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Case Report

Tremor as an early sign of hereditary spastic paraplegia due to mutations in *ALDH18A1*

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Abstract

Background: The *ALDH18A1* gene is located at 10q24.1 and encodes delta-1-pyrroline-5-carboxylate synthetase (P5CS), a mitochondrial bifunctional enzyme that catalyzes the first two steps in *de novo* biosynthesis of proline, ornithine, citrulline, and arginine. *ALDH18A1*-related disorders have been classified into four groups, such as autosomal dominant and recessive hereditary spastic paraplegia (SPG9A and SPG9B, respectively), as well as autosomal dominant and recessive cutis laxa (ADCL3 and ARCL3A, respectively). Neurodegeneration is a characteristic feature of all groups.

Case report: Here, we report a girl with compound heterozygous disease-causing variants (c.-28-2A>G and c.383G>A, p.Arg128His) in the *ALDH18A1* gene, revealed by whole exome sequencing. The c.-28-2A>G variant in intron 1, inherited from the mother, is a novel mutation, while the c.383G>A variant in exon 4, inherited from the father, has already been reported. The patient presented with vigorous infantile tremor preceding progressive spastic paraplegia. Dysmorphic features included elongated face, deep-set ears, upturned nose, long philtrum and pointed chin. Intrauterine and postnatal growth retardation, microcephaly, global developmental delay and profound intellectual disability were also noticed. Blood fasting ammonia level, plasma proline, ornithine and arginine levels were normal, while citrulline level was slightly decreased. Brain MRI revealed moderate hypoplasia of the corpus callosum and reduction of white matter volume.

Conclusions: The patient represents SPG9B, a rare form of autosomal recessive hereditary spastic paraplegias. The early onset tremor, preceding lower limb spasticity appears to be a unique early manifestation of neurodegeneration in this case.

Keywords: *ALDH18A1*-related disorders; Neurodegeneration; Hereditary spastic paraplegia type 9B; Growth retardation; Intellectual disability

1. Introduction

Monoallelic and biallelic mutations in *ALDH18A1* (OMIM 138250), located at 10q24.1, can cause neurodegeneration in association with various non-neurological features [1–3]. Based on genotypic and phenotypic features the *ALDH18A1*-related disorders have been classified into four groups, such as autosomal dominant and recessive hereditary spastic paraplegia (SPG9A, OMIM 601162 and SPG9B, OMIM 616586,

Abbreviations: P5CS, delta-1-pyrroline-5-carboxylate synthetase; G5K, glutamate 5-kinase; G5PR, glutamate 5-phosphate reductase; SPG9A, autosomal dominant hereditary spastic paraplegia; SPG9B, autosomal recessive hereditary spastic paraplegia; ADCL3, autosomal dominant cutis laxa; ARCL3A, autosomal recessive cutis laxa; GnomAD, The Genome Aggregation Database

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respectively), as well as autosomal dominant and recessive cutis laxa (ADCL3, OMIM 616603 and ARCL3A, OMIM 219150, respectively) [1–3]. *ALDH18A1* encodes delta-1-pyrroline-5-carboxylate synthetase (P5CS, EC 1.2.1.41 and 2.7.2.11), a mitochondrial bifunctional enzyme that catalyzes the first two steps in the biosynthesis of proline, ornithine, citrulline, and arginine from glutamate. It comprises two domains, with different enzymatic activities: an N-terminal glutamate 5-kinase (G5K) domain, responsible for the glutamate phosphorylation to gamma-glutamyl phosphate, and a C-terminal glutamate 5-phosphate reductase (G5PR) domain, which catalyzes the reduction and conversion to gamma-glutamyl semialdehyde, which is further metabolized to proline and ornithine [1–3]. Two isoforms of P5CS are generated, differing only by 2 amino acid insert in the G5K domain. The short P5CS isoform has high activity in gut, where it catalyzes an essential step in the arginine biosynthetic pathway. The long isoform of P5CS is expressed in multiple tissues and is necessary for the synthesis of proline from glutamate [4]. Although P5CS expression in the brain is not strong, it has a measurable activity [4].

Each of the *ALDH18A1*-related disorders are rare [1]. We extend the genotypic and phenotypic spectrum of SPG9B by reporting a girl with compound heterozygous *ALDH18A1* mutations who had intense tremor in infancy, preceding the development of spastic paraplegia.

