Neurology Asia 2022; 27(1) : 131 – 136

ATG7 polymorphisms rs7625184 and rs2606750 are not associated with Parkinson's disease

[1,2,3]Ming Zou, [3]Jian-Yong Wang, [2]Ren-Jun Lv, [2,4,5]Shan-Jing Nie, [3]Lan-Bing Zhu, [1,2,4,5]Xue-Ping Liu

[1]Department of Geriatric Neurology, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong; [2]Department of Geriatric Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong; [3]Department of Geriatrics & Neurology, the Second Affiliated Hospital and Yuying Children's Hospital, Wenzhou Medical University, Wenzhou, Zhejiang; [4]Anti-Aging Monitoring Laboratory, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong; [5]Department of Anti-Aging, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong, China.

Abstract

Background & Objective: 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 investigating 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 to reveal the links between *ATG7* and PD.

Keywords: Parkinson's disease, polymorphism, autophagy-related gene 7, autophagy, association

INTRODUCTION

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 selective 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 include 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 decrease 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 whether *ATG7* single nucleotide polymorphisms (SNPs) are associated

Address correspondence to: Xue-ping Liu, PhD, Department of Geriatric Neurology, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong, 250021, China. Tel: +8613455131453, E-mail: Liuxueping1962@163.com

Date of Submission: 19 April 2021; Date of Acceptance: 17 October 2021

https://doi.org/10.54029/2022nkt

with PD risk. In this study, we aimed to explore whether *ATG7* genetic variations are associated with PD susceptibility in a large Chinese cohort.

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 atypical and secondary parkinsonism, as well as those with family history of PD were excluded from our study. Healthy controls were volunteers, who underwent brain magnetic resonance examination and laboratory examinations that included complete blood count, comprehensive metabolic panel, liver function tests, thyroid function test, and ceruloplasmin. All the controls were free of neurological and psychotic disorders according to their medical history, physical examinations, laboratory examinations and brain magnetic resonance imaging. The PD and control groups were comparable by gender and age (P =0.843 and P =0.158, respectively; Table 1). 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.

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: r2 ≥ 0.8, and mean allele frequency (MAF) ≥ 0.1 in Han Chinese population from Beijing, China. We finally selected rs7625184 (T>C) and rs2606750 (T>C) in our study, because both of them could be digested by restriction enzymes, when their allele is "C".

Genotyping

Genomic DNA was extracted from the peripheral blood samples of participants using a DNA blood kit (Tiangen, Beijing, China), as described before.[16] SNPs were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The primer pairs, restriction enzymes, and fragment length of SNPs were presented in Table 2. PCRs were conducted according to the manufacturer's protocol (Tiangen, Beijing, China). The annealing temperature were 52 °C for rs7625184, and 56 °C for rs2606750. PCR products were digested by restriction endonucleases according to manufacturer's protocol (New England BioLabs, Beverly, MA; Table 2). The digested fragments were separated and visualized as described previously.[16] In brief, the 561 bp PCR products of rs7625184 were digested at 37 °C, using AccI restriction enzyme, and the 532 bp PCR products of rs2606750 were digested at 65 °C, using BstBI restriction enzyme. The products of RFLPs were separated and visualized under a gel imaging system. The representative gel pictures of the RFLPs were showed in Figure 1. Twenty PCR samples from each SNPs were verified by direct sequencing (BGI Tech, Shanghai, China), and all of them were consistent with the enzymatic genotyping.

Table 1: Characteristics of the PD patients and controls

		Control	PD	*P*
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.

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
				CC: 497+64
rs2606750	BstBI	Forward: 5'- AAGACTTGTCCCTTCACATT -3'	532	TT: 532
		Reverse: 5'- CCTTTCCCATCCCACTCCA -3'		TC: 532+358+174
				CC: 358+174

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

Data analysis

All of the analyses in our study were performed by using statistical package of Predictive Analytics Software 18.0 (PASW, version 18.0) for windows. The 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).

