No association of the LRRK2 genetic variants with Alzheimer's disease in Han Chinese individuals

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Negative results

The leucine-rich repeat kinase 2 (LRRK2) gene has been regarded as 1 of the most common genetic causes of Parkinson's disease (PD). We hypothesized that LRRK2-susceptible allele(s) for PD might pose a risk for Alzheimer's disease (AD). In this study, we screened 12 LRRK2 gene variants in 2 independent cohorts from southwestern China (341 AD patients and 435 normal individuals) and eastern China (297 AD patients and 384 normal individuals), to discern the potential association between this gene and AD. No variant was identified to be associated with AD in either case-control sample. As both of the cohorts were of Han Chinese origin, we combined the LRRK2 variant data for the 2 sample sets together (a total of 638 AD patients and 819 normal individuals) and still found no association between the LRRK2 gene and AD, suggesting that LRRK2 gene variants may not affect the development of AD in Han Chinese individuals.

1. Introduction

Alzheimer’s disease (AD) is a prevalent neurodegenerative disease that leads mainly to severe memory loss in elderly persons (>60 years of age) (Querfurth and LaFerla, 2010). The pathogenesis of AD is multifactorial; both environmental and genetic factors can affect the development of AD (Querfurth and LaFerla, 2010). Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been reported to be responsible for Parkinson’s disease (PD) (Kett and Dauer, 2012). Because of the observed tau pathology in patients with LRRK2 mutation and the very close location between the LRRK2 gene and the reported AD-associated genomic region (Pericak-Vance et al., 1997; Zimprich et al., 2004), this gene was regarded as a potential candidate for AD in recent studies (Lee et al., 2006; Santos-Reboucas et al., 2008; Tan et al., 2009; Toft et al., 2005; Zhao et al., 2011). However, only 2 independent studies have identified potential risk variants in AD patients (Santos-Reboucas et al., 2008; Zhao et al., 2011), whereas the other studies showed that LRRK2 mutations are not common in AD patients. As only 1 or 2 LRRK2 mutations were analyzed in these previous studies, it remains elusive as to whether other genetic variants of the LRRK2 gene are susceptible to AD.

To clarify the potential association between the LRRK2 variants and AD, we screened 8 reported PD-associated variants and 4 Tag single nucleotide polymorphisms (SNPs) of the LRRK2 gene in 341 AD patients and 435 healthy subjects from southwestern China. We did not identify any association of the LRRK2 variants with AD. This result was further validated in an independent sample set (297 AD patients and 384 normal individuals) from eastern China, indicating that LRRK2 gene variants may not affect the development of AD in individuals of Han Chinese origin.

2. Methods

Two independent sample sets, 1 from southwestern China and the other from eastern China, were recruited for this study. The
cohort from southwestern China was composed of 341 AD patients and 435 normal individuals who were collected at the Shanghai Mental Health Center. The cohort from eastern China was composed of 297 AD patients and 384 normal individuals who were collected at the Mental Health Center of West China Hospital. The cohort from southwestern China was composed of 341 AD patients and 435 normal controls from southwestern China, and in 297 AD patients and 384 normal controls from eastern China.

Eight previously reported disease-associated variants and 4 Tag SNPs (HapMap, http://hapmap.ncbi.nlm.nih.gov/, phase 3, CHB) of the LRRK2 gene were investigated. The information for each SNP is shown in Table S1. All 12 SNPs were detected by SNaPshot assay, which was composed of a multiplex polymerase chain reaction (PCR) of all of the SNPs and followed by a single-base extension process. The SNaPshot assay was

### Table 1

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Populations</th>
<th>Allele</th>
<th>No. of samples</th>
<th>p Value</th>
<th>Genotype</th>
<th>No. of samples</th>
<th>p Value</th>
<th>Adjusted p Value</th>
<th>HWE p Value (control)</th>
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<tr>
<td>Rs732374&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Southwestern China</td>
<td>A/G</td>
<td>207/437</td>
<td>288/582</td>
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<td>AA/GC/AG</td>
<td>38/171/131</td>
<td>46/193/196</td>
<td>0.18</td>
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<tr>
<td></td>
<td>Eastern China</td>
<td>A/G</td>
<td>218/342</td>
<td>252/516</td>
<td>0.33</td>
<td>AA/GC/AG</td>
<td>43/129/124</td>
<td>50/182/152</td>
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<tr>
<td></td>
<td>Combined</td>
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<td>425/780</td>
<td></td>
<td></td>
<td></td>
<td>86/311/165</td>
<td></td>
<td></td>
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<tr>
<td>Rs34594498&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>Southwestern China</td>
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<td>861/9</td>
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<td>CC/TT/CT</td>
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<td>Rs4473003&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>245/437</td>
<td>288/582</td>
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<td>CC/TT/CT</td>
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<td>CC/TT/CT</td>
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#### Key:
- **AD**: Alzheimer’s disease
- **HWE**: Hardy–Weinberg equilibrium
- **SNP**: Single nucleotide polymorphism

