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RESEARCH ARTICLE

POLR3B is associated with a developmental and epileptic encephalopathy with myoclonic-atonic seizures and ataxia

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Abstract

Objective: *POLR3B* encodes the second largest subunit of RNA polymerase III, which is essential for transcription of small non-coding RNAs. Biallelic pathogenic variants in *POLR3B* are associated with an inherited hypomyelinating leukodystrophy. Recently, *de novo* heterozygous variants in *POLR3B* were reported in six individuals with ataxia, spasticity, and demyelinating peripheral neuropathy. Three of these individuals had epileptic seizures.

The aim of this article is to precisely define the epilepsy phenotype associated with *de novo* heterozygous *POLR3B* variants.

Methods: We used online gene-matching tools to identify 13 patients with *de novo POLR3B* variants. We systematically collected genotype and phenotype data from clinicians using two standardized proformas.

Results: All 13 patients had novel *POLR3B* variants. Twelve of 13 variants were classified as pathogenic or likely pathogenic as per American College of Medical Genetics (ACMG) criteria. Patients presented with generalized myoclonic, myoclonic-atonic, atypical absence, or tonic-clonic seizures between the ages of six months and 4 years. Epilepsy was classified as epilepsy with myoclonic-atonic seizures (EMAtS) in seven patients and "probable EMAtS" in two more.

Seizures were treatment resistant in all cases. Three patients became seizure-free. All patients had some degree of developmental delay or intellectual disability. In

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most cases developmental delay was apparent before the onset of seizures. Three of 13 cases were reported to have developmental stagnation or regression in association with seizure onset.
Treatments for epilepsy that were reported by clinicians to be effective were: sodium valproate, which was effective in five of nine patients (5/9) who tried it; rufinamide (2/3); and ketogenic diet (2/3).
Additional features were ataxia/incoordination (8/13); microcephaly (7/13); peripheral neuropathy (4/13), and spasticity/hypertonia (6/13).
Significance: *POLR3B* is a novel genetic developmental and epileptic encephalopathy (DEE) in which EMAtS is the predominant epilepsy phenotype. Ataxia, neuropathy, and hypertonia may be variously observed in these patients.

KEYWORDS
Encephalopathy, Epilepsy, Genetic, Myoclonic-atonic seizures, POLR3B

1 | INTRODUCTION

One in 400 children is diagnosed with epilepsy before their third birthday.[1] In this age group, neuroimaging and genetic testing allow a precise underlying etiology to be identified in more than half of patients. It is now clear that genetic causation accounts for the majority of identifiable etiology.[1] Consequently, there has been a reconceptualization of the early childhood epilepsies as a collection of rare, mostly genetic, diseases. Genetic etiology is more likely to be identified in children with early onset of seizures (<1 year),[2,3] and in children with therapy-resistant seizures.[4]

Establishing a precise genetic etiology for a person's epilepsy can guide therapeutic decision-making,[5,6] prevent further costly and invasive investigations,[7] guide counseling about recurrence risk for future offspring,[4] and end lengthy diagnostic odysseys for patients and their families.[8] Knowledge of a rare genetic diagnosis may empower families to make contact with other families with the same condition to share experiences and support. Collating genotypic and phenotypic data about patients with a specific genetic etiology for their epilepsy helps progress clinical and scientific research. A major goal of such research is to develop and implement effective disease-modifying treatments.

In the current era, first-line diagnostic genetic testing in the epilepsies often involves high-throughput technologies to look for rare genetic variants in numerous genes simultaneously. This may involve testing large panels of specific disease-associated genes, larger panels of nonspecific disease-associated genes, or even the entire exome or genome, which will include many genes not known to have any association with disease. The wider the genetic testing net that is cast, the more dependent we become on interpreting variants in the context of phenotype. Thus it has become increasingly important to understand the full phenotypic spectrum associated with each specific genetic etiology. Knowledge of phenotype guides both variant interpretation and genetic counseling. It is not uncommon for genes that were found initially to be associated with one specific phenotype to be subsequently identified with either a wider spectrum of disease, a distinct phenotype, or even a novel phenotype linked to a new mechanism of disease such as a "gain of function" property, as recently described in *SCN1A*-associated epilepsies.[9]

Here we report 13 patients with rare heterozygous variants in a gene that was reported initially in association with a recessive hypomyelinating leukodystrophy: *POLR3B*. The majority of these patients presented with an

Key points

- The *POLR3B* gene encodes a ribosomal polymerase, responsible for transcribing small noncoding RNA.
- *POLR3B*-DEE (developmental and epileptic encephalopathy) is a newly described developmental and epileptic encephalopathy.
- Epilepsy with myoclonic-atonic seizures (EMAtS) is the most common epilepsy phenotype.
- Electroencephalography in patients with *POLR3B*-DEE usually shows generalized spike/polyspike and slow wave discharges.
- Associated features may include ataxia, neuropathy, hypertonia, and microcephaly.

early onset developmental and epileptic encephalopathy (DEE) with myoclonic-atonic seizures.

POLR3B encodes the second largest subunit of RNA polymerase III. RNA polymerase III is essential for the transcription of small non-coding RNAs.[10] Small non-coding RNAs include 5S ribosomal RNA, transfer RNA, and U6 small nuclear RNA. Despite the apparently ubiquitous roles of these small RNA molecules in RNA maturation and transcription, mutations in *POLRB3* and other genes encoding ribosomal polymerase III subunits have been associated with a range of organ-specific diseases. Examples of ribosomal polymerase III–associated (Pol III) diseases include hypomyelinating leukodystrophy (*POLR3A*,[11] POLR3B,[12] *POLR3C*,[13] *POLR3D*,[14] and *POLR3K*[15]); susceptibility to varicella zoster virus–induced encephalitis and pneumonitis (*POLR3A*, *POLR3C*, *POLR3E* and *POLR3F*)[16,17]; primary ovarian insufficiency (*POLR3H*)[18]; and endosteal hyperostosis and oligodontia (*POLR3GL*).[19] Precise disease mechanisms remain poorly understood. Currently it is not clear what underpins gene–disease relationships in Pol III disorders.[10]

2 | METHODS

2.1 | *POLR3B* patient identification and phenotype capture

The index patient (Patient 1), was recruited to a whole genome sequencing study, which aimed to identify novel genetic etiologies in DEEs of early childhood.[1] A second patient at the same center, presenting with a very similar phenotype, was identified on clinical diagnostic testing using a panel including genes associated with developmental disorders, the DDG2P exome.[20] Data relating to four further previously published patients was obtained from the corresponding authors.[21,22] Seven further patients were identified via the online gene-matching tool GeneMatcher.[23]

Inclusion criteria for the current study were:

1. Patient found to have a variant in *POLR3B* that is either *de novo* or of undetermined inheritance.
2. No other etiological diagnosis for epilepsy or developmental encephalopathy identified.
3. Patient has epilepsy.
4. Parents or carers have given consent to inclusion in the study and publication of findings.

Contributing clinicians for each patient were identified and each completed two structured proformas detailing the genotype and phenotype of their patient.

The phenotype proformas required completion of the following details:

- Age at onset of seizures, presenting seizure type, subsequent seizure types, and epilepsy classification as per the 2022 International League Against Epilepsy (ILAE) position paper on classification and definition of epilepsy syndromes with onset in childhood.[24]
- Anti-seizure therapy history. Clinicians were asked to highlight, but not quantify, if any therapies were perceived subjectively to be particularly efficacious for seizure control.
- Full developmental history across all domains of development, with results of validated developmental assessments where available. Clinicians were asked whether there was any developmental slowing or regression observed at the time of epilepsy onset.
- Full systemic, neurological, and dysmorphology examination, and height, weight, and occipital frontal circumference (OFC).
- Results of all neurological investigations, including electroencephalography (EEG), brain magnetic resonance imaging (MRI), and nerve conduction studies, and the age of the patient when investigations were performed.

