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Association between *PARK16* gene polymorphisms and susceptibility of Parkinson's disease in a Chinese population

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ABSTRACT. Recent genome-wide association studies identified 11 risk loci in different populations of familial and sporadic Parkinson's disease (PD) patients. Few loci have been verified in different European and Asian populations. We also validated 2 new single-nucleotide polymorphisms, rs947211 and rs823144, in *PARK16* to explore their association with susceptibility to PD in the Xinjiang Uygur and Han populations. This case-control study included 312 PD patients (130 Uygur and 182 Han) and 359 control subjects (179 Uygur and 180 Han). Polymerase chain reaction-restriction fragment length polymorphism analysis and DNA sequencing were used to detect the rs947211 and rs823144 polymorphism in the *PARK16* gene between the Xinjiang Uygur and Han populations. Frequencies of the A allele and AA genotype (42.1 and 15.7%, respectively) of rs947211 in PD patients were significantly lower than those in the control group (54.7 and 28.7%, respectively, $P < 0.01$). A allele and AA genotype frequencies of

rs823144 were 56.8 and 31.8% in the PD patients group and were 54.1 and 29.3% in the control group; no significant difference was found ($P > 0.05$). In both the Han and Uygur groups, the rs947211 polymorphism was associated with PD. Haplotype analysis also indicated that the A-A and G-A haplotypes were associated with PD. We found that the rs947211 polymorphism may be a susceptibility marker for PD in the Chinese population; the A-A and G-A haplotypes may be a protective factor and a risk factor, respectively, for PD in the Chinese population.

Key words: Genetic polymorphism; Parkinson's disease; PARK16

INTRODUCTION

Parkinson's disease (PD) is a complex neurodegenerative disease; the main pathological changes include depigmentation of substantia nigra dopaminergic neurons and formation of Lewy bodies. Most PD patients are sporadic and exhibit no clear genetic history (Lang and Lozano, 1998; Goedert et al., 2012). Some genetic variants appear to increase the susceptibility to sporadic PD in the general population. Recent genome-wide association studies conducted in North America, Europe, and Asia showed that the *PARK16* gene polymorphism was associated with PD (Satake et al., 2009; Simón-Sánchez et al., 2011; Liu et al., 2011).

PARK16 (OMIM 613164) is located at chromosome 1q32, which includes the transcribed regions of 5 related genes: *RAB7L1*, *SLC41A1*, *NUCKS1*, *SLA45A3*, and *PM20D1*. The *SLC41A1*, *NUCKS1*, and *RAB7L1* genes were shown to be associated with PD (Tucci et al., 2010). However, studies in Asia, including China, Singapore, and Taiwan, have shown inconsistent results. Furthermore, additional studies have shown that genetic polymorphisms in *PARK16* have significant regional and ethnic differences (Tan et al., 2010; Vilariño-Guell et al., 2010; Chang et al., 2011; Yan et al., 2011; Chiang et al., 2012). In this study, we selected Uygur and Han ethnic patients with PD and healthy controls in Xinjiang to explore the relationship between the *PARK16* gene rs949211 and rs823144 polymorphisms and PD.

MATERIAL AND METHODS

Subjects and methods

The PD patient and control groups were selected from the First Affiliated Hospital of Xinjiang Medical University from June 2010 to April 2013. A total of 312 PD patients (all sporadic) who visited the neurology specialist clinic were enrolled. Diagnosis was based on the PD diagnostic criteria of the UK Brain Bank. Exclusion criteria were as follows: Parkinson's syndrome caused by cerebrovascular disease, encephalitis, trauma, drugs, Parkinson's plus syndrome, and severe systemic disease. A total of 359 volunteers from the same area with no family history of PD and PD clinical manifestations were included as the control group. Gender, age, and ethnicity were matched between the 2 groups. The study was approved by the First Affiliated Hospital of Xinjiang Medical Ethics Committee, and all subjects signed informed consent.

DNA extraction

First, 2 mL fasting blood was acquired from all subjects. Next, using ethylenediaminetetraacetic acid as an anticoagulant, genomic DNA was extracted using a kit (Shanghai Tiangen Bio Co., Ltd., Shanghai, China).

