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BRIEF COMMUNICATION

Novel *SPTBN2* gene mutation and first intragenic deletion in early onset spinocerebellar ataxia type 5

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Abstract

In the present study, we describe two novel cases of SCA5 with early onset. The first one, carrying a novel heterozygous de novo missense mutation in *SPTBN2* gene, showed a striking very severe cerebellar atrophy and reduction of volume of the pons at a very young age (16 months). The latter, carrying the first de novo intragenic deletion so far reported in *SPTBN2* gene, showed a mild cerebellar atrophy involving the hemispheres and a later onset. In both cases, for the first time, a hyperintense signal of the dentate nuclei was observed.

Introduction

Inherited ataxias are a clinically rare and genetically heterogeneous group of disorders characterized by progressive cerebellar ataxia, incoordination, dysarthria, and difficulty in swallowing, due to pathogenic variants in more than 100 genes identified so far.[1–3] Two major groups of ataxia are described: autosomal dominant cerebellar ataxias (also known as spinocerebellar ataxia, SCA) leading to an adult-onset ataxia and autosomal recessive cerebellar ataxias (also known as SCARs). In recent years, it has become clear that the monoallelic or biallelic state of the pathogenic variant is responsible for adult- or infantile-onset cerebellar ataxia.[3] Among these, SCA type 5 (SCA5) is a dominant ataxia associated with spectrin beta non-erythrocytic 2 (*SPTBN2*) gene mutations, characterized by a slowly progressive ataxia with typical adult-onset and global cerebellar atrophy at brain magnetic resonance image (MRI).[2,4–7] The *SPTBN2* gene (11q13.2) encodes the b-III spectrin protein, which is primarily expressed in the Purkinje cells of the cerebellum, and it is involved in trafficking and anchoring of crucial

Table 1. Features of the two patients herein described and patients described up to date with early onset SCA5

Patient	Sex	*SPTBN2* gene mutation	Inheritance	Clinical onset	Developmental delay	Pyramidal signs	Cerebellar findings	Dystonia/ Dyskinesia	Ocular findings	Brain MRI
This Study										
ID 91618	M	c.185C > A p. Thr62Asn het	de novo	5 mo	+	Brisk reflexes	Hypotonia, ataxia	–	Nystagmus, strabismus	16 mo: severe CA involving both the hemispheres and vermis, with abnormal hyperintense signal of the dentate nuclei. The pons was reduced in volume
ID 14020	F	c.1653 + 697_4278+353; p.(Lys551_Gln1426del) g.66463395_66474289del10895 het	de novo	10 mo	+	Brisk reflexes	Ataxia, tremor	–	Strabismus	3 yrs: mild CA involving the hemispheres associated with hyperintense signal of the dentate nuclei. The brainstem and supratentorial brain were normal
Other studies										
Jacob	F	c.1438C > T p. Arg480Trp het	*na*	12 mo	+	Brisk reflexes	Ataxia, tremor	–	Nystagmus	1 yrs: diffuse cerebellar hypoplasia 2 yrs and 9 yrs: mild progression of the CA
Parolin-		Schnekenberg	F		c.1438C > T p. Arg480Trp het	de novo	8 mo	+	–	Ataxia
–		Strabismus	Mild CA							
Mizuno	F	c.1309C > G p. Arg437Gly het	de novo	10 mo	+	–	Ataxia, tremor, dysarthria	–	Nystagmus	10 mo and 2 yrs: moderate CA and mild pontine atrophy
Nuovo	F	c.1438C > T p. Arg480Trp het	de novo	12 mo	+	–	Ataxia, dysarthria	–	Nystagmus	1 yrs$^{10/12}$: global cerebellar hypoplasia
Nicita	M	c.479C > T p. Phe160Cys het	de novo	5 mo	+	–	Ataxia	–	Strabismus	Progressive CA
	F	c.185C > T p. Thr62Ile het	*na*	8 mo	+	–	Ataxia	–	Nystagmus	CA
	F	c.1309C > T p. Arg437Trp het	de novo	10 mo	+	–	Ataxia	–	–	CA
	M	c.1310C > A p. Arg437Gln het	de novo	5 mo	+	Brisk reflexes	Ataxia, tremor	–	Nystagmus	Progressive CA
Accogli	F	c.1310G > A p. Arg437Gln	de novo	2 yrs	+	Brisk reflexes	Ataxia, dysarthria	–	Nystagmus	13 mo: CA (vermis > hemispheres) 4 yrs: progressive CA

(*Continued*)

Table 1 Continued.

