Exploring the mechanism of acupuncture for Parkinson's disease based on network pharmacology techniques

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Abstract. Objective: To explore the potential mechanism of action of acupuncture for Parkinson's disease using network pharmacology. Methods: Firstly, Parkinson's disease-related targets were collected from the DisGeNET disease database, followed by the GEO database to obtain the possible targets of acupuncture for Parkinson's disease, and the targets of the active substance-dopamine produced after acupuncture were predicted in the SuperPred database. All target IDs were converted into corresponding gene IDs in the UniProt database. Finally, the disease targets of Parkinson's disease were intersected with the potential targets of acupuncture treatment to obtain the key targets of acupuncture for Parkinson's disease, and these key targets were analyzed for GO and KEGG pathway enrichment. Results: A total of 2079 targets for Parkinson's disease were obtained, 3193 targets for acupuncture treatment of Parkinson's disease, and 24 key targets. GO and KEGG enrichment analysis was performed on the key targets, and the most significant GO entries and key pathways were obtained for targets in cellular components, molecular functions and biological processes. Conclusion: Acupuncture may treat Parkinson's disease via activating cAMP signaling pathway, IL−17 signaling pathway and dopaminergic synapse.

Keywords: Acupuncture; Parkinson's disease; Network pharmacology.

1. Introduction

Parkinson's disease (PD), also known as tremor palsy, is a common neurodegenerative disease of the middle-aged and elderly, with an average age of onset of about 60 years and a prevalence of 1-2%, and has become the third most prevalent disease of the elderly in the world, and the incidence gradually increases with age [1]. The cause of the disease is still unclear, but the industry now generally believes that the pathogenesis of the disease is related to reduced levels of the neurotransmitter dopamine in the nigrostriatal system of the brain. Parkinson's disease is more severe and can cause limb tremors and muscle stiffness, resulting in limited mobility, frequent falls, and the formation of various traumatic injuries. In the late stage, due to long-term bed rest, loss of self-care ability and decreased body resistance, it is easy to cause complications such as pneumonia and heart failure, which can be life-threatening in serious cases. At present, PD is mainly treated clinically with drugs, such as levodopa, MAO-B (monoamine oxidase type B) inhibitors, amantadine, COMT (catechol-O-methyltransferase) inhibitors, anticholinergic drugs, etc. In severe cases, symptoms can be treated with deep brain electrical stimulation (DBS). However, current treatments can only control early symptoms, and drug efficacy may gradually decrease as the disease progresses.

Acupuncture therapy has a long history, accumulated by the ancient people during their long medical practice, and is very popular among people. First, its action is relatively safe, compared to drugs, acupuncture treatment has no toxic side effects, safety can be guaranteed, when using acupuncture treatment, there is no need to consider more about its toxic side effects this aspect. In addition, acupuncture treatment is short and effective, and patients can have a clear diagnosis and clear treatment. In the treatment, the indications are wide, including internal and external, gynecological and pediatric, oral, and ENT diseases, all of which can be treated by acupuncture. At

this stage, acupuncture therapy is gradually coming onto the international stage, playing an increasing role in the health care of the world's people.

Acupuncture has obvious advantages in treating PD, relieving patients' pain and muscle spasm symptoms, etc., and alleviating their pain. According to clinical studies currently known [2], acupuncture of Yanglingquan can enhance neuronal responses in the substantia nigra, caudate nucleus, and thalamus, and alter the protein expression level of dopaminergic neuronal areas in the substantia nigra [3]. Acupuncture of Zusanli, Dazhui and Baihui significantly suppressed rotational asymmetry and reduced nigrostriatal dopamine neuron deficiency and mitochondrial ultrastructural damage [4]. Meanwhile, electroacupuncture of Yanglingquan and Taichong could activate 16 genes with degenerative effects on striatal dopaminergic neurons and play a neuroprotective role [5]. High frequency 100Hz electroacupuncture of Yanglingquan and Sanjian points revealed increased tyrosine hydroxylase activity in the striatum and substantia nigra, suggesting that the increase in the number of dopamine neurons is an indication that acupuncture inhibits the degeneration of dopamine neurons [6-8]. The iron content in the substantia nigra was significantly reduced after acupuncture at Taichong and Yanglingquan points on alternate days compared with the model group, and acupuncture also had a significant effect on ferritin heavy chain levels, but not on Fe2+ and ferritin light chains, based on which it can be concluded that acupuncture intervenes in iron-related oxidative stress induced by PD and prevents toxic injuries induced by iron metabolism [9]. Scalp acupuncture in synergy with methyldopa was found to significantly increase blood glucose metabolism levels after five weeks of treatment, and to some extent alleviate impaired mitochondrial ATP production and improve mitochondrial function [10].

