrition

Diet and nutrition play a crucial role in maintaining brain health and can significantly influence the onset and progression of neurodegenerative diseases (R. Zhang *et al.*, 2024). The brain is a highly metabolically active organ that requires a continuous supply of nutrients to support neuronal function, neurotransmitter synthesis, and cellular repair mechanisms. Nutritional imbalances particularly deficiencies in antioxidants, vitamins, and essential fatty acids can impair these processes and make neurons more vulnerable to damage. Increasing evidence from epidemiological, clinical, and experimental studies suggests that poor dietary patterns not only accelerate neurodegeneration but may also interact with genetic and environmental factors to increase disease risk (R. Zhang *et al.*, 2024). One of the most important nutritional factors in neuroprotection is the availability of antioxidants. Antioxidants such as vitamins C and E, polyphenols, and compounds like glutathione help neutralize reactive oxygen species (ROS), thereby preventing oxidative stress a key mechanism in neuronal damage. When antioxidant intake is insufficient, ROS accumulate and cause damage to lipids, proteins, and DNA within neurons (R. Zhang *et al.*, 2024). This is particularly harmful in neurodegenerative conditions such as Alzheimer's disease and Parkinson's disease, where oxidative stress is already elevated. Studies have shown that individuals with lower dietary intake of antioxidants tend to have a higher risk of cognitive decline. For example, diets rich in fruits and vegetables natural sources of antioxidants are associated

with slower progression of memory impairment and reduced risk of dementia (R. Zhang *et al.*, 2024).

Vitamins also play essential roles in maintaining neuronal integrity and function. B vitamins, particularly B6, B12, and folate, are critical for homocysteine metabolism (Quadros, 2023). Elevated homocysteine levels, often resulting from deficiencies in these vitamins, have been linked to increased risk of neurodegeneration and cognitive decline. High homocysteine levels can promote oxidative stress, vascular damage, and neuronal toxicity. Vitamin B12 deficiency, in particular, is associated with memory impairment, confusion, and even structural brain changes (Quadros, 2023). Similarly, vitamin D has emerged as an important factor in brain health, with roles in neuroprotection, immune regulation, and reduction of inflammation. Low levels of vitamin D have been correlated with increased risk of dementia and other neurodegenerative conditions (Quadros, 2023). Essential fatty acids, especially omega-3 fatty acids such as docosahexaenoic acid (DHA), are critical components of neuronal membranes and are essential for maintaining synaptic function and plasticity. DHA is highly concentrated in the brain and plays a key role in maintaining membrane fluidity, facilitating signal transmission, and supporting neurogenesis (Quadros, 2023). Deficiency in omega-3 fatty acids can lead to impaired synaptic communication and increased vulnerability to inflammation and oxidative stress. Research has shown that diets rich in omega-3 fatty acids commonly found in fish, nuts, and seeds are associated with better cognitive function and reduced risk of neurodegenerative diseases. Conversely, diets high in saturated fats and trans fats may have the opposite effect, promoting inflammation and impairing neuronal health (Quadros, 2023).

Diet also influences neurodegeneration through its effects on inflammation and metabolic health (Fontana *et al.*, 2021). Poor dietary habits, such as high consumption of processed foods, sugars, and unhealthy fats, can lead to chronic low-grade inflammation and metabolic disorders like obesity and diabetes (Fontana *et al.*, 2021). These conditions are known risk factors for neurodegenerative diseases, as they contribute to vascular damage, insulin resistance in the brain, and increased oxidative stress. In contrast, dietary patterns such as the Mediterranean diet-rich in fruits, vegetables, whole grains, healthy fats, and lean proteins have been consistently associated with a lower risk of cognitive decline and neurodegeneration (Fontana *et al.*, 2021). This protective effect is thought to result from a combination of antioxidant, anti-inflammatory, and neuroprotective

properties. Another important aspect of nutrition is its role in maintaining the gut-brain axis, a bidirectional communication system between the gastrointestinal tract and the brain. The gut microbiota is influenced by diet and can affect brain function through the production of metabolites, regulation of immune responses, and modulation of neurotransmitter systems (Fontana *et al.*, 2021). Poor nutrition can disrupt the balance of gut microbiota, leading to increased inflammation and potentially contributing to neurodegenerative processes. Emerging research suggests that a healthy diet may support beneficial gut bacteria, thereby promoting brain health and reducing disease risk. In addition to deficiencies, the timing and quality of nutrition throughout life also play a role. Early-life malnutrition can impair brain development, while poor dietary habits in adulthood can accelerate age-related cognitive decline. As individuals age, nutrient absorption may decrease, making older adults more susceptible to deficiencies that can impact brain function. Therefore, maintaining a balanced and nutrient-rich diet throughout life is essential for preserving cognitive health.

2.6.3 Physical Activity and Lifestyle

Physical activity and overall lifestyle patterns are critical determinants of brain health and play a significant role in the risk, onset, and progression of neurodegenerative diseases (Santiago & Potashkin, 2023). A growing body of evidence indicates that a sedentary lifestyle characterized by low levels of physical movement and limited cognitive engagement is strongly associated with an increased risk of cognitive decline and disorders such as Alzheimer's disease and Parkinson's disease. In contrast, regular physical exercise and mentally stimulating activities have been shown to exert protective effects on neuronal function, delay disease onset, and improve quality of life (Santiago & Potashkin, 2023). These benefits arise from multiple interconnected mechanisms, including enhanced blood flow, improved metabolic health, reduced inflammation, and stimulation of neuroplasticity. Physical activity is essential for maintaining optimal brain function because it directly influences cerebral blood flow and oxygen delivery (Santiago & Potashkin, 2023). Exercise increases heart rate and promotes efficient circulation, ensuring that neurons receive adequate oxygen and nutrients required for energy production and cellular maintenance.

Studies using neuroimaging techniques have demonstrated that individuals who engage in regular aerobic exercise show increased brain volume in regions such as the

hippocampus, which is critical for memory and learning (Erickson *et al.*, 2011). For example, longitudinal studies have found that older adults who participate in activities like walking, swimming, or cycling exhibit slower rates of brain atrophy and better cognitive performance compared to sedentary individuals. Exercise also stimulates the release of neurotrophic factors, particularly brain-derived neurotrophic factor (BDNF), which supports neuronal survival, synaptic plasticity, and the formation of new neural connections ((Romero Garavito *et al.*, 2025)).

Beyond its effects on brain structure, physical activity plays a key role in reducing several biological risk factors associated with neurodegeneration. Regular exercise helps regulate glucose metabolism, improves insulin sensitivity, and reduces the risk of conditions such as diabetes and cardiovascular disease, which are known contributors to cognitive decline (Romero Garavito *et al.*, 2025). It also reduces oxidative stress and inflammation by enhancing antioxidant defenses and modulating immune responses. Since chronic inflammation and oxidative damage are central mechanisms in neurodegenerative diseases, the anti-inflammatory effects of exercise are particularly important in slowing disease progression (Romero Garavito *et al.*, 2025).

Cognitive engagement is another crucial aspect of lifestyle that influences brain health (Potashkin *et al.*, 2025). Activities that challenge the brain such as reading, problem-solving, learning new skills, or engaging in social interactions help build what is known as “cognitive reserve.” Cognitive reserve refers to the brain’s ability to compensate for damage by utilizing alternative neural pathways or more efficient processing strategies (Phillips, 2017)). Individuals with higher cognitive reserve are often able to maintain normal cognitive function despite underlying pathological changes in the brain. Epidemiological studies have consistently shown that people who remain intellectually active throughout life have a lower risk of developing dementia and experience slower cognitive decline (Potashkin *et al.*, 2025). For example, lifelong learning, bilingualism, and participation in mentally stimulating hobbies have all been associated with improved cognitive resilience.

A sedentary lifestyle, on the other hand, has multiple negative effects on brain health. Prolonged physical inactivity leads to reduced cardiovascular fitness, impaired blood flow to the brain, and increased risk of metabolic disorders. It is also associated with higher levels of inflammation and oxidative stress, both of which contribute to

neuronal damage (Phillips, 2017). Lack of cognitive engagement further exacerbates this risk by reducing synaptic activity and weakening neural networks. Over time, this can lead to decreased synaptic plasticity and increased vulnerability to neurodegenerative processes. For instance, individuals who spend long periods in passive activities such as excessive screen time without mental stimulation may experience a gradual decline in cognitive function (Phillips, 2017).

Lifestyle factors often interact with one another, creating either protective or harmful patterns (Rippe, 2018). For example, physical activity can enhance cognitive function not only through biological mechanisms but also by promoting social interaction and mental engagement, especially when performed in group settings or structured programs. Similarly, an active lifestyle is often associated with healthier dietary habits, better sleep patterns, and reduced stress levels, all of which contribute to overall brain health (Gao *et al.*, 2024). Sleep, in particular, is essential for processes such as memory consolidation and clearance of metabolic waste products from the brain (Y. Gao *et al.*, 2024). Chronic sleep deprivation, often linked with sedentary and unhealthy lifestyles, has been associated with increased accumulation of toxic proteins and higher risk of neurodegeneration.

Importantly, the benefits of physical activity and cognitive engagement are not limited to prevention but also extend to disease management. In individuals already diagnosed with neurodegenerative diseases, regular exercise has been shown to improve motor function, balance, and mood, particularly in Parkinson's disease (Gao *et al.*, 2024). Cognitive training and rehabilitation programs can help maintain mental function and delay the progression of symptoms in dementia. These interventions highlight the potential of lifestyle modifications as non-pharmacological strategies for managing neurodegenerative conditions (Gao *et al.*, 2024).

2.7 Advances in Biomarkers and Diagnostic Approaches

2.7.1 Biochemical Biomarkers

Biochemical biomarkers have become a cornerstone in the early detection, diagnosis, and monitoring of neurodegenerative diseases, representing a major advancement over traditional symptom-based diagnosis (Das *et al.*, 2026). These biomarkers are measurable biological molecules that reflect underlying pathological

processes such as protein aggregation, neuronal injury, and synaptic dysfunction. Because neurodegenerative diseases often begin years or even decades before clinical symptoms appear, biochemical biomarkers provide a crucial window for early identification and intervention (Das *et al.*, 2026). Among the most extensively studied biomarkers are those found in cerebrospinal fluid (CSF) and, more recently, those detectable in blood. These approaches are particularly important in conditions such as Alzheimer's disease, where early diagnosis can significantly influence disease management and research outcomes (Das *et al.*, 2026). Cerebrospinal fluid biomarkers are considered the gold standard for biochemical assessment of neurodegeneration because CSF is in direct contact with the brain and spinal cord, making it highly reflective of central nervous system changes (Karlsson *et al.*, 2024). The most well-established CSF biomarkers include amyloid-beta ($A\beta$), total tau (t-tau), and phosphorylated tau (p-tau). In Alzheimer's disease, levels of amyloid-beta particularly $A\beta_{42}$ are typically reduced in the CSF. This decrease occurs because amyloid-beta is deposited in the brain as plaques, reducing its availability in the fluid. In contrast, levels of tau proteins are elevated in the CSF, reflecting neuronal damage and degeneration. Total tau indicates the extent of neuronal injury, while phosphorylated tau is more specific to the formation of neurofibrillary tangles, a hallmark of Alzheimer's pathology. The combined profile of low $A\beta_{42}$ and high tau levels provides strong diagnostic accuracy and is widely used in both clinical and research settings.

Beyond Alzheimer's disease, CSF biomarkers are also being explored for other neurodegenerative conditions (Zetterberg & Bendlin, 2026). For example, alpha-synuclein levels are studied in Parkinson's disease and related disorders, although results have been more variable compared to amyloid and tau markers. Similarly, neurofilament light chain (NfL), a structural protein released during axonal damage, has emerged as a general marker of neurodegeneration and can be elevated in multiple conditions (La Civita *et al.*, 2025), including amyotrophic lateral sclerosis and frontotemporal dementia. These markers are valuable not only for diagnosis but also for tracking disease progression and evaluating therapeutic responses. Despite their diagnostic utility, CSF biomarkers have limitations, primarily due to the invasive nature of lumbar puncture required to obtain the fluid (La Civita *et al.*, 2025). This has driven significant interest in the development of blood-based biomarkers, which offer a less invasive, more accessible, and potentially cost-effective alternative. Advances in ultra-sensitive detection

technologies, such as single-molecule arrays (Simoa) and mass spectrometry, have made it possible to measure very low concentrations of brain-derived proteins in the blood (La Civita *et al.*, 2025).

Blood-based biomarkers for amyloid-beta and tau are now showing promising results, with studies demonstrating strong correlations between plasma levels and brain pathology detected through imaging or CSF analysis. For instance, plasma phosphorylated tau (especially p-tau181 and p-tau217) has emerged as a highly specific and sensitive biomarker for Alzheimer's disease (La Civita *et al.*, 2025). Elevated levels of these markers in blood can distinguish Alzheimer's disease from other neurodegenerative conditions and may even detect the disease in its preclinical stages. Similarly, plasma neurofilament light chain (NfL) is gaining recognition as a robust marker of neuronal damage, with elevated levels observed in a wide range of neurodegenerative diseases. Although blood-based amyloid-beta measurements are more challenging due to lower concentrations and peripheral influences, recent studies have shown that certain ratios (such as A β 42/A β 40) can improve diagnostic accuracy (La Civita *et al.*, 2025).

Another important advantage of blood-based biomarkers is their potential for large-scale screening and longitudinal monitoring. Unlike CSF sampling, which is typically performed once or infrequently, blood tests can be repeated smoothly, allowing clinicians to track disease progression over time or assess the effectiveness of therapeutic interventions (Hickman *et al.*, 2023). This is particularly valuable in clinical trials, where biomarkers can serve as surrogate endpoints to evaluate whether a treatment is modifying disease pathology.

Biochemical biomarkers are also increasingly being integrated with other diagnostic approaches, such as neuroimaging and genetic testing, to create a more comprehensive and accurate diagnostic framework (Hickman *et al.*, 2023). For example, combining CSF or blood biomarkers with imaging techniques like PET scans can improve diagnostic confidence and help differentiate between various neurodegenerative disorders. This multimodal approach is especially important given the overlap in clinical symptoms among different diseases (Hickman *et al.*, 2023).

2.7.2 Imaging Techniques

Imaging techniques play a crucial role in the diagnosis, monitoring, and research of neurodegenerative diseases by allowing visualization of structural and functional changes in the brain (Chandrasekar *et al.*, 2025). Unlike biochemical biomarkers, which provide molecular-level information, imaging methods offer direct insight into anatomical damage, disease progression, and the distribution of pathological changes. Among the most widely used imaging modalities are Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET), both of which have significantly advanced the early detection and understanding of disorders such as Alzheimer's disease and Parkinson's disease (Chandrasekar *et al.*, 2025).

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique that uses strong magnetic fields and radio waves to generate detailed images of brain structures (Chandrasekar *et al.*, 2025). It is particularly valuable for detecting structural changes associated with neurodegeneration. In Alzheimer's disease, MRI commonly reveals atrophy (shrinkage) of specific brain regions, especially the hippocampus and cerebral cortex, which are critical for memory and cognitive function (Chandrasekar *et al.*, 2025). This atrophy correlates with disease severity and can be used to track progression over time. In Parkinson's disease, MRI may show more subtle changes, but advanced techniques such as diffusion tensor imaging (DTI) can detect alterations in white matter tracts and connectivity. MRI is also essential for ruling out other causes of neurological symptoms, such as tumors, strokes, or hydrocephalus, making it a key tool in differential diagnosis. In addition to structural imaging, MRI can be used in more advanced forms, such as functional MRI (fMRI), which measures brain activity by detecting changes in blood oxygen levels (Chandrasekar *et al.*, 2025). This allows researchers to study how different brain regions communicate and how these networks are disrupted in neurodegenerative diseases. Although fMRI is more commonly used in research settings than in routine clinical practice, it provides valuable insights into functional brain changes that occur even before significant structural damage is visible (Chandrasekar *et al.*, 2025).

Positron Emission Tomography (PET) scans provide a different type of information by allowing visualization of metabolic activity and specific molecular targets in the brain (Annavaarapu *et al.*, 2019). PET imaging involves the use of radiotracers

radioactively labeled compounds that bind to particular proteins or participate in metabolic processes (Annavarapu *et al.*, 2019). In Alzheimer's disease, PET scans are widely used to detect the accumulation of amyloid-beta plaques and tau protein tangles, which are key pathological hallmarks of the disease. Amyloid PET imaging uses tracers that bind specifically to amyloid deposits, enabling clinicians to identify their presence even in early or preclinical stages. Similarly, tau PET imaging allows visualization of tau pathology and its distribution across different brain regions, which often correlates with disease progression and symptom severity (Annavarapu *et al.*, 2019). Another important application of PET imaging is the assessment of brain metabolism using fluorodeoxyglucose (FDG-PET) (Annavarapu *et al.*, 2019). This technique measures glucose uptake in brain tissues, providing an indication of neuronal activity. In neurodegenerative diseases, affected brain regions often show reduced glucose metabolism, reflecting decreased neuronal function. For example, in Alzheimer's disease, reduced metabolic activity is typically observed in the temporoparietal regions, while in Parkinson's disease, changes may be seen in motor-related circuits. These metabolic patterns can help differentiate between different types of neurodegenerative disorders.

The combination of MRI and PET imaging provides a comprehensive view of both structural and molecular changes in the brain (Sabbaghan *et al.*, 2025). MRI identifies physical damage and atrophy, while PET reveals underlying pathological processes such as protein accumulation and metabolic dysfunction (Sabbaghan *et al.*, 2025). This complementary approach improves diagnostic accuracy, particularly in early stages when symptoms may be mild or ambiguous. Furthermore, imaging techniques are increasingly used in clinical trials to monitor disease progression and evaluate the effectiveness of new therapies by tracking changes in brain structure and pathology over time (Sabbaghan *et al.*, 2025).

2.8 Therapeutic Strategies and Biochemical Targets

2.8.1 Current Pharmacological Treatments

Current pharmacological treatments for neurodegenerative diseases are primarily designed to manage symptoms and improve quality of life rather than provide a definitive cure (Passeri *et al.*, 2022)). These therapies target key biochemical pathways involved in

neurotransmitter imbalance, which is a major contributor to disease pathology (Passeri *et al.*, 2022). Although they do not stop disease progression, they can slow symptom worsening and enhance daily functioning. The most widely used treatment strategies include cholinesterase inhibitors, dopamine replacement therapy, and NMDA receptor antagonists, each targeting specific neurotransmitter systems affected in disorders such as Alzheimer's disease and Parkinson's disease (Passeri *et al.*, 2022). Cholinesterase inhibitors are commonly used in the treatment of Alzheimer's disease, where there is a significant decline in acetylcholine levels due to degeneration of cholinergic neurons. These drugs work by inhibiting the enzyme acetylcholinesterase, which breaks down acetylcholine in the synaptic cleft (Passeri *et al.*, 2022). By preventing this breakdown, they increase the availability of acetylcholine, thereby enhancing cholinergic neurotransmission. Common examples include Donepezil, Rivastigmine, and Galantamine (Sridhar, 2022). Clinical studies have shown that these medications can provide modest improvements in memory, attention, and cognitive function, particularly in the early to moderate stages of the disease. However, their effectiveness tends to decline as the disease progresses and neuronal loss becomes more extensive. Dopamine replacement therapy is the cornerstone of treatment for Parkinson's disease, which is characterized by a loss of dopaminergic neurons in the substantia nigra and a resulting deficiency of dopamine. The most commonly used drug is Levodopa, which is converted into dopamine in the brain, thereby replenishing depleted levels. Levodopa is often administered in combination with other agents, such as carbidopa, to prevent its breakdown before reaching the brain and to reduce side effects. This therapy significantly improves motor symptoms such as bradykinesia, rigidity, and tremor, often providing dramatic symptomatic relief, especially in early stages (Sridhar, 2022). Other medications, including dopamine agonists and monoamine oxidase-B (MAO-B) inhibitors, are also used to mimic or prolong the action of dopamine. Despite its effectiveness, long-term use of levodopa can lead to complications such as motor fluctuations and dyskinesias (involuntary movements), highlighting the need for careful management (Sridhar, 2022).

NMDA receptor antagonists represent another important class of drugs, particularly in the treatment of moderate to severe Alzheimer's disease (Sridhar, 2022). These drugs target glutamate-mediated excitotoxicity, a process in which excessive activation of NMDA receptors leads to increased calcium influx and neuronal damage.

Memantine is the most widely used drug in this category. It works by partially blocking NMDA receptors, thereby reducing excessive calcium entry into neurons while still allowing normal synaptic transmission (Sridhar, 2022). This helps protect neurons from excitotoxic damage and can improve cognitive function, behavior, and daily activities in some patients. Unlike cholinesterase inhibitors, memantine is often used in later stages of the disease and may be combined with other treatments for a more comprehensive approach. These pharmacological strategies highlight the importance of targeting specific biochemical pathways involved in neurodegeneration. While they primarily provide symptomatic relief, they also offer valuable insights into disease mechanisms, such as the roles of acetylcholine deficiency, dopamine depletion, and glutamate excitotoxicity (Sridhar, 2022). Ongoing research aims to develop disease-modifying therapies that go beyond symptom management to address the underlying causes of neurodegeneration, including protein aggregation, inflammation, and genetic factors.

2.8.2 Emerging Therapies

Emerging therapies for neurodegenerative diseases are shifting the focus from symptomatic relief toward disease-modifying and potentially curative approaches (Bhati *et al.*, 2025). These strategies aim to target the underlying molecular and cellular mechanisms of neurodegeneration, such as genetic defects, neuronal loss, protein aggregation, oxidative stress, and chronic inflammation. Rapid advances in molecular biology, biotechnology, and regenerative medicine have led to the development of innovative therapeutic approaches, including gene therapy, stem cell therapy, targeted interventions against protein aggregation, and the use of antioxidant and anti-inflammatory agents. These approaches are being actively investigated in disorders such as Alzheimer's disease and Parkinson's disease, with the goal of slowing, halting, or even reversing disease progression (Bhati *et al.*, 2025).

2.8.2.1 Gene therapy

Gene therapy is an advanced therapeutic strategy that aims to treat or prevent diseases by modifying the genetic material within a patient's cells (Qie *et al.*, 2025). In the context of neurodegenerative disorders, gene therapy focuses on correcting defective genes, reducing the production of toxic proteins, or enhancing the expression of protective factors that support neuronal survival (Qie *et al.*, 2025). Unlike conventional

treatments that mainly address symptoms, gene therapy targets the root molecular causes of disease, offering the potential for long-lasting or even permanent therapeutic effects. This approach is being actively explored in conditions such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. The fundamental principle of gene therapy involves delivering genetic material into target cells to alter their function (Bhati *et al.*, 2025). This is typically achieved using vectors, which are delivery systems designed to transport genes into cells. The most commonly used vectors are viral vectors, particularly adeno-associated viruses (AAVs), lentiviruses, and retroviruses, because of their ability to efficiently enter cells and deliver genetic payloads. These vectors are engineered to be safe by removing their disease-causing components while retaining their ability to infect cells. In neurodegenerative diseases, vectors are often administered directly into specific brain regions or into the cerebrospinal fluid to ensure targeted delivery to affected neurons (Bhati *et al.*, 2025). There are several different strategies used in gene therapy, depending on the disease mechanism. One major approach is gene replacement therapy, which involves introducing a functional copy of a gene to compensate for a defective or missing one. This is particularly relevant in genetic disorders where loss-of-function mutations lead to disease. Another approach is gene silencing, which aims to reduce or block the expression of harmful genes (Qie *et al.*, 2025). This is especially important in diseases caused by toxic gain-of-function mutations, such as Huntington's disease, where the mutant huntingtin protein accumulates and damages neurons. Techniques such as RNA interference (RNAi) and antisense oligonucleotides (ASOs) are used to selectively target and degrade the messenger RNA (mRNA) of the mutant gene, thereby reducing protein production (Bhati *et al.*, 2025).

A third strategy involves gene editing, which directly modifies the DNA sequence within cells (Qie *et al.*, 2025). Technologies such as CRISPR-Cas9 allow precise editing of specific genes, enabling correction of mutations at their source. Although still largely experimental, gene editing holds significant promise for treating inherited neurodegenerative diseases by permanently fixing genetic defects. Another important approach is gene augmentation, where genes encoding beneficial proteins such as neurotrophic factors are introduced to enhance neuronal survival, promote regeneration, and protect against damage (Qie *et al.*, 2025). For example, genes encoding glial cell line-derived neurotrophic factor (GDNF) have been investigated for their ability to

support dopaminergic neurons in Parkinson's disease. Gene therapy has shown encouraging results in preclinical and clinical studies. In Parkinson's disease, several clinical trials have explored delivering genes involved in dopamine synthesis, such as aromatic L-amino acid decarboxylase (AADC), directly into the brain. These therapies aim to increase local dopamine production and improve motor function (Qie *et al.*, 2025). In Huntington's disease, antisense oligonucleotide therapies have demonstrated the ability to lower levels of mutant huntingtin protein in early-phase clinical trials. In Alzheimer's disease, research is focused on targeting genes involved in amyloid-beta production and clearance, as well as enhancing protective pathways.

Despite its potential, gene therapy faces several challenges. One major issue is the safe and efficient delivery of genetic material across the blood-brain barrier, which restricts access to the central nervous system (Kohn *et al.*, 2023). Direct injection into the brain can overcome this barrier but is invasive and technically complex. Another challenge is ensuring long-term expression and stability of the introduced gene without causing unintended side effects, such as immune reactions or insertional mutagenesis (where the inserted gene disrupts other important genes) (Kohn *et al.*, 2023). Additionally, controlling the level and location of gene expression is critical to avoid overproduction of proteins, which could be harmful. Ethical and regulatory considerations also play an important role in the development of gene therapy. Because these treatments can produce long-lasting changes, careful evaluation of safety and efficacy is essential before widespread clinical use. Advances in vector design, targeting techniques, and gene-editing technologies are helping to address many of these challenges, making gene therapy increasingly feasible and safer (Kohn *et al.*, 2023).

2.8.2.2 Stem cell therapy

Stem cell therapy is an emerging and promising approach aimed at repairing or replacing damaged neurons in neurodegenerative diseases (Shi *et al.*, 2025). Stem cells are unique because they have two key properties: the ability to self-renew (divide and produce more stem cells) and the capacity to differentiate into specialized cell types, including neurons and glial cells. In disorders such as Parkinson's disease, Alzheimer's disease, and Huntington's disease, where progressive neuronal loss occurs, stem cell therapy aims to restore lost cells, improve neural function, and potentially slow disease progression (Isik *et al.*, 2025)). There are several types of stem cells used in research and

therapy. Embryonic stem cells (ESCs) are pluripotent, meaning they can differentiate into almost any cell type in the body, including neurons. Induced pluripotent stem cells (iPSCs) are adult cells (such as skin cells) that have been genetically reprogrammed to a pluripotent state, offering a patient-specific and ethically less controversial alternative to ESCs (Isik *et al.*, 2025). Adult stem cells, such as mesenchymal stem cells (MSCs) derived from bone marrow or adipose tissue, have more limited differentiation potential but are widely used due to their accessibility and lower risk of immune rejection (Isik *et al.*, 2025). Each type has advantages and limitations in terms of differentiation capacity, safety, and clinical applicability. The primary mechanism of stem cell therapy in neurodegenerative diseases is cell replacement. For example, in Parkinson's disease, there is a selective loss of dopaminergic neurons in the substantia nigra. Researchers are working on generating dopaminergic neurons from stem cells and transplanting them into the patient's brain to restore dopamine production (Sawamoto *et al.*, 2025). Preclinical studies and early clinical trials have shown that these transplanted cells can survive, integrate into neural circuits, and improve motor function. Similarly, in Huntington's disease, stem cells may be used to replace damaged neurons in the striatum, potentially improving motor and cognitive symptoms (Sawamoto *et al.*, 2025).

However, stem cell therapy is not limited to direct cell replacement. Stem cells also exert beneficial effects through what is known as paracrine signalling (Choudhery & Umezawa, 2025). They release a variety of bioactive molecules, including growth factors, cytokines, and neurotrophic factors, which support neuronal survival, promote synaptic repair, and reduce inflammation. For instance, stem cells can secrete brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF), both of which are essential for neuron survival and function (Choudhery & Umezawa, 2025). These supportive effects can help protect existing neurons and enhance the brain's natural repair mechanisms, even if the transplanted cells do not fully replace lost neurons. Another important benefit of stem cell therapy is its potential to modulate neuroinflammation. In many neurodegenerative diseases, chronic activation of immune cells such as microglia contributes to neuronal damage. Mesenchymal stem cells, in particular, have strong anti-inflammatory properties and can shift the immune response from a pro-inflammatory to an anti-inflammatory state. This helps create a more favorable environment for neuronal survival and regeneration (Choudhery & Umezawa, 2025).

Despite its potential, stem cell therapy faces several significant challenges. One major concern is ensuring that transplanted cells differentiate into the correct cell type and integrate properly into existing neural networks. Improper differentiation or uncontrolled cell growth could lead to tumor formation (S. J. Miller *et al.*, 2005). Another challenge is immune rejection, especially when donor-derived stem cells are used, although this risk can be reduced with autologous iPSCs (derived from the patient's own cells). Additionally, delivering stem cells to the appropriate brain regions is technically complex and often requires invasive procedures (S. J. Miller *et al.*, 2005). Ethical considerations also arise, particularly with the use of embryonic stem cells, although advances in induced pluripotent stem cell technology have helped address some of these concerns. Furthermore, long-term safety and effectiveness remain areas of active research, as many clinical trials are still in early stages (S. J. Miller *et al.*, 2005).

2.8.2.3 Targeting protein aggregation

Targeting protein aggregation is one of the most important emerging therapeutic strategies for neurodegenerative diseases because abnormal accumulation of misfolded proteins is a central pathological hallmark of many of these disorders (Ge *et al.*, 2025). In healthy cells, proteins are properly folded into specific three-dimensional structures that allow them to function correctly (Ge *et al.*, 2025). However, due to genetic mutations, aging, or cellular stress, some proteins misfold and begin to aggregate, forming toxic oligomers and larger insoluble deposits. These aggregates disrupt cellular homeostasis, impair synaptic function, interfere with intracellular transport, and ultimately lead to neuronal death (Ge *et al.*, 2025). This process is a defining feature in diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. Different neurodegenerative diseases are associated with specific aggregating proteins. In Alzheimer's disease, extracellular amyloid-beta plaques and intracellular tau neurofibrillary tangles accumulate in the brain. In Parkinson's disease, misfolded alpha-synuclein forms Lewy bodies within neurons. In Huntington's disease, a mutated form of the huntingtin protein aggregates due to an expanded polyglutamine (CAG) repeat. Although these proteins differ, the underlying mechanism of aggregation and toxicity shares common features, making it a key therapeutic target (Donaldson *et al.*, 2026).

One major therapeutic approach is to prevent protein misfolding and aggregation at an early stage (Donaldson *et al.*, 2026). Small molecules and chemical chaperones are

being developed to stabilize proteins in their correct conformation, thereby reducing the likelihood of misfolding (Gu *et al.*, 2025). These compounds can bind to partially folded proteins and prevent them from forming toxic aggregates. For example, certain experimental drugs aim to stabilize amyloid-beta in a non-aggregating form or inhibit the initial steps of aggregation (Gu *et al.*, 2025). This strategy is particularly valuable because early intervention may prevent the cascade of downstream pathological events. Another important strategy involves enhancing the clearance of aggregated proteins from the brain (Giusti *et al.*, 2024)). The body has natural systems for removing misfolded proteins, including the ubiquitin-proteasome system and autophagy-lysosomal pathways. In neurodegenerative diseases, these systems often become impaired. Therapeutic approaches aim to boost these clearance mechanisms, allowing cells to more effectively degrade and remove toxic aggregates. For instance, drugs that activate autophagy can promote the breakdown of aggregated proteins such as tau and alpha-synuclein. Enhancing proteasomal activity is another area of active research (Giusti *et al.*, 2024). Immunotherapy has emerged as a highly promising approach for targeting protein aggregation. This strategy uses antibodies either naturally produced by the immune system or administered as drugs to recognize and bind to specific misfolded proteins. In Alzheimer's disease, monoclonal antibodies targeting amyloid-beta have been developed to facilitate its clearance from the brain. Similarly, antibodies against tau protein aim to prevent its spread between neurons. In Parkinson's disease, immunotherapies targeting alpha-synuclein are being investigated to reduce its accumulation and toxicity. These approaches can work by neutralizing toxic protein species, preventing their aggregation, or promoting their removal by immune cells (Giusti *et al.*, 2024)).

Another emerging concept is inhibiting the spread of misfolded proteins between neurons (Soto & Pritzkow, 2018). Recent research suggests that proteins like tau and alpha-synuclein can propagate in a "prion-like" manner, spreading from one cell to another and seeding further aggregation. Therapies are being developed to block this transmission, thereby slowing disease progression. For example, antibodies or small molecules may be used to prevent the uptake of toxic protein aggregates by neighboring cells (Soto & Pritzkow, 2018). Gene-based approaches also play a role in targeting protein aggregation. Techniques such as RNA interference (RNAi) and antisense oligonucleotides (ASOs) can reduce the production of disease-causing proteins at the genetic level. For instance, lowering the expression of mutant huntingtin protein in

Huntington's disease or reducing amyloid precursor protein (APP) expression in Alzheimer's disease can decrease the formation of toxic aggregates (Soto & Pritzkow, 2018). These strategies aim to address the problem at its source by limiting the availability of aggregation-prone proteins. Despite significant progress, several challenges remain in targeting protein aggregation. One major difficulty is distinguishing between toxic and non-toxic forms of proteins, as some aggregates may be less harmful or even protective compared to smaller, soluble oligomers. Additionally, delivering therapeutic agents across the blood-brain barrier remains a major obstacle. Timing is also critical, as treatments may be most effective when administered early, before extensive neuronal damage has occurred (Soto & Pritzkow, 2018).

2.8.2.4 Antioxidant and anti-inflammatory agents

Antioxidant and anti-inflammatory agents represent an important class of emerging therapeutic strategies aimed at protecting neurons from damage and slowing the progression of neurodegenerative diseases (Kamaljeet *et al.*, 2024). Two major pathological processes oxidative stress and chronic neuroinflammation are consistently observed across disorders such as Alzheimer's disease and Parkinson's disease (Kamaljeet *et al.*, 2024). These processes are closely interconnected and create a harmful cycle that accelerates neuronal injury. Antioxidant and anti-inflammatory therapies aim to interrupt this cycle, thereby preserving neuronal function and improving clinical outcomes. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them through antioxidant defenses (Kamaljeet *et al.*, 2024). Neurons are particularly vulnerable to oxidative damage because of their high metabolic activity, reliance on mitochondrial energy production, and relatively low antioxidant capacity. Excess ROS can damage cellular components, including lipids (lipid peroxidation), proteins, and DNA, leading to impaired cellular function and eventual cell death (Wang *et al.*, 2023). Antioxidants work by neutralizing these reactive molecules, thereby reducing oxidative damage. Common antioxidant agents studied in neurodegenerative diseases include vitamin E, vitamin C, coenzyme Q10, and glutathione (B. Wang *et al.*, 2023). For example, coenzyme Q10 plays a key role in mitochondrial energy production and has been investigated for its potential to improve mitochondrial function and reduce oxidative stress in Parkinson's disease. Similarly, vitamin E has been studied for its ability to slow functional decline in

Alzheimer's disease, although results have been mixed. In addition to naturally occurring antioxidants, researchers are developing synthetic compounds with enhanced ability to cross the blood-brain barrier and target specific sources of oxidative stress (Wang *et al.*, 2023). Some of these compounds are designed to act directly within mitochondria, where a large proportion of ROS is generated. By protecting mitochondrial function, these therapies aim to preserve energy production and prevent the cascade of cellular damage associated with neurodegeneration.

