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The rs3756063 polymorphism is associated with *SNCA* methylation in the Chinese Han population

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ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Genome-wide association studies have confirmed the association of single nucleotide polymorphisms (SNPs) located in the *SNCA* gene with the risk of PD. While hypomethylation of the *SNCA* intron-1 was observed in patients with sporadic PD, an association between *SNCA* SNPs and *SNCA* methylation levels has been identified. To investigate whether these SNPs are associated with the level of *SNCA* methylation in the Chinese population, we genotyped *SNCA* SNPs and analyzed the relationship between *SNCA* SNPs and *SNCA* DNA methylation status from peripheral blood mononuclear cells of Chinese Han PD patients. Our results revealed that the rs3756063 polymorphism could contribute to the risk of PD in the Chinese Han population and confirmed the effect of this polymorphism on *SNCA* DNA methylation. Further studies will be needed to gain a better understanding of the mechanisms underlying the associations between SNPs, methylation and PD pathogenesis.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (AD) and is characterized by the progressive loss of the dopaminergic neurons of the substantia nigra [1,2]. It is generally considered that aging, genes and environmental factors influence the etiology of PD. Mutations in the alpha-synuclein gene (*SNCA*), encoding alpha-synuclein protein, the first to be identified in monogenic PD, account for approximately 2% of the autosomal dominant forms of early-onset PD [3,4]. Braak et al. later showed that the accumulation of alpha-synuclein protein in the brain is a hallmark of PD [5]. While most PD cases are sporadic, several case-control studies have reported that *SNCA* might be a susceptibility gene for sporadic PD and that its increased expression results in parkinsonian syndrome [6–9]. Genome-wide association studies have further confirmed that certain single nucleotide polymorphisms (SNPs) located in *SNCA* are associated with the risk of PD [10–17]. With the progress of study, researchers disproved the view that alpha-synuclein protein as a 'neuron-specific' protein in PD and indicated that high levels of alpha-synuclein expression were found in peripheral [18,19]. Further studies showed that *SNCA* variants associated with alpha-synuclein levels in the blood as well as brain [20–23].

DNA methylation is the most intensely studied epigenetic mechanism and is characterized by the transfer of a methyl group from *S*-adenosylmethionine (SAM) to cytosine residues at the CpG dinucleotides on the DNA, which alters gene expression [24,25]. Because methylation status is related to the expression of gene transcripts, abnormal methylation patterns in the CpG islands of disease-associated genes might be involved in disease pathogenesis. Eliezer et al. identified that the level of genome-wide DNA methylation in brain and blood was consistent in PD patients [26]. The hypomethylation of *SNCA* intron-1 was observed in patients with sporadic PD, and the hypomethylation of this region could be associated with an increase in *SNCA* expression in vitro [27–29].

As we all known that *SNCA* variants associated with a-synuclein levels, and there was closely link between *SNCA* methylations level and *SNCA* expressions. We evaluated whether *SNCA* variants are associated with the SNCA methylation level, thus affecting the *SNCA* expression levels. In our previous study, we confirmed the hypomethylation of *SNCA* in the Chinese Han PD population and revealed the Rep1 polymorphism, which is located ~10 kb upstream of the translational start site of *SNCA* and was associated with *SNCA* DNA methylation [30]. Recently, Pihlstrøm et al. investigated the relationship between *SNCA*

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Table 1
Fundamental data, mean SNCA methylation levels and SNCA mRNA levels in PD patients and controls.

	PD (n = 91)	Controls (n = 92)	p
Gender (male/female)	47/44	49/43	0.827
Age (years)	62.00 ± 9.72	62.27 ± 9.54	0.849
Mean SNCA methylation levels (%)	14.27 ± 8.65	17.17 ± 8.89	0.011
Mean SNCA-mRNA levels	0.0015 ± 0.0007	0.0014 ± 0.0007	0.84

SNPs (including rs3756063, rs356165 and rs2245801) and determined that rs3756063 was associated with *SNCA* methylation levels [31]. However, it remains unknown whether these SNPs are associated with the *SNCA* methylation level in the Han population from mainland China. In this study, we genotyped *SNCA* SNPs and investigated the relationship between *SNCA* SNPs and *SNCA* DNA methylation status in Chinese Han PD patients.

