Epileptic Disord 2016; 18 (3): 324-8

Electroclinical phenotypes and outcomes in *TBC1D24*-related epilepsy

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Received February 23, 2016; Accepted June 17, 2016

ABSTRACT – *TBC1D24* is a newly recognized gene in which variations lead to variable clinical phenotypes including drug-resistant epilepsy. We report four patients with novel variants of *TBC1D24* demonstrating drug-resistant focal epilepsy, developmental delays, and head growth deceleration. All patients had seizure semiologies consisting of prolonged, unilateral, focal clonic activity of the arm, leg or face, in addition to generalized clonic or myoclonic seizures. Ictal EEG characteristics included *epilepsia partialis continua*, *epilepsy of infancy with migrating focal seizures*, and other focal seizures with indiscrete interictal-ictal transitions. Two seemingly unrelated Navajo patients with identical variations experienced super-refractory status epilepticus at 9 months of age, with one achieving resolution with ketogenic diet therapy. Our series suggests that *TBC1D24*-related epilepsy can manifest with hypotonia, developmental delays, and a variety of focal-onset seizures prone to electroclinical dissociation.

Key words: *TBC1D24*, epilepsy, epilepsia partialis continua, malignant migrating partial seizures in infancy, super refractory status epilepticus

TBC1D24 is an autosomal recessive gene associated with variable clinical phenotypes including non-syndromic hearing loss and drug-resistant epilepsy. The *TBCD124* gene encodes a member of the Tre2-Bub2-Cdc16 (TBC) domain-containing RAB-specific GTPase activating protein expressed in the CNS. It functions to coordinate Rab proteins and other GTPases for normal transport of intracellular vesicles (Campeau *et al*., 2014). *In utero TBC1D24* knockdown studies in rats have revealed neurons with abnormal maturation. The protein interacts with ADP ribosylation factor (ARF6), a GTPase enzyme critical for membrane trafficking. *In vivo* work suggests that the TBC1D24 protein prevents ARF6 activation and is critical to normal neuronal migration and maturation (Falace *et al*., 2014). Epilepsy phenotypes described with *TBC1D24* mutations include familial infantile myoclonic epilepsy, epilepsy of infancy with migrating focal seizures (EIMFS), and DOORS (deafness, onychodystrophy, osteodystrophy,

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doi:10.1684/epd.2016.0849

intellectual disabilities, and seizures) syndrome. We report four patients with novel *TBC1D24* variations, each presenting with unique seizure characteristics.

Materials and methods

The Institutional Review Board at Phoenix Children's Hospital approved this study. A retrospective case review was performed on patients at Phoenix Children's Hospital between 0 to 18 years of age, diagnosed with *TBC1D24*-related epilepsy between 2011 and 2015. Data obtained included patient demographics, genetic testing results, EEG results, treatments utilized, developmental outcomes, and seizure burden.

Patient I

A girl born to non-consanguinous Navajo parents experienced focal seizures two days after birth, consisting of unilateral hand twitching and ipsilateral eye deviation, lasting several hours. By two months, she started experiencing generalized clonic convulsions. Her seizures remained intractable to levetiracetam, clobazam, and phenobarbital.

Brain MRI at five months was normal. EEG monitoring captured a five-hour focal seizure, consisting clinically of right-hand twitching, which correlated with increased rhythmic delta activity over the left hemisphere and infrequent multifocal sharp waves. Transitioning towards sleep, her hand twitching persisted with increased delta activity in the left hemisphere, consistent with epilepsia partialis continua (EPC) (*supplementary figure 1*).

A comprehensive epilepsy panel revealed two highly evolutionarily conserved *TBC1D24* variants inherited *in trans*: a previously unreported nonsense variant (c.121C>T; p.Gln41Ter) in exon 2 (ExAC prevalence 0.00%, maximal subpopulation frequency 0.00%; SIFT function: benign, MutationTaster: deleterious) from her father, and a previously unreported missense variant (c.321T>A; p.Asn107Lys) in exon 2 (ExAC prevalence 0.00%, maximal subpopulation frequency 0.00%; SIFT function: equivocal, Polyphen2: deleterious, MutationTaster: deleterious) from her mother.

At nine months, she was babbling, but not sitting. She was normocephalic (OFC: 43.0 cm; 20%) with decelerating head growth, and still remained with drug-resistant epilepsy. At 9.5 months, she experienced non-convulsive super-refractory status epilepticus (SRSE), characterized electrographically by left-hemispheric periodic lateralizing epileptiform discharges refractory to midazolam, ketamine, pentobarbital, and hypothermia (*supplementary figure 2*). She passed away in hospice care at that age in the context of SRSE.

