Alu elements mediate large *SPG11* gene rearrangements: further spatacsin mutations

Maria Conceição Pereira, MSc^{1,2}, José Leal Loureiro, MD^{1,3}, Jorge Pinto-Basto, MD^{1,2,4}, Eva Brandão, MD^{1,3}, Ana Margarida Lopes, BSc², Georgina Neves, MD⁵, Pureza Dias, MD⁶, Ruth Gerales, MD⁷, Isabel Pavão Martins, MD, PhD⁷, Vitor Tedim Cruz, MD³, Erik-Jan Kamsteeg, MD, PhD⁸, Han G. Brunner, MD, PhD⁸, Paula Coutinho, MD, PhD^{1,3}, Jorge Sequeiros, MD, PhD^{1,2,4} and Isabel Alonso, PhD^{1,2,4}

Purpose: Hereditary spastic paraplegias compose a group of neurodegenerative disorders with a large clinical and genetic heterogeneity. Among the autosomal recessive forms, spastic paraplegia type 11 is the most common.

Methods: To better understand the spastic paraplegia type 11 mutation spectrum, we studied a group of 54 patients with hereditary spastic paraplegia. Mutation screening was performed by PCR amplification of *SPG11* coding regions and intron boundaries, followed by sequencing. For the detection of large gene rearrangements, we performed multiplex ligation-dependent probe amplification.

Results: We report 13 families with spastic paraplegia type 11 carrying either novel or previously identified mutations. We describe a complex entire *SPG11* rearrangement and show that large gene rearrangements are frequent among patients with spastic

paraplegia type 11. Moreover, we mapped the deletion breakpoints of three different large *SPG11* deletions and provide evidence for *Alu* microhomology-mediated exon deletion.

Conclusion: Our analysis shows that the high number of repeated elements in *SPG11* together with the presence of recombination hotspots and the high intrinsic instability of the 15q locus all contribute toward making this genomic region more prone to large gene rearrangements. These findings enlarge the amount of data relating repeated elements with neurodegenerative disorders and highlight their importance in human disease and genome evolution.

Genet Med 2012;14(1):143–151

Key Words: *Alu*-mediated nonallelic homologous recombination; hereditary spastic paraplegia; large gene rearrangements, spatacsin; *SPG11*

INTRODUCTION

Spastic paraplegia type 11 (SPG11; OMIM# 604360) is the most frequent form of autosomal recessive spastic paraplegias (HSP).^{1,2} It is mainly characterized by rapidly progressive spasticity and muscle weakness of early onset³ and a thin corpus callosum on brain magnetic resonance imaging (MRI); it is often associated with mental impairment and progressive cognitive decline.³ Nonetheless, cases of SPG11 without thin corpus callosum^{4,5} have been reported and, as more families are studied, the clinical spectrum is broadening.

The *SPG11* gene encodes a ubiquitous, highly conserved protein named spatacsin.³ This protein is highly expressed in the central nervous system; recent immunocytochemistry assays showed that spatacsin is distributed throughout the cytoplasm and is probably involved in axonal transport.⁶ A possible involvement in DNA repair has also been suggested; in fact, a new HSP disease-causing gene was recently identified (*KIAA0415*, responsible for SPG48) and its product was found to be involved in DNA repair.⁷ Moreover, this protein is an interactor of two other HSP proteins, spatacsin and spastizin, whose overlapping brain expression patterns⁸ and clinical traits

(SPG11 and SPG15) suggest an involvement of these three proteins in the same pathway.

Among HSP genes, *SPG11* has a high mutation rate, and notwithstanding its size, it is actually the most frequently mutated gene in autosomal recessive spastic paraplegia with thin corpus callosum. To date, more than 100 different mutations^{1–5,9–23} have been reported worldwide, in a total of 128 families with complex autosomal recessive spastic paraplegia.

In this study, we report 13 new families with SPG11 presenting novel or previously described mutations, as well as the molecular mechanism responsible for large deletions in this gene; we also discuss the high frequency of the large gene rearrangements identified in this gene.

