Multi-chip Meta-analysis of Parkinson's Disease for Clinical Validation of Small Samples of Key Genes in Disease

Unit in charge of clinical research： Zhujiang Hospital of Southern Medical University

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Confidentiality statement

Research plan confirmation signature page

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Consent from the principal investigator on the protocol:

I have read the program carefully, agree with all the necessary information in the program to conduct the research, and agree to execute as described in the program. I understand that the research should not be initiated without the approval of the ethics committee and should fully comply with the relevant regulations of the unit.

Informed consent and appropriate documentation are required for all participants in the study. After obtaining the written informed consent signed by the subjects, the study will be carried out according to the declaration of Helsinki and the requirements of laws and regulations related to clinical research.

_____	_____	_____
Name of principal investigator	signature	date

Summary of research programme

Title	Multi-chip Meta-analysis of Parkinson's Disease for Clinical Validation of Small Samples of Key Genes in Disease
Investigator	Gao Xiaoya, deputy chief physician
Main research units	Zhujiang Hospital of Southern Medical University
research center	Department of Neurology, Zhujiang Hospital, Southern Medical University
research objective	The research team used meta-analytical statistical methods to integrate the results of different research groups on Parkinson's disease, using meta-analysis to find key genes related to the pathogenesis and development of Parkinson's disease, and to make small clinical results. The verification of the sample, the internal mechanism of the pathogenesis of Parkinson's disease and provide guidance and reference for subsequent experimental research.
research hypothesis	Synaptic related functions may be closely related with the development of Parkinson's disease(PD). Alpha - synuclein (alpha alpha-synuclein), PPP3CB (protein phosphorylase 3 catalytic subunit beta) and other genes may be key genes in the pathogenesis of Parkinson's disease, and their expression may be closely related to the occurrence and development of PD.
research design	Observational case-control clinical study
Subject population	Inclusion criteria: 1. PD group: Patients with primary PD; 2. Patients with Parkinsonism-Plus syndrome (PPS): Patients with Progressive superanuclear palsy (PSP) and multiple system atrophy (MSA); 3. Healthy controls (HC);
diagnostic criteria	2015 International Parkinson's and Movement Disorders Association (MDS) Parkinson's disease diagnostic criteria. 2017 clinical diagnostic criteria for MDS progressive supra palsy; Giman standard, the 2008 American board of neurology (AAN) MSA diagnostic consensus.
sample size	The study was divided into PD group, PPS group and healthy control

	group, at least 80 cases, 23 cases and 110 cases, respectively.
Main exposure factors	1. After admission, all patients and the control group took 2 tube venous blood samples of 12 ml and saliva samples of 20 ml respectively and sent them to the laboratory for testing. 2. Unified Parkinson's Disease Rating Scale (UPDRS), non motor symptom rating scale (NMSS), Hamilton Depression Scale(HAMD), Hamilton Anxiety Scale (HAMA), Montreal Cognitive Assessment(MoCA) and Pittsburgh sleep quality index (PSQI) were used to assess the motor and non-motor symptoms of PD patients; The clinical symptoms of patients with PSP were evaluated by Progressive superanuclear palsy Rating Scale (PSPRS) and MSA by Unified multiple system atrophy Rating Scale (UMSARS).
Outcome variable	1. The primary end point: the expression level of alpha synuclein, PPP3CB and miRNA-ceRNA (microRNA-endogenous competitive RNA) network and its target protein in the disease group and the control group; 2. Secondary end point: correlation between gene, transcriptome , target protein expression and clinical indicators of patients and analysis of risk factors for Disease.
Data collection and management	After the completion of the experiment, the case observation table and experimental test data will be reviewed by the researcher and then recovered, and data processing will be carried out. The data of the case report form was input in double copies, and the American clinical trial database was locked after the examination and confirmation.
Statistical methods	Statistical hypothesis: it is assumed that the expression of alpha synuclein, PPP3CB and other genes in PD patients is different among PD, PPS and healthy controls, and has a good correlation with clinical indicators such as the severity of PD. Main statistical analysis methods: SPSS 10.0 software was used to analyze the data. The normal distribution of the measurement data is represented by mean ± SD, and the comparison among the three groups is represented by one-way ANOVA; the non-normal distribution data is represented by median (quartile), and Kruskal Wallis rank test; the count data is represented by chi-square test; the grade data is

	represented by Ridit analysis or chi-square test.The value of $P < 0.05$ was considered statistically significant.ROC curve is used to analysis genes diagnostic value, when $AUC > 0.5$, the closer AUC is to 1, the better the diagnosis effect.The accuracy of AUC is lower when it is 0.5-0.7, medium when it is 0.7-0.9 and higher when it is above 0.9.When $AUC=0.5$, the diagnostic method is completely ineffective and has no diagnostic value.$AUC<0.5$ is not consistent with the real situation.The risk factors of disease were analyzed by logistic regression.

