Diffuse hypomyelination is not obligate for POLR3-related disorders

Roberta La Piana, MD*
Ferdy K. Cayami, MD*
Luan T. Tran, MSc
Kether Guerrero, MSc
Rosalina van Spaendonk, PhD
Katrin Õunap, MD, PhD
Sander Pajusalu, MD
Tobias Haack, PhD
Evangeline Wassmer, MD, PhD
Dagmar Timmann, MD, PhD
Hanna Mierzewska, MD, PhD
Bwee T. Poll-Thé, MD, PhD
Chirag Patel, MD
Helen Cox, MD
Tahir Atik, MD
Huseyin Onay, MD, PhD
Ferda Ozkınay, MD
Adeline Vanderver, MD
Marjo S. van der Knaap, MD, PhD
Nicole I. Wolf, MD, PhD‡
Geneviève Bernard, MD, MSc, FRCPc‡

Correspondence to Dr. Bernard: genevieve.bernard@mcgill.ca

Supplemental data at Neurology.org

ABSTRACT

Objective: To report atypical MRI patterns associated with *POLR3A* and *POLR3B* mutations.

Methods: This was a multicenter retrospective study to collect neuroradiologic, clinical, and molecular data of patients with mutations in *POLR3A* and *POLR3B* without the classic MRI phenotype, i.e., diffuse hypomyelination associated with relative T2 hypointensity of the ventrolateral thalamus, globus pallidus, optic radiation, corticospinal tract at the level of the internal capsule, and dentate nucleus, cerebellar atrophy, and thinning of the corpus callosum.

Results: Eight patients were identified: 6 carried mutations in *POLR3A* and 2 in *POLR3B*. We identified 2 novel MRI patterns: 4 participants presented a selective involvement of the corticospinal tracts, specifically at the level of the posterior limbs of the internal capsules; 4 patients presented moderate to severe cerebellar atrophy. Incomplete hypomyelination was observed in 5 participants.

Conclusion: Diffuse hypomyelination is not an obligatory feature of POLR3-related disorders. Two distinct patterns, selective involvement of the corticospinal tracts and cerebellar atrophy, are added to the MRI presentation of POLR3-related disorders. ***Neurology*® 2016;86:1622–1626**

GLOSSARY

ExAC = Exome Aggregation Consortium; **TOP** = terminal oligopyrimidine tract; **WES** = whole-exome sequencing.

POLR3-related leukodystrophy is a rare autosomal recessive disease characterized by hypomyelination often accompanied by dental abnormalities and hypogonadotropic hypogonadism.[1–5] In its classical form, the association of these features is referred to as 4H syndrome.[1,2] Mutations in the *POLR3A* and *POLR3B* genes, which encode for the 2 largest subunits of the RNA polymerase III (POLR3) complex, as well as in *POLR1C,* also encoding a POLR3 subunit, are responsible for this disease.[6–11] With the identification of the causative genes, patients with suggestive clinical or MRI picture can undergo genetic testing, confirming the diagnosis.[12] The MRI pattern of POLR3-related leukodystrophy is suggestive and characterized by diffuse hypomyelination associated with relative T2 hypointensity of the ventrolateral thalamus, globus pallidus, optic radiation, corticospinal tract at the level of the internal capsule and dentate nucleus, cerebellar atrophy, and thinning of the corpus callosum.[12–14] Recognition of this pattern was proven effective in detecting patients with 4H leukodystrophy caused by *POLR3A-B* or *POLR1C* mutations and is therefore

*These authors contributed equally to this work.

‡Dual last authors.

