

Spastic paraplegia gene 7 in patients with spasticity and/or optic neuropathy

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Mutations in the spastic paraplegia 7 (SPG7) gene encoding paraplegin are responsible for autosomal recessive hereditary spasticity. We screened 135 unrelated index cases, selected in five different settings: SPG7-positive patients detected during SPG31 analysis using SPG31/SPG7 multiplex ligation-dependent probe amplification ($n = 7$); previously reported ambiguous SPG7 cases ($n = 5$); patients carefully selected on the basis of their phenotype (spasticity of the lower limbs with cerebellar

signs and/or cerebellar atrophy on magnetic resonance imaging/computer tomography scan and/or optic neuropathy and without other signs) ($n = 24$); patients with hereditary spastic paraparesis referred consecutively from attending neurologists and the national reference centre in a diagnostic setting ($n = 98$); and the index case of a four-generation family with autosomal dominant optic neuropathy but no spasticity linked to the *SPG7* locus. We identified two *SPG7* mutations in 23/134 spastic patients, 21% of the patients selected according to phenotype but only 8% of those referred directly. Our results confirm the pathogenicity of Ala510Val, which was the most frequent mutation in our series (65%) and segregated at the homozygous state with spastic paraparesis in a large family with autosomal recessive inheritance. All *SPG7*-positive patients tested had optic neuropathy or abnormalities revealed by optical coherence tomography, indicating that abnormalities in optical coherence tomography could be a clinical biomarker for *SPG7* testing. In addition, the presence of late-onset very slowly progressive spastic gait (median age 39 years, range 18–52 years) associated with cerebellar ataxia (39%) or cerebellar atrophy (47%) constitute, with abnormal optical coherence tomography, key features pointing towards *SPG7*-testing. Interestingly, three relatives of patients with heterozygote *SPG7* mutations had cerebellar signs and atrophy, or peripheral neuropathy, but no spasticity of the lower limbs, suggesting that *SPG7* mutations at the heterozygous state might predispose to late-onset neurodegenerative disorders, mimicking autosomal dominant inheritance. Finally, a novel missense *SPG7* mutation at the heterozygous state (Asp411Ala) was identified as the cause of autosomal dominant optic neuropathy in a large family, indicating that some *SPG7* mutations can occasionally be dominantly inherited and be an uncommon cause of isolated optic neuropathy. Altogether, these results emphasize the clinical variability associated with *SPG7* mutations, ranging from optic neuropathy to spastic paraplegia, and support the view that *SPG7* screening should be carried out in both conditions.

Keywords: *SPG7*; hereditary spastic paraparesis; optic neuropathy; cerebellar atrophy, optical coherence tomography

Abbreviations: AAA = ATPases associated with a variety of cellular activities; MLPA = multiplex ligation-dependent probe amplification; OCT = optical coherence tomography

Introduction

Mutations in spastic paraplegia gene 7 (*SPG7*) (MIM 602783) are responsible for *SPG7* hereditary spastic paraparesis, the first autosomal hereditary spastic paraplegia for which the causative gene was identified (Casari *et al.*, 1998). However, hereditary spastic paraplegia are widely known to be clinically and genetically heterogeneous diseases with >50 different chromosomal loci with autosomal dominant, autosomal recessive or X-linked forms reported. In the last few years, at least 20 genes responsible for hereditary spastic paraplegia have been identified (Blackstone *et al.*, 2011). Gene testing is consequently more and more complex, and the establishment of genotype–phenotype correlations is of great importance. In daily practice, most cases with spasticity of the lower limbs appear to be sporadic. Even when a family history is reported, the small size of the families does not always allow the mode of inheritance to be determined. Indeed, the reduced penetrance and variable expression observed in autosomal dominant forms of hereditary spastic paraplegia can mimic recessive inheritance. Although identification of the mutation in a patient has no implications for treatment as yet, it is essential for appropriate genetic counselling.

SPG7 is composed of 17 exons that contain a high frequency of rare nucleotide variants of unknown status (Elleuch *et al.*, 2006), complicating interpretation of the results of molecular testing. Paraplegin, the protein encoded by *SPG7*, is a member of the AAA (ATPases associated with a variety of cellular activities) protein family (Casari *et al.*, 1998), which is localized to the inner mitochondrial membrane (Atorino *et al.*, 2003; Koppen *et al.*, 2007). Paraplegin assembles with the paralogous AFG3L2 protein

(MIM 604581) to form the oligomeric mAAA protease complex involved in mitochondrial protein maturation and degradation (Leonhard *et al.*, 2000; Nolden *et al.*, 2005; Rugarli and Langer, 2006; Tatsuta and Langer, 2009). Interestingly, heterozygous mutations in the *AFG3L2* gene are responsible for the autosomal dominant spinocerebellar ataxia SCA28, which is characterized by young adult onset, slowly progressive gait and limb ataxia, dysarthria, increased reflexes and ophthalmoparesis (Edener *et al.*, 2010).

Mutations in *SPG7* were initially reported in three families, two with isolated spasticity and one with a complex phenotype (Casari *et al.*, 1998); however, the clinical characteristics and typical time course of the *SPG7* hereditary spastic paraplegia subtype remains unclear. Cerebellar involvement and/or mild cerebellar atrophy observed on MRI scans are the most frequent additional features (Casari *et al.*, 1998; Wilkinson *et al.*, 2004; Elleuch *et al.*, 2006; Warnecke *et al.*, 2007; Brugman *et al.*, 2008), followed by optic neuropathy (Casari *et al.*, 1998; De Michele *et al.*, 1998; McDermott *et al.*, 2001), ptosis (Tzoulis *et al.*, 2008; Warnecke *et al.*, 2010) and supranuclear palsy (Warnecke *et al.*, 2007). Age at onset ranges from 10 to 45 years (McDermott *et al.*, 2001; Elleuch *et al.*, 2006; Warnecke *et al.*, 2007; Brugman *et al.*, 2008; Tzoulis *et al.*, 2008). The frequency of *SPG7* mutations has varied from 1.5% to 12% among studies due to differences in inclusion criteria (Wilkinson *et al.*, 2004; Elleuch *et al.*, 2006; Brugman *et al.*, 2008). The role of single heterozygous mutations in *SPG7* is still unresolved at this time (McDermott *et al.*, 2001; Elleuch *et al.*, 2006) but a recent publication suggests a dominant effect without clinical analysis of the carriers (Sanchez-Ferrero *et al.*, 2012).

We present here the results of a *SPG7* analysis, including sequencing and the search for micro-rearrangements in 134 patients with spastic features independently of their mode of transmission and in a four-generation family segregating autosomal dominant optic neuropathy with no spastic gait linked to the *SPG7* locus. This allowed us to define the clinical and mutation spectrum of the disease associated with *SPG7* and to propose guidelines as to when *SPG7* should be tested in patients with hereditary spastic paraplegia and patients with autosomal dominant optic neuropathy.

