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POLR3B-associated leukodystrophy: clinical, neuroimaging and molecular-genetic analyses in four patients: clinical heterogeneity and novel mutations in *POLR3B* gene

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ABSTRACT

Introduction and aim of the study. White matter disorders represent a spectrum of neurological diseases frequently associated with an unfavourable prognosis and a delay in diagnostics. We report the broad phenotypic spectrum of a rare hypomyelinating leukodystrophy and three novel mutations. Further, we aim to explore the role of the combined clinical and neuroimaging diagnostic approach in the era of whole exome sequencing.

Materials and methods. We present a clinical, neuroimaging and molecular-genetic characterisation of four patients from three families suffering from a rare genetic leukoencephalopathy. Two severely affected siblings (P1, P2) manifested a profound developmental delay, cerebellar symptomatology, microcephaly, failure to thrive, short stature and delayed teeth eruption with oligodontia. The other two patients (P3, P4), on the contrary, suffer from substantially less serious impairment with mild to moderate developmental delay and cerebellar symptomatology, delayed teeth eruption, or well-manageable epilepsy. In all four patients, magnetic resonance revealed cerebellar atrophy and supratentorial hypomyelination with T2-weight hypointensities in the areas of the ventrolateral thalamic nuclei, corticospinal tract and the dentate nuclei.

Results. Using whole-exome sequencing in P1, P2 and P3, and targeted sequencing in P4, pathogenic variants were disclosed in *POLR3B*, a gene encoding one of 17 subunits of DNA-dependent RNA polymerase III — all patients were compound heterozygotes for point mutations. Three novel mutations c.727A>G (p.Met243Val) and c.2669G>A (p.Arg890His) (P1, P2), and c.1495G>A (p.Met499Val) (P3) were found. Magnetic resonance revealed the characteristic radiological pattern of POLR3-leukodystrophies in our patients.

Conclusion and clinical implications. The diagnosis of POLR3-associated leukodystrophies can be significantly accelerated using the combined clinical and neuroradiological recognition pattern. Therefore, it is of crucial importance to raise the awareness of this rare disorder among clinicians. Molecular-genetic analyses are indispensable for a swift diagnosis confirmation in cases of clear clinical suspicion, and for diagnostic search in patients with less pronounced symptomatology. They represent an invaluable tool for unravelling the complex genetic background of heritable white matter disorders.

Key words: POLR3B, DNA-dependent RNA polymerase III, leukodystrophy, hypomyelination, hypodontia

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Introduction

Leukodystrophies represent a heterogenous group of heritable neurological disorders affecting the white matter of the central nervous system (CNS) with or without peripheral nervous system involvement [1]. They can be differentiated into hypomyelinating and demyelinating leukodystrophies using CNS magnetic resonance imaging (MRI) recognition patterns. CNS MRI in demyelinating leukodystrophies shows prominent hyperintensity of the white matter in the T2-weighted (T2-W) sequence and hypointensity in the T1-weighted (T1-W) sequence, as opposed to hypomyelinating leukodystrophies in which mild white matter hyperintensity on T2-W sequence and mild hypo-, iso- or hyperintensity on T1-W sequence can be observed [1, 2, 3]. The radiological criteria for hypomyelination further require an unchanged pattern of deficient myelination on two successive MRI scans at least six months apart, and at least one of them performed after the age of one year, separating thus delayed myelination and hypomyelination [2]. At least 91 heritable disorders affecting the white matter are known, 30 of which are the classic leukodystrophies with primary myelin infliction, the rest being formed by genetic leukoencephalopathies in which myelin homeostasis disruption is part of a systemic metabolic disorder [1].

