



POLR3A-related spastic ataxia: new mutations and a look into the phenotype

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Abstract

Adolescent-onset spastic ataxia is a proposed novel phenotype in compound heterozygous carriers of an intronic mutation (c.1909 + 22G > A) in the *POLR3A* gene. Here, we present ten new cases of *POLR3A*-related spastic ataxia and discuss the genetic, clinical and imaging findings. Patients belonged to six pedigrees with hereditary spastic paraplegia or cerebellar ataxia of unknown origin. All affected subjects presented with compound heterozygous variants, comprising c.1909 + 22G > A in combination in each pedigree with one of the following novel mutations (Thr596Met, Tyr665LeufsTer11, Glu198Ter, c.646-687_1185 + 844del). The new mutations segregated with the phenotype in all families. The phenotype combined variable cerebellar ataxia, gait and lower limb spasticity, involvement of central sensory tracts and in some cases also intention tremor. The reportedly characteristic hyperintensity along the superior cerebellar peduncle on MRI was observed in ~80% of the cases. Our study extends the clinical and molecular phenotype further supporting the pathogenic role of the c.1909 + 22G4A intronic mutation and identifying four novel causative mutations in *POLR3A*-related spastic ataxia. Certain characteristic MRI features may be useful to guide genetic diagnosis.

Keywords Hereditary spastic paraplegia · Hereditary ataxia · Spastic ataxia · *POLR3A*

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Introduction

POLR3-related disorders comprise a number of clinically overlapping disease entities caused by recessive mutations in *POLR3A*, *POLR3B* and *POLR1C* genes [1]. The best recognized phenotypes consist of an early-childhood-onset hypomyelinating leukodystrophy manifesting with variable combinations of cerebellar ataxia, tremor, spasticity, mental retardation, oligodontia and hypogonadotropic hypogonadism [1–6]. Recently, a novel and milder phenotype consisting of adolescent-onset spastic ataxia has been proposed to be specific of an intronic mutation (c.1909 + 22G4A) in the *POLR3A* gene [7]. In these subjects the typical signs of hypomyelinating leukodystrophy on MRI would be absent and a characteristic hyperintensity along the superior cerebellar peduncle (SCP) in T2 and FLAIR sequences would be observed instead [8–10]. Whether or not the reported intronic mutation is specific for this clinical and MRI phenotype is still a matter of debate [11–13]. Here, we present ten new cases of *POLR3A*-related adolescent-onset spastic ataxia, all of them presenting with the c.1909 + 22G > A variant in compound heterozygosity with four novel mutations.

Our study extends the clinical and molecular phenotype further supporting the pathogenic role of the c.1909+22G4A intronic mutation within the *POLR3A* gene in this syndrome.

Patients and methods

Subjects

The ten patients reported here belonged to six different pedigrees and were studied at the University Hospital Marqués de Valdecilla in Santander, Spain. The individuals had a previous diagnosis of either cerebellar ataxia or hereditary spastic paraplegia (HSP) of unknown origin. In the setting of clinical practice, a diagnostic multigenic panel was conducted. All patients and relatives signed an informed consent and the study was approved by the local ethical committee.

Genetic analysis

Sequencing was performed using a custom-targeted NGS approach designed to study 187 related ataxia and spastic paraplegia genes using SureSelect Capture Library reagents (Agilent Technologies, Santa Clara, CA, USA) and probes custom designed to capture coding exons, following Agilent protocols and recommendations in combination with CytoScan XON Suite (Applied Biosystems, Waltham, MA, USA). Genetic methodology, primers and PCR conditions are detailed in Supporting information (genetic methodology and Table S1).

MRI imaging

The MRI studies were performed on a 3.0-T scanner (Achieva 3.0T; Philips Healthcare, Best, The Netherlands) with an 8-channel head coil. The protocol included an axial T2-weighted sequence, 3DFLAIR (TR 8000, slice thickness: 0.6 mm), and 3DT1 (slice thickness 1 mm).

