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Novel and functional ABCB1 gene variant in sporadic Parkinson's disease

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HIGHLIGHTS

- ABCB1 gene promoter was analyzed in sporadic PD patients and controls.
- A novel and heterozygous DSV was only identified in one PD patient.
- This DSV significantly changed ABCB1 gene promoter activity.
- ABCB1 gene variants may contribute to PD development as a rare risk factor.

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ABSTRACT

Parkinson's disease (PD) is a common progressive neurodegenerative disease. Most cases of PD are sporadic, which is caused by interaction of genetic and environmental factors. To date, genetic causes for sporadic PD remain largely unknown. ATP-binding cassette sub-family B member 1 (ABCB1) is a membrane-associated protein that acts as an efflux transporter for many substrates, including chemotherapeutic agents, anti-epilepsy medicine, antibiotics and drugs for PD. ABCB1 gene is widely expressed in human tissues, including endothelial cells of capillary blood vessels at blood–brain barrier sites. In PD patients, decreased ABCB1 levels have been reported. We speculated that misregulation of ABCB1 gene expression, caused by DNA sequence variants (DSVs) within its regulatory regions, may be involved in PD development. In this study, we genetically and functionally analyzed the proximal promoter of the human ABCB1 gene, which is required for constitutive expression, in sporadic PD patients and healthy controls. The results showed that a novel and heterozygous DSV g.117077G>A was identified in one PD patient, but in none of the controls. This DSV significantly altered the transcriptional activity of the ABCB1 gene promoter in transiently transfected HEK-293 cells. A heterozygous DSV g.116347T>C was only found in one control. Four single-nucleotide polymorphisms, g.116154T>C (rs28746504), g.117130A>G (rs2188524), g.117356C>G (rs34976462) and g.117372T>C (rs3213619), and one heterozygous deletion

Abbreviations: ABCB1, ATP-binding cassette sub-family B member 1; ATP13A2, Lysosomal *P*-type transmembrane cation-transporting ATPase, PARK9; BBB, Blood–brain barrier; DJ-1, PARK7 gene; DSVs, DNA sequence variants; FOXO1, Forkhead box factor O1; FOXO3a, Forkhead box factor O3a; GBA, Lysosomal β-glucocerebrosidase; LRRK1, Leucine-rich repeat kinase 1; PD, Parkinson's disease; PINK1, PTEN-induced kinase 1; SNCA, Alpha-synuclein gene; SNPs, Single-nucleic acid polymorphisms; SP1, Specificity protein 1; SP3, Specificity protein 3; UCHL-1, Ubiquitin-C-terminal hydrolase 1.

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DSV g.116039del were found in PD patients and controls with similar frequencies. Therefore, our findings suggest that ABCB1 gene promoter DSVs may contribute to PD development as a rare risk factor.

1. Introduction

Parkinson's disease (PD) is a common and progressive neurodegenerative disease. Most cases are sporadic PD, which is caused by interactions of genetic and environmental factors. The pathological characteristics of PD are the loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and the intracellular deposition of Lewy bodies due to the accumulation of α-synuclein aggregates. To date, a number of genetic loci and genes, such as alpha-synuclein (SNCA), PTEN-induced kinase 1(PINK1), Parkin, leucine-rich repeat kinase 1 (LRRK1), ubiquitin-C-terminal hydrolase 1 (UCHL-1), PARK7 (DJ-1), lysosomal *P*-type transmembrane cation-transporting ATPase (ATP13A2, also known as PARK9) and lysosomal β-glucocerebrosidase (GBA) genes, have been associated with familiar PD cases [1–3]. However, genetic causes and underlying molecular mechanisms for sporadic PD remain largely unknown.

ATP-binding cassette sub-family B member 1 (ABCB1), also known as *P*-glycoprotein or multidrug resistance protein 1 (MDR1), is a member of the ABC transporter superfamily and plays an important role in the development of multidrug resistance (MDR) in human cancer cells [4]. ABCB1 is a membrane-associated protein and acts as an efflux transporter for many substrates, including chemotherapeutic agents, anti-epilepsy medicine, antibiotics and drugs for PD [5–7]. The ABCB1 gene is widely expressed in human organs and tissues, including liver, kidney, adrenal gland, colon, small intestine and capillaries of brain and testis [8–10]. In circulating leukocytes, ABCB1 gene expression has been detected [11,12]. Moreover, ABCB1 is expressed in endothelial cells of capillary blood vessels at blood–brain barrier (BBB) sites with relative high levels [8,9]. Recent studies have suggested that ABCB1 plays an important role in the functions of the BBB and pathogenesis of neurological diseases [6,13].