Design of primers and amplification of the target gene

According to previous genome-wide association studies (Satake et al., 2009) and the SNP information for the *PARK16* gene in National Center for Biotechnology Information SNP database (NCBI, http://www.ncbi.nlm.nih.gov/SNP), we chose rs947211 and rs823144. Primers were designed based on the DNA sequences (Table 1). For DNA amplification, the total polymerase chain reaction (PCR) volume was 20 μL, including 0.5 μL 100 ng/μL upstream and downstream primers, 10 μL 2X Power Taqman Master Mix (Beijing Baitaike Biotechnology Co., Ltd., Beijing, China), 3.0 μL 50 ng/μL DNA, and 11 μL ddH_2O. Primers were synthesized by Sangon Shanghai Co., Ltd. (Shanghai, China). Each sample was amplified for 35 cycles in a 9700 amplification system (Applied Biosystems, Foster City, CA, USA).

Table 1. Primers and annealing temperatures.

SNPs	Primers	Products (bp)	Annealing temperatures (°C)
rs947211	F: 5'TTTCTGCTTTTCTCACCTCCT3' R: 5'ATTTGGGCCTGGGAGTATCT3'	307	58.7
rs823144	F: 5'CTGACTGCCGAGAAACTGGAC3' R: 5'GATGAGGCTTGGGCTTTTACTA3'	571	53.6

PCR product detection

For PCR product detection, 7 μL PCR products were mixed with 2X bromo buffer and separated by 4% agarose gel electrophoresis. Nucleic acid dyes were used to stain the samples after 4 V/cm voltage electrophoresis for 1 h. Bands were observed and photographed under ultraviolet light.

Enzyme digestion genotyping

For genotyping, 8 μL PCR products and 2 μL 10X buffer were combined with 5 U restriction endonucleases *Hpy*188I and *Bsa*HI (New England Biolabs, Ipswich, MA, USA) and 20 μL sterile double-distilled water at 37°C overnight (16 h). Next, 9 μL digestion products were separated on a 4% agarose gel for ethidium bromide staining at 110 V for 1.5 h. A Gel Doc 1000 gel imaging analysis system (Bio-Rad, Hercules, CA, USA) was used to observe the electrophoretic bands.

Direct sequencing

To determine the accuracy of our results, we randomly selected 10% of the samples (31 in the patient group, 36 in the control group) for direct sequencing.

Statistical analysis

Genotype frequencies were calculated using the gene-counting method. The Hardy-Weinberg equilibrium test of genotypes was performed using the Arlequin software. The ages between the 2 groups were compared using the independent sample *t*-test, and gender, allele and genotype frequencies between the 2 groups were compared using the χ^2 test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Hardy-Weinberg equilibrium test

The χ^2 values for the rs947211 and rs823144 genotypes of the *PARK16* gene in the case and control groups were 1.97 and 0.78 and 0.099 and 0.002, respectively, with all $P > 0.05$. The sample population was found to be in Hardy-Weinberg equilibrium and thus represented the whole population.

Enzyme digestion of the *PARK16* gene

As shown in Figures 1 and 2, the PCR products (307 bp) of rs947211 were digested with *Hpy*188I. The AA genotype showed 1 band (307 bp), the GG genotype showed 2 bands (147 and 160 bp), and the AG genotype produced 3 bands (307, 147, and 160 bp). The PCR products of rs823144 were 571 bp; after *Bsa*HI digestion, the 25-bp fragment was too small to be observed on the agarose gel. Therefore, the AA genotype had 1 band (546 bp), the CC genotype had 2 bands (383 and 163 bp), and the AC genotype had 3 bands (163, 383, and 546). Direct sequencing verified the genotyping results.

Genotype and allele distribution frequencies of rs947211 and rs823144 in the 2 groups

As shown in Table 2, the genotype and allele frequencies of the rs947211 polymorphism were compared between the case and control groups; the difference was statistically significant [$P < 0.01$, odds ratio (OR) = 0.60, 95% confidence interval: 0.48-0.76]. The rs823144 genotype and allele frequencies were not significantly different between the case and control groups ($P > 0.05$). After ethnicity grouping, the genotype and allele frequencies of the rs947211 were significantly different between the case and control groups, both in the Han and Uygur populations (both $P < 0.05$).

Haplotype analysis of *PARK16*

SHEsis (http://analysis.bio-x.cn) analysis was performed to construct the haplotypes, as shown in Table 3. The G-A haplotype was very common in the case group compared to the control group ($P < 0.01$; OR = 1.82, 95% confidence interval: 1.44-2.30). However, the frequency of the A-A haplotype was higher in the control group than in the case group ($P < 0.01$; OR = 0.48, 95% confidence interval: 0.36-0.66).

