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Original Research

Integrative Nutrigenomic Systems Biology Analysis of Traditional Chinese Medicine Interventions in Parkinson's Disease: Nutrient-Gene-Disease Network Perspectives

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Abstract

The pathology of PD is characterized by progressive degeneration of dopaminergic neurons, although the full regulatory network involved in this process is not yet fully established. The present research employed a multi-omic systems biology design, integrating transcriptomic, functional, epigenetic, and microRNA analyses to develop a mechanistic model of neurodegeneration in the substantia nigra. We have determined six differentially expressed genes, such as tyrosine hydroxylase (TH), solute carrier family 18 member 2 (SLC18A2/VMAT2), and engrailed 1 (EN1), that are of critical interest in the disruption of the dopaminergic synapse and the inability to load vesicular neurotransmitters (fold enrichment: 2164.22). Notably, we have identified a candidate dual regulatory axis underlying the silencing of these neuroprotective genes. In this mechanism, repressive histone marks (H3K27me3 and H3K9me3) are concurrent, and post-transcriptional repression via specific microRNAs, in

particular, hsa-miR-431-3p (EN1) and mmu-miR-362-5p (SLC18A2), is involved. The combined model thus finds a mutual dysregulation of epigenetics and microRNA as the main cause of gene silencing. Besides, the Traditional Chinese Medicine (TCM) components were analyzed to identify compounds that can interact with the core targets (TH and SLC18A2), thereby providing translational potential. The results identify candidate TCM compounds predicted to interact with core targets (TH and SLC18A2), providing hypothesis-generating leads for multi-target interventions that may modulate the repressive epigenetic landscape, suppress regulatory microRNAs, and engage dopaminergic pathways. Predicted interactions require experimental validation to distinguish beneficial modulation from potential inhibition. This would seek to reverse severe neuronal activity and halt the advancement of Parkinson's disease. These findings illustrate a neuro-nutrigenomic application in which dietary-derived and herbal compounds may modulate gene expression and epigenetic marks in Parkinson's disease.

Keywords
Parkinson's disease; epigenetic regulation; SLC18A2 (VMAT2); microRNA; dopaminergic synapse

1. Introduction

Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra and α-synuclein aggregation forming Lewy bodies [1, 2]. Patients present with motor symptoms (bradykinesia, rigidity, tremor, postural instability) and non-motor symptoms including depression, anxiety, and autonomic dysfunction [3].

Deep brain stimulation (DBS) is an established surgical treatment for advanced PD [4], while spinal cord stimulation (SCS) has been investigated for specific symptoms, particularly gait impairment [3, 5]. Vibroacoustic therapy has also been explored as an adjunctive intervention for improving motor symptoms [6]. More recently, focused ultrasound (FUS) has emerged as a clinically approved non-invasive treatment option for selected patients with advanced PD [7].

Although levodopa remains the first-line therapy, its efficacy over time does not halt disease progression, highlighting the need for novel therapeutic approaches that target underlying neurodegenerative mechanisms [6, 8-10]. PD has a multifactorial etiology involving complex genetic, environmental, and biochemical interactions [11-13]. Systems biology approaches, particularly those incorporating nutrigenomics, are essential for understanding these interactions and identifying novel biomarkers and therapeutic targets by integrating multi-omics data [11, 14-18].

In addition, the utilization of sophisticated computational models provides deeper insight into the complex processes involved in PD, paving the way for new therapeutic approaches that go beyond traditional paradigms [11, 13]. Traditional Chinese Medicine (TCM) adopts a holistic, multi-targeted paradigm consistent with systems biology principles [19, 20]. TCM constituents can simultaneously modulate multiple signaling cascades and gene networks relevant to PD [21, 22]. Network pharmacology approaches enable exploration of TCM active constituents and their biological targets, providing mechanistic insights for therapeutic development [23-25].

However, despite the established strengths of a systems-level viewpoint and the multitarget capabilities of TCM, a systematic, integrative nutrigenomic systems biology analysis of how PD-related gene expression patterns directly correspond to predicted TCM treatments is a significant literature gap [26, 27]. Published Gene Expression Omnibus (GEO) sets offer a powerful tool for discovering robust and reproducible gene signatures across diverse patient groups, yielding a set of differentially expressed genes with high confidence in PD pathology. Future integration of this genomic information with enrichment software such as DAVID, GeneMANIA, and specific TCM databases, e.g., BATMAN-TCM 2.0, can help close the knowledge gap between molecular processes and therapeutic interventions. The research design was thus to conduct an integrative systems-biological study, with the first step being to identify shared DEGs across PD patient samples obtained from GEO databases. These genes were then characterised by functional enrichment and network analyses. Lastly, a nutrigenomic tool, employing TCM network pharmacology, was used to forecast possible specific herbs and active constituents with the potential to adjust the identified core PD gene network, which provided new evidence-based insights into TCM-based therapeutic interventions in PD. While individual epigenetic or miRNA alterations have been implicated in PD, an integrated model combining repressive histone modifications with specific miRNA-mediated repression remains underexplored. This study addresses this gap by suggesting a candidate dual regulatory axis that may contribute to dopaminergic neurodegeneration, with TCM predictions providing complementary hypothesis-generating leads.

2. Materials and Methods

The overall workflow consisted of: (1) data acquisition and DEG identification using GEO2R from four GEO datasets, (2) identification of core overlapping DEGs via Venn analysis, (3) functional enrichment with DAVID and Enrichr, (4) network analysis with GeneMANIA, and (5) TCM network pharmacology using BATMAN-TCM 2.0. A schematic summary of the workflow will be provided as Figure S1. Full lists of DEGs from each dataset and GEO2R analysis parameters are provided in Supplementary Materials. All analyses were performed using the publicly available web-based tools described; no custom scripts were generated.