#### Notes:
- <sup>a</sup> p Value was calculated by Fisher’s exact test.
- <sup>b</sup> Binary logistic regression analysis was performed to assess the association of each SNP in the control population was set as reference. Minor allele frequencies of rs34594498, rs33939927, rs35801418, rs34637584, rs35870237, and rs732374 were too low and these SNPs were not included in the logistic regression analysis.
- <sup>c</sup> One patient from southwestern China failed to be genotyped for SNPs rs732374, rs34594498, and rs7307310.
- <sup>d</sup> One patient from eastern China failed to be genotyped for SNPs rs732374, rs34594498, and rs4473003.
- <sup>e</sup> One patient from eastern China failed to be genotyped for SNPs rs732374, rs34594498, and rs4473003.
- <sup>f</sup> Minor allele frequencies of SNPs rs33939927, rs35801418, rs34637584, and rs35870237 were less than 0.01, and we did not include these SNPs in the analysis.

### Table 2

<table>
<thead>
<tr>
<th>Haplotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Southwestern China</th>
<th>Eastern China</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
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<td>Patients</td>
<td>Controls</td>
<td>Patients</td>
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<td>CGCCGATCA</td>
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<td>164</td>
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<tr>
<td>ACTCCGCG</td>
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<td>GCTGACAG</td>
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<td>CTCGCCGG</td>
<td>58</td>
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<td>CTCGCCGG</td>
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<td>GCTCAGCG</td>
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<tr>
<td>ACTCCGAC</td>
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<td>20</td>
<td>14</td>
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<td>ACTCCGCG</td>
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<td>18</td>
<td>-</td>
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<td>Rare haplotypes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44</td>
<td>55</td>
<td>42</td>
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</tbody>
</table>

#### Key:
- **AD**: Alzheimer’s disease
- **SNP**: Single nucleotide polymorphism

#### Notes:
- <sup>a</sup> Order of SNPs for each haplotype: rs732374-rs34594498-rs4473003-rs3393990-rs7298930-rs7307310-rs3477834-rs3761863.
- <sup>b</sup> Haplotypes with a frequency of less than 3% were pooled together.
performed following the detailed, step-by-step procedure described in our recent study (Wang et al., 2012). The ε4 allele of the apolipoprotein E (APOE) gene, which is considered to be a risk factor for AD, was also genotyped by using the SNaPshot assay. Primers for PCR and single-base extension are listed in Table S1. Binary logistic regression analysis was performed to assess the associations of LRRK2 SNPs with the risk of AD, with adjustment for APOE4 allele status. The statistical power was calculated by using Quanto software (Gauderman, 2002) with log-additive inheritance mode. With a false-positive result rate controlled as 0.05 for minor allele frequency (MAF) from 0.1 to 0.5, the statistical power to detect the odds ratio (OR) as 1.5 for risk alleles was expected to be from 67.6% to 95.8%. A detailed Methods section is available in the online Supplementary data.

Fig. 1. Linkage disequilibrium (LD) pattern of 8 single nucleotide polymorphisms (SNPs) in the LRRK2 gene in Alzheimer’s disease (AD) patients and controls. Value in each square refers to $r^2 \times 100$. (A) LD pattern of 8 SNPs in the LRRK2 gene in AD patients and controls from southwestern China. (B) LD pattern of 8 SNPs in the LRRK2 gene in AD patients and controls from eastern China. (C) LD pattern of 8 SNPs in the LRRK2 gene in AD patients and controls in the combined populations from southwestern China and eastern China.
3. Results

