ATP6V1B2-related disorders featuring Lennox-Gastaut-Syndrome: a case-based overview

Greta Amore[1,2], Elisa Calì[1], Maria Spanò[2], Giorgia Ceravolo[1,3], Giuseppe Donato Mangano[4], Giovanna Scorrano[5], Stephanie Efthymiou[1], Vincenzo Salpietro[1,6], Henry Houlden[1], Gabriella Di Rosa[2#]

[1]Department of Neuromuscular Disorders, UCL Institute of Neurology, Queen Square, London, WC1N 3BG, United Kingdom.

[2]Unit of Child Neurology and Psychiatry, Department of Human Pathology of the Adult and Developmental Age "Gaetano Barresi", University of Messina, Via C. Valeria 1, 98125 Messina, Italy.

[3]Unit of Emergency Pediatrics, Department of Human Pathology and Evolutive Age "Gaetano Barresi", University of Messina, Via C. Valeria 1, 98125 Messina, Italy.

[4]Department of Biomedicine, Neuroscience 9 and Advanced Diagnostics (BiND), Human Anatomy Section, Via del Vespro 129, 90127, Palermo, Italy.

[5]Department of Pediatrics, "G. D'Annunzio", University of Chieti-Pescara, Chieti, Italy.

[6]Department of Pediatrics, University of L'Aquila, 67100, L'Aquila, Italy.

#Corresponding to: Gabriella Di Rosa, Unit of Child Neurology and Psychiatry, Department of Human Pathology of the Adult and Developmental Age, "Gaetano Barresi" University of Messina, 98125 Messina, Italy. E-mail: g.dirosa@unime.it. Fax: 00390902212906. Phone: 00390902212915.

Keywords: ATP6V1B2; ATP6V1B2-related disorders; Deafness-onycodystrophy-osteodystrophy-mental retardation-seizures; Developmental epileptic encephalopathy;

Dominant deafness-onycodystrophy; Lennox-Gastaut syndrome; Zimmermann-Laband syndrome 2.

ABSTRACT

Background*: ATP6V1B2* (ATPase, H+ transporting, lysosomal VI subunit B, isoform 2) encodes for a subunit of a ubiquitous transmembrane lysosomal proton pump, implicated in the acidification of intracellular organelles and in several additional cellular functions. Variants in *ATP6V1B2* have been related to a heterogeneous group of multisystemic disorders sometimes associated with variable neurological involvement. However, our knowledge of genotype-phenotype correlations and the neurological spectrum of *ATP6V1B2*-related disorders remain limited due to the few numbers of reported cases.

Case study: We hereby report the case of an 18-year-old male Sicilian patient affected by a global developmental delay, skeletal abnormalities, and epileptic encephalopathy featuring Lennox-Gastaut syndrome (LGS), in which exome sequencing led to the identification of a novel *de novo* variant in *ATP6V1B2* (NM_001693.4: c.973G>C, p.Gly325Arg).

Conclusions: Our report provides new insights on the inclusion of developmental epileptic encephalopathies (DEEs) within the continuum group of *ATP6V1B2*-related disorders, expanding the phenotypic and molecular spectrum associated with these conditions.

INTRODUCTION

ATP6V1B2 (OMIM #606939) encodes for a subunit of a vacuolar ATPase (V-ATPase), a multi-subunit transmembrane lysosomal proton pump implicated in the acidification of intracellular

organelles, essential for several cellular and sub-cellular functions [1]. Given its key-role, *ATP6V1B2* has been associated with a heterogeneous group of syndromic disorders that include (a) "dominant deafness-onycodystrophy" (DDOD) syndrome (OMIM #124480), (b) "deafness-onycodystrophy-osteodystrophy-mental retardation and seizures" (DOORS) syndrome (OMIM #220500) and (c) Zimmermann-Laband syndrome 2 (ZLS2) (OMIM #616455). These phenotypes, though well-distinguishable, present overlapping features (*e.g.,* sensorineural deafness, nail-phalanges abnormalities, oro-buccal anomalies) and could be regarded as part of the same phenotypic continuum [2][Figure 1].

Epileptic seizures, albeit inconstant, are accounted among the potential clinical features shared by both DOORS and ZLS2, though not common in DDOD [2]. Recently, data from the literature have suggested that refractory epilepsies and developmental epileptic encephalopathies (DEEs) may be part of the phenotypes associated with heterozygous *ATP6V1B2* variants, potentially expanding the spectrum of *ATP6V1B2*-related disorders [3].