MATERIALS AND METHODS

Patients

We selected a group of 54 unrelated Portuguese patients with HSP to screen for *SPG11* mutations: 19 cases with a suggestive pattern of autosomal recessive inheritance and 35 isolated cases. Twenty-one patients presented with pure HSP, whereas 33 had a complex phenotype, with mental impairment, neuropathy,

¹UnIGENE, IBMC, Porto, Portugal; ²CGPP, IBMC, Porto, Portugal; ³Serviço Neurologia, Hospital São Sebastião, Feira, Portugal; ⁴ICBAS, University of Porto, Porto, Portugal;

⁵Serviço Neurologia, Centro Hospitalar de Vila Real/Peso da Régua, Vila Real, Portugal; ⁶Serviço Neurologia, Hospital Santo André de Leiria, Leiria, Portugal; ⁷Serviço Neurologia, Hospital de Santa Maria, Lisboa, Portugal; ⁸Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. Correspondence: Isabel Alonso (ialonso@ibmc.up.pt)

Submitted 12 August 2011; accepted 6 September 2011; advance online publication 5 January 2012. doi:10.1038/gim.2011.7

Table 1 Clinical and imagiologic features of SPG11 patients identified

Case	Gender	Familial/ isolated	csg	HSP	Age at onset (yrs)	Present age (yrs)	TCC	Other signs	Origin	Mutation	Protein
1	M	Familial	+	Complex	22	34	+	Mental impairment	PT	[c.3809T>A] + [c.3809T>A]	[p.V1270D] + [p.V1270D]
2	M	Familial	-	Complex	18	31	+	Cutaneous hyperpigmentation	PT	[c.529_533del] + [c.529_533del]	[p.I177SfsX2] + [p.I177SfsX2]
3	F	Isolated	-	Complex	18	34	+	Mental impairment	PT	[c.529_533del] + c.529_533del]	[p.I177SfsX2] + [p.I177SfsX2]
4	F	Familial	+	Complex	23	50	NA	Neuropathy	PT	[c.529_533del] + [c.529_533del]	[p.I177SfsX2] + [p.I177SfsX2]
5	M	Familial	+	Complex	15	31	+	Slight mental impairment	PT	[c.529_533del] + [c.733_734del]	[p.I177SfsX2] + [p.M245VfsX2]
6	M	Familial	-	Complex	Childhood	42	NA	Mental impairment and neuropathy	PT	[c.733_734del] + [c.733_734del]	[p.M245VfsX2] + [p.M245VfsX2]
7	F	Isolated	+	Complex	13	36	+	Mental impairment	PT	[c.5989_5992del] + [c.5989_5992del]	[p.L1997MfsX60] + [p.L1997MfsX60]
8	M	Familial	-	Complex	15	23	+	Mental impairment	PT	[c.4907-524_5121+317del] + [c.4907-524_5121+317del]	[p.G1636DfsX11] + [p.G1636DfsX11]
9	M	Familial	+	Complex	10	32	NA	Mental impairment	PT	[c.733_734del] + [c.6206-160_6344-833del]	[p.M245VfsX2] + [p.G2069AfsX329]
10	F	Isolated	-	Complex	6	30	+	Mental impairment, ataxia, and neuropathy	PT	[c.5867-3237_6478-450del] + [c.6832_6833del]	[p.T1956SfsX17] + [p.S2278LfsX61]
11	M	Isolated	-	Complex	13	26	+	Mental impairment	PT	[c.4907-524_5121+317del] + [c.4907-524_5121+317del]	[p.G1636DfsX11] + [p.G1636DfsX11]
12	M	Familial	-	Complex	Childhood	18	NA	Mental impairment	NL	[c.5867-3237_6478-450del] + [c.5867-3237_6478-450del]	[p.T1956SfsX17] + [p.T1956SfsX17]
13	M	Familial	-	Complex	Childhood	17	NA	Mental impairment	NL	[c.1348dupA] + [c.5867-3237_6478-450del]	[p.I450NfsX26] + [p.T1956SfsX17]
14	M	Familial	-	Complex	1	30	NA	Mental impairment, epilepsy, and neuropathy	PT	[c.2996_2999delACAG] + [?]	[p.H999LfsX37] + [?]