2. Case report

The proband, a girl was born from the first uneventful pregnancy to heathy, non-consanguineous Caucasian parents on the 37th gestational week. Her birth weight was 1950 g (−2.2 SD), head circumference 30 cm (−2.0 SD) and length 44 cm (−1.4 SD). Dysmorphic features included elongated face, deep-set ears, upturned nose, long philtrum and pointed chin (Fig. 1A, B). She did not have any cutaneous involvement. At about 2 months of age fast head and hand tremor appeared in the form of rhythmic back-and-forth involuntary movements with low amplitudes both at rest and during action. Fasting did not worsen these movements. The tremor became quite vigorous with waxing amplitudes during infancy and gradually waned later. There was no tremor at rest by the age of 5 years; however, emotional distress, particularly fear still provoked it. She had hypotonia and was unable to sit at age of 10 months, or stand at 12 months of age. She had very short attention span and limited interest in her surroundings. Developmental Quotient (DQ) of 45 was found by Brunet-Lézine test at the age of 2 years.

Fasting ammonia level was normal. Plasma proline, ornithine and arginine levels were also within the normal range, while citrulline level was slightly decreased (9 μmol/L, normal: 10–50 μmol/L, borderline). The results of other blood tests were normal.

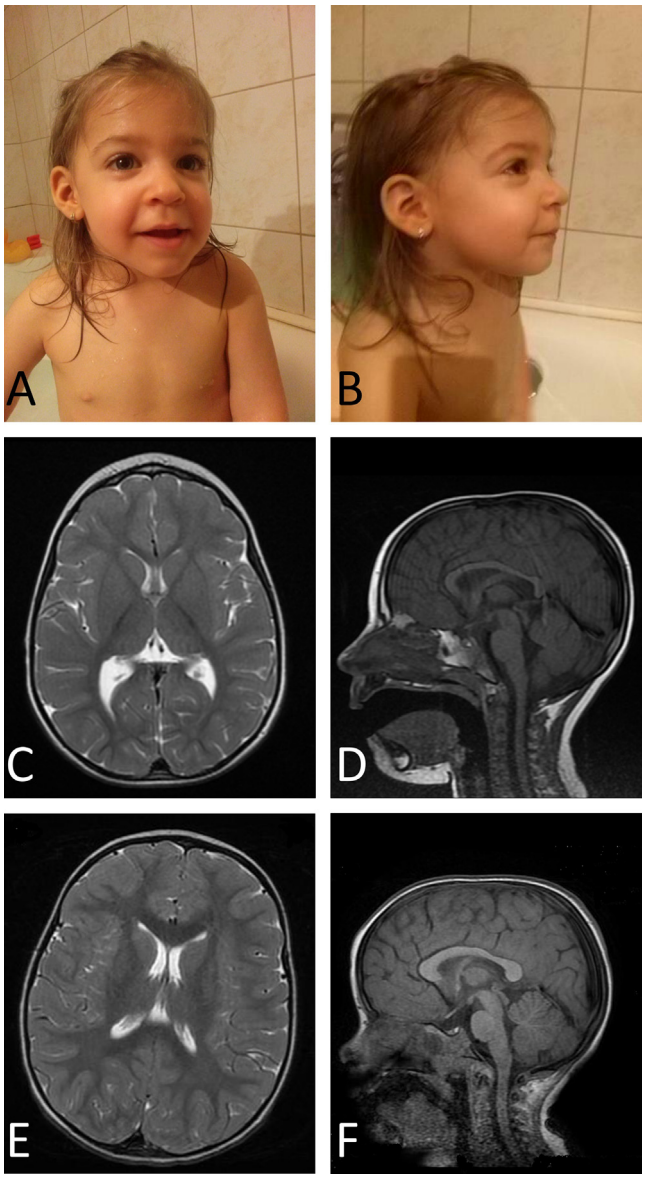

Fig. 1. Photos and MR images of the patient. The patient at the age of 2.5 years (A and B). Dysmorphic features can be seen: elongated face, deep-set ears, upturned nose, long philtrum and pointed chin. Parental written permission has been gained to publish the patient's photos. T2-weigthed axial (C) MR image of the patient shows the paucity of white matter and thinning of the genu of the corpus callosum compared to age-matched control (E). T1 weighted sagittal MR image of the patient (D) demonstrates the hypoplasia of the corpus callosum (D) compared to control (F).

