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Letter to the Editor

SPG4-related hereditary spastic paraplegia: frequency and mutation spectrum in Brazil

To the Editor:

Hereditary spastic paraplegia (HSP) is a heterogeneous group of neurodegenerative disorders characterized by progressive lower limb spasticity and weakness. Mutations in *SPAST*/*SPG4* are the major cause of autosomal dominant (AD-HSP) in Europe accounting for 40–50% of all patients (1). However, there are few studies on SPG4-HSP from Latin America (2), which has a distinctive ethnic background (populations from European, African and Native American descent). Therefore, our aim was to investigate the frequency and mutation spectrum of SPG4-HSP in a cohort of 55 Brazilian patients with AD-HSP from 34 unrelated families.

Patients were recruited from three centers in the south and southeast of Brazil: Universidade Estadual de Campinas (23 families), Universidade Federal do Paraná (7) and Universidade Federal do Rio Grande do Sul (4). This study was approved by our institution Ethics Committee and written informed consent was obtained from all participants.

Genomic DNA was used in polymerase chain reactions with primers designed to cover the 17 exons of *SPAST*/*SPG4* (3). Mutation screening was performed by automatic Sanger sequencing and multiplex-ligand probe amplification (MLPA). All variants found were checked in human single nucleotide polymorphism (SNP) and mutation databases: Ensembl (www.ensembl.org), Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and 1000 genomes project (www.1000genomes.org). New variants were subsequently screened in 200 control chromosomes using Sanger sequencing. To evaluate pathogenicity, we performed *in silico* analyses using POLYPHEN-2 (http://genetics.bwh.harvard.edu/pph2/), Mutation taster (http://www.mutationtaster.org/), human splicing finder-v2.4.1 (http://www.umd.be/HSF/) and ESEfinder.

In our cohort, seven subjects from two unrelated families had a complicated phenotype (two with dementia and five with epilepsy/peripheral neuropathy). The remaining 48 patients had pure phenotypes, with the mean age at onset of 32 years. Twenty-four patients from 12 unrelated families presented mutations in *SPAST*/*SPG4* (Table 1). All these subjects had a pure phenotype. Each mutation was found in a single pedigree. A synonymous SNP c.878G>A (rs145264166) was also found in one family. MLPA revealed no copy number variation (CNV) in our patients.

We show that mutations in *SPAST*/*SPG4* are frequent in Brazilian families with AD-HSP (35% in this cohort). Our patients with *SPAST*/*SPG4* mutations present a pure phenotype, with early adulthood onset. Eight of these mutations have been already described (3–8). The non-stop mutation c.1849C>T reported by Alvarez et al. (4) did segregate with the phenotype in family PR4. Since c.1849C>T abolishes the stop codon and results in a 46aa longer transcript, this suggests that a

Table 1. Mutations identified in the *SPAST*/*SPG4* gene in 12 Brazilian families with AD HSP

Family	Age at onset (index case; years)	# Patients[a] (men/women)	Mutation	Gene location	Protein change	Mutation type	Reference
PR1	18	1 (1/0)	c.162C>T	Ex 1	Ex1 skipping	Splice site	This study
PR2	29	3 (1/2)	c.839_840delAG	Ex 5	p.Q280Rfs289	Frameshift deletion	(7)
CP1	25	2 (2/0)	c.1255G>T	Ex 10	p.G419X	Nonsense	This study
PA1	23	1 (0/1)	c.1267G>T	Ex 10	p.V423L	Missense	(5)
CP2	7	1 (0/1)	c.1378C>T	Ex 11	p.R460C	Missense	(5)
CP3	34	1 (0/1)	c.1413+5G>A	In 11	Ex11skipping + frameshift	Splice site	(3)
PR3	1	2 (0/2)	c.1495C>T	Ex 13	p.R499C	Missense	(3)
CP4	26	2 (1/1)	c.1535_1535delA	Ex 13	p.E512Gfs529	Frameshift deletion	This study
CP5	30	4 (3/1)	c.1651G>C	Ex 15	p.A551P	Missense	(6)
CP6	32	2 (1/1)	c.1667_1668delCA	Ex 15	p.A556Gfs575	Frameshift deletion	This study
PA2	25	1 (1/0)	c.1741C>T	Ex 17	p.R581X	Nonsense	(8)
PR4	30	4 (3/1)	c.1849T>G	Ex 17	p.*616Eext*46	Non-stop change	(4)

[a]Patients evaluated by a neurologist at one of the centers.