From the Laboratory of Neurogenetics of Motion (R.L.P.) and Department of Neuroradiology (R.L.P.), Montreal Neurological Institute and Hospital, and the Departments of Neurology and Neurosurgery (R.L.P., L.T.T., K.G., G.B.) and Pediatrics (R.L.P., L.T.T., K.G., G.B.), McGill University, Montreal, Canada; Departments of Child Neurology (F.K.C., M.S.v.d.K., N.I.W.) and Clinical Genetics (F.K.C., R.v.S.), VU University Medical Center, Amsterdam, the Netherlands; Department of Genetics (K.Õ.) United Laboratories, Tartu University Hospital, Tartu; Department of Pediatrics (K.Õ.), University of Tartu, Estonia; Institute of Human Genetics (T.H.), Technische Universität München; Institute of Human Genetics (T.H.), Helmholtz Zentrum München, Munich, Germany; Department of Neurology (E.W.), Birmingham Children's Hospital, UK; Department of Neurology (D.T.), University Clinic Essen, University of Duisburg-Essen, Germany; Department of Child and Adolescent Neurology (H.M.), Institute of Mother and Child, Warsaw, Poland; Department of Child Neurology (B.T.P.), AMC Academic Medical Center, Amsterdam, the Netherlands; West Midlands Regional Clinical Genetics Unit (C.P., H.C.), Birmingham Women's Hospital, UK; Departments of Pediatrics (T.A.) and Medical Genetics (H.O.), Faculty of Medicine (F.O.), Ege University, Izmir, Turkey; Department of Neurology (A.V.), Children's National Health System, Washington, DC; Neuroscience Campus Amsterdam (M.S.v.d.K., N.I.W.), the Netherlands; and Department of Medical Genetics (G.B.), Montreal Children's Hospital, McGill University Health Center, Montreal, Canada.

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Table 1 *POLR3A* and *POLR3B* mutations identified in our cohort

	Family	Gene	Status	gDNA	Chromosomal location	Protein	Region	Previously reported	MAF	Method	Clinical features	MRI features
1	I	*POLR3A*	C Het	c.1048+1G>A	chr10:79781617C>T	—	Intron 7	No	NA	Gene testing	A, HD, SSt	PLIC
				c.1289+3A>C	chr10:79778917T>G	—	Intron 9	No	NA			
2	II	*POLR3A*	Hom	c.2710 G>A	chr10:79753032C>T	G904R	Exon 20	No	8.24E-06	Gene testing	A, HD, DD, Sp	PLIC
3	III	*POLR3A*	C Het	c.1771-6C>G	chr10:79769439C>G	—	Intron 13	rs115020338	3.30E-05	Gene testing	A, HD, DD, Sp	PLIC
				c.3205C>T	chr10:79744965G>A	R1069W	Exon 24	No	—			
4	IV	*POLR3B*	C Het	c.2084-6A>G	chr12:106848274A>G	—	Intron 19	No	0.0001769	Gene testing	A, M, DD	CA
				c.2302C>T	chr12:106850924C>T	R768C	Exon 22	rs371453512	2.48E-05			
5	V[a]	*POLR3A*	C Het	c.2381A>C	chr10:79760831T>G	Q794P	Exon 18	No	0.0001237	WES	A, M, DD, Sp	CA
				c.-35C>G	chr10:79789200 G>C	—	Exon 1 (5′UTR)	rs201700756	0.00128			
6	V[a]	*POLR3A*	C Het	c.2381A>C	chr10:79760831T>G	Q794P	Exon 18	No	0.0001237	WES	A, M, DD, Sp	CA
				c.-35C > G	chr10:79789200 G>C	—	Exon 1 (5′UTR)	rs201700756	0.00128			
7	VI	*POLR3B*	C Het	c.1244T>C	chr12:106821117T>C	M415T	Exon 13	rs199504211	0.0006182	WES	A, M, DD, SSt	CA
				c.2774C>T	chr12:106889893C>T	P925L	Exon 24	No	8.24E-06			
8	VII	*POLR3A*	C Het	c.1909 + 22G>A	chr10:79769273C>T	—	Intron 14	rs191875469	0.001326	Gene testing	A, HD	PLIC
				c.2549A>G	chr10:79759806T>C	H850R	Exon 19	No	NA			

Abbreviations: A = ataxia or other cerebellar signs; C Het = compound heterozygous; CA = cerebellar atrophy; DD = developmental delay; HD = hypodontia or dental abnormalities; Hom = homozygous; M = myopia; MAF = minor allelefrequency; NA = not available; PLIC = abnormal signal of the posterior limb of the internal capsule; Sp = spasticity; SSt = short stature; WES = whole-exome sequencing.
[a] Siblings.

used to orient the diagnostic process.[12–14] Patients without this pattern showing nonspecific hypomyelination are unlikely to carry mutations in *POLR3A* or *POLR3B*.[15]

METHODS We performed a multi-institutional cross-sectional observational study of the clinical, radiologic, and molecular data of patients who fulfilled the following inclusion criteria: presence of recessive *POLR3A* or *POLR3B* mutations and absence of typical POLR3-related MRI features.[13,14]

We identified 8 patients from 7 nonconsanguineous families, all of Caucasian ethnicity, fulfilling these criteria. Five participants underwent *POLR3A* and *POLR3B* sequencing because they presented suggestive clinical features (hypodontia or dental abnormalities, short stature, and myopia, either associated or not). In 3 participants from 2 families, *POLR3A* and *POLR3B* mutations were identified by whole-exome sequencing (WES). For all families for which DNA was available, segregation was verified. Analysis for the potential pathogenicity of novel mutations was performed, including in silico analysis. For the novel splicing mutations, we sequenced cDNA when RNA was available.

