Neuroscience Letters 646 (2017) 30–35

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Genetic analysis of the ATG16L1 gene promoter in sporadic Parkinson's disease

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HIGHLIGHTS

- ATG16L1 gene promoter was genetically analyzed in sporadic PD patients.
- Eight DSVs including six SNPs were found in this study population.
- One novel heterozygous DSV was only identified in a sporadic PD patient.
- This DSV did not significantly affected the ATG16L1 gene promoter activity.
- Genetic variants in ATG16L1 gene promoter may not contribute to PD development.

ARTICLE INFO

Article history:
Received 7 February 2017
Accepted 3 March 2017
Available online 6 March 2017

Keywords:
Parkinson's disease
Autophagy
ATG16L1
Promoter
DNA sequence variant

ABSTRACT

Parkinson's disease (PD) is a common and progressive neurodegenerative disease in which the majority of cases arise sporadically. Sporadic PD is caused by the interactions of genetic and environmental factors. To date, genetic causes for sporadic PD remain largely unknown. Autophagy, a highly conserved cellular process, has been implicated in PD pathogenesis. We speculated that genetic variants in autophagy-related genes (ATG) that regulate gene expression may contribute to PD development. In our previous studies, we have identified several functional DNA sequence variants (DSVs) in the ATG5, ATG7 and LC3 genes in sporadic PD patients. In this study, we further genetically and functionally analyzed the promoter of the ATG16L1 gene, a critical gene for autophagosome formation, in groups of sporadic PD patients and ethnic-matched healthy controls. One novel heterozygous DSV, 233251432C>T, was found in one PD patient. Functionally, this DSV did not affect the transcriptional activity of the ATG16L1 gene promoter in human dopaminergic SH-SY5Y cells. Two heterozygous DSVs including one SNP, 233251286G>A (rs539735288) and 233251582C>T, were found only in controls. In addition, five other SNPs were found in both PD patients and controls. Taken together, the data suggested that genetic variants within the ATG16L1 gene promoter were not a risk factor for sporadic PD development.

Abbreviations: ATG, autophagy-related gene; CNTNAP3, contactin associated protein-like 3; CSNK2, casein kinase 2; DSVs, DNA sequence variants; EVA1A, eva-1 homolog A; FIP200, focal adhesion kinase family interacting protein of 200 kD; GBA, glucocerebrosidase; LC3B, microtubule associated protein 1 light chain 3 beta; LRRK2, leucine-rich repeat kinase 2; PD, Parkinson's disease; PPP1, protein phosphatase 1; SH-SY5Y, human dopaminergic neuroblastoma cells; TECPR1, TECtonin β-Propeller Repeat containing 1; TMEM166, transmembrane protein 166.

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http://dx.doi.org/10.1016/j.neulet.2017.03.007
0304-3940/

1. Introduction

Parkinson's disease (PD) is a common and progressive neurodegenerative disease in which the majority of cases are sporadic. The clinical features of PD are resting tremor, postural instability, rigidity and bradykinesia. Lewy body formation and dopaminergic neuronal loss in the substantia nigra are the two pathological characteristics of PD. Sporadic PD is caused by the interactions of genetic and environmental factors. Recent genome-wide association, next generation sequencing and exome sequencing studies have associated several genetic variants with sporadic PD, including leucine-rich repeat kinase 2 (LRRK2) and lysosomal β-glucocerebrosidase (GBA) genes [1,2]. However, genetic causes for sporadic PD remain largely unknown. Autophagy is a highly conserved cellular pathway that delivers long-lived proteins and organelles to lysosomes for digestion, including macroautophagy, microautophagy and chaperone-mediated autophagy. Human studies and animal experiments have confirmed that dysfunctional macroautophagy and chaperone-mediated autophagy contribute to PD pathogenesis [3,4]. Many known PD genes affect the turnover of mitochondria and other vesicular structures by the autophagy-lysosomal system and lysosomal function [5–7].