Contributing clinicians were asked to provide details of the *POLR3B* variant identified in their patient including genomic coordinates (GRCh38) and inheritance.

All variants were evaluated for pathogenicity by assessing frequency in the asymptomatic population (gnomAD) and in silico pathogenicity scores (REVEL, Align, SIFT, PolyPhen-2, Splice AI). All variants were classified as per the American College of Medical Genetics (ACMG) criteria for pathogenicity.[25]

POLR3B missense variants were modeled by SWISS-MODEL[26] using 7ae1 and 7fjj as wild-type template.[27,28] Structural consequences were predicted with dynamut2[29] and missense3D.[30] Figure 2 was made with Mol*.[31] Missense tolerance landscape is from MetaDome.[32]

3 | RESULTS

Phenotypic findings in each case are detailed in Table 1. Genetic findings and testing platform are detailed in Table 2.

3.1 | Clinical phenotypes of *POLR3B* cohort

3.1.1 | Presenting seizures (see Table 1)

Age at first epileptic seizure ranged from six months to four years (median = 12 months). For eight of the 13 patients the first seizure was classified as a myoclonic-atonic

TABLE 1 Epilepsy phenotypes of the *POLR3B* cohort.

Patient	Age at fist seizure	First seizure type	Subsequent seizures (age of onset)	Interictal EEG findings (age at EEG)	Were seizures captured on EEG? (age when captured)	Type of seizure captured?
Patient 1 – female 9 years at most recent review	10 months	M-A	M-A (10 m) GM (18 m) GC (15 m) AA (7 years)	Frequent bursts of high amplitude sharp/spike and slow wave, increasing frequency at night (24 months)	Yes (17 months)	M-A
Patient 2 – male 4 years at most recent review	11 months	M-A	M-A (11 m)	Frequent 1–2 second bursts of high amplitude sharp/spike and slow wave (11 months)	Yes (14 months)	M-A
Patient 3 – male 6 years at most recent review	18 months	M-A	M-A (18 m)	N/A	Yes (20 months)	M-A
Patient 4 – female 7.5 years at most recent review	18 months	Myoclonic absence	Myoclonic absence (18 m)	Bursts of bifrontal spikes/ spikes-waves, more abundant during sleep (3 years)	Yes (3 years)	Myoclonic absence
Patient 5 – female 7 years at most recent review	25 months	CSE	CSE (25 m) AA (unknown)	Bifrontal spikes (3 years)	No	N/A
Patient 6 – female* 11 years at most recent review	12 months	GM	GM (12 m) AA (unknown)	Generalized and multifocal epileptiform discharges (6 years)	No	N/A
Patient 7 – male* 22 years at most recent review	6 months	M-A	M-A (6 m) GM (unknown) AA (unknown) GTC (unknown)	N/A	Yes (2 years)	M-A
Patient 8 – male 12 years at most recent review	4 years	AA	AA (4y) M-A (4y) GM (4y) GTC (5y) NCSE (5y)	Generalized spike and wave discharges with bifrontal predominance (4 years)	Yes (4 years)	M-A
Patient 9 – female 10 years at most recent review	6 months	M-A	M-A (6 m)	N/A	Yes (2 years)	M-A
Patient 10 – male* 9 years at most recent review	12 months	M-A	M-A (12 m)	N/A	Yes (3 years)	M-A
Patient 11 – female 9 years at most recent review	39 months	AA	AA (39 m)	Generalized burst-suppression pattern (39 months)	Yes (39 months)	AA
Patient 12 – male* 19 months at most recent review	6 months	M-A	M-A (6 m)	Generalized spike/polyspike-slow wave discharges (1–2 Hz, posterior maximum) were seen either as a single burst or in longer runs up to 9 seconds with no clinical correlate (23 months)	No	N/A
Patient 13 – male 19 months at most recent review	11 months	M-A	M-A (11 m)	Generalized polyspike wave discharge (12 months)	No	N/A

Note: *Included in previous publication.

Abbreviations: AA, atypical absence; ACC, anterior corpus callosotomy; CBD, cannabidiol; CLB, clobazam; CNB, cenobamate; CSE, convulsive status epilepticus; CZP, clonazepam; EMA, Epilepsy with myoclonic absences. EMAtS, Epilepsy with myoclonic-atonic seizures; ETX, ethosuximide; GBP, gabapentin; GC, Generalized clonic; GM, generalized myoclonic; GT, generalized tonic; GTC, generalized tonic–clonic; KD, ketogenic diet, KV, K.Vita; LCS, lacosamide; LEV, levetiracetam; LTG, lamotrigine; M-A, myoclonic-atonic; MAD, modified Atkins diet; NCSE, non-convulsive status epilepticus; OXC, oxcarbazepine; PB, phenobarbital; PRED, prednisolone; RUF, rufinamide; TPM, topiramate; VNS, vagal nerve stimulation; VPA, sodium valproate; ZNS, zonisamide.

EEG correlate	Epilepsy classification	Anti-seizure treatments tried	Current anti-seizure treatments	Anti-seizure treatments reported to be effective	Current seizure frequency (age at which reported)
High amplitude bisynchronous slow with marked spikes	EMAtS	LEV, VPA, CLB, RUF, Pyridoxine, KD (6)	VPA	KD, VPA	Infrequent AA (9 years)
High amplitude bisynchronous spike and slow wave	EMAtS	CLB, VPA, KD, RUF, LEV, CBD, KV, LTG, CNB (9)	LTG, CNB	VPA, KD, CNB	6–9 M-A per day (3.5 years)
High amplitude 2–3 Hz irregularly generalized epileptiform discharges with bifrontal predominance. These last about 1–1.5 seconds.	EMAtS	LEV, TPM, ZNS, MAD (4)	None	ZNS	Seizure free since aged 5.5 years (8 years)
Bursts of bifrontal spikes/ spikes-waves	EMA	LTG, ETX, ZNS, CLB, VPA (5)	ZNS, CLB, VPA	ETX	Multiple myoclonic absences per day (4 years)
N/A	Unclassified epilepsy	LCS, OXC (2)	LCS, OXC	LCS, OXC	1–2 episodes of CSE per year (3 years)
N/A	Unclassified epilepsy	VPA, PRED, LEV, CLB, KD, TPM (6)	TPM	PRED, TPM	Very infrequent GM and AA (10 years)
High amplitude bisynchronous poly-spike wave	EMAtS	PB, VPA, ETX, LEV, OXC, PRED, ZNS, KD, VNS, ACC, GBP (11)	VPA, OXC, GBP	None	Daily GM and AA (22 years)
Burst of irregular generalized spike and wave and polyspike lasting 1 second	EMAtS	RUF, ETX, VPA, CLB, LEV, LTG, CBD (7)	RUF, LTG, ETX, CBD	RUF	Multiple daily AA, one GTCS per month, one M-A per month (12 years)
Generalized polyspike discharge	EMAtS	PB, LEV, VPA, CLB, RUF (5)	CLB, RUF	CLB, RUF	Seizure-free since aged 7 years (9 years)
High amplitude bisynchronous spike and slow wave	EMAtS	VPA, LEV (2)	VPA, LEV	VPA, LEV	Very infrequent M-A (9 years)
Suppression of EEG	Unclassified epilepsy	VPA (1)	VPA	VPA	Seizure-free since aged 5 years (12 years)
N/A	Probable EMAtS	LEV, CLB, CZP (3)	CZP	CZP	3–4 M-A per day (19 months)
N/A	Probable EMAtS	VPA (1)	VPA	VPA	10 x M-A and 10 x AA per day (2 years)

TABLE 2 Developmental phenotypes of the *POLR3B* cohort.