+, presence; -, absence; csg, consanguinity; HSP, hereditary spastic paraplegia; NA, information not available; PT, Portugal; NL, Netherlands; TCC, thin corpus callosum.

and/or epilepsy in addition to the spasticity of the lower limbs. Some patients presented with a thin corpus callosum, white matter hyperintensity, or medullar atrophy on brain MRI. In addition, *SPG11* mutation screening was also performed in two Dutch families.

Mutation screening

Peripheral blood was collected from patients and their relatives after written informed consent was obtained. Genomic DNA was extracted from peripheral blood leukocytes through salting out,²⁴ and specific primers were designed for all coding regions (including splice site boundaries and intronic flanking regions) of the *SPG11* gene (RefSeq NM_025137.3), using PrimerQuest. Each exon was amplified by PCR with HotStarTaq Master Mix (Qiagen, Hilden, Germany), directly sequenced with the Big Dye Terminator Kit v1.1 (Applied Biosystems, Carlsbad, CA), and loaded on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA).

Multiplex ligation-dependent probe amplification

To detect large gene rearrangements (deletions or duplications), multiplex ligation-dependent probe amplification (MLPA) was performed using the Salsa MLPA kit P306-A1 (MRC-Holland, Amsterdam, The Netherlands), according to the manufacturer's instructions. Fragments were analyzed on an ABI 3130xl Genetic Analyzer using 250-LIZ (Applied Biosystems, Carlsbad, CA), as a size standard, and GeneMarker v1.90 (SoftGenetics, State College, PA).

Long-range PCR and breakpoint analysis

Long-range PCR was performed on genomic DNA from probands to confirm the large *SPG11* deletions detected by MLPA; breakpoints were determined by direct sequencing. The reaction used the Expand Long template PCR system (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. Primers for the exons upstream and downstream of the deletion were used and the deletion breakpoints were afterwards narrowed down by primer walking. Finally, deletion junctions were amplified with specific primer sequences, followed by direct sequencing for breakpoint confirmation.

In silico analysis

All new mutations found were analyzed with Alamut v2.0 (Interactive Biosoftware, Rouen, France). The entire *SPG11* gene (NCBI36:15:42641587:42743768;-1), including 600 bp upstream and downstream of the 5' and 3' untranslated regions, was analyzed using both RepeatMasker (<http://www.repeatmasker.org>) and CENSOR (<http://www.ebi.ac.uk/Tools/censor>) to identify interspersed repeats. Sequence identities of *Alu* repeat signatures of interest were evaluated with the National Center for Biotechnology Information (NCBI) BLASTN tool; the homologous genes of four primate species (*Gorilla gorilla*, *Pan troglodytes*, *Pongo pygmaeus*, and *Macaca mulatta*) were also analyzed with RepeatMasker and NCBI BLASTN for comparison with the human gene regarding the presence or absence

of the particular *Alu* elements of interest. Finally, COMPASS²⁵ was used to search for complex motifs, degenerated from the sequence CCNCCNTNNCCNC, in the human *SPG11* gene.

RESULTS

SPG11 mutations in patients with spastic paraplegia

Mutation screening and gene dosage analysis of *SPG11* allowed the identification of disease-causing mutations in 14 of 54 unrelated HSP patients tested. Through *SPG11* direct sequencing, we identified 6 point mutations (mostly small deletions) including one novel missense and a 4-bp deletion. MLPA allowed us to detect three new large gene rearrangements—two different single-exon deletions, as well as multiple exon deletions, and an interesting case of a heterozygous complex gene rearrangement. The main clinical features and the mutations of the diagnosed patients are summarized in [Table 1](#); the presence of the identified mutations was confirmed in additional affected family members as well as its parental segregation.

