Three Novel Mutations of the Spastin Gene in Chinese Patients With Hereditary Spastic Paraplegia

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Background: Hereditary spastic paraplegia is a group of genetically heterogeneous neurodegenerative disorders characterized by progressive spasticity of the lower limbs. The most common form of hereditary spastic paraplegia is caused by mutations in the spastin gene (SPG4), which encodes spastin, an adenosine triphosphatase associated with various cellular activities.

Objective: To investigate the Chinese patients with hereditary spastic paraplegia for mutations in SPG4.

Methods: DNA samples from 31 unrelated patients were analyzed for mutations in SPG4 by single-strand conformation polymorphism analysis and direct sequencing. All DNA samples were screened for mutations by the polymerase chain reaction, followed by electrophoresis and silver staining. Each new variant identified was analyzed in 50 control subjects to determine whether it is a polymorphism or a mutation.

Results: Three novel mutations were detected in 4 affected individuals, including 2 missense mutations (T1258A and A1293G) and 1 deletion mutation (1668-1670delCTA).

Conclusions: To our knowledge, this is the first report of SPG4 mutations in the People’s Republic of China. The percentage of involved Chinese families with autosomal dominant hereditary spastic paraplegia with an SPG4 mutation is 18% (4/22), lower than the estimated 40% linked to this locus.

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HEREDITARY SPASTIC paraplegia (HSP) comprises a group of inherited neurodegenerative disorders with the shared characteristics of slowly progressive spasticity and weakness of the lower limbs. Conventionally, they are divided into pure or complicated forms depending on whether the paraparesis exists in isolation or with other major clinical features, such as dementia, mental retardation, epilepsy, extrapyramidal disturbances, ataxia, deafness, retinopathy, optic neuropathy, peripheral neuropathy, and skin lesions. Hereditary spastic paraplegia can be inherited in an autosomal dominant (AD), autosomal recessive, or X-linked manner.

To date, 19 loci (SPG1-SPG17, SPG19, and SPG20) that play a role in the development of HSP have been mapped. Eight genes for these loci have been identified. The 4 dominant forms, at SPG3A, SPG4, SPG10, and SPG13, are the results of mutations in the genes that encode atlastin, spastin, kinesin heavy chain, and heat shock protein 60, respectively. The 2 recessive forms, at SPG7 and SPG20, are caused by mutations in the genes that encode paraplegin and spartin, respectively. The 2 X-linked forms, at SPG1 and SPG2, are the results of mutations in the genes that encode the L1 cell adhesion molecule and the proteolipid protein, respectively.

Forty percent of all AD families are linked to the AD locus (SPG4). The defective gene for SPG4 encodes a 616-amino acid protein named spastin. Spastin is an adenosine triphosphatase associated with various cellular activities (AA) protein, with characteristic AAA cassettes in the C-terminal from amino acid 342 to amino acid 599. According to computer predictions, spastin possesses a nuclear localization signal, Walker motif A and B, an AAA minimal consensus sequence, leucine zipper motifs, and a helix-loop-helix domain. It is thought to act as a molecular chaperone and to play essential roles in many cellular
activities, including cell cycle regulation, gene expression, vesicle-mediated protein transport, and protein degeneration. Recent evidence indicates that spastin is distributed within the cytoplasm and interacts with microtubules. However, the function of spastin remains unclear.

There have been 124 different SPG4 mutations reported in North America, Europe, Japan, and Korea. To our knowledge, research on spastin mutations in Chinese patients with HSP has not been reported previously. To investigate the Chinese patients with HSP for mutations in SPG4, we analyzed SPG4 mutations in 31 apparently unrelated Chinese kindreds with HSP.

RESULTS

CLINICAL DATA

Of the 31 kindreds, 22 inherited as AD traits and 9 as sporadic. Patients in most cases presented first with hyperreflexia and spasticity of the lower limbs. The age at onset of symptoms showed a wide range, from 2 to 51 years. In the 31 probands, 22 were pure HSP and 9 were complicated HSP (characterized by spasticity and weakness of the lower limbs combined with cognitive deficit, ataxia, muscular atrophy, or ichthyosis) (Table 1).