In total, we analyzed 12 LRRK2 SNPs and the APOE genotypes by using SNAPSHOT in 2 independent sample sets from southwestern China (341 AD patients and 435 normal individuals) and eastern China (297 AD patients and 384 normal individuals), respectively (Fig. S1). The APOE genotyping result confirmed the previous finding that APOE allele ε4 is a risk factor for AD (Table S2). The allele frequency of ε4 in our patient population from southwestern China (13.3%) was, in general, similar to those in previous studies in a Chinese population (11.8%) (Liu et al., 1999) and a Mongolian population (17%) (Hurlitelemuer et al., 2010), whereas the patient population from eastern China presented with a higher allele frequency of ε4 (24.6%). Four of the 12 LRRK2 SNPs (rs33939927, rs35801418, rs34637584, and rs35870237) had a MAF of less than 0.01 (Table 1). The genotype, allele, and haplotype frequency of the other 8 SNPs were calculated (Tables 1 and 2). None of these 8 SNPs showed any deviation from Hardy–Weinberg equilibrium in the control population of either sample set (Table 1). In both cohorts, LD structures of the 8 SNPs were similar in the patient and control populations (Fig. 1A and B), and no significant difference was observed between patients and controls for the genotype, allele, and haplotype frequency of the 8 SNPs (Tables 1 and 2). Logistic regression analysis revealed that, after adjustment for APOE4 status, there was still no statistically significant difference for the LRRK2 SNPs between the patient and control populations in either cohort (Table 1).

As both of the cohorts were of Han Chinese origin, we further pooled the LRRK2 variant data of the 2 sample sets together (a total of 638 AD patients and 819 normal controls). There was still no variant identified to be associated with AD (Tables 1 and 2, Fig. 1C).

4. Discussion

Previous studies of the LRRK2 mutations in AD patients have yielded controversial results. Some studies showed an infrequent occurrence of the LRRK2 mutations in AD (Lee et al., 2006; Tan et al., 2009; Toft et al., 2005), whereas others argued for an important role of the LRRK2 gene in AD (Santos-Reboucas et al., 2008; Zhao et al., 2011). Specifically, mutation p.G2019S was identified in a Brazilian man who presented with clinical features of both AD and PD (Santos-Reboucas et al., 2008), which suggested that a certain part of the neurodegenerative pathway overlapped in the 2 diseases. Variant p.R1628P was regarded as a risk factor for AD in patients from Singapore (Zhao et al., 2011). It should be noted that most of those studies screened for only 1 or 2 LRRK2 variants, and a thorough analysis considering more LRRK2 variants is necessary to clarify the potential association between the LRRK2 gene and AD.

In this study, we used the SNAPSHOT assay to genotype 12 LRRK2 variants (including 8 reported disease-associated variants and 4 Tag SNPs) in 2 independent cohorts from southwestern China and eastern China, respectively. These genetic variants are located in the entire LRRK2 gene region. Analysis for the matri-lineal genetic components in the patient and control populations showed that our samples were well matched, and there was no apparent population stratification in our sample (author’s unpublished data). Consistent with some previous studies (Lee et al., 2006; Toft et al., 2005), we found no AD patient with mutations p.R1441C (rs33939927), p.Y1699C (rs35801418), p.G2019S (rs34637584), and p.I2020T (rs35870237), indicating that these PD pathogenic mutations were very rare in Chinese patients with AD. For the other 8 disease-associated SNPs or Tag SNPs with a higher MAF, there was no significant difference between AD patients and controls with regard to the allele, genotype, or haplotype frequency in either sample set. After adjustment for the APOE allele ε4 status, no statistically significantly different result was obtained. Our results indicate that genetic variants in the LRRK2 gene may not affect AD in Han Chinese individuals.

Our study has 2 limitations. First, the sample size was relatively small, which may preclude us from drawing a firm conclusion. Nonetheless, we remedied this limitation by screening the LRRK2 genetic variants in 2 independent cohorts. Consistent results were obtained in these 2 independent sample sets in that no LRRK2 variant was identified to be associated with AD. Moreover, we performed a combined analysis with the LRRK2 variant data of the 2 cohorts, and still found no positive association between LRRK2 variants and AD in the combined Han Chinese sample set (composed of 638 AD patients and 819 normal controls). Second, we lacked some demographic data, such as age, sex, and years of education in the study groups (AD and control groups), which prevented us from retrieving more information in the association analyses. Further study with a large sample size and sufficient demographic data shall be carried out to solidify our conclusion.