2.1 Data Acquisition and Curation

Gene expression datasets related to PD in Homo sapiens were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) repository. A preliminary search using the keyword “Parkinson disease” OR “Parkinson’s diseases” in the title provided the following list of datasets: GSE165082, GSE100054, GSE75249, GSE72267, GSE54536, GSE54282, GSE49036, GSE42966, GSE20314, GSE20186, GSE20164, GSE20141, GSE20146, To achieve the greatest possible data quality and comparability, the datasets were culled based on the following eligibility criteria: (1) the presence of raw/properly normalized data; (2) appropriate identification of PD and control samples; and (3) sufficient sample size. This yielded four datasets for the following analyses: GSE8397-GPL96, GSE20186, GSE42966, and GSE165082.

2.2 Determination of Differentially Expressed Genes (DEGs)

A powerful meta-analytical method was used to find a high-confidence set of genes recurrently modified in PD. All four curated GEO datasets were analyzed using the GEO2R web-based tool to perform differential gene expression analysis. GEO2R helped to compare PD samples against control ones and to identify DEGs by statistically testing them, with an option of the Benjamini-Hochberg method of multiple testing correction, which is widely presented in bioinformatics literature studies [28, 29]. The analyses were performed using default settings, and genes were considered differentially expressed (DEGs) when their adjusted p-value was < 0.05. The independent DEG lists for the four datasets that met the eligibility criteria enabled subsequent comparisons and the identification of genes dysregulated across all studies. This was realized by Venn diagram analysis that was used earlier to find overlapping gene signatures. The resulting core set of common DEGs served as the input for all further functional and network analyses.

To evaluate the robustness of the six-gene core signature, we performed an independent validation using the substantia nigra microarray dataset GSE20164 (6 PD and 5 control samples), which was not included in the original meta-analysis. Differential expression analysis was conducted using GEO2R with default parameters and Benjamini-Hochberg adjustment for multiple testing. The direction and nominal significance of the six core genes were examined.

2.3 Functional Enrichment Analysis

The core DEGs were analyzed through functional annotation and pathway analysis to outline their biological functions in PD pathology. The enrichment analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) web tool (Version 6.8) [30, 31]. DEGs were cross-compared to Gene Ontology (GO) terms-Biological Process (BP) and Cellular Component (CC)-and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Enrichment analysis provided a comprehensive picture of the biological processes and pathways associated with the identified DEGs. The statistical significance level of 0.05 was used to label those terms with overrepresentation. Regulatory insights were also derived from enrichment in Epigenomics Roadmap histone modification (ChIP-seq) and miRTarBase annotations within enrichr [32-35].

2.4 Functionality Analysis and Gene-Network

The GeneMANIA website was used in order to visualize functional associations and interactions between the core DEGs. A web-based gene interaction identification and visualization tool, GeneMANIA, was used to construct a full protein-protein interaction (PPI) network [36]. The network integrated information from several types of interactions, including physical interactions and co-expression, to ensure the reliability of the identified interactions. The resulting network could then be analyzed to identify potential functions, interactions, and associated genes (via network expansion), thus providing a comprehensive picture of the biological context of the detected PD signature.

2.5 Intervention Prediction in TCM

Network pharmacology methodology was used to predict possible TCM interventions based on the identified core DEGs. The database (Bioinformatics Analysis Tool of Molecular Mechanism of TCM) (BATMAN-TCM 2.0) offered the needed framework [37]. BATMAN-TCM 2.0 predicts ingredient-target interactions based on structural similarity and protein similarity to known ligands, ranking them by enrichment score. These predictions indicate potential target engagement but do not specify functional effects (e.g., activation vs. inhibition). The selected DEGs were simulated into BATMAN-TCM 2.0 to find statistically significant correlations between them and TCM herbs and their active ingredients [37]. The analysis resulted in Enrichedherbsresult and Enrichedingredientsresult, which are consistent with the current pharmacological studies that make use of special TCM databases. The tool further enabled the formation of a gene-drug interaction network that graphically presented the relationships between the core PD DEGs and the predicted TCM compounds. The resulting interaction data were stored as a file named genedruginteractionresults-1018-2025.

3. Results

3.1 Determination of Common Differentially Expressed Genes (DEGs)

3.1.1 Selection and Characteristics of Data Set

The four selected datasets comprised post-mortem substantia nigra (SN) and peripheral blood samples. GSE8397 (GPL96) included 30 Parkinson's disease (PD) and 17 control SN samples. GSE20186 (GPL96) included 14 PD and 14 control SN samples. GSE42966 (GPL4133) included 9 PD and 6 control SN samples. GSE165082 (GPL11154) included 12 PD and 14 control whole-blood samples (RNA-Seq). A summary of all dataset characteristics is provided in Table S1.

3.1.2 Discovery and Regulation of Core DEGs

Each of the four datasets was analyzed by individual differential-expression analyses, and the lists of statistically significant DEGs (adjusted $p < 0.05$) were subsequently intersected in a Venn diagram (Table S2). This strict meta-analysis methodology led to a set of final meta-analyzed core signatures of six differentially expressed genes (DEGs) that were consistently altered in all four independent studies: EN1, PCDH8, PCSK1, RET, SLC18A2, and TH (Figure 1C). The direction of regulation of these six core DEGs was analyzed in the four datasets. The down-regulation of five out of six genes in PD samples was consistently observed compared to the controls in all cohorts: EN1, PCDH8, PCSK1, RET and SLC18A2, the latter being essential in storing vesicular dopamine and preventing cytosolic toxicity [38]. The rest of the gene, TH (tyrosine hydroxylase), was down-regulated in three SN cohorts (GSE8397, GSE20186) and the entire whole-blood cohort (GSE165082), which suggests a mixed regulation that is dominated by down-regulation.

