Neuroscience Letters 500 (2011) 31–35

Contents lists available at ScienceDirect

Neuroscience Letters

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

Genetic analysis of lysosomal alpha-galactosidase A gene in sporadic Parkinson's disease

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ARTICLE INFO

Article history:
Received 5 May 2011
Received in revised form 24 May 2011
Accepted 30 May 2011

Keywords:
Parkinson's disease
Lysosome
Alpha-galactosidase A
Single-nucleotide polymorphisms

ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disease. Majority of PD cases are sporadic, resulting from interaction of genetic and environmental factors. Accumulating evidence indicates that autophagy, which delivers alpha-synuclein to lysosomes for degradation, is involved in the PD pathogenesis. Some lysosomal hydrolases, such as glucocerebrosidase gene and ATP13A2, a lysosomal ATPase gene, have been implicated in PD. We have previously screened the activities of a group of lysosomal hydrolases in sporadic PD patients and found that alpha-galactosidase A (GLA) activities are significantly decreased. In this study, we analyzed GLA gene in sporadic PD patients by sequencing its promoter and exon regions. One single-nucleotide polymorphism (SNP) in the promoter region, rs3027580 (NG_007119.1:g.4292G>C), and two SNPs in the GLA 5′-untranslated region, rs2071225 (NM_000169.2:c.−10C>T) and rs3027585 (NM_000169.2:c.−12G>A), were identified with similar frequencies in sporadic PD patients and healthy controls. A novel variant (NG_007119.1:g.4488C>G) within the promoter region, at the −573 site upstream of the translation start codon (ATG), was found in one male PD patient, but not in female PD patients or healthy controls. Our data suggest that the sequence variant may affect GLA gene expression by altering transcription factor binding sites, contributing to the pathogenesis of sporadic PD.

Parkinson's disease (PD) is a common neurodegenerative disease that affects 1–2% people of over 65 years old. The clinical features of PD are bradykinesia, resting tremor, rigidity and postural instability. PD is pathologically characterized with the loss of dopaminergic neurons in the substantia nigra and formation of intraneuronal inclusions (Lewy bodies). More than a dozen of genetic loci related to PD have been identified with case–control, genetic linkage and association, and genome-wide association studies. Several genes, including SNCA, PINK1, Parkin, LRRK1, UCHL-1, DJ-1 and GBA genes, have been associated with familiar PD [18,19]. Clinically, majority of PD cases are sporadic, which is caused by interaction of genetic and environmental factors. To date, genetic genes and variants for sporadic PD remain largely unknown.

Alpha-synuclein (α-syn) protein is the major component of Lewy bodies and plays a central role in the PD pathogenesis [27,32]. Autophagy–lysosome system is a conserved cellular process to remove macromolecules and damaged organelles, which includes three subtypes, macroautophagy (hereafter referred to as autophagy), microautophagy and chaperone-mediated autophagy (CMA) [20]. Accumulating evidence has demonstrated that autophagy and CMA are implicated in the degradation of α-syn protein and dysfunctional autophagy and CMA lead to the degradation, accumulation and aggregation of α-syn protein [5,11,17,21,31,33,35]. In human PD patients, cellular and animal PD models, increased autophagic vacuoles and altered expression autophagic marker genes have been documented [2,3,37].

More than 60 lysosomal hydrolases and proteins have been identified. Lysosomal dysfunction and deficient lysosomal hydrolases have been also involved in α-syn degradation. Lysosomal cathepsin D has been shown to affect α-syn degradation and aggregation in cultured cells [12]. In PD mouse model, α-syn aggregates are formed accompanied with abnormal lysosomes [22]. Accumulation of α-syn is observed in the brain of a GM2 gangliosidosis mouse model and human lipidosis cases [28,29]. Mutations of lysosomal genes, ATP13A2 (a lysosomal ATPase) and glucocerebrosidase genes, genetic cause of Gaucher's disease, have been

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doi:10.1016/j.neulet.2011.05.238

linked to PD patients [1,24]. Therefore, decreased activities of lysosomal hydrolases and proteins may contribute to the pathogenesis of sporadic PD.

Alpha-galactosidase A (GLA) is a lysosomal hydrolase that involves in glycosphingolipid metabolism. Deficient GLA activity causes Fabry disease, a lysosomal storage disease with intracellular accumulation of globotriaosylceramides and glycosphingolipids [13]. Parkinsonism has been observed in the patients with Fabry disease [7,8]. We have previously screened a group of lysosomal hydrolases and found that GLA activity is significantly decreased in the peripheral leukocytes of sporadic PD patients [38]. To investigate the genetic causes of decreased GLA activity, we perform a genetic analysis of the GLA gene in patients with sporadic PD and healthy controls in this study.

