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Clinical and genetic analysis of four Taiwanese families with autosomal dominant hereditary spastic paraplegia

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Background/Purpose: Hereditary spastic paraplegias (HSPs) are clinically and genetically heterogeneous neurodegenerative disorders. Defects in the SPG4 and SPG3A genes are the two leading causes of HSPs with autosomal dominant inheritance (AD-HSPs). The purpose of this study was to investigate the clinical features and associated genetic mutations in Taiwanese families with AD-HSP.
Methods: Four kindreds with AD-HSP were recruited, and clinical data were collected from the affected individuals. Genetic studies were conducted in the following order: sequence analysis of the SPG4 gene (SPAST) exons, multiplex ligation-dependent probe amplification to detect genetic rearrangements in SPAST, and sequence analysis of the SPG3A gene exons.
Results: Four different SPAST mutations were detected, including a novel small deletion, a missense mutation, and two gross deletions involving exon 17. Although all symptomatic cases manifested as uncomplicated phenotypes, considerable intrakindred and inter-kindred variations in terms of age at onset, rate of progression, and severity of disease were observed.

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Conclusion: Mutation patterns and phenotypic expressivity are heterogeneous in Taiwanese patients with *SPG4*-related HSP. Genetic rearrangements could be a significant cause of *SPG4*-related HSP in the Taiwanese population. Assessment of the large deletions that could present in *SPAST* is warranted when direct sequencing is uninformative.

Introduction

Hereditary spastic paraplegias (HSPs) are a heterogeneous group of neurodegenerative disorders that are characterized by progressive spasticity and weakness of the lower limbs.[1] Genetically, all modes of inheritance, including autosomal dominant, autosomal recessive, and X-linked, have been characterized as HSPs. Autosomal dominant HSPs (AD-HSPs) represent around 70% of cases of uncomplicated HSPs (also referred to as "pure HSPs" when spastic paraplegia is the sole manifestation).[2] For all AD-HSP families, mutations in *SPG4* and *SPG3A* are the two most common causes, accounting for around 40% and 10% of pedigrees, respectively.

Several clinical and genetic studies on *SPG3A*- and *SPG4*-related HSP have been reported in the Han Chinese population.[3–5] In the present study, we investigated the clinical features and performed genetic analysis on the *SPG4* and *SPG3A* genes in Taiwanese AD-HSP patients. To

the best of our knowledge, this is the first report on genetic mutations in Taiwanese families with AD-HSP.

Methods and materials

Four unrelated ethnic Han Taiwanese kindreds with AD-HSP were included in this study (Fig. 1). The diagnosis of AD-HSP is based on the following diagnostic criteria: (1) pure spastic paraplegia; (2) spastic tetraparesis with earlier and more severe effects to lower limbs; (3) spastic paraplegia as an early and prominent sign of a degenerative disease affecting the nervous system; (4) positive family history of spastic gait disturbance with affected members in at least two generations; and (5) exclusion of other causes that could explain the presenting symptoms.[2] Clinical information and family histories were collected and neurological examinations were performed by a neurologist according to a standard protocol. The study protocol was approved by
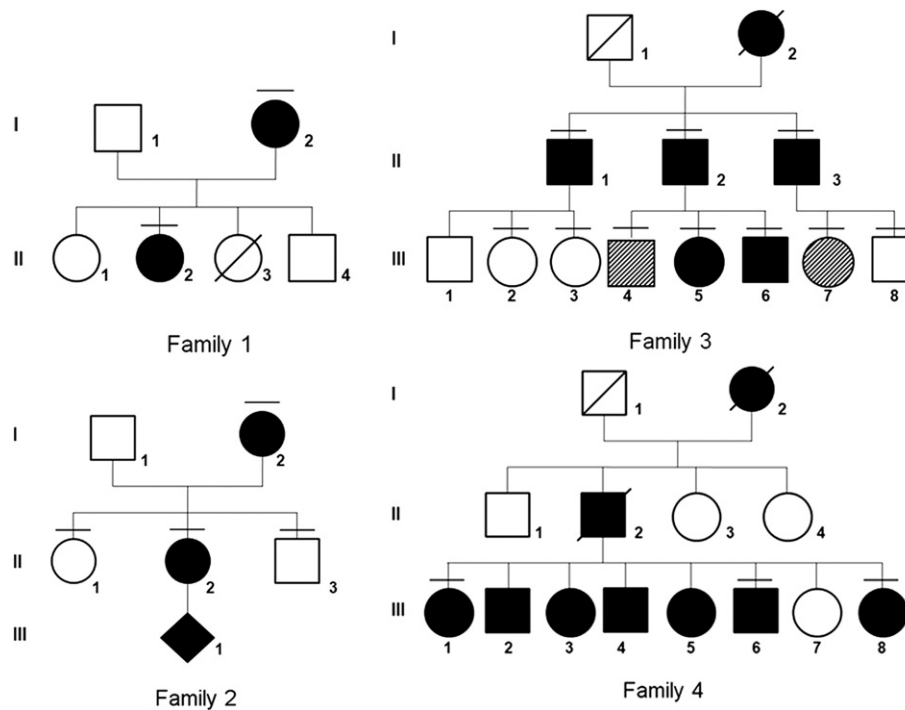

