ATG7 Polymorphisms rs7625184 and rs2606750 are Not Associated with Parkinson's Disease: A Case Control Study

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1 ATG7 polymorphisms rs7625184 and rs2606750 are not associated with Parkinson's
2 disease: a case control study

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Abstract

Background: Deregulation of autophagy is involved in the development and progression of Parkinson's disease. ATG7, an E1 like enzyme, plays a key role in autophagy. This study aimed to investigate the association between ATG7 polymorphisms and PD susceptibility.

Methods: Single nucleotide polymorphisms of ATG7, including rs7625184 and rs2606750, were identified by polymerase chain reaction-restriction fragment length polymorphism in a Han Chinese population consisting of 312 PD patients and 309 healthy controls.

Results: Genotyping analyses showed that none of the 2 SNPs was significantly associated with PD risk.

Conclusions: Our results suggest that rs7625184 and rs2606750 are not associated with PD susceptibility. Further studies are warranted in revealing the links between ATG7 and PD.

Keywords: Parkinson's disease, Polymorphism, Autophagy-related gene 7, Autophagy, Association

Background

Parkinson's disease (PD) is the second most common neurodegenerative disease, that occurs in 1.7% of people over 65 in China [1]. PD is characterized by selectively loss of dopaminergic neurons and Lewy body formation in midbrain substantia nigra, and it is believed to be caused by many genetic and environmental factors [2, 3]. Alpha-synuclein (α -syn), the main component of Lewy bodies, plays an important role in PD pathogenesis [4]. A growing body of evidence have linked α -syn accumulation to the dysfunction of autophagy lysosomal pathway [5].

Autophagy is an essential degradation pathway for cell survival, which including three types: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy (hereafter called autophagy) [6]. Autophagy impairment generates dopamine neuron loss and α -syn aggregation in substantia nigra [7]. Mutation of genes, such as Parkin, PINK1, ATP13A2 and FBXO7, that produce familial PD, have been found to be related with autophagy [6, 8].

The process of autophagy include initial steps, vesicle elongation, vesicle completion, membrane retrieval, docking and fusion, vesicle breakdown and degradation [9]. These events are regulated by proteins called autophagy-related genes (ATG). ATG7 is an E1 like enzyme, and it is a key molecule in vesicle elongation and vesicle completion processes [9]. Mouse models with deletion of ATG7 in midbrain dopamine neurons lead to reduced striatal dopamine content and ubiquitinated aggregate formation in neurons [10]. What's more, mice that lack ATG7 in nervous system presented a decreasing in coordinated movement [11].

To date, limited studies have been performed to investigate the association between ATG7 polymorphisms and PD [12, 13]. There was still no definitive conclusion if ATG7 single nucleotide polymorphisms (SNPs) are associated with PD risk. In this study, we aimed to explore whether ATG7 genetic variations are associated with PD susceptibility in a large Chinese cohort.

Materials and methods

Subjects

A total of 621 subjects of Han Chinese ethnicity Participated in this study, including 309 healthy controls (157 men and 152 women) and 312 sporadic PD patients (151 men and 161 women). The median age of PD patients and healthy controls were 67 (interquartile range: 60-75) and 70 (interquartile range: 57.5-78) years old respectively. The idiopathic PD patients were diagnosed according to the UK Parkinson's Disease Society Brain Bank Criteria by 2 movement disorder specialists [14]. Patients with secondly parkinsonism or with a family history of PD were excluded from our study. Healthy controls were free of neurological and psychotic disorders according to their medical history, physical and laboratory examinations. The PD and control groups were comparable by gender and age ($P=0.843$ and $P=0.158$, respectively; Table 1).

Table 1. Characteristics of the PD patients and controls

		Control	PD	<i>P</i>
Subject number		309	312	
Gender	Male, n (%)	157 (50.8)	151 (48.4)	0.843 ^a
	Female, n (%)	152 (49.2)	161 (51.6)	
Age median (IR)		70 (57.5-78)	67 (60-75)	0.158 ^b

PD, Parkinson's disease; IR, interquartile range.

^a Analyzed by Chi square test.

^b Analyzed by Mann-Whitney Test.