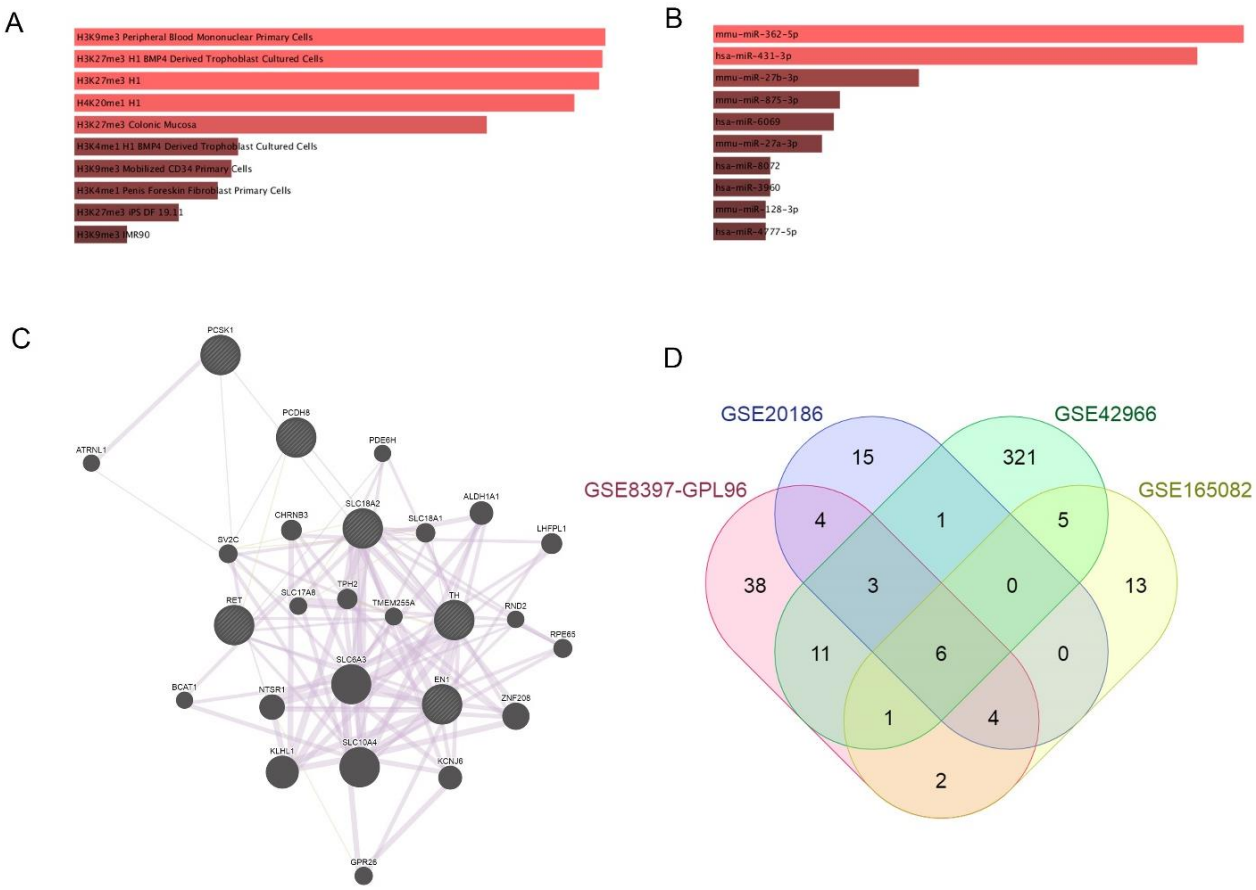

Figure 1 A candidate dual epigenetic and microRNA regulatory axis drives downregulation of neuroprotective genes in PD. (A) Repressive histone marks (H3K9me3, H3K27me3) are enriched at core gene loci. (B) Specific microRNAs (mmu-miR-362-5p targeting SLC18A2/PCDH8; hsa-miR-431-3p targeting EN1) are predicted to post-transcriptionally repress core genes. (C) Associated genes with the six candidate PD genes. (D) Venn diagram showing the six core DEGs consistently downregulated across four independent GEO datasets. The integrated analysis suggests a coordinated mechanism where repressive chromatin marks and miRNA-mediated repression synergistically silence dopaminergic and neuroprotective genes in PD.

3.1.3 Validation in Independent Datasets

To further evaluate the robustness of the six-gene core signature, we tested its performance in an independent substantia nigra dataset (GSE20164, 6 PD and 5 controls). All six genes (EN1, PCDH8, PCSK1, RET, SLC18A2, and TH) showed consistent directional downregulation in PD samples with nominal statistical significance (raw $p < 0.05$) (Table S3). However, none reached significance after multiple testing correction (adjusted $p > 0.05$), which is expected given the small sample size and limited statistical power of this dataset (Table S2). These results provide additional supportive evidence for the coordinated downregulation of this gene module in PD, complementing the strong meta-analysis findings from the four discovery datasets.

3.2 Functional Enrichment Analysis

Functional enrichment analysis of the six differentially expressed genes (EN1, PCDH8, PCSK1, RET, SLC18A2, and TH) was performed using the DAVID tool to identify the major biological functions and pathways associated with these genes in PD.

3.2.1 Gene Ontology (GO) Enrichment

It was found that the analysis was significantly enriched with the terms that refer to neuronal structure and functions. In the case of Cellular Component (CC), the terms with the highest enrichment were connected directly with the morphology and structure of neurons, such as dendrite ($P = 3.22 \times 10^{-8}$, 5 genes), terminal bouton ($P = 6.23 \times 10^{-5}$, 2 genes) and axon ($P = 9.74 \times 10^{-5}$, 3 genes). This suggests that the shared DEGs are most likely to be concentrated in the signalling and connection domains of the neuronal system.