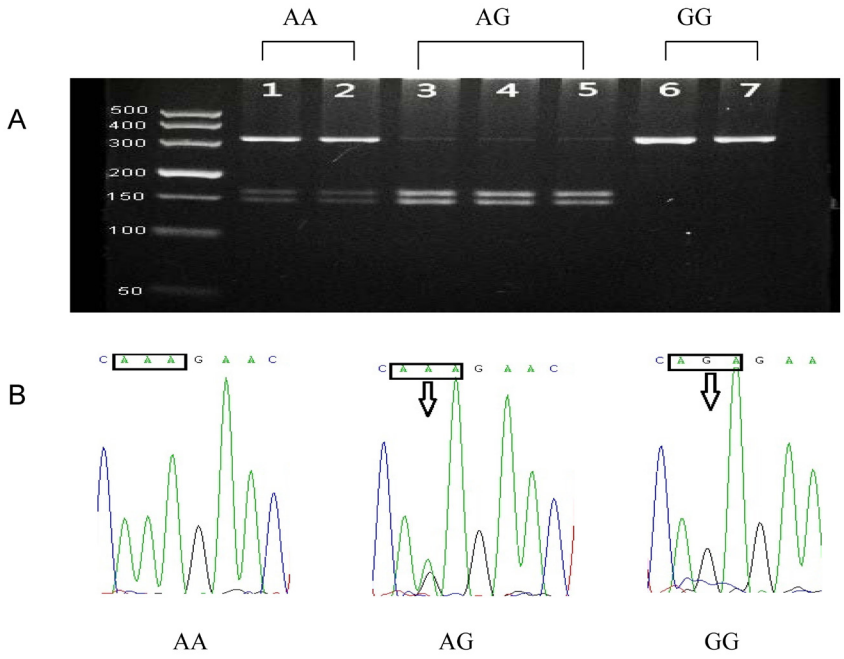

Figure 1. Genotyping results of rs947211. **A.** Result of RFLP. **B.** Result of sequencing.

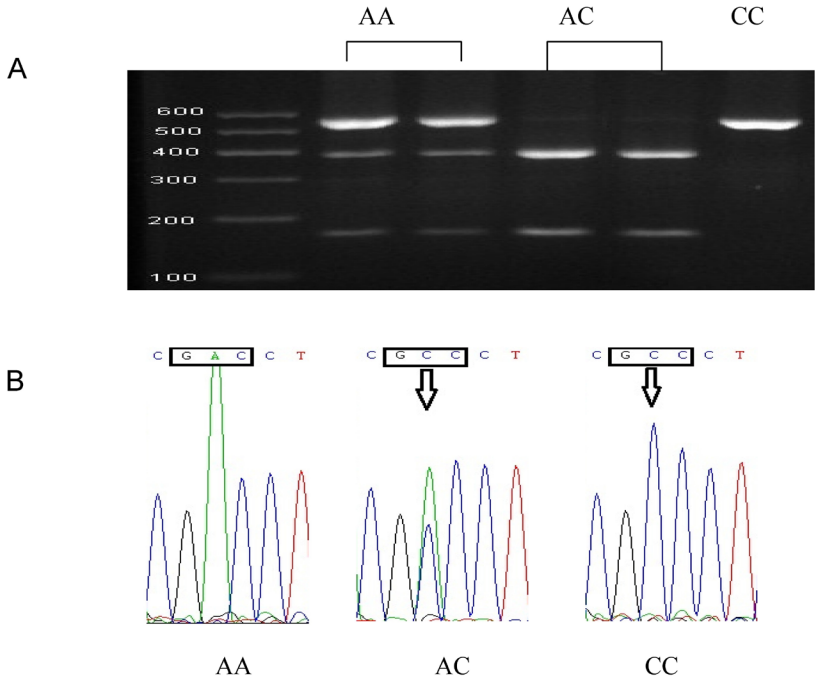

Figure 2. Genotyping results of rs823144. **A.** Result of RFLP. **B.** Result of sequencing.

Table 2. Genotype and allele frequencies of rs947211 and rs823144.