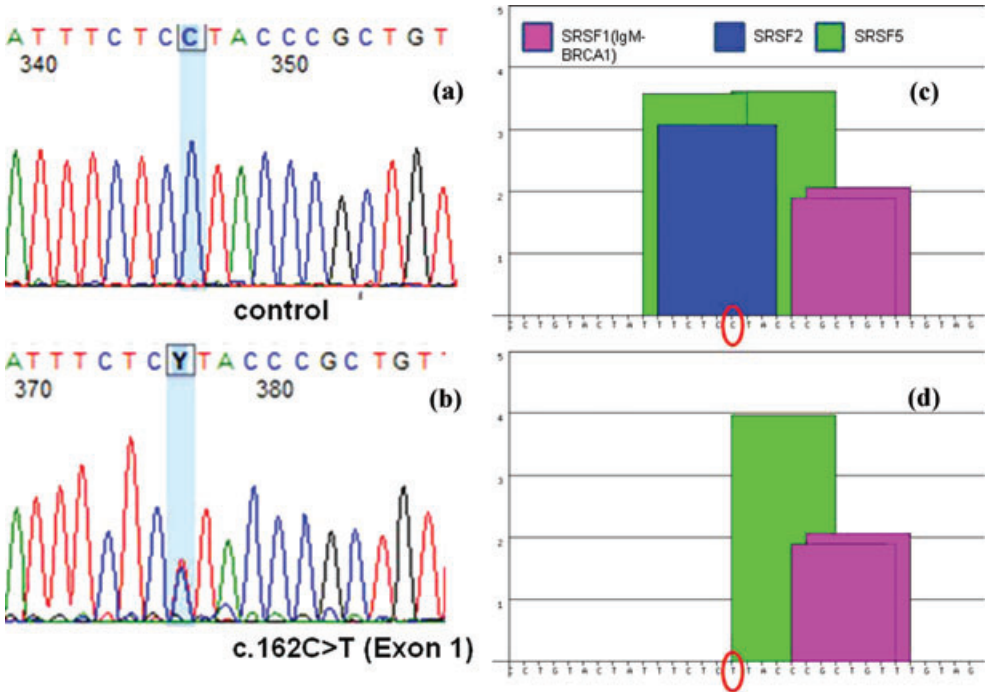

Fig. 1. Left column. Sequence analysis of the first exon of *SPG4/SPAST* in a healthy control (**a**) and patient PR1 showing the c.162C>T mutation (**b**). Right column. Graphical representation of *in silico* ESE finder analysis. Using the normal *SPAST/SPG4* sequence surrounding the c.162C>T variant, a number of different splice enhancer binding sites are predicted. When the synonymous change (surrounded by red circle) is introduced, the splice enhancer sites SRSF2 and SRSF5 are lost (blue and green bars, lower panel) and the scores of other ESEs are altered. The nucleotides that were analyzed with ESE finder correspond to nucleotides 147–178 of SPAST/SPG4 cDNA.

gain-of-function mechanism may operate in this case if the extended protein is synthesized and toxic, although evidence so far is mostly consistent with a loss-of-function mechanism.

Patient CP2 had the known missense mutation c.1378C>T, but in apparent homozygosis (both parents were deceased, making it impossible to confirm their genotype). This patient had an otherwise mild phenotype, but with very early onset, thus suggesting that age at onset rather than disease severity may be associated to homozygous *SPAST*/*SPG4* mutations.

We were able to identify four novel mutations in *SPAST*/*SPG4*. The c.162C>T variant is a synonymous SNP that is predicted to remove two exon splicing enhancer sites thus producing a transcript lacking exon1 (Figure 1). It would have been important to have additional family members to confirm segregation of the mutation; however, DNA samples of other relatives were not available. The remaining three novel mutations (c.1255G>T, c.1667_1668delCA and c.1535_1535delA) are all located in the highly conserved AAA ATPase domain and are predicted to result in early truncation of spastin (Fig. 1).

The only previous report on SPG4-HSP in Brazil is that of a large pedigree segregating a duplication spanning exons 10, 11 and 12 of *SPAST*/*SPG4* (2). Despite this, we did not find any copy number aberrations in *SPAST*/*SPG4* in our cohort. Further studies are needed to certify that CNV are indeed rare in Brazilian patients with SPG4-HSP.

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References

1. Finsterer J, Loscher W, Quasthoff S, Wanschitz J, Auer-Grumbach M, Stevanin G. Hereditary spastic paraplegias with autosomal dominant, recessive, X-linked, or maternal trait of inheritance. J Neurol Sci 2012: 318: 1–18.
2. Mitne-Neto M, Kok F, Beetz C et al. A multi-exonic SPG4 duplication underlies sex-dependent penetrance of hereditary spastic paraplegia in a large Brazilian pedigree. Eur J Hum Genet 2007: 15: 1276–1279.
3. Fonknechten N, Mavel D, Byrne P et al. Spectrum of SPG4 mutations in autosomal dominant spastic paraplegia. Hum Mol Genet 2000: 9: 637–44.
4. Alvarez V, Sánchez-Ferrero E, Beetz C et al. Mutational spectrum of the SPG4 (SPAST) and SPG3A (ATL1) genes in Spanish patients with hereditary spastic paraplegia. BMC Neurol 2010: 10: 89.
5. McDermott CJ, Burness CE, Kirby J et al. Clinical features of hereditary spastic paraplegia due to spastin mutation. Neurology 2006: 67: 45–51.
6. Sauter S, Miterski B, Klimpe S et al. Mutation analysis of the spastin gene (SPG4) in patients in Germany with autosomal dominant hereditary spastic paraplegia. Hum Mutat 2002: 20: 127–132.
7. Duning T, Warnecke T, Schirmacher A et al. Specific pattern of early white-matter changes in pure hereditary spastic paraplegia. Mov Disord 2010: 25: 1986–1992.
8. Patrono C, Scarano V, Cricchi F et al. Autosomal dominant hereditary spastic paraplegia: DHPLC-based mutation analysis of SPG4 reveals eleven novel mutations. Hum Mutat 2005: 25: 506.

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