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Mutation analysis of *SPAST*, *ATL1*, and *REEP1* in Korean Patients with Hereditary Spastic Paraplegia

Tae-Hyoung Kim,[a,b] Jae-Hyeok Lee,[a,b] Young-Eun Park,[a] Jin-Hong Shin,[a,b] Tai-Seung Nam,[c] Hyang-Sook Kim,[b] Ho-Jung Jang,[b] Artem Semenov,[b] Sang Jin Kim,[d] Dae-Seong Kim[a,b]

[a]Department of Neurology, Pusan National University School of Medicine, Yangsan, Korea
[b]Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, Korea
[c]Department of Neurology, Chonnam National University Hospital, Gwangju, Korea
[d]Department of Neurology, Busan Paik Hospital, Inje University College of Medicine, Busan, Korea

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Correspondence
Jae-Hyeok Lee, MD, PhD
Department of Neurology, Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, 20 Geumo-ro, Mulgeum-eup, Yangsan 626-770, Korea
Tel +82-55-360-2453
Fax +82-55-360-2152
E-mail jhlee.neuro@pusan.ac.kr

Dae-Seong Kim, MD, PhD
Department of Neurology, Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, 20 Geumo-ro, Mulgeum-eup, Yangsan 626-770, Korea
Tel +82-55-360-2450
Fax +82-55-360-2152
E-mail dskim@pusan.ac.kr

Background and Purpose Hereditary spastic paraplegia (HSP) is a genetically heterogeneous group of neurodegenerative disorders that are characterized by progressive spasticity and weakness of the lower limbs. Mutations in the spastin gene (*SPAST*) are the most common causes of HSP, accounting for 40−67% of autosomal dominant HSP (AD-HSP) and 12−18% of sporadic cases. Mutations in the atlastin-1 gene (*ATL1*) and receptor expression-enhancing protein 1 gene (*REEP1*) are the second and third most common causes of AD-HSP, respectively.

Methods Direct sequence analysis was used to screen mutations in *SPAST*, *ATL1*, and *REEP1* in 27 unrelated Korean patients with pure and complicated HSP. Multiplex ligation-dependent probe amplification was also performed to detect copy-number variations of the three genes.

Results Ten different *SPAST* mutations were identified in 11 probands, of which the following 6 were novel: c.760A>T, c.131C>A, c.1351_1353delAGA, c.376_377dupTA, c.1114A>G, and c.1372A>C. Most patients with SPAST mutations had AD-HSP (10/11, 91%), and the frequency of SPAST mutations accounted for 66.7% (10/15) of the AD-HSP patients. No significant correlation was found between the presence of the *SPAST* mutation and any of the various clinical parameters of pure HSP. No *ATL1* and *REEP1* mutations were detected.

Conclusions We conclude that *SPAST* mutations are responsible for most Korean cases of genetically confirmed AD-HSP. Our observation of the absence of *ATL1* and *REEP1* mutations needs to be confirmed in larger series.

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Key Words hereditary spastic paraplegia, *SPAST*, *ATL1*, *REEP1*, Korea.

Introduction

Hereditary spastic paraplegia (HSP) is a genetically heterogeneous group of neurodegenerative disorders that are characterized by progressive spasticity and weakness of the lower limbs.[1] Clinically, HSP can be classified as either "pure" or "complicated" depending on the presence of additional features such as ataxia, extrapyramidal signs, severe amyotrophy, peripheral neuropathy, optic atrophy, pigmentary retinopathy, mental retardation, dementia, and epilepsy.[1,2]

Hereditary spastic paraplegia can be inherited as autosomal dominant (AD, AD-HSP), autosomal recessive, or an X-linked trait, and at least 52 loci have been mapped and 31 genes identified to date.[3] Mutations in the spastin gene (*SPAST*, SPG4) are the most common causes of HSP, accounting for up to 40−

67% of AD-HSP cases and 12−18% of sporadic cases.[4-7] Mutations in the atlastin-1 gene (*ATL1*, SPG3A) and receptor expression-enhancing protein 1 gene (*REEP1*, SPG31) are the second and the third most common causes of AD-HSP, respectively.[1] More than half of all clinically diagnosed AD-HSP cases result from mutations in these three genes.[8] Therefore, mutation analysis of these genes will allow more-focused and cost-effective investigations of potential cases of HSP.