Results

Clinical features

The main clinical features of the affected subjects are summarized in Fig. 1 and Videos 1–4. Age at onset of first symptoms ranged from 10 to 31 years. Gait clumsiness or unsteadiness was the presenting symptom in all cases but in subjects from Fam 2 who presented with upper limb tremor. All cases were able to walk independently at the age of examination (mean disease duration 25.8 years), four of them requiring support. A predominantly spastic-type gait was observed in Fam 1 and 3, whereas in subjects

from Fam 2 and 4 ataxia predominated over spasticity. Subjects from Fam 5 and 6 showed mixed features. Dysarthria, when present (Fam 2 and 4), was mild. Interestingly, ocular movements were normal in nearly all cases except for Fam 3: II-4, who showed mild gaze-evoked nystagmus. Half of the subjects showed tremor affecting upper limbs, both postural and predominantly kinetic, in one of the subjects also affecting the head. Deep tendon reflexes were conserved in upper limbs and either normal or brisk in lower limbs. All patients showed Babinski sign as well as reduced vibration sense at the lower limbs and Romberg's sign. Pes cavus was present in three cases and eight also showed genu recurvatum, requiring orthopedic measures in one case (Fam 4: II-3). Distal lower-limb erythrocyanosis was noted in five patients, iris diaphragm sign obtained by pressing on the skin with the fingertip being negative. Two patients from the same pedigree (Fam 4) reported dental abnormalities and another one suffered myopia (Fam 6: II-1). No patient showed clinically overt signs of gonadal dysfunction nor cognitive abnormalities. Electrophysiological studies showed identical findings in all patients consisting of abnormal lower-limb somatosensory evoked potentials (SEP) and motor evoked potentials, whereas motor and sensory nerve conduction studies were normal.

Genetic findings

Genetic diagnosis was obtained for six independent index cases, all of them presenting with compound heterozygous variants identified in the *POLR3A* gene. PCR and Sanger sequencing confirmed the compound heterozygosity of the identified *POLR3A* variants and the segregation with the phenotype in all families. The identified variants in *POLR3A* are summarized in Fig. 1 and Table S2 including five of them not previously described. For index cases for whom NGS only identified an heterozygous variant in *POLR3A*, analysis with a CytoScan Xon microarray and ChAs allowed to identify a deletion (c.646–687_c.1185+844del; NG_029648.1: g.78020702_78023071del; p.Glu216_Lys395del) (Fig. S1) including exons 6, 7 and 8, not previously described. NGS, PCR and Sanger sequencing with specific sequencing primers with 5' poly(A) and poly(T) were carried out to avoid sequencing errors due to the presence of several homopolymer regions (Fig. S2 and S3). Remarkably, the deleted breakpoint region is surrounded by two identical 21 bp repetitive sequences, suggesting that an unequal crossing over event occurred. This region of the protein codifies partially for an RNA pol N domain (IPR006592 InterPro), which is conserved and characteristic motif for RNA polymerases that bind to DNA for the synthesis of RNA [14].

