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Inferring microarray datasets reveals critical biomarkers and potential drug targets of Parkinson's disease

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Abstract

Parkinson's disease (PD) is a critical neurological disorder characterized by loss of voluntary motor control and substantial slowing of movement. While traditionally attributed to environmental factors, recent studies underscore the significant role of genetics in the onset and progression of PD. This study aimed to identify differentially expressed genes (DEGs) and relevant pathways in PD by analyzing gene expression data from four datasets (83 PD and 53 control substantia nigra samples) sourced from the Gene Expression Omnibus (GEO) database. Using GEO2R, we identified common DEGs and performed functional annotation and KEGG pathway enrichment analysis through Enrichr. We constructed a protein–protein interaction (PPI) network using StringDB and identified hub genes via CytoHubba. Results revealed 18 critical DEGs enriched in pathways such as dopaminergic synapse and cocaine addiction. Key hub genes included Tyrosine Hydroxylase (TH), Solute Carrier Family 18 Member A2 (SLC18A2), and Potassium Inwardly Rectifying Channel Subfamily J Member 6 (KCNJ6). These findings provide insights into the molecular mechanisms of PD, highlighting potential biomarkers and therapeutic targets. This study offers a robust framework for future research and the development of effective treatment strategies for Parkinson's disease.

Keywords: Microarray, Parkinson's disease, enrichment analysis, PPI network, KEGG pathway.

INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disorder that causes impairment of voluntary motor control and extensive slowness of movements (bradykinesia) often including tremor, muscle rigidity, and posture abnormalities.[1] Parkinson's disease is thought to afflict 1 to 2 persons per 1000 persons, but the frequency grows with age. Across the globe, 1% people over the age of 60 years are affected.[2] The fundamental pathophysiology of Parkinson's disease is the loss of dopaminergic neurons (DA, or tyrosine hydroxylase positive, TH+) in the substantia nigra (SN) pars compacta, as well as the appearance of Lewy bodies and Lewy neurites formed by the accumulation of α-synuclein (SNCA) in afflicted areas of brain.[3] For years, environmental variables such as exposure to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine) and paraquat (N, N′-dimethyl-4,4′-bipyridinium dichloride) were believed to be the origin of Parkinson's disease[4], but with the advancements in technology, genetic factors

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are also investigated for novel biomarkers for diagnostics and therapeutic interventions. Due to the limited knowledge about the disease's etiology and pathophysiology it is not yet possible to cure Parkinson's disease.[5] It is therefore essential to investigate the biochemical processes of Parkinson's disease to identify its causative factors and biomarkers.

Several studies have contributed significantly to the understanding of PD biomarkers. For instance, the LRRK2 gene, mutations of which are a common cause of familial PD, has been extensively studied.[6] DJ-1, another gene, is associated with both familial and sporadic PD, and its loss of function leads to increased oxidative stress and neurodegeneration.[7] The α-synuclein gene (SNCA) has been implicated in both familial and sporadic PD, with its mutations leading to protein aggregation and Lewy body formation.[8] Biomarkers such as cerebrospinal fluid (CSF) levels of α-synuclein, DJ-1, and tau proteins are being evaluated for their potential to diagnose and monitor PD progression.[9] By focusing on early-stage PD and integrating data from these diverse studies, we aim to provide a comprehensive understanding of the molecular mechanisms underlying PD and identify biomarkers that could be crucial for early diagnosis and therapeutic intervention.