Brain MRI at the age of 2 years revealed reduced volume of white matter and moderate hypoplasia of the corpus callosum (Fig. 1C–F).

At the age of 5.5 years her head circumference was 46 cm (−3.5 SD), weight 15 kg, (−1.9 SD), and height 98 cm (−2.8 SD). By this age, marked spasticity through most of the range of motion (Modified Ashworth scale

2) developed in her lower limbs with brisk deep tendon reflexes. Wide based spastic gait was also observed. There was no speech and intellectual disability was evident. She had disruptive behavior hindering us from taking formal Intelligence Quotient (IQ) test.

2.1. Genetic analysis

Routine chromosomal analysis by G-banding showed normal 46,XX karyotype. Genomic DNA was extracted from peripheral blood samples with the Puregene kit (Gentra). Array comparative genomic hybridization (aCGH) showed normal genomic copy number (Quantitative Genomic Medicine Laboratories, S.L., Barcelona, Spain).

Trio analysis by whole exome sequencing (WES) was performed with CentoXome® Gold at Centogene AG (Rostock, Germany) as described earlier [5]. A heterozygous variant in intron 1 (NM_002860.4:c.-28-2A>G) and another heterozygous variant in exon 4 (NM_002860.4:c.383G>A, NP_002851.2:p.Arg128His) of the *ALDH18A1* gene were detected (Fig. 2A, B). The c.-28-2A>G variant is likely pathogenic because it changes the acceptor splice site of intron 1 in the 5′-UTR, causing skipping of exon 2 (the start codon is in exon 2). The possible molecular effect of this variant was tested *in silico* using MutationTaster. It predicted that c.-28-2A>G variant is disease causing (prob: 0.969300883264394); the wild type splice site (tgca|GATA c.-28) has been lost. This variant is absent in the databases [GnomAD (The Genome Aggregation Database), dbSNP, Exome Variant Server, ClinVar]. However, the p.Arg128His variant has been previously reported in compound heterozygous state as disease-causing for autosomal recessive spastic paraplegia [3]. The c.-28-2A>G variant was also detected in the mother (Fig. 2A) in a heterozygous state, whereas the c.383G>A was detected in the father (Fig. 2B) also in a heterozygous state.

3. Discussion

The patient in this report is compound heterozygous for two *ALDH18A1* disease-causing mutations. Both mutations affect the G5K domain of the P5CS enzyme. The phenotypic characteristics, such as intrauterine growth retardation, dysmorphic features, short statue, microcephaly, global developmental delay, cognitive impairment, progressively developing spastic paraplegia and lack of cutaneous manifestations in association with biallelic *ALDH18A1* mutations meet the criteria of SPG9B [1–3,6–8].Fifteen patients in 8 families have been described so far with this disorder, including the patient in this report (Table 1). The compound heterozygous mutations in 5 families and homozygous mutations in 3 families distributed randomly in the *ALDH18A1* gene, affecting both the G5K and G5PR domains (Table 1). Corpus callosum hypoplasia and thin white matter, found in our patient, have been described in *ALDH18A1*-related disorders, however they are rare in SPG9B (Table 1). Autopsy findings or neuropathology have never been reported.

The vigorous infantile tremor, the presenting sign in our patient, was unusual and has never been reported so early in SPG9B. Tremor, reported in other cases of SPG9B, started later, at around the 7th and 15th years of age [2,3] (Table 1). It can be regarded as a manifestation of neurodegeneration [9]. Tremor has also been seen in patients with ARCL3 due to biallelic *ALDH18A1* mutations [10,11]. Tremor can occur in other types of hereditary spastic paraplegias as well, like in SPG4 due to *SPAST* mutation [12] and SPG11 due to mutations in the *spatacsin* gene [13,14].

