DOORS syndrome and a recurrent truncating *ATP6V1B2* variant

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Purpose: Biallelic variants in *TBC1D24*, which encodes a protein that regulates vesicular transport, are frequently identified in patients with DOORS (deafness, onychodystrophy, osteodystrophy, intellectual disability [previously referred to as mental retardation], and seizures) syndrome. The aim of the study was to identify a genetic cause in families with DOORS syndrome and without a *TBC1D24* variant.

Methods: Exome or Sanger sequencing was performed in individuals with a clinical diagnosis of DOORS syndrome without *TBC1D24* variants.

Results: We identified the same truncating variant in *ATP6V1B2* (NM_001693.4:c.1516C>T; p.Arg506*) in nine individuals from eight unrelated families with DOORS syndrome. This variant was already reported in individuals with dominant deafness onychodystrophy (DDOD) syndrome. Deafness was present in all individuals, along with onychodystrophy and abnormal fingers and/or

toes. All families but one had developmental delay or intellectual disability and five individuals had epilepsy. We also describe two additional families with DDOD syndrome in whom the same variant was found.

Conclusion: We expand the phenotype associated with ATP6V1B2 and propose another causal gene for DOORS syndrome. This finding suggests that DDOD and DOORS syndromes might lie on a spectrum of clinically and molecularly related conditions.

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INTRODUCTION

Biallelic variants in *TBC1D24* (MIM 613577), which encodes a protein that regulates vesicular transport, have been identified in half of the patients with DOORS syndrome (MIM 220500); however, no causal gene has been identified in the remaining half.[1] DOORS syndrome stands for deafness, onychodystrophy, osteodystrophy, developmental delay and/ or intellectual disability—previously referred as mental retardation—and seizures. Dominant deafness onychodystrophy (DDOD; MIM 124480) is a condition that partially

overlaps with DOORS syndrome, without intellectual disability and seizures. The mode of inheritance—autosomal recessive in DOORS syndrome and autosomal dominant in DDOD syndrome—also differs. DDOD syndrome is caused by a truncating variant in *ATP6V1B2* (MIM 606939), encoding a subunit of the vacuolar (V)-ATPase responsible for the acidification of intracellular organelles.[2,3] We had previously reported a different variant in *ATP6V1B2* causing Zimmermann–Laband syndrome (MIM 616455), characterized by gingival hyperplasia, intellectual disability, and nail

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and phalangeal abnormalities.[4] Two other *ATP6V1B2* variants were reported: one in a patient with intellectual disability, hypotonia, microcephaly, and seizures,[5] and another with epilepsy and mild gingival and nail anomalies. However, their phenotypes were otherwise not consistent with DOORS syndrome.[6]

Here, we report nine individuals from eight unrelated families with a clinical diagnosis of DOORS syndrome and no variant in *TBC1D24*, in whom we identified the same truncating variant in *ATP6V1B2* that causes DDOD syndrome (NM_001693.4:c.1516C>T; p.Arg506*). We also studied two families already reported with DDOD syndrome[7,8] for whom we performed molecular studies. We thus expand the phenotype associated with *ATP6V1B2* variants.

MATERIALS AND METHODS

Ethics statement
This project was approved by the institutional review board of CHU Sainte-Justine. All experiments were performed in accordance with relevant guidelines and regulation. Written informed consent for research was obtained from each participant in the study or their legal representative. Consent for publication of photos included in this article were also obtained.

Participants
Families were identified in a cohort comprising 46 families with DOORS syndrome (based on the presence of three of the five main features forming the acronym) and 2 with DDOD syndrome recruited in a study led by P.M.C. Families in whom no variant in *ATP6V1B2* was detected were excluded from this report.

Exome sequencing
Exome sequencing was performed in four individuals with DDOD syndrome and in four with DOORS syndrome, including three previously reported by Campeau et al.[1] Sanger sequencing was used to confirm the presence of the variant in other individuals with DOORS syndrome and its absence in healthy relatives.