MRI were reviewed collegially by our team. At least axial T2-weighted and sagittal T1- or T2-weighted images were available.

Standard protocol approvals, registrations, and patient consents. The institutional review boards of each participating institution approved the use of clinical data for the study.

RESULTS **Mutation findings.** Six participants carried mutations in *POLR3A* and 2 in *POLR3B* (table 1). Among the 13 mutations identified, 8 were novel, and not reported in public databases (Exome Variant Server, NHLBI GO Exome Sequencing Project [ESP], Seattle, WA [http://evs.gs.washington.edu/EVS/] [June 2015]; Exome Aggregation Consortium [ExAC], Cambridge, MA [http://exac.broadinstitute.org] [June 2015]). Five mutations were intronic and affected a splice site or induced a new donor site. All new mutations were rare and predicted to be pathogenic by in silico tools[16–18] (table e-1 on the *Neurology*® Web site at Neurology.org) besides for the c.-35C>G change, which had been reported in homozygous state in 2 out of over 60,000 participants (ExAC [http://exac.broadinstitute.org] [July 2015]) in patients 5 and 6. Segregation analysis in this family revealed that each of the parents carried one variant, and the healthy brother the paternal variant. WES failed to uncover other possible causal variants. Table 1 reports detailed information about the genetic status and mutations found in our cohort.

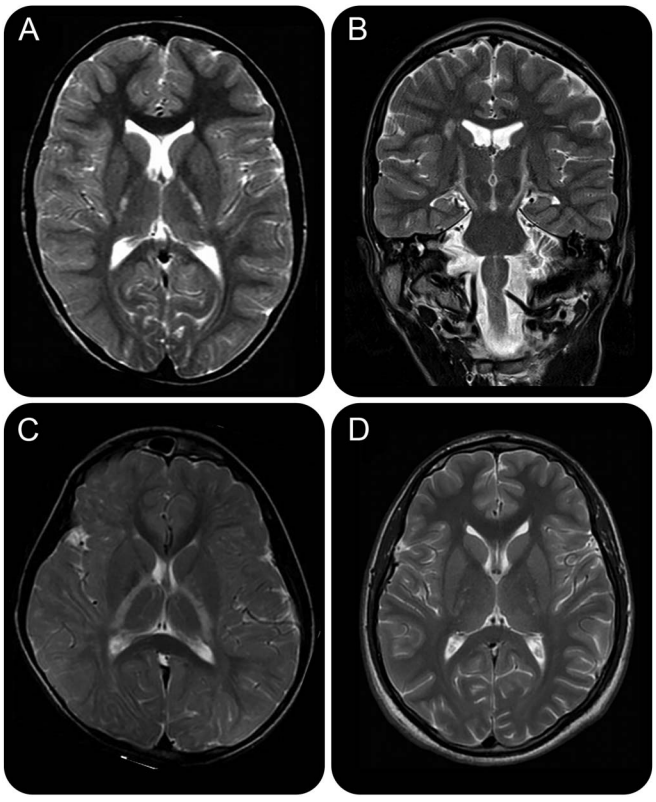
Clinical findings. Age at onset ranged from 6 weeks to 10 years (mean age at onset 52.3 months). The symptoms at disease onset were gait ataxia, dysarthria, and tremor in 3 participants (cases 1, 4, and 8). Two participants (cases 2 and 3) presented with spasticity and diplegic gait. The patient with the earliest disease onset, at 1.5 months, presented with failure to thrive (case 7). Clinical examination revealed ataxia of

variable severity in all patients; cerebellar tremor was documented in 4 participants (cases 1, 2, 3, and 4); pyramidal signs and spasticity were present in 4 participants (cases 2, 3, 5, and 6). One patient had severe dystonic tremor (case 3) (table 1).

