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Case Report

Disrupted oxidative stress resistance: A homozygous mutation in the catalytic (TLDc) domain of *TBC1D24* gene associated with epileptic encephalopathy

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1. Introduction

The *TBC1D24* gene produces a protein that is required for normal development, survival, and vesicle trafficking in mammalian neurons [1]. The protein consists of TBC and TBC/Lysin Motif Domain/Catalytic (TLDc) domains. The TBC domain is responsible for vesicular membrane trafficking to synapses. Although less is known regarding the function of the TLDc domain, it is involved in oxidative stress sensing and resistance, as demonstrated in cell cultures [1]. Disorders associated with *TBC1D24* mutations include non-syndromic hearing loss, deafness, onychodystrophy, osteodystrophy, mental retardation, seizures (DOORS syndrome), progressive myoclonus epilepsy, familial infantile myoclonic epilepsy, and early infantile epileptic encephalopathy-16 (EIEE16; MIM 615338) [2]. EIEE16 is characterized by infantile-onset, long-lasting myoclonic or focal seizures intractable to medical treatment, which result in neuromotor regression or psychomotor development failure with occasional extrapyramidal signs [2]. For unknown reasons, the TLDc domain mutates less frequently than the TBC domain in various epilepsy syndromes [2]. Although compound heterozygous mutations have been identified, only two patients with homozygous mutations in the TLDc domain have been reported [1].

Here, we describe a patient with a homozygous missense mutation in the TLDc domain of *TBC1D24*, who was diagnosed with EIEE16. This is the first report of a homozygous form of this mutation; to the best of our knowledge, this is the third report of a patient with a homozygous mutation in the TLDc domain.

2. Case report

The patient's parents were consanguineous, first-degree cousins paternally and second-degree cousins maternally. Her mother's aunt had epilepsy. The son of the patient's maternal uncle had febrile seizures (Fig. 1). The patient was born via normal spontaneous delivery. Her birth weight was 3300 g, length was 48 cm, and head circumference was 35 cm. She was 15 days old at the time of her first seizure: her extremities jerked and her eyelids blinked for several minutes. She began treatment with phenobarbital. She had subsequent seizures approximately once every 1–2 weeks; thus, she began treatment with levetiracetam. Her metabolic work-up (carnitine, acyl carnitine, and amino acid profiles by tandem mass spectrometry and urine organic acids analysis) was normal. Cranial magnetic resonance imaging at the age of 5 months revealed prominent sulci, subarachnoid enlargement, and a cavum septum pellucidum. Interictal electroencephalography showed fast rhythms over the frontal region, without epileptic activity.

The patient's seizures lengthened as she became older, lasting from a few hours to 2–3 days; they were intractable to both of her medications. Multifocal myoclonic twitching occurred most commonly in her eyelids, head, face, and lips; when severe, it occasionally affected her extremities. The amplitude of her myoclonia was variable. She was often conscious and maintained her posture during the course of

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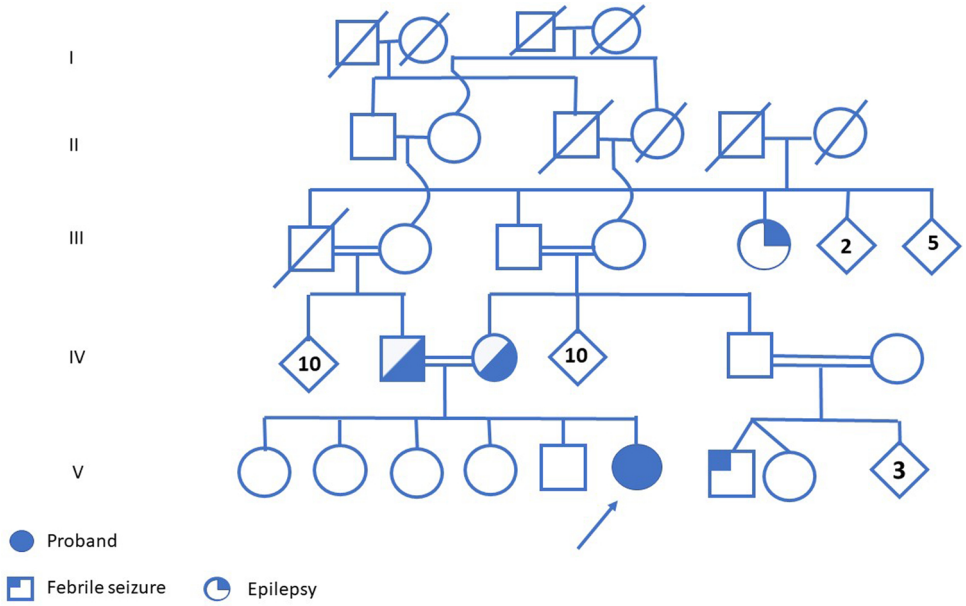