The first patient studied (case 1) carried a novel homozygous 1-bp exchange, c.3809T>A in exon 22, resulting in a missense substitution of a valine at protein position 1270 for an aspartic acid ([Figure 1a](#)), a very conserved residue. Of interest, patients 8 and 11 were found to be homozygous for a novel large deletion encompassing exon 29. PCR of this exon did not result in fragment amplification, and MLPA confirmed the homozygous deletion. Although they share the same mutation, the two patients originate from different geographical regions. Patient 9 was found to be a compound heterozygous carrier of the c.733_734delAT, in exon 4, and a novel large deletion comprising exon 33 detected by MLPA. Patient 10 presented a more challenging case as she was found to be compound heterozygous for the c.6832_6833delAG deletion in exon 37 and a complex entire gene rearrangement comprising the duplication of exons 1 to 30 and 35 to 40 in combination with a deletion that encompasses exons 31 to 34 ([Figure 1b](#)). The duplicated area extended at least to the flanking genes *CASC4* and *B2M*, also detected by the MLPA probe mix. This four-exon deletion was also present in two Dutch families, although not associated with this complex rearrangement. Last, we identified a novel frameshift mutation in patient 14, c.2996_2999delACAG, in exon 16, changing a histidine to a leucine at position 999, leading to an altered reading frame and a premature stop codon after 36 residues (p.His999LeufsX37) ([Figure 1c](#)). This patient had a complex form of spastic paraplegia, with neuropathy, epilepsy, and mental impairment, and a family history of the disease; however, after mutation screening and MLPA, we could find only this small deletion in the heterozygous state, thereby being unable to establish a diagnosis of *SPG11*.

Breakpoint deletion determination and deletion mechanism

The breakpoints of the 1056-bp deletion (exon 29) were mapped by primer walking to 524 bp upstream and 317 bp downstream of exon 29 (c.4907-524_5121+317del) in both patients. This deletion ([Figure 2a](#)) leads to a frameshift, generating a premature

Figure 2 Characterization of the spastic paraplegia type 11 (SPG11) intragenic deletions found in this study and schematic representation of its breakpoints. (a) Breakpoint deletion for exon 29 found in two Portuguese families; (b) representation of the exon 33 deletion found in one Portuguese family (showing contamination with wild-type allele in the sequencing product; nevertheless, sequence deconvolution confirms the deletion breakpoints); (c) exon 31 to 34 deletion breakpoint from two Dutch and one Portuguese patient; (d) breakpoint analysis of a previously reported SPG11 deletion shows the same rearrangement mechanism. An *Alu*-mediated mechanism appears to be responsible for all these deletions.

described, both 5' and 3' breakpoints also coincide with *Alu* elements (with the 5' breakpoint within an *AluSc8* in intron 32, and the 3' breakpoint within an *AluY* in intron 33), with high degree of homology (86% identity) between them. Furthermore, this breakpoint matches a 19-bp microhomology domain.

The breakpoints for the four-exon deletion (Figure 2c) were also mapped (c.5867-3237_6478-450del) in the Portuguese and both Dutch families; likewise, it also results in a frameshift and a premature stop codon after 16 residues (p.T1956SfsX17). The boundaries of this 8323-bp deletion, again, overlap *Alu* elements (5' within an *AluY*

Figure 3 Evolutionary conservation of the *Alu* sequences involved in spastic paraplegia type 11 (*SPG11*) deletions in nonhuman primates. The *Alu* elements found in this study to be involved in *SPG11* deletions are boxed. Additional regions within the *SPG11* gene showing these microhomology domains, as well as the evolutionary conservation of the different *Alus* in nonhuman primates, are also presented.

in intron 30 and the 3' coinciding with an *AluSx* in intron 34), possessing an adjacent 42-bp region of microhomology and a high degree of identity (79%). All results found with RepeatMasker for interspersed repeats were confirmed with the CENSOR software; a total of 189 masked elements were found, representing 55% of the *SPG11* gene. Also, COMPASS allowed the identification of 13 degenerate sequences from the CCNCCNTNNCCNC motif, distributed throughout the gene, and totaling 23 occurrences.

DISCUSSION

Spatacin mutation frequency in patients with spastic paraplegia

The *SPG11* mutational spectrum of our cohort comprises mostly small deletions, together with three large-scale rearrangements (six different families), all distributed throughout the *SPG11* gene.

Of interest, we found two recurrent mutations, c.529_533delATATT and c.733_734delAT, which together account for 41% of the mutated alleles. A founder effect has been suggested for a few mutations in *SPG11* patients from the Mediterranean basin.⁵ In particular, a haplotype analysis has shown that both Portuguese and Brazilian patients with the c.529_533delATATT mutation share the same haplotype, pointing toward a common mutational origin.⁴ The 2-bp deletion in exon 4 (c.733_734delAT) is one of the two most frequent

mutations worldwide^{1,3-5,11-13,16,22}, which might suggest either the presence of a mutational hotspot or a significantly old mutational event. Another interesting finding in this study is the frequency of large deletions, which appear in almost half of our index cases.

