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Mutation analysis of *PARK2* in a Uyghur family with early-onset Parkinson's disease in Xinjiang, China

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ABSTRACT

Background: The *PARK2* gene was recently identified as a causative gene for autosomal recessive early-onset Parkinson's disease (EOPD). Studies on how specific *PARK2* mutations are manifested on different genetic backgrounds may benefit prognosis and clinical management. Until now, there have been no reports of *PARK2* mutations in a Uyghur family with EOPD.
Methods: We identified a large Uyghur EOPD family with *PARK2* mutations, and analyzed genealogical, clinical, and genetic data from the family.
Results: Three of 15 members were diagnosed with EOPD, and two point mutations, c.951G>C (p.G284R) and c.924C>T (p.R275W), were found in six family members. Among the mutation-positive members, the three affected members were compound heterozygote, while the three unaffected members were single heterozygote.
Conclusion: This is the first report describing a Uyghur family with *PARK2* mutations. The compound heterozygous mutation c.951G>C (p.G284R) and c.924C>T (p.R275W) is the pathogenic factor in this EOPD Uyghur family.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder of the central nervous system. The core pathology is loss of dopaminergic neurons in the substantia nigra [1], but the etiology of most sporadic PD cases remains unclear. Interactions among age, environment, and genetic factors may underlie sporadic PD [2,3]. A variety of gene mutations are associated with familial early-onset PD (EOPD) [4]. Like other inherited diseases, the common inherited forms of PD include both autosomal recessive and autosomal dominant forms. The three most common mutated genes in autosomal recessive EOPD are *PARK2* (parkin), PINK1, and *PARK*7 [5], of which mutant *PARK2* is the most common genetic cause of familial and EOPD, accounting for up to 50% of familial and 18% of sporadic EOPD cases [6,7]. However, a recent systematic review by Kilarski et al. concluded that the weighted pooled proportion of *PARK2* mutation-positive cases was only 8.6% in EOPD [8]. Like other genetic diseases, the frequencies and penetrance of specific parkin mutations vary among nationalities. *PARK2* mutation frequencies among Caucasians and Asians are similar at 7.7% and 10.5%, but significantly lower in Latin Americans [8]. *PARK2* gene mutations are also common in Chinese EOPD patients, and are mainly exon rearrangements, deletions, or missense mutations [9,10]. The Uyghur populate the Xinjiang region of China and are a distinct ethnic group from the dominant Han race. To date, no report has examined *PARK2* mutations in Uyghur familial EOPD.

Xinjiang, where Uyghur prefer to settle, connects the people from East Asia and Central Asia. Historically, Uyghur hail from Turkey, but until 2008 there was no genetic evidence to support this origin. Xu et al. reported that Uyghur have a European and East Asian ancestry, 60% and 40%, respectively [11]. Thus it needs to be identified whether *PARK2* mutation in Uyghur familial EOPD has both European and Asian genetic characteristics.

In this study, we report PCR and DNA sequencing results from a large Uyghur family from Xinjiang with *PARK2* mutations and inherited EOPD. We collected the clinical data of three affected members and analyzed the family pedigree for 15 members from three generations.

2. Patients and methods

2.1. PD family

We studied 15 members of a large Uyghur family from Toksun County, Xinjiang, China exhibiting a high incidence of EOPD. Three members were identified with early onset (<40 years of age)

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Parkinson's disease, while other studied family members were unaffected. The patients with PD were evaluated by neurology specialists according to the UKPD Brain Bank criteria. We collected clinical information from these three patients, including medical history, neurological examination results, brain magnetic resonance imaging (MRI), Unified PD Rating Scale (UPDRS), Hoehn and Yahr stage, psychiatric assessment, and Montreal Cognitive Assessment (Uyghur edition). This study was approved by local ethics committees, and written consent was obtained from all participants. All procedures were conducted strictly according to the Administrative Regulations on Medical Institution, issued by the State Council of the People's Republic of China.

2.2. Polymerase chain reaction amplification and sequencing

Peripheral blood samples were collected from 10 selected subjects for genomic DNA analysis (including three patients and seven healthy family members). Genomic DNA was extracted following the standard protocol of the TIANamp Genomic DNA Extraction Kit. Specific primers for *PARK*2 exons 3–7 were synthesized by Beijing Biomed Co., Ltd. (Beijing, China; primer sequencing is presented in Table 1). The PCR protocol was as follows: denaturation at 95 °C for 2 min, then 34 cycles of annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and denaturation at 95 °C for 20 s. Cycles were followed by a final round of annealing at 55 °C for 30 s and extension at 72 °C for 7 min. After PCR, 10% of the PCR reaction mixture volume was run on 2% agarose gels. The remaining PCR products were sent to Beijing Genomics Institute for DNA sequencing. The DNA sequencing data were analyzed by APE (Bio-soft, (http://biologylabs.utah.edu/jorgensen/wayned/ape/)).