RESULTS
We identified the same truncating variant in *ATP6V1B2* (NM_001693.4:c.1516C>T; p.Arg506*) in two families with DDOD syndrome and in nine individuals from eight unrelated families with a clinical diagnosis of DOORS syndrome. The variant was absent in healthy relatives. Table 1 shows other genetic etiologies identified in a total of 46 families assessed ultimately by exome sequencing for DOORS syndrome, including the cohort of 26 families previously reported by Campeau et al.[1] The most frequent cause is biallelic variants in *TBC1D24*, while no genetic cause was identified in 6 families (13%). Our previous publications discuss clinical overlap between DOORS and most conditions mentioned in Table 1.[4,9–13] Table 2 and Fig. 1 provide details about phenotypes described in the present report. Figures 2 and 3 show the location of the p.Arg506* variant and previously reported missense variants.

Individuals from families 1 and 2 had a diagnosis of DDOD syndrome and typical features of the condition were found in

Table 1 Genetic etiology identified in a total of 46 families affected by DOORS syndrome.

Genetic etiology	Number of families (%)
TBC1D24	13 (28%)
GPI biosynthesis genes	8 (17%)
ATP6V1B2	8 (17%)
SWI/SNF complex genes (Coffin–Siris syndrome)	5 (11%)
Potassium channel genes (Zimmermann–Laband syndrome)	3 (7%)
Other genes (*UBE3B, TANGO2, SON*)	3 (7%)
Unidentified genetic etiology	6 (13%)

Table 2 Clinical features of individuals with *ATP6V1B2* variants.

Individual/family	1 (*n* = 3)	2 (*n* = 3)	3 (*n* = 2)	4 (*n* = 1)[a]	5 (*n* = 1)[a]	6 (*n* = 1)[a]	7 (*n* = 1)	8 (*n* = 1)	9 (*n* = 1)	10 (*n* = 1)
ATP6V1B2 variant c.1516C>T	+	+	+	+	+	+	+	+	+	+
DDOD	+	+	–	–	–	–	–	–	–	–
DOORS	–	–	+	+	+	+	+	+	+	+
Deafness	+	+	+	+	+	+	+	+	+	+
Onychodystrophy	+	+	+	+	+	+	+	+	+	+
Brachydactyly	+	+	+	+	+	+	–	+	+	+
Bulbous swelling of terminal phalanges	+	+	+	–	–	–	–	–	–	–
Finger-like thumb	+	+	–	–	–	–	+	–	–	+
Triphalangeal thumb	–	–	+	+	+	+	–	–	+	–
Dental anomalies	+[b] (2/3)	–	–	+[b]	+[c]	–	–	–	–	–
DD/ID	–	–	–	+[d]	+[e]	+[f]	+[d]	+[f]	+[e]	+[f]
Seizures	–	+ (1/3)	+ (1/2)	+	+	+	–	–	+	+

