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SNCA rs356182 variant increases risk of sporadic Parkinson's disease in ethnic Chinese

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ABSTRACT

Purpose: A genome-wide association study (GWAS) has recently identified a novel single nucleotide polymorphism (SNP) rs356182 at *SNCA* that can modulate the risk of Parkinson's disease (PD) in Caucasian ancestry. The present study was designed to clarify the strength of the association in ethnic Chinese population.
Methods: Using a case–control methodology, we genotyped the SNP rs356182 to investigate the association with risk of PD. A total of 2205 ethnic Han Chinese study subjects comprising 1053 sporadic PD patients (581 males, 472 females) and 1152 controls (604 males, 548 females) were recruited from Mainland China. Additionally, the SHEsis software platform was applied for linkage disequilibrium (LD) analysis between rs356182 and another PD-associated synuclein SNP rs356219 we previously reported.
Results: The frequency of *SNCA* rs356182-G allele was significantly higher in PD group than that in controls (odds ratio (OR) = 1.470, 95% confidence interval (CI): 1.284–1.683, $P = 2.306E-8$). Subjects carrying GG/AG genotype had an increased risk compared with the AA carriers (OR = 1.162, 95% CI: 1.143–2.274, $P = 0.006$). Among all the genotypes of rs356182, GG genotype showed the strongest association with risk of PD (GG vs. AG/AA, OR = 1.620, 95% CI: 1.368–1.919, $P = 2.001E-8$). However, the gender, onset age, disease duration, Hoehn-Yahr stage, UPDRS scores and other clinical features were similar between GG genotype carriers and non-carriers. No LD between rs356182 and rs356219 was found in our population ($r^2 = 0.016$ and $D' = 0.163$).
Conclusion: Our study firstly demonstrates that *SNCA* rs356182 variant has an increased risk of susceptibility to PD in Han Chinese population. Further functional analysis is required to determine the role of this SNP in development of PD.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting over 1% of the elderly population and leading to a variety of motor and non-motor symptoms, and currently we do not have a treatment that prevents or arrests the disease process [20]. PD is characterised by a specific pattern of neuronal loss with accompanying Lewy pathology—the deposition of abnormal fibrillar alpha-synuclein (SNCA) in Lewy bodies and Lewy neuritis. The exact etiology of PD still remained elusive, but increasing knowledge about the genetic architecture of PD has demonstrated that genetic factors play an important role in the pathogenesis of disease [23].

SNCA, located at chromosome 4q21, encoding alpha-synuclein, which plays a central role in the pathophysiology of Parkinson's disease, was the first autosomal dominant gene to be identified for PD. Up until 2015, five missense mutations (A53T, A30P, E46K, H50Q and G51D) as well as genomic multiplications in *SNCA* have been identified as causes for rare familial forms of PD, mediating disease through a change in protein fibrillation or increased expression of the protein product, alpha-synuclein [17,19,32–34,37]. However, these are thought to account for only a small proportion of PD. Additional common variants, although neither necessary nor sufficient to lead to disease, also have a small effect on increasing the transcription of *SNCA*, and collectively they comprise a larger portion of genetic component responsible for the development of PD [5,16]. Genome-wide association studies (GWAS), designed to capture common variation in candidate genes, have demonstrated association between *SNCA* common variants and susceptibility to sporadic Parkinson's disease [4,29]. Recently, a published GWAS meta-analysis from the USA and Europe identified *SNCA* rs356182 significantly associated with increased risk for PD in a group with Caucasian ancestry including 13,708 cases and 95,282 controls in the discovery phase, and replicated the strong association in an independent Caucasian set of 5353 cases and 5551 controls [29].

Abbreviations: GWAS, genome-wide association study; SNP, single nucleotide polymorphism; PD, Parkinson's disease; SNCA, alpha-synuclein; OR, odds ratio; CI, confidence interval; MAF, minor allele frequency; UTR, untranslated region; UPDRS, Unified Parkinson's Disease Rating Scale; PDQ-39, The 39-Item Parkinson's Disease Questionnaire; MMSE, Mini-Mental State Examination; HAMD, Hamilton Depression Scale.

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However, the role of rs356182 variant in the pathogenesis of PD is not clear among Han Chinese population. Considering ethnic differences in background risk allele frequency, population-specific heterogeneity in the pathogenesis of the disease and power of studies, independent replication is needed to confirm genetic association in a large cohort from the Chinese population. Furthermore, the potential difference of the clinical characteristics among different genotype carriers has not been examined. To address these questions, we performed a large case-control study on the association between *SNCA* rs356182 and PD in a Han Chinese population. In addition, we analyzed the linkage disequilibrium (LD) between rs356182 and another synuclein SNP rs356219 we previously published as associated with PD in our Han Chinese population of 685 patients and 569 controls. We also summarized the synuclein SNPs reported in Chinese PD patients.

2. Material and methods

2.1. Subjects

A total of 2205 ethnic Han Chinese study subjects comprising 1053 incident sporadic PD patients (581 males, 472 females) and 1152 population controls (604 males, 548 females) were enrolled from Mainland China. All of them were recruited at the Department of Neurology, West China Hospital, Sichuan University and had no family history of PD. All patients (the mean age 56.84 ± 10.58 years, range 20–86) were examined and followed-up longitudinally by 2 movement disorders neurologists and diagnosed with idiopathic PD according to the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria [13]. The mean age at onset of the patients was 52.23 ± 10.65 years (range 17–85) and mean course was 4.62 ± 4.02 years. The controls (the mean age 54.45 ± 16.44 years, range 18–92) were healthy volunteers without neurodegenerative diseases and generally matched with patients for age and gender. Written informed consent was obtained from all subjects. The Ethics Committee of Sichuan University approved the study. DNA was extracted from blood leukocytes by standard procedures.

