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ARTICLE

SPG7 mutations explain a significant proportion of French Canadian spastic ataxia cases

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Hereditary cerebellar ataxias and hereditary spastic paraplegias are clinically and genetically heterogeneous and often overlapping neurological disorders. Mutations in *SPG7* cause the autosomal recessive spastic paraplegia type 7 (SPG7), but recent studies indicate that they are also one of the most common causes of recessive cerebellar ataxia. In Quebec, a significant number of patients affected with cerebellar ataxia and spasticity remain without a molecular diagnosis. We performed whole-exome sequencing in three French Canadian (FC) patients affected with spastic ataxia and uncovered compound heterozygous variants in *SPG7* in all three. Sanger sequencing of *SPG7* exons and exon/intron boundaries was used to screen additional patients. In total, we identified recessive variants in *SPG7* in 22 FC patients belonging to 12 families (38.7% of the families screened), including two novel variants. The p.(Ala510Val) variant was the most common in our cohort. Cerebellar features, including ataxia, were more pronounced than spasticity in this cohort. These results strongly suggest that variants affecting the function of SPG7 are the fourth most common form of recessive ataxia in FC patients. Thus, we propose that *SPG7* mutations explain a significant proportion of FC spastic ataxia cases and that this gene should be considered in unresolved patients.
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INTRODUCTION

Hereditary cerebellar ataxias and hereditary spastic paraplegias (HSPs) are clinically and genetically heterogeneous and often overlapping neurological disorders. HSPs are characterized by a predominant progressive spasticity and weakness in the lower limbs due to degeneration of the corticospinal tracts,[1] whereas the main feature of cerebellar ataxias is progressive cerebellar degeneration leading to impaired balance, gait and speech.[2] Both HSP and hereditary ataxias can be associated with other neurological and non-neurological features, resulting in complex phenotypes with frequent intra- and inter-familial variability. Significantly, cerebellar ataxias are very often associated with pyramidal involvement leading to $>50\%$ of recessive ataxias manifesting as spastic ataxias.[3] Because of the individual rarity and genetic heterogeneity of these conditions, their molecular diagnosis remains challenging and time-consuming.

Mutations in the gene *SPG7* were the first identified genetic cause of autosomal recessive HSP in 1998 (MIM602783).[4] Since then, a significant number of causative mutations were found in several HSP cohorts from different populations.[5–14] SPG7 can be characterized by a pure or complex HSP phenotype. Increasingly, reports documented that cerebellar ataxia and cerebellar atrophy on magnetic resonance imaging (MRI) are the most frequent additional features in complex SPG7 cases.[6,7,9,12,15] In a study of a large Dutch cohort, cerebellar ataxia was found in 57% of cases and it was even the dominating clinical symptom in some of these patients.[6] In addition, a recent report suggests that *SPG7* mutations are a frequent cause of adult-onset undiagnosed cerebellar ataxia in patients of British descent.[16] The sequencing of *SPG7* in next-generation sequencing panels has further identified cases of spastic ataxia carrying compound heterozygous variants, supporting that SPG7 may be one of the most common forms of recessive ataxias worldwide.[8] Thus, a growing number of studies indicate that SPG7 should be considered in the differential diagnosis of recessive cerebellar ataxia.[6,16,17]

In Quebec, Friedreich ataxia (MIM229300), autosomal recessive spastic ataxia of Charlevoix–Saguenay (MIM270550) and autosomal recessive spinocerebellar ataxia type 8 (MIM610743) account for the majority of autosomal recessive cerebellar ataxia cases and, except for the latter one, they usually present as spastic ataxias.[18] However, a large number of French Canadian (FC) cerebellar ataxia cases remain unresolved, many of which have associated spasticity and often milder adult-onset phenotypes. Only recently were three SPG7 FC cases reported from the province of Ontario.[19] The relative prevalence of SPG7 in the FC population is unknown.

