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FBXO7 gene mutations may be rare in Chinese early-onset Parkinsonism patients

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ABSTRACT

A recent study has shown that *FBXO7* is a causative gene for PARK15-linked autosomal recessive early-onset Parkinsonism which was described by Davison for the first time in 1954 and known as Pallido-Pyramidal Disease or Parkinsonia-Pyramidal Syndrome in the past. In order to investigate the characteristics of *FBXO7* gene mutations in Chinese early-onset Parkinsonism patients, we performed polymerase chain reaction and DNA direct sequencing on 135 patients and 200 controls. In this study, we found 10 polymorphisms including two novel polymorphisms (−274G → C, c.A155G), but no pathogenetic mutations in the *FBXO7* gene were detected. This suggests that *FBXO7* mutations may be rare in Chinese early-onset Parkinsonism patients.

Introduction

Parkinson's disease (PD) is a progressive degenerative disease associated with loss of dopaminergic neurons in the substantia nigra pars compacta, and presents intraneuronal cytoplasmic inclusion bodies called Lewy bodies. The pathogenesis of PD is unclear, but genetic deficits, environmental exposure, oxidative stress, mitochondrial dysfunction, defective handling of misfolded synuclein and failure of the ubiquitin proteasome system may be responsible for it [21,4,18]. Most PD patients are sporadic; only 5–10% patients are familial [22]. Since *α-synuclein* was reported be associated with PD [16], disease causing genes got more and more attention on pathogenetic study of it. Previous studies have identified at least 15 loci responsible for Parkinsonism, which were named PARK1–PARK15. Among these 15 loci, mutations of six genes including *parkin* at PARK2 [12] (OMIM#600116), *PINK1* at PARK6 [23] (OMIM#605909), *DJ-1* at PARK7 [1] (OMIM#606324), *ATP13A2* at PARK9 [17] (OMIM*610563), *PLA2G6* at PARK14 [14] (OMIM#256600), and *FBXO7* at PARK15 [3] (OMIM:*605648) were found to be associated with autosomal recessive early-onset Parkinsonism (AREP).

Here, we will focus on PARK15-linked early-onset autosomal recessive Parkinsonism. This subtype of Parkinsonism is caused by mutations of the *FBXO7* gene [3], and used to be called Pallido-Pyramidal Disease (PPD) or Parkinsonian-Pyramidal Syndrome (PPS). It is associated with a series of symptoms that includes spasm state, hyperreflexia, and positive pyramidal signs besides the three classical PD symptoms (static tremor, bradykinesia, and rigidity). Nearly 20 cases of pedigree have been reported [2,3,9,11,13,15,19,20] since 1954. However, this disease is still a rare subtype of Parkinsonism, and no statistical study has been done to report the frequency of PPD in Parkinsonism. In 2008, Shojaee et al. [19] reported an Iranian pedigree, in which all affected individuals exhibited equinovarus deformity since childhood. The genome-wide linkage analysis on that pedigree with 500 K SNP Arrays mapped the locus to chromosome 22, and a new disease-associated missense mutation (c.1132C → G) was found resulting in the nonconservative amino acid substitution of glycine for arginine at position 378(R378G) in the F-box protein 7. In 2009, Di Fonzo et al. [3] found an *FBXO7* homozygous truncating mutation (Arg498Stop) in an Italian autosomal recessive early-onset parkinsonian pedigree, while compound heterozygous mutations (a splice-site IVS7+1G/T mutation and a missense Thr22Met mutation) presented in a Dutch family. Since then, gene *FBXO7* was officially designated as PARK15.

Until now, no investigation of the mutation of *FBXO7* in Chinese early-onset Parkinsonism patients has been done. In this study, we executed polymerase chain reaction (PCR) combined with DNA direct sequencing on 23 unrelated probands with autosomal recessive early-onset Parkinsonism and 112 sporadic patients with early-onset Parkinson disease, in order to screen the *FBXO7* mutation. Since patients affected with PPD usually exhibit special

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Table 1
FBXO7 gene variants detected in this study.

Position	Ref. no.	Nucleotide change	Protein change	Variant frequency (%)	
				Case	Control
Exon1	rs2072813	−329C → T	None	49.6296	30.5000
Exon1	**None**	**−274G → C/G**	**None**	**3.7037**	**3.5000**
Exon1	rs2072814	−221C → T	None	77.0370	85.0000
Exon1	rs8136485	Ivs1 + 116C → T	None	17.7778	21.0000
Exon1	rs9621461	Ivs1 + 254G → A	None	20.7407	10.5000
Exon1	rs8137714	Ivs1 + 272T → G	None	17.7778	21.0000
Exon2	**None**	**c.A155G/A**	**Tyr52Cys**	**0.7407**	**2.0000**
Exon2	rs11107	c.G345A	pMet115Ile	88.8889	90.5000
Exon6	rs738982	Ivs6-75T → C	None	88.8889	90.5000
Exon6	rs9726	c. C949T	pLeu317Leu	88.8889	90.5000

Novel variants detected in our study are in bold. Accession number (rs) is given for each known *FBXO7* polymorphism. The nucleotide numbers are according to the *FBXO7* cDNA sequence deposited in Genbank (accession number BC008361). For each polymorphism, the variant allele is reported after the → symbol. In this study, the variant frequency has no significant difference between case and control (*P* value > 0.05).

symptoms such as spasm state, hyperreflexia, and positive pyramidal signs, some of the PD patients in this group were chosen because they exhibited such symptoms.

