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REVIEW

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TRIT1 defect leads to a recognizable phenotype of myoclonic epilepsy, speech delay, strabismus, progressive spasticity, and normal lactate levels

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Abstract

TRIT1 defect is a rare, autosomal-recessive disorder of transcription, initially described as a condition with developmental delay, myoclonic seizures, and abnormal mitochondrial function. Currently, only 13 patients have been reported. We reviewed the genetic, clinical, and metabolic aspects of the disease in all known patients, including two novel, unrelated *TRIT1* cases with abnormalities in oxidative phosphorylation complexes I and IV in fibroblasts. Taken together the features of all 15 patients, *TRIT1* defect could be identified as a potentially recognizable syndrome including myoclonic epilepsy, speech delay, strabismus, progressive spasticity, and variable microcephaly, with normal lactate levels. Half of the patients had oxidative phosphorylation complex measurements and had multiple complex abnormalities.

KEYWORDS

lipidomics, mitochondrial tRNA, myoclonic seizures, OXPHOS, spasticity

Ewout Muylle and Huafang Jiang equally contributed to this study.

1 | INTRODUCTION

TRIT1 deficiency is a rare genetic disorder of transcription with an autosomal-recessive inheritance pattern. The *TRIT1* gene, located on chromosome 1p34, encodes for a tRNA isopentenyl transferase (ITPase). The transfer of an isopentenyl group from dimethylallyl pyrophosphate to N6 of adenine at position 37 of the tRNA is catalyzed by this enzyme.[1,2] This post-transcriptional modification of tRNAs is essential for folding, stability and maintaining the correct reading frame during protein translation.[2,3] Moreover, the activity of the tRNA toward its codon increases four times due to this modification.[3] The TRIT protein is shown to be targeted to both the cytoplasm and the mitochondria after its transcription and translation.[3,4]

The first two patients with pathological *TRIT1* variants were described in 2014.[3] The two affected individuals had severe developmental delay, myoclonic seizures, and abnormal mitochondrial function.

Truncating *TRIT1* variants have been associated with a different, more severe phenotype, including intrauterine growth retardation, neonatal microcephaly, polymicrogyria, sensorineural hearing loss, and visual loss.[1] The link between these neurodevelopmental symptoms and the abnormal modification of the tRNA is not yet well understood.

Here, we review all patients with pathogenic variants in *TRIT1* described in the literature, compare the clinical, genetic, and metabolic characteristics including the effect of the gene on the oxidative phosphorylation (OXPHOS) and report on two novel cases.

2 | CLINICAL REVIEW OF REPORTED PATIENTS

Including our two novel patients (see Supporting Information), 15 patients have been described in the medical literature so far (five males, eight females, for two patients, the gender was not reported[1,3–9]). Age of symptom onset was between 3 and 14 months, and one patient had symptom onset antenatally (for four patients, age of symptom onset was not reported). Age at diagnosis was not reported for a part of the patients, the remaining eight (including our two new patients) were diagnosed at the age between 1 and 16 years. There was consanguinity with homozygous mutations in four cases (additionally, there was one case with homozygous missense mutations, but consanguinity was not reported).

All 15 patients, including our two new patients, presented with a primarily neurological symptom spectrum, mainly characterized by developmental delay and seizures. All patients presented with seizures, and 12 were reported to suffer from myoclonic jerks. Abnormal electroencephalogram (EEG) recordings were reported in 12 patients.

The majority of the patients developed cognitive delay (11/15), and 11 patients presented with microcephaly. Eight patients had abnormalities on magnetic resonance imaging, including cerebral atrophy, delayed myelination, reduced periventricular white matter, megacisterna magna, abnormalities of the corpus callosum, Dandy–Walker-malformation, hydrocephalus, polymicrogyria, vermis hypoplasia, and septo-optic dysplasia. Hypotonia was present in seven patients, while spasticity (4/15) had only been described in two other patients before we described this symptom in our cases as well.

Hearing was typically not affected, only one patient presented with sensorineural hearing loss. Optic disc hypoplasia was present in four patients, one of them also had pigmentary retinopathy, and one additionally suffered from retinal hypoplasia and cataract. One patient had myopia and astigmatism with left esotropia. Beyond one of our patients, a previously reported patient also suffered from strabismus.

Gastrointestinal symptoms were reported in four patients (one with gastroesophageal reflux disease [GERD], one with constipation, one needed a G-tube at 4 years of age and one had malnutrition and constipation).