The frequency of SPG4 is higher and the incidence of SPG3A is lower in Koreans than in other ethnic groups,[9-13] which might be attributable to an ethnic difference in the genetic background of Korean patients with HSP. However, this needs to be confirmed in a larger series. No previous study has performed mutation screening of *REEP1* in Korean patients with HSP. The present study performed mutation analysis of *SPAST*, *ATL1*, and *REEP1* in 27 unrelated Korean patients with pure and complicated HSP in order to assess the role of these three genes in the occurrence of HSP in a Korean population.

Methods

In total, 27 unrelated Korean probands with HSP were included in this study. All of these patients were evaluated neurologically and genetically after giving informed consent. This study was reviewed and approved by the Institutional Review Board of Pusan National University Yangsan Hospital. HSP was diagnosed by qualified neurologists on the basis of Harding's criteria.[14] Patients with other neurological conditions were excluded based on the clinical, radiological, and biochemical findings.

Clinical parameters included age at onset, family history, and the presence of hyperreflexia, spasticity, weakness, and sensory abnormalities. Additional neurological signs that are suggestive of complicated cases, as mentioned above, were also described. Disability stage was assessed on the 5-point scale described by Fonknechten et al.[15]

Direct sequence analyses of whole coding regions of *SPAST, ATL1*, and *REEP1* were performed in order to identify mutations. Sequence-specific primer pairs covering the entire coding region of all three genes were used, as described elsewhere[16-18] with minor modifications (available upon request). Polymerase chain reaction (PCR) was performed in a Gene Atlas Thermal Cycler (ASTEC, Seoul, Korea). PCR-amplified products were separated and purified using 2% agarose gels and SolGent Agarose Gel Extraction Kits (SolGent, Daejeon, Korea), cycle-sequenced with PCR primers using the BigDye Terminator Sequencing Kit (Applied Biosystems, Foster, CA, USA), and electrophoresed using an ABI PRISM 3730XL DNA analyzer (Applied Biosystems, Foster, CA, USA).

To confirm the pathogenicity of novel mutations identified in the sequence analysis, and to differentiate them from benign polymorphisms, PCR and restriction fragment length polymorphism (PCR-RFLP) analysis were performed using DNA from the included patients and 100 normal controls. Multiple sequence alignment of spastin orthologs was also conducted to evaluate two novel missense mutations and a novel in-frame deletion mutation for evolutionary conserved residues in the protein. Genomic and mRNA reference sequences of the three genes (*SPAST*: NG_008730.1, NM_014946.3; *ATL1*: NG_009028.1, NM_015915.4; and *REEP1*: NG_013037.1, NM_001164730.1) were used to describe the sequence variants.

Among patients in whom direct sequencing analysis did not identify mutations, multiplex ligation-dependent probe amplification (MLPA) was performed to detect copy-number variations of the three genes. MLPA reactions were performed according to the manufacturer's protocol using MLPA kit P165 (*SPAST*, *ATL1*) and P213 (*REEP1*) from MRC-Holland (Amsterdam, The Netherlands).

Statistical significance was assessed using nonparametric tests (the Mann-Whitney U test and Fisher's exact test) to establish any associations between the presence of a *SPAST* mutation and particular clinical parameters in patients with pure HSP. Spearman's rank correlation coefficient was used to measure the statistical significance between disease duration and disability score. All statistical analyses were performed using SPSS (version 18.0, SPSS Inc., Chicago, IL, USA).

Results

The patients' clinical data are given in Supplementary Table 1. The age of the patients ranged from 14 to 64 years (mean ±SD=36.6±13.4 years); 16 patients were male and 11 were female. Fifteen of the 27 probands exhibited an AD inheritance pattern, and 9 appeared to be sporadic. The exact inheritance pattern could not be determined in three patients. The age at onset varied widely from 1 to 59 years (24.8±14.5 years). Nineteen and 8 patients were classified as pure and complicated HSP, respectively. The complicated forms included peripheral neuropathy on nerve conduction studies (n=2), mental retardation (n=3), epilepsy (n=1), cerebellar ataxia (n=1), saccadic pursuit (n=1), and dysarthria (n=6). Most of the complicated HSP patients had more than one additional feature.