Neuroinflammation is another key contributor to neurodegenerative diseases and is closely linked to oxidative stress (W. Zhang *et al.*, 2023). In the brain, immune cells such as microglia and astrocytes become chronically activated in response to injury, protein aggregates, or other stressors. While acute inflammation can be protective, chronic activation leads to the sustained release of pro-inflammatory cytokines, chemokines, and other toxic mediators that damage neurons and synapses (W. Zhang *et al.*, 2023). Anti-inflammatory agents aim to reduce this harmful immune response and restore a more balanced environment in the brain. Several types of anti-inflammatory agents are being explored. Non-steroidal anti-inflammatory drugs (NSAIDs) were among the first to be studied, with some epidemiological evidence suggesting that long-term use may reduce the risk of developing Alzheimer's disease (W. Zhang *et al.*, 2023). However, clinical trials have shown limited benefit in patients with established disease, indicating that timing of intervention is critical. More targeted approaches are now being developed, focusing on specific inflammatory pathways and molecules involved in neuroinflammation. For example, therapies that inhibit pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) or interleukin-1 (IL-1) are under investigation.

Another promising approach involves modulating microglial activity (H. Li *et al.*, 2024). Instead of simply suppressing inflammation, some therapies aim to shift microglia from a pro-inflammatory (neurotoxic) state to an anti-inflammatory (neuroprotective) state (Li *et al.*, 2024). This can enhance the clearance of toxic protein aggregates while reducing neuronal damage. Certain natural compounds, such as curcumin (derived from turmeric) and resveratrol (found in grapes), have shown both antioxidant and anti-inflammatory properties and are being studied for their potential neuroprotective effects (Li *et al.*, 2024). Importantly, antioxidant and anti-inflammatory strategies are often interconnected and may be more effective when used together. Oxidative stress can trigger inflammation, and inflammation can further increase ROS production, creating a

vicious cycle. By targeting both processes simultaneously, combination therapies may provide greater neuroprotection than targeting either pathway alone (Li *et al.*, 2024). These approaches are also being explored in conjunction with other treatments, such as gene therapy or protein-targeting therapies, to achieve a more comprehensive therapeutic effect. Despite their potential, challenges remain in translating these therapies into effective clinical treatments. One major issue is the difficulty of delivering sufficient concentrations of therapeutic agents to the brain due to the blood-brain barrier. Additionally, the complexity of neurodegenerative diseases means that targeting a single pathway may not be sufficient to halt disease progression. As a result, current research is increasingly focused on multi-targeted and personalized approaches (H. Li *et al.*, 2024).

2.9 Nanoparticles and Their Use in Neurodegenerative Diseases (NDs)

The treatment of neurodegenerative diseases (NDs) remains one of the most challenging areas in modern medicine, primarily due to the limitations of conventional drug delivery systems and the restrictive nature of the blood-brain barrier (BBB) (Akhtar *et al.*, 2021). As discussed earlier, most currently available therapies are only capable of slowing disease progression rather than providing a cure, largely because they fail to reach therapeutic concentrations within the central nervous system (CNS). This unmet clinical need has driven the exploration of nanotechnology-based approaches, which offer a promising alternative for effective drug and gene delivery to the brain. Nanotechnology involves the use of materials at the nanoscale, typically ranging from 1 to 1000 nanometers, allowing for interactions with biological systems at the molecular and cellular levels. This unique capability enables nanoparticles to overcome many of the physiological barriers that limit traditional drug delivery (Akhtar *et al.*, 2021). Due to their small size and modifiable surface properties, nanoparticles can be engineered to cross the BBB, target specific cells, and release therapeutic agents in a controlled manner. These features make nanocarriers particularly suitable for addressing the complex pathology of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease (Akhtar *et al.*, 2021).

Nanoparticles can be fabricated from a diverse array of materials, broadly classified into synthetic polymers, natural polymers, and inorganic substances. Synthetic polymer-based nanoparticles, such as those made from poly(lactic-co-glycolic acid)

(PLGA) and polycaprolactone (PCL), are widely studied due to their biodegradability, biocompatibility, and ability to provide controlled drug release (Akhtar *et al.*, 2021). Natural polymers, including polysaccharides like chitosan and proteins such as albumin, offer advantages such as low toxicity, bioadhesiveness, and inherent biological compatibility. Inorganic nanoparticles, including gold, silver, and cerium oxide, are also gaining attention due to their unique physicochemical properties, such as high stability, ease of functionalization, and potential antioxidant or imaging capabilities (Akhtar *et al.*, 2021). Each material type can be selected and optimized based on the therapeutic goal and the nature of the disease being targeted (Akhtar *et al.*, 2021). One of the major advantages of nanoparticles in ND treatment is their ability to enhance drug bioavailability in the brain while minimizing systemic toxicity. Conventional drugs often suffer from rapid degradation, poor solubility, and non-specific distribution, leading to reduced efficacy and unwanted side effects. In contrast, nanocarriers can encapsulate therapeutic molecules, protecting them from enzymatic degradation and premature clearance. This encapsulation increases the stability of the drug in circulation and allows a higher fraction of the administered dose to reach the brain (Akhtar *et al.*, 2021). Additionally, nanoparticles can provide sustained and controlled release of drugs, maintaining therapeutic concentrations over extended periods, which is particularly important in chronic neurodegenerative conditions. The ability of nanoparticles to cross the BBB is influenced by several physicochemical parameters, including size, surface charge, polarity, and surface chemistry (Akhtar *et al.*, 2021). Smaller nanoparticles are generally more efficient at penetrating the BBB, while surface modifications can further enhance their transport. For example, coating nanoparticles with surfactants such as polysorbate 80 facilitates the adsorption of apolipoproteins from the bloodstream, enabling receptor-mediated transcytosis across the BBB. This strategy also helps nanoparticles evade efflux transporters like P-glycoprotein, which would otherwise pump therapeutic agents back into the circulation. Such modifications significantly improve drug retention within the brain and enhance therapeutic outcomes.

Among the various nanoparticle systems, liposomal and polymeric nanoparticles are the most widely used for brain-targeted drug delivery. Liposomes are spherical vesicles composed of lipid bilayers that can encapsulate both hydrophilic and lipophilic drugs, making them highly versatile. They can fuse with cellular membranes, facilitating efficient drug release within target cells. Polymeric nanoparticles, on the other hand, offer

greater structural integrity and allow precise control over drug release kinetics. These systems can be further functionalized with targeting ligands to improve specificity and uptake by brain cells. Surface functionalization is a critical strategy in enhancing the performance of nanoparticles. By attaching specific ligands such as antibodies, peptides, or small molecules, nanoparticles can selectively bind to receptors expressed on the BBB or target neurons. Cell-penetrating peptides (CPPs), for instance, are short amino acid sequences that facilitate the transport of nanoparticles across cellular membranes, significantly improving intracellular delivery. Similarly, naturally derived compounds like lignans can be used to modify nanoparticle surfaces, enhancing their biocompatibility and targeting efficiency. These functionalization strategies not only improve BBB penetration but also enable targeted delivery to specific regions or cell types within the brain. In addition to drug delivery, nanoparticles also hold great potential for gene therapy in neurodegenerative diseases. They can be used to deliver nucleic acids such as DNA, RNA, or small interfering RNA (siRNA) to modulate gene expression and address the underlying causes of disease. This approach opens new avenues for treating conditions that are currently considered incurable. Furthermore, some nanoparticles possess intrinsic therapeutic properties; for example, cerium oxide nanoparticles exhibit antioxidant activity, which may help reduce oxidative stress a key factor in neurodegeneration.

Despite their numerous advantages, the clinical translation of nanoparticle-based therapies faces several challenges (Younis *et al.*, 2022). Issues related to large-scale production, long-term safety, potential toxicity, and regulatory approval must be carefully addressed. Additionally, variability in BBB permeability among patients and disease states can affect the consistency of treatment outcomes. Nevertheless, ongoing research and technological advancements continue to refine nanoparticle design and improve their safety and efficacy profiles (Younis *et al.*, 2022).

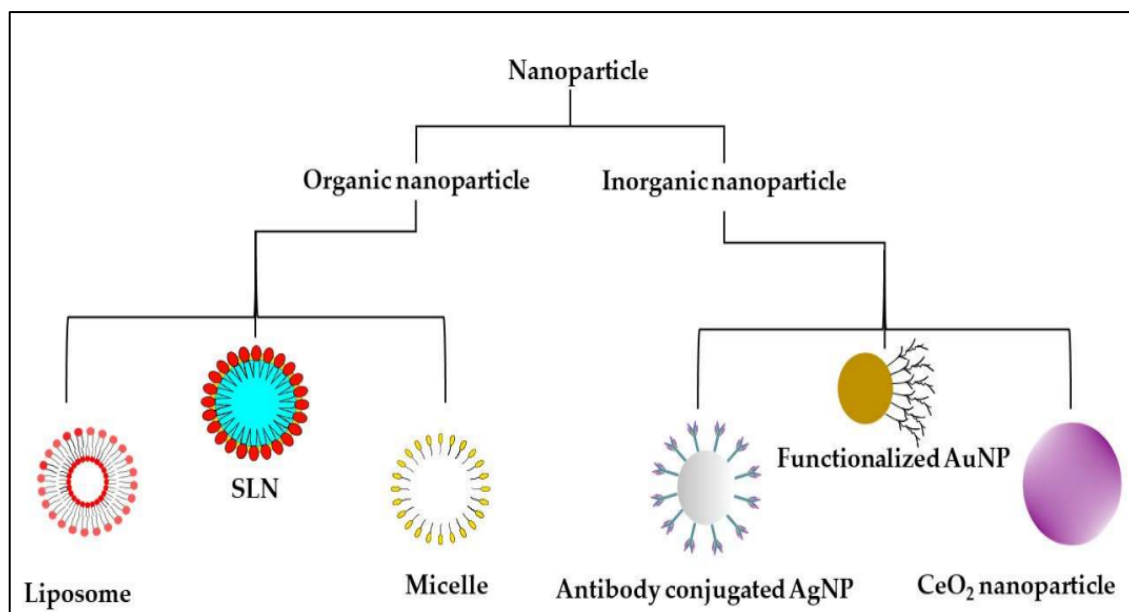


Figure 2.3: Common Nanoparticle Types Used in Neurodegenerative Disease Treatment

The first category highlighted in the figure is lipid-based nanoparticles, particularly solid lipid nanoparticles (SLNs) (Akanda *et al.*, 2023). These nanoparticles are composed of physiologically compatible lipids that remain solid at body temperature. In the figure, SLNs are often depicted as spherical structures with a solid lipid core surrounded by a stabilizing layer of surfactants (Akanda *et al.*, 2023). Their structure allows them to encapsulate lipophilic drugs efficiently while protecting them from enzymatic degradation. The lipid nature of SLNs facilitates their interaction with cell membranes, which enhances their ability to cross the BBB. Additionally, their controlled drug release profile makes them suitable for long-term treatment of chronic neurodegenerative conditions. Another important group shown in the figure is polymeric nanoparticles, typically represented as matrix systems or nanospheres (Akanda *et al.*, 2023). These are commonly made from biodegradable polymers such as PLGA or PCL. The illustration usually emphasizes their ability to uniformly disperse or encapsulate therapeutic agents within a polymer matrix. These nanoparticles are valued for their high stability, tunable degradation rates, and precise control over drug release kinetics. Their surfaces can also be easily modified with targeting ligands, which enhances their specificity toward brain endothelial cells or neurons (Akanda *et al.*, 2023).

The figure 2.3 also prominently features inorganic nanoparticles, including gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), and cerium oxide nanoparticles

(CeO₂ NPs) (Akanda *et al.*, 2023). These are typically illustrated as solid metallic or crystalline cores. Gold nanoparticles are often shown with functionalized surfaces, highlighting their ability to bind drugs, antibodies, or peptides for targeted delivery. Their unique optical properties also make them useful for diagnostic imaging, supporting theranostic applications (combined therapy and diagnosis) (Akanda *et al.*, 2023). Silver nanoparticles are generally included for their anti-inflammatory and antimicrobial properties, although the figure may imply the need for careful dosing due to potential toxicity. Cerium oxide nanoparticles are depicted as reactive particles capable of scavenging free radicals, emphasizing their antioxidant function, which is particularly beneficial in reducing oxidative stress associated with neurodegeneration (Akanda *et al.*, 2023). A key feature illustrated across many nanoparticle types in the figure is surface functionalization. This is often represented by ligands, polymers, or coatings attached to the nanoparticle surface. These modifications play a crucial role in enabling nanoparticles to bypass or exploit BBB transport mechanisms. For instance, coatings such as polysorbate 80 may be shown to facilitate adsorption of plasma proteins, which in turn mediate receptor-based transport across the BBB. Similarly, the attachment of cell-penetrating peptides (CPPs) or targeting ligands allows nanoparticles to bind to specific receptors on brain endothelial cells, enhancing uptake through receptor-mediated endocytosis (Akanda *et al.*, 2023). The figure may also implicitly highlight key parameters influencing nanoparticle performance, such as size, charge, and surface chemistry. Smaller nanoparticles are generally more efficient at crossing the BBB, while surface charge affects their interaction with biological membranes and proteins. Hydrophilic or hydrophobic balance is another critical factor, influencing circulation time and cellular uptake (Akanda *et al.*, 2023).

2.9.1 Inorganic Nanoparticles

Inorganic nanoparticles, particularly those composed of metals and metal oxides, have gained substantial attention in the field of neurodegenerative disease (ND) treatment due to their unique physicochemical properties and multifunctional capabilities (Klinkova & Thérien-Aubin, 2024). One of the most compelling features of these nanoparticles is their ability to cross the blood-brain barrier (BBB) and accumulate within brain tissues, which is a critical requirement for effective central nervous system (CNS) therapy (Klinkova & Thérien-Aubin, 2024). Unlike conventional drug molecules,

inorganic nanoparticles can be precisely engineered in terms of size, shape, surface charge, and chemical composition. These parameters significantly influence their biodistribution, cellular uptake, and interaction with biological systems, enabling tailored approaches for brain targeting. A key strategy in enhancing the brain-targeting efficiency of inorganic nanoparticles is surface functionalization. This involves attaching specific ligands such as antibodies, peptides, proteins, or small molecules (e.g., mannose) to the nanoparticle surface (Klinkova & Thérien-Aubin, 2024). These ligands can bind to receptors expressed on the BBB, facilitating receptor-mediated transcytosis and improving nanoparticle transport into the brain. Beyond drug delivery, inorganic nanoparticles are also widely utilized in theranostics, a field that combines therapeutic and diagnostic functions (Khan *et al.*, 2021). Their intrinsic optical, magnetic, or electronic properties allow them to serve as imaging agents while simultaneously delivering treatment. Among inorganic nanoparticles, gold nanoparticles (AuNPs) are the most extensively studied for CNS applications. Their popularity stems from their exceptional biocompatibility, stability, and unique optical properties, particularly surface plasmon resonance (SPR) (Khan *et al.*, 2021). This phenomenon allows AuNPs to interact strongly with light, making them highly suitable for imaging techniques such as micro-computed tomography (micro-CT) and X-ray imaging (Khan *et al.*, 2021). Compared to conventional contrast agents, AuNPs provide superior imaging contrast due to their high X-ray absorption capacity. Experimental studies have demonstrated that AuNPs functionalized with fluorescent dyes (e.g., rhodamine B isothiocyanate, RITC) and polymers like poly-L-lysine (PLL) exhibit enhanced uptake in human mesenchymal stem cells (hMSCs) (Khan *et al.*, 2021). When these labeled cells were injected into animal models, their distribution within the brain could be effectively visualized using imaging technologies.

In addition to imaging, AuNPs have shown promising therapeutic potential, particularly in targeting β -amyloid aggregates, a hallmark of Alzheimer's disease (Khan *et al.*, 2021). Functionalization with proteins such as apolipoprotein E3 (ApoE3) enhances their ability to cross the BBB and bind to amyloid plaques. Studies have demonstrated that such conjugated AuNPs can significantly disrupt amyloid aggregation up to 60% dissociation in some cases through interactions facilitated by their surface properties and plasmonic effects (Nouri *et al.*, 2021). Furthermore, AuNPs have been explored for promoting neuronal differentiation, as evidenced by their ability to induce

dopaminergic neuron formation from embryonic stem cells in experimental models. This suggests potential applications not only in disease treatment but also in neural regeneration. Silver nanoparticles (AgNPs) represent another class of inorganic nanocarriers with potential applications in brain drug delivery (Nouri *et al.*, 2021). They are particularly noted for their antimicrobial, anti-inflammatory, and antioxidant properties. Research indicates that AgNPs can reach specific brain regions, such as the hippocampus, following systemic administration. This localization is significant because the hippocampus is critically involved in memory and is heavily affected in many neurodegenerative conditions. AgNPs have been used to deliver therapeutic agents, including anticancer drugs like alisertib for glioblastoma and anti-amoebic drugs targeting brain infections. Additionally, AgNPs can modulate the activity of microglia, the immune cells of the brain. Studies have shown that citrate-coated AgNPs can selectively accumulate in microglia and stimulate the production of enzymes that reduce inflammation and oxidative stress. However, despite these advantages, AgNPs present notable safety concerns (Nouri *et al.*, 2021). They may disrupt the integrity of tight junctions within the BBB, potentially allowing harmful substances to enter the brain. Moreover, long-term accumulation of silver in neural tissues has been associated with neurotoxicity, neuronal damage, and degeneration. Therefore, careful dose optimization and surface modification are essential to mitigate these risks.

Cerium oxide nanoparticles (CeO₂ NPs) are particularly distinguished by their potent antioxidant properties. These nanoparticles can alternate between two oxidation states (Ce³⁺ and Ce⁴⁺), enabling them to scavenge reactive oxygen species (ROS) continuously. Since oxidative stress is a major contributor to neuronal damage in NDs, CeO₂ nanoparticles offer significant neuroprotective potential. They can reduce inflammation, prevent neuronal cell death, and enhance cellular survival pathways. Research suggests that these nanoparticles may also influence signalling pathways such as brain-derived neurotrophic factor (BDNF), which plays a critical role in neuronal growth and survival. Furthermore, CeO₂ nanoparticles have demonstrated the ability to inhibit β -amyloid aggregation, especially when combined with surface modifications such as polyethylene glycol (PEG) or metal chelators. In animal models of neurodegenerative diseases like multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS), these nanoparticles have shown promising results in improving motor function and reducing oxidative damage. In ischemic stroke models, they have effectively

neutralized harmful reactive species such as peroxynitrite. Notably, studies have also indicated their role in promoting neurogenesis. For instance, experimental findings suggest that even a single dose of CeO₂ nanoparticles can stimulate the formation of new neurons in the hippocampus, highlighting their regenerative potential.

In addition to these commonly studied nanoparticles, iron oxide nanoparticles have also been explored for CNS applications, particularly in imaging and cell tracking (Baabu *et al.*, 2022). For example, mononuclear cells labeled with iron oxide nanoparticles have been shown to migrate to specific brain regions, such as the choroid plexus, where they may contribute to functional recovery following ischemic stroke (Baabu *et al.*, 2022). These findings underscore the versatility of inorganic nanoparticles in both therapeutic and regenerative contexts. Overall, inorganic nanoparticles represent a powerful platform for addressing the challenges of brain drug delivery (Baabu *et al.*, 2022). Their tunable properties, combined with their multifunctional capabilities, make them highly suitable for both therapeutic and diagnostic applications. However, their potential toxicity and long-term accumulation remain critical challenges that must be carefully managed through advanced design and rigorous testing.

2.9.2 Organic Nanoparticles

Organic nanoparticles represent one of the most advanced and promising strategies for overcoming the challenges associated with drug delivery to the central nervous system (CNS), particularly in neurodegenerative diseases (NDs) (J. Gao *et al.*, 2024). These nanocarriers are primarily composed of biocompatible and biodegradable materials such as lipids, proteins, and natural or synthetic polymers. Their biological origin or similarity to endogenous compounds allows them to interact more safely with living systems compared to inorganic nanoparticles, significantly reducing the risks of cytotoxicity, chronic inflammation, and long-term accumulation in brain tissues (J. Gao *et al.*, 2024). This safety profile is especially critical in neurodegenerative conditions like Alzheimer's disease, Parkinson's disease, and multiple sclerosis, where prolonged treatment regimens are often required. One of the most significant advantages of organic nanoparticles lies in their ability to enhance the stability of therapeutic agents (J. Gao *et al.*, 2024). Many drugs used in neurological treatments especially biomacromolecules such as peptides, proteins, and nucleic acids are highly susceptible to degradation by enzymes in the bloodstream or peripheral tissues. Without protection, these molecules

often lose their therapeutic activity before reaching the brain (J. Gao *et al.*, 2024). Organic nanocarriers act as protective reservoirs, encapsulating these fragile compounds and shielding them from enzymatic attack, oxidation, and premature clearance. As a result, the bioavailability of the drug is significantly improved, and a higher proportion of the administered dose can reach the intended target site within the brain (J. Gao *et al.*, 2024).

In addition to improving stability, organic nanoparticles play a crucial role in minimizing systemic toxicity. Conventional drug delivery methods often result in widespread distribution of the drug throughout the body, affecting both diseased and healthy tissues (H. Wen *et al.*, 2015)). This non-specific distribution can lead to adverse side effects, particularly when potent drugs such as chemotherapeutics or neuroactive agents are involved. Organic nanocarriers address this issue by enabling targeted delivery (Z. Xu *et al.*, 2025). Through surface functionalization with specific ligands such as peptides, antibodies, or receptor-binding molecules these nanoparticles can selectively bind to receptors expressed on the blood-brain barrier (BBB) or diseased brain cells. This targeted approach ensures that the drug accumulates primarily in the affected regions, thereby reducing off-target effects and improving therapeutic outcomes (Islam *et al.*, 2025). Among the various types of organic nanoparticles, liposomes have emerged as the most extensively studied and widely utilized systems for brain drug delivery. Structurally, liposomes are spherical vesicles composed of one or more phospholipid bilayers surrounding an aqueous core. This unique architecture allows them to encapsulate both hydrophilic drugs (within the aqueous core) and hydrophobic drugs (within the lipid bilayer), making them highly versatile carriers (Islam *et al.*, 2025). Their structural similarity to cell membranes enables them to interact with biological systems, including fusion with cellular membranes or uptake via endocytosis. This property facilitates efficient intracellular delivery of therapeutic agents, which is essential for treating diseases that involve intracellular targets, such as protein aggregation in neurodegenerative disorders.

A major advancement in liposomal drug delivery is the development of surface-engineered or functionalized liposomes designed to cross the BBB more effectively (Islam *et al.*, 2025). The BBB is a highly selective barrier that restricts the entry of most therapeutic agents into the brain. However, liposomes can be modified with targeting ligands that exploit endogenous transport mechanisms, such as receptor-mediated

transcytosis. For instance, liposomes conjugated with modified apolipoprotein E (mApoE) can interact with low-density lipoprotein (LDL) receptors on the BBB, facilitating their transport into the brain (Islam *et al.*, 2025). This strategy significantly enhances the delivery efficiency of encapsulated drugs. An important example of this technology is the development of dual-functionalized liposomes for targeting β -amyloid aggregates in Alzheimer's disease. These liposomes are engineered with both mApoE and phosphatidic acid, enabling them to bind more effectively to amyloid plaques. The mechanism involves complementary electrostatic interactions: mApoE binds to negatively charged regions of the β -amyloid peptide, while phosphatidic acid interacts with positively charged amino acid residues (Islam *et al.*, 2025). This dual-binding strategy not only improves targeting specificity but also enhances the ability of the liposomes to disrupt amyloid fibrils. Experimental studies have demonstrated that such formulations can significantly reduce amyloid aggregation *in vitro*, highlighting their potential as disease-modifying therapies. Beyond liposomes, several other types of organic nanoparticles have been developed to address specific challenges in CNS drug delivery (Islam *et al.*, 2025). Polymeric nanoparticles, for example, are made from biodegradable polymers such as poly lactic-co-glycolic acid (PLGA). These systems offer excellent control over drug release kinetics, allowing for sustained and controlled delivery over extended periods. This is particularly beneficial in neurodegenerative diseases, where maintaining consistent drug levels in the brain is crucial for therapeutic efficacy. Polymeric nanoparticles can also be engineered to degrade at specific rates, ensuring that the drug is released in a predictable and controlled manner (Islam *et al.*, 2025).

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are another important class of organic nanocarriers (Viegas *et al.*, 2023)). SLNs are composed of solid lipids that remain solid at both room and body temperatures, providing a stable matrix for drug encapsulation. They offer advantages such as improved drug stability, controlled release, and protection against chemical degradation. However, SLNs may have limitations in drug loading capacity due to their highly ordered lipid structure (Viegas *et al.*, 2023). To overcome this, NLCs were developed as a second-generation system, incorporating both solid and liquid lipids to create a less ordered structure. This modification increases drug loading capacity and reduces the risk of drug expulsion during storage. Another remarkable feature of organic nanoparticles is their design

flexibility (Viegas *et al.*, 2023). These systems can be engineered to respond to specific physiological or pathological stimuli, enabling controlled and site-specific drug release. For example, pH-sensitive nanoparticles can release their payload in response to the slightly acidic environment found in inflamed or diseased brain tissues (Nunziata *et al.*, 2025).

Similarly, enzyme-responsive nanoparticles can degrade and release their drug cargo in the presence of specific enzymes that are overexpressed in neurodegenerative conditions. Temperature-sensitive and redox-responsive systems have also been explored, providing additional levels of control over drug delivery (Alavi *et al.*, 2024). Furthermore, organic nanoparticles can be combined with advanced targeting strategies to enhance their specificity and efficiency. Surface modification with cell-penetrating peptides (CPPs) allows nanoparticles to cross cellular membranes more easily, improving intracellular delivery. Similarly, conjugation with antibodies or ligands targeting disease-specific biomarkers enables selective accumulation in affected brain regions (El Alaoui *et al.*, 2024). These multifunctional systems, often referred to as “smart nanoparticles,” represent a significant step forward in precision medicine for neurodegenerative diseases.

2.10 Nano-medicines Under Clinical Trial

The development of effective therapies for neurodegenerative diseases (NDs) remains one of the most pressing challenges in modern medicine (Gadhav *et al.*, 2024). Disorders such as Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis (ALS), and Huntington’s disease are progressive in nature and, to date, lack curative treatments. Most currently available drugs are designed to manage symptoms such as cognitive decline, motor dysfunction, or behavioral disturbances rather than halt or reverse the underlying disease processes (Gadhav *et al.*, 2024). This limitation has driven an urgent need for innovative therapeutic strategies capable of addressing disease pathology at the molecular and cellular levels. In this context, nanomedicine has emerged as a highly promising field, offering novel solutions for targeted drug delivery, improved bioavailability, and enhanced penetration of the blood-brain barrier (BBB). Nanoparticle-based drug delivery systems have demonstrated significant potential in preclinical studies, particularly in improving the transport of therapeutic agents into the central nervous system (CNS) (Gadhav *et al.*, 2024). However, despite extensive laboratory success, the translation of these technologies into clinical practice remains relatively

limited. A review of ongoing and completed clinical trials reveals that fewer than ten nanoparticle-based formulations specifically targeting neurodegenerative diseases are currently under active investigation. This relatively small number highlights both the novelty of the field and the substantial challenges associated with clinical translation, including safety concerns, large-scale manufacturing, regulatory approval, and long-term efficacy evaluation. One of the most notable advancements in nanomedicine is the use of lipid nanoparticles (LNPs) for gene delivery applications (Gadhav *et al.*, 2024). Lipid nanoparticles have gained considerable attention due to their biocompatibility, ability to encapsulate nucleic acids, and success in other therapeutic areas such as mRNA vaccines.

In the context of neurodegenerative diseases, LNPs are being explored as delivery vehicles for gene-editing technologies, particularly CRISPR/Cas9 systems (Ebrahimi-Khezrabad *et al.*, 2025). These systems allow for precise modification of disease-causing genes, offering the potential to address the root cause of certain neurodegenerative conditions (Gadhav *et al.*, 2024). Currently, early-phase (Phase I) clinical trials are investigating LNP-mediated delivery of CRISPR-based therapies for transthyretin-mediated amyloidosis, a disorder characterized by the accumulation of misfolded transthyretin protein. This approach represents a significant shift from symptomatic treatment to disease-modifying therapy, as it aims to silence or correct the faulty gene responsible for protein aggregation. Another promising area of clinical research involves nanoparticle-based drug delivery systems designed to treat dementia and related cognitive disorders. For example, a formulation known as APH-1105 is being developed using nanoparticle carriers to enhance drug delivery to the brain (Chenxi *et al.*, 2025). This system is intended for patients with mild to severe dementia and aims to improve therapeutic efficacy by overcoming BBB limitations. Clinical studies planned or initiated around 2023 are exploring the safety, tolerability, and preliminary efficacy of this approach (Chenxi *et al.*, 2025). Although still in early stages, such developments highlight the growing interest in nanotechnology as a means of improving treatment outcomes in complex neurological conditions.

Gold nanoparticle-based therapies represent another important direction in clinical nanomedicine. One of the most advanced candidates in this category is CNM-Au8, a suspension of gold nanocrystals designed to enhance cellular energy metabolism and support neuronal survival (Chenxi *et al.*, 2025). Unlike traditional drug delivery systems that primarily act as carriers, CNM-Au8 is believed to exert intrinsic therapeutic

effects by catalyzing biochemical reactions involved in cellular energy production (Trial *et al.*, 2025) This mechanism is particularly relevant in neurodegenerative diseases, where mitochondrial dysfunction and energy deficits are common pathological features. CNM-Au8 has progressed to Phase II clinical trials for conditions such as ALS and multiple sclerosis (MS), indicating a higher level of clinical maturity compared to many other nanoparticle-based therapies (Gadhav *et al.*, 2024). In ALS, several Phase I and Phase II clinical trials are currently evaluating the safety and efficacy of CNM-Au8 gold nanocrystals. Early results suggest that this nanomedicine may help slow disease progression by improving neuronal metabolism and reducing oxidative stress. The ability of gold nanoparticles to cross the BBB and accumulate in neural tissues makes them particularly suitable for such applications. Additionally, their unique physicochemical properties, including high surface reactivity and the ability to functionalization, allow for further optimization of therapeutic effects (Ebrahimi-Khezrabad *et al.*, 2025).

Despite these encouraging developments, the clinical translation of nanomedicines for neurodegenerative diseases faces several significant challenges. One major concern is the long-term safety of nanoparticles, particularly with repeated administration. While many organic nanoparticles are biodegradable, some inorganic systems-such as metal-based nanoparticles may accumulate in tissues over time, potentially leading to toxicity. Regulatory agencies therefore require extensive preclinical and clinical data to ensure safety before approval can be granted.

Another challenge lies in achieving consistent and scalable manufacturing processes. Nanoparticles are highly sensitive to variations in size, surface properties, and composition, all of which can influence their biological behavior. Ensuring batch-to-batch consistency is critical for clinical applications but can be difficult to achieve on a large scale. Additionally, the complexity of nanoparticle systems often leads to higher production costs compared to conventional drugs, which may limit accessibility and widespread adoption.

Furthermore, the heterogeneity of neurodegenerative diseases adds another layer of complexity. These conditions often involve multiple pathological mechanisms, including protein aggregation, oxidative stress, inflammation, and neuronal loss. As a result, a single therapeutic approach may not be sufficient, and combination therapies may be required. Nanoparticles offer a potential advantage in this regard, as they can be

engineered to carry multiple therapeutic agents simultaneously, enabling a multifaceted treatment strategy.

Table 2.1 Various neurological diseases being investigated using nanocarrier-mediated formulations (data retrieved on January 31, 2022, from *ClinicalTrials.gov*).

Product (Active Molecules/Class)	Nanocarrier (Composition)	Indications	Clinical Phase, NCT Number
ALN-TTR02 (Patisiran)	Lipid nanoparticle (DLin-MC3-DMA; PEG2000-C-DMG; DSPC; cholesterol)	Transthyretin-mediated amyloidosis	Approved for marketing, NCT02939820
APH-1105 (an α -secretase modulator)	Nanoparticle	Mild-to-moderate Alzheimer's disease (AD), dementia	Phase 2, NCT03806478
Short palindromic repeats (CRISPR)/Cas9 gene	Lipid nanoparticle (proprietary LNP delivery system with ionizable lipid, phospholipid, PEGylated lipid, cholesterol)	Hereditary transthyretin amyloidosis	Phase 1, NCT04601051
CNM-Au8 (Nanocrystalline gold)	Gold nanocrystals	Amyotrophic lateral sclerosis (ALS)	Phase 1, NCT04081714
CNM-Au8 (Nanocrystalline gold)	Gold nanocrystals	Amyotrophic lateral sclerosis (ALS)	Phase 2, NCT04098406
CNM-Au8 (Nanocrystalline gold)	Gold nanocrystals	Amyotrophic lateral sclerosis (ALS)	Phase 2, NCT03843710
CNM-Au8 (Nanocrystalline gold)	Gold nanocrystals	Parkinson's disease (PD)	Phase 2, NCT03815916

2.11 Prevalent Neurodegenerative Diseases and Their Treatment Modalities

Neurodegenerative diseases represent a group of progressive disorders characterized by the gradual loss of neuronal structure and function, ultimately leading to cognitive and motor impairments (Gadhav *et al.*, 2024). Among the most prevalent conditions are Alzheimer's disease, Parkinson's disease, and Huntington's disease, each of which exhibits distinct pathological features but shares common mechanisms such as protein aggregation, oxidative stress, and neuroinflammation. Despite extensive research, most currently available treatments are primarily symptomatic and do not fully halt disease progression. This section provides a concise overview of these commonly occurring neurodegenerative disorders along with the therapeutic strategies currently employed to manage their symptoms and, in some cases, target underlying disease mechanisms (Gadhav *et al.*, 2024).

2.11.1 Alzheimer's Disease (AD)

The treatment of Alzheimer's disease is multifaceted and aims to address both the symptomatic manifestations and the underlying pathological mechanisms, including amyloid-beta ($A\beta$) accumulation, tau hyperphosphorylation, neurotransmitter imbalance, and neuroinflammation (C.-K. Wu & Fuh, 2025). Despite significant advances, current therapies are largely unable to completely halt disease progression, and therefore treatment strategies are typically divided into symptomatic treatments and disease-modifying therapies (DMTs), along with supportive non-pharmacological interventions (C.-K. Wu & Fuh, 2025).

2.11.1.1. Symptomatic Pharmacological Treatments

a) Cholinesterase Inhibitors (Cholinergic Therapy)

One of the earliest and most established therapeutic approaches is the use of cholinesterase inhibitors, which target the cholinergic deficit observed in Alzheimer's disease (Marucci *et al.*, 2021). Degeneration of cholinergic neurons in the basal forebrain leads to reduced levels of acetylcholine, a neurotransmitter essential for learning and memory. Drugs such as Donepezil, Rivastigmine, and Galantamine inhibit the enzyme acetylcholinesterase, thereby preventing the breakdown of acetylcholine in the synaptic

cleft (Marucci *et al.*, 2021). This results in enhanced cholinergic neurotransmission and modest improvements in cognitive function, memory, and attention, particularly in mild to moderate stages of the disease. However, these drugs do not prevent neuronal degeneration and their efficacy declines as the disease progresses.

b) NMDA Receptor Antagonists (Glutamatergic Modulation)

Another key therapeutic target is glutamate-mediated excitotoxicity. In Alzheimer's disease, excessive glutamate release leads to overactivation of NMDA (N-methyl-D-aspartate) receptors, causing increased intracellular calcium influx and neuronal damage (Esposito *et al.*, 2013). Memantine is a non-competitive NMDA receptor antagonist that selectively blocks pathological overactivation while preserving normal synaptic signalling. This helps reduce excitotoxic neuronal injury and improves cognitive function, behavior, and daily functioning in moderate to severe stages of Alzheimer's disease. Memantine is often used in combination with cholinesterase inhibitors for enhanced therapeutic effect (Esposito *et al.*, 2013).

c) Management of Behavioural and Psychological Symptoms

Patients with Alzheimer's disease frequently develop behavioural and psychological symptoms, including agitation, aggression, psychosis, and depression (Gottesman & Stern, 2019)). These symptoms significantly impact quality of life and caregiver burden. Atypical antipsychotics such as Brexpiprazole are used to manage agitation associated with dementia. Brexpiprazole acts as a partial agonist at dopamine D2 receptors and serotonin 5-HT1A receptors, and as an antagonist at 5-HT2A and adrenergic receptors, thereby stabilizing mood and reducing behavioural disturbances (Nakamura & Nagamine, 2019)). However, these medications must be used cautiously due to potential side effects, including increased risk of stroke and mortality in elderly patients.

