Epileptic Disord 2020; 22 (3): 317-22

ATP6V1B2-related epileptic encephalopathy

Luciana Midori Inuzuka [1,2], Lúcia Inês Macedo-Souza [2], Bruno Della-Rippa [2], Fabiola Paoli Monteiro [3], Daniel de Souza Delgado [4], Luis Filipe Godoy [4], Luiza Ramos [3], Larissa Sampaio de Athayde Costa [3], Eliana Garzon [1,2], Fernando Kok [3,2]

[1] Epilepsy Clinic, Hospital Sírio-Libanês,
[2] Department of Neurology, University of São Paulo School of Medicine,
[3] Mendelics Genomic Analysis,
[4] Radiology Department, Hospital Sírio-Libanês, São Paulo, Brazil

Received August 22, 2019; Accepted February 24, 2020

ABSTRACT – *ATP6V1B2* encodes a subunit of the lysosomal transmembrane proton pump necessary for adequate functioning of several acid hydrolases. *De novo* monoallelic variants of this gene have been associated with two distinct phenotypes: Zimmermann-Laband syndrome 2 (ZLS2), an intellectual deficiency/multiple malformation syndrome, and dominant deafness onychodystrophy (DDOD), a multiple malformation syndrome without cognitive involvement. Epilepsy is not observed in DDOD, is variably present in ZLS2, but is a common feature in Zimmermann-Laband syndrome 1 (ZLS1) (caused by monoallelic pathogenic variants in *KCNH1*) and Zimmermann-Laband syndrome-like (ZLSL) (associated with *KCNK4* variants).
Herein, we report a case of an infant with severe epileptic encephalopathy with microcephaly and profound developmental delay, associated with a novel *de novo* loss-of-function variant in *ATP6V1B2*, diagnosed by whole-exome sequencing. This finding expands the spectrum of *ATP6V1B2*-associated disorders and adds *ATP6V1B2* as a new member for the growing list of early-onset epileptic encephalopathy genes. [*Published with video sequence*].

Key words: *ATP6V1B2*, Zimmermann-Laband syndrome 1, Zimmermann-Laband syndrome 2, epileptic encephalopathy, dominant deafness onychodystrophy, epilepsy

VIDEO ONLINE

Correspondence:
Luciana Midori Inuzuka
Department of Neurology,
University of São Paulo School of Medicine,
Rua Haddock Lobo, 131,
cj 1309, São Paulo, 01414-001, Brazil
<lminuzuka@gmail.com>

doi:10.1684/epd.2020.1166

*ATP6V1B2 (*ATPase, H+ transporting, lysosomal VI subunit B, isoform 2*)*, that encodes for one of the subunits of the cross-membrane proton pump, is responsible for keeping the lysosomal pH acidic (Marshansky *et al.,* 2014). This activity is necessary for adequate functioning of several lysosomal hydrolases. Two *de novo* variants of this gene have been associated with Zimmermann-Laband 2 (ZLS2, OMIM # 616455): a missense variant, p.(Arg485Pro) (Kortüm *et al*., 2015), clinically characterized by intellectual disability, a dysmorphic face, gingival hyperplasia, hypertrichosis, and nail aplasia or hypoplasia; and the p.(Glu374Gln) variant, reported by Popp *et al*. (2017), in a patient with microcephaly and intellectual disability, who had three seizures.