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

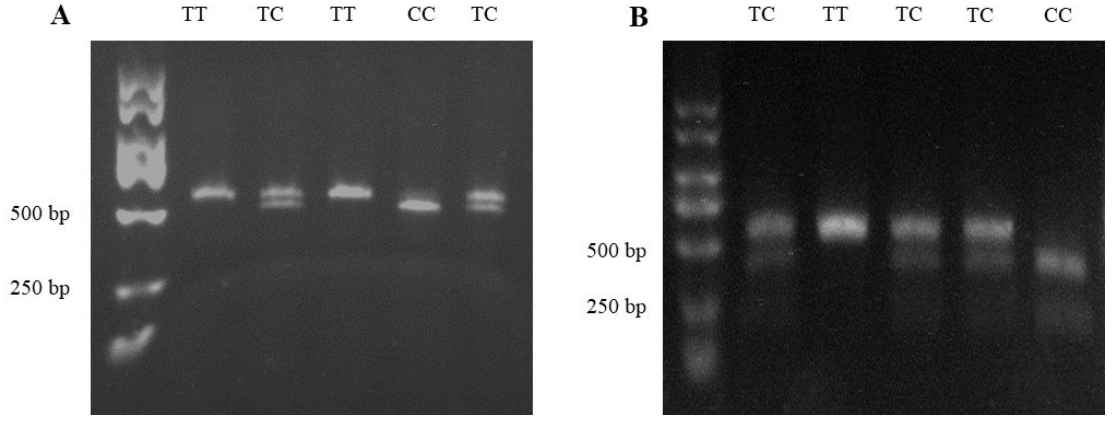

Figure 1: Representative gel picture of the RFLP. A. RFLP picture of rs7625184. B. RFLP picture of rs2606750

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

SNPs	Genotype, n (%)			*P*	Allele, n (%)		*P*	OR (95% CI)
rs7625184	TT	TC	CC		T	C		
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	*P*	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.

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 limit the extent of our conclusion. Future investigations are warranted to further uncover the association between ATG7 and PD.

In conclusion, the current study suggest that 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.

ACKNOWLEDGEMENTS

The authors are grateful to all of the subjects for participating in this study.

DISCLOSURES

Financial support: The study was supported in part by funding from National Natural Science Foundation of China (81801271), and Wenzhou Municipal Science and Technology Bureau (Y2020065).