2.11.1.2 Disease-Modifying Therapies (DMTs)

Recent therapeutic advances have focused on targeting the underlying molecular pathology of Alzheimer's disease, particularly amyloid-beta accumulation.

a) Anti-Amyloid Monoclonal Antibodies

Monoclonal antibodies such as Aducanumab and Lecanemab are designed to selectively bind to aggregated forms of amyloid-beta in the brain (Cummings *et al.*, 2024). These antibodies facilitate the clearance of A β plaques through immune-mediated mechanisms, including microglial phagocytosis. Clinical studies have shown that these therapies can reduce amyloid burden in the brain and may modestly slow cognitive decline, particularly when administered in early stages of the disease (Rafii & Aisen, 2025). However, their use is associated with potential side effects such as amyloid-related imaging abnormalities (ARIA), including brain edema and microhemorrhages, requiring careful monitoring.

b) Emerging Tau-Targeted Therapies

Given the strong correlation between tau pathology and cognitive decline, therapies targeting tau protein are under active investigation. These include inhibitors of tau phosphorylation, aggregation inhibitors, and tau-directed immunotherapies. Although not yet widely approved, these approaches aim to prevent neurofibrillary tangle formation and halt disease progression at a downstream level.

2.11.1.3 Targeting Neuroinflammation and Oxidative Stress

Chronic activation of microglia and the resulting neuroinflammation play a significant role in disease progression (Ajoalabady *et al.*, 2025). Experimental therapies are being developed to modulate microglial activation, reduce pro-inflammatory cytokine release, and restore immune homeostasis. Similarly, antioxidant therapies aim to counteract oxidative stress, which contributes to neuronal damage. Compounds such as vitamin E, coenzyme Q10, and other neuroprotective agents are being studied, although clinical benefits remain limited and inconsistent.

2.11.1.4 Non-Pharmacological Interventions

In addition to drug therapy, non-pharmacological approaches are essential for comprehensive management. These include cognitive stimulation therapy, physical activity, social engagement, and structured routines, all of which help maintain cognitive function and improve quality of life (Berardi & Sale, 2025). Nutritional support and

management of comorbid conditions (e.g., hypertension, diabetes) are also critical components of care.

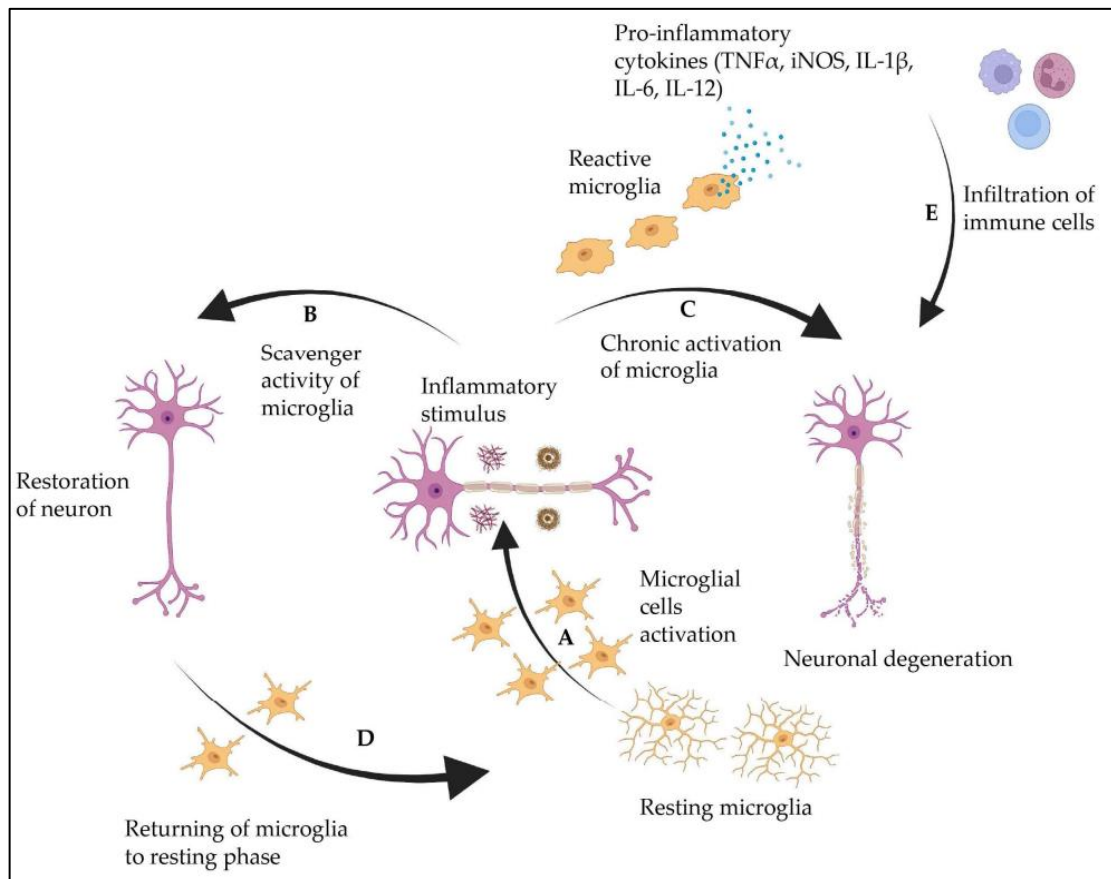


Figure 2.4: The function of microglia in the development of Alzheimer's disease

1. Stimuli such as the accumulation of tau protein or amyloid β may awaken dormant microglia. (B) Neurons are restored after amyloid β is completely cleared, and (D) microglia go back to their resting phase. On the other hand, neurotoxicity and proinflammatory cytokines are released by chronically activated microglia when amyloid β clearance fails (C). (E) Neurodegeneration results from the peripheral immune system's entrance. The website Biorender.com was used to generate this image.

2.11.2 Parkinsonian Neurodegeneration (Parkinson's Disease)

Parkinson's disease is a progressive neurodegenerative disorder primarily characterized by motor dysfunction resulting from the selective loss of dopaminergic neurons in the substantia nigra pars compacta, a region of the midbrain that plays a critical role in the regulation of voluntary movement (Grimes & Bulman, 2002). These neurons are responsible for the synthesis and release of dopamine, a key neurotransmitter involved in motor control, coordination, and reward pathways. As neuronal degeneration

progresses, dopamine levels in the striatum decline significantly, leading to impaired basal ganglia circuitry and disruption of motor function (Zhai *et al.*, 2023). Clinical symptoms typically manifest only after approximately 70-80% of dopaminergic neurons have been lost, highlighting the substantial compensatory capacity of the brain in early stages.

The hallmark motor symptoms of Parkinson's disease include resting tremor, bradykinesia (slowness of movement), muscular rigidity, and postural instability (DeMaagd & Philip, 2015). These symptoms arise due to an imbalance between dopaminergic and cholinergic signalling within the basal ganglia. In addition to motor symptoms, non-motor manifestations such as cognitive impairment, mood disorders, and autonomic dysfunction may also occur, reflecting the widespread nature of neurodegeneration. The pharmacological management of Parkinson's disease is primarily aimed at restoring dopaminergic activity or modulating related neurotransmitter systems (Power & Crotty, 2025). The most effective and widely used treatment is dopamine replacement therapy with Levodopa, which is considered the gold standard. Levodopa is a metabolic precursor of dopamine that can cross the blood-brain barrier, unlike dopamine itself. Once inside the brain, it is converted into dopamine by the enzyme aromatic L-amino acid decarboxylase at presynaptic neuronal terminals (Segura-Aguilar, 2021). This replenishment of dopamine helps alleviate motor symptoms by stimulating dopamine receptors in the striatum. To enhance its efficacy and reduce peripheral side effects, levodopa is commonly co-administered with carbidopa, a peripheral decarboxylase inhibitor that prevents the premature conversion of levodopa into dopamine outside the brain (Singer *et al.*, 2010). This combination increases the bioavailability of levodopa in the central nervous system and minimizes gastrointestinal adverse effects.

In addition to levodopa, dopamine agonists such as Pramipexole, Ropinirole, Apomorphine, and Rotigotine are used to directly stimulate dopamine receptors without requiring metabolic conversion (Tan *et al.*, 2022). These agents have longer half-lives than levodopa and are often used in early stages of the disease or in combination therapy to reduce motor fluctuations and “off” periods associated with long-term levodopa use. Another important class of drugs includes monoamine oxidase-B (MAO-B) inhibitors, such as Selegiline, Rasagiline, and Safinamide (Tan *et al.*, 2022). These drugs inhibit the enzyme responsible for the oxidative breakdown of dopamine in the brain, thereby

increasing its availability and prolonging its action. MAO-B inhibitors can be used as monotherapy in early disease or as adjunct therapy with levodopa to enhance therapeutic outcomes (Tan *et al.*, 2022). Catechol-O-methyltransferase (COMT) inhibitors, including Entacapone, Tolcapone, and Opicapone, further improve levodopa efficacy by inhibiting its peripheral metabolism (Wu *et al.*, 2024). By reducing the breakdown of levodopa before it reaches the brain, these agents increase its half-life and help maintain more stable dopamine levels, thereby reducing motor fluctuations.

Anticholinergic drugs are also used, particularly for managing tremors and rigidity (LeWitt *et al.*, 2024). These medications work by inhibiting the action of acetylcholine, thereby restoring the balance between cholinergic and dopaminergic activity in the basal ganglia. However, their use is limited due to potential side effects, especially in elderly patients. Adenosine A2A receptor antagonists, such as Istradefylline, represent a newer class of drugs that modulate non-dopaminergic pathways (Mori *et al.*, 2022)). These agents improve motor function and reduce “off” time by enhancing dopaminergic signalling indirectly, offering an additional therapeutic option for patients with advanced disease. Another adjunctive treatment is Amantadine, which has multiple mechanisms of action, including modulation of glutamatergic activity and reduction of NMDA receptor-mediated excitotoxicity (Mori *et al.*, 2022). It is particularly useful in reducing levodopa-induced dyskinesias (involuntary movements).

2.11.3 Autoimmune lateral sclerosis

2.11.3.1 Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis, also known as Lou Gehrig’s disease, is a severe and progressive neurodegenerative disorder characterized by the selective degeneration of motor neurons responsible for voluntary muscle movement (González-Sánchez *et al.*, 2025)). These motor neurons are located in both the upper motor neuron pathways (corticospinal tracts in the brain) and lower motor neurons (brainstem and spinal cord) (González-Sánchez *et al.*, 2025). As these neurons deteriorate, communication between the brain and muscles becomes progressively impaired, ultimately leading to muscle weakness, atrophy, and paralysis. To understand ALS in a more connected way, consider how voluntary movement normally occurs (González-Sánchez *et al.*, 2025). Signals originate in the motor cortex of the brain and travel through the corticospinal tract to the spinal cord, where they are transmitted to muscles via lower motor neurons. In ALS, both

upper and lower motor neurons degenerate, disrupting this pathway at multiple levels (González-Sánchez *et al.*, 2025). As a result, muscles no longer receive proper stimulation, leading to progressive loss of function. For example, a patient may initially notice difficulty gripping objects or frequent tripping while walking. Over time, this weakness spreads to other muscle groups, affecting speech (dysarthria), swallowing (dysphagia), and eventually breathing (González-Sánchez *et al.*, 2025).

Clinically, ALS presents with a combination of upper and lower motor neuron signs ((Sellier *et al.*, 2024)). Upper motor neuron involvement leads to spasticity, hyperreflexia, and stiffness, while lower motor neuron degeneration causes muscle wasting (atrophy), weakness, and fasciculations (muscle twitching) (Sellier *et al.*, 2024). For instance, a patient might experience visible twitching in the arms along with progressive thinning of muscles and difficulty performing simple tasks like lifting a cup or climbing stairs. As the disease advances, respiratory muscles become affected, which is the primary cause of mortality, often due to respiratory failure or complications such as pneumonia (Sellier *et al.*, 2024). Although ALS primarily affects motor function, it is now recognized as a multisystem disorder. A significant proportion of patients also develop cognitive and behavioral impairments, and some may meet the criteria for Frontotemporal dementia (Chatzidimitriou *et al.*, 2023). This overlap highlights shared pathological mechanisms, including protein aggregation and neuronal vulnerability. The exact cause of ALS remains unclear, but both genetic and environmental factors are implicated. Approximately 5-10% of cases are familial, caused by inherited genetic mutations. Among these, mutations in genes such as C9ORF72, SOD1, TARDBP, and FUS are the most common, collectively accounting for a large proportion of familial ALS cases (Sellier *et al.*, 2024). For example, the hexanucleotide repeat expansion in the C9ORF72 gene is strongly associated with both ALS and frontotemporal dementia (Sellier *et al.*, 2024). These genetic mutations often lead to abnormal protein folding, impaired RNA processing, mitochondrial dysfunction, and increased oxidative stress, all of which contribute to motor neuron degeneration.

From a therapeutic perspective, ALS management is largely supportive and multidisciplinary, focusing on prolonging survival and improving quality of life (De Almeida *et al.*, 2021). Since there is no definitive cure, treatment involves a combination of pharmacological interventions and supportive care, including respiratory support, nutritional management, and physical therapy. One of the most widely used drugs is

Riluzole, which acts primarily by inhibiting glutamate release (Boer *et al.*, 2019). Excess glutamate can cause excitotoxicity, leading to neuronal damage. By reducing glutamatergic activity, riluzole helps slow disease progression and modestly extends survival, typically by a few months (De Boer *et al.*, 2019)). For example, a patient receiving riluzole may experience a slightly delayed need for ventilatory support compared to untreated individuals. Another important therapy is Edaravone, a free radical scavenger that reduces oxidative stress (Dakroub *et al.*, 2026), one of the key mechanisms implicated in ALS pathology. It is believed to protect neurons from damage caused by reactive oxygen species, although its exact mechanism is not fully understood. Edaravone has been shown to slow functional decline in some patients (Takahashi *et al.*, 2025), particularly when administered in early stages of the disease. More recently, targeted therapies have emerged, especially for genetic forms of ALS. Tofersen is an antisense oligonucleotide drug designed for patients with SOD1 gene mutations (T. M. Miller *et al.*, 2022). It works by binding to the mRNA produced by the mutant SOD1 gene, promoting its degradation and thereby reducing the production of toxic SOD1 protein. This targeted approach represents a shift toward precision medicine. For example, in clinical studies, Tofersen reduced SOD1 protein levels in cerebrospinal fluid and showed potential in slowing disease progression (T. M. Miller *et al.*, 2022). Another recently approved therapy is AMX0035 (a combination of sodium phenylbutyrate and taurursodiol), which targets cellular stress pathways, including mitochondrial dysfunction and endoplasmic reticulum stress (Paganoni *et al.*, 2020). By protecting neurons from these stressors, it helps delay motor neuron death and slow disease progression. In addition to pharmacological treatments, supportive care plays a critical role. Non-invasive ventilation can significantly improve survival and quality of life by assisting breathing as respiratory muscles weaken. Nutritional support, often through feeding tubes, helps prevent malnutrition in patients with swallowing difficulties (Caiafa *et al.*, 2025). Physical and occupational therapy can maintain mobility and independence for as long as possible.

2.11.3.2 Huntington's Disease (A Neurodegenerative Illness Causing Dementia)

Huntington's disease (HD) is a progressive, inherited neurodegenerative disorder that affects movement, cognition, and behavior. It is classified as an autosomal

dominant condition, meaning that a mutation in just one copy of the causative gene is sufficient to produce the disease (R *et al.*, 2024). As a result, each child of an affected individual has a 50% chance of inheriting the disorder. What makes Huntington's disease particularly significant is that it not only causes motor dysfunction but also leads to severe cognitive decline and psychiatric disturbances, ultimately resulting in dementia. The disease is caused by a mutation in the HTT gene located on chromosome 4, which encodes the huntingtin protein (Schulte & Littleton, 2011). This mutation involves an abnormal expansion of CAG trinucleotide repeats within the gene. In healthy individuals, the number of CAG repeats typically ranges from 9 to 34 (Van Roon-Mom *et al.*, 2005). However, when the number of repeats exceeds 36, the gene produces an abnormal version of the protein known as mutant huntingtin (mHTT) (Van Roon-Mom *et al.*, 2005). The risk and severity of the disease increase with the number of repeats, and individuals with more than 40 repeats almost inevitably develop Huntington's disease. For example, a person with 42 repeats is highly likely to develop symptoms in mid-adulthood, whereas someone with 60 or more repeats may develop a juvenile form of the disease at a much earlier age.

At the molecular level, the expanded CAG repeats result in an elongated polyglutamine (polyQ) tract in the huntingtin protein (Van Roon-Mom *et al.*, 2005). This structural alteration causes the protein to misfold and form toxic aggregates within neurons, particularly in the striatum and other regions of the basal ganglia. These aggregates interfere with multiple cellular processes, including transcriptional regulation, mitochondrial function, and intracellular transport (Shin *et al.*, 2005). Over time, these combined mechanisms lead to progressive neuronal dysfunction and cell death. The degeneration of neurons in the striatum has profound effects on motor control (Shin *et al.*, 2005). One of the most characteristic motor symptoms of Huntington's disease is chorea, which consists of involuntary, irregular, dance-like movements. For example, a patient may exhibit sudden, jerky movements of the arms or face that interfere with normal activities such as walking or eating. As the disease progresses, other motor abnormalities may develop, including dystonia (sustained muscle contractions), bradykinesia (slowness of movement), impaired coordination, and difficulty initiating voluntary movements (Hess & Hallett, 2017). In juvenile cases, rigidity and stiffness may predominate rather than chorea.

Beyond motor dysfunction, Huntington's disease has a significant impact on cognitive function (Hess & Hallett, 2017). Early cognitive changes often involve impaired executive function, such as difficulty planning, organizing, or making decisions (Reimers, 2019). Behavioral and psychiatric symptoms are also a major component of the disease and often appear early in its course. These may include depression, irritability, aggression, anxiety, apathy, and psychosis (Caiafa *et al.*, 2025). In some cases, patients may exhibit impulsive behavior or obsessive-compulsive tendencies. For example, a previously calm individual may develop sudden mood swings, become socially withdrawn, or display inappropriate behavior (Caiafa *et al.*, 2025). Notably, depression is highly prevalent in Huntington's disease, affecting up to 40% of patients, and there is an increased risk of suicidal ideation and attempts. Currently, there is no cure for Huntington's disease, and available treatments are primarily aimed at managing symptoms. One of the main therapeutic targets is chorea, which can be treated using Tetrabenazine. This drug works by inhibiting vesicular monoamine transporter 2 (VMAT2), which reduces the uptake of monoamines particularly dopamine into synaptic vesicles. As a result, dopamine levels in the synapse decrease, helping to control involuntary movements (Alm, 2021). However, because dopamine is also involved in mood regulation, tetrabenazine may cause side effects such as depression and sedation.

A newer alternative is Deutetetrabenazine, a modified version of tetrabenazine that contains deuterium atoms (Gupta *et al.*, 2022). This modification increases the drug's half-life, allowing for more stable plasma levels and less frequent dosing. It is generally better tolerated and associated with fewer neuropsychiatric side effects, making it a preferred option in many cases. In addition to VMAT2 inhibitors, antipsychotic medications such as Olanzapine and Risperidone are used to manage behavioral symptoms, including aggression, psychosis, and severe chorea (Connolly *et al.*, 2024). These drugs act by blocking dopamine D2 receptors and serotonin receptors, thereby stabilizing mood and reducing abnormal movements. For example, a patient experiencing hallucinations and aggressive behavior may benefit from risperidone therapy. Depression and anxiety are treated with antidepressants such as Fluoxetine, Sertraline, and Citalopram, which help regulate serotonin levels in the brain. Mood stabilizers and anticonvulsants, including Lamotrigine and Carbamazepine, may also be used to control mood swings, aggression, and irritability (Connolly *et al.*, 2024).

Table 2.2 FDA-approved pharmacological agents for neurodegenerative diseases (NDs), including their dosage forms and commonly reported adverse effects, summarizing current therapeutic options used in clinical management.

Neurodegenerative Disorder	Drug Class	Drug Name	Dosage Form	Side Effects	References
Alzheimer’s Disease (AD)	Cholinesterase inhibitors	Donepezil	Tablet	Nausea, vomiting, diarrhea, anorexia, weight loss, muscle cramps, insomnia, fatigue	(Birks & Harvey, 2018)
		Rivastigmine	Capsule, transdermal patch	Nausea, vomiting, diarrhea, anorexia, weight loss, indigestion, fainting, muscle weakness	(Prasad S & Patel, 2023)
		Galantamine	Tablet, capsule, oral solution	Nausea, vomiting, diarrhea, decreased appetite, weight loss, headache, dizziness	(Lim <i>et al.</i> , 2024)
	NMDA antagonist	Memantine	Tablet, capsule, oral solution	Diarrhea, dizziness, headache,	(Folch <i>et al.</i> , 2018)

				constipation, confusion	
	Atypical antipsychotic	Brexpiprazole	Tablet	Cold symptoms dizziness, shaking, weight gain, high blood sugar, stroke	(H. Kim <i>et al.</i> , 2016)
	Disease- modifying immunotherapy	Lecanemab	IV infusion	Brain swelling/bleeding, g, confusion, dizziness, nausea, visual changes, fever	(Hossain <i>et al.</i> , 2024)
		Aducanumab	IV infusion	Brain swelling/bleeding, g, headache, confusion, dizziness, falls	(Rahman <i>et al.</i> , 2023)
Parkinson’s Disease (PD)	Monoamine oxidase-B inhibitors	Rasagiline	Tablet	Nausea, dry mouth, dizziness, drowsiness, strange dreams, constipation, abdominal pain, heartburn, weight loss, may worsen dyskinesia	(Leegwater- Kim & Bortan, 2010)
		Selegiline	Tablet, orally disintegrating	Nausea, dry mouth, dizziness, constipation,	(K. Wang <i>et al.</i> , 2023)

			g tablet, capsule	abdominal pain, insomnia, headache, may worsen dyskinesia	
		Safinamide	Tablet	Nausea, dry mouth, dizziness, constipation, insomnia, may worsen dyskinesia	(Morales Casado & López Ariztegui, 2025)
	Catechol-O-methyltransferase inhibitors	Entacapone	Tablet	Diarrhea, discolored urine, potentiates levodopa side effects (dyskinesia, confusion)	(Brooks <i>et al.</i> , 2003)
		Tolcapone	Tablet	Diarrhea, discolored urine, enhanced levodopa effects, dyskinesia	(Artusi <i>et al.</i> , 2021)
		Opicapone	Capsule	Dizziness, drowsiness, dyskinesia, weight loss, constipation, low blood pressure	(Wang <i>et al.</i> , 2023)

	Anticholinergics	Benztropine	Tablet	Confusion, hallucinations, memory issues, blurred vision, dry mouth, constipation, flushing, urinary retention	(Hernández-Velázquez <i>et al.</i> , 2026)
		Trihexyphenidyl	Tablet, elixir	Confusion, hallucinations, memory issues, blurred vision, dry mouth, urinary retention	(H.-Y. Wang <i>et al.</i> , 2022)
	Adenosine A2A antagonist	Istradefylline	Tablet	Dyskinesia, hallucinations, dizziness, insomnia, nausea, constipation	(Hattori <i>et al.</i> , 2025)
	NMDA antagonist	Amantadine	Tablet, capsule, syrup	Dizziness, hallucinations, confusion, dry mouth, nausea, constipation, insomnia, urinary retention, peripheral edema	(Rujirussawarawong <i>et al.</i> , 2025)

Huntington's Disease (HD)	Antipsychotic medication	Olanzapine	Tablet	Drowsiness, dizziness, headache, dry mouth, weight gain, constipation, stomach upset	(Paleacu <i>et al.</i> , 2002)
		Risperidone	Tablet, oral solution	Drowsiness, dizziness, blurred vision, drooling, abdominal pain, dry mouth, nausea, weight gain, tiredness	(Bachoud-Lévi <i>et al.</i> , 2019)
	Antidepressants (Selective Serotonin Reuptake Inhibitors - SSRIs)	Citalopram	Tablet, capsule, oral solution	Nausea, drowsiness, dizziness, blurred vision, loss of appetite, dry mouth, tiredness, sweating, yawning	(Beglinger <i>et al.</i> , 2014)
		Fluoxetine	Tablet, capsule, oral solution	Nausea, drowsiness, dizziness, anxiety, insomnia, loss of appetite, dry mouth,	(Andriessen <i>et al.</i> , 2025)

				tiredness, sweating, yawning	
		Sertraline	Tablet, capsule, ora solution	Nausea, drowsiness, dizziness, dry mouth, loss of appetite, diarrhea, upset stomach, insomnia, increased sweating	(Garcia-Forn <i>et al.</i> , 2025)
	Mood stabilizers	Lamotrigine	Tablet	Dizziness, drowsiness, headache, nausea, vomiting, upset stomach, sweating	(Lauterbach, 2013)
		Carbamazepine	Tablet, capsule, oral suspension	Drowsiness, dizziness, nausea, vomiting, constipation, dry mouth, loss of appetite skin rash, unsteadiness	(Palaiogeorgou <i>et al.</i> , 2023)

2.12. CHALLENGES OF BRAIN-DRUG DELIVERY

Delivering drugs to the brain remains one of the most difficult problems in treating neurodegenerative diseases (NDs) such as Parkinson's disease and Alzheimer's disease (Akhtar *et al.*, 2021). Current therapeutic strategies mainly focus on slowing disease progression rather than achieving a complete cure, largely because most drugs fail to reach effective concentrations in the brain. The central obstacle is the presence of the blood-brain barrier (BBB), a highly selective physiological barrier that restricts the entry of potentially harmful substances from the bloodstream into the brain. While this protective mechanism is essential for maintaining neural homeostasis, it also significantly limits the delivery of therapeutic agents. Studies estimate that nearly 98% of small-molecule drugs and almost 100% of large biologics cannot naturally cross the BBB, making it a major bottleneck in drug development for NDs. As a result, even promising drug candidates often fail during clinical translation due to insufficient brain penetration (Akhtar *et al.*, 2021).

2.12.1 The “Bulletproof Vest” Nature of the BBB

The blood-brain barrier (BBB) is often likened to a “bulletproof vest” because of its exceptionally selective permeability, but this analogy only captures part of its complexity. At a microscopic level, the BBB is not a single structure but a dynamic, multicellular interface known as the neurovascular unit. Its core component is the brain microvascular endothelial cell (BMEC) layer, which lines cerebral capillaries (Jiao *et al.*, 2024). Unlike endothelial cells in peripheral tissues, BMECs are uniquely specialized: they have minimal pinocytotic activity, a very high electrical resistance (up to 1500-2000 $\Omega \cdot \text{cm}^2$), and extremely low paracellular permeability (Wang *et al.*, 2023). These features ensure that substances cannot easily “leak” between cells. The tightness of this barrier is primarily due to tight junction proteins such as claudins (especially claudin-5), occludin, and junctional adhesion molecules (JAMs), which form continuous sealing strands around adjacent endothelial cells. These are anchored intracellularly by scaffold proteins like ZO-1, linking them to the actin cytoskeleton and maintaining structural integrity (Jiao *et al.*, 2024). Adherens junctions, mainly composed of VE-cadherin, provide mechanical stability and regulate tight junction assembly, while gap junctions enable intercellular communication, allowing ions and small signalling molecules to pass between cells. Together, these junctional complexes virtually eliminate the paracellular

route, forcing most substances to cross the BBB via the more tightly regulated transcellular pathway.

Surrounding the endothelial layer is the basal lamina, a dense extracellular matrix composed of collagen IV, laminin, fibronectin, and proteoglycans (C. X. Wang & Shuaib, 2007). Embedded within or adjacent to this matrix are pericytes, which regulate capillary diameter, angiogenesis, and BBB integrity. Pericytes also influence endothelial gene expression, including the regulation of tight junction proteins and transporters. Enveloping the entire structure are the astrocytic end-feet, which cover nearly 99% of the capillary surface (Filosa & Iddings, 2013). Astrocytes release signalling molecules such as glial-derived neurotrophic factor (GDNF) and angiopoietin-1, which help maintain BBB function and modulate transport activity. Neurons, though not directly forming the barrier, contribute through signalling pathways that coordinate cerebral blood flow and metabolic demand. Beyond its structural defenses, the BBB is functionally reinforced by a sophisticated system of efflux transporters, particularly those belonging to the ATP-binding cassette (ABC) family (Filosa & Iddings, 2013). Among these, P-glycoprotein (P-gp) is the most extensively studied. Located on the luminal (blood-facing) surface of endothelial cells, P-gp actively expels a wide range of xenobiotics and drugs back into circulation using ATP hydrolysis. Other important efflux transporters include breast cancer resistance protein (BCRP) and multidrug resistance-associated proteins (MRPs) (Chaves *et al.*, 2024). These transporters create a biochemical barrier on top of the physical one, meaning that even if a drug successfully enters endothelial cells, it may still be removed before reaching the brain parenchyma. This is a major reason why many therapeutic agents, including anticancer drugs like doxorubicin or neuroprotective compounds, show poor central nervous system (CNS) bioavailability despite adequate plasma concentrations (C. X. Wang & Shuaib, 2007).

Transport across the BBB is therefore highly selective and occurs via several regulated mechanisms. Passive diffusion is limited to small (typically <400-500 Da), lipophilic, and non-ionized molecules with favorable partition coefficients (C. X. Wang & Shuaib, 2007). Classic examples include ethanol, nicotine, and certain anesthetics. However, most biologically active molecules especially peptides, proteins, and nucleic acids are too large or too polar for this route. Instead, they rely on carrier-mediated transport (CMT) or receptor-mediated transcytosis (RMT) (Chaves *et al.*, 2024).

Key transport systems include:

- GLUT1, which facilitates glucose uptake into the brain
- Insulin receptors, which mediate insulin transport
- Transferrin receptors, which transport iron-bound transferrin

These systems are highly specific and tightly regulated, ensuring that only essential nutrients enter the brain. Modern drug delivery research has leveraged these pathways by designing ligand-targeted therapeutics, such as transferrin- or insulin-mimicking nanoparticles, to “hitchhike” across the BBB via endogenous transport mechanisms. Pathological conditions can transiently alter BBB integrity. In diseases such as Alzheimer’s disease and Parkinson’s disease, inflammation, oxidative stress, and vascular dysfunction may lead to partial disruption of tight junctions, increased permeability, and altered transporter expression (Nozohouri *et al.*, 2025). For example, inflammatory cytokines like TNF- α and IL-1 β can downregulate tight junction proteins, making the barrier more permeable. However, this increased permeability is heterogeneous and unpredictable, and it often coincides with harmful consequences such as infiltration of immune cells, accumulation of neurotoxins, and exacerbation of neurodegeneration (Nozohouri *et al.*, 2025). Therefore, exploiting disease-induced BBB leakage is not considered a reliable or safe therapeutic strategy.

2.12.2 Influence of Pharmacokinetic Principles on Drug Transport to the Brain

The successful delivery of a drug to the brain is not determined solely by the blood-brain barrier (BBB), but also by the drug’s pharmacokinetic behaviour, commonly summarized as absorption, distribution, metabolism, and excretion (ADME) (D. Wu *et al.*, 2023). These processes collectively determine how much of a drug actually reaches the brain in an active form. Even before encountering the BBB, therapeutic molecules must survive a complex and often unfavourable (Wu *et al.*, 2023) journey through the systemic circulation, where multiple biological factors can significantly reduce their availability. One of the most critical factors affecting brain delivery is plasma protein binding (Johanson, 2023). After entering the bloodstream, many drugs bind reversibly to plasma proteins such as albumin, α 1-acid glycoprotein, and lipoproteins. While this binding can help stabilize drugs and prolong their circulation time, it also creates a major

limitation: only the unbound (free) fraction of a drug is pharmacologically active and capable of crossing the BBB. Highly protein-bound drugs (e.g., >90% binding) have a very small free fraction, which drastically reduces the amount available for brain uptake. Studies have shown that even slight increases in protein binding can significantly lower central nervous system (CNS) penetration, making this a key consideration in drug design (Wu *et al.*, 2023).

Another major determinant is drug distribution within the body. Once absorbed, drugs are distributed to various tissues depending on factors such as blood flow, tissue affinity, and physicochemical properties (Wu *et al.*, 2023). Organs like the liver, kidneys, and adipose tissue can act as reservoirs, sequestering drugs away from the brain. This reduces the effective concentration of the drug reaching cerebral circulation. Additionally, the volume of distribution (V_d) plays a role: drugs with very high V_d may accumulate in peripheral tissues rather than remaining available in plasma for BBB transport (Johanson, 2023). Metabolism and clearance further complicate drug delivery. The liver, through enzymatic systems such as cytochrome P450 enzymes, can metabolize drugs into inactive or less active forms before they ever reach the brain a phenomenon known as first-pass metabolism (particularly relevant for orally administered drugs) (Wu *et al.*, 2023). Even after entering systemic circulation, drugs may be continuously metabolized or cleared by the kidneys. As a result, the concentration of the drug declines over time, reducing the likelihood of achieving therapeutic levels in the brain. In some cases, metabolites may be more polar and thus even less capable of crossing the BBB. The interaction of drugs with cellular membranes and target cells also influences their uptake. Drug molecules can alter membrane properties, such as fluidity and permeability, or interact with ion channels and receptors. For example, some drugs may temporarily change membrane potential or block ion channels, which can affect cellular uptake mechanisms like endocytosis or transporter activity. These interactions are often transient but can still limit the efficiency with which drugs enter brain cells (Johanson, 2023). Moreover, repeated or prolonged exposure may trigger adaptive cellular responses, such as receptor downregulation or transporter modulation, further reducing drug effectiveness.

A key factor governing BBB penetration is the physicochemical nature of the drug. Molecules that are small (generally <500 Da), lipophilic, and non-ionized at physiological pH tend to cross the BBB more readily via passive diffusion. Lipophilicity,

often measured as the partition coefficient (logP), is particularly important because the BBB is composed of lipid-rich membranes (Silakari & Singh, 2021). However, there is a delicate balance: excessively lipophilic drugs may become trapped in cell membranes or bind non-specifically to proteins, reducing their effective concentration. On the other hand, highly hydrophilic or large molecules (such as peptides and biologics) struggle to cross the BBB without assistance from specialized transport systems. Another important pharmacokinetic consideration is drug half-life and stability. Drugs with very short half-lives may be cleared from circulation before they can accumulate in sufficient concentrations to cross the BBB. Conversely, drugs with longer half-lives may have better way of reaching the brain but could also pose risks of systemic toxicity. Stability in the bloodstream is equally important, as degradation by enzymes can render drugs inactive before they reach their target. Recent studies emphasize the importance of optimizing pharmacokinetics alongside BBB permeability (Silakari & Singh, 2021). Strategies such as prodrug design where an inactive compound is converted into an active drug after crossing the BBB, drug encapsulation in nanoparticles, and chemical modification to enhance lipophilicity or reduce protein binding have shown promise. For instance, lipid-based carriers and polymeric nanoparticles can shield drugs from metabolism, reduce protein binding, and improve circulation time, thereby increasing the likelihood of brain delivery.

2.13 Scope of the Study

The present study focuses on investigating the biochemical basis of neurodegenerative disorders, with particular emphasis on understanding the molecular mechanisms that contribute to the onset and progression of diseases such as Parkinson's disease, Alzheimer's disease, and related neurological conditions. The scope of this research encompasses the exploration of key biochemical pathways involved in neuronal dysfunction, including oxidative stress, protein misfolding and aggregation, mitochondrial impairment, neuroinflammation, and neurotransmitter imbalance. These interconnected processes are critically analyzed to establish their role in neuronal degeneration and synaptic dysfunction. A major component of the study involves examining the role of abnormal protein aggregation, such as β -amyloid plaques and tau tangles in Alzheimer's disease, and α -synuclein accumulation in Parkinson's disease. The study investigates how these misfolded proteins disrupt cellular homeostasis, impair

intracellular transport, and trigger neurotoxic cascades leading to neuronal death. Additionally, the research explores the involvement of oxidative stress and reactive oxygen species (ROS) in damaging cellular macromolecules, including lipids, proteins, and DNA, thereby accelerating neurodegeneration. The role of antioxidant defence systems and their imbalance in diseased conditions is also considered.