Materials and methods

Patients

After obtaining informed written consent, we studied 134 unrelated hereditary spastic paraplegia index cases and 7 out of 10 affected members of a four-generation French family with isolated autosomal dominant optic neuropathy without spasticity unambiguously linked to the *SPG7* locus (Figs 1 and 2). All patients with hereditary spastic paraplegia had previously tested negative in an analysis of the *SPAST* (*SPG4*) gene including direct sequencing of the coding sequence and multiplex ligation probe-dependent amplification (MLPA, kit P165). Patients with ages at onset <10 years were also negative for the *ATL1* (*SPG3A*) gene. Seven patients tested *SPG7*-positive in a screen for *SPG31* mutations including a search for rearrangements in the *SPG7/SPG31* genes using the P213 MLPA kit (MRC-Holland) (Goizet *et al.*, 2011). Five patients with autosomal recessive inheritance were previously reported by us as having only one clearly

deleterious heterozygous mutation or homozygous Ala510Val mutations (Elleuch *et al.*, 2006). One hundred and twenty-two patients were selected for *de novo SPG7* screening. They were divided into two groups. One group ($n = 98$), designated as the 'routine' diagnostic group, included patients with spastic paraplegia referred consecutively by the attending neurologists. The other group ($n = 24$), designated the research group, included carefully selected patients, sampled by the clinical research network SPATAX (spastic paraplegia and cerebellar ataxia network), who consented to participate in research. The selection criteria for the research group were the presence of spastic paraparesis with cerebellar signs and/or cerebellar atrophy on MRI/CT scans and/or optic neuropathy. Patients in both groups originated mostly from France [64/98 (65%) and 17/24 (70%), respectively]. The remaining patients originated from North Africa ($n = 18$), Portugal ($n = 7$), Spain ($n = 2$), the Middle East ($n = 2$), Great Britain ($n = 1$), Poland ($n = 1$), Serbia ($n = 1$), Italy ($n = 1$), Columbia ($n = 1$), the West Indies ($n = 2$), Guinea ($n = 1$) and Chechnya ($n = 1$). Unfortunately, the origin of three patients was unknown. The age at onset was defined as first symptom noticed by the patient and/or relatives. The mean age at onset was 26.6 ± 19.1 years (range 1–65 years), and the age at examination 42.5 ± 18.3 years (range 2–85 years). The overall severity was moderate, but 34% of the patients needed help walking or used wheelchairs (7%).

In the diagnostic group ($n = 98$), the patients were mostly isolated ($n = 56$, 57%); 32 had an affected sib compatible with autosomal recessive transmission (33%) and six (6%) had a family history of dominant inheritance. No information on the family history was available for four patients (4%). In the research group ($n = 24$), 12 patients (50%) were isolated; six patients (25%) had family histories compatible with autosomal recessive transmission and six (25%) had dominant inheritance.

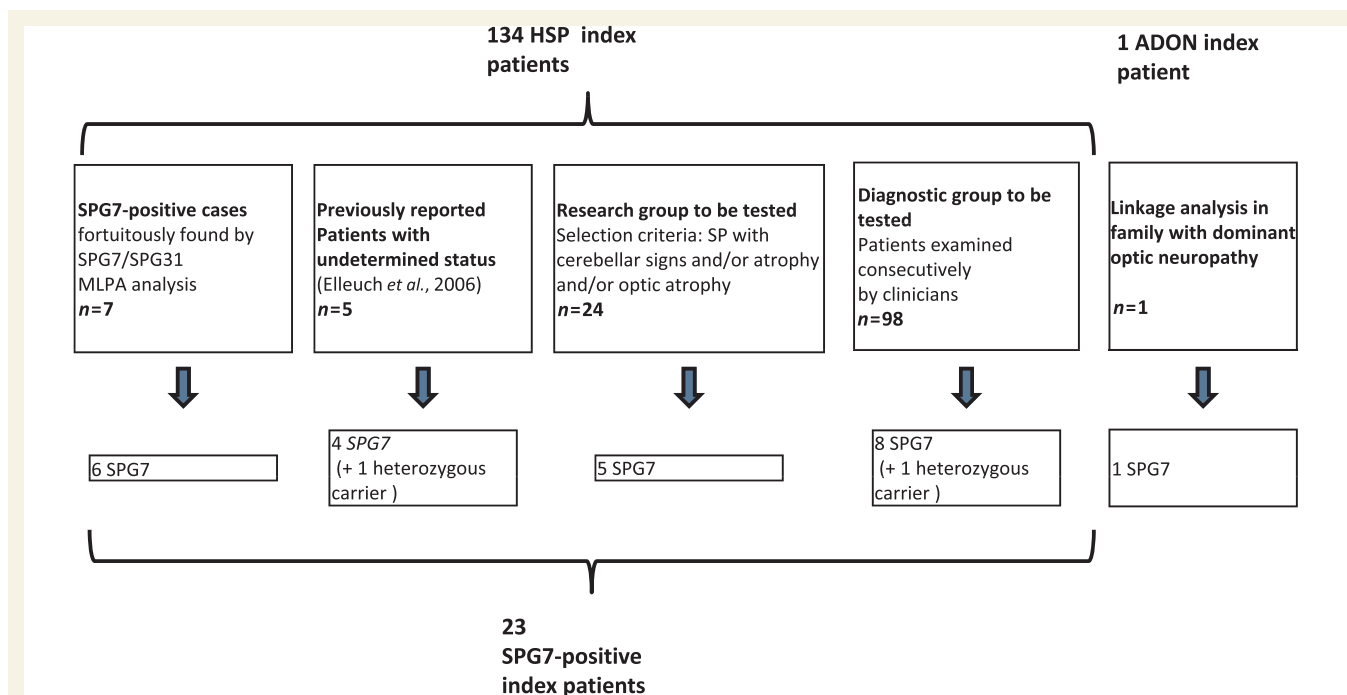


Figure 1 Study design. Five different patients groups were screened for *SPG7* mutations; 23 *SPG7*-positive patients were identified among 134 probands with spastic paraplegia (SP). ADON = isolated autosomal dominant optic neuropathy; HSP = hereditary spastic paraplegia.

