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REPORT

Genetic Analysis of *FBXO2*, *FBXO6*, *FBXO12*, and *FBXO41* Variants in Han Chinese Patients with Sporadic Parkinson's Disease

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Abstract Parkinson's disease (PD) is the second most common neurodegenerative disorder and has an elusive etiology. It is likely multifactorial, and genetic defects contribute to its pathogenesis. At least 25 genetic loci and 20 monogenic genes have been identified in monogenic PD. Recessive F-box protein 7 gene (*FBXO7*) mutations reportedly cause hereditary parkinsonism. To explore the roles of four paralogs (*FBXO2*, *FBXO6*, *FBXO12*, and *FBXO41*) in PD development, their variants (rs9614, rs28924120, rs6442117, and rs61733550, respectively) were analyzed in 502 Han Chinese patients with PD and 556 age, gender, and ethnicity-matched normal participants in mainland China. Statistically significant differences in genotypic and allelic frequencies were detected only in the *FBXO2* variant rs9614 ($P = 0.001$ and 0.023, respectively; odds ratio 0.819, 95% confidence interval 0.690–0.973) between patients and controls. These results suggest that the *FBXO2* variant rs9614 C allele may decrease the PD risk in mainland Han Chinese and may be a biomarker for PD.

Keywords Parkinson's disease · *FBXO2* · *FBXO6* · *FBXO12* · *FBXO41* · Variant

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Introduction

Parkinson's disease (PD, OMIM 168600) is a common, progressive, multifactorial, neurodegenerative disease caused by genetic and environmental risk factors [1–3]. It is age-related, affecting ~1% of the population over 60 years of age, rising to 4% in people over 85 [4, 5]. Motor parkinsonism, defined as bradykinesia plus rigidity or rest tremor, is the cardinal clinical feature [6]. Other motor and non-motor symptoms are also frequently observed in PD [7]. Pathologically, PD cases have dopaminergic neuronal loss in the substantia nigra pars compacta. Lewy bodies, which are substantial intracytoplasmic inclusions with ubiquitylated alpha-synuclein, form in surviving neurons [7, 8]. Degeneration and death of dopaminergic neurons in PD are postulated to result from pivotal cellular system impairments, including oxidative stress, mitochondrial dysfunction, disrupted proteolysis referring to the ubiquitin-proteasome system or lysosomal autophagy, neuroinflammation, and/or excitotoxicity [9–11]. Although the pathogenic mechanisms remain unclear, at least 25 loci and 20 disease-linked genes have been identified to be responsible for monogenic PD [7, 12–14]. It has been reported that mutations in a critical domain of a gene such as leucine-rich repeat kinase 2 (*LRRK2*) can cause monogenic PD, whereas variants in a non-crucial region may either increase the risk or play a protective role [15–17]. Known heritable components are responsible for 5%–10% of PD patients. Most cases are sporadic forms resulting from a combination of multiple factors [13, 18]. It has been proposed that variants, particularly nucleotide alterations

causing amino-acid changes in disease-causing genes or their biologically relevant paralogous genes, play a role in the risk of sporadic PD [12, 19–21]. It has been reported that homozygous or compound heterozygous mutations in the F-box protein 7 gene (*FBXO7*, OMIM 605648) cause hereditary parkinsonism including parkinsonian-pyramidal syndrome and typical PD (PARK15, OMIM 260300). This aroused our interest in evaluating the roles of the F-box gene family [22–25]. In this study, four variants (rs9614, rs28924120, rs6442117, and rs61733550) in the paralogs of *FBXO7* (*FBXO2*, *FBXO6*, *FBXO12*, and *FBXO41*), which putatively alter amino-acids and affect protein functions, were studied in Han Chinese PD patients.

Materials and Methods

Participants

We recruited 502 patients with sporadic PD (65.7 ± 10.2 years; onset age, 62.3 ± 7.6 years, 63 cases with onset age ≤50 years; disease duration, 1–26 years; male/female ratio, 302/200) and 556 age-, sex-, and ethnicity-matched healthy individuals (65.9 ± 10.1 years, male/female ratio, 335/221) from the Third Xiangya Hospital of Central South University, Changsha, China. All patients resided in south China and were not related. All were examined by experienced neurologists, and the diagnosis of PD was established according to clinical criteria [6, 26]. Written informed consent was given by all participants prior to enrollment. This study was approved by the Institutional Review Board of the Third Xiangya Hospital of Central South University, Changsha, China.