Subjects and methods

Subjects

A total of 135 patients with early-onset Parkinsonism were studied including 23 patients who are probands with onset age ≤40 years [mean age (28.91 ± 12.40) years] from AREP family constellation. Of those, 3 patients presented hyperreflexia, 2 patients presented positive pyramidal signs, and 1 patient presented both symptoms. 112 others are sporadic patients with onset age ≤40 years [mean age (31.72 ± 8.42) years]. Of those, 8 patients presented hyperreflexia, 5 patients presented positive pyramidal signs, and 3 patients presented both symptoms. All of the 135 patients met the following criteria: (1) having at least two of the three cardinal motor signs (resting tremor, bradykinesia, rigidity); (2) having an excellent response following L-Dopa therapy; (3) having previously been excluded from the homozygous mutations of the *parkin*, *PINK1*, *DJ-1*, *ATP13A2*, and *PLA2G6* genes via direct sequencing or real-time quantitative PCR analysis [6,7]; (4) having been excluded from secondary Parkinsonism, Hepatolenticular Degeneration, Hereditary Spastic Paraplegia, and Spinocerebellar Ataxias. All patients are mainland Chinese and have signed the informed consent. All patients were collected by the Neurology Department of Xiangya Hospital affiliate to Xiangya Medical Academy of Central South University and the National Lab of Medical Genetics of China, and all came from various provinces of the Chinese mainland such as Hunan, Hubei, Jiangxi, Zhejiang, Yunnan, Shandong, Guangdong, etc.

Methods

Genomic DNA was isolated from peripheral blood of patients and 200 normal Chinese individuals by standard protocols. Nine exons and introns boundaries of the *FBXO7* gene, as well as fragments of *FBXO7* cDNA were amplified by PCR. Primers and reaction conditions were designed routinely [3]. PCR reactions were performed in 10 μl total volume containing 0.8 μl of 10× TaKaRa PCR buffer with $MgCl_2$, 25 μM of each dNTPs, 3 μM forward primer, 3 μM reverse primer, 0.05 units of HortStart taq Polymerase (Takara Biotechnology, DaLian, Co., Ltd.) and 25 ng genomic DNA or 0.5 μl total cDNA, ddH_2O up to 10 μl. The PCR products were separated by 6% polyacrylamide gels. Each PCR product was purified and directly sequenced in both forward and reverse directions on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). DNAStar was used for sequence alignment and analysis (DNAStar, Inc., Madison, WI).

Results

We found 10 sequence changes (Table 1); of those, 8 polymorphisms have been previously reported, 2 others are newly discovered: −274G → C (Fig. 1), c.A155G (Fig. 2). No pathogenic mutation was found. All 10 base changes have been found in normal people. The difference between the frequency of those 10 polymorphisms in PD patients and the frequency in the control group was non-significant (*P* value > 0.05).

3 of the 10 polymorphisms were located at coding regions and changed the expression of proteins, including c.A155G (Tyr52Cys, novel polymorphism) and c.G345A (pMet115Ile, rs11107) in exon2, and c. C949T (pLeu317Leu, rs9726) in exon6. 7 others were found in introns, with 6 of them located at non-coding regions of exon1, and another polymorphism at a non-coding region of exon6.

We found that two polymorphisms are complete linkage with each other: Ivs1 + 116C → T (rs8136485) and Ivs1 + 272T → G (rs8137714). And three polymorphisms: c.G345A (pM115I, rs11107) in exon2, Ivs6-75T → C (rs738982) and c.C949T (pL317L, rs9726) in exon6 are complete linkage too. The D' = 1 and the r^2 = 1 when we analyzed them in this study.

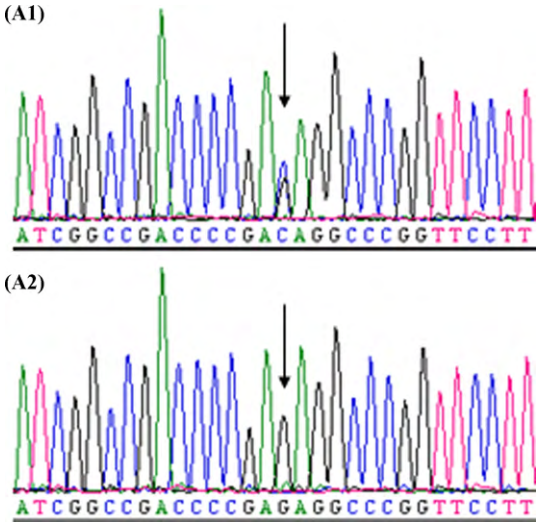

Fig. 1. (A1) A heterozygous polymorphism: −275G → C (arrow points) and (A2) wild type (arrow points).