Single nucleotide polymorphisms (SNPs)

Thirteen tag-single nucleotide polymorphisms (tag-SNPs) of ATG7, including rs11707842, rs7625184, rs2454476, rs2606750, rs2447607, rs2594992, rs17034276, rs2305295, rs4684776, rs4684787, rs6442260, rs9818393, and rs9873812, were identified according to the HapMap project and Haploview v.4.2 [15]. The parameters are as follows: $r^2 \geq 0.8$, and mean allele frequency (MAF) ≥ 0.1 in Han Chinese

93 population from Beijing, China. We finally selected rs7625184 (T>C) and rs2606750
 94 (T>C) in our study, because both of them could be digested by restriction enzymes,
 95 when their allele is “C”.

96 **Genotyping**

97 Genomic DNA was extracted from the peripheral blood samples of participants using
 98 a DNA blood kit (Tiangen, Beijing, China), as described before [16]. SNPs were
 99 determined by polymerase chain reaction-restriction fragment length polymorphism
 100 (PCR-RFLP) analysis. The primer pairs, restriction enzymes, and fragment length of
 101 SNPs were presented in table 2. PCRs were conducted according to the
 102 manufacturer's protocol (Tiangen, Beijing, China). The annealing temperature were
 103 52°C for rs7625184, and 56°C for rs2606750. PCR products were digested by
 104 restriction endonucleases according to manufacturer's protocol (New England
 105 BioLabs, Beverly, MA; Table 2). The digested fragments were separated and
 106 visualized as described previously [16]. Twenty PCR samples from each SNPs were
 107 verified by direct sequencing (BGI Tech, Shanghai, China), and all of them were
 108 consistent with the enzymatic genotyping.

109

110 Table 2. PCR primers and RFLP products

SNPs	Restriction enzyme	Primers	PCR product, bp	RFLP size, bp
rs7625184	AccI	Forward: 5'- GCATAATCTTACCACTGG -3'	561	TT: 561
		Reverse: 5'- CTCTCCATTCCCACTGCTAC -3'		TC: 561+497+64
rs2606750	BstBI	Forward: 5'- AAGACTTGTCCTTCACATT -3'	532	CC: 497+64
		Reverse: 5'- CCTTTCCCATCCCACTCCA -3'		TT: 532
				TC: 532+358+174
				CC: 358+174

111 PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNPs, single
 112 nucleotide polymorphisms.

113

114

115 **Data analysis**

116 All of the analyses in our study were performed by using statistical package of
 117 Predictive Analytics Software 18.0 (PASW, version 18.0) for windows. The
 118 Hardy-Weinberg equilibrium (HWE) and Kolmogorov-Smirnov (KS) tests were used

to evaluate the genotype distribution of the population and normality respectively. The differences in gender, and genotype and allele frequencies between PD and control groups were assessed by χ^2 test. The difference in age between the two groups was assessed by Mann-Whitney Test. Multivariate analysis was performed by binary logistic regression model with gender, age and genotypes as covariates. A two-tailed *P* value <0.05 was considered statistically significant.

Results

The ATG7 variants, rs7625184 and rs2606750 were not associated with PD susceptibility

The genotype distribution of rs7625184 and rs2606750 in PD patients and healthy controls met with HWE (*P* >0.05). For both rs7625184 and rs2606750, no statistical difference in genotype distribution was found between PD and controls groups (*P* =0.904 and *P* =0.280, respectively; Table 3). There was also no statistical difference in their allele frequencies between the 2 groups (*P* =0.659 and *P* =0.128, respectively; Table 3). We further performed a logistic regression analysis with gender, age, and the two SNPs as covariates. The result showed that neither rs7625184 nor rs2606750 was risk factor for PD (Table 4).

Table 3. Genotype and allele frequencies of rs7625184 and rs2606750 in PD patients and controls

SNPs	Genotype, n (%)			<i>P</i>	Allele, n (%)		<i>P</i>	OR (95% CI)
	TT	TC	CC		T	C		
rs7625184								
Controls	153 (49.5)	127 (41.1)	29 (9.4)	0.904	433 (70.1)	185 (29.9)	0.659	1.056 (0.829-1.345)
PD	149 (47.8)	132 (42.3)	31 (9.9)		430 (68.9)	194 (31.1)		
rs2606750								
	TT	TC	CC		T	C		
Controls	43 (13.9)	162 (52.4)	104 (33.7)	0.280	248 (40.1)	370 (59.9)	0.128	0.840 (0.670-1.052)
PD	54 (17.3)	169 (54.2)	89 (28.5)		277 (44.4)	347 (55.6)		

CI, confidence interval; OR, odds ratio; PD, Parkinson's disease; SNPs, single nucleotide polymorphisms.