			Total				Han				Uygur			
			PD (N, %)	Control (N, %)	P value	OR (95%CI)	PD (N, %)	Control (N, %)	P value	OR (95%CI)	PD (N, %)	Control (N, %)	P value	OR (95%CI)
			N = 312	N = 359			182	180			130	179		
rs947211	Allele	A	236 (0.421)	362 (0.547)	<0.01	0.604 (0.481-0.758)	149 (0.44)	185 (0.58)	<0.01	0.57	87 (0.40)	177 (0.52)	<0.01	0.61
		G	324 (0.579)	300 (0.453)			191 (0.56)	135 (0.42)		(0.42-0.78)	133 (0.61)	165 (0.48)		(0.43-0.86)
	Genotype	AA	44 (0.157)	95 (0.287)	<0.01		28 (0.17)	52 (0.33)	<0.01		16 (0.15)	43 (0.25)	<0.05	
		AG	148 (0.529)	172 (0.520)			93 (0.55)	81 (0.51)			55 (0.50)	91 (0.53)		
		GG	88 (0.314)	64 (0.193)			49 (0.29)	27 (0.17)			39 (0.36)	37 (0.22)		
rs823144	Allele	A	318 (0.568)	358 (0.541)	0.343	1.116 (0.890-1.400)	190 (0.56)	164 (0.51)	0.23	1.21	128 (0.58)	194 (0.57)	0.73	1.06
		C	242 (0.432)	304 (0.459)			150 (0.44)	156 (0.49)		(0.89-1.64)	92 (0.42)	148 (0.43)		(0.75-1.50)
	Genotype	AA	89 (0.318)	97 (0.293)	0.615		50 (0.29)	37 (0.23)	0.41		39 (0.36)	60 (0.35)	0.87	
		AC	140 (0.500)	164 (0.495)			90 (0.53)	90 (0.56)			50 (0.46)	74 (0.43)		
		CC	51 (0.182)	70 (0.211)			30 (0.18)	33 (0.21)			21 (0.19)	37 (0.22)		

Table 3. Haplotype analysis.

SNPs	Haplotype	PD (%)	Control (%)	χ^2	P value	OR (95%CI)
rs947211-rs823144	A-A	69.48 (0.12)	149.15 (0.23)	21.16	<0.01	0.48 (0.36-0.66)
	A-C	166.52 (0.30)	212.85 (0.32)	0.83	0.36	0.89 (0.70-1.14)
	G-A	248.52 (0.44)	201.85 (0.31)	25.14	<0.01	1.82 (1.44-2.30)
	G-C	75.48 (0.14)	98.15 (0.15)	0.45	0.5	0.90 (0.65-1.24)

DISCUSSION

Satake et al. (2009) found that rs947211 showed the most significant association with PD. This polymorphism is located 8.5 kb upstream of the *RAB7L1* gene and 5.6 kb downstream of the *SLC41A1* gene in *PARK16*. However, linkage disequilibrium analysis showed that the locus was closely associated with *NUSKS1* transcript levels, which encodes a nuclear protein containing common phosphorylation sites of casein kinase II and cyclin-dependent kinase (Ostvold et al., 2001). When we explored the association of rs947211 in the *PARK16* gene with PD in Uygur and Han Chinese subjects in Xinjiang, we found that individuals with the A allele had a significantly lower risk of suffering from PD than individuals carrying the G allele ($P < 0.01$, OR = 0.60). Our results were consistent with several studies in Asian population (Satake et al., 2009; Tan et al., 2010). However, similar studies including Europeans showed that this locus was not associated with the occurrence of PD (Ramirez et al., 2011; Mata et al., 2011), further suggesting that the presence of polymorphic loci differs among various races.

The rs823144 locus is located in the *RAB7L1* promoter region, and full-length *RAB7L1* cDNA encodes the RAB7 protein, which contains 203 amino acids. RAB7 is a member of the renin-angiotensin system oncogene family. More than 60 members of this family are considered to play an important role in regulating material exchange on both sides of the membrane through protein structure (Shimizu et al., 1997; Verhoeven et al., 2003). Although our results showed that the polymorphism was not associated with PD in Uygur and Han subjects in Xinjiang, Gan-Or et al. (2012) used the online software TFSEARCH to predict its association with PD and found that the polymorphism may exclude the binding sites of transcription factor c-Ets-1 and increase binding to 3 other sites (p300, GATA-1, and Sp1), altering PD susceptibility between individuals. MacLeod et al. (2013) found that the *LRRK2* (G2019S) mutation drastically reduced neurite length in rats. Overexpression of *RAB7L1* can suppress this phenomenon, suggesting that the gene's function may be related to the pathogenesis of PD caused by *LRRK2* mutations. Our haplotype results showed that 2 SNPs had weak lineage, and that the A-A combination may be a protective factor against Parkinson's disease ($P < 0.05$, OR = 0.36-0.66). The G-A combination significantly increased the risk of Parkinson's disease ($P < 0.05$, 1.44-2.30).

In summary, we verified that rs947211 was associated with PD in the Uygur and Han Chinese populations in Xinjiang. Although the results showed that rs823144 was not associated with PD, analysis of a larger sample study is necessary.

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