LETTER TO JMG

Exon deletions of *SPG4* are a frequent cause of hereditary spastic paraplegia

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Background: Point mutations in *SPG4*, the gene encoding spastin, are a frequent cause of autosomal dominant hereditary spastic paraplegia (AD-HSP). However, standard methods for genetic analyses fail to detect exonic microdeletions.

Methods: 121 mutation-negative probands were screened for rearrangements in *SPG4* by multiplex ligation-dependent probe amplification.

Results: 24 patients with 16 different heterozygotic exon deletions in *SPG4* (20%) were identified, ranging from one exon to the whole coding sequence. Comparison with 78 patients with point mutations showed a similar clinical picture but an earlier age at onset.

Conclusions: Exon deletions in *SPG4* are as frequent as point mutations, and *SPG4* is responsible for 40% of AD-HSP.

Hereditary spastic paraplegias (HSPs) are a genetically heterogeneous group of neurodegenerative disorders clinically characterised by progressive stiffness and weakness of the lower limbs that result from axonal neurodegeneration in the corticospinal tract. Pure and complicated forms of HSP have been described depending on whether spasticity occurs in the absence or presence of other clinical features such as cerebellar ataxia, neuropathy, retinal degeneration, cognitive impairment, dementia or epilepsy. The clinical heterogeneity of HSP is partly explained by its genetic heterogeneity. To date, 35 loci have been identified, associated with autosomal dominant, autosomal recessive and X linked modes of inheritance (for additional information, see the HUGO site at <http://www.gene.ucl.ac.uk/nomenclature/>).

The most common form of autosomal dominant HSP (AD-HSP) is caused by mutations in the *SPG4/SPAST* gene (MIM 604277), encoding spastin, a member of the AAA family of ATPases.¹ *SPG4* has been shown to account for 15–40% of all AD-HSP families, depending on the population.^{2–5} HSP due to *SPG4* mutations (MIM 182601) is generally described as a pure form of the disease—that is, as spastic paraparesis often associated with a decreased sense of vibration in the lower limbs and urinary problems—but has also occasionally been described in association with additional features, including cognitive impairment, peripheral neuropathy, cerebellar signs or epilepsy.^{6–8} Age at onset is highly variable, ranging from early infancy up to the eighth decade. More than 150 different mutations have been identified in all exons of the *SPG4* gene except for exon 4, which is alternatively spliced. All types of DNA alterations are observed, including missense, non-sense, splice-site mutations, and small insertions or deletions.⁹ This wide spectrum of mutations, combined with the observation that many mutations reduce the abundance of the normal full-length transcript and/or functionally normal spastin protein,

suggests that the pathogenic mechanism is haploinsufficiency—that is, the disease occurs once the level of functional spastin falls below a critical level.^{10–11} Although larger deletions in *SPG4* have been described in two cases^{9–12} and should theoretically lead to HSP, they are missed by standard screening methods such as direct sequencing and denaturing high-performance liquid chromatography and have not yet been systematically sought. In this study, we screened 121 patients for micro-rearrangements in the *SPG4* gene by multiplex ligation-dependent probe amplification (MLPA) to assess their frequency in families with HSP.

METHODS

Patients

We selected 121 European, mostly French (105/121), probands with HSP, in whom mutations in the *SPG4* gene were not detected by denaturing high-performance liquid chromatography. Dominant transmission was observed in 120 families. The remaining family had two affected sibs. Most of the probands presented pure HSP ($n = 114$). The complicated forms ($n = 7$) included signs of peripheral neuropathy ($n = 3$), and one each with cerebellar signs, mental retardation, cognitive impairment and parkinsonism. Informed written consent was obtained from each individual before blood sampling. This study was approved by the ethical committee Paris-Necker (CCPRB, number 03-12-07, 2/10/2004).

Multiplex ligation-dependent probe amplification

The autosomal dominant spastic paraplegia MLPA kit (P165) designed to search for deletions or duplications in *SPG4* was purchased from MRC-Holland (Amsterdam, The Netherlands). MLPA reactions were carried out according to the manufacturer's instructions. Electrophoresis of polymerase chain reaction products was performed using an ABI 3730 sequencer and MLPA data were analysed using the GeneMapper V.4.0 software (PE Applied Biosystems, Foster City, California, USA). Relative ratios were calculated for each peak using the formula $r = \text{mean}(\text{peak area}_{\text{patient}}/\text{control area}_{\text{patient}})/(\text{peak area}_{\text{control individual}}/\text{control area}_{\text{control}})$.