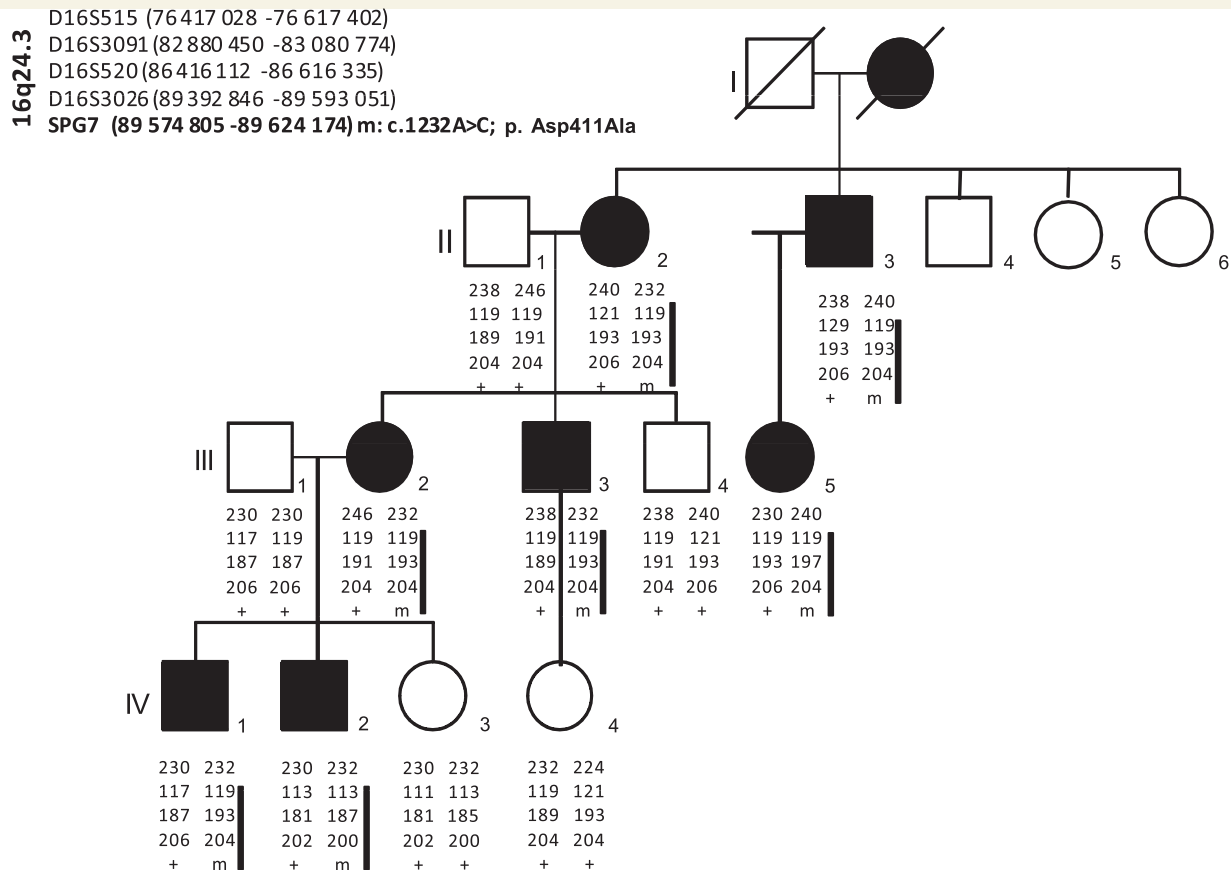


Figure 2 Pedigree of the family with autosomal dominant optic neuropathy, showing haplotypes at the SPG7 locus. Genome-wide search for linkage was performed in seven affected members using Hap300Duov2SNP Arrays (Illumina). Several candidate regions were pointed but analyses using microsatellite markers in both affected and unaffected members identified—under the hypothesis of complete penetrance—a unique candidate region on chromosome 16q24.3 (maximum multipoint LOD-score $Z_{\max} = 3$). All affected individuals segregate a common haplotype harbouring the SPG7 p.Asp411Ala mutation (black bar), absent in all unaffected individuals.

The clinical phenotype was defined as ‘isolated’ or ‘pure’ spastic paraparesis in the diagnostic group (40/98) more often than in the research group (0/24). Most patients with complicated spastic paraplegia had cerebellar signs or atrophy (51%), 15% had optic neuropathy and 40% (37/92) decreased vibration sense. Additional associated symptoms included cognitive impairment ($n = 14$ patients, including 12 with mental retardation and two with dementia), polyneuropathy ($n = 13$ patients, including three demyelinating sensorimotor, two axonal sensorimotor, one axonal sensitive and seven demyelinating sensitive polyneuropathy without clear information), generalized epilepsy ($n = 5$), hearing impairment ($n = 6$), atrophy of the corpus callosum ($n = 5$), extrapyramidal symptoms ($n = 3$) and tetraspasticity ($n = 1$).

Neurological examination of patients with isolated autosomal dominant optic neuropathy

The index case (Case III3) of the four-generation family with isolated autosomal dominant optic neuropathy who has the Asp411Ala mutation in SPG7 at the heterozygous state (Family 24, Fig. 2) underwent a standardized neurological examination, conduction velocity and

electromyography as well as brain MRI. In addition to the index case, none of the six affected individuals with optic neuropathy reported any gait or walking difficulties or instability.

Ophthalmological examination

Ten SPG7-positive patients with hereditary spastic paraplegia underwent a standardized ophthalmological examination of the fundus, visual field and visual acuity. It included optical coherence tomography (OCT), which provides an image of the optic disc and peripapillary region, and generates reproducible measurements of the nerve fibre layer. OCT imaging was performed with Stratus® Carl Zeiss–Meditec OCT machine, based on time-domain detection system (Carl Zeiss Meditec, Inc.).

SPG7 analysis

We amplified the whole coding region of the paraplegin gene from genomic DNA by 17 PCRs, with a primer pair for each exon, as previously described (Elleuch *et al.*, 2006). Amplicons were directly sequenced in both directions using the Big Dye® Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). The sequence

products were run on an ABI 3730 automated sequencer (PE Applied Biosystems) and the sequences analysed with SeqScape 2.5 software (PE Applied Biosystems). Mutations found in patients were searched for directly in available relatives by direct sequencing of the corresponding exon. In addition, 180 European controls (healthy spouses of patients with other neurological diseases) were included to test new variants in the *SPG7* gene.

The MLPA P213 kit, designed to search for *SPG7/SPG31* rearrangements, was used for all patients with hereditary spastic paraplegia (MRC-Holland). MLPA reactions were carried out according to the manufacturer's instructions. Electrophoresis of PCR products was performed using an ABI 3730 sequencer and MLPA data were analysed using the GeneMapper 3.5 software (Applied Biosystems). Relative ratios were calculated using the formula $r = \text{mean}(\text{peak area}_{\text{patient}}/\text{control area}_{\text{patient}})/(\text{peak area}_{\text{control individual}}/\text{control area}_{\text{control individual}})$.

SCA28 analysis

Exons 10, 15, 16 of *AFG3L2* (SCA28), mapping to the AAA-domain (exon 10) and M41-protease domain of the *AFG3L2* protein were screened in patients with hereditary spastic paraplegia for phenotype modifiers by direct sequencing, as previously described (Cagnoli *et al.*, 2010). All mutations so far reported in patients with SCA28 are located in one of these exons (Cagnoli *et al.*, 2010; Di Bella *et al.*, 2010; Edener *et al.*, 2010).

Statistical and bioinformatic analyses

Frequency calculations and comparisons of means were performed with SPSS software. Identification of mutations and analyses of amino acid conservation in orthologues were performed with Alamut 2.0 Software (Interactive Biosoftware).

Results

Genetic analyses

Patients with spasticity of the lower limbs

While screening patients with dominant hereditary spastic paraplegia for *SPG31* mutations with the P213 MLPA kit (MRC-Holland), which also contains probes for *SPG7*, we identified seven patients with an apparent heterozygous or homozygous deletion of one or two *SPG7* exon(s). Direct sequencing of the corresponding exons and screening of the remaining *SPG7* coding sequence in these patients revealed deleterious *SPG7* mutations or deletions in six out of seven cases (Table 1, Patients 5–10); a synonymous polymorphism was identified in the seventh patient. In three out of the six positive patients, a pathogenic heterozygous mutation was associated with the heterozygous c.1529C>T/p.Ala510Val variant, previously described as a polymorphism and recently recognized as a hypomorphic and possibly pathogenic variant (Bonn *et al.*, 2010).

To gain further insight into the frequency and mode of inheritance of *SPG7* mutations in hereditary spastic paraplegia, we screened 122 patients divided into a routine diagnostic and a research group (see 'Materials and Methods' section) for *SPG7* mutations. Five previously reported patients with undetermined status were also included (Elleuch *et al.*, 2006). Four out of these five

patients had one homozygous or two heterozygous mutations. The mutation (Met1?, Gln507X) was associated in *trans* with the Ala510Val mutation in two cases or with a mutation that had been missed (Leu78X) in one case (index Patient 2). In the fourth case, no other mutation or variant was found (Gln82del). In the remaining family (index Patient 3), the Ala510Val mutation perfectly segregated at the homozygous state with spastic paraplegia (Supplementary Fig. 1).

A homozygous *SPG7* mutation ($n = 2$) or two heterozygous *SPG7* mutations ($n = 11$) were identified in 13 of the 122 newly screened patients (10.7%) (Table 1). Ala510Val was associated with another mutation in 9 out of 13 families. Arguments in favour of the pathogenic status of the identified changes are listed in Supplementary Table 1. In addition, none of the mutations were found in our 180 European controls. The mutations segregated perfectly with the disease in all families where this could be tested ($n = 7$) (Supplementary Fig. 1). Overall, this study reports 23 confirmed *SPG7*-positive patients: six were found fortuitously by MLPA analysis, four were previously considered to be of undetermined status (Elleuch *et al.*, 2006), five patients were newly identified in the research group and eight in the diagnostic group (Fig. 1). Two patients with single heterozygous variants were still considered to have undetermined *SPG7* status.

Remarkably, the proportion of patients with hereditary spastic paraplegia with mutations was comparable in families including two affected siblings (5/44, 11%) and in isolated cases (7/71, 10%).

The percentage of *SPG7*-positive patients with hereditary spastic paraplegia was higher in the selected research group (20.8%, 5/24) than in the consecutively examined diagnostic group (8.1%, 8/98), although the difference was not significant ($P = 0.84$).