Patient	Sex	*SPTBN2* gene mutation	Inheritance	Clinical onset	Developmental delay	Pyramidal signs	Cerebellar findings	Dystonia/ Dyskinesia	Ocular findings	Brain MRI
		het								
Rea	M	c.812C > T	de novo	6 mo	+	Brisk reflexes	Ataxia	Dystonia	–	1 yr: normal 7 yrs: mild hemisphere CA 8 yrs: hemisphere and vermis CA
		p. Thr271Ile								
		het								
Zonta	F	c.1438C > T	de novo	17 mo	+	+	Hypotonia, ataxia,		intentional tremor	–
		p. Arg480Trp								
		het								
Strabismus		2 yrs: cerebellar and vermian hypoplasia with enlargement of cerebrospinal fluid spaces and IV ventricle								

+, present; –, absent; CA, cerebellar atrophy; F, female; M, male; mo, months; MRI, magnetic resonance imaging; na, not available; yrs, years.

neurotransmitter transporters and ion channels to neuronal cell membranes.[5,8–12] Recently, 11 cases of infantile-onset SCA5 have been reported, all showing global developmental delay, eye movement anomalies, and cerebellar ataxia, carrying either missense or truncating heterozygous mutations in *SPTBN2* gene.[2,3,7,13–17] In the present study, we describe two new cases of SCA5 with early onset. The first one, carrying a novel heterozygous de novo missense mutation in *SPTBN2* gene, shows a very severe cerebellar atrophy at onset. The latter carries the first de novo intragenic deletion so far reported in *SPTBN2* gene.

Methods

The patients' parents provided written informed consent to the research and to the publication of the results. Study approval by the Ethics committee was provided by the E. Medea Scientific Institute Ethic Committee.

The probands' DNA were screened using a targeted next-generation sequencing (NGS) approach with a panel of 231 genes. The deletion was identified using ExomeDepth, a powerful bioinformatic tool realized to identify CNVs in exome samples and in NGS data from gene panels. Further details are provided in File S1.

Patients underwent cerebral MRI at 1.5 and 3T. In both cases, multiplanar T1- and T2-weighted images were acquired with age-appropriate TR and TE values. Sedation was required to perform the MRI.

Results

The clinical, genetic, and neuroradiological features of the two patients herein described and the 11 patients described up to date with early onset SCA5 are summarized in Table 1. For a detailed description of the two novel patients, see File S1.

Patient ID 91618

The patient was the only child (male) of non-consanguineous healthy parents. Perinatal history was normal. Since the first year of life, main developmental milestones appeared delayed and an impairment in social and communication skills was evident. At the age of 16 months, neurological examination showed poor social and communicative interaction, absence of expressive language, axial hypotonia, ataxia, increased deep tendon reflexes, clumsiness, and crawling. Brain MRI at 16 months of age documented a severe cerebellar atrophy involving both the hemispheres and vermis, with abnormal hyperintense signal of the dentate nuclei (Fig. 1A). The pons showed reduced volume. NGS of a panel of 231 genes (see File

S1) showed a novel heterozygous variant c.185C > A (p. Thr62Asn) in *SPTBN2* gene. The variant was not present in any of the parents suggesting a de novo origin. The variant was not present in any public database. Predictions of possible pathogenicity were obtained with different software as detailed in File S1. The Thr62 residue is already known to be involved in a mutation, p. Thr62Ile (c.185C > T)[12]. The amino acid change falls within the calponin homology domain of b-III spectrin protein (Fig. 2A and B).