As for many other rare diseases, the implementation of whole-exome sequencing (WES) in research and in clinical settings has greatly accelerated the identification of disease-causing genes in the field of ataxias. Here we report on the identification of causative *SPG7* variants in 22 unresolved FC spastic ataxia cases belonging to 12

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families, making it the fourth most common recessive ataxia in this population.

SUBJECTS AND METHODS

Subjects

Patients presenting with cerebellar ataxia, spasticity and a family history suggestive of autosomal recessive or sporadic inheritance were seen at several neuromuscular clinics in the province of Quebec and Eastern Ontario between 2002 and 2014. Mutations in *SACS* and *FRDA* were ruled out in the majority of cases. All participating family members signed an informed consent form approved by the institutional ethics committee of the Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) or the Children's Hospital of Eastern Ontario.

Molecular analyses

Genomic DNA was extracted from peripheral blood cells using standard methods. WES was performed on individuals 1, 5 and 9 using the SureSelect exome capture kit v.5 (Agilent Technologies, Santa Clara, CA, USA). The libraries were sequenced on an Illumina HiSeq 2000 (Illumina, San Diego, CA, USA) with paired-end 100-bp reads at the McGill University and Genome Quebec Innovation Center (Montreal, Quebec, Canada). Sequences were aligned to the human reference genome (UCSC hg19) using the BWA (Burrows–Wheeler Aligner) algorithm, variant calling was performed using SAMtools[20] and annotation was done using ANNOVAR[21] and custom scripts, as previously described. For Sanger sequencing, PCR was used to amplify selected individual exons and intron–exon boundaries of *SPG7*. PCR products were sent to McGill University and Genome Quebec Innovation Center for sequencing, using a 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Mutation detection analysis was performed using SeqMan v.4.03 (DNASTAR Inc., Madison, WI, USA) and 4Peaks (A. Griekspoor and Tom Groothuis, mekentosj.com).

Patient 22 was analyzed using the HSP Sanger panel at the Hospital for Sick Children (Toronto, Ontario, Canada), as previously described.[19] Variants identified were submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/).

For complementary DNA (cDNA) sequencing, skin fibroblasts were derived from individual 19 and a healthy control and were grown according to standard protocols. Total RNA was extracted using Trizol reagent (Ambion, Foster City, CA, USA) and reverse transcribed into cDNA using the Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Exons 7 and 8 of *SPG7* were amplified by PCR from the cDNA and sequenced. Sequencing and variant detection analysis were performed as described above.

RESULTS

Exome sequencing

A group of nine unrelated patients with unresolved autosomal recessive spastic ataxia were sent for WES. As there was some phenotypic heterogeneity within the cohort regarding age of onset, severity and associated neurological symptoms, it was expected that causative variants in different genes would be uncovered. We searched for homozygous or compound heterozygous variants with a minor allele frequency $<3\%$ in the 1000 Genomes and Exome Variant Server (EVS) databases. As expected, we identified distinct candidate genes in several individuals, confirming the genetic heterogeneity within our cohort. Nevertheless, in three patients (1, 5 and 9), we uncovered compound heterozygous variants in *SPG7* (NM_003119, NG_00808.2.1; Table 1, Supplementary Table S1 and S2). The three cases carried the most frequent previously reported variant c.1529C>T (p.(Ala510Val)) on one allele.[15] To assess the possibility that the p.(Ala510Val) variant arose from a single event, we looked at genotypes of known SNPs surrounding this shared variant. Single-nucleotide polymorphisms were selected if they were reported in dbSNP138, had a mapping quality >50 and coverage higher than $\times 10$. Carriers of this variant shared a common 1.33-Mb haplotype (rs1107678-rs7196459), suggesting that p.(Ala510Val) derives from a single ancestral event (Supplementary Table S3).

In addition, patients 1 and 5 each carried a different previously reported pathogenic missense variant on the second allele (c.2249C>T (p.(Pro750Leu)) and c.1715C>T (p.(Ala572Val)), respectively).[5,7] In patient 9, we found a novel intronic variant c.988-1G>A located one base pair before exon 8. This variant was absent from the 1000 Genomes and EVS databases and present at a frequency of 1.66e-05 in the Exome Aggregation Consortium (ExAC). In addition, it was found at a frequency of 2/2000 in our in-house exome database. Furthermore, it was predicted to be disease causing due to the loss of an acceptor splice site by MutationTaster. Sanger sequencing validated the presence of the corresponding variants in the three patients and co-segregation with disease status was confirmed in families A and B.