Many epidemiological studies support the fact that PD is influenced by complex interactions between genetic and environmental factors. For instance, exposure to pesticides and a history of head trauma have been associated with an increased risk of PD[10], whereas caffeine and cigarette smoking have been linked to a reduced risk.[11] These interactions highlight the multifactorial nature of PD, necessitating a deeper understanding of the genetic components involve.[12]

Recent research has reported over 20 genetic mutations correlated with Parkinson's disease, providing a framework to better characterize pathogenesis mechanism of disease. Notable genes include SNCA, LRRK2, PARK2, PARK7, PINK1, and GBA These genes are implicated in multiple cellular pathways such as ubiquitin–proteasome degradation (e.g., PARK2, PINK1), chaperone activities (e.g., DNAJC13), endosomal–lysosomal dynamics (e.g., GBA), as well as mitochondrial maintenance and mitophagy (e.g., PARK7, PINK1). The diversity of these pathways underscores the complexity of PD pathogenesis.[13]

These genes are associated in multiple cellular pathways like ubiquitin–proteasome degradations[14], chaperone activities and endosomal–lysosomal dynamics[15], as well as mitochondrial maintenance and mitophagy.[16] Parkinson's disease etiology has a significant contribution of synuclein (aSyn) RNA transcript isoform with an expanded 3′ untranslated region has been established.[17] Despite these genetic ties, fewer than 10% of the condition is presently attributed as a monogenic disorder, and much remains unknown about the multifactorial nature of the disease.

Modern high-throughput data has improved our understanding of the fundamental processes behind complicated disorders. To investigate interactions among significantly co-expressed genes, many network strategies have been explored.[18] Regulatory networks of genes and proteins are an effective way for deciphering the biochemical basis underlying complicated disorders like Parkinson's disease.[19] Using PPI methodology of known protein activities and patterns of coordination, the Presenilin-associated rhomboid-like protein (PARL) was characterized as pathogenic in Parkinson's disease.[20]

PARL is involved in mitochondrial function and maintenance, and its dysfunction can lead to mitochondrial fragmentation, a hallmark of PD pathophysiology.[21] Furthermore, other pathogenic genes, such as LRRK2 and SNCA, have been identified and are known to play significant roles in PD.[22]

Significant advancement in high-throughput technologies, as well as in-silico bioinformatics tools for processing this huge data, has provided an excellent opportunity to better understand Parkinson's Disease. Our study specifically targets early-stage Parkinson's disease (PD) to identify potential biomarkers and therapeutic targets. In this study, we performed bioinformatic analysis of microarray gene expression data by combining data from numerous sources, avoiding small data bias to discovering potential target genes in Parkinson's disease. This research will help researchers better understand the mechanisms that govern pathogenicity and regulation of Parkinson's disease and will provide novel insights for future research.

METHODS

Microarray data source

The microarray data used in this study was extracted from the GEO database (https://www.ncbi.nlm.nih.gov/geo) with the query "Parkinson

disease in humans tissue substantia nigra". After a critical review, the gene expression data with GEO ID: GSE7621[11] based on GPL570 Platform (Affymetrix Human Genome U133 Plus 2.0 Array), GSE49036[12] based on GPL570 Platform (Affymetrix Human Genome U133 Plus 2.0 Array), GSE20186[13] based on the GPL96 Platform (Affymetrix Human Genome U133A Array) and GSE8397[14] based on GPL96 platform (Affymetrix Human Genome U133A Array) was selected for the further analysis.

Screening of the differentially expressed genes (DEGs)

We used GEO2R web-based tool of NCBI (https://www.ncbi.nlm.nih.gov/geo/geo2r/) to screen the differentially expressed genes among 4 datasets analyzing the samples as control vs disease. Genes that satisfied the threshold |logFC| >1.0 and p-value <0.05 were shortlisted for further analysis. To demonstrate the intersection of 4 datasets and to find common DEGs among these datasets, a venn diagram was created using a web tool Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

Functional enrichment and pathway analysis

The Enrichr classification system (https://maayanlab.cloud/Enrichr) was used to do an enrichment analysis of the key differentially expressed genes.[15] Using the Enrichr classification system, the DEGs were categorized based on their molecular function (MF), biological process (BP), and cellular component (CC). Further, the pathway analysis was carried out using the KEGG database in Enricher. KEGG database is enriched with wide information about gene function, biological pathways, genomes, and diseases.

