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RESEARCH ARTICLE
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EXPLORING IMMUNE-RELATED HUB GENES IN PARKINSON'S DISEASE USING INTEGRATIVE BIOINFORMATICS APPROACH

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor dysfunction and the loss of dopaminergic neurons. Emerging evidence suggests that immune system dysregulation and neuroinflammation play key roles in PD pathogenesis. In this study, we aimed to explore immune-related hub genes and associated signaling pathways using bioinformatics analysis of the GSE20141 dataset. We identified 250 differentially expressed genes (DEGs) and constructed a protein-protein interaction (PPI) network to pinpoint key hub genes. Four hub genes, FGF17**,** MED26**,** LCK**,** and RPS12, were identified as central players in the network, and functional enrichment analysis revealed significant involvement of several immune-related pathways.Key pathways enriched in our analysis included the JAK-STAT signaling pathway**,** NF-kappa B signaling pathway**,** and T cell receptor signaling pathway, all of which are known to regulate immune responses and inflammation in neurodegenerative diseases. Interestingly, our study also identified pathways less commonly associated with PD, such as the adipocytokine signaling pathway and osteoclast differentiation, suggesting a potential link between metabolic dysregulation and neuroinflammation. Our findings highlight both established and novel immune mechanisms in PD, suggesting potential therapeutic targets aimed at modulating immune responses. This study provides valuable insights into the complex immune landscape of PD and emphasizes the importance of immune modulation in understanding and treating this debilitating disease. Further research is needed to validate the role of the identified hub genes and pathways and to explore their potential as targets for PD therapy.

Keywords: PD; GSE20141; DEGs; Hub genes

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder primarily characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra, leading to motor symptoms such as tremor, bradykinesia, rigidity, and postural instability (1, 2). In addition to these hallmark motor symptoms, PD is also associated with a wide range of non-motor symptoms, including cognitive impairment, autonomic dysfunction, mood disorders, and sleep disturbances, which significantly impact the quality of life of patients (3). The precise etiology of PD is multifactorial, involving a complex interplay of genetic, environmental, and cellular factors (4). The underlying causes of PD are not fully understood, but several contributing factors have been identified. Genetic mutations account for 5-10% of PD cases, with mutations in genes such as SNCA, LRRK2, PINK1, PARK7 (DJ-1), and GBA being implicated in familial forms of the disease (5). However, the majority of PD cases are sporadic, where no single genetic factor can be pinpointed. In sporadic PD, environmental risk factors such as exposure to pesticides, heavy metals, and other toxins, as well as lifestyle factors like head injury and aging, are believed to contribute to the development of the disease (6).

At the cellular level, PD is characterized by the accumulation of misfolded alpha-synuclein protein, which aggregates into Lewy bodies, leading to neuronal toxicity and death (7). Mitochondrial dysfunction, oxidative stress, and impaired protein clearance pathways, including autophagy and the ubiquitin-proteasome system, have also been implicated in PD pathology (8). These cellular dysfunctions disrupt normal neuronal activity, ultimately causing neurodegeneration.

In addition to genetic and environmental factors, growing evidence suggests that neuroinflammation plays a pivotal role in PD pathogenesis (9). Chronic activation of the immune system, both centrally within the brain and peripherally in the systemic circulation, has been observed in PD patients (10). Microglial activation, increased levels of pro-inflammatory cytokines, and T-cell infiltration into the brain are key features of PD-related neuroinflammation (11). This persistent inflammatory response can exacerbate neurodegeneration, creating a vicious cycle of immune activation and neuronal loss (11).

With advancements in high-throughput genomic technologies, it has become possible to identify key molecular players involved in PD by analyzing large-scale transcriptomic datasets (12). The Gene Expression Omnibus (GEO) database provides a valuable repository of gene expression data, allowing for in-depth exploration of differentially expressed genes (DEGs) in neurodegenerative diseases like PD. By analyzing these datasets, researchers can identify hub genes and pathways that may be critical in PD pathology, particularly those involved in immune responses.

