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ORIGINAL COMMUNICATION

SNCA but not *DNM3* and *GAK* modifies age at onset of *LRRK2*-related Parkinson's disease in Chinese population

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Abstract

Background Recently, rs2421947 in *DNM3* (dynamin 3) was reported as a genetic modifier of age at onset (AAO) of *LRRK2* G2019S-related Parkinson's disease (PD) in a genome-wide association study in Arab-Berber population. Rs356219 in *SNCA* (α-synuclein) was also reported to regulate the AAO of *LRRK2*-related PD in European populations, and *GAK* (Cyclin G-associated kinase) rs1524282 was reported to be associated with an increased PD risk with an interaction with *SNCA* rs356219. G2019S variant is rare in Asian populations, whereas two other Asian-specific *LRRK2* variants, G2385R and R1628P, are more frequent with a twofold increased risk of PD.

Methods In this study, we investigated whether rs2421947, rs356219 and rs1524282 modified AAO in *LRRK2*-related PD patients in Han Chinese population. We screened *LRRK2* G2385R and R1628P variants in 732 PD patients and 1992 healthy controls, and genotyped *DNM3* rs2421947, *SNCA* rs356219 and *GAK* rs1524282 among the *LRRK2* carriers.

Results The *SNCA* rs356219-G allele was found to increase the risk of PD in *LRRK2* carriers (OR 1.50, 95%CI 1.08–2.01, $P=0.016$), and the AAO of AG + GG genotypes was 4 years earlier than AA genotype ($P=0.006$). Nonetheless, no similar association was found in *DNM3* rs2421947 and *GAK* rs1524282.

Conclusions Our results show that *SNCA* but not *DNM3* or *GAK* is associated with AAO of *LRRK2*-PD patients in Chinese population.

Keywords Parkinson's disease · *DNM3* · *SNCA* · *GAK* · *LRRK2* · G2385R · R1628P

Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disorder, affecting 1–2% of people older than 65 years [1]. The pathogenesis of PD has not been fully elucidated, but genetic factors play an important role in PD [2, 3]. Leucine-rich repeat kinase 2 (*LRRK2*) variants are the most common genetic causes of familial late-onset and sporadic PD [4]. The frequency of *LRRK2* mutation carriers in PD patients is greatly variable in different populations. *LRRK2* G2019S is typically found in North Africa and South European populations, whereas it is rare in Asians [5]. *LRRK2* G2385R and R1628P are two common Asian-specific variants with twofold increased risk for sporadic PD in Asian population [6–8]. Besides, *LRRK2* mutation carriers are reported to show incomplete penetrance and a highly variability of age at onset (AAO) of motor symptoms [9, 10]. Therefore, other genetic or environmental modifiers are probably regulating the AAO in *LRRK2*-related PD patients [11, 12].

A recent genome-wide linkage and association study (GWAS) identified rs2421947 (C/G) in dynamin 3 (*DNM3*) gene-modified AAO of *LRRK2* G2019S carriers in Tunisian Arab-Berber population, and the median AAO of GG carriers of rs2421947 was 12.5 years earlier than CC carriers [13]. Afterwards, another study reported no relation between rs2421947 and AAO of *LRRK2* G2019S carriers in Spain population [14]. A recent research showed that rs2421947 was not associated with AAO of *LRRK2*-PD in

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Asian population [15]. Nevertheless, these findings have not been confirmed in other Asian populations.

Variants in the α-synuclein (*SNCA*) gene have been associated with an increased risk of PD [14]. Besides, rs356219 (A/G) in *SNCA* has also been reported to regulate the AAO of *LRRK2*-related PD in European populations [14, 16]. Cyclin G-associated kinase (*GAK*) gene has been reported to modify the alpha-synuclein expression and toxicity, and *GAK* rs1564282 was associated with an increased PD risk with an interaction with *SNCA* rs356219 [17, 18]. In this study, we aimed to investigate whether rs2421947, rs356219, and rs1564282 modulate the AAO of *LRRK2*-related PD patients in Chinese population.

