Association analysis of *NUCKS1* and *INPP5K* polymorphism with Parkinson's disease

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Genome-wide association studies have reported numerous candidate loci associated with Parkinson's disease (PD). *NUCKS1* and *INPP5K* are two such candidate loci, although they have rarely been reported in Asian populations. To explore these potential genes for PD susceptibility, we investigated the association between PD and two SNPs, rs823114 and rs1109303, located on the *NUCKS1* and *INPP5K* genes, respectively, in the Han population of northern China. We genotyped the two SNPs using the multiplex PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) technique. A total of 685 subjects including 322 sporadic PD patients and 363 healthy controls were recruited from the population. After Bonferroni correction, our results suggested that there was a significant association of a minor allele (G) in rs823114 with reduced risk of PD development ($P = 0.017$, OR = 0.768, 95%CI = 0.618 – 0.955), and the difference in genotypes between the PD patients and healthy controls was significant under the dominant model (GA + GG vs. AA). After stratification by gender, males had a lower risk than females ($P = 0.008$, OR = 0.666, 95%CI = 0.495 – 0.898). However, the distribution of genotype frequency exhibited no significant differences between the PD and control groups ($P > 0.025$) in *INPP5K* rs1109303 ($P = 0.048$, OR = 0.806, 95%CI = 0.650 – 0.998). We conclude that *NUCKS1* rs823114 indicates a decreased risk of susceptibility to PD and shows a male genetic distribution bias in the Han Chinese population.

Key words: Parkinson's disease, single nucleotide polymorphism, association, *NUCKS1*, *INPP5K*

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by a large number of motor and non-motor features that can impact on function to a variable degree, which affects about 1% of individuals over 65 years of age (Spencer et al., 2011). There are four cardinal features of PD that can be grouped under the acronym TRAP: tremor at rest, rigidity, akinesia (or bradykinesia) and postural instability; these are caused by accelerated loss of dopaminergic neurons in the substantia nigra and abnormal formation of Lewy bodies in residual neurons (Jankovic, 2008). The etiology of PD is very complex, probably involving a combination of

polygenic inheritance, environmental exposures, oxidative stress, neuroinflammation and apoptosis (Klein and Schlossmacher, 2007; Cicchetti et al., 2009; Schapira and Jenner, 2011). However, increasing knowledge related to the genetic architecture of PD has demonstrated that hereditary factors play an important role in PD pathogenesis (Hamza and Payami, 2010).

The *NUCKS1* (nuclear casein kinase and cyclin-dependent kinase substrate 1) gene in the *PARK16* locus located on chromosome 1q32 was recognized as a new risk factor for PD in a Japanese cohort (Satake et al., 2009). The *NUCKS1* gene, which encodes the nuclear protein casein kinase, plays an important role in the DNA damage response (Parpys et al., 2015) and is also used for DNA repair (Grundt et al., 2004); however, its involvement in the pathogenesis of PD has not been addressed. A recent study has shown that a novel single nucleotide polymorphism (SNP), rs823114, at *NUCKS1* is

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associated with PD in an Ashkenazi population (Vacic et al., 2014).

Uric acid (UA) possesses potent antioxidant activity and is the enzymatic end product of purine metabolism in vivo (Weisskopf et al., 2007). UA exerts a neuroprotective effect by reducing oxidative stress and scavenging oxygen radicals against dopaminergic neuron death in PD (Duan et al., 2002). Epidemiological studies have established that high levels of serum urate reduced the risk of PD (de Lau et al., 2005; Ascherio et al., 2009). Serum UA levels associated with the inositol phosphoinositide-5-phosphatase K (*INPP5K*) gene can be used to predict disease severity and progression in potential PD patients. The interaction between the SNP rs1109303 on the *INPP5K* gene and serum urate level in PD patients was significant ($P = 2.01 \times 10^{-8}$), and may be associated with the risk of PD (Nazeri et al., 2015).

In the current study, because rs823114 and rs1109303 have not yet been reported in any Chinese population with reference to PD, we genotyped the *NUCKS1* SNP rs823114 and the *INPP5K* SNP rs1109303 by the multiplex polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique to assess their association with the risk of PD in the northern Han Chinese population.

MATERIALS AND METHODS

Subjects and sample collection As PD subjects, a total of 322 ethnic Han Chinese PD patients from northern China were recruited (161 males, 161 females). All patients were diagnosed by neurological specialists from the First Affiliated Hospital of China Medical University according to the guidelines of the British PD Society Brain Bank (Hughes et al., 1992), excluding cerebrovascular diseases, encephalitis, trauma, and other patients with severe systemic disease (Table 1).

For the control group, a total of 363 unrelated healthy participants who did not have a family history of PD were recruited from the local community (205 males, 158 females), and were matched to the PD group for race, age and gender (Table 1).