Figure 1 Pedigrees of four families with hereditary spastic paraplegia. The circles represent female subjects, the squares represent male subjects, and the diamond represents the patient whose gender was withheld for confidentiality reasons. The filled symbols indicate affected individuals with a detected mutation. The hatched symbols denote asymptomatic mutation carriers. The cases that were involved in the clinical and genetic studies are indicated with a cross bar above the individual symbol. The phenotypes of the cases that were not examined are defined according to the information provided by other family members.

the Institutional Review Board of Chang Gung Memorial Hospital (No. 99-2103B). Written informed consent was obtained from all patients who participated in this study.

Mutation screenings were conducted in the following order. First, we used polymerase chain reaction (PCR) and direct sequencing to analyze the mutations caused by nucleotide substitutions or small deletions or insertions into any of the exons, including adjacent splice sites in the *SPG4* gene, *SPAST*. If no mutations were detected, multiplex ligation-dependent probe amplification (MLPA) was performed to detect exonic deletions and duplications in *SPAST*. For the remaining kindreds that did not demonstrated any detected mutations in *SPAST*, sequence analysis of the exons of the *SPG3A* gene, *ATL1*, was performed.

Genomic DNA was extracted from peripheral blood leukocytes for genetic analysis. All exons and flanking intronic regions of *SPAST* and *ALT1* were amplified by PCR. The amplified fragments were directly sequenced. S44L and P45Q polymorphisms in *SPAST* were genotyped by direct sequence analysis. MLPA was performed using the SALSA MLPA kit (P165-B1 HSP/Spastin; MRC-Holland, Amsterdam, Netherlands) according to the manufacturer's instructions, and data were analyzed as previously described.[6] For each sample, a normalized ratio of the relative peak area between 0.8—1.2 was considered normal. Heterozygous deletions were indicated by a ratio between 0.3—0.7.

mRNA analysis was performed to assess the extent of the deletions and their probable effects on protein translation when an exonic deletion was detected by MLPA. Total mRNA was extracted from the blood leukocytes using Trizol (Invitrogen, Carlsbad, CA, United States) and converted to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, United States) according to the manufacturer's instructions. Primers were designed for PCR amplification of the cDNA segments that encompassed the putative deleted region. The amplified fragments were then subjected to direct sequencing.