The DNA-dependent RNA polymerase III-associated (POLR3-associated) leukodystrophies comprise a set of clinically, radiologically and genetically defined, yet heterogeneous, hypomyelinating leukodystrophies, for which a common aetiopathogenesis has been disclosed recently – mutations in the *POLR3A, POLR3B,* and *POLR1C* genes encoding subunits of the DNA-dependent RNA polymerase III. The prominent clinical symptoms of POLR3-associated leukodystrophies include progressive motor dysfunction or regression with hypotonia and marked cerebellar and pyramidal or extrapyramidal signs with a variable degree of intellectual impairment. Dental abnormalities constitute a distinct clinical sign. The severity of impairment may vary greatly among individual patients, and the disease may present in several distinct phenotypic forms. A specific radiological pattern exists offering a highly sensitive and specific diagnostic tool. Currently, only supportive symptomatic treatment exists [4–9].

Here, we present a thorough clinical, neuroimaging and molecular-genetic characterisation of four patients from three unrelated families. The sibling patients (P1, P2) presented severe both neurological and non-neurological phenotypes with early onset and rapid disease progression with profound developmental delay, various intriguing clinical features, and hypomyelination on CNS MRI. The other two patients (P3, P4) developed similar MRI findings, although they were much less dramatically affected with mild to moderate intellectual disability and other somatic symptoms that were fewer and less distinctly pronounced. Compound heterozygosity for mutations in *POLR3B* gene was found in all the patients, and three previously unreported mutations were revealed.

Case reports

Two affected siblings (P1, P2) and two other unrelated patients (P3, P4) born to healthy non-consanguineous parents are presented (Tab. 1).

P1, P2: The older sister (P1) was born prematurely in the 34th week of gestation with adequate birth weight of 2,300 g and length of 44 cm. The imminent postnatal adaptation was uneventful with an Apgar score of 9-10-10. Due to an early postnatal infection, the girl had had to be temporarily treated with antibiotics since her second day of life; however, recovery occurred within days, and discharge from hospital in a good clinical condition followed. Psychomotor milestones achievement was satisfactory until the age of six months, when a developmental deceleration and arrest were observed. Subsequently, the girl's clinical state started to deteriorate markedly. During regular clinical evaluations, she began to present with profound psychomotor retardation, central hypotonic syndrome, and a progressive cerebellar symptomatology. The prominent evolving features were dysmetria, dyskinetic choreatic movements, lower extremities spasticity, failure to thrive (17 kg, < 1st percentile at 10 years), short stature (114 cm, < 1st percentile at 10 years), microcephaly (45 cm, < 1st percentile at 10 years), convergent strabismus, central nystagmus, optic nerve atrophy, hypertelorism, inverted mammils and oligodontia with atypical teeth shape, and delayed eruption of both deciduous and permanent teeth (Fig. 1). Her clinical state is now still slowly progressive, predominantly concerning the cerebellar symptomatology. At the age of 11, she is neither able to sit nor to walk, and her cognitive development corresponds to the 3rd trimenone.

The second sister (P2) was born at term with a birth weight of 3,220 g and birth length of 49 cm. The clinical course and disease progression were very similar to her sister. At the age of seven months, developmental slowdown and practical arrest occurred. Progressively, substantial psychomotor retardation, hypotonic syndrome and cerebellar symptomatology evolved, with tremor, acral dyskinesias, failure to thrive (12.5 kg, < 1st percentile at six years), short stature (100 cm, < 1st percentile at 5.5 years), microcephaly (45 cm, < 1st percentile at six years), craniofacial dysmorphia (hypertelorism, enlarged auricles, mild macroglossia), strabismus, hypermetropia, inverted mammils and abnormal dental findings with oligodontia and delayed deciduous and permanent teeth eruption (Fig. 1). At the age of seven years, showing mild intrafamilial difference, she is able to sit unassisted and recently began to stand up and walk around the furniture, milestones her older sister never achieved . Her cognitive development is approximately at the level of eight months. Similarly to her sister, the cerebellar symptomatology is currently slowly progressive.

