De novo missense variants in the PPP2R5D gene associated with Parkinson's disease in Chinese Han population

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Research Article

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Abstract

Recent studies have reported an association between PPP2R5D mutations and early-onset levodopa-responsive parkinsonism. However, there was lack of comprehensive analysis of this gene in large Parkinson's disease(PD) cohort.The aim of this study was to examine the frequency and spectrum of PPP2R5D mutations in a Han Chinese cohort with early- or late-onset PD. We sequenced all 17 exons of PPP2R5D and their flanking intron regions in 668 sporadic PD patients. Novel variants detected were validated based on genomic databases and verified using genetic data from unrelated controls. Three of the 668 PD patients carried three novel, unique PPP2R5D exonic variants(p.R91S,p.E8A,p.R523L).These variants were predicted to be "disease- causing" by mutation taster and all three mutations were found to be highly conserved across species. Our study identified three novel, rare PPP2R5D variants among Han Chinese PD patients, which broadens the spectrum of PPP2R5D mutations potentially associated with the disease.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease among elderly[1].The prevalence and global burden of PD continues to increase at an exponential rate, severely threatening patients' quality of life. The histopathological hallmark of PD is the loss of dopaminergic neurons in the substantia nigra pars compacta and concomitant aggregation of Lewy bodies containing α-synuclein in surviving neurons[2]. Although researchers have proposed that genetic and environmental factors can play a role in PD[2], the precise pathophysiology of this neurodegenerative disease is not well understood.

In addition to causing familial PD, genetic mutations have been reported to account for sporadic disease onset and for increased risk of PD. So far, more than 20 genes have been identified as proximate causes of PD[3].The discovery of such disease-causing genes can enhance our understanding of how PD arises and progresses[4]. A recent study involving three European patients reported an association between an exonic mutation in the PPP2R5D gene, p.E200K, and early-onset levodopa-responsive parkinsonism[5]. Another study reported signs of levodopa-responsive parkinsonism in a young woman carrying a PPP2R5D mutation previously associated with neurodevelopmental disorders[6].Additionally, a study involving a patient with juvenile-onset parkinsonism reported a pathogenic variant, p.E250K, in exon 7 of the PPP2R5D gene[7].

The identification of these potential pathologic mutations in the PPP2R5D gene motivated our assessment of the association between PPP2R5D and PD in Han Chinese patients diagnosed with sporadic PD. Therefore, we sequenced the coding region, exon-intron boundaries, as well as untranslated and flanking regions of the PPP2R5D gene in order to help clarify the potential role of PPP2R5D mutations in patients with early- or late-onset PD.

Methods

2.1 Study subjects

We recruited consecutive Han Chinese patients with sporadic PD who were treated between 1th,Jan 2016 and 31th Dec 2020 at the Movement Disorder Center in the West China Hospital in Chengdu, China. All patients were diagnosed by two independent movement disorder specialists based on idiopathic PD criteria recommended by the United Kingdom Parkinson's Disease Society Brain Bank or the Movement Disorder Society. Patients were stratified into two cohorts based on age at onset: those with early-onset PD (EOPD) had an age at onset ≤ 50 years, and those with late-onset PD (LOPD) patients had an age at onset > 50 years. 600 unrelated healthy Han Chinese subjects without a history of neurodegenerative disease were recruited as controls.

This study was approved by the Ethics Committee of the West China Hospital, Sichuan University. Written informed consent was obtained from all patients or their legal guardians for their anonymized clinical and genetic data to be analyzed and published for research purposes.

2.2 PPP2R5D sequencing and statistical analysis

The methods of sequencing PPP2R5D (GenBank NM_006245) was reported before.

Chromatograms were double-checked by two independent researchers to ensure that no variants were overlooked. Variants identified were designated as "known" or "novel" after comparing them to variants indexed in the following databases: ESP6500(http://evs.gs.washington.edu/EVS/), gnomAD_exome (https://gnomad. broadinstitute.org/), and the 1000 Genomes

Project(https://www. genome.gov/ 27528684/1000-genomes-project). The function of the mutations was predicted by Mutation Taster(http://www.mutationtaster.org/) and SIFT(http://sift.jcvi.org/) online software. The mutations also be verified in 600 unrelated healthy Han Chinese controls.

If a potential exonic mutation was detected in a patient, whole-exon sequencing was performed to exclude variants relevant to parkinsonism. We used the STRING database(https://www.string-db.org/) to predict the proteins that interact with PPP2R5D.