Although there have been many reports on the treatment of Parkinson's disease by acupuncture, the mechanism has not been elucidated. In this paper, we firstly summarize the rules of acupuncture points in the treatment of Parkinson's disease, and elucidate the potential mechanism of acupuncture in the treatment of Parkinson's disease through network pharmacology technology, in order to provide a basis and reference for the clinical treatment of Parkinson's disease.

2. Methods

2.1 Literature research summarizes the pattern of acupuncture point extraction

2.1.1 Data sources. The clinical research literature on acupuncture for Parkinson's disease from January 2000 to March 2023 was retrieved from China Knowledge Network Infrastructure (CNKI), VIP database (VIP), and Wan Fang data knowledge service platform (Wan Fang). The search terms were "Parkinson's OR Parkinson's disease OR senile tremor OR tremor palsy" and "acupuncture OR moxibustion OR electroacupuncture".

2.1.2 Inclusion criteria. (1) the type of literature was a clinical randomized controlled trial (RCT); (2) the sample size of literature was ≥ 30 cases and the total effective rate was ≥ 80%; (3) the prescription of acupuncture was clear and the treatment means was acupuncture, including moxibustion, electroacupuncture and acupoint injection; (4) the diagnosis of treatment subjects was clear and met the definition and clinical manifestations of Parkinson's disease.

2.1.3 Exclusion criteria. (1) duplicate publications only keep the latest published one, and delete the rest; (2) literature of empirical, case-by-case, animal experiments or systematic evaluation type that are not clinical trials; (3) literature with incomplete full text; (4) literature using personal experience acupuncture points or unrecognized acupuncture points; (5) literature using head acupuncture and auricular acupuncture points as the main treatment means [11].

2.2 Acupuncture for Parkinson's disease potential targets

The GEO database at (https://www.ncbi.nlm.nih.gov/) was searched with the keyword "Acupuncture Parkinson's Disease" at, and the human species was selected to obtain the transcriptomic data of acupuncture for Parkinson's disease. Transcriptomic data of Parkinson's patients were obtained from [12], and their associated GSE178470 matrix and platform files were

downloaded and saved as Excel datasheets, the obtained data were organized and summarized. By taking the mean and logarithm, the data with different change trends after 5 and 8 treatment sessions were removed, and the targets with |log2FC| > 1 were screened as potential targets of acupuncture action. In addition, it has been reported in the literature that dopamine secretion increases in the body after acupuncture treatment, and then the therapeutic effect is produced by dopamine [13,14], so we predicted the targets of dopamine by SuperPred database [15], and the targets with Probability and Model accuracy greater than 80% will be used as potential targets. The above targets were pooled and the duplicate values were removed as potential targets for acupuncture treatment of Parkinson's disease.

2.3 Parkinson's disease target mining

Search the DisGeNET database (https://www.disgenet.org/search) with "Parkinson's Disease" as the keyword, and keep the targets related to Parkinson's disease. As disease-related targets, the names of the genes corresponding to the targets were retrieved and de-duplicated.

2.4 Key targets of acupuncture for Parkinson's disease

By using UniProt database (https://www.uniprot.org/), the above target IDs could be converted into corresponding gene IDs. Then Venny2.1 (http://bioinfogp.cnb.csic.es/) was used to intersect the Parkinson's disease targets with the potential therapeutic targets of acupuncture after de-duplication to obtain acupuncture treatment. The key targets of Parkinson's disease were obtained.

2.5 GO and KEGG enrichment analysis

The key targets and eligible log2FC values obtained above were entered, human species were selected, and GO and KEGG enrichment analyses were performed with the help of the microbiology letter platform (http://www.bioinformatics.com.cn/). The enrichment results were saved and the top 10 positions of each of the biological process (BP), cell component (CC) and molecular function (MF) that the gene may be involved in, the cell component and the molecular function that the gene may perform were selected to draw bubble maps. The KEGG enrichment analysis was performed in the same way, and the top 10 enriched pathways were histogrammed and a KEGG relationship network was constructed at [16].