In both sisters, biochemical work-up did not reveal any abnormalities. Genetic analyses including micro-array hybridisation assays (aCGH) and targeted gene (*MECP2, SMN1*) analyses were negative. Thorough metabolic evaluation was also normal.

Table 1. Clinical symptoms in two severely (P1, P2) and two moderately (P3, P4) impaired patients with three novel variants in *POLR3B* gene and their comparison to literature

Clinical feature	Patient 1	Patient 2	Patient 3	Patient 4	Wolf et al., 2014 n = 103
Age of onset	6 months	7 months	3 years	2 years	< 10 years (90%)
Age at referral	10 years	6 years			n.d.
Developmental delay	+	+	+	+	most patients
Hypotonia	+	+	-	+	n.d.
Ataxia	+	+	+	+	almost all patients
Tremor	-	+	-	+	many patients
Dyskinesias	-	+	+	+	a few patients
Acral spasticity	+	-	+	+	+
Epilepsy	-	-	+	-	19%
Dental abnormalities	+	+	-	+	87%
Delayed dentition	+	+	-	+	71%
Hypo- /oligodontia	+	+	-	+	72%
Abnormal teeth shape	+	+	-	+	+
Microcephaly	+	+	-	-	n.d.
Short stature	+	+	-	n.d.	51 %
Failure to thrive	+	+	-	-	n.d.
Facial dysmorphy	+	+	-	-	n.d.
Inverted mammils	+	+	-	-	n.d.
Strabismus	+	+	-	-	n.d.
Nystagmus	+	+	-	-	most patients
Optic atrophy	+	-	+	-	+
Myopia	-	-	+	+	87%
Hypermetropia	-	+	-	-	n.d.

n.d. — not determined

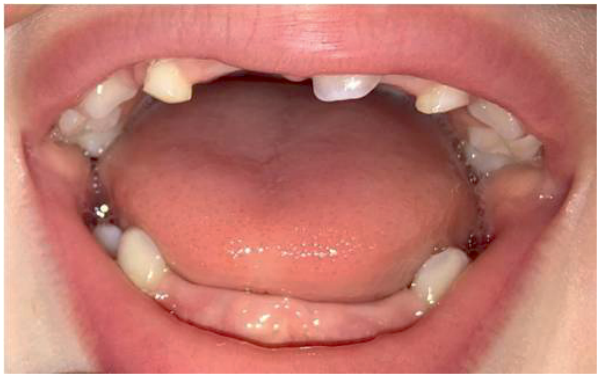

Figure 1. Oligodontia of P2 at the age of 6 years due to delayed eruption of both the deciduous and permanent teeth with atypically shaped teeth and enamel hypoplasia

P3: The perinatal data of the currently 28 year-old male P3 document delayed imminent postnatal adaptation necessitating resuscitation. Nevertheless, further development was unremarkable and appropriate until three years of age when slow retardation of psychomotor milestones achievement started to become apparent. Subsequently, apart from in the long term almost stationary mild psychomotor delay, clumsiness and behavioural problems began to develop. The clinical state then remained for a long time without any substantial progress. The patient attended a regular elementary school initially. Later on, however, he had to be assigned to a special educational programme. At the age of 11 years, the first epileptic seizure of a generalised tonic-clonic nature appeared. Antiepileptic therapy was initiated with a satisfactory effect, and epilepsy has since then been well-managed. In subsequent years, however, the patient's communication skills deteriorated mildly, and mood oscillations were noted. Furthermore, cerebellar and extrapyramidal symptomatology developed during the second decade of life and started to dominate the clinical picture, including ataxia, dyskinetic movements of the extremities and the neck, and hyperreflexia with hyp-/paresthesias of the lower limbs. Also, the patient's behavioural disorder then progressed with sexually inappropriate behaviour being noted. Psychological examination objectified a below average performance in the majority of the areas tested.

