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

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**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

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**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.2 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).9 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
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. 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 imaging. 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. 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. 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 ℃ for rs7625184, and 56 ℃ 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. In brief, the 561 bp PCR products of rs7625184 were digested at 37 ℃, using AccI restriction enzyme, and the 532 bp PCR products of rs2606750 were digested at 65 ℃, 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

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

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

a Analyzed by Chi square test.

b Analyzed by Mann-Whitney Test.
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 χ² 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 pathway6,9,20, 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

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Restriction enzyme</th>
<th>Primers</th>
<th>PCR product, bp</th>
<th>RFLP size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7625184</td>
<td>AccI</td>
<td>Forward: 5’- GCATAATCTTACCACTGG -3’</td>
<td>561</td>
<td>TT: 561</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: 5’- CTCTCCATTCCCACTGCTAC -3’</td>
<td>TC: 561+497+64</td>
<td>CC: 497+64</td>
</tr>
<tr>
<td>rs2606750</td>
<td>BstBI</td>
<td>Forward: 5’- AAGACTTGTCCCCTCACATT -3’</td>
<td>532</td>
<td>TT: 532</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: 5’- CTTTTCCCATCCCACTCCA -3’</td>
<td>TC: 532+358+174</td>
<td>CC: 358+174</td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNPs, single nucleotide polymorphisms.
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 promotor by luciferase reporter.\footnote{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 promotor) 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.\footnote{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.\footnote{23} \(ATG7\) polymorphism (V471A) has been identified to be related with age at onset of Huntington’s disease.\footnote{23,24} In addition, deletion of \(ATG7\) has been widely used in PD research as an autophagy model.\footnote{25,26} And it has also been explored as a potential drug therapy target of PD.\footnote{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 were not associated with PD susceptibility.

<table>
<thead>
<tr>
<th>Factors</th>
<th>B</th>
<th>P</th>
<th>OR</th>
<th>95% CI for OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.010</td>
<td>0.904</td>
<td>1.010</td>
<td></td>
</tr>
</tbody>
</table>

\(\text{CI, confidence interval; OR, odds ratio; PD, Parkinson’s disease.}\)

\(\text{a Binary logistic regression with gender, age, and 2 SNPs as covariates.}\)
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