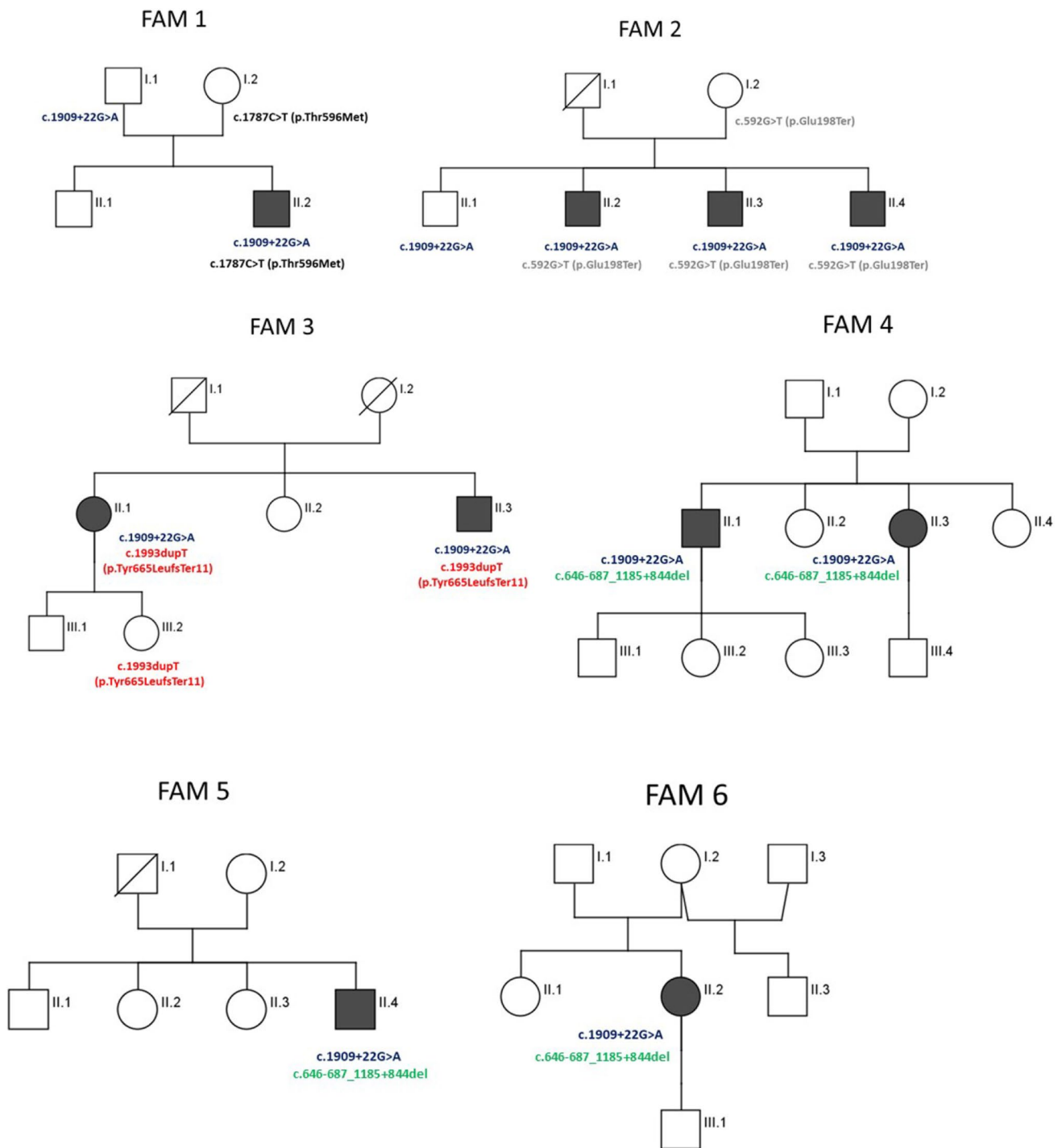


Fig. 1 *POLR3A*-related spastic ataxia pedigrees. Segregation analysis of the mutations and clinical phenotype. *DTR* Deep tendon reflexes, *UL* upper limbs, *SCP* superior cerebellar peduncle, *SEP* somatosen-

sory evoked potentials, *MEP* motor evoked potentials, + present, - absent, + mild, ++ moderate, +++ severe, *n.a.* not assessed

