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RAPID COMMUNICATION

Rare variant analysis of *PLXNA1* in Parkinson's disease in the Chinese population

Recently, p.Glu1121Ter in *PLXNA1* was identified as potential cause for a patient with parkinsonism. However, no further replication has been conducted in a wider range of Parkinson's disease (PD) cohorts. To evaluate the genetic association of *PLXNA1* with PD, we systematically analyzed the rare protein-coding variants in 1,245 Chinese patients with whole exome sequencing. Fisher's exact test was performed between each variant and the risk of PD, while over-representation of rare variants in patients was examined with optimized sequence kernel association test at gene level. Totally, 42 rare variants were identified. At variant level, p.V172M was significantly associated with PD, while p.L12M and p.R571C were nominally associated with PD. Burden analysis showed enrichment of ultra-rare variants of *PLXNA1* in PD. In addition, one patient carried a variant in the neighboring amino acid, and diaplayed clinical characteristics similar to those of the patient in the original study. Our study explored the rare variant of *PLXNA1* in PD in the Asian population and paved the way for future research.

PD is a common neurodegenerative disorder caused by the degeneration of dopaminergic neurons in the substantia nigra pars compacta. The exact pathogenesis of PD is complex, and mounting evidence has supported the important role of genetic factors in the susceptibility of PD. Recently, post-mortem exome sequencing on a 38-year-old patient with developmental delay who developed parkinsonism later identified a heterozygous nonsense mutation p.Glu1121Ter in *PLXNA1* as the potential disease cause.[1] *PLXNA1* is a member of a large family of cell-surface receptor proteins which are involved in developing cortical axons within the central nervous system,[2] and was suggested to be involved in several neurological disorders.[3,4] However, no further replication study has been conducted to explore the role of *PLXNA1* in a wider range of PD populations.

In this context, we aimed to systematically evaluate the genetic associations of *PLXNA1* in a Chinese PD cohort. A total of 1,245 unrelated Chinese PD patients were recruited from the Department of Neurology of West China Hospital of Sichuan University. The patients were diagnosed by two neurologists specializing in movement disorders according to either the Movement Disorder Society clinical diagnostic criteria for PD or the United Kingdom PD Society Brain Bank Clinical Diagnostic Criteria. The genomic DNA of each patient was extracted from peripheral blood mononuclear cells using standard phenol-chloroform procedures. Then whole exome sequencing was conducted on the Illumina NovaSeq 6000 system following the manufacturer's instructions. Controls were 2,657 individuals of Chinese ancestry from the SG10K pilot study. Paired-end 151-bp whole genome sequencing was performed on the Illumina HiSeq 4000 platform or HiSeq X platform.

The rare variants which met the following criteria were analyzed[5]: (1) minor allele frequency (MAF) was lower than 0.01; (2) variants were annotated as missense, splice donor, splice acceptor, start-lost, stop-gained, stop-loss or frameshift substitution by ANNOVAR. Allelic association analysis was performed using standard Fisher's exact test with default parameters. The summary data of the East Asian population from gnomAD v2.1.1 was also used as population controls.

Gene-based rare variant burden analysis was conducted to evaluate the aggregate association of rare variants in *PLXNA1* with PD using the optimized sequence kernel association test (SKAT-O, R package). We adjusted sex and the first three principal components. Considering that variants with different MAF might have various consequences, we categorized variants into rare variants (MAF < 0.01) and ultra-rare variants (MAF < 0.001). For each category, we tested the association for all rare variants and rare

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damaging variants, which were predicted as damaging or pathogenic by at least 5 of 10 *in-silico* tools. Power calculation was performed with the Power_Logistic function with default parameters provided in the SKAT R package. Given the number of samples, the disease frequency of 0.001 and the significance level of 0.05, the cohort has 91.6% power to detect association for variants with MAF $<$ 0.01 under 1,000 simulations.

We further tried to explore whether rare *PLXNA1* variants were associated with specific clinical features. Trained interviewers collected detailed information on demographic and clinical characteristics at baseline when the patients were enrolled at the hospital. Patients with unknown clinical characteristics were excluded. Student's *t*-test and Fisher's exact test were used for continuous variables and categorical variables respectively in the association analysis. We first analyzed patients with *PLXNA1* rare variants and those without. Then we analyzed patients with variants which showed association with PD (p.V172M, p.L12M, and p.R571C) and those without.

The average age at onset (SD) of the patients was 46.24 (12.11) with a sex ratio of 1.06 (male/female: 640/605). A total of 42 rare variants with MAF $<$ 1% were identified, all of which were missense variants (Table S1, and Fig. S1). Among these variants, 38 were ultra-rare (MAF $<$ 0.001), and 25 were predicted as pathogenic by at least 5 *in-silico* prediction tools (Table S2). One variant p.V172M was significantly associated with an increased risk of PD after the Bonferroni correction (Table S1). Another two variants were nominally associated with PD, namely p.L12M and p.R571C. One patient with homozygote of p.V172M was identified, and her mother was diagnosed with PD in her fifties as well. At the gene level, ultra-rare variants and rare damaging variants in *PLXNA1* were significantly enriched in PD patients (Fig. 1). Notably, one patient carried a variant in the neighboring amino acid p.R1122W of the variant in the original study.[1] Similar to the patient in the original study, this patient was with severe motor symptoms at baseline.

