Short communication

SNP rs356219 of the α-synuclein (SNCA) gene is associated with Parkinson's disease in a Chinese Han population

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ABSTRACT

Background: Over the last decades, increasing knowledge about the genetic architecture of Parkinson’s disease (PD) has provided novel insights into the pathogenesis of the disorder. Recently, several studies in different populations have found a strong association between idiopathic PD and the single-nucleotide polymorphism (SNP) rs356219, which is located in the 3’UTR of the SNCA gene. In this study, we aimed to verify these findings and to explore further the nature of the association in a subset of Chinese Han PD patients.

Methods: Four hundred and three unrelated patients with sporadic PD and 315 healthy ethnically matched control subjects were recruited consecutively for the study. Patients and normal controls were genotyped for SNCA rs356219 variant by ligase detection reaction (LDR).

Results: A statistically significant difference was found in the frequencies of the single alleles of rs356219 (χ² = 12.986, P = 0.002) between PD patients and normal subjects. The distribution of A > G genotypes was different between patients and controls (χ² = 13.243, P < 0.001). The OR for subjects with the variant genotypes (AG and GG) was 1.88 (95% CI = 1.27–2.78, P = 0.001). The frequencies of the homozygous genotype for this variant was 42.2% (170 patients), which was significantly higher than that in controls (32.4%, P < 0.001).

Conclusion: The results suggested that SNCA rs356219 variant might have an increased risk of susceptibility to PD in a Chinese Han population. Further studies are needed to replicate the association that we found.

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1. Introduction

Parkinson’s disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopamine-producing neurons in the substantia nigra and other brain stem nuclei. Clinical manifestations include: resting tremor, rigidity, bradykinesia and postural instability. The incidence of PD rises steeply with age, affecting 2% of the people older than 65 years [1]. The etiology of PD remains unclear, both genetic susceptibility and environmental factors are considered to contributing factors [2]. Genetic studies have shown that several mutations in seven genes are linked to PD. It is speculated that α-synuclein, LRRK-2, and GBA are implicated in a common biochemical pathway that is important in the pathogenic process [2].

α-Synuclein is one part of a highly conserved protein family called synucleins: it is a major component of Lewy bodies, a pathological hallmark of sporadic PD [3]. Recent studies have suggested that levels of α-synuclein in extracellular biological fluid are associated with PD and they implicated synuclein as a potential biomarker for diagnosing PD and estimating severity [3]. α-Synuclein is encoded by SNCA, which is located on chromosome 4q22. Mutations in the SNCA gene mainly result in rare familial forms of...
PD, while genetic variability in the SNCA gene modulates susceptibility to sporadic PD [4]. Over the last decades, an association between common variants in SNCA and risk of sporadic PD has been established through numerous studies [5]. Genetic variability in the promoter and 3′ region of the SNCA gene coding z-synuclein modulates the risk of developing sporadic PD [6]. Single nucleotide polymorphism (SNP) rs356219, a tagging SNP for a disease-associated haplotype in the 3′ region of the SNCA gene, has a significant effect on SNCA mRNA level in the substantia nigra and the cerebellum [4].

Recently, Elbaz et al in a high resolution whole genome association study of PD by evaluated rs356219 and other three SNPs in a group with Caucasian ancestry [7]. All 4 SNPs displayed a highly significant association with PD at the significance level adjusted for multiple comparisons. The stronger associations were detected for rs356219 (OR 1.22, p = 2.6 × 10−16), located at the 3′ end of the gene. Wider et al. [8] examined rs356219 and other two genotypes in three Caucasian PD patient-control series from the United States, Ireland, and Norway. They found that the SNCA rs356219-G allele was associated with an increased risk of PD. Refenes et al. [9] analyzed MAPT haplotypes and performed SNP genotyping with Taqman assays for the SNCA rs356219 marker in Greek and Italian cohorts of 352 patients and 417 controls, however, they were unable to confirm an association between the SNCA rs356219 G allele or GG homozygote with PD. Therefore, an increased risk for PD with rs356219 of SNCA genotypes is not reproducible in all PD populations.

In our case-control study, we selected SNP rs356219, located in the 3′-region of the SNCA gene, to determine whether the allele frequencies of rs356219 are significantly different in PD patients compared to healthy controls in Chinese Han population.