Patient	Age at first developmental concern	Developmental slowing or plateau following onset of seizures?	Age at most recent validated developmental assessment	Developmental assessment used	Summary findings of validated developmental assessment	Age at most recent general assessment	Degree of intellectual disability or developmental delay	Autism spectrum disorder (ASD) or autistic features (age at diagnosis)	Verbal ability	Feeding
Patient 1-female	12 months (1st sz 10 m)	Yes, and development improved with seizure control	4 years 11 months	Ages and stages questionnaire	Locomotor skills at 24-month level Visual skills at 36-month level Speech and language at 30-month level Interactive social at 30-month level Self-care social skills at 24-month level	9 years	Moderate intellectual disability	No	Short sentences only	Orally fed
Patient 2-male	9 months (1st sz 11 m)	Yes, and development improved with seizure control	32 months	Schedule of growing skills	Global function at 18 months Motor skills at 15 months	4 years	Moderate intellectual disability	No	No words, but sounds with meaning e.g. "toot toot" for car	Orally fed
Patient 3-male	6 months (1st sz 18 m)	No, but development improved with seizure control	5 years	WPPSI-IV	Full Scale IQ = 64, 1st percentile	6 years	Mild intellectual disability	ASD (19 months)	Verbal with full sentences	Orally fed
Patient 4-female	12 months (1st sz 18 m)	No	Not done	N/A	N/A	7.5 years	Severe intellectual disability	ASD (5 years)	No words Limited non-verbal communication	Orally fed
Patient 5-female	18 months (1st sz 25 m)	Yes, with speech particularly	25 months	VABS-3	Receptive communication, age equivalent 14 months Expressive communication, age equivalent 12 months Gross motor skills, age equivalent 14 months Fine motor skills 13, age equivalent 24 months	7 years	Mild intellectual disability	ASD (3 years)	Verbal	Orally fed
Patient 6 – female*	12 months (1st sz 12 m)	No	7 years	WPPSI-III	Full Scale IQ = 55	11 years	Mild intellectual disability	No	Verbal Dysarthria	Orally fed

TABLE 2 (Continued)

Patient	Age at first developmental concern	Developmental slowing or plateau following onset of seizures?	Age at most recent validated developmental assessment	Developmental assessment used	Summary findings of validated developmental assessment	Age at most recent general assessment	Degree of intellectual disability or developmental delay	Autism spectrum disorder (ASD) or autistic features (age at diagnosis)	Verbal ability	Feeding
Patient 7 – male*	6 months (1st sz 6 m)	No	17 years	RIAS	Verbal IQ = 43 Nonverbal IQ = 66 Combined IQ = 46	22 years	Moderate intellectual disability	No	Verbal Dysarthria	Orally fed
Patient 8-male	2 years (1st sz 4y)	No	N/A	N/A	N/A	12 years	Mild intellectual disability	Some ASD features but no formal diagnosis	Verbal	Orally fed
Patient 9-female	8 months (1st sz 6 m)	Unknown	N/A	N/A	N/A	10 years	Moderate intellectual disability	No	Verbal (80–90 words)	Orally fed
Patient 10 – male*	33 months (1st sz 12 m)	No	9 years	KTEA-3	Significant weaknesses across all other areas assessed including his understanding of language, pattern recognition, visuo-constructional skills, fine motor planning and speed, learning and memory, and academic skills	9 years	Moderate intellectual disability	No	Verbal with 3–4 word sentences Dysarthria	Orally fed
Patient 11 – female	21 months (1st sz 39 m)	No	N/A	N/A	N/A	9 years	Mild intellectual disability	No	Verbal	Orally fed
Patient 12 – male*	9 months (1st sz 6 m)	No	12 months	Bayley III	Extremely low or borderline in all domains except expressive language was low average	19 months	Global developmental delay	No	No meaningful syllables	Orally fed
Patient 13 – male	6 months (1st sz 11 m)	No	18 months	ET 6-6-R	Strongly below average	19 months	Global developmental delay	No	No words	Orally fed

Note: *Included in previous publication.

Abbreviations: Bayley – III, Bayley Scales of Infant and Toddler Development, Third Edition; ET 6-6-R, Entwicklungstest für Kinder von 6 Monaten bis 6 Jahren (Developmental test for children from 6 months to 6 years) – Revision; KTEA-3, Kaufman Test of Educational Achievement, Third Edition; M-CHAT – R, Modified Checklist for Autism in Toddlers, Revised; RIAS, Reynolds Intellectual Assessment Scales; sz, seizure; VABS-3, Vineland Adaptive Behavior Scales, Third Edition; WPPSI-III, Wechsler Preschool and Primary Scale of Intelligence, Third Edition; WPPSI-IV, Wechsler Preschool and Primary Scale of Intelligence, Fourth Edition.

seizure. The presenting seizure appeared to be related to age. All patients who presented with myoclonic-atonic seizures were under the age of 18 months at the time of first seizure. Those presenting at relatively later ages presented with the following seizure types: one with myoclonic absence seizures (18 months); one with convulsive status epilepticus (25 months); two with atypical absences (four years and 39 months of age, respectively). One patient, who presented at 12 months, was described as having generalized myoclonic seizures without an atonic component.

3.1.2 | Epilepsy evolution and classification (see Table 1)

Patients who only had one seizure type

Five of the eight patients who presented with myoclonic-atonic seizures continued to have myoclonic-atonic as their only seizure type—these patients were 19 months, 19 months, four years, six years, and 10 years of age at the most recent epilepsy review. One patient (age 7.5 years at the most recent review) had myoclonic absence seizures only; and one patient (age nine years at the most recent review) had atypical absences only.

Patients who had multiple seizure types

The remaining six patients all had multiple seizure types. Seizure types observed were atypical absence seizures (six patients); generalized tonic-clonic/clonic seizures (four patients, of whom one had convulsive status epilepticus); generalized myoclonic seizures (four patients); and non-convulsive status epilepticus (one patient). Neither focal seizures nor epileptic spasms were reported in any of the patients.

Epilepsy classification as per ILAE 2022 Criteria

Seven patients had their epilepsy classified as epilepsy with myoclonic-atonic seizures (EMAtS). On review of the diagnostic criteria for EMAtS[24] all seven patients satisfied these criteria. Two further patients had "probable EMAtS" due to myoclonic-atonic seizures not being definitively captured on EEG. In all but one of the patients with EMAtS/probable EMAtS, age at onset was at the early end of the spectrum for this syndrome (range six months to four years, median 11 months). One patient had their epilepsy classified as epilepsy with myoclonic absences (EMA). Three patients had unclassified epilepsy.

3.1.3 | EEG (see Table 1)

EEG data were provided for all 13 patients. All patients had generalized ictal or interictal epileptiform discharges on EEG. Just one patient (Patient 6) was also reported to have multifocal epileptiform discharges. One patient (Patient 11) had a generalized burst suppression EEG pattern at the age of 39 months. She had atypical absence seizures in correlation with EEG suppressions. Background EEG was within normal limits for the other 12 patients.