Three patients had an atrial septal defect (one along with a ventricular septal defect), and one patient had a bicuspid aortic valve. Other internal organ symptoms were not reported. Two patients were diagnosed with diabetes and one patient suffered from ketotic hypoglycemia. Recurrent respiratory infections were reported in only one patient.

OXPHOS results showed deficiency of different complexes (mainly I and IV) in 4/5 of the reported patients[3] and complex III in the family described by Kernohan et al.[4] For the two novel patients we detected mildly abnormal Complex I and IV activity in patient 2, and abnormal complex I, III, and IV expressions in patient 1 in fibroblasts (see also Supporting Information for methods).

From the reported cases, nine had normal lactic acid (for the other six patients, lactic acid levels were not reported), while carnitine levels were normal in three reported patients but lowered in one patient. Three patients had normal pyruvic acid. Pyruvic acid was found to be decreased in two patients (for the other 10 patients, levels were not reported). The two novel patients had normal lactic acid, normal pyruvate, and carnitine levels.

Detailed information can be found summarized in Table 1.

TABLE 1. Molecular, metabolic and clinical features of 15 patients with TRIT1 defect

			Yıldırım et al.[9]	Yoo et al.[5]		Forde et al.[1]	Takenouchi et al.[6]	Balciuniene et al.[7]	Kernohan et al.[4]				Whittaker et al.[8]	Yarham et al.[3]	
Patients	**Patient 1**[a]	**Patient 2**[a]	**Patient 3**[b]	**Patient 4**[c]	**Patient 5**[c]	**Patient 6**[a]	**Patient 7**[a]	**Patient 8**[a]	**Patient 9**[a]	**Patient 10**[a]	**Patient 11**[a]	**Patient 12**[a]	**Patient 13**[a]	**Patient 14**[a]	**Patient 15**[a]
Genotype	c.[967C>T]; [882_883del]	c.[326T>C]; [979C>T]	c.[246G>C]; [246G>C]	c.[979G>A]; [682+2T>C]	c.[979G>A]; [682+2T>C]	c.[1171_1172del]; [334del]	c.[244A>G]; [1034A>G]	c.[334del]; [326T>C]	c.[1256A>C]; [848T>G]	c.[1256A>C]; [1204C>T]	c.[856A>G]; [22C>T]	c.[856A>G]; [22C>T]	"Homozygous missense v."	c.[968G>A]; [968G>A]	c.[968G>A]; [968G>A]
Effect on protein	p.[Arg323Tyr]; [Glu295Glyfs*8]	p.[Ile109Thr]; [Arg327*]	p.[Met82Ile]; [Met82Ile]	p.[Glu327Lys]; [?]	p. [Glu327Lys]; [?]	p.[Lys391Glufs*7]; [Arg112Glufs*36]	p.[Met82Val]; [Tyr345Cys]	p.[Arg112fs]; [Ile109Thr]	p. [His419Pro]; [Ile283Ser]	p.[His419Pro]; [Arg402*]	p.[Lys286Glu]; (Arg8*]	p. [Lys286Glu]; [Arg8*]	p. [Arg323Gln]; (Arg323Gln]	p. [Arg323Gln]; [Arg323Gln]	p.[Arg323Gln]; [Arg323Gln]
Functional studies supporting pathogenicity	+	+	−	+	+	−	+	−	(+)	+	−	−	−	+	+
ACMG classification based on ClinVar	(Likely) pathogenic; NR	VUS (likely) pathogenic	(VUS; VUS)	Likely pathogenic; NR	Likely pathogenic; NR	NR; (likely) pathogenic	NR; VUS	(Likely) pathogenic; (likely) pathogenic	VUS; VUS	VUS; VUS	VUS; (likely) pathogenic	VUS; (likely) pathogenic	NR	Pathogenic Pathogenic	Pathogenic Pathogenic
General patient information															
Sex	Female	Male	Male	Female	Male	Female	Female	NR	Female	Female	Female	Male	NR	Male	Female
Age of onset of symptoms	3 months	4 months	6 months	3 months	3 months	Antenatal	14 months	NR	3 months	6 months	5 months	NR	NR	13.5 months	NR
Age of diagnosis	1 year	3 years	6 years	16 years	13 years	NR	NR	NR	4 years	9 years	NR	NR	NR	NR	16 years
Consanguin.	−	−	+	−	−	+	−	NR	−	−	−	−	NR	+	+
Central nervous system															
Microcephaly	+	+	+	+	NR	+	−	NR	+	+	+	+	NR	+	+
Cognitive delay	+	+	+	+	+	+	+	NR	+	+	NR	NR	NR	+	+
Myoclonic jerks	+	+	+	−	+	+	+	NR	+	+	+	NR	+	+	+
Seizures	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hypotonia	−	+	+	NR	NR	+	+	NR	+	+	+	NR	NR	NR	NR
Hearing	Normal	Recurrent ear infections	Normal	NR	NR	Axial SNHL		NR	NR	Normal	Normal	Normal	NR	NR	Normal	Normal
Vision	Esotropia	Strabismus	Strabismus	Retinal and optic disc hypoplasia Cataract	Optic disc hypoplasia	Optic nerve hypoplasia pigmentary retinopathy		NR	NR	Optic disc hypoplasia	Normal	Myopia, left esotropia	NR	NR	Normal	Normal
Spasticity	+	+	+	NR	NR	+		NR	NR	NR	NR	NR	NR	NR	NR	NR
Organ systems															
Cardiovascular	−	NR	Bicuspid aortic valve	−	−	Moderate atrial septic defect		NR	NR	Atrial septal defect	Atrial and ventricular septal defect		NR	NR	NR	−	−
Respiratory	−	NR	NR	NR	NR	Recurrent respiratory tract infections		NR	NR	NR	NR		NR	NR	NR	NR	NR
Endocrine	−	NR	Ketotic hypoglycemia	NR	NR	NR		NR	NR	TSH ↑ and normal T4	Normal		NR	NR	NR	Diabetes	Diabetes
Musculo-skeletal	−	NR	NR	NR	NR	NR		NR	NR	NR	NR		NR	NR	NR	NR	NR
Gastro-intestinal	−	−	Malnutrition, constipation	NR	NR	GERD, enteral feeding		NR	NR	−	G-tube at age of 4 years		Constipation	NR	NR	NR	NR
Clinical tests															
EEG	Diffuse epileptiform discharges with absence seizures	Localized and generalized seizures	Abnormal	Abnormal		Abnormal	Asymmetric background, focal discharges	Epileptic spike and wave complex during myoclonic jerks	NR	Abnormal	Abnormal	Abnormal	NR	Normal background with focal epileptiform abnormalities over temporal lobes	Slow background myoclonic epilepsy	NR