Marked myopia required ophthalmological correction. Due to the development of gynaecomastia, the patient underwent a thorough endocrinological examination, which yielded no abnormal findings. Neither dental abnormalities, nor growth impairment, failure to thrive, nor any other conspicuous symptoms were observed.

For the time being, the patient's clinical state seems to remain stationary, except for mild worsening of his cognitive functions.

Complex biochemical and metabolic workup were inconclusive, as were genetic analyses including the common microdeletion syndromes and X-linked mental retardations panel.

P4: Patient 4 is a 19 year-old woman born without any perinatal burden and with physiological birth anthropometric data. The early developmental milestones achievement remained within broader limits until two years of age, when mild psychomotor impairment started to become apparent. Teeth eruption was already delayed at that time. Simultaneously, growth velocity slowdown occurred and insufficient growth hormone production was confirmed. Substitution treatment was therefore initialised, with a satisfactory response. At around three years of age, apart from the developmental delay, intentional tremor with clumsiness and ataxia with frequent falls came to the forefront of the phenotypic picture and set off a broad diagnostic process. The patient's clinical condition subsequently slowly progressed, dominantly in the cerebellar component; choreiform dyskinesias and hyperreflexia together with pyramidal signs were noted further on. The absence of any signs of puberty initiation provoked endocrinology examinations disclosing borderline levels of both central and peripheral sex-hormones. Thus, hormonal substitution therapy was commenced. However, psychiatric problems including depression, food refusal and pseudohallucinations occurred thereafter. These were judged however to be a possible adverse consequence of the hormonal therapy. Another CNS MR at 16 years of age revealed mild progression of the cerebellar atrophy; yet the neurological findings of the cerebellar symptomatology seem to be long-term stationary. In summary, the current clinical state of P4 is non-progressive and dominated by mild intellectual disability, cerebellar symptomatology, optic atrophy and markedly disrupted dental eruption resulting in oligodontia with abnormally shaped teeth.

As with the other patients, biochemical and metabolic examinations remained unremarkable. Genetic testing, aiming at e.g. various spinocerebellar ataxias, yielded no results.

Material and methods

Exome sequencing of the trio (proband(s) and unaffected parents; P1, P2, P3) was performed using an Illumina HiSeq 2000 system (Illumina, USA) and SeqCap EZ Exome Enrichment kit v3.0 (Roche NimbleGen, USA) [10]. Sanger sequencing of all exons and exon–intron boundaries of *POLR3B* gene (NG_031837.1, NM_018082.5) was used in P4. *POLR3B* mutations identified by exome sequencing were confirmed by Sanger sequencing in probands and their parents.

Ethics

This study was approved by the ethics committee of the General University Hospital in Prague and was conducted in agreement with institutional guidelines. Written informed consent was obtained from parents for genetic analysis.

Results and Discussion

Mutations affecting POLR3 subunits, in particular the two largest subunits POLR3A (DNA-dependent RNA-polymerase III, subunit A) and POLR3B (DNA-dependent RNA-polymerase III, subunit B), are causative of a continuous spectrum of allelic and clinically, radiologically and genetically defined group of hypomyelinating leukodystrophic disorders categorised as POLR3-associated leukodystrophies. This group includes five disorders initially described as individual entities: Hypomyelination with Oligodontia (HO), Tremor-Ataxia with Central Hypomyelination (TACH), Hypomyelination with Cerebellar Atrophy and Hypoplasia of the Corpus Callosum (HCAHCC), Ataxia, Delayed Dentition and Hypomyelination (ADDH) and Hypomyelination, Hypodontia, Hypogonadotropic Hypogonadism (4H). With the expansion of molecular genetic testing, these disorders have been revealed to have a similar genetic basis: mutations in genes encoding for POLR3 subunits. Therefore, rather than separate diseases, they represent a broad phenotypic spectrum with distinct phenotypes of the so-called POLR3-associated leukodystrophies [11, 12, 13]. They represent an extremely rare disorder with single case reports reported worldwide. However, as there is no database gathering these patients, neither the precise number of patients suffering from POLR3-associated leukodystrophies nor other epidemiological data are currently available. No more than a few hundred patients have been reported in the literature, the largest cohort amounting to 105 patients [11]. Genetic testing in our patient cohort revealed that all patients were compound heterozygotes for point mutations in *POLR3B* gene, a gene encoding one of the 17 subunits of the DNA-dependent RNA polymerase III.

