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 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 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 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.

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. Xinjiang 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.

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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 (χ² test for 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 ddH₂O. The PCR was performed on the PE9600 thermal cycler (PE. 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 AccI enzyme and 7.5 µl ddH₂O. 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 χ² test. P<0.05 was regarded as statistically significant.

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, χ²=0.320, P>0.05; control group, χ²=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...
those in the control group ($\chi^2$=6.720, $P=0.01$ and $\chi^2$=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 ($\chi^2$=16.95, $P=0.000$ and $\chi^2$=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 ($\chi^2$=7.873, $P=0.005$ and $\chi^2$=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 ($\chi^2$=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 ($\chi^2$=4.437, $P=0.035$ and $\chi^2$=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 ($\chi^2$=2.456, $P=0.117$ and $\chi^2$=2.405, $P=0.121$).

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases (n)</th>
<th>Genotype frequency, n (%)</th>
<th>Allele frequency, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>354</td>
<td>GG: 333 (94.1), GA: 21 (5.9), AA: 0 (0)</td>
<td>G: 687 (97), A: 21 (3)</td>
</tr>
<tr>
<td>Control</td>
<td>340</td>
<td>GG: 333 (97.9), GA: 7 (2.1), AA: 0 (0)</td>
<td>G: 673 (99), A: 7 (1)</td>
</tr>
</tbody>
</table>

PD, Parkinson’s disease.

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

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

PD, Parkinson’s disease.

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

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

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 female PD groups ($\chi^2=3.471, P=0.062$ and $\chi^2=3.413, P=0.065$) and the male control and female control groups ($\chi^2=3.341, P=0.068$ and $\chi^2=3.256, P=0.071$).

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases (n)</th>
<th>GG</th>
<th>GA</th>
<th>AA</th>
<th>G</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male PD</td>
<td>202</td>
<td>192 (95)</td>
<td>10 (5)</td>
<td>0 (0)</td>
<td>394 (97.5)</td>
<td>10 (2.5)</td>
</tr>
<tr>
<td>Male control</td>
<td>190</td>
<td>187 (98.4)</td>
<td>3 (1.6)</td>
<td>0 (0)</td>
<td>377 (99.2)</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td>Female PD</td>
<td>152</td>
<td>141 (92.8)</td>
<td>11 (7.2)</td>
<td>0 (0)</td>
<td>293 (96.4)</td>
<td>11 (3.6)</td>
</tr>
<tr>
<td>Female control</td>
<td>150</td>
<td>146 (97.3)</td>
<td>4 (2.7)</td>
<td>0 (0)</td>
<td>296 (98.7)</td>
<td>4 (1.3)</td>
</tr>
</tbody>
</table>

No statistically significant differences were detected in the genotypic and allelic frequencies between these two groups, suggesting that the G2385R polymorphism does not correlate 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 ethnic 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 mutation 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.

### Acknowledgements

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