In addition, we have also found a novel missense mutation (V1270D) that replaces a highly conserved hydrophobic residue by a negatively charged hydrophilic amino acid (Figure 1a). All Web-based variant scoring algorithms used by Alamut (PolyPhen-2, SIFT, Align GVG), as well as PANTHER,²⁶ SNAP,²⁷ PMut,²⁸ and PhD-SNP,²⁹ predicted this mutation to be pathogenic. This substitution is located adjacent to the third highly conserved α -helix³ and thus could have a major impact on protein folding.

The novel 4-bp deletion results in a premature termination codon in exon 16, and although we could not confirm *SPG11* diagnosis in the patient carrying this deletion, we cannot fully exclude it either, since the second disease-causing mutation may be located in regions that our genetic screen does not cover: the promoter, the 3' or 5' untranslated regions (intronic flanking regions required for splicing were covered by our analysis). As a general rule, only premature termination codons (PTCs) located <50–55 nucleotides upstream, or downstream of the last exon–exon junction, manage to escape nonsense-mediated decay (NMD).³⁰ Hence, the mutant transcript in question is probably targeted for degradation, because it is located closer to the 5' end of the mRNA and upstream of several exon–exon

junctions. Nevertheless, there are exceptions to this rule. Namely, it was recently shown that a PTC-causing mutation in the middle of the GABA_A receptor GABRA1 escapes NMD;³¹ thus, we can neither confirm nor exclude *SPG11* diagnosis.

In contrast, the large deletion found in patient 9, which causes exon 33 skipping, leads to a frameshift and a premature termination codon after 328 amino acids, meaning that, if translated, the protein would have almost a full-length size (2,397 amino acids), i.e., only 46 amino acids shorter than the wild type. Assuming a correct splicing of the remaining exons downstream of the deleted portion, and considering that the PTC is positioned after the last exon–exon junction, it is highly probable that this transcript escapes NMD, originating an almost full-length protein with a mutant C-terminal. This novel spatacin C-terminus may behave differently from the normal protein, by abnormal protein interaction or trafficking, which could result in a more severe phenotype. In fact, this patient shows a phenotype more severe than average, with a classical age-at-onset (10 years), but presenting an unusually fast progression to wheelchair by the age of 18; this may be due to the presence/aberrant trafficking of the mutant protein.

The large duplicated region found in patient 10 (at least from *B2M* to *CASC4*, i.e., ~300 kb) does not seem to overly contribute to the patient's phenotype. Here, the four-exon deletion (31–34), in combination with the 2 bp point mutation, appears to be responsible for the disease, resulting in the clinical signs compatible with an *SPG11* phenotype. It appears that this complex rearrangement has arisen from an *SPG11* allele missing exons 31–34, which was afterwards duplicated, encompassing a considerably large flanking region as MLPA has allowed us to determine.

There are several disorders associated with the 15q locus involving copy number variations that can comprise deletions, duplications, and even triplications—Angelman/Prader-Willi syndrome, 15q duplication syndrome, and 15q triplication³²—which altogether illustrate the high degree of instability in this chromosomal region.

Alu-mediated *SPG11* rearrangements

Regarding the single or multiple exon deletions found in our set of patients, the identification of the same microhomology domains in other *Alu* elements spread throughout the *SPG11* gene (Figure 3) gives rise to the hypothesis that different, yet similar, large-scale rearrangements may occur through recombination mediated by the various *Alu* elements.

Although the microhomology sequence involved in the deletion of exons 31–34 does not appear frequently in other *Alu* elements, as it happens in other cases, the same deletion has been found so far in five additional families of German and Dutch descent.^{4,10,22} This could suggest that the genomic architecture of this particular gene region is prone to the occurrence of this specific recombination, as we also found it in Portuguese patients.

Besides the deletions reported here, another has been mapped involving the loss of exons 37 to 39 in an Italian patient.¹¹ Our analysis shows that this large gene rearrangement also overlaps with *Alu* elements and its breakpoints involve a

microhomology sequence that is also present in five other *Alu* elements (Figure 2d).

