

Association between Parkinson's disease and G2019S and R1441C mutations of the LRRK2 gene

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Received March 13, 2014; Accepted November 6, 2014

DOI: 10.3892/etm.2015.2659

Abstract. In recent genome-wide association studies (GWAS), 11 risk loci were identified in patients with familial and sporadic Parkinson's disease (PD) in different populations. The LRRK2 gene was found to be a mutation hot spot in European and Asian populations. The aim of the present study was to investigate the incidence of G2019S and R1441C mutations in the LRRK2 gene in individuals from the Xinjiang region of China, and to explore the associations between LRRK2 gene single nucleotide mutations and susceptibility to PD in the Uyghur and Han populations of Xinjiang. A case-control study was conducted with a group of 312 patients with PD, including 130 Uyghur and 182 Han individuals. The control group comprised 359 subjects, including 179 Uyghur and 180 Han individuals. Polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing methods were used to detect the G2019S and R1441C mutations in the LRRK2 gene in the Uyghur and Han populations. No known mutations or new hybrids were found. Thus, there was no evidence that Uyghur and Han patients with PD possess the G2019S or R1441C mutations of the LRRK2 gene. This does not exclude the possibility of the presence other LRRK2 gene mutations that are associated with PD in the Uyghur and Han populations. In the future, the association of the LRRK2 gene with PD development in different regions and populations requires further study, in addition to the regulatory effects of the G2019S and R1441C mutations on gene expression.

Introduction

Parkinson's disease (PD) is a common degenerative disease of the central nervous system in the elderly, which is primarily associated with environmental and genetic factors. There are a number of genes that have been established to be associated with PD, which include LRRK2. Mutations of LRRK2 are considered to be the most prevalent in the pathogenesis of PD; in studies of North American and European PD patients, 5% had a mutation of LRRK2 and a family history of PD, while 1-2% had sporadic PD (1-4). The LRRK2 gene is the causative gene of the autosomal dominant hereditary type 8 PD (5,6). It is composed of five structural domains, namely, the ankyrin repeat (ANK), leucine-rich repeat (LRR), Ras of complex proteins (Roc) C-terminal of Roc (COR), mitogen activated kinase kinase kinase (MAPKKK) and WD40 regions (7-9). Major mutations of LRRK2 include R1441C, R1441G, R1441H, R1514Q, Y1699C, G2019S, I2020T, I2012T and G2385R (10-12). Mutations of LRRK2 have been associated with a number of diseases, in particular with familial PD and sporadic PD, and the G2019S mutation is one of the most common mutations in PD (13). Clear racial and regional differences exist in the incidence of PD.

Xinjiang is an autonomous region located in Central Asia, which has two predominant populations with different genetic backgrounds, namely, the Uyghur and Han populations. The current case-control study selected PD patients and healthy individuals from the Uyghur and Han populations of the Xinjiang region for the analysis of LRRK2 gene mutations. To the best of our knowledge, this is the first time that the association between the G2019S and R1441C mutations of the LRRK2 gene and PD susceptibility has been investigated in different ethnicities and regions.

Subjects and methods

Diagnostic criteria and study subjects. From June 2010 to April 2013, 312 patients with PD (all sporadic) visiting the specialist neurology clinic of the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China) were enrolled in the study. The diagnosis was in line with the UK Brain Bank diagnostic criteria for PD. Cerebrovascular disease, encephalitis, trauma, drug-induced Parkinson's syndrome, Parkinson's plus syndrome and other severe systemic diseases were excluded. The control group consisted of 359 volunteers

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Key words: Uyghur, Parkinson's disease, G2019S, R1441C, mutation

Table I. G2019S genotype and allele frequency comparison/cases (%) of Parkinson's disease and control groups of Uyghur and Han individuals from Xinjiang.

Ethnic group	PD group	Genotype frequency						Allele frequency			Control group						Allele frequency		
		GG	GA	AA	G	A	Control group	GG	GA	AA	G	A	GG	GA	AA	G	A		
Uyghur	130	130 (100)	0 (0)	0 (0)	0 (0)	260 (100)	0 (0)	0 (0)	0 (0)	179	179 (100)	0 (0)	0 (0)	0 (0)	358 (100)	0 (0)			
Male	76	76 (100)	0 (0)	0 (0)	0 (0)	152 (100)	0 (0)	0 (0)	0 (0)	104	104 (100)	0 (0)	0 (0)	0 (0)	208 (100)	0 (0)			
Female	54	54 (100)	0 (0)	0 (0)	0 (0)	108 (100)	0 (0)	0 (0)	0 (0)	75	75 (100)	0 (0)	0 (0)	0 (0)	150 (100)	0 (0)			
Han	182	182 (100)	0 (0)	0 (0)	0 (0)	364 (100)	0 (0)	0 (0)	0 (0)	181	181 (100)	0 (0)	0 (0)	0 (0)	362 (100)	0 (0)			
Male	109	109 (100)	0 (0)	0 (0)	0 (0)	218 (100)	0 (0)	0 (0)	0 (0)	109	109 (100)	0 (0)	0 (0)	0 (0)	218 (100)	0 (0)			
Female	73	73 (100)	0 (0)	0 (0)	0 (0)	146 (100)	0 (0)	0 (0)	0 (0)	72	72 (100)	0 (0)	0 (0)	0 (0)	144 (100)	0 (0)			