Statistical tests

Frequencies were compared with the χ^2 test or Fisher's exact test when appropriate. Quantitative variables were compared with the Mann-Whitney U test. Statistical analysis was performed using SPSS software (v 13.0). Values are expressed as mean (SD).

Abbreviations: AD-HSP, autosomal dominant hereditary spastic paraplegia; HSP, hereditary spastic paraplegia; MLPA, multiplex ligation-dependent probe amplification

Table 1 SPG4 exon deletions found in 24 hereditary spastic paraplegia families

Family number	Deletion	Nomenclature	Predicted effect on protein	Segregation	Ages at onset (years)*
165	Exon 1	c.1-?_415+?del	Unknown	ND	31
172	Exon 1	c.1-?_415+?del	Unknown	Yes (n=5)	2, 2, 30
366	Exon 1	c.1-?_415+?del	Unknown	Yes (n=5)	10 , 37, 47, 50, 50
774	Exons 1 to 4	c.1-?_682+?del	Unknown	ND	1
628	Exons 1-17	c.1-?_1851+?del	Absence of protein	ND	14
202	Exons 1-17	c.1-?_1851+?del	Absence of protein	ND	26
253	Exons 1-17	c.1-?_1851+?del	Absence of protein	Yes (n=3)	10 , 14, 35
282	Exons 1-17	c.1-?_1851+?del	Absence of protein	ND	29
237	Exons 4-17	c.587-?_1851+?del	Unknown	ND	38
742	Exons 5-6	c.683-?_1004+?del	p.Glu228ValfsX237	Yes (n=4)	40
530	Exons 5-7	c.683-?_1098+?del	p.Glu228ValfsX233	Yes (n=2)	40 , 77
167	Exon 6	c.871-?_1004+?del	p.Gly291TrpfsX295	Yes (n=2)	1, 15
374	Exons 8-12	c.1099-?_1493+?del	p.Leu367AlafsX379	ND	37
629	Exons 8-17	c.1099-?_1851+?del	Unknown	ND	40
152	Exons 8-17	c.1099-?_1851+?del	Unknown	Yes (n=5)	25 , 30, 32, 52
157	Exon 9	c.1174-?_1245+?del	p.Ala492_Tyr415del	ND	17
235	Exons 9-12	c.1174-?_1493+?del	p.Lys393PhefsX404	ND	40
335	Exons 10-16	c.1246-?_1728+?del	p.Val416_Glu576del	ND	33
12	Exon 13	c.1494-?_1536+?del	p.Arg499GlnfsX515	Yes (n=5)	14, 18, 25 , 54
333	Exon 13	c.1494-?_1536+?del	p.Arg499GlnfsX515	Yes (n=3)	1, 26
236	Exon 16	c.1688-?_1728+?del	p.Glu563AspfsX570	Yes (n=2)	1, 53
803	Exon 16	c.1688-?_1728+?del	p.Glu563AspfsX570	Yes (n=4)	20, 36
193	Exons 16-17	c.1688-?_1851+?del	Unknown	ND	30
168	Exon 17	c.1729-?_1851+?del	Unknown	Yes (n=4)	30 , 50

HSP, hereditary spastic paraplegia; n, number of affected patients tested; ND: not determined.

*Ages at onset were undetermined for 12 patients. Ages in bold correspond to those of the index cases.

RESULTS

Results of MLPA analysis

We identified 24 index patients (22 French, 1 Portuguese and 1 Spanish) with microdeletions in the *SPG4* gene. This corresponds to a frequency of 20% (24/121). Sixteen different exon deletions were detected, spanning a single exon to the whole coding sequence of the gene (ie, exons 1-17). Five of the deletions (exon 1, exons 1-17, exons 8-17, exon 13 and exon 16) were detected in more than one family, but the remaining 11 were private rearrangements. Segregation of the microdeletion with the disease was analysed when DNA from other affected individuals was available (table 1). All affected relatives (n = 32) of probands from 12 families also had the corresponding exon deletion, confirming that these rearrangements are responsible for the disease. In addition, we tested six asymptomatic relatives and examined one deletion carrier who was still asymptomatic at age 46 years. These observations confirm that the penetrance is age dependent and incomplete in patients with *SPG4* deletions, as described previously for *SPG4* point mutations.

Clinical features of patients with deletions

Age at onset in patients with *SPG4* deletions, including the 24 index cases and 32 affected relatives, ranged from 1 to 77 years (mean (SD) age at onset 28.7 (17.4) years, n = 44). Age at onset could not be determined for 12 patients, including 3 who presented minor signs only detected on examination. All index cases with exon deletions had pure HSP. However, cognitive impairment was present in a single affected relative. One index patient (FSP-774) had cerebellar atrophy on cerebral MRI but no cerebellar signs at examination. The age at onset for the four index patients with the largest deletion spanning exons 1-17 was between 4 and 35 years, similar to the mean age at onset of the whole group.