2. Materials and methods

2.1. Subjects

Four hundred and three unrelated patients with sporadic PD were recruited consecutively from the Neurology Department of six hospitals between June 2009 and May 2011. Patients were examined and observed longitudinally by two neurologists and diagnosed with PD according to published criteria [10]. This study included 315 healthy control subjects randomly selected from outpatients who underwent regular physical examinations during the same time in the same six hospitals. They were free from signs of parkinsonism, matched to the patients’ group in gender, age and ethnicity and they had no family history of Parkinsonism. All the subjects were of Han ancestry (Table 1). The ethical review board at each institution approved the study, and all participants or their legal surrogates signed informed consent. Venous blood samples for DNA extraction were collected by using standard techniques.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from leukocyte pellets by UltraPure™ Genome DNA extraction kit (SBS Genetech Technology Co., LTD, Shanghai, China). The rs356219 polymorphism was genotyped by the PCR-LDR Sequencing method: A 263 bp DNA fragment containing the polymorphic site was amplified by PCR using the forward primer 5′-CATGCTATGCTTGCTGTTCT-3′ and the reverse primer 5′-TCATTCAGTGCTGTTCGTCT-3′ and the reverse primer 5′-TCATTCAGTGCTGTTCGTCT-3′ and the reverse primer 5′-TCATTCAGTGCTGTTCGTCT-3′ and the reverse primer 5′-TCATTCAGTGCTGTTCGTCT-3′ and the reverse primer 5′-TCATTCAGTGCTGTTCGTCT-3′ the PCR was carried out in a total volume of 15 μl containing 1.5 μl 10 × PCR buffer, 1.5 μl 25 mmol magnesium chloride, 0.25 μl 10 pmol each primer, 0.3 μl dNTP, 0.2 μl Taq polymerase (MBI fermentas), 1 μl of genomic DNA and 10 μl H2O. The PCR cycling parameters were 35 cycles of 20 s at 94 °C, 56 °C for 20 s and 72 °C for 40 s. Ligase Detection Reaction (LDR) was performed in a total volume of 10 μl containing 2 μl PCR product, 1 μl 10 × Taq DNA ligase buffer, 0.125 μl 40 U/μl Taq DNA ligase (NEB), 1 μl 10 pmol probes (0.01 μl each of probe), and 5.875 μl H2O. LDR probes were composed of 1 common probe Rs356219-TG-TG-GATTTCGTTGTCTTTTCTCT-3′ and 2 discriminating probes, Rs356219-TC 5′-TCTATTATTTCGCTGAAATATAGGACA-3′ and Rs356219-TT 5′-AGCTCT- TATTATTTCGCTGAAATATAGGACA-3′ (designed by the Shanghai Generay, Biotech Co., Ltd.). Subsequently, LDR products were analyzed by DNA sequencing (Model 377, Applied Biosystems). All assays were conducted blindly without the knowledge of case or control status. Additionally, about 10% of the samples were randomly selected and retested by direct DNA sequencing on a 3730xl DNA analyzer (Applied Biosystems) and the results were 100% concordant.

2.3. Statistical data analysis

Differences of continuous variables were evaluated using Student’s t test. Data were expressed as mean ± SD. Pearson χ²-test was used to compare allele distribution and qualitative variables represented as frequencies. Hardy–Weinberg equilibrium was assessed for both subjects and controls by a χ² goodness-of-fit test. Odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the correlation between the SNP 356219 A > G polymorphism and the risk of PD by using a binary logistic analysis. Two-tailed P < 0.05 were considered as statistical significance. All the statistical analyses were performed with SPSS13.0 software (SPSS Inc., Chicago, IL).

3. Results

We analyzed the frequency of the SNP variant with a A > G transition (rs356219) in 403 patients with sporadic idiopathic PD and in 315 healthy controls. The frequencies of the genotypes and alleles were summarized in Table 2. The allele frequencies of SNP rs356219 A > G in PD group and control group was 35.6%/64.4% and 45.1%/54.9% for the control group. There was a significant difference between these two groups (χ² = 12.986, P = 0.002). The distribution of A > G genotypes was different between cases and controls (χ² = 13.243, P < 0.001). The OR for subjects with the variant genotypes (AG and GG) was 1.88 (95%CI = 1.27–2.78, P = 0.001). The frequencies of the homozygous genotype for this variant were 42.2% (70 patients), which was significantly higher than that in controls (32.4%, P < 0.001). We found that GG genotype carriers had a 119% increase in risk of PD compared with the AA carriers. The genotype frequencies of the SNP in patients and controls were in Hardy–Weinberg equilibrium (P = 0.478 and P = 0.112).

