Correlation between LRRK2 gene G2385R polymorphisms and Parkinson's disease

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Abstract. The aim of the current study was to determine the correlation between the G2385R polymorphism of the LRRK2 gene and Parkinson's disease (PD) and the differences in genotypic and allelic frequencies between the Uyghur and Han Chinese populations. A case-control study was performed in which the genotypic and allelic frequencies of the LRRK2 gene G2385R polymorphism were analyzed using a polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing. Results showed the frequency of the GG genotype to be the highest, whereas that of the GA-type heterozygote was the lowest. No AA genotype was identified. The frequency of the GA genotype among Han patients was higher compared with that of the control group. Han individuals who carry the A allele have a higher risk of PD than non-carriers. In the present study, the frequencies of the GA genotype and A allele among Han patients were found to be higher compared with those in the Uyghur group. Moreover, Han individuals who carry the A allele exhibited a higher risk of PD than the Uyghur individuals. No statistically significant differences in genotypic and allelic frequencies were observed between the control and PD groups who were >50 years of age. The risk of PD was higher among individuals carrying the A allele than among non-carriers. The PD (≤50 years of age), the male and the female groups were compared with the control group, but no statistically significant differences were identified in allelic or genotypic frequencies. The genotypic and allelic frequencies of the LRRK2 gene G2385R polymorphism between the Uyghur and Han populations were significantly different. The A allele of the LRRK2 gene G2385R polymorphism is correlated with an increased risk of PD, particularly at an age of ≥50 years.

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Introduction

Parkinson's disease (PD) is a degenerative disease of the nervous system that occurs during middle age. Previous studies showed that genetic factors are important in the etiology of PD (1,2), with LRRK2 described as a significant susceptibility gene. Approximately 20 mutations in the LRRK2 gene have been confirmed to be associated with PD, with the mutations having significant regional and ethnic variations. Few studies have been performed on G2385R sites, which are considered to be specific genetic risk factors for the East Asian population (3-6).

Xinjiang Uyghur individuals are known to have a different genetic background compared with Xinjiang Han Chinese. A study on the LRRK2 gene polymorphism in the Xinjiang region, located in Central Asia, is the first to be performed concerning the LRRK2 gene polymorphism of PD patients of various ethnicities and regional backgrounds. Xinjian Uyghur and Han Chinese individuals with PD and healthy controls were enrolled in the current study. The correlation between the LRRK2 gene polymorphism G2385R and PD in Uyghur and Han Chinese individuals was examined.

Subjects and methods

Subjects. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. Written informed consent was obtained from all participants.

PD patients in the patient group were confirmed based on epidemiological survey (sporadic), whereas the healthy individuals without PD in the control group were selected from the survey population who were of identical age, gender, ethnicity and background as PD patients, but not genetically related to the patient group. The PD patients were screened using the diagnostic criteria by BrainBank (United Kingdom) (7). The patients were examined by specialists from the Neurological Department of the First Affiliated Hospital of Xinjiang Medical University, China, in cases of difficult diagnosis. When necessary, diagnosis was confirmed using a head MRI or CT scan. Patients who were 50 years old were divided into early- and late-onset PD groups. Secondary PD, Parkinson's syndrome, hyperthyroidism and other genetic or neural diseases were excluded.

General data. There were 354 cases in the PD group, comprising 171 Uyghur and 183 Han individuals. For the Uyghur individuals, the male:female ratio was 97:74 and their ages ranged from 31 to 95 (mean, 62.1±12.3) years. For the Han individuals, the male:female ratio was 105:78 and their ages ranged from 25 to 85 (mean, 61.9±11.5) years. There were 340 cases in the control group, comprising 160 Uyghur and 180 Han individuals. The Uyghur male:female ratio was 90:70 and their ages ranged from 33 to 90 (mean, 61.1±11.4) years. The Han male:female ratio was 100:80 and their ages ranged from 27 to 86 (mean, 60.9±11.4) years. There was no significant difference in gender and age between the PD and control groups (χ^2 gender, 0.098; P>0.05; t-test for age = 1.104; P>0.05).

DNA extraction. The patients and normal controls provided informed consent prior to genomic DNA extraction from 2 ml of peripheral venous blood using the conventional phenol/chloroform method. DNA purity was 1.7-1.9 at ≥10 ng/μl and was stored at -20˚C.

