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Novel *POLR1C* mutation in RNA polymerase III-related leukodystrophy with severe myoclonus and dystonia

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Abstract

Introduction: RNA polymerase III (Pol III)-related leukodystrophies are a group of autosomal recessive neurodegenerative disorders caused by mutations in *POLR3A* and *POLR3B*. Recently a recessive mutation in *POLR1C* causative of Pol III-related leukodystrophies was identified.

Methods: We report the case of a Tunisian girl of 14 years of age who was referred to our department for evaluation of progressive ataxia that began at the age of 5. Genetic diagnosis was performed by NGS and Sanger analysis. In silico predictions were performed using SIFT, PolyPhen-2, and Mutation Taster.

Results: Neurological examination showed cerebellar and tetrapyramidal syndrome, mixed movement disorders with generalized dystonia and severe myoclonus leading to death at 25 years. Brain MRI scans showed diffuse hypomyelination associated with cerebellar atrophy. It also showed bilateral T2 hypointensity of the ventrolateral thalamus, part of the posterior limb of the internal capsule, the substantia nigra and the subthalamic nucleus. Next generation sequencing leukodystrophy panel including *POLR3A* and *POLR3B* was negative. Sanger sequencing of the coding regions of *POLR1C* revealed a novel homozygous mutation.

Ichraf Kraoua, Adnane Karkar, Ilhem Ben Youssef-Turki and Imen Dorboz equal contribution.

Conclusion: The clinical and imaging findings of patients with *POLR1C* hypomyelinating leukodystrophy are reviewed. Interestingly, severe myoclonic dystonia and T2 hypointensity of the substantia nigra and the subthalamic nucleus are not reported yet and could be helpful for the diagnosis of *POLR1C* hypomyelinating leukodystrophy.

KEYWORDS

dystonia, hypomyelination, leukodystrophy, myoclonus, *POLR1C*

1 | INTRODUCTION

RNA polymerase III (Pol III)-related leukodystrophies are a group of autosomal recessive neurodegenerative disorders caused by mutations in *POLR3A* and *POLR3B* (Bernard et al., 2011; Tétreault et al., 2011). They are characterized by childhood onset of progressive motor decline manifesting as progressive cerebellar dysfunction and mild cognitive regression. Other features may include hypo/oligodontia and hypogonadotropic hypogonadism. In addition, Thiffault et al., (2015) have identified a new gene, *POLR1C,* encoding shared Pol I and Pol III complexes and related to RNA polymerase III (Pol III)-related leukodystrophies. This gene is also mutated in the Treacher Collins syndrome (TCS; MIM_248390; POLR1D; MIM_613717; Dauwerse et al., 2011). We report a novel *POLR1C* mutation in a patient with hypomyelinating leukodystrophy associated with severe nonepileptic myoclonus and dystonia.

2 | RESULTS

A Tunisian girl of 14 years of age was referred to our department for evaluation of progressive ataxia that began at the age of 5. She was followed overtime until the age of 25. She was born to first-degree consanguineous healthy parents, after an uneventful pregnancy and delivery. She had two healthy brothers aged 30 and 34. There were no similar cases in her family. Psychomotor development was normal up until the age of 5: she was able to pronounce sentences at the age of 3.

At the age of 5, she developed tremor of upper limbs. Two months later, she developed an unsteady gait, frequent falls with progressive worsening. The first neurological examination performed at 6 years of age showed cerebellar signs: ataxic gait, tremor of upper limbs, and mild dysmetria. CT scan showed bilateral and symmetric periventricular white matter hypodensity and brain MRI showed diffuse T2-weighted hyperintensity of the white matter. Electroencephalography (EEG) recording, nerve conduction velocities, amino acids and organic acids chromatography, lactacidemia, cupper tests, vitamin E, IgA, α-fetoprotein, and cerebrospinal fluid analysis (CSF) were normal. Gradual worsening of gait disturbance with dysarthria and appearance of movement disorders marked the outcome. At the age of 14, examination showed a spastic ataxic gait, a severe static and kinetic cerebellar syndrome with dysarthria, tetrapyramidal signs and generalized myoclonus. Fundus examination was normal. Brain MRI showed diffuse hyperintensity of the supratentorial and cerebellar white matter on the T2-weighted images. Optic radiations were spared. T1 sequence showed diffuse isointensity of the supratentorial white matter suggesting a hypomyelinating process. Bilateral T2-hypointensity of the ventrolateral thalamus, part of the posterior limb of the internal capsule, the substantia nigra, the subthalamic nucleus, and the dentate nuclei was also noted. Furthermore, cerebellar atrophy and a thin corpus callosum were observed (Figure 1). Visual evoked potentials showed a delayed left P100 wave with bilateral reduced amplitude. Somatosensory evoked potentials were altered in lower limbs. Auditory brainstem response was normal. EEG was controlled and was normal and electromyography confirmed the nonepileptic myoclonus on deltoid.

