Short Communication

Four mutations of the *spastin* gene in Japanese families with spastic paraplegia

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Abstract

Hereditary spastic paraplegia (HSP) is a group of genetically heterogeneous neurodegenerative disorders characterized by slowly progressive spasticity and weakness of the lower limbs. HSP is caused by failure of development or selective degeneration of the corticospinal tracts, which contain the longest axons in humans. The most common form of HSP is caused by mutations of the *spastin* gene (*SPAST*), which is located on chromosome 2p21-p22 that encodes spastin, one of the ATPases associated with diverse cellular activities (AAA). In this study, we detected four causative mutations of *SPAST* among 14 unrelated patients with spastic paraplegia (SPG) patients. Two missense mutations (1447A→G, 1207C→G) and two deletion mutations (1465delT, 1475-1476delAA) were located in the AAA cassette region. Three of these four mutations were novel. Previous reports and our results suggest that the frequency of *SPAST* mutations is higher among Japanese patients with autosomal dominant hereditary spastic paraplegia (ADHSP), although *SPAST* mutations are also observed in patients with sporadic spastic paraplegia.

Keywords: spastic paraplegia; spastin; SPAST; hereditary spastic paraplegia

Introduction

Hereditary spastic paraplegia (HSP) is a group of clinically and genetically heterogeneous neurodegenerative disorders characterized by weakness, spasticity, and loss of vibration sensation in the lower limbs (Harding 1983). HSPs were conventionally classified on the basis of the pattern of inheritance, and by the presence (complicated HSP) or absence (pure HSP) of additional neurological features (e.g., mental retardation, epilepsy, optic atrophy, ataxia, amyotrophy, extrapyramidal

features, sensory neuropathy, and retinopathy). HSP can be inherited in an autosomal dominant (AD-HSP), autosomal recessive (AR-HSP), or x-linked recessive (X-HSP) fashion. Among these modes of inheritance, AD-HSP is the most common. To date, at least 29 genetic loci causing HSP have been identified, including 13 for AD-HSP, 13 for AR-HSP, and 3 for X-HSP (HUGO, http://www.gene.ucl.ac.uk/nomenclature). The majority of families with AD-HSP (40% or more) have mutations on chromosome 2p21-p22. Hazan *et al*. (1999) reported that the *spastin* gene (*SPAST*) occupies approximately 90 kb of genomic DNA and contains 17 putative exons, which encode a new member of the AAA (ATPases associated with diverse cellular activities) protein family named spastin. The open reading frame encodes 616 amino acids and there is marked similarity of the carboxy terminus (residues 342-599) with that of other AAA proteins. The members of this family are characterized by the possession of walker motifs A and B, a AAA minimal consensus sequence, a leucine zipper motif, and a helix –loop domain (Sauter *et al*. 2002). These proteins act as molecular chaperones and play an essential roles in many cellular activities, including cell cycle regulation, gene expression, and vesicle-mediated protein transport and protein degradation (Patel and Latterich 1998). More than 150 mutations of *SPAST* have been identified (Burger J *et al*. 2000; Fonknechten *et al*. 2000; Tang *et al*. 2004; Depienne *et al*. 2006), including point mutations (missense, nonsesse, and splice site) insertions, and deletions. It is noteworthy that *SPAST* mutations are more frequently associated with AD-HSP in Japanese patients (Namekawa *et al*.2001; Namekawa *et al*.2002; Yabe *et al*.2002; Fukunaga *et al*.2005; Iwanaga *et al*.2005).

Here we present clinical and genetic data on new Japanese families with AD-HSP. In a previous report, Yabe *et al*. (2002) showed that five out of 12 probands with AD-HSP (41.6%) had *SPAST* mutations. This time, we screened 14 unrelated

Japanese patients, including 8 with AD-HSP and 6 sporadic cases of unknown origin, to assess the frequency of SPG4. As a result, we identified four *SPAST* mutations, including 3 in patients with AD-HSP and 1 in a sporadic case. Three of these four mutations were novel.