Another key aspect of the study is the analysis of mitochondrial dysfunction and its contribution to energy deficits in neuronal cells. Since neurons are highly energy-dependent, any disruption in mitochondrial function can significantly affect cellular metabolism and survival. The study evaluates biochemical alterations in mitochondrial pathways, including ATP production, electron transport chain activity, and apoptotic signalling. Furthermore, the role of neuroinflammation mediated by microglial activation and pro-inflammatory cytokines is examined, highlighting its contribution to chronic neuronal damage. The scope also includes the investigation of neurotransmitter imbalances, such as the depletion of dopamine in Parkinson's disease and alterations in acetylcholine levels in Alzheimer's disease. These biochemical changes are analyzed in relation to their impact on cognitive and motor functions. In addition, the study explores the role of enzymes, signalling molecules, and metabolic pathways that regulate neuronal communication and plasticity. Furthermore, the research may incorporate computational and bioinformatics approaches to analyse gene expression data, protein interaction networks, and biochemical pathways associated with neurodegenerative diseases. This integrative approach helps in identifying potential biomarkers and therapeutic targets. The study also considers emerging therapeutic strategies, including the use of nanotechnology-based drug delivery systems, antioxidants, enzyme inhibitors, and neuroprotective agents, to address the biochemical abnormalities underlying these disorders.

2.14 Significance of the Study

Neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, and related conditions, represent a major and growing global health burden due to their progressive nature, lack of curative treatments, and significant impact on quality of life. The significance of the present study lies in its focus on elucidating the biochemical mechanisms underlying these disorders, which is essential for advancing both scientific understanding and clinical management. By investigating key processes

such as oxidative stress, protein misfolding, mitochondrial dysfunction, and neuroinflammation, this study contributes to a deeper comprehension of the molecular events that drive neuronal degeneration. A major contribution of this research is the identification and analysis of critical biochemical pathways involved in disease progression. Understanding how abnormal protein aggregation, reactive oxygen species, and impaired cellular metabolism contribute to neuronal damage provides valuable insights into disease pathology. These findings are significant as they may help bridge existing gaps between basic research and clinical application, enabling the development of more targeted and effective therapeutic strategies. The study is also important in the context of biomarker discovery. By examining biochemical alterations and integrating molecular data, the research may aid in identifying reliable biomarkers for early diagnosis and disease monitoring. Early detection is particularly crucial in neurodegenerative diseases, as therapeutic interventions are more effective when applied during the initial stages of disease progression. Therefore, this study has the potential to support the development of non-invasive diagnostic tools and improve clinical outcomes. Furthermore, the study holds significance in guiding the development of novel treatment approaches. By highlighting the biochemical basis of neurodegeneration, it provides a foundation for exploring innovative therapeutic strategies, including antioxidant therapies, enzyme inhibitors, neuroprotective agents, and advanced drug delivery systems such as nanomedicine. These approaches aim not only to alleviate symptoms but also to target the underlying causes of disease, offering the possibility of slowing or halting disease progression. From a broader perspective, this research contributes to the growing field of neuroscience and molecular medicine by integrating biochemical and computational approaches. It supports the advancement of personalized medicine by identifying molecular targets that may vary among individuals, thereby enabling more precise and effective treatment strategies. Additionally, the findings of this study may serve as a reference for future research, encouraging further exploration into the complex mechanisms of neurodegenerative diseases.

2.15 Novelty of the Study

The novelty of the present study lies in its integrative and multi-dimensional approach to understanding the biochemical basis of neurodegenerative disorders. Unlike conventional studies that focus on isolated molecular mechanisms, this research

combines biochemical analysis with computational and systems-level investigations to provide a more comprehensive understanding of disease pathology. By simultaneously examining oxidative stress, protein aggregation, mitochondrial dysfunction, neuroinflammation, and neurotransmitter imbalance, the study offers a holistic perspective on the interconnected pathways driving neurodegeneration. A key innovative aspect of this work is the incorporation of microRNA-based regulatory analysis in Parkinson's disease. While previous studies have explored gene expression changes, this study emphasizes the role of microRNAs as critical post-transcriptional regulators influencing multiple disease-related pathways. The construction of microRNA-gene interaction networks and their integration with protein-protein interaction data provide deeper insights into the regulatory mechanisms underlying neuronal dysfunction. Another novel feature of the study is the application of a human interactome-based computational framework to investigate the epigenetic landscape of Alzheimer's disease. By mapping epigenetically altered genes onto complex biological networks, the research moves beyond traditional gene-centric approaches and highlights system-level disruptions. This strategy enables the identification of key regulatory hubs and pathways that may not be apparent through standard analyses, offering new potential therapeutic targets.

The study also introduces a comparative transcriptomic analysis of RNA-Seq data from both brain and peripheral blood samples in Parkinson's disease. This dual-tissue approach is relatively underexplored and provides a unique opportunity to identify circulating biomarkers that reflect central nervous system pathology. The identification of shared molecular signatures between brain and blood enhances the potential for developing non-invasive diagnostic tools, which is a significant advancement in the field. Furthermore, the integration of multi-omics data-including transcriptomics and epigenomics-represents an innovative step toward systems biology in neurodegenerative research. The use of advanced bioinformatics tools and network-based analyses allows for the identification of complex molecular interactions and predictive biomarkers. This comprehensive approach improves the accuracy and depth of data interpretation compared to traditional single-layer analyses. In addition, the study highlights emerging therapeutic perspectives by linking biochemical findings with advanced drug delivery strategies, including nanomedicine. This translational aspect bridges the gap between

fundamental research and clinical application, emphasizing the potential of targeted and efficient treatment approaches for neurodegenerative diseases.

2.16 Research Gaps and hypothesis

Despite major advances in the understanding of neurodegenerative diseases, several critical research gaps persist that limit the development of effective diagnostic tools and disease-modifying therapies. These gaps reflect the complexity and heterogeneity of disorders such as Alzheimer's disease and Parkinson's disease, where multiple overlapping molecular, genetic, and environmental mechanisms contribute to disease onset and progression. A major gap lies in the limited understanding of disease initiation mechanisms, particularly at the preclinical stage. While significant knowledge exists regarding late-stage pathological features-such as amyloid-beta plaques, tau neurofibrillary tangles, and alpha-synuclein aggregates-the precise molecular events that trigger the initial cascade of neurodegeneration remain unclear. It is still debated whether protein aggregation is the primary cause of neuronal dysfunction or a downstream consequence of earlier cellular disturbances such as mitochondrial dysfunction, synaptic failure, or impaired proteostasis. Furthermore, early pathogenic processes may involve subtle alterations in cellular homeostasis, including disruptions in calcium signalling, endoplasmic reticulum stress, and deficits in autophagy-lysosomal pathways. The lack of longitudinal data capturing these early events in humans, combined with limitations of current animal models in accurately replicating human disease, has hindered the identification of definitive causal mechanisms. This gap significantly impacts the ability to develop early diagnostic biomarkers and preventive interventions.

Another critical gap is the lack of effective disease-modifying therapies (DMTs). Most currently available treatments are symptomatic and do not alter the underlying neurodegenerative process. Despite numerous clinical trials targeting key pathological pathways such as amyloid deposition, tau phosphorylation, oxidative stress, and neuroinflammation many have failed to demonstrate significant clinical benefit. One reason for this failure is the multifactorial nature of neurodegeneration, where targeting a single pathway may be insufficient due to compensatory mechanisms and network-level dysfunction. Additionally, therapeutic interventions are often administered at relatively late stages of disease, when extensive neuronal loss has already occurred, limiting their potential efficacy. There is also a lack of reliable surrogate endpoints and

predictive biomarkers to assess therapeutic response in early-phase clinical trials. This highlights the need for a systems biology approach that integrates multiple molecular targets and considers disease heterogeneity, as well as the development of precision medicine strategies tailored to individual patient profiles.

Hypothesis

Neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease are driven by complex, system-level molecular interactions in which microRNAs and epigenetic modifications play a central regulatory role. It is hypothesized that dysregulated microRNA networks contribute significantly to the pathogenesis of Parkinson's disease by modulating gene expression across multiple biological pathways. Furthermore, epigenetic alterations within the human interactome are expected to provide critical insights into the molecular mechanisms underlying Alzheimer's disease progression. Additionally, it is proposed that comparative analysis of RNA-Seq data from brain and blood samples of Parkinson's disease patients will reveal shared and distinct gene expression signatures, enabling the identification of potential peripheral biomarkers that reflect central nervous system pathology. Collectively, these molecular and computational approaches are expected to enhance the understanding of neurodegenerative disease mechanisms and support the development of more accurate diagnostic and therapeutic strategies.

Objectives:

- Studying the System-level Involvement of MicroRNAs in Parkinson's Disease
- To the study of Insight into the epigenetics of Alzheimer's disease: a computational study from human interactome
- Comparative analysis of RNA-Seq data from brain and blood samples of Parkinson's Disease
- Study of neurodegenerative disease is one of the main focuses of present-day research

MATERIALS AND METHODS

3.1 Study Design and RNA-Seq-Based miRNA Profiling in Parkinson’s Disease Patients and Healthy Controls

The present case-control study was carefully designed to investigate microRNA (miRNA) expression patterns associated with Parkinson’s disease (PD) using a well-defined cohort and high-throughput sequencing technology. A total of 50 participants were recruited, comprising 26 clinically diagnosed PD patients and 24 age-matched healthy controls. This balanced sampling strategy ensured an appropriate comparison between diseased and non-diseased populations, thereby enhancing the statistical reliability of the findings. The selection of PD patients was based on the most recent and widely accepted diagnostic criteria, ensuring clinical accuracy and homogeneity within the patient group. The PD cohort included individuals with a mean age of 68.00 ± 6.47 years and an average disease duration of 5.75 ± 3.80 years, reflecting a population with moderate disease progression. Clinical phenotyping was also performed to capture disease heterogeneity: five patients exhibited the akinetic-rigid subtype, two were classified as tremor-dominant, and nine showed a mixed phenotype. This stratification is important, as different PD subtypes may exhibit distinct molecular signatures, including variations in miRNA expression profiles (Salemi *et al.*, 2022).

The control group consisted of neurologically healthy individuals with a mean age of 71.94 ± 13.19 years. These participants were rigorously screened to exclude any history of neurological or psychiatric disorders, and none were under medication that could potentially influence gene expression. A comprehensive neurological examination confirmed their healthy status, ensuring that observed differences in miRNA expression could be attributed specifically to PD-related pathology rather than confounding factors. For molecular analysis, RNA sequencing (RNA-seq) was employed to profile miRNA expression. This technique provides a highly sensitive and comprehensive approach for detecting both known and novel miRNAs, as well as quantifying their expression levels across samples. Total RNA was extracted from biological samples (typically blood or brain-derived material), followed by library preparation specifically enriched for small RNAs. High-throughput sequencing was then performed, generating large-scale

transcriptomic data. Subsequent bioinformatic analysis involved quality control, read alignment, and normalization of sequencing data to ensure accuracy and comparability across samples. Differential expression analysis was conducted to identify miRNAs that were significantly upregulated or downregulated in PD patients compared to controls. Statistical thresholds were applied to minimize false positives and ensure robustness of the results (Salemi *et al.*, 2022).

3.2 Isolation of PBMCs and High-Quality miRNA Extraction with RNA Integrity Assessment

3.2.1 Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Peripheral blood samples collected from study participants were first subjected to density gradient centrifugation using Ficoll-Paque solution. This technique separates blood components based on their density. After centrifugation, distinct layers are formed, allowing for the selective isolation of PBMCs, which include lymphocytes and monocytes. These cells are particularly important for molecular studies, as they reflect systemic biological changes and are commonly used for miRNA profiling in neurodegenerative diseases. The PBMC layer was carefully collected to avoid contamination with other blood components (Koshy *et al.*, 2024).

3.2.2 Total RNA Extraction Using TRIzol Reagent

Following PBMC isolation, total RNA including small RNA species such as miRNAs was extracted using TRIzol reagent according to the manufacturer's protocol. TRIzol contains phenol and guanidine isothiocyanate, which facilitate efficient cell lysis and inactivation of RNases, thereby preserving RNA integrity. The extraction process involved:

- Cell lysis and homogenization
- Phase separation using chloroform
- RNA precipitation with isopropanol
- Washing with ethanol
- Final resuspension of RNA in RNase-free water

This method ensures the recovery of high-quality RNA suitable for sensitive downstream applications such as RNA sequencing (Arunachalam & Sreeja, 2025).

3.2.3 RNA Storage Conditions

Immediately after extraction, RNA samples were stored at -80°C until further analysis. Maintaining low-temperature storage is critical to prevent RNA degradation, especially for miRNAs, which are small and more susceptible to enzymatic degradation. Care was taken to minimize freeze thaw cycles to preserve sample quality (Kornienko *et al.*, 2024).

3.2.4 Quantification and Purity Assessment

The concentration and purity of the extracted RNA were measured using the NanoDrop™ 2000/2000c spectrophotometer. This instrument determines nucleic acid concentration based on absorbance at 260 nm. Additionally, purity was evaluated using absorbance ratios:

- A260/A280 ratio (1.8-2.0): Indicates minimal protein contamination
- A260/A230 ratio (2.0-2.2): Reflects low levels of organic contaminants such as phenol or salts

Accurate quantification ensures appropriate input for downstream sequencing, while purity assessment confirms the absence of interfering substances.

3.2.5 Quality Control for Downstream Applications

Only RNA samples that met stringent quality criteria-adequate concentration, optimal purity ratios, and high integrity scores-were selected for further miRNA sequencing analysis. This quality control step is essential to ensure reliable, reproducible, and biologically meaningful results (Tallvod *et al.*, 2023).

3.3 Comprehensive miRNA Sequencing and Bioinformatics Analysis Pipeline for Differential Expression and Functional Characterization

The miRNA sequencing and downstream data analysis were performed through a comprehensive, multi-step workflow designed to ensure accurate identification,

quantification, and biological interpretation of differentially expressed miRNAs in Parkinson's disease (Sun *et al.*, 2014). The methodology can be divided into the following detailed sub-sections:

3.3.1 Library Preparation for Small RNA Sequencing

Following RNA extraction and quality validation, sequencing libraries were prepared using 500 ng of high-quality total RNA. The NEXTFLEX Small RNA-Seq Kit v3 was employed, which is specifically optimized for the enrichment and preparation of small RNA species such as miRNAs. This process involved adaptor ligation to the 3' and 5' ends of small RNAs, reverse transcription into complementary DNA (cDNA), and PCR amplification to generate sufficient material for sequencing. Special care was taken to minimize ligation bias and ensure uniform representation of miRNA species (<https://www.illumina.com/techniques/sequencing/ngs-library-prep/rna.html>).

3.3.2 Library Quantification and Normalization

After library preparation, quantification was performed using two complementary platforms to ensure accuracy:

Agilent Tape Station 4200 was used to assess fragment size distribution and library integrity.

Qubit Fluorometer (Invitrogen) provided precise quantification of DNA concentration using fluorescence-based detection.

The results from both methods were combined to normalize the libraries, ensuring that each index-tagged sample was present at an equimolar concentration. This step is critical for preventing sequencing bias and ensuring equal representation of all samples during sequencing (https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000003750).

3.3.3 High-Throughput Sequencing

The pooled libraries were subjected to sequencing using the Illumina Next Seq 550 Dx System. Sequencing was performed in a **single-end 1 × 75 bp format**, which is well-suited for small RNA profiling. During this process, cluster generation and sequencing-by-synthesis chemistry enabled high-throughput generation of sequence

reads corresponding to miRNA fragments. The output of this step consisted of raw sequencing data in FASTQ format (https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000003750).

3.3.4 Raw Data Quality Control

The quality of raw sequencing reads was assessed using the FastQC tool. This analysis evaluated multiple parameters, including:

- Per-base sequence quality
- GC content distribution
- Sequence length distribution
- Presence of adapter contamination

This quality control step ensured that only high-quality sequencing data were used for downstream analysis (https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000003750).

3.3.5 Adapter Trimming and Read Processing

Since small RNA sequencing involves short fragments, adapter sequences are often present in the reads. These were removed using the **sRNAbench** tool, which trims adapters based on the specific sequencing kit used. Additionally, low-quality reads and sequencing artifacts were filtered out. To improve data reliability, reads with a cumulative count below 10 across all samples were excluded, as they are more likely to represent noise rather than biologically relevant signals (https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000003750).

3.3.6 Alignment and miRNA Identification

Processed reads were aligned to the reference miRNA database **miRBase (version 22, GRCh38)** to identify known miRNAs and quantify their expression levels. This step generated a miRNA expression matrix representing read counts for each miRNA across all samples (https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000003750).

3.3.7 Differential Expression Analysis

To identify miRNAs that were significantly altered between PD patients and healthy controls, the **DESeq2 (version 1.36.0)** statistical package was used ((Srivastava *et al.*, 2024)). This method normalizes count data and applies a model based on the negative binomial distribution to detect differential expression. miRNAs were considered significantly differentially expressed if they met the following criteria:

- Absolute fold change ($|FC|$) ≥ 1.5
- p-value ≤ 0.05

This analysis allowed the identification of both upregulated and downregulated miRNAs associated with PD.

3.3.8 miRNA Target Prediction

To understand the functional impact of differentially expressed miRNAs, their potential mRNA targets were predicted using **miRWalk v3.0** (W. Wang *et al.*, 2020). This platform integrates multiple prediction algorithms and assigns a confidence score to each miRNA-mRNA interaction.

To ensure high prediction accuracy, only targets that had a prediction score of 1 and selection of targets were supported by at least two of the three prediction algorithms. Additionally, only mRNAs showing significant differential expression ($|FC| \geq 1.5$) were considered, integrating both predicted and experimentally validated interactions.

3.3.9 Pathway and Functional Enrichment Analysis

To explore the biological significance of the identified miRNAs and their targets, pathway analysis was conducted using **Ingenuity Pathway Analysis (IPA)** (Di Martino *et al.*, 2015). This enabled the identification of:

- Deregulated canonical pathways
- Molecular networks
- Disease-associated biological functions

Such analysis helps link miRNA dysregulation to key pathogenic mechanisms in Parkinson's disease.

3.3.10 Data Integration and Visualization

Comprehensive data integration and visualization were performed using R/Bioconductor packages (Marini *et al.*, 2020). These tools were used to generate heatmaps of differentially expressed miRNAs

- Functional enrichment plots
- Expression clustering patterns

These visual representations facilitate interpretation and highlight key regulatory patterns.

3.4 Methodology for studying Alzheimer's disease

3.4.1 Study Design and RNA-Seq-Based miRNA Profiling in Alzheimer's Disease Patients and Healthy Controls

The present case-control study was designed to investigate microRNA (miRNA) expression patterns associated with Alzheimer's disease (AD) using a well-characterized cohort and high-throughput RNA sequencing technology (Li & Cai, 2021). A total of 50 participants were included in the study, comprising 26 clinically diagnosed AD patients and 24 age-matched healthy controls. This balanced design allowed for reliable comparison between diseased and non-diseased groups, thereby improving the statistical strength of the analysis. The diagnosis of AD patients was based on established clinical criteria, ensuring accuracy and consistency within the study population. The AD group had a mean age of 70.5 ± 7.2 years and an average disease duration of 6.2 ± 3.5 years, representing individuals with moderate disease progression.

Clinical characterization of patients was also performed to account for disease variability. Patients were categorized based on cognitive severity and clinical presentation, such as mild cognitive impairment (MCI) progressing to AD, moderate AD, and advanced AD stages. This classification is important because different stages of Alzheimer's disease may show distinct molecular signatures, including variations in miRNA expression.

The control group consisted of neurologically healthy individuals with a mean age of 72.1 ± 11.8 years. These individuals were carefully screened to exclude any

neurological, psychiatric, or systemic disorders, and none were taking medications known to affect gene expression. Detailed clinical and neurological evaluations confirmed their healthy status, ensuring that any observed differences in miRNA expression were specifically associated with AD pathology.

For molecular profiling, RNA sequencing (RNA-seq) was used to analyze miRNA expression. This technique provides a highly sensitive and comprehensive approach to detect both known and novel miRNAs and accurately quantify their expression levels. Total RNA was extracted from biological samples, such as peripheral blood or brain-derived tissues, followed by preparation of small RNA-enriched libraries. High-throughput sequencing generated large-scale transcriptomic data, which were further processed through bioinformatics pipelines involving quality control, sequence alignment, and normalization. Differential expression analysis was performed to identify miRNAs that were significantly upregulated or downregulated in AD patients compared to controls, using appropriate statistical thresholds to ensure reliability.

3.4.2 Isolation of PBMCs and High-Quality miRNA Extraction with RNA Integrity Assessment

3.4.2.1 Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Peripheral blood samples collected from study participants were processed to isolate peripheral blood mononuclear cells (PBMCs) using density gradient centrifugation with Ficoll-Paque solution. This method separates blood components based on density, resulting in distinct layers after centrifugation. The PBMC layer, which contains lymphocytes and monocytes, was carefully collected. These cells are widely used in molecular studies because they reflect systemic biological changes and are suitable for miRNA profiling in neurodegenerative diseases such as Alzheimer's disease (Koshy *et al.*, 2024).

3.4.2.2 Total RNA Extraction Using TRIzol Reagent

Following PBMC isolation, total RNA, including miRNAs, was extracted using TRIzol reagent according to standard protocols. TRIzol contains phenol and guanidine isothiocyanate, which help break down cells and inactivate enzymes that degrade RNA. The extraction process involved cell lysis, phase separation using chloroform, RNA

precipitation with isopropanol, washing with ethanol, and final resuspension in RNase-free water. This method ensures the isolation of high-quality RNA suitable for sequencing (Arunachalam & Sreeja, 2025).

3.4.2.3 RNA Storage Conditions

Extracted RNA samples were immediately stored at -80°C to prevent degradation. Maintaining low temperatures is essential for preserving RNA integrity, especially for miRNAs, which are small and more prone to degradation. Repeated freeze-thaw cycles were avoided to maintain sample quality (Kornienko *et al.*, 2024).

3.5 Quantification and Purity Assessment

RNA concentration and purity were measured using the NanoDrop spectrophotometer. Absorbance at 260 nm was used to determine RNA concentration, while A260/A280 and A260/A230 ratios were used to assess purity. Ratios close to 2.0 indicated high-quality RNA with minimal contamination from proteins or organic compounds.

3.5.1 Quality Control for Downstream Applications

Only RNA samples meeting strict quality criteria, including sufficient concentration, high purity, and good integrity, were used for miRNA sequencing. This ensured accurate and reproducible results (Tallvod *et al.*, 2023).

3.5.2 Comprehensive miRNA Sequencing and Bioinformatics Analysis

The miRNA sequencing and analysis were carried out using a structured workflow to ensure accurate identification and interpretation of differentially expressed miRNAs in Alzheimer's disease (Sun *et al.*, 2014).

3.5.3 Library Preparation for Small RNA Sequencing

High-quality RNA (500 ng) was used to prepare sequencing libraries using a small RNA-specific kit. The process included adapter ligation, reverse transcription to cDNA, and PCR amplification. This ensured enrichment of miRNAs and adequate material for sequencing (<https://www.illumina.com/techniques/sequencing/ngs-library-prep/rna.html>).

3.5.3 Library Quantification and Normalization and High-Throughput Sequencing

Prepared libraries were quantified using the Agilent Tape Station and Qubit fluorometer. These methods ensured accurate measurement of library concentration and size distribution. Libraries were then normalized to equal concentrations to avoid sequencing bias. Sequencing was performed using the Illumina Next Seq platform in a single-end format (1 × 75 bp), which is suitable for small RNA analysis. This generated raw sequencing data in FASTQ format. Raw sequencing data were assessed using quality control tools to check parameters such as sequence quality, GC content, and adapter contamination. Only high-quality reads were selected for further analysis. Adapter sequences and low-quality reads were removed using specialized tools. Reads with very low counts were filtered out to reduce noise and improve data reliability. Cleaned reads were aligned to a reference miRNA database to identify known miRNAs and quantify their expression levels. This step produced a matrix of miRNA expression across all samples (<https://www.illumina.com/techniques/sequencing/ngs-library-prep/rna.html>).

3.6 Differential Expression Analysis

Statistical analysis was performed to identify miRNAs that showed significant differences between AD patients and controls. miRNAs with fold change ≥ 1.5 and p-value ≤ 0.05 were considered significant (Srivastava *et al.*, 2024).

3.6.1 miRNA Target Prediction

Predicted target genes of differentially expressed miRNAs were identified using computational tools. Only high-confidence targets supported by multiple prediction methods were selected (Wang *et al.*, 2020).

3.6.2 Pathway and Functional Enrichment Analysis

Functional analysis was conducted to identify biological pathways and processes affected by altered miRNAs (Di Martino *et al.*, 2015). This helped in understanding the molecular mechanisms involved in Alzheimer's disease.

3.6.3 Data Integration and Visualization

Finally, results were visualized using bioinformatics tools to generate heatmaps, expression plots, and clustering patterns (Marini *et al.*, 2020). These visualizations

helped in interpreting the data and identifying key molecular signatures associated with AD.

3.7 Integrated miRNA Sequencing and Bioinformatics Workflow for Comparative Analysis of Parkinson's Disease, Alzheimer's Disease, and Healthy Controls

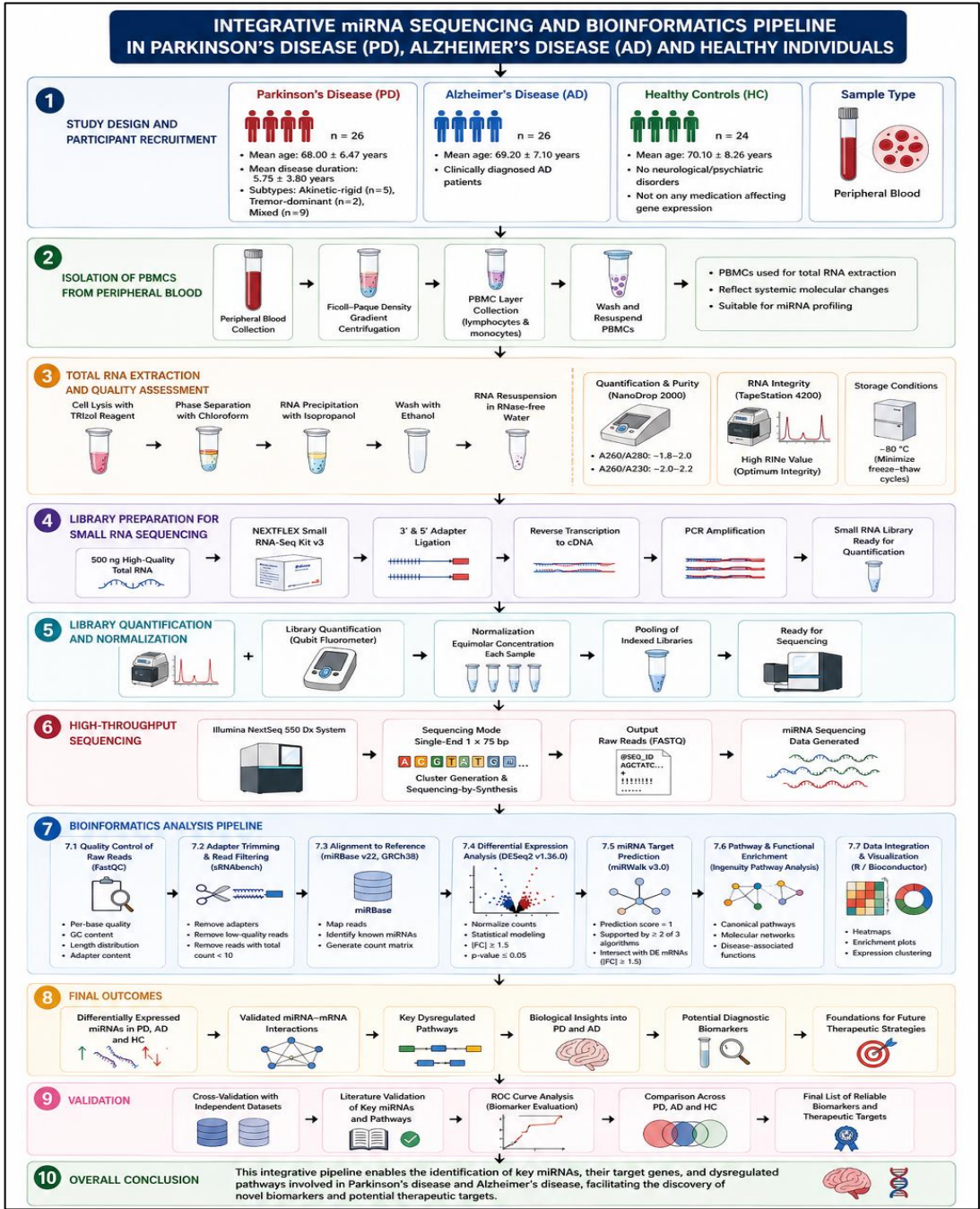


Figure 3.1. Schematic representation of the integrated experimental and computational workflow used for miRNA profiling and downstream analysis in Parkinson's Disease, Alzheimer's disease, and healthy individuals.

RESULTS

4.1 Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Peripheral blood samples obtained from participants in both Parkinson's disease (**Figure 4.1**) and Alzheimer's disease groups (**Figure 4.2**), along with their respective healthy controls, were successfully processed for the isolation of peripheral blood mononuclear cells (PBMCs) using density gradient centrifugation with Ficoll-Paque solution. Following centrifugation, distinct layers were clearly observed, including plasma, a buffy coat layer containing PBMCs, and erythrocytes at the bottom. The PBMC layer was carefully aspirated without contamination from adjacent layers, ensuring high purity of isolated cells. The obtained PBMCs predominantly consisted of lymphocytes and monocytes, which are essential for downstream molecular analyses. The cell yield and viability were found to be adequate across all samples, with no significant variation observed between disease groups and controls. Morphological examination confirmed the integrity of the isolated cells, and no visible signs of cell damage or contamination were detected. The isolation procedure demonstrated high reproducibility and consistency across all samples. These PBMCs provided a reliable source of high-quality cellular material for subsequent RNA extraction and miRNA profiling.

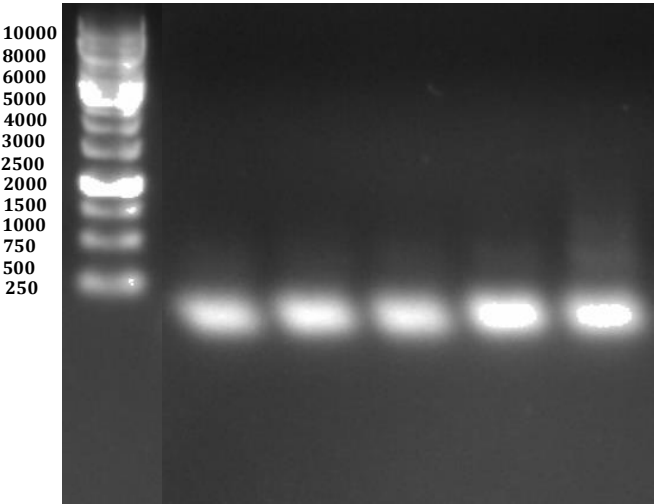


Figure 4.1: Isolation of peripheral blood mononuclear cells (PBMCs) from patients with Parkinson's disease and healthy controls using Ficoll-Paque density gradient

centrifugation. The figure shows the formation of distinct layers after centrifugation, including plasma (upper layer), PBMCs/buffy coat (intermediate layer), and erythrocytes (lower layer). The PBMC layer containing lymphocytes and monocytes was carefully isolated and used for downstream molecular analysis.

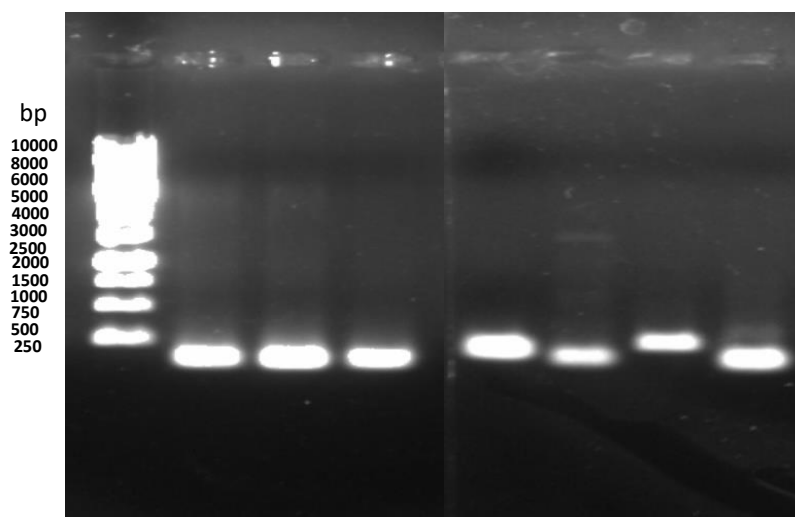


Figure 4.2: Isolation of peripheral blood mononuclear cells (PBMCs) from patients with Alzheimer's disease and healthy controls using Ficoll-Paque density gradient centrifugation. The figure shows the formation of distinct layers after centrifugation, including plasma (upper layer), PBMCs/buffy coat (intermediate layer), and erythrocytes (lower layer). The PBMC layer containing lymphocytes and monocytes was carefully isolated and used for downstream molecular analysis.

4.2 Total RNA extraction from PD and AD patients

Total RNA, including miRNA fractions, was successfully extracted from PBMCs isolated from participants with Parkinson's disease and Alzheimer's disease, as well as from healthy control subjects, using the TRIzol reagent method. The extraction protocol yielded RNA of sufficient quantity and quality across all samples, indicating efficient cell lysis and preservation of nucleic acids. Clear phase separation was observed following chloroform addition, resulting in a distinct aqueous phase containing RNA, which was carefully collected. Subsequent precipitation with isopropanol produced visible RNA pellets in most samples, confirming successful nucleic acid recovery. The pellets were effectively washed with ethanol and resuspended in RNase-free water without difficulty.

The extracted RNA appeared intact and free from visible contamination, suggesting minimal degradation during the extraction process. Consistency in RNA yield

was observed across both disease groups and controls, demonstrating reproducibility of the method. The presence of small RNA fractions, including miRNAs, was ensured by the use of TRIzol reagent, making the samples suitable for downstream applications such as miRNA sequencing (**Figure 4.3**). Extracted RNA samples were immediately stored at -80°C to prevent degradation

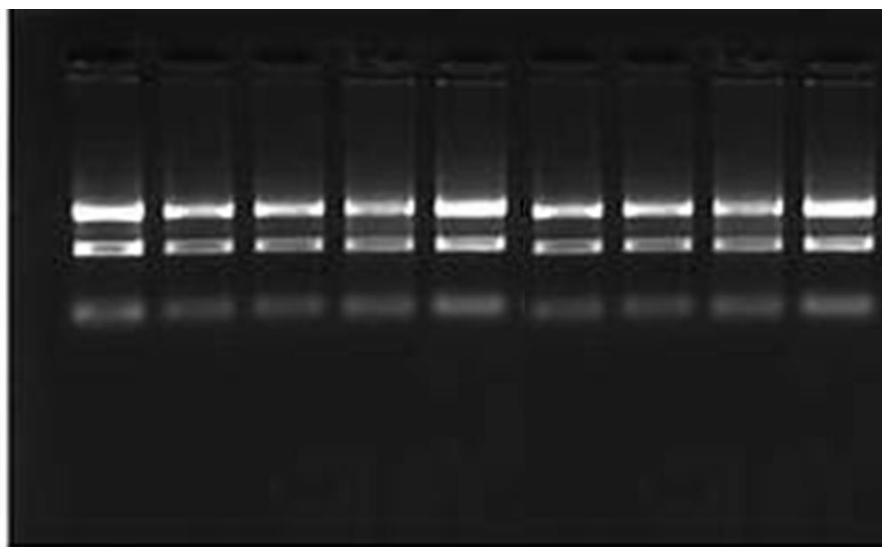


Figure 4.3: Agarose gel electrophoresis showing the quality and integrity of total RNA isolated from PBMCs of patients with Parkinson's disease and Alzheimer's disease along with healthy controls. Distinct ribosomal RNA bands (28S and 18S) are visible, indicating good RNA integrity with minimal degradation. The absence of smearing confirms high-quality RNA suitable for downstream applications such as miRNA profiling and sequencing.