In P1 and P2, with the severe phenotype, two novel heterozygous mutations — maternally inherited c.727A>G (p.Met243Val) and paternally inherited c.2669G>A (p.Arg890His) — were found. Using exome sequencing, P3 was found to bear a novel variant c.1495G>A (p.Met499Val) inherited from his mother and a previously characterised pathogenic variant c.2084-6A>G leading to a frameshift and a premature stop codon (p.Gly695Valfs*5) [5]. The novel variants change evolutionally conserved amino acids. The impact of these three missense mutations on *POLR3B* was assessed with MutationTaster [14], PolyPhen2 [15] and SIFT [16] programmes and mutations were predicted to be pathogenic. Sequence analysis

Table 2. MRI findings in two severely (P1, P2) and two moderately (P3, P4) impaired patients with three novel variants in *POLR3B* gene

MRI feature	Patient 1	Patient 2	Patient 3	Patient 4
Age at MRI examination (years)	10	5	15	16
Diffuse supratentorial hypomyelination	+	+	+	+
T2-weight hypointensities				
ventrolateral thalamus	+	+	+	+
globus pallidus	-	-	+	+
corticospinal tracts (internal capsule)	+	+	+	+
nucleus dentatus	+	+	+	+
optic radiation	-	-	-	-
Cerebellar atrophy	+	+	+	+
Cortical dysplasia	+	+	-	-
Arachnoideal cysts	-	+	-	-

of the *POLR3B* in P4 revealed compound heterozygosity for two reported mutations, a frameshift variant c.2570+1G>A leading to a premature truncated protein (p.Gly818fs), and a common missense mutation c.1568T>A (p.Val523Glu) found in patients from a European background, homozygosity of which causes a milder phenotype. Apart from this exception, patients with compound heterozygous mutations show no difference in disease progression from those bearing homozygous mutations [17].

POLR3 is one of the three DNA-dependent RNA polymerases found in eukaryotic cell nuclei, each of them being responsible for the transcription of a specific set of genes [5, 18]. POLR3 transcribes a number of various non-coding RNAs involved in essential cellular processes such as translation, RNA processing or transcription regulation [19]. POLR3 is composed of 17 subunits, the two largest of which, POLR3A and POLR3B, form the catalytic centre of the enzyme and are responsible for the vast majority of POLR3-associated leukodystrophies [11, 18]. A mutation in POLR1C – a POLR1 (DNA-dependent RNA polymerase I) and POLR3 shared subunit previously associated with the autosomal recessive Treacher Collins syndrome, has recently been proved to also be causative of a minority of cases of POLR3-associated leukodystrophies [20].

The pathophysiological pathway underlying POLR3-leukodystrophies remains elusive. However, it is presumed that either defective POLR3 assembly, stability or nuclear import or decreased enzymatic activity could affect the levels of various RNAs, which in turn are indispensable for embryonic CNS myelin formation and subsequent myelin homeostasis [5]. POLR3 function is cell-type and cell-cycle dependent. With certain transcripts being brain-specific, this might be the mechanism partially explanatory of the profound CNS impairment and the relative sparing of other tissues and organs [21]. However, the cause of the common dental impairment still remains unclear, as does the endocrine infliction frequently seen in POLR3 patients.