Comparison of probands with microdeletions (n = 24) and probands with point mutations (n = 78, unpublished data) showed that the former were significantly younger at onset (25.4 (12) vs 31.8 (16.8) years, p = 0.033; table 2). All other features were strikingly similar in both groups, except for a tendency towards less severe walking disability in the deleted

group compared with the other group (9% vs 14%), despite similar disease durations.

DISCUSSION

We identified 24 patients with partial exonic deletions in *SPG4* among 121 patients who were negative for point mutations. These findings demonstrate that a large proportion (20%) of patients with mutation-negative HSP in fact carry *SPG4* microdeletions and confirm that haploinsufficiency of *SPG4* is a major cause of AD-HSP.

Previous estimates of the proportion of patients with *SPG4* mutations who had family histories of the disease ranged between 15% and 40%. The percentage of patients with point mutations in *SPG4* in our large series of French HSP families (which include patients with unknown linkage status) is, however, approximately 25% (A Durr, C Depienne and A Brice, personal communication), a proportion also confirmed in another large European series.¹³ When taking into account only the French families with autosomal dominant inheritance and excluding families with *SPG4* point mutations in this study, the proportion of cases with exon deletions reaches 16% of AD-HSP. These results indicate that *SPG4* could indeed be responsible for 40% of HSP families in the French population. This proportion might even be greater considering that mutations or deletions in the *SPG4* promoter have not yet been systematically sought.

Many different combinations of exon deletions are observed, indicating that dosage should be applied to all *SPG4* exons. This can easily be performed in a single reaction using MLPA. A few deletions were found more than once, and it will be interesting to determine whether they descend from a common founder or are recurrent mutations.

Globally, the clinical picture of the patients with exon deletions is similar to that of patients with point mutations: most patients present pure HSP which includes, in addition to spasticity in the lower limbs, brisk reflexes, decreased sense of vibration at ankles, proximal or generalised weakness in the lower limbs, as well as frequent sphincter disturbances as part of the "SPG4 phenotype". However, the age at onset seemed to be significantly earlier in patients with microdeletions who

Table 2 Clinical comparison of index patients with *SPG4* exon deletions and *SPG4* point mutations

	<i>SPG4</i> exon deletions	n	<i>SPG4</i> point mutations	n	p Value
Mean (SD) age at examination (years)	45.4 (15.9)	24	51.2 (14.9)	78	0.075
Range	18–76		6–85		
Mean (SD) age at onset (years)	25.4 (12)	23	31.8 (16.8)	78	0.033
Range	1–40		1–63		
Women : men	8:16	24	34:44	78	0.479
Severe disability walking (needs two canes or is a wheelchair user) (%)	9	2/23	14	10/71	0.501
Gait spasticity (%)	87.5	21/24	96	71/74	0.134
Spasticity at rest (%)	86	19/22	85.5	47/55	0.918
Increased reflexes LL (%)	87.5	21/24	87.3	62/71	0.982
Increased reflexes UL (%)	33	8/24	34	23/68	0.965
Extensor plantar reflex (%)	91	21/23	90	63/70	0.847
Proximal motor deficit LL (%)	42	10/24	58	42/72	0.156
Distal wasting LL (%)	17	4/24	13	9/69	0.659
Decreased vibration sense at ankles (%)	59	13/22	59	40/68	0.982
Decreased touch and prick sense LL (%)	0	0/22	3	2/68	0.416
Urinary urgency (%)	39	9/23	56	39/70	0.167

LL, lower limb; UL, upper limb.

Key points

- In all, 121 patients with hereditary spastic paraplegia negative for point mutations in *SPG4* were screened for rearrangements in *SPG4* by multiplex ligation-dependent probe amplification.
- We identified 20% of exonic microdeletions.
- Clinical features of patients with *SPG4* deletions were similar to those with point mutations with an earlier age at onset.

tended to be less severely affected than patients with point mutations. Disease progression had indeed been previously shown to be more rapid in late-onset than in early-onset cases.³ Patients with a deletion of the whole gene also had a clinical phenotype and an age at onset similar to patients with other deletion types or point mutations. The functional consequence of *SPG4* mutation is therefore the loss of spastin function.

This study confirms that HSP is caused by *SPG4* haploinsufficiency, attested by the similar phenotypes associated with different types of mutations including whole-gene deletions. The fact that exon deletions are almost as frequent as point mutations in this large series of families with AD-HSP justifies the inclusion of gene dosage experiments in future genetic tests for *SPG4* so that appropriate genetic counselling can be given.