In macroautophagy (hereafter referred to as autophagy), autophagosome formation is mediated by a number of core autophagy-related (ATG) proteins. Several subgroups of core ATG proteins are involved in the process, including the ATG1/ULK complex, the ATG9 cycling system, the PtdIns 3-kinase complex, the ATG12 conjugation system and the ATG8/LC3 conjugation system [8,9]. ATG16L1, as well as ATG5, ATG7, ATG10 and ATG12, is a major component of ATG12 conjugation system, and plays an essential role in the elongation of the phagophore, a precursor of the autophagosome [10]. ATG16L1 forms an oligomeric complex with ATG12-ATG5 conjugates to facilitate LC3/ATG8-conjugation to phosphatidylethanolamine by recruiting an LC3-ATG3 intermediate [11,12]. In this capacity, ATG16L1 is an important determinant that specifies the site of LC3 lipidation [13]. A recent study also reported that ATG16L1 is involved in plasma membrane trafficking and endocytosis during autophagosome biogenesis [14]. Most ATG16L1-deficient mice die within 1 day of delivery, indicating an essential role of ATG16L1 in animal survival during neonatal starvation. Furthermore, autophagic disruption and enhanced production of IL-1β and IL-18 have been observed, implicating ATG16L1 in the inflammatory immune response [15]. In mice with a hypomorphic mutation in ATG16L1, developmental retention of early-stage cells in various tissues was observed, including impaired differentiation toward neurons [16]. In addition, ATG16L1 exhibits other non-autophagic functions, such as in cellular secretion and exocytosis [17–21].

It has been demonstrated that autophagy is regulated at transcriptional, post-transcriptional, and post-translational levels [22]. Deregulated autophagy has been observed in the brain tissues of PD patients and model animals [23]. Mutations in the ATG genes have been associated with various human diseases [24]. We speculated that genetic variants in ATG genes may impair autophagic function, contributing to the sporadic PD development. In our previous studies, genetic variants within the regulatory regions of the autophagy genes, such as microtubule associated protein 1 light chain 3 beta (LC3B), ATG5 and ATG7, were identified and functionally analyzed in sporadic PD patients [25–27]. In this study, we further genetically analyzed the essential core autophagy gene – ATG16L1 in groups of sporadic PD patients and ethnic-matched controls.

2. Materials and methods

2.1. Study subjects

A total of 151 patients with sporadic PD (mean age 66.29 years, male 83, female 68) were recruited from the Division of Neurology, Affiliated Hospital of Jining Medical University, Jining Medical University, Jining, Shandong, China. All PD patients were diagnosed by two neurologists. Ethnic-matched healthy controls ($n = 174$, mean age 59.31 years, male 93, female 81) were recruited from the same hospital. PD patients and controls with a family PD history were excluded. This study was approved by the Human Ethics Committee of Affiliated Hospital of Jining Medical University. Informed consents were obtained.

2.2. Direct DNA sequencing

Peripheral leukocytes were isolated and genomic DNAs were extracted with DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA). The ATG16L1 gene promoter, from −1095 bp to +151 bp, was generated by PCR and directly sequenced. Two overlapping DNA fragments, −1095 bp to −377 bp (719 bp) and −451 bp to +151 bp (602 bp), were generated to cover the ATG16L1 promoter region. PCR primers were designed according to the genomic sequence of the human ATG16L1 gene (GenBank access number, NC_000002.12) (Table 1). DNA fragments were sequenced on a 3750 DNA Analyzer (Applied Biosystems, Foster city, CA, USA) and compared with the wild type ATG16L1 gene promoter.

2.3. Functional analysis with dual luciferase assay

Wild-type and variant ATG16L1 gene promoters (1075 bp, from −923 bp to +152 bp) were generated by PCR, which were subcloned into KpnI and HindIII sites of a luciferase reporter vector (pGL3-basic). The PCR primers are shown in Table 1. The constructed expression vectors were transiently transfected into human dopaminergic cells (SH-SY5Y) and luciferase activities were measured using a dual-luciferase reporter assay. Relative transcriptional activities of variant ATG16L1 gene promoters were obtained. All experiments were repeated at least three times, in triplicates.

2.4. Statistical analysis

Quantitative data are represented as mean ± SEM and analyzed by Student's t-test. The distributions of DNA sequence variants (DSVs) were compared by χ^2 test using SPSS v13.0. $P < 0.05$ was considered statistically significant.

3. Results

A total of eight DSVs including six single-nucleotide polymorphisms (SNPs) were identified in this study group. The locations and frequencies of which are shown in Table 2 and Fig. 1A. The DNA sequence chromatograms of the DSVs are depicted in Fig. 1B. One novel heterozygous DSV 233251432C>T was identified in one PD patient, but in none of the controls. One novel heterozygous DSV 233251582C>T and one SNP 233251286G>A (rs539735288) were only found in controls. Five SNPs, 233250873G>A (rs146693112), 233250963T>C (rs1816753), 2332511039T>C (rs12476635), 233251112A>T (rs74599577) and 233251563C>T (rs77820970), were found in both PD patients and controls with similar frequencies ($P > 0.05$).