The CNS MRI findings of POLR3-associated leukodystrophies comprise a set of features forming together a highly sensitive (84.6%) and specific (92.9%) recognition pattern [9]. The characteristic POLR3-leukodystrophy CNS MRI features include diffuse supratentorial hypomyelination accompanied by relative T2-W hypointensities in the areas of the globus pallidus, ventrolateral nuclei of the thalamus, corticospinal tract (at the level of the posterior limb of the internal capsule) and in the dentate nucleus and the optic radiation. Cerebellar atrophy and thinning of the corpus callosum complete the neuroradiological pattern characteristic of POLR3-leukodystrophies (Tab. 2). Patients not bearing this MRI pattern are unlikely to suffer from POLR3-associated leukodystrophy [3, 9, 22].

Following standard procedures, CNS MRI was performed at the age of 32 months and at seven and 10 years in P1, at 15 months, 28 months and five years in P2, at 15 years at P3, and at eight and 16 years in P4. All our patients meet the majority of the criteria of the POLR3-associated leukodystrophy recognition pattern, with diffuse non-progressive symmetrical supratentorial hypomyelination and cerebellar atrophy (Fig. 2), affecting both vermis and the hemispheres. Cerebellar atrophy is slightly more common in POLR3B cases compared to POLR3A patients [11]. The degree of white matter hypomyelination reached approximately similar levels among all the patients. Cerebellar atrophy seems to be less prominent in P3 (Fig. 2F), arguably corresponding to a milder cerebellar symptomatology in this patient. The typical T2-W hypointensities in the regions of the ventrolateral thalami (Fig. 2E, H), the dentate nuclei (Fig. 2I) and the corticospinal tract at the level of the posterior limb of the internal capsule (Fig. 2E, H, Fig. 3B) were observed in all our patients. Hypointensities in the globi pallidi (Fig. 2H, K) were only seen in P3 and P4 (Tab. 2). Optic radiation involvement, also often accompanying

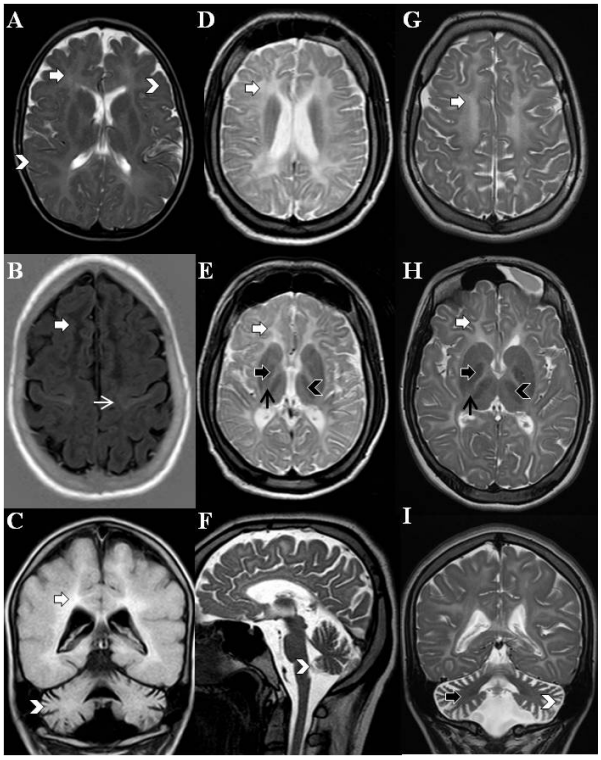

Figure 2. CNS MRI of P1, P2, P3 and P4. Diffuse hypomyelination (white arrows) visible as white matter T2-weight or FLAIR (A, C, D, G, E, H) hyperintensity and T1-weight hypointensity (B) is documented in P1 (B), P2 (A, C), P3 (D, E, F) and P4 (G, H, I). Mildly asymmetric gyrification compatible with the diagnosis of nonlissencephalic cortical dysplasia with polymicrogyria (A; white arrowheads) is depicted in P2. Cerebellar atrophy, affecting both vermis and the hemispheres, can be observed in FLAIR (C; white arrowheads) and T2-weight sequences (F, I; white arrowheads). T2-weight hypointensity in the area of nucleus dentatus (I; black arrow) can be observed in P4. T2-weight hypointensities in globi pallidi (E, H; black arrows), ventrolateral thalami (E, H; black arrowheads) and the corticospinal tract at the level of the posterior limb of the internal capsule (E, H; black thin arrows) are demonstrated in axial images of P3 and P4. Preserved myelination of gyrus praecentralis and of the corticospinal tract upstream of internal capsule (B; white thin arrow) corresponding to T1-weight hyperintensity is observed in P1