Ten different mutations of *SPAST*−comprising 4 missense, 4 nonsense, 1 in-frame deletion, and 1 frameshift mutations−were identified in 11 probands (Table 1, Fig. 1). Six of these (c.760A>T, c.131C>A, c.1351_1353delAGA, c.376_377dupTA, c.1114A>G, and c.1372A>C) were novel, and the others (c.734C>G, c.1496G>A, c.1741C>T, and c.1196C>T) have been described previously.[7,16,19,20] Two novel missense mutations and one in-frame deletion mutation (c.1114A>G,

c.1372A>C, and c.1351_1353delAGA) were located in the ATPases Associated with a wide variety of Activities (*AAA*) cassette domain. Two novel nonsense mutations and one frameshift mutation led to a premature termination codon, resulting in the production of a truncated protein. None of the mutation variants were found in 100 normal controls in PCR-RFLP analysis. No mutations in *ATL1* and *REEP1* were found, and no copy-number variants were detected among all three genes.

SPAST mutations were present in 66.7% (10/15) of the AD-HSP patients and 57.9% (11/19) of those with pure HSP. All of the *SPAST* mutations were associated with pure HSP. The clinical features of patients with pure HSP with *SPAST* mutations were compared to those without mutations (Table 2). In the *SPAST*-mutation-positive group, although most patients (90.9%, 10/11) had an AD inheritance pattern, the correlation was not statistically significant (p=0.111). Furthermore, various clinical parameters did not differ significantly between the *SPAST*-mutation-positive and -negative groups, and there was no significant correlation between disease duration and disability stage (Spearman's rho=0.239, p=0.324).

Discussion

This study identified *SPAST* mutations in ten AD-HSP patients and one sporadic HSP patient among 27 unrelated pure and complicated HSP probands. Six of these mutations have not been described previously. The frequency of *SPAST* mutations in our AD-HSP patients (66.7%) is higher than those reported previously (range, 18−42%).[15,21-23] Park et al.[7] also reported a similar frequency of *SPAST* mutations in Korean patients with uncomplicated AD-HSP. However, they did not perform MLPA to identify exon deletions or duplications. According to previous studies, exon deletions of *SPAST* are not rare and have been found in 18% and 20% of the point-mutation-negative patients.[24,25] However, in our patients MLPA analysis of *SPAST* revealed no copy-number variants.

Mutations of *ATL1* and *REEP1* are relatively common in

Table 1. SPG4 mutations identified in the study

Patient number	Location	Mutation type	Nucleotide change	Predicted protein change	Reference
1	Exon 17	Nonsense	c.1741C>T	p.Arg581*	19
2	Exon 5	Nonsense	c.760A>T	p.Lys254*	Novel
3	Exon 1	Nonsense	c.131C>A	p.Ser44*	Novel
4	Exon 5	Nonsense	c.734C>G	p.Ser245*	16
5	Exon 11	In-frame deletion	c.1351_1353delAGA	p.Arg451del	Novel
6	Exon 1	Frameshift	c.376_377dupTA	p.Ile127Thrfs*35	Novel
7	Exon 8	Missense	c.1114A>G	p.Arg372Gly	Novel
8	Exon 11	Missense	c.1372A>C	p.Ser458Arg	Novel
9	Exon 13	Missense	c.1496G>A	p.Arg499His	7
10	Exon 8	Missense	c.1114A>G	p.Arg372Gly	Novel
11	Exon 9	Missense	c.1196C>T	p.Ser399Leu	20

*Nonsense mutations.