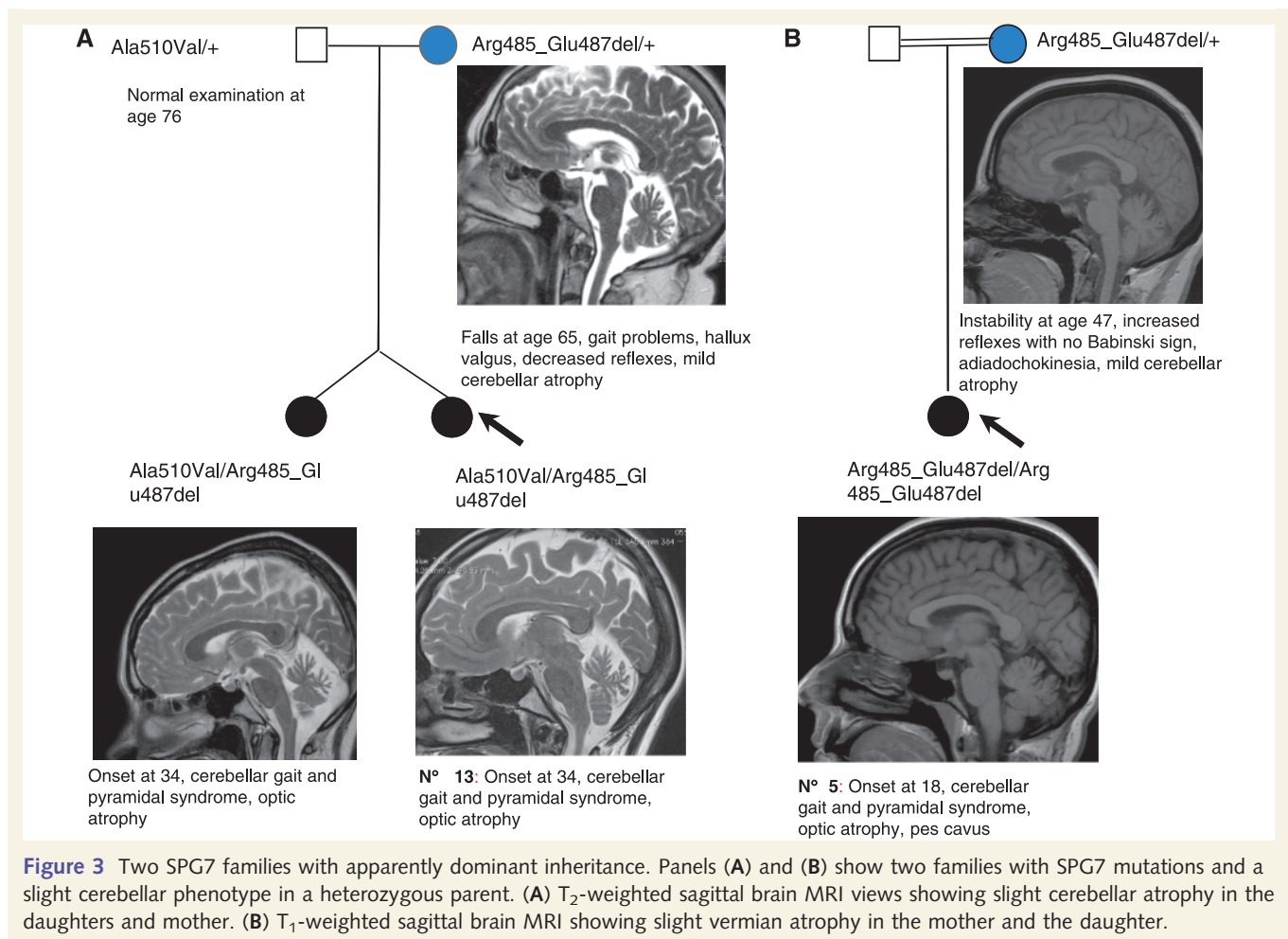
Compound heterozygous *SPG7* mutations were found in three hereditary spastic paraplegia families with possible autosomal dominant inheritance (index Cases 6, 8 and 13). In Family 13, the mother had a heterozygous Arg485_Glu487del mutation. When examined at the age of 72 years, she had a very slight cerebellar phenotype without spasticity or pyramidal signs (Fig. 3A). The mother of Patient 5, who also had a heterozygous Arg485_Glu487del mutation, had a cerebellar gait and cerebellar atrophy on MRI (Fig. 3B). In addition, the brother of the index case in Family 3 with a heterozygous Ala510Val mutation developed a late-onset peripheral neuropathy (Supplementary Fig. 1). The patient had abolished reflexes at the age of 59 years, with pyramidal signs. Electrophysiological investigation at the age of 65 years showed that motor nerve conduction velocities and amplitudes were reduced and sensory nerve action potentials were more markedly reduced or even abolished; neurogenic changes were present. This suggested a sensory motor neuropathy of the axonal type, more marked in the lower limbs. A second examination at the age of 76 years revealed increased axonal degeneration. No common causes, such as diabetes, were found.

Overall, homozygous mutations were found in 17% (4/23) of the cases and compound heterozygous mutations in 83% (19/23). The most frequent mutation was the Ala510Val variant that was

Table 1 Homozygous, compound heterozygous and single heterozygous variants in SPG7

Patient	Family history	Patient code	Group	Exon/intron	Base change	Effect on protein	State
Homozygous and compound heterozygous mutations in SPG7							
1	AR	N 09 2089 (FSP-608)	Elleuch <i>et al.</i> , 2006	Exon 11	c.1519 C>T	p.Gln507X	Compound heterozygous
2	AR	N 09 2097 (FSP-190)	Elleuch <i>et al.</i> , 2006	Exon 2	c.233 T>A	p.Leu78X	Compound heterozygous
3	AR	N10 0603 (FSP-623)	Elleuch <i>et al.</i> , 2006	Exon 11	c.1529C>T	p.Ala510Val	Homozygous
4	AR	N 09 2088 (FSP-135)	Elleuch <i>et al.</i> , 2006	Exon 1	c.1 A>T	p.Met1?	Compound heterozygous
5	Spo	N 10 0271 (FSP-554)	SPG31/SPG7	Exon 11	c.1450_1458del	p.Arg485_Glu487del	Homozygous
6	AD	N 09 2090 (SAL-640)	SPG31/SPG7	Exon 11	c.1450_1458del	p.Arg485_Glu487del	Compound heterozygous
7	Spo	N 09 2092 (FSP-171)	SPG31/SPG7	Exon 6	c.1 A>G	p.Met1?	Compound heterozygous
8	AD	N 09 2091 (AAD-088)	SPG31/SPG7	Exon 11	c.1450_1458del	p.Arg485_Glu487del	Compound heterozygous
9	Spo	N 09 2096 (FSP-159)	SPG31/SPG7	Exon 2	c.233 T>A	p.Leu78X	Compound heterozygous
10	AR	N 09 2235	SPG31/SPG7	Exon 1-2	c.1-?.286+?	p.0?	Compound heterozygous
11	Spo	N 09 2505 (FSP-1099)	R	Exon 2	c.233 T>A	p.Leu78X	Homozygous
12	Spo	N 09 2535 (399-598)	R	Intron 4	c.618+3G>C	p.?	Compound heterozygous
13	AD	N 09 2492 (FSP-796)	R	Exon 11	c.1450_1458del	p.Arg485_Glu487del	Compound heterozygous
14	AR	N 09 2531 (FSP-476)	R	Exon 8	c.1049_1077del	p.Pro350GlnfsX36	Compound heterozygous
15	Spo	N 09 2537 (FSP-659)	R	Exon	c.2216dupA	p.Asp739LysfsX3	Homozygous
16	AR	N 09 2528 (FSP-859)	D	Exon 8	c.1053dup	p.Gly352ArgfsX44	Compound heterozygous
17	Spo	N 09 2213	D	Intron 3	c.376+1G>T	p.?	Compound heterozygous
18	AR	N 09 2532 (AAR-275)	D	Exon 11	c.1529C>T	p.Ala510Val	Compound heterozygous
19	Spo	N 07 0674	D	Exon 11	c.1529C>T	p.Ala510Val	Compound heterozygous
20	AR	FSP-932 (N 09 2542)	D	Exon 8	c.1053dup	p.Gly352ArgfsX44	Compound heterozygous
21	AR	N 08 0132	D	Exon 11	c.1529C>T	p.Ala510Val	Compound heterozygous
22	Spo	N 10 0935	D	Exon 10	c.1369 C>T	p.Arg457X	Compound heterozygous
23	Spo	N 10 0094	D	Exon 6	c.861dup	p.Asn288X	Compound heterozygous
Single heterozygous variants in SPG7							
24	AR	N 09 2087	Elleuch <i>et al.</i> , 2006	Exon 2	c.246_248del	p.Gln82del	Heterozygous
25	Spo	N 10 0577	D	Exon 11	c.1457G>A	p.Arg486Gln	Heterozygous
26	AD	ADON	R	Exon 9	c.1232A>C	p.Asp411Ala	Heterozygous

