Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Expression characteristics of peripheral blood genes reveal potential biomarkers and candidate therapeutic targets for Parkinson's disease

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Received January 25, 2020; **Accepted** May 5, 2020; **Published** May 15, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.2.19

Abstract: In neurodegenerative disease, Parkinson's disease is the second most common one. Current demographic trends tell that by 2030, the risk of prevalence is close to 4% and the incidence is expected to double. Understanding the detailed process of Parkinson's disease can help us to figure out new biomarkers and candidate therapeutic targets for the diagnosis and progression of PD. This study is based on modularity for in-depth analysis and exploration of critical genes in the pathogenesis of Parkinson's disease, intended to identify the molecular processes of Parkinson's disease. According to the hypergeometric test, by performing differential analysis, enrichment analysis, co-expression module analysis, network connectivity analysis and finally, the ncRNA (non-coding RNA) and transcription factor that regulate the module were predicted. Based on the above methods, we obtained ten co-expression modules, including 2180 differential genes. Among them, RB1, IL7, and other genes were significantly differentially expressed in PD patients, and they had existing regulation in dysfunction modules, which was identified as Key genes in PD. The biological processes involved in the modular genes, for example, regulate lymphocyte activation, signal release, cellular calcium homeostasis, regulation of inflammatory responses, and regulation of exocytosis. This behavior significantly regulates signaling pathways such as cytokine-cytokine receptor interactions. Further, we identified ncRNA pivot including miR-25-3p. Also, transcription Factors pivot such as RELA, STAT1 significantly regulate dysfunction modules. This study can help to reveal all Parkinson's core dysfunction modules and potential regulatory factors as well as essential genes and the study assists to improve our understanding of its pathogenesis. The study can also be used to determine treatment goals and measure the effectiveness of interventions to provide predictive biomarkers and candidate therapeutic targets.

Key words: Parkinson's disease; Dysfunction module; Biomarkers; Therapeutic targets; Essential genes.

Introduction

Parkinson's disease (PD) has been known as a progressive neurodegenerative disease (1). Earlier, Parkinson's disease usually starts with asymmetry or unilateral motor symptoms. As the disease progresses, patients develop more common signs of bradykinesia including sound volume reduced, fewer activities daily and so on. Patients with advanced Parkinson's disease may have disabling manifestations, for instance, impaired balance, gait freezing, falls, speech disorders, and cognitive impairment (2). In addition to the above main symptoms, there are some complications of Parkinson's disease, such as depression and reversible toxic psychosis (3, 4). The presence of psychiatric complications is indicative of the age, duration, and stage of the disease, the dose of the drug, and occasionally the contextual factors (4). Parkinson's causes and genetic factors, environmental factors such as long-term exposure to pesticides, people have a greater chance of suffering from Parkinson's. Further, comprehensive factors such as head trauma, excessive smoking, too much intake of coffee and alcohol will lead to Parkinson (5, 6).

Pathologically, α-synuclein is the main component of Lowy body and is also a pathological marker of Parkinson's disease (7). In genetics, the single nucleotide polymorphism rs1805874 in the Calbindin1 gene has connections with Parkinson's disease in East Asians, but it could not affect Caucasians (8). In the SEPT14 locus, there are two SNPs which are rs11981883 and rs10241628, related to a reduced risk of PD (9). Simultaneously, in the MTHFR gene, a meta-analysis showed that the functional SNP rs1801133 was significantly related to susceptibility to PD among people in Europe and Asia, and MTHFR may be a risk gene for PD (10). Besides, SNCA genes that are significantly related to PD, such as rs181489, rs356186, rs356219, rs894278 rs2583988, rs2619364, rs10005233, and rs11931074. Among them, rs356186 may be the only protective SNP in Parkinson's disease (11). On the other hand, in the gene, the low-density lipoprotein receptor-related protein 8 (LRP8) gene and the single nucleotide polymorphism (SNP) rs5174 have a significant relationship with the risk of Parkinson's disease (PD) in the Caucasian population (12). In the meantime, scientists have identified some potential Parkinson's therapeutic targets. For example, miR-7 and miR-433 (13), miRNA 155 (14), microRNA-181a (15), HOTAIR (16) and MALAT1 (17). So far, the clinical diagnosis of PD has been based primarily on the late onset of dyskinesia. Therefore, in the early stage, the identification of Parkinson's disease in the Caucasian population was considered as the main challenge, especially for the diagnosis and management of the disease (18). At present, levodopa is a pharmaco-