3. Results

3.1 Acupuncture point pattern of acupuncture treatment

The top 5 acupoints were Taichong (20 times), Baihui (18 times), Hegu (17 times), Zusanli (15 times), and Fengfu (13 times). The results are shown in Table 1.

Table 1 Statistics on the frequency of use of high-frequency acupoints (used ≥ 5 times) in acupuncture for Parkinson's disease.

Index	Acupoints	Frequency/times	Channel tropism
1	Taichong (LR3)	20	Liver channel of foot jueyin
2	Baihui (DU20)	18	Du meridian
3	Hegu (LI4)	17	Large intestine channel of hand yangming
4	Zusanli (ST36)	15	Stomach channel of foot yangming
5	Fengfu (DU16)	13	Du meridian
6	Yanglingquan (GB34)	12	Gallbladder channel of foot shaoyang
7	Qihai (RN6)	10	Ren meridian
8	Taixi (KI3)	10	Kidney channel of foot shaoyin
9	Quchi (LI11)	9	Large intestine channel of hand yangming
10	Sanyinjiao (SP6)	9	Spleen channel of foot taiyin
11	Neiguan (PC6)	9	Pericadium channel of hand jueyin

12	Wangu (GB12)	8	Gallbladder channel of foot shaoyang
13	Fenglong (ST40)	7	Stomach channel of foot yangming
14	Yamen (DU15)	7	Du meridian
15	Zhongwan (RN12)	7	Ren meridian
16	Guanyuan (RN4)	6	Ren meridian
17	Tianshu (ST25)	5	Stomach channel of foot yangming
18	Waiguan (SJ5)	5	Tri-jiao channel of hand shaoyang

Summarizing the selected acupoints, it was found that all 12 main meridians and the ren and du meridians were involved, and the most frequently used and the number of selected acupoints was the du meridian. Nine acupoints were involved and used 42 times, followed by the stomach channel of foot yangming, bladder channel of foot taiyang, and large intestine channel of hand yangming, respectively. The results are shown in Table 2.

Table 2 Statistical table of selected acupuncture points attributed to meridians.

Index	Channel tropism	Frequency/times	Acupoints/pc
1	Du meridian	42	9
2	Stomach channel of foot yangming	36	8
3	Bladder channel of foot taiyang	29	8
4	Large intestine channel of hand yangming	24	4
5	Liver channel of foot jueyin	19	6
6	Gallbladder channel of foot shaoyang	15	6
7	Spleen channel of foot taiyin	12	2
8	Kidney channel of foot shaoyin	9	4
9	Pericadium channel of hand jueyin	8	2
10	Ren meridian	8	2
11	Tri-jiao channel of hand shaoyang	5	3
12	Heart channel of hand shaoyin	3	5
13	Small intestine channel of hand taiyang	3	1
14	Lung channel of hand taiyin	1	1

The 58 acupoints used were mainly concentrated on the extremities, head and face, and neck, in order of frequency: 63 times on the extremities, 35 times on the head and face, 17 times on the neck, 14 times on the chest and abdomen, and 13 times on the lower back [17-20]. In addition, it is known from the literature that the combination of acupuncture points can give full play to the comprehensive effect of acupuncture points on the body and can effectively relieve the symptoms of Parkinson's disease patients.

3.2 Targets of acupuncture for Parkinson's disease

3.2.1 Potential targets. The GEO database (https://www.ncbi.nlm.nih.gov/) was searched with "Acupuncture Parkinson's Disease" as the keyword, and a total of 3193 targets were obtained according to the screening criteria of $|\log2FC| > 1$ screening criteria to obtain a total of 3193 targets for acupuncture treatment of Parkinson's disease, where each target corresponds to the heat map before treatment, five courses of treatment, and eight courses of treatment as shown in Figure 1.