In this study, we aimed to identify immune-related hub genes associated with PD using GEO datasets. By analyzing differentially expressed genes between PD patients and healthy controls, we focused on immune-related genes and their potential roles in PD pathogenesis. Our findings provide new insights into the molecular mechanisms linking immune dysregulation and neurodegeneration, which may inform the development of immunomodulatory therapies for PD.

Methodology

Data Acquisition and Preprocessing

In this study, we utilized the publicly available GEO dataset GSE20141, which contains transcriptomic data from PD patients and healthy controls. This dataset was selected based on its relevance to neurodegenerative disease research and its inclusion of both PD and control samples. The dataset was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) (13), and the raw expression data were processed for further analysis.

The GSE20141 dataset was first normalized using the Robust Multi-array Average (RMA) method to minimize technical variability and ensure comparability across samples. Probes corresponding to multiple genes were collapsed to the median value for each gene, and probes without corresponding gene annotations were excluded. After preprocessing, the dataset was log-transformed to stabilize variance across genes.

Differential Gene Expression Analysis

To identify DEGs between PD patients and healthy controls, we applied the limma (linear models for microarray data) package in R. The dataset was divided into two groups: PD samples and control samples. A model was fitted to the expression data to estimate fold changes in gene expression between the two groups. The statistical significance of the differences was assessed using moderated t-tests, and the resulting p-values were adjusted for multiple comparisons using the Benjamini-Hochberg method to control the false discovery rate (FDR).

Genes were considered differentially expressed if they met the following criteria

1. |log2 fold change (logFC)| > 1.0
2. Adjusted p-value < 0.05

These thresholds were selected to identify genes that showed both biologically meaningful changes in expression and statistical significance.

Functional Enrichment Analysis

After identifying the DEGs, we performed functional enrichment analysis to determine the biological pathways and processes associated with these genes. A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses was conducted using the clusterProfiler package in R. Significantly enriched KEGG pathways were identified using a hypergeometric test, and results with an adjusted p-value < 0.05 were considered statistically significant.

Identification of Immune-Related Hub Genes

To identify immune-related hub genes among the DEGs, we used a network-based approach. First, the DEGs were cross-referenced with a curated list of immune-related genes obtained from the ImmPort database (https://www.immport.org/). Genes that overlapped between the DEGs and immune-related genes were selected for further analysis.

We then constructed a protein-protein interaction (PPI) network using the STRING database (https://string-db.org/), which provides known and predicted interactions based on experimental evidence, co-expression, and computational predictions. The PPI network was visualized and analyzed using Cytoscape software (v3.8.2). Hub genes were identified using the cytoHubba plugin in Cytoscape, which ranks genes based on centrality measures such as degree, betweenness, and closeness. The top-ranked immune-related hub genes were prioritized for further analysis.

Statistical Analysis

All statistical analyses were conducted using R software (v4.1.0). Differential expression analysis was performed using the limma package, while functional enrichment and PPI network analyses were carried out using clusterProfiler, STRING, and Cytoscape. P-values were adjusted for multiple testing using the Benjamini-Hochberg method, and a threshold of adjusted p-value < 0.05 was considered statistically significant for all analyses.

Results

Dataset Analysis and Identification of DEGs and Hub Genes

The GSE20141 dataset was utilized in the study to explore DEGs and hub genes in PD patients (Figure 1). Figure 2 presents the PPI networks generated from DEGs identified in the GSE20141 dataset. In both panels A and B, the pink-colored nodes represent the 250 DEGs that were identified through differential expression analysis, while the yellow-colored nodes highlight the four hub genes identified as central players within the network. In Figure 2A, the PPI network shows the interconnections between the DEGs, with a wide distribution of genes involved in various pathways. The presence of numerous connections in the network suggests that these DEGs are part of complex molecular interactions that may contribute to PD pathogenesis. These interactions involve genes associated with immune responses, signaling pathways, and cellular functions, reflecting the multi-faceted nature of neurodegenerative processes in PD. In Figure 2 B, the yellow nodes—FGF17**,** MED26**,** LCK**,** and RPS12**—**represent the identified hub genes within the PPI network. Hub genes

are typically highly connected and are thought to play central roles in the regulation of biological processes. The identification of these hub genes suggests their potential importance in the progression of PD, especially in terms of immune regulation and neuroinflammation, given their prominent connections to other DEGs in the network.