Patient II

A girl with hypotonia and sensorineural hearing loss, born to non-consanguineous Navajo parents, experienced seizures at 2 months of age, consisting of left-handed twitching that progressed to involve the leg with left eye deviation and generalized clonic or myoclonic activity. These seizures could last up to five hours. Her seizures were intractable to levetiracetam, topiramate, clobazam, and oxcarbazepine.

Brain MRI at two months was normal, and a BAER test revealed bilateral sensorineural hearing loss. Interictal EEG studies revealed multifocal spikes. An EEG at three months revealed right hemispheric status epilepticus (SE), electrographically correlating with rhythmic delta activity in the right temporal region (T6) and subsequent evolution to right hemispheric discontinuity, followed by repetitive spike-wave discharges in the right temporal region (*supplementary figure 3*). This clinically correlated with left eye deviation and left upper extremity clonic movements. Genetic testing revealed two *TBC1D24* variants inherited *in trans*. As in Patient I, a nonsense mutation (c.121C>T; p.Gln41Ter) in exon 2 was derived from her father, and a missense variant (c.321T>A; p.Asn107Lys) in exon 2 from her mother.

At 8 months, she experienced motor and language regression. Shortly thereafter, she suffered from myoclonic SRSE. Transitioning towards SRSE, her interictal pattern revealed abundant central midline low-amplitude spikes that evolved to persistent higher-amplitude qualities, later infrequently associated with cortical leg myoclonus. This evolved into myoclonic SE, clinically accompanied by periodic leg movements, often bilaterally synchronous at 0.5 Hz (*supplementary figure 4*). SRSE remained refractory to midazolam, ketamine, and pentobarbital drips, and lasted four days. It was ultimately aborted with ketogenic diet therapy, although her residual seizure burden remained poor.

Patient III

A girl born to non-consanguineous Hispanic parents experienced focal seizures at 3 months of age, consisting of unilateral clonic twitching of the arm or leg with ipsilateral eye deviation, lasting up to an hour and including perioral cyanosis. She remained refractory to levetiracetam, lamotrigine, topiramate, and clobazam. At three months, brain MRI and interictal EEG were normal. A captured non-convulsive ictal event with oxygen desaturation consisted of rhythmic left-temporal theta activity with evolution to delta activity (*supplementary figure 5*). Genetic testing revealed a maternally inherited *TBC1D24* missense variant

Table 1. Clinical characteristics in Patients I-IV.

	Patient I	Patient II	Patient III	Patient IV
DNA variants	c.121C>T, c.321T>A	c.121C>T, c.321T>A	c.845C>G, c.919A>G	c.845C>G, c.919A>G
Protein variants	p.Gln41Ter, p.Asn107Lys	p.Gln41Ter, p.Asn107Lys	p.Pro282Arg, p.Asn307Asp	p.Pro282Arg, p.Asn307Asp
Onset of seizures	2 days old	2 months old	3 months old	3 months old
Seizure Classification	EPC, SRSE (PLEDs)	Focal-onset seizures, cortical myoclonus	MMPSI	Focal-onset seizures
Developmental Delays	Yes	Yes	Yes	Yes
Head Circumference Regression	Yes	Yes	Yes	Yes

EPC: Epilepsia partialis continua; SRSE: super-refractory status epilepticus; PLEDs: periodic lateralizing epileptiform discharges.

(p.Pro282Arg; c.845C>G) (ExAC prevalence 0.02%, maximal subpopulation frequency 0.18%; SIFT function: deleterious, Polyphen2: deleterious MutationTaster: deleterious) and a paternally inherited missense variant (p.Asn307Asp; c.919A>G) (ExAC prevalence 0.00%, maximal subpopulation frequency 0.00%; SIFT function: equivocal, Polyphen2: deleterious MutationTaster: deleterious). Both mutations were to highly conserved amino acids and inherited *in trans*. At 19 months, she was walking, babbling though without words, and was normocephalic (OFC: 46.0 cm; 36%), but with mildly decelerating head growth. At that age, she remained with drug-resistant epilepsy, with the same seizure types as before.

Patient IV

A brother of Patient III without hand preference had seizures at 3 months of age, consisting of behavioural arrest and oxygen desaturations, accompanied by unilateral clonic activity of the arms or legs with ipsilateral eye deviation. Breathing pattern changes were observed with most of his events, lasting upwards of 30 minutes, approximately four times a month. He remained refractory to topiramate, levetiracetam, lamotrigine, and clobazam.

Brain MRI at 19 months of age revealed left hippocampal volume loss with associated T2 hyperintense signal and slight left cerebral atrophy, consistent with left mesial temporal sclerosis (*supplementary figure 6*). EEG monitoring at 18 months of age revealed interictal diffuse background slowing without epileptiform discharges and no captured events. At that age, he remained with drug-resistant epilepsy, with the same seizure types as before. Genetic testing revealed identical mutations as his sister. Developmentally, he could pull to stand, cruise, and babble at 23 months. His head circumference at 31 months was normocephalic (47.0 cm; 7%) with deceleration of head growth.