Results

We analyzed clinical and genetic data collected from 203 patients with EOPD (age at onset, 44.2 ± 3.8 years) and 465 patients with LOPD (age at onset, 61.6 ± 7.3 years). After sequencing all 17 PPP2R5D exons in all recruited patients, we identified three men with PD, each carrying a unique exonic missense variant: Patient 1 carried the p.R91S variant, Patient 2 carried the p.E8A variant, and Patient 3 carried the p.R523L variant (Table 1, Fig. 1A).

We calculated a PPP2R5D exonic mutation rate of 0.9% for the EOPD patients and 0.2% for the LOPD patients. All three variants identified in this study were “novel” since they had not been previously recorded in the databases examined, nor were they observed in the 600 healthy controls. In addition, all three mutations were found to be highly conserved across species (Fig. 1B).

Based on Mutation Taster, all three variants were predicted to be “disease-causing”. Based on SIFT, the p.R91S and p.R523L variants were predicted to be “damaging” and “tolerable”, while the p.E8A variant was predicted to be “tolerable”. Additionally, whole-exon sequencing revealed no other potential variants relevant to parkinsonism in these three patients. The STRING database predicted an association between PPP2R5D and Glucogen Synthase Kinase 3 Bate(GSK3β) (Fig. 1C).

Discussion

In this study, we sequenced the PPP2R5D gene in 203 EOPD and 465 LOPD Han Chinese patients, and identified three de novo exonic variants, one in a LOPD patient and two in EOPD patients. All three variants are novel and potentially pathogenic with respect to PD. The exonic mutation rate of PPP2R5D in our Han Chinese patients was 0.9% among those with EOPD and 0.2% among those with LOPD. As far as we know, this is the first study to assess genetic sequences of exonic variants in the PPP2R5D gene based on data from a large cohort of EOPD and LOPD patients.

The PPP2R5D gene encodes the B subunit (B56) of protein phosphatase 2A (PP2A). PP2A plays a role in key neuronal and developmental regulation processes by regulating PI3K/AKT and GSK3β-mediated cell growth, chromatin remodeling, and gene transcription[8,9] (Fig. 1C). The E420K mutation of PPP2R5D has been shown in cell culture studies to constitutively activate AKT/mTOR signaling, leading to larger cells and uncoordinated cell growth[10] . Following phosphorylation of S129, PP2A can also regulate α-synuclein, which is an integral part of the Lewy bodies and a pathophysiological hallmark of PD[11]. Increased activity of PP2A activates tyrosine hydroxylase and, consequently, dopamine synthesis[12]. Knocking out the PPP2R5D gene in mice leads the microtubule-associated protein tau to become progressively phosphorylated[13]; such tau can aggregate in PD, either contributing to the disease or simply reflecting its progression[14]. Our study justifies further research into the mechanisms behind the apparent association between PPP2R5D and PD.

A recent study involving three men carrying the p.E200K mutation in the PPP2R5D gene and one patient reported severe atrophy of the substantia nigra but the absence of Lewy body pathology (Patients 4 in Table 1)[5]; similar pathologic characteristics were observed in PD patients with mutations in the parkin gene. All three patients with the p.E200K mutation showed levodopa response, sporadic early-onset parkinsonism, and mild intellectual disability. In another study, a patient with a PPP2R5D p.E250K mutation showed levodopa-responsive, early-onset parkinsonism[6]: FP-CIT (DaTscan) SPECT imaging performed in his early 20s showed

lower dopamine uptake in the right basal ganglia than in the left, and the patient rapidly developed motor fluctuations and dyskinesias. Furthermore, a 29-year-old woman with a p.E198K mutation in the PPP2R5D gene showed PPP2R5D-related neurodevelopmental disorder as well as levodopa-responsive, early-onset parkinsonism, indicating that PPP2R5D can play a role in both PPP2R5D-related neurodevelopmental disorder[15,16] and parkinsonism[7]. Compared to patients who carry the E200K mutation, those with the E198K mutation tend to experience more severe intellectual disability and developmental delay[16]. This reflects the critical role of E198K in subunit interaction and binding.

Our study identified three patients with three unique exonic mutations in the PPP2R5D gene. Patient 1 was a 40-year-old man carrying the R91S mutation in exon 3. He developed the first symptoms of PD when he was 39 years old; he had no familial history of neurodegenerative disease.Although the patient experienced mild anxiety, he showed no obvious signs of depression, and his Moca and MMSE scores were normal. Furthermore, he experienced no motor complications, had a H-Y stage of 1.0, and responded well to a rotigotine transdermal patch (6 mg). Patient 2 was a 47-year-old man carrying the R523L mutation in exon 13. He reported no familial history of neurodegenerative disease. He began showing bradykinesia of his left arm when he was 43 years old, followed by the development of serious gait disorder, Freezing of gate(FOG), and wearing-off phenomenon over the next three years. These symptoms responded to treatment using levodopa.Patient 3 was a 59-year-old man carrying the E8A mutation in exon 1. The patient developed PD when he was 56 years old. After treatment with levodopa and piribedil sustained-release tablets, he was able to achieve 70% remission, resulting in the wearing-off phenomenon at approximately three years after his initial diagnosis.

