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

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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, 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 (Wamecke 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).

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
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.
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 neuritis 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 tetraparesis (n=1).

Neurological examination of patients with isolated autosomal dominant optic neuropathy

The index case (Case III-3) 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 \( r = \frac{\text{peak area}_{\text{patient}}}{\text{peak area}_{\text{control}}} \), where peak area is the patient/control area. 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 \( r = \frac{\text{peak area}_{\text{patient}}}{\text{peak area}_{\text{control}}} \), where peak area is the patient/control area.

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 heterogeneous 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 heterogeneous mutation was associated with the heterozygous c.1529C>T/p.Ala510Val variant, previously described as a polymorphism and recently reclassified 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 heterogeneous mutations. The mutation (Met17, 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 heterogeneous 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 heterogeneous 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 heterogeneous mutations in 83% (19/23). The most frequent mutation was the Ala510Val variant that was...
<table>
<thead>
<tr>
<th>Patient Family history</th>
<th>Patient code</th>
<th>Group</th>
<th>Exon/intron</th>
<th>Base change</th>
<th>Effect on protein</th>
<th>State</th>
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<tr>
<td>Homozygous and compound heterozygous mutations in SPG7</td>
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<tr>
<td>3</td>
<td>AR</td>
<td>N10 0603 (FSP-623)</td>
<td>Elleuch et al., 2006</td>
<td>Exon 11</td>
<td>c.1529C&gt;T</td>
<td>p.Ala510Val</td>
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<tr>
<td>5</td>
<td>Spo</td>
<td>N10 0271 (FSP-954)</td>
<td>SPG31/SPG7</td>
<td>Exon 11</td>
<td>c.1450_1458del</td>
<td>p.Arg485_Glu487del</td>
</tr>
<tr>
<td>7</td>
<td>Spo</td>
<td>N09 2092 (FSP-171)</td>
<td>SPG31/SPG7</td>
<td>Exon 1 Exon 6</td>
<td>c.1 A&gt;G c.850_851delins</td>
<td>p.Met1? p.Phe284ProfsX45</td>
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<tr>
<td>9</td>
<td>Spo</td>
<td>N09 2096 (FSP-159)</td>
<td>SPG31/SPG7</td>
<td>Exon 2 Exon 11</td>
<td>c.233 T&gt;A c.1450_1458del</td>
<td>p.Leu78X p.Arg485_Glu487del</td>
</tr>
<tr>
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<td>AR</td>
<td>N09 2235</td>
<td>SPG31/SPG7</td>
<td>Exon 1-2 Exon 11</td>
<td>c.1715 C&gt;T c.1529C&gt;T</td>
<td>p.Ala510Va</td>
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<td>11</td>
<td>Spo</td>
<td>N09 2505 (FSP-1099)</td>
<td>R</td>
<td>Exon 2</td>
<td>c.233 T&gt;A</td>
<td>p.Leu78X</td>
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<td>N09 2535 (399-598)</td>
<td>R</td>
<td>Exon 4 Exon 17</td>
<td>c.618 +3G&gt;C c.2240 T&gt;C</td>
<td>p.? p.Ile747Thr</td>
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<td>15</td>
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<td>N09 2537 (FSP-659)</td>
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<td>Exon</td>
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<td>p.Asp739GlyfsX3</td>
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<tr>
<td>17</td>
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<td>N09 2213</td>
<td>R</td>
<td>Exon 3 Exon 8</td>
<td>c.376 +1G&gt;T c.1045G&gt;A</td>
<td>p.? p.Gly349Ser</td>
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</tbody>
</table>

| Single heterozygous variants in SPG7 |
| 24 | AR | N09 2087 | Elleuch et al., 2006 | Exon 2 | c.246_248del | p.Gln82del | 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 $Z_{\text{max}} = 3$). 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 impair-
ment, 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
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
II13, 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 hori-
zontally 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 mu-
tations 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 rec-
escusive transmission. Interestingly, the recurrent Ala510Val
mutation was identified in 15 out of 23 patients with hereditary
spastic paraplegia at the compound heterozygous state (in com-
bination with another mutation) and, in one family, at the homo-
zygous 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 com-
plementation 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 nega-
tive for SPG7. Overall, the disease progressed slowly in SPG7 pa-
tients, 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 pre-
sent with cerebellar signs (Klebe et al., 2006; Depienne et al.,
2007; Salinas et al., 2008). Interestingly, one SPG7-positive pa-
tient in this study already had cerebellar atrophy in the first 2 years
of the disease (Patient 1) whereas others, without cerebellar atro-
phy, 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
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 tetraparesis. 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 dys executive 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 metalloprotease 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., 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

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

<table>
<thead>
<tr>
<th>Patients</th>
<th>Elleuch et al., 2006</th>
<th>SPG31/SPG7</th>
</tr>
</thead>
<tbody>
<tr>
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<td>France</td>
</tr>
<tr>
<td>Gender</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Familial history</td>
<td>AR</td>
<td>AR</td>
</tr>
<tr>
<td>Age at examination (follow-up)</td>
<td>38</td>
<td>45</td>
</tr>
<tr>
<td>Age at onset</td>
<td>36</td>
<td>39</td>
</tr>
<tr>
<td>Symptoms at onset</td>
<td>Stiff legs</td>
<td>Stiff legs</td>
</tr>
<tr>
<td>Severity</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Gait/lower limb spasticity</td>
<td>Mild</td>
<td>Moderate</td>
</tr>
<tr>
<td>Lower limb weakness</td>
<td>None</td>
<td>Proximal LL</td>
</tr>
<tr>
<td>Lower limb reflexes</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Muscle wasting</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Extensor plantar reflex</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Decreased vibration at ankles</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Axial ataxia</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Lower limb</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Upper limb</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Saccadic pursuit</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ophthalmologic examination*</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Impaired oculomotoricity/ ophthalmoplegia</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cerebral MRI</td>
<td>CA</td>
<td>n.a.</td>
</tr>
<tr>
<td>optic neuropathy</td>
<td>Yes</td>
<td>n.a.</td>
</tr>
<tr>
<td>EMG</td>
<td>Normal</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

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

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

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.
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

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

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

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

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

**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.

ND = not determined.
mental retardation in addition to spastic paraplegia, suggesting that a gene other than \textit{SPG7} could be responsible for this complex phenotype.

Nevertheless, at least three index cases with \textit{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 \textit{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 \textit{et al}., 2001). These observations suggest that a single \textit{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 \textit{SPG7} mutation carriers (Warnecke \textit{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 \textit{SPG7} hereditary spastic paraplegia; their ataxia was also less severe.

**Conclusion and decision-making**

Our study has shown that \textit{SPG7} mutations are a frequent cause of middle-aged onset of spastic gait when strict inclusion criteria are applied. \textit{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 neuritis, or if OCT reveals the presence of pathology even in the absence of decreased visual acuity (Fig. 4). Our data also demonstrate that \textit{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 \textit{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 \textit{SPG7}-positive patients.
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Supplementary material

Supplementary material is available at Brain online.

References