logical alternative to dopamine as a treatment. Effective agents contain catechol-O-methyltransferase (COMT), dopamine agonists, monoamine oxidase-B (MAO-B) and anticholinergic medicine and amantadine (19). Deep brain stimulation, such as the subthalamic nucleus or Globus pallidus, can be treated with neurosurgery for advanced and disabling symptoms (19). These findings deepened our understanding of the pathogenesis and treatment mechanisms of PD and guided us in the direction of further research. Relevant researchers have reported Parkinson's research results, but for deeper research in the future, the corresponding effects of research results are still vague. To gain a comprehensive and in-depth understanding of Parkinson's pathogenesis, we conducted a systematic modular analysis to determine the dysfunction modules and core molecules between them to exploit Parkinson's key genes further.

Materials and Methods

Data resource

The NCBI Gene Expression Omnibus database (GEO Dataset) (20) includes a broad classification of high-throughput experimental data, including single-channel and dual-channel microarray-based assays for mRNA abundance, genomic DNA and protein molecules. In addition, it includes data from non-array-based high-throughput functional genomics and proteomics technologies. We first collected a set of untreated Phase 1 Parkinson's peripheral blood gene expression profiles from GEO, numbered GSE54536 (21). The data set included five untreated stage 1 Parkinson and five normal controls. Then, we screened the ncRNA-mRNA interaction pairs with score >= 0.5 from the RAID v2.0 database (22), including 431937 interaction pairs involving 5431 ncRNAs. The RAID v2.0 database enrolls more than 5.27 million RNA-related interactions, including more than 4 million RNA-RNA interactions and more than 1.2 million RNA-protein interactions, almost referring to 130,000 of the 60 species. RNA/protein symbols, which can help us comprehensively, observe various RNA-related interactions. At the same time, all human transcription factor target data were downloaded and used in the general database TRRUST v2 database of transcription studies (23), involving 2492 transcription factors and 9396 interaction pairs.

Differentially expressed gene

The analysis of differential expression of the gene expression profile data of this study was performed using the R language limma package (24-26). Primarily, we use the correct background function to accomplish background correction and normalization of the data. Secondly, the control probe and the low-expressed probe were filtered using the normalize Between Arrays function quantile normalization. Moreover, the differentially expressed genes of the data set were identified based on the lmFit and eBayes functions, by defaulting parameters.

Co-expression analysis

To explore Parkinson's potential biomarkers and candidate therapeutic targets, we accomplished a differential analysis of untreated Parkinson's and healthy controls to obtain a differential gene expression profile for Parkinson's disease. Simultaneously, to explore the synergistic expression of Parkinson's differential genes, we used WGCNA (27) to analyze Parkinson's differential expression profile matrix to discovery a gene module for synergistic expression. At first, the correlation coefficient weighting value was used, that is, the gene correlation coefficient was taken to the power of N, and the correlation coefficient between any two genes was calculated. The connections between genes in the network are subject to scale-free networks, allowing the algorithm more significant. Further, depending on the correlation coefficient between the genes, in the cluster tree, the branches represent individual gene modules, but the colors also represent different modules. According to each of the dysfunctional modules, observing the size of the gene in regulation, leading to a critical gene in the dysfunctional module is considered to be an essential gene leading to Parkinson's.

Enrichment analysis

Exploring the functions and signaling pathways involved in gene involvement can help scientists study the molecular mechanisms of disease. For the dysfunctional module, enrichment analysis of gene function and gene pathway is an effective means to explore the potential mechanism of Parkinson's. Therefore, we used the R language Cluster profiler package (28) for Go function and KEGG pathway enrichment analysis in the 10 module genes of breast cancer.

Cluster Profiler is a Bioconductor software package that that can perform statistical analysis and visualization of the functional clustering of gene sets and gene clusters. In addition, we use Cytoscape's BinGO (29) application to perform path analysis on the integrated module network.

Transcription factors and ncRNAs that regulate dysfunctional modules

The transcription and post-transcriptional regulation of genes often consider non-coding genes and transcription factors as the core driving force. Therefore, we have scientifically predicted and tested the role of Parkinson's dysfunctional module. Pivot regulators are defined as modulators that have significant regulatory functions in the process of Parkinson's disease, including ncRNA and TF. We believe that the control connection between the regulator and the module requires more than two, and based on the hypergeometric test, the module's enrichment target p-value is less than 0.01.