Primer design. According to the DNA sequence of exon 48 in the G2385R of the LRRK2 gene from the National Center for Biotechnology Information (NCBI) and ensemble, primers for G2385R were designed. The upstream primer was 5'-TAGCCCTGTTGTGGAAGTG-3' and the downstream primer was 5'-TTCAGAGGCAGAAAGGAAG-3'. The length of the amplified fragment was 170 bp. The primers were synthesized by the Beijing Huata Company (Beijing, China).

Polymerase chain reaction (PCR) amplification and identification. The total PCR volume was 25 μl, including 0.5 μl of 100 ng/μl upstream and downstream primers, 10 μl MIX, 3.0 μl of 50 ng/μl gDNA and 11 μl ddH_2O. The PCR was performed on the PE9600 thermal cycler (P.E. USA Inc., Cincinnati, OH, USA). The reactions were incubated at 94˚C for 2 min, followed by 35 cycles of 94˚C for 30 sec, annealing at 55.5˚C for 30 sec and 72˚C for 45 sec, then extended to 72˚C for 5 min and preserved at 4˚C. Subsequently, 7 μl of the mixture was combined with loading buffer and the sample was analyzed in 2% agarose gel. Subsequent to staining, the gel was observed under a UV instrument and images were captured. The amplified fragment length was 170 bp.

Restriction fragment length polymorphism (RLFP). The enzyme digestion reaction was performed using a 20-μl reaction system containing 10 μl PCR product, 2 μl 10X buffer, 5 units *Acc*I enzyme and 7.5 μl ddH_2O. The reaction system was incubated at 37˚C and was digested overnight (16-24 h). The digestion products of the G2385R genotypes were confirmed using 6% neutral polyacrylamide gel with D50 Marker as the standard, certain samples were confirmed through DNA sequencing. Only 170 bp was identified in the GG homozygote (wild-type, no restriction site), three fragments (170, 123 and 47 bp) were identified in the heterozygous GA-type and two fragments (123 and 47 bp) were identified in the AA homozygotes.

Statistical analysis. SPSS 17.0 software was used for data analysis. The frequencies of the genotypes and alleles were analyzed using the gene counting method. Rates (%) were used to represent the counting data. The allelic and genotypic frequencies of the two groups were analyzed using a χ^2 test. P<0.05 was regarded as statistically significant.

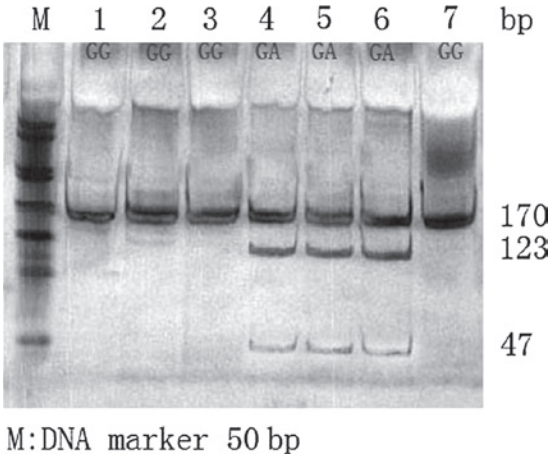

Figure 1. Genotypes of the G2385R polymorphism of the LRRK2 gene.

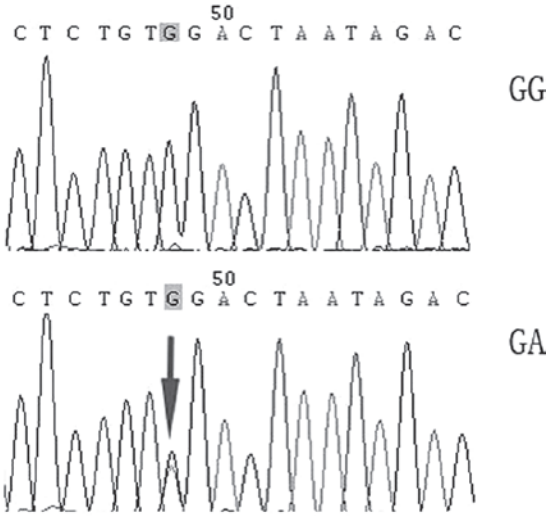

Figure 2. Sequence of the G2385R polymorphism of the LRRK2 gene.

Results

Goodness-of-fit test for Hardy-Weinberg equilibrium. The distribution of the two genotypic polymorphisms in the case and control groups were consistent with the Hardy-Weinberg genetic equilibrium and results of the goodness-of-fit test were regarded as excellent (PD group, χ^2=0.320, P>0.05; control group, χ^2=0.036, P>0.05).