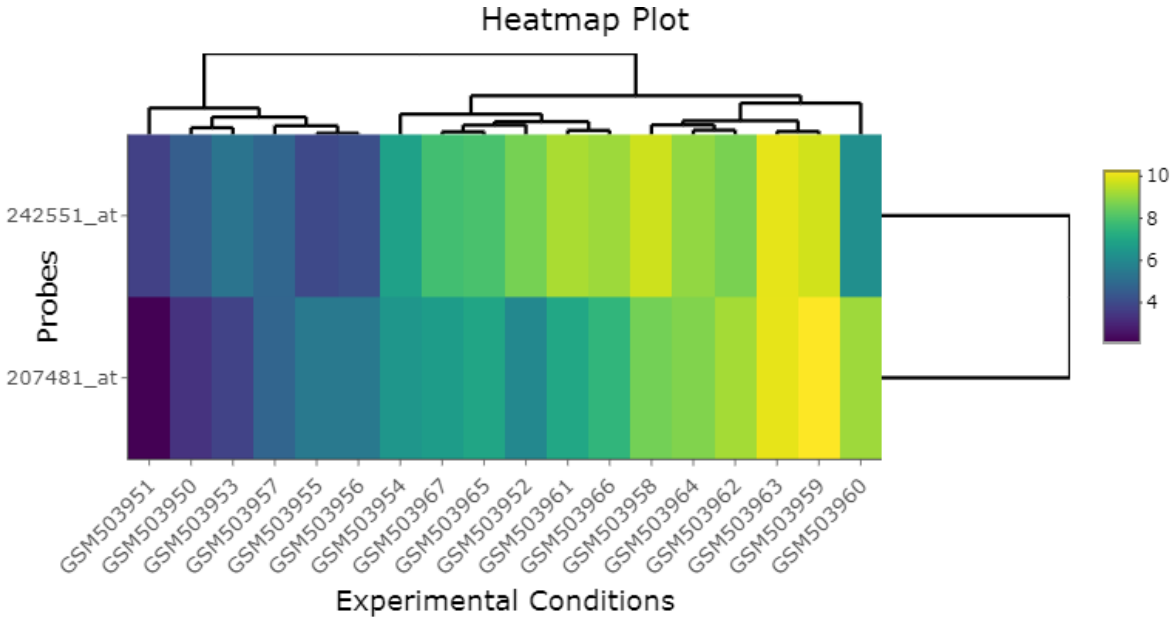

Figure 1: Overall expression profile of PD and normal samples in GSE20141 dataset.