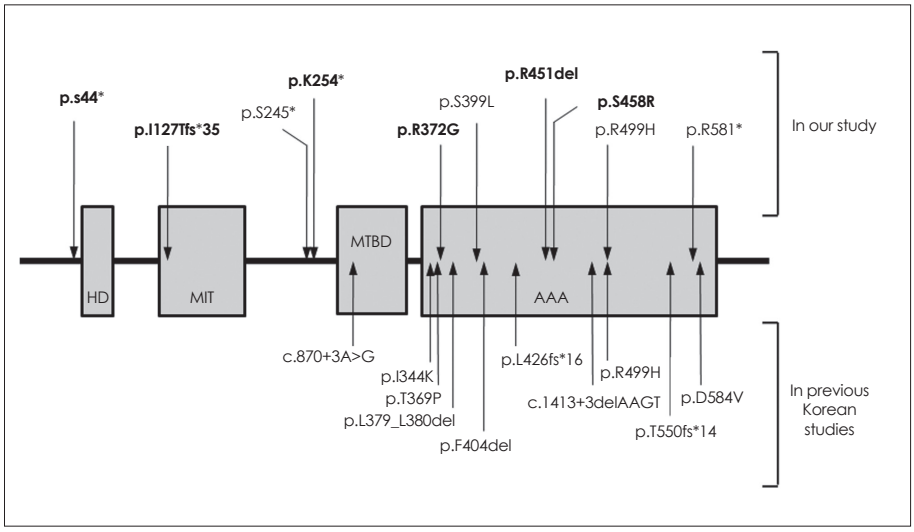

Fig. 1. Organization of spastin and location of *SPAST* mutations identified in Korean HSP patients. *Nonsense mutations. AAA: ATPases Associated with a wide variety of Activities, HD: hydrophobic domain, HSP: hereditary spastic paraplegia, MIT: microtubule interacting and trafficking domain, MTBD: microtubule binding domain.

Table 2. Comparison of clinical features between pure HSP groups with and without SPAST mutations

	SPAST-positive (*n*=11)	SPAST-negative (*n*=8)	*p*-value
Gender (male:female)	7:4	5:3	
Age at onset (mean±SD)	21.3±15.2	23.9±11.8	0.657
Disease duration (mean±SD)	17.1±14.4	10.3±7.9	
Inheritance (*n*, %)			
AD	10 (90.9)	4 (50)	0.111
Non-AD	1 (9.1)	4 (50)	
Clinical findings (*n*, %)			
Decreased sensation	1 (9.1)	3 (37.5)	0.262
Upper limb involvement	6 (54.5)	2 (25)	0.352
Urinary dysfunction	1 (9.1)	4 (50)	0.111

Statistical significance test was done by Mann-Whitney U test and Fisher's exact test.
AD: autosomal dominant, HSP: hereditary spastic paraplegia.

other ethnic groups and have been variously identified in up to 9% and 6.5% of HSP patients.[13,26] Copy-number variations in *ATL1* and *REEP1* have also been reported.[27-30] However, we did not identify any pathogenic mutations in *ATL1* and *REEP1*. To date, only one Korean family with a missense mutation in *ATL1* has been reported. These results suggest that *SPAST* mutations are responsible for most cases of genetically confirmed AD-HSP in Korean patients. The relatively high rate of *SPAST* mutations and the lower incidence of *ATL1* and *REEP1* mutations among Korean HSP patients imply the existence of ethnic differences in the subtype of HSP. However, our observation of the absence of *ATL1* and *REEP1* mutations needs to be confirmed in larger series.

Previous studies have demonstrated that most *SPAST* mutations are located in the *AAA* cassette domain, regardless of ethnicity.[15,16,21-23,31,32] Six of the causative mutations identified in this study are located in the *AAA* cassette domain. It is remarkable that three of the nonsense mutations and the single frameshift mutation in our patients were outside of this domain. All of these mutations are predicted to affect the *AAA* cassette domain indirectly. Nonsense and frameshift mutations are expected to cause premature truncation before and within the *AAA* cassette domain, thereby producing dysfunctional spastin.

Mutations in *SPAST*, *ATL1*, and *REEP1* are predominantly associated with pure forms of AD-HSP.[1,2] However, some of them may have other neurologic deficits seen in complicated HSP.[1,2] They can also appear sporadically due to low penetrance, de-novo mutations, premature death of transmitting parents, or underrecognition of family history.[2] All of the *SPAST* mutations in our patients were associated with pure HSP. Most patients with *SPAST* mutations belonged to AD-HSP. The low incidence of *SPAST* mutations in the sporadic cases is consistent with the findings of previous studies.[19,21] Among various clinical parameters of pure HSP, no significant correlation was found with the presence of a *SPAST* mutation in this study. Previous studies have found the onset age to be lower in *SPAST*-mutation-negative groups,[32,33] and another study found that the disease progression was faster in patients with late-onset *SPAST* HSP than in those with early-onset HSP.[15] In addition, wheelchair use and abnormal vibration sense in the lower limbs were more common in a *SPAST*-mutation-positive group.[34] Further studies with larger numbers of patients are needed to elucidate the relationship between *SPAST* mutations and clinical phenotypes of Korean patients with HSP.