A third *de novo* variant in *ATP6V1B2* was associated with dominant deafness onychodystrophy (DDOD, OMIM # 124480): nonsense p.(Arg506*) (Yuan *et al*., 2014; Menendez *et al*., 2017), leading to congenital deafness and hypoplastic nails, associated with malformation of fingers and toes. The morphological changes similar to those seen in human DDOD have been reproduced in a murine model of this variant (Zhao *et al*., 2019). Recently, Shaw *et al*. (2019) reported the variant, p.(Leu398Val), in *ATP6V1B2* in a multigenerational family with six evaluated individuals showing epilepsy of variable severity, intellectual disability (present in three individuals), dystrophic nails (in two individuals), and gingival hyperplasia (found in three individuals). Epileptic encephalopathy, as seen in our patient, has not been reported in any *ATP6V1B2*-related phenotypes. Another unrelated gene, *KCNH1,* has been associated with two conditions associated with epilepsy as one of the symptoms: Temple-Baraitser syndrome (OMIM # 6118216) and Zimmermann-Laband 1 syndrome (ZLS1, OMIM # 135500). Both of these conditions are phenotypically similar to ZLS2, except for epilepsy and, occasionally, microcephaly, seen only in *KCNH1*-related syndromes (Bramswig *et al*., 2015; Mastrangelo *et al*., 2016; Mégarbané *et al*., 2016). A similar phenotype was recently reported with monoallelic *de novo* variants of *KCNN3* (Potassium Channel, Calcium-Activated, Intermediate/Small Conductance, Subfamily N, Member 3, OMIM * 602983) (Bauer *et al*., 2019) and *KCNK4* (Potassium Channel, Subfamily K, Member 4, OMIM * 605720) (Bauer *et al*., 2018), expanding the genetic heterogeneity of ZLS. Epilepsy was seen in only two of three reported cases with *KCNK3* variants (*table 1*). Herein, we report a patient with a novel *de novo* nonsense variant of *ATP6V1B2* associated with severe epileptic encephalopathy and microcephaly, accompanied by all of the other expected clinical features associated with ZLS2. This study was approved by the institutional review board, and the patient's family consented to this publication.

Case study

A two-year-old female was born at term via Caesarean section after an uncomplicated pregnancy, in good general health condition (birth weight: 3.570 g, percentile 25-50, z score:-0.4; OFC = 35 cm, percentile 25, z score:-0,7). She was the first child of non-consanguineous parents. Infantile spasms began at four months and tended to occur mostly in clusters upon awakening. The patient was treated with several antiepileptic drugs, including vigabatrin, levetiracetam, oxcarbazepine, clonazepam, topiramate, valproic acid, and phenobarbital, which failed to control her seizures. She currently has two or three isolated tonic seizures per day, usually upon awakening. She has microcephaly (OFC = 43.5 cm, z score <-2), with a metopic suture of neural crest origin, and frontal narrowing; she has small and wide-spaced teeth, with remarkable gingival hypertrophy. Hypertrichosis (mostly frontal and affecting the upper and lower limbs), hypoplastic distal phalanges of fingers and toes, and small toenails are also present (*figure 1*). She has profound developmental delay and became unable to support her head, sit alone, roll over onto her back, or grasp objects. Her vision and hearing are apparently normal. Brain magnetic resonance imaging (MRI) showed encephalic volume reduction. Karyotyping, chromosomal microarray analysis, metabolic work-up, hand X-ray, and brainstem evoked potential were all normal. At 22 months of age, a video electroencephalogram (video-EEG) showed disorganized background activity (excessive delta and theta waves during wakefulness and sleep) and epileptiform discharges characterized by bilateral anterior multispikes. (*figures 2, 3*). Four asymmetric tonic seizures were recorded during sleep. The seizures were electrographically characterized by suppression of background activity, lasting for three seconds, followed by rhythmic delta activity over the bilateral anterior regions mixed with sharp waves (*figure 4*). Whole-exome sequencing (WES) detected a nonsense variant [(Chr8:20,077,842A>T; c.1465A>T

Table 1. Zimmermann-Laband related genes and associated clinical findings.

Condition	Gene	Gingival	Hypertrichosis	Onychodystrophy	DD/ID	Facial	Epilepsy	Hyperplasia	Dysmorphism
ZLS1	*KCN1*	+	+	+	+	+	+	+	+
ZLS2	*ATP6V1B2*	+	+	+	+	+	-/+	+	+
ZLS2-associated	*KCNN3*	+	+	+	+	+	+	+	-
ZLS-like	*KCNK4*	+	+	+	+	+	+	+	+

ZLS: Zimmermann-Laband Syndrome; DD/ID: development delay/ intellectual deficiency; +: present; -: absent; –/+: absent or present.