We hereby describe the case of an 18-year-old Sicilian male presenting with a complex phenotype, predominantly featuring a DEE meeting the electro-clinical criteria for a Lennox-Gastaut syndrome (LGS), found to carry a novel *de novo* missense variant in *ATP6V1B2*. Written informed consent was obtained by patient's parents to publish this case, including the patient's photographs.

CASE PRESENTATION

Our patient is the son of non-consanguineous parents. He was born via a programmed caesarean section at term after an uneventful pregnancy. Perinatal history was unremarkable.

At 3 months, after Diphtheria-Tetanus-Pertussis vaccine, he presented with hyperthermia and generalized epileptic seizures, well controlled by phenobarbital (Pb). The MRI showed slight white matter hyperintensities, mostly affecting the paraventricular areas, making suspect an immune-mediated condition (such as an acute disseminated encephalomyelitis - ADEM) [Figure 2 A]. Cerebrospinal fluid examination could not be performed due to parents' refusal; hence the diagnosis could not be definitively confirmed. The EEG was unremarkable.

After several months of seizure-freedom, he presented with an unprovoked epileptic seizure, characterized by impaired awareness, periorbital cyanosis, and limbs hypertonia, lasting overall 5 minutes. EEG did not show significant abnormalities, whereas an MRI confirmed the previous findings (with a significant reduction at following MRIs). By that time, the child presented only with a mild global neurodevelopmental delay.

Given the reoccurrence of analogue epileptic attacks during his second year of life, valproate (VPA) was administered in add-on to Pb and to Dexamethasone cycles, achieving seizure-freedom for 5 years.

In the meantime, the child developed a global neurodevelopmental delay, characterized by motor impairment, absent speech, impaired interaction and moderate-to-severe intellectual disability (ID).

Since the age of 7, despite an adequate treatment with antiepileptic drugs (AEDs), he experienced new types of seizures (atypical absences with motor involvement, tonic seizures during sleep, drop attacks, and status epilepticus during infections/fever), together with the onset of behavioral abnormalities (opposition and restlessness), left hemiplegic gait (in the absence of new MRI abnormalities), and an overall worsening of the neurocognitive domain.

The EEGs performed over the years showed an abnormal background activity with interictal generalized polymorphic abnormalities, mostly expressed on the precentral and right areas of the brain, especially during sleep, whereas a follow-up MRI additionally detected a right hippocampal mesial temporal sclerosis [Figure 2 B-E].

By the age of 13 years, several AEDs had already been trialled unsatisfactorily in terms of seizure control [Table 1], delineating a drug-resistant epileptic encephalopathy consistent with the criteria of an LGS.

At his last follow up (18 years old), the patient presents with several polymorphic seizures per week and his EEG has not significantly improved. He cannot speak and shows stereotyped movements and repetitive behaviour. At physical examination, he presents significant scoliosis, wide-spaced teeth and coarse facies [Figure 2 F-G].

Laboratory testing, including plasmatic and urinary amino-acids and urinary organic acids, and genetic testing (karyotype, array-comparative genomic hybridization, sequencing of *SCN1A*, *MECP2*, *STXBP1* and *POLG1*), were unremarkable.

Written informed consent was obtained from the parents. The study and associated research protocols received approval from the Review Boards and Bioethics Committees at University College London Hospital (project 06/N076). Trio exome sequencing was performed and revealed a heterozygous mutation in the gene *ATP6V1B2* (NM_001693.4): c.973G>C (p.Gly325Arg). Sanger sequencing confirmed that the variant occurred *de novo* (being present in the proband and not in the healthy parents). This variant, not yet reported in the literature, is classified as "likely pathogenic" according to the "American College of Medical Genetics" criteria (PM2, PM6, PP3). *In silico* analysis performed with VarMap, which includes protein predictors and evolutionary conservation, supports a deleterious effect (Residue

report). The location of the identified variant is likely to affect the multi-subunit enzyme function, and therefore a high disease propensity of the variant (score 1.78). Thus, we considered the p.Gly325Arg variant in the *ATP6V1B2* gene the most plausible explanation for the disease pathogenesis in our child based on (*i*) its *de novo* occurrence, (*ii*) the absence of this variant in the heterozygous state from exome or genome sequencing data part of available datasets that we screened, such as 1000 Genomes Project, NHLBI Exome Variant Server, the Genome Aggregation Database (GnomAD) and 100K Genome Project, (*iii*) the protein predictors and evolutionary conservation scores indicating an high likelihood of deleterious impact as a result of this aminoacidic substitution, (*iv*) the consistency of the phenotype of our patient with previously described individuals carrying *ATP6V1B2* pathogenic variants, and (*v*) the exclusion of any other known or pathogenic variants in genes (implicated in neurological and/or paediatric disorders) within the exome sequencing data.