Patient ID 14020

The patient was the only child (female) of distant consanguineous healthy parents (third-degree cousins). Main developmental milestones appeared delayed. At the age of 20 and 29 months, simple febrile seizures occurred (2–3 min). Repeated EEGs showed during sleep poor organization of background activity without epileptic abnormalities. Brain MRI at 3 years of age showed only mild cerebellar atrophy involving the hemispheres associated with hyperintense signal of the dentate nuclei (Fig. 1B). The brainstem and supratentorial brain were normal. At the age of 37 months, neurological examination showed microcephaly (head circumference 40.9 cm, <3rd centile), poor verbal language (few words), diffuse hypotonia, increased deep tendon reflexes, presence of bilateral Babinski sign, mild dysmetria and tremor, and gait ataxia. A mild developmental delay was documented. Targeted NGS did not lead to any pathogenic variant, but the analysis with ExomeDepth detected a putative heterozygous deletion in *SPTBN2* spanning from exon 13 to 20, confirmed by quantitative PCR (qPCR) (data not shown). We subcloned and sequenced the junction fragment from patient genomic DNA (Fig. 2A and B) and we identified two AluSx elements flanking the two breakpoints in intron 12 and 20, which represent the microhomology domains mediating a deletion of 10895bp (g.66463395_66474289del10895) as shown in Figure 3A and B. The deletion was not present in any of the parents suggesting a de novo origin in the patient. Several attempts were made to confirm the deletion at cDNA level starting from RNA extracted from patient's blood. Due to the lack of *SPTBN2* gene expression in blood, no amplification products were obtained. Patient's parents refused skin biopsy, therefore we can only suppose that the genomic deletion generates an in-frame cDNA deletion from exons 13 to 20, producing a putative protein

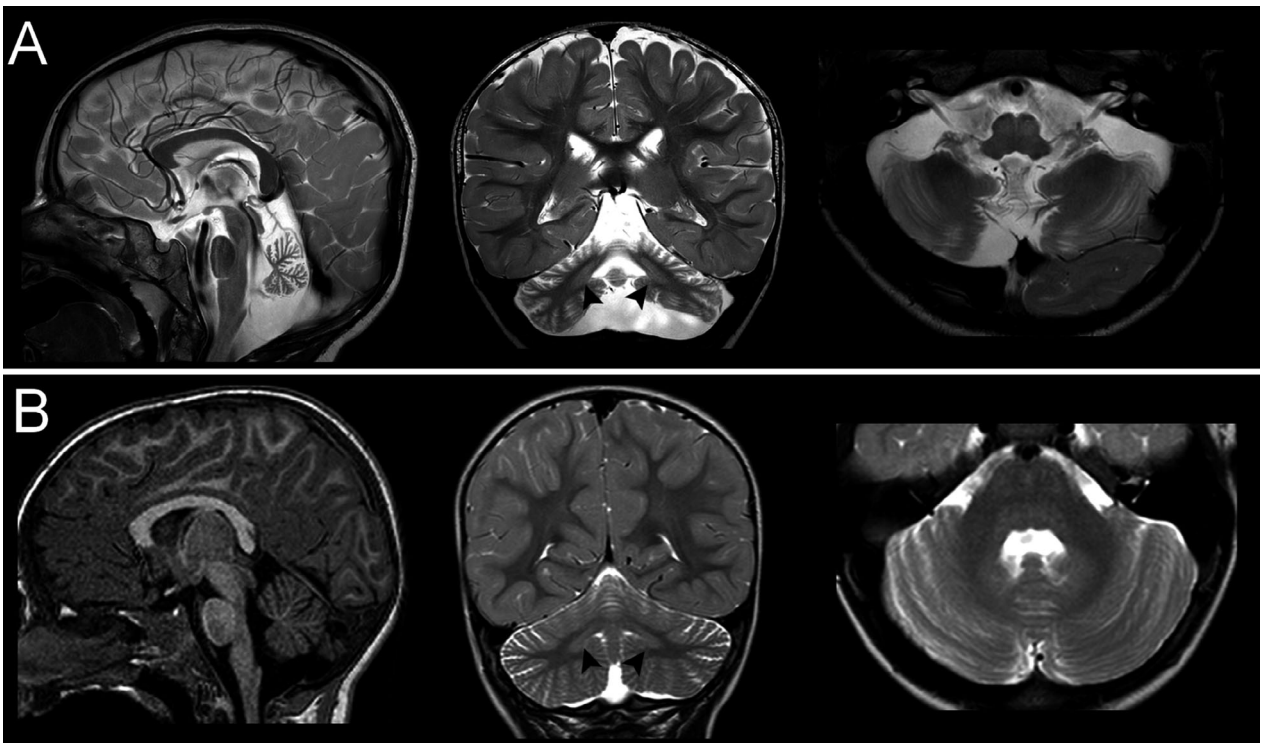

Figure 1. MRI findings. Patient 1 (row A) at 16 months of age shows a severe vermian and cerebellar atrophy with associated relative pontine atrophy. Patient 2 (row B) at 3 years of age has a milder degree of cerebellar atrophy with a normal pons. In both subjects, dentate nuclei have a hyperintense abnormal signal on T2-weighted sequences (arrowheads).