AD = autosomal dominant; ADON = isolated autosomal dominant optic neuropathy; AR = autosomal recessive; D = Diagnostic testing sample; R = Research testing sample; Spo = isolated index cases.



identified in 65% (15/23) of the patients including one family in which the mutation was homozygous.

Patients with autosomal dominant optic neuropathy

A genome-wide linkage analysis was performed in seven affected members in a large French family with autosomal dominant optic neuropathy using Hap300Duov2SNP Arrays (Illumina). Several candidate regions were pinpointed, but analyses using microsatellite markers in both affected and unaffected members identified—under the hypothesis of complete penetrance—a unique candidate region on chromosome 16q24.3 (maximum multipoint LOD-score $Z_{\max} = 3$). After excluding the *OPA1* gene virtue of both the absence of mutations and the segregation of different haplotypes with the disease and the *OPA4* (18q12, MIM605293) and *OPA5* (22q12 MIM610708) loci by linkage, screening of *SPG7* in the index case of the family identified a novel missense mutation c.1232A>C, p.Asp411Ala at the heterozygous state. This mutation, which affects an evolutionary conserved aspartic acid located two amino acids downstream of the Walker B motif of the AAA domain of *SPG7*, is predicted to be deleterious by both SIFT and PolyPhen-2 prediction algorithms. It was present neither in patients with hereditary spastic paraplegia nor in 200 control individuals or databases (HapMap, 1000Genomes, Exome variant

server). The analysis of other family members confirmed the perfect segregation of the mutation. All affected individuals segregate a common haplotype harbouring the *SPG7* p.Asp411Ala mutation with the disease: all seven available affected members were heterozygote for the mutation whereas the two available unaffected individuals all had the normal allele (Fig. 2).

Subsequently, *SPG7* was screened for mutation in an extended cohort of 152 unrelated patients affected with isolated optic neuropathies of various mode of transmission, but no additional *SPG7* mutation was identified, indicating that *SPG7* mutations are a rare cause of autosomal dominant optic neuropathy.

Clinical features

Neurological and ophthalmological phenotype of patients with two *SPG7* mutations

Twenty-three index cases and five affected sibs had spasticity of the lower limbs. The age at onset known for 26 patients was surprisingly late, with a mean of 37.5 ± 8.4 years, ranging from 18 to 52 years, and a median of 39 years. Onset was significantly later in *SPG7* than in non-*SPG7* index cases (36.8 ± 8 versus 24.8 ± 20 , $P = 0.008$). The overall phenotype was isolated spastic gait in 14 (50%), spasticity with overt cerebellar signs in 11 (39%), with

ophthalmoplegia in two and with symptomatic optic neuropathy in one patient. Interestingly, optic neuropathy was found, either by the ocular fundus examination or OCT analysis, in all *SPG7*-positive patients ($n = 10$) (Table 5). Only one patient (Patient 17), who used a microscope for her work, complained of problems with vision at onset of the disease. Of note, none of the *SPG7*-positive patients had mental retardation, polyneuropathy, epilepsy, hearing impairment, atrophy of the corpus callosum, extrapyramidal signs or tetraspasticity.

Follow-up examinations were performed in five patients after 3 (Patients 11 and 13), 7 (Patient 12), 9 (Patient 14) and 17 (Patient 3) years. Functional disability progressed slightly or moderately in all patients but one (Patient 11), but all were still able to walk without help. Optic neuropathy was detected during follow-up in two patients (Patients 11 and 14). Patient 3 developed axonal-sensorimotor polyneuropathy and subcortical dementia, 7 years after the initial examination. Results are presented in Tables 2, 3 and 4 ($n = 28$).

Ophthalmological and neurological phenotype of patients with autosomal dominant optic neuropathy carrying the *SPG7* Asp411Ala mutation

The presenting sign of all affected members ($n = 8/10$) was progressive and isolated visual loss in the first decade of life with a final visual acuity varying between 1/10 and 3/10 in the second decade. None complained of any walking difficulties or stiffness of the legs. The identification of a *SPG7* mutation segregating with the disease, prompted us to carry out a complete neurological work up of the 41-year-old index case (Patient III3, Fig. 2). He had decreased reflexes in the lower limbs without evident Babinski sign, nor spasticity at rest. His gait was unremarkable and he exhibited mild cerebellar stance and a slight tremor at the heel–shin test. Ocular gaze was limited horizontally and vertically. Conduction velocity studies showed axonal peripheral polyneuropathy and unambiguous cerebellar atrophy on MRI.

Is the *AFG3L2* gene (*SCA28*) a possible modifier of the *SPG7* phenotype?

To explain the cerebellar predominance and the presence of ophthalmoplegia, nearly constant finding in *SCA28*, in several *SPG7*-positive patients, we screened exons 10, 15 and 16 of the *AFG3L2* gene encoding a protein that interacts with paraplegin in search of variants that might act as modifiers. However, no variants or mutations were found in these exons.

Discussion

We report 23 index cases and five affected sibs with two *SPG7* mutations from 134 families (17%) with hereditary or sporadic spastic paraparesis identified in different settings. Homozygous or compound heterozygous *SPG7* mutations were identified in 20.8% (5/24) of our research group patients with spasticity, but in only 8.1% (8/98) of the diagnostic group (Fig. 1). In all 23 cases with spastic paraparesis, an *SPG7* mutation or variant was

found on each copy of the gene, compatible with autosomal recessive transmission. Interestingly, the recurrent Ala510Val mutation was identified in 15 out of 23 patients with hereditary spastic paraplegia at the compound heterozygous state (in combination with another mutation) and, in one family, at the homozygous state. The pathogenicity of Ala510Val has already been suspected (McDermott *et al.*, 2001; Wilkinson *et al.*, 2004; Elleuch *et al.*, 2006; Arnoldi *et al.*, 2008; Brugman *et al.*, 2008; Sanchez-Ferrero *et al.*, 2012), and was confirmed by a yeast complementation assay (Bonn *et al.*, 2010). If this variant is considered to be pathogenic, the frequency of *SPG7* mutations is far greater than previously thought (at least 10% in most studies) and is comparable to the frequency determined in sporadic cases (Brugman *et al.*, 2008).