Table II. R441C genotype and allele frequency comparison/cases (%) of Parkinson's disease and control groups of Uyghur and Han individuals from Xinjiang.

Ethnic group	PD group	Genotype frequency						Allele frequency			Control group						Allele frequency		
		CC	CT	CG	GT	TT	GG	C	T	Control group	CC	CT	CG	GT	TT	GG	C	T	
Uyghur	130	130 (100)	0 (0)	0 (0)	0 (0)	0 (0)	260 (100)	0 (0)	0 (0)	179	179 (100)	0 (0)	0 (0)	0 (0)	0 (0)	358 (100)	0 (0)		
Male	76	76 (100)	0 (0)	0 (0)	0 (0)	0 (0)	152 (100)	0 (0)	0 (0)	104	104 (100)	0 (0)	0 (0)	0 (0)	0 (0)	208 (100)	0 (0)		
Female	54	54 (100)	0 (0)	0 (0)	0 (0)	0 (0)	108 (100)	0 (0)	0 (0)	75	75 (100)	0 (0)	0 (0)	0 (0)	0 (0)	150 (100)	0 (0)		
Han	182	182 (100)	0 (0)	0 (0)	0 (0)	0 (0)	364 (100)	0 (0)	0 (0)	181	181 (100)	0 (0)	0 (0)	0 (0)	0 (0)	362 (100)	0 (0)		
Male	109	109 (100)	0 (0)	0 (0)	0 (0)	0 (0)	218 (100)	0 (0)	0 (0)	109	109 (100)	0 (0)	0 (0)	0 (0)	0 (0)	218 (100)	0 (0)		
Female	73	73 (100)	0 (0)	0 (0)	0 (0)	0 (0)	146 (100)	0 (0)	0 (0)	72	72 (100)	0 (0)	0 (0)	0 (0)	0 (0)	144 (100)	0 (0)		

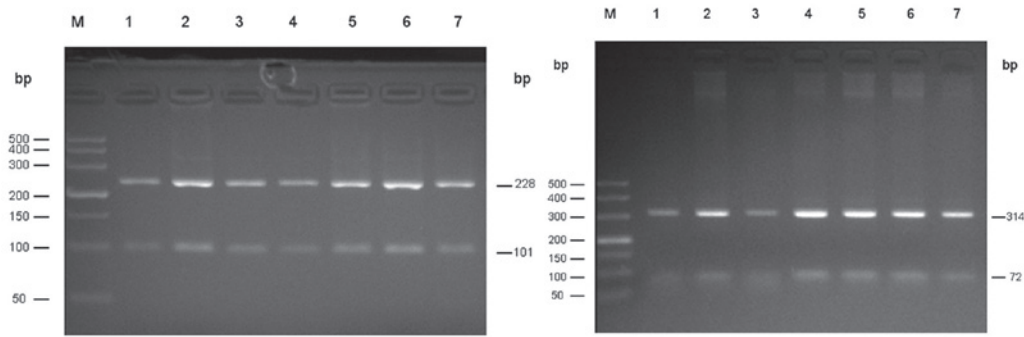


Figure 1. Electrophoresis results of the G2019S mutation in exon 41 of the LRRK2 gene digested with ScfI. Electrophoresis results of the R1441C mutation in exon 31 of the LRRK2 gene digested with BstU1. M, DNA marker 500 bp in length; 1-7, dominant hereditary sporadic PD patients.

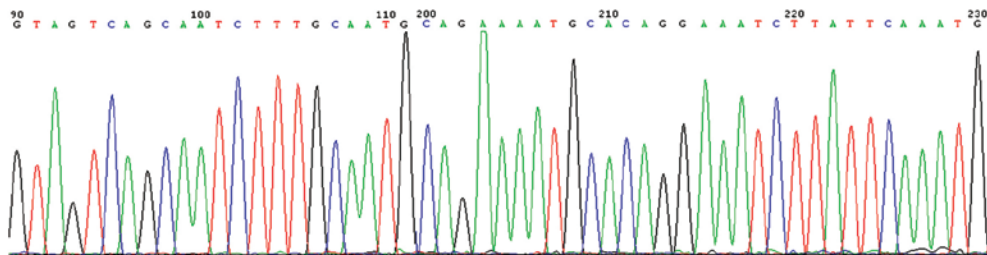


Figure 2. Partial sequencing figure of exon 41 in the LRRK2 gene.