Fig. 1. Family pedigree of the proband.

myoclonic seizures (Video 1); however, when her myoclonia increased in amplitude, she lost consciousness (Video 2). With longer seizures, she exhibited autonomic signs, such as pupil dilatation and facial flushing. Her seizures worsened when she had a fever or viral illness, which caused different types of seizures, such as focal onset clonic seizures or focal onset behavior arrest. She did not have any extrapyramidal signs. Clobazam was added to the phenobarbital and levetiracetam, but it was ineffective. The patient was negative for *SCN1A* gene mutations. Her eye and hearing tests were normal. At the age of 11 months, she could sit without support, but showed no evidence of crawling. She did not produce a syllable. She had no dysmorphic features, with normal nails and terminal phalanges. Her head circumference was 45 cm (50–75 percentile). Topiramate was added to the levetiracetam, phenobarbital, and clobazam polytherapy with no permanent benefit. At 17 months, she could stand with support, but could not walk or speak. Her most recent interictal electroencephalography showed no epileptic discharges.

She was tested for epileptic encephalopathies using clinical exome sequencing. Genomic DNA was extracted from peripheral venous blood using a QIAamp® DNA Mini Kit (QIAGEN, Ankara, Turkey). The Clinical Exome Solution V2 kit (Sophia Genetics, Saint-Sulpice, Switzerland) was used for exome enrichment, in accordance with the manufacturer's protocol. This capture-based target enrichment kit covers 4490 genes with mutations causing known inherited diseases. This revealed a homozygous missense mutation [NM_001199107.2:c.1499C > T, (p.Ala500Val)] in the *TBC1D24* gene. Sanger sequencing showed that the patient was homozygous and her parents were heterozygous for the mutation, which seems to have been responsible for the patient's phenotype.

3. Discussion

TBC1D24 mutations causing epilepsy have a severity spectrum ranging from familial infantile myoclonic epilepsy with normal intellect that is responsive to medication to early infantile epileptic encephalopathy leading to developmental delay and death [2]. Extending the spectrum, Campeau et al. first identified *TBC1D24* mutations in the etiology of DOORS syndrome in 2010 [3]. Our patient presented with neonatal seizures, and eventually with multifocal myoclonic seizures refractory to treatment. Multifocal or migrating myoclonic seizures with focal seizures, as described by Zhang et al. in 19 patients, seem to be a hallmark of the severe epileptic phenotype.