Protein-protein interaction network construction and identification of hub genes

To generate the PPI networks, the DEGs were processed via a web-based PPI network generation tool, the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org/). After that, the PPI pairings with a total score > 1.0e-16 were selected, eliminating the repetitive interactions. The topology of the PPI network was then examined using the Cytoscape program (http://www.cytoscape.org/). CytoHubba, a Cytoscape plugin program, was used to compute the degree of each protein node. Hub genes were defined as those with scores of 10 or more gene degrees in the PPI network.

RESULTS

Identification of DEGs

The selected datasets GSE7621, GSE49036, GSE20186 and GSE8397 comprised 25 (16 cases and 9 controls), 28 (20 cases and 8 controls), 36 (18 cases and 18 control) and 47 (29 cases and 18 controls) samples for substantia nigra (SN) Table 1.

GEO2R was used to compare and screen the DEGs between control and cases with the set threshold criteria of |logFC| ≥1.0 and p-value <0.05. A Venn analysis was carried out to show the intersection of up and down regulated DEGs, that depicted 18 critical genes which were common in amongst four datasets (Figure 1a and Figure 1b).

Functional enrichment analyses of DEGs

The enrichment analysis for GO function and KEGG pathway for DEGs were performed using the Enrichr. The GO terms with enrichment scores included molecular function (MF), biological process (BP), and cellular component (CC) ontologies. The results indicated that DEGs enriched for MF, were majorly enriched with sodium chloride symporter activity (GO:0015378), followed by monoamine transmembrane transporter activity (GO:0008504), oxidoreductase activity and olfactory receptor binding (GO:0031849) (Figure 2a). The GO BP analysis revealed that DEGs were significantly enriched with catechol-containing compound biosynthetic process (GO:0009713) followed by cation transport (GO:0006812), synaptic transmission, dopaminergic (GO:0001963), dopamine transport (GO:0015872) and monoamine transport (GO:0015584) (Figure 2b). The CC gene ontologies showed that DEGs were enriched in the neuron projection (GO:0043005), axon (GO:0030424), synaptic vesicle membrane (GO:0030672) and exocytic vesicle membrane (GO:0099501) (Figure 2c). The KEGG pathway analysis in Enrichr indicated DEGs to be significantly enriched in dopaminergic synapse, cocaine addiction and amphetamine addiction (Figure 2d).

PPI network construction and hub gene identification

Protein interactions and associations among the different differentially expressed genes were identified using the STRING-DB online software

Table 1: Table detailing the selected datasets/patients

Dataset ID	Total Samples	Cases	Controls	Tissue	Rationale
GSE7621	25	16	9	Substantia Nigra (SN)	This dataset was chosen to investigate the role of polymorphisms in the axon guidance pathway in Parkinson's disease (PD). By analyzing SNPs within axon-guidance genes, it helps in predicting PD susceptibility and progression, highlighting the importance of common gene variants in complex diseases.
GSE49036	28	20	8	Substantia Nigra (SN)	This dataset was selected to explore the transcriptomic changes in the substantia nigra (SN) across different stages of PD and incidental Lewy body disease (iLBD). It provides insights into the early deregulation of pathways related to axonal degeneration and immune response, and the consistent impairment of mTOR, EIF2, and eIF4/p70S6K signaling throughout PD progression.
GSE20186	36	18	18	Substantia Nigra (SN)	This dataset was included to perform a genome-wide meta-analysis of gene sets in PD, identifying early defects in mitochondrial function and glucose metabolism. It underscores PGC-1α as a potential therapeutic target for early intervention, offering new avenues for treatment strategies.
GSE8397	47	29	18	Substantia Nigra (SN)	This dataset was utilized to establish the transcriptomic expression profile of the substantia nigra in sporadic PD cases. It identified differentially regulated genes and new candidate genes mapping to PARK loci, emphasizing the role of the DNAJ family of chaperones in PD pathogenesis.