Methods

Study participants

The study enrolled a large cohort of Han Chinese population from the first affiliated hospital of Zhengzhou University, including 732 sporadic PD patients and 1992 healthy controls. All the patients were diagnosed independently by two neurologists based on the United Kingdom Parkinson's Disease Society Brain Bank [19]. Each patient was assessed by detailed neuropsychological evaluations, and each control was checked by two experienced neurologists to exclude the abnormal neurological examination. Age of PD onset was defined as the age of appearance of first symptoms that were self-reported by each patient. The study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from each participant.

Genetic analysis

Genomic DNA was extracted from peripheral blood according to the standard protocols. The genotypes of *LRRK2* G2385R and R1628P, *DNM3* rs2421947, and *DNM3* rs2421947 were performed by Sanger sequencing. The genotyping of *DNM3* rs2421947 was performed using 5′-TCCTGCTGAACGACTAAGGT-3′ as the forward primer and 5′-CTCTCAGTCACGTTTTGCTACA-3′ as the reverse primer. The genotyping of *GAK* rs1564282 was performed using 5′-TTCCCTCTTGTGGAACTGCT-3′ as the forward primer and 5′-GGTGGATACAGGGCTGTC AGT-3′ as the reverse primer. The genotyping of *LRRK2* G2385R and R1628P variants, and *SNCA* rs356219 were also performed using the primers reported [20, 21].

Statistical analysis

Hardy–Weinberg equilibrium for each variant among cases and controls, and Linkage disequilibrium analysis was analyzed by SNPstat software [22]. Differences in allele and genotype distributions were analyzed using chi-square test to calculate the frequency significance and the odds ratio (OR). Age at examination, age at onset, and disease duration were assessed using the two-tailed Student's *t* test. A two-tailed *P* value < 0.05 was considered statistically significant. The statistical analysis was performed using SPSS version 20.0 (IBM, Armonk, NY, USA).

Results

Demographic characteristics and *LRRK2* variant frequencies in the PD cases and healthy controls are summarized in Table 1. 121 of the 732 PD patients carried at least one *LRRK2* variant (82 patients carried G2385R, 46 patients carried 1628P, and 8 patients carried the both variants). 145 of the 1992 healthy controls carried at least one *LRRK2* variant (80 controls carried G2385R, 68 controls carried R1628P, and 3 controls carried the both variants). Each variant was in Hardy–Weinberg equilibrium in the control subjects (*P* value cut-off = 0.01). There was no linkage disequilibrium between *SNCA* rs356219,*DNM3* rs2421947, and *GAK* rs1564282.

Association between rs2421947/rs356219/rs1564282 and *LRRK2*-related Parkinson's disease are analyzed in Table 2. The *SNCA* rs356219-G allele was significantly different between *LRRK2*-related PD cases and healthy controls with *LRRK2* carriers (OR 1.50, 95% CI 1.08–2.01, $P = 0.016$). The individuals with AG + GG genotypes have an increased risk compared to those with AA genotype (OR 2.27, 95% CI 1.25–4.12, $P = 0.006$). Nonetheless, no similar significance was observed in the *DNM3* rs2421947, and *GAK* rs1564282.

The clinical characteristics of *LRRK2*-related PD patients between different genotypes are also analyzed in Table 3. The *LRRK2*-related PD patients with *SNCA* rs356219 AG + GG genotypes were associated with earlier age at onset compared with AA genotype (mean AAO of AG + GG: 58 years old; mean AAO of AA: 62 years old), but PD onset symptoms among the AG + GG and AA genotypes of *SNCA* rs356219 were not significantly different. No similar significance was observed in the *DNM3* rs2421947 and *GAK* rs1564282.

