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Original Article

SPG3A gene polymorphisms in hereditary spastic paraplegia

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Abstract: Objective: This study aimed to analyze the hereditary spastic paraplegia (HSP)/spastic paraplegia 3A (*SPG3A*) genomic structure as well as the polymorphisms in SPG3G genomic structure by comparing with the normal subjects. Methods: A total of 66 sporadic cases with HSP were collected from April 2014 to September 2016. Genomic DNA extraction was performed, and all coding exons and junction region in the *SPG3A* gene were sequenced. Genetic mutations were identified and DNA sequence alignment was performed against 80 normal subjects without blood relationship. The polymorphism in *SPG3A* gene was analyzed. Results: The coding sequence of the *SPG3A* gene consisted of 14 exons and two polymorphisms were detected at exons 2 and 3 compared with the normal subjects; one polymorphism was detected at exons 3, 4 and 6, respectively. Conclusion: The two coding exons in the *SPG3A* gene in normal subjects were polymorphic and highly conservative. The intron consisted of 3 polymorphic coding sequences. Understanding the polymorphism and genetic mutations in the *SPG3A* gene will contribute to the diagnosis and treatment of HSP.

Keywords: Hereditary spastic paraplegia, gene, polymorphism, *SPG3A*

Introduction

Hereditary spastic paraplegia (HSP) is a group of inherited neurological disorders caused by axonal degeneration [1, 2]. This disease usually affects the motor ability and the patients may need the wheelchair. *SPG3A*-related HSP may appear in childhood, and the symptoms are inconspicuous and progress slowly [3, 4]. The autosomal dominant HSP is associated with the mutation at the SPG3 locus on 14q11-q21 chromosome [5, 6]. In this study, we analyzed the genetic mutation and polymorphism in the *SPG3A* gene in HSP patients and normal subjects.

Materials and methods

Baseline data

A total of 66 sporadic cases with HSP were collected from April 2014 to September 2016. None of them had blood relationship with each other, and the diagnosis was jointly made by 3 neurologists with rich experience. In the meantime, 80 normal subjects without blood relationship were chosen as controls. The informed consent was obtained from all subjects before DNA analysis. **Table 1** shows the comparison of baseline data between the two groups.

Methods

From all subjects 100 ml of peripheral venous blood was collected for genomic DNA extraction by using phenol-chloroform extraction. The *SPG3A* gene sequences were aligned between the two groups. Primers were designed using the software (ABI), and their sequences are shown in **Table 2**. The PCR reaction system had a volume of 25 μl, and Tag DNA polymerase and dNTPs were provided by Beijing Biosynthesis Biotechnology Co., Ltd. PCR procedures were as follows: preheating at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, 34 cycles. After PCR, 10 μl of the amplified product

Table 1. Comparison of baseline data and general biochemical indicators between the two groups

Characteristics	Control group (n=80)	Research Group (n=66)	X^2/t	P
Age (years)	43.7±11.9	44.1±12.3	1.035	0.377
Sex (male/female)	43/37	35/31	0.713	0.409
BMI (kg/m2)	25.7±3.5	25.6±3.4	0.851	0.396
TG (mmol/L)	1.6±0.8	0.6±0.8	0.233	0.739
TC (mmol/L)	4.3±0.7	4.3±0.6	0.391	0.685
LDL-C (mmol/L)	2.5±0.6	2.6±0.7	1.149	0.351
HDL-C (mmol/L)	1.2±0.2	1.2±0.2	0.447	0.655
SBP (mmHg)	125.4±15.1	126.4±16.9	1.637	0.247
DBP (mmHg)	79.8±10.6	79.2±10.1	1.553	0.272