Extraneurologic features were found in 6 patients. Specifically, 4 participants had hypodontia, delayed dentition, or other dental abnormalities (cases 1, 2, 3, and 8) suggestive of POLR3-related leukodystrophy. Two participants presented short stature (cases 1 and 7), and 4 had myopia (cases 4, 5, 6, and 7), both frequent findings in patients with mutations in *POLR3A* or *POLR3B*.

MRI findings. Mean age at the last MRI was 15 years (range 4–35 years). We identified 2 distinct MRI patterns (figures 1, 2, e-1, and e-2). Four participants, all with *POLR3A* mutations, presented a selective involvement of the corticospinal tracts, which was particularly evident at the level of the posterior limb of the internal capsule as T2-hyperintense signal (cases 1, 2, 3, and 8) (figures 1 and e-1). In the other 4 patients (2 with *POLR3A* and 2 with *POLR3B* mutations), moderate to severe cerebellar atrophy was variably associated with nonspecific T2-hyperintense white matter abnormalities, as specified below, or thinning of the corpus callosum (figures 2 and e-2) (cases 4, 5, 6, and 7). We documented focal, partially confluent, T2-hyperintense white matter abnormalities located in the deep frontal and parietal white matter, suggesting partial hypomyelination, in 5 participants (cases 2, 3, 4, 5, and 6), while the remaining 3 presented adequate myelination for age.

Figure 1 **Involvement of the corticospinal tracts**



Axial (A, C, D) and coronal (B) T2-weighted images in patients 1 (A, B), 1 (C), and 8 (D) with *POLR3A* mutations show the presence of bilateral and symmetric T2-hyperintense signal at the level of the posterior limb of the internal capsules. Incomplete hypomyelination is seen in (B).

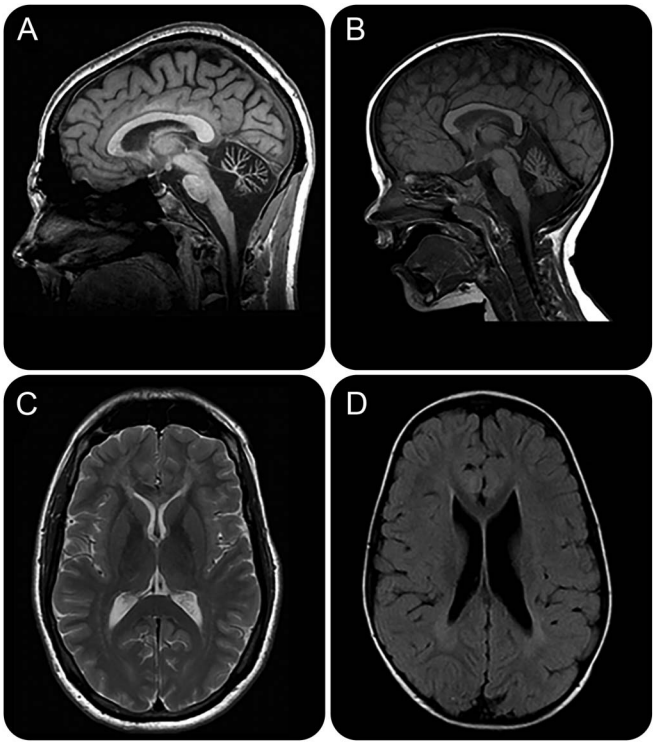
DISCUSSION Our work broadens the MRI spectrum of POLR3-related leukodystrophy by describing 2 new MRI patterns in this disease that has been known as a hypomyelinating disorder. Our results indicate that diffuse hypomyelination is not an obligatory feature. We also documented the presence of 6 *POLR3A* and 2 *POLR3B* mutations not reported before in public databases. Interestingly, 5 of the 13 mutations in our cohort were noncoding, 4 predicted to affect splicing. Pathogenicity of the variant in the 5′ untranslated region in patients 5 and 6 could not be unambiguously resolved as it had been reported in homozygous state in 2 participants in a large database. However, this variant is situated in a terminal oligopyrimidine tract (TOP). The change of a pyrimidine (C) for a purine (G) shortens the TOP, and it has been shown that deletions or substitutions in this region result in unregulated translation.[19,20] Its effect might be mild, and homozygous carriers indeed might be unaffected, but in combination with a pathogenic mutation this variant could lead to disease. Segregation analysis and the absence of other possible causal variants in WES in this family indeed support a causal role for this variant, as does the identification of other families with isolated cerebellar atrophy.