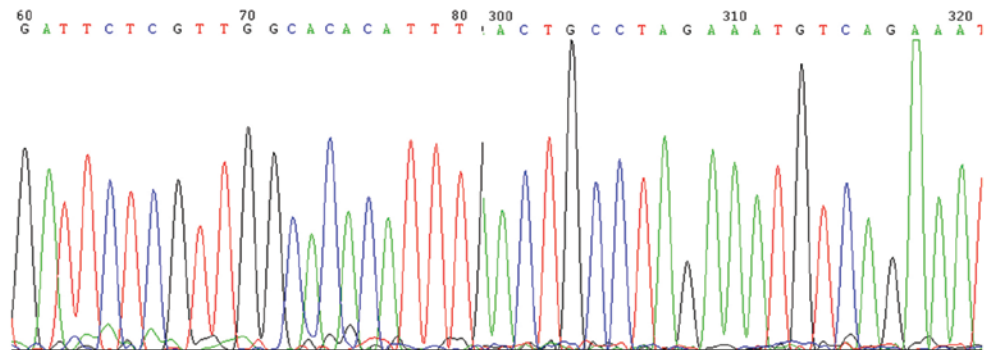


Figure 3. Partial sequencing figure of exon 31 in the LRRK2 gene.

from the same region, who had no family history or clinical manifestations of PD. Gender, age and ethnicity were matched between the two groups. The study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University, and all subjects provided informed consent.

DNA extraction. A sample of blood (2 ml) was collected from each subject, subsequent to the provision of informed consent. Following EDTA anticoagulation, a non-centrifugal-type DNA Extraction kit (Shanghai Tiangen Biotech Co., Ltd., Shanghai, China) was used to extract the genomic DNA Tris-borate buffer (Sangon Biotech Co., Ltd., Shanghai, China) was added and the DNA was maintained at -80°C .

Design of primers and amplification of the gene. The primers used for G2019S were based on those used in a previous study by Thaler *et al* (14) and the sequences were as follows: upstream, 5'-CCTGTGCATTTTCTGGCAGATA-3' and

downstream, 5'-CCTCTGATGTTTTTATCCCCATTC-3'. According to the study by Paisán-Rauíz *et al* (7), the primer sequences for R1441C were as follows: upstream, 5'-TCAACAGGAATGTGAGCAGG-3' and downstream 5'-CCCACAATTTTAAGTGAGTTGC-3'. DNA amplification was carried out as follows: The total volume of the quantitative polymerase chain reaction (qPCR) was 20 μl , including 100 ng/ μl upstream and downstream primers (0.5 μl), 2X Power Taqman Master Mix (10 μl ; Beijing Baitaie Biotechnology Co., Ltd., Beijing, China), 50 ng/ μl DNA (3.0 μl) and ddH₂O (11 μl). Primers were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). A GeneAmp System 9700 thermal cycler (Applied Biosystems Corporation, Foster City, CA, USA) was used. PCR was performed after the first denaturation at 95°C for 2 min; each cycle consisted of denaturation at 95°C for 20 sec, annealing at 62°C for 20 sec and extension at 72°C for 30 sec. The number of total PCR cycles was 35.

qPCR product detection. Equal volumes of PCR products (7 μ l) were taken, sample buffer [2X bromophenol blue; Sangon Biotech (Shanghai) Co., Ltd.] was added and the solution was mixed. The sample was placed on a 4% agarose gel for nucleic acid staining and 4 V/cm electrophoresis was performed for one hour.

Enzyme digestion genotyping. PCR products (8 μ l), 10X endonuclease buffer (2 μ l; New England Biolabs, Inc., Ipswich, MA, USA) and restriction endonucleases *ScaI* and *BstUI* (5 units; New England Biolabs Inc., Ipswich, MA, USA) were added to 20 μ l sterile double distilled water and incubated at 37°C overnight (16 h). The digestion products (9 μ l) were placed on a 4% agarose gel for ethidium bromide staining, and underwent electrophoresis at 110 V for 1.5 h. A Gel Doc 1000 gel imaging analysis system (Bio-Rad, Hercules, CA, USA) was used to detect the electrophoretic bands (Fig. 1).