Consistent with previous studies, our study identified two EOPD patients carrying PPP2R5D mutations [6-8]. The present study appears to be the first to detect a PPP2R5D mutation in an LOPD patient. In silico analyses predict that the p.E8A and the p.R91S variants are “disease-causing” mutations. Since these mutations occur at the 5’ promoter region of PPP2R5D, they could potentially affect transcription. In contrast, the R523L mutation is located at the armadillo-type fold of PP2R5D; this structure has been reported to be functionally important for other genes(eg.beta-catenin)[17]. Further studies should be performed to understand the functions of these mutations in vivo.

We must consider the results of our study in the light of certain limitations. The related small number of patients in the EOPD cohorts, as well as our exclusion of patients with a familial history of PD may have caused a bias in our understanding of the pathophysiology of PD. Furthermore, we could not examine the effects of other genes on PD because we restricted our sequencing analysis to the PPP2R5D gene and conducted whole-exome sequencing only for the three patients who carried the variants. Since we only predicted the function of the three mutations using in silico tools, functional experiments are needed to understand the role of these variants in PD.

Despite these limitations, our findings suggest that mutations in the PPP2R5D gene may associated with PD in Han Chinese. Further studies must be conducted to understand the effects of these three exonic variants of PPP2R5D in PD patients, as well as identify other mutations that might be associated with the disease. Based on previous studies and our findings, we recommend adding the PPP2R5D gene to the gene panel sequencing analysis conducted to detect early-onset PD.

Author Declarations

Ethical Approval and consent to participate : The protocol of this study was approved by the Ethics Committee of West China Hospital of Sichuan University (Chengdu, China), and the study was performed in accordance with the ethical standards in the 1964 Declaration of Helsinki and its amendments.

Consent to publication : All authors have approved the contents of this manuscript.

All the authors Data Availability of data and materials: The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

Competing interests: The authors declare no competing interests.

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Authors' contributions:XLY,PPN,KLL concept the idea,acquisition,interpretation of the data, and drafted the manuscript. HR and YMX contributed to the study , acquisition of the data, statistical analysis, and critical revision of the manuscript for important intellectual content.

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Compliance with Ethical Standards

Disclosure of potential conflicts of interest: The authors declare that they have no competing interests.

Research involving Human Participants : The study was performed in accordance with the ethical standards in the 1964 Declaration of Helsinki and its amendments.

Informed consent: On admission, each participant signed written informed consent for their data to be analyzed and published anonymously for research purposes.

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Tables

Table 1. Clinical characteristics of Han Chinese patients with PD carrying PPP2R5D mutations

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Reference	Our study	Our study	Our study	Ref. 6	Ref. 6	Ref. 6	Ref. 7	Ref. 8
Ethnicity	Han Chinese	Han Chinese	Han Chinese	European	European	European	NA	European
Mutation	p.R91S	p.E8A	p.R523L	p.E200K	p.E200K	p.E200K	p.E250K	p.E198K
Sex	Male	Male	Male	Male	Male	Female	Male	Female
Age at recruitment in years	41	60	47	61	34	44	NA	29
Age at PD onset in years	39	56	43	40	27	22	20	20
Onset symptom	Right arm, bradykinesia	Right arm, rest tremor	Left arm, bradykinesia and rigidity	Gait difficulty	Right arm, rest tremor	Asymmetric, rest tremor	Left arm, rest tremor and bradykinesia	Tremor
Motor symptom	B,R	T,R	B,T,R,P	B,P,	T,R,P	T,P	B,T	B,T,R
Motor complications	No	No	Fluctuations	Fluctuations	Fluctuations	No	Fluctuations and dyskinesias	NA
ICD	-	-	-	+	+	NA	NA	NA
RBD	-	-	-	-	+	-	-	NA
Levodopa response	+	+	+	+	+	+	+	+
Neuroimaging results	Normal	Normal	Normal	MRI: subcortical white matter hyperintensities (T2)	Normal	None	MRI□hydrocephalus and aqueductal stenosis; FP-CIT (DaTscan) SPECT imaging: reduced dopamine uptake	SPECT imaging: bilateral reduction of the presynaptic dopamine transporter in the striatum (more pronounced on the left side)
Comorbidity	No	No	No	No	No	No	Attention deficit hyperactivity disorder, and an auditory processing disorder	Delayed psychomotor development, muscular hypotonia, macrocephaly, seizures, and dysmorphic facial features