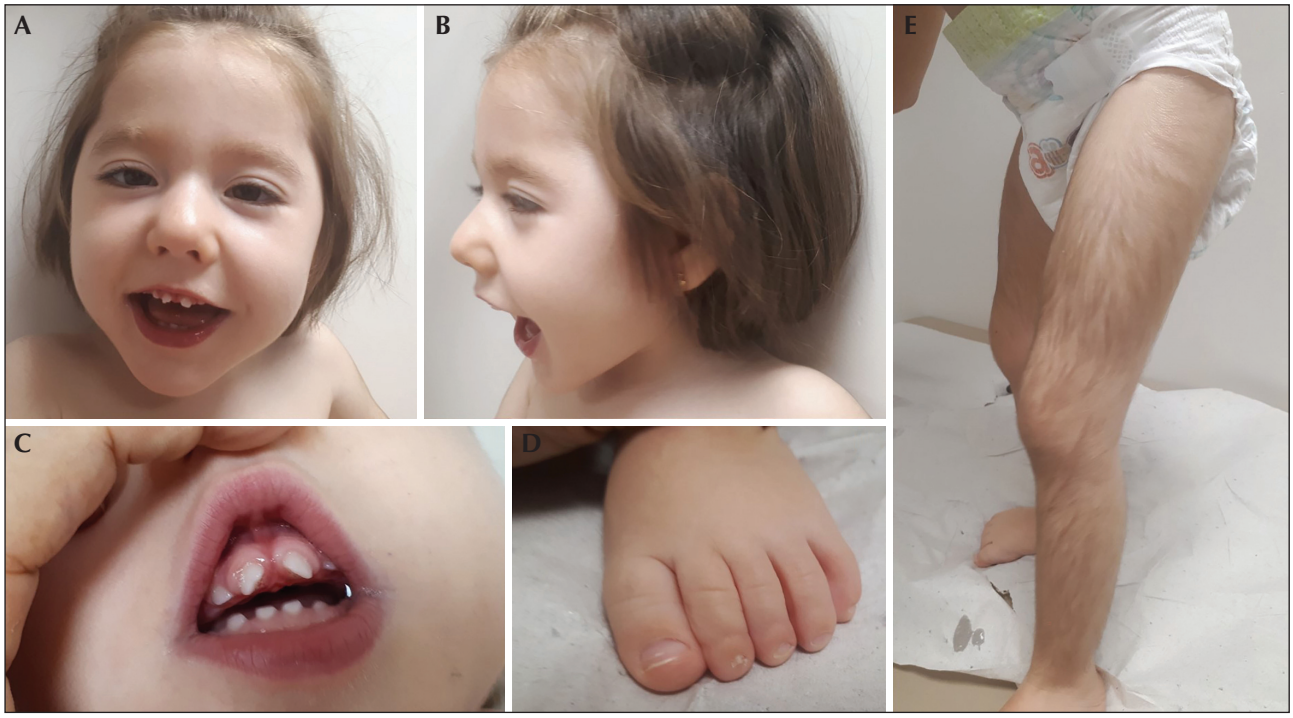

Figure 1. (A, B) Front and profile facial features; (C) gingival hyperplasia; (D) onychodystrophy of the toes; and (E) hypertrichosis.