Results

SPAST mutations were discovered in all four kindreds. In family 1, a novel mutation consisting of a two-base deletion in exon 16 (c.1714_1715 del AT) caused a shift in the open reading frame and the premature termination of translation (p.M572VfsX3). In family 3, we identified a point mutation in exon 11 that lead to an amino acid substitution (c.1382T>C, p.L461P), which has been previously reported (The Human Gene Mutation Database website, http://www.hgmd.cf.ac. uk/ac/index.php; Wolfenbuttel, German).[7] The residue leucine 461 is highly evolutionarily conserved according to the public genome database (see HomoloGene on the National Center for Biotechnology Information website, http://www.ncbi.nlm.nih.gov/homologene; Bethesda, MD, United States; and ClustalW2 on the European Bio-informatics Institute website, http://www.ebi.ac.uk/ Tools/clustalw2/; Hinxton, Great Britain).[8,9] These two mutations were not detected in 100 unrelated ethnic Han Taiwanese individuals who served as controls. All of the individuals in the four kindreds carried the common alleles (S and P) for the S44L and P45Q polymorphisms.

In families 2 and 4, no mutation in *SPAST* was detected by direct sequencing of the PCR products. In these two families, MLPA showed aberrant profiles that suggested a gross deletion involving *SPAST* exon 17. In family 2, the signal of the exon 17A probe (its ligation site is located at c.2031-2032, about 40 base pairs upstream of the stop codon) was reduced, whereas the signal of the exon 17B probe (its ligation site is located at c.3961-3962) was within normal range, indicating a deletion that affected exon 17 at the 5'-end (Fig. 2). cDNA analysis revealed the deletion of the first 1090 bases of exon 17 at the mRNA level (Fig. 3). According to the nucleotide sequence, it was predicted that the aberrant mRNA transcript would cause the replacement of the last 40 normal amino acids with 16 erroneous residues at the C-terminus of spastin (M577DfsX16). In family 4, the signals of both the exon 17A and exon 17 B probes were reduced (Fig. 2). However, the effects of the deletion on translation could not be determined due to the failure to amplify the mutant transcript in 3'-untranslated region of *SPAST* by cDNA analysis. We speculate that this deletion may involve the entire exon 17 and extend to outside of the *SPAST* gene. Because *SPAST* mutations were discovered in all four families, further analysis of the *SPG3A* gene was not performed.

The clinical characteristics of the affected members in the four families are shown in Table 1. This study included 15 cases (female:male ratio of 8:6; the gender of one patient was omitted for confidentiality reasons due to young age; age range: 6—71 years old). All symptomatic cases manifested as uncomplicated phenotypes and presented as motor dysfunction due to weakness or severe spasticity of the lower limbs. A wide range of age at symptom onset, from 1—50 years old, was noted. Neurological examinations of the cranial nerves and upper limbs were normal except for accentuated jaw-jerk and tendon reflex in some patients. There was no correlation between duration of the disease and lower limb weakness, sphincter dysfunction, distal sensory abnormalities, or ambulation function. Considerable intrafamilial variation in terms of age of onset, rate of progression, and severity of disease were observed in families 2 and 3. Family 2 was remarkable in terms of the progressively earlier disease onset in successive generations, mimicking the phenomenon of "anticipation." Two members of family 3 (III-4 and III-7), who were normal on neurological examination, also carried the mutation found in the other symptomatic familial members. Generally, there is no clear phenotypic correlation with respect to the different patterns of mutations.