4. Discussions

Our case–control study analyzed the association between the risk for PD and genetic polymorphism at a position approximately 9 kilobases downstream from the SNCA gene. According to our data, the SNP rs356219 A–G variant was associated with an increased risk for PD in a Chinese Han population. Several studies have confirmed that SNCA is one of the main contributors to genetic susceptibility for PD among a Chinese population [6,11]. More recently, Yu et al. [6] performed a case-control study and concluded

Table 2

Distribution of rs356219 genotype and the PD risk estimates for rs356219 genotypes.

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 403)</th>
<th>Controls (n = 315)</th>
<th>OR(95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>54(13.4)</td>
<td>71(22.5)</td>
<td>1.00(refer)</td>
<td>0.12</td>
</tr>
<tr>
<td>AG</td>
<td>179(44.4)</td>
<td>142(45.1)</td>
<td>1.66(0.9–2.51)</td>
<td>0.18</td>
</tr>
<tr>
<td>GG</td>
<td>170(42.2)</td>
<td>102(32.4)</td>
<td>2.19(1.42–3.37)</td>
<td>0.00</td>
</tr>
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</tr>
</tbody>
</table>

Abbreviations: PD, Parkinson’s disease.
that the SNP rs7684318, located in the intron region of SNCA gene, is associated with sporadic PD in a Chinese Han population. Almost at the same time, Hu et al. [12] conducted a case–control study and suggested that the SNP rs356165, located in the 3’UTR of the SNCA gene, was not associated with PD in Chinese population. The present reports provided evidence to an association between an SNP variant and sporadic PD in the 3’ untranslated region (3’UTR) of the SNCA gene in our population.

In our hospital-based case–control study, we investigated the association of SNP rs356219 with the risk of PD in a local population with Chinese Han ancestry including 403 patients and 315 healthy controls. Our data showed both the frequencies of the genotypes and alleles of the SNP rs356219 were associated with sporadic PD in a Chinese Han population. We observed an 88% increased risk of PD in subjects with rs356219 AG/GG compared with the AA carriers (OR = 1.88, 95% CI = 1.27–2.78, P = 0.001). The G allele of rs356219 was found to contribute to PD susceptibility with odds ratios (ORs) similar to previous reports previously [13]. Our findings are consistent with earlier observations in a Korean PD case–control cohort [14]. Therefore, our study confirms that, in addition to the European and Caucasian population, SNP rs356219 is also a potential risk factor for PD in at least two Asian populations. Indeed, it may be a general risk factor for Asians, though it seems unlikely to be specific.

SNCA is well established as a causative gene for PD. Variants in multiple regions of this gene are associated with susceptibility to sporadic PD [5]. Altered expression levels of α-synuclein are regarded as a potential mechanism contributing to the association between SNCA variants and PD. Associations between rs356219 polymorphisms and α-synuclein expression have been reported [13,14]. Mata et al. [11] found SNP rs356219 was significantly associated with PD (OR 1.41; 95% CI, 1.28–1.55; P = 1.6 × 10(−12)) and its risk-associated allele was correlated with higher transformed plasma α-synuclein levels in patients. However, Westerlund et al. in a Swedish study, found that their cohort of PD patients had markedly reduced levels of α-synuclein in the cerebellum, and this decrease appeared to be independent of SNCA genotype. They found significant association of SNP rs2737029 (P = 0.003; χ² = 9.07) and SNP rs356204 (P = 0.048; χ² = 3.91) of SNCA with PD. However, SNP rs356219 (P = 0.218; χ² = 1.516) was not associated with the risk of PD [14]. These studies yielded conflicting results, and the function of rs356219 remains unclear. Well-designed phenotypic studies, and further functional assays should be designed to elucidate the underlying mechanisms of PD pathogenesis associated with this genetic variant.

Some possible limitations of our study should be acknowledged. Firstly, all case–control association studies carry the risk of false-positive findings due to population stratification, although the extent to which such stratification actually contributes to false-positive findings is controversial. Further studies are needed to circumvent these problems and to confirm our results [6]. Secondly, our sample size was not big enough. Further replication studies in independent larger populations are necessary to corroborate our results.

5. Conclusion

Our study confirms that, in addition to the intron regions of the SNCA gene, the 3’ untranslated region is also a potential risk factor for PD in the Chinese population and the G allele of rs356219 might confer a risk factor. Further replication studies in independent and larger populations are necessary to corroborate our results.

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