Identification of *SPG7* variants in additional spastic ataxia patients

To identify additional patients with *SPG7* variants, we screened 21 additional unrelated patients from our cohort of unresolved cases. Because of the genetic homogeneity observed in FC, we focused our analysis on *SPG7* exons in which we had previously identified variants in patients. Strikingly, we uncovered rare variants in eight additional unrelated patients (38.1%, 8/21). We confirmed the presence of the identified variants in four affected relatives, which brought the total to 12 cases belonging to eight families (Table 1). In parallel, we also identified variants in *SPG7* in subject 22 using a Sanger sequencing HSP panel. This included five missense variants that were previously reported to affect the protein function,[5,7,10,15,22] and two novel variants (Table 1). In fact, five families (eight individuals) carried the novel splice site variant c.988-1G>A described above, making it the second most common variant in our cohort. Furthermore, patient 22 carried a novel variant (c.473_474del, p.(Leu158GlnfsTer30)), which is predicted to induce a frameshift and a premature stop codon at position 187 of *SPG7*. We confirmed co-segregation of all variants with the disease status in family members for which DNA was available. Thus, we uncovered rare homozygous or compound heterozygous variants in *SPG7* in 38.7% of the families screened (12/31).

cDNA sequencing

To confirm the pathogenicity of the splice site variant c.988-1G>A, we sequenced exons 7 and 8 of the cDNA of patient 19, who was homozygous for this variant. The results confirm that the substitution of G to A causes the loss of the acceptor splice site and leads to the use of two alternative cryptic acceptor splice sites within exon 8 (Figure 1). This leads to frameshifts and premature stop codons that likely produce two truncated SPG7 proteins (p.Ser330ProfsTer65 and p.Ser330LeufsTer460). Thus, these results strongly suggest that the *SPG7* variant c.988-1G>A is indeed a mutation because it affects the function of the SPG7 protein.

Clinical features of patients with *SPG7* variants

Despite the allelic heterogeneity, this FC cohort supports a more homogeneous core phenotype where spasticity and ataxia are both a constant feature (Table 1). The fact that 100% of cases presented with ataxic features explains why *SPG7* was not screened initially in these patients. This cohort confirms the variable but generally adult age of onset (mean 34.2, 15–55) and the phenotypic intra-familial variability.[15] Urinary urgency was a very common symptom that required medical treatment in many cases (14/22; 63.6%). Compared with other series, there was never any chronic external