Under the Biological Process (BP)-group, the most high-ranking term, aminergic neurotransmitter loading into synaptic vesicle ($P = 2.37 \times 10^{-7}$, 2 genes) closely associates the gene signature to the central pathophysiologic defect of PD-dopaminergic neurotransmission. Other important terms were pigmentation and amphetamine response.

3.2.2 KEGG Pathway Analysis

KEGG pathway enrichment provided a close relationship to addiction and synaptic pathways. The most important pathways were cocaine addiction ($P = 9.65 \times 10^{-5}$, 2 genes) and amphetamine addiction ($P = 1.92 \times 10^{-4}$, 2 genes), as they have molecular mechanisms in common with the dopamine system. The direct pathway implicated in PD pathology, the dopaminergic synapse ($P = 7.05 \times 10^{-4}$, 2 genes) was highly significant as well, which proved that the identified gene signature has a direct impact on the biological processes of dopaminergic neurons (Figure 2).

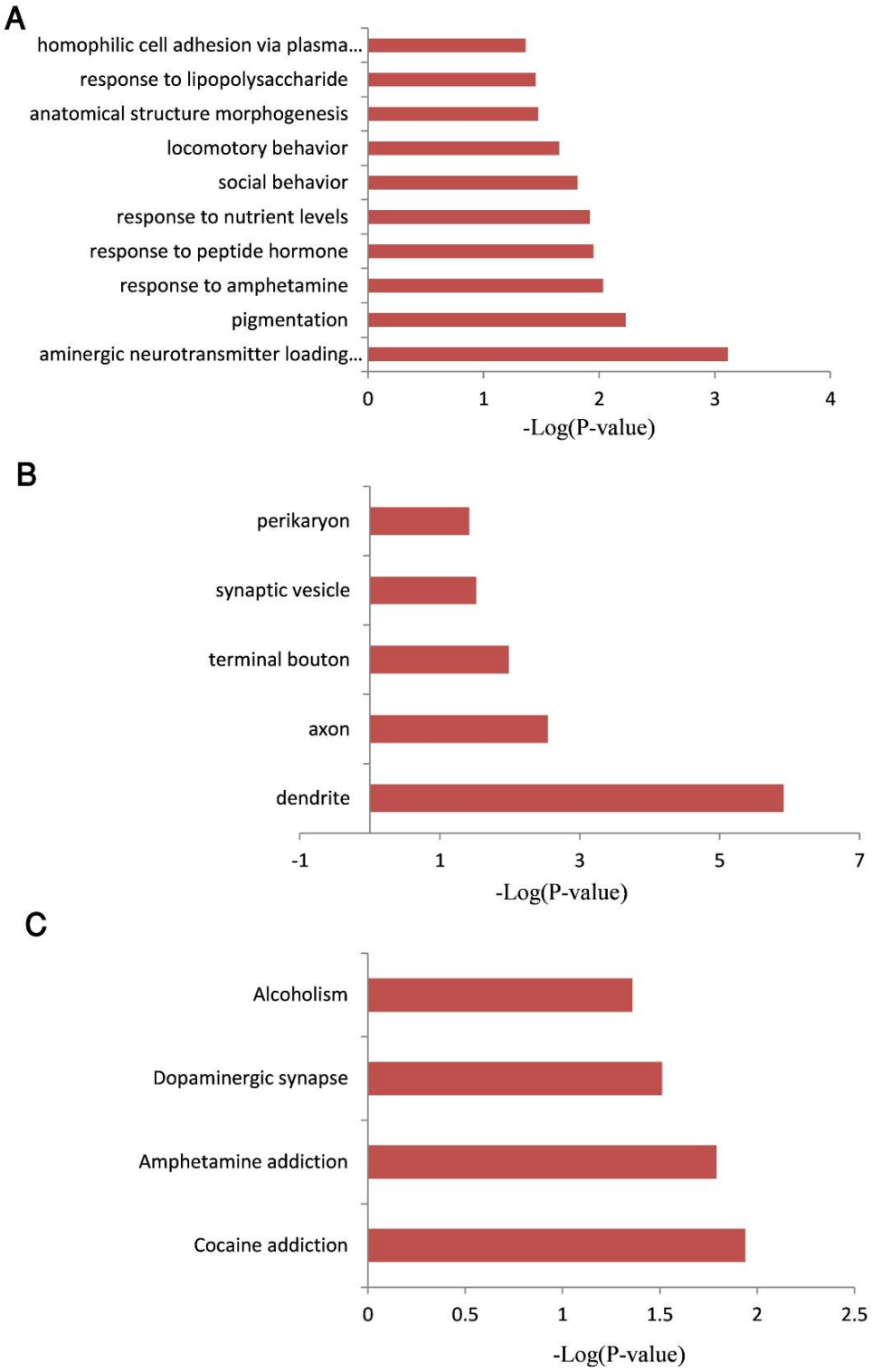

Figure 2 Functional enrichment analysis reveals disruption of dopaminergic synaptic machinery in Parkinson's disease. (A) Top enriched Biological Processes. (B) Top enriched Cellular Components. (C) Top enriched KEGG pathways for the six core genes (EN1, PCDH8, PCSK1, RET, SLC18A2, TH). The gene signature is strongly enriched in aminergic neurotransmitter loading into synaptic vesicles (fold enrichment: 2164.22, $P = 2.37 \times 10^{-7}$), dopaminergic synapse ($P = 7.05 \times 10^{-4}$), and neuronal structures (dendrites, axons, terminal boutons), highlighting the central role of these genes in dopamine neurotransmission-the primary pathophysiological defect in PD.