At the age of 17, the patient's condition worsened with marked exacerbation of myoclonus and the onset of generalized dystonia with axial and laryngeal predominance and swallowing problems. She could no longer walk unsupported. Comprehension of her speech was increasingly difficult. However, her cognitive profile and the comprehension were stable during overtime. On examination, we also noted oculomotor abnormalities with nystagmus and limitation in the vertical gaze. In order to make differential diagnosis between the known progressive ataxias with leukoencephalopathy, many diagnoses were discussed as mitochondriopathies, ceroid lipofuscinosis, sialidosis, GM2 gangliosidosis, Niemann Pick type C disease, and *PLA2G6*-associated neurodegeneration. Redox couples, CSF lactate, urinary oligosaccharides analysis were normal. The Filipin test was normal and the *NPC1* sequencing was negative excluding the diagnosis of Niemann Pick type C disease.

At 18 years of age, she presented four tonic-clonic seizures initially treated with phenobarbital that was stopped for drowsiness. She then received levetiracetam that was rapidly interrupted by the family without recurrence of seizures.

At the age of 21, the patient became wheelchair-bound and developed cognitive regression. A follow-up brain and

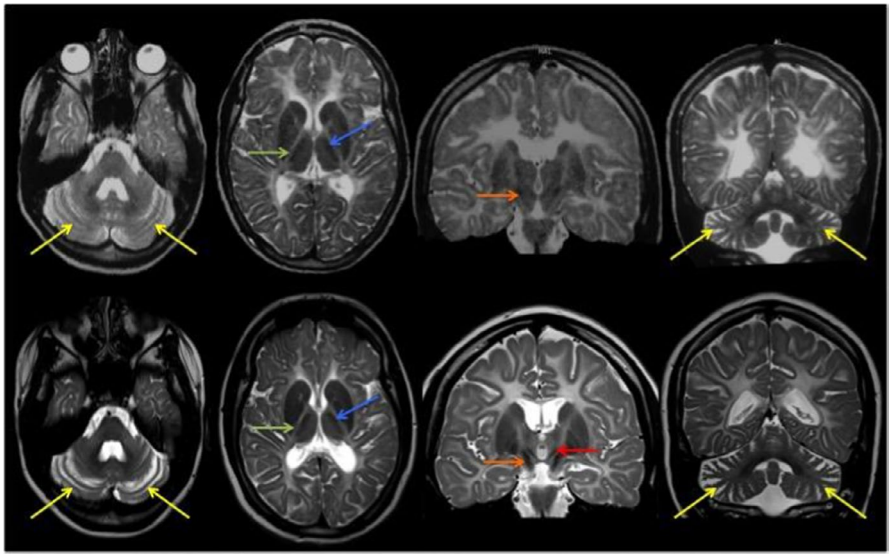

FIGURE 1 Magnetic Resonance Imaging (MRI) findings. T2-weighted images obtained at the age of 14 (a to d) and 21 (e to h). These images show diffuse T2 hyperintensity of the supratentorial and cerebellar white matter, cerebellar atrophy (yellow arrows), bilateral hypointensity of a part of the posterior limb of the internal capsule (green arrows), the ventrolateral thalamus (blue arrows), the substantia nigra (orange arrows), and the subthalamic nucleus (red arrow). Abnormal T2-hypointensities were best seen on the second brain MRI performed on a 3-Tesla machine (vs. 1 Tesla for the first MRI)

spinal MRI was performed and showed the same findings with additional cortical and subcortical atrophy and increased cerebellar atrophy (Figure 1). Spinal cord was normal.

At the age of 23, the dystonia worsened with additional facial and tongue dystonia, hypersalivation, and dysphagia. She also developed urinary and fecal incontinence. The patient was tested at that time at the French reference center for leukodystrophies, LeukoFrance at the Robert Debré hospital. Hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) and Pol III-related leukodystrophy was suspected. Ethical approval was obtained. The patient and his family provided written informed consent for clinical and genetic investigations. DNA was extracted from peripheral blood and sent for sequencing using the next generation sequencing leukodystrophy-panel containing 26 of the most frequently involved genes including *TUBB4A, POLR3A,* and *POLR3B*. No pathological variant was found. The diagnosis of *POLR1C* mutation was subsequently discussed. Sequence analysis revealed a homozygous mutation in *POLR1C* (NM_203290.2; GRCh38). This mutation is located in exon 8, and induces a missense variation NG_028283.3 (NM_203290.2):c.863T > C; p.Phe288Ser. Cosegregation analysis confirmed that the mutation was inherited from heterozygous carrier parents. In silico analysis using SIFT, PolyPhen-2, and Mutation Taster predicted this variation to be deleterious. Furthermore, it is located in the following protein domains, RBP11-like dimerization (IPR009025) domain and RpoA/D/Rpb3-type (IPR011263), which are involved in the protein dimerization activity and DNA-directed RNA polymerase activity. According to the Genome Aggregation Database (gnomAD), only 45 out of 277,248 alleles carried this variation but never in a homozygous state, supporting the diagnosis of *POLR1C*.