SPG7 phenotype and diagnostic criteria

The age at onset in all *SPG7* patients was late, with a median of 39 years, which confirms the finding of nearly all *SPG7* studies (De Michele *et al.*, 1998; McDermott *et al.*, 2001; Elleuch *et al.*, 2006; Arnoldi *et al.*, 2008; Tzoulis *et al.*, 2008). Interestingly, despite the fact that the age at onset was <20 years in 36/98 (37%) in the diagnostic and 12/24 (50%) in the research group we did not find any patients with *SPG7* in the early-onset group. All patients in our series who had onset in childhood or adolescence were negative for *SPG7*. Overall, the disease progressed slowly in *SPG7* patients, most of whom were able to walk even after long disease durations. This was confirmed in patients who had follow-up examinations up to 17 years after the initial examination, and is consistent with previous studies (De Michele *et al.*, 1998; Brugman *et al.*, 2008; Tzoulis *et al.*, 2008).

SPG7-positive patients are described as pure or complex. The major associated features in complex *SPG7* patients are cerebellar signs or atrophy of the cerebellum observed by MRI imaging (Elleuch *et al.*, 2006; Brugman *et al.*, 2008; Tzoulis *et al.*, 2008). In addition, bilateral optic disc pallor has been seen in several *SPG7*-positive families (Casari *et al.*, 1998; De Michele *et al.*, 1998; McDermott *et al.*, 2001).

The traditional clinical distinction between pure and complex hereditary spastic paraplegia is difficult to maintain; however, patients might have a pure phenotype on examination, but be complex *in fine*, if cerebellar atrophy is detected by brain MRI or optic neuropathy by OCT.

Cerebellar involvement is not specific to *SPG7* hereditary spastic paraplegia; many other hereditary spastic paraplegia subtypes present with cerebellar signs (Klebe *et al.*, 2006; Depienne *et al.*, 2007; Salinas *et al.*, 2008). Interestingly, one *SPG7*-positive patient in this study already had cerebellar atrophy in the first 2 years of the disease (Patient 1) whereas others, without cerebellar atrophy, have only slight clinical signs 19 years after disease onset (Patient 5). Remarkably, most (5/8) of the *SPG7*-positive patients in the diagnostic group had neither cerebellar signs nor cerebellar atrophy (4/7). However, slight cerebellar signs can be overlooked in patients with prominent spasticity.

With respect to optic neuropathy, all patients with hereditary spastic paraplegia who underwent OCT after the *SPG7* result was available had detectable pathology. Yet, only 40% (4/10) of this

Table 2 Clinical features of *SPG7*-positive probands with spastic gait [groups Elleuch *et al.*, (2006) and *SPG31/SPG7*]

Patients	Elleuch <i>et al.</i> , 2006				<i>SPG31/SPG7</i>					
	1	2	3	4	5	6	7	8	9	10
Geographic origin	France	France	France	France	France	France	France	France	France	France
Gender	F	M	M	F	F	M	M	F	F	F
Familial history	AR	AR	AR	AR	Spo	AD	Spo	AD	Spo	AR
Age at examination (follow-up)	38	45	56 (73)	47	37	52	60	61	57	56
Age at onset	36	39	40	46	18	25	37	40	42	44
Symptoms at onset	Stiff legs	Stiff legs	Stiff legs	US	Stiff legs	Stiff legs	Fatigue	US	US	Stiff legs; US
Severity	Moderate	Moderate	Moderate	Moderate	Moderate	Severe	Severe	Moderate	Unable to walk	Severe
Gait/lower limb spasticity	Mild	Moderate	Mild	Mild	Mild	Severe	Moderate	Moderate	n.a.	Moderate
Lower limb weakness	None	Proximal LL	None	None	None	Proximal LL	None	Yes	n.a.	n.a.
Lower limb reflexes	Increased	Increased	Normal	Increased	Increased	Increased	Increased	Increased	n.a.	Increased
Muscle wasting	None	None	None	None	None	None	Proximal LL	Mild LL	n.a.	n.a.
Extensor plantar reflex	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	n.a.	Yes
Decreased vibration at ankles	No	No	Yes	Yes	Yes	No	No	Yes	No	No
Axial ataxia	No	No	No	Yes	No	No	No	No	No	No
Lower limb	No	No	No	No	No	No	No	No	No	No
Upper limb	No	No	No	No	No	No	No	No	No	No
Nystagmus	No		No	No	No				No	
Saccadic pursuit	No	No	No	No	No	No	No	No	Yes	No
Dysarthria	No	No	No	No	Yes	No	No	No	No	No
Ophthalmologic examination*		n.a.				n.a.	n.a.	n.a.		Normal
Impaired oculomotricity/ ophthalmoplegia	No	No	Vertical gaze limited	No	No	No	No	No	n.a.	No
Cerebral MRI	CA	n.a.	CA (CT Scan)	Normal	Normal	n.a.	Normal	Normal	n.a.	Normal
Optic neuropathy	Yes	n.a.	Yes	n.a.	Yes	n.a.	Yes	n.a.	n.a.	n.a.
EMG	Normal	n.a.	n.a.	Normal	Normal	n.a.	Normal	Axonal PNP	n.a.	n.a.

AD = autosomal dominant; AO = Age at onset; AR = autosomal recessive; CA = cerebellar atrophy; F = female; LL = lower limb; M = male; n.a. = not applicable; Spo = sporadic; UL = upper limb; US = unsteadiness.

*Routine ophthalmologic examination.

subgroup had decreased visual acuity and 50% (5/10) had optic disc pallor at the fundus (Table 5). OCT, therefore, is useful for detecting subclinical optic neuropathy, which is suggestive of an *SPG7* mutation. However, OCT was performed in patients who had disease durations of at least 10 years. It would thus be interesting to determine whether optic atrophy already exists at early stages of the disease. In order to show that this is specific to *SPG7* we need to screen other patients with non-*SPG7* hereditary spastic paraplegia. Nevertheless, the identification of a *SPG7* mutation responsible for clinically isolated optic neuropathy in a large multi-generational pedigree supports the view that the loss of retinal ganglion cells in patients with hereditary spastic paraplegia with *SPG7* mutations is not coincidental. The implication of *SPG7* in isolated optic neuropathies may, however, be uncommon as suggested by the absence of mutations in additional cases of hereditary optic neuropathies with various mode of inheritance and no mutation in major genes for optic neuropathy, namely mitochondrial genes responsible for Leber's hereditary optic neuropathy (*LHON*, OMIM 535000) and optic atrophy 1 (*OPA1*, OMIM 165500).

The *SPG7*-negative patients with complex hereditary spastic paraplegia had a large number of associated symptoms, including cognitive impairment, polyneuropathy, generalized epilepsy, hearing impairment, atrophy of the corpus callosum, extrapyramidal symptoms and tetraspasticity. Additional symptoms other than cerebellar involvement and/or optic neuropathy are exceptional

in *SPG7*-positive patients. Warnecke and collaborators (2007) described a family with cognitive deficits including a dysexecutive syndrome and supranuclear palsy (Arnoldi *et al.*, 2008). In our series, only one *SPG7*-positive patient (Patient 3) had subcortical dementia; it was observed on follow-up, at the age of 56 years, 17 years after the initial examination, and could just be coincidental.