Neurodegeneration seems to be a common feature of autosomal recessive and autosomal dominant *ALDH18A1*-related disorders [1]. A unifying view for these disorders has been hypothesized, claiming that the different presentations conform to a disease continuum of decreasing severity from the cutis laxa forms ARCL3A and ADCL3 to the motor syndromes SPG9B and SPG9A [1]. Global developmental delay and intellectual disability are major manifestations of the central nervous system involvement with equal frequency in both autosomal recessive forms, i.e. in ARCL3A and SPG9B [1]. While hypotonia is a consistent feature in ARCL3A, occasionally followed by pyramidal signs [1,10,15], hypotonia seems to be rare in SPG9B; progressive hypertonia, spasticity and pyramidal signs prevail instead [1,3], as in our patient. A transition between these autosomal recessive conditions might be represented by patients reported with biallelic mutations without both cutis laxa and spastic paraplegia [[16], Patient 2 in [17]].

Data has been collected and reviewed by Marco-Marin and coworkers in favor of the view that the severity of the various syndromes in *ALDH18A*-related disorders would correspond to higher or lower degrees of loss of P5CS function [1]. Decreased serum P5CS activity was found in a patient with SPG9B due to homozygous p.Ser242Asn mutation in the G5K domain; however, the P5CS protein level and its mitochondrial localization in HeLa cells transfected with the mutant *ALDH18A1* plasmids remained unchanged [7]. In another patient with SPG9B due to compound heterozygous mutations in the G5PR domain of *ALDH18A1* (p.Arg371Gln and p.Ser497Asn) residual activity of the P5CS was also observed [8]. Indeed, these findings may suggest that the SPG9B phenotype could be associated with residual P5CS activity, while some patients with biallelic null mutations in *ALDH18A1* and cutis laxa phenotype exhibited pronounced reduction or total absence of P5CS protein [1,11,18,19].

