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SHORT REPORT

A multi-exonic *SPG4* duplication underlies sex-dependent penetrance of hereditary spastic paraplegia in a large Brazilian pedigree

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***SPG4* mutations are the most frequent cause of autosomal-dominant hereditary spastic paraplegia (HSP). *SPG4* HSP is characterized by large inter- and intrafamilial variability in age at onset (AAO) and disease severity. The broad spectrum of *SPG4* mutations has recently been further extended by the finding of large genomic deletions in *SPG4*-linked pedigrees negative for 'small' mutations. We had previously reported a very large pedigree, linked to the *SPG4* locus with many affected members, which showed gender difference in clinical manifestation. Screening for copy number aberrations revealed the first case of a multi-exonic duplication (exon10_12dup) in the *SPG4* gene. The mutation leads to a premature stop codon, suggesting that the protein product is not functional. The analysis of 30 individuals who carry the mutation showed that males have on average an earlier AAO and are more severely affected. The present family suggests that this HSP pathogenesis may be modulated by factors related to individual background and gender as observed for other autosomal dominant conditions, such as facio-scapulohumeral muscular dystrophy or amyloidosis. Understanding why some individuals, particularly women, are 'partially protected' from the effects of this and other pathogenic mutations is of utmost importance.**
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Introduction

The major feature of the heterogeneous condition hereditary spastic paraplegia (HSP) is progressive weakness and spasticity of the lower limbs. The most frequent cause is mutation of the *SPG4* gene, associated with autosomal-dominant inheritance.[1] *SPG4* HSP shows large inter- and intrafamilial variability in age at onset (AAO) and disease severity, suggesting the existence of modifying factors.[2] The broad spectrum of *SPG4* mutations has recently been further extended by the finding of large genomic deletions

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in *SPG4*-linked pedigrees, which had screened negative for 'small' mutations.[3] We previously reported on another such *SPG4*-linked but apparently mutation-negative pedigree, which, in addition, appeared unique in almost exclusively men expressing the phenotype.[4]

Subjects and methods

DNA of three definitely affected individuals was analyzed using *SPG4*-specific multiplex ligation-dependent probe amplification (MLPA – MRC Holland) as described previously.[5] Long-range PCR on genomic DNA followed by sequencing was used to define the limits of the aberration identified. Mutation-specific PCR was used to establish the carrier status of 52 individuals.

The polymorphic variants c.131C>T or c.134C>A were screened as described previously.[6] The samples were sequenced in a Mega Bace 1000 DNA Sequencer – Amersham Bioscience (GE-Healthcare). cDNA to be used in RT-PCR was prepared from peripheral blood of affected family members.

Fifty-two available family members (30 women and 22 men) from three generations were clinically re-investigated and classified by a team of neurologists, who were blind about the genotypes; these included most of the formerly reported[4] as well as 14 additional individuals (Figure 1 and Table 1).

Results

Using MLPA, we identified a partial *SPG4* duplication (exon10_12dup) in DNA of three definitely affected individuals. Long-range PCR followed by sequencing of the junction revealed that this duplication was in tandem (c.1246-2896_1493 + 523dup) with non-homology of the sequences contributing to the novel fusion (Supplementary Figure 1). None of the family members carried the polymorphic variants c.131C>T or c.134C>A. RT PCR-based cDNA analysis showed the duplication to also be present at mRNA level. From the total of 52 individuals investigated, 30 (14 women) carried the duplication, 12 of whom (9 women) had no clinical complaints. AAO data compiled during the re-investigation showed a strong difference between men and women ($P = 0.017$, Figure 2).