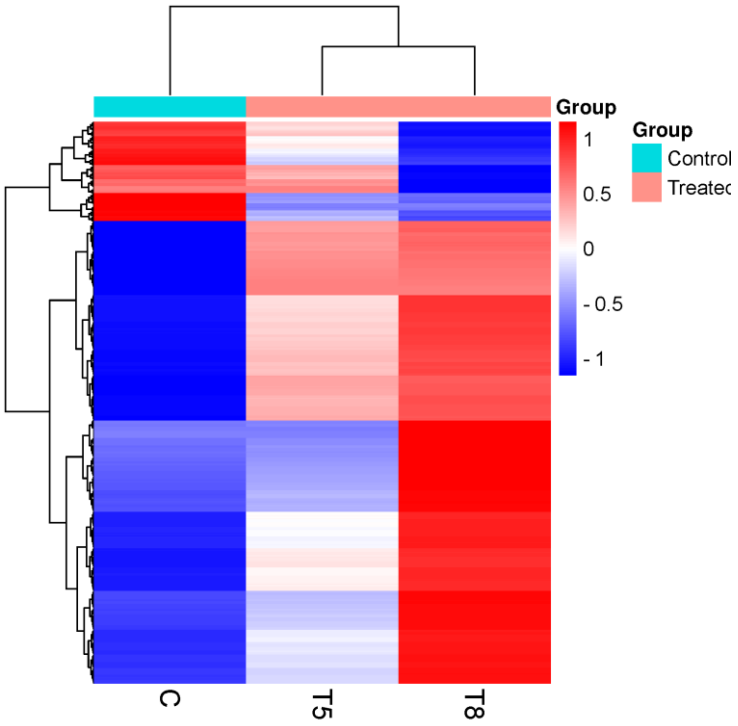


Figure 1 Heat map of acupuncture for Parkinson's disease target before treatment, five courses of treatment, and eight courses of treatment. C: control group; T5: after 5 treatment sessions; T8: after 8 treatment sessions.

3.2.2 Key targets. Searching the Disgenet database (https://www.disgenet.org/search) with "Parkinson's Disease" as the keyword and eliminating the duplicate disease. The targets of Parkinson's disease were intersected with the potential therapeutic targets of acupuncture to obtain a total of 24 key targets of acupuncture for Parkinson's disease. The Venn diagram of Parkinson's disease targets and potential therapeutic targets of acupuncture is shown in Figure 2.

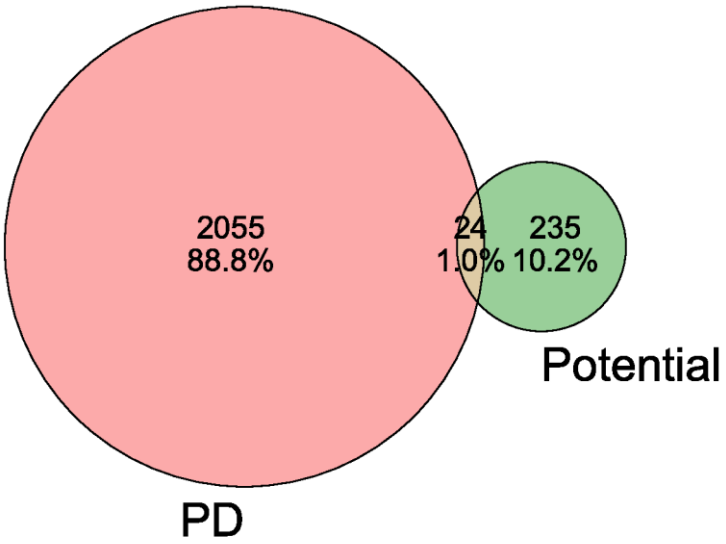


Figure 2 Parkinson's disease targets and potential targets of acupuncture for Parkinson's disease Venn diagram. PD: Parkinson's disease target; Potential: potential target of acupuncture for Parkinson's disease.