Results of the LRRK2 gene G2385R polymorphism assay showed the frequency of the GG genotype to be the highest, whereas the frequency of the GA-type heterozygote was the lowest. No AA genotype was identified (Figs. 1 and 2).

Comparison of the G2385R genotypic and allelic frequency distribution. The comparison of the G2385R polymorphism genotypic and allelic frequencies between the PD and control groups are shown in Table I. The GA genotypic and A allelic frequencies in the PD group were significantly higher than

Table I. Comparison of the G2385R polymorphism allele and genotype frequency between the PD and control groups.

		Genotype frequency, n (%)			Allele frequency, n (%)	
Group	Cases (n)	GG	GA	AA	G	A
PD	354	333 (94.1)	21 (5.9)	0 (0)	687 (97)	21 (3)
Control	340	333 (97.9)	7 (2.1)	0 (0)	673 (99)	7 (1)

PD, Parkinson's disease.

Table II. Comparison of the G2385R polymorphism allele and genotype frequency between the Uyghur and Han ethnicities.

		Genotype frequency, n (%)			Allele frequency, n (%)	
Groups	Cases (n)	GG	GA	AA	G	A
Uyghur PD	171	170 (99.4)	1 (0.6)	0 (0)	341 (99.7)	1 (0.3)
Uyghur control	160	159 (99.4)	1 (0.6)	0 (0)	319 (99.7)	1 (0.3)
Han PD	183	163 (89.1)	20 (10.9)	0 (0)	346 (94.5)	20 (5.5)
Han control	180	174 (96.7)	6 (3.3)	0 (0)	354 (98.3)	6 (1.7)

PD, Parkinson's disease.

Table III. Comparison of G2385R polymorphism allele and genotype frequency in the PD and control groups with different age.

		Genotype frequency, n (%)			Allele frequency, n (%)	
Groups	Cases (n)	GG	GA	AA	G	A
Early-onset (≤50 years of age)						
PD	77	72 (93.5)	5 (6.5)	0 (0)	149 (96.8)	5 (3.2)
Control	71	70 (98.6)	1 (1.4)	0 (0)	141 (99.3)	1 (0.7)
Late-onset (>50 years of age)						
PD	277	261 (94.2)	16 (5.8)	0 (0)	538 (97.1)	16 (2.9)
Control	269	263 (97.8)	6 (2.2)	0 (0)	532 (98.9)	6 (1.1)

PD, Parkinson's disease.

those in the control group (χ^2=6.720, P=0.01 and χ^2=6.582, P=0.01). The risk of occurrence of PD was higher for individuals carrying the A allele than those without the A allele (OR, 2.94; 95% CI, 1.29-6.69).

The comparison of the G2385R genotypic and allelic frequencies between the Uyghur and Han PD and control groups are shown in Table II. The GA genotypic and A allelic frequencies in the Han PD group were significantly higher than those in the Uyghur PD group (χ^2=16.95, P=0.000 and χ^2=16.432, P=0.000). The risk of PD occurrence was higher among the Han Chinese individuals carrying the A allele than for Uyghur individuals (OR, 19.71; 95% CI, 4.66-83.43). The GA genotypic and allelic frequencies in the Han PD group were significantly higher than those in the Han control group (χ^2=7.873, P=0.005 and χ^2=7.581, P=0.006). The risk of PD occurrence among the Han Chinese individuals carrying the A allele was significantly higher than that in the Han Chinese without the A allele (OR, 3.41; 95% CI, 1.42-8.19). The difference in genotypic and allelic frequencies was not statistically significant between the Uyghur PD and control groups (χ^2=0.002, P=0.962).

A comparison was conducted of the G2385R genotypic and allelic frequency distributions between the PD patients and the controls with various (Table III). The GA genotypic and A allelic frequencies were higher in the late-onset PD group than in the control group (>50 years of age) and the difference was statistically significant (χ^2=4.437, P=0.035 and χ^2=4.436, P=0.037). The risk of PD occurrence was significantly higher among individuals carrying the A allele than in individuals without the A allele (OR, 2.64; 95% CI, 1.07-6.50). No statistically significant difference was detected in the genotypic and allelic frequencies between the early-onset PD and the control group >50 years old (χ^2=2.456, P=0.117 and χ^2=2.405, P=0.121).

Table IV. Comparison of G2385R polymorphism allele and genotype frequency in PD group and control group with different gender.

