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*), located on chromosome 2p21-p22, which 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. 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 HSP, although *SPAST* mutations are also observed in patients with sporadic spastic paraplegia.

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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). Most 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 that encode spastin, a new member of the ATPases associated with the diverse cellular activities (AAA) protein family. The open reading frame encodes 616 amino acids, and there is marked similarity between the carboxy terminus (residues 342–599) of spastin and those 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). The proteins act as molecular chaperones and play 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 et al. 2000; Fonknechten et al. 2000; Tang et al. 2004; Depienne et al. 2006), including point mutations (missense, nonsense, 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 5 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 with sporadic cases of unknown origin, to assess the frequency of the *SPG4* gene. As a result, we identified four *SPAST* mutations, including three in patients with AD-HSP and one 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±SD age: 51.67±4.51 years, 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 clinical status, which include lower-limb spasticity in the absence of any evidence of structural lesions or demyelination. AD inheritance was present in eight cases and the

remaining six 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 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 s, annealing at 51°C for 40 s, 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 4 of the 14 unrelated SPG patients (Table 1). There were 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 starting at 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 sibling 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 and does not have spastic paraparesis.

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	48	3	+	+	+	Moderate	+
4	+	47	F	39	3	+	+	+	Mild	+

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)

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 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 years, and required assistance at 75 years, while his brother (48 years old) 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 had 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, 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 with glycine at position 441, while 1207C → G leads to replacement of proline with 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). Associa-

tion 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 al. 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 paraplegia 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, Proukakakis 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 in their Cambridge cognitive examination (CAMCOG) score over 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) five Japanese families with *SPAST* mutations who represented 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 other large deletion mutations other than this one 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 indicated that each pedigree had a different founder.

In summary, we reported four *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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