For all *SPG11* rearrangements mapped to date, the microhomology region adjacent to both breakpoints is located in the same relative position, closer to one or the other of the *Alu* extremities. The recombination event excises complementary parts of the *Alu* elements so that an *Alu* is left in the recombination spot and another leaves with the deleted portion; thus, these exchange genetic material with each other, allowing for an inter-/intrachromosomal form of nonallelic homologous recombination to take place (Figure 2). Our findings indicate that, although each deletion appears to be unique, *Alu* sequences are determinant for the instability observed at the *SPG11* locus. The high frequency of *Alu* elements in *SPG11* suggests a locus prone to de novo rearrangements; however, among six patients carrying these large deletions, parental segregation excluded a de novo event in five of the families. Notwithstanding, no information is available regarding the presence of *SPG11* rearrangements in the general healthy population.

Evolutionary conservation of *Alus* involved in human *SPG11* deletions in nonhuman primates

To look at these *Alu* elements in the *SPG11* gene from an evolutionary point of view, which could help to explain the genetic rearrangements we found, the *SPG11* gene sequence of four nonhuman primates was analyzed (Figure 3). Our results show that the *Alu* elements containing the microhomology regions that are involved in the large deletions found in *SPG11* patients are also present in nonhuman primates and conserved across evolution. These repetitive elements appear to have increased in number, at the *SPG11* locus, throughout evolution, with the exception of the Gorilla, though this may be due to the gene not being fully sequenced in this species. Apparent to the observer is also the increase in elements belonging to the newest *Alu* family, *AluY*.³³ Altogether, the increasing number of *Alus* present in the gene ultimately favors recombinatory events that might lead to a loss of segments of the *SPG11* gene through an intra-/interchromosomal mechanism of nonallelic homologous recombination.

Transposable elements are highly frequent in the human genome, constituting up to 40% of its sequence.³⁴ The majority of these elements lost their transposition ability a long time ago, although some of them, in particular, LINE-1 and *Alu*, have been shown to maintain this capacity, thus contributing to recent evolutionary history of the primate genomes³⁵ and also to the genomic instability found to be involved in cancer and several genetic disorders.³⁶

Similar to what happens with the *BRCA1* gene, often known to suffer *Alu*-mediated deletions,^{37,38} analysis of *SPG11* also shows a high density of interspersed repeats, namely *Alu* elements, which together with the large size of this gene (~101 kb) favors the occurrence of various rearrangements. In addition, and similar to what seems to be happening with the *SPG11* deletions, the *Alu*-mediated *BRCA1* rearrangements arise from nonallelic homologous recombination.

Recombination hotspots are apparently unrelated to spatacsin rearrangements

Recently, a degenerate 13-bp motif (CCNCCNTNNCCNC) was associated with recombination hotspots and genome instability in humans.³⁹ Moreover, this motif was present in breakpoint regions of disease-causing nonallelic homologous recombination hotspots⁴⁰ and could, in theory, also be involved in *SPG11* deletions. Through bioinformatic analysis, we found 13 degenerate sequences that occur 23 times over the gene's length, although none of them is located near any of the deletions identified in *SPG11* patients. Nevertheless, other recombination events mediated by clusters of this motif might occur, more specifically in introns 18 and 20, where these elements seem to cluster.

Conclusions

In this study, we report 13 new families with *SPG11*, carrying both novel and previously reported mutations. We describe a complex entire *SPG11* rearrangement and show that large gene rearrangements are frequent among patients with *SPG11*. In addition, we present the molecular characterization of three large *SPG11* deletions together with their mechanisms and show a high frequency for *Alu*-mediated deletions in this gene. Our analysis suggests that the high number of repeated elements in *SPG11* together with the presence of recombination hotspots and the high intrinsic instability of the 15q locus all contribute toward making this genomic region prone to large gene rearrangements.

Our findings enlarge the amount of data relating repeated masked elements to neurodegenerative disorders and highlight the importance of these elements in human disease and in evolution.