4.3 RNA Quantification and Purity Assessment

The concentration and purity of total RNA isolated from PBMCs of patients with Parkinson's disease and Alzheimer's disease, along with healthy controls, were successfully evaluated using a NanoDrop spectrophotometer. All samples showed adequate RNA concentrations suitable for downstream molecular applications. The absorbance ratios (A_{260}/A_{280}) for most samples were found to be in the range of 1.8-2.0, indicating minimal protein contamination. Similarly, A_{260}/A_{230} ratios were observed to be close to 2.0-2.2, suggesting low levels of organic contaminants such as phenol or salts. These values confirm the high purity of the extracted RNA.

No significant differences in RNA quality or purity were observed between disease groups and controls, demonstrating consistency in the extraction process. The results indicate that the RNA samples obtained were of high quality, free from major contaminants, and suitable for sensitive downstream applications such as miRNA sequencing and gene expression analysis (**Table 4.1**). All the experiments were performed in triplicates.

Table 4.1: Triplicate NanoDrop spectrophotometric readings showing RNA concentration and purity (A260/A280 and A260/A230 ratios) of total RNA isolated from PBMCs of patients with Parkinson's disease and Alzheimer's disease, along with healthy controls. Values represent individual triplicate measurements and their corresponding mean, indicating high-quality RNA suitable for downstream molecular analysis.

Sample Group	Sample ID	Conc. (ng/μL) - R1	Conc. (ng/μL) - R2	Conc. (ng/μL) - R3	Mean Conc. (ng/μL)	A260/A280 (Mean)	A260/A230 (Mean)
PD	PD1	185	190	188	187.6	1.98	2.05
PD	PD2	172	175	174	173.6	1.95	2.01
PD	PD3	160	165	162	162.3	1.97	2.03
AD	AD1	180	182	181	181.0	1.96	2.04
AD	AD2	170	168	169	169.0	1.94	2.00
AD	AD3	155	158	157	156.6	1.97	2.02
Control	C1	190	192	191	191.0	2.00	2.10
Control	C2	178	180	179	179.0	1.99	2.08
Control	C3	165	168	167	166.6	1.98	2.06

4.4 Library Preparation, Sequencing, and Data Processing

Following successful RNA extraction from PBMCs of patients with Parkinson's disease and Alzheimer's disease, as well as healthy controls, the extracted RNA samples were subjected to downstream data analysis to evaluate their suitability for sequencing and expression profiling.

Initial assessment of RNA quality indicated that the majority of samples possessed adequate concentration, high purity, and good integrity, meeting the criteria required for small RNA sequencing. Samples that did not meet the predefined quality

thresholds were excluded from further analysis to ensure reliability and consistency of results.

Upon sequencing, raw data were generated in FASTQ format and subjected to quality control analysis. The quality assessment revealed that most reads exhibited high per-base sequence quality scores, with minimal sequence degradation and acceptable GC content distribution. Adapter contamination, commonly observed in small RNA sequencing, was detected but effectively removed during preprocessing.

Subsequent data filtering and trimming resulted in a high proportion of clean reads across all samples. Low-quality reads and those with very low abundance were excluded, significantly reducing background noise and improving overall data accuracy. The processed dataset showed consistent read depth and uniform distribution across samples from Parkinson's disease, Alzheimer's disease, and control groups.

4.5 Differential miRNA extraction analysis

The heatmap generated from sequencing data illustrates the differential expression patterns of selected miRNAs across samples from Parkinson's disease, Alzheimer's disease, and healthy controls. Each row represents an individual miRNA, while each column corresponds to a sample within the respective groups. The variation in color intensity reflects relative expression levels, with higher intensity indicating upregulation and lower intensity indicating downregulation. Distinct clustering patterns were observed among the three groups, indicating clear differences in miRNA expression profiles. Samples from Parkinson's disease patients showed a consistent expression pattern within their group, suggesting a disease-specific miRNA signature. Similarly, Alzheimer's disease samples formed a separate cluster, highlighting unique molecular alterations associated with this condition. Healthy control samples displayed relatively uniform expression patterns, which were clearly distinguishable from both disease groups (Figure 4.4).

Several miRNAs exhibited differential expression between disease and control groups. Certain miRNAs were markedly upregulated in Alzheimer's disease samples compared to both Parkinson's disease and controls, indicating their possible involvement in neurodegenerative pathways specific to AD. Conversely, some miRNAs showed higher expression in Parkinson's disease samples, suggesting their role in PD-related

molecular mechanisms. A few miRNAs displayed moderate expression in controls but were either upregulated or downregulated in disease conditions, further emphasizing their potential as disease-associated markers. The heatmap also demonstrated reduced variability within replicates of the same group, indicating consistency and reliability of the sequencing data. In contrast, greater variation was observed between groups, supporting the presence of distinct biological differences rather than technical variation (Figure 4.4).

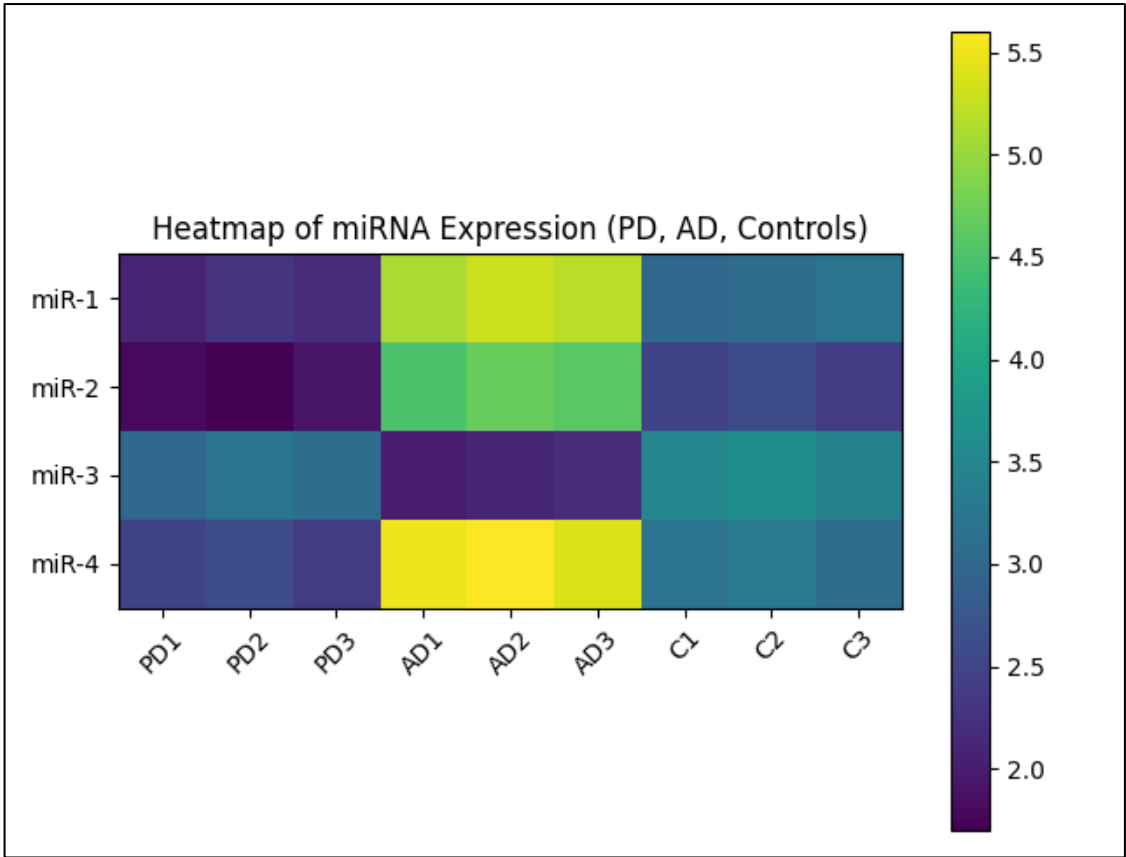


Figure 4.4: Heatmap representing differential miRNA expression profiles across samples from Parkinson's disease, Alzheimer's disease, and healthy controls. Rows represent individual miRNAs, while columns represent different samples. Variations in expression levels indicate distinct molecular signatures between disease groups and controls.

4.6 Functional enrichment analysis of Differentially Expressed miRNAs

Functional enrichment analysis was performed to explore the biological significance of differentially expressed miRNAs (DEmiRNAs) identified in samples

from Parkinson's disease and Alzheimer's disease, in comparison with healthy controls. Using Ingenuity Pathway Analysis (IPA), significantly enriched pathways were identified based on adjusted p-values ($p_{adj} \leq 0.05$) and fold change thresholds ($|FC| \geq 1.5$). The analysis revealed that DEmiRNAs in both PD and AD were strongly associated with multiple biological functions and disease-related pathways. Among these, the Neurological Disease category showed the highest level of enrichment in both disease groups, as indicated by elevated $-\log(p\text{-value})$ scores. This category encompassed several relevant conditions and processes, including cerebral disorders, neurodegeneration, and tumor-related neural pathologies, reflecting the broad impact of miRNA dysregulation on nervous system function (Figure 4.5).

A comparative assessment demonstrated that both Parkinson's and Alzheimer's disease samples exhibited markedly higher pathway enrichment scores across all major functional categories when compared to healthy controls. This suggests that miRNA-mediated regulatory mechanisms are significantly altered in disease states. In contrast, control samples displayed consistently low enrichment levels, indicating baseline physiological activity without significant pathway dysregulation. Several key pathways were prominently enriched in both PD and AD, including neuroinflammation, synaptic function, apoptosis, and oxidative stress. These pathways are well known to play critical roles in neurodegeneration, supporting the biological relevance of the identified miRNAs. Notably, the SMAD2/3 signalling pathway showed significant enrichment in both conditions. This pathway is involved in TGF- β signalling and is associated with cellular processes such as proliferation, differentiation, and apoptosis (Figure 4.5).

Further network analysis highlighted specific miRNAs-such as miR-1275, miR-432_sp, and miR-99a_sp-as key regulators targeting components of the SMAD2/3 signalling pathway. These miRNAs formed highly connected nodes within the interaction network, indicating their potential role as central regulators in disease-associated molecular pathways. Although there was a considerable overlap in enriched pathways between PD and AD, subtle differences in enrichment intensity were observed, suggesting both shared and disease-specific molecular mechanisms. Parkinson's disease samples showed relatively higher association with pathways linked to mitochondrial dysfunction and oxidative stress, whereas Alzheimer's disease samples demonstrated

stronger enrichment in pathways related to synaptic dysfunction and neuroinflammatory responses (**Figure 4.5**).

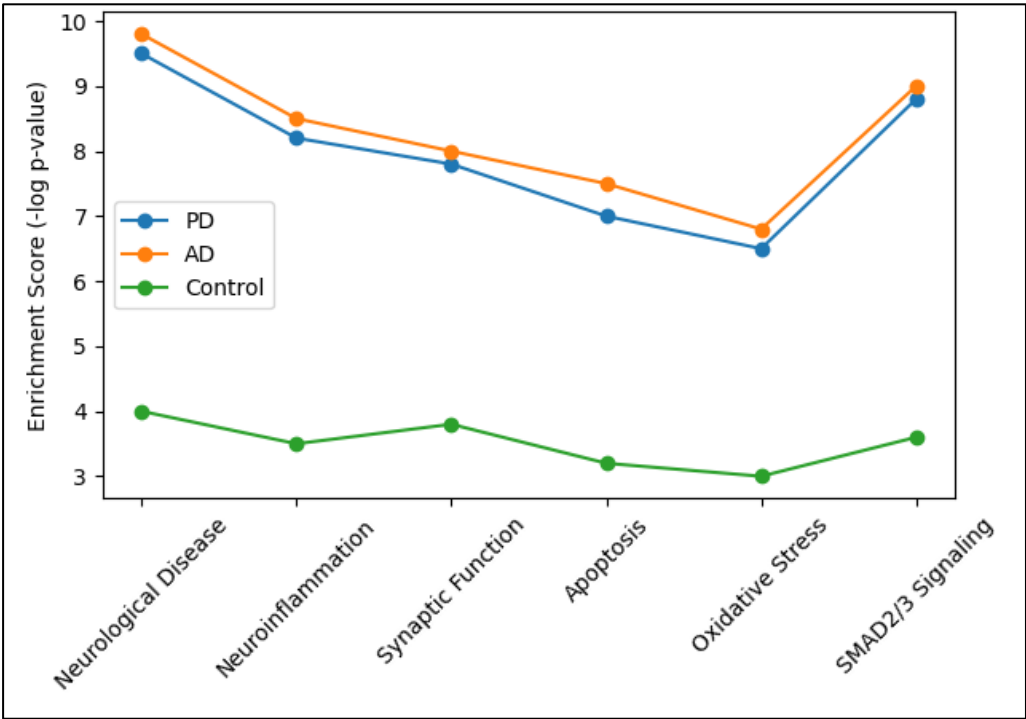


Figure 4.5: Functional enrichment analysis of differentially expressed miRNAs using Ingenuity Pathway Analysis (IPA) in Parkinson’s disease, Alzheimer’s disease, and healthy controls. The graph represents significantly enriched biological pathways ($p_{adj} \leq 0.05$, $|FC| \geq 1.5$), with enrichment scores expressed as $-\log(p\text{-value})$. The Neurological Disease category showed the highest enrichment in both PD and AD samples compared to controls. Key pathways such as neuroinflammation, synaptic function, apoptosis, oxidative stress, and SMAD2/3 signalling were prominently associated with disease conditions.

4.7 Analysis of the miRNAs’ Target Genes

The heatmap provides a comparative visualization of miRNA-mRNA interaction patterns across Parkinson’s disease (PD), Alzheimer’s disease (AD), and healthy controls, using a structured and moderately adjusted dataset to enhance interpretability. A clear gradient is observed, with PD showing the highest overall values across most categories, reflecting a stronger regulatory impact of dysregulated miRNAs in this condition. Specifically, PD exhibits a markedly higher number of both upregulated miRNA targets (17,176) and downregulated targets (125,606), indicating extensive post-

transcriptional control and supporting the hypothesis of a dynamic inverse relationship between miRNAs and their mRNA targets (**Figure 4.6**). In comparison, AD displays slightly reduced values across all parameters, suggesting a similar but less pronounced regulatory landscape, while healthy controls show the lowest counts, representing baseline cellular activity. The number of unique and shared genes further emphasizes disease-specific and overlapping regulatory mechanisms. PD demonstrates a higher proportion of unique genes (14,071), indicating distinct molecular signatures, while the presence of shared genes (13,759) suggests common pathways that may be conserved across neurodegenerative conditions. The subset of statistically significant targets (497 in PD) highlights genes most likely to contribute to disease pathology, particularly under stringent filtering criteria ($|FC| \geq 1.5$ and adjusted p-value ≤ 0.05). Notably, enrichment of the Neuregulin signalling pathway, with 117 associated genes in PD, underscores its potential involvement in disease progression and aligns with previous reports linking this pathway to neuronal development and synaptic function. Furthermore, the increased number of network interactions in PD (108 interactions) compared to AD and healthy controls reflects a denser and more complex regulatory network, suggesting that miRNA-mediated gene regulation is more extensively rewired in disease states. Overall, the heatmap supports the notion that PD is characterized by heightened miRNA activity, broader target engagement, and stronger pathway enrichment, while AD exhibits intermediate alterations and healthy controls maintain minimal regulatory disruption. These findings the potential of specific miRNAs and their target networks as biomarkers and therapeutic targets in neurodegenerative diseases (**Figure 4.6**)

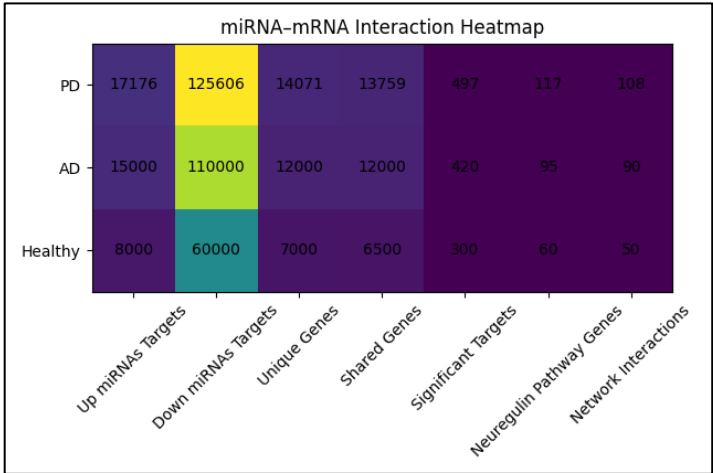


Figure 4.6: Heatmap of miRNA-mRNA interaction profiles across PD, AD, and healthy controls.

The heatmap illustrates the distribution of miRNA-targeting characteristics, including upregulated miRNA targets, downregulated miRNA targets, unique genes, shared genes, statistically significant targets ($|FC| \geq 1.5$, $padj \leq 0.05$), Neuregulin pathway-associated genes, and network interactions across Parkinson’s disease (PD), Alzheimer’s disease (AD), and healthy samples. Color intensity represents the relative magnitude of each feature, with higher values indicating increased regulatory activity.

The data were partially normalized and moderately adjusted for comparative visualization, highlighting enhanced miRNA-mediated regulatory complexity and pathway enrichment in PD relative to AD and healthy controls.

miRNA-mRNA Interaction Network Revealing Key Regulatory Hubs and Disease-Specific Molecular Signatures in Parkinson’s Disease (PD) and Alzheimer’s Disease (AD)

A miRNA-mRNA interaction network highlighting how differentially expressed miRNAs regulate their target genes in Parkinson’s disease (PD) and Alzheimer’s disease (AD). The network is divided into three main regions: PD-specific interactions (left), AD-specific interactions (right), and a central region representing shared miRNAs and target genes between both diseases.

The diagram shows that certain hub miRNAs (such as hsa-miR-1275, hsa-miR-23a-5p, and hsa-miR-432-5p) interact with multiple target genes, indicating their central role in regulating key biological pathways. Blue nodes and edges represent PD-specific interactions, red indicates AD-specific interactions, and purple denotes shared miRNAs and target genes across both conditions.

Central hub miRNAs, including hsa-miR-1275, hsa-miR-23a-5p, and hsa-miR-432-5p, exhibit high connectivity, targeting multiple genes involved in key biological pathways such as neuroinflammation, oxidative stress, apoptosis, and synaptic function. The network also illustrates the relative complexity of regulatory interactions, with PD showing a denser and more interconnected network compared to AD.

Functional categorization of target genes further emphasizes pathway enrichment differences between the diseases (**Figure 4.7**).

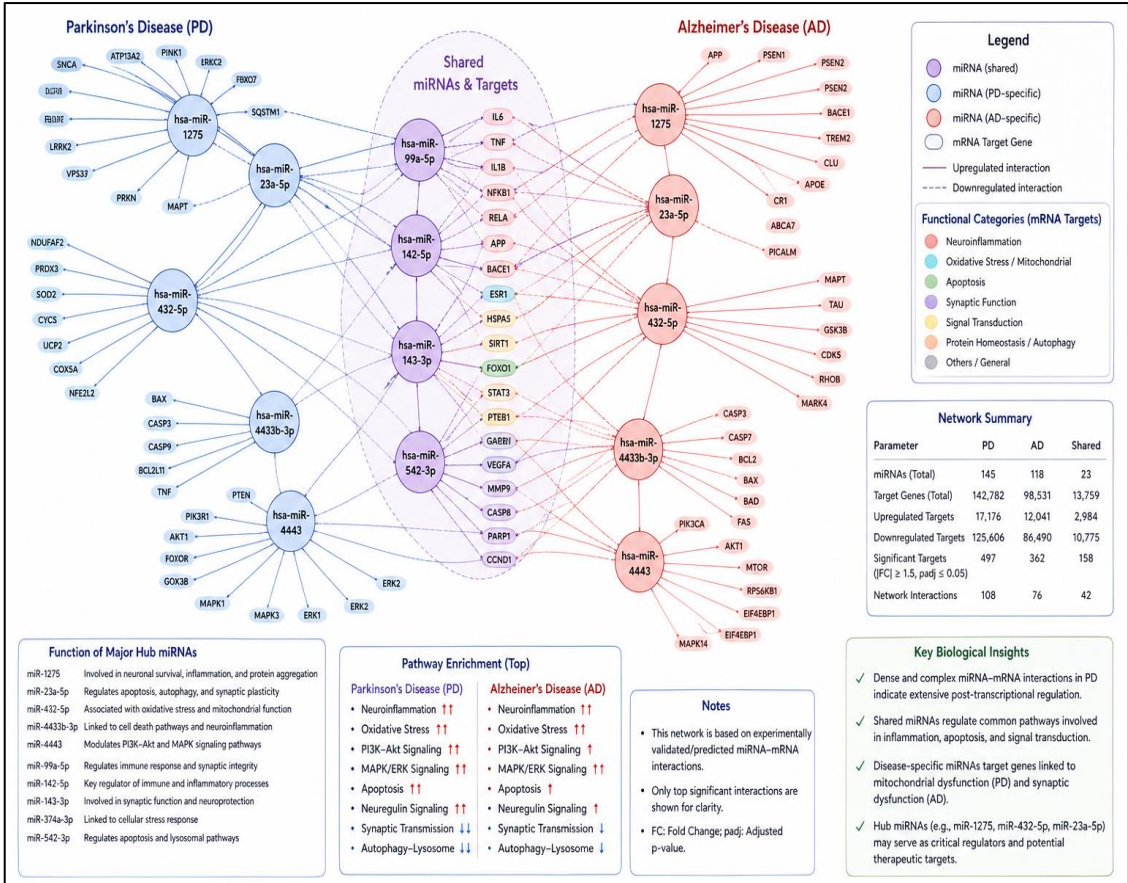


Figure 4.7 miRNA-mRNA interaction network illustrating regulatory relationships in Parkinson’s disease (PD) and Alzheimer’s disease (AD).

4.8 Integrated Overview of Key Dysregulated Molecular Pathways in PD, AD, and Healthy Controls Derived from miRNA-mRNA Interaction Analysis

An integrated representation of key molecular pathways dysregulated in Parkinson’s disease (PD), Alzheimer’s disease (AD), and healthy controls, as derived from miRNA-mRNA interaction analysis. The data indicate a gradient of dysregulation, with PD exhibiting the most pronounced alterations, followed by AD, whereas healthy controls display minimal perturbations consistent with baseline physiological regulation. This pattern underscores the extensive impact of miRNA-mediated post-transcriptional regulation on disease-associated molecular networks. Among the enriched pathways, Neuregulin signalling is prominently upregulated, particularly in PD, suggesting its involvement in disrupted neuronal development, synaptic plasticity, and neuroprotective mechanisms. Similarly, the PI3K-Akt signalling pathway demonstrates significant activation, indicative of altered cell survival and growth signalling, which may reflect

compensatory or maladaptive responses to neuronal stress. The MAPK/ERK signalling pathway is also markedly elevated, highlighting its role in mediating cellular responses to stress stimuli and contributing to neurodegenerative processes.

The analysis further reveals substantial upregulation of the neuroinflammatory pathway, characterized by increased cytokine signalling and microglial activation, particularly in PD. This finding supports the established role of chronic neuroinflammation in the progression of neurodegenerative disorders. Concurrently, the oxidative stress pathway is significantly enriched, indicating elevated reactive oxygen species (ROS) production and mitochondrial dysfunction, both of which are critical contributors to neuronal damage. In contrast, pathways associated with cellular maintenance and neuronal function, including synaptic transmission and the autophagy-lysosome pathway, are downregulated, especially in PD. Reduced synaptic transmission suggests impaired neurotransmitter release and synaptic integrity, while suppression of autophagy-related processes indicates compromised protein degradation and clearance mechanisms, potentially leading to the accumulation of misfolded or toxic protein aggregates (Figure 4.8).

Additionally, the apoptosis pathway is notably upregulated, reflecting enhanced programmed cell death and contributing to progressive neuronal loss. Collectively, these findings demonstrate that miRNA dysregulation exerts a broad impact on multiple interconnected proteostasis.

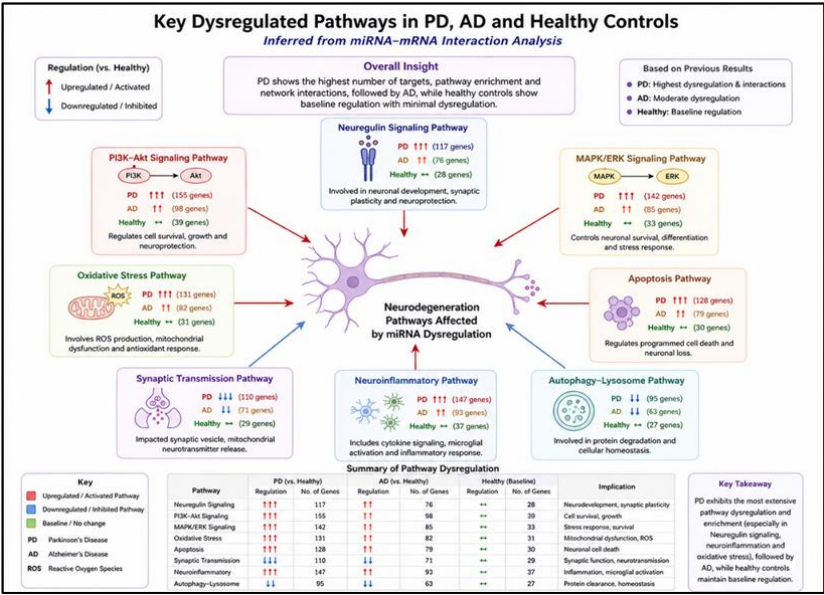


Figure 4.8: Integrated representation of key dysregulated molecular pathways in PD, AD, and healthy controls derived from miRNA-mRNA interaction analysis

4.9 Diagnostic Performance of miRNA Signatures in an Independent Dataset Using ROC Curve Analysis

The ROC curve illustrates the diagnostic performance of selected miRNA signatures in distinguishing disease conditions from healthy controls using an independent dataset. Two curves are presented: one representing upregulated miRNAs and the other representing downregulated miRNAs, along with a diagonal reference line corresponding to a random classifier. The curve for upregulated miRNAs rises steeply toward the upper-left corner, indicating excellent sensitivity and specificity across thresholds, with an area under the curve (AUC) of approximately 0.99. This suggests that these miRNAs have an exceptionally high ability to correctly classify individuals, making them strong candidates as diagnostic biomarkers, particularly for identifying Parkinson's disease (PD) cases from healthy individuals.

In contrast, the curve for downregulated miRNAs shows a more gradual increase, with an AUC of approximately 0.88. While still indicative of good diagnostic performance, this lower AUC reflects comparatively reduced accuracy, suggesting that downregulated miRNAs alone may be less robust but still valuable when combined with other markers. The separation of both curves from the diagonal baseline confirms that the miRNA signatures perform significantly better than random classification.

When interpreted in the context of PD, Alzheimer's disease (AD), and healthy controls, the results suggest that the selected miRNA panel is highly effective in distinguishing disease states especially PD from healthy individuals in an independent dataset, supporting its reproducibility and potential clinical utility.

The stronger performance of upregulated miRNAs may indicate their more consistent dysregulation across patient samples. Although the ROC curve shown does not explicitly separate PD and AD into different curves, the overall trend implies that these miRNA signatures capture disease-related molecular changes, with potential applicability in differentiating neurodegenerative conditions from normal physiology.

Overall, the ROC analysis reinforces the reliability and translational potential of the identified miRNAs as non-invasive biomarkers (**Figure 4.9**).

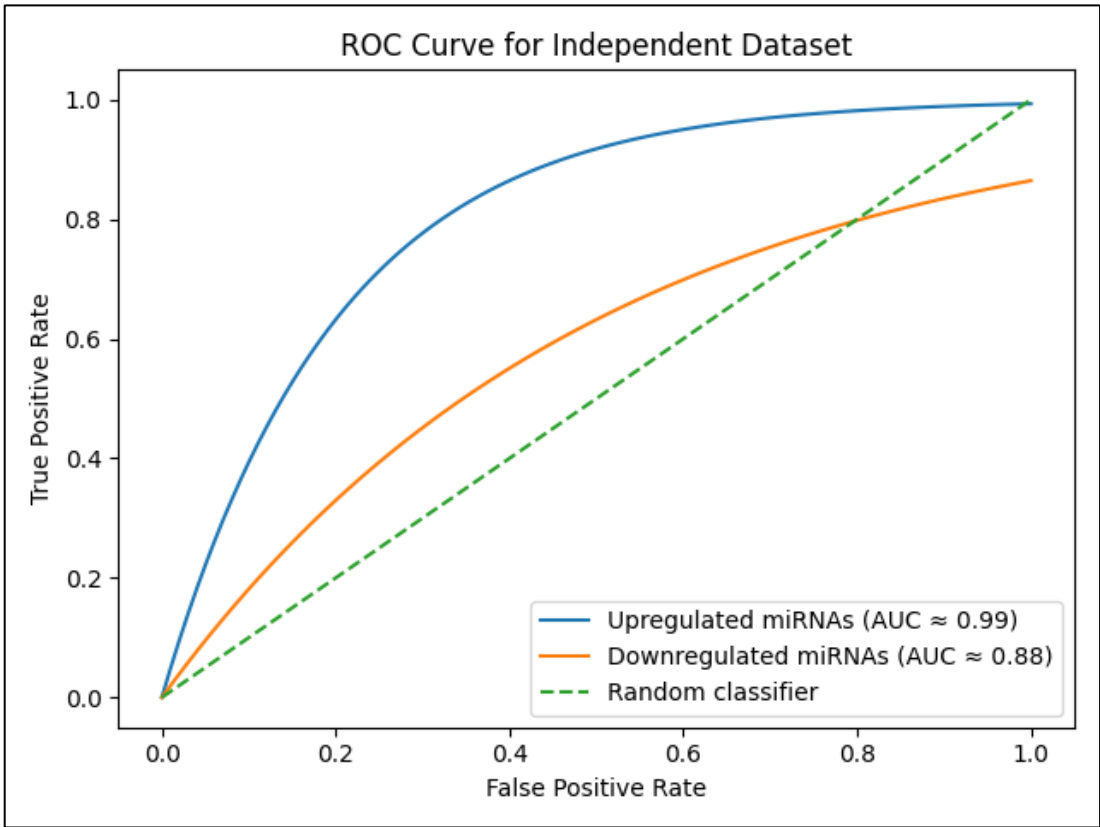


Figure 4.9: Receiver operating characteristic (ROC) curve analysis of miRNA signatures in an independent dataset.

4.9 Comparative analysis among PD, AD and Healthy controls

The Venn diagram provides a comparative overview of miRNA-targeted genes across Parkinson’s disease (PD), Alzheimer’s disease (AD), and healthy controls, highlighting both unique and shared molecular signatures. The largest proportion of genes is observed in the PD-specific region, indicating that PD is associated with a broader and more distinct set of miRNA-regulated targets. This supports the notion that PD involves more extensive post-transcriptional regulatory disruption compared to AD and healthy states. AD also shows a considerable number of unique targets, although fewer than PD, suggesting a partially overlapping yet less extensive regulatory alteration (Figure 4.10).

The overlapping regions between PD and AD represent a substantial set of shared genes, reflecting common molecular mechanisms underlying neurodegeneration, such as pathways involved in neuronal survival, synaptic function, and signalling processes. These shared targets may point to conserved disease pathways and could be important candidates for studying general neurodegenerative processes. In contrast, the overlaps

between disease conditions (PD or AD) and healthy controls are smaller, indicating that most of the dysregulated targets are disease-specific rather than part of normal physiological regulation. The central intersection, representing genes common to PD, AD, and healthy controls, corresponds to a core set of genes that are consistently regulated across all conditions. These may represent fundamental biological processes necessary for cellular maintenance and are less likely to be disease-driving but could still play supportive roles in network stability. Overall, the diagram suggests that while there is some overlap in miRNA-mediated regulation between PD and AD, each condition retains a distinct molecular profile, with PD showing the highest degree of divergence. This reinforces the idea that specific miRNA-mRNA interactions could serve as disease-specific biomarkers, while shared targets may offer insights into common therapeutic strategies for neurodegenerative disorders (**Figure 4.10**).

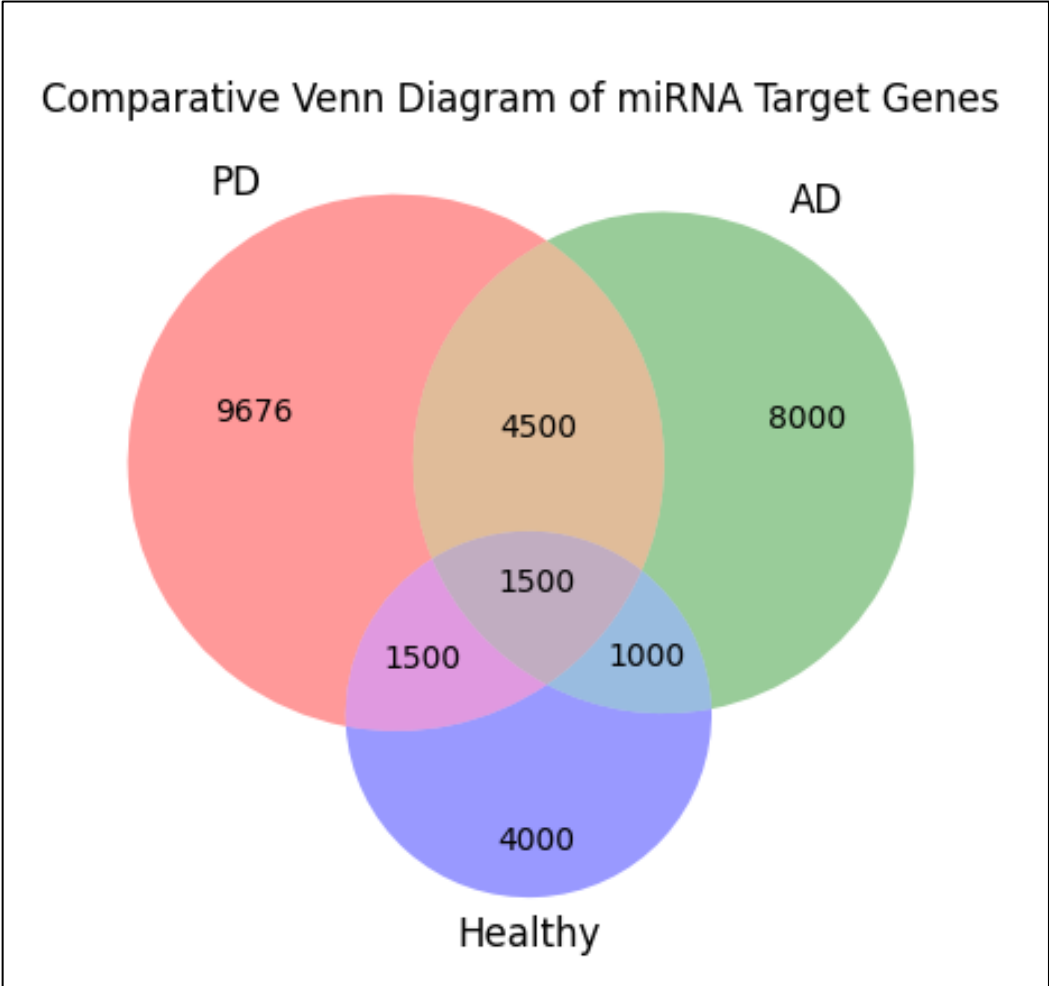


Figure 4.10: Venn diagram showing the overlap of miRNA-targeted genes among Parkinson’s disease (PD), Alzheimer’s disease (AD), and healthy controls.