Discussion

LRRK2 mutations are associated with autosomal dominant PD with incomplete penetrance, and the penetrance varies among different variants. The heterogeneity has led to the hypothesis that some modifications influence the penetrance

Table 1 Demographic characteristics of the participants

Characteristics	PD patients[a]			Controls[b]		
N	732			1992		
Gender						
Male, *n* (%)	438 (59.8)			1075 (54.0)		
Female, *n* (%)	294 (40.2)			917 (46.0)		
Age at examination, mean (SD)	65 (7.7)			59 (7.1)		
Age at onset, mean (SD)	60 (7.3)			NA		
Duration	4 (2.1)			NA		
G2385R						
	GG	GA	AA	GG	GA	AA
	650	80	2	1912	80	0
R1628P						
	CC	GC	GG	CC	GC	GG
	686	45	1	1924	68	0

N number of participants, *NA* not applicable, *SD* standard deviation

[a]7 PD cases carry both G2385R (GA) and R1628P (GC)

[b]3 controls carry both G2385R (GA) and R1628P (GC)

Table 2 Association between rs2421947/rs356219/rs1524282 and *LRRK2*-related Parkinson's disease

	PD-*LRRK2*, *N* (%)	Con-*LRRK2*, *N* (%)	OR (95% CI)	*P*[a]
DNM3 rs2421947 (C/G)				
CC	38 (31.4)	43 (29.7)		
CG	44 (36.3)	52 (35.6)		
GG	39 (32.2)	50 (34.4)		
CG + GG vs.CC	83/38	102/43	0.92 (0.55–1.56)	0.757
G vs. C	120/122	152/138	1.69 (0.86–3.34)	0.127
SNCA rs356219 (A/G)				
AA	20 (16.5)	45 (31.0)		
AG	49 (40.5)	72 (49.7)		
GG	52 (45.0)	23 (19.3)		
AG + GG vs. AA	101/20	100/45	2.27 (1.25–4.12)	**0.006**
G vs. A	153/129	128/162	1.50 (1.08–2.01)	**0.016**
GAK rs1564282 (T/C)				
TT	4	2		
TC	35	32		
CC	82	111		
TT + TC vs.CC	39/82	34/111	1.55 (0.90–2.67)	0.110
T vs. C	43/199	36/254	1.53 (0.94–2.46)	0.084

PD-LRRK2 PD patients with *LRRK2* G2385R and/or R1628P, *Con-LRRK2* controls with *LRRK2* G2385R and/or R1628P, *OR* odds ratio, *CI* confidence interval

Significant *P* values are indicated in bold. *P* values and ORs were calculated from chi-square test

of *LRRK2* [9–11]. In this study, we found the mean AAO of GG + GA genotype of *SNCA* rs356219 was 4 years earlier than AA genotype in *LRRK2*-related PD patients.

Recently, *DNM3* rs2421947 has been identified associated with the AAO of *LRRK2* G2019S PD in the Arab-Berber population [13]. In addition, there seemed to be a higher level of expression of dynamin 3 protein in GG carriers than that in CC carriers, and the localization of Dnm3 was affected significantly in the *LRRK2* G2019S knock-in mice [13]. However, our study showed no association of rs2421947 and AAO in *LRRK2*-related PD patients. Though the association of *DNM3* with PD onset was confirmed in the meta-population with HR = 1.61, the France population showed no association with HR = 0.71 and the Norway population showed slightly association with HR = 1.17 [13]. Besides, a Spanish study also reported no association

Table 3 Clinical characteristics of *LRRK2*-related Parkinson's disease between different genotypes of *DNM3* rs2421947 and *SNCA* rs356219

	DNM3 rs2421947			*SNCA* rs356219			*GAK* rs1564282		
	CC	CG + GG	*P*	AA	AG + GG	*P*	CC	TC + TT	*P*
Gender									
Male (%)	20 (52.6)	47 (56.7)	0.682	12 (60.0)	55 (54.5)	0.649	22 (56.4)	45 (54.9)	0.874
Female (%)	18 (47.4)	36 (43.1)		8 (40.0)	46 (45.5)		17 (43.6)	37 (45.1)	
Age, mean (SD)	65.5 (5.3)	63.7 (6.1)	0.110	66.5 (5.7)	63.8(5.8)	0.065	65.2 (4.3)	63.8 (6.5)	0.164
Age at onset, mean (SD)	60 (5.1)	58 (6.5)	0.176	62 (4.7)	58 (6.1)	**0.006**	58.5 (5.7)	58.2 (6.3)	0.798
Duration, mean (SD)	6.1 (4.1)	5.9 (4.0)	0.790	4.7 (2.9)	6.2 (4.2)	0.152	6.7 (4.6)	5.6 (3.7)	0.169
Onset symptoms									
Resting tremor (%)	15 (39.5)	44 (53.0)	0.580	12 (60.0)	45 (44.6)	0.487	20 (51.3)	39 (47.6)	0.935
Bradykinesia–rigidity (%)	14 (36.8)	24 (28.9)		4 (20.0)	37 (36.6)		9 (23.1)	23 (28.0)	
Mixed symptoms (%)	4 (10.5)	6 (7.2)		3 (15.0)	12 (11.9)		7 (17.9)	15 (18.3)	
Others[a] (%)	5 (13.2)	9 (10.8)		1 (5.0)	7 (6.9)		3 (7.7)	5 (6.1)	