2. Materials and methods

2.1. Subjects and SNCA transcript analysis

A 30-ml sample of venous peripheral blood from 91 sporadic PD patients (mean ± SD age = 62.00 ± 9.72 years, female = 44, male = 47) and 92 controls (mean ± SD age = 62.27 ± 9.54 years, female = 49, male = 43) were collected in ethylenediamine tetra-acetate (EDTA) vacutainer tubes. The Department of Neurology, Xiangya Hospital, Central South University, the National Laboratory of Medical Genetics of China and the Neurodegenerative Disorders Research Center, Central South University approved the inclusion of the 91 sporadic PD patients, who were diagnosed by United Kingdom PD Brain Bank Criteria [32]. The Health Examine Center of Second Xiangya Hospital approved the inclusion of the 92 controls. All patients and controls signed an informed consent to participate in the project. The genomic DNA was prepared using the TIA Namp Genomic DNA blood kit (Tiangen Biotech, Beijing, China), total RNA was isolated using the standard Trizol method (Qiagen), and all of the samples were stored at −80 °C. The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Central South University.

2.2. Sodium bisulfite sequencing

Briefly, an Epitect Bisulfite Kit (Qiagen) was used for the bisulfite conversion. The converted product was purified and amplified by polymerase chain reaction (PCR) using primer sequences described previously [33]. The PCR products were then cloned into the pGEM-T easy vector (Promega, Madison, WI, U.S.A.), and for each subject, >10 independent clones were sequenced to study the CpG site methylation levels. A BiQ analyzer (quality control software for DNA methylation data from bisulfite sequencing) served as a control.

2.3. cDNA synthesis and real-time quantitative PCR (RT-PCR)

The mRNA levels of *SNCA* determined by RT-PCR were used to analyze *SNCA* expression. First, cDNA was synthesized from total RNA using the RevertAid™ First Strand cDNA synthesis Kit (Fermentas, Burlington, Canada). We then performed RT-PCR using a ABI 7900 HT Fast Real-time PCR system (Applied Biosystems, Inc., Foster City, CA), and the SYBR Premix Ex Taq™ real-time PCR Kit (Takara Biotech, Co., Dalian, China); primer sequences have been described previously [26].

2.4. SNCA rs356182/rs356165/rs2245801/rs3756063 genotyping

Four *SNCA* SNPs (rs356182/rs356165/rs2245801/rs3756063) were reported in genome-wide association studies and were studied. All of the SNPs were genotyped by PCR.

2.5. Statistical analyses

The Statistical Package for Social Sciences (SPSS, version 17.0) was used for all statistical analyses. A $p < 0.05$ was considered to be statistically significant. The frequencies and percentages of allelic genotypes and the mRNA and DNA methylation levels between patients and controls were compared by using a Chi-square test. The association between mRNA and DNA methylation levels was assessed by using a Pearson correlation. To compare the association of mRNA or DNA methylation levels with gender, age, disease status and SNP genotypes, a linear regression analysis was used.

3. Results

There were no significant differences in gender or age distribution between the patients and controls. The mean DNA methylation level of *SNCA* intron-1 in PD patients was lower than that of controls (p =

Table 2
Allele frequencies of four SNPs in PD patients and controls.

	PD (n^a = 182)	Controls (n^a = 184)	P[b]	OR	95% CI
rs3756063[c]					
G	19 (10.4%)	33 (17.9%)	0.042	1.875	1.022–3.438
C	163 (89.6%)	151 (82.1%)			
rs356182					
G	63 (34.6%)	69 (37.5%)	0.566	0.939	0.759–1.163
A	119 (65.4%)	115 (62.5%)			
rs356165					
G	87 (47.8%)	94 (51.1%)	0.832	0.956	0.635–1.440
A	95 (52.2%)	90 (48.9%)			
rs2245801					
T	20 (11.0%)	32 (17.4%)	0.082	1.705	0.935–3.110
C	162 (89.0%)	152 (62.6%)			

[a] n = 2 * case numbers.
[b] p values presented were corrected for by linear regression analysis, adjusting for gender, age and disease status.
[c] rs3756063 C allele had a significantly higher frequency among the patients.