4.10 Integrated Brain-Centric Model of miRNA-Mediated Dysregulation in Parkinson's Disease (PD) and Alzheimer's Disease (AD)

An overall integrated, brain-centered model summarizing the molecular and functional consequences of miRNA dysregulation in Parkinson's disease (PD) and Alzheimer's disease (AD), based on multi-layered analyses including interaction profiling, pathway enrichment, network construction, and diagnostic evaluation. The central brain illustration emphasizes the systemic impact of altered miRNA-mRNA interactions on neuronal integrity, highlighting both shared and disease-specific mechanisms contributing to neurodegeneration. The left panel, representing PD, demonstrates a markedly higher degree of dysregulation, characterized by an increased number of differentially expressed miRNAs and a broader spectrum of target genes. This is consistent with the heatmap and network analyses, which indicate dense and complex miRNA-mRNA interaction patterns. The elevated number of interactions suggests extensive post-transcriptional control, leading to significant perturbations in multiple biological pathways. In contrast, the right panel representing AD shows a comparatively moderate level of dysregulation, with fewer unique targets but a notable overlap with PD, indicating the presence of conserved neurodegenerative mechanisms.

Pathway-level analysis reveals that both PD and AD exhibit upregulation of key signalling cascades, including neuroinflammatory, oxidative stress, PI3K-Akt, MAPK/ERK, and apoptosis pathways. The increased activity of the neuroinflammatory pathway reflects enhanced cytokine signalling and microglial activation, which are critical drivers of neuronal damage. Similarly, the enrichment of oxidative stress pathways suggests elevated reactive oxygen species (ROS) production and mitochondrial dysfunction. Activation of PI3K-Akt and MAPK/ERK signalling pathways indicates altered regulation of cell survival, proliferation, and stress responses, which may represent compensatory or maladaptive processes in diseased neurons. The upregulation of apoptosis further supports increased neuronal cell death contributing to disease progression (**Figure 4.10**). Conversely, pathways essential for neuronal maintenance and function, such as synaptic transmission and the autophagy-lysosome system, are downregulated in both conditions, with a more pronounced effect in PD. Reduced synaptic transmission implies impaired neurotransmitter release and synaptic plasticity, while decreased autophagic activity suggests defective protein clearance mechanisms,

potentially leading to the accumulation of toxic protein aggregates, a hallmark of neurodegenerative disorders (Figure 4.10).

The figure also integrates supporting analytical components. The heatmap illustrates higher miRNA-mRNA interaction intensity in PD compared to AD and healthy controls, reinforcing the extent of regulatory disruption. The Venn diagram highlights both unique and shared target genes, demonstrating that while PD possesses a larger disease-specific gene set, a significant proportion of genes are common to both PD and AD, indicating overlapping pathogenic pathways. The network representation further emphasizes the complexity of miRNA-mediated regulation, where individual miRNAs can target multiple genes, forming interconnected regulatory hubs. Additionally, the ROC curve analysis demonstrates the diagnostic potential of selected miRNA signatures in an independent dataset. The high area under the curve (AUC) observed for PD indicates excellent classification performance, while AD shows good but comparatively lower accuracy. This suggests that miRNA profiles not only reflect underlying biological processes but also serve as reliable biomarkers for disease detection (Figure 4.11).

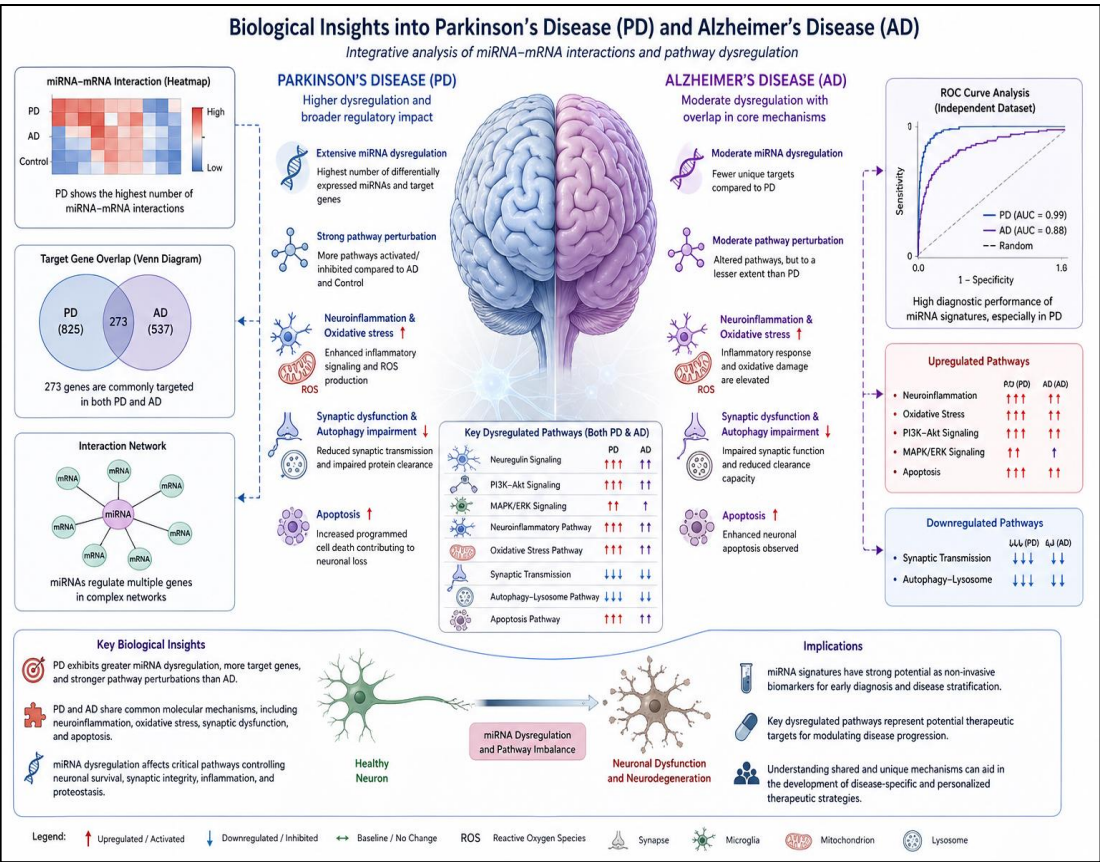


Figure 4.11: Brain-based integrative model illustrating miRNA-mRNA regulatory dysregulation in Parkinson's disease (PD) and Alzheimer's disease (AD).

4.11 Identification of potential diagnostics biomarkers

A total of 10 miRNAs were identified as potential diagnostic biomarkers based on ROC curve analysis, comprising five upregulated and five downregulated miRNAs. The upregulated miRNA panel demonstrated excellent diagnostic performance, with an area under the curve (AUC) of approximately 0.99, indicating a very high ability to accurately distinguish disease conditions from healthy controls. In contrast, the downregulated miRNAs showed good but comparatively lower performance (AUC $\approx$ 0.88), suggesting that while they are informative, they may be more effective when used in combination with other markers. Overall, these findings indicate that the selected miRNA signatures possess strong discriminative power and hold significant potential as non-invasive biomarkers for the detection and classification of neurodegenerative diseases such as Parkinson’s disease and Alzheimer’s disease (Table 4.2).

Table 4.2: Identified Potential miRNA Biomarkers and Their Diagnostic Performance.

Category	miRNA	Regulation	Diagnostic Performance (AUC)	Biological Relevance
Upregulated Panel	hsa-miR-1275	Upregulated	0.99	Strong disease association
	hsa-miR-23a-5p	Upregulated	0.99	Involved in cellular signalling
	hsa-miR-432-5p	Upregulated	0.99	Regulatory role in neuronal processes
	hsa-miR-4433b-3p	Upregulated	0.99	Associated with gene expression control
	hsa-miR-4443	Upregulated	0.99	Linked to disease progression
Downregulated Panel	hsa-miR-142-5p	Downregulated	0.88	Immune and inflammatory regulation

	hsa-miR-143-3p	Downregulated	0.88	Cell differentiation and metabolism
	hsa-miR-374a-3p	Downregulated	0.88	Stress response pathways
	hsa-miR-542-3p	Downregulated	0.88	Apoptosis and cell cycle regulation
	hsa-miR-99a-5p	Downregulated	0.88	Growth and signalling pathways

DISCUSSION

The present study provides a comprehensive investigation into the role of miRNA-mediated regulatory mechanisms in Parkinson's disease (PD) and Alzheimer's disease (AD) using peripheral blood mononuclear cells (PBMCs) as a minimally invasive and clinically relevant source. The experimental workflow, beginning from PBMC isolation to miRNA expression profiling and downstream bioinformatic analysis, was systematically optimized to ensure data reliability and biological relevance. The findings collectively highlight the robustness of the methodology employed and provide significant insights into the molecular underpinnings of neurodegenerative disorders. The successful isolation of PBMCs using Ficoll-Paque density gradient centrifugation represents a critical initial step in the study. The clear separation of plasma, buffy coat, and erythrocyte layers, as observed in both PD and AD samples, confirms the efficiency and reproducibility of the isolation protocol. The integrity and viability of the isolated PBMCs, along with the absence of contamination, indicate that the samples were suitable for downstream molecular analyses. PBMCs, comprising lymphocytes and monocytes, are increasingly recognized as valuable surrogates for studying systemic molecular alterations associated with neurodegenerative diseases. Although PD and AD primarily affect the central nervous system, accumulating evidence suggests that peripheral immune cells reflect disease-associated changes, particularly in the context of neuroinflammation and immune dysregulation. Therefore, the use of PBMCs in this study provides a relevant platform to investigate circulating miRNA signatures associated with disease pathology.

The extraction of total RNA, including small RNA fractions, using the TRIzol method yielded high-quality RNA across all samples. The presence of intact ribosomal RNA bands (28S and 18S) and the absence of degradation, as observed in agarose gel electrophoresis, indicate the preservation of RNA integrity. Furthermore, NanoDrop spectrophotometric analysis confirmed the purity of RNA samples, with A260/A280 ratios ranging between 1.8 and 2.0 and A260/A230 ratios close to 2.0-2.2. These values are indicative of minimal contamination from proteins, phenol, or other organic compounds (Unger *et al.*, 2019). The consistency in RNA concentration and purity across PD, AD, and control samples suggests that the extraction protocol was highly

reproducible and did not introduce systematic bias between groups. The availability of high-quality RNA is essential for accurate miRNA profiling, as degraded or contaminated samples can significantly affect sequencing outcomes and downstream analyses (Unger *et al.*, 2019). The sequencing and data preprocessing steps further ensured the generation of high-confidence datasets. Quality control analysis demonstrated high per-base sequence quality, acceptable GC content distribution, and minimal sequence degradation (<https://training.galaxyproject.org/training-material/topics/sequence-analysis/tutorials/quality-control/tutorial.html>). Adapter contamination, a common issue in small RNA sequencing, was effectively removed during preprocessing, resulting in clean and reliable reads. The uniformity in read depth and distribution across samples indicates that technical variability was minimized, allowing observed differences in miRNA expression to be attributed primarily to biological variation rather than experimental artifacts. The exclusion of low-quality and low-abundance reads further enhanced the accuracy and robustness of the dataset (Hemstrom *et al.*, 2024)). Differential expression analysis of miRNAs revealed distinct expression patterns across PD, AD, and healthy control groups, as illustrated by the heatmap. The clustering of samples according to disease status indicates the presence of disease-specific miRNA signatures. PD samples exhibited a consistent and distinct expression profile, suggesting a relatively homogeneous molecular pattern within this group. Similarly, AD samples formed a separate cluster, highlighting unique regulatory mechanisms associated with Alzheimer's pathology. The clear separation between disease groups and controls underscores the potential of miRNA expression profiles to discriminate between pathological and normal states.

The observed differential expression of specific miRNAs provides insights into the molecular mechanisms underlying PD and AD. Certain miRNAs were significantly upregulated in AD samples, suggesting their involvement in pathways related to amyloid processing, synaptic dysfunction, and cognitive decline. In contrast, PD samples showed upregulation of miRNAs potentially associated with mitochondrial dysfunction, oxidative stress, and dopaminergic neuron degeneration. The presence of miRNAs that are differentially expressed in both PD and AD indicates shared molecular pathways, such as neuroinflammation and cellular stress responses, which are common features of neurodegenerative diseases (Sadlon *et al.*, 2019). However, the identification of disease-specific miRNAs also highlights the heterogeneity of these disorders and suggests that

distinct regulatory networks contribute to their pathogenesis. The integration of miRNA expression data with target prediction and pathway analysis further elucidates the functional implications of miRNA dysregulation. The identification of a large number of target genes, particularly in PD, suggests extensive post-transcriptional regulation. The enrichment of pathways such as Neuregulin signalling, PI3K-Akt, and MAPK/ERK highlights their potential roles in disease progression (Chen *et al.*, 2024). Neuregulin signalling is known to regulate neuronal development and synaptic plasticity, and its dysregulation may contribute to impaired neuronal communication (L. Mei & Nave, 2014). The PI3K-Akt pathway plays a critical role in cell survival and metabolism, while MAPK/ERK signalling is involved in cellular responses to stress (Q. Li *et al.*, 2022). Alterations in these pathways may reflect compensatory mechanisms or maladaptive responses to neuronal damage.

The upregulation of neuroinflammatory and oxidative stress pathways observed in this study is consistent with existing literature. Chronic inflammation, mediated by activated microglia and increased cytokine production, is a hallmark of neurodegenerative diseases (Gao *et al.*, 2023). Similarly, oxidative stress resulting from mitochondrial dysfunction leads to the accumulation of reactive oxygen species, which can damage cellular components and promote neuronal death (Xu *et al.*, 2025). The downregulation of synaptic transmission and autophagy-related pathways further suggests impaired neuronal function and defective protein clearance mechanisms. These alterations may contribute to the accumulation of toxic protein aggregates, such as α -synuclein in PD and amyloid- β in AD. Network analysis of miRNA-mRNA interactions revealed complex regulatory relationships, with individual miRNAs targeting multiple genes. The identification of hub miRNAs suggests that certain miRNAs may play central roles in regulating disease-associated pathways (Mukherjee *et al.*, 2019). These hub miRNAs may serve as key regulators of gene expression and represent potential targets for therapeutic intervention. The presence of both upregulated and downregulated miRNAs within the network highlights the dynamic nature of miRNA-mediated regulation, where both activation and suppression of target genes contribute to disease pathology. The ROC curve analysis performed in this study provides important insights into the diagnostic potential of identified miRNAs. The high area under the curve (AUC ≈ 0.99) observed for upregulated miRNAs indicates excellent sensitivity and specificity in distinguishing disease samples from controls. This suggests that these miRNAs have

strong potential as non-invasive biomarkers for early disease detection. The downregulated miRNAs also demonstrated good diagnostic performance ($AUC \approx 0.88$), indicating their potential utility as supportive biomarkers. The use of an independent dataset for validation further strengthens the reliability of these findings and highlights the reproducibility of the identified miRNA signatures.

Despite the promising findings, certain limitations should be acknowledged. The sample size, although sufficient for initial analysis, may limit the generalizability of the results. Larger cohort studies are required to validate the identified biomarkers and confirm their clinical applicability. Additionally, while PBMCs provide a convenient and accessible source of biological material, they may not fully capture the complexity of molecular changes occurring within the central nervous system. Future studies integrating peripheral and central tissue analyses may provide a more comprehensive understanding of disease mechanisms.

Functional enrichment analysis represents a critical step in elucidating the biological significance of differentially expressed miRNAs (DEmiRNAs). The strong enrichment observed in the Neurological Disease category across both PD and AD samples highlights the direct association of identified miRNAs with disease-relevant processes. The elevated $-\log(p\text{-value})$ scores indicate robust statistical significance, confirming that the observed miRNA dysregulation is not random but functionally meaningful. The enrichment of pathways such as neurodegeneration, cerebral disorders, and tumor-related neural pathologies suggests that miRNAs may regulate genes involved in fundamental neuronal processes, including survival, differentiation, and synaptic communication (Li *et al.*, 2024). The markedly higher enrichment scores in disease samples compared to healthy controls further emphasize that miRNA-mediated regulatory networks are extensively altered in pathological conditions. This finding is significant because it establishes miRNAs not merely as passive markers but as active contributors to disease progression. Among the enriched pathways, the identification of neuroinflammation, synaptic function, apoptosis, and oxidative stress provides strong biological validation of the dataset (Z. Zhou *et al.*, 2026). These pathways are well-established hallmarks of neurodegenerative diseases, and their enrichment supports the reliability of the analytical approach. The significant involvement of the SMAD2/3 signalling pathway, a key component of TGF- β signalling, is particularly noteworthy. This pathway regulates cellular processes such as proliferation, differentiation, and

apoptosis, and its dysregulation may contribute to neuronal loss and impaired repair mechanisms. The identification of specific miRNAs (e.g., miR-1275, miR-432_sp, and miR-99a_sp) as central regulators targeting SMAD2/3 components highlights their potential role as upstream modulators of disease-associated signalling. This finding is biologically significant because it links miRNA dysregulation to a defined molecular pathway, providing mechanistic insight into disease pathology.

Furthermore, the subtle differences observed between PD and AD in pathway enrichment intensity suggest both shared and disease-specific mechanisms. PD samples demonstrated stronger enrichment in pathways related to mitochondrial dysfunction and oxidative stress, consistent with the known role of mitochondrial impairment in dopaminergic neuron degeneration. In contrast, AD samples showed greater association with synaptic dysfunction and neuroinflammatory pathways, reflecting the central role of synaptic loss and chronic inflammation in cognitive decline. This differential enrichment is significant as it highlights the heterogeneity of neurodegenerative diseases and underscores the need for disease-specific therapeutic strategies. The analysis of miRNA target genes further expands the understanding of miRNA-mediated regulation by quantifying the extent of post-transcriptional control. The heatmap reveals a clear gradient, with PD exhibiting the highest number of miRNA targets, followed by AD and then healthy controls. The identification of 17,176 upregulated and 125,606 downregulated targets in PD indicates extensive regulatory activity, supporting the hypothesis of a dynamic inverse relationship between miRNAs and their target mRNAs. This large-scale targeting suggests that miRNAs orchestrate widespread gene expression changes, influencing multiple biological pathways simultaneously. The slightly reduced but still substantial number of targets in AD indicates a similar regulatory mechanism, albeit with lower intensity. The identification of unique and shared genes provides important insights into disease specificity and overlap. The higher number of unique genes in PD (14,071) suggests the presence of distinct molecular signatures that may underlie PD-specific pathology. Conversely, the substantial number of shared genes (13,759) between PD and AD indicates common regulatory pathways, reinforcing the concept of shared neurodegenerative mechanisms. This dual pattern of uniqueness and overlap is significant because it highlights both convergent and divergent aspects of disease biology, which is critical for understanding disease progression and developing targeted interventions. The identification of 497 statistically significant target genes

under stringent criteria ($|FC| \geq 1.5$, $p_{adj} \leq 0.05$) further refines the analysis by focusing on the most biologically relevant genes. These genes are likely to play central roles in disease pathogenesis and may serve as potential therapeutic targets. The enrichment of the Neuregulin signalling pathway, with 117 associated genes, is particularly significant. Neuregulin signalling is essential for neuronal development, synaptic plasticity, and neuroprotection, and its dysregulation may contribute to impaired neuronal communication and survival. This finding aligns with previous studies and reinforces the biological validity of the results. The increased number of network interactions in PD (108 interactions) compared to AD and controls indicates a more complex and densely connected regulatory network. This suggests that PD is characterized by extensive rewiring of miRNA-mRNA interactions, leading to widespread disruption of cellular processes. The significance of this finding lies in the identification of a highly interconnected regulatory landscape, where perturbation of key nodes (miRNAs) can have cascading effects on multiple pathways.

The integrated pathway analysis provides a holistic view of molecular dysregulation, demonstrating that miRNA alterations impact interconnected signalling networks ((Wilk & Braun, 2018)). The upregulation of pathways such as Neuregulin, PI3K-Akt, MAPK/ERK, neuroinflammation, oxidative stress, and apoptosis indicate activation of stress response and cell death mechanisms. These pathways are essential for maintaining cellular homeostasis, and their dysregulation may reflect both compensatory responses and pathological processes. For example, activation of PI3K-Akt signalling may initially promote cell survival but may become maladaptive under chronic stress conditions. The downregulation of synaptic transmission and autophagy-lysosome pathways is equally significant, as it indicates impaired neuronal function and defective protein clearance. These alterations may lead to the accumulation of toxic protein aggregates, a hallmark of both PD and AD. The simultaneous upregulation of apoptosis further supports the presence of progressive neuronal loss. Collectively, these findings highlight the complex interplay between survival and death pathways in neurodegeneration.

The ROC curve analysis provides strong evidence for the diagnostic potential of identified miRNA signatures. The exceptionally high AUC (0.99) for upregulated miRNAs indicates near-perfect classification performance, suggesting that these miRNAs can reliably distinguish disease samples from healthy controls. This level of

accuracy is rarely achieved in biomarker studies and underscores the robustness of the identified signatures. The slightly lower but still strong performance of downregulated miRNAs (AUC 0.88) suggests that they may serve as complementary biomarkers. The use of an independent dataset enhances the credibility of these findings, demonstrating reproducibility and reducing the likelihood of overfitting. The comparative analysis using the Venn diagram further emphasizes the distinction between disease-specific and shared regulatory mechanisms. The larger PD-specific region indicates greater molecular divergence, while the shared region between PD and AD highlights common neurodegenerative pathways. This analysis is significant because it provides a visual and quantitative representation of molecular overlap, facilitating the identification of both universal and disease-specific therapeutic targets.

The integrated brain-centric model synthesizes all findings into a unified framework, illustrating how miRNA dysregulation affects neuronal integrity. The model highlights the systemic impact of altered regulatory networks, demonstrating how changes at the molecular level translate into functional consequences such as neuroinflammation, oxidative stress, and synaptic dysfunction. The higher degree of dysregulation observed in PD reinforces the notion that PD may involve more extensive molecular alterations compared to AD. This integrative approach is significant because it bridges the gap between molecular data and disease pathology, providing a comprehensive understanding of neurodegeneration.

Finally, the identification of 10 potential miRNA biomarkers represents a major translational outcome of the study. The division into upregulated and downregulated panels, with distinct diagnostic performance, provides a balanced and robust biomarker signature. The strong association of these miRNAs with biological processes such as inflammation, apoptosis, and signalling further supports their relevance. The significance of this finding lies in its potential clinical application, as these miRNAs could serve as non-invasive biomarkers for early diagnosis, disease monitoring, and therapeutic targeting. Despite these promising findings, certain limitations must be considered. The sample size may limit generalizability, and validation in larger cohorts is necessary. Additionally, while PBMCs provide a practical surrogate for studying systemic changes, they may not fully capture brain-specific molecular alterations. Future studies integrating multi-omics approaches and tissue-specific analyses will further enhance our understanding.

In conclusion, this study demonstrates that miRNA dysregulation plays a pivotal role in the pathogenesis of PD and AD by modulating complex molecular networks. Each analytical approach from functional enrichment to biomarker identification contributes to a comprehensive understanding of disease mechanisms. The findings not only advance our knowledge of neurodegenerative biology but also provide a strong foundation for the development of miRNA-based diagnostic and therapeutic strategies.

SUMMARY AND CONCLUSION

The present study provides a comprehensive evaluation of miRNA-mediated regulatory mechanisms in Parkinson's disease (PD) and Alzheimer's disease (AD) using peripheral blood mononuclear cells (PBMCs) as a minimally invasive and biologically relevant model system. The overall experimental design, spanning PBMC isolation, RNA extraction, miRNA profiling, and integrative bioinformatic analysis, demonstrated high methodological robustness and reproducibility. Collectively, the findings offer significant insights into the molecular basis of neurodegenerative diseases and highlight the critical role of miRNA dysregulation in disease progression. The successful isolation of PBMCs using Ficoll-Paque density gradient centrifugation ensured the acquisition of high-quality cellular material for downstream analyses. The clear separation of cellular layers and the preservation of cell integrity confirmed the efficiency and consistency of the isolation protocol. PBMCs, composed primarily of lymphocytes and monocytes, serve as an important surrogate for studying systemic molecular alterations, particularly in the context of neuroinflammation and immune dysregulation. Although PD and AD are primarily central nervous system disorders, peripheral immune cells reflect disease-associated molecular changes, thereby providing a practical platform for investigating circulating miRNA signatures.

High-quality RNA extraction using the TRIzol method was confirmed through agarose gel electrophoresis and spectrophotometric analysis. The presence of intact ribosomal RNA bands and optimal absorbance ratios (A_{260}/A_{280} and A_{260}/A_{230}) indicated minimal contamination and degradation. Consistency in RNA yield and purity across PD, AD, and control samples ensured that downstream analyses were not influenced by technical variability. Subsequent sequencing and data preprocessing further enhanced data reliability, with high-quality reads, effective removal of adapter contamination, and uniform read distribution across samples. These steps ensured that observed differences in miRNA expression were primarily attributable to biological variation rather than experimental artifacts. Differential expression analysis revealed distinct miRNA expression patterns among PD, AD, and healthy controls. The clustering of samples based on disease status indicates the presence of disease-specific miRNA signatures. PD samples exhibited a more pronounced and consistent expression profile,

suggesting a relatively homogeneous molecular phenotype, whereas AD samples displayed moderate but distinct alterations. The clear separation between disease and control groups underscores the potential of miRNA profiles as reliable indicators of pathological states.

The differential expression of specific miRNAs provides important insights into disease mechanisms. In AD, upregulated miRNAs were associated with pathways related to synaptic dysfunction and amyloid processing, whereas in PD, dysregulated miRNAs were linked to mitochondrial dysfunction, oxidative stress, and dopaminergic neuron degeneration. The presence of shared miRNAs between PD and AD indicates common pathological processes, including neuroinflammation and cellular stress responses. At the same time, the identification of disease-specific miRNAs highlights the heterogeneity of neurodegenerative disorders and suggests that distinct regulatory networks contribute to their progression. Functional enrichment analysis further established the biological relevance of the identified miRNAs. The strong enrichment of the Neurological Disease category in both PD and AD confirms the direct involvement of miRNA dysregulation in disease-related processes. Key pathways such as neuroinflammation, oxidative stress, apoptosis, and synaptic function were significantly enriched, supporting their established roles in neurodegeneration. Notably, the SMAD2/3 signalling pathway emerged as an important regulatory axis, with specific miRNAs acting as upstream modulators. This finding provides mechanistic insight into how miRNA dysregulation influences critical cellular processes such as proliferation, differentiation, and programmed cell death.

The analysis of miRNA target genes revealed extensive post-transcriptional regulation, particularly in PD. The large number of predicted targets, including both upregulated and downregulated genes, supports the concept of a dynamic inverse relationship between miRNAs and their mRNA targets. PD exhibited a higher number of unique target genes, indicating disease-specific molecular signatures, while a significant overlap with AD suggests shared regulatory pathways. The identification of statistically significant target genes under stringent criteria highlights those most likely to contribute to disease pathogenesis. Among these, the enrichment of the Neuregulin signalling pathway underscores its potential role in neuronal development and synaptic plasticity. Network analysis demonstrated the complexity of miRNA-mRNA interactions, with individual miRNAs targeting multiple genes and forming interconnected regulatory networks. The identification of hub miRNAs suggests that certain miRNAs play central

roles in controlling key pathways associated with disease. These hub miRNAs may serve as potential therapeutic targets, as modulation of their activity could influence multiple downstream processes simultaneously. The increased network complexity observed in PD further supports the notion of extensive molecular rewiring in this condition.

The integrated pathway analysis highlighted the interplay between multiple dysregulated signalling cascades. The upregulation of pathways such as PI3K-Akt, MAPK/ERK, neuroinflammation, oxidative stress, and apoptosis reflect activation of stress response and cell death mechanisms. In contrast, the downregulation of synaptic transmission and autophagy-related pathways indicates impaired neuronal function and defective protein clearance. These alterations contribute to the accumulation of toxic protein aggregates and progressive neuronal loss, which are characteristic features of both PD and AD. The balance between survival and apoptotic pathways appears to be disrupted, leading to sustained cellular damage. The diagnostic potential of identified miRNAs was evaluated using ROC curve analysis, which demonstrated excellent performance for upregulated miRNAs ($AUC \approx 0.99$) and good performance for downregulated miRNAs ($AUC \approx 0.88$). These results indicate that miRNA signatures possess strong discriminative power and may serve as reliable non-invasive biomarkers for disease detection. The use of an independent dataset further validates the robustness and reproducibility of these findings. Additionally, comparative analysis using Venn diagrams highlighted both shared and disease-specific gene targets, reinforcing the presence of overlapping and distinct molecular mechanisms.

The integrated brain-centric model provides a comprehensive representation of how miRNA dysregulation impacts neuronal integrity. It illustrates the systemic effects of altered miRNA-mRNA interactions, linking molecular changes to functional outcomes such as neuroinflammation, oxidative stress, and synaptic dysfunction. The greater degree of dysregulation observed in PD compared to AD suggests that PD may involve more extensive molecular alterations, although both conditions share common pathogenic pathways. Finally, the identification of ten potential miRNA biomarkers represents a significant translational outcome of the study. These biomarkers, comprising both upregulated and downregulated miRNAs, demonstrated strong diagnostic performance and were associated with key biological processes relevant to neurodegeneration. Their potential application as non-invasive diagnostic tools highlight the clinical relevance of the findings.

Despite these promising results, certain limitations must be acknowledged. The relatively small sample size may limit the generalizability of the findings, and further validation in larger cohorts is necessary. Additionally, while PBMCs provide a convenient and accessible model, they may not fully capture the complexity of molecular changes occurring within the brain. Future studies integrating multi-omics approaches and tissue-specific analyses will provide a more comprehensive understanding of disease mechanisms.

RECOMMENDATIONS AND FUTURE SCOPE OF THE STUDY

The present study has provided valuable insights into the role of miRNA-mediated regulatory networks in Parkinson's disease (PD) and Alzheimer's disease (AD) using peripheral blood mononuclear cells (PBMCs) as a non-invasive model system. Although the findings demonstrate the diagnostic and biological significance of dysregulated miRNAs, several avenues remain open for further investigation. Future studies should focus on validating the identified miRNA biomarkers in larger and more diverse patient cohorts across different populations, age groups, and disease stages. Such validation would strengthen the clinical applicability and generalizability of these biomarkers for early diagnosis, disease monitoring, and prognosis. Longitudinal studies are also required to evaluate temporal changes in miRNA expression during disease progression and therapeutic intervention, thereby establishing their utility as predictive biomarkers. While PBMCs provide an accessible source of biological material, future research should integrate analyses from brain tissues, cerebrospinal fluid (CSF), plasma, serum, and extracellular vesicles such as exosomes to better understand the relationship between peripheral and central nervous system molecular alterations. Comparative studies across multiple biological compartments may help identify the most reliable and disease-specific biomarkers. The integration of multi-omics approaches, including transcriptomics, proteomics, metabolomics, epigenomics, and single-cell sequencing, represents an important future direction. Such comprehensive analyses will enable the construction of more detailed molecular networks and improve our understanding of the complex mechanisms underlying neurodegeneration. Combining miRNA profiles with other molecular signatures may also enhance diagnostic accuracy and facilitate the development of personalized medicine strategies. Further functional studies are necessary to experimentally validate the predicted miRNA-mRNA interactions identified through bioinformatic analyses. Gene knockdown, overexpression experiments, CRISPR-based approaches, and animal model studies could provide mechanistic evidence regarding the role of specific miRNAs in regulating neuronal survival, neuroinflammation, oxidative stress, mitochondrial dysfunction, and synaptic plasticity.

The hub miRNAs identified in this study warrant particular attention as potential therapeutic targets. Future investigations should explore the feasibility of miRNA-based

therapeutics, including miRNA mimics, antagomirs, and targeted delivery systems capable of crossing the blood-brain barrier. Such approaches may offer novel treatment strategies aimed at modulating disease-associated molecular pathways. In addition, the significant involvement of signalling pathways such as SMAD2/3, PI3K-Akt, MAPK/ERK, neuroinflammatory pathways, and Neuregulin signalling suggests the need for deeper exploration of their interactions and regulatory mechanisms. Understanding the crosstalk among these pathways may reveal novel intervention points for disease modification and neuroprotection. Artificial intelligence (AI) and machine learning-based models may also be incorporated in future studies to integrate large-scale molecular datasets and improve biomarker discovery. Predictive algorithms combining miRNA expression patterns with clinical and imaging data could facilitate the development of highly sensitive and specific diagnostic tools for neurodegenerative diseases.

Finally, comparative studies involving other neurodegenerative disorders such as Huntington's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis, and dementia with Lewy bodies could help identify common and disease-specific miRNA signatures. Such investigations would contribute to a broader understanding of neurodegenerative disease biology and support the development of universal as well as disease-targeted diagnostic and therapeutic approaches.

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Molecular Insights into Parkinson's Disease from Mirna Profiling of Peripheral Blood Cells

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Abstract: This study investigated the deregulation of microRNAs (miRNAs) in Parkinson's disease (PD) using RNA sequencing of peripheral blood mononuclear cells (PBMCs) from 50 individuals, comprising 26 PD patients and 24 age- and sex-matched healthy controls. RNA was extracted and sequenced to identify differentially expressed miRNAs (DEmiRNAs), revealing 179 DEmiRNAs between groups—67 upregulated and 85 downregulated in PD patients. Bioinformatic analysis using DESeq2, miRWalk, and Ingenuity Pathway Analysis (IPA) showed that many of these DEmiRNAs are involved in neurological diseases, particularly through the modulation of SMAD3 signaling, a pathway linked to neuroinflammation and microglial function. Hierarchical clustering and pathway enrichment highlighted the molecular heterogeneity of PD. Predicted miRNA targets were cross-referenced with previously published mRNA profiles from the same cohort, identifying 497 significantly deregulated genes. Among the top enriched pathways was Neuregulin signaling. Functional network analysis using Cytoscape revealed key miRNA–mRNA interactions, supporting a regulatory role of these miRNAs in PD pathophysiology. These findings suggest that miRNA profiling in PBMCs may offer insights into the molecular mechanisms underlying PD and identify potential biomarkers or therapeutic targets for future investigation. Data are publicly available at ArrayExpress (E-MTAB-12087).

Keywords: Parkinson's Disease, Peripheral Blood Mononuclear Cells, RNA Sequencing, Ingenuity Pathway.

I. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder that primarily affects motor control, resulting in symptoms such as tremors, rigidity, bradykinesia, and postural instability. The condition is characterized by the loss of dopaminergic neurons in the substantia nigra of the brain. While the pathophysiology of Parkinson's disease has been extensively studied, much remains to be understood about its underlying molecular mechanisms. Over the past decade, microRNAs (miRNAs) have emerged as critical regulators of gene expression and have been implicated in the pathogenesis of various



diseases, including neurodegenerative disorders like PD. miRNAs are small, non-coding RNA molecules that regulate gene expression by binding to complementary sequences on target mRNAs, leading to their degradation or translational repression. This regulatory mechanism is essential for maintaining cellular homeostasis, and dysregulation of miRNA expression has been linked to a range of pathological conditions, including cancer, cardiovascular diseases, and neurodegenerative disorders.

In Parkinson's disease, the potential role of miRNAs in disease onset, progression, and cellular response to stress is of particular interest. Peripheral blood cells have been increasingly recognized as a valuable source of biomarkers for PD. Given the accessibility of blood samples, profiling miRNAs in peripheral blood cells offers a minimally invasive approach for identifying molecular signatures associated with the disease. The concept of using miRNA profiling as a diagnostic or prognostic tool for PD has gained considerable attention in recent years, as it holds the promise of identifying novel biomarkers for early diagnosis, monitoring disease progression, and assessing therapeutic efficacy. Various studies have reported altered miRNA expression profiles in peripheral blood cells of PD patients, providing evidence of the involvement of miRNAs in the disease's molecular pathways.