Discussion

The molecular defect we identified corroborates the recent suggestion of copy number errors to underlie all cases of *SPG4* HSP negative for small mutations.[3] However, only deletions have been reported so far, whereas we now present the first instance of a partial *SPG4* duplication. The lack of sequence identity at the novel junction in our case (Supplementary Figure 1) argues against non-allelic homologous recombination underlying the duplication event. The duplication is in tandem and results in an mRNA containing two adjacent exon 10–12 blocks. This arrangement causes a frameshift and creates a pre-terminal stop codon (PTC) in the second exon 10, thereby disrupting the enzymatic domain. Lack of enzymatic activity has been proposed as the disease-causing mechanism for numerous missense and truncating mutations showing the same primary effect.[2,7]

The ascertainment of carrier status and AAO data by the present study allowed statistical confirmation of highly sex-dependent penetrance in our pedigree (Figures 1 and 2 and Table 1). Since the median AAO of SPG4 HSP is approximately 30 years,[8] we observed more women than

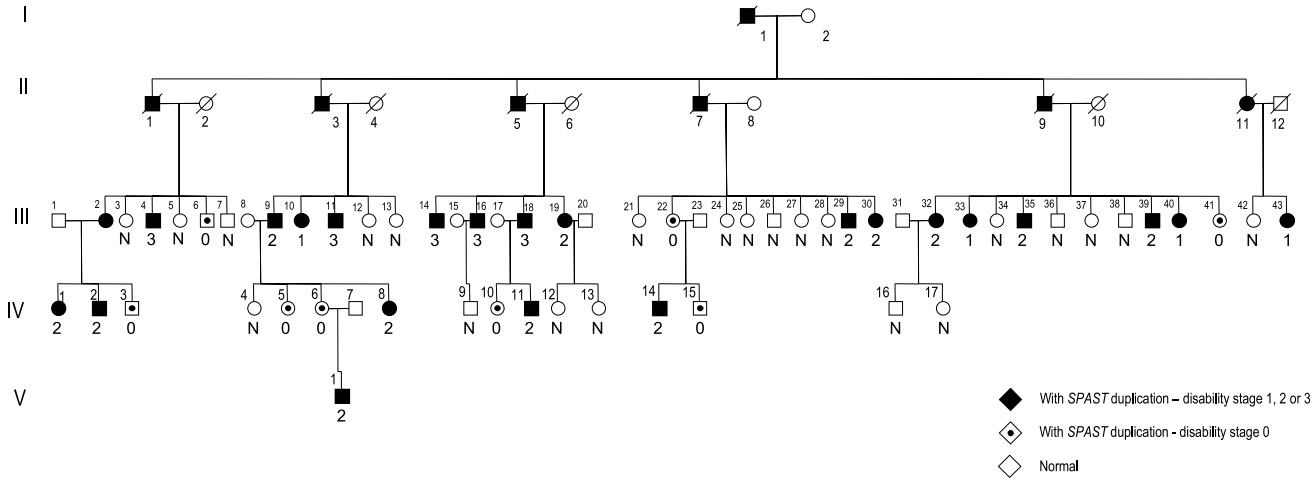

Figure 1 Pedigree for the 52 individuals included in this analysis. Symbol below individuals corresponds to molecular and neurological condition. N: no mutation detected; 0: asymptomatic at neurological examination; 1: asymptomatic with mild neurological abnormalities; 2: moderate neurological abnormalities, able to walk without support; 3: severe neurological abnormalities, only able to walk with cane or walker.