Table 4. Multivariate analysis of risk factors for PD by binary logistic regression

Factors	B	<i>P</i>	OR	95% CI for OR	
				Lower	Upper
Constant	0.010	0.904	1.010		

CI, confidence interval; OR, odds ratio; PD, Parkinson's disease.

^a Binary logistic regression with gender, age, and 2 SNPs as covariates.

Discussion

Accumulating evidence showed that autophagy dysfunction plays an essential role in α -syn degradation and PD pathology [5, 17-19]. ATG7 is a key enzyme in autophagy pathway [6, 9, 20], and it is associated with PD and dementia with lewy bodies (DLB) [10, 11, 21, 22]. In the present study, we performed a case-control study to investigate the relationship between ATG7 SNPs and PD risk in a currently largest cohort. However, our results showed that both rs7625184 and rs2606750 were not associated with PD susceptibility.

Both rs7625184 and rs2606750 are located in intron of ATG7. There was no known clinical case has been reported relating to these two sites. In our cohort, C allele of rs7625184 and T allele of rs2606750 are the minor alleles (30.5% and 42.3%). It is in accordance with the frequency of Asian population in NCBI dbSNP (35.0% and 17.0%). As far as we know, our research is the first attempt to explore the relationship between the two SNPs and PD, though we got a negative result.

Two previous studies have been carried out to analyse the association between ATG7 SNPs and sporadic PD. Chen et al. sequenced ATG7 promotor region in 101 PD patients and 148 healthy controls. They identified four novel heterozygous variants (11313449G>A, 11313811T>C, 11313913G>A and 11314041G>A) in PD patients, and found that these mutations decreased transcriptional activities of the ATG7 gene promoter by luciferase reporter [13]. However, due to the limited sample size, it remains unclear whether the four mutations affect the autophagic activity and PD susceptibility. The other study analyzed rs1375206 (a SNP in promoter) and plasma ATG7 levels in 124 PD patients and 105 comparable healthy controls. They found the plasma ATG7 levels were higher in PD patients, but no significant difference in genotype distribution was found between two groups [12]. Further studies should be performed to elucidate the association between ATG7 SNPs and PD susceptibility.

As we know, dysfunctions in autophagy have been observed in Huntington disease and PD[23]. ATG7 polymorphism (V471A) has been identified to be related

with age at onset of Huntington's disease [23, 24]. In addition, deletion of ATG7 has been widely used in PD research as an autophagy model [25, 26]. And it has also been explored as a potential drug therapy target of PD [27-29]. Therefore, it is of great significance to further explore the relationship between ATG7 and PD, as well as the underlying mechanism.

However, in our study, we selected rs7625184 and rs2606750 instead of all the tag-SNPs, because they could be digested by restriction enzymes. This may limited the objectivity of our conclusion. Future investigations are warranted to further uncover the association between ATG7 and PD.

Conclusions

In conclusion, the current study suggest in a Chinese cohort that rs7625184 and rs2606750 of ATG7 were not associated with PD susceptibility. Further studies are needed to define the role of ATG7 in PD as well as the causality of the polymorphisms.

List of abbreviations

ATG: Autophagy-related genes; CMA: Chaperone-mediated autophagy; DLB: Dementia with lewy bodies; HWE: Hardy-Weinberg equilibrium; KS: Kolmogorov-Smirnov; MAF: Mean allele frequency; PCR: polymerase chain reaction; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; PD: Parkinson's disease; SNPs: Single nucleotide polymorphisms

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Second Affiliated Hospital and Yuying Children's Hospital, Wenzhou Medical University. All subjects signed written informed consents prior to participation in the study. All methods were carried out in accordance with relevant guidelines and regulations.

Availability of data and materials

The data that support the findings of this study are available from the corresponding

author upon reasonable request.

Competing interests

The authors declare that there is no potential conflict of interest.

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Authors' contributions

MZ, JYW and XPL designed the study. MZ, JYW and LBZ examined the patients, and collected blood samples. JYW and LBZ analyzed blood samples and interpreted the genetic data. RJL and SJN provided statistical support. XPL and JYW drafted the manuscript. XPL supervised the study. All authors read, revised and approved the final version of the manuscript.

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