The p.Ala500Val mutation in our patient has been described previously in combination with other mutations in a compound heterozygous state [1,2,4,5]. All of the patients in prior reports developed epilepsy before 12 months of age, generally before 6 months of age. No patient with the mutation, including our patient, had hearing loss or dysmorphic features resembling DOORS syndrome. Although the ancestry of patients with p.Ala500Val has not been described, 6 of 10 patients were from China; therefore, this mutation might be relatively common in East Asian populations. Table 1 summarizes the reported clinical characteristics and mutation status of patients with p.Ala500Val mutations emphasizing seizure details. Zhang et al. described a p.Ala500Val/p.Ala515Val compound heterozygous mutation in a patient with a Dravet syndrome phenotype with generalized tonic clonic and focal seizures, fever-sensitive myoclonus, and status epilepticus [4]. Although the clinical features resembled those of our patient, we presume that the patient described by Zhang et al. had more generalized tonic clonic seizures, compared to our patient. Although they described other patients with similar seizures triggered by febrile episodes, only one patient was considered to have Dravet syndrome [4]. The involvement of the TLDc domain, particularly between amino acid residues 500 and 511, has also been implicated in an exercise-induced dystonia phenotype that begins at as early as 2 years [1]; our patient has not shown dystonia yet.

Disorders related to *TBC1D24* are presumably due to a loss of protein function, because frameshift, nonsense, or splice site variants result in more severe epilepsy phenotypes. Furthermore, there is functional evidence regarding some missense variants in *Drosophila*. Introduction of the human pathogenic missense variants p.Arg40 and p.Arg242 into *Drosophila* led to impaired synaptic vesicle trafficking and seizures, supporting the hypothesis that missense variants result in reduced *TBC1D24* function. Functional studies are needed to examine the effects of other missense variants [3].

The TLDc domain is involved in oxidative stress resistance. When the TLDc domain is destabilized or its structural conformation is disturbed locally, reactive oxygen species can access critical regions of the protein domain; endogenous reactive oxygen species levels may already be sufficient to affect the TLDc domain [1]. Potentially, the inflammation that occurs with a fever or infection precipitates seizures in some individuals [2], as in our patient.

Only two other homozygous mutations have been reported in the

Table 1
Clinical characteristics of patients with p.Ala500Val mutation as compound heterozygous.

Patient no	1	2	3	4	5	6	7	8	9	10
Authors reporting the patients	Zhang et al Patient 1	Zhang et al Patient 2	Zhang et al Patient 3	Zhang et al Patient 4	Zhang et al Patient 5	Zhang et al Patient 6	Lüthy et al Patient 1	Lüthy et al Patient 2	Balestrini et al Patient	Li et al Patient
Mutation	c.116C > T (p.Ala39Val) c.1499C > T (p.Ala500Val)	c.116C > T (p.Ala39Val) c.1499C > T (p.Ala500Val)	c.1153C > T (p.Gln385Ter) c.1499C > T (p.Ala500Val)	c.725 G > A (p.Arg242His) c.1499C > T (p.Ala500Val)	c.1544C > T (p.Ala515Val) c.1499C > T (p.Ala500Val)	c.241_252del (p.Ile81_Lys84del) c.1499C > T (p.Ala500Val)	c.[241_252del]; p.[I81_K84del]; [1499C4T] [A500 V]	c.[241_252del]; p.[I81_K84del]; [1499C4T] [A500 V]	2546835T > C (p.Phe229Ser) 2550465C > T (p.Ala500Val)	c.1416_1437del (p.Ser473Argfs_43) c.1499C > T (p.Ala500Val)
Seizure type	Focal (staring, flushing, or cyanosis of the face) last for minutes, Mc	Focal (eye deviation to one side and cyanosis of the face), Mc	GTCS; focal (eyes deviation to one side and one limb jerks), Mc	Focal (staring, eye and head deviation to one side, cyanosis of the face), Mc	GTCS, focal (staring for minutes), Mc	Focal (eye deviation to one side and cyanosis of the face), Mc	Myoclonic jerks exacerbated by fever Rolandic-type focal motor seizures	Rare focal motor seizure, last up to 20 minutes	Daily myoclonus Rare GTCS	Generalized tonic-clonic seizure, rhythmic unilateral facial spasms
Myoclonus	Multifocal, unilateral, migrating from one limb to another, affecting eyelids, limbs and peri-oral region, without loss of consciousness, triggers: febrile episodes and common infections	Multifocal, migrating, affecting eyebrows, face, peri-oral region, and left or right upper limbs without loss of consciousness, triggers: febrile episodes and common infection	Affecting tongue, face, peri-oral region, and either the right or left limbs, triggers: febrile episodes	Affecting eyelids, face, limbs, and toes, without loss of consciousness, triggers: no	Affecting eyes, limbs without loss of consciousness, triggers: febrile episodes	Multifocal, migrating, affecting eyelids, head, and left or right upper limbs without loss of consciousness, triggers: febrile episodes	Involving face or hand with drooling dysarthria	NA	Involving peri-oral region and/or limbs, rhythmic, bilateral, lasting up to 30 min, no loss of consciousness	Rhythmic unilateral facial spasms fixation of the eyes to one side. spasms in the craniofacial region
Clinical diagnosis	EIMFS	EIMFS	EIMFS	EIEE	Dravet syndrome	Unclassified	RE-EID	RE-EID	Infantile myoclonic	Infantile myoclonic
Age at last follow up	3 years 4 months	5 years	2 years 1 month	2 years 3 months	6 years 2 months	4 years 1 months	8 years	12 years	2 years	4 years
Gender	Male	Male	Female	Female	Female	Female	Female	Male	Male	Female
Age at first seizure	2,5 months	1,5 months	1 month	1 day	3 months	6 months	11 months	6 months	3 months	3 months
EPC	+	+	+	+	+	+	NA	NA	–	+
Neuroimaging	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Mild cerebral cerebellar atrophy
Hearing screening	ND, clinically normal	Normal	Normal	Normal	ND, clinically normal	ND, clinically normal	ND, clinically normal	ND, clinically normal	NA	NA
Neurological examination	Neuromotor Delay	Neuromotor delay	Neuromotor delay	Neuromotor delay	Neuromotor delay	Normal	Normal	Normal	Neuromotor delay Hypotonia Nystagmus	Neuromotor delay
Additional problems	NA	NA	NA	NA	NA	NA	EID	EID	–	Left eye abducted
Response to therapy	NA	NA	NA	NA	NA	NA	EID - Still present	EID -Still present	Moderate response	NA