Table 2. Primer sequences for 14 exons in the *SPG3A* gene

Number	Forward	Reverse
1	5'-GAGGGTGTGACGCTGGTATC-3'	5'-AAGTGGAGGGCCAGAAGACC-3'
2	5'-CTGTGTCGGATGTTTGAGAG-3'	5'-TGGAATGGTTACACCACAGC-3'
3	5'-TCGAATTGGAGAGGGATAAG-3'	5'-AAGTGCAACTTCAAGGATCC-3'
4	5'-TGGTAACCCTAATGACCTAG-3'	5'-ATGATTCCCAATTTCTGTTG-3'
5	5'-GTAGGGAATGATGAAGTAAG-3'	5'-CTAATTGGGCCAATAGTTCC-3'
6	5'-GTTATACCTAGAGGGAAAAG-3'	5'-GACCCTAATTAATATACCTGG-3'
7	5'-GGCACCTTAAAGTCCTCATA-3'	5'-CACCAAATGATCCAACAGA-3'
8	5'-TTAGTAGCAGCCCTGTCGTG-3'	5'-CATCAGCCTCCTATCAGTGG-3'
9	5'-TGGAGGACTGGGAAGGATTC-3'	5'-TTCCTCGTACCTTTGCTCCC-3'
10	5'-GCATTTCAGGAAAGGGAAAC-3'	5'-ATTTCTGACAGCCAGAAATC-3'
11	5'-GAAATGTGAACTGCCTGTGG-3'	5'-AGTTGCATGAAGGATACTGG-3'
12	5'-GCAGGCTCCTGATTATTAAC-3'	5'-TCTAATGCAGTGGCTGGCAC-3'
13	5'-CTGCAGGAGTATCTGTTCTG-3'	5'-CACCAAAGATTGTTCTAATC-3'
14	5'-ATGCACACATTGAGGAGTTG-3'	5'-TACTCCGTTCTGATGGAAGC-3'

was taken and evaluated by 1.2% agarose gel electrophoresis. The remaining portion was first purified and then sequenced.

Statistical method

Data were processed by SPSS 20.0 software. The data were assessed by mean ± standard deviation (SD), count data by using chi-square test, t test measurement data. Pearson test was performed to detect the correlation between the gene mutation and the baseline data, and the correlation between the polymorphism of the gene α=0.05.

Results

SPG3A genomic structure

The gene associated with *SPG3A* HSP was localized. It was revealed that this coding sequence consisted of 14 exons, and the relevant acceptor splicing site was sequenced, as shown in **Table 3**.

Polymorphism analysis

Polymorphisms were detected at exons 2 and 3 and introns 3 and 4 and 6, as shown in **Table 4**.

Correlation analysis between genetic mutation and some baseline data

The baseline data were compared between the two groups, and no apparent correlation was detected between the disease and age, gender, BMI and blood pressure (**Table 5**). The results of correlation tests for polymorphic loci are given in **Table 6**.

Discussion

HSP is characterized by hidden onset and slow progression, and its complexity is manifested as other concurrent symptoms. This feature can easily lead to misdiagnosis, and genetic testing is usually required to confirm the diagnosis [7-9]. HSP can be found across all ages, but it is relatively rare in the elderly people. There are only very limited reports on the onset above the age of 60 years old [10, 11]. However, we know little about the pathogenesis of HSP, and non-genetic influence factors have not been identified yet. According to our study, there was no significant difference in age, gender and BMI between the two groups (P>0.05). Further correlation tests did not reveal apparent correlations between the baseline data and polymorphisms in HSP.

HSP is clinically divided into simple and complex type HSP. The former usually presents as progressive spasm and weakness in the lower limbs, with or without impairment of seismesthesia and functional disorders of bladder [12,