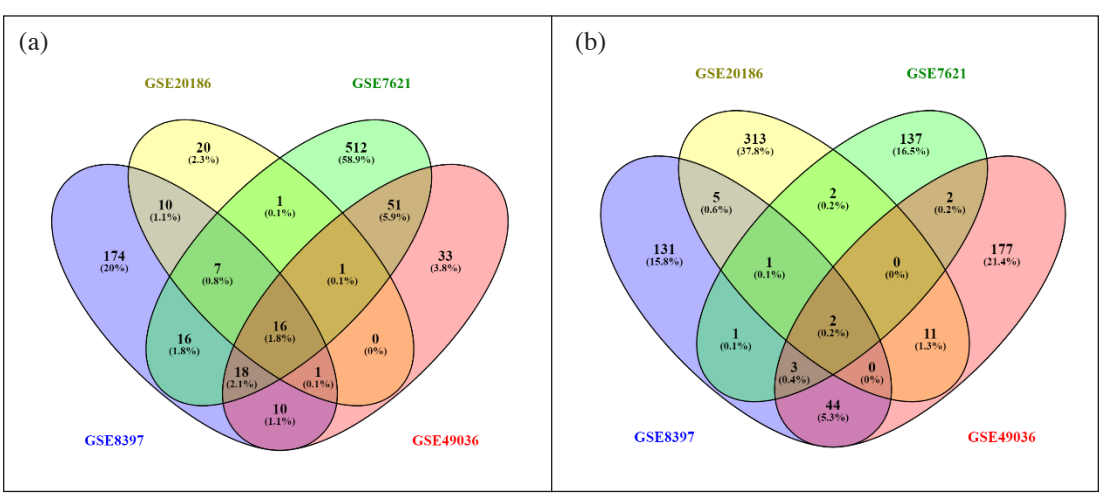


Figure 1. A Venn analysis was carried out to show the intersection of a) up regulated and b) down regulated DEGs, that depicted 18 critical genes which were common in amongst four datasets

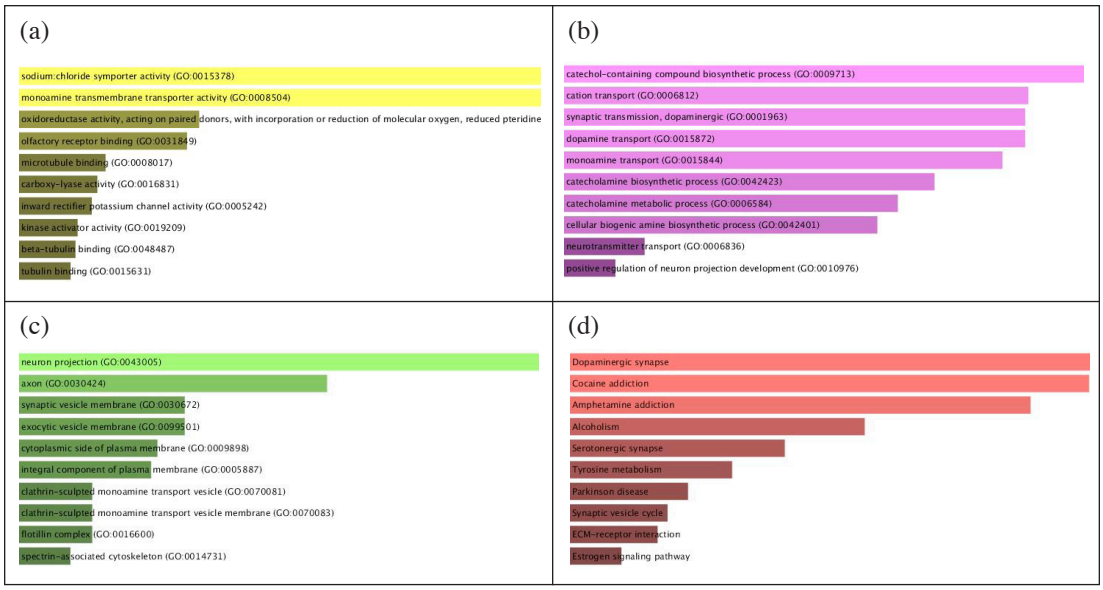


Figure 2. The enrichment analysis for GO function and KEGG pathway for DEGs were performed using the Enrichr. The GO terms with enrichment scores included a) molecular function (MF), b) biological process (BP), c) cellular component (CC) ontologies and d) KEGG pathway analysis.