Motor symptoms were classified as “B”,bradykinesia; “P”, postural instability and gait difficulty; “R”, Rigidity, or “T”,Tremor.
ICD, Impulse control disorder; MRI,magnetic resonance imaging; NA, not available; PD, Parkinson’s disease; RBD, Rapid-eye-movement sleep disorders; SPECT, Single photon emission computed tomography

Figures

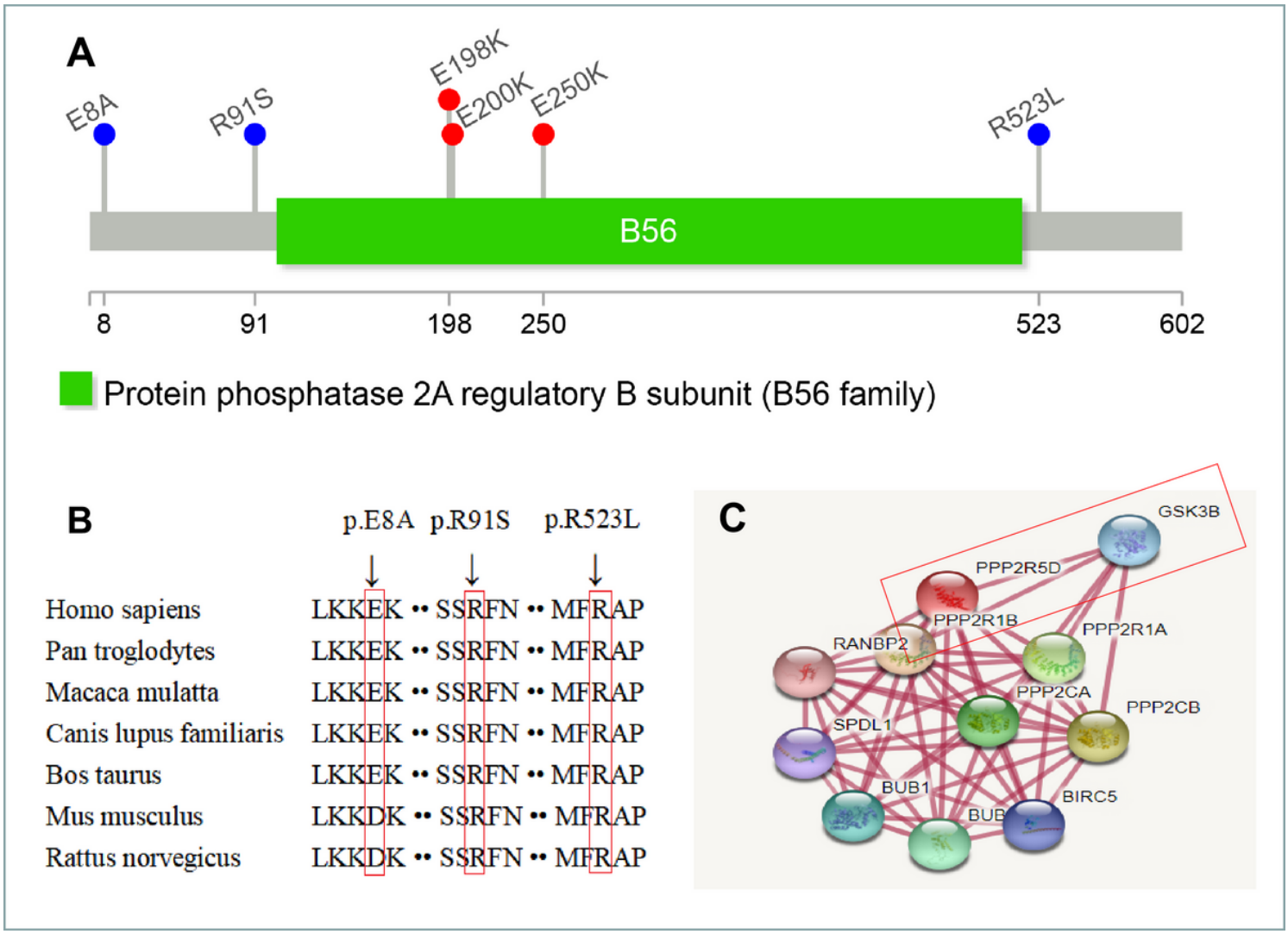

Figure 1

(A) PPP2R5D mutations detected in patients with Parkinson's disease (blue indicates mutations detected in our study; red, mutations reported in previous studies). (B) Conservative analysis of PPP2R5D mutations identified in our Han Chinese PD patients. (C) Relationship between PPP2R5D and other interaction proteins based on the STRING database.