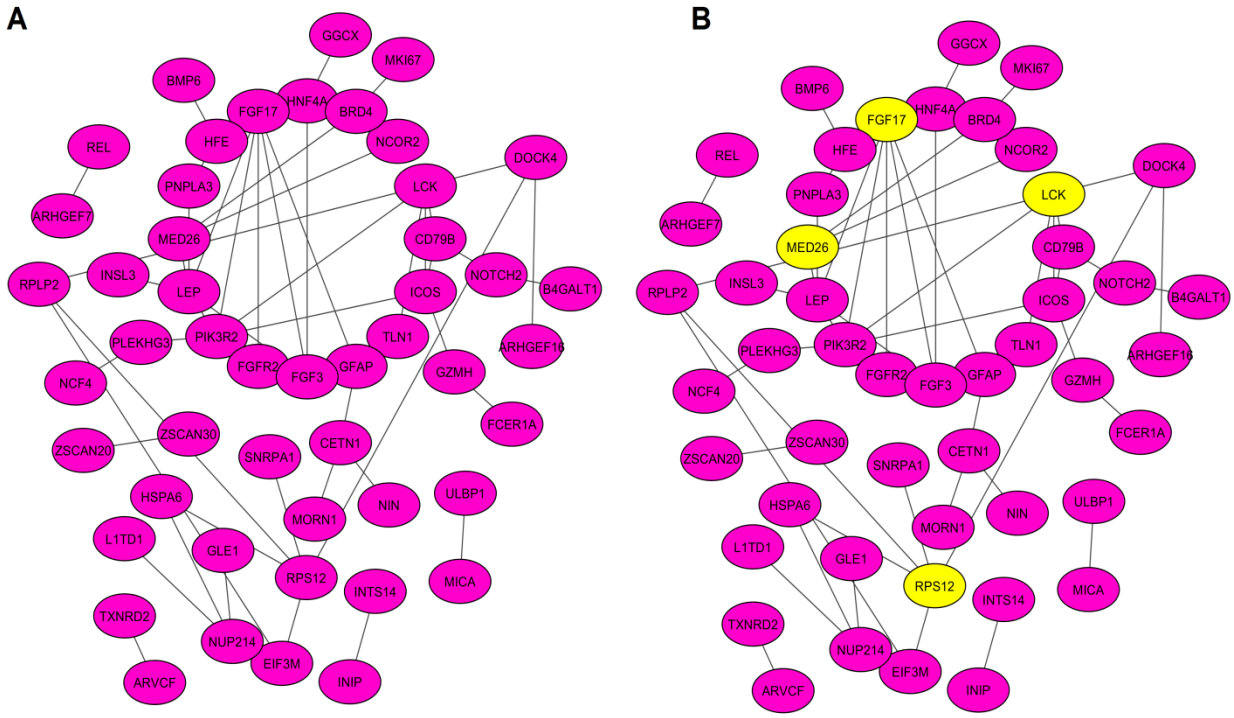

Figure 2: Identified DEGs and Hub genes GSE20141 dataset. (A) DEGs. (B) Hub genes within DEGs.

Hub Genes-Associated Important Pathways

Figure 3 and 4 highlights the enriched pathways related to the identified hub genes in the study. The x-axis represents the fold enrichment, which indicates the overrepresentation of genes from each pathway in the dataset compared to what would be expected by chance. The y-axis lists the pathways that are significantly enriched, with the colors representing the significance of enrichment (adjusted

p-value or FDR). Warmer colors (yellow to red) represent higher statistical significance based on the -log10(FDR) values.

Pathway Interpretation

Adipocytokine Signaling Pathway: This pathway is associated with the release of signaling molecules (adipokines) from adipose tissue, which can modulate immune responses, metabolism, and inflammation (14). In the context of neurodegenerative diseases like Parkinson's, dysregulation of adipokines may contribute to systemic inflammation, impacting disease progression.

Melanoma: Although this pathway is classically associated with cancer, several components of cancer-related signaling pathways overlap with immune signaling (15). The connection to melanoma in this case could indicate altered cellular proliferation or immune dysregulation in Parkinson's disease, reflecting shared pathways between cancer and neurodegeneration.

JAK-STAT Signaling Pathway: This is a critical pathway for immune cell activation, differentiation, and proliferation (16). The JAK-STAT pathway is particularly significant in regulating inflammatory responses. Its enrichment suggests that immune responses regulated by cytokines through JAK-STAT signaling may play a vital role in the pathogenesis of Parkinson's disease, especially in neuroinflammation and immune activation in the brain.

Th1 and Th2 Cell Differentiation: Th1 and Th2 cells are subsets of T-helper cells that mediate different immune responses (17). Th1 cells are involved in pro-inflammatory responses, while Th2 cells are associated with anti-inflammatory responses. The enrichment of this pathway suggests an imbalance between Th1 and Th2 cells, contributing to neuroinflammatory mechanisms in PD.

T Cell Receptor Signaling Pathway: T cell receptor (TCR) signaling is essential for T cell activation and function (17). The involvement of this pathway indicates that T cells, which are known to infiltrate the central nervous system in neurodegenerative diseases, may play a crucial role in PD's immune-mediated neurodegeneration.