3.3 Analysis of Regulatory Mechanism

Integration of ChIP-seq and miRTarBase data revealed a coordinated dual mechanism underlying downregulation of the core DEGs. The core DEGs were then investigated using the transcriptional and post-transcriptional regulatory factors in the specialised functional annotation tools of the database DAVID to determine the underlying mechanisms behind the observed differences in expression.

3.3.1 Epigenetic Regulation (ChIP-seq)

Examination of Epigenomics Roadmap Histone Marks (HM) ChIP-seq data determined that a number of notable histone modification marks were associated with the core DEGs. The most enriched words were associated with repressive chromatin marks. As an illustration, the H3K9me3 peripheral blood mononuclear primary cells ($P = 3.83 \times 10^{-3}$) were enriched, including PCSK1 and EN1 genes. Moreover, the H3K27me3 marker, which is typically associated with gene silencing, was strongly enriched in H1 embryonic stem cell settings, indicating that RET, PCSK1, PCDH8, and SLC18A2 were subject to the most common repression protocol. This indicates that the down-regulation of the PD gene signature could be caused by epigenetic silencing, especially in immune and developmental contexts.

3.3.2 MicroRNA (miRNA) Regulation

The miRTarBase analysis revealed possible post-transcriptional regulation of the core DEGs by special microRNAs (miRNAs). The highest hits were mmu-miR362-5p ($P = 2.50 \times 10^{-3}$), which is expected to target PCDH8 and SLC18A2. Also, hsa-miR-431-3p ($P = 3.00 \times 10^{-3}$) and hsa-miR-6069 ($P = 1.25 \times 10^{-2}$) were predicted to target EN1. These results indicate that the destabilisation or lower translation of the mRNA of the core down-regulated genes in PD may be due to altered miRNA expression (Figure 1). This concurrent epigenetic silencing and miRNA-mediated repression represents a previously candidate integrated regulatory axis that may synergistically suppress neuroprotective gene expression in PD.

3.4 Gene-Network and Function Analysis

GeneMANIA was used to create a functional PPI network to examine interactions among the six core DEGs and relationships among highly related neighbouring genes that may be involved in PD pathology. The network was mainly constructed on the co-expression information with a strong functional correlation between the genes.

3.4.1 Topology and Major Neighbors on a Network

Network analysis was able to incorporate the main signature of EN1, PCDH8, PCSK1, RET, SLC18A2 and TH. The analysis observed various functionally relevant neighbor genes that showed high co-expression weights with the core DEGs. The three most appropriate candidate genes introduced into the network were:

- SLC6A3 (dopamine transporter, DAT): co-expressed to a significant extent with TH and SLC18A2. Dopamine reuptake into the presynaptic terminal is directly facilitated by SLC6A3, which plays an essential role in the pathogenesis of PD.
- SLC10A4 (sodium-discovered bile acid transporter): co-expressed with EN1, TH and SLC18A2, indicating a surprising role or complicated functional relationship in the monoamine system.
- SV2C (synaptic vesicle glycoprotein 2C): plays a significant role in synaptic vesicle activity and release, directly associated with the action of SLC18A2 (VMAT2).

These extensions support the hub of dopaminergic synapses and neurotransmitter transportation in the disease molecular signature.

3.4.2 Enriched Network Functions

The mapped combined core and neighbor genes within the TCM network were highly functional in terms of their enrichment, and thus strongly supported the results of the DAVID analysis (Section 3.2). The enriched functions were concentrated on membrane transport and synaptic processes the most.

The most enriched words were:

- sodium symporter activity (FDR = 3.04×10^{-3})
- synaptic vesicle (FDR = 3.04×10^{-3})
- transmembrane transporter activity-organic hydroxy compound (FDR = 3.04×10^{-3})

All of these results highlight the fact that the genes of interest in the network have a direct role in the nature and stability of the synaptic vesicle and the general process of neurotransmitter packaging and release.

3.5 Prediction of the Intervention in TCM

The BATMAN-TCM database was used to predict potential therapeutic agents of TCM, which could be used to intervene with the pathogenesis of PD by taking effect on the core six DEGs including EN1, PCDH8, PCSK1, RET, SLC18A2, and TH.

3.5.1 Single Therapeutic Herbs

The analysis revealed the presence of several TCM herbs with high enrichment ratios, indicating a high likelihood of modulating the activity of the core PD genes (Table S4). Table S4 shows the top five enriched herbs, which are mostly targeted at the major dopaminergic genes TH and SLC18A2.

The brightest potential herb was the genus Pinus koraiensis (HAI SONG ZI), and then the genus Rauwolfia (e.g., R. latifrons and R. perakensis), which are sources of alkaloids (e.g. reserpine, below) that influence monoamine signalling.

3.5.2 Anticipated Active Ingredients

An additional comparison of the central DEGs to the TCM compound database revealed selected active ingredients that provide candidate interactors for further validation. Table S5 shows the top five compounds according to their high enrichment ratios. BATMAN-TCM 2.0 analysis predicted several TCM-derived compounds with high enrichment scores for interaction with the core targets TH and SLC18A2 (see Table S5, Table S6, Figure 3). Notably, some top-enriched compounds such as

reserpine and rotenone are well-known VMAT2 inhibitors that can deplete dopamine and induce parkinsonian symptoms. These results demonstrate predicted target binding but do not imply beneficial activation or therapeutic efficacy. All predictions are in silico and require experimental validation to determine functional outcomes (activation, inhibition, or toxicity).