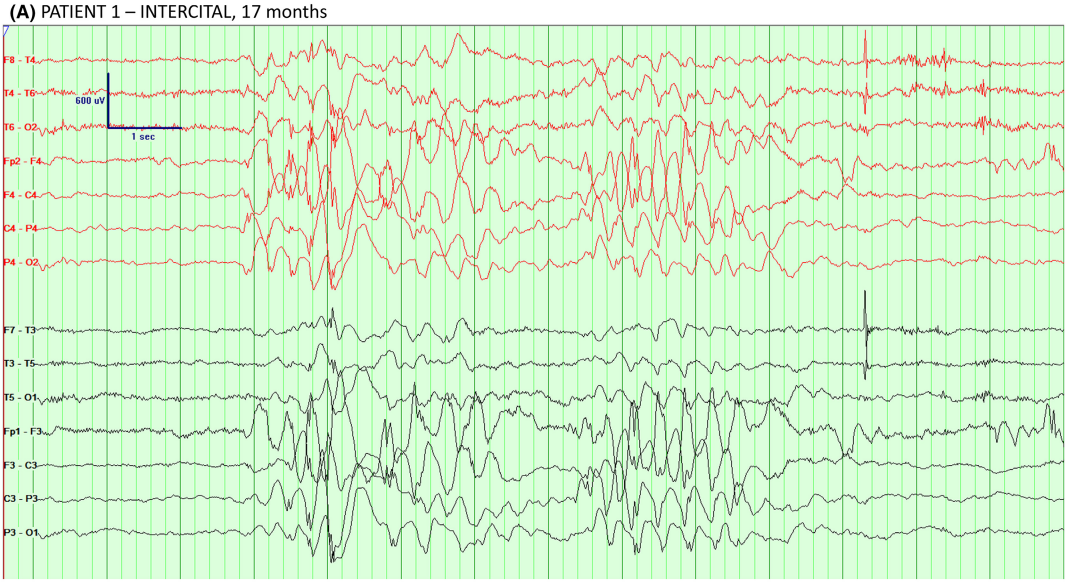
Eight patients had seizures captured on EEG—in all cases the seizure type captured was a myoclonic-atonic seizure and the EEG correlate was a high-amplitude burst of generalized spike/polyspike and wave. The EEG studies of Patient 1 and Patient 2, capturing myoclonic-atonic seizures, are shown in Figure 1.

3.1.4 | Anti-seizure treatments and effectiveness (see Table 1)

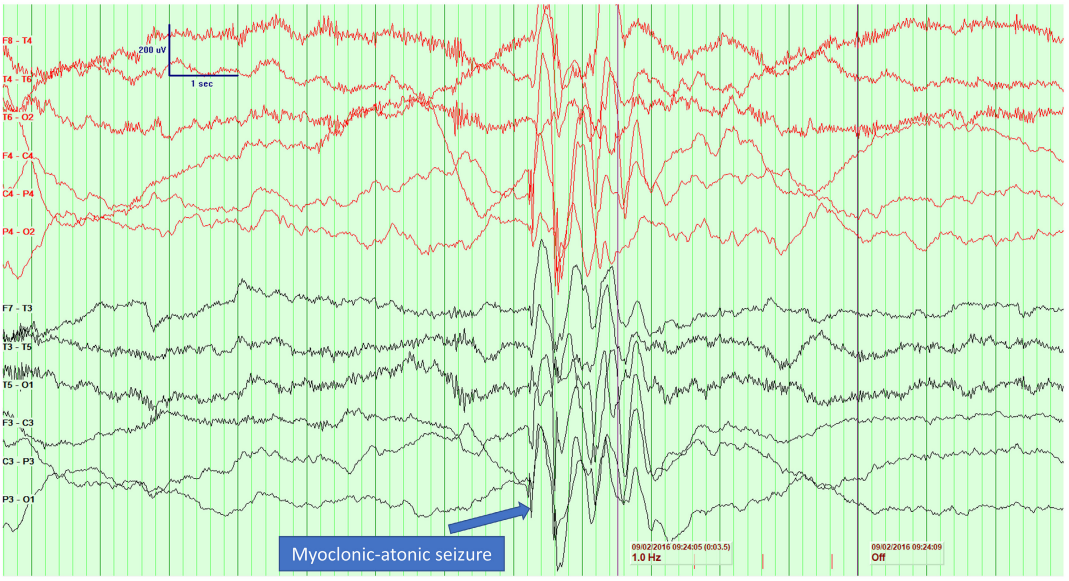
Eleven of the 13 patients satisfied diagnostic criteria for therapy-resistant seizures, meaning that they continued to have epileptic seizures despite adequate trials of two tolerated, appropriately chosen, and used anti-seizure medicine (ASM) schedules (whether as monotherapies or in combination).[33] The median number of ASMs trialed per patient in our cohort was five. No ASM was reported consistently to be efficacious. Sodium valproate was reported to be effective for five of the nine (5/9) patients for whom it was tried. Ketogenic diet was reported to be effective for two of the three (2/3) patients. Rufinamide was reported to be effective in two of the three (2/3) patients. Cenobamate (1/1), zonisamide (1/3), ethosuximide (1/3), lacosamide (1/1), oxcarbazepine (1/2), topiramate (1/2), clobazam (1/4), prednisolone (1/2), levetiracetam (1/9), and clonazepam (1/1) were all reported to be efficacious in individual patients. One patient had an unsuccessful trial of oral pyridoxine treatment. Outcomes, in terms of epileptic seizure control, were variable. Three patients had been seizure-free for at least six months at the time of reporting; four

FIGURE 1 EEG study Right hemisphere electrodes (red) over left hemisphere electrodes (black) montage with frontopolar and electrocardiogram channels hidden to improve figure clarity. (A) Interictal EEG at the age of 17 months in Patient 1. (B) Ictal EEG at the age of 17 months in Patient 1, capturing a myoclonic-atonic seizure. (C) Ictal EEG at the age of 14 months in Patient 2, capturing a myoclonic-atonic seizure.

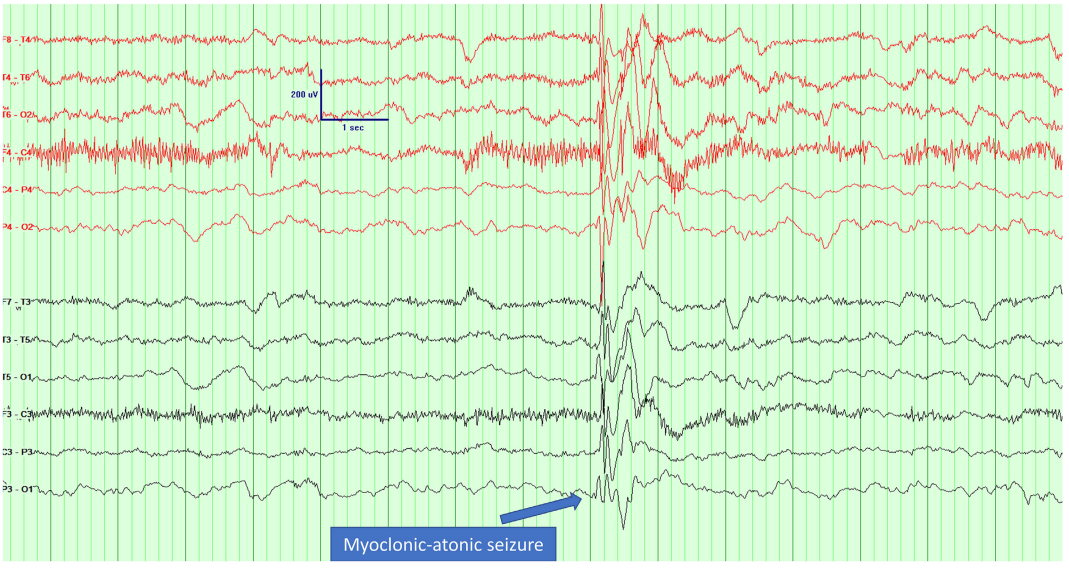
(A) PATIENT 1 – INTERCITAL, 17 months



(B) PATIENT 1 – ICTAL, 17 months



(C) PATIENT 2 – ICTAL – 14 months



patients were having infrequent seizures; and six patients were having multiple seizures per day.