Table 3. Sequence of exon-intron junction in the *SPG3A* gene

Number	Exon			Intron		
	Length (bp)	Start stop position	Receptor sequence	Length (bp)	Start stop position	Donor sequence
1	202	126383-126584	-	-	-	GTGAGTAGCAAATGAGAACT
2	248	101064-101311	GTCACTGCTCTGTTCAACAG	25067	101312-126379	GTATGCAGGAAGTACTTTAA
3	135	98067-98201	TAGACTTTATCATTTTATAG	2862	98202-101063	GTATGATGCTAACTTCCTAA
4	105	97503-97607	TTTACTCTTCTTGCCTAGTAG	459	97608-98066	GTATGAAATAAGCCCATTTT
5	51	95241-95291	AATTTTATTTCTTTATCAAG	2211	95292-97502	GTAACAATATTTATTTTCTT
6	57	93509-93565	GTGTGTAATTTTGTGACTAG	1675	93566-95240	GTGAGCGAGTGTTAAATGAT
7	93	75729-75821	CCTTTCTTATTATTTGACGAG	17687	75822-93508	GTTTGTTAGATATTTAGGTA
8	139	74569-74707	TTCAGAATGATTTACTGCAG	1021	74708-75728	GTTTGTGTCTTTAATGAAT
9	128	68360-68487	GGATTTGCTTTTACTTGTAG	6081	68488-74568	GTATCACTCTCATTTCTAGA
10	57	67188-67244	ATCTTTTCTTTTATTCTTAG	1115	67245-68359	GTATTTATTAATGAGGAGGC
11	72	65839-65910	ATTTTGTACTTTGTCCAAAG	1277	65911-67187	GTAAGAGTTAAATATTTTAA
12	432	60629-61060	TACTTCTCTATCTGATACAG	4778	61061-65838	GTAAGAACACCTTTAATTCA
13	15	60124-60138	TAATCTGCCTTTTGCCACAG	490	60139-60628	GTAAGTTAAATTTTAGACGAA
14	444	57462-57905	TTTTGATGCTTTTATTCTAG	2218	57906-60123	-

Table 4. Polymorphisms in *SPG3A* gene

Sequence	Gene locus	Polymorphic change	Frequency
Exon 2	74	A-G	0.12
Exon 3	351	A-G	0.21
Intron 3	19	T-G	0.22
Intron 4	14	A-C	0.34
Intron 6	7	G-A	0.08

Table 5. Correlation analysis between genetic mutation and some baseline data

Stratification	OR	95% CI	P
Age	0.797	-0.447~0.935	0.406
Sex	1.366	0.338~7.531	0.392
BMI	1.293	0.931~4.035	0.114
Hypertension	0.968	-3.001~1.403	0.765

Table 6. Correlation test for polymorphic loci

Sequence	OR	95% CI	P
Exon 2	1.767	0.927~2.813	0.077
Exon 3	1.082	0.626~1.871	0.074
Intron 3	1.436	0.959~2.153	0.125
Intron 4	1.687	0.089~2.629	0.785
Intron 6	1.077	0.634~1.828	0.094

13]; the latter is also combined with retinal degeneration, optic nerve atrophy, muscular atrophy, ataxia and mental retardation in addition to the above [14, 15]. In terms of genetics, HSP is divided into autosomal dominant, autosomal recessive and sex-linked recessive types, all of which have genetic heterogeneity. So far over 20 HSP-related genotypes have been identified, and *SPG4* and *SPG6* are the most common, followed by *SPG3A* [16, 17]. The genetic features of *SPG3A* are closely related to the loci on chromosome 14. The majority of the mutations are localized to exons 4, 7, 8 and 12, and the probability of mutation at exon 12 is the highest, accounting for about 35% [18, 19]. *SPG3A* is expressed in the central nervous system, and the protein encoded contains a conservative functional region, with the formation of gamma-glutamyl transpeptidase (GGT) loci. Little is known about the functions of the protein encoded by the *SPG3A* gene. It is generally believed that this protein shares some similarities with *SPG3A* and the development of HSP is caused by the mutations in the *SPG3A*, which act via changing the activity of GGT. This hypothesis is supported by the fact that atlastin is highly homologous with GNRP-1 [20].

The coding sequence of the *SPG3A* gene which consists of 14 exons is considered to be highly conservative. By DNA sequence alignment with normal subjects we detected two polymorphisms at exons 2 and 3 and one polymorphism at introns 3, 4 and 6, respectively. The frequency of exon 2 mutation was 0.12 and that of exon 3 mutation was 0.21; the frequency of intron 3, 4 and 6 mutations was 0.22, 0.34 and

0.08, respectively. Understanding the mutated loci in the *SPG3A* gene and their relative frequencies will benefit the genetic analysis of HSP, which is significant for HSP related study.

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Disclosure of conflict of interest

None.

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