Results

Identify Parkinson's dysregulated molecules

Biologists have conducted many experiments and studies on the pathogenesis of Parkinson, and thus identified the potential genes of Parkinson. However, the complex molecular connections and overall effects of these genes are unclear. To observe molecular changes in the course of Parkinson's disease, we performed differential expression analysis using disease samples and normal samples based on microarray data. To identify Parkinson's differential gene expression (DEG), we obtained potential biomarkers and candidate therapeu-

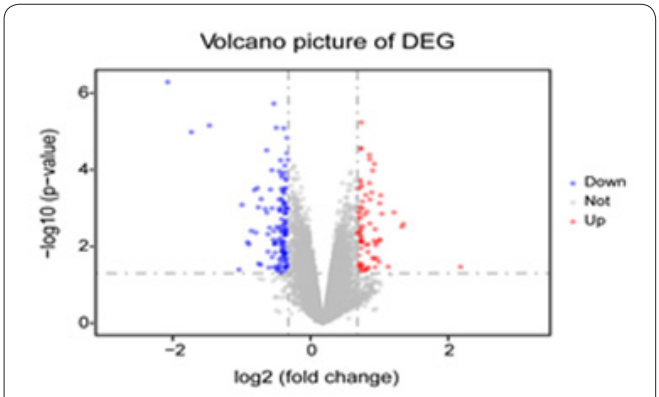

Figure 1. Volcano map of differential genes.

tic targets for Parkinson's disease. The results showed that a total of 2180 differential genes (Figure 1), and we believe that Parkinson's potential biomarkers and candidate therapeutic targets exist in these differential genes.

Identify Parkinson's functional disorder module

Firstly, based on 2180 differential genes with Parkinsonian expression disorders and their interaction genes, an expression profile matrix was constructed in patient samples. What's more, as reported by the Weighted Gene Co-Expression Network Analysis (WGCNA), from disease samples, we know that the genes significantly exhibit group co-expression (Figure 2). Modularity is not only a globally complex system but also a well-organized classification of subsystems. However, each subsystem has its characteristics. From the perspective of elemental genes, the module is a collection of genes that can be expressed synergistically, while in the same module, the genes have consistent expression behavior. In addition, there are interactions between modules. These relationships bring about the overall effect of characterizing global characteristics. Interactions act as bridges to help elemental genes to play a relevant role in the worldwide network. In patient samples, Parkinson's expression behavior is classified into different modules, and this behavior is useful for understanding the complex synergistic relationship between genes, from the perspective of expression behavior. Therefore, the co-expression team was used as a module, and we studied ten functional barrier modules for Parkinson (Figure 2A and B). Based on the functional disorder module, we identified the critical genes for each module and finally obtained core genes such as RB1, IL7.

Functions and pathways involved in the gene of interest

Function and pathway are essential mediators of the physiological response of the disease. In the dysfunctional module, exploring the features and pathways involved in related genes not only helps to determine the upstream and downstream relationships of the pathway genes within the module but also facilitates the establishment of molecular bridges between modules and diseases. This behavior can deepen the understanding of the underlying molecular mechanisms of the disease. According to 10 modules, for GO function and KEGG pathway, enrichment analysis was performed, and we finally obtained 18902 biological processes, 2419 cells, 3597 molecular functions and 1132 KEGG pathways