A few common single-nucleotide polymorphisms (SNPs) in the ABCB1 gene, including 1236C/T in exon 12 (rs1128503), 2677G/T/A in exon 21 (rs2032582) and 3435C/T in exon 26 (rs1045642), have been extensively studied in PD patients [6,13]. These ABCB1 gene SNPs, alone or in conjunction with exposure to insecticides and pesticides, are suggested to be important risk factors for PD development in different populations [14–18]. However, no significant association between ABCB1 gene SNPs and PD has been found in some studies, though frequencies of the ABCB1 gene SNPs are higher in PD patients than controls [19–22]. Therefore, the effects of ABCB1 gene SNPs on PD need to be further established.

Recent studies have shown that the function of ABCB1 at BBB sites is significantly decreased in PD patients compared to controls, which may facilitate the accumulation of toxic compounds in the brain [23,24]. Reduced levels of ABCB1 mRNA in the brain tissues have also been observed in PD patients [25]. Thus, it is speculated that misregulation of ABCB1 gene expression, caused by DNA sequence variants (DSVs) within its regulatory regions, may be involved in PD development. In this study, we genetically and functionally analyzed the proximal promoter of the human ABCB1 gene, which is required for constitutive expression, in sporadic PD patients and healthy controls.

2. Materials and methods

2.1. Study subjects

All patients with sporadic PD ($n = 115$, mean age 67.12 years, male 59, female 56) were recruited from the Division of Neurology, Jining Medical University Affiliated Hospital, Jining Medical University, Jining, Shandong, China. Ethnic-matched healthy controls ($n = 147$, mean age 59.36 years, male 73, female 74) were from the Health and Physical Examination Center in the same hospital. All PD patients were diagnosed by two neurologists. Subjects with a family history of PD were excluded. This study was approved by the Human Ethics Committee of Jining Medical University Affiliated Hospital. Informed consent was obtained.

2.2. DNA sequencing

Peripheral leukocytes were isolated and genomic DNAs were extracted with QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). The proximal promoter of the human ABCB1gene was analyzed, from −1553bp to +262bp encompassing the major transcription start site at 117365 of the human ABCB1 genomic sequence (NG_011513.1). Three overlapping DNA fragments, −1553bp to −784bp (770bp), −836bp to −134bp (703bp) and −342bp to +262bp (604bp), were generated by PCR and directly sequenced. PCR primers were designed with the genomic sequence of the human ABCB1 gene (GenBank accession number, NG_011513.1) and shown in Table 1. DNA fragments were sequenced on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). All the DNA sequences were aligned and compared with the wild-type human ABCB1 gene proximal promoter.

Table 1
PCR primers for the proximal promoter of the human ABCB1 gene.[a]

PCR primers	Sequences	Location	PCR products
Sequencing			
ABCB1-F1	5′-AGATGGACCACAGGGTTGTT-3′	115812	770bp
ABCB1-R1	5′-TGCAATCTGCACAATTCAAA-3′	116581	
ABCB1-F2	5′-AAGCTCTGATGTGAGTTAGCATTG-3′	116529	703bp
ABCB1-R2	5′-CGAGAAACTGCGAAACAGGT-3′	117231	
ABCB1-F3	5′-AATGCGAATCCCGAGAAAAT-3′	117023	604bp
ABCB1-R3	5′-GGACTTGCCAGAGGACTTCA-3′	117626	
Functional analysis			
ABCB1-F	5′-(KpnI) AGCTCACGCCTGTAATCCCTG-3′	115970	1630bp
ABCB1-R	5′-(HindIII) CACGCCTCAAGAAGCCCTTCT-3′	117599	

[a] PCR primers were designed with the genomic sequence of the human ABCB1 gene (NG_011513.1). The major transcription start site is at position 117365.