Mutation Analysis of SPG4 and SPG3A Genes and Its Implication in Molecular Diagnosis of Korean Patients With Hereditary Spastic Paraplegia

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Background: Hereditary spastic paraplegia (HSP), a genetically and clinically heterogeneous group of neurodegenerative disorders, is characterized by progressive lower limb weakness and spasticity. Among the 8 loci associated with the autosomal dominant uncomplicated HSP (AD-HSP), the spastin (SPG4) and atlastin (SPG3A) genes have been known to account for approximately 40% and 10% of all cases, respectively.

Objective: To investigate the contribution of these 2 genes in the occurrence of HSP in Korean patients.

Design: Clinical and genetic study.

Setting: Tertiary care center.

Patients: Eighteen patients with uncomplicated HSP (11 AD and 7 sporadic) underwent screening for gene mutation.

Main Outcome Measures: Mutations in the SPG4 and SPG3A genes as detected by direct sequencing of all coding exons and flanking intronic sequences.

Results: We identified 8 different SPG4 mutations, 7 of which have not been reported elsewhere. Among the detected mutations were 3 missense mutations, 2 in-frame deletions, 2 frameshift mutations, and 1 splice-site mutation. No mutation was found in the SPG3A gene.

Conclusion: Compared with previous studies, a higher frequency of SPG4 gene mutations in AD-HSP (7/11; 64%) was observed, suggesting that a mutation analysis for the SPG4 gene might be helpful for molecular diagnosis of AD-HSP in Korean patients.

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Eighteen patients with HSP from a tertiary care center were included in the present study. All patients were neurologically and genetically evaluated after giving informed consent. Genomic DNA was extracted from peripheral blood leukocytes using a Wizard Genomic DNA Purification kit following the manufacturer's instructions (Promega Corporation, Madison, Wis). All coding exons and flanking introns of the SPG3A and SPG4 genes were amplified by polymerase chain reaction using primers designed by the authors (available on request). Sequencing was performed with a BigDye Terminator Cycle Sequencing Ready Reaction kit, version 2.0 (Applied Biosystems). Mutations detected were analyzed by direct sequencing of the corresponding exons. Mutations were confirmed by sequencing of 100 control chromosomes.

**METHODS**

**CLINICAL FINDINGS**

Among the 18 probands, the disease was inherited as AD in 11 and was sporadic in 7 (Table 1). The first presenting symptoms in the patients were weakness and/or spasticity; 12 of 18 patients had weakness and spasticity, 1 patient had only weakness, and 5 patients had only spasticity. The age at onset of symptoms showed a wide range, from 1 to 59 years (mean ± SD, 34.6 ± 15.8 years). All patients were diagnosed as having uncomplicated HSP characterized by spasticity and hyperreflexia of the lower limbs. Nine patients had accompanying bladder function disturbances, and 4 patients showed decreased vibratory sense in the lower extremities without any other sensory impairment. Most patients, except for 2 who displayed hyperreflexia of the arm, exhibited no upper extremity symptoms. The disability status of the patients also showed a considerable variability. The disability staging could not be assessed in 2 children who had not started walking (patients 3 and 5).

**MUTATION ANALYSIS**

Eight different mutations of the SPG4 gene, including 3 missense, 2 in-frame deletion, 2 frameshift deletion, and 1 splicing mutation, were detected (Table 2). Except for 1 splice-site mutation (c.1413 + 3delAAGT), all were novel mutations not described previously. None of the novel variants were observed in 100 control chromosomes by direct sequencing of the corresponding exons. Seven patients with mutations had 1 or more affected members in the family, suggesting an AD inheritance pattern, and 1 patient had no family history of HSP (patient 7 in Table 1 and Table 2). All mutations detected were heterozygous and located in the highly conserved AAA cassette-encoding region of the SPG4 gene. However, no mutation was found in the SPG3A gene.

**COMMENT**

Molecular genetic analysis of the SPG4 and SPG3A genes in 18 unrelated Korean patients with uncomplicated HSP revealed 8 different mutations in the SPG4 gene and no mutation in the SPG3A gene. Of the 11 patients with a familial background of AD inheritance, 7 had SPG4 mutations (64%), which is higher than in previous reports (Table 3). Possible explanations for the higher rate of

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Table 1. Clinical Findings of 18 Korean Patients With Uncomplicated Hereditary Spastic Paraplegia

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age at Examination, y</th>
<th>Age at Onset, y</th>
<th>Family History</th>
<th>Lower Extremity</th>
<th>Sensory Impairment</th>
<th>Upper Extremity</th>
<th>Sensory Impairment</th>
<th>Weakness</th>
<th>Babinski Sign</th>
<th>Disability Stage</th>
<th>SPG4 Mutation</th>
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<td>-</td>
<td>+</td>
<td>2</td>
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<td>-</td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not assessed; –, negative; +, positive.