Involvement of the cerebellum and the optic nerve in *SPG7*-positive patients might be explained by recent pathophysiological findings concerning paraplegin, the protein encoded by *SPG7*. Paraplegin contains an M41 metallopeptidase domain and an ATPase domain characteristic of the AAA family of ATPases (Langer, 2000). With its paralogous AFG3L2 (OMIM 604581) subunits, it forms the oligomeric mAAA protease complex, which localizes to the inner membrane of mitochondria (Atorino *et al.*, 2003; Koppen *et al.*, 2007). The AFG3L2 protein has 69% and 49% sequence similarity to the yeast orthologous proteins Yta12 and Yta10, respectively (Banfi *et al.*, 1999). The mAAA protease mediates protein degradation and activation in mitochondria (Leonhard *et al.*, 2000; Rugarli and Langer, 2006; Tatsuta and Langer, 2009). Very recently, heterozygous missense mutations in the AFG3L2 gene were found to cause spinocerebellar ataxia type 28 (SCA28; OMIM 610246) (Di Bella *et al.*, 2010). The associated clinical characteristics of heterozygous SCA28 patients include pyramidal signs, ptosis and ophthalmoplegia (Cagnoli *et al.*, 2010; Di Bella *et al.*, 2010). Patients with homozygous

Table 3 Clinical features of SPG7-positive probands with spastic gait (Research group)

Patient	11	12	13	14	15
Geographic origin	France	Maroc	France	France	France
Gender	F	M	F	F	M
Familial history	Spo	Spo	AD	AR	Spo
Age at examination (follow up)	54 (57)	42 (49)	42 (45)	43 (52)	45
Age at onset	44	32	34	37	40
Symptoms at onset	US	US	visual blurring	Stiff legs	Stiff legs
Severity	Severe	Severe	Moderate	Moderate	Mild
Gait/LL spasticity	Moderate	Moderate	Mild	Moderate	Mild
LL weakness	None	None	None	n.a.	None
LL reflexes	Increased	Increased	Increased	Increased	Increased
Muscle wasting	None	Distal LL	None	n.a.	None
Extensor plantar reflex	Yes	Yes	Yes	n.a.	Yes
Decreased vibration at ankles	Yes	Normal	Yes	Normal	Yes
Axial ataxia	No	No	Yes	Yes	No
Lower limb	No	No	No	No	No
Upper limb	No	Yes	No	No	No
Nystagmus	No	No	No	Yes	No
Saccadic pursuit	No	No	Yes	Yes	Yes
Dysarthria	No	No	Yes	No	Yes
Ophthalmologic examination*					
Impaired oculomotricity/ ophthalmoplegia	No	Vertical gaze limited	No	No	No
Cerebral MRI	CA	CA, global atrophy pons	CA	Normal	Upper vermis atrophy
Optic neuropathy	Yes	n.a.	Yes	Yes	Yes
EMG	n.a.	n.a.	n.a.	n.a.	n.a.

AD = autosomal dominant; AR = autosomal recessive; CA = cerebellar atrophy; F = female; LL = lower limb; M = male; n.a. = not applicable; Spo = sporadic; UL = upper limb; US = unsteadiness.

*Routine ophthalmologic examination.

AFG3L2 mutations have a more severe phenotype that includes oculomotor apraxia, dystonia and progressive myoclonic epilepsy (Pierson *et al.*, 2011). This suggests that some of the clinical heterogeneity and the variable severity of cerebellar involvement in *SPG7* might be due to variants in *AFG3L2* that act as genetic modifiers. Therefore, in *SPG7*-positive patients we analysed exons 10, 15 and 16 of *AFG3L2*, in which all of the reported mutations are located (Cagnoli *et al.*, 2010; Di Bella *et al.*, 2010; Pierson *et al.*, 2011). No variants were found, although variants in other exons or non-coding regions of the gene might have been missed.

The underlying pathophysiology in *SPG7* and the multisystem involvement observed in some *SPG7*-positive patients are typical of mitochondrial disorders, particularly the presence of optic neuropathy. The loss of retinal ganglion cells is indeed a hallmark of mitochondrial disorders including neurodegenerative diseases due to mitochondrial defects like Friedreich ataxia or Charcot–Marie–Tooth type 2A2 (Fortuna *et al.*, 2009; Rouzier *et al.*, 2012). The optic neuropathy may even be the unique symptom of mitochondrial dysfunction as in Leber's hereditary optic neuropathy (OMIM 535000), OPA1 (OMIM 165500) or OPA7 (Hanein *et al.*, 2009). The sensitivity of retinal ganglion cells to mitochondrial dysfunction is thought to result from a particularly high energy dependence and vulnerability (Marelli *et al.*, 2011; Yu-Wai-Man *et al.*, 2011a). Interestingly, the mAAA proteases have been reported to

play a role in the processing of the OPA1 protein (Martinelli and Rugarli, 2010). Therefore, it is possible that mutations in *SPG7* cause loss of retinal ganglion cells through alteration of the OPA1 function. With respect to the Asp411Ala mutation segregating with isolated autosomal dominant optic neuropathy with neuropathy but no hereditary spastic paraplegia, it is important to note that it is located in the AAA domain of the protein, two amino acids downstream of the Walker B motif (residues 405–409). This motif is involved with the upstream Walker A motif in the fixation and hydrolysis of ATP. To our knowledge this is the only naturally occurring missense mutation identified in this region of the protein. Interestingly, a human *SPG7* protein harbouring the p.E408Q mutation in the Walker B motif has been expressed in addition to wild-type *AFG3L2* in a *Saccharomyces cerevisiae* strain lacking both YAT11 and YTA12. This mutation had a dominant negative effect on both the *AFG3L2* homohexamers and the paraplegin/*AFG3L2* heterohexamer with reduced ATPase and proteolytic activities (Augustin *et al.*, 2009). It is likely that the Asp411Ala mutation interferes with the binding and/or hydrolysis of ATP by the neighbouring Walker B domain leading to decreased proteolytic activity of mAAA proteases in retinal ganglion cells. The retinal-restricted expression of the disease in patients harbouring the mutation is consistent with exacerbated vulnerability of retinal ganglion cells (Marelli *et al.*, 2011; Yu-Wai-Man *et al.*, 2011b).

Table 4 Clinical features of SPG7-positive probands with spastic gait (Diagnostic group)

Patient	16	17	18	19	20	21	22	23
Geographic origin	France	France	France	France	UK	France	France	France
Gender	M	F	F	M	F	F	M	F
Familial history	AR	Spo	AR	Spo	AR	AR	Spo	Spo
Age at examination	71	55	57	55	43	37	36	57
Age at onset	n.a.	52	45	20	30	30	31	43
Symptoms at onset	n.a.	Stiff legs; US	US	n.a.	Stiff legs	Stiff legs	Stiff legs; cramps	Stiff legs; US
Severity	n.a.	Moderate	Mild	Moderate	n.a.	Moderate	Severe	Moderate
Gait/LL spasticity	n.a.	Moderate	None	n.a.	n.a.	Moderate	Moderate	Moderate
LL weakness	n.a.	Proximal LL	None	None	n.a.	Proximal/distal LL	Proximal/distal LL	Proximal/distal LL
LL reflexes	n.a.	Increased	Increased	n.a.	n.a.	Increased	Increased	Increased
Muscle wasting	n.a.	None	None	None	n.a.	None	None	None
Extensor plantar reflex	n.a.	Yes	No	Yes	n.a.	Yes	Yes	Yes
Decreased vibration at ankles	Yes	No	Normal	n.a.	n.a.	Yes	No	Yes
Axial ataxia	n.a.	No	Yes	No	No	Yes	No	No
Lower limb	n.a.	No	No	No	No	Yes	No	No
Upper limb	n.a.	No	No	No	No	No	No	No
Nystagmus			No			No		
Saccadic pursuit	n.a.	No	No	No	No	No	No	No
Dysarthria	n.a.	No	No	Yes	No	Yes	No	No
Ophthalmologic examination*	n.a.	Normal		n.a.	Normal	Normal	Normal	Optic atrophy
Impaired oculomotricity/ ophthalmoplegia	n.a.	No	No	n.a.	No	No	No	No
Cerebral MRI	n.a.	Normal	CA	CA	Normal	CA	Normal	Normal
Optic neuropathy	n.a.	n.a.	Yes	n.a.	n.a.	n.a.	n.a.	n.a.
EMG	n.a.	Normal	n.a.	n.a.	n.a.	Normal	n.a.	n.a.

AD = autosomal dominant; AR = autosomal recessive; CA = cerebellar atrophy; F = female; LL = lower limb; M = male; n.a. = not applicable; Spo = sporadic; UL upper limb; US = unsteadiness.