Table 1 Clinical features and variants identified in French Canadian SPG7 cases

Individual ID	Family ID	Mutations	Age of onset	Age at exam	Urinary urgency	CPEO	Optic atrophy	Nystagmus	Dysarthria	Spasticity	Hyper-reflexia	Babinski	Ataxia	Dysmetria	Proximal weakness	Age documented proximal weakness	Cerebellar atrophy on MRI
1	A	Ex 11c.1529C>T p.(Ala510Val) Ex 17c.2249C>T p.(Pro750Leu)	20	50	–	–	–	+	++	++	+	+	++	+	–		++
2			55	67	–	–	–	–	+	+	+	+	+	–	LE	67	++
3			40	57	+	–	–	+	–	++	+	+	+	–	–		+
4			32	44	+	–	–	+	+	+	+	+	+	+	–		–
5	B	Ex 11c.1529C>T p.(Ala510Val) Ex 13c.1715C>T p.(Ala572Val)	15	58	+	–	–	++	++	++	+		++	+	–		++
6			43	66	+	–	–	++	+	++	+	–	++	+	–		++
7			50	74	+	–	–	–	++	++	+	–	++	+	–		++
8			45	68	+	–	–	–	+	++	+	+	++	+	–		+
9	C	Ex 11c.1529C>T p.(Ala510Val) **Ex 8c.988 – 1G>A**	25	43	–	–	–	+	++	++	+	+	++	++	–		+
10	D	Ex 11c.1529C>T p.(Ala510Val) Ex 13c.1715C>T p.(Ala572Val)	28	61	–	–	–	+	+++	+++	+	+	++	+	UE/LE	61	++
11	E	Ex 8c.1045G>A p.(Gly349Ser) Ex 17c.2249C>T p.(Pro750Leu)	32	48	+	–	–	–	+	++	+	+	++	+	LE	48	–
12			40	48	–	–	–	+	++	+	+	+	++	+	–		NA
13	F	Ex 8c.1045G>A p.(Gly349Ser) Ex 17c.2249C>T p.(Pro750Leu)	48	51	+	–	–	+	+	+	+	+	++	+	–		+
14	G	Ex 11c.1529C>T p.(Ala510Val) Ex 2c.233T>A p.(Leu78Ter)	30	51	+	–	–	+	++	++	+	+	++	++	–	58	++
15	H	Ex 11c.1529C>T p.(Ala510Val) (hmz)	20	29	–	–	–	+	+	+	+	+	++	+	–		–
16	I	**Ex 8c.988 – 1G>A** Ex 17c.1715C>T p.(Ala572Val)	25	45	–	–	–	–	++	++	+	+	++	+	–		++
17			37	43	–	–	–	–	+	+	+	+	+	+	–		++
18	J	Ex 2c.233T>A p.(Leu78Ter) **Ex 8c.988 – 1G>A**	25	33	+	–	–	–	+	++	+	+	+	+	–	41	–
19	K	**Ex 8c.988 – 1G>A** (hmz)	40	45	+	–	–	+	++	+	+	–	++	+	–	54	NA
20			35	39	+	–	–	+	+	++	+	+	+	+	–		NA
21			39	44	+	–	–	+	–	++	+	+	+	–	–	52	+
22	M	**Ex 4c.473_474del** **p.(Leu158GlnfsTer30)** **Ex 8c.988 – 1G>A**	30	50	+	–	+	+	++	++	+	+	+	+	–	NA	–

Abbreviations: +, mild; ++; moderate; +++, severe; Ex, exon; CPEO, chronic external ophtalmoplegia; LE, lower extremities; MRI, magnetic resonance imaging; UE, upper extremities.
Exons are numbered according to reference NG_00808.2.1.
Novel variants are indicated in bold characters.