Materials and Methods

The clinical and genetic features of the 14 unrelated patients with spastic paraplegia {mean age: 51.67±4.51 years (S.D.), range: 44-56 years} were evaluated by neurologists after informed consent was given. Hokkaido University Ethics Committee approved this study. The affected individuals were selected using the Harding (1983) criteria for definition of the clinical status, which include lower limb spasticity in the absence of any evidence of structural lesions or demyelination. AD inheritance was present in 8 cases and the remaining 6 cases had no family history. The procedure used for DNA sequence analysis has been described elsewhere (Yabe *et al* .2002). In addition, a large deletion mutation of *SPAST* that was previously found in a Japanese family (Iwanaga *et al*.2005) was analyzed by long - acting PCR (in a total volume of 20 µl) using an LA PCR^{TM} Kit ver2.1 (Takara, Japan; catalogue RR013A). With this Kit, it was possible to amplify large alleles up to about 48 Kbp. The PCR reaction mixture was initially denatured at 96°C for 2 min, followed by 30 cycles of denaturation at 94°C for 40 sec, annealing at 51°C for 40 sec, and extension at 72°C for 3 min. Final elongation was done at 72°C for 10 min, after which LA-PCR products were separated by 1% agarose gel electrophoresis (the size of the normal allele was 3 Kbp).

Results

Four different mutations of *SPAST* were identified in four of the 14 unrelated SPG patients (Table 1). There were summarizes the two missense mutations, including (1447A→G) in exon 11 and (1207C→G) in exon 7, as well as two deletion mutations in exon 11 (1465delT and 1475delAA). Three of these four mutations were novel. We detected these mutations in DNA of the affected individuals, but not in 50 normal controls (data not shown). No large deletion mutations were found in any of these patients. The clinical features of the four patients are summarized in Table 2. The initial symptom of all four patients was unsteadiness of the gait. Their cranial nerves and the muscle tone and power of the upper limbs were normal. Spinal MRI showed no abnormalities or atrophy of the spinal cord.

Case 1

A 56-year-old woman had developed gait unsteadiness from 8 years of age. At the age of 36 years, she noted difficulty with running. At the age of 50 years, she required aids for walking. Neurological examination revealed generalized hyperreflexia, bilateral sustained ankle clonus, diminished vibration sensation in the lower limbs, and a spastic gait. She was well oriented and had normal cognitive function without evidence of optic atrophy, nystagmus, or dysarthria. All of these neurological findings suggested that she had pure HSP. Her also had a spastic gait and was mentally retarded. Her father died of colon cancer at the age of 82 years. He did not have gait disturbance. Her mother is still alive at the age of 84 years old and does not have spastic paraparesis.

Case 2

A 52-year-old man first noted gait unsteadiness and difficulty in bending his legs at the age of 49 years, which had progressed over the last 3 years. Neurological examination revealed generalized hyperreflexia (particularly in the lower limbs) and a spastic gait. Other neurological findings (such as vibration sensation in the lower limbs) were

normal. His mother had developed gait disturbance at the age of 55 year, and required assistance at 75 years, while his brother (43 years old) also had similar neurological symptoms. The clinical phenotype of this family was pure HSP without complicating features such as optic atrophy or sensory impairment.

Case 3

A 52-year-old man presented to the Neurology Department with a history of unsteady gait and frequent falls for 3 years. Neurological examination revealed generalized hyperreflexia, lower limb spasticity, bilateral ankle clonus, and diminished vibration sensation in the lower limbs, but no extensor plantar reflex. Other family members had no neurological diseases. His father had died at the age of 86 years, and his mother had died at 68 years. Neither of them had gait disturbance or any other neurological problems.

Case 4

A 47-year-old woman had first noted weakness of the lower limbs and difficulty bending her legs from the age of 39 years. Currently, she has difficulty running and climbing stairs, but can walk unaided. Neurological examination revealed generalized hyperreflexia, bilateral spasticity, and diminished vibration sensation in the lower limbs. There was increased ankle clonus, but no extensor plantar reflex. Her father, an older sister, and one elder brother also has similar neurological problems, with the age at onset being 46, 48, and 49 years, respectively. Her cognitive function was normal and there was no evidence of optic atrophy.

Discussion

In this study, three new mutations of *SPAST* were detected. All of these new mutations affected the highly conserved coding region of the AAA cassette. The two novel