DISCUSSION

V-ATPases play a central role in determining and maintaining the acidic environment of intracellular organelles and are thus fundamental for several cellular functions [1]. V-ATPases consist of a multi-subunit complex arranged in two domains: V1 (peripheral), responsible for ATP-hydrolysis, and V0 (integral membrane domain), involved in proton translocation. In mammals, V1 has eight different subunits (A-H) [4]. Two isoforms of the B subunit have been identified in humans: B1, present in kidneys, epididymis, eyes, and inner ears; B2, known as the brain isoform, which is ubiquitous and is able to replace B1 when necessary. Therefore, it is not surprising that V-ATPases have been related to a wide variety of multisystemic diseases [4].

Accordingly, *ATP6V1B2* is involved in distinct phenotypes (albeit the underpinning mechanism remain widely unknown), such as renal clear cell carcinoma [5], bladder cancer [6], follicular lymphoma [7], and the previously mentioned DDOD, DOORS and ZLS2. Epilepsies and DEEs are emerging as novel *ATP6V1B2*-related phenotypes [8] [Figure 2].

Particularly, a recurrent *ATP6V1B2* variant, p.Arg506, has been identified in cases of DDOD and DOORS, either as *de novo* or inherited in an autosomal dominant manner[9–13]. Interestingly, the same variant has been detected in an aged case of DOORS syndrome with a demonstrated tauopathy, suggesting a correlation between lysosomal dysfunction and tauopathies [14]. Also, a *de novo* p.Arg485Pro variant has been described in two families with ZLS2 [15] [Table 1].

In regard to the epileptic phenotypes, the *de novo* variants p.Lys489 and p.Glu374Gln have been identified respectively in a case of *ATP6V1B2*-related epileptic encephalopathy (drug-resistant infantile spasms, later followed by tonic seizures upon awakening) associated with microcephaly, gingival hypertrophy, hypertrichosis and finger-nail abnormalities, and in a patient with epilepsy, ID, hypotonia and microcephaly [3,16]; whereas a segregating familial variant, p.Leu398Val, has been found by Shaw et al. [8] as causative of different epileptic phenotypes in two families [Table 1].

We hereby report an 18-year-old individual carrying a novel *de novo* missense *ATP6V1B2* variant associated with a complex phenotype inclusive of skeletal abnormalities, a global neurodevelopmental delay, and an overall drug-resistant DEE, featuring LGS. LGS is a severe type of childhood-onset epileptic encephalopathy, defined by the triad: 1) multi-drug-resistant polymorphic seizures (mostly tonic seizures, atypical absences and drop attacks); 2)

a hallmark EEG pattern consisting of bursts of slow spike-wave complexes on an abnormal background activity coupled with generalized paroxysmal fast activity (poly-spikes) during sleep; 3) ID [17].

To the best of our knowledge, no other LGS have been reported in association with *ATP6V1B2* variants before. Our patient also had a clinical history which might suggest the co-occurrence of an immune-mediated condition in infancy, with a later onset of LGS. Anyhow, although the link between the *ATP6V1B2* variant and our patient's clinical signs and symptoms is still widely unknown, our case remains without precedents and, together with recent data proving an involvement of *ATP6V1B2* in different cancer and immunological processes, as well as in the mTOR pathway, provides a hint for a possible interplay between genetics and immune-mediated mechanisms, worthy of additional investigations [5].

In this regard, recent studies focusing on *ATP6V1B2* role in follicular lymphoma have demonstrated that pathogenic variants of this gene may affect the lysosomal V-ATPase activity, causing an elevation of lysosomal pH and an impairment of protein hydrolysis, eventually reducing the cytoplasmic amino-acid concentrations. This would result in compensatory activation of the autophagic flux and mTOR/TOR pathway, eventually promoting cell survival and cancerogenesis [7]. Moreover, mTOR-signaling pathway is an important regulator of developmental myelination, and increasing evidence is emerging on its role in autoimmunity and, particularly, in the etiopathogenesis of demyelinating disorders of the central nervous system, such as Multiple Sclerosis [18].