*Disability stage: 1, normal gait or very slight stiffness in the legs; 2, moderate gait stiffness; 3, unable to run but able to walk alone; 4, walk with help; and 5, wheelchair bound.

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SPG4 mutation detection in our series include (1) the small number of cases in the present study; (2) different methods of mutation detection: direct sequencing analysis in the present study vs mutation screening in the previous reports; and (3) an ethnic difference in the genetic background of AD-HSP.

Among 7 patients without any family history (sporadic cases), the mutation detection rate was low (1/7; 14%), in line with previous studies.22,23 Nevertheless, mutation analysis of the SPG4 gene in patients with sporadic spastic paraplegia is of significance in the diagnosis and genetic counseling of patients and family members because identification of the mutation can confirm the diagnosis of spastic paraplegia, and patients with sporadic spastic paraplegia with an SPG4 mutation have the same chance of passing the mutation to their offspring as patients with AD-HSP.

The mutations identified in our patients include 3 missense mutations, 2 in-frame deletions, 2 frameshift deletions, and 1 splice-site mutation. The 1 splice-site mutation (c.1413 + 3delAAGT) was previously reported, and the remaining 7 mutations have not been described previously.22 The I344K mutation in exon 7 was the first novel mutation reported in a Korean patient with HSP by the authors and now appears in the Human Gene Mutation Database.21 As for the remaining 2 missense mutations (D584V and R499H), different amino acid changes in the same codons (D584H and R499C) have been reported.7,14,24 The DNA samples from family members were available in 4 of the 7 families with SPG4 mutations. Each of these 4 families showed cosegregation of the phenotype and mutation identified. Although most reports of SPG4 mutations show a significant proportion of splice-site mutations (30%-50%), our study revealed only 1 splice-site mutation (12.5%), with missense mutation (37.5%) being the most frequent type.14,24 Since we used primers that covered approximately 60 base pairs of the flanking intronic regions, mutations in the splice sites could not be missed. The exact function of the spastin protein is still not known; however, levels of spastin messenger RNA in patients with SPG4 mutations were reported to be uniformly reduced, suggesting a loss of protein function by inactivation of the AAA domain.7,9,11,14

Among the common features of uncomplicated HSP, 73% of patients have hyperreflexia in the upper limbs; 38% to 59% may be affected by urinary frequency, urgency, or hesitancy; and 58% to 68% may be affected by sensory impairment such as abnormal vibration sensa-
The upper extremity functions were relatively well preserved in our cases, and 4 patients showed decreased vibratory sense in the lower extremities as the sole sensory function impairment. On the other hand, 9 (50%) of 18 patients had urinary function disturbances.

Although approximately 10% of uncomplicated AD-HSP is known to be caused by mutations in the SPG3A gene, none of our patients had SPG3A gene mutations. This might be attributable to a small number of patients with AD-HSP who did not have the SPG4 mutation in the present study or to an ethnic difference. It is necessary to recruit more patients with AD-HSP to investigate the contribution of SPG3A mutations in AD-HSP in Korea.

Among the 4 patients with AD-HSP, family samples were available for linkage analysis in only 1 patient, and we could exclude linkage to the SPG4 or SPG3A locus in this family. Linkage analysis was performed with 4 microsatellite markers (D2S165, D2S35, D2S367, and D2S2163) for the SPG4 locus and 2 microsatellite markers (D14S288 and D14S276) and 1 single nucleotide polymorphism (S179Y) for the SPG3A locus.

In conclusion, to our knowledge, this is the first report on SPG4 and SPG3A mutations in a series of Korean patients with uncomplicated HSP, and we identified 8 different mutations in the SPG4 gene, including 7 novel mutations. The mutations detected were unique to each patient, in line with previous studies that show that most families carry a unique mutation. Our observation of the higher rate of mutation detection in the AD cases needs to be confirmed in a larger series. Nevertheless, this observation indicates that a molecular genetic test for SPG4 is promising in mutation detection. Finally, the clinical findings described in the present study may be useful in elucidating clinical variations associated with different mutations in the future.

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REFERENCES