(Figure 3). An association of 16 nodes and 37 edges with an PPI enrichment p-value of 2.08e-09 were identified in the protein-protein interaction network. We then evaluated the top ten genes ranking them by their connectivity degree in the protein-protein interaction network using Cytohubba, as represented in Figure 4.

The degree metric measures the connectivity of a node within a network by counting its direct connections to other nodes. This approach is particularly relevant because nodes with high degrees, or hub genes, typically play central roles

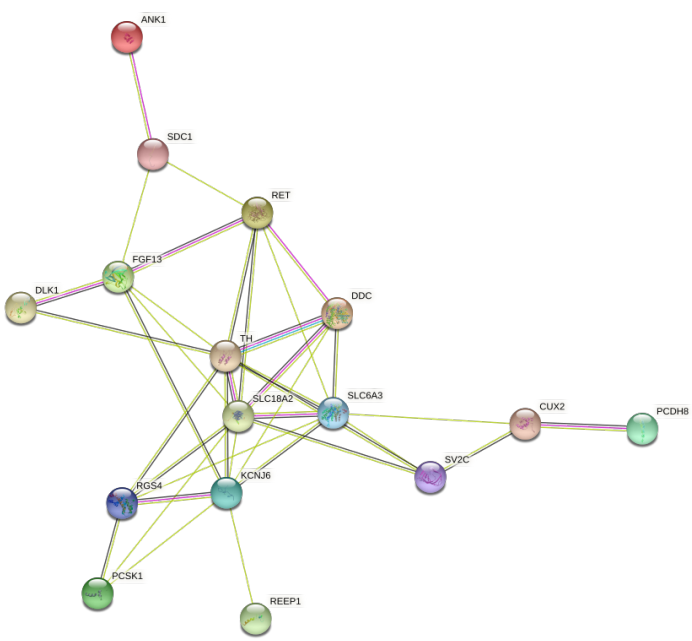
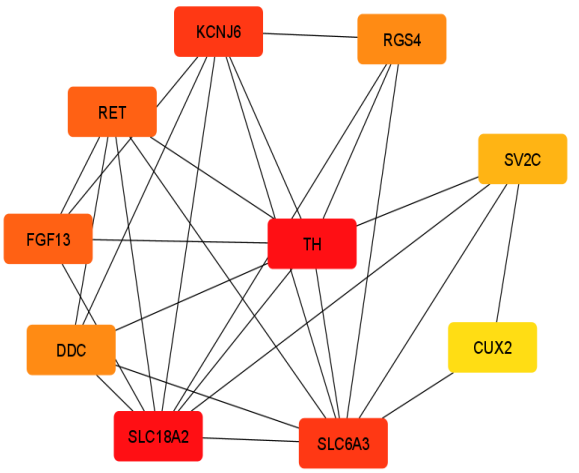


Figure 3. Protein interactions and associations among the different differentially expressed genes were identified using the STRING-DB online software



Rank	Name	Score
1	TH	9
1	SLC18A2	9
3	KCNJ6	8
3	SLC6A3	8
5	FGF13	6
5	RET	6
7	DDC	5
7	RGS4	5
9	SV2C	4
10	CUX2	3

Figure 4. Top ten genes ranking them by their connectivity degree in the protein-protein interaction network using Cytohubba.

in maintaining the structure and functionality of biological networks. In the context of Parkinson's disease, identifying these highly connected genes is crucial, as they often serve as key regulators involved in multiple pathways and essential cellular functions. Their high connectivity suggests that they are integral to the network's stability, and their dysfunction can have profound effects on biological processes. Additionally, focusing on hub genes simplifies the analysis of complex networks, enabling researchers to prioritize genes with substantial biological and clinical relevance. Therefore, the degree algorithm was chosen for its ability to highlight these critical nodes, providing a robust and insightful approach to understanding the underlying mechanisms of disease.