		Genotype frequency, n (%)			Allele frequency, n (%)	
Groups	Cases (n)	GG	GA	AA	G	A
Male PD	202	192 (95)	10 (5)	0 (0)	394 (97.5)	10 (2.5)
Male control	190	187 (98.4)	3 (1.6)	0 (0)	377 (99.2)	3 (0.8)
Female PD	152	141 (92.8)	11 (7.2)	0 (0)	293 (96.4)	11 (3.6)
Female control	150	146 (97.3)	4 (2.7)	0 (0)	296 (98.7)	4 (1.3)

PD, Parkinson's disease.

A comparison was conducted of the G2385R genotypic and allelic frequency distributions in the PD patient and control groups with different gender (Table IV). No statistically significant differences were found in the genotypic and allelic frequencies between the male PD and the male control groups (χ^2=3.471, P=0.062 and χ^2=3.413, P=0.065) and the female PD and the female control group (χ^2=3.341, P=0.068 and χ^2=3.256, P=0.071).

Discussion

Among the neurodegenerative diseases, the incidence of PD ranks second to Alzheimer's disease. Genetic factors are significant etiological factors. The LRRK2 gene, also known as PARK8, causes disease and possesses the highest frequency of gene mutation in autosomal dominant PD (8). It is also the disease-causing gene most common in late-onset PD.

The LRRK2 gene mutation has significant geographic and ethnic variations. G2019S and R1441C are common among Caucasian populations in Europe and North Africa (9-13). By contrast, this mutation is extremely rare in Asia, accounting for only 0.1% of LRRK2 mutations. In previous studies, no G2019S, R1441G or R1441C mutations were identified in a Chinese population (14,15). Mata *et al* first reported that G2385R is a Chinese-specific PD risk factor (16), making the study of this mutation popular. The mutation frequency of G2385R in Malay PD patients was 2.0%, but the mutation was not correlated with the incidence of PD (17). This type of polymorphism is not detected in other ethnicities, including Southern Asian and Caucasian populations (18-21). This point mutation is common in the sporadic PD patients in East Asian countries and regions, with a frequency of 10% in mainland China, 7.27% in Singapore, 11.6% in Japan and 8% in Taiwan (22). A study from mainland China revealed that the mutation frequencies in the G2385R PD populations from Shanghai and Sichuan were at 5.96 and 11.8%, respectively. The mutation had a marked correlation with the incidence of PD (4,22). A previous study performed by Ross *et al* also confirmed that the mutation of G2385R increased the PD risk of East Asian ethnicities (1.73, 1.20-2.49; P=0.0026) (6).

The current study revealed that the mutation rate of the G2385R polymorphism is extremely low among the Uyghur population in the Xinjiang region. The mutation frequency of the GA genotype was 0.58 (1/171) and 0.62% (1/160) in the Uyghur PD and the Uyghur control groups, respectively. No statistically significant differences were detected in the genotypic and allelic frequencies between these two groups, suggesting that the G2385R polymorphism does not correlation with PD in the Xinjiang Uyghur population. Previous studies have confirmed that the G2385R polymorphism is a common mutation among the Chinese PD population and is, therefore, a specific genetic risk factor in East Asian populations (17,23,24). Uyghur and Han individuals in the Xinjiang region in Central Asia have various genetic, geographic and ethinic backgrounds, leading to varying results. The GA mutation frequencies were 10.9 (20/183) and 3.3% (6/180) in the Han PD and Han control groups, respectively. Statistically significant differences were found in the genotypic and allelic frequencies between these two groups. The risk of PD was higher among individuals carrying the A allele than those without the A allele, suggesting that the G2385R polymorphism is correlated with the occurrence of PD among the Han population in the Xinjiang region. In the age subgrouping, statistically significant differences were detected in the genotypic and allelic frequencies between the late-onset PD group (>50 years old) and the control group. The risk of PD was higher among individuals carrying the A allele than those without the A allele, suggesting that the G2385R polymorphism is correlated with late-onset PD. This is consistent with reports that the LRRK2 gene is a common disease-causing gene in late-onset PD. In the gender subgrouping, there were no significant differences in the G2385R genotypic and allelic frequencies between the male PD and control groups and the female PD and control groups, respectively. The results contrast with those reported by Li *et al* (22) where the frequency of the G2385R mutation among female patients was significantly higher than that among male patients. The varying results are caused by sampling errors, inadequate sample size, regional differences and other environmental and lifestyle differences.

In conclusion, the G2385R polymorphism is correlated with PD among the Han population in Xinjiang, particularly among those individuals >50 years old. However, the polymorphism is not correlated with the incidence of PD among the Uyghur population. The LRRK2 gene mutation has geographic and ethnic variations. Expanding the sample size in other populations and ethnic groups is necessary for further studies.