Discussion

SPG4-related dysfunction is the most common type of AD-HSP and frequently manifests with uncomplicated phenotypes. The product of the *SPG4* gene (*SPAST*), spastin, is a member of the AAA (*A*TPase *a*ssociated with diverse cellular *a*ctivities) family, which is characterized by a common domain which mediates ATPase function called the AAA cassette.[10] Spastin can sever and disassemble microtubules and regulate the functions in an ATPase-dependent manner.[11] Spastin also possesses an MIT
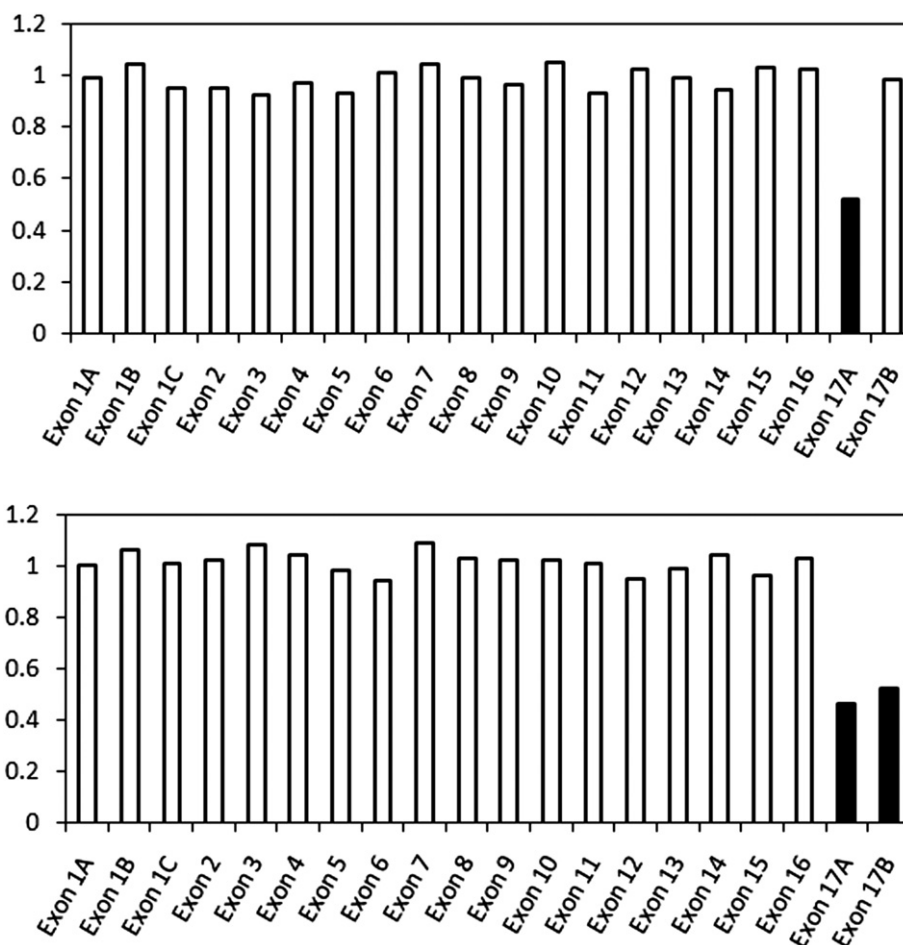
Figure 2 *SPAST*-specific multiple ligation-dependent probe amplification profiles of family 2 (upper panel) and family 4 (lower panel). The values along the vertical axis represent the normalized ratios of the relative peak areas of the exons. A value between 0.8—1.2 is considered normal while a value between 0.3—0.7 indicates a heterozygous deletion of the exon. Exon-specific probes with reduced signals (17A in family 2 and 17A and 17B in family 4) are highlighted by the black bars.

(*m*icrotubule *i*nteracting and *t*rafficking) domain in the N-terminus, which may be involved in microtubule and membrane interactions.[12] Thus, *SPAST* mutations may cause deleterious effects on the microtubule dynamics
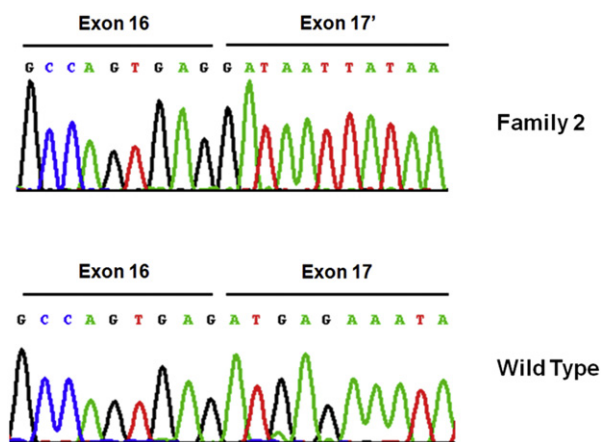

Figure 3 Sequence analysis of the mutant transcript of family 2. Deletion of the 5'-region of exon 17 is indicated.

involved in cytoskeletal stability, axonal transport, and intracellular trafficking; these effects may underlie the axonal degeneration observed in HSP.