Conflict of interests: None

REFERENCES

1. Zhang ZX, Roman GC, Hong Z, *et al*. Parkinson's disease in China: prevalence in Beijing, Xian, and Shanghai. *Lancet* 2005; 365:595-7. doi: 10.1016/S0140-6736(05)17909-4.
2. Greenamyre JT, Hastings TG. Biomedicine. Parkinson's--divergent causes, convergent mechanisms. *Science* 2004; 304:1120-2. doi: 10.1126/science.1098966.
3. McCulloch CC, Kay DM, Factor SA, *et al*. Exploring gene-environment interactions in Parkinson's disease. *Hum Genet* 2008; 123:257-65. doi: 10.1007/s00439-008-0466-z.
4. Luk KC, Kehm V, Carroll J, *et al*. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science* 2012; 338:949-53. doi: 10.1126/science.1227157.
5. Xilouri M, Brekk OR, Stefanis L. Autophagy and alpha-synuclein: Relevance to Parkinson's disease and related synucleopathies. *Mov Disord* 2016; 31:178-92. doi: 10.1002/mds.26477.
6. Karabiyik C, Lee MJ, Rubinsztein DC. Autophagy impairment in Parkinson's disease. *Essays Biochem* 2017; 61:711-20. doi: 10.1042/EBC20170023.
7. Hunn BHM, Vingill S, Threlfell S, *et al*. Impairment of macroautophagy in dopamine neurons has opposing effects on parkinsonian pathology and behavior. *Cell Rep* 2019; 29:920-31 e7. doi: 10.1016/j.celrep.2019.09.029.
8. Gusdon AM, Zhu J, Van Houten B, Chu CT. ATP13A2 regulates mitochondrial bioenergetics through macroautophagy. *Neurobiol Dis* 2012; 45:962-72. doi: 10.1016/j.nbd.2011.12.015.
9. Levine B, Kroemer G. SnapShot: Macroautophagy. *Cell* 2008; 132:162 e1- e3. doi: 10.1016/j.cell.2007.12.026.
10. Friedman LG, Lachenmayer ML, Wang J, *et al*. Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of alpha-synuclein and LRRK2 in the brain. *J Neurosci* 2012; 32:7585-93. doi: 10.1523/JNEUROSCI.5809-11.2012.
11. Komatsu M, Waguri S, Chiba T, *et al*. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* 2006; 441:880-4. doi: 10.1038/nature04723.
12. Zhao X, Chen Y, Wang L, *et al*. Associations of ATG7 rs1375206 polymorphism and elevated plasma ATG7 levels with late-onset sporadic Parkinson's disease in a cohort of Han Chinese from southern China. *Int J Neurosci* 2020:1-9. doi: 10.1080/00207454.2020.1731507.
13. Chen D, Pang S, Feng X, *et al*. Genetic analysis of the ATG7 gene promoter in sporadic Parkinson's disease. *Neurosci Lett* 2013; 534:193-8. doi: 10.1016/j.neulet.2012.12.039.
14. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992; 55:181-4. doi: 10.1136/jnnp.55.3.181.
15. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21:263-5. doi: 10.1093/bioinformatics/bth457.
16. Zhang X, Cheng X, Hu YB, *et al*. Serotonin transporter polymorphic region 5-HTTLPR modulates risk for Parkinson's disease. *Neurobiol Aging* 2014; 35:1957 e9- e14. doi: 10.1016/j.neurobiolaging.2014.03.002.
17. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science* 2004; 305:1292-5. doi: 10.1126/science.1101738.
18. Pan T, Kondo S, Le W, Jankovic J. The role of autophagy-lysosome pathway in neurodegeneration associated with Parkinson's disease. *Brain* 2008; 131:1969-78. doi: 10.1093/brain/awm318.
19. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. Alpha-synuclein is degraded by both autophagy and the proteasome. *J Biol Chem* 2003; 278:25009-13. doi: 10.1074/jbc.M300227200.
20. Ichimura Y, Imamura Y, Emoto K, *et al*. In vivo and in vitro reconstitution of Atg8 conjugation essential for autophagy. *J Biol Chem* 2004; 279:40584-92. doi: 10.1074/jbc.M405860200.
21. Crews L, Spencer B, Desplats P, *et al*. Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of alpha-synucleinopathy. *PLoS One* 2010; 5:e9313. doi: 10.1371/journal.pone.0009313.
22. Sato S, Hattori N. Dopaminergic neuron-specific autophagy-deficient mice. *Methods Mol Biol*. 2018; 1759:173-5. doi: 10.1007/7651_2018_156.
23. Metzger S, Saukko M, Van Che H, *et al*. Age at onset in Huntington's disease is modified by the autophagy pathway: implication of the V471A polymorphism in Atg7. *Hum Genet* 2010; 128:453-9. doi: 10.1007/s00439-010-0873-9.
24. Metzger S, Walter C, Riess O, *et al*. The V471A polymorphism in autophagy-related gene ATG7 modifies age at onset specifically in Italian Huntington disease patients. *PLoS One* 2013; 8:e68951. doi: 10.1371/journal.pone.0068951.
25. Zhou J, Song J, Wu S. Autophagic degradation of stromal interaction molecule 2 mediates disruption of neuronal dendrites by endoplasmic reticulum stress. *J Neurochem* 2019; 151:351-69. doi: 10.1111/jnc.14712.
26. Ahmed I, Liang Y, Schools S, *et al*. Development and characterization of a new Parkinson's disease model resulting from impaired autophagy. *J Neurosci* 2012; 32:16503-9. doi: 10.1523/JNEUROSCI.0209-12.2012.
27. Niu XY, Huang HJ, Zhang JB, *et al*. Deletion of autophagy-related gene 7 in dopaminergic

neurons prevents their loss induced by MPTP. *Neuroscience* 2016; 339:22-31. doi: 10.1016/j.neuroscience.2016.09.037.
28. Xie C, Ginet V, Sun Y, *et al*. Neuroprotection by selective neuronal deletion of Atg7 in neonatal brain injury. *Autophagy* 2016; 12:410-23. doi: 10.1080/15548627.2015.1132134.
29. Han B, Wang L, Fu F, *et al*. Hydroxysafflor yellow A promotes alpha-synuclein clearance via regulating autophagy in rotenone-induced Parkinson's disease mice. *Folia Neuropathol* 2018; 56:133-40. doi: 10.5114/fn.2018.76618.