(Continues)

TABLE 1. (Continued)

Clinical tests																
ECG	Normal	NR	NR	NR	NR	Atrial septal defect	Normal	NR	NR	NR	NR	NR	NR	NR	NR	NR
MRI brain	Delayed myelination, corpus callosum dysplasia, and an enlarged cisterna magna	Brain atrophy	Normal	Reduced periventricular white matter around the temporal horn Megacisterna magna Hypoplastic CC	Dandy–Walker malformation Hydrocephalus	Bilateral perisylvian polymicrogyria Thin CC Cortical atrophy Hypoplasia of inferior cerebellar vermis	Normal	NR	Septo-optic dysplasia Partial agenesis of CC	Generalized cerebral atrophy	Frontal atrophy and increased fluid	NR	NR	Normal	NR
Metabolic tests																
Lactic acid	Normal		Normal	12.5 mg/dl	11.7 mg/dl	NR	NR	Normal	NR	Normal	Normal		NR	NR	Normal		NR
Pyruvic acid	Normal		Normal	0.53 mg/dl	0.5 mg/dl	NR	NR	Normal	NR	NR	NR		NR	NR	Normal		NR
Carnitine	Normal		Normal	9.3 nmmol/ml	NR	NR	NR	NR	NR	NR	NR		NR	NR	Normal		NR
OXPHOS results	Complex I, II, and IV deficiency		Decreased Complex I and IV	NR	NR	NR	NR	NR	NR	NR	Complex III and IV deficiency		NR	NR	Complex I and IV deficiency		Complex I and IV deficiency

Abbreviation: NR, not reported.
[a]RefSeq: NM_017646.4.
[b]RefSeq: NM_017646.6.
[c]RefSeq: NM_001312692.1.