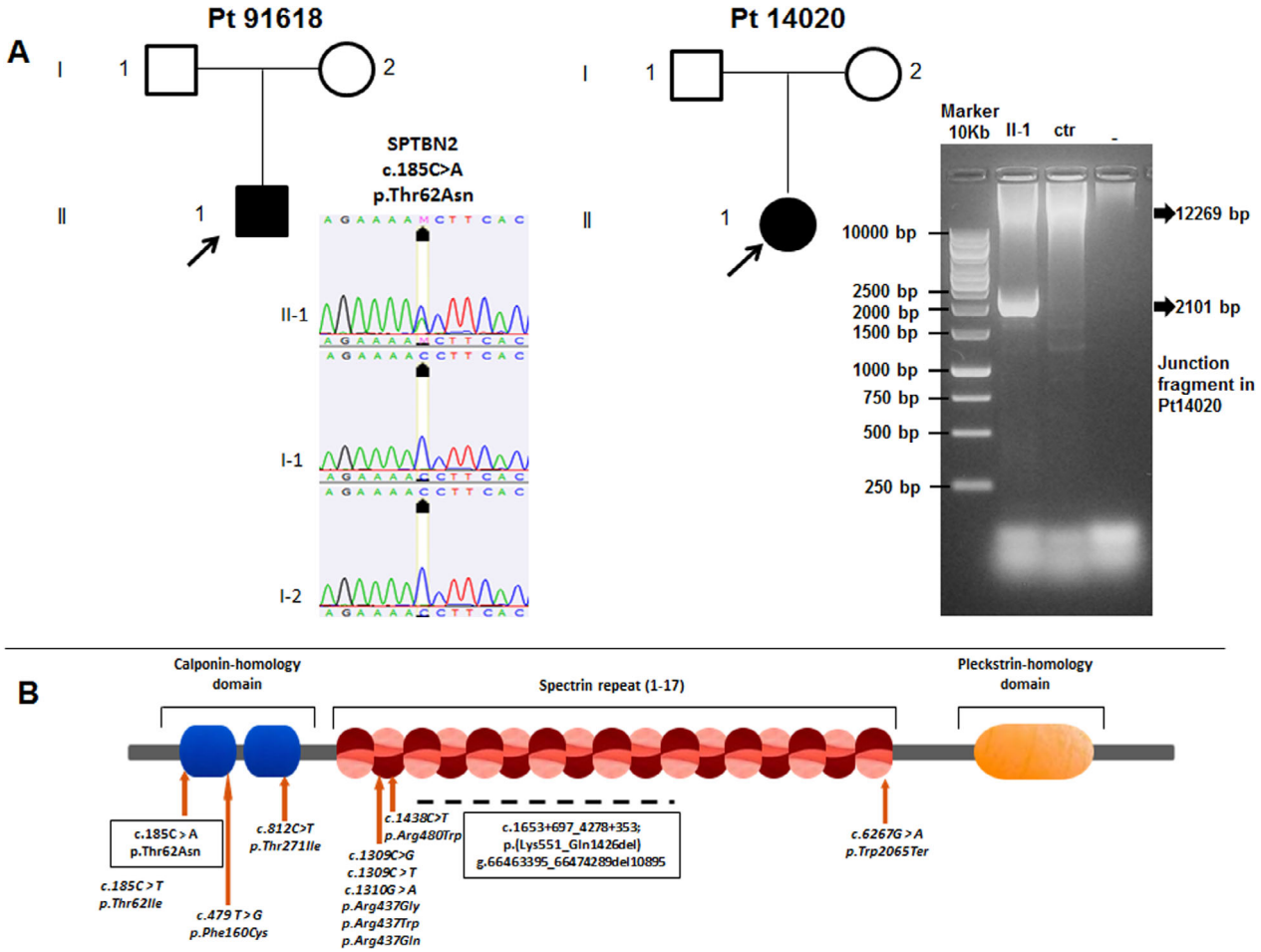

Figure 2. Patient's pedigrees and mutations. Panel (A) represents the patients 91,618 and 14,020 pedigrees. Electropherograms of the mutant and wild-type sequence in patient and in the parents, respectively, are shown. On the right, gel electrophoresis of genomic amplification product in patient 14,020 and control using primer encompassing the putative deleted region. A fragment of approximately 2000 bp is amplified in patient's DNA only containing the deletion breakpoint. Panel (B) shows a schematic representation of the *SPTBN2* protein with the known functional domains, the known mutations (italics character), and the mutations identified in the patients herein described (boxed). The dashed line indicates the deleted region. Acc. N. of the *SPTBN2* genomic and protein sequences are NM_006946.2 and NP_008877.1, respectively.

lacking 875 amino acid residues (p.(551Lys_Gln1426del)) within the spectrin repeats of the SPTBN2 protein (Fig. 3C).