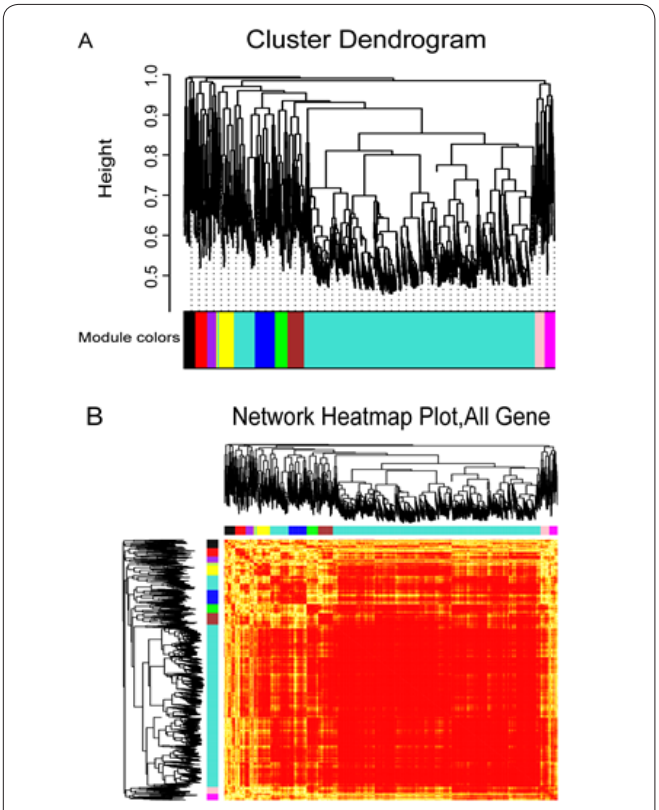

Figure 2. Synergistic expression of Parkinson's differential genes in patient samples. **A:** The intersection gene identified eight modules based on collaborative expression behavior. Each color of the X-axis represents a module, and the Y-axis represents the weight of the corresponding module. **B:** Module gene co-expression heat map. As shown in the figure, the expression behavior of the same module gene is synergistic, and the expression of the non-identical gene is different.

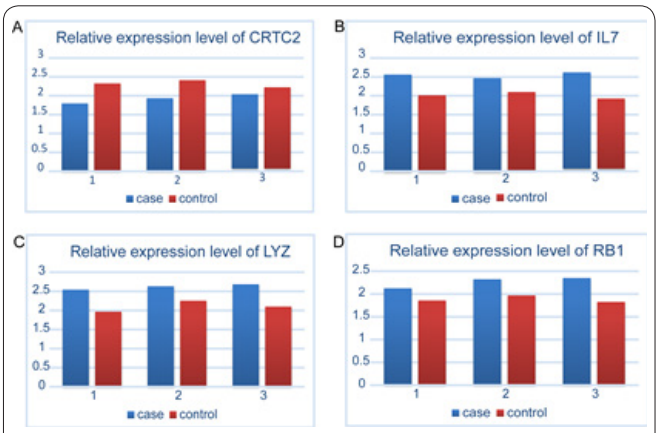

Figure 3. Functional and pathway enrichment analysis excerpts of the module gene. **A:** Module gene GO function enrichment analysis excerpt. The color increased from blue to purple, and the concentration increased significantly. The larger the circle, the more significant the proportion of the gene in the module that accounts for the GO function. **B:** Module gene KEGG pathway enrichment analysis excerpt. The color increases from blue to purple, and the enrichment rises significantly. The larger the circle, the more significant the proportion of the gene in the KEGG pathway entry.

(Figure 3). These functions were found to focus on the regulation of lymphocyte activation, signal release, cellular calcium homeostasis, regulation of inflammatory responses, and regulation of exocytosis. Equally important, the enrichment of the KEGG pathway reflects the signaling pathways of the significant cytokine-cytokine

Figure 4. The path analysis results of the integrated module network.

receptor interactions in Parkinson's differential genes. Conclusively, with 10 module networks, BinGO was processed for path analysis (Figure 4).

TF and ncRNA driving the Parkinson process

Considering the viewpoint of systems biology and systems genetics, transcription and post-transcriptional regulation in genes have been documented as critical regulators for disease development. Meanwhile, transcription factors and ncRNAs are common regulators as well. Although many biologists have valued the management of TF and ncRNA on the pathogenesis of Parkinson, few investigations have concentrated on their overall global effects on dysfunctional mechanisms and the role of bridges in development. Thus, in this study, based on the targeted regulatory relationship of TF and ncRNA to the module genes, we performed a pivotal analysis of the co-modules to explore critical transcriptional regulators that regulate the progression of Parkinson's disease. The predicted results (Figure 5A and B) showed that a total of 682 ncRNAs involved 741 ncRNA-module regulatory pairs and 21 transcription factors entangled 23 TF-module target pairs. Furthermore, the number of pivot control modules was statistically analyzed, and the most dysfunctional modules with ncRNA such as miR-25-3p and TF including RELA and STAT1 were found. These transcription factors and ncRNAs may regulate the progression of Parkinson's disease by mediating dysfunctional modules. Thus, we recognized these potential regulatory factors as dysfunctional molecules in the Parkinsonian process.