3 | NOVEL PATIENTS AND METHODS

Patients were recruited to the rare and undiagnosed research repository (IRB 19-005839) and genomic profiling of mitochondrial disease studies (IRB 19-003389). Deidentified fibroblast samples were analyzed for biomarker discovery, including functional and expression studies and lipidomics (IRB 16-004682). Patient matching was accomplished upon completing genetic studies by using GeneMatcher.[10]

3.1 | Patient 1

The patient is a 4-year-old girl, with motor and speech developmental delay, esotropia, microcephaly, corpus callosum agenesis, and poor weigh gain, known with the history of febrile seizures, who developed intermittent myoclonic jerks at the age of 13 months. She improved in her seizure control significantly after more than a year of ketogenic diet. She just started to walk and is known with spasticity. She can say five words (Supporting Information).

Whole exome sequencing detected compound heterozygous variants in *TRIT1*: NM_017646.4c.[967C>T];[882_883del] (p.[Arg323Trp];[Glu295Glyfs*8]), respectively paternally and maternally.

A skin biopsy was performed as part of the clinical evaluation to assess the expression of respiratory enzyme complexes (REC) and oxygen consumption rate (OCR) by Seahorse. OXPHOS complexes activity analysis and lipidomics (trans-boarder sample sharing) were not possible in fibroblasts.

3.2 | Patient 2

The patient is a 4-year-old boy. He is known with a history of motor developmental delay, speech delay, strabismus, and seizures (first generalized convulsion) presented at the age of 2 years. He had no failure to thrive. He was well treatable on antiepileptic therapy. He started to walk and had his first words around 30 months. He is known with spasticity, and he has developed microcephaly (Supporting Information).

Whole exome sequencing detected compound heterozygous variants in *TRIT1*, NM_017646.4c.[326T>C];[979C>T] (p.[Ile109Thr];[Arg327*]), respectively maternally and paternally inherited.

As part of the standard of care fibroblasts were established from this patient for mitochondrial functional studies. REC analysis in fibroblasts showed mild decrease in the activity of the OXPHOS protein complexes I and

IV compared to citrate synthase.[11] Additional studies in fibroblasts included OCR by seahorse, and lipidomic studies (Figures 3 and S1).[12-15]

For cell culturing, Western blot, mitochondrial respiration, and lipidomics analysis. See Supporting Information/methods.

4 | RESULTS

4.1 | Western blot

TRIT1 protein appeared as a double band on Western blot in both patients correlating with two of its different isoforms. The upper band is representing isoform 1, whereas the lower band is representing isoform 4. (Other isoforms of TRIT1 were not detectable on Western blot.) Isoform 1 of TRIT1 was deficient in both patients, confirming the diagnosis. In the first patient, both isoforms 1 and 4 were found to be decreased compared to three age matched controls (Figure 1A). In the second patient, isoform 1 of TRIT1 was not detected. However, the signal of isoform 4 was found to be increased compared to age-matched controls (Figure 1B).

In patient 1, the expression of complexes I (based on NDUSF2 expression), III (based on UQCRC2 expression), and IV (based on COX IV expression) were significantly reduced in comparison with three controls. SDHA and ATPB corresponding to complex II and V expression were normal. No Western blot for REC expression was done in patient 2 (Figure 2A).

4.2 | OCR in fibroblasts

In order to confirm the impaired mitochondrial function, we performed OCR experiments on P1 fibroblasts and two controls and in P2 and 3 controls. Basal, maximal, and ATP-linked OCR were all significantly reduced in P1 compared to controls (Figure 2B). Basal respiration was mildly decreased while proton leak and ATP-linked respiration were reduced in P2 compared to three controls (Figure S1).

4.3 | Lipidomic analysis

A total of 929 species across 26 subclasses of phospholipids, sphingolipids, glycerides, cholesteryl esters, and neutral lipids were identified and quantified in the sample from patient 2 using high-resolution LC–MS/MS. The total level of each subclass of lipids was calculated by adding the peak areas of individuals species within them. Several phospholipids involved in the cytidine diphosphate-diacylglycerol (CDP-DAG) pathway of phospholipids showed changes (Figure 3A). While phosphatidic acid (PtdOH) levels were elevated in the patient, the levels of phosphatidylglycerol (PtdGlycerol), phosphatidylserine (PtdSerine), and phosphatidylethanolamine (PtdEthanolamine) were decreased (Figure 3B). Diacylglycerol (DAG) is utilized in the CDP-ethanolamine and CDP-choline pathways, and it was also decreased in patient 2 (Figure 3A,B). Changes in the sphingolipid pathways were noted as well (Figure 3C); while dihydrosphingosine and dihydroceramide from de novo synthesis pathway were within the range of controls, ceramide, and sphingomyelin were decreased (Figure 3C,D).