Discussion

In recent years, an increasing number of infantile-onset autosomal recessive cerebellar ataxias associated with heterozygous *SPTBN2* gene mutations (SCA5) were described. The SPTBN2 protein, expressed in soma and dendrites of cerebellar Purkinje cells, is required for the maintenance of dendritic architecture and for the trafficking and stabilization of several membrane proteins. Mutations in *SPTBN2* gene alter dendritic morphology and density and cause changes in Purkinje cells' intrinsic excitability. This reduces sodium currents and causes deficits in glutamatergic neurotransmission.[12] To date, 11 infantile-onset cases of dominant *SPTBN2* gene mutations have been reported, four with the same p. Arg480Trp mutation, all showing the common phenotype of developmental delay in early infancy, evolving into intellectual disability and ataxia. Hyperreflexia, dystonia, and eye movement anomalies can be variously present. Herein we describe two novel SCA5 cases with early onset. Clinical findings of both our cases are completely in line with literature. Nevertheless, at brain MRI, the first one showed a striking, very severe cerebellar atrophy at a very young age (16 months) with a global volume reduction of hemispheres and vermis associated with a marked deepening and enlargement of folia sulci and cortical subarachnoid

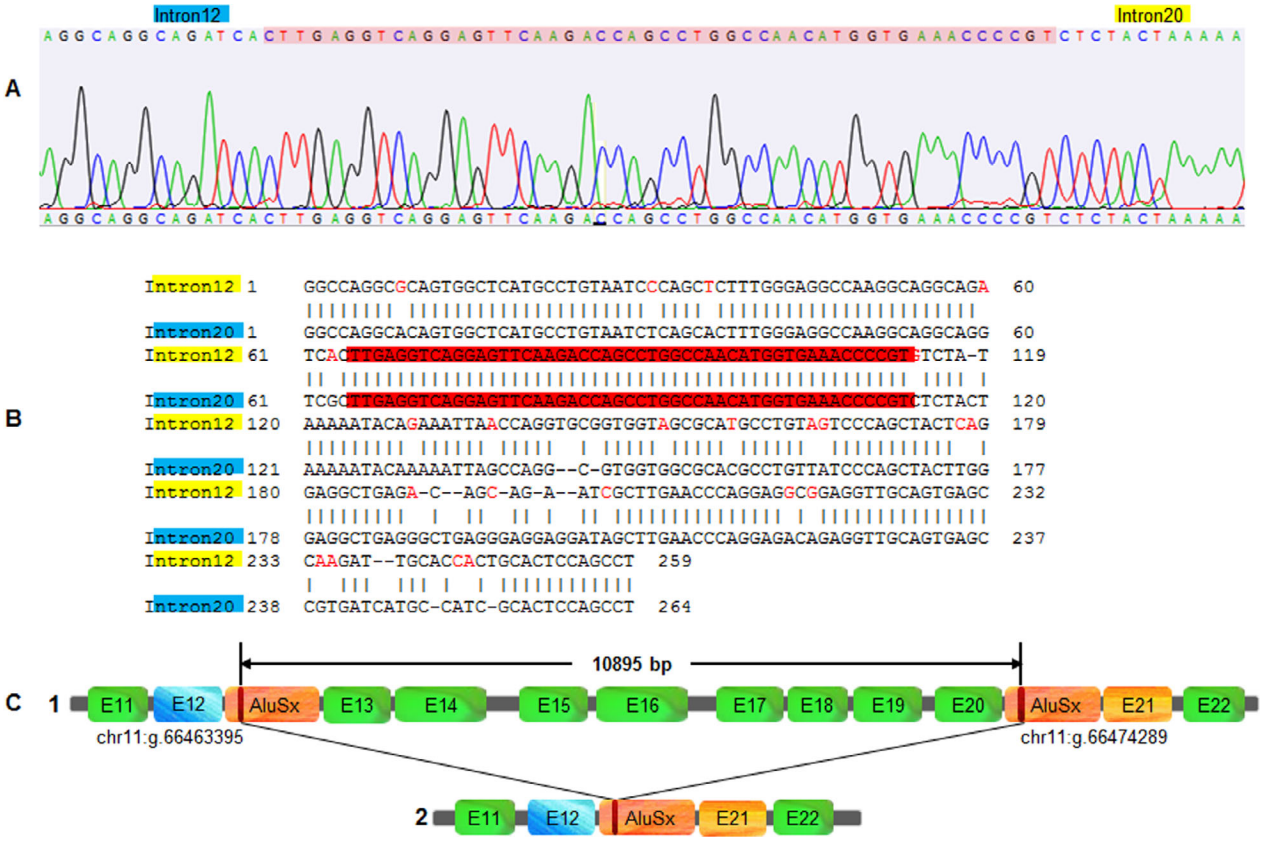

Figure 3. Characterization of the deletion breakpoint in Pt 14020. Panel (A) indicates the patient's genomic DNA sequence encompassing the breakpoint. Panel (B) shows the patient's genomic DNA sequence alignment between the two intronic regions flanking the breakpoint and containing the AluSx repeats. Panel (C) is a schematic representation of the genomic *SPTBN2* deletion of 10895 bp, which generates a putative in-frame cDNA deletion encompassing exons 13 to 20 of *SPTBN2* gene thereby leading to a putative protein missing 875 aa residues.

spaces, pons hypoplasia, and a normal-appearing supratentorial brain. The second one showed only a mild cerebellar atrophy. These findings are quite in line with literature reports in which a cerebellar vermis and hemispheric hypoplasia or atrophy of varying degrees with no evidence of cortical involvement are described.[2,3,7,13–17] Further, this is the report of the second case of brainstem involvement (pons hypoplasia) so far documented.