NF-kappa B Signaling Pathway: NF-kappa B is a major transcription factor regulating immune and inflammatory responses. Its activation leads to the expression of cytokines, chemokines, and adhesion molecules (17). Dysregulation of NF-kappa B signaling can result in chronic inflammation, which has been implicated in the progression of PD.

Th17 Cell Differentiation: Th17 cells are another subset of T-helper cells that produce pro-inflammatory cytokines, particularly IL-17. Th17-mediated responses are associated with autoimmunity and chronic inflammation (17). The enrichment of this pathway may suggest a role for Th17 cells in the autoimmune or inflammatory aspects of PD.

AMPK Signaling Pathway: AMP-activated protein kinase (AMPK) is a key regulator of cellular energy homeostasis (18). In neurodegeneration, AMPK can influence autophagy and mitochondrial function. The enrichment of this pathway may indicate metabolic dysfunctions or altered energy balance in neuronal cells contributing to PD pathogenesis.

Osteoclast Differentiation: Osteoclasts are involved in bone resorption, but this pathway may reflect immune interactions since osteoclast differentiation shares regulatory molecules with immune responses (18). This could imply a broader dysregulation in signaling pathways beyond classical immune functions, impacting cellular health in PD.

Natural Killer Cell Mediated Cytotoxicity: Natural killer (NK) cells are part of the innate immune system and are involved in recognizing and destroying infected or damaged cells (18). The involvement of NK cell cytotoxicity may indicate their role in targeting stressed or damaged neurons, contributing to neurodegeneration in PD.

Ribosome: The ribosomal pathway indicates that translational control may be altered in PD, which could affect protein synthesis and lead to the accumulation of misfolded proteins, a hallmark of neurodegenerative diseases.

Yersinia Infection: Although typically associated with bacterial infection, this pathway's enrichment could reflect immune dysregulation linked to pathogen recognition, suggesting that molecular mimicry or immune responses to infections could be relevant in PD's progression.

Breast Cancer and Gastric Cancer: These pathways could indicate shared molecular mechanisms between neurodegeneration and cancer, particularly those related to cellular signaling, apoptosis, and immune evasion, which may also play roles in PD pathophysiology.

Non-alcoholic Fatty Liver Disease (NAFLD): NAFLD has been associated with metabolic dysregulation and systemic inflammation. The connection to this pathway could suggest that metabolic disturbances and inflammatory responses in PD may parallel those observed in conditions like NAFLD.