Clinical features of the 4 AD families with SPG4 mutations are summarized in Table 2. Cranial nerves and power in the upper limbs were normal in all. In the lower limbs, spasticity in all was far more severe than any weakness present. There were only pure HSP patients in families H209 and H981, while pure and complicated HSP patients were in families H216 and H228. The age of onset in families H209 and H216 was much later than in the other 2 families. The mean±SD age of onset in family H209 was 34.60±6.69 years; in family H216, 34.50±7.78 years; in family H228, 4.50±5.00 years; and in family H981, 17.00±2.83 years. The mean±SD duration of the disease in family H209 was 15.80±10.64 years; in family H216, 13.50±9.19 years; in family H228, 25.25±18.59 years; and in family H981, 29.50±14.85 years. The son of the proband in family H981, aged 6 years, who was a mutation carrier, was asymptomatic and might be a presymptomatic patient.

MUTATION DETECTION

We detected 3 novel SPG4 mutations in 4 unrelated AD HSP families, including 2 missense mutations (T1258A and A1293G) and 1 deletion mutation (1668-1670delCTA) (Table 3 and Figures 1, 2, and 3). The mutation T1258A presented in 2 families simultaneously. All detected mutations were heterozygous and located in the highly conserved AAA cassette-encoding region of SPG4.

The mutation T1258A in exon 8 led to a replacement of leucine (a hydrophobic amino acid) with glutamine (a polar and hydrophilic amino acid) at position 378. The mutation A1293G in exon 8 replaced methionine (a polar and hydrophilic amino acid) at position 390 with valine (a hydrophobic amino acid). The 2 missense mutations occur close to Walker motif A, a conserved adenosine triphosphatase domain in the AAA cassette. The deletion mutation (1668-1670delCTA) in exon 14 resulted in a leucine deletion at position 515, which occurs in a leucine zipper motif. This is the second example of the deletion of an amino acid in spastin. All variants were analyzed in 100 control chromosomes, and were not detected by single-strand conformation polymorphism analysis.

STATISTICAL ANALYSIS

There was no significant difference between the average age at onset of all the probands with and without SPG4 mutations (F=0.001, P=.98).

METHODS

PATIENTS

Thirty one unrelated Chinese kindreds with HSP were involved in this study. All probands were examined by a neurologist (B.T., G.Z., W.L., L.S., X.Z., or H.J.) and selected based on the Harding criterion for the definition of clinical status, which was lower limb spasticity in the absence of any evidence of a structural lesion or demyelination. Disability was assessed on a 5-point scale: 1, normal or very slight stiffness in the legs; 2, moderate gait stiffness; 3, unable to run but able to walk alone; 4, able to walk with help; and 5, wheelchair bound. All patients were of Han nationality, from Hunan, Shandong, Heilongjiang, Zhejiang, and Guizhou in the People's Republic of China. Blood specimens were obtained from family members and control subjects after informed consent. Genomic DNA from peripheral blood leukocytes or lymphoblast lines was obtained by standard extraction methods.

MUTATION ANALYSIS

Single-Strand Conformation Polymorphism Analysis

According to the methods of Hentati et al. the 17 coding exons of SPG4 (GenBank accession AJ246003) were amplified by the polymerase chain reaction using the original primer pairs. The reaction was performed in thermocyclers (Perkin-Elmer, Inc, Foster City, Calif), starting with an initial denaturation of 3 minutes at 94°C, followed by 30 cycles of 30 seconds' denaturation, 30 seconds' annealing at the primer-specific temperature, and 30 seconds' extension at 72°C. Electrophoresis of the polymerase chain reaction products was performed on 8% polyacrylamide (29:1 ratio of acrylamide-bisacrylamide) gels in 0.5% Tris acetate buffer (pH 8.3) at 200 V for 30 seconds. The polymorphism analysis was performed on 8% polyacrylamide (29:1 ratio of acrylamide-bisacylamide) gels in 0.5% Tris borate EDTA, followed by silver staining. Samples showing altered migration patterns were selected for further analysis.