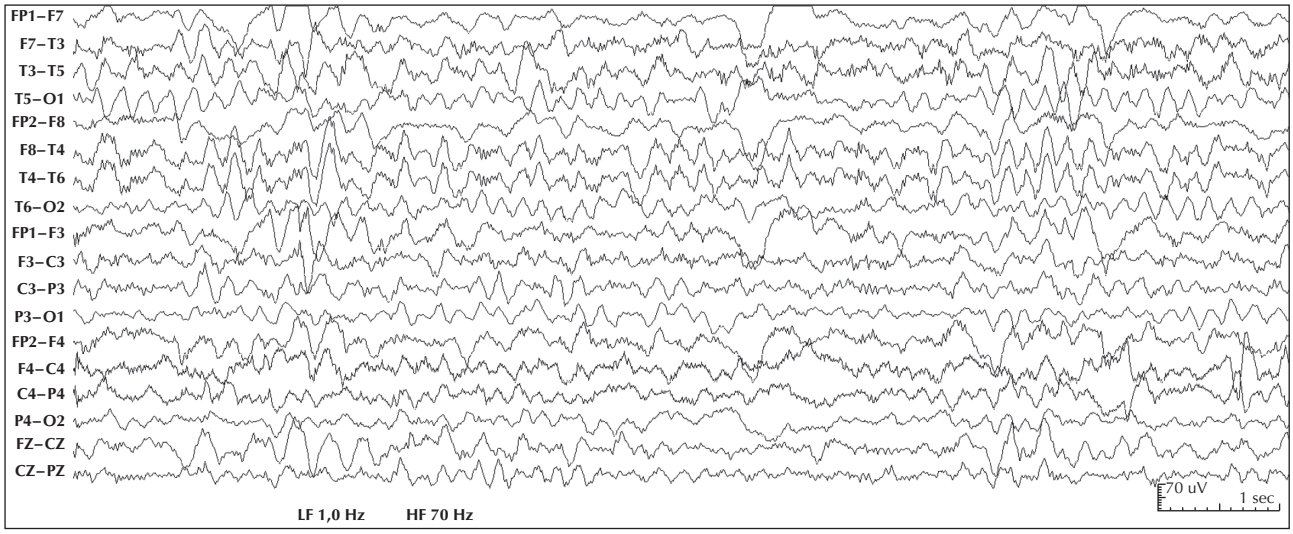

Figure 2. Video-EEG of the patient at 22 months of age showing disorganized background activity (excessive theta and delta waves) during wakefulness.

ENST0000027390 p.(Lys489*)] in *ATP6V1B2*. This variant, which leads to a premature protein truncation, is not found in population databases (1000 Genomes, GnomAD) and has not been reported in the literature or other databases (ClinVar, HGMD). The presence of this variant was confirmed by Sanger sequencing in the patient (but not in the parents), occurring as a *de novo* event. According to the American College of Medical Genetics (ACMG) criteria, this variant is classified as pathogenic (PVS1, PM2 and PM6) (Richards *et al*., 2015). Consistent with GnomAD, *ATP6V1B2* is highly intolerant to loss-of-function (pLI=0.99), a finding that supports the pathogenicity of the p.(Lys489*) variant detected in our patient. Additionally, it is located in exon 14, the last exon of *ATP6V1B2,* and might escape mRNA nonsense-mediated decay, leading to translation of an abnormal truncated protein, shortened by 23 amino acids. No other potentially pathogenic