The results showed that the genes Tyrosine Hydroxylase (TH), and Solute Carrier Family 18 Member A2 (SLC18A2) were the most outstanding genes with connectivity degree of 9, followed by Potassium Inwardly Rectifying Channel Subfamily J Member 6 (KCNJ6) and Solute Carrier Family 1 Member 3 (SLCA3) with degree of 8. The genes Fibroblast Growth Factor 13 (FGF13) and Ret Proto-Oncogene (RET) showed a connectivity degree 6, while the Dopa Decarboxylase (DDC), Regulator of G Protein Signaling 4 (RGS4) and Synaptic Vesicle Glycoprotein (SV2C) showed connectivity degree of 5, 5 and 4 respectively. The gene Cut Like Homeobox 2 (CUX2) had a least connectivity degree of 3 (Table 2).

DISCUSSION

Several scientific and clinical investigations have been undertaken over the years to uncover the etiology and underlying mechanisms of Parkinson's disease, which are still unknown.

Table 2: Indicating genes are upregulated or downregulated among the identified hub genes

Gene Symbol	Description	Regulation
SLC18A2	Solute carrier family 18 member A2	Down
SLC6A3	Solute carrier family 6 member 3	Down
KCNJ6	Potassium inwardly rectifying channel subfamily J member 6	Down
FGF13	Fibroblast growth factor 13	Down
RET	Ret Proto-Oncogene	Up
TH	Tyrosine hydroxylase	Down
DDC	Aromatic L-amino acid decarboxylase	Up
RGS4	Regulator of G protein signaling 4	Up
SV2C	Synaptic vesicle glycoprotein 2C	Up
CUX2	Cut like homeobox 2	Up

It's difficult to get similar conclusions among researchers because of the disease's complexity and diversity, data bias, and the outcomes of a single study with limited subjects.

Our study delves into the intricate molecular mechanisms underlying Parkinson's disease (PD) through a rigorous analysis of multiple gene expression datasets sourced from diverse cohorts. By harnessing advanced bioinformatic tools, we identified 18 differentially expressed genes (DEGs) that significantly contribute to PD pathogenesis. This comprehensive approach unveiled novel biological pathways and processes, shedding light on previously unexplored facets of PD etiology. We searched and retrieved data from four different cohort profiles from GEO database and, then used bioinformatic approaches to evaluate these datasets to combine data from diverse research and produce consistent results. A significant breakthrough of our study lies in the discovery of 10 hub genes, such as Tyrosine Hydroxylase (TH), SLC18A2, SLC6A3, and RET, pivotal in dopamine metabolism, neurotransmission, and neuronal survival. The dysregulation of these genes underscores their potential as biomarkers and therapeutic targets for PD. Notably, upregulation of RET and downregulation of TH and SLC18A2 highlight their critical roles in mitochondrial function and neurotransmitter balance, suggesting promising avenues for therapeutic strategies.[23]

These overlapping 18 DEGs were analyzed for enrichment and then classified into three datasets based on their Gene Ontologies (GO) functional annotation: Molecular Function (MF), Biological Process (BP), and Cellular Component (CC). Significant DEGs engaged in the GO for MF were elevated in sodium-chloride symporter activity, monoamine transmembrane transporter activity, oxidoreductase activity, and olfactory receptor binding, according to the results of functional enrichment analysis. These 18 DEGs were discovered to be enriched in signaling pathways such as dopaminergic synapse, cocaine addiction, and amphetamine addiction using GO KEGG pathway analysis. Using a DEG-encoding proteins PPI network, the most closely linked genes were screened, and 10 hub genes, including TH, SLC18A2, KCNJ6, SLCA6, FGF13, RET, DDC, RGS4, SV2C, and CUX2, were found.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the dopamine production pathway, needed to convert tyrosine to L-DOPA. The nigrostriatal dopaminergic system is involved in the pathogenesis of Parkinson's disease (PD), with a decrease in TH activity, TH production, and TH mRNA in the striatum of PD patients and animal models. As a result, TH is one of the key targets for gene therapy in Parkinson's disease. The effects of L-DOPA and novel antiparkinsonian therapies on TH activity have been widely explored.[24]

For decades, it has been recognized that Parkinson's disease is caused by a loss of dopaminergic neurons in the substantia nigra, and that supplementation with levodopa, the metabolic precursor of dopamine, has a dramatic effect. KCNJ6, DDC, TH, SLC6A3 and, SLC18A2 were identified as genes involved in dopaminergic metabolism as an outcome of our research.