Discussion

Our series demonstrates four children with previously unreported variants in the *TBC1D24* gene. Clinical phenotypes observed include drug-resistant focal epilepsy, myoclonic seizures and myoclonic status epilepticus, developmental regression, and head growth deceleration (*table 1*).

The *TBC1D24* gene was originally recognized in human epilepsy in 2010 when two separate families were reported. A series of developmentally normal adult patients from an Italian family were described with compound heterozygous *TBC1D24* missense variants in exon 8 and exon 2, presenting with familial infantile myoclonic epilepsy (FIME) and normal MRI brain imaging, except for a periventricular nodular heterotopia in one individual (Zara *et al*., 2000; Falace *et al*., 2010). Four siblings in a consanguineous Arab-Israeli family with a homozygous *TBC1D24* missense variant in exon 2 presented with focal epilepsy, intellectual disability, and MRI brain imaging with cortical thickening, cerebellar atrophy, and high signal to the ansiform lobule (Corbet *et al*., 2010; Afawi *et al*., 2013). Contrasting these patients, later reports have described a more severe phenotype. Two siblings in one family with a nonsense

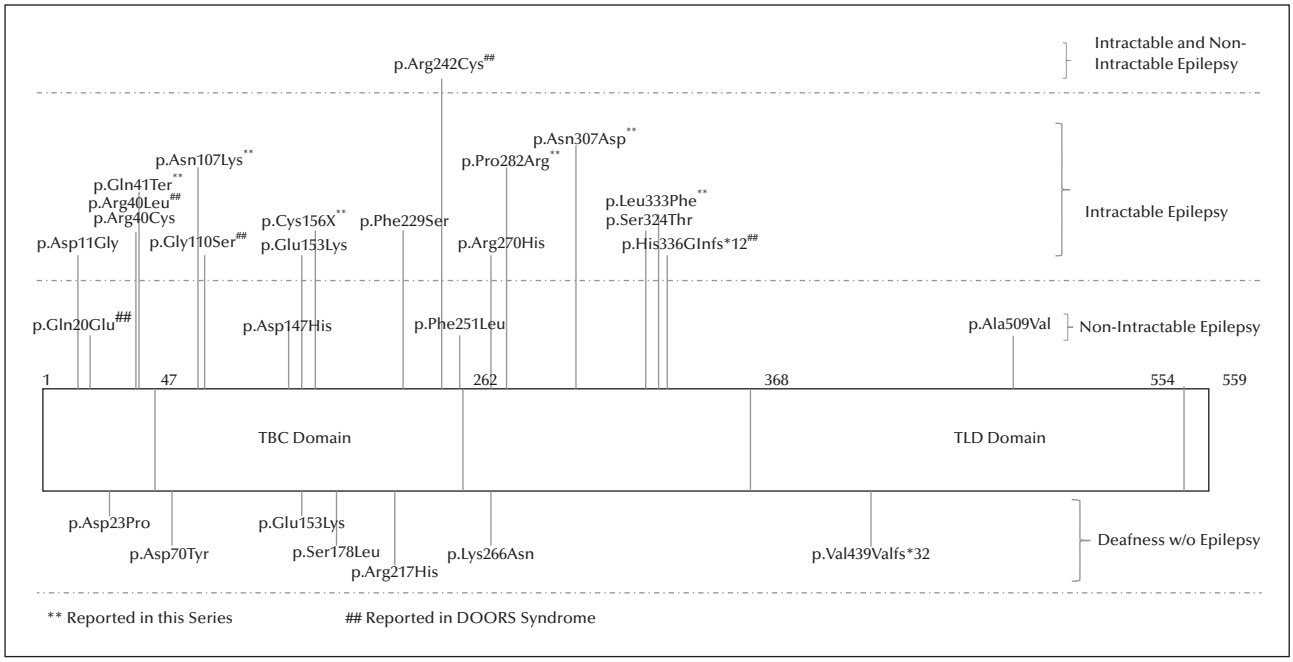

Figure 1. Clinical phenotypes of patients with *TBC1D24* mutations described in the literature.

variant and missense variant in *TBC1D24* presented with EIMFS, and had brain atrophy present on neuroimaging within 9 months of age, with one sibling passing away from SUDEP at 18 months (Milh *et al*., 2013). Five members of a Turkish family were identified with a homozygous nonsense variant in exon 3, presenting with refractory myoclonic epilepsy, episodic dystonia, dysautonomia, and neurological retardation. One such patient had moderate cerebral atrophy at 13 months of age, and secondary ventricular enlargement at 37 months (Guven and Tolun, 2013). Recently, eleven patients from nine families with DOORS syndrome were identified with *TBC1D24* variants. Among them, seizures were described as generalized tonic-clonic, complex partial, focal clonic seizures, and infantile spasms, and neuroimaging varied from normal imaging to cortical atrophy as young as four months (Campeau *et al*., 2014).