	Fam 1	Fam 2	Fam 2	Fam 2	Fam 3	Fam 3	Fam 4	Fam 4	Fam 5	Fam 6
	II:2	II-2	II-3	II-4	II-1	II-3	II-1	II-3	II-4	II-2
Sex	M	M	M	M	F	M	M	F	M	F
Age (ys)	40	48	50	44	60	33	61	58	53	32
Age at onset (ys)	18	30	14	30	30	10	31	13	18	27
Symptom at onset	Gait clumsiness	Hand tremor	Hand tremor	Hand tremor	Gait clumsiness	Gait clumsiness	Gait instability	Gait instability	Gait clumsiness	Gait clumsiness
Gait ataxia	+	+	++	+	+	++	++	+++	+	+
Spastic gait	+++	+	-	-	++	+++	+	++	++	+
Upper limb ataxia	-	++	+++	+	-	+	-	++	+	-
Lower limb ataxia	++	++	++	+	++	+	+++	+++	++	+
Dysarthria	-	+	+	-	-	-	+	+	-	-
Nystagmus	-	-	-	-	-	+	-	-	-	-
Upper limb DTR	-	+	+	+	+	-	+	+	++	+
Patellar reflex	+	++	+	+	+++	+	++	+	++	+
Aquiles reflex	++	+	+	+	++	-	+	+	+++	-
Babinski's sign	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral
Hypopallesthesia	+++	++	+++	++	++	+	++	+++	++	+
Romberg's sign	+	+	+	-	+	++	+	n.a.	+	-
Pes cavus	-	Bilateral	-	-	-	Bilateral	-	Bilateral	-	Bilateral
Postural/kinetic tremor UL	-	++/+++	+++/>+++	++/++	-	-	-	+/++	-	+/-
Dystonia	-	-	-	-	-	-	-	Cervical	UL	UL
SARA score	8.5	11	15.5	6.5	8	-	12	20	9	3.5
Genu recurvatum	++	-	+/-	-	+	n.a.	+	+++	+	+
SCP hypersignal (MRI)	-	+	+	+	+	+	+	+	+	n.a.
Spinal cord atrophy (MRI)	+/-	-	+/-	+	+	+/-	+	+	¿	n.a.
Abnormal SEP	+	+	+	+	+	+	+	+	+	+
Abnormal MEP	+	+	+	+	+	+	+	+	+	+
Acrocyanosis	+	-	-	+	-	n.a.	+	+	-	+
Dental abnorm.	-	-	-	-	-	n.a.	+	+	-	-

Fig. 1 (continued)

MR imaging

The MRI demonstrated on 3D FLAIR MRI images a striking bilateral hyperintensity along the superior cerebellar peduncles extending from the dentate nucleus up to the

midbrain, just below the red nucleus in eight out of nine cases (Fig. 2). In addition, roughly half cases showed mild cervical spinal cord atrophy. No signs of hypomyelinating leukodystrophy were observed on MRI.