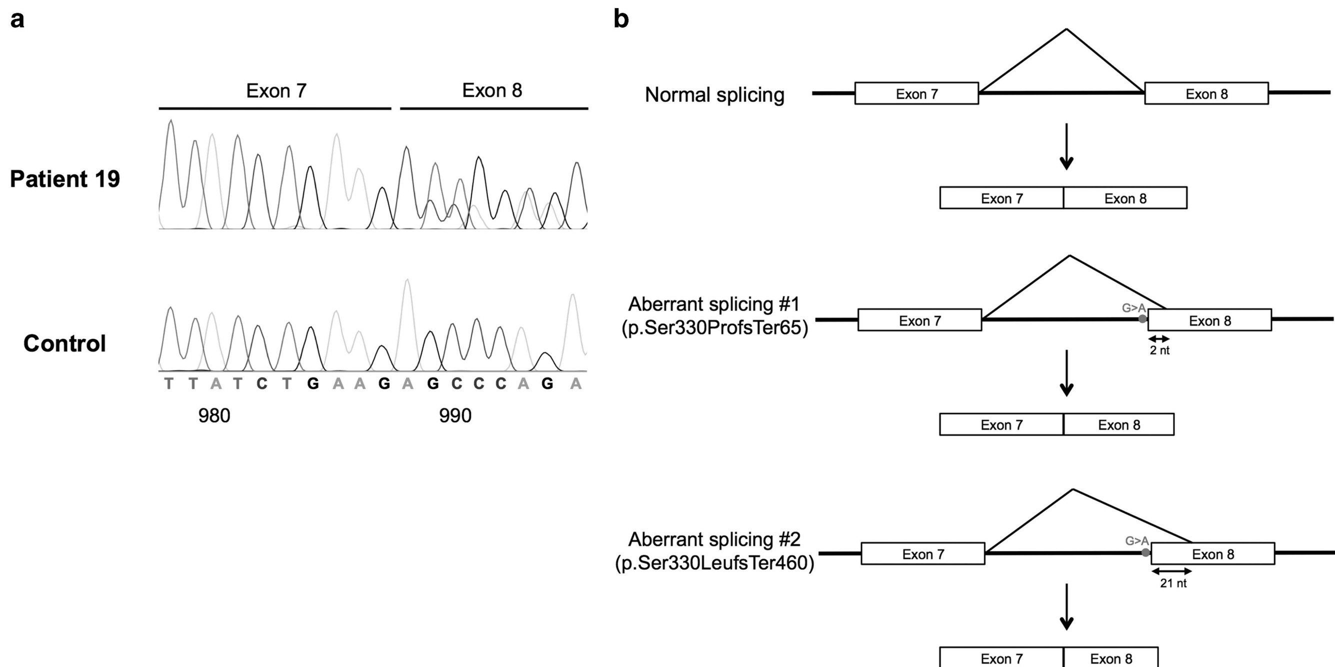

Figure 1 cDNA analysis of the splice site variant c.988-1G > A. (**a**) Complementary DNA sequence chromatograms are shown for a non-affected control and patient 19 who is homozygous for the c.988-1G > A variant. The G to A substitution leads to the loss of the acceptor splice site and the use of two alternate cryptic acceptor sites located within exon 8, causing the deletion of 2 and 21 nucleotides from the beginning of exon 8, respectively, as well as a frameshift and premature stop codon. (**b**) Schematic representation of normal splicing of *SPG7* (upper panel) and aberrant splicing as seen in case 19 (middle and lower panel).

ophthalmoplegia documented even in the older cases, though clearly some pursuit difficulties above abnormal saccades and nystagmus (15/22; 68.1%) seem to appear with age.[23] Only one patient demonstrated optic atrophy as has been reported in other cases.[23,24] Although proximal lower limb weakness was a rare finding at initial evaluation (3/22; 13.6%), it clearly develops with time (mean age 54.4, 7/21; 33.3%). Ambulatory loss appears to be exceedingly rare in patients. MRI data were available for 16 patients. Various degrees of cerebellar atrophy were present in the majority of them (14/16; 87.5%) and it was associated with mild supratentorial atrophy in a few cases (Table 1, Figure 2). Intra-familial variability was also observed on MRI, as cerebellar atrophy was moderate in the more ataxic siblings, but absent or milder in others with less ataxia (Table 1, Figure 2). Follow-up MRI was available for one case presenting mild cerebellar atrophy over a period of 7 years (age 51–58); subject 13 did not show marked progression of cerebellar atrophy despite clinical progression of his ataxia without loss of independent ambulation (Figure 2). However, more serial MRI data would be necessary to establish a correlation between the increasing ataxia and the cerebellar atrophy. The evolution of the gait difficulty in patients is clearly due to both the progression of the ataxia and the spasticity. Most patients were followed in a rehabilitation clinic on a yearly basis. Lioresal in doses of 10–90 mg per day in divided doses were given to many of the more spastic patients. In a few rare cases, Botox injections in the lower extremities were given. Urinary urgency was medically treated in the > 50% of cases presenting this symptom.