*Routine ophthalmologic examination.

Table 5 Ophthalmological findings in 10 SPG7-positive patients with spastic gait

Patient	Age at examination (disease duration)	Papillary aspect on funduscopy	OCT (normal > 110 μ m, Left-Right)	Eye movements	Visual field	Visual acuity/10	Conclusion
1	58 (22)	normal	106–103	limited gaze horizontal bilateral	ND	10	Optic sectorial atrophy
3	74 (34)	mild pallor	83–83	slow saccades, vertical limited gaze	normal	7	Optic neuropathy
5	37 (19)	mild pallor	79–80	normal	ND	10	Optic neuropathy
7	60 (23)	normal	97 sectorial	normal	ND	10	Optic neuropathy
11	56 (12)	mild pallor	70–76	limited gaze horizontal bilateral	normal	10	Optic neuropathy
13	44 (10)	mild pallor	93–94	ND		10	Optic neuropathy
14	52 (15)	normal	103–102	normal	ND	8	Optic neuropathy
15	52 (12)	normal	94–109	slow saccades, hypometric	ND	10	Optic neuropathy
18	66 (21)	mild pallor	95–100	normal	normal	8 Left 9 Right	Optic neuropathy
23	59 (16)	mild pallor	93 sectorial	normal	abnormal	6	Optic neuropathy

ND = not determined.

Do heterozygous mutations predispose to a cerebellar syndrome or atrophy?

Two patients in this study had only one heterozygous variant (Glu82del and Arg486Gln). Arg486Gln is a rare variant

(rs111475461) that was found in healthy individuals at a low frequency (minor allele frequency of 0.002 in dbSNP) (McDermott *et al.*, 2001), suggesting that it is not pathogenic. Although Glu82del was not found in controls (Elleuch *et al.*, 2006), the patient with this variant had nystagmus, hearing impairment and

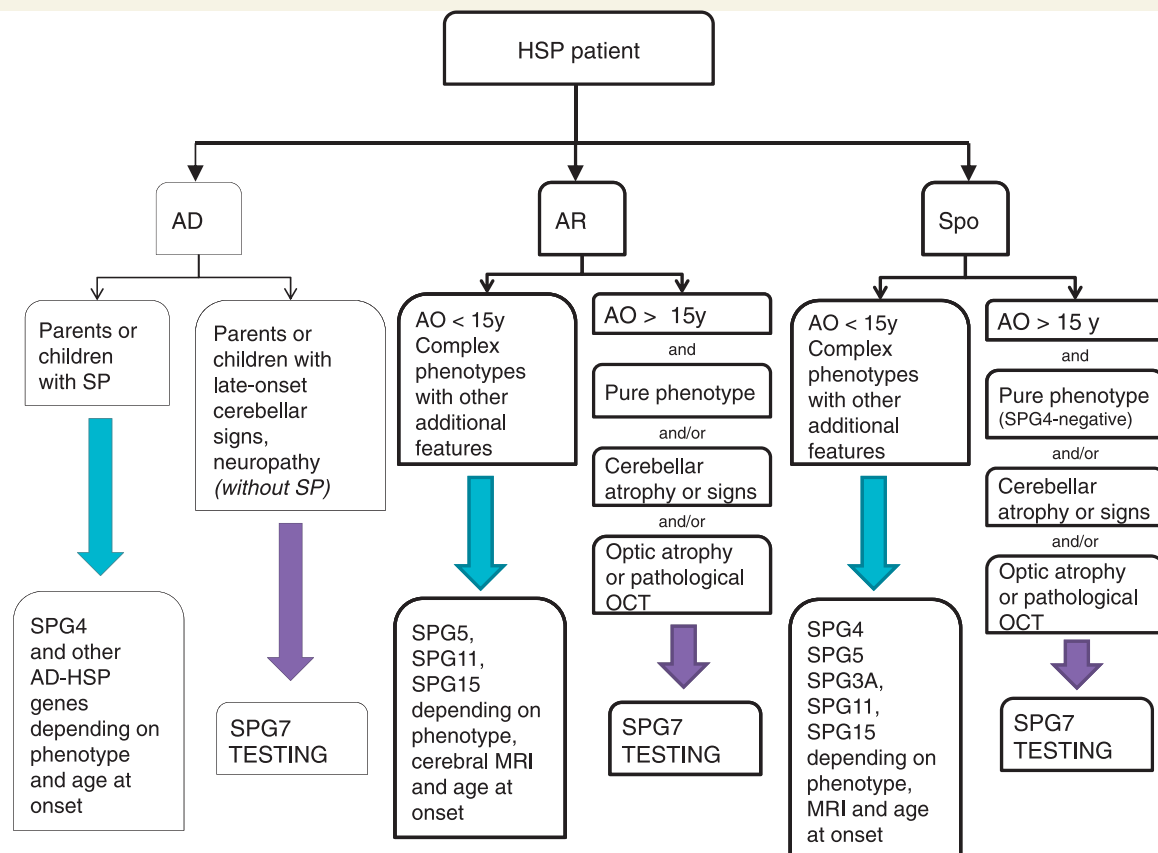


Figure 4 Flow chart to guide testing for SPG7 in patients with spastic paraparesis. AD = autosomal dominant; AO = age at onset; AR = autosomal recessive; HSP = hereditary spastic paraplegia; SP = spastic paraplegia; Spo = sporadic; y = years.

mental retardation in addition to spastic paraplegia, suggesting that a gene other than *SPG7* could be responsible for this complex phenotype.

Nevertheless, at least three index cases with *SPG7* mutations had a family history compatible with autosomal dominant transmission (Patients 6, 8 and 13; Table 1 and Fig. 3). The mother of index Case 13, who carried a heterozygous Arg485_Glu487del mutation, had a slight cerebellar phenotype on examination (Fig. 3A). The mother of Patient 5, who also carried a heterozygous Arg485_Glu487del mutation, had cerebellar gait and cerebellar atrophy on MRI (Fig. 2B). Finally, one individual in Family 3 with a heterozygous Ala510Val mutation developed an axonal sensory motor neuropathy with pyramidal signs. A patient with a compound heterozygous Arg485_487del and Ala510Val mutations was previously reported to have an affected father with a heterozygous Arg485_487del mutation (McDermott *et al.*, 2001). The authors concluded that the Ala510Val mutation might act as genetic modifier, whereas the Arg485_487del mutation is a disease causing dominant allele (McDermott *et al.*, 2001). These observations suggest that a single *SPG7* mutation may constitute a susceptibility factor for a late-onset neurodegenerative disorder the nature of which would depend on the identity of the mutation. Interestingly, diffusion tensor imaging recently confirmed subclinical abnormalities in heterozygous *SPG7* mutation carriers (Warnecke *et al.*, 2010). It

should be noted, in any case, that cerebellar ataxia always developed later in carriers of a single heterozygous mutation than in their relatives with *SPG7* hereditary spastic paraplegia; their ataxia was also less severe.

Conclusion and decision-making

Our study has shown that *SPG7* mutations are a frequent cause of middle-aged onset of spastic gait when strict inclusion criteria are applied. *SPG7* should, therefore, be tested in autosomal recessive or sporadic hereditary spastic paraplegia, particularly if it is associated with cerebellar atrophy/cerebellar signs and/or optic neuropathy, or if OCT reveals the presence of pathology even in the absence of decreased visual acuity (Fig. 4). Our data also demonstrate that *SPG7* mutations are unlikely to be found in young-onset hereditary spastic paraplegia and in patients with a complicated phenotype that differs from the proposed inclusion criteria. Finally, although uncommon, we show here that *SPG7* mutations may account for isolated optic neuropathy with peripheral neuropathy. This suggests that systematic neurological examinations in individuals with clinically isolated optic atrophy could help in identifying *SPG7*-positive patients.

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Supplementary material

Supplementary material is available at *Brain* online.

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