SLC6A3 and SLC18A2 are two genes that have recently received a lot of attention. SLC6A3 is involved in dopamine reuptake, or clearance, from the synaptic cleft in the striatum, which is important for preserving the integrity of dopamine neurons. SLC6A3 influences corticosteroid brain activity patterns, which may alter the cognitive profile and prognosis of Parkinson's disease patients.[25] Four SNPs in the SLC6A3 gene (rs6347, rs3756450, rs2652510, and rs2550956) were linked to PD risk in a meta-analysis.[26] SLC18A2 is an H+-ATPase antiporter that packages neurotransmitters into vesicles for release from monoaminergic neurons. SLC18A2 has two main functions in the brain: it mediates monoamine neurotransmission, and it protects cells against toxicity. Because cytosolic dopamine is cytotoxic, SLC18A2 is required for dopamine neuron survival.[27] SLC18A2 knockout animals have dopamine, norepinephrine, and serotonin depletion as well as progressive neurodegeneration in numerous monoaminergic areas.[28] In humans, an SLC18A2 mutation that severely lowers vesicular function has been linked to an infantile parkinsonism-like syndrome with monoamine deficiencies.[29]

In addition, many genes associated to cell growth and proliferation components of nerve cells, such as RET, were discovered in our studies. As a member of the cadherin superfamily, RET encodes a receptor tyrosine kinase that communicates via the Src/Ras/MAPK, PI3K/Akt, NF-B, JNK, and PLC pathways.[30] Mature mesencephalic nerve cells, primarily with in midbrain, cerebellum, pons, and thalamus, express RET robustly at both the mRNA and protein levels.[31] In dopaminergic neurons, RET can interact with PINK-1 and parkin to retain mitochondrial physiological and morphological stability.[32] RET is not required for prenatal or postnatal development. In animal studies,

RET expression in neural cells causes PD-like symptoms, indicating that it has a significant contribution in the survival of mesencephalic dopamine cells.

Despite its strengths, our study faces several limitations that warrant consideration. Firstly, the inherent variability among datasets and differences in study designs across cohorts may introduce biases and confounding factors. Although we applied stringent criteria for data selection and analysis, disparities in sample sizes, demographics, and experimental protocols could influence the reproducibility and applicability of our findings. Furthermore, the observational nature of our study limits definitive causal inference regarding the identified DEGs and their mechanistic roles in PD progression. Future research employing functional studies, including experimental validation in animal models or cellular assays, is crucial to elucidate the precise biological functions and therapeutic potential of these genes. Additionally, bioinformatic analyses, while powerful, are susceptible to false positives or false negatives. Despite employing rigorous statistical corrections and validation methods, the complexity of PD as a multifactorial disease necessitates cautious interpretation of gene expression data and careful translation to clinical implications.

In conclusion, while our study provides valuable insights into the molecular landscape of PD and identifies promising candidate genes for further investigation, addressing these limitations is essential for validating our findings and translating them effectively into clinical practice.

In this study, we identified multiple hub genes that are critical in Parkinson's disease using integrated bioinformatics approach. After screening DEGs, we constructed a complex PPI network of 16 potential genes with 37 crucial connecting edges. We found that the most altered hub genes and significantly enriched sub-networks were associated to dopamine metabolism, nerve conduction and neuronal toxicity and proliferation. These discoveries could help us better understand the cause and molecular events that lead to PD, and these prospective gene targets could be used for effective diagnostics and therapeutics.

DISCLOSURE

Conflict of interest: None

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