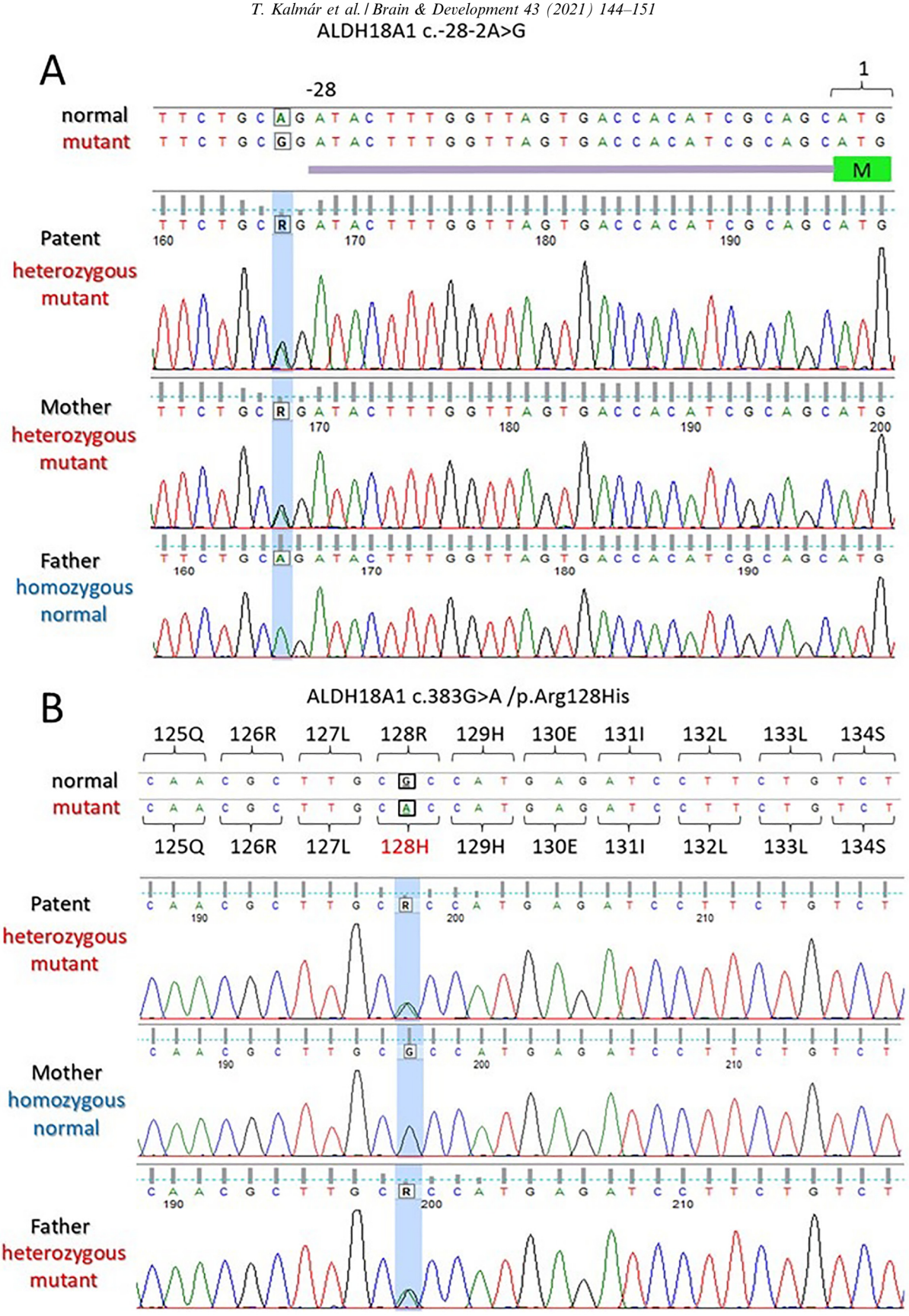

Fig. 2. Mutations in the *ALDH18A1* gene. (A) A single nucleotide change (boxed) near the 5′ UTR in the mutant compared to normal (wild type) sequence. The patient inherited this mutation from her mother (R = A/G). (B) A single nucleotide change (boxed) in the exon 4 in the mutant compared to normal (wild type) sequence. The patient inherited this mutation from her father (R = A/G). The mutated positions are highlighted blue in the DNA chromatograms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Reported biallelic *ALDH18A1* variants associated with autosomal recessive spastic paraplegia (SPG9B).

References	**This study**	[6]		[2]			[3]	
Number of affected siblings	**1**	2		1			4	
Gender	**F**	M	F	M	F	F	M	F
Ethnicity	**Caucasian**	Japanese	Japanese	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Intron/exon location	**Intron 1**	Exon 2	Exon 2	Exon 3	Exon 4	Exon 4	Exon 4	Exon 4
	Exon 4	Exon 4	Exon 4	Exon 14	Exon 15	Exon 15	Exon 15	Exon 15
Nucleotide variation	**c.-28-2A>G**	c.30C>A	c.30C>A	c.251G>A	c.383G>A	c.383G>A	c.383G>A	c.383G>A
	c.383G>A	c.383G>A (two homozygous mutations)	c.383G>A (two homozygous mutations)	c.1741G>A	c.1910T>C	c.1910T>C	c.1910T>C	c.1910T>C
Protein variation	**?**	p.Phe10Leu p.	p.Phe10Leu	p.Arg84Gln	p.Arg128His	p.Arg128His	p.Arg128His	p.Arg128His
	p.Arg128His	Arg128His (two homozygous mutations)	p.Arg128His (two homozygous mutations)	p.Glu581Lys	p.Leu637Pro	p.Leu637Pro	p.Leu637Pro	p.Leu637Pro
Protein domain	**G5K/G5K**	G5K/G5K	G5K/G5K	G5K/G5PR	G5K/G5PR	G5K/G5PR	G5K/G5PR	G5K/G5PR
Dysmorphic features	**Yes**	NA	NA	NA	Yes	No	Yes	Yes
Microcephaly	**Yes**	NA	NA	NA	Yes	Yes	No	No
Growth retardation	**Yes**	NA	NA	NA	Yes	Yes	No	Yes
Developmental delay Cognitive impairment	**Yes**	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lower limb spasticity	**Yes**	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ataxia	**No**	No	No	No	No	No	No	NA
Cerebellar signs								
Tremor (age at onset, years)	**Yes (<1)**	**No**	**No**	**Yes (15)**	**No**	**No**	**No**	**NA**
Epilepsy	**No**	No	No	Yes	No	No	No	No
Cutaneous findings	**No**	No	No	No	No	No	No	No
Ocular findings	**No**	No	No	No	NA	NA	Probable cataract	NA
Brain MRI	**Corpus callosum hypoplasia Thin white matter**	Normal	Normal	Normal	NA	NA	Thin corpus callosum Periventricular white matter anomalies Mild cortical atrophy	NA
Plasma amino acids (proline, citrulline, ornithine, arginine)	**Normal**	NA	NA	Normal	NA	NA	NA	NA

(*continued on next page*)

Table 1 (*continued*)
Reported biallelic *ALDH18A1* variants associated with autosomal recessive spastic paraplegia (SPG9B).