5 | DISCUSSION

Our two novel patients, presenting with mild to moderate developmental delay (including significant speech delay) and seizures (including tonic–clonic, as well as myoclonic seizures in patient 1), with symptom onset during the first months of life (3–4 months of age) fit very well to the previously described clinical spectrum of developmental delay and myoclonic seizures. In both cases, seizures were treatable.

In addition, early growth delay was also common in TRIT1 deficiency, and both neonatal and progressive microcephaly were observed in the 15 patients. Delayed myelination, corpus callosum dysplasia and brain atrophy in our two patients demonstrate the rather heterogenic phenotypical spectrum of brain abnormalities in TRIT1 deficiency.

Some patients with TRIT1 deficiency present with severe eye findings, even visual loss. Both of our patients had strabismus/esotropia, but without visual or hearing loss.

Biochemically, our two patients, and four out of five patients in the literature showed respiratory chain abnormalities, but other laboratory markers were in most cases within normal limits. Unfortunately, in many of the previously described cases, OXPHOS complex expression or activity measurements were not performed, or results were not reported; these should be investigated in future diagnosed cases.[16]

Overall, our new patients present at the rather mild end of the phenotypical spectrum, with only mild to moderate developmental delay and seizures that have been treatable with anti-epileptic medication and/or ketogenic diet. Spasticity was a symptom in both of our patients, which had only been described in two other patients before (although in many, this may just not have been reported).

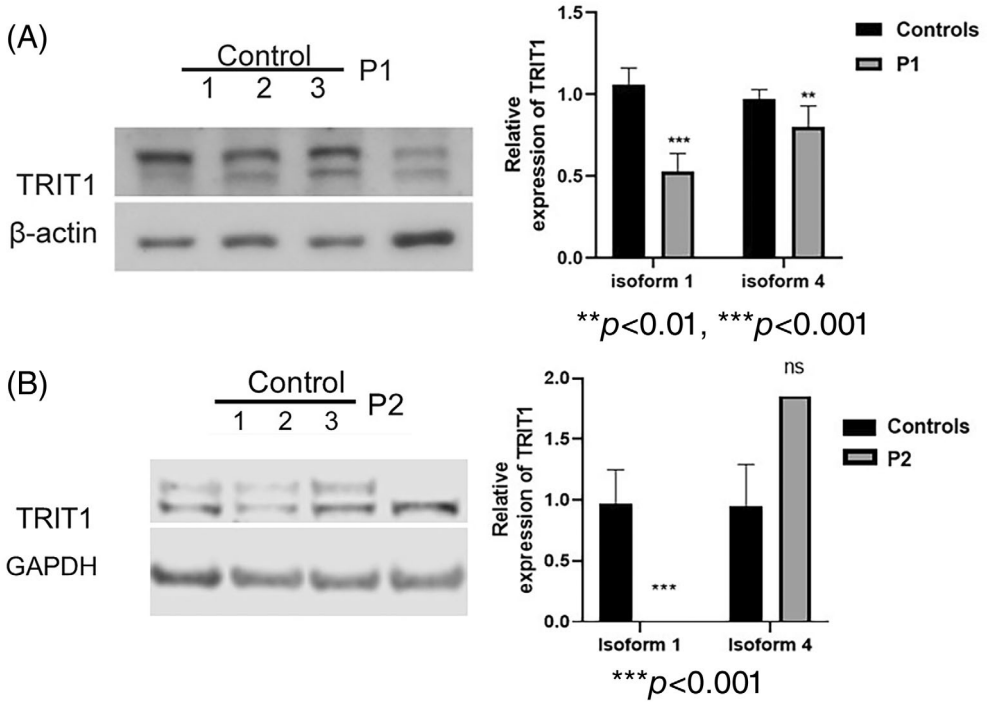

FIGURE 1 TRIT1 protein appeared as a double band on Western blot in both patients correlating with two of its different isoforms. The upper band is representing isoform 1, whereas the lower band is representing isoform 4. Isoform 1 of TRIT1 was deficient in both patients, confirming the diagnosis (Patient 1: A, Patient 2: B). Relative expression of TRIT1 is compared to beta-actin (BACT) expression in patient 1, and to GADPH expression in patient 2.