Discussion

Parkinson's disease has been known as a progressive neurodegenerative disease that causes motor symptoms and cognitive deficits. Performing dysfunction is a core symptom of cognitive deficits in Parkinson's disease (30). Currently, a medication that is used to cure Parkinson's disease could affect thermoregulation, so drugs can

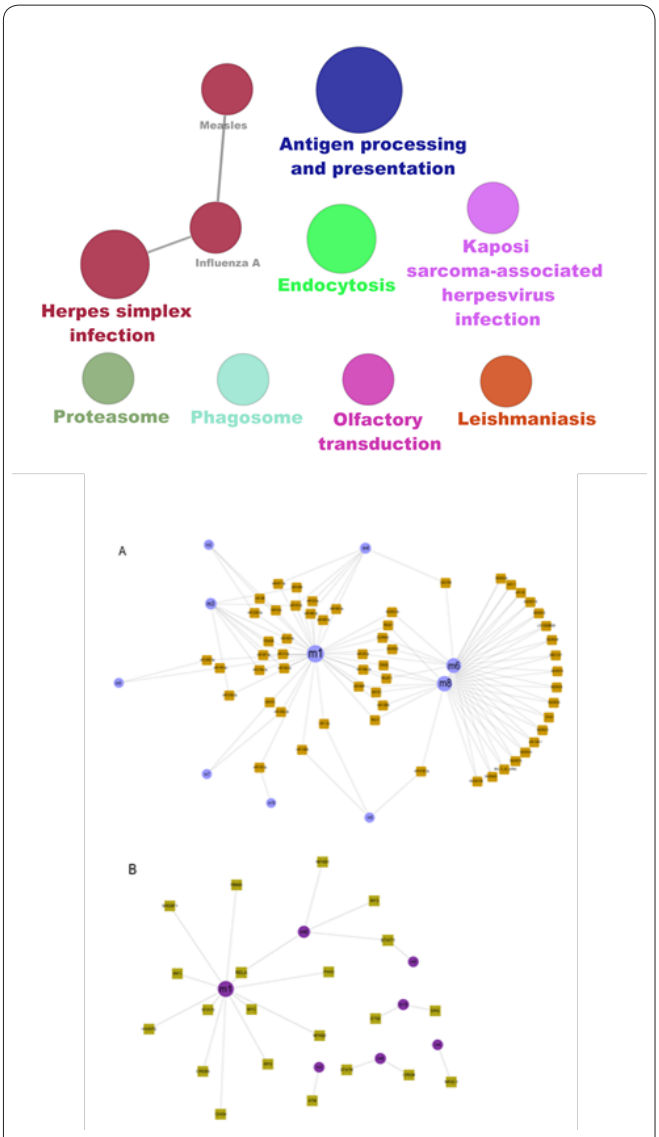

Figure 5. Modulatory influence of the regulator on the dysfunction module. **A:** The purple circle indicates the module, and the yellow square shows the ncRNA. **B:** The module has been showed as purple circles, and the yellow square represents the TF.

seriously affect patients and may worsen or even life-threatening (31). Therefore, it is very vital to understand its pathogenic mechanism. Although researchers have studied Parkinson's research in various aspects from the NCBI-GEO database, its specific potential biomarkers and candidate therapeutic targets remain unclear. In this study, we collected peripheral blood genes based on the NCBI Gene Expression Omnibus database (GEO Dataset) for patients with Parkinson's disease, healthy individuals. Based on Parkinson's differential gene expression profile data, the Parkinson's gene dysfunction module driven by transcription factors and ncRNA regulators was analyzed to understand the potential biological targets and candidate therapeutic targets of Parkinson. At the module level, the module significantly regulates lymphocyte activation, signal release, cellular calcium homeostasis, regulation of inflammatory responses, and regulation of exocytosis and the module is also considerably involved in signaling pathways such as cytokine-cytokine receptor interactions. Among them, studies have found that the signaling of dopamine receptor D3 on CD4+T cells is beneficial to Th1 and Th17-mediated immune function, and it is also necessary for pro-

moting neuroinflammation in the Parkinsonian mouse model (32). Alpha-synuclein-induced ER-mitochondrial contact loosening affects the destruction of Ca^{2+} exchange between mitochondria and endoplasmic reticulum and the production of mitochondrial ATP, which may be heavily dependent on correct Ca^{2+} signaling and ATP neurons are particularly harmful(33, 34).

At the same time, neuroinflammation is a neurodegenerative disease that has a direct influence on age. However, neuroinflammation is also a vital contributor to the pathogenesis, so anti-inflammatory agents become a new therapeutic focus (35). However, point mutations and overexpression of α-synuclein are responsible for the abnormal degradation of neurons and microglia, which are related to the autophagy-lysosomal pathway and the endosomal-lysosomal system. It directly leads to pathological intracellular accumulation, abnormal externalization, and re-internalization cycle and exocytosis (36). More importantly, interleukin-3 (IL-3) binds to its receptor and inductees a series of signaling processes that regulate hematopoietic cell proliferation and differentiation (37). The module's genes are also involved in the lysosomal, PI3K-Akt, Wnt signaling pathways. In Parkinson's patients, autophagic dysfunction happens in several phases of the autophagy/lysosomal degradation mechanism, which leads to the creation of intracellular protein aggregates and eventual neuronal cell death (38). At the same time, Purmorphamine activates sonic hedgehog signaling in a mouse model of Parkinson's disease, which protects dopaminergic neurons and attenuates inflammatory responses by mediating the PI3K/Akt signaling pathway (39).