Catalog

1 research background and purpose

1.1 research background

Parkinson's disease (PD) is a relatively common degenerative disease of the central nervous system. As society gradually becomes aging, the number of PD patients is increasing, but its exact pathogenesis is still not fully understood. May be related to genetic factors, environmental factors, immunological abnormalities, mitochondrial dysfunction and oxidative stress, ageing, apoptosis and other factors; the current genetic diagnosis is in the ascendant, making the understanding of the etiology and pathogenesis of Parkinson's disease more In-depth, provide more basis and means for the pathogenesis and development of Parkinson's disease, but due to the number of individual samples, operational norms and platform differences, different research groups have great differences in the results of gene chip research on Parkinson's mechanism, resulting in the reliability is poo; in order to improve the reliability of Parkinson's disease onset and development gene diagnosis,We will carry out the research of integrating the results of different research groups by meta-analysis to find the key genes of Parkinson's disease, and carry out the clinical verification.

1.2 purpose of the study

The research group uses the statistical method of meta-analysis to integrate the research results of different research groups on Parkinson's disease chip together, uses meta-analysis to find the key genes related to the pathogenesis and development of Parkinson's disease, and carries out small sample verification in clinical, excavates the internal mechanism of Parkinson's disease and provides guidance and reference for the follow-up experimental research.

1.3 research hypothesis

Synaptic function may be closely related to the occurrence and development of PD. Alpha synuclein, PPP3CB and other genes may be the key genes in the pathogenesis of PD, and their expression is related to the pathogenesis and development of PD patients.

2 Study design and procedure

Meta analysis and clinical retrospective case-control design were used in this study.The research period is about 2 years.

3 subjects (cohort selection)

PD patients, PPS patients in the inpatient department or outpatient department of Neurology Department of Zhujiang Hospital who meet the requirements and the healthy control population in the physical examination center of Zhujiang Hospital from November 2018 .

3.1 diagnostic criteria

PD group: patients with Parkinson's disease were diagnosed according to 2015 International Association of Parkinson's and Movement Disorders Association (MDS);

PPS group: patients with Parkinson-plus syndrome were diagnosed according to 2017 MDS progressive supranuclear paralysis and 2017 clinical diagnostic criteria for MDS progressive supra palsy and Giman standard, the 2008 American board of neurology (AAN) MSA diagnostic consensus.

3.2 inclusion criteria

Inclusion criteria:PD group: Patients with primary PD according to the 2015 International Association of Parkinson's and Movement Disorders Association (MDS); 2. Patients with Parkinson-plus syndrome:according to 2017 MDS progressive supranuclear paralysis and 2017 clinical diagnostic criteria for MDS progressive supra palsy and Giman standard, the 2008 American board of neurology (AAN) MSA diagnostic consensus. 3. Healthy controls.

3.3 Exclusion criteria

1.Other causes of Parkinson's syndrome, including cerebrovascular disease, encephalopathy, trauma and drugs;

2.people with ther neurodegenerative diseases, such as huntington's disease, lewy body dementia, alzheimer's disease, etc.

3.The person have other disabilities or diseases, such as aphasia, severe dementia or consciousness disorders, malignant tumors, liver and kidney dysfunction, serious heart disease or other acute or chronic diseases or life-threatening diseases.

4.someone who refuse to participate in the study.

3.4 Patient recruitment

Patients with Parkinson's disease or Parkinson-plus syndrome who met the requirements after 2018-11 in the Department of Neurology of Zhujiang Hospital, and healthy controls in the health examination center of Zhujiang Hospital.

4 observation indicators

General clinical data, CT, MRI and other imaging examinations within one week after admission, and PD group patients, MDS-UPDRS,NMSS, HAMD, HAMA, Moca, PSQI, H & Y rating scale and other scales were used to assess the movement and non-movement symptoms of PD patients, PSPRS was used to assess the clinical symptoms of PSP patients, UMSARS was used to assess the clinical symptoms of MSA patients.

4.1 General clinical characteristics (including demographic indicators)

Age, sex, admission diagnosis, comorbidity, routine examination, etc.

4.2 main exposure factors/factors affecting prognosis(including control selection)

All the participants took 3 tubes of venous blood for gene detection; this study did not interfere with the clinical treatment of patients, and the clinical treatment of patients was carried out according to the guidelines and relevant specifications.

4.3 study end point

The primary end points: The expression levels of alpha-synuclein, PPP3CB and other genes and proteins in peripheral blood and saliva.