DISCUSSION

We report the identification of causative variants in *SPG7* in 22 FC patients from 12 families affected with autosomal recessive spastic ataxia. This is only the second report of *SPG7* mutations in FC patients,[19] and the first report of a large cohort. These results strongly suggest that homozygous or compound heterozygous *SPG7* variants explain a significant proportion of FC spastic ataxia cases (38.7% of families in our cohort). Sanger sequencing detected a slightly higher frequency of SPG7 cases (9/22, 40.9%) than WES (3/9, 33.3%). This may be due to a larger phenotypic heterogeneity in the patients sent for WES compared with the ones screened by Sanger sequencing. SPG7 should be considered in spastic ataxia patients lacking a genetic diagnosis. The *SPG7* c.1529C > T (p.(Ala510Val)) variant was present in six families (12 patients), including one homozygote, making it the most frequent variant identified in our cohort. It was also found to be the most common *SPG7* variant in several other populations.[9,15,16,25] Whereas it was first thought to be a benign variant, its pathogenicity was later demonstrated[15,22] and its presence in our FC spastic ataxia cohort supports this claim. In addition, this variant was present at a frequency slightly above 1% in our in-house exome database. This is significantly higher than public databases (1000 Genomes, EVS and ExAc), but is comparable to what has been reported in the literature for British, Spanish, Italian and German populations.[9,10,15,22] A threshold of one percent is frequently used for the filtering of recessive variants. However, 'not so rare' variants have been identified as causing recessive diseases and this should be taken into account when searching for disease variants altering protein function. Stringent filtering parameters might prevent the uncovering of disease variants, as would have been the case in our SPG7 cohort. In addition to this more common variant, we identified two previously unreported *SPG7* variants, including a novel splice site variant present in five families (eight patients), making it the second most frequent variant in our cohort. Despite the two more common variants in our cohort, the identification of seven distinct *SPG7* variants supports the growing documentation that there is allelic heterogeneity in the FC founder population, even for rare diseases.[26] Additional patients are currently being investigated for *SPG7* mutations in a clinical setting, which may