Four of six patients who were having daily seizures were younger than five years at the time of reporting, whereas six of the seven patients who were either seizure-free or having infrequent seizures were nine years or older. Hence there is a suggestion that seizure control may improve over time. Of the nine patients with EMAtS/probable EMAtS, six were older than seven years at the most recent reporting. For all six patients, their myoclonic-atonic seizures had become very infrequent or had stopped entirely, and two patients had become free of all seizure types.

3.1.5 | Developmental profiles and progress (see Table 2)

Age at the first developmental concern ranged from six to 33 months. For seven patients, the first developmental concern was identified prior to presentation with seizures; for two patients, the first developmental concern coincided with seizure presentation; and for four patients, first developmental concern was highlighted after presentation with seizures. In three patients (Patients 1, 2, and 5), seizure onset was felt to be associated with developmental slowing or plateau. In Patients 1 and 2, plus one further patient (Patient 3), improved seizure control was associated with developmental progress.

For nine patients we were provided with reports from validated developmental or neuropsychological testing. A variety of different assessments were used. Assessments were consistent with mild intellectual disability in three patients and moderate intellectual disability in four patients. Two patients were younger than two years of age at the time of formal developmental assessment, but findings were consistent with global developmental delay. Of the four patients who did not have formal developmental assessment reports available, two were felt to have mild intellectual disability, one was felt to have moderate intellectual disability, and one was felt to have severe intellectual disability.

Of the 11 patients who were older than two years of age at their most recent assessment, one (9%) had severe intellectual disability, five (46%) had moderate intellectual disability, and five (46%) had mild intellectual disability. Motor, cognitive, and speech and language development appeared to be equally affected. Three patients (23%) were diagnosed with autism spectrum disorder. All patients who were older than two years at most recent assessment gained the ability to walk independently, nine gained the ability to talk, and all were orally fed.

3.1.6 | Motor features: Ataxia, hypertonia, and neuropathy (see Table 3)

Six patients were reported to have ataxia (three mild and three moderate). The age at onset of ataxia ranged between 19 months and 11 years. Three of these patients had additional cerebellar signs (intention tremor and dysmetria). An additional three patients had an unstable or uncoordinated gait but were not felt to have frank ataxia. Of the four patients with no reported ataxia, two were 19 months at the most recent assessment and were not yet walking. Four patients had abnormal nerve conduction studies, of whom two were felt clinically to have peripheral neuropathy and two had no signs of neuropathy on neurological examination but had asymptomatic nerve conduction studies done. Two further patients had asymptomatic nerve conduction studies done and these were reported as normal. Of interest, all four of the patients who had abnormal nerve conduction studies also had signs of hypertonia on neurological examination. One of these was using ankle-foot orthoses. Two further patients (Patient 1 and Patient 4) had signs of lower limb hypertonia on neurological examination but did not have nerve conduction studies performed.

3.1.7 | Growth parameters (see Table 3)

We were provided with height, weight, and occipital frontal circumference (or OFC) for all 13 patients. Seven patients had microcephaly and two had short stature (both of whom also had microcephaly). No patient had macrocephaly and all 13 patients had an OFC that was below the mean for age. We did not have serial measurements or parental measures for comparison.

3.1.8 | Imaging (see Table 3)

All 13 patients had MRI brain scan data available. MRI was reported as normal in eight patients, showed global cerebral or white matter volume loss in three, and nonspecific white matter changes (without volume loss) in two.

3.1.9 | Additional features

Astigmatism, myopia, or hypermetropia were reported in four patients and a further patient had cortical visual impairment and attended a school for the blind. There were no consistent dysmorphic features reported. Non-neurological findings on physical examination are reported in Table 3.

TABLE 3 Motor features, growth parameters, neuroimaging, and other findings in the *POLR3B* cohort.

Patient	Current mobility (age) and age at walking	Ataxia? (age at onset, age at assessment)	Clinical peripheral neuropathy? (age at assessment)	Nerve conduction study findings? (age at assessment)	Muscle tone (age at assessment)	Growth parameters (age at assessment)	MRI brain (age at time of imaging)	Any other features
Patient 1-female	Ambulant with ataxia (9 year) Walked at 15 months.	Moderate gait ataxia (onset 18 months) but no other cerebellar signs (9 years)	No overt neuropathy (9 years)	Not done	Left lower limb hypertonia, wears ankle-foot orthosis (9 years)	H = −1.05 SD (8 years) W = 0.86 SD (8 years) OFC = −0.51 SD (8 years)	Normal (21 months)	Hypermetropia, astigmatism
Patient 2-male	Ambulant with ataxia (4 years) Walked at 28 months	Moderate gait ataxia (onset at 2 years) but no other cerebellar signs (4 years)	No overt neuropathy (4 years)	Normal nerve conduction studies (3 years)	Normal (4 years)	H = −0.303 SD (3 years) W = −1.13 SD (3 years) OFC = −0.57 SD (3 years)	Normal (14 months)	None
Patient-male	Ambulant with normal gait (6 years) Walked at 15 months	Incoordination but not frank ataxia and no cerebellar signs (6 years)	No overt neuropathy (6 years)	Not done	Normal (6 years)	H = 0.27 SD (4 years) W = 0.07 SD (4 years) OFC = −0.47 SD (4 years)	Bilateral patchy T2 hyperintense signal abnormality, most conspicuous in the biatrial and frontal white matter. Mild cortical irregularity of the superior vermian gyri (22 months)	Dysarthria, toe walking Down slanting eyes Depressed nasal bridge Overriding 2–3 toes with mild syndactyly
Patient 4-female	Ambulant with unstable gait (7 years) Walked at 2.5 years.	Unstable gait but not frank ataxia and no cerebellar signs (7 years)	No overt neuropathy (7 years)	Not done	Normal tone but brisk deep tendon reflexes (7 years)	H = 0.34 SD (4 years) W = 0.6 SD (4 years) OFC = −2.1 SD (4 years) (microcephaly)	Normal (26 months)	Hypertelorism, epicanthic folds
Patient 5-female	Ambulant with normal gait (7 years). Walked at 18 months	Incoordination but not frank ataxia and no cerebellar signs (7 years)	No overt neuropathy (7 years)	Not done	Normal (7 years)	H = −1.01 SD (7 years) W = −0.32 SD (7 years) OFC = 0 SD (7 years)	Global cerebral atrophy (24 months and 48 months)	Hyperpigmented macules Cortical visual impairment

(Continues)

TABLE 3 (Continued)