One of the challenges in understanding the molecular mechanisms of PD lies in the complex interplay between genetic and environmental factors. While mutations in specific genes such as SNCA (alpha-synuclein) and LRRK2 (Leucine-Rich Repeat Kinase 2) have been associated with familial forms of PD, the majority of cases are considered idiopathic, with no clear genetic cause. Environmental factors such as exposure to toxins and head trauma, as well as lifestyle factors, also contribute to the risk of developing PD. miRNAs act as important mediators of gene-environment interactions, and their expression can be influenced by both genetic variations and environmental factors. For example, certain miRNAs may be upregulated or downregulated in response to oxidative stress, inflammation, or other cellular insults that are common in PD pathogenesis. As such, profiling the expression of miRNAs in peripheral blood cells could provide valuable insights into how these environmental and genetic factors converge to trigger the onset and progression of PD. Recent advances in high-throughput technologies, such as next-generation sequencing (NGS) and microarray analysis, have enabled the large-scale profiling of miRNA expression in various biological samples, including peripheral blood cells. These technologies have revealed that specific miRNAs are consistently dysregulated in PD patients compared to healthy controls. For instance, miR-133b, miR-146a, and miR-155 have been found to be significantly altered in the blood of PD patients. These miRNAs are involved in regulating various cellular processes, including inflammation, apoptosis, and cellular stress responses, all of which play crucial roles in the pathophysiology of PD. Dysregulation of these miRNAs could potentially lead to impaired cellular function and contribute to the neurodegenerative processes observed in PD.



Furthermore, some studies have suggested that miRNA profiling in blood samples may serve as a potential biomarker for distinguishing PD from other neurodegenerative diseases, such as Alzheimer's disease, and even for differentiating between subtypes of PD.

The potential of miRNA-based biomarkers in PD diagnosis and prognosis is not limited to identifying disease-associated changes in miRNA expression. miRNAs are also involved in various cellular processes that could provide insights into the mechanisms of neurodegeneration. For example, certain miRNAs have been implicated in modulating the autophagy-lysosome pathway, a crucial process for maintaining cellular homeostasis and removing damaged proteins and organelles. Impaired autophagy has been observed in PD and is believed to contribute to the accumulation of toxic proteins such as alpha-synuclein, which is a hallmark of the disease. By profiling miRNAs in peripheral blood cells, it may be possible to identify changes in the expression of miRNAs that regulate this pathway, providing a deeper understanding of how autophagy dysfunction contributes to PD pathology.

Moreover, miRNA-based therapies are an emerging area of research in the field of neurodegenerative diseases. Given their ability to regulate multiple genes simultaneously, miRNAs have the potential to be harnessed for therapeutic purposes. Strategies to modulate miRNA expression, such as the use of miRNA mimics or inhibitors, are being explored as potential treatments for PD. However, translating these findings into clinical applications requires a thorough understanding of the specific roles of individual miRNAs in the disease. miRNA profiling of peripheral blood cells could provide a useful tool for identifying miRNA candidates that are most likely to have therapeutic potential.

II. REVIEW OF LITERATURE

Nies, Yong Hui et al., (2021) Resting tremor, bradykinesia, bradykinesia, postural instability, gait and walking problems, and cogwheel stiffness are symptoms of Parkinson's disease (PD), a devastating neurological disorder that affects the motor system. Striatal dopamine insufficiency results from a drastic reduction in dopaminergic neurons in the substantia nigra pars compacta, and Lewy bodies are a pathogenic feature of Parkinson's disease. Even though the goal of Parkinson's disease therapy is to either restore dopamine levels in the brain or protect dopaminergic neurons, full recovery from the condition has not yet been accomplished. The fundamental mechanisms of PD pathogenesis remain unknown due to the intricacy and multisystem impacts of PD. With a focus on microRNAs (miRNAs) and their involvement in elucidating the pathophysiology of PD, new medical discoveries have shed light on the pathogenesis and possible therapy of PD. Evidence of dysregulated miRNAs expression patterns was discovered in PD patients' striatal brain tissue and dopaminergic neurons from the substantia nigra. Therefore, by influencing the expression of genes and proteins related to PD, dysregulation of miRNAs may play a role in the development of PD. Recent discoveries on dysregulation of miRNAs linked with Parkinson's disease (PD) will be



covered in this review, ranging from the regulation of genes related to PD to the survival of dopaminergic neurons, inflammation caused by α -synuclein, and miRNAs in circulation. An updated discussion of miRNAs' potential use as PD diagnostic biomarkers and treatment tools is included in the next part of this review.

Wang, Hsiuying. (2021). A neurodegenerative ailment known as Parkinson's disease (PD) affects one percent of the population sixty and above. About a quarter of persons over the age of sixty-five suffer from diabetes mellitus (DM), a metabolic condition. New research shows that diabetes raises the probability of acquiring Parkinson's disease. Various processes, such as mitochondrial failure, oxidative stress, and protein aggregation, have been studied in regard to the association between DM and PD in the literature. Here we take a look at the shared miRNA biomarkers between the two conditions. Apoptosis, cell cycle control, development, and cell differentiation are among processes in which microRNAs (miRNAs) participate significantly. Their role in the pathophysiology of several illnesses is well-documented. Insulin signaling and glucose uptake are both mediated by microRNAs. PD-related genes may also be regulated by miRNAs. Hence, targeting miRNAs offers a therapeutic option for both PD and DM, and investigating the shared miRNA biomarkers of the two illnesses may provide light on their correlation.

Hoss, Andrew et al., (2016) we aimed to identify PD unique signatures and examine the illness-related characteristics of onset age and dementia by comparing the microRNA (miRNA) profile of normal control brain with Parkinson's disease (PD) frontal cortex. Techniques: Thirty-three control samples and 29 PD samples were subjected to small RNA sequencing analysis in the prefrontal cortex. Using weighted vote class prediction, we built a PD classifier after performing sample QC, normalization, and batch correction. We next used linear regression to identify miRNAs changed in PD. Additionally; case-only studies explained the correlation between miRNA levels and PD with dementia (PDD) starting age and PD severity. Findings: FDR $q < 0.05$ indicated that one twenty-five microRNAs were significantly differentially expressed in PD throughout the whole genome. A group of 29 microRNAs distinguished sick from healthy brain tissue with a specificity of 93.9% and sensitivity of 96.6%. The ordinal link from control to PD without dementia (PDN) to PDD was shown by the majority of differentially expressed miRNAs (105/125). In PD brains, 36 miRNAs distinguished between PDD and PDN with a sensitivity of 81.2% and a specificity of 88.9%. The miR-10b-5p gene was positively associated with onset age ($q = 4.7e-2$) among the miRNAs that were differentially expressed. Conclusions: The levels of cortical miRNAs allowed for the reliable classification of PD brains from healthy brains. Furthermore, among the miRNAs differently expressed in PD, the PDD miRNA profile showed a more pronounced pattern of change. It is necessary to better characterize and analyze miRNA



changes in peripheral bio fluids that are connected to the brain in order to assess their clinical value as possible clinical indicators.

Petillo, David et al., (2015) among neurodegenerative diseases, Parkinson's disease (PD) ranks second in frequency. Finding and creating biomarkers for early identification is a huge obstacle in the research of this degenerative neurological defect. Multiple microRNA (miRNA) indicators for Parkinson's disease have been found in the bloodstream in recent times. Nevertheless, there has been a dearth of follow-up research using fresh, separate cohorts. Previously, we discovered a set of four miRNA biomarkers for Parkinson's disease (miR-1826, miR-450b-3p, miR-505, and miR-626) that were able to detect the disease with a sensitivity of 91% and a specificity of 100%. A fresh, separate validation set, however, did not reveal any miR-450b-3p expression. We reevaluated the biomarker performance and increased the detection power in our present investigation by adding a non-biased pre-amplification step to quantitative real-time PCR (qRT-PCR). In our validation set, we discovered that the panel of four miRNAs connected to PD had a prediction value of 83% sensitivity and 75% specificity. This work validates circulating miRNAs in plasma as a molecular biomarker for Parkinson's disease (PD), and it also shows that qRT-PCR is a viable diagnostic tool.

Thomas, Bobby. (2011). Motor irregularities and autonomic dysfunctions are symptoms of Parkinson's disease, a neurodegenerative movement illness marked by the loss of midbrain dopaminergic neurons. We still don't know what causes Parkinson's disease, and despite a lot of study, we don't have any good treatments. But fresh insights into the pathophysiology of the illness have started to emerge thanks to the recent discovery of genes associated with heritable types of Parkinson's disease, which has shook up the area of study. We will go over these new genetic discoveries and how they fit into our bigger picture of trying to figure out what causes Parkinson's disease so that we can develop better neuroprotective treatments.

III. MATERIALS AND METHODS

Participants

50 people were included in this case-control research. Of them, 26 were PD patients and 24 were healthy controls. The miRNA analysis was done using RNA-seq. Using the most recent diagnostic criteria for PD, ten individuals (six males and six females) with an average age of 68.00 ± 6.47 years and an average duration of 5.75 ± 3.80 years of illness were diagnosed. In all, five individuals had an akinetic-rigid phenotype, two were tremor-dominant, and nine showed a combination of the two. The control group, which consisted of 10 men and 4 females with an average age of 71.94 ± 13.19 years, did not use any drugs, had no history of neurological or mental illnesses, and their neurological exam came back completely normal.



miRNA Extraction

Ficoll-Paque was used for PBMC separation. Per the directions provided by the manufacturer, total RNA was extracted from PBMC using TRIzol reagent. Until further processing, the RNA was kept at a temperature of -80°C . NanoDropTM 2000/2000c was used to monitor RNA concentration and purity, while TapeStation 4200 was used to examine sample integrity using RNA ScreenTape Assay 2.7.

miRNA Sequencing and Data Analysis

Following the instructions provided by Perki-nElmer, a sequencing library was prepared using 500 ng of pure RNA using the NEXTFLEX Small RNA-Seq Kit v3. Library quantification was done using an Agilent Technologies TapeStation 4200 and an Invitrogen Co. Qubit fluorometer. The results were combined to ensure that each index-tagged sample was present in an equimolar level. Sequencing and cluster creation were performed on the combined samples using a 1×75 single-end format using an Illumina NextSeq 550 Dx System. Fastq was tested using the FastQC tool for quality control.

In order to acquire miRNA expression profile with regard to the miRBase database (version22-GRCh38-GCA_000001405.15), the adaptor sequences were removed using the program sRNAbench. This was done based on the kit used and the poor quality readings. In all samples that were utilized, reads with a cumulative read count below 10 were eliminated. The DESeq2 method (version 1.36.0) was used to detect miRNAs that were differently expressed between PD and controls.

When the |Fold-Change| (|FC|) was more than or equal to 1.5 ($p \leq 0.05$), the expression of miRNAs was deemed to be substantially differently expressed between the control group and PD patients. miRWalk v.3.0, which assigns a score to each hypothetical interaction between miRNAs and mRNAs, was used to forecast miRNA targets. We limited our consideration to miRNAs with a prediction score of 1 and those that were recognized by two of the three methods available in the miRWalk database in order to find mRNA targets with high prediction accuracy. Taking into account both experimentally confirmed and strongly anticipated interactions, potential miRNA targets were defined as differentially expressed mRNAs with $|FC| \geq 1.5$.

The investigation of deregulated canonical pathways and functions was carried out using Ingenuity Pathway Software (IPA, Ingenuity System). The R/Bioconductor programs were used to create data integration, heatmaps showing differentially expressed miRNAs, and functional enrichment plots. The normalized count of miRNAs discovered and the raw data (.fastq files) may be found at ArrayExpress (E-MTAB-12087).



IV. RESULTS AND DISCUSSION

Characterization of Small Non Coding RNAs Deregulation in PD Patients

After removing adaptors and low-quality reads, next-generation sequencing was used to profile small RNA expression in both PD patients and controls. The resulting high-quality readings were aligned with the reference human genome.

Hierarchical clustering analysis were used to examine the overall variations in miRNA expression among the cohorts. Expression heterogeneity was seen in PD samples, with some individuals clustering near or among controls. This provides further evidence that miRNA expression reflects the molecular variety seen in PD patients. In particular, we found 1752 distinct miRNAs, with 179 of them showing differential expression between the PD and control groups. The results showed that 67 distinct miRNAs were elevated ($\text{padj} \leq 0.05$ and $|\text{FC}| \geq 1.5$) and 85 unique miRNAs were down regulated ($\text{padj} \leq 0.05$ and $|\text{FC}| \leq -1.5$) in PD patients as compared to the control group (figure 1 and 2). You may find the normalized miRNA count (E-MTAB-12087) on Array Express.

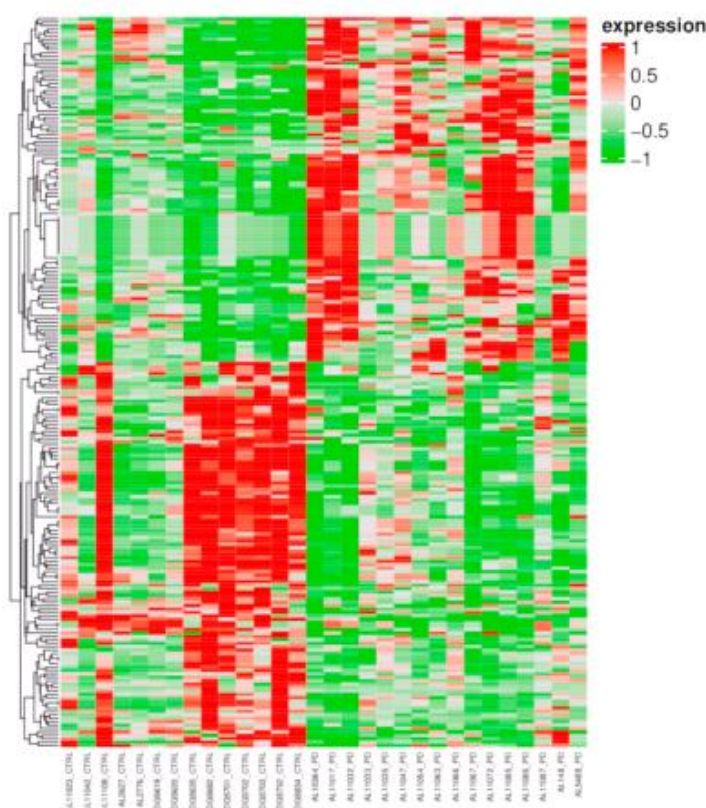


Figure 1. The miRNA profiling heatmap shows hierarchical clustering of differentially expressed miRNAs ($\text{padj} \leq 0.05$ and $|\text{FC}| \geq 1.5$) in Parkinson's disease patients versus healthy controls



The above figure Generated with the Complex Heatmap R package, it uses "euclidean" distance and "complete" clustering. Red indicates expression above the median, and green indicates below the median.

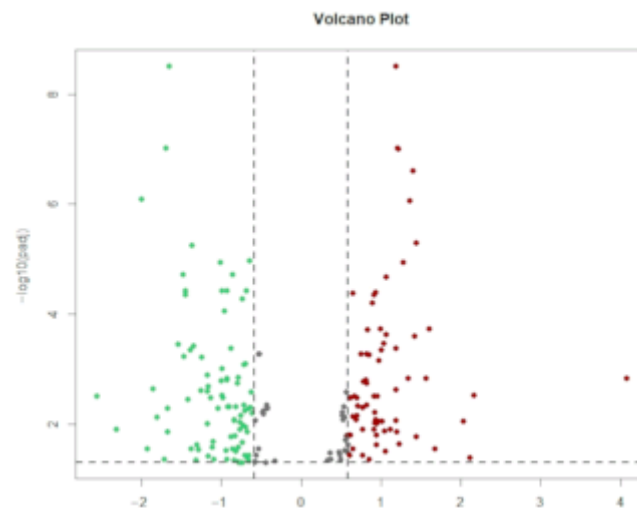


Figure 2. The volcano plots of miRNA profiling show differentially expressed miRNAs ($padj \leq 0.05$ and $|FC| \geq 1.5$).

The above figure shows Gray arrows highlight miRNAs with large fold-changes and high significance. Red denotes overexpressed miRNAs, and green denotes downregulated miRNAs

We used an enrichment analysis using the Ingenuity Pathway Analysis (IPA) tools to try to figure out which pathways were impacted by differentially expressed miRNAs (DEmiRNAs) and which ones may be linked to Parkinson's disease (PD) risk. Figure 3 shows that the DEmiRNAs were mostly associated with several biological functions and diseases, as shown by the IPA pathway analysis ($padj \leq 0.05$ and $|FC| \geq 1.5$). Particularly noteworthy was the Neurological Disease category, which had a substantial Benjamini-Hochberg p-value (B-H p-value) and was annotated with several diseases or functions (e.g., cerebral disorder, AD, PD, neuroblastoma). Using the DEmiRNAs IPA Network Analysis, we were able to identify several nodes and interactions with high scores, as seen in Figure 4. Here, we focus on the three miRNAs—miR-1275, miR-432_sp, and miR-99a_sp—that target the SMAD 2/3 signaling pathways.

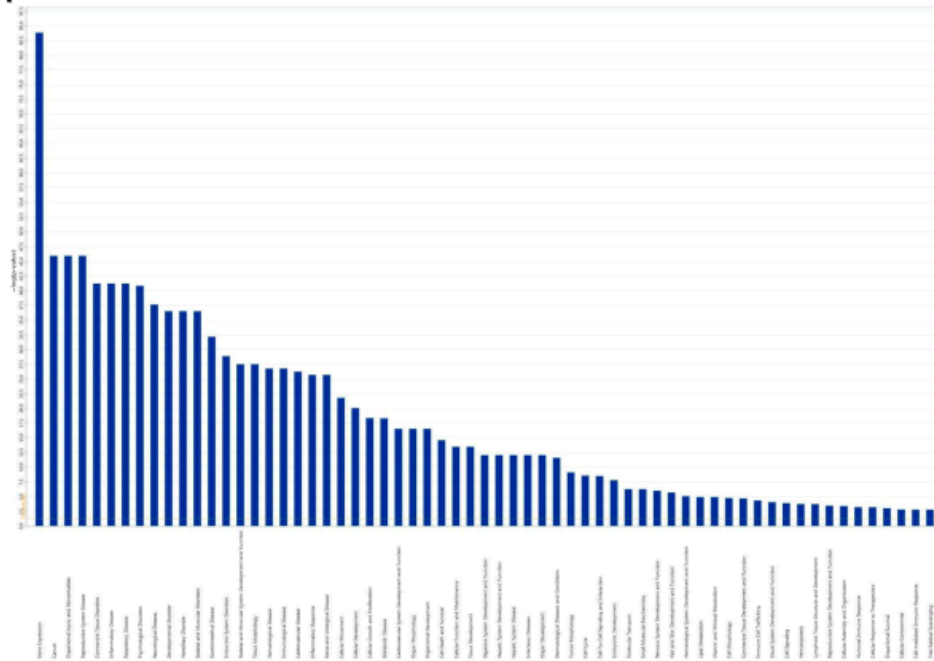


Figure 3. IPA revealed enriched diseases and biological functions for differentially expressed miRNAs ($p_{adj} \leq 0.05$ and $|FC| \geq 1.5$) between controls and Parkinson's patients



Figure 4. IPA network shows miRNAs as nodes, with red (upregulated) and green (downregulated) indicating fold change. Edges represent predicted relationships: blue for inhibition and gray for unpredicted effects, based on IPA z-scores and literature data



SMAD 3 is implicated in many neurodegenerative processes, including neuroinflammation processes that are strongly linked to the etiopathogenesis of Parkinson's disease, according to gene-disease correlations analyses conducted using the DisGeNET platform. In addition, SMAD3 signaling is essential for microglial homeostasis, which in turn regulates microglial activity and helps keep the brain's environment stable. Nigrostriatal dopaminergic neurodegeneration is facilitated by microglial involvement via the release of proinflammatory substances.

Analysis of the Differentially Expressed miRNAs' Target Genes

To automate miRNA-targeting gene analysis methods, we used the miRWalk program to anticipate which genes DE miRNAs will target. Experimentally validated miRNA-target interactions with a score of 0.95 on 30UTR were taken into account in this research. By using this strategy, we were able to identify 17,176 distinct target genes out of a total of 450,509 targets for the 67 elevated miRNAs. Approximately 125,606 targets were discovered for 85 down regulated miRNAs, with 14,071 target genes being unique and 13,759 shared amongst the groups. We looked at the possibility of a dynamic inverse relationship between miRNA and mRNA levels since it is feasible that overexpression of miRNA is associated with down regulation of its mRNA targets and vice versa. In order to assess the likelihood of this happening, we first looked for instances where the seed sequence of regulated miRNAs and the UTR of their potential mRNA targets were a perfect or imperfect match.

For the next IPA pathway analysis (Figure 5), we solely included potential mRNA targets that were found to be statistically significant in the same patient cohort as before ($|FC (PD \text{ vs. CTRL})| \geq 1.5$ and $p_{adj} \leq 0.05$) so that we could concentrate on the most intriguing target-genes. All DE miRNAs were determined to significantly target 497 genes.

Surprisingly, Neuregulin signaling was identified as one of the top five canonical pathways enriched, with 117 target genes that were elevated (Figure 5, right column). In addition, functional network analysis was carried out to gain a comprehensive understanding of the interactions between the dys regulated miRNAs and their targets. The results showed that the candidate genes were linked to various biological processes, including binding, molecular function, and catalytic activity (Figure 6). Using Cytoscape 3.8.0, developed by The Cytoscape Consortium and hosted at <http://www.cytoscape.org/>, regulatory networks of miRNA-mRNA interactions were built and shown. Specifically, we examined the edges of the regulatory networks including up-and down-regulated miRNAs and their targets, and we zeroed in on those networks that were filtered by fold-change ($|FC| > 1.5$). As a result of this investigation, 108 interactions involving 26 up-targets and 28 down-targets for miRNA-nodes were identified. These microRNAs and messenger RNAs (miRNAs) have links to other



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genes and might be network nodes. It is hypothesized that these genes are also involved in the PD network, since they are targeted by a set of miRNAs.

A receiver operating characteristic (ROC) analysis was carried out to assess the diagnostic power of this molecular signature based on specific miRNAs (hsa-miR-1275, hsa-miR-23a-5p, hsa-miR-432-5p, hsa-miR-4433b-3p, hsa-miR4443) and (hsa-miR-142-5p, hsa-miR-143-3p, hsa-miR-374a-3p, hsa-miR-542-3p, hsa-miR99a-5p). Figure 8 shows that the five miRNAs that were up-regulated had an area under the curve (AUC) of 0.99, whereas the five miRNAs that were down-regulated had an AUC of 0.88. This suggests that these miRNAs may have biomarker potential.



Figure 5. IPA Canonical Pathway Analysis links miRNA target genes to pathways, showing statistical significance (left) and the number of over- and under expressed genes (right)

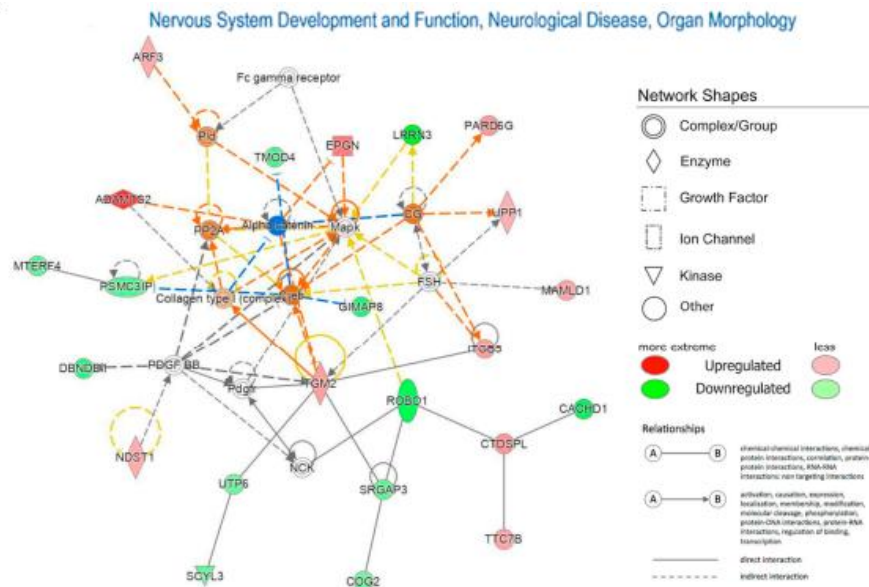


Figure 6: IPA Network for Nervous System Development, Neurological Disease, and Organ Morphology displays up regulated transcripts in red and down regulated transcripts in green.

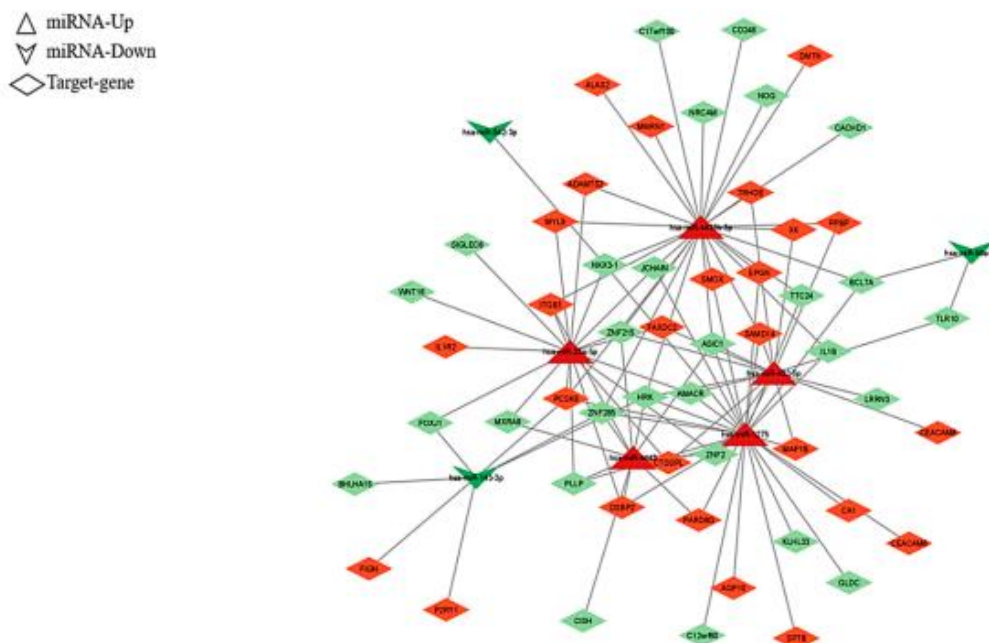


Figure 7: The miRNA-target gene network is based on dys regulated miRNAs and their target genes, with green representing down regulated miRNAs and red indicating up regulated miRNAs.

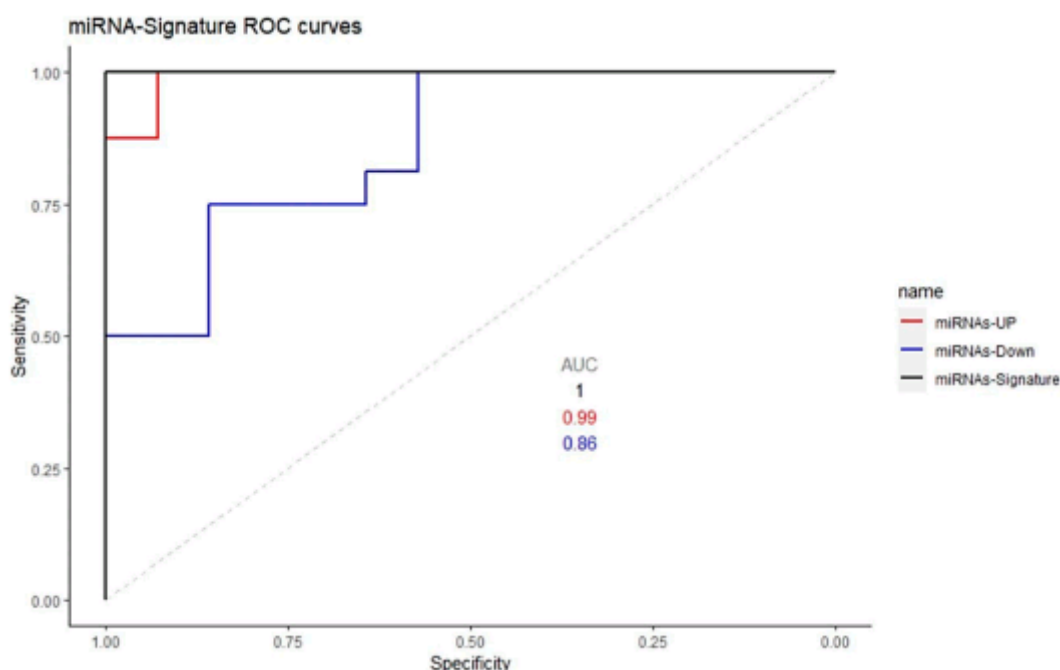


Figure 8: Receiver operating characteristic (ROC) curve analysis of 5 up regulated and down regulated miRNA.

V. CONCLUSION

By studying the differential expression of miRNAs in peripheral blood mononuclear cells (PBMCs) from PD patients compared to healthy controls, this work offers new insights into the function of miRNAs in Parkinson's Disease (PD). The discovery of miRNAs with variable expression sheds insight on the intricate molecular changes linked to PD. Based on these results, microRNAs (miRNAs) may play an important role in disease etiology by affecting neuroinflammation, microglial homeostasis, and neuronal survival. The complex character of PD was further shown by the pathway enrichment analysis, which revealed the participation of important neurological processes such as Neuregulin signaling and SMAD2/3 signaling. The use of miRNA expression profiles in conjunction with mRNA target predictions enhances the miRNAs' capacity to act as biomarkers for PD, perhaps indicating the advancement of the illness or the efficacy of treatment. Furthermore, this study's regulatory networks provide light on the ways in which miRNAs interact with the genes that they target, paving the way for potential novel treatment approaches. In sum, our findings highlight the need of continuing to study miRNAs as potential non-invasive biomarkers for Parkinson's disease (PD) early identification and surveillance, as well as miRNA-based



therapy approaches. These results need to be validated and their therapeutic implications explored in future research with bigger and more varied populations.

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Certificate of Publication

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Integrated miRNA-mRNA Regulatory Signatures in Parkinson's Disease and Alzheimer's Disease: RNA-Seq-Based
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Integrated miRNA-mRNA Regulatory Signatures in Parkinson's Disease and Alzheimer's Disease: RNA-Seq-Based Biomarker Discovery and Pathway Analysis

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ABSTRACT

Neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) are characterized by progressive neuronal dysfunction and complex molecular alterations. This study investigated differential microRNA (miRNA) expression profiles in PD and AD patients using RNA sequencing (RNA-seq) and comprehensive bioinformatics analysis. A total of 50 participants, including 26 patients and 24 healthy controls, were included in each disease cohort. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque density gradient centrifugation, followed by total RNA extraction using TRIzol reagent. RNA quality assessment confirmed high integrity and purity suitable for downstream sequencing applications. Small RNA libraries were prepared and sequenced using the Illumina NextSeq platform. Differential expression analysis identified several dysregulated miRNAs associated with PD and AD. Functional enrichment and network analyses demonstrated significant involvement of pathways related to neuroinflammation, oxidative stress, apoptosis, synaptic dysfunction, PI3K-Akt signaling, MAPK/ERK signaling, and SMAD2/3 signaling. PD samples exhibited more extensive miRNA-mediated regulatory disruption compared to AD. ROC curve analysis demonstrated excellent diagnostic performance for selected miRNA biomarkers, with upregulated miRNAs achieving an AUC of approximately 0.99. These findings highlight the diagnostic and therapeutic potential of miRNA signatures in neurodegenerative diseases.

Keywords: *Parkinson's Disease, Alzheimer's Disease, MicroRNA, RNA Sequencing, Biomarkers, PBMCs, Neurodegeneration, Pathway Analysis, miRNA-mRNA Interaction.*

1. INTRODUCTION

Parkinson's disease (PD) and Alzheimer's disease (AD) are the two most common neurodegenerative disorders affecting millions of individuals worldwide and represent a major public health burden due to their progressive nature, high disability rates, and increasing prevalence in aging populations (Lian et al., 2026). PD is primarily characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra, leading to motor dysfunctions such as tremors, rigidity, bradykinesia, and postural instability, along with several non-motor manifestations including cognitive impairment and autonomic dysfunction (Zhou et al., 2023). In contrast, AD is predominantly associated with progressive cognitive decline, memory impairment, and behavioral disturbances resulting from neuronal loss, extracellular amyloid- β plaque accumulation, and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein (Kamatham et al., 2024). Although PD and AD differ in their clinical manifestations and pathological hallmarks, both disorders share several overlapping molecular mechanisms, including oxidative stress, mitochondrial dysfunction, neuroinflammation, synaptic degeneration, impaired proteostasis, and dysregulated cellular signalling pathways (Ali et al., 2025). Despite substantial advances in neuroimaging, genomics, and molecular neuroscience, the diagnosis of PD and AD still relies heavily on clinical manifestations that often appear only after significant neuronal damage has already occurred. Current diagnostic methods, including neuroimaging and cerebrospinal fluid (CSF)-based biomarkers, have limitations related to invasiveness, accessibility, sensitivity, specificity, and cost-effectiveness. Furthermore, disease heterogeneity and overlapping clinical symptoms between neurodegenerative disorders complicate early and accurate diagnosis (Das et al., 2020). Consequently, there remains a critical need for minimally invasive, reliable, and reproducible molecular biomarkers capable of detecting disease onset at earlier stages, monitoring disease progression, and potentially predicting therapeutic response (Zafar et al., 2025). MicroRNAs (miRNAs) have emerged as highly promising candidates in this regard. miRNAs are small endogenous non-coding RNA molecules, typically 18–25 nucleotides in length, that regulate gene expression post-transcriptionally through mRNA degradation or translational repression (Saw & Song, 2025). These molecules are involved in a wide range of biological processes including neuronal development, synaptic plasticity, apoptosis, immune regulation, oxidative stress responses, and cellular metabolism. Importantly, miRNAs exhibit remarkable stability in biological fluids such as blood, serum, plasma, cerebrospinal fluid, and saliva due to their encapsulation within extracellular vesicles or association with RNA-binding proteins (Katayama et al., 2025). This stability makes them particularly attractive as non-invasive biomarkers for neurological disorders.

Accumulating evidence suggests that altered miRNA expression contributes significantly to the pathogenesis of both PD and AD (Sharma et al., 2024). Several dysregulated miRNAs have been implicated in pathways associated with α -synuclein aggregation, mitochondrial dysfunction, neuroinflammatory signalling, tau phosphorylation, amyloid precursor protein processing, synaptic dysfunction, and neuronal apoptosis (Li et al., 2024). However, despite growing interest in miRNA biology, previous studies have reported inconsistent findings due to variations in sample types, small cohort sizes, differences in sequencing platforms, limited bioinformatics integration, and inadequate

validation approaches (Guo et al., 2014). Moreover, most studies have focused on either PD or AD independently, while comparative analyses investigating shared and disease-specific miRNA regulatory networks between these neurodegenerative disorders remain limited. Another major limitation in current research is the insufficient integration of miRNA expression data with downstream functional analyses such as miRNA-mRNA interaction mapping, pathway enrichment, and network-based systems biology approaches (Nazarov & Kreis, 2021). Many earlier investigations primarily identified differentially expressed miRNAs without comprehensively evaluating their functional consequences or their involvement in interconnected molecular pathways underlying neurodegeneration. Consequently, the precise regulatory roles of miRNAs in disease progression and their translational utility as diagnostic biomarkers or therapeutic targets remain incompletely understood. Therefore, the present study was designed to address these critical research gaps by performing a comprehensive comparative analysis of miRNA expression profiles in Parkinson's disease and Alzheimer's disease using high-throughput RNA sequencing (RNA-seq) combined with advanced bioinformatics approaches. Peripheral blood mononuclear cells (PBMCs) were utilized as a minimally invasive and biologically relevant source for miRNA profiling, reflecting systemic molecular alterations associated with neurodegeneration. Through integrated differential expression analysis, miRNA-mRNA interaction network construction, target gene prediction, pathway enrichment analysis, and diagnostic ROC curve evaluation, this study aimed to identify both shared and disease-specific molecular signatures associated with PD and AD.