In the current study, *SPAST* mutations were found in all four of the Taiwanese HSP kindreds by using a genetic testing protocol to determine AD-HSP. These four mutations, including a novel deletion, have not been reported in ethnic Han Chinese persons.[3,4] Furthermore, all of the mutations affected the AAA cassette-encoding region of *SPAST*. To date, more than 200 different mutations in *SPAST* have been described (please see the HGMD website). Interestingly, nonsense, splice site, and frameshift mutations, which create premature termination codons, may affect any exon while the majority of missense mutations are located in the AAA cassette-encoding region. These findings suggest the crucial role of the AAA cassette in the functions of spastin. We also identified partial and entire deletions of exon 17 in the *SPAST* gene of two kindreds. One of the large deletions was predicted to cause a defect equivalent to a frameshift with the premature termination of translation by cDNA analysis. Genetic rearrangements, including exonic deletions and duplications, have been increasingly recognized as causes of *SPG4*-related HSP.[6,13] It

Table 1 Clinical characteristics and identified mutations of hereditary spastic paraplegia patients.

Family	Case	Sex	Age (years)	Age at onset (years)	Muscle power[a] (P/D)		Extensor plantar reflex	Distal lower limb vibratory sensation loss	Sphincter dysfunction[b]	Ambulation function[c]	Detected mutation
					UE	LE					
1	I-2	F	52	12	5/5	4/5	+	+	+	2	SPAST
	II-2	F	31	11	5/5	4/5	+	+	+	2	c.1714_1715 del AT
2	I-2	F	61	42	5/5	5/5	+	+	+	3	SPAST
	II-2	F	38	11	5/5	5/5	+	+	+	2	Deletion of 5'- region of exon 17
	III-1	NA[d]	6	1	5/5	5/5	+	+	+	1	NA[d]
3	II-1	M	62	40	5/5	1/0	+	+	−	4	SPAST
	II-2	M	58	53	5/5	5/5	−	−	−	2	c.1382T>C
	II-3	M	52	42	5/5	4/5	+	−	+	3	
	III-4[e]	M	30	−	5/5	5/5	−	−	−	1	
	III-5	F	27	24	5/5	5/5	+	−	−	1	
	III-6	M	25	19	5/5	4/2	+	+	−	3	
	III-7[e]	F	25	−	5/5	5/5	−	−	−	1	
4	III-1	F	71	50	5/5	4/3	−	+	+	4	SPAST
	III-6	M	56	48	5/5	5/5	−	−	−	3	Deletion of exon 17
	III-8	F	48	44	5/5	5/5	−	−	−	2	

Abbreviations: D, distal; F, female; LE, lower extremities; M, male; P, proximal; UE, upper extremities.
[a] MRC grading.
[b] Urinary retention, frequency or incontinence.
[c] 1, able to run; 2, unable to run, walking independently; 3, walking with aid; 4, wheelchair bound.
[d] NA, not applicable (gender data omitted for confidentiality; mutation not tested due to ethical consideration).
[e] Asymptomatic mutation carrier.

is estimated that exonic deletions could be found in around 15—20% of AD-HSP families that test negative for SPAST point mutations. Despite the small sample size of the current study, our findings suggest that exonic deletion is a significant cause of SPG4-related HSP in ethnic Han Taiwanese individuals. Whether the genetic rearrangement of SPAST also makes an important contribution to HSP in Han Chinese individuals warrants further investigation.

Clinically, marked interkindred variability with respect to age of onset, rate of progression, and disease severity have been noted in SPG4-related HSP,[14] similar to the families described in this report. Apparent anticipation, a special pattern of intrakindred variability occasionally found in association with SPG4,[15] was observed in our study (family 2). We also found two asymptomatic mutation carriers in family 3, suggesting the incomplete penetrance of this mutation. However, long-term clinical follow-up examinations are needed to confirm these conditions because these two individuals are still young. Despite the different types of SPG4 mutations that have been described, no clear phenotype-genotype correlation can be established. It is postulated that genetic modifiers may account for the variability in SPG4 phenotypes. Missense variants in the N-terminus of SPAST, including S44L and P45Q, have been suggested to be rare polymorphisms that may aggravate clinical features when they present in trans to a classic SPAST mutation located in the AAA cassette-encoding region.[16] However, these polymorphisms, which could be rare in ethnic Han Chinese individuals, were not detected in our patients.