Sequencing

Abnormally migrating fragments were gel purified and automatically sequenced on a sequencer (model ABI 377; Applied Biosystems, Shanghai, Hunan).

Variants identified by sequencing were analyzed for cosegregation in family members. All newly identified disease-associated variants were examined for presence in 50 controls (100 chromosomes) by single-strand conformation polymorphism analysis.

STATISTICAL ANALYSIS

Comparison of means was performed using a 2-tailed t test and an analysis of variance test.
Table 1. Clinical Information for the 31 Probands*

<table>
<thead>
<tr>
<th>Proband</th>
<th>AAO, y</th>
<th>Inheritance</th>
<th>Phenotype</th>
<th>DS‡</th>
<th>BD</th>
<th>TC</th>
<th>ULH</th>
<th>Other Comments</th>
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<td>−</td>
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<td>0090</td>
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<td>P</td>
<td>3</td>
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<td>AD</td>
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<tr>
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Abbreviations: AAO, age at onset; AD, autosomal dominant; BD, bladder disturbance; C, complicated; DS, disability stage; P, pure; S, sporadic; TC, talipes cavus; ULH, upper limb hyperreflexia; −, absent; +, present.
*All probands experienced lower limb hyperreflexia.
†Data are given as number of DNA samples.
‡Stages are as follows: 1, normal or very slight stiffness in the legs; 2, moderate gait stiffness; 3, unable to run but able to walk alone; 4, able to walk with help; and 5, wheelchair bound.14
§Probands with spastin gene mutations.

Table 2. Clinical Information for the Patients With Spastin Gene Mutations*

<table>
<thead>
<tr>
<th>Family</th>
<th>Member</th>
<th>AAO, y</th>
<th>Duration, y</th>
<th>DS‡</th>
<th>BD</th>
<th>TC</th>
<th>DV</th>
<th>ULH</th>
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<td>+</td>
<td>−</td>
<td>+</td>
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</tr>
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<td></td>
<td>II:1</td>
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<td>1</td>
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<td>+</td>
<td>+</td>
<td>−</td>
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<td>IV:1</td>
<td>29</td>
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<td>+</td>
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<td>4</td>
<td>+</td>
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<tr>
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Abbreviations: AAO, age at onset; BD, bladder disturbance; DS, disability stage; DV, decreased vibration sense; TC, talipes cavus; ULH, upper limb hyperreflexia; −, absent; +, present.
*All patients experienced lower limb hyperreflexia.
†Stages are explained in the fourth footnote to Table 1.
‡Relevant clinical data for the other 3 affected members were unavailable.
Three mutations of SPG4 were detected in 22 apparently unrelated Chinese AD HSP kindreds; to our knowledge, none have been described previously. In this study, SPG4 mutations accounted for 18% (4/22) of the mutations in AD HSP families, which is lower than the estimated 40% linked to this locus. The lower percentage may be correlated with racial differentiation and dissimilar genetic backgrounds because our methods are similar to those used in other SPG4 mutation analyses. No mutation (0/9) was detected in sporadic cases. Some sporadic cases could be de novo mutations of AD HSP, which would decrease the percentage of our families linked to SPG4. To our knowledge, this is the first report of SPG4 mutations in Chinese patients with HSP.

The 124 different SPG4 mutations reported previously are scattered along the coding region of the gene, except for exon 4, and include all types of DNA alterations, including 33 missense mutations (26.6%), 16 nonsense mutations (12.9%), 37 splice site mutations (29.8%), 10 insertions (8.1%), and 28 deletions (22.6%).4,12-29 The frequency of spastin splice site mutations was significantly higher than in patients with other human genetic disorders.14,15,21-23,29 A schematic diagram of SPG4 complementary DNA, including the position of the mutations identified in this study and in previous studies, is shown in Figure 4. The 3 novel mutations detected in the study and most of the previously reported mutations affected the AAA cassette of the spastin protein, which suggests this region is crucial for its function. All missense mutations, except 3, occur in the AAA cassette and could impair the spastin function through either a dominant negative effect11 or a loss of function. The other types of mutations located inside or outside the AAA cassette-encoding region directly result in a stop codon or cause a frameshift with the subsequent introduction of a premature termination codon, which could lead to the production of a