Table 1 Clinical data for 30 mutation carriers

			Age (years)				*Pyramidal signs*					
Disability stage	*Individual*	*Sex*	*At onset*	*At examination*	*Disease duration (years)*	*Disease severity*	*LL reflexes*	*Plantar response*	*Vibration sense diminished in LL*	*Pes cavus*	*Spasticity*	*Sphincteric disturbance*
0	IV-15	M		31		None	Normal	Flexor	No	No	No	None
0	IV-3	M		42		None	Normal	Flexor	No	No	No	None
0	III-6	M		55		None	Normal	Flexor	No	No	No	None
0	III-22	F		58		None	Normal	Flexor	No	No	No	Micturition urgency
0	III-41	F		55		None	Normal	Flexor	No	No	No	None
0	IV-10	F		38		None	Normal	Flexor	No	No	No	None
0	IV-5	F		38		None	Normal	Flexor	No	No	No	Micturition urgency
0	IV-6	F		32		None	Normal	Flexor	No	No	No	Micturition urgency
1	III-40	F		52		No motor complain	Normal	Extensor	No	No	No	Micturition urgency
1	III-33	F		62		No motor complain	Brisk	Extensor	No	No	No	Micturition urgency
1	III-43	F		45		No motor complain	Brisk	Extensor	No	No	Mild	None
1	III-10	F		65		No motor complain	Brisk	Extensor	No	No	Mild	None
2	IV-2	M	29	38	9	Mild	Brisk	Extensor	Yes	No	Mild	None
2	III-39	M	41	46	5	Mild	Brisk	Extensor	No	No	Mild	Micturition urgency
2	IV-14	M	27	32	5	Mild	Brisk	Extensor	No	No	Mild	None
2	III-9	M	50	67	17	Mild	Brisk	Extensor	Yes	No	Mild	Micturition urgency
2	V-1	M	1	10	9	Mild	Brisk	Extensor	No	No	Mild	Micturition urgency
2	III-29	M	12	46	34	Mild	Brisk	Extensor	Yes	Yes	Mild	Micturition urgency
2	III-35	M	54	58	4	Mild	Brisk	Extensor	Yes	Yes	Mild	Micturition incontinent
2	IV-11	M	1	33	32	Mild	Brisk	Extensor	Yes	Yes	Moderate	None
2	III-30	F	38	43	5	Mild	Brisk	Extensor	No	No	Mild	Micturition urgency
2	III-32	F	46	66	20	Mild	Brisk	Extensor	No	No	Mild	Micturition urgency
2	IV-1	F	48	49	1	Mild	Brisk	Extensor	No	No	Mild	None
2	IV-8	F	23	25	2	Mild	Brisk	Absent	No	No	Mild	Micturition incontinent
2	III-19	F	17	59	42	Mild	Brisk	Extensor	No	No	Mild	None
3	III-11	M	26	63	37	Moderate	Brisk	Extensor	Yes	No	Moderate	None
3	III-14	M	18	73	55	Moderate	Brisk	Extensor	Yes	No	Moderate	Micturition incontinent
3	III-18	M	25	67	42	Moderate	Brisk	Extensor	ND	No	Moderate	Micturition incontinent
3	III-16	M	49	68	19	Moderate	Brisk	Extensor	ND	No	Moderate	Micturition incontinent
3	III-4	M	38	63	25	Moderate	Brisk	Extensor	Yes	Yes	Moderate	Micturition incontinent

F, Female; LL, lower limbs; M, Male; UL, upper limbs; ND, not done.

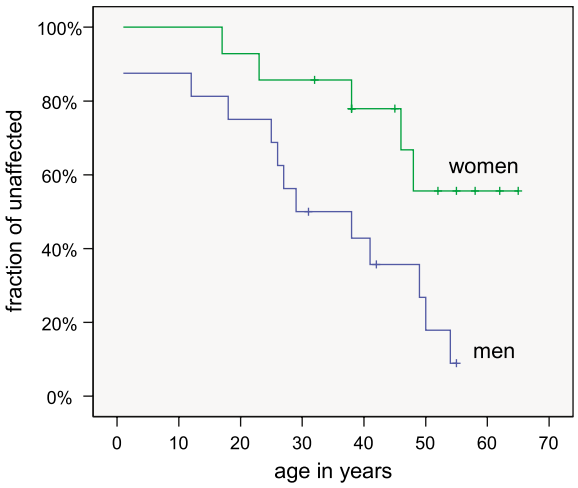

Figure 2 Kaplan–Meier curves. AAO for women (green, $n=14$) and men (blue, $n=16$) mutation carriers. Outcome differs significantly between male and female mutation carriers ($P=0.017$, Log Rank Mantel-Cox).

men showing an unusual course of the disease (Figure 2). The presence of known intragenic disease modifiers,[6] potentially contributing to the distribution of AAO, was excluded. Further efforts are needed to resolve why a seemingly special mutation leads to the 'protection' of a defined group of carriers in this pedigree. Interestingly, a similar gender difference in clinical course has been observed for other autosomal-dominant neuromuscular disorders, such as facioscapulohumeral muscular dystrophy[9] and amyloidosis.[10] Understanding why some individuals, particularly women, are 'partially protected' from the effects of this and other pathogenic mutations remains a great challenge that might open new avenues for treating these disorders.

Acknowledgements

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)