The current study was approved by the Ethics Committee on Human Research of China Medical University. All participants were provided with an informed

consent form.

DNA extraction and genotyping Peripheral blood samples were collected from the participants, and genomic DNA was extracted using the sodium dodecyl sulfate - protease K - phenol-chloroform method. According to published sequences in GenBank, forward and reverse primers were designed and are shown in Table 2.

Multiplex PCR-RFLP was used to identify polymorphisms of the SNPs. A final volume of 20 μ l was used, comprising 1 μ l genomic DNA (about 20–50 ng/ μ l), 10 μ l 2 $\times$ Power Taq PCR MasterMix (Bioteke Corporation, Beijing, China), 7 μ l ddH₂O and primer concentrations as shown in Table 2. PCR amplification was performed under the following conditions: initial denaturation of 94 °C for 1 min, followed by 33 cycles of 94 °C for 30 s, annealing at 65 °C for 30 s and elongation at 72 °C for 30 s, and a final extension at 72 °C for 5 min. For restriction enzyme digestion, 2 μ l PCR product, 5 U HaeIII and BanII (TaKaRa, Dalian, China) were mixed in 10 μ l TaKaRa M buffer and incubated at 37 °C for 2 h. Digestions were separated on an 8% polyacrylamide gel, and fragments were visualized by the Molecular Imager Gel Doc XR+ System (Bio-Rad, Hercules, CA, USA) after ethidium bromide staining.

Statistical data analysis The data were analyzed using Power Marker v3.25, Statistical Package of Social Sciences v20.0 (IBM, Chicago, IL, USA) and Stata version 10.0 (StataCorp, College Station, TX, USA). A Hardy-Weinberg equilibrium test was performed using a χ^2 test for both patients and control groups. The strength of

Table 1. Clinical features of PD patients and healthy controls in the northern Chinese Han population

Variable	Controls	Patients with PD
	N = 363	N = 322
Male	205 (56.47%)	161 (50%)
Female	158 (43.53%)	161 (50%)
AAE	67.15 $\pm$ 10.60	64.09 $\pm$ 8.63
AAO	NA	58.62 $\pm$ 9.52

The sample mean $\pm$ SD is given for age at enrollment (AAE) and age at PD onset (AAO); NA = not applicable.

Table 2. Primer sequences and parameters for PCR amplification of SNPs rs823114 and rs1109303

SNP	Direction	Sequence (5'-3')	Primer concentration (μ M)	Restriction enzyme	Allele size (base pairs)
rs823114	forward	ATAGGCTCCACCTTCACCGT	0.40	HaeIII	194 (136, 58)
	reverse	AAACTAACACTGAGCAACGCTGAAC	0.40		
rs1109303	forward	TGCCTCCAGAATAAAGTTAAAGTCC	0.60	BanII	168 (121, 47)
	reverse	AAGGTGTTCCAGGCAGCAAAT	0.60		

the association between the two loci and PD was measured by odds ratios (ORs) with 95% confidence intervals (CIs). We explored the association for a homozygous co-dominant model, a heterozygous co-dominant model, a dominant model and a recessive model. Power analysis was conducted by PS program statistical software. The degree of heterogeneity between males and females was determined by Q-statistic. $P > 0.05$ for the Q-test indicated a lack of heterogeneity and $P < 0.05$ indicated heterogeneity. Probability values were adjusted for multiple comparisons using the Bonferroni correction. The significance threshold for single SNP tests was set as 0.025 (0.05/2) since two independent SNPs were included in the association analyses.

RESULTS

Genotyping the two SNPs Multiplex PCR-RFLP analysis was performed to genotype the two SNPs. PCR product sizes were 194 bp (rs823114) and 168 bp (rs1109303). PCR products were simultaneously digested using HaeIII and BanII and separated by electrophoresis. Undigested fragments represent rs823114-A and rs1109303-T alleles, while the fragments of 136 and 121 bp represent rs823114-G and rs1109303-G alleles, respectively (Fig. 1). Fragments shorter than 47 bp ran off the gel under our electrophoresis conditions. These results indicated that the multiplex PCR-RFLP assay was successful for genotyping.

Genetic parameters and association analysis The actual Hardy-Weinberg P values for rs823114 and rs1109303 in the control and PD groups were 0.7060, 0.2932, 0.0723 and 0.1428, respectively. Genotypic and allelic frequencies of PD and control groups are summarized in Table 3. The allelic frequency of rs823114 on the *NUCKS1* gene was significantly different between PD cases and control samples. The G-allele ($P = 0.017$) and the G-allele carriers ($P = 0.011$) were both more frequent in the healthy control group than in PD patients. Minor allele frequency of rs823114 was 0.368 and 0.431 in patients and controls, respectively (Table 3). Association analysis of rs1109303 on the *INPP5K* gene showed that there were no significant differences among allelic

Fig. 1. Genotyping patterns of rs823114 and rs1109303 loci determined by multiplex PCR-RFLP. M: size marker.