Our patient shares some overlapping clinical features with previously described *ATP6V1B2* subjects, including coarse facies, oro-buccal abnormalities, hypotonia, and scoliosis; however,

he does not present with other peculiar DDOD/DOORS characteristics (*e.g.*. deafness, gingival hypertrophy, nail-finger abnormalities, hypertrichosis), nor with microcephaly (reported in 4 *ATP6V1B2*-mutated subjects) [3,10,14–16] [Table 1]. Noteworthy he also shows behavioral abnormalities and, particularly, stereotyped movements and repetitive behavior.

Additionally our subject had a hippocampal mesial temporal sclerosis. To date only another subject [14] has been described with an *ATP6V1B2*-related phenotype (DOORS with epilepsy and behavioral disturbances) and structural hippocampal abnormalities (moderate atrophy). This is particularly interesting considering not only that *ATP6V1B2* is expressed in the brain (hence in hippocampus too), but also that recent studies revealed a role of *ATP6V1B2* in the hippocampal function in mammals, suggesting a potential role on its development and functioning [4,19]

Taken together, our data further support the involvement of *ATP6V1B2* in variable clinical presentations and also corroborate the inclusion of refractory epilepsies and DEEs within the broad spectrum of *ATP6V1B2*-related conditions. Further studies are needed to unravel the underpinning mechanisms and the molecular and phenotypic impact of different *ATP6V1B2* variants on brain developmental processes, to understand the impact of non-genetic triggers (such as inflammation) on phenotypic outcomes of *ATP6V1B2*-related disorders and to delineate a potential genotype-phenotype correlation within this ultra-rare and neglected group of conditions.

REFERENCES

1. Marshansky V, Rubinstein JL, Grüber G. Eukaryotic V-ATPase: Novel structural findings and functional insights. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 2014; 1837:857–79.

2. Gao X, Dai P, Yuan YY. Genetic architecture and phenotypic landscape of deafness and onychodystrophy syndromes. Hum Genet. 2022; 141:821–38.

3. Inuzuka LM, Macedo-Souza LI, Della-Rippa B, Monteiro FP, Delgado D de S, Godoy LF, et al. ATP6V1B2-related epileptic encephalopathy. Epileptic Disord. 2020; 22:317–22.

4. Zhao W, Gao X, Qiu S, Gao B, Gao S, Zhang X, et al. A subunit of V-ATPases, ATP6V1B2, underlies the pathology of intellectual disability. EBioMedicine. 2019 ;45:408–21.

5. Li X, Li H, Yang C, Liu L, Deng S, Li M. Comprehensive Analysis of ATP6V1s Family Members in Renal Clear Cell Carcinoma With Prognostic Values. Front Oncol. 2020;10:567970.

6. Fang ZQ, Zang WD, Chen R, Ye BW, Wang XW, Yi SH, et al. Gene expression profile and enrichment pathways in different stages of bladder cancer. Genet Mol Res. 2013;12:1479–89.

7. Wang F, Yang Y, Klionsky DJ, Malek SN. Mutations in V-ATPase in follicular lymphoma activate autophagic flux creating a targetable dependency. Autophagy. 2022, in press. doi: 10.1080/15548627.2022.2071382

8. Shaw M, Winczewska-Wiktor A, Badura-Stronka M, Koirala S, Gardner A, Kuszel Ł, et al. EXOME REPORT: Novel mutation in ATP6V1B2 segregating with autosomal dominant epilepsy, intellectual disability and mild gingival and nail abnormalities. Eur J Med Genet. 2020;63:103799.

9. Yuan Y, Zhang J, Chang Q, Zeng J, Xin F, Wang J, et al. De novo mutation in ATP6V1B2 impairs lysosome acidification and causes dominant deafness-onychodystrophy syndrome. Cell Res. 2014; 24:1370–3.

10. Beauregard-Lacroix E, Pacheco-Cuellar G, Ajeawung NF, Tardif J, Dieterich K, Dabir T, et al. DOORS syndrome and a recurrent truncating ATP6V1B2 variant. Genet Med. 2021; 23:149–54.

11. Li Y, Xiong J, Zhang Y, Xu L, Liu J, Cai T. Case Report: Exome Sequencing Identified Variants in Three Candidate Genes From Two Families With Hearing Loss, Onychodystrophy, and Epilepsy. Front Genet. 2021; 12:728020.

12. Gao X, Qiu SW, Feng ML, Huang SS, Kang DY, Han MY, et al. Establishment of human induced pluripotent stem cell line (CPGHi002-A) from a 10-month-old female patient with DDOD syndrome carrying a heterozygous c.1516 C > T mutation in ATP6V1B2. Stem Cell Res. 2020; 48:101986.