Some patients were negative for causative mutations in PD-associated genes. The following occurred in the following percentages of patients: no vacuolar protein sorting 35 gene (*VPS35*) mutations in 24.1% (121/502), no F-box protein 48 gene (*FBXO48*) mutations in 65.7% (330/502), or no mutations in the S100 calcium binding protein B gene (*S100B*), the alpha-synuclein gene (*SNCA*), or the *RAB39B* gene (RAB39B, member of RAS oncogene family) in 74.9% (376/502) [27]. Of those tested, 59.2% (297/502) were negative for point mutations (p.A502V and p.R1205H) in the eukaryotic translation initiation factor 4 gamma 1 gene (*EIF4G1*), and 97.6% (490/502) had no rs10788972 or rs12046178 variants in the transcription elongation factor A N-terminal and central domain containing 2 gene (*TCEANC2*) [21, 28].

Variant Genotyping

The National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/) was used to search for F-box genes, including the *FBXO7* gene. Four variants in the paralogs of the *FBXO7* gene with a frequency >5%, rs9614 (c.353A>C, p.K118T), rs28924120 (c.840G>C, p.E280D), rs6442117 (c.496T>C, p.W166R), and rs61733550 (c.1568G>A, p.R523H), were obtained from the public database of single-nucleotide polymorphisms (http://www.ncbi.nlm.nih.gov/SNP/). They were described as putatively damaging by the bioinformatics tools Sorting Intolerant from Tolerant (http://sift.jcvi.org/), Polymorphism Phenotyping version 2 (http://genetics.bwh.harvard.edu/pph2/), or MutationTaster (http://www.mutationtaster.org/). No *FBXO7* gene variants matched the criteria. The four variants were genotyped using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry following protocols in the iPLEX^{TM} Gold Application Guide (Agena Bioscience, San Diego, CA) to reveal any potential involvement in PD pathogenesis [20, 29]. Genomic DNA was extracted from peripheral blood using standard protocols [30]. Primers amplifying short segments for mass spectrometry were designed using Sequenom Assay Design 3.1 software (Sequenom Inc., San Diego, CA) and are shown in Table 1. Multiplex and single-base extension reactions generated allele-specific short products for the MALDI-TOF mass spectrometry assay. MassARRAY Typer 4.0 software (Sequenom) obtained the genotype of samples from spectral peaks [31]. Mass spectrometric genotyping concordance was tested by performing Sanger sequencing in 8% of randomized samples with the Applied Biosystems 3500 Genetic Analyzer (Life Technologies, Foster City, CA) [20, 30]. Primer pairs for locus-specific amplification and sequencing were designed using online Primer3 (http://primer3.ut.ee/, Table 1), and the specificity was checked using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast).

Statistical Analysis

Statistical analysis was performed using Predictive Analytics Software Statistics 18.0 (SPSS Inc., Chicago, IL). Genotype and allele frequencies were calculated, and Pearson's χ^2 test was applied to genotypic and allelic frequency differences. A two-sided P value of 0.05 was set as the threshold for statistical significance [20].

Results and Discussion

The genotypic and allelic frequencies are summarized in Table 2. No deviation from Hardy-Weinberg equilibrium was evident in the normal control cohort (P >0.05). Statistically significant differences in genotypic and allelic frequencies were identified in the *FBXO2* variant rs9614 between patients and controls (P = 0.001 and 0.023,

Table 1 Primer sequences for rs9614, rs28924120, rs6442117, and rs61733550.

	rs9614	rs28924120	rs6442117	rs61733550
Forward primer sequence for amplicon 1[a] (5′→3′)	acgttggatgACTGGCA GCAGTTCTACTTC	acgttggatgTCTGGACAA CAGCTCGGTAG	acgttggatgAGATGCATCT CGCCATCACT	acgttggatgTGATGACCTCATTGGAGCGT
Reverse primer sequence for amplicon 1[a] (5′→3′)	acgttggatgTTTCGGCC CATTTCGCAGCA	acgttggatgAACAGCAG CATTGTCGTCAG	acgttggatgATGCAATAAC AGGGCTGTGG	acgttggatgTCCACGCTCACTTTTGAACC
Amplicon 1 length (bp)	121	121	109	110
Extending primer sequence[a] (5′→3′)	GTTCTACTTCCTGAGCA	GCATGGACAGGAGGA	CCACACTTTGATAGTTTTTTTCC	cccgaCCACTGCCTCCCTCGGGG
Forward primer for amplicon 2[b] (5′→3′)	GTACCTGGACGAGCTGCC	CCCGGCCTGAAAACA TTTATTAC	GGGCAGAATGAAAGCACAGT	CCAGGTAGGTGAAGATGCAGA
Reverse primer for amplicon 2[b] (5′→3′)	CCAGGCGCTTAATCCCATTT	AACAGCAGCATTG TCGTCAG	TCAGGGCTCACTTACCATCA	CAAAGGACAGGGAGGCAGAG
Amplicon 2 length (bp)	356	307	293	291

[a] Lower case letters in the primer sequences are 5′-end tags (forward and reverse primers for amplicon 1) or non-homologous sequences (extending primer) to increase the mass of primers.