ACKNOWLEDGMENTS

We thank all patients and their families who participated in this study. We also thank all the physicians who referred HSP patients to our center. This work was supported by research grant PIC/IC/83232/2007, FCT (Fundação para a Ciência e Tecnologia), cofunded by FEDER (Fundo Europeu de Desenvolvimento Regional) and COMPETE (Programa Operacional Factores de Competitividade) and by Financiamento Plurianual de Unidades de Investigação (FCT). I.A. is funded by Programa Ciência, POPH – QREN – Tipologia 4.2 – Promoção do Emprego Científico, cofunded by ESF, and national funds from the MCTES (Ministério da Ciência e Ensino Superior).

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

- Boukhris A, Stevanin G, Feki I, et al. Hereditary spastic paraplegia with mental impairment and thin corpus callosum in Tunisia: SPG11, SPG15, and further genetic heterogeneity. *Arch Neurol* 2008;65:393–402.
- Erichsen AK, Stevanin G, Denora P, Brice A, Tallaksen CM. SPG11—the most common type of recessive spastic paraplegia in Norway? *Acta Neurol Scand, Suppl* 2008;188:46–50.
- Stevanin G, Santorelli FM, Azzedine H, et al. Mutations in SPG11, encoding spatacin, are a major cause of spastic paraplegia with thin corpus callosum. *Nat Genet* 2007;39:366–372.
- Denora PS, Schlesinger D, Casali C, et al. Screening of ARHSP-TCC patients expands the spectrum of SPG11 mutations and includes a large scale gene deletion. *Hum Mutat* 2009;30:E500–E519.
- Stevanin G, Azzedine H, Denora P, et al. Mutations in SPG11 are frequent in autosomal recessive spastic paraplegia with thin corpus callosum, cognitive decline and lower motor neuron degeneration. *Brain* 2008;131(Pt 3):772–784.
- Murmu RP, Martin E, Rastetter A, et al. Cellular distribution and subcellular localization of spatacin and spastizin, two proteins involved in hereditary spastic paraplegia. *Mol Cell Neurosci* 2011;47:191–202.
- Slabicki M, Theis M, Krastev DB, et al. A genome-scale DNA repair RNAi screen identifies SPG48 as a novel gene associated with hereditary spastic paraplegia. *PLoS Biol* 2010;8:e1000408.
- Hanein S, Martin E, Boukhris A, et al. Identification of the SPG15 gene, encoding spastizin, as a frequent cause of complicated autosomal-recessive spastic paraplegia, including Kjellin syndrome. *Am J Hum Genet* 2008;82:992–1002.
- Anheim M, Lagier-Tourenne C, Stevanin G, et al. SPG11 spastic paraplegia. A new cause of juvenile parkinsonism. *J Neurol* 2009;256:104–108.
- Bauer P, Winner B, Schüle R, et al. Identification of a heterozygous genomic deletion in the spatacin gene in SPG11 patients using high-resolution comparative genomic hybridization. *Neurogenetics* 2009;10:43–48.
- Crimella C, Arnoldi A, Crippa F, et al. Point mutations and a large intragenic deletion in SPG11 in complicated spastic paraplegia without thin corpus callosum. *J Med Genet* 2009;46:345–351.
- Del Bo R, Di Fonzo A, Ghezzi S, et al. SPG11: a consistent clinical phenotype in a family with homozygous spatacin truncating mutation. *Neurogenetics* 2007;8:301–305.
- Hehr U, Bauer P, Winner B, et al. Long-term course and mutational spectrum of spatacin-linked spastic paraplegia. *Ann Neurol* 2007;62:656–665.
- Kim SM, Lee JS, Kim S, et al. Novel compound heterozygous mutations of the SPG11 gene in Korean families with hereditary spastic paraplegia with thin corpus callosum. *J Neurol* 2009;256:1714–1718.
- Lee MJ, Cheng TW, Hua MS, et al. Mutations of the SPG11 gene in patients with autosomal recessive spastic paraparesis and thin corpus callosum. *J Neurol Neurosurg Psychiatr* 2008;79:607–609.
- Liao SS, Shen L, Du J, et al. Novel mutations of the SPG11 gene in hereditary paraplegia with thin corpus callosum. *J Neurol Sci* 2008;275:92–99.
- Orlén H, Melberg A, Raininko R, et al. SPG11 mutations cause Kjellin syndrome, a hereditary spastic paraplegia with thin corpus callosum and central retinal degeneration. *Am J Med Genet B Neuropsychiatr Genet* 2009;150B:984–992.
- Paisan-Ruiz C, Dogu O, Yilmaz A, Houlden H, Singleton A. SPG11 mutations are common in familial cases of complicated hereditary spastic paraplegia. *Neurology* 2008;70(16 Pt 2):1384–1389.
- Paisan-Ruiz C, Nath P, Wood NW, Singleton A, Houlden H. Clinical heterogeneity and genotype-phenotype correlations in hereditary spastic paraplegia because of spatacin mutations (SPG11). *Eur J Neurol* 2008;15:1065–1070.
- Pippucci T, Panza E, Pompili E, et al. Autosomal recessive hereditary spastic paraplegia with thin corpus callosum: a novel mutation in the SPG11 gene and further evidence for genetic heterogeneity. *Eur J Neurol* 2009;16:121–126.
- Samaranch L, Riverol M, Masdeu JC, et al. SPG11 compound mutations in spastic paraparesis with thin corpus callosum. *Neurology* 2008;71:332–336.
- Schüle R, Schlipf N, Synofzik M, et al. Frequency and phenotype of SPG11 and SPG15 in complicated hereditary spastic paraplegia. *J Neurol Neurosurg Psychiatr* 2009;80:1402–1404.
- Southgate L, Dafou D, Hoyle J, et al. Novel SPG11 mutations in Asian kindreds and disruption of spatacin function in the zebrafish. *Neurogenetics* 2010;11:379–389.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- Maccari G, Gemignani F, Landi S. COMPASSS (Complex Pattern of Sequence Search Software), a simple and effective tool for mining complex motifs in whole genomes. *Bioinformatics* 2010;26:1777–1778.
- Thomas PD, Campbell MJ, Kejariwal A, et al. PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res* 2003;13:2129–2141.