In addition, disorders of the Wnt/β-catenin pathway are associated with a variety of diseases, including neurodegenerative diseases and cancer, such as Parkinson's disease (40). At the molecular level, we have explored the core genes of dysfunctional dysfunction of RB1, IL7, and other ten regulatory modules through co-expression modules. These core genes are not only significantly differentially expressed, but also play a regulatory role in the dysfunction module. Among them, RB1 inhibits glutamate excitotoxicity and regulates synaptic transmission to improve motor function damage of MPTP model of PD, indicating that Rb1 can be used as a potential therapeutic agent for PD (41). At the same time, interleukin-7 (IL-7) is a long-known cytokine known in immunology, which is essential for B cell and T cell proliferation, and the lack of IL-7 leads to Immune immature cells are stagnant (42). The impact on Parkinson's disease was not found in studies of other viral genes. Therefore, Parkinson's driving genes have yet to be further explored by scientists. Essential genes, as driving genes for dysfunction modules, encourage the development and progression of a disease and can be considered as potential therapeutic targets for Parkinson's.

Next, we predicted that 682 ncRNAs participate in Parkinson's pathogenesis through a mediator module. Based on statistical analysis, we determined that miR-25-3p has a significant effect on the three dysfunctional modules and is the gene that regulates the most modules. To observe cervical cancer studies, we learned that miR-25-3p reverses epithelial-mesenchymal transition by targeting Sema4C in cisplatin-resistant cervical cancer cells, which would be a new approach for patients with cervical cancer who are resistant to chemotherapy (43). In triple-negative breast cancer, new diagnostic and therapeutic targets were identified because miR-25-3p promotes proliferation by targeting the tumor suppressor BTG2 (44). At the same time, by targeting the Aktl gene, the metabolic function of C2C12 cells is enhanced, and the transcription factor AP-2α can positively regulate miR-25-3p (45).

Additionally, MiR-25-3p inhibits the proliferation of tongue squamous cell carcinoma cells and regulates the expression of cell cycle-associated proteins, which plays an essential role in the development of tongue squamous cell carcinoma (46). Even so, no effect on the pathogenesis of Parkinson's disease was found in the research of miR-25-3p. However, the results of our analysis show that its significant regulation of dysfunction modules is an essential factor leading to the pathogenesis of Parkinson's disease and further exploration will be required. At the same time, other ncRNAs that significantly regulate the Parkinson's dysfunction module may also participate in the primary process of Parkinson's, which can be used as a candidate for further molecular experiments (46-52).

Finally, we identified 21 transcription factors with different degrees of differential expression, and they have a regulatory Parkinson's dysfunction module. According to regulatory analysis, RELA, STAT1 significantly regulates two modules, those are essential in the pathogenesis of Parkinson's disease. Among them, reducing the expression of RelA to alleviate NF-κB inhibition, NF-κB inhibition but not activation is the basis of MPP + toxicity after cell death mechanism, and has significance for the pathogenesis of PD (53).

To improve the defects of oxidative phosphorylation in Parkinson's disease, firstly prevent MPP (+) -induced cell death, and at the same time use methods such as microRNA-7 to down-regulate RelA, increase Glut3 expression, and promote glycolysis (54). In addition, H_2O_2 produced by microglia NOX_2 can regulate the immune function of astrocytes in a STAT1/3 dependent manner, providing additional evidence for the immunogenic pathogenesis and therapeutic studies of PD (55). On the other hand, inhibition of the JAK/STAT pathway disrupts the circuits of neuroinflammation and neurodegeneration, thereby attenuating the pathogenesis of PD (56). At the same time, other transcription factors that significantly regulate the Parkinson's dysfunctional module may also participate in the primary process of Parkinson's, which needs to be confirmed by experiments (56-61).

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This paper was supported by project name: Clinical Research on the Integrated Model of the Prevention and Control of Alzheimer's Disease by the Epidemiological Investigation of Mild Cognitive Impairment Based on Big Data and Intervention and Detection of Intestinal Microflora, and Lishui City Science and Technology, Grant Number: 2018zdhz07.

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