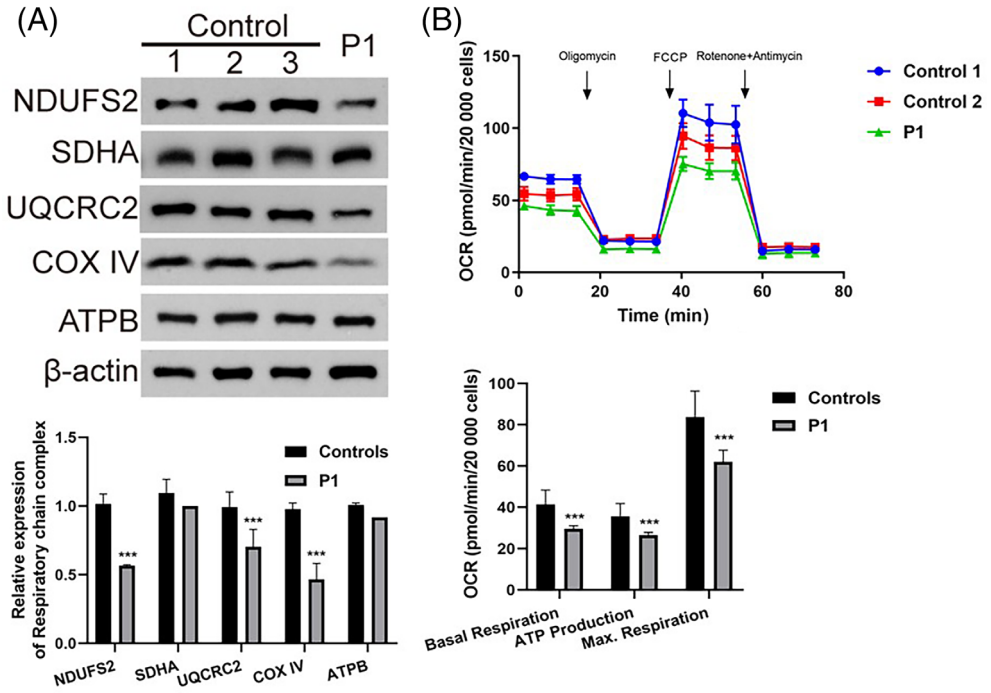

FIGURE 2 (A) Western blot for specific subunits of the different OXPHOS subcomplexes in patient 1. Beta-actin was used as internal control for relative expression. In patient 1, the expression of complexes I (based on NDUSF2 expression), III (based on UQCRC2 expression), and IV (based on COX IV expression) were significantly reduced in compared to three controls. SDHA and ATPB corresponding to complex II and V expression were normal (Figure 2). No Western blot for REC expression was done in patient 2. For the abnormal REC activity, measurements in fibroblasts in patient 2, see Figure S1. (B) Analysis of OCR in two fibroblasts isolated from controls and patient 1. (A) OCR graph in fibroblasts from P1 as compared to controls ($n = 22$–24). (B) Graphs of basal respiration, ATP production, and maximal respiration calculated from OCR (A). Data are indicated as mean ± SD. Significance is calculated using unpaired Student's *t*-test, $^{***}p < 0.001$ (vs. Controls). FCCP, carbonyl cyanide phenylhydrazone; OCR, oxygen consumption rate.