In conclusion, our findings suggest that both mutation patterns and phenotypic expressivity are heterogeneous in Taiwanese patients with SPG4-related HSP. In light of the increasing number of reported cases with genetic rearrangements in SPAST, assessment of large deletions using, for instance, MLPA is warranted when direct sequencing is negative or inconclusive.

Acknowledgments

References

1. Harding AE. Hereditary spastic paraplegias. *Semin Neurol* 1993; **13**:333—6.
2. Fink JK, Heiman-Patterson T, Bird T, Cambi F, Dubé MP, Figlewicz DA, et al. Hereditary Spastic Paraplegia Working group. Hereditary spastic paraplegias: advances in genetic research. *Neurology* 1996;**46**:1507—14.
3. Qin W, Zhang T, Han J, Tang L, Li X, Feng G, et al. A novel insertion mutation in spastin gene is the cause of spastic paraplegia in a Chinese family. *J Neurol Sci* 2003;**210**:35—9.
4. Tang B, Zhao G, Xia K, Pan Q, Luo W, Shen L, et al. Three novel mutations of the spastin gene in Chinese patients with hereditary spastic paraplegia. *Arch Neurol* 2004;**61**:49—55.

5. Chan KY, Ching CK, Mak CM, Lam CW, Chan AY. Hereditary spastic paraplegia: identification of an SPG3A gene mutation in a Chinese family. *Hong Kong Med J* 2009;**15**:304—7.
6. Depienne C, Fedirko E, Forlani S, Cazeneuve C, Ribaï P, Feki I, et al. Exon deletions of SPG4 are a frequent cause of hereditary spastic paraplegia. *J Med Genet* 2007;**44**:281—4.
7. The Human Gene Mutation Database website. http://www.hgmd.cf.uk/ac/index.php.
8. HomoloGene on the National Center for Biotechnology Information website. http://www.ncbi.nlm.nih.gov/homologene.
9. ClustalW2 on the European Bioinformatics Institute website. http://www.ebi.ac.uk/Tools/clustalw2/.
10. Hazan J, Fonknechten N, Mavel D, Paternotte C, Samson D, Artiguenave F, et al. Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. *Nat Genet* 1999;**23**:296—303.
11. Errico A, Ballabio A, Rugarli EI. Spastin, the protein mutated in autosomal dominant hereditary spastic paraplegia, is involved in microtubule dynamics. *Hum Mol Genet* 2002;**11**:153—63.
12. Ciccarelli FD, Proukakis C, Patel H, Cross H, Azam S, Patton MA, et al. The identification of a conserved domain in both spartin and spastin, mutated in hereditary spastic paraplegia. *Genomics* 2003;**81**:437—41.
13. Beetz C, Nygren AO, Schickel J, Auer-Grumbach M, Bürk K, Heide G, et al. High frequency of partial SPAST deletions in autosomal dominant hereditary spastic paraplegia. *Neurology* 2006;**67**:1926—30.
14. Fonknechten N, Mavel D, Byrne P, Davoine CS, Cruaud C, Bönsch D, et al. Spectrum of SPG4 mutations in autosomal dominant spastic paraplegia. *Hum Mol Genet* 2000;**9**: 637—44.
15. Reddy PL, Seltzer WK, Grewal RP. Possible anticipation in hereditary spastic paraplegia type 4 (SPG4). *Can J Neurol Sci* 2007;**34**:208—10.
16. Svenson IK, Kloos MT, Gaskell PC, Nance MA, Garbern JY, Hisanaga S, et al. Intragenic modifiers of hereditary spastic paraplegia due to spastin gene mutations. *Neurogenetics* 2004; **5**:157—64.