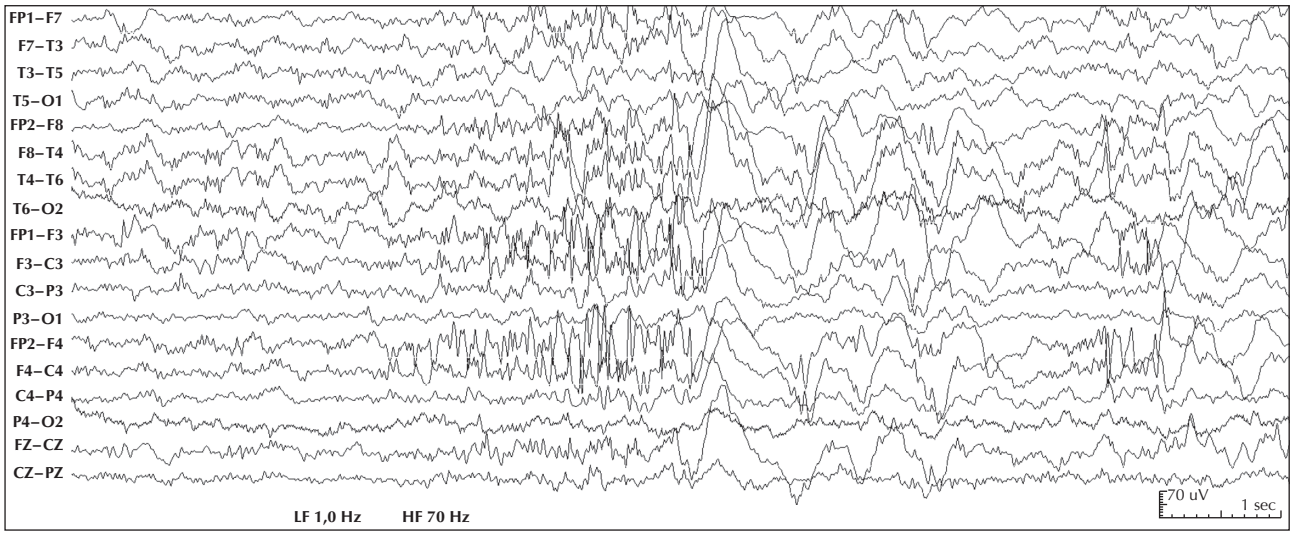

Figure 3. Video-EEG of the patient at 22 months of age showing epileptiform discharges characterized by bilateral anterior multispikes.

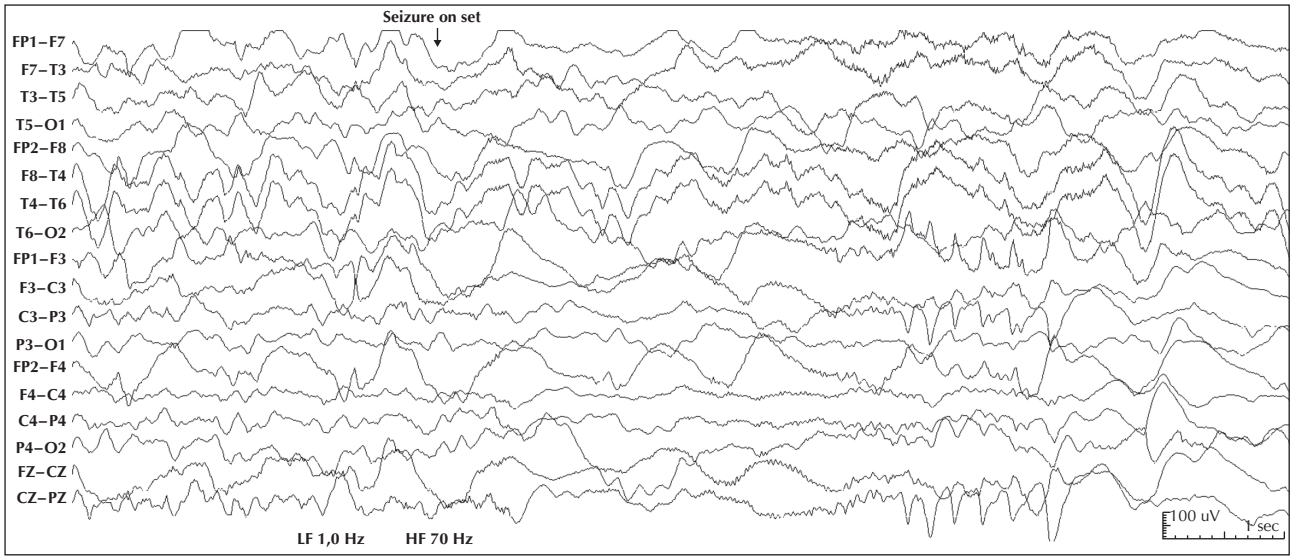

Figure 4. Video-EEG at 22 months of age showing a tonic seizure with suppression of background activity, followed by rhythmic delta activity over the bilateral anterior regions mixed with sharp waves.

variants in genes known to be associated with epileptic encephalopathy were identified by WES.

Discussion

Only three *de novo* pathogenic variants have been reported in *ATP6V1B2*: the two missense variants, c.1454G>C p.(Arg485Pro) (Körtum *et al*., 2015) and p.(Glu374Gln) (Popp *et al*., 2017), leading to ZLS2, and the recurrent nonsense variant c.1516C>T p.(Arg506*), reported in four patients with DDOD (Menendez *et al*., 2017; Yuan *et al*., 2014). The nonsense p.(Lys489*) variant reported in our patient is the fourth known pathogenic *de novo* change detected in *ATP6V1B2*, leading to a more complex phenotype, which combines developmental and physical features of ZLS2 with infantile-onset epileptic encephalopathy and microcephaly, a characteristic that might be seen in *KCNH1*-associated epilepsy. A summary of the clinical presentation of all *ATP6V1B2* patients reported is presented in *table 2*. The nonsense variants p.(Lys489*) and p.(Arg506*) might escape nonsense-mediated decay, as they are located in exon 14, the last exon of the gene. Nevertheless, they are associated with significantly different phenotypes, with a much more severe presentation for the p.(Lys489*) vari-

Table 2. Clinical and genetic characteristics in *ATP6V1B2 de novo* mutation.