Disclosure statement

The authors declare no conflicts of interest.

Acknowledgements

We are grateful to the subjects who donated DNA samples. We thank Miss Hui-Zhen Wang and Mr. Qin Xiang for technical assistance. This study was supported by the Ministry of Science and Technology of China (2011CB910900), National Natural Science Foundation of China (30925021), and the Strategic Priority Research Program (B) of the Chinese Academy of Sciences (XDB02020000).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.08.013.

References


Supplementary Materials

No association of the *LRRK2* genetic variants with Alzheimer’s disease in Han Chinese

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1. Introduction
Alzheimer’s disease (AD) is a prevalent neurodegenerative disease, which mainly lead to severe memory loss in elderly people over 60 years old (Querfurth and LaFerla, 2010). The pathogenesis of AD is multifactorial, both environmental and genetic factors can affect the development of AD (Querfurth and LaFerla, 2010).

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been reported to be responsible for Parkinson’s disease (PD) (Kett and Dauer, 2012). Because of the observed tau pathology in patients with LRRK2 mutation and the very close location between the LRRK2 gene and the reported AD-associated genomic region (Pericak-Vance et al., 1997; Zimprich et al., 2004), this gene was regarded as a potential candidate for AD in recent studies (Chang et al., 2010; Lee et al., 2006; Li et al., 2012; Santos-Reboucas et al., 2008; Santos-Reboucas et al., 2009; Tan et al., 2009; Tedde et al., 2007; Toft et al., 2005; Zabetian et al., 2006; Zhao et al., 2011). However, only two independent studies had identified potential risk variants in AD patients (Santos-Reboucas et al., 2008; Zhao et al., 2011), whereas most of the other studies showed that LRRK2 mutations were not common in AD patients. As only one or two LRRK2 mutations were analyzed in these previous studies, it remains elusive whether other genetic variants of the LRRK2 gene were susceptible to AD.

In order to clarify the potential association between the LRRK2 variants and AD, we screened 8 reported PD-associated variants and 4 Tag SNPs of the LRRK2 gene in 341 AD cases and 435 healthy subjects from Southwest China. We identified no association of the LRRK2 variants with AD. This result was further validated in an independent sample set (297 AD cases and 384 normal individuals) from East China, indicating that LRRK2 gene variants may not affect the development of AD in Han Chinese.

2. Materials and methods
2.1. Samples
A total of two independent sample sets, one was from Southwest China and the other one was from East China, were recruited in this study. The cohort from Southwest China was composed of 341 AD patients and 435 normal individuals which were collected at the Mental Health Center of West China Hospital. The cohort from East China, which was composed of 297 AD patients and 384 normal individuals, were collected at the Shanghai Mental Health Center and Tongde Hospital of Zhejiang Province. All subjects were of Han Chinese origin. The diagnosis of AD was performed following the DSM-IV and the NINCDS-ADRDA criteria. The healthy controls were confirmed to have normal cognitive function. Written informed consents conforming to the tenets of the Declaration of Helsinki were obtained from all participants prior to this study. The institutional review board of the Kunming Institute of Zoology, Chinese Academy of Sciences approved this study.

2.2. SNP selection and genotyping
Eight previously reported disease-associated mutations/variants and four Tag SNPs (HapMap, http://hapmap.ncbi.nlm.nih.gov/, phase 3, CHB) of the LRRK2 gene were investigated in this study. The information of each SNP is shown in Table S1. All of the 12 SNPs were detected by SNaPshot assay which was composed of a multiplex PCR of all the SNPs and followed with a single-base extension process. The SNaPshot assay was performed following the detailed step-by-step
procedure described in our recent study (Wang et al., 2012). In brief, multiplex PCR was conducted in a volume of 8 μL reaction solution containing 20-50 ng template DNA, 0.4 mM dNTPs, 0.2-0.5 μM of each primer (Table S1), 2.0 mM MgCl₂ and 1.0 U of AmpliTaq Gold polymerase (Applied Biosystems). The PCR reaction was performed with the following procedures: a pre-denaturation cycle at 94 °C for 2 min; 40 amplification cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min. After being cleaned up with 1.0 U of shrimp alkaline phosphatase (SAP) and 0.5 U of Exonuclease I (TaKaRa Biotechnology Co. Ltd., Dalian, China), 4 μL of the PCR products were used as DNA template for the single-base extension reaction, which was further supplemented with 5 μL SNaPshot Multiplex Ready Reaction Mix and 0.4-0.8 μM pooled SNP-specific oligonucleotide primers (Table S1) to a total volume of 10 μL. Twenty five cycles of single-base extension reaction were carried out with the following condition per cycle: 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 30 s. Products were purified by SAP (1.0 U) at 37°C for 40 min, followed by a heat inactivation at 75°C for 20 min. Mixture with 4 μL of products and 9 μL of Hi-DiTM formamide was analyzed with capillary electrophoresis on ABI PRISM TM 3730xl DNA analyzer (Applied Biosystems). The GeneMarker software was used to read the genotyping result (Holland and Parson, 2011).