deletion mutations (1465delT and 1475-76delAA) led to frameshift and a premature termination codon, thus resulting in the production of a truncated protein. We also detected two missense mutations (1447A→G and 1207A→G) in the highly conserved AAA cassette coding region and one of these (1207A→G) was novel. The 1447A→G mutation results in the replacement of aspartic acid by glycine at position 441, while 1207C→G leads to replacement of proline by arginine at position 361. Mutations of the AAA cassette coding region may lead to loss of protein activity (Patel and Latterich 1998). Most of the *SPAST* missense mutations within the AAA domain of *SPAST* alter the ability of the protein to regulate interactions with its target and thus lead to constitutive binding to microtubules (Errico *et al*. 2002). Association of spastin with the microtubule cytoskeleton is mediated by the N-terminal region of this protein and is regulated through the ATPase activity of the AAA domain (Reid *et al*. 2003). It was recently demonstrated that spastin is a microtubule-severing enzyme and that several disease-associated mutations impair its enzymatic activity (Evans et at. 2005). Impairment of the fine regulation of the microtubule cytoskeleton in long axons by mutations of *SPAST* may underlie the pathogenesis of HSP. Unfortunately, we could not confirm cosegregation with the disease and obtain clinical information on other members of our families since we did not have their cooperation. However, these mutations were considered to be critical because they were located in the AAA cassette and could not be found in healthy controls.

In a previous study, no common mutations were identified. Therefore, allelic heterogeneity of *SPAST* was assumed to contribute to variability in the clinical phenotype of SPG patients, although some AD-HSP families had *SPAST* mutations linked to 2p (Fonknechten *et al*. 2000; Tang *et al*. 2004). Recently, Depienne *et al*. (2006) reported that genetic, epigenetic, and environmental factors could also influence

disease expression in carriers of sporadic *SPAST* mutations. In our study, we identified one patient (case 3) who had a sporadic *SPAST* mutation and no family history. In such circumstances, we provide genetic counseling to the affected individuals.

All of the patients in this series had pure spastic parpalegia without other neurological findings, reticular changes, cataract, or skin disease. Previous reports have described that *SPAST* mutations most often cause pure HSP, although there are exceptions (Tallaksen *et al*. 2001, Proukakis *et al*. 2003). However, it was recently demonstrated that patients with SPG4-linked AD-HSP and an average age of 67 years showed a mean 9-point decrease of the Cambridge cognitive examination (CAMCOG) score during a 3-year period (McMonagle *et al*. 2004). In contrast, there was no evidence of definite cognitive impairment or dementia in our HSP patients with *SPAST* mutations, even in the sporadic case.

We previously reported (Yabe *et al*. 2002) 5 Japanese families with *SPAST* mutations represented who 41.6 % (5/12) of the AD-HSP cases in that series. In addition, we found new *SPAST* mutations accounting for 37.5% (3/8) of our current AD-HSP cases. Only one patient (case 3) had the *SPAST* mutation associated with sporadic spastic paraplegia. The present findings suggest that structural *SPAST* mutations account for approximately 30%-50% of AD-HSP in Japanese families. Iwanaga *et al*. (2005) reported one patient who had a large deletion involving the 5'-UTR of *SPAST,* and we also investigated their large deletion mutation in our cases. However, we could not rule out all of large deletion mutations except this mutation because direct sequencing was not suitable to analyze them (Sauter *et al.* 2002) . Although the frequency of large deletion mutations of *SPAST* is unknown, it is possible that the frequency of *SPAST* mutations in Japanese patients with AD-HSP would be increased somewhat by detecting such mutations. We also found a *SPAST* mutation in a

sporadic elderly patient with moderate spasticity. Some of the mutations occurring in sporadic cases might have a lower penetrance than those identified in families (Depienne *et al*.2006). In addition, the variety of mutations in our cases indicates that each pedigree had a different founder.

In summary, we reported 4 *SPAST* mutations that caused AD-HSP in Japanese patients. Testing patients with spastic paraplegia for *SPAST* mutations, even in the absence of a positive family history, may provide more information about the functions of spastin protein.

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References

Burger J, Fonknechten N, Hoeltzenbein M, Neumann L, Bratanoff E, Hazan J, Reis A (2000) Hereditary spastic paraplegia caused by mutations in the SPG4 gene. Eur J Hum Genet 8: 771-776

Depienne C, Tallaksen C, Lephay JY, Bricka B, Poea-Guyon S, Fontatine B, Labauge P, Brice A, Durr A (2006) Spastin mutations are frequent in sporadic spastic paraparesis and their spectrum is different from the one observed in familial cases. J Med Genet 43: 259-265

Errico A, Ballabio A, Rugarli EI (2002) Spastin, the protein mutated in autosomal dominant hereditary spastic paraplegia, is involved in microtubule dynamics. Hum Mol Genet 11:153-163

Evans KJ, Gomes ER, Reisenweber SM, Gundersen GG, Lauring BP (2005) Linking axonal degeneration to microtubule remodeling by spastin-mediated microtubule severing. J Cell Biol 168:599-606