A total of 69 unrelated patients with sporadic PD with age range from 41 to 85 years (male 38, median age 66.9 years; female 31, median age 66.6 years) were recruited from Division of Neurology, Jining Medical College Affiliated Hospital, Jining Medical College, Jining, Shandong, China. The clinical diagnosis was made by two neurologists. PD patients with family history were excluded from this study. A total of 81 healthy controls with age range from 41 to 84 years (male 42, median age 60.3 years; female 39, median age 59.7 years) were recruited from the Physical Examination Center in the same hospital. This study was approved by the Human Ethic Committee of Jining Medical College Affiliated Hospital. Informed consents were obtained from all the participants.

Genomic DNA and total RNA were prepared from the peripheral leukocytes. The promoter and coding regions of GLA gene were generated with PCR. Primers for the GLA coding regions were designed based on the GLA cDNA sequence in the GeneBank (NM_000169.2) and primers for the GLA promoter region on the GLA DNA sequence (NG_007119.1) (Table 1). All the PCR products were analyzed by bi-directional sequencing with BigDye® Terminator v 3.0 reagents and a 3730xl DNA Analyzer (Applied Biosystems, Foster city, CA, USA). SPSS v13.0 was used for statistical analysis to compare frequencies of gene variants between PD patients and controls.

As GLA gene is located at Xq22 [6], the male and female subjects were grouped separately for statistical analysis. The genotype

Table 1
PCR primers for sequenceing GLA promoter and coding regions.

PCR primers	DNA sequences	Locations	Product size (bp)
Promoter			
GLA-P-F	5′-AAGCACGCATTTGCCTAGAT-3′	4210	845
GLA-P-R	5′-GGTGACCGGACAGCATAAAT-3′	5054	
Exons			
GLA-F1	5′-GGTTACCCGCGGAAATTTAT-3′	71	371
GLA-R1	5′-GAGGGTCTGCCTGAAGTCTG-3′	441	
GLA-F2	5′-AACCTTGACTGCCAGGAAGA-3′	267	700
GLA-R2	5′-GGGCCATCTGAGTTACTTGC-3′	966	
GLA-F3	5′-GACCAGGGGGTTGGAATG-3′	883	501
GLA-R3	5′-ATGACATCTGCATTGTATTTTCTAGC-3′	1383	

Primers for GLA promoter are designed based on the DNA sequence from NG_007119.1. Primers for GLA exons are designed based on the cDNA sequence of NM_000169.2.

Table 2
The genotypes and alleles in the GLA gene in PD patients and controls.

SNPs/variant	Genotype			*P* values	Allele		*P* values
	CC	CT	TT		C	T	
5′-untranslated region rs2071225 (NM_000169.2:c.−10C>T)							
Male							
PD (*n* = 38)	–	–	–		36	2	0.653
Control (*n* = 42)	–	–	–		40	2	
Female							
PD (*n* = 31)	29	2	0	0.609	60	2	0.608
Control (*n* = 39)	36	3	0		75	3	
SNPs/variant	Genotype			*P* values	Allele		*P* values
	GG	GA	AA		G	A	
5′-untranslated region rs3027585 (NM_000169.2:c.−12G>A)							
Male							
PD (*n* = 38)	–	–	–		34	4	0.443
Control (*n* = 42)	–	–	–		39	3	
Female							
PD (*n* = 31)	31	0	0	0.167	62	0	0.170
Control (*n* = 39)	36	3	0		75	3	
SNPs/variant	Genotype			*P* values	Allele		*P* values
	GG	GC	CC		G	C	
Promoter region rs3027580 (NG_007119.1:g.4292G>C)							
Male							
PD (*n* = 38)	–	–	–		36	2	0.653
Control (*n* = 42)	–	–	–		40	2	
Female							
PD (*n* = 31)	29	2	0	0.609	60	2	0.608
Control (*n* = 39)	36	3	0		75	3	
SNPs/variant	Genotype			*P* values	Allele		*P* values
	CC	CG	GG		C	G	
Novel variant (NG_007119.1:g.4488C>G)							
Male							
PD (*n* = 38)	–	–	–		37	1	
Control (*n* = 42)	–	–	–		42	0	