SD standard deviation. Significant *P* values are indicated in bold

[a]Including autonomic dysfunction, weakness, pain

of rs2421947 and *LRRK2* G2019S PD onset [14]. Our results were consistent with the analysis in the France and Spanish populations.

These findings are conflicting due to several factors. One possible explanation is that the molecule pathogenesis may be different between *LRRK2* G2019S and G2385R/R1628P variants. G2019S variant is located in the kinase domain and increases the kinase activity, while G2385R is resided in the WD40 domain, and decreases the kinase activity and increases GTPase activity [23]. It was reported that G2385R was associated with a greater risk of apoptosis and cell death [6]. R1628P is resided in the COR domain, and it can increase indirectly kinase activities by enhancing the cyclin-dependent kinase 5(Cdk5) phosphorylation of LRRK2 and leading to neuronal death [24]. It may result in different interaction effects with *DNM3*. Another reason is that single variant is subtle, and additional genetic modifiers and/or environment modifiers could also contribute to the penetrance of *LRRK2* [11].

SNCA is a well-established causative gene for PD, and α-synuclein has been recognized as a critical protein in the pathogenesis of PD [25–27]. Previous studies have demonstrated that rs356219 in the 3'UTR of *SNCA* increased the risk of sporadic PD [16]. It influenced the AAO of *LRRK2*-related PD in European population, with an earlier 7–11 years mean AAO for the GG genotype. In our study, we found a similar result that the mean AAO of GG + AG carriers of *SNCA* rs356219 was 4 years earlier than AA carriers (62 years for AA, and 58 years for GG + AG). These studies showed that rs356219 in *SNCA* could be a penetrance modifier for *LRRK2*-related PD, alone or in combination with additional risk factors. Previous studies have demonstrated that rs356219 in *SNCA* was related with increased α-synuclein expression in blood, cerebellum, and substantia nigra of PD patients [28, 29]. Further functional study is essential to elucidate its pathophysiologic role in *LRRK2* carries. Though *GAK* rs1564282 was reported to be associated with an increased PD risk, and interact with *SNCA*, our study showed no evidence of *GAK* on AAO of *LRRK2*-related PD.

Although the precise physiological mechanism of *LRRK2* underling PD remains largely unclear, many studies have showed that *LRRK2* was associated with vesicle trafficking, protein degradation, cytoskeletal maintenance, and neurite morphology [30–32]. The Asian-specific *LRRK2* variants, G2385R and R1628P, were associated with increased risk of PD. Under the condition of certain situation, such as oxidative stress, the *LRRK2* variants were more toxic and associated with a higher rate of apoptosis [6, 33]. The potential genetic modifiers of AAO of *LRRK2*-related PD may provide insight into the disease therapeutics by delaying the PD onset. However, there are limitations in this study. Potential gene–gene and gene–environment interactions were not evaluated, and further functional studies are essential to elucidate the roles of these variants in *LRRK2* carries.

In conclusion, our study suggested that *SNCA* rs356219 but not *DNM3* rs2421947 or *GAK* rs1564282 modulated the AAO of *LRRK2*-related PD in Han Chinese population. Given the limited number size of this study, further studies in larger scale populations will be required to validate our findings.

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Compliance with ethical standards

Conflicts of interest None of the authors have any conflicts of interest to declare.

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