Table 3. Distribution of genotypic and allelic frequencies of SNPs rs823114 and rs1109303 in PD patients and controls in the northern Chinese Han population

SNP	Variants	PD (%)	Control (%)	OR (95%CI)	P value	Power
rs823114	A	407 (63.20)	413 (56.89)	0.768 (0.618–0.955)	0.017	0.664
	G	237 (36.80)	313 (43.11)			
	n	644	726			
	AA	133 (41.31)	116 (31.96)	NA	0.038	
	AG	141 (43.79)	181 (49.86)			
	GG	48 (14.90)	66 (18.18)			
	n	322	363			
rs1109303	G	381 (59.16)	391 (53.86)	0.806 (0.650–0.998)	0.048	0.504
	T	263 (40.84)	335 (46.14)			
	n	644	726			
	GG	119 (36.96)	114 (31.41)	NA	0.165	
	GT	143 (44.41)	163 (44.90)			
	TT	60 (18.63)	86 (23.69)			
	n	322	363			

Two polymorphic markers were in Hardy-Weinberg equilibrium.

OR: odds ratio; CI: confidence interval.

Table 4. Genotypic and allelic frequencies of SNP rs823114 between sexes

Gender	Variants	PD (%)	Control (%)	OR (95%CI)	<i>P</i> value	Power
Male	A	204 (63.30)	220 (53.66)			
	G	118 (36.70)	190 (46.34)	0.666	0.008	0.764
	n	322	410	(0.495–0.898)		
	AA	66 (40.99)	62 (30.25)			
	AG	72 (44.72)	96 (46.83)			
	GG	23 (14.29)	47 (22.92)	NA	0.037	
	n	161	205			
Female	A	203 (63.04)	193 (61.08)			
	G	119 (36.96)	123 (38.92)	0.92	0.609	0.08
	n	322	316	(0.668–1.267)		
	AA	67 (41.61)	54 (34.17)			
	AG	69 (42.86)	85 (53.80)			
	GG	25 (15.53)	19 (12.03)	NA	0.146	
	n	161	158			

Table 5. Analysis of genotype distributions for rs823114 polymorphism between PD and control groups under four genetic models

Genetic model		PD	Control	<i>P</i> value	OR (95%CI)	Power
Homozygous co-dominant	AA	133	116	0.045	0.634	0.808
	GG	48	66		(0.405–0.992)	
Heterozygous co-dominant	AG	141	181	0.022	0.679	0.821
	AA	133	116		(0.487–0.947)	
Dominant	AA	133	116	0.011	0.667	0.953
	GG + GA	189	247		(0.488–0.913)	
Recessive	AA + GA	274	297	0.251	0.788	0.452
	GG	48	66		(0.525–1.184)	

and genotypic frequency distribution between patient and control groups (Table 3).

Male genetic distribution bias and heterogeneity analysis We performed gender stratification to understand associations between SNPs and PD. We found a substantial difference in the rs823114 G-allele in the male cohort ($P = 0.008$), which establishes that the rs823114 G-allele is a protective factor (Table 4). The value of heterogeneity chi-square was 1.06 and the P value was 0.304 based on the respective allele frequency of both sexes in rs823114. There was no significant difference between the female and male groups. No association between the SNP rs1109303 locus and PD was observed in male or female populations (data not shown).

Analysis of genotype distributions of rs823114 between PD and control groups under four genetic models The difference in genotypes between the PD

patients and healthy controls was significant under the heterozygous co-dominant model ($P = 0.022$) and the dominant model ($P = 0.011$), but not in the homozygous co-dominant model ($P = 0.045$) or the recessive model ($P = 0.251$) (Table 5). No associations were found among the four genetic models for the *INPP5K* rs1109303 with PD (data not shown).

DISCUSSION

Sporadic PD has a complex etiology, and most studies indicate that the pathogenesis of PD involves interactions between environmental and complex genetic factors (Schapira and Jenner, 2011). Up to now, hundreds of candidate SNPs have been identified in PD susceptibility genes, and their associations with PD have been widely explored. To investigate the association between PD susceptibility and genes, we genotyped two SNPs located on *NUCKS1* and *INPP5K* genes in the northern Han

Chinese population.