[2] In addition, in both our cases, a hyperintense signal of the dentate nuclei was observed, a finding not described in literature up to now associated with SCA5, but found in association with cerebrotendinous xanthomatosis and methotrexate toxicity, both conditions clinically excluded in our patients. From a genetic point of view, the first patient herein reported carried a novel heterozygous de novo missense mutation in *SPTBN2* gene, while the second one harbored the first intragenic deletion of 10895 bp that spans from intron 12 to 20 of the *SPTBN2* gene and leads to a putative protein lacking 875 amino acid within the spectrin repeat region. AluSx sequences were found to flank the breakpoint and likely mediate the deletion event. It is well known that homologous recombination between dispersed Alu sequences can lead to genetic changes, such as duplications, deletions, and translocations, in different disease genes.[18] These sequences of about 300 base pairs share a great level of identity and allow more efficient homologous recombination than sequences with an imperfect identity which would represent an inefficient target.[18,19] The suggested minimum requirement for favorable homologous recombination is 75% of identity between Alu elements.[20,21] In our study, we hypothesize that the de novo deletion of 10895 bp in *SPTBN2* gene was generated by an unequal homologous recombination event, due to a misalignment between Alu Sx in intron 12 (5′ deletion boundary) of *SPTBN2* gene and Alu Sx in intron 20 (3′ deletion boundary). Both Alu Sx show 86% sequence identity (91% without gaps) and a microhomology domain of 50 bp. This homology is comparable to that previously reported by Rossetti et al in the factor VIII coagulation gene and that reported by Zhang et al in the mitochondrial acetoacetyl-CoA thiolase (T2) gene.[22,23] These

studies let us speculate that, also in our case, unequal homologous recombination between Alus was the likely mechanism for this deletion, adding the dominant SCA due to *SPTBN2* mutations to the group of the genetic disorders having Alu/Alu recombination as underlying genetic mechanism. Of note, the second patient, carrying the intragenic deletion of *SPTBN2* gene, showed a milder clinical (later onset) and neuroradiological phenotype than the patient with the missense mutation.

In conclusion, the authors present two novel cases of infantile-onset SCA associated with a novel *SPTBN2* mutation—the first with an early severe cerebellar involvement and the latter with the first intragenic deletion so far reported.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Romaniello performed acquisition of data and funding; drafted the manuscript. Arrigoni performed neuroradiological study and was involved in revising critically the manuscript. Citterio performed molecular data. De Rinaldis performed acquisition of data. Trabacca performed acquisition of data. Panzeri performed molecular data and was involved in revising critically the manuscript. Bassi was involved in revising critically the manuscript and in acquisition of funding. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. Material and Methods and the two Patients herein described.