In relation to signs associated with Pol III-related leukodystrophies, she had neither hypogonadism nor oligodentia. FSH, LH, and pelvic ultrasound were normal. The panoramic radiography showed no dental abnormalities. The patient died in March 2018 (at the age of 25 years and 10 months) after a severe respiratory infection.

3 | DISCUSSION

We report on the ninth patient with *POLR1C* hypomyelinating leukodystrophy. Our patient had a novel mutation which is the fourtheenth described.

POLR1C is well known for its association with autosomal recessive TCS (MIM248390), a disorder of craniofacial development characterized by a combination of bilateral downward slanting of the palpebral fissures, colobomas of the lower eyelids with a paucity of eyelashes medial to the defect, hypoplasia of the facial bones, cleft palate, malformation of the external ears, atresia of the external auditory canals, and bilateral conductive hearing loss (Dauwerse et al., 2011). Leukodystrophy due to *POLR1C* mutation is extremely rare. Indeed, so far only eight patients have been reported (Thiffault et al., 2015). Demographic, genetic, clinical, and radiological characteristics of our patient and those of the eight published cases by Thiffault et al., (2015) are summarized in Tables 1, 2 and 3.

TABLE 1 Demographic and genetic data of index patient and reported patients with *POLR1C* mutations (Tétreault et al., 2011)

Patients	Families	Gender	Ethnicity	Consanguinity	Age at last assessment	Age of onset	Genetic characteristics	
Number	Number	M/F		Yes/no	Years	Years	Mutation 1	Mutation 2
1	I	M	Libyan	Yes	8	0.5	c.95A > T; p.Asn32Ile	c.95A > T; p.Asn32Ile
2	II	M	Hungarian	No	10	1	c.221A > G; p.Asn74Ser	c.221A > G; p.Asn74Ser
3	III	M	Asian (China)	No	4	1	c.436T > C; p.Cys146Arg	c.883_885delAAG; p.Lys295del
4	IV	F	Caucasian (Armenia/Russia)	No	6	2.5	c.77C > T; p.Thr26Ile	c.326G > A; p.Arg109His+
5	V	F	Caucasian	No	9	1.5	c.193A > G; p.Met65Val	c.572G > A; p.Arg191Gln
6	VI	F	Caucasian (Turkey)	Suspected	18	4	c.326G > A; p.Arg109His	c.970G > A; p.Glu324Lys
7	VII	M	Caucasian	No	33	2	c.395G > A; p.Gly132Asp	c.461_462delAA; p.Lys154Argfs*4
8	VIII	F	Caucasian	No	2	1	c.281T > C; p.Val94Ala	c.785T > C; p.Ile262Thr
9 **INDEX PATIENT**	IX	F	Tunisian	Yes	25	5	c.863T > C; p.Phe288Ser	c.863T > C; p.Phe288Ser

Abbreviations: M, male; F, female; +, present; −, absent.

TABLE 2 Clinical data of index patient and reported patients with *POLR1C* mutations (Tétreault et al., 2011)

Patients	Symptoms at onset	Developmental delay	Age at walking without support (months)	Abnormal cognition	Cerebellar signs	Tremor	Pyramidal signs	Dystonia	Myoclonus	Age at wheelchair (years)	Myopia	Dental abnormalities	Hypogonadotropic hypogonadism
1	Delayed motor development	+	22	+	+	+	+	Unspecified	−	3	−	+	Too young
2	Ataxia, tremor, head Titubation	+	18	+	+	+	+	Unspecified	−	9*	−	−	Too young
3	Delayed motor development and failure to thrive	+	Never independently	+	+	+	+	Unspecified	−	Always	−	−	Too young
4	Tremor, dysmetria	+	15	−	+	+	+	Unspecified	−	−*	−	+	Too young
5	Delayed motor development	+	24	+	+	+	+	Unspecified	−	Always*	+	−	Too young
6	Clumsy gait, frequent falls	−	18	+	+	+	+	Unspecified	−	9 (long distances)*	+	−	−
7	Delayed motor development	+	Never independently	+	+	+	_	Unspecified	+	Puberty	+	−	−
8	Delayed motor development	+	24 (with support)	−	+	+	_	Unspecified	−	−	−	+	Too young
9 **INDEX PATIENT**	Tremor, ataxia	−	12	−	+	+	+	+++	+++	21*	−	−	−

Abbreviations: +, present; −, absent.