2.2. Genetic analysis

We performed genotyping using Sequenom iPLEX matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass-spectrometry technology (Sequenom iPLEX Assay, San Diego, USA) according to manufacturer's instructions. Approximately 15 ng of genomic DNA was used to genotype each sample. Briefly, locus-specific polymerase chain reaction (PCR) and detection primers were designed using the MassArray Assay Design 3.0 software (Sequenom, San Diego, USA). The sample DNAs were amplified by primers flanking the targeted sequence, followed by dephosphorylation and allele-specific primer extension. Cleaned extension products were loaded into a 384-format Spectro-Chip, and subjected to MALDI-TOF mass spectrometry. The resultant data were analyzed by the Sequenom MassArray Typer software (Sequenom).

2.3. Statistical analysis

We assessed Hardy–Weinberg equilibrium (HWE) for the SNP rs356182 in cases and controls with an exact test. The frequencies of the alleles and genotypes in the patients and control groups were analyzed using the Chi-square test. Age at onset and clinical characteristics in different genotypes were assessed by the two-tailed Student's *t*-test. A two-tailed *P*-value ≤0.05 was considered statistically significant. Statistical analysis was carried out using the Statistical Package for the Social Sciences version 21.0 (SPSS, version 21.0) for Windows. The SHEsis software platform was applied for linkage disequilibrium analysis.

3. Results

Data from a total of 2205 subjects including 1053 PD cases and 1152 healthy controls were analyzed. Distributions of the SNP genotype in cases and controls were in Hardy–Weinberg equilibrium ($P = 0.832$ and $P = 0.164$, respectively). Genotype and allele frequencies between cases and controls were summarized in Table 1. The frequency of G allele was significantly higher in PD group than that in controls (OR = 1.470, 95% CI: 1.284–1.683, $P = 2.306E-8$). Subjects carrying GG/AG genotype had an increased risk compared with the AA carriers (OR = 1.162, 95% CI: 1.143–2.274, $P = 0.006$). Among all the genotypes of rs356182, GG genotype showed the strongest association with risk of PD (GG vs. AG/AA, OR = 1.620, 95% CI: 1.368–1.919, $P = 2.001e-8$; GG vs. AA, OR = 1.950, 95% CI: 1.372–2.772, $P = 0.000163$).

In an exploratory analysis, we compared the clinical characteristics of GG individuals with AG/AA individuals (Table 2). However, no significant association was observed in the clinical presentation for gender, current age, onset age, disease duration, initial symptoms, UPDRS, Hoehn-Yahr stage, PDQ-39, MMSE and HAMD.

Using the SHEsis software platform, no linkage disequilibrium was found between SNP rs356182 and rs356219 in our Han Chinese population ($r^2 = 0.016$ and $D' = 0.163$).

In addition to SNP rs356182, a number of genetic polymorphisms of *SNCA* have been identified to be associated with PD in Chinese population, so we accomplished a summary of the synuclein SNPs reported in Chinese PD patients (Table 3) [2,3,9–12,14,21,22,30,31,35,36].

4. Discussion

In our case-control study, SNP rs356182, at a position approximately 19 kb downstream from the *SNCA* gene, displayed a highly significant association with susceptibility to sporadic PD among a Han Chinese population from mainland China. According to our data, the G allele of rs356182 was associated with an increased risk for PD (OR = 1.470, 95% CI: 1.284–1.683, $P = 2.306E-8$). Our finding is consistent with results of the latest PD GWAS in Caucasian population [29], which found on *SNCA* gene, rs356182 was the most strongly associated (G vs. A OR = 1.34, 95% CI: 1.30–1.38, $P = 1.85E-82$). Our study confirms that SNP rs356182 is also a potential risk factor for PD in Han Chinese population.

SNP rs356182 is located in the 3′ untranslated region (3′ UTR) of the *SNCA* Gene. Recently, accumulating evidence strongly suggests that, in addition to genetic variability in the 5′ promoter region, variations in the 3′ UTR of *SNCA* also play an important role in the development of PD [7,25–28]. Associations between several *SNCA* SNPs (rs356219, rs11931074, rs356220, rs356221 and rs356165) in the 3′ UTR and PD

Table 1
Association between rs356182 and Parkinson's disease.

	PD cases N (%)	Controls N (%)	OR (95%CI)	*P* value
Genotype				
GG	632 (60.0)	554 (48.1)		
AG	366 (34.8)	504 (43.7)		
AA	55 (5.2)	94 (8.2)		
GG + AG	998 (94.8)	1058 (91.8)		
AG + AA	421 (40.0)	598 (51.9)		
GG + AG vs. AA			1.162 (1.143–2.274)	0.006
GG vs. AG + AA			1.620 (1.368–1.919)	2.001E−08
GG vs. AA			1.950 (1.372–2.772)	0.000163
Allele				
G	1630 (77.4)	1612 (70.0)		
A	476 (22.6)	692 (30.0)		
G vs. A			1.470 (1.284–1.683)	2.306E−08