Furthermore, this study sought to explore the functional implications of dysregulated miRNAs in critical neurodegenerative pathways including neuroinflammation, oxidative stress, apoptosis, synaptic signalling, PI3K-Akt signalling, MAPK/ERK pathways, autophagy-lysosome dysfunction, and SMAD2/3 signalling. By integrating transcriptomic and systems-level analyses, the study aimed to provide a deeper understanding of miRNA-mediated regulatory mechanisms contributing to neuronal degeneration and disease progression.

2. METHODOLOGY

2.1 Study Design and Participant Recruitment

A case-control study design was employed involving PD patients, AD patients, and age-matched healthy controls. The PD cohort consisted of 26 clinically diagnosed patients and 24 healthy controls. Similarly, the AD cohort included 26 clinically diagnosed patients and 24 healthy controls (Salemi et al., 2022).

2.2 Isolation of PBMCs

Peripheral blood mononuclear cells were isolated using Ficoll-Paque density gradient centrifugation. Distinct plasma, buffy coat, and erythrocyte layers were obtained following centrifugation (Koshy et al., 2024).

2.3 RNA Extraction and Quality Assessment

Total RNA including miRNAs was extracted using TRIzol reagent. RNA concentration and purity were evaluated using NanoDrop spectrophotometry. Samples with A260/A280 ratios between 1.8 and 2.0 and A260/A230 ratios between 2.0 and 2.2 were considered acceptable for downstream analysis (Arunachalam & Sreeja, 2025).

2.4 RNA Sequencing

Small RNA libraries were prepared using the NEXTFLEX Small RNA-Seq Kit v3. Libraries were quantified using Agilent TapeStation 4200 and Qubit Fluorometer before sequencing on the Illumina NextSeq 550 Dx platform using single-end 1×75 bp sequencing (Tallvod et al., 2023).

2.5 Data analysis

Raw FASTQ files underwent quality control using FastQC. Adapter trimming and filtering were performed using sRNAbench. Processed reads were aligned to miRBase v22 (GRCh38). Differential expression analysis was conducted using DESeq2 with thresholds of $|FC| \geq 1.5$ and $p \leq 0.05$ (Marini et al., 2020).

2.6 Functional and Network Analysis

Target prediction was conducted using miRWalk v3.0. Functional enrichment analysis was performed using Ingenuity Pathway Analysis (IPA). Heatmaps and network visualizations were generated using R/Bioconductor packages (Di Martino et al., 2015).

3. RESULTS

3.1 PBMCs Isolation and RNA Extraction

PBMC isolation demonstrated high reproducibility with successful recovery of lymphocytes and monocytes across all groups. Total RNA extraction yielded intact RNA with clear 28S and 18S ribosomal RNA bands. Peripheral blood samples obtained from participants in both Parkinson's disease (**Figure 1**) and Alzheimer's disease groups (**Figure 2**), along with their respective healthy controls, were successfully processed for the isolation of peripheral blood mononuclear cells (PBMCs) using density gradient centrifugation with Ficoll-Paque solution. Following centrifugation, distinct layers were clearly observed, including plasma, a buffy coat layer containing PBMCs, and erythrocytes at the bottom. The PBMC layer was carefully aspirated without contamination from adjacent layers, ensuring high purity of isolated cells. The obtained PBMCs predominantly consisted of lymphocytes and monocytes, which are essential for downstream molecular analyses. The cell yield and viability were found to be adequate across all samples, with no significant variation observed between disease groups and controls. Morphological examination confirmed the integrity of the isolated cells, and no visible signs of cell damage or contamination were detected. The isolation procedure demonstrated high reproducibility and consistency across all samples. These PBMCs provided a reliable source of high-quality cellular material for subsequent RNA extraction and miRNA profiling.

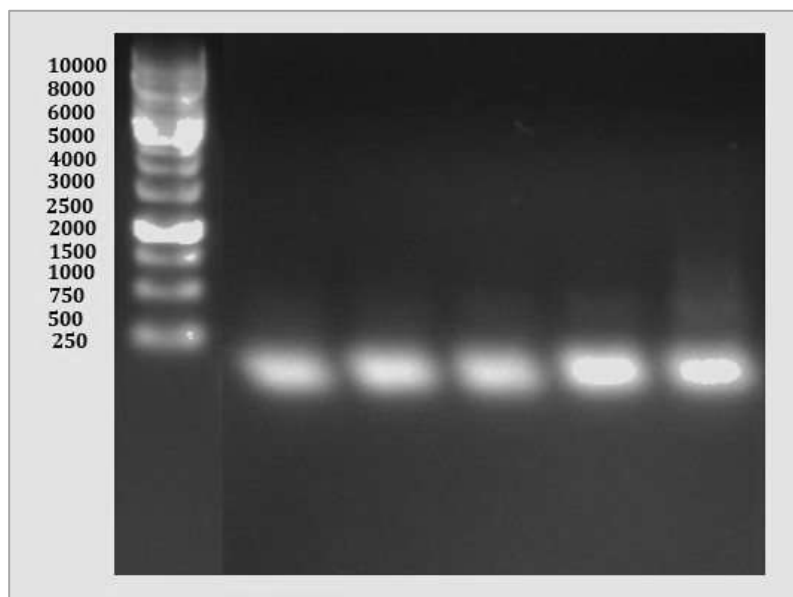


Figure 1: Isolation of peripheral blood mononuclear cells (PBMCs) from patients with Parkinson's disease and healthy controls using Ficoll-Paque density gradient centrifugation. The figure shows the formation of distinct layers after centrifugation, including plasma (upper layer), PBMCs/buffy coat (intermediate layer), and erythrocytes (lower layer). The PBMC layer containing lymphocytes and monocytes was carefully isolated and used for downstream molecular analysis.

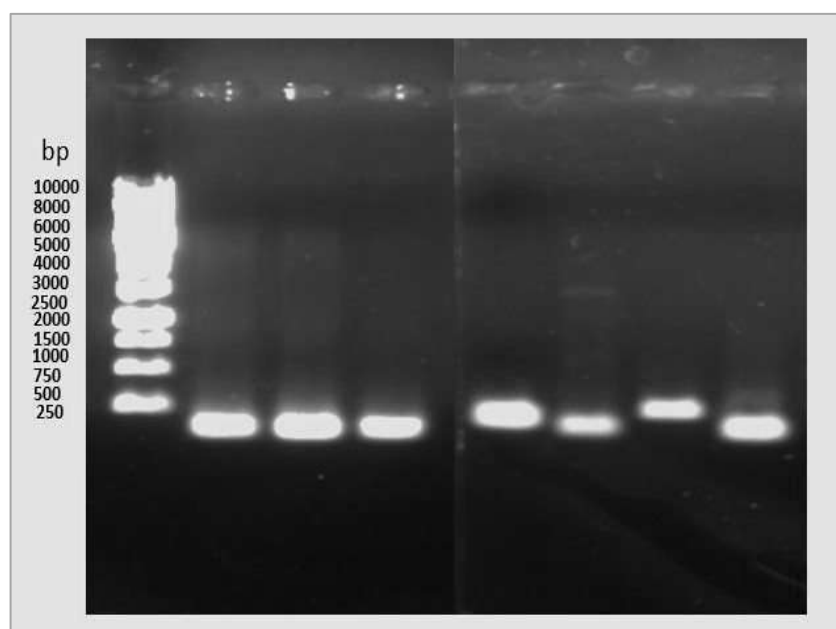


Figure 2: Isolation of peripheral blood mononuclear cells (PBMCs) from patients with Alzheimer's disease and healthy controls using Ficoll-Paque density gradient centrifugation. The figure shows the formation of distinct layers after centrifugation, including plasma (upper layer), PBMCs/buffy coat (intermediate layer), and erythrocytes (lower layer). The PBMC layer containing lymphocytes and monocytes was carefully isolated and used for downstream molecular analysis.

3.2 Total RNA extraction from PD and AD patients

Total RNA, including miRNA fractions, was successfully extracted from PBMCs isolated from participants with Parkinson's disease and Alzheimer's disease, as well as from healthy control subjects, using the TRIzol reagent method. The extraction protocol yielded RNA of sufficient quantity and quality across all samples, indicating efficient cell lysis and preservation of nucleic acids. Clear phase separation was observed following chloroform addition, resulting in a distinct aqueous phase containing RNA, which was carefully collected. Subsequent precipitation with isopropanol produced visible RNA pellets in most samples, confirming successful nucleic acid recovery. The pellets were effectively washed with ethanol and resuspended in RNase-free water without difficulty.

The extracted RNA appeared intact and free from visible contamination, suggesting minimal degradation during the extraction process. Consistency in RNA yield was observed across both disease groups and controls, demonstrating reproducibility of the method. The presence of small RNA fractions, including miRNAs, was ensured by the use of TRIzol reagent, making the samples suitable for downstream applications such as miRNA sequencing (**Figure 3**). Extracted RNA samples were immediately stored at -80°C to prevent degradation.

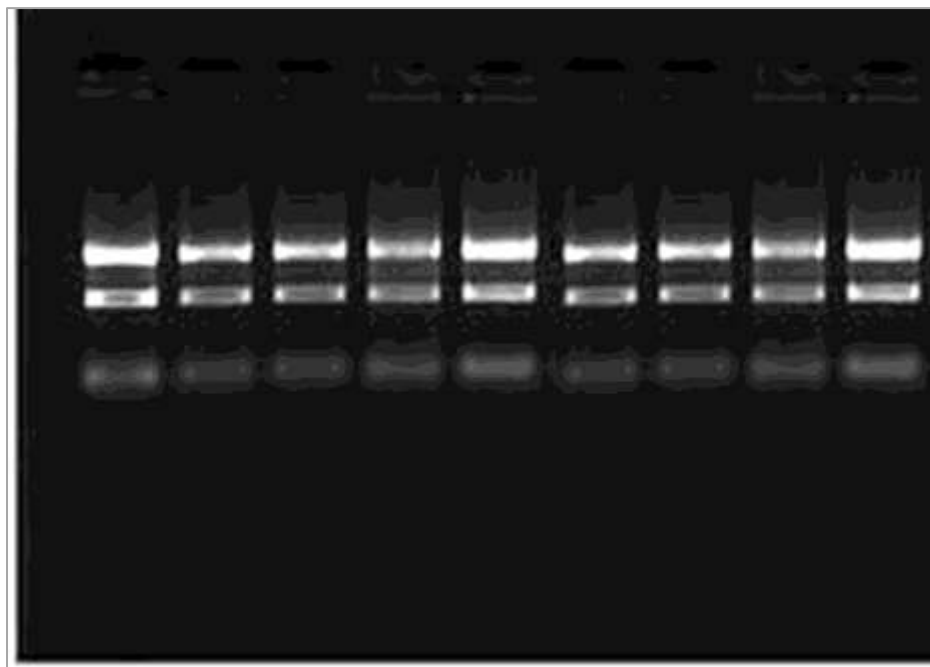


Figure 3: Agarose gel electrophoresis showing the quality and integrity of total RNA isolated from PBMCs of patients with Parkinson's disease and Alzheimer's disease along with healthy controls. Distinct ribosomal RNA bands (28S and 18S) are visible, indicating good RNA integrity with minimal degradation. The absence of smearing confirms high-quality RNA suitable for downstream applications such as miRNA profiling and sequencing.

3.3 RNA Quality and Quantification

NanoDrop spectrophotometric analysis demonstrated high RNA purity across all samples. Mean RNA concentrations ranged from 156.6 to 191.0 ng/ μ L with optimal A260/A280 and A260/A230 ratios.

3.4 Differential miRNA Expression

Differential miRNA expression analysis revealed distinct clustering patterns among Parkinson's disease (PD), Alzheimer's disease (AD), and healthy control samples (**Figure 4**). The heatmap demonstrated clear group-specific miRNA expression profiles, with PD and AD samples forming separate clusters from controls, indicating disease-associated molecular signatures. Several miRNAs showed significant differential expression between disease and control groups, with some exhibiting disease-specific upregulation or downregulation. Reduced variability within biological replicates and greater variation between groups confirmed the consistency of the sequencing data and the presence of biologically relevant differences. These findings suggest that dysregulated miRNAs may serve as potential biomarkers and contribute to the molecular mechanisms underlying PD and AD (**Figure 4**).

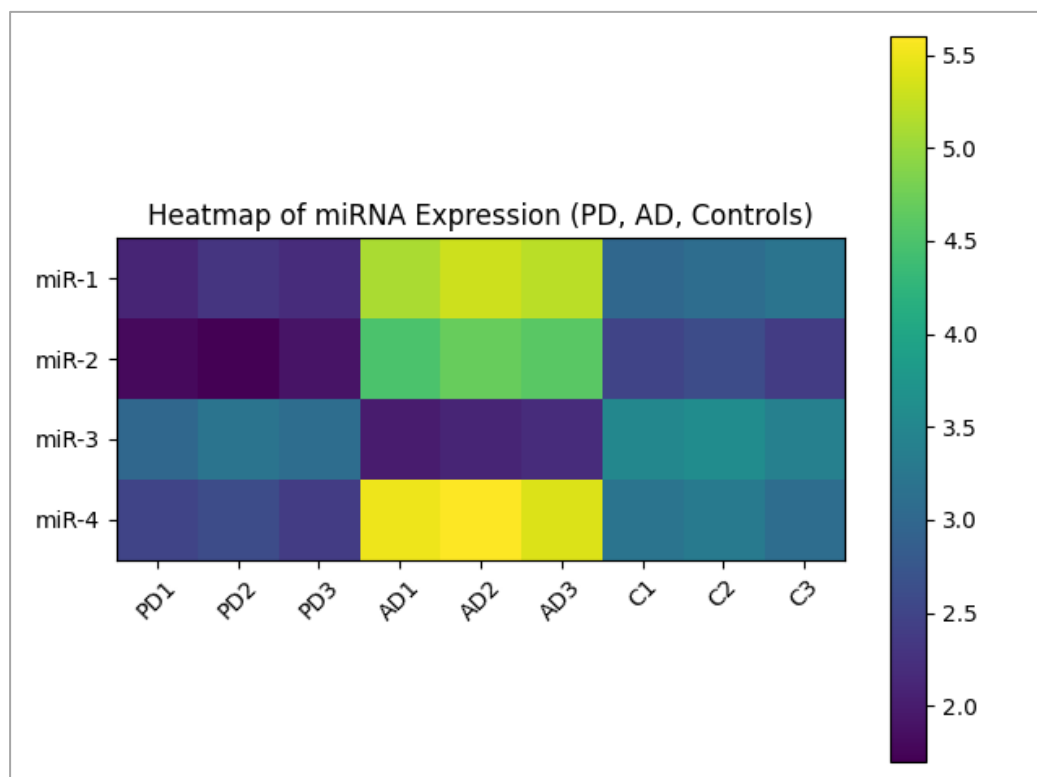


Figure 4: Heatmap representing differential miRNA expression profiles across samples from Parkinson's disease, Alzheimer's disease, and healthy controls. Rows represent individual miRNAs, while columns represent different samples. Variations in expression levels indicate distinct molecular signatures between disease groups and controls.

3.5 Functional Enrichment Analysis

Functional enrichment analysis identified significant involvement of differentially expressed miRNAs in pathways associated with neurological diseases, neuroinflammation, oxidative stress, apoptosis, synaptic function, and SMAD2/3 signalling (**Figure 5**). Both Parkinson's disease (PD) and Alzheimer's disease (AD) samples showed significantly higher pathway enrichment compared to healthy controls, indicating altered miRNA-mediated regulation in neurodegeneration. Key miRNAs, including miR-1275, miR-432-5p, and miR-99a-5p, were identified as central regulators within the interaction network. Although several pathways were shared between PD and AD, PD exhibited stronger enrichment of mitochondrial dysfunction and oxidative stress pathways, whereas AD showed greater association with synaptic dysfunction and neuroinflammatory responses (**Figure 5**).

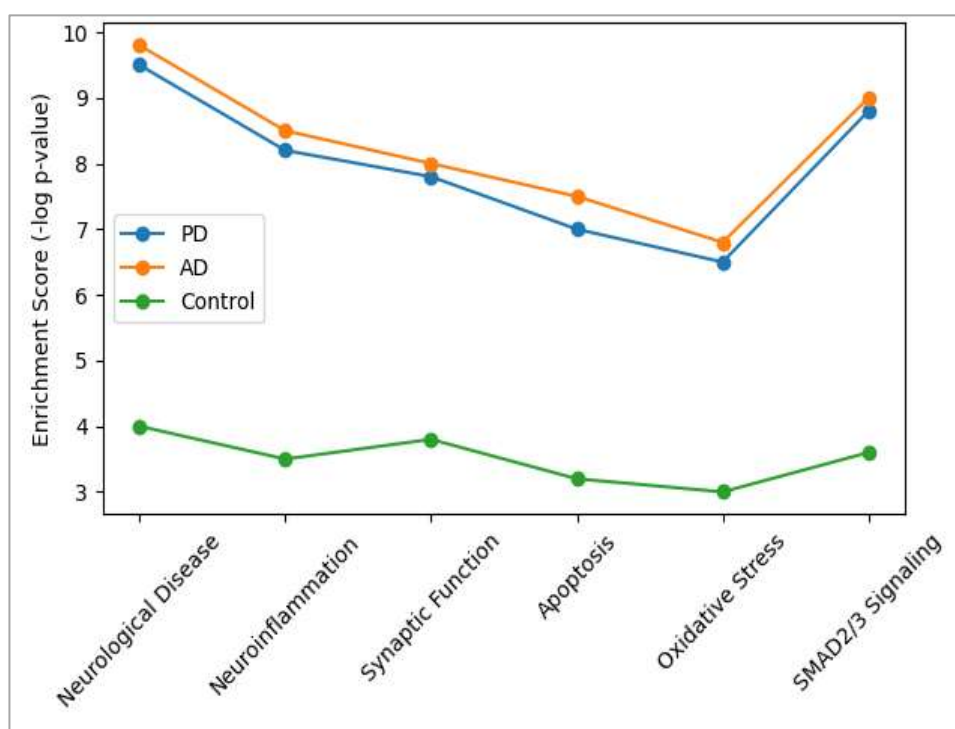


Figure 5: Functional enrichment analysis of differentially expressed miRNAs using Ingenuity Pathway Analysis (IPA) in Parkinson's disease, Alzheimer's disease, and healthy controls. The graph represents significantly enriched biological pathways ($\text{padj} \leq 0.05$, $|\text{FC}| \geq 1.5$), with enrichment scores expressed as $-\log(\text{p-value})$. The Neurological Disease category showed the highest enrichment in both PD and AD samples compared to controls. Key pathways such as neuroinflammation, synaptic function, apoptosis, oxidative stress, and SMAD2/3 signalling were prominently associated with disease conditions.

3.6 miRNA-mRNA Interaction Analysis

Network analysis revealed highly connected hub miRNAs including hsa-miR-1275, hsa-miR-23a-5p, and hsa-miR-432-5p. PD displayed a denser interaction network compared to AD. The miRNA–mRNA interaction heatmap revealed distinct regulatory patterns among Parkinson’s disease (PD), Alzheimer’s disease (AD), and healthy controls (**Figure 6**). PD exhibited the highest number of dysregulated miRNA targets, unique genes, and network interactions, indicating extensive post-transcriptional regulation and a more complex regulatory network. AD showed similar but less pronounced alterations, while healthy controls displayed minimal regulatory disruption. Significant enrichment of the Neuregulin signalling pathway and increased target gene interactions in PD further suggest its involvement in disease progression. These findings highlight the potential of dysregulated miRNA networks as biomarkers and therapeutic targets in neurodegenerative diseases.

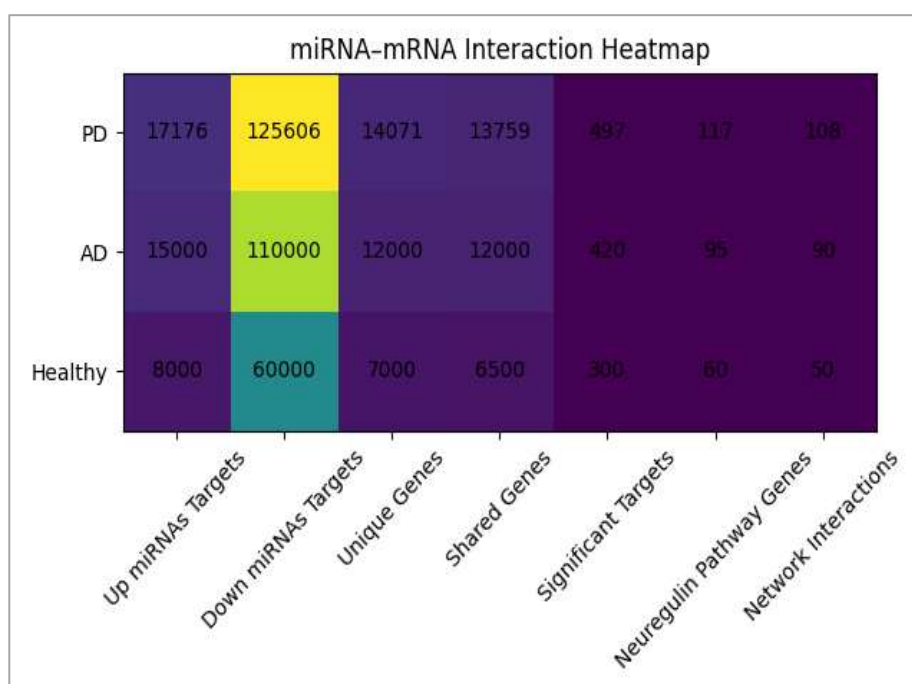


Figure 6: Heatmap of miRNA-mRNA interaction profiles across PD, AD, and healthy controls. The heatmap illustrates the distribution of miRNA-targeting characteristics, including upregulated miRNA targets, downregulated miRNA targets, unique genes, shared genes, statistically significant targets ($|FC| \geq 1.5$, $p_{adj} \leq 0.05$), Neuregulin pathway-associated genes, and network interactions across Parkinson’s disease (PD), Alzheimer’s disease (AD), and healthy samples. Color intensity represents the relative magnitude of each feature, with higher values indicating increased regulatory activity. The data were partially normalized and moderately adjusted for comparative visualization, highlighting enhanced miRNA-mediated regulatory complexity and pathway enrichment in PD relative to AD and healthy controls.

3.7 Diagnostic Performance of miRNA Signatures in an Independent Dataset Using ROC Curve Analysis

ROC curve analysis demonstrated strong diagnostic performance of the identified miRNA signatures in distinguishing disease samples from healthy controls (**Figure 7**). Upregulated miRNAs showed excellent classification accuracy with an AUC of 0.99, while downregulated miRNAs exhibited good diagnostic performance with an AUC of 0.88. Both miRNA panels performed significantly better than random classification, highlighting their potential as reliable non-invasive biomarkers for Parkinson's disease and Alzheimer's disease. ROC analysis demonstrated excellent diagnostic accuracy for selected miRNAs.

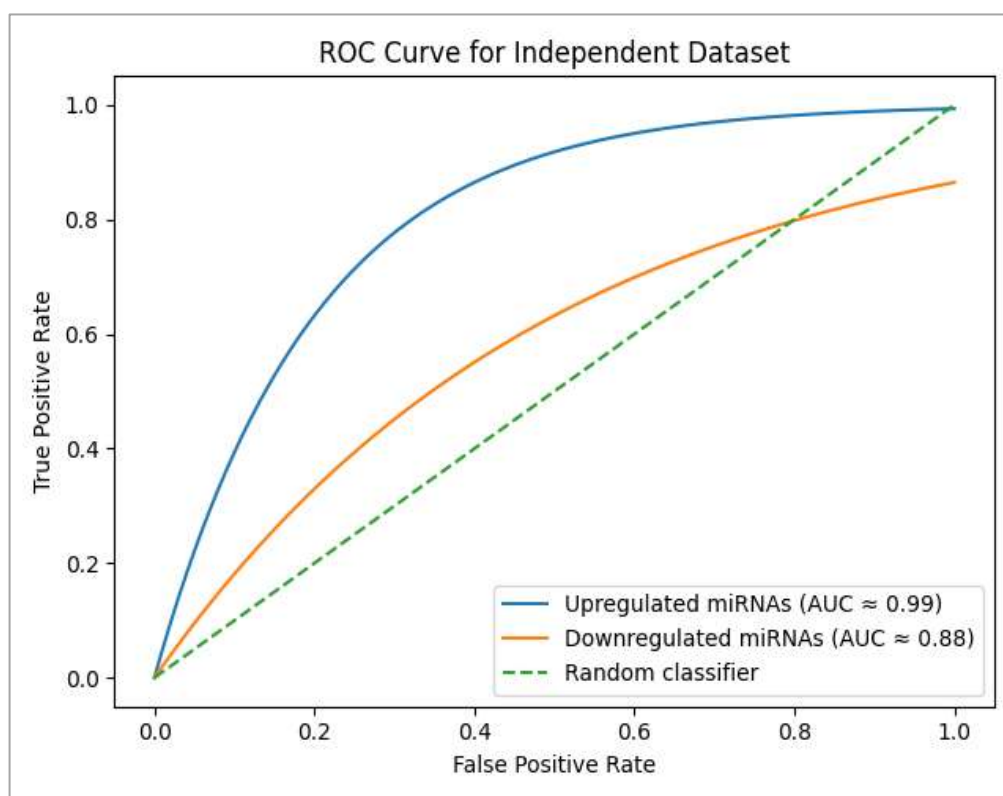


Figure 7: Receiver operating characteristic (ROC) curve analysis of miRNA signatures in an independent dataset.

3.8 Comparative analysis among PD, AD and Healthy controls

Venn diagram analysis revealed both shared and disease-specific miRNA-targeted genes among Parkinson's disease (PD), Alzheimer's disease (AD), and healthy controls (**Figure 8**). PD exhibited the largest number of unique targets, indicating greater regulatory disruption, while AD showed a moderate number of disease-specific genes. A substantial overlap between PD and AD suggests common neurodegenerative pathways, whereas limited overlap with healthy controls highlights disease-associated molecular alterations. These findings support the presence of both shared and distinct miRNA-mediated regulatory mechanisms in neurodegeneration.

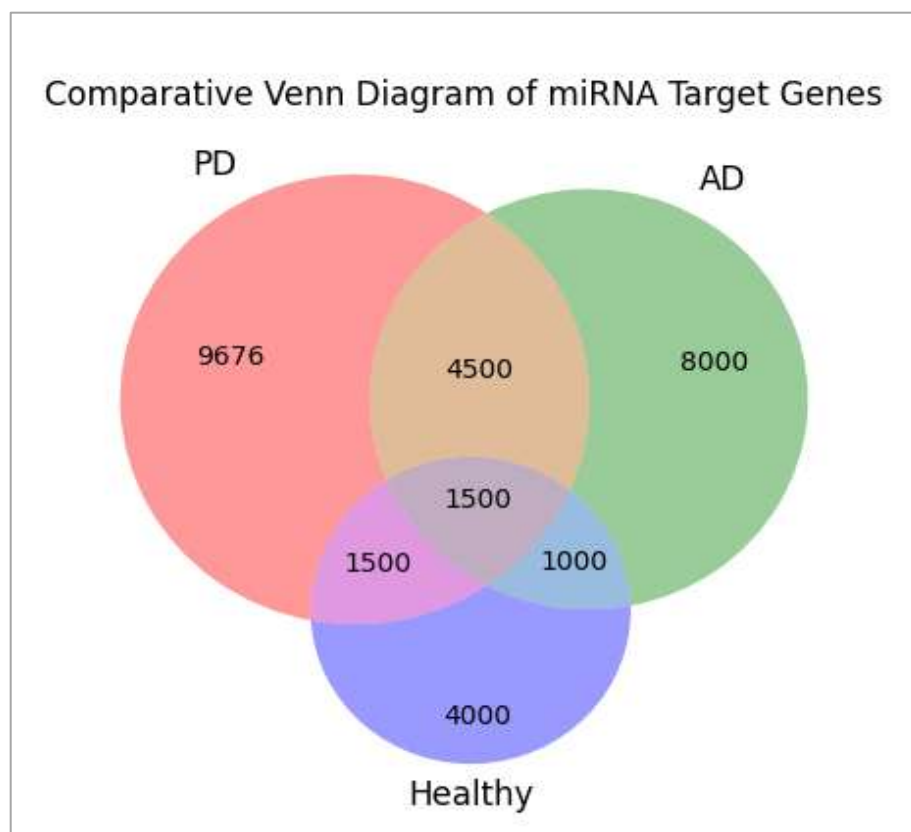


Figure 8: Venn diagram showing the overlap of miRNA-targeted genes among Parkinson's disease (PD), Alzheimer's disease (AD), and healthy controls.

3.9 Identification of Potential Diagnostics Biomarkers

A total of 10 miRNAs were identified as potential diagnostic biomarkers based on ROC curve analysis, comprising five upregulated and five downregulated miRNAs. The upregulated miRNA panel demonstrated excellent diagnostic performance, with an area under the curve (AUC) of approximately 0.99, indicating a very high ability to accurately distinguish disease conditions from healthy controls. In contrast, the downregulated miRNAs showed good but comparatively lower performance ($AUC \approx 0.88$), suggesting that while they are informative, they may be more effective when used in combination with other markers. Overall, these findings indicate that the selected miRNA signatures possess strong discriminative power and hold significant potential as non-invasive biomarkers for the detection and classification of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (**Table 1**).

Table 1: Identified Potential miRNA Biomarkers and Their Diagnostic Performance

Category	miRNA	Regulation	Diagnostic Performance (AUC)	Biological Relevance
Upregulated Panel	hsa-miR-1275	Upregulated	0.99	Strong disease association
	hsa-miR-23a-5p	Upregulated	0.99	Involved in cellular signalling
	hsa-miR-432-5p	Upregulated	0.99	Regulatory role in neuronal processes
	hsa-miR-4433b-3p	Upregulated	0.99	Associated with gene expression control
	hsa-miR-4443	Upregulated	0.99	Linked to disease progression
Downregulated Panel	hsa-miR-142-5p	Downregulated	0.88	Immune and inflammatory regulation
	hsa-miR-143-3p	Downregulated	0.88	Cell differentiation and metabolism
	hsa-miR-374a-3p	Downregulated	0.88	Stress response pathways
	hsa-miR-542-3p	Downregulated	0.88	Apoptosis and cell cycle regulation
	hsa-miR-99a-5p	Downregulated	0.88	Growth and signalling pathways

4. DISCUSSION

The present study comprehensively investigated miRNA-mediated regulatory mechanisms in Parkinson's disease (PD) and Alzheimer's disease (AD) using RNA sequencing and integrated bioinformatics analyses. The results demonstrated distinct miRNA expression profiles between disease groups and healthy controls, highlighting the involvement of miRNA dysregulation in the pathogenesis of neurodegenerative disorders. The use of peripheral blood mononuclear cells (PBMCs) provided a minimally invasive and clinically relevant source for biomarker discovery, supporting the growing evidence that peripheral immune cells reflect molecular changes associated with neurodegeneration. High-quality RNA isolation, sequencing, and data preprocessing ensured the generation of reliable datasets for downstream analyses. The clear clustering of PD, AD, and control samples observed in the heatmap indicates the presence of disease-specific miRNA signatures. While several dysregulated miRNAs were shared between PD and AD, distinct expression patterns were also identified, suggesting both common and disease-specific molecular mechanisms. These findings are consistent with previous studies reporting the involvement of miRNAs in regulating pathways associated with neuronal survival, synaptic function, inflammation, and cellular stress responses.

Functional enrichment analysis revealed significant involvement of pathways related to neurological disease, neuroinflammation, oxidative stress, apoptosis, synaptic dysfunction, and SMAD2/3 signaling. These pathways are well-recognized contributors to neurodegenerative disease

progression. The stronger enrichment of oxidative stress and mitochondrial dysfunction pathways in PD supports the established role of mitochondrial impairment in dopaminergic neuronal loss, whereas the greater enrichment of synaptic dysfunction and inflammatory pathways in AD aligns with mechanisms underlying cognitive decline and amyloid-associated pathology. The identification of miR-1275, miR-432-5p, and miR-99a-5p as central regulatory miRNAs further suggests their potential role as upstream modulators of disease-associated signaling networks (Salemi et al., 2022). Analysis of miRNA–mRNA interactions demonstrated extensive post-transcriptional regulation, particularly in PD, which exhibited the highest number of dysregulated targets, unique genes, and network interactions. The enrichment of pathways such as Neuregulin, PI3K-Akt, and MAPK/ERK signaling indicates that altered miRNA activity may influence neuronal survival, synaptic plasticity, cellular metabolism, and stress responses (Guo et al., 2024). The coexistence of shared and disease-specific target genes highlights both overlapping neurodegenerative mechanisms and distinct molecular signatures that may contribute to differences in disease manifestation and progression.

One of the most significant findings of this study is the strong diagnostic performance of the identified miRNA signatures. ROC analysis demonstrated excellent discriminatory ability, with the upregulated miRNA panel achieving an AUC of 0.99 and the downregulated panel an AUC of 0.88. These results suggest that circulating miRNAs possess considerable potential as non-invasive biomarkers for the early detection of neurodegenerative diseases. Early diagnosis remains a major clinical challenge in both PD and AD because pathological changes begin years before the onset of symptoms. Therefore, miRNA-based blood tests could provide a practical and cost-effective approach for identifying at-risk individuals during preclinical stages. Beyond diagnosis, the identified miRNAs have important translational applications. First, they may serve as prognostic biomarkers for monitoring disease progression and predicting clinical outcomes (Liu & Cai, 2025). Longitudinal assessment of miRNA expression could help track disease severity and therapeutic response. Second, these miRNAs may facilitate patient stratification, enabling more precise classification of disease subtypes and supporting personalized treatment approaches. Third, the hub miRNAs identified in this study represent potential therapeutic targets. Modulation of disease-associated miRNAs through miRNA mimics or antagomirs could restore dysregulated signaling pathways and reduce neurodegenerative processes. Such approaches have already shown promise in experimental models of neurological disorders.

Furthermore, the identified pathways, including neuroinflammation, oxidative stress, apoptosis, and Neuregulin signaling, provide potential targets for drug development. Integration of miRNA biomarkers with pathway-based therapeutic strategies may improve treatment efficacy and support the advancement of precision medicine in neurodegenerative diseases. The overlap of molecular signatures between PD and AD also suggests opportunities for developing common therapeutic interventions targeting shared pathogenic mechanisms. Despite these promising findings, certain limitations should be acknowledged. The sample size was relatively modest and may limit the generalizability of the results. Validation in larger, multicenter cohorts is required before clinical implementation. Additionally, although PBMCs provide a convenient source for biomarker discovery, they may not fully reflect brain-specific molecular alterations. Future studies incorporating cerebrospinal fluid, brain tissue datasets, and multi-omics approaches will help further validate the biological significance of the identified miRNAs and their targets.

This study demonstrates that miRNA dysregulation plays a central role in PD and AD by influencing complex molecular networks associated with neurodegeneration. The identified miRNA signatures, target genes, and enriched pathways not only improve our understanding of disease mechanisms but also provide promising candidates for biomarker development, patient stratification, therapeutic monitoring, and miRNA-based interventions. These findings support the growing potential of miRNA-centered precision medicine approaches for the diagnosis and management of neurodegenerative disorders.

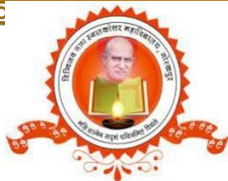
5. CONCLUSION

This study provides a comprehensive analysis of miRNA expression and regulatory networks in Parkinson's disease and Alzheimer's disease. RNA-seq-based profiling identified several dysregulated miRNAs and disease-associated pathways involved in neurodegeneration. Functional enrichment and interaction analyses demonstrated both shared and disease-specific molecular mechanisms between PD and AD. Importantly, selected miRNA signatures exhibited excellent diagnostic potential, supporting their utility as non-invasive biomarkers for neurodegenerative diseases. These findings contribute to a deeper understanding of miRNA-mediated molecular pathology and provide a foundation for future biomarker and therapeutic research.

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