Clinical findings	**Our patient**	**Kortüm, 2015 (family 7)**	**Kortüm, 2015 (family 8)**	**Menendez, 2017**	**Yuan, 2014 (patient 1)**	**Yuan, 2014 (patient 2)**	**Yuan, 2014 (patient 3)**	**Popp, 2017**
Diagnosis	*ATP6V1B2*-related epileptic encephalopathy	ZL2	ZL2	DDOD	DDOD	DDOD	DDOD	*ATP6V1B2*-related epileptic encephalopathy?
Variant	p.Lys489* (c.1465A>T)	p.Arg485Pro (c.1454G>C)	p.Arg485Pro (c.1454G>C)	p.Arg506* (c.156C>T)	p.Arg506* (c.156C>T)	p.Arg506* (c.156C>T)	p.Arg506* (c.156C>T)	p.Glu374Gln (c.1120G>C)
Age at last evaluation (y)	2	22	5	12	2.5	2	18	NR
Sex	F	M	F	M	F	M	F	NR
Epilepsy	+	-	-	-	-	-	-	+
Microcephaly	+	-	-	-	NR	NR	NR	+
Hypertrichosis	+	+	+	-	NR	NR	NR	NR
Scoliosis		+	NR	-	NR	NR	NR	NR
Gingival enlargement	+	+	+	-	NR	NR	NR	NR
Craniofacial dysmorphism	+	+	+	-	-	-	-	NR
Deafness	-	Unilateral	-	Bilateral	Bilateral	Bilateral	Bilateral	NR
Onychodystrophy	+	+	+	+	+	+	+	NR
Osteodystrophy	-	+	+	+	+	+	+	NR
Developmental delay	Global	Global	Global	-	-	-	-	+
Intellectual disability	+	+	+	-	-	-	-	+
Hypotonia	+	+	+	-	NR	NR	NR	+
Brain MRI	Reduced brain volume	Normal	NR	Normal	NR	NR	NR	NR
EEG	Multifocal paroxysms	Encephalopathy	Normal	Normal	NR	NR	NR	NR

DDOD: dominant deafness-onychodystrophy; ZL2; Zimmermann-Laband 2; NR: not reported; -: absent; +: present; EEG: electroencephalogram; MRI: magnetic resonance image.

ant. One might speculate that protein function is more severely affected by the loss of the last 23 amino acids for p.(Lys489*) compared to the loss of six amino acids for p.(Arg506*). Epilepsy was unusually severe in our patient, with onset at infancy and refractoriness to several antiepileptic drugs. Profound developmental delay associated with post-natal microcephaly was also part of the clinical presentation, as well as characteristic dysmorphic features, including gingival hypertrophy, hirsutism, and onychodystrophy. *ATP6V1B2* should be included in the expanding list of genes associated with early-infantile epileptic encephalopathy. □

Legend for video sequence

Video-EEG at 22 months of age showing an asymmetric tonic seizure.

Key words for video research on www.epilepticdisorders.com
Phenomenology: tonic seizure
Localisation: unknown
Syndrome: epileptic encephalopathy not otherwise classified
Aetiology: ATP6V1B2-related epileptic encephalopathy

Acknowledgements and disclosures.
We are grateful to the unrestricted collaboration of the patient's family.
None of the authors have any conflict of interest to declare.

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TEST YOURSELF

(1) What are the main clinical characteristics of *ATP6V1B2*-related phenotypes?

(2) Mutations in which other gene determine an *ATP6V1B2*-like phenotype?

(3) What is the known function of *ATP6V1B2*?

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".