

ONLINE MUTATION REPORT

Spastin gene mutation in Japanese with hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) is a cluster of genetically heterogeneous disorders that has spastic paraplegia as the central feature.¹ Autosomal dominant HSP (AD-HSP) is also genetically heterogeneous and seven loci have been identified so far on chromosomes 14q (SPG3), 2p (SPG4), 15q (SPG6), 8q (SPG8), 12q (SPG10), 19q (SPG12), and 2q (SPG13).²⁻⁹ Among them, the SPG4 gene named *spastin* (GenBank accession No AJ246001) has recently been identified; it is composed of 17 exons and encodes a putative nuclear member of the AAA (ATPases associated with diverse cellular activities) protein family.¹⁰ In the original report, five different mutations were identified in seven families.¹⁰

HSP is a rare disorder in the Japanese population and the prevalence of SPG4 among Japanese AD-HSP patients is unknown.¹¹ Only one Japanese family has been reported with an insertion mutation in exon 8 of *spastin*.¹² In order to assess the frequency of *spastin* mutations in the Japanese, we analysed mutations in probands from 12 Japanese AD-HSP pedigrees.

SUBJECTS AND METHODS

All of the subjects were neurologically evaluated by the present authors. The diagnosis was based on the neurological findings, a progressive course with an insidious onset, and a positive family history indicating dominant inheritance. Neuroimaging studies were done to exclude other causes of spastic paraplegia. Among the subjects, one was a member of an HSP family that was previously reported to have 2p linked AD-HSP (maximum lod score 3.53).¹³

All patients had progressive spasticity in the lower limbs bilaterally and their reflexes were hyperactive. All the patients showed an extensor plantar response. None of them showed extrapyramidal signs, ophthalmoplegia, retinal degeneration, skeletal muscle atrophy, or intellectual disturbance. Superficial sensation was intact, although some patients showed a slight reduction of vibration sensation. The mean age at onset was 35.3 years (SD 15.6, n=12), the range being 10-57 years. Spinal MRI showed no abnormalities or atrophy of the spinal cord.

After obtaining informed consent, high molecular weight genomic DNA was extracted from white blood cells or from lymphoblastoid cell lines. According to the method of Hazan *et al*,¹⁰ the 17 coding exons of the *spastin* gene were amplified by the polymerase chain reaction (PCR) using the original primer pairs, except those for exon 14 (http://www.genoscope.cns.fr/externe/English/Projets/Projet_U/primers.html). Since exon 14 was not amplified effectively using the reported pair of primers, we used two other primers (forward primer: 5'-TAACAGCACAAAGACCCTGTC and reverse primer: 5'-GCTGTAAGATAAACCAATCCA). The 5' upstream 4.8 kb region from the initiator ATG codon and the 3' non-coding region from the stop codon to the polyadenylation site were also amplified using six pairs of primers designed from clone D and three pairs of primers from clone G (http://www.genoscope.cns.fr/externe/English/Projets/Projet_U/status.html).

Table 1 SPG4 mutations in 12 Japanese probands with AD-HSP

Patient No	Location	Mutation	Amino acid change	Effect
98-175	Exon 13	1620C→T	R499C	Missense
99-15	Intron 14	1742-1g→t	?	Splicing mutation
99-33	Exon 7	1164C→A	Q347K	Missense
99-116	Exon 8	1288A→G	K388R	Missense
99-175	Intron 9	1370+1g→t	?	Splicing mutation

Amplified PCR products were purified with Microcon® (Amicon Inc, Beverly, MA, USA) and were sequenced directly using an ABI PRISM 377 DNA sequencing system (Applied Biosystems, Tokyo, Japan).

RESULTS

Five of the 12 AD-HSP probands were found to have *spastin* gene mutations (table 1). The five mutations responsible for HSP included three missense mutations (R499C, Q347K, and K388R) and two splice site mutations (1370+1g-t and 1742-1g-t). The proband of the 2p linked family had no mutations in any of the exons or splice junction sites, 5' upstream 4.8 kb region from the initiator ATG codon or the 3' non-coding region from the stop codon to the polyadenylation site. In the subjects with *spastin* mutation, the mean age at onset was 31.8 years (SD 18.1, n=5), the range being 10-54 years. There was no significant difference in the mean age at onset between the probands with and without *spastin* mutation by Student's *t* test (p=0.53).

DISCUSSION

The present study showed that five out of 12 probands with AD-HSP (41.6%) had *spastin* gene mutations. In the remaining AD-HSP probands (58.4%), such mutations were not detected. This is the first report on the frequency of *spastin* gene mutation among Japanese patients with AD-HSP. Three out of five mutations, including Q347K and the two splice site mutations, were novel mutations.^{10 14-20} Each of these novel mutations was segregating with affected members in each family (data not shown). The variety of mutations in our subjects indicates that each pedigree had a different founder.

Recently, Fonknechten *et al*¹⁴ analysed 17 exons of the *spastin* gene in 87 AD-HSP probands from around the world, but mainly from Europe. They reported that SPG4 accounted for 37% of all AD-HSP. Our estimation of the prevalence of SPG4 in Japanese AD-HSP patients is compatible with their finding.

As in previous reports, the three missense mutations that we found were localised within the functional domain of *spastin* (the AAA cassette) and were therefore considered to interfere with the functioning of *spastin*. Two splice site mutations were also detected in the present study. Fonknechten *et al*¹⁴ suggested that the frequency of *spastin* splice site mutations

was significantly higher than in other human genetic disorders. These mutations are considered to be deleterious since the result is unstable aberrant transcripts that may lead to a deficiency of spastin.¹⁴⁻²⁰

In the present study, we could not detect any specific mutation of the *spastin* gene in the proband of a 2p linked family. Svenson *et al*²⁰ and Fonknechten *et al*¹⁴ also described similar cases where no mutation was detected in some 2p linked families. These findings may indicate that these AD-HSP patients have mutations in other regions of the *spastin* gene, for example, introns, or have mutations of other genes located nearby. Recently, Higgins *et al*²¹ reported that spastic paraplegia could be caused by a 4 base deletion (del TAAT) near the splice junction in intron 3 of the *spastin* gene, but this mutation was not detected in our subjects. Further studies are needed to clarify the molecular mechanisms underlying SPG4.

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