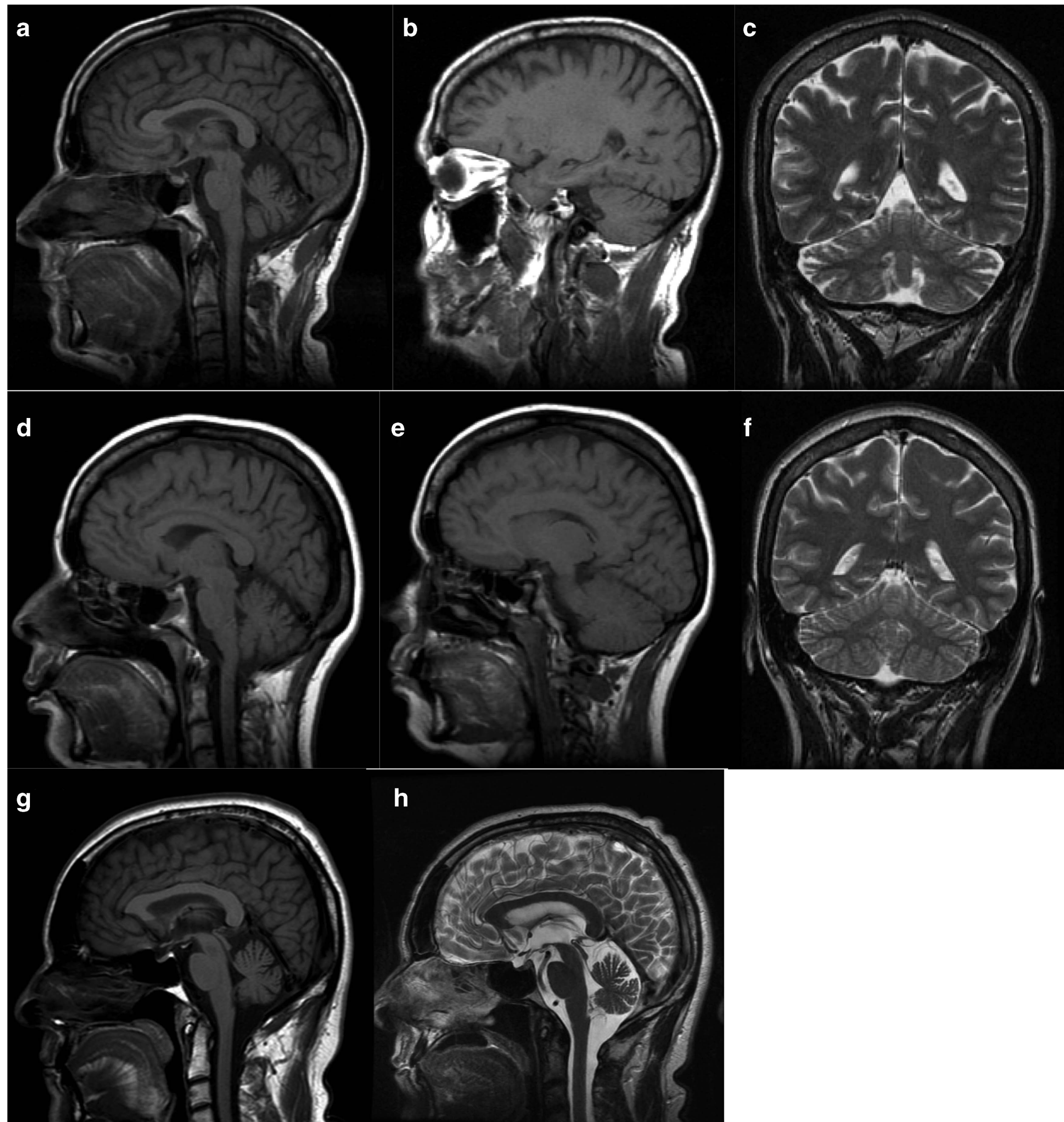

Figure 2 Brain MRI of FC cases with *SPG7* variants. (**a**–**f**) Sagittal T1-weighted (**a**, **b**, **d**, **e**) and coronal T2-weighted (**c**, **f**) images show moderate atrophy of the cerebellar vermis and hemispheres in subject 2 at age 57 (**a**–**c**), whereas cerebellar atrophy is milder in subject 3 at age 51 (**d**–**f**). Sagittal T1- (**g**) and T2- (**h**) weighted images show mild cerebellar atrophy on the initial MRI of subject 13 at age 51 (**g**) without significant progression at the follow-up MRI at age 58.

lead to the identification of more variants in the FC population. Furthermore, considering the recurrence of several variants in our cohort and the relatively high frequency of the p.(Ala510Val) variant in the FC population, our results highlight the importance of genetic counseling for *SPG7* variant carriers.

Variants in three of the patients were identified through WES, again illustrating the efficiency of exome sequencing for the diagnosis of inherited ataxias, as it identified the genetic cause in patients for whom *SPG7* would not have been considered based solely on clinical symptoms. This study, as well as more recent publications on *SPG7* mutation carriers, supports that cerebellar ataxia is a very frequent feature.[5–7,9,15] When present in milder adult-onset cases with spasticity, it suggests this diagnosis if not associated with cognitive deficit or peripheral neuropathy. In fact, cerebellar features, including ataxia, are more pronounced than spasticity in our cohort. This agrees with more recent literature suggesting that cerebellar ataxia can be the dominating clinical symptom in SPG7 and that SPG7 should be considered in the differential diagnosis of inherited cerebellar ataxia. In conclusion, we show for the first time that mutations in *SPG7* are an important cause of autosomal recessive spastic ataxia in the FC population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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WEB RESOURCES

EVS: https://evs.gs.washington.edu/EVS/. ExAC: Cambridge, MA, USA (http://exac.broadinstitute.org; March 2015). MutationTaster: www.mutationtaster.org

ACCESSION CODES

Gene: SPG7. Variants/accession numbers: c.1529C > T, p.(Ala510Val)/SCV000245719; c.2249C > T, p.(Pro750Leu)/SCV000245720; c.1715C > T, p.(Ala572Val)/SCV000245721; c.988-1G > A/SCV000245722; c.1045G > A, p.(Gly349Ser)/SCV000245723; c.233T > A, p.(Leu78Ter)/SCV000245724; c.473_474del, p.(Leu158QfsTer30)/SCV000245726.

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