*Fast deterioration with infection.

TABLE 3 MRI findings of index patient and reported patients with *POLR1C* mutations (Tétreault et al., 2011)

Patients	Age at last MRI (years)	Diffuse hypomyelination	Myelination of optic radiations	Myelination of posterior limb of internal capsule	Hypointense ventrolateral thalami	Hypointense dentate nucleus	Relatively hypointense pallidi	Cerebellar atrophy	Thin corpus callosum	Supratentorial atrophy
1	6	+	+	–	+	+	+	–	+*	+*
2	4.5	+	+	+	+	–	–	–	+*	–
3	4.5	+	+	+	+	+	–	–	+	–
4	6	+	+	–	+	+	–	+	+*	–
5	8	+	+	–	+	+	–	++*	+*	–
6	18	+	+	+	+	+	–	++*	+++*	–
7	33	+	+	+	+	–	+	+++*	+++*	++*
8	2.75	+	+	+	+	–	–	++*	+*	–
9	21	+	+	+	+	+	+	++*	+++*	+*
INDEX PATIENT										

Abbreviations: +, present; –, absent; +*, mild; ++*, moderate; +++*, severe.

Overall, by pooling our data with those from the literature, all patients with *POLR1C* mutations share clinical and radiological features of Pol III-related leukodystrophies. They all presented in early childhood; the mean age at onset was 2 years (range 0.5–5 years). Psychomotor development was delayed in seven cases. Two patients never walked independently, one patient walked at 24 months with support and the six others walked without support at a mean age of 18 months. Mental retardation was reported in six patients. All patients developed progressive cerebellar ataxia and tremor. Seven patients developed progressive spasticity. Dystonia, reported in few patients with *POLR3A* mutations was not specified in patients with mutations in *POLR1C* (Bernard et al., 2011). Myoclonus was reported once (patient 7). In our patient, myoclonus and dystonia were the prominent and the most severe symptoms. Disease progression was variable. A wheelchair was required in five patients, at the age of 3 years in one patient, 9 years in patients 2 and 6, later (puberty) in individual 7 and at 21 years in our patient. Deterioration triggered by infection was reported in four cases and was the cause of death only in our patient. Compared to reported cases, our patient had a slower progression even at the beginning of the disease and progressively accelerated in the adolescence with the appearance of the movement disorders that were very disabling with wheelchair-bound at 21 years. Abnormal smooth pursuit nystagmus without signs of optic atrophy is the main ocular feature of Pol III-related leukodystrophy. Vertical gaze limitation found in our patient has not been reported in the previous cases with *POLR1C* mutations but was reported in patients with *POLR3A* mutations (Bernard et al., 2011).

Extraneurological signs characteristic of Pol III-related leukodystrophies such as dental abnormalities and hypogonadotropic hypogonadism seem to be less common in patients with *POLR1C* mutations. Six patients were too young, hypogonadotropic hypogonadism was not reported and dental abnormalities were found in only three patients out of nine.

Patients with *POLR1C* mutations share characteristic radiological features classically found in patients mutated for *POLR3A* and *POLR3B* (La Piana et al., 2014).

The mean age at last good quality MRI was 11.5 years (range 2.75–33).

Constant imaging findings in patients with *POLR1C* mutations were diffuse hypomyelination with preserved myelination of the optic nerve radiations (T2 hypointensity), T2 hypointensity of the anterolateral nuclei of the thalami and a thin corpus callosum. Preserved myelination of the dentate nuclei (T2 hypointensity) and part of the pyramidal tracts within the posterior limb of the internal capsule was seen in six patients. Cerebellar atrophy was also present in six cases. Five out of the 6 years and above, suggesting an evolutive

feature. Bilateral T2 hypointensity of the globi pallidi was reported in three cases. Our patient presented additional bilateral T2 hypointensity of the substantia nigra and the subthalamic nucleus. This feature has not been reported before and could be of diagnostic value, even more when movement disorders are present.

Overall, the association of all these radiological findings was present in the large majority of Pol III-related leukodystrophies and can be helpful in the clinical setting to distinguish Pol III-related leukodystrophies among other hypomyelinating disorders and, thus, guide the molecular diagnostic workup.

4 | CONCLUSION

Clinical and radiological spectrum of *POLR1C* hypomyelinating leukodystrophy is delineated in this report. Our observation report a novel mutation and it is the first to describe nonepileptic severe myoclonus and dystonia and T2 hypointensity of the substantia nigra and the subthalamic nucleus in *POLR1C* hypomyelinating leukodystrophy.

CONFLICT OF INTEREST

None declared.

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