The autosomal recessive inheritance pattern of *TBC1D24*-related epilepsies is unique among known genetic epilepsies (*figure 1*). While many demonstrate broad phenotypic variability, they typically arise out of *de novo* mutations or an autosomal dominant inheritance pattern. The phenotypic variability with *TBC1D24* has been postulated to be related to mutation severity or in relation to exon 2, which encodes the TBC domain critical for ARF6 interaction. Knockout mouse studies suggest that disruption of the TLDc domain *in vivo* is sufficient to cause neurodegeneration (Finelli *et al*., 2016). In human studies of *TBC1D24*, a predictive genotype-phenotype profile has not yet emerged. Within our series, despite Patients III and IV having identical variants, both siblings had different seizure types and developmental outcomes.

Focal clonic seizures observed in all of our patients were clinically characterized by prolonged unilateral clonic jerking of the arm, leg, or face. Ictal patterns appeared diverse, and interictal-ictal transitions were often indiscrete and prone to electroclinical dissociation. These characteristics represent unique challenges to the recognition of seizure onset in these patients and the appropriate treatment escalation. The presence of two unrelated Navajo children with identical *TBC1D24* variants is notable, and suggests the potential for founder variations within this population. We recommend all infants with EPC or EIMFS of unknown aetiology be considered for testing of *TBC1D24* variants. Further clinical and electrographic descriptions of patients with *TBC1D24*-related epilepsies, as well as in-depth biochemical studies of the TBC1D24 protein, may shed further insight into the pathophysiology of this epilepsy gene and provide guidance in terms of appropriate prognostication and therapies. □

Supplementary data.

Summary didactic slides and supplementary figures are available on the www.epilepticdisorders.com website.

Acknowledgements and disclosures.
We are grateful to Michelle Wotowiec, MA for critical reading of this manuscript, and to Dr. Jeffrey Miller, MD for providing neuroradiology consultation for our manuscript.
There are no funding agencies to disclose in correspondence with this study.
None of the authors have any conflict of interest to disclose.

References

Afawi Z, Mandelstam S, Korczyn AD, *et al*. TBC1D24 mutation associated with focal epilepsy, cognitive impairment and a distinctive cerebro-cerebellar malformation. *Epilepsy Res* 2013; 105: 240-4.

Campeau P, Kasperaviciute D, Lu JT, *et al*. The genetic basis of DOORS syndrome: an exome-sequencing study. *Lancet Neurol* 2014; 13: 44-58.

Corbet MA, Bahlo M, Jolly L, *et al*. A focal epilepsy and intellectual disability syndrome is due to a mutation in TBC1D24. *Am J Hum Genet* 2010; 87: 371-5.

Falace A, Filipello F, La Padula V, *et al*. TBC1D24, an ARF6-interacting protein, is mutated in familial infantile myoclonic epilepsy. *Am J Hum Genet* 2010; 87: 365-70.

Falace A, Buhler E, Fadda M, *et al*. TBC1D24 regulates neuronal migration and maturation through modulation of the ARF6-dependent pathway. *Proc Natl Acad Sci U S A* 2014; 111: 2337-42.

Finelli MJ, Sanchez-Pulido L, Liu KX, *et al*. The evolutionary conserved Tre2/Bub2/Cdc16 (TBC), Lysin motif (LysM). Domain catalytic (TLDc) domain is neuroprotective against oxidative stress. *J Biol Chem* 2016; 291: 2751-63.

Guven A, Tolun A. TBC1D24 truncating mutation resulting in severe neurodegeneration. *J Med Genet* 2013; 50: 199-202.

Milh M, Falace A, Villeneuve N, *et al*. Novel compound heterozygous mutations in *TBC1D24* cause familial malignant migrating partial seizures in infancy. *Hum Mutat* 2013; 34: 869-72.

Zara F, Gennaro E, Stabile M, *et al*. Mapping of locus for a familial autosomal recessive idiopathic myoclonic epilepsy of infancy to chromosome 16p13. *Am J Hum Genet* 2000; 66: 1552-7.

TEST YOURSELF

(1) What is the known role of the TBC1D24 protein?

(2) What are established epilepsy and seizure phenotypes observed in patients with *TBC1D24*-related epilepsy?

(3) Electrographically, what phenomenon makes characterizing and localizing ictal onset challenging in patients with *TBC1D24*-related epilepsy?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".