In addition, we analyzed whether the identified *PLXNA1* rare variants in PD patients were associated with specific clinical characteristics, including the motor section of the Unified Parkinson Disease Rating Scale III to assess the severity of motor symptoms, the Hoehn-Yahr stage to assess the severity of the symptom, the Hamilton Depression Rating Scale to evaluate depression, the Hamilton Anxiety Rating Scale to evaluate anxiety, the Frontal Assessment Battery to evaluate executive function, and the Montreal Cognitive Assessment (MOCA) to measure cognitive ability. However, no significant association was identified (Table S3).

PLXNA1 is a transmembrane receptor for semaphorins that act as axonal guidance cues during nervous system development. Pathogenic variants in *PLXNA1* have been originally reported in individuals with neurological disorders like epileptic encephalopathies, epilepsy, and neurodevelopmental syndrome mainly comprising developmental delay, brain, and eye anomalies.[3,4] Recently, a heterozygous nonsense *PLXNA1* variant p.Glu1121Ter was identified as the candidate disease cause for a 38-year-old patient with developmental delay and parkinsonism,[1] suggesting a potential association between *PLXNA1* and parkinsonism. In the current PD cohort, we identified one patient who carried a variant in the neighboring amino acid p.R1122W, which was absent from controls and the East Asian population from gnomAD. This variant was predicted as damaging by six prediction tools, and has a GERP score of 4.4, suggesting evolutionary conservation. Whole exome sequencing did not identify mutations in known PD genes for this patient. The patient developed parkinsonism at the age of 52, and his father was diagnosed with PD as well. However, we could not perform segregation analysis in this

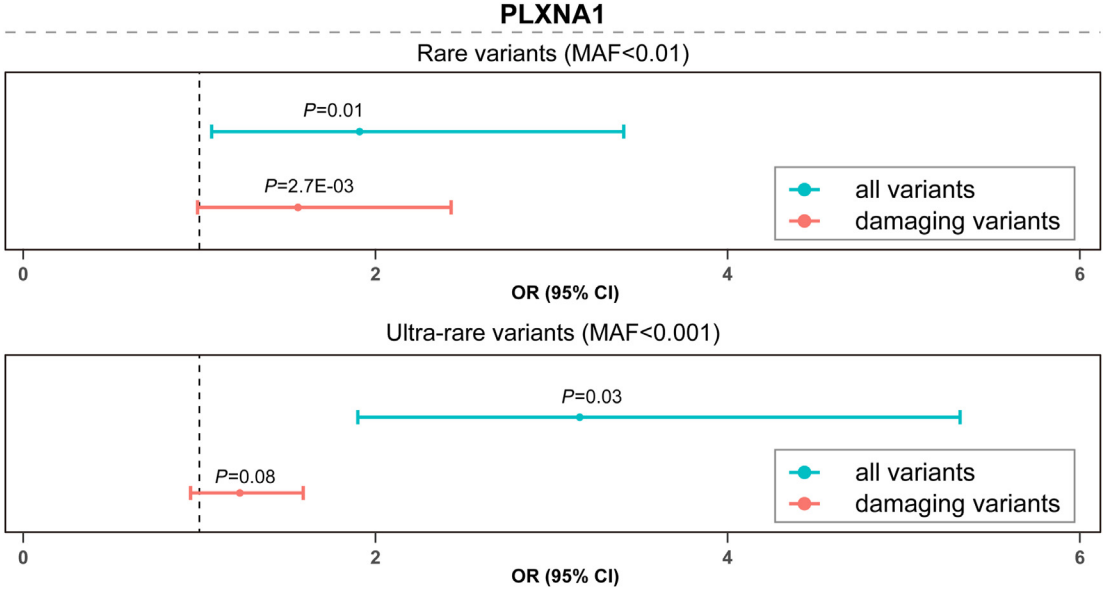

Figure 1 Enrichment analysis of rare variants in *PLXNA1* in PD. All rare variants and rare damaging variants with MAF $<$ 1% and MAF $<$1‰ were analyzed separately. OR and 95% confidence intervals (CI) were calculated using Fisher's exact test. *p* values were calculated with optimized sequence kernel association test (SKAT-O) under 1,000 permutations.

family since the blood sample of the father was not available. Similar to the patient in the original study,[1] he suffered severe motor symptoms at baseline, with the Unified Parkinson's Disease Rating Scale part III (UPDSR3) score of 30. The patient did not have cognitive impairment, with a MOCA score of 26. At variant level, p.V172M was significantly associated with an increased risk of PD. This variant was predicted as damaging by six prediction tools, and had a GERP score of 3.56, suggesting potential pathogenicity. At gene level, the burden test showed enrichment of ultra-rare variants of *PLXNA1* in PD.

In conclusion, we systematically analyzed the rare protein-coding variants of *PLXNA1* with association analyses at allele and gene levels. We demonstrate that ultra-rare variants of *PLXNA1* were enriched in PD patients, but further replication was still warranted. Our work explores the rare variant of *PLXNA1* in PD in the Chinese population, and paves way for future research on *PLXNA1* in PD.

Author contributions

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript: A. Writing of the first draft, B. Review and Critique; (4) A. Patients enrollment and clinical data collection C. Li: 1 A, 1C, 2 A, 2 B, 2C, 3 A; R. Ou: 2 B, 3 B, 4 A; Y. Hou: 3 B, 4 A; J. Lin: 3 B, 4 A; K. Liu: 3 B, 4 A; Q. Wei: 3 B, 4 A; X. Chen: 3 B, 4 A; W. Song: 3 B, 4 A; B. Zhao: 3 B, 4 A; H. Shang: 1 B, 2C, 3 B.

Conflict of interests

Authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.08.001.

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