Fonknechten N, Mavel D, Byrne P, Davoine C, Cruaud CS, Boentsch D, Samson D, Coutinho P, Hutchinson M, McMonagle P, Burgunder JM, Tartaglione A, Heinzlef O, Feki I, Deufel T, Parfrey N, Brice A, Fontaine B, Prud'homme JF, Weissenbach J, Durr A, Hazan J (2000) Spectrum of SPG4 mutations in autosomal dominant spastic paraplegia. Hum Mol Genet 9:637–644

Fukunaga S, Ikezoe K, Ohyagi Y, Taniwaki K, Kira J, Morita M (2005) A case of sporadic SPG4 with leu 534 pro. Rinsho Shinkeigaku 45:618 (Japanese)

Harding AE (1983) Classification of the hereditary ataxias and paraplegias. Lancet 21:1151–1155

Hazan J, Fonknechten N, Mavel D, Paternotte C, Samson D, Artiguenave F, Davoine CS, Cruaud C, Durr A, Wincker P, Brottier P, Cattolico L, Barbe V, Burgunder JM, Prud'homme JF, Brice A, Fontaine B, Heilig R, Weissenbach J (1999) Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. Nat Genet 23:296-303

Iwanaga H, Tsujino A, Shirabe S, Eguchi H, Fukushima N, Niikawa N, Yoshiura K I, Eguchi K (2005) Large deletion involving the 5'-UTR in the spastin gene caused mild phenotype of autosomal dominant hereditary spastic paraplegia. Am J Med Genet 133A:13-17

McMonagle P, Byrne P, Hutchinson M (2004) Further evidence of dementia in SPG4-linked autosomal dominant hereditary spastic paraplegia. Neurology 62:407-410

Namekawa M, Takiyama Y, Sakoe K, Nagaki H, Shimazaki H, Yoshimura M, Ikeguchi K , Nakano I, Nishizawa M (2002) A Japanese SPG4 family with a novel missense mutation of the SPG4 gene: intrafamilial variability in age at onset and clinical severity. Acta Neurol Scand 106:387-391

Patel S, Latterich M (1998) The AAA team: related ATPase with diverse functions. Trends Cell Biol 8:65–71

Proukakis C, Auer-Grumbach M, Wagner K, Wilkinson PA, Reid E, Patton MA, Warner TT, Crosby AH (2003) Screening of patients with hereditary spastic paraplegia reveals seven novel mutations in the SPG4 (Spastin) gene. Hum Mutat 21(2):170-174

Reid E (2003) Science in motion: common molecular pathological themes emerge in the hereditary spastic paraplegias. J Med Genet 40:81-86

Sauter S, Miterski B, Klimpe S, Bonsch D, Schols L, Visbeck A, Papke T, Hopf HC, Engel W, Deufel T, Epplen JT, Neesen J (2002) Mutation analysis of the spastin gene (SPG4) in patients in Germany with autosomal dominant hereditary spastic paraplegia. Hum Mutat 20:127-132

Tallaksen CM, Durr A, Brice A (2001) Recent advances in hereditary spastic paraplegia. Curr Opin Neurol 14:457–463

Yabe I, Sasaki H, Tashiro K, Matsuura T, Takegami T, Satoh T (2002) Spastin gene mutation in Japanese with hereditary spastic paraplegia. J Med Genet 39:e46

Legends for tables

Table 1: *SPAST* mutations in our patients with spastic paraplegia

Table 2: Clinical characteristics of our SPG patients

Disability was scored as (1) Normal or very slight stiffness; (2) Moderate gait disturbance; (3) Unable to run, but able to walk; (4) Able to walk with assistance; (5) Wheelchair-bound; UL: upper limb, LL: lower limb (Fonknechten *et al*. 2000)

Table 1 *SPAST* mutations in our patients with spastic paraplegia

Patient no.	Exon location	Nucleotide change	Mutation type	Amino acid change
1.	Exon 11	1447A→G	Missense	D441G
2.	Exon 11	1465delT	Frameshift	-
3.	Exon 7	1207 C→G	Missense	P361R
4.	Exon 11	1475-1476 delAA	Frameshift	-

Table 2 Clinical characteristics of our SPG patients

Patient No.	Family history	Age	Sex	Age at onset	Disability	Clinical features				
						UL Hyperreflexia	LL Hyperreflexia	Ankle Clonus	Vibration sensation	Spasticity
1.	+	56	F	8	4	+	+	+	Mild	+
2.	+	52	M	49	2	+	+	-	Normal	+
3.	-	52	M	47	3	+	+	+	Moderate	+
4.	+	47	F	40	3	+	+	+	Mild	+