Patient	Current mobility (age) and age at walking	Ataxia? (age at onset, age at assessment)	Clinical peripheral neuropathy? (age at assessment)	Nerve conduction study findings? (age at assessment)	Muscle tone (age at assessment)	Growth parameters (age at assessment)	MRI brain (age at time of imaging)	Any other features
Patient 6 – female*	Ambulant, with ataxia and spasticity (11 years) Walked at 2.5 years	Mild gait ataxia (onset at 2 years) Mild intention tremor and dysmetria Unable to balance on one foot or perform tandem gait (11 years)	Peripheral neuropathy (7 years)	Combined axonal and demyelinating polyneuropathy of both motor and sensory nerves (7 years)	Hypertonia with pyramidal signs. Deep tendon reflexes: Arms, 3+; legs, 4+. Crossed adductor reflex. sustained ankle clonus. Extensor plantar responses.	H = −2.2 SD (9 years) (short stature) W = 1.46 SD (9 years) OFC = −2.44 SD (9 years) (microcephaly)	Normal (5 years)	Partial growth hormone deficiency
Patient 7 – male*	Ambulant, with ataxia (22 years) Walked at 18 months	Moderate gait ataxia (unknown onset) Bilateral circumduction Unable to perform tandem gait or stance Slow finger to nose movements (22 years)	Peripheral neuropathy (12 years)	Predominantly demyelinating sensory motor neuropathy Slow motor conduction velocities Markedly decreased amplitudes in tibial nerve Absent sural responses	Mild hypertonia with 3+ deep tendon reflexes throughout except 1+ at the ankles	H = −1.8 SD (22 years) W = 1.8 SD (22 years) OFC = 2.2 SD (22 years) (microcephaly)	Mild non-specific white matter T2 signal hyperintensity in the posterior periventricular regions which were stable over time (had 10 MRIs between ages 2 and 20 years)	Impulsive behavior, myopia
Patient 8-male	Ambulant with normal gait (6 years) Walked at approx. 15 months	No ataxia or incoordination (6 years)	No overt neuropathy (6 years)	Not done	Normal (6 years)	H = 1.39 SD (7 years) W = 0.58 SD (7 years) OFC = −0.9 SD (7 years)	Normal (4 years)	
Patient 9-female	Ambulant with ataxia and spasticity (6 years) Walked at 5 years	Mild gait ataxia (onset at 2 years) and truncal instability Dysmetria (6 years)	No overt neuropathy (6 years)	Consistent with peripheral neuropathy (6 years)	Lower limb spasticity (wears ankle foot orthoses)	H = −0.52 SD (6 years) W = −0.02 SD (6 years) OFC = −2.38 SD (6 years) (microcephaly)	Normal (1 year)	Strabismus, astigmatism, hypermetropia Bilateral ptosis, mild synophrys with bushy eyebrows and long eyelashes Bilateral 5th toe clinodactyly

TABLE 3 (Continued)

Patient	Current mobility (age) and age at walking	Ataxia? (age at onset, age at assessment)	Clinical peripheral neuropathy? (age at assessment)	Nerve conduction study findings? (age at assessment)	Muscle tone (age at assessment)	Growth parameters (age at assessment)	MRI brain (age at time of imaging)	Any other features
Patient 10 – male*	Ambulant with mild ataxia and collapsing foot arches (11 years) Walked at 22 months	Mild gait ataxia (onset at 10 years)	No overt neuropathy (10 years)	Normal (4 years)	Normal (10 years)	H = −2.93 SD (9 years) (short stature) W = −3.30 SD (9 years) OFC = −3.41 SD (9 years) (microcephaly)	Normal (7 years)	Myopia, astigmatism, short stature, hypothyroidism, constipation, hypotonia
Patient 11 – female	Ambulant with normal gait (9 years) Walked at 21 months	No ataxia or incoordination (9 years)	No overt neuropathy (9 years)	Not done	Normal (9 years)	H = 0.67 SD (9 years) W = −0.09 SD (9 years) OFC = −3.33 SD (9 years) (microcephaly)	Normal (3 years)	Astigmatism hypermetropia strabismus, prognathia, plagiocephaly, smooth philtrum, large and protruding ears
Patient 12 – male*	Not yet walking (19 months)	No ataxia or incoordination (19 months)	No overt neuropathy (19 months)	Reduced conduction velocity with normal amplitude values in the sensory and motor responses from the upper and lower limbs (18 months)	Increased tone in upper and lower limbs with brisk reflexes at knee and ankle Catch in both lower limbs Toe walking when placed on feet (19 months)	H = −0.63 SD (19 months) W = 0.81 SD (19 months) OFC = −2.28 SD (19 months) (microcephaly)	Diffuse white matter volume reduction, thin corpus callosum (21 months)	Brachycephaly, thin upper lip
Patient 13 – male	Not yet walking (19 months)	No ataxia or incoordination (19 months)	No overt neuropathy (19 months)	Not done	Normal	H = −1.54 SD (19 months) W = 0.67 SD (19 months) OFC = −1.16 SD (19 months)	Global cerebral volume loss (13 months)	

*Included in previous publication.

Abbreviations: H, height; OFC, occipital-frontal circumference; W, weight.

3.2 | Genetic findings of POLR3B cohort (see Table 4)

Twelve of the 13 variants in *POLR3B* were confirmed *de novo*. Patient 8 did not have parental DNA available, but the variant was absent from two unaffected siblings. All 13 variants were novel and absent from large databases of healthy population. Twelve of 13 variants were classified as pathogenic or likely pathogenic as per ACMG criteria.[25] Patient 6, Patient 7, Patient 10,[21] and Patient 12[22] have been published previously. All the variants were missense, and they clustered in the region of the gene encoding the part of the polymerase where transcribed DNA is melted. There was in silico evidence to suggest that all 13 variants are damaging to protein function (Table 4). Splice prediction tools predict that the variant in Patient 8 will create a new splice acceptor site at c.1730. This prediction is also supported by SpliceAI (https://spliceailookup.broadinstitute.org/). The consequence of this splicing change is difficult to predict without cDNA analysis from this patient, but will possibly interfere with the correct splicing of this exon.

3.3 | *POLR3B* missense tolerance landscape and structural modeling

The *POLR3B* variants found in this cohort were found in regions of the gene that are intolerant of genetic variation.[32] There was a clear clustering of the epilepsy-associated variants within or nearby the DNA-binding domain of *POLR3B*[29,30] (see Figure 2) For further details of the modeling, see the Supplementary Material.

4 | DISCUSSION

We describe the detailed phenotypes of 13 patients with epilepsy and missense variants in *POLR3B*, three of which have been reported previously by Djordevic et al.[21] and one of which has been published previously by D'Gama.[22] There is strong evidence that these variants are causative because all variants appear to be absent from the general population; 11 of the 13 variants were predicted as damaging by all in silico tools tested; and in all the cases where we had parental samples available (12/13), the variants were confirmed as *de novo*. *POLR3B* is highly constrained to missense variation in DECIPHER (Missense Constraint 0.64, p-Value 2.20×10^{-12}), gnomAD v 2.1.1 (Z score = 3.2), and gnomAD v 4.0.0 (Z score = 3.48).

Despite variability in other aspects of the phenotype—including neuroimaging findings, growth parameters, dysmorphic features, neurocutaneous features, and comorbid medical conditions—there were some highly consistent features in this cohort. Nine of the patients were classified as having EMAtS or probable EMAtS, and the majority of those with EMAtS had onset of seizures at the younger end of the range for this epilepsy syndrome. All patients had generalized discharges on EEG. Ataxia/incoordination, hypertonia, and peripheral neuropathy were identified in many of these patients.

More than 350 monogenic causes of epilepsy have been described.[4] The majority of monogenic DEEs are associated with early neonatal seizures, epileptic spasms, or multifocal epilepsy.[1,34,35,36,37] The number of genes associated with DEEs in which generalized seizures are the predominant seizure type are relatively few, the most established being *SLC2A1*,[2] *SYNGAP*,[38] *CHD2*,[39] and *SLC6A1*.[40] *CHD2-DEE* is distinctive for the high proportion of patients with clinical photosensitivity, which is present in 80%. None of the patients in our *POLR3B* cohort were reported to have photosensitivity. Other rarer DEEs in which generalized seizures appear to be the most prominent seizure type are *NEXMIF*,[41] *HNRNPU*,[42] and *EEF1A2*.[43] Overall, the diagnostic yield of genetic testing in patients presenting with myoclonic-atonic seizures is relatively low,[44] but all these genes, along with *POLR3B*, should be considered as potential etiologies.