EPC: epilepsia partialis continua; EIMFS: epilepsy of infancy with migrating focal seizures; EEG: electroencephalography; EID: exercise induced dystonia; EIEE: early infantile epileptic encephalopathy; GTCS: Generalized tonic clonic seizure; Mc: myoclonus; NA: not available; ND: not determined; RE-EID: Rolandic epilepsy-exercise induced dystonia.

TLDc domain [1]. Muoana et al. reported the novel NM_001199107.2:c.1079 G > T, (p.Arg360Leu) mutation as likely pathogenic in a patient with progressive myoclonus; Atli et al. identified a NM_001199107.2:c.1433 G > A (p.Arg478His) homozygous mutation in a patient with DOORS syndrome [1]. It is not known how such mutations alter the diverse functions of this gene in neuronal cell survival and synaptic function, or why some patients with a *TBC1D24* gene mutation have DOORS syndrome, while others (e.g., our patient) develop epileptic encephalopathy.

Duru et al. were the first to describe a family with early infantile epileptic encephalopathy related to *TBC1D24* mutations [3]. Their patients had myoclonic epilepsy unresponsive to treatment, with initially intermittent dystonia that later became permanent. They regarded the disorder as progressive myoclonic epilepsy with dystonia, although they did not detect the causative gene. Movement disorders, extrapyramidal signs, episodic ataxia, and alternating hemiplegia have been reported in the phenotypic pleiotropy of *TBC1D24* mutations [1].

4. Conclusion

Using clinical exome sequencing, we diagnosed TBC1D24-related epileptic encephalopathy in a patient with neonatal-onset seizures. We believe this is the first reported homozygous p.Ala500Val mutation; it may help to elucidate the epileptic phenotypes associated with *TBC1D24* gene mutations. Screening for *TBC1D24* mutations should be considered in patients with infantile-onset long-lasting myoclonic seizures intractable to treatment.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.clineuro.2020.106080.

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