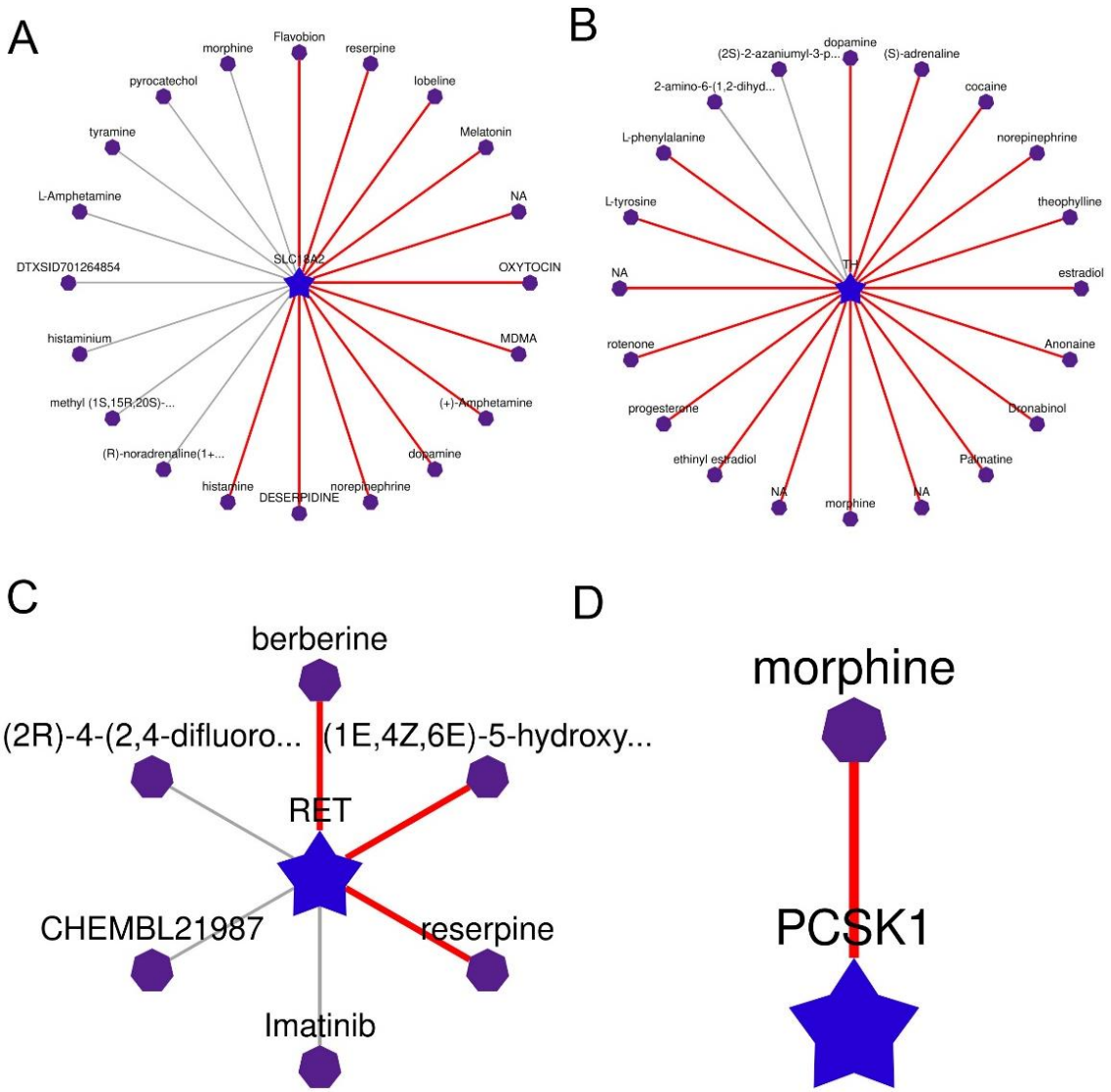

Figure 3 Network pharmacology predicts TCM compounds targeting core PD genes (TH and SLC18A2). (A-D) Interaction networks between core genes and TCM-derived compounds. Some top-enriched compounds (e.g., reserpine, rotenone) are known VMAT2 inhibitors with neurotoxic potential, demonstrating that BATMAN-TCM predicts binding only, not directionality. These are hypothesis-generating leads requiring experimental validation, not therapeutic recommendations.

4. Discussion

This paper used a systematic bioinformatics methodology that combined various publicly available microarray datasets of post-mortem brains of PD patients to identify robustly core differentially expressed genes (DEGs) that relate to the pathogenesis of PD. Our meta-analysis converged on a signature of six highly significant, consistently downregulated genes: TH, SLC18A2,

EN1, RET, PCSK1, and PCDH8. Follow-up functional enrichment and consistently implicated them in the dopaminergic synapse and the translocation of synaptic vesicles, establishing their significant role in the neurochemical phenotype of PD. Furthermore, we identified candidate epigenetic regulators and predicted TCM compounds that may interact with these core genes, providing additional insights into PD molecular mechanisms and generating hypotheses for future therapeutic investigation. Downregulation of the vesicular monoamine transporter 2 (VMAT2), which is encoded by the gene SLC18A2, and of tyrosine hydroxylase (TH) is a critical event in the neurobiological processes that drive Parkinson disease, and specifically in the substantia nigra. The storage and release of dopamine in the synaptic vesicles depends on the transporter called SLC18A2 (VMAT2). A significant decrease in VMAT2 levels in PD results in a decrease in the ability to store dopamine in vesicles. This decreases dopamine availability and exacerbates its cytosolic toxicity, leading to increased oxidative stress and neurodegeneration in the substantia nigra [39]. Moreover, animal models with VMAT2 deficiency have motor impairment and pathology similar to human PD [40]. The accumulation of misfolded proteins, including α-synuclein, adds to the toxicity of reduced VMAT2 levels, causing progressive neuronal loss [41]. Enhancement of VMAT2 expression has been proposed as a potential neuroprotective strategy [42]. The primary contribution of this study is the identification of a candidate dual regulatory axis-combining repressive histone modifications (H3K27me3 and H3K9me3) with specific miRNA targeting- that may cooperatively contribute to silencing key neuroprotective and dopaminergic genes in PD. This integrated model extends beyond individual regulatory layers reported previously. It suggests that concurrent repressive marks may establish a closed chromatin state while miRNAs (e.g., hsa-miR-431-3p on EN1; mmu-miR-362-5p on SLC18A2 and PCDH8) provide additional post-transcriptional repression. Such synergy offers a plausible mechanistic hypothesis for the robust downregulation observed across independent cohorts and highlights potential high-order control points for therapeutic intervention. This dual axis aligns with emerging evidence of epigenetic-miRNA crosstalk in neurodegeneration but adds to the growing systems-level evidence in PD substantia nigra linking these layers to a concise set of core dopaminergic targets.