3.3 Potential therapeutic mechanisms of acupuncture for Parkinson's disease

3.3.1 GO functional enrichment analysis. GO functional enrichment analysis of key targets of dopamine-acupuncture for Parkinson's disease was performed by the microbiology platform, and the top 10 most significantly enriched GO entries in cellular components, molecular functions and biological processes were obtained, as shown in Figure 3. The top 10 most significantly enriched GO entries in cellular components were: synaptic transmission dopaminergic transmission dopaminergic, dopamine uptake involved in synaptic transmission, catecholamine uptake involved in synaptic transmission, dopamine uptake, catecholamine uptake, neurotransmitter reuptake, neurotransmitter uptake, response to cocaine, dopamine transport, neutrophil The top 10 most significantly enriched molecular function GO entries were: membrane raft, membrane microdomain, membrane region, presynaptic membrane, secretory granule lumen, cytoplasmic vesicle lumen, and cytoplasmic vesicle lumen, The top 10 most significantly enriched GO entries for biological processes were: catechumen, membrane microdomain, membrane region, presynaptic membrane, secretory granule lumen, cytoplasmic vesicle lumen, vesicle lumen, dopaminergic synapse, ciliary membrane, integral component of presynaptic membrane. Entries were: catecholamine binding, postsynaptic neurotransmitter receptor activity, neurotransmitter receptor activity, organic hydroxy compound transmembrane transporter activity, sodium: chloride symporter activity, anion: sodium symporter activity, cation: chloride symporter activity, and neurotransmitter transmembrane transporter activity, anion: cation symporter activity, pattern recognition receptor activity.

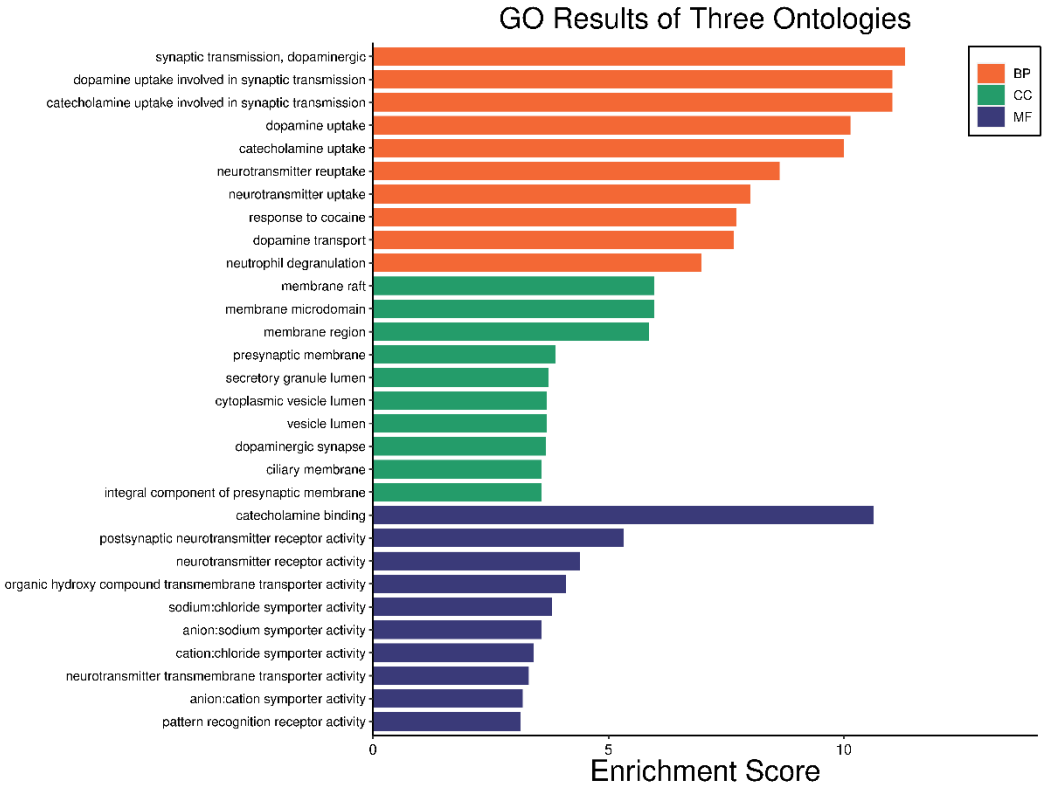


Figure 3 Dopamine-Key Target for Acupuncture in Parkinson's Disease GO functional enrichment analysis of the top 10 most significantly enriched biological processes, cellular components and molecular functional GO entries. BP: biological process; CC: cell component; MF: molecular function.

3.3.2 KEGG pathway enrichment analysis. The KEGG pathway enrichment analysis was performed on the key targets of dopamine-acupuncture for Parkinson's disease, and the top 10 pathways were selected in descending order of p-value and plotted in bubble diagrams, see Figure 4, where the vertical coordinates represent the names of the pathways, and the bubbles from small to large represent the increasing number of genes enriched in the pathways. The color of the bubbles from blue to red indicates that the enrichment is more and more significant, i.e., the higher the

relevance of the pathway to the disease, and it is likely to be a key pathway. The results showed that the core pathways mainly include dopaminergic synapse、cocaine addiction、IL−17 signaling pathway、tuberculosis、hematopoietic cell lineage and so on.