Table 3. Mutations Detected in the Spastin Gene

<table>
<thead>
<tr>
<th>Family</th>
<th>Exon</th>
<th>Location*</th>
<th>Mutation</th>
<th>Protein Change†</th>
<th>Consequence</th>
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<td>T1258A</td>
<td>Leu378Gln</td>
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<td>1668-1670delCTA</td>
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*Numbers refer to the spastin gene complementary DNA sequence. †Numbers refer to the spastin peptide sequence.

Figure 1. A, Family H209. The proband is indicated (arrow). B, Single-strand conformation polymorphism analysis of exon 8 of the spastin gene in family H209 members. The abnormal migration alteration is indicated (arrow). C, Family H216. The proband is indicated (arrow). D, Single-strand conformation polymorphism analysis of exon 8 of the spastin gene in family H216 members. The abnormal migration alteration is indicated (arrow). E, Wild-type sequence. F, Sequence analysis identified a 1258T→A mutation in exon 8 of the spastin gene in families H209 and H216. The novel mutation is indicated (arrow). Squares indicate males; circles, females; shaded symbols, individuals with hereditary spastic paraplegia (HSP); unshaded symbols, individuals without HSP; symbols with a slash mark, deceased individuals; and N, healthy control subject.
truncated protein lacking the adenosine triphosphatase domain. However, Charvin et al31 did not find a truncated protein when they performed protein analysis of lymphoblastoid cell lines of HSP patients carrying either nonsense or frameshift spastin mutations. Spastin transcript analysis provided strong evidence that transcripts were unstable in vivo, resulting in the absence or marked reduction of mutated spastin protein. These data strongly suggest that a dosage effect underlies the molecular mechanism.31

From all those previously described mutations, we find that most patients had unique SPG4 mutations, ie, recurring mutations are uncommon.3,14-16,20,23,24 We found a recurring mutation of T1258A in 2 families, suggesting that the mutation may have a higher frequency in the Chinese population, which could be helpful for future SPG4 diagnostic testing in the population. But the 2 kindreds came from the Hunan province, so we cannot exclude the possibility that a founder effect exists.

Figure 2. A, Family H228. The proband is indicated (arrow). B, Single-strand conformation polymorphism analysis of exon 8 of the spastin gene in family H228 members. The abnormal migration alteration is indicated (arrows). C, Wild-type sequence. D, Sequence analysis identified a 1293A→G mutation in exon 8 of the spastin gene. The novel mutation is indicated (arrow). Pedigree symbols are explained in the legend to Figure 1.

Figure 3. A, Family H981. The proband is indicated (arrow). B, Single-strand conformation polymorphism analysis of exon 14 of the spastin gene in family H981 members. The abnormal migration alteration is indicated (arrows). C, Wild-type sequence. D, Sequence analysis identified a 1668-1670delCTA mutation in exon 14 of the spastin gene. The novel mutation is indicated (arrow). Pedigree symbols are explained in the legend to Figure 1.
The number of different mutations found, together with the low yield of mutations in the small families and sporadic cases, suggests that mutation detection in spastin may not always by itself be a logical way forward for molecular diagnosis in patients with HSP. Future diagnosis of SPG4 mutations would benefit if we find some featured phenotypes associated with SPG4. Unfortunately, most patients in this study had a pure HSP phenotype with remarkable variation in age at onset and severity of the disease. For example, comparison of average age at onset between all the probands with and without SPG4 mutations did not reveal a significant difference ($P = .98$). Even in the same family, patients had phenotype variation, particularly age at onset.

In summary, 3 novel mutations we detected widened the SPG4 mutation spectrum, and we concluded that the percentage of SPG4 mutations in Chinese AD HSP patients is lower than estimated. The molecular mechanism underlying SPG4 should be further clarified, which would be helpful for the future treatment of HSP.

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