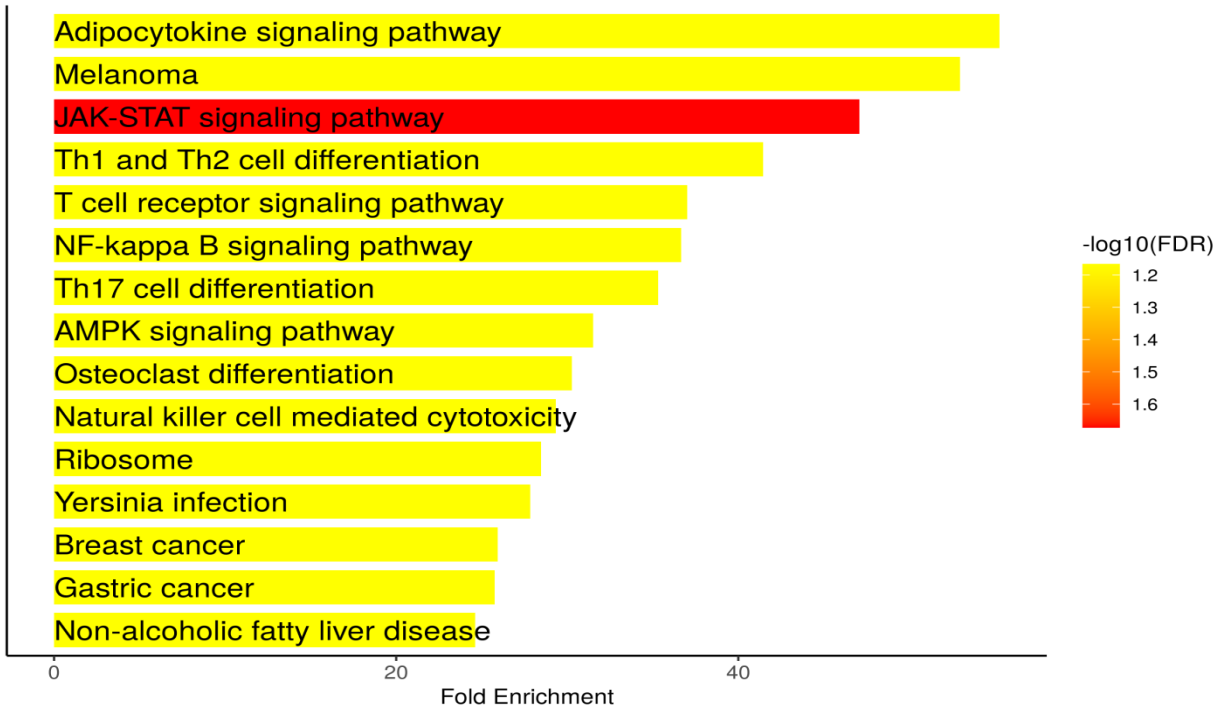

Figure 3: Hub genes-associated important signaling pathways.