3. Results

3.1. Family pedigree map

A Uyghur family showing EOPD with autosomal recessive inheritance was studied for mutations in *PARK2*. The family pedigree was constructed based on genotyping of peripheral blood samples from 10 members of three generations, including seven unaffected members (1, 3, 7, 10, 11, 14, and 15) and three with EOPD (6, 8, and 9; Fig. 1).

3.2. Clinical features

The three affected cases met the diagnostic threshold for EOPD. The proband was a 35-year-old male with a 3 year history of PD, manifested as resting tremor of the right limb, rigidity, gait dysfunction, and facial stiffness. Routine blood examination showed no coagulation abnormalities. Blood copper was lower than normal, but ceruloplasmin and liver ultrasound were normal, and no K–F rings were observed. Head computed tomography (CT) and MRI scans were negative. The patient was responsive to Levodopa. The other two patients had signs and symptoms resembling the proband. The clinical features of these three patients are summarized in Table 2.

Table 1
Primers used to amplify exons 3–7 of *PARK2*.

Primer	Sequence	Length
Exon 3	F: ACATGTCACTTTTGCTTCCCT	
	R: AGGCCATGCTCCATGCAGACTGC	427
Exon 4	F: ACAAGCTTTTAAAGAGTTTCTTGT	
	R: AGGCAATGTGTTAGTACACA	261
Exon 5	F: ACATGTCTTAAGGAGTACATTT	
	R: TCTCTAATTTCCTGGCAAACAGTG	227
Exon 6	F: AGAGATTGTTTACTGTGGAAACA	
	R: GAGTGATGCTATTTTTAGATCCT	268
Exon 7	F: TGCCTTTCCACACTGACAGGTACT	
	R: TCTGTTCTTCATTAGCATTAGAGA	239

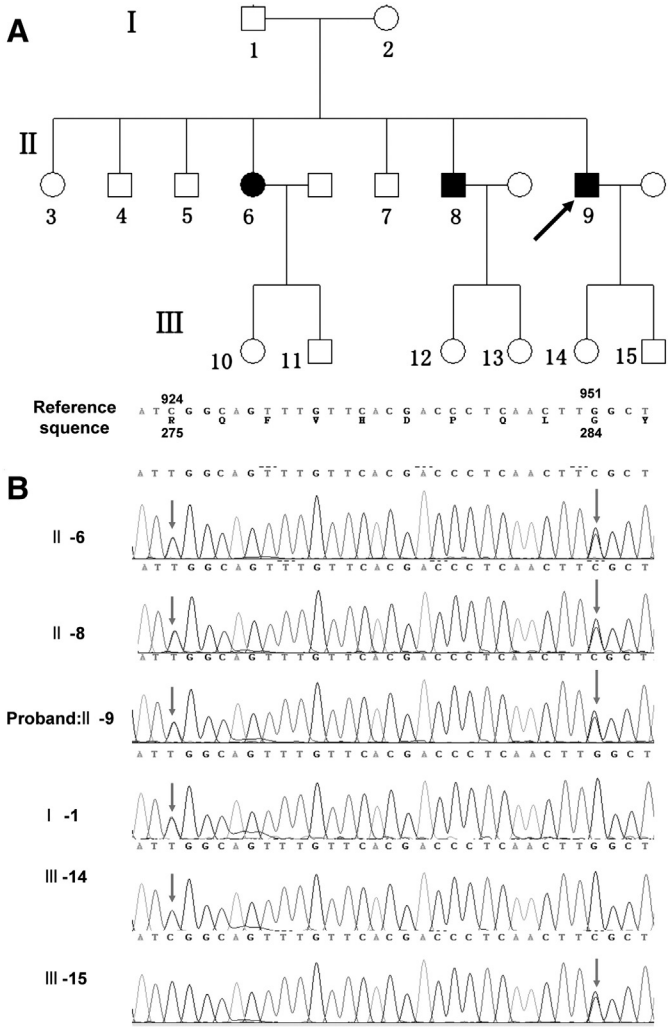

Fig. 1. A: Pedigrees of this Uyghur family with PD: Fifteen members of three generations, blackened elements denote a family member affected by PD. Arrow indicates the proband (9). B: Direct sequencing analysis of the parkin gene in this Uyghur PD family.

Table 2
Clinical features of patients with *PARK2* mutations.

	Patient ID		
	II-6	II-8	II-9
Age at disease onset (y)	35	34	31
Disease duration (y)	5	4	4
Resting tremor	+	+	+
Bradykinesia	+	+	+
Rigidity	+	+	+
Gait disturbance	−	−	−
Postural instability	+	+	+
Clinical response to levodopa	+	+	+
Wearing off	+	−	−
Asymmetry at onset	−	+	−
Urinary urgency	−	−	−
Levodopa-induced dyskinesia	−	−	−
Dystonia at onset	+	+	+
Hyperreflexia	+	+	+
Hallucination	−	−	−
Other neuro-psychiatric manifestations[a]	+	+	+
Dementia	−	−	−
Brain MRI	−	−	−
UPDRS score	25	35	27
Hoehn & Yahr	1	2	1
MoCA score	24	26	26

[a] Other neuro-psychiatric manifestations include insomnia and depression.

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