POLR3-leukodystrophies, was not present in either of these patients [9]. Thin hypoplastic corpus callosum, described in approximately 50% of children younger than 10 years and more frequently associated with *POLR3B* mutations, was found in all four patients (Fig. 3A). Interestingly, both P1 and P2 expressed additional findings of dysmyelination of the white matter with asymmetric gyrification compatible with a diagnosis of nonlissencephalic cortical dysplasia in both sisters - cortical dysplasia with pachygyria in P1, and cortical dysplasia and polymicrogyria in P2 (Fig. 2B). The aetiology of these findings was not clarified; nevertheless, cortical dysplasia has never been described in any POLR3 patient before. Given its simultaneous occurrence in both siblings, the presence of another underlying genetic cause cannot be excluded, despite negative findings in the whole exome sequencing. P2 was also noted to possess a temporobasal enlargement of the external cerebrospinal fluid spaces due to an arachnoid cyst (30 x 25 x 22 mm). However, as arachnoid cysts are not an especially rare finding even in healthy asymptomatic populations, they cannot be related to POLR3-leukodystrophies. In a subset of patients, a small cyst within the splenium has been observed [11].

The onset of POLR3-associated leukodystrophies ranges from early childhood to adolescence, with only a minority (10%) of patients presenting after 10 years of age. The prominent clinical features include progressive motor dysfunction or regression with hypotonia and marked cerebellar and pyramidal or extrapyramidal signs, presenting as gait abnormalities, ataxia, tremor, dysmetria, abnormal eye smooth pursuit, nystagmus or other gaze limitations, dystonia, diskinetic movements, spasticity or hyperreflexia and others [11] (Tab. 1).

In spite of the few exceptions documented, POLR3A-associated disease has been found to be associated with a more rapid disease progression, more severe disease course, and shorter life expectancy, despite having a slightly later disease onset compared to patients bearing mutations in *POLR3B*. Patients from a European background are more likely to have mutations in *POLR3B* [11, 23].

The clinical presentation in both our severely (P1, P2) and moderately (P3, P4) impaired patients is in accordance with the hitherto described broad phenotypic spectrum of POLR3-associated leukodystrophies.

As with the majority of patients, both siblings manifested early and similarly with developmental delay, cerebellar and pyramidal symptomatology and a corresponding dental abnormality pattern, which to a lesser extent was also the clinical presentation of P4. On the other hand, P3 manifested with unprogressive mild to moderate intellectual disability with behavioural problems and unspecified clumsiness in early childhood, with a subsequent onset of epilepsy at the beginning of his second decade and further deterioration of cognitive skills and the slow progression of other symptoms. No dental abnormalities have been detected in P3 so far, although they manifest in around three quarters of patients [11] (Tab. 1). Seizures occurred neither in the sibling patients (P1, P2), nor in P4. Because epilepsy is present in only approximately 20% of patients, these patients may remain without seizures. However, if epilepsy eventually evolves, it is usually well-controllable by pharmacotherapy [11]. Cognitive impairment of POLR3 patients can be of variable severity. Our sibling patients manifested a profound intellectual disability, while most described patients have developed only a mild to moderate impairment, just like P3 and P4. Some patients may even have normal cognitive abilities, or only variably expressed learning difficulties [11]. Intercurrent infections have been noted to worsen the disease course, with not all patients