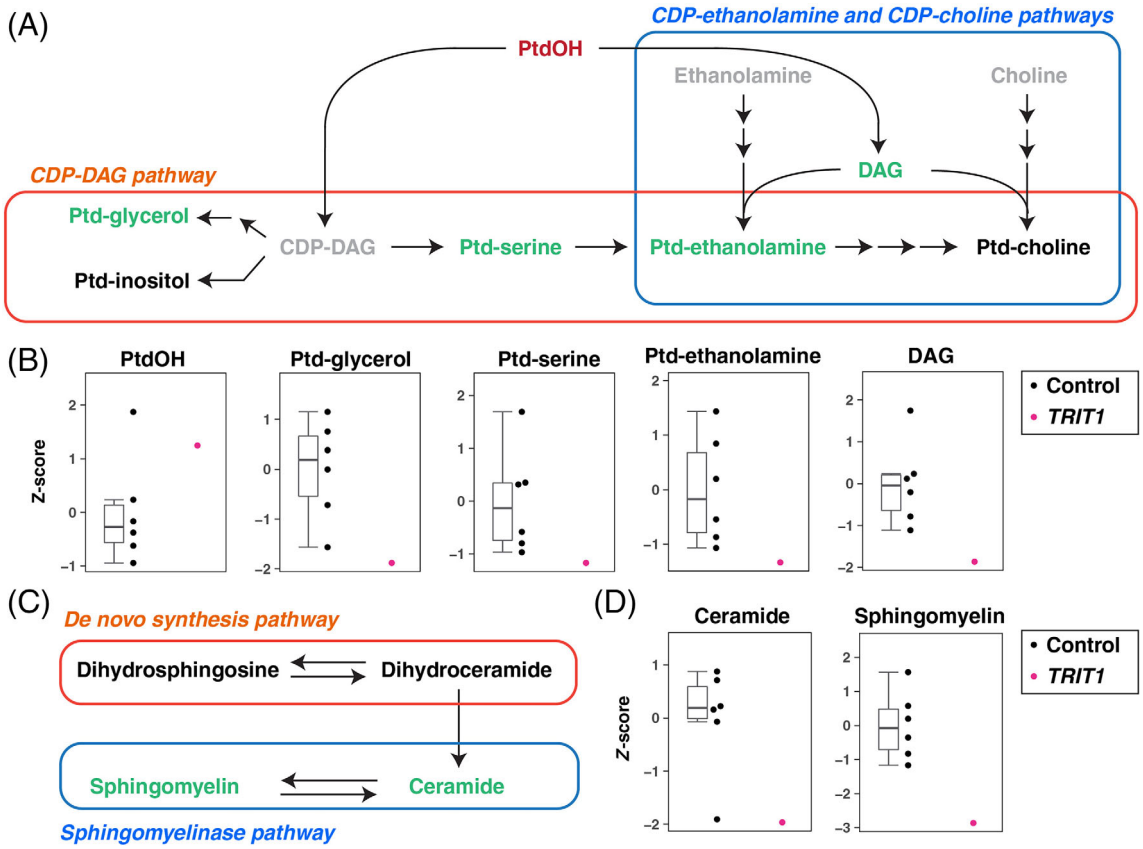

FIGURE 3 Lipidomic alterations in fibroblasts from patient 2 compared to six controls (A) Pathways for the synthesis of phospholipids via CDP-DAG, CDP-ethanolamine, and CDP-choline pathways. Lipid names colored in red and green indicate those with increased or decreased levels in patient 2, respectively. Lipids with names in black had levels in the patient within the range of controls. Molecules in gray indicate the metabolites that were not analyzed. (B) *Z*-score scatter plots of lipids that showed changes in the pathways of phospholipid synthesis with boxplots accompanying control data. Black dots indicate the *z*-scores of controls and pink dot represents the *z*-score for that analyte from patient 2. The middle line in the box represents the average *z*-score from the distribution of control data points, and top and bottom whiskers represent the maximum and minimum values in the control data. (C) Overview of de novo synthesis of sphingolipids and sphingomyelinase pathways. Lipid names colored in green indicate those with decreased levels in patient 2. Lipids with names in black had levels in the patient within the range of controls. Lipid names in black indicate lipids whose levels were within the range of controls. (D) *Z*-score scatter plots of sphingolipids that were altered in patient 2. The lipids are indicated as in panel B. Black dots indicate the *z*-scores of controls and pink dot represents the *z*-score for that analyte from patient 2. The middle line in the box represents the average *z*-score from the distribution of control data points, and top and bottom whiskers represent the maximum and minimum values in the control data.

Phospholipids play a critical role in mitochondrial function as essential structural components of the mitochondrial membrane.[17] They are also involved in various cellular functions including mitochondrial fusion and apoptosis.[18] PtdOH is a key precursor of phospholipids. It is converted into CDP-DAG, which is a precursor of PtdSerine, PtdGlycerol, PtdInositol, PtdEthanolamine, and PtdCholine. Alternatively, through the CDP-ethanolamine and CDP-choline pathways, PtdOH is also converted to DAG, which is then utilized to synthesize PtdEthanolamine and PtdCholine. Higher levels of PtdOH and lower levels of its downstream lipids (PtdGlycerol, PtdSerine, PtdEthanolamine, and DAG) in patient 2 are suggestive of a block in phospholipid synthesis.

Sphingolipids are very important for normal mitochondrial bioenergetics due to their roles in mitochondrial network, mitochondrial fission, ATP production, and reactive oxygen species production.[19] Thus, decreased levels of sphingomyelin and ceramide observed in patient 2 are suggestive for changes in mitochondrial bioenergetics.