Secondary end point: The correlation between the expression level of alpha synuclein, PPP3CB in peripheral blood and saliva and the clinical characteristics of PD and PPS, and the analysis of risk factors of PD and PPS.

4.4 potential confounding variables

Patients may have unknown diseases that affect the expression level of alpha synuclein, PPP3CB and other genes and proteins or take related drugs.

5 sample size estimation

The patients were divided into PD group (an least 80 cases), PPS group (at least 23 cases) and control group (at least 110 cases).

6 data collection and data management

6.1 data source

The data came from general population data and clinical data, peripheral blood and saliva test results, PD and PPS patients' related scale evaluation results.

6.2 data collection

1.Make a case observation table, and record the age, gender, race, admission diagnosis, complications, neurological physical examination, laboratory examination, craniocerebral imaging and other general demographic, clinical symptoms and signs as well as routine examination and examination data of the patients.

2. Expression level of alpha-synuclein, PPP3CB and other genes and proteins in peripheral blood and saliva.

3. In PD group, MDS-UPDRS, NMSS, HAMD, HAMA, MoCA, PSQI, HOEHN&YAHR (H-Y) grading were used to assess the motor and non motor symptoms and disease severity of PD patients, PSPRS were used to assess the clinical symptoms of PSP patients, UMSARS was used to assess the clinical symptoms of MSA patients.

6.3 database

The study data will be updated in real time in the US clinical trials database (Clinical Trials. Gov).

6.4 data Management plan and quality control

The completed case observation form, gene and protein detection data shall be reviewed by the supervisor and then recovered, and handed over to Chaohao Guan and Cailing Feng for data processing. The data in the case report form shall be entered in double copies (Chaohao Guan and Cailing Feng). After the inspection and confirmation, the data shall be locked in the US clinical trial database.

7 statistical analysis

(1) statistical software: use SAS 22.0 statistical software for statistical analysis.

(2) basic principle: Bilateral test was used for all statistical inferences. The test level with statistical significance was set at 0.05, and the confidence interval of parameters was estimated to be 95%.As far as possible, parameter method can be adopted. When the data does not meet the condition of parameter method, data transformation method can be adopted to make it meet the condition. If it still does not meet the condition, non-parameter method can be considered.

(3) minimum, maximum, P25, median and p75 are given when necessary;Matching measurement data to mean and standard deviation of travel value; the median and average rank are given by the nonparametric method.The frequency distribution and the corresponding percentage are given by counting data.The frequency distribution and the corresponding percentage, as well as the median and average rank are given in the rank data.Qualitative data showed positive rate, positive number and number of cases in the denominator.

(4) analysis of baseline data: description and analysis of baseline data (including demographic indicators, etc.).

(5) one way ANOVA was used to analyze the differences among groups.

(6) ROC curve was used to analyze the correlation between gene and diagnosis. when AUC > 0.5, the closer AUC is to 1, the better the diagnosis effect. The accuracy of AUC is lower when it is 0.5-0.7, medium when it is 0.7-0.9 and higher when it is above 0.9. When AUC=0.5, the diagnostic method is completely ineffective and has no diagnostic value. AUC<0.5 is not consistent with the real situation. The risk factors of disease were analyzed by logistic regression.

(7) Logestic regression analysis is used to analyze the risk factors of PD and PPS , and the differences among each group.

8 ethical considerations

To ensure that this clinical study complies with the declaration of Helsinki and relevant Chinese regulations on clinical research. Subjects can be selected for clinical study only after signing the informed consent. The researchers guaranteed to maintain the privacy of the subjects.

9 quality control and quality assurance

Xiaoya Gao, the head of clinical trial, Qing Wang, Chaohao Guan, Xiaobo Wei , Cailing Feng and Xiaomei Liang, the fixed members of the project, carried out the study strictly according to the requirements of the clinical trial plan. The applicant appointed 1-2 supervisors (Lingling Zhang) to supervise and cooperate with the work of the experimental unit at any time. The person in charge of the project checked the case record at any time and solved the possible problems in time.

10 organization and Implementation (time schedule)

Two years of clinical trials, statistical processing of clinical trial data and writing and publishing papers for about one year.

11 responsibilities of all parties involved

Xiaoya Gao, deputy chief physician: project leader, responsible for the design and development of clinical trial implementation plan and clinical evaluation.

Qing Wang, Chief Physician: CO researcher, participated in the design of clinical research program.

Chaohao Guan: Assistant Researcher, responsible for sample collection, data collection and statistics.

Xiaobo Wei: Assistant Researcher, in charge of sample testing, etc.

Cailing Feng postgraduate: Assistant Researcher, responsible for sample testing and other work.

Xiaomei Liang, postgraduate: Assistant Researcher, in charge of sample testing and other work.

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