The *NUCKS1* gene is located in the *PARK16* locus together with four other genes, *RAB7L1*, *SLC41A1*, *SLC45A3* and *PM20D1*, and the *RAB7L1*, *SLC41A1* and *NUCKS1* genes are located on the same linkage disequilibrium block (Tucci et al., 2010). The *PARK16* locus has been found to be associated with risk of PD and shows population differences (Gan-Or, 2015), but the role of the susceptibility region within *PARK16* in the pathogenesis of PD is still a mystery. Analysis of CpG methylation in the frontal cortex and cerebellum tissues revealed that *NUCKS1* and *RAB7L1* were promising candidate genes for PD (Plagnol et al., 2011). Although *RAB7L1* gene polymorphisms in relation to PD have been studied extensively (Khaligh et al., 2017), few studies have examined the association between the *NUCKS1* gene and PD in Chinese populations. In our previous study, we investigated SNPs located in the susceptibility region of *PARK16*, including rs823076 on the *SLC41A1* gene, rs708723 on the *RAB7L1* gene and rs4951261 on the *NUCKS1* gene, but none of these SNPs were associated with PD (Cui et al., 2013). The rs823114 locus on the *NUCKS1* gene was initially found to be closely related to the transcript levels of *NUCKS1* ($P = 2.7 \times 10^{-34}$) in a Japanese population (Satake et al., 2009). Subsequently, a study based on Ashkenazi Jews confirmed that *NUCKS1* was a promising candidate for PD ($P = 6.12 \times 10^{-4}$) (Liu et al., 2011). We observed the mutation in the *NUCKS1* rs823114 locus as a protective factor for PD ($P = 0.017$, OR = 0.768, 95%CI = 0.618 – 0.955), which is consistent with the Ashkenazi Jewish population ($P = 2.76 \times 10^{-6}$, OR = 0.75, 95%CI = 0.68 – 0.83) (Vacic et al., 2014). Based on the available data, we also performed a meta-analysis (Supplementary Fig. S1). However, it is known that sample size and race background for population studies can interfere with a meta-analysis result. Thus, further association studies in China and Asia will undoubtedly be helpful. In this study, we found that the G-allele of rs823114 on the *NUCKS1* gene was significantly associated with reduced risk of PD in our subject population. However, the genotype of rs823114 did not remain associated after Bonferroni correction. In addition, results for the heterozygous codominant model (AG vs. AA) and dominant model (GA+GG vs. AA) demonstrated that the G-allele of rs823114 played a protective role for PD patients. Besides that, the result from gender stratification showed that rs823114 was strongly associated with PD in males ($P = 0.008$). Previous studies have reported that the prevalence of PD in males is higher than in females (Benito-Leon et al., 2003; Van Den Eeden et al., 2003), so this study provides meaningful data for the association of *NUCKS1* gene polymorphism with PD in a Chinese Han population, especially by suggesting that male genetic distribution bias on *NUCKS1* occurs in PD patients.

Hyperuricemia has a protective effect on the development and progression of neurodegenerative diseases such as PD and Alzheimer's disease (Mandal and Mount, 2015). Large prospective studies suggested that higher uric acid levels could reduce the risk of PD (Weisskopf et al., 2007) and slow down disease progression (Ascherio et al., 2009). In PD-related cell culture and animal model studies, treatment with uric acid can reduce oxidative stress levels and protect DA neurons (Cipriani et al., 2012; Chen et al., 2013). The expressed product of the *INPP5K* gene inhibits the PI3K/Akt pathway by hydrolyzing intracellular phosphatidylinositol (3,4,5)-trisphosphate, and the PI3K/Akt pathway has been demonstrated to be neuroprotective in PD (Nakano et al., 2017). The SNP rs1109303 on *INPP5K* was found to cause this inhibition, leading to a risk of PD occurrence (Gong et al., 2012). A recent study has shown that a relationship between rs1109303 on the *INPP5K* gene and serum urate level in patients with PD was significant (Nazeri et al., 2015). Unfortunately, studies on *INPP5K* genetic polymorphisms in PD have rarely been reported among Asian populations, especially in Chinese populations. Identification of this genetic polymorphism will support the use of urate-based intervention in PD (The Parkinson Study Group SURE-PD Investigators, 2014). In this study, the allelic and genotypic frequencies of rs1109303 were not significantly different between the PD and control groups ($P > 0.025$). Therefore, we hypothesized that the SNP rs1109303 shows no association with PD in the Han population of northern China. One possible reason is the limited experimental sample size enrolled in this investigation. Thus, increasing sample size and selecting more populations in mainland China are required to reasonably assess whether there is a significant association between PD and this SNP in future studies.

CONCLUSIONS

This study showed an association between *NUCKS1* rs823114 and sporadic PD in a Chinese population for the first time, but a lack of association with PD for the rs1109303 locus of *INPP5K*. Meanwhile, the genetic parameters and association analysis between *NUCKS1* rs823114 and PD also displayed a male genetic distribution bias in the northern Han Chinese population.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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