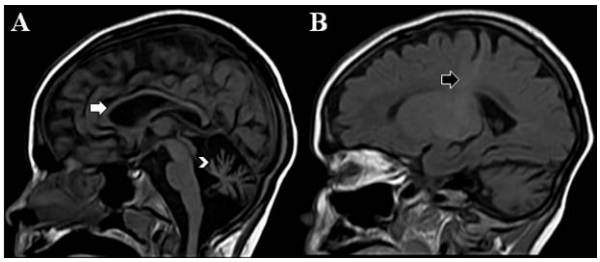

Figure 3. CNS MRI in P1, T1-weight sequence. Atrophy of the corpus callosum (A; white arrow) and of the cerebellum (A; white arrowhead) is demonstrated, together with preserved myelinisation of the corticospinal tract and its proximity upstream of the internal capsule (B; black arrow). Similar findings have been found in P2, the sister of P1

regaining their previous level [11], although this phenomenon has not so far been observed in our patients.

The non-neurological symptoms are dominated by dental abnormalities, with an abnormal or delayed deciduous and definitive teeth eruption, and hypo- /oligodontia and dysmorphic teeth, as seen in P1, P2 and P4. Other non-neurological symptoms, as observed in P4, may include endocrine dysfunctions such as prolactin or growth hormone deficiencies or hypogonadotropic hypogonadism, which leads to delayed puberty in three quarters of POLR3B patients [11, 24, 25]. Short stature, affecting about 50% of patients, was a dominant feature in our siblings, as well as microcephaly and failure to thrive. Worthy of note concerning the POLR3-leukodystrophies is the substantial diversity in disease onset and clinical course severity and progression, including even intrafamilial heterogeneity [11]. Progressive myopia seems to be a frequent part of the syndrome, as observed in our moderately affected P3 and P4; cataracts can sometimes develop. Interestingly, convergent strabismus has not been mentioned yet, while optic atrophy, as seen in P1 and P3, has only been described occasionally and mostly in older patients [23].

The precise pathophysiological mechanism leading to the substantial difference in the clinical phenotype severity between P1, P2 with two novel mutations and the other two patients, P3 and P4, remains currently elusive and cannot be correlated purely to CNS MRI findings, which are of approximately similar severity. Additional analyses need to be performed. Since *POLR3B* possesses a high degree of mutational heterogeneity with mutational sites distributed throughout the whole gene, further studies on more patients are necessary in order to determine a precise genotype-phenotype correlation, something which cannot be drawn for the time being [5].

Clinical implications and conclusion

Leukodystrophies are a growing group of heritable white matter disorders, for which comprehensive diagnostic algorithms have been created to assist in reaching the proper diagnosis as early as possible [1].

In certain cases, the clinical and neuroimaging signs and symptoms may assist in narrowing the differential diagnosis and focusing the diagnostic process, including the molecular-genetic analyses, as witnessed by POLR3-associated leukodystrophies [3, 9].

Therefore, awareness of POLR3-associated leukodystrophies ought to be increased among clinicians, as this particular entity offers highly specific and sensitive clinical and neuroimaging recognition patterns, making thereby targeted sequencing a method of choice, rather than whole exome sequencing. This may be of substantial significance not only for clinicians, but also for patients and the general healthcare system.

However, there still are a number of mono-/oligosymptomatic phenotypes, some of them especially with the non-neurological presenting symptoms, which may pose a more challenging diagnostic task and thus require broader molecular-genetic analyses, such as whole exome sequencing [26].

Such sequencing, whether genome, exome or targeted panel sequencing, offers a valuable diagnostic tool if properly used. Concomitantly, it offers an opportunity to further unravel the underlying genetic and pathophysiological background of these disorders, and shift today's mostly symptomatic care towards a search for disease-specific targeted therapies and novel therapeutic modalities (for an overview of novel therapies see Helman et al. [27]).

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