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REFERENCES

- 1 Hazan J, Fonknechten N, Mavel D, Paternotte C, Samson D, Artiguenave F, Davoine CS, Cruaud C, Durr A, Wincker P, Brottier P, Catalico L, Barbe V, Burgunder JM, Prud'homme JF, Brice A, Fontaine B, Heilig B, Weissenbach J. Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. *Nat Genet* 1999;23:296–303.
- 2 McDermott CJ, Burness CE, Kirby J, Cox LE, Rao DG, Hewamadduma C, Sharrack B, Hadjivassiliou M, Chinnery PF, Dalton A, Shaw PJ. Clinical features of hereditary spastic paraplegia due to spastin mutation. *Neurology* 2006;67:45–51.
- 3 Fonknechten N, Mavel D, Byrne P, Davoine CS, Cruaud C, Bonsch D, Samson D, Coutinho P, Hutchinson M, McMonagle P, Burgunder JM, Tartaglione A, Heinzle O, Feki I, Deufel T, Partfey N, Brice A, Fontaine B, Prud'homme JF, Weissenbach J, Durr A, Hazan J. Spectrum of *SPG4* mutations in autosomal dominant spastic paraplegia. *Hum Mol Genet* 2000;9:637–44.
- 4 Meijer IA, Hand CK, Cossette P, Figlewicz DA, Rouleau GA. Spectrum of *SPG4* mutations in a large collection of North American families with hereditary spastic paraplegia. *Arch Neurol* 2002;59:281–6.
- 5 Sauter S, Mitterski B, Klimpe S, Bonsch D, Schols L, Visbeck A, Papke T, Hopf HC, Engel W, Deufel T, Epplen JT, Neesen J. Mutation analysis of the spastin gene (*SPG4*) in patients in Germany with autosomal dominant hereditary spastic paraplegia. *Hum Mutat* 2002;20:127–32.
- 6 Nielsen JE, Johnsen B, Koefoed P, Scheuer KH, Gronbech-Jensen M, Law I, Krabbe K, Norremolle A, Eiberg H, Sondergaard H, Dam M, Rehfeld JF, Krarup C, Paulsen OB, Hasholt L, Sorensen SA. Hereditary spastic paraplegia with cerebellar ataxia: a complex phenotype associated with a new *SPG4* gene mutation. *Eur J Neurol* 2004;11:817–24.
- 7 Tang B, Zhao G, Xia K, Pan Q, Luo W, Shen L, Long Z, Dai H, Zi X, Jiang H. Three novel mutations of the spastin gene in Chinese patients with hereditary spastic paraplegia. *Arch Neurol* 2004;61:49–55.

- 8 **Mead SH**, Proukakis C, Wood N, Crosby AH, Plant GT, Warner TT. A large family with hereditary spastic paraparesis due to a frame shift mutation of the spastin (SPG4) gene: association with multiple sclerosis in two affected siblings and epilepsy in other affected family members. *J Neurol Neurosurg Psychiatry* 2001;**71**:788–91.
- 9 **McDermott CJ**, Burness CE, Kirby J, Cox LE, Rao DG, Hewamadduma C, Sharrack B, Hadjivassiliou M, Chinnery PF, Dalton A, Shaw PJ. Clinical features of hereditary spastic paraplegia due to spastin mutation. *Neurology* 2006;**67**:45–51.
- 10 **Patrono C**, Casali C, Tessa A, Cricchi F, Fortini D, Carrozzo R, Siciliano G, Bertini E, Santorelli FM. Missense and splice site mutations in SPG4 suggest loss-of-function in dominant spastic paraplegia. *J Neurol* 2002;**249**:200–5.
- 11 **Burger J**, Fonknechten N, Hoeltzenbein M, Neumann L, Bratanoff E, Hazan J, Reis A. Hereditary spastic paraplegia caused by mutations in the SPG4 gene. *Eur J Hum Genet* 2000;**8**:771–6.
- 12 **Iwanaga H**, Tsujino A, Shirabe S, Eguchi H, Fukushima N, Niikawa N, Yoshiura K, Eguchi K. Large deletion involving the 5'-UTR in the spastin gene caused mild phenotype of autosomal dominant hereditary spastic paraplegia. *Am J Med Genet A* 2005;**133**:13–17.
- 13 **McDermott CJ**, Burness CE, Kirby J, Cox LE, Rao DG, Hewamadduma C, Sharrack B, Hadjivassiliou M, Chinnery PF, Dalton A, Shaw PJ. Clinical features of hereditary spastic paraplegia due to spastin mutation. *Neurology* 2006;**67**:45–51.

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