2.3. APOE genotyping
The ε4 allele of the apolipoprotein E (APOE) gene, which is considered to be a risk factor for AD (Tsai et al., 1994), was also examined. The first nucleotide in the 112th and 158th amino acid of the APOE protein were analyzed by SNaPshot assay, primers for PCR and single-base extension were listed in Table S1.

2.4. Data analysis
The linkage disequilibrium (LD) structure of 8 SNPs (rs732374, rs34594498, rs4473003, rs33949390, rs7298930, rs7307310, rs34778348 and rs3761863) was constructed by using Haploview software version 4.2 (Barrett et al., 2005). Haplotypes consisting of these 8 SNPs were constructed through PHASE2.0 program (Stephens et al., 2001). The allele frequency, genotype frequency and haplotype frequency were compared between AD cases and controls by Fisher exact test (two tailed) or Pearson's chi-squared test by SPSS16.0 (SPSS Inc., Chicago, Illinois). Chi-square test was utilized to estimate the deviation from the Hardy–Weinberg equilibrium (HWE). Binary logistic regression analysis was performed with SPSS16.0 (SPSS Inc., Chicago, Illinois) to assess the associations of the LRRK2 SNPs with the risk of AD, with an adjustment for APOE4 status (APOE4+, APOE4-). The statistical power was calculated by using the Quanto software (Gauderman, 2002) with log-additive inheritance mode. With false positive rate controlled as 0.05, for minor allele frequency (MAF) from 0.1 to 0.5, the statistical power to detect the odds ratio (OR) value as 1.5 for risk allele was expected to be from 67.6% to 95.8%.

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variants of the MRC1 gene and the IFNG gene are associated with leprosy in Han

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156-157.

Table S1. Primers for genotyping 12 LRRK2 SNPs and APOE alleles by using SNaPshot assay

