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Research article

Analysis of several loci from genome-wide association studies in Parkinson's disease in mainland China

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HIGHLIGHTS

- To investigate the association between several GWAS loci and sporadic PD in Chinese individuals.
- We find RAB7L1 rs708723 is associated with PD in Chinese population.
- GPNMB rs156429 polymorphism may be associated with male PD in Chinese individuals.
- NMD3 rs34016896 and STBD1 rs6812193 may not be associated with PD in Chinese Han population.

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ABSTRACT

Large-scale meta-analyses of genome-wide association studies in Parkinson's disease (PD) have identified a number of susceptibility loci in sporadic PD. Since the characteristics of those loci in a Han Chinese population from mainland China were unknown, we performed a case-control replication study in this population and evaluated several single nucleotide polymorphisms (SNPs) identified in a recent GWAS-meta-analysis. In total, 933 subjects comprised of 460 PD patients and 473 controls were genotyped. We found strong evidence of an association for rs708723 in *RAB7L1* in the total sample (genotype $p = 0.01$, allele $p = 0.01$, OR = 0.78, 95% CI = 0.65–0.94). With rs156429 in *GPNMB*, there was a significant difference in genotype and allele distribution between male PD patients and the control subgroup (genotype $p = 0.01$, allele $p = 0.01$, OR = 0.67, 95% CI = 0.49–0.92). However, we did not observe any significant difference in genotype or allele distribution between PD and control for rs34016896 in *NMD3* and rs6812193 in *STBD1*.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder and affects approximately 1.7 million individuals (age ≥65) in China [1]. The exact pathogenesis of PD remains poorly understood, but mounting evidence has revealed that genetic factors play an important role. Moreover, genetic insights are helping to define the molecular causes of PD, allowing for the development of personalized risk modeling.

Genome-wide association studies (GWAS) have provided tangible gains in understanding the genetic architecture of PD [2]. Common variants in *SNCA, MAPT, GBA, GAK,* and *HLA-DRA* genes have been shown to be associated with sporadic PD [2–4]. Meanwhile, meta-analysis of PD GWAS datasets has yielded varying results. The International Parkinson's Disease Genomics Consortium (IPDGC) conducted the first large-scale meta-analysis of GWAS in PD and identified 11 PD associated loci [5]. Using a two-stage meta-analysis in large cohorts of PD cases and controls, IPDGC nominated several new PD risk loci (*RAB7L1*/1q32, *STX1B*/16p11, *FGF20*/8p22, *STBD1*/4q21, and *GPNMB*/7p15) [6]. These same loci

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Table 1
Genotype and allele frequencies of rs708723 in *RAB7L1* in patients and control individuals.

RAB7L1 rs708723		*N*	Genotype				Allele			
			C/*C*	*C*/*T*	*T*/*T*	*p* value[a]	*C*	*T*	*p* value[a]	OR(95%CI)[a]
All	Case 0.78 (0.65–0.94)	460	63(13.6%)	219(47.6%)	178(38.8%)	0.01	345(37.4%)	575(62.6%)	0.01	0.78 (0.65–0.94)
	Control	473	90(19.0%)	230(48.6%)	153(32.3%)	–	410(43.3%)	536(56.7%)	–	–
Male	Case	236	34(14.5%)	116(49.1%)	86(36.3%)	0.25	184(39.1%)	288(60.9%)	0.25	0.86(0.64–1.11)
	Control	237	46(19.4%)	110(46.4%)	81(36.3%)	–	202(42.6%)	272(57.4%)	–	–
Female	Case	224	28(12.6%)	103(45.9%)	93(41.4%)	0.02	159(35.6%)	289(64.4%)	0.02	0.79(0.55–0.94)
	Control	236	40(18.6%)	120(50.8%)	82(30.5%)	–	208(44.1%)	264(55.9%)	–	–
≤50	Case	137	17(12.4%)	64(46.7%)	56(40.9%)	0.12	98(35.8%)	176(64.2%)	0.12	0.74(0.51–1.08)
	Control	103	19(18.4%)	28(0.538)	34(33.0%)	–	88(42.7%)	118(57.3%)	–	–
>50	Case	323	45(14.1%)	156(48.0%)	122(37.9%)	0.03	246(38.1%)	400(61.9%)	0.03	0.79(0.63–0.98)
	Control	370	71(0.0%)	180(48.6%)	119(32.2%)	–	322(43.5%)	418(56.5%)	–	–

[a] The estimated odds ratios (ORs) and relative 95% confidence intervals (95% CI) were adjusted for gender and age at enrollment.

were also revealed by another large-scale meta-analysis of PD GWAS [7]. When analyzing GWAS-linked loci, the genetic heterogeneity across a given population is extremely important. To gain further insight into genetic factors contributing to PD, we performed a case-control study to investigate those loci in a cohort of Han Chinese.