The core signature identified here shows strong overlap with prior PD transcriptomic studies, particularly for TH and SLC18A2, which are among the most consistently downregulated genes in the substantia nigra across multiple meta-analyses [43-47]. EN1 has been implicated in dopaminergic neuron maintenance and vulnerability in both human and mouse studies [48]. RET and PCSK1 appear in several meta-analyses as downregulated genes, while PCDH8 was recently proposed as a potential biomarker [46]. The consistent co-downregulation of all six genes across our four independent datasets suggests they may function as part of a coordinated module in PD pathogenesis, even if some members are less frequently reported as top hits individually.

TH is the rate-limiting enzyme of dopamine production; the downregulation of TH plays a role in the depletion of dopamine. It has been shown that the expression of TH in the substantia nigra of PD patients is vastly reduced, which is associated with the loss of dopaminergic cells, as well as the classic motor symptoms of the condition. This under-regulation has been attributed to symptomatic manifestations such as bradykinesia and rigidity [49, 50]. Degradation of both TH and VMAT2 would suggest more extensive dysregulation of dopaminergic signaling pathways [51, 52].

Engrailed-1 (EN1) is an essential transcription factor used in survival and maintenance of dopaminergic neurons. EN1 defects have been associated with the gradual degeneration of these neurons and are a prerequisite for the continued survival of dopaminergic neurons in adult mice.

EN1 heterozygous mouse model has a severe and progressive degeneration of dopaminergic cells in the substantia nigra [53-55]. EN1 preserves dopaminergic neurons against apoptosis and increased expression correlates with protective mechanisms against mitochondrial apoptosis [56, 57]. EN1 has also been implicated in pathways associated with α-synuclein biology [58]. RET enhances the effects of GDNF on RET neurotrophic signaling and may enhance the resilience of dopaminergic neurons in PD [59, 60].

Proprotein convertase Subtilisin/Kexin Type 1 (PCSK1) is involved in the processing of pro-neurotransmitters and pro-neuropeptides [61] and has been identified as a candidate gene in PD [26]. Protocadherins, including PCDH8, are involved in synaptic adhesion and stability [62]; PCDH8 may contribute to impaired synaptic stability and neuronal vulnerability in PD [26, 46].

H3K27me3 and H3K9me3 are known as repressive histone marks in the substantia nigra that contribute to the establishment of closed chromatin and inactive transcription states [63]. Studies have demonstrated cooperative repressive functions of H3K27me3 and H3K9me3 during chromatin silencing [63]. These marks have been found to increase in PD neurons and are associated with decreased expression of neuroprotective genes and activation of apoptotic pathways [64, 65].

Bioinformatic analysis predicts that hsa-miR-431-3p targets the engrailed homeobox 1 (EN1) gene. More broadly, altered miRNA regulation has been widely implicated in PD [66], and hsa-miR-431-3p has been reported among dysregulated miRNAs in PD cerebrospinal fluid [67]. Altered expression of circulating miRNAs, including potential dysregulation of hsa-miR-431-3p, has been observed in PD and may serve as a source of diagnostic biomarkers [67, 68].

mmu-miR-362-5p is predicted to target both PCDH8 and SLC18A2 (VMAT2), which is consistent with the critical role of SLC18A2 in dopaminergic function [12, 69]. Altered miRNA expression, including potential dysregulation of hsa-miR-431-3p, has been observed in PD and may serve as a source of diagnostic biomarkers [69].

The core signature shows strong enrichment in dopaminergic synapse function, synaptic vesicle loading, and neuronal structure, consistent with the central pathophysiology of impaired dopamine neurotransmission in PD.

The core gene signature is central to dopaminergic neuron dysfunction in the substantia nigra. The reduced dopamine machinery involves Tyrosine Hydroxylase (TH) and Vesicular Monoamine Transporter 2 (SLC18A2/VMAT2), which are strongly downregulated. Their downregulation leads to reduced dopamine availability, impaired vesicular storage, and increased cytosolic dopamine toxicity, contributing to oxidative stress and neurodegeneration [69, 70]. EN1, a critical transcription factor for dopaminergic neuron survival, is also consistently downregulated, with its depletion worsening cell loss and apoptosis [12]. RET, PCSK1, and PCDH8 further contribute to neurotrophic signaling, neuropeptide processing, and synaptic stability.

The enrichment analysis supports the conclusion that the identified gene changes are focused on synaptic transmission. The term with the highest enrichment level (Fold Enrichment: 2164.22) in the biological process category is amine-neurotransmitter loading into synaptic vesicle. The cellular-location analysis concentrates the genes in neuronal structures, especially the dendrite (Fold Enrichment: 37.27), axon, and synaptic vesicle. In addition, KEGG pathway analysis reveals that the Dopaminergic synapse and Parkinson disease pathways are significantly enriched. TCM network analysis provides both graphical and statistical support for the high degree of co-expression and direct functional connections among the core genes TH, SLC18A2, and EN1.