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References

1. Gosal D, Ross OA and Toft M: Parkinson's disease: the genetics of a heterogeneous disorder. Eur J Neurol 13: 616-627, 2006.
2. Pankratz N and Foroud T: Genetics of Parkinson disease. Genet Med 9: 801-811, 2007.
3. Di Fonzo A, Wu-Chou YH, Lu CS, *et al*: A common missense variant in the LRRK2 gene, Gly2385Arg, associated with Parkinson's disease risk in Taiwan. Neurogenetics 7: 133-138, 2006.
4. An XK, Peng R, Li T, *et al*: LRRK2 Gly2385Arg variant is a risk factor of Parkinson's disease among Han Chinese from mainland China. Eur J Neurol 15: 301-305, 2008.
5. Tan EK, Zhao Y, Skipper L, *et al*: The LRRK2 Gly2385Arg variant is associated with Parkinson's disease: genetic and functional evidence. Hum Genet 120: 857-863, 2007.
6. Ross OA, Soto-Ortolaza AI, Heckman MG, *et al;* Genetic Epidemiology Of Parkinson's Disease (GEO-PD) Consortium: Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study. Lancet Neurol 10: 898-908, 2011.
7. Hughes AJ, Daniel SE, Kilford L and Lees AJ: Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55: 181-184, 1992.
8. Lesage S, Dürr A and Brice A: LRRK2: a link between familial and sporadic Parkinson's disease. Pathol Biol (Paris) 55: 107-110, 2007.
9. Deng H, Le WD, Guo Y, *et al*: Genetic and clinical identification of Parkinson's disease patients with LRRK2 G2019S mutation. Ann Neurol 57: 933-934, 2005.
10. Lesage S, Dürr A, Tazir M, *et al*; French Parkinson's Disease Genetics Study Group: LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. N Engl J Med 354: 422-423, 2006.
11. Ozelius LJ, Senthil G, Saunders-Pullman R, *et al*: LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. N Engl J Med 354: 424-425, 2006.
12. Paisán-Ruíz C, Jain S, Evans EW, *et al*: Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 44: 595-600, 2004.
13. Simón-Sánchez J, Martí-Massó JF, Sánchez-Mut JV, *et al*: Parkinson's disease due to the R1441G mutation in Dardarin: a founder effect in the Basques. Mov Disord 21: 1954-1959, 2006.
14. Patra B, Parsian AJ, Racette BA, *et al*: LRRK2 gene G2019S mutation and SNPs [haplotypes] in subtypes of Parkinson's disease. Parkinsonism Relat Disord 15: 175-180, 2009.
15. Cookson MR and Bandmann O: Parkinson's disease: insights from pathways. Hum Mol Genet 19: R21-R27, 2010.
16. Mata IF, Kachergus JM, Taylor JP, *et al*: Lrrk2 pathogenic substitutions in Parkinson's disease. Neurogenetics 6: 171-177, 2005.
17. Tan EK, Zhao Y, Tan L, *et al*: Analysis of LRRK2 Gly2385Arg genetic variant in non-Chinese Asians. Mov Disord 22: 1816-1818, 2007.
18. Haubenberger D, Bonelli S, Hotzy C, *et al*: A novel LRRK2 mutation in an Austrian cohort of patients with Parkinson's disease. Mov Disord 22: 1640-1643, 2007.
19. Toft M, Haugarvoll K, Ross OA, *et al*: LRRK2 and Parkinson's disease in Norway. Acta Neurol Scand Suppl 187: 72-75, 2007.
20. Biskup S, Mueller JC, Sharma M, *et al*: Common variants of LRRK2 are not associated with sporadic Parkinson's disease. Ann Neurol 58: 905-908, 2005.
21. Paisán-Ruíz C, Lang AE, Kawarai T, *et al*: LRRK2 gene in Parkinson disease: mutation analysis and case control association study. Neurology 65: 696-700, 2005.
22. Li C, Ting Z, Qin X, *et al*: The prevalence of LRRK2 Gly2385Arg variant in Chinese Han population with Parkinson's disease. Mov Disord 22: 2439-2443, 2007.
23. Farrer MJ, Stone JT, Lin CH, *et al*: Lrrk2 G2385R is an ancestral risk factor for Pakinson's disease in Asia. Parkinsonism Relat Disord 13: 89-92, 2007.
24. Funayama M, Li Y, Tomiyama H, *et al*: Leucine-rich repeat kinase 2 G2385R variant is a risk factor for Parkinson disease in Asian population. Neuroreport 18: 273-275, 2007.