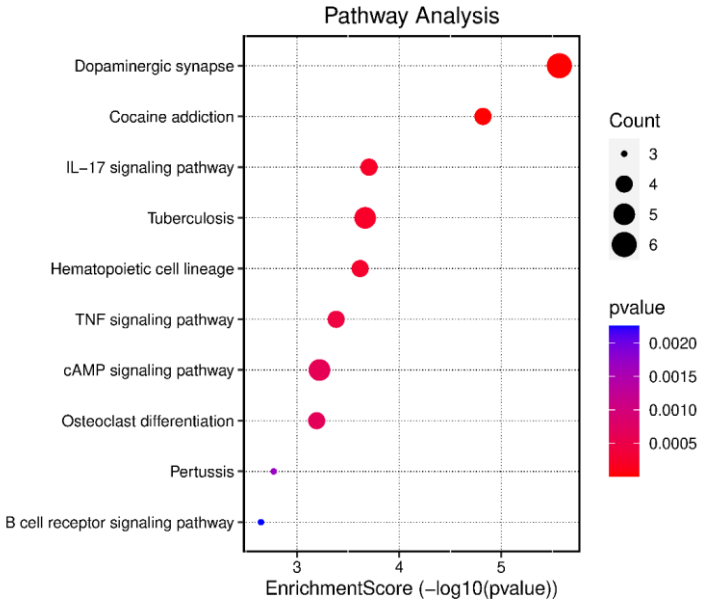


Figure 4 KEGG signaling pathway enrichment analysis of the top 10 most significantly enriched KEGG pathways.

4. Discussion

Although Parkinson's disease does not have a clear name in Chinese medicine, it is mostly classified as "tremor" or "spasm" in Chinese medicine. The pathogenesis of Parkinson's disease is mostly caused by wind, fire, phlegm and blood stasis, resulting in internal movement of liver and wind and loss of nourishment of the tendons and veins. The location of the disease is mainly in the tendons and veins, and is closely related to the liver, spleen and kidney. Treatment is to clear heat, resolve phlegm and calm the wind, nourish the liver and kidney, and benefit qi and nourish blood.

As can be seen from Table 2, the acupoints selected for acupuncture treatment of Parkinson's disease, the most frequently used and the number of points selected are the Governor's Vessel. Hua Boren's "Fourteen Scriptures in play" says: "The Governor is the word for all, and the line in the back, for the Yang Vessel of the capital outline." It is called the "Sea of Yang Vessels" and has the role of supervising the Yang energy of the whole body and directing the Yang meridians. Plain Questions - Bone and Space Theory" says: "The Governor's Vessel, the upper forehead crosses the top and enters the brain. The main trunk of the Governor's Vessel travels in the middle of the back and enters the brain, which is the house of the vital energy, so the Governor's Vessel plays an important role in the treatment of brain diseases.

In recent years, a large number of studies have been conducted on the mechanism of action of acupuncture in the treatment of Parkinson's disease, and many experimental evidences have shown that acupuncture can affect dopamine neurons, produce anti-excitotoxic effects, improve oxidative stress, regulate immune abnormalities, and modulate mitochondrial dysfunction to alleviate Parkinson's disease symptoms. And in this paper cAMP signaling pathway, IL-17 signaling pathway and dopaminergic synapse is consistent with the literature, suggesting that acupuncture for Parkinson's disease may be effective by activating the cAMP signaling pathway, IL -17 signaling pathway and dopaminergic synapse [21,22]. Further validation through experiments is required for follow-up.

5. Summary

In this paper, we first summarized the acupuncture point pattern of acupuncture for Parkinson's disease through literature review, and used the GEO database and target prediction of active substances produced by acupuncture as potential targets of acupuncture for Parkinson's disease. Then, the above potential targets were intersected with the targets of Parkinson's disease to obtain the key targets of acupuncture for the treatment of Parkinson's disease. Finally, by GO and KEGG enrichment analysis, it was clarified that acupuncture may treat Parkinson's disease by activating cAMP signaling pathway, IL-17 signaling pathway and dopaminergic synapse.

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