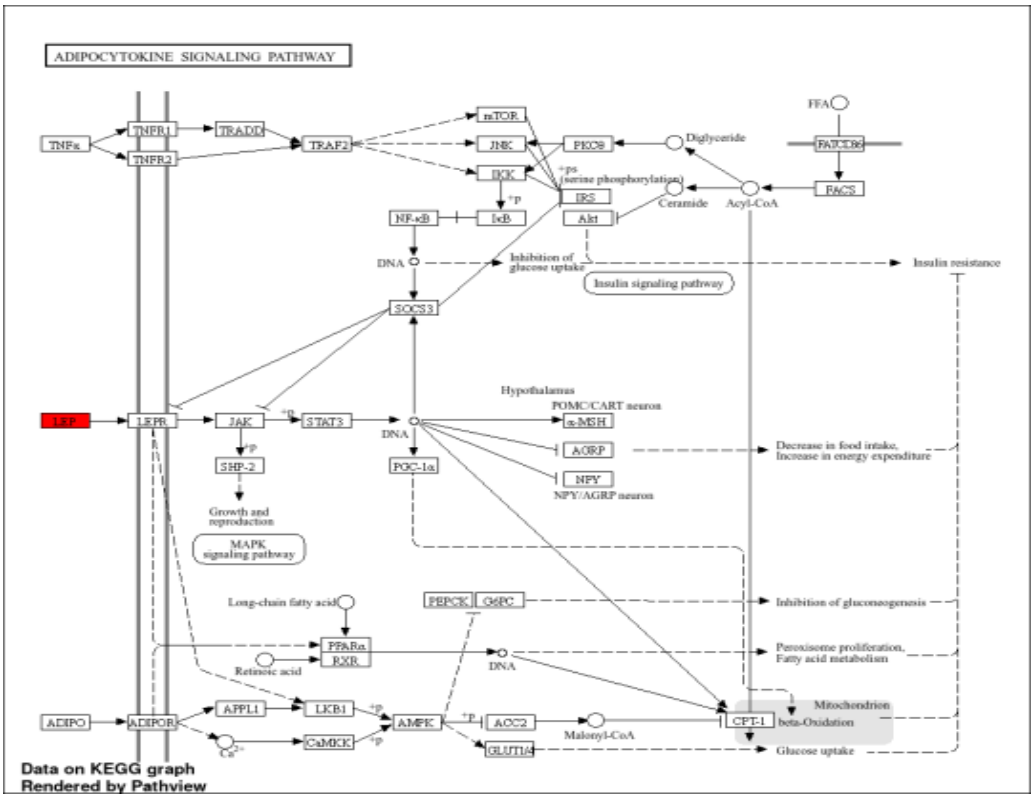

Figure 4: Hub genes-associated most significant pathways

Discussion

PD is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, resulting in motor dysfunction, including tremors, rigidity, and bradykinesia (2). Over recent decades, significant research has revealed that the pathophysiology of PD is not limited to dopaminergic neuronal loss, but involves a multifactorial process, including genetic, environmental, and immune-mediated factors. Increasing evidence highlights the critical role of neuroinflammation and immune system dysregulation in the development and progression of PD (2). In this study, we explored the immune-related hub genes in PD using the GSE20141 dataset, identifying key DEGs and enriched signaling pathways. Our findings provide novel insights into the immune mechanisms involved in PD and suggest potential therapeutic targets.

Several prior studies have explored the molecular underpinnings of PD, with many emphasizing the role of immune responses. Our findings are consistent with earlier reports that highlight the involvement of immune-related pathways in PD pathogenesis, particularly the JAK-STAT signaling pathway, T cell receptor signaling, and NF-kappa B signaling (19). These pathways have been implicated in neuroinflammation and microglial activation in PD, which are considered to be key drivers of neuronal death in the disease. A study reported that NF-kappa B signaling plays a pivotal role in the inflammatory response in PD, driving the production of pro-inflammatory cytokines that exacerbate neurodegeneration (20). Our findings align with this view, as the NF-kappa B signaling pathway was significantly enriched in our analysis, underscoring its continued relevance in PD research.

In addition to confirming these known pathways, our study also identified less explored pathways, such as the adipocytokine signaling pathway and natural killer (NK) cell-mediated cytotoxicity, as being associated with PD. The involvement of the adipocytokine signaling pathway in PD suggests that metabolic dysfunction may play a larger role in the disease than previously recognized. While metabolic disturbances have been implicated in other neurodegenerative diseases, the direct link between adipocytokines and PD is relatively novel. Recent study has suggested that metabolic and inflammatory dysfunctions may coalesce to exacerbate neurodegenerative processes (21). Our study supports this by showing the enrichment of pathways that regulate metabolic processes, such as the AMPK signaling pathway, highlighting the potential interplay between energy dysregulation and immune activation in PD.

Interestingly, our study also identified hub genes, including FGF17, MED26, LCK, and RPS12, which were found to be central players in the protein-protein interaction network. These hub genes have not been widely studied in the context of PD, suggesting new directions for future research. For example, FGF17 (Fibroblast Growth Factor 17) has been associated with neurodevelopment and neuronal survival but has not been extensively explored in neurodegeneration. Our identification of FGF17 as a hub gene suggests that it may have a critical role in modulating immune responses and neuroprotection in PD. This is supported by earlier research showing that members of the fibroblast growth factor family are involved in brain development and repair mechanisms (21).

In contrast to earlier studies that focused predominantly on T cells and microglial activation, our results also underscore the importance of natural killer (NK) cells in PD. The natural killer cell-mediated cytotoxicity pathway was enriched in our analysis, pointing to the potential role of NK cells in PD. While NK cells are known to play a role in the innate immune system, their involvement in PD has been relatively underexplored. Recent work suggests that NK cells may contribute to the clearance of damaged neurons or participate in neuroinflammation in PD (22). Our findings add further evidence to this hypothesis and warrant additional studies to clarify the specific role of NK cells in neurodegeneration.

Conclusion

In conclusion, our study provides a comprehensive analysis of immune-related hub genes and pathways involved in PD, with several novel insights. The identification of key immune signaling pathways, such as JAK-STAT, NF-kappa B, and T cell receptor signaling, confirms their critical roles in neuroinflammation and PD pathogenesis. Our discovery of less commonly associated pathways,

such as adipocytokine signaling and osteoclast differentiation, offers new avenues for future research. Additionally, the identification of novel hub genes like FGF17, MED26, LCK, and RPS12 highlights potential therapeutic targets. Further investigation into these pathways and genes may yield important insights into PD's pathogenesis and lead to the development of novel therapeutic strategies aimed at modulating immune responses in PD.

Conflict of Interest

None

Acknowledgement

None

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