Direct sequencing. To determine the accuracy of the results, 10% of the samples were randomly selected (31 from the patient group and 36 from the control group) for direct sequencing, which was performed by Shanghai Invitrogen Biotechnology Co., Ltd. (Shanghai, China).

Statistical methods. The age difference between the two groups was compared using an independent samples t-test; the differences in gender, allele and genotype frequencies between the two groups were compared using a χ^2 test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

qPCR. Using the template DNA, qPCR amplification products of 329 bp were obtained from Uyghur (n=186) and Han (n=127) patients with PD. All the products were digested with *ScaI* overnight and 228 and 101 bp fragments were obtained as presented in Fig. 1 (left). Following amplification, PCR products of 386 bp were obtained, the resulting products were digested overnight using *BstUI*, and two fragments of 314 and 72 bp were obtained as presented in Fig. 1 (right).

Sequence analysis. Following random sequencing comparison, no G2019S (Fig. 2) and R1441C (Fig. 3) mutations or novel heterozygotes were found. For G2019S, only the GG genotype and no mutant genotypes were identified in all subjects of the Han and Uyghur populations. For R1441C, only one genotype (CC) was identified in the Han and Uyghur populations. There were no differences between PD patients and the control group in these two single nucleotide polymorphisms (Tables I and II).

Discussion

In recent years, an increasing number of studies have focused on PD, and in particular on the genes associated with PD. By far, the LRRK2 gene is the most frequently mutated gene in autosomal dominant hereditary PD patients, and its incidence gradually increases with age (1,15-17). The LRRK2 gene is located on chromosome 12p11.2-q13.1 and has a total length of 1,441 nucleotides, containing 51 exons and encoding 2,527 amino acids, with a relative molecular weight of 286,000. The product

protein LRRK2/dardarin is rich in leucine (18). In the study of the molecular activity of LRRK2, two regions associated with GTP enzymes and kinases have been found. The most common LRRK2 mutations in the two regions affect the enzymatic activity, suggesting that their functionality is important (19). Different LRRK2 mutations have been reported in patients with familial and sporadic PD. Genome-wide association studies (GWAS) have found that common mutations in SNCA, LRRK2, MAPT and HLA are risk factors for PD, and have demonstrated that a loss of chromosome 18 can significantly increase the development of PD (20-33). G2019S is the most common causative mutation of PD, and ~2% of PD cases are caused by the G2019S mutation (34). G2019S is located in exon 41 of the LRRK2 gene; it increases LRRK2 kinase activity and accelerates the phosphorylation of ezrin/radixin/moesin protein (11); however, while it induces neuronal apoptosis it has no effect on the GTP carrier or GTP activity. G2019S reduces the interaction of LRRK2 with the 14-3-3 proteins and increases the aggregation of and interaction with FADD (35). Healy *et al* (17) found that in 19,376 PD patients across 21 regions, the G2019S mutation was highest in North African populations, and that it accounted for 39% of sporadic PD and 36% of familial PD. In the Jewish population of Northern Europe, G2019S accounted for 10% of cases of sporadic and 28% of cases of familial PD. However, in Asia this locus mutation is very rare, only accounting for 0.1% of LRRK2 mutations. In Japan, India, Singapore, China, Taiwan and the mainland, Gly2019Ser and Arg1441Cys/Gly mutations were not found in PD patients during LRRK2 gene detection.

R1441C is another common mutation locus in LRRK2, it is located in exon 31 of the LRRK2 gene, and it induces neuronal apoptosis. It has no kinase activity, or at least the effect is negligible; however, it stabilizes the LRRK2 dimer, reduces GTP activity and participates in FADD aggregation (35). R1441G, found in northern Spain, has the same codon as R1441H, which primarily occurs in the Caucasian population (36). Of 304 patients with PD in Belgium, 18.1% had familial PD, and the R1441C mutation accounted for 10.7% (37). R1441C is in the Roc functional domain, with GTP enzyme sequences to participate in regulatory activities, including signal transduction, cell differentiation and cell growth (38). R1441C impairs dopamine neurotransmission and D2 receptor function, leading to degeneration of the dopaminergic nervous system in patients with PD (38,39). To the best of our knowledge, there has been no relevant report of the mutation in Han PD patients.

In the current study, the G2019S and R1441C mutations of the LRRK2 gene, or any novel variant of these, were not found to be present in PD patients from the Han and Uyghur populations. This is consistent with previous studies concerning the Chinese population (40,41). These mutations may not be hot spot mutations in PD; or the small sample size of this study may explain why the mutations were not found. Future studies of these populations may investigate recent-onset PD patients, larger sample sizes and other mutations of the LRRK2 gene.

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