POLR3B disease–associated variants were first identified in biallelic state in patients with a progressive hypomyelinating leukodystrophy. Abnormal dentition and hypogonadotrophic hypogonadism were observed in some affected individuals, leading to the term 4H leukodystophy syndrome (hypomyelination, hypodontia, and hypogonadotrophic hypogonadism).[11,12] None of these seven patients reported initially had epilepsy as a feature of their phenotype. Since this initial description, many other patients have been reported with *POLR3B*-related leukodystrophy, but also with biallelic pathogenic variants in genes encoding other subunits of the RNA polymerase III, including *POLR3A*,[11,12] *POLR3C*,[13] *POLR3K*,[15] and *POLR3D*.[14] Epilepsy has never been reported as a presenting or predominant feature in these patients. It is well recognized that biallelic pathogenic variants in 4H-causing genes are hypomorphic. As an example, one *POLR3B* variant associated with the leukodystrophy phenotype demonstrates impairment of polymerase III biogenesis in a mouse model.[45]

In 2021, Djordevic et al.[21] reported six patients with *de novo* heterozygous missense variants in *POLR3B*. These patients had a distinct phenotype from those with biallelic variants. There was no evidence of leukodystrophy on the MRI brain scans, and abnormal dentition and hypogonadotrophic hypogonadism were not observed. Five of the patients had a demyelinating peripheral neuropathy. Three of them had epilepsy. Proteomic

TABLE 4 Genetic findings in the *POLR3B* cohort.

Patient ID	Identification method	Testing platform	Genomic coordinates (GRCh38)	*POLR3B* variant (NM_018082.5)	Inheritance	gnomAD (v2.1.1 and v4.1.0)	REVEL	Other in silico predictions	ACMG evidence and classification
1	Index patient	Research based trio WGS	chr12:106430313G > T	c.1304G > T p.Gly435Val	*De novo*	Absent	0.93	No splicing change predicted. Align GVGD: C65. SIFT: DELETERIOUS. PolyPhen-2: HumVar Probably Damaging	PM2, PS2, PP3, PP2, PM1_Sup Likely Pathogenic
2	Known to clinician of index patient	DDG2P exome	chr12:106427219A > G	c.1124A > G p.Asp375Gly	*De novo*	Absent	0.598	No splicing change predicted. Align GVGD: C65. SIFT: DELETERIOUS. PolyPhen-2: HumVar Probably Damaging	PM2, PS2, PP2 Likely Pathogenic
3	GeneMatcher	Whole exome	chr12:106410931G > T	c.1072G > T p.Gly358Cys	*De novo*	Absent	0.808	No splicing change predicted. Align GVGD: C65. SIFT: DELETERIOUS. PolyPhen-2: HumVar Probably Damaging	PM2, PS2, PP2, PP3, PM5_Sup Likely Pathogenic
4	GeneMatcher	Clinical trio exome	chr12:106380055A > T	c.639A > T p.Arg213Ser	*De novo*	Absent	0.454	No splicing change predicted. Align GVGD: C35. SIFT: DELETERIOUS. PolyPhen-2: HumVar Probably Damaging	PM2, PS2, PP2, PM1_Sup Likely Pathogenic
5	GeneMatcher	Epilepsy panel	chr12:106430294T > G	c.1285T > G p.Phe429Val	*De novo*	Absent	0.829	No splicing change predicted. Align GVGD: C45. SIFT: DELETERIOUS. PolyPhen-2: HumVar Probably Damaging	PM2, PS2, PP2, PP3, PM1_Sup Likely Pathogenic
6	Previous publication[21]	Clinical trio exome	chr12:106410946G > A	c.1087G > A p.Glu363Lys	*De novo*	Absent	0.706	No splicing change predicted. Align GVGD: C55. SIFT: DELETERIOUS. PolyPhen-2: HumVar Probably Damaging	PM2, PS2, PP2, PP3 Likely Pathogenic
7	Previous publication[21]	Clinical trio exome	chr12:106410953C > T	c.1094C > T p.Ala365Val	*De novo*	Absent	0.829	No splicing change predicted. Align GVGD: C65. SIFT: DELETERIOUS. PolyPhen-2: HumVar Probably Damaging	PM2, PS2, PP2, PP3 Likely Pathogenic
8	GeneMatcher	Clinical exome	chr12:106433820A > G	c.1729A > G p.Asn577Asp	Unknown, but absent from 2 unaffected siblings	Absent	0.349	Possible new splice acceptor site at c.1730 in 4/4 tools. Align GVGD: C0. SIFT: TOLERATED. PolyPhen-2: HumVar Benign	PM2, PP2, PP3 Variant of Uncertain Significance

(Continues)

TABLE 4 (Continued)

Patient ID	Identification method	Testing platform	Genomic coordinates (GRCh38)	*POLR3B* variant (NM_018082.5)	Inheritance	gnomAD (v2.1.1 and v4.1.0)	REVEL	Other in silico predictions	ACMG evidence and classification
9	GeneMatcher	Clinical trio exome	chr12:106430382A > C	c.1373A > C p.Gln458Pro	*De novo*	Absent	0.762	No splicing change predicted. Align GVGD: C65. SIFT: DELETERIOUS. Polyphen-2: HumVar Probably Damaging	PM2, PS2, PP2, PP3 Likely Pathogenic
10	Previous publication[21]	Clinical trio exome	chr12:106430286T > C	c.1277T > C p.Leu426Ser	*De novo*	Absent	0.716	No splicing change predicted. Align GVGD: C65. SIFT: DELETERIOUS. PolyPhen-2: HumVar Benign	PM2, PS2, PP2, PP3, PM1_Sup Likely Pathogenic
11	Known to clinician of Patient 4	Research based trio WGS	chr12:106378318A > G	c.548A > G p.Gln183Arg	*De novo*	Absent	0.734	No splicing change predicted. Align GVGD: C35, SIFT: DELETERIOUS. Polyphen-2: HumVar Probably Damaging	PM2, PS2, PP2, PP3 Likely Pathogenic
12	Previous publication[22]	Research based trio WGS	chr12:106433856G > A	c.1765G > A p.Gly589Arg	*De novo*	Absent	0.733	Possible new splice acceptor site at c.1767. Align GVGD: C65. SIFT: DELETERIOUS. Polyphen-2: HumVar Possibly Damaging	PM2, PS2, PP2, PP3 Likely Pathogenic
13	GeneMatcher	Research based trio WGS	Chr12:106433848C > T	c.1757C > T p.Ser586Phe	*De novo*	Absent	0.463	No splicing change predicted. Align GVGD: C0. SIFT: DELETERIOUS. PolyPhen-2: HumVar Benign	PM2, PS2, PP2 Likely Pathogenic