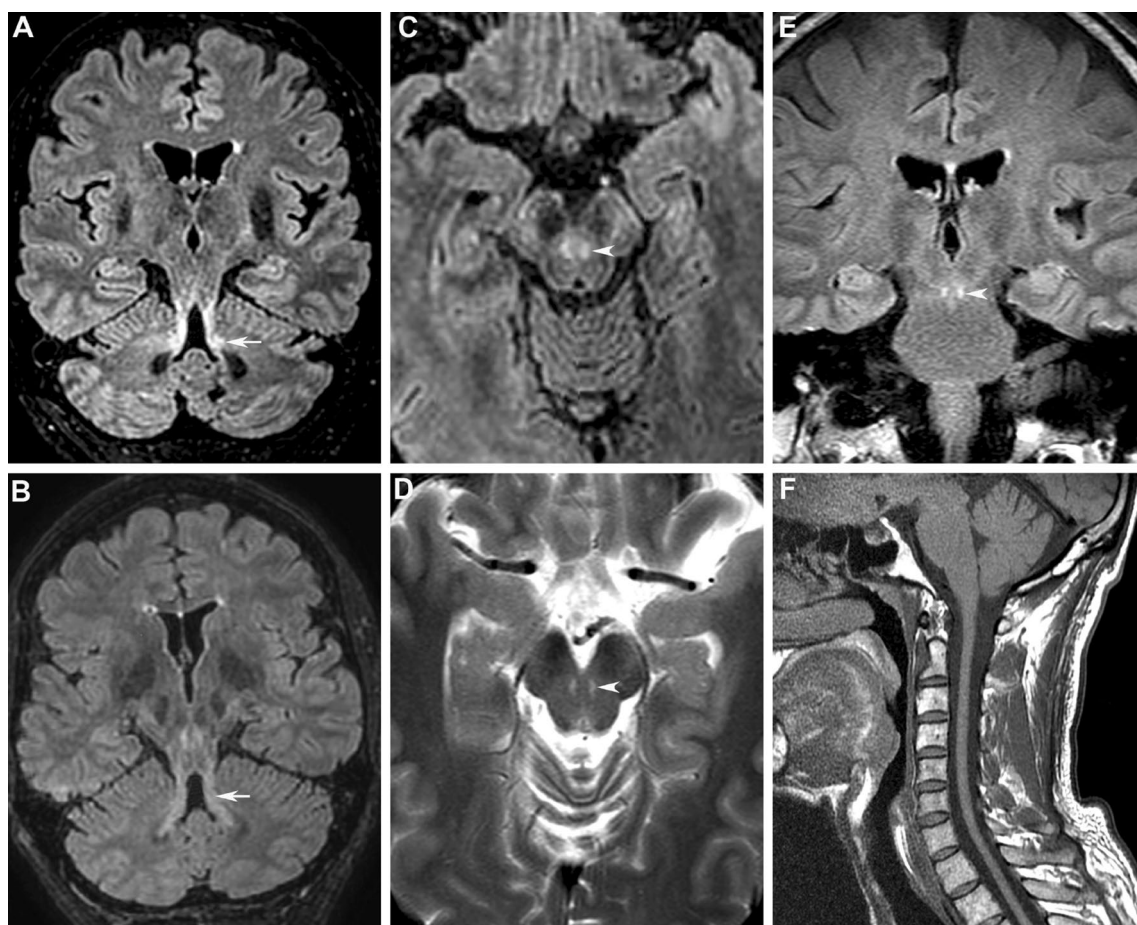


Fig. 2 MRI findings in affected individuals with *POLR3A*-related spastic ataxia. Striking bilateral hyperintensity along the entire superior cerebellar peduncle (SCP) ranging from the cerebellar dentate nucleus (white arrows in **a** and **b**) to the midbrain just below the red nucleus (white arrowheads in **c**, **d** and **e**). 3D FLAIR paracoronal

view angulated along the course of the SCP (**a**, **b**) and standard axial (**c**), axial T2 (**d**) and coronal FLAIR (**e**). Sagittal-T1 (**f**) showing mild but evident atrophy of the spinal cord without cerebellar involvement. **a**, **d**, **e**, **f** (Fam 2-II:4); **b** (Fam 3-II:1); **c** (Fam 2-II:2)

Discussion

Spastic ataxia of adolescent onset is a recently proposed new phenotype observed in compound heterozygous carriers with an intronic mutation (c.1909+22G>A) within the *POLR3A* gene [7–13, 15]. In this article, we provide ten additional cases that support this association and also report four novel mutations in this gene.

The phenotypic spectrum of hypomyelinating leukodystrophies, caused by recessive mutations in *POLR3* genes, comprises a number of clinically overlapping entities [1–6]. Recently, Minnerop et al. identified a new intronic mutation in *POLR3A* gene (c.1909+22G>A) in a pedigree with recessive spastic ataxia of unknown origin. From this finding, a large cohort of patients with a diagnosis of HSP or cerebellar ataxia of unknown origin was tested, and *POLR3A* mutations were identified in ~3.1% of cases, 80% of which were compound heterozygous for the intronic

c.1909+22G>A [7]. The uniform phenotype consisted of a progressive spastic ataxia initiated in adolescence with a high occurrence of tremor and involvement of the central sensory pathways. This phenotype differs from that knowingly attributed to *POLR3A*-related leukodystrophies because of a later age at onset, absence or scarcity of non-neurological manifestations such as endocrine dysfunction or hypo/oligodontia, absence of the characteristic features of MRI (hypomyelination, cerebellar atrophy and corpus callosum hypoplasia), and the presence of bilateral hyperintensity along the superior cerebellar peduncles in MRI FLAIR sequences [8–10]. More recently, Rydning et al. replicated this association in an independent Norwegian cohort [15]. Some authors, however, assert that given the overlap of clinical and imaging characteristics between the different *POLR3A*-related syndromes, the spastic ataxia phenotype should not be considered as one separate but rather a part of the spectrum [11].