The main pathway that may contribute to the repression of neuroprotective and dopamine-related genes involves chromatin remodeling and post-transcriptional regulation. Consistent with previous studies demonstrating cooperative repressive functions of H3K27me3 and H3K9me3 [63, 64], the concurrent enrichment of these marks may facilitate the establishment of a closed chromatin state in PD neurons. The concurrent enrichment of these marks may facilitate the development of closed chromatin and non-transcriptional states in PD neurons. In particular, H3K27me3 can target RET, PCSK1, PCDH8, SLC18A2, and EN1. Besides changes in the chromatin, certain microRNAs (miRNAs) are also predicted to have a direct action on the core DEGs; that is, hsa-miR-431-3p is predicted to target the neuroprotective transcription factor EN1 (Combined Score: 2580.41), which also leads to the loss of dopaminergic neurons by disrupting the balance in the expression of neurons to survive. On its own, mmu-miR-362-5p is predicted to target both the dopamine storage gene, SLC18A2, and the adhesion protein PCDH8.

Importantly, the BATMAN-TCM tool predicts potential binding interactions based on structural similarity but does not indicate the functional direction of the effect (i.e., whether a compound activates, inhibits, or has no net effect on the target). This is particularly relevant because several top-enriched compounds, such as reserpine and rotenone, are known VMAT2 inhibitors that can induce parkinsonism. Therefore, these results should be regarded strictly as hypothesis-generating.

To prioritize promising candidates for future experimental testing, we propose the following criteria: (1) exclusion of known VMAT2 inhibitors and neurotoxins; (2) presence of prior literature demonstrating neuroprotective effects in PD cellular or animal models; (3) favorable toxicity and safety profiles; and (4) potential to modulate multiple components of the dopaminergic-epigenetic-miRNA network.

Collectively, these TCM predictions serve as hypothesis-generating leads for future multi-target nutrigenomic strategies in PD, rather than direct therapeutic recommendations. Rigorous experimental validation is essential before any translational development.

5. Limitations

A key consideration is that BATMAN-TCM predictions reflect target interaction potential rather than directional effects. Several top-enriched compounds (e.g., reserpine from Rauwolfia species, rotenone) are established VMAT2 inhibitors and dopaminergic toxins, which validate the tool's sensitivity for SLC18A2/TH but warrant caution in direct therapeutic interpretation. Future studies should prioritize experimental screening to identify activators or neuroprotective modulators among lower-ranked or herb-derived mixtures.

6. Conclusions

This integrative nutrigenomic systems-biology study identifies a highly robust core set of six differentially expressed genes central to Parkinson's disease pathophysiology and, most importantly, suggests a candidate dual epigenetic-miRNA regulatory axis that may coordinately contribute to their silencing. The concomitant enrichment of repressive histone marks (H3K27me3 and H3K9me3) with specific post-transcriptional repression predicted for hsa-miR-431-3p (predicted targeting of EN1) and mmu-miR-362-5p (predicted targeting of SLC18A2 and PCDH8) provides an integrated systems-level mechanistic model for the sustained downregulation of neuroprotective and dopaminergic genes in the substantia nigra.

Functional enrichment analysis showed a strong correlation between these genes and impairment of the dopaminergic system, particularly aminergic neurotransmitter packaging into synaptic vesicles and dopaminergic synapse function. The significant downregulation of tyrosine hydroxylase (TH) and the vesicular monoamine transporter 2 (SLC18A2/VMAT2) appears to be a leading factor in reduced dopamine storage and increased cytosolic toxicity in the substantia nigra.

A key mechanistic insight of this study is the identification of a candidate dual regulatory network involving overlapping repressive histone marks (H3K27me3 and H3K9me3) and specific microRNAs. This joint epigenetic and microRNA dysregulation likely contributes to the transcriptional silencing observed in PD neurons.

In addition, network pharmacology analyses using BATMAN-TCM identified TCM-derived candidates predicted to interact with core targets (TH and SLC18A2). These findings provide hypothesis-generating leads for future multi-targeted nutrigenomic approaches. Overall, this integrative framework illustrates how nutrigenomics can reveal nutrient-gene interactions that influence epigenetic and microRNA-mediated regulation in neurological disorders.

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Author Contributions

EA carried out the gathering and analyzing of studies. BS participated in the design of the study and drafted the manuscript. EA and BS conceived of the study and participated in its design and coordination as well as the analyses of data. All authors read and approved the final manuscript.

Competing Interests

The authors have declared that no competing interests exist.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Figure S1: Schematic overview of the integrative systems-biology workflow for identifying core Parkinson's disease genes and predicting TCM interventions.
2. Table S1: Characteristics of the four GEO datasets included in the meta-analysis.
3. Table S2: Full lists of differentially expressed genes identified in each GEO dataset (GSE8397, GSE20186, GSE42966, GSE165082) with adjusted p-values and $\log_2$ fold changes.
4. Table S3: Expression of the six-gene core signature in the independent validation dataset GSE20164 (6 PD vs 5 controls).
5. Table S4: Enriched TCM herbs targeting Parkinson's disease genes, ranked by enrichment ratio. Columns include Latin/English/Pinyin names, number of TCM ingredients, number of genes, and enrichment ratio.

6. Table S5: Known and predicted compounds interacting with key Parkinson's disease genes (SLC18A2, TH, RET, PCSK1). Includes compound IDs, gene symbols, and confidence scores (e.g., known targets or predicted with scores like 0.99).
7. Table S6: Detailed TCM-derived compound interactions with key Parkinson's disease target genes. Columns include Entrez Gene ID, Gene Symbol (SLC18A2, TH, RET, PCSK1), and associated TCM ingredients by PubChem ID with interaction confidence ("known target" or predicted probability score in parentheses).

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