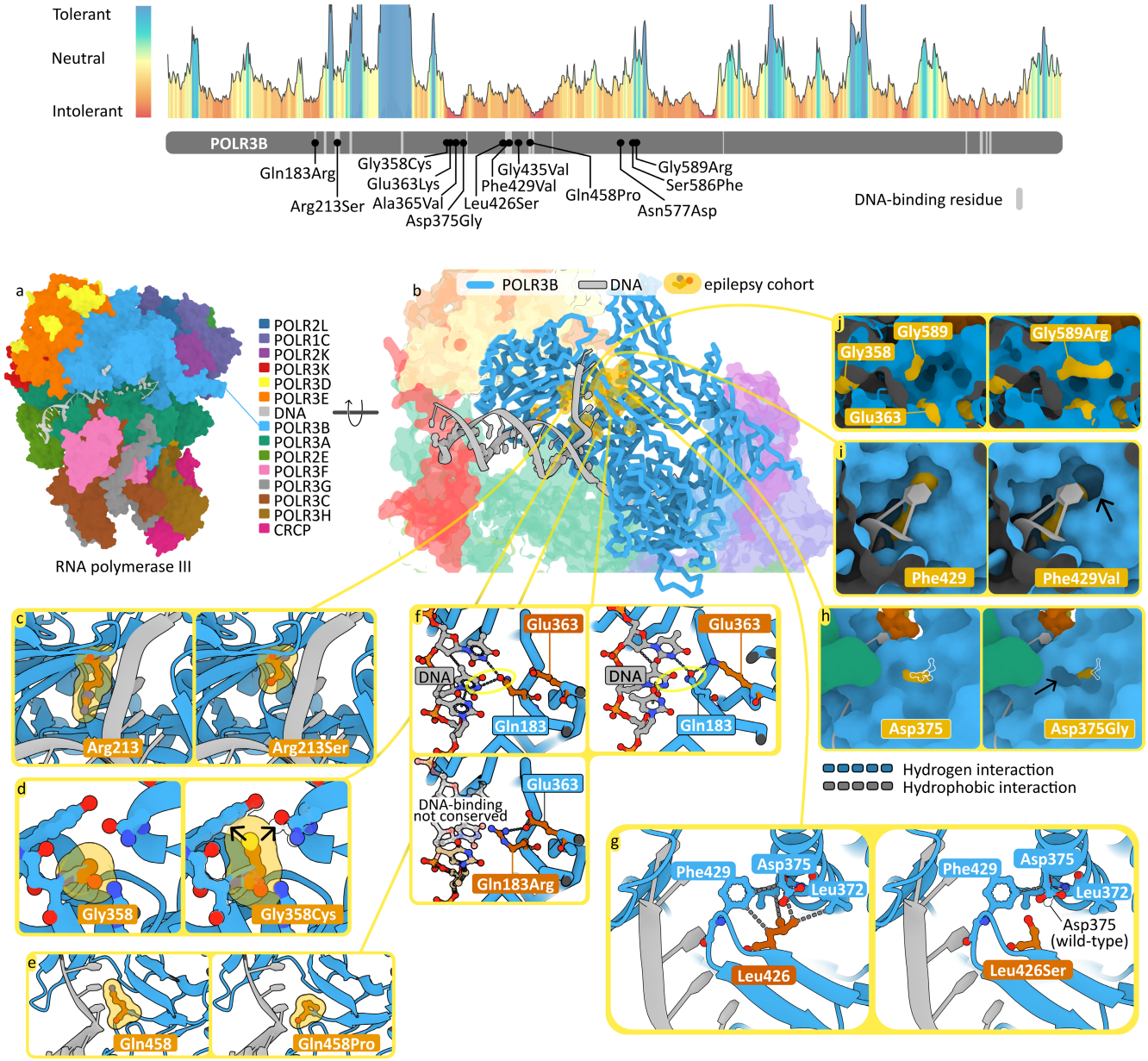


FIGURE 2 Localization and predicted effects of *POLR3B* variants. Above: MetaDome missense tolerance landscape. Below, structural modeling with *POLR3B* in blue, DNA in light gray. (A) RNA polymerase III complex, with its DNA template. (B) Cluster of the variants (yellow) around the DNA binding region. (C–J) Captions of the predicted structural effects of 9 of 13 missense variants. (For further details see Supplementary Material.)

analysis of the *POLR3B* variants identified in these patients revealed a disease mechanism different from that of the hypomyelinating leukodystrophy cases. Rather than impairing polymerase III biogenesis, the variants caused aberrant association of individual enzyme subunits. Ando et al.[46] reported two further patients with Charcot–Marie–Tooth disease associated with *de novo* variants in *POLR3B*. Neither of these patients had intellectual disability, ataxia, or seizures. Hence, *de novo* variants in *POLR3B* appear to be associated with a spectrum of disease presentations, involving various combinations of peripheral neuropathy, epileptic seizures, ataxia, and developmental impairment/intellectual disability. At present, the case numbers are too few to start investigating genotype–phenotype relationships.

What is clear is that the mechanism of disease is distinct from the global reduction of polymerase III activity that is observed in the hypomyelinating leukodystrophy cases. It is possible that these *de novo* missense variants confer a gain-of-function property on the polymerase III complex, or have other downstream effects on other specific proteins. If overactivity of *POLR3B* is a contributor to epileptogenesis, then this opens potential avenues for therapeutic intervention. Wholesale inhibition of

polymerase III would not be advisable, since there is an established association with progressive leukodystrophy. However, certain licensed medications, which are known to inhibit RNA polymerase, could be considered as candidates for therapeutic trials. Examples of ribosomal polymerase inhibitors are melatonin[47] and rapamycin/mTOR inhibitors.[10] Mammalian target of rapamycin (mTOR) inhibitors are effective anti-seizure medicines and licensed for epilepsy treatment in tuberous sclerosis complex.[48] There are small scale human data to suggest that melatonin may have an anti-seizure effect.[49]

5 | CONCLUSION

POLR3B-DEE is a newly described generalized DEE. Pathogenic variants causing this condition are *de novo* missense variants, and they cluster in the DNA-binding domain of the gene. Seizures are resistant to conventional ASMs and the only treatments reported to be effective in more than one patient were sodium valproate and the ketogenic diet.

AUTHOR CONTRIBUTIONS

Conceived the study, collected and assimilated the data, and wrote the draft manuscripts: Joseph D Symonds. Identified novel *POLR3B* variants through research-based genomic sequencing: Joseph D Symonds, Martin Armstrong, Houman Ashrafian, Ioana Cutcutache, Katherine S Elliott, Marc Planes, Konrad Platzer, Sylvia Redon, Matthew Page, Christian Stockhaus, Marie-Laure Vuillaume, Emma L Wakeling, and Julian C Knight. Collected and submitted clinical data on patients presented in the manuscript: Joseph D Symonds, Kristen L Park, Cyril Mignot, Stewart MacLeod, Geneviève Bernard, Kathleen Brown, Andreas Brunklaus, Mary Callaghan, Georg Classen, Julie S Cohen, David Dyment, Amy McTague, James Reese, Constance Smith-Hicks, Nicole I Wolf, Grace Yoon, AND Sameer M Zuberi. Collected genetic data on patients presented in the manuscript: Joseph D Symonds, Kristen L Park, Cyril Mignot, Geneviève Bernard, Arnaud Isapof, Shelagh Joss, Boris Keren, Michael Marble, Matthew Osmond, Margarita Saenz, Christian Stockhaus, and Marie-Laure Vuillaume. Produced in silico models of the *POLR3B* variants presented in this manuscript (Figure 2): Jean-Madeleine de Sainte Agathe. Assessed all variants for pathogenicity, as per ACMG guidelines (Table 2): Daniel Stobo. Reviewed and helped refine the manuscript: Joseph D Symonds, Kristen L Park, Cyril Mignot, Stewart MacLeod, Martin Armstrong, Houman Ashrafian, Geneviève Bernard, Kathleen Brown, Andreas Brunklaus, Mary Callaghan, Georg Classen, Julie S Cohen, Ioana Cutcutache, Jean-Madeleine de Sainte Agathe, David Dyment, Katherine S Elliott, Arnaud Isapof, Shelagh Joss, Boris Keren, Michael Marble, Matthew Osmond, Amy McTague, Marc Planes, Konrad Platzer, Sylvia Redon, Matthew Page, James Reese, Margarita Saenz, Constance Smith-Hicks, Daniel Stobo, Christian Stockhaus, Marie-Laure Vuillaume, Nicole I Wolf, Grace Yoon, Sameer M Zuberi, Emma L Wakeling, and Julian C Knight.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any conflicts of interest that have influenced their participation in this research or production of this manuscript.

DATA AVAILABILITY STATEMENT

All relevant data in this publication are presented in the tables within the main manuscript.

ETHICS STATEMENT

All authors confirm that they have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

CONSENT

Verbal consent was obtained from the parents/legal guardians of each patient presented in this article. Consent was taken by the clinician (neurologist or clinical geneticist) involved.

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SUPPORTING INFORMATION

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

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