[b] Primers used for locus-specific polymerase chain reaction amplification (amplicon 2) and Sanger sequencing.

Table 2 Genotypic and allelic distributions of rs9614 and rs6442117 in patients with sporadic PD and normal controls.

Variants	Genotype/ Allele	Total PD (n = 502)	Total controls (n = 556)	Male PD (n = 302)	Male controls (n = 335)	Female PD (n = 200)	Female controls (n = 221)	P value (χ^2)[a]	P value (χ^2)[b]	P value (χ^2)[c]
rs9614	AA	178	142	111	77	67	65			
	AC	219	298	116	167	103	131			
	CC	105	116	75	91	30	25	**0.001** (13.949)	**<0.001** (15.213)	0.247 (2.795)
	A	575	582	338	321	237	261			
	C	429	530	266	349	163	181	**0.023** (5.181)	**0.004** (8.243)	0.953 (0.003)
rs6442117	TT	228	264	148	158	80	106			
	TC	214	237	122	144	92	93			
	CC	60	55	32	33	28	22	0.529 (1.272)	0.797 (0.453)	0.190 (3.321)
	T	670	765	418	460	252	305			
	C	334	347	186	210	148	137	0.311 (1.028)	0.833 (0.045)	0.066 (3.381)

Significant P values <0.05 are shown in bold.

PD Parkinson's disease.

[a] All PD compared with total controls.

[b] Male PD compared with male controls.

[c] Female PD compared with female controls.

respectively; odds ratio = 0.819, 95% confidence interval = 0.690–0.973), especially between male patients and male controls ($P < 0.001$ and $P = 0.004$, respectively; odds ratio = 0.724, 95% confidence interval = 0.580–0.903). The frequency of the minor C allele was 0.427 in patients and 0.477 in controls. No significant differences were found in genotypic and allelic frequencies in the *FBXO12* variant rs6442117 between PD patients and total controls ($P = 0.529$ and 0.311, respectively; odds ratio = 1.099, 95% confidence interval = 0.916–1.319). Monomorphism (genotype CC) was found in the genotypes of rs28924120 and rs61733550, and no further evaluation was performed.

PD is a genetically heterogeneous disorder, and great progress has been achieved in identifying disease-causing genes for monogenic PD since 1997. However, the genetic basis is yet to be described. This is particularly true regarding variants that either exert an increased risk or play a protective role against sporadic PD development [1, 4, 5]. The *FBXO7* gene, located in chromosome 22q12.3, was first identified as the disease-causing gene for autosomal recessive early-onset parkinsonian-pyramidal syndrome (PARK15) in a large Iranian pedigree [22]. To date, at least five mutations have been reported to be associated with the development of parkinsonism [22–25].

Here, we performed a case-control study in a Han Chinese population cohort with 1058 participants to assess the role of four variants (*FBXO2* rs9614, *FBXO6* rs28924120, *FBXO12* rs6442117, and *FBXO41* rs61733550) in the development of sporadic PD. Statistically significant differences were only identified in the genotype and allele of the *FBXO2* variant rs9614 (p.K118T), which was predicted to be "probably damaging" by Polymorphism Phenotyping version 2, indicating that it is associated with PD. The minor C allele of rs9614 may result in a decreased risk of PD in mainland Han Chinese, and it may be a biomarker for PD, especially in males. However, the other three variants, rs6442117, rs28924120, and rs61733550, play no apparent role in PD development.

The *FBXO2* gene (OMIM 607112), located in chromosome 1p36.22, lies in tandem with the *FBXO6* gene and spans over 6 kb. It consists of six exons, encodes a protein with 296 amino-acids, and is specifically expressed in the brain [32]. The protein, similar to FBXO7, belongs to the F-box protein family, which is defined by the presence of an F-box domain [33]. It is a component of the Skp1-cullin-F-box ubiquitin E3 ligases, and plays important roles in the ubiquitin-proteasome protein-degradation pathway and mitochondrial maintenance, both of which are robustly associated with PD pathogenesis [8, 9, 22, 25, 34, 35]. In this scenario, the primary hypothesis was confirmed and we reasoned that the rs9614 variant exerted a protective role in PD development in our participants, by sharing a common or similar molecular pathway with *FBXO7*. Many factors such as sample size limitations and the rarity of genotype or allele in the Han Chinese population may account for the negative results in the other three variants. Furthermore, cohort composition, clinical heterogeneity, methodology, synergistic or antagonistic interaction with other variants and environmental factors, and other confounding factors should be taken into account with regard to the findings in this study.

In summary, our findings suggest an association between the *FBXO2* gene and development of PD. Given that different populations are genetically heterogeneous and variants have population-specific frequencies, additional gene variant studies in independent larger cohorts and in cohorts with different geographical origins, along with functional investigations of the candidate gene, are warranted to confirm any risk or protective roles of the four variants in PD development.

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