27. Bromberg Y, Rost B. SNAP: predict effect of non-synonymous polymorphisms on function. *Nucleic Acids Res* 2007;35:3823–3835.
28. Ferrer-Costa C, Gelpí JL, Zamakola L, Parraga I, de la Cruz X, Orozco M. PMUT: a web-based tool for the annotation of pathological mutations on proteins. *Bioinformatics* 2005;21:3176–3178.
29. Capriotti E, Calabrese R, Casadio R. Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. *Bioinformatics* 2006;22:2729–2734.
30. Holbrook JA, Neu-Yilik G, Hentze MW, Kulozik AE. Nonsense-mediated decay approaches the clinic. *Nat Genet* 2004;36:801–808.
31. Kang JQ, Shen W, Macdonald RL. Two molecular pathways (NMD and ERAD) contribute to a genetic epilepsy associated with the GABA(A) receptor GABRA1 PTC mutation, 975delC, S326fs328X. *J Neurosci* 2009;29:2833–2844.
32. Kumar D. Disorders of the genome architecture: a review. *Genomic Med* 2008;2:69–76.
33. Witherspoon DJ, Watkins WS, Zhang Y, et al. Alu repeats increase local recombination rates. *BMC Genomics* 2009;10:530.
34. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860–921.
35. Deininger PL, Batzer MA. Mammalian retroelements. *Genome Res* 2002;12:1455–1465.
36. Cordaux R, Batzer MA. The impact of retrotransposons on human genome evolution. *Nat Rev Genet* 2009;10:691–703.
37. Mazoyer S. Genomic rearrangements in the BRCA1 and BRCA2 genes. *Hum Mutat* 2005;25:415–422.
38. Ticha I, Kleibl Z, Stribrna J, et al. Screening for genomic rearrangements in BRCA1 and BRCA2 genes in Czech high-risk breast/ovarian cancer patients: high proportion of population specific alterations in BRCA1 gene. *Breast Cancer Res Treat* 2010;124:337–347.
39. Myers S, Freeman C, Auton A, Donnelly P, McVean G. A common sequence motif associated with recombination hot spots and genome instability in humans. *Nat Genet* 2008;40:1124–1129.
40. Roehl AC, Vogt J, Mussotter T, et al. Intrachromosomal mitotic nonallelic homologous recombination is the major molecular mechanism underlying type-2 NF1 deletions. *Hum Mutat* 2010;31:1163–1173.