5.1 | Recommendations

In conclusion, TRIT1 deficiency is a disorder that is characterized by mild to moderate developmental delay, speech delay, myoclonic seizures, possible muscular hypotonia, spasticity, growth delay, microcephaly, and

mild dysmorphic features, and in case of truncating mutations, visual or hearing abnormalities, as well as cardiac abnormalities (mainly atrial septal defects). Lactic acidemia is not characteristic. These features make this disorder a potentially recognizable syndrome, although many symptoms overlap with other neurogenetic disorders. The combination of myoclonic seizures, speech delay, growth delay, microcephaly, mild dysmorphic features, especially with strabismus, should, however, prompt clinicians include TRIT1 deficiency into their differential diagnosis. In any case, genetic testing will be necessary to confirm the defect and to exclude other genetic factors contributing to the phenotype.

Even though, besides cardiac abnormalities, other internal organ manifestations have not been described, we recommend screening of all organ systems in all patients at the time of diagnosis, especially since the group of diagnosed patients is still small and the full phenotypical spectrum may include more features than the ones that have already been described in the literature so far. At the very least, cardiac evaluations, by electrocardiogram and echocardiogram, should be part of routine screenings in newly diagnosed cases of TRIT1 deficiency. Follow-up investigations should again include cardiac evaluations, as well as ophthalmological and hearing evaluations to detect impairments early in the disease course.

Routine laboratory abnormalities have not been detected in TRIT1 patients, but due to the two siblings that have been reported with diabetes, patients should be screened for possible diabetic changes (HbA1c and glucose profile) on a regular basis. Notably, a decrease of the expression of OXPHOS pathway protein complexes has been described in four of the five patients where this has been performed (including our patient 1). OXPHOS complex measurements in patient 2, who is a patient with a rather mild phenotype, showed a mild decrease in complex I and in complex IV activity, and close to normal basal respiration, which may indicate that these measurements may result normal in milder patients. Interestingly, he has been noted to show signs of increased fatigue and problems with temperature regulation, pointing to a potential defect of energy metabolism. Since OXPHOS complexes have only been studied for a few of the known patients, but almost all measured cases had abnormalities, we recommend OXPHOS expression or activity measurements in fibroblast tissue (which is relatively easily accessible) for all newly diagnosed patients, if possible.

Importantly, it is worth noting that ketogenic diet, but not anti-epileptic medical therapy, led to significant improvement of neurological and developmental features in patient 1. This adds TRIT1 deficiency to the list of disorders that potentially benefit from ketogenic diet. Due to the known problems of developmental and speech delay, altered muscle tone (hypotonia, spasticity), and potential failure to thrive with poor weight gain (and potential need for G-tube feeding), physical therapy, ergotherapy, speech therapy, as well as dietary evaluations by a metabolic dietitian, should be part of the treatment plan.

The number of suspected TRIT1 deficient patients is much higher than the cases we reported on here. There are several *TRIT1* variants of uncertain significance submitted to the ClinVar database without any previous functional assessment. It would be of great interest to facilitate connecting families to researchers/clinicians having interest for TRIT1 functional characterization and natural history.

AUTHOR CONTRIBUTIONS

Ewout Muylle, Huafang Jiang, Christin Johnsen, Eva Morava, and Seul Kee Byeon wrote the manuscript; Wasantha Ranatunga, Kishore Garapati, Roman M. Zenka, and Graeme Preston collected data, Akhilesh Pandey, Fang Fang, and Tamas Kozicz reviewed the data and the manuscript.

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CONFLICT OF INTEREST

Ewout Muylle, Huafang Jiang, Christin Johnsen, Seul Kee Byeon, Wasantha Ranatunga, Kishore Garapati, Roman M. Zenka, Graeme Preston, Akhilesh Pandey, Tamas Kozicz, Fang Fang, and Eva Morava are all in agreement with the manuscript and declare no conflict of interest; Did not receive reimbursements/fees/funds/salaries from an organization that may in any way gain or lose financially from the results reported in the reviewed manuscript in the last 5 years and have no other competing financial or nonfinancial interests, as outlined in the JIMD Conflict of Interest form.

ETHICS STATEMENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human

experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study. This study was approved by Hospital Institutional Ethics Committee. Written informed consent was obtained from the parents of the patients for collection of samples and publication of medical data.

INFORMED CONSENT

Consent was obtained from both patients for publication of deidentified clinical data.

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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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