References	[7]	[8]		[6]		[3]	
Number of affected siblings	1	2		2		2	
Gender	F	M	M	M	M	M	M
Ethnicity	Chinese	Caucasian	Caucasian	Japanese	Japanese	Caucasian	Caucasian
Intron/exon location	Exon 7	Exon 10 Exon 10	Exon 10 Exon 10	Exon 12 Exon 16	Exon 12 Exon 16	Exon 17	Exon 17
Nucleotide variation	c.725G>A (homozygous)	c.1112G>A c.1490G>A	c.1112G>A c.1490G>A	c.1321C>T c.1994G>A	c.1321C>T c.1994G>A	c.2143G>C (homozygous)	c.2143G>C (homozygous)
Protein variation	p.Ser242Asn (homozygous)	p.Arg371Gln p.Ser497Asn	p. Arg371Gln p.Ser497Asn	p.Arg441Ter p.Arg665Gln	p.Arg441Ter p.Arg665Gln	p.Asp715His (homozygous)	p.Asp715His (homozygous)
Protein domain	G5K	G5PR/G5PR	G5PR/ G5PR	G5PR/GSPR	G5PR/GSPR	GSPR	GSPR
Dysmorphic features	NA	Yes	Yes	NA	NA	Yes	Yes
Microcephaly	NA	Yes	Yes	NA	NA	Yes	Yes
Growth retardation	NA	Yes	Yes	NA	NA	NA	NA
Developmental delay Cognitive impairment	No	Yes	Yes	Yes	Yes	Yes	Yes
Lower limb spasticity	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ataxia Cerebellar signs	No	No	No	Yes	Yes	NA	No
Tremor (age at onset, years)	**No**	**No**	**No**	**No**	**No**	**Yes (7)**	**Yes (7)**
Epilepsy	No	Yes	Yes	No	No	No	No
Cutaneous findings	No	No	No	No	No	No	No
Ocular findings	No	No	No	No	No	NA	NA
Brain MRI	Normal	Increase in the prominence of the cortical sulci	NA	Mild cerebellar atrophy	Mild cerebellar atrophy	Normal	NA
Plasma amino acids (proline, citrulline, ornithine, arginine)	NA	NA	NA	Normal	Normal	Normal	NA

Abbreviations: M: male, F: female, G5K: glutamate 5-kinase domain, G5PR: gamma-glutamyl phosphate reductase domain, NA: not available.

Measurements of blood ammonia and amino acid levels led to inconstant results. Increased blood ammonia and low plasma proline, ornithine, citrulline and arginine were described in both SPG9A and ARCL3A [3,10]. In contrast, the ammonia and amino acid levels were in the normal or low normal range in SPG9B patients regardless of whether the mutation affected the G5K, as in our case, the G5PR, or both domains [2,3,6]. The patient population with ALDH18A1-related disorders is small, hampering the comparison of the sometimes contradictory results gained by various methods in singular cases, or families with different phenotypes.

The pathophysiology of the neurological impairment in *ALDH18A*-related disorders remains to be clarified. Reduced cerebral proline and/or creatine synthesis might have a role [1,19], however moonlighting of P5CS protein cannot be ruled out [19,20]. Further research warranted to elucidate the mechanism of neurodegeneration in these conditions.

4. Conclusion

We report a girl with a rare form of autosomal recessive hereditary spastic paraplegia (SPG9B) due to compound heterozygous mutations in the *ALDH18A1* gene. The c.-28-2A>G variant in intron 1 is a novel mutation; it was inherited from her mother. The other, c.383G>A variant in exon 4, inherited from her father, has already been published. Vigorous infantile tremor preceding progressive spastic paraplegia was a unique clinical manifestation of the disease. Intrauterine and postnatal growth retardation, dysmorphic features, microcephaly, delayed development and intellectual disability were the other characteristic features of the disorder.

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Ethical approval

Written informed parental consent has been obtained.

Written permission has been gained from the parents to publish the patient's photos in a scientific journal.

The study was approved by the Human Investigation Review Board at Albert Szent-Györgyi Clinical Centre, University of Szeged, Hungary.

Conflict of Interest Disclosures

The authors declare no competing interests.

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