The cases presented here share most of the clinical and MRI characteristics with those reported by Minnerop and Rydning and likewise had received a previous diagnosis of hereditary ataxia or HSP of unknown origin [7, 15]. The mean age at onset was around adolescence in half of the cases, the remaining half beginning at age 30. The phenotype combined variable cerebellar ataxia, gait and lower extremity spasticity, central sensory pathway involvement and, in some cases tremor, predominantly of intention type. It was not as homogeneous, however, as some cases would fall into a clinical diagnosis of HSP and others had received a diagnosis of cerebellar tremor–ataxia syndrome. Our patients mostly lacked non-neurological manifestations. Only 20% presented dentition abnormalities, contrary to Minnerop and Rydning who reported a frequency of 65% and 85%, respectively [7, 15]. Other features such as hypogonadism, myopia, or mental impairment were absent in our series. Peripheral nerve conduction studies showed no abnormalities, similar to the cases reported by Gauquelin, and contrary to the findings of Minnerop and Rydning who reported signs of neuropathy in 30% and 20% of the cases, respectively. Central sensory tracts, however, showed a constant impairment, with abnormal SEP observed in all patients. There is a clinical sign we have observed with unusual frequency and severity, the presence of genu recurvatum. It affected 80% of our cases and in at least one case (Fam 4: II-1, Video 4) at a severe degree. As there is no mention of its incidence in other series, we do not know if it could be a characteristic sign in this entity. The same applies to the presence of distal lower-limb acrocyanosis, observed in half of the patients in our series. Normal iris diaphragm sign points to the absence of sympathetic denervation [16]; in any case, similar findings have been reported in HSP [17].

In accordance with previous series, more than 80% of our patients showed hyperintensity along the SCP on 3D FLAIR MRI; conversely, there were no signs of hypomyelination [7]. Spinal atrophy, or mild cerebellar atrophy, was also an inconstant feature in this series.

Regarding genetics, all the cases were compound heterozygous carriers of the intronic c.1909 + 22G > A variant which has been previously described in spastic ataxia cases [7]. All cases also carried a second genetic variation not previously described that segregated with the phenotype and was predicted to be pathogenic by bioinformatics. Pedigrees 4, 5 and 6 shared a deletion (c.646–687_c.1185 + 844del; NG_029648.1: g.78020702_78023071del p.Glu216_Lys395del) (Supplementary Fig. 3) including exons 6, 7 and 8, while pedigrees 1, 2 and 3 showed single nucleotide changes. Since functional analyses were not carried out in our study, we cannot completely exclude a linkage imbalance with other pathogenic mutations.

Our data support the idea that hereditary spastic ataxia should be considered a distinct phenotype within the

spectrum of *POLR3A*-related disorders. We agree with the authors who propose that the c.1909 + 22G > A mutation would be the main determinant in this phenotype. This mutation has been shown to cause an incomplete loss of the wild allele, which in turn can modulate the phenotype, moving it away from the classical hypomyelination phenotype towards a milder spastic ataxia phenotype. As a nuance, since cases beginning over age 20 are not as uncommon, the term early adolescence would be best omitted when naming this phenotype.

From the perspective of clinical practice, we consider truly useful to classify these cases as *POLR3A*-related HSP, ataxia or spastic ataxia. In this clinical context, the very characteristic features of MRI can be of great help in guiding genetic diagnosis.

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Compliance with ethical standards

Conflicts of interest JI received honoraria as speaker from Zambon and financial support for attending conferences from Abbie. KMSC, MCI, XF, IS, EML, AGG, JLMG, JB and AM report no conflict of interests.

Ethical approval This study was conducted according to the ethical principles for medical research involving human subjects according to the Declaration of Helsinki.

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