13. Menendez I, Carranza C, Herrera M, Marroquin N, Foster J, Cengiz FB, et al. Dominant deafness-onychodystrophy syndrome caused by an ATP6V1B2 mutation. Clin Case Rep. 2017;5:376–9.

14. Zádori D, Szalárdy L, Reisz Z, Kovacs GG, Maszlag-Török R, Ajeawung NF, et al. Clinicopathological Relationships in an Aged Case of DOORS Syndrome With a p.Arg506X Mutation in the ATP6V1B2 Gene. Front Neurol. 2020;11:767.

15. Kortüm F, Caputo V, Bauer CK, Stella L, Ciolfi A, Alawi M, et al. Mutations in KCNH1 and ATP6V1B2 cause Zimmermann-Laband syndrome. Nat Genet. 2015; 47:661–7.

16. Popp B, Ekici AB, Thiel CT, Hoyer J, Wiesener A, Kraus C, et al. Exome Pool-Seq in neurodevelopmental disorders. Eur J Hum Genet. 2017;25:1364–76.

17. Asadi-Pooya AA. Lennox-Gastaut syndrome: a comprehensive review. Neurol Sci. 2018; 39:403–14.

18. Mammana S, Bramanti P, Mazzon E, Cavalli E, Basile MS, Fagone P, et al. Preclinical evaluation of the PI3K/Akt/mTOR pathway in animal models of multiple sclerosis. Oncotarget. 2018; 9:8263–77.

19. Jung HY, Kim W, Hahn KR, Kang MS, Kwon HJ, Choi JH, et al. Changes in the expression of the B subunit of vacuolar H+-ATPase, in the hippocampus, following transient forebrain ischemia in gerbils. Iran J Basic Med Sci. 2021; 24:1482–7.

ACKNOWLEDGMENTS

This study was supported by the Wellcome Trust (WT093205MA and WT104033AIA), Medical Research Council (H.H.), European Community's Seventh Framework Programme (FP7/2007-2013, under grant agreement No. 2012- 305121 to H.H.) We are also supported by the National Institute for Health Research (NIHR) University College London Hospitals (UCLH) Biomedical Research Centre (BRC).

AUTHOR CONTRIBUTION STATEMENT

Conceptualisation: G.A.; writing—original draft preparation, G.A.; writing—review and editing, E.C., V.S., and G.D.R.; supervision, V.S., H.H., and G.D.R.; figure and table preparation, G.A; data collection, M.S., G.C., G.S., S.E., G.D.M.; analysis and interpretation of the results: G.A., and E.C. All authors contributed to manuscript revision, read, and approved the submitted version.

CONFLICTS OF INTEREST

The authors disclose no conflicts of interest.

FIGURE LEGEND

Figure 1. ATP6V1B2-related disorders: Although the underpinning mechanisms remain widely unknown, the ubiquitous transmembrane lysosomal proton pump ATP6V1B2 **is** today acknowledged to be involved in different disorders. Among these, "Dominant deafness-onycodystrophy" (DDOD), deafness-onycodystrophy-osteodystrophy-mental retardation-seizures" (DOORS) and "Zimmermann-Laband syndrome 2 (ZLS2) are three well-distinguishable phenotypes, that present significant overlapping clinical features. Neurodevelopmental disorders and developmental epileptic encephalopathies (DEEs) are also emerging as ATP6V1B2-related disorders. Finally, given its several functions, ATP6V1B2 has also been related to other disorders, such as renal clear cell carcinoma, bladder cancer and follicular lymphoma.

Figure 2. Instrumental investigations and patient's photographs:

Brain MRI images: Brain MRI performed over time showed: A) bilateral white matter hyperintensities, particularly evident in the paraventricular areas in fluid attenuated inversion recovery (FLAIR) images; B) a right hippocampal mesial temporal sclerosis.

EEG recordings: C) Video-EEG recording of a tonic seizure during sleep, characterized by various amplitude multifocal spikes, sharp waves and diffuse fast activities, on a poorly organized background. **D-E)** Interictal EEGs show several generalized polymorphic abnormalities (such as high-voltage spikes-/polispikes-waves complexes, slow waves and fast poli-spikes) mostly evident on the precentral areas of the brain, bilaterally, on an abnormal background, both in wakefulness (D) and during sleep (E).

Patient's photographs: F) Full-length picture of our patient demonstrating the presence of a significant scoliosis. G) Close-up photo showing wide-spaced and misaligned teeth.