2. Methods

2.1. Patients and controls

In total, 460 mainland Chinese sporadic PD patients were recruited from the outpatient neurology clinic of Xiangya Hospital. The diagnosis of PD was made by two or more experienced neurologists according to the United Kingdom brain bank criteria [8]. Of these patients, 236 (51.3%) were men, and 224 (48.7%) were women. The mean age at disease onset in the patient group was 55.6 years (SD 10.4 years). Information regarding family history, demographic characteristics, clinical data, and neurological examination were recorded for each patient. Patients with an age of onset ≤50 years were defined as early onset PD (EOPD) and comprised 29.8% of all cases. None of the patients had a reported family history of parkinsonism or neurologic and psychiatric conditions other than PD in one or more first- or second-degree relatives. A control group of 473 healthy mainland Chinese subjects from the same geographic areas was obtained, matched for age and sex (50.1% men, mean age 58.3 ± 12.0 years) with the PD patients. There was no statistically significant difference in the age and gender between patient and control groups ($p > 0.05$, Chi-square test). Blood samples were obtained from all subjects, and genomic DNA was extracted from peripheral blood lymphocytes using standard phenol–chloroform procedures. The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Central South University. All subjects provided informed consent prior to participation in the study.

2.2. SNPs Selection

We selected PD-associated SNPs previously reported independently by two GWAS-meta-analysis studies, including: rs708723 in *RAB7L1*, rs156429 in *GPNMB,* rs34016896 in *NMD3*, rs6812193 in *STBD1,* and rs591323 in *FGF20*.

2.3. Genotyping

DNA was extracted from full blood or buffy coat using standard techniques. All five selected SNPs were genotyped by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) using the MassArray system (Sequenom, San Diego, CA, USA) as described [9]. For quality control, the positive and negative controls (no DNA) were included on every 96-well assay plate, and 50 patients and 50 controls were randomly selected for Sanger sequencing. Briefly, locus-specific polymerase chain reaction and detection primers were designed using the MassArray Assay Design 3.0 software (Sequenom). Sample genomic DNA (approximately 15 ng) was amplified by primers flanking the targeted sequence, followed by dephosphorylation and allele-specific primer extension. Cleaned extension products were loaded into a 384-format SpectroChip and subjected to MALDI-TOF MS. Finally, the resultant

Table 2
Genotype and allele frequencies of rs156429 in *GPNMB* in patients and control individuals.

GPNMB	rs156429	*N*	Genotype			*v*				
			G/*G*	*G*/*A*	*A*/*A*	*p* value[a]	*G*	*A*	*p* value[a]	OR(95%CI)[a]
All	Case 0.87(0.70–1.09)	460	22(4.8%)	156(33.9%)	282(61.3%)	0.21	200(21.8%)	720(78.2%)	0.22	0.87(0.70–1.09)
	Control	473	21(4.4%)	187(39.7%)	265(55.9%)	–	229(24.3%)	715(75.7%)	–	–
Male	Case	236	10(4.1%)	71(30.2%)	155(65.8%)	0.01	91(19.1%)	381(80.9%)	0.01	0.67(0.49–0.92)
	Control	237	10(4.2%)	102(43.0%)	125(52.7%)	–	122(25.7%)	352(74.9%)	–	–
Female	Case	224	12(5.7%)	84(37.7%)	128(56.6%)	0.41	108(24.5%)	340(75.5%)	0.41	0.87(0.64–1.20)
	Control	236	11(4.7%)	86(36.2%)	139(59.1%)	–	108(22.8%)	364(77.2%)	–	–
≤50	Case	137	7(5.4%)	43(31.5%)	87(63.1%)	0.57	57(21.2%)	217(78.8%)	0.57	0.88(0.57–1.37)
	Control	103	4(3.9%)	40(31.5%)	59(63.1%)	–	48(23.5%)	158(76.5%)	–	–
>50	Case	323	15(4.6%)	112(34.9%)	196(60.5%)	0.32	142(22.0%)	504(78.0%)	0.33	0.88(0.68–1.20)
	Control	370	17(4.6%)	147(39.7%)	206(55.7%)	–	181(24.5%)	559(75.5%)	–	–

[a] The estimated odds ratios (ORs) and relative 95% confidence intervals (95% CI) were adjusted for gender and age at enrollment.