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Location (in LRRK2) and potential function</th>
<th>Primer (5’-3’)</th>
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<td>rs732374</td>
<td>intron 7</td>
<td>F: CTATTTACATCAATAATTTTTGGAATGTTG</td>
<td>R: ATTGTCTAAGTAAATATTCAAGACAGC</td>
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<td>E: (gact)_1AATTAAATAGCTTATAGTGAATAAA</td>
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<tr>
<td>rs34594498</td>
<td>c.C1256T, p.A419V</td>
<td>F: TCCATGCTGATGCCTTT</td>
<td>R: CCACCGTTACATTTTGTCTTT</td>
</tr>
<tr>
<td></td>
<td>Disease associated</td>
<td>E: (gact)_1CTTAATGATATTTTTGACGATCCTG</td>
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<tr>
<td>rs4473003</td>
<td>intron 15</td>
<td>F: TTAATTATCGATCTACTAGTGAAGG</td>
<td>R: AAAATAAGAGATACACACAGCAGACTTAG</td>
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<td></td>
<td>Tag SNP</td>
<td>E: (gact)_2GACTTGGAAAGGAAATAGAATATTTTCCA</td>
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<td>Disease associated</td>
<td>E: ct(gact)_7AACAGGTTTGATCTTTTCCAAGGCT</td>
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<tr>
<td></td>
<td>Disease associated</td>
<td>E: t(gact)_2GTCTAAACACCTAAAGGGTGATATTTCCGC</td>
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<tr>
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<td>E: act(gact)_5GACTTTGAAAGGAAAAATAGAAATATTCTC</td>
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<td>rs7298930</td>
<td>intron 38</td>
<td>F: AAATAGGTTGTATCTCTTCTAAAAACATT</td>
<td>R: AAATGGGAAAGGAAATTTG</td>
</tr>
<tr>
<td></td>
<td>Tag SNP</td>
<td>E: (gact)_4CAAGGTTACGACTCCAGCATCTTTATATTAG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disease associated</td>
<td>E: (gact)_1TTTACCAATCTACAGCTACTGAGCA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disease associated</td>
<td>E: (gact)_2TTTACCAATCTACAGCTACTGAGCA</td>
<td></td>
</tr>
<tr>
<td>rs7307310</td>
<td>intron 43</td>
<td>F: AAAGAGTATAACGCACTTTTAAAAATATC</td>
<td>R: TTTAACTGGGAAACCAACTAGATCA</td>
</tr>
<tr>
<td></td>
<td>Tag SNP</td>
<td>E: act(gact)_3TACATATTTTGTCTTTCTAGTAATTTTAT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disease associated</td>
<td>E: act(gact)_2GTGTTGGAAGAAACTGAAAAACTCTG</td>
<td></td>
</tr>
<tr>
<td>rs3761863</td>
<td>c.T7190C, M2397T</td>
<td>F: TTGCTAGGAGGAGGTTT</td>
<td>R: TAAATAGCATTATCTTATAATGTTG</td>
</tr>
<tr>
<td></td>
<td>Disease associated</td>
<td>E: TTGATTACCTGGTTTCTTATTACC</td>
<td></td>
</tr>
<tr>
<td>APOE-112-158</td>
<td></td>
<td>F: ACAAAATCGGAAACTGAGGAA</td>
<td>R: GGCAGGGAGCCCACTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E-112: act(gact)_2GCTGGGGCGCGACATGGGAAGACGTG</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>E-158: t(gact)_2CCGCGATGCCGATACGCTGCAGAAG</td>
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*a* In the “(gact)_n”, n means repeats of “gact”. F: forward primer; R: reverse primer; E: extension primer.
Table S2. Genotyping results of the *APOE* gene

<table>
<thead>
<tr>
<th>Haplotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Isoform</th>
<th>Southwest China</th>
<th>P-value</th>
<th>OR (95%CI)</th>
<th>East China</th>
<th>P-value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Case</td>
<td>Control</td>
<td></td>
<td>Case</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>ε4</td>
<td>88</td>
<td>86</td>
<td>0.04</td>
<td>1.39 (1.02-1.91)</td>
<td>144</td>
<td>67</td>
</tr>
<tr>
<td>TC</td>
<td>ε3</td>
<td>515</td>
<td>716</td>
<td></td>
<td>403</td>
<td>591</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>ε2</td>
<td>61</td>
<td>68</td>
<td></td>
<td>39</td>
<td>66</td>
<td></td>
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<tr>
<td>Total</td>
<td></td>
<td>664</td>
<td>870</td>
<td></td>
<td>586</td>
<td>724</td>
<td></td>
</tr>
<tr>
<td>ε4+</td>
<td>82</td>
<td>81</td>
<td>0.05</td>
<td>1.43 (1.01-2.03)</td>
<td>124</td>
<td>63</td>
<td>2.03x10^-10</td>
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<td>ε4-</td>
<td>250</td>
<td>354</td>
<td></td>
<td></td>
<td>169</td>
<td>299</td>
<td></td>
</tr>
<tr>
<td>Total&lt;sup&gt;b&lt;/sup&gt;</td>
<td>332</td>
<td>435</td>
<td></td>
<td></td>
<td>293</td>
<td>362</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The order of variants for each haplotype: the first nucleotides of the codon that coded the 112th and 158th amino acid of the *APOE* protein, respectively.

<sup>b</sup> Nine patient samples from Southwest China, four patient samples and 22 control samples from East China failed to be genotyped.
Fig. S1. SNaPshot profile of 12 LRRK2 SNPs analyzed in this study. 1. rs3761863; 2. rs732374; 3. rs7307310; 4. rs7298930; 5. rs4473003; 6. 33949390; 7. rs34594498; 8. rs33939927; 9. rs35801418; 10. rs34637584; 11. rs35870237; 12. rs34778348.