In conclusion, we report herein a mutational analysis of *SPAST*, *ATL*, and *REEP1* in Korean patients with HSP. *SPAST* mutations were the found in most of the genetically confirmed AD-HSP patients. The findings of this study highlight the importance of *SPAST* mutation screening among Korean patients.

Conflicts of Interest

The authors have no financial conflicts of interest.

Acknowledgements

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Supplementary Table 1. Clinical data of HSP patients

Patient no.	Age/sex	AO	D	DS	FH	IP	HLL	SLL	WLL	VLL	E	U	HUL	SUL	WUL	Other
Pure HSP patients with SPG4 mutation (*n*=11)																
1	54/F	40	14	4	+	AD	+	+	-	-	+	-	+	-	-	None
2	20/M	19	1	1	+	AD	+	+	-	-	+	-	-	-	-	None
3	51/M	30	21	2	+	AD	+	+	-	-	+	-	-	-	-	None
4	32/F	5	27	4	+	AD	+	+	+	+	+	-	+	+	-	None
5	19/M	1	18	2	+	AD	+	+	+	-	+	-	+	-	-	None
6	36/M	13	23	3	+	AD	+	+	-	-	+	-	+	-	-	None
7	42/M	26	16	2	+	AD	+	+	-	-	+	+	-	-	-	None
8	33/M	25	8	5	+	AD	+	+	+	-	+	-	+	-	-	None
9	53/F	1	52	5	+	AD	+	+	-	-	+	-	-	-	-	None
10	33/M	27	6	3	+	AD	+	+	+	-	+	-	+	-	-	None
11	49/F	47	2	3	-		+	+	+	-	+	-	-	-	-	None
Pure HSP patients without SPG4 mutation (*n*=10)																
12	32/M	15	18	2	+	AD	+	+	-	+	+	-	+	-	-	None
13	25/F	5	20	2	+	AD	+	+	-	-	+	-	-	-	-	None
14	30/F	20	10	3	+	?	+	+	+	-	+	+	+	-	-	None
15	24/M	20	4	1	+	AD	+	+	+	-	+	-	-	-	-	None
16	51/M	31	20	2	+	AD	+	+	-	-	+	-	-	-	-	None
17	47/F	42	5	3	+	?	+	+	-	-	+	+	-	-	-	None
18	37/M	35	3	2	-		+	+	-	+	+	+	-	-	-	None
19	25/M	23	2	3	-		+	+	-	+	+	+	-	-	-	None
Complicated HSP patients without SPG4 mutation (*n*=8)																
20	28/M	20	8	5	+	AD	+	+	+	+	+	+	+	-	-	Dysarthria, peripheral neuropathy
21	14/F	12	2	3	+	?	+	+	-	-	+	-	+	-	-	Epilepsy, mental retardation
22	33/M	32	1	4	-		+	+	+	+	+	+	+	-	-	Cerebellar ataxia
23	20/M	20	1	3	-		+	+	+	-	-	-	-	-	-	Dysarthria, mental retardation
24	60/F	50	10	3	-		+	+	+	+	+	+	-	-	+	Dysarthria
25	64/F	59	5	3	-		+	+	-	-	+	+	+	-	-	Dysarthria
26	45/F	25	20	5	-		+	+	+	+	+	+	-	-	-	Saccadic pursuit, dysarthria, sensorimotor polyneuropathy
27	31/M	26	5	4	-		+	+	+	-	+	+	-	-	-	Dysarthria, mental retardation

+: present, -: absent, ?: uncertain inheritance pattern.
AO: age at onset, AD: autosomal dominant, D: duration, DS: disability stage (1=normal gait or mild stiffness in the legs, 2=moderate gait stiffness, 3=unable to run, but able to walk without support, 4=walk with support, 5=wheelchair-bound), E: extensor plantar reflexes, F: female, FH: family history, GEN: gaze evoked nystagmus, HLL: hyperreflexia in lower limbs, HUL: hyperreflexia in upper limbs, IP: inheritance pattern, LL: lower limbs, LT: light touch sense, M: male, PP: pinprick test, SLL: spasticity in lower limbs, SUL: spasticity in upper limbs, TP: temperature sense, U: urinary dysfunction, VLL: impaired vibration sense in lower limbs, WLL: weakness in lower limbs, WUL: weakness in upper limbs.