A specific involvement of the corticospinal tracts, particularly evident at the level of the internal capsule as T2-hyperintense signal, was the most striking finding in a subgroup of patients. Interestingly, in typical cases, more commonly with *POLR3B* mutations,[12] the corticospinal tracts are usually one of the better myelinated structures.

The second pattern is the presence of cerebellar atrophy in the absence of diffuse hypomyelination. Cerebellar atrophy was previously known to be associated with *POLR3A* or *POLR3B* mutations in more than 80% of the participants, always in combination with diffuse hypomyelination.[12–14,21]

Focal, partly confluent T2-hyperintense white matter changes were present in some participants of both groups and located in the deep frontal and parietal white matter. The signal intensity of the abnormal areas corresponds to the one seen in hypomyelination, focal hypomyelination therefore being the most likely interpretation. These changes are obviously reminiscent of the classical MRI of 4H leukodystrophy, but sufficiently different to make a straightforward diagnosis

Figure 2 Cerebellar atrophy



(A, B) Sagittal T1-weighted images from participant 4 with *POLR3B* (A) and participant 5 with *POLR3A* (B) mutations show the presence of severe (A) and moderate cerebellar atrophy. (C, D) Axial T2-weighted and T2-fluid-attenuated inversion recovery images of the patients presented respectively in (A, B) show the presence of partial hypomyelination.

challenging. Our results confirm white matter involvement when *POLR3A* or *POLR3B* is mutated; however, different pathogenic processes could be responsible for the variable and expanded MRI phenotypes of brain abnormalities associated with *POLR3A* or *POLR3B* mutations. Further insight into the role of POLR3 in myelin formation and maintenance as well as in axonal integrity is needed to explain the heterogeneity of the radiologic patterns.

The diagnosis of POLR3-related leukodystrophy relies both on MRI findings and clinical signs. In our cohort, the presence of typical extraneurologic features oriented the clinicians towards the testing of *POLR3A* and *POLR3B* genes in 5 participants. Therefore, our study confirms the importance of the classical clinical criteria—particularly hypodontia and hypogonadotropic hypogonadism—in the diagnostic process of POLR3-related disorders, especially when cardinal MRI features are lacking. WES allowed the discovery of *POLR3A* and *POLR3B* mutations in the remaining cases, thus highlighting the role of next-generation sequencing in expanding the phenotypes of already known disorders.

AUTHOR CONTRIBUTIONS

Roberta La Piana collected, analyzed, and interpreted patient data, reviewed the article, and wrote the draft of the manuscript. Ferdy K. Cayami collected, analyzed, and interpreted patient data, reviewed the article, and wrote the draft of the manuscript. Luan T. Tran collected, analyzed, and interpreted patient data and reviewed the article. Kether Guerrero was responsible for molecular genetic analysis and reviewed the article. Rosalina van Spaendonk was responsible for molecular genetic analysis and reviewed the article. Katrin Õunap collected, analyzed, and interpreted patient data and reviewed the article. Sander Pajusalu collected, analyzed, and interpreted patient data, reviewed the article. Tobias Haack was responsible for molecular genetic analysis, reviewed the article. Evangeline Wassmer collected, analyzed, and interpreted patient data and reviewed the article. Dagmar Timmann collected, analyzed, and interpreted patient data and reviewed the article. Hanna Mierzewska collected, analyzed, and interpreted patient data and reviewed the article. Bwee T. Poll-Thé collected, analyzed, and interpreted patient data and reviewed the article. Chirag Patel collected, analyzed, and interpreted patient data and reviewed the article. Helen Cox collected, analyzed, and interpreted patient data and reviewed the article. Tahir Atik collected, analyzed, and interpreted patient data and reviewed the article. Huseyin Onay collected, analyzed, and interpreted patient data and reviewed the article. Ferda Ozkınay collected, analyzed, and interpreted patient data and reviewed the article. Adeline Vanderver collected, analyzed, and interpreted patient data and reviewed the article. Marjo S. van der Knaap collected, analyzed, and interpreted patient data and reviewed the article. Nicole I. Wolf designed and supervised the study, collected data, and reviewed the article. Genevieve Bernard designed and supervised the study, collected data, and reviewed the article.

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DISCLOSURE

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