DD developmental delay, *DDOD* dominant deafness onychodystrophy, *ID* intellectual disability.
[a]Individuals 4, 5, and 6 were reported by Campeau et al.[1] and referred as individuals 24, 25, and 26, respectively in the previous publication.
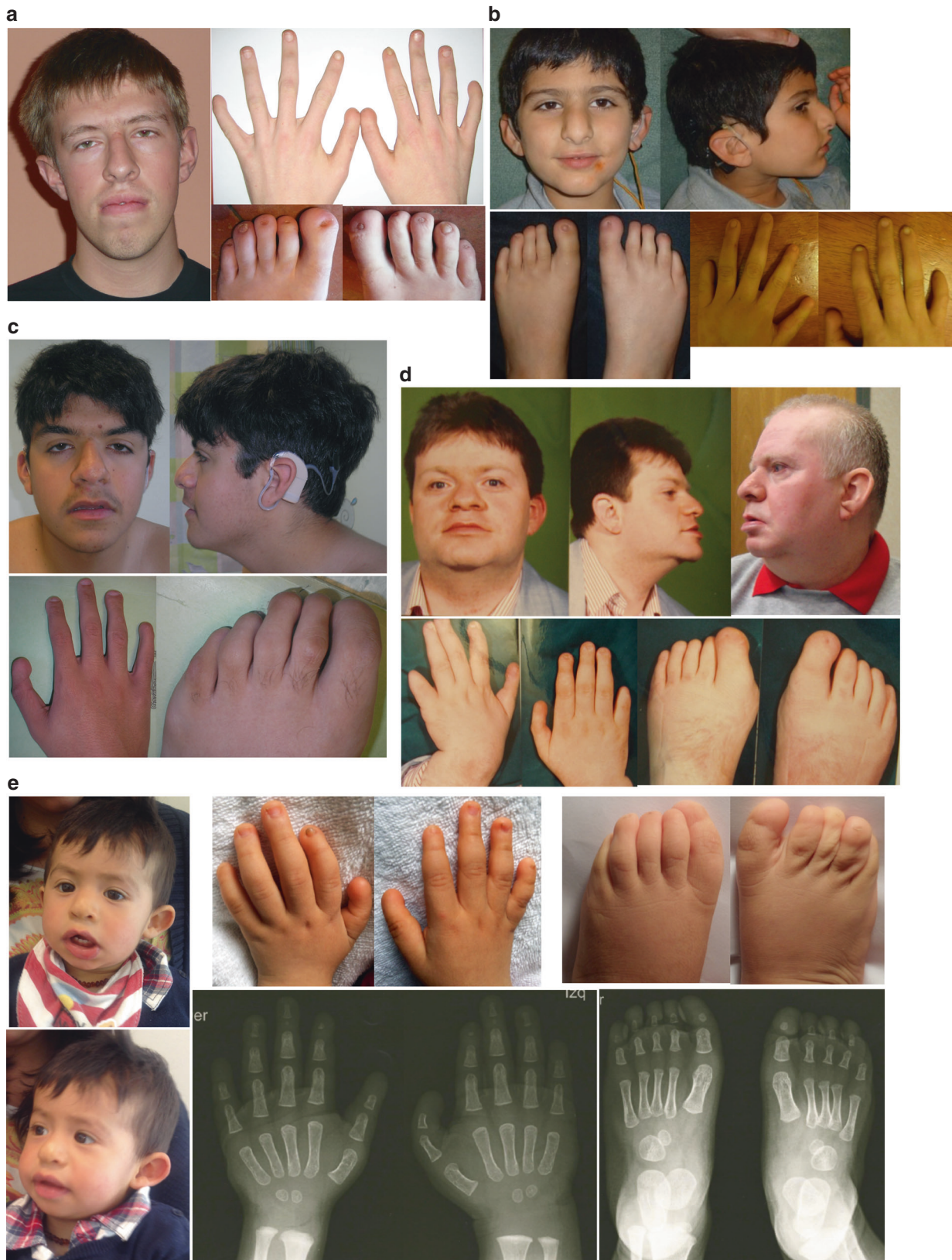[b]Late dentition.
[c]Misalignment.
[d]Mild ID.
[e]Severe ID.
[f]Severity of ID not assessed. See Supplement for other dysmorphisms noted.

Fig. 1 Physical features of individuals with DOORS syndrome. Note onychodystrophy and brachydactyly in all. (**a**) Individual 4: prognathism, broad nasal ridge, low-set ears, downslanted palpebral fissures, and slight ptosis. (**b**) Individual 5: broad nasal ridge, downslanted palpebral fissures, and low-set ears. (**c**) Individual 6: wide base of the nose, downslanted palpebral fissures, long philtrum, and prominent fingerpads. (**d**) Individual 10: broad nasal ridge and prognathism. (**e**) Individual 7: bulbous nose, low-set and cupped ears. Radiographs show smaller development and/or delayed ossification of the distal phalanges in both hands and feet.
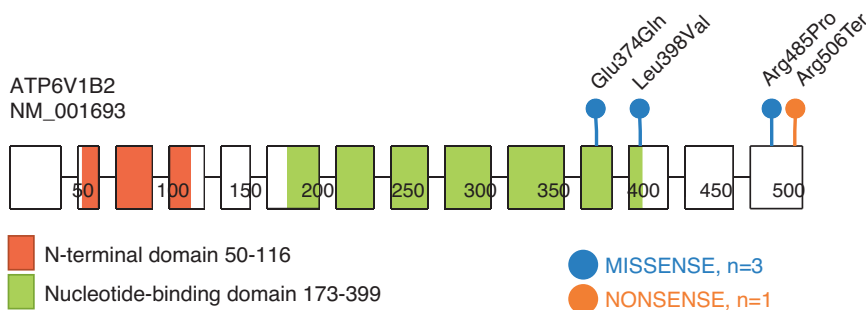
Fig. 2 Location of the known pathogenic variants in _ATP6V1B2_. Figure made using ProteinPaint, introns not drawn to scale, and untranslated regions are not shown. We have reported the p.Arg485Pro variant in Zimmermann–Laband syndrome,[4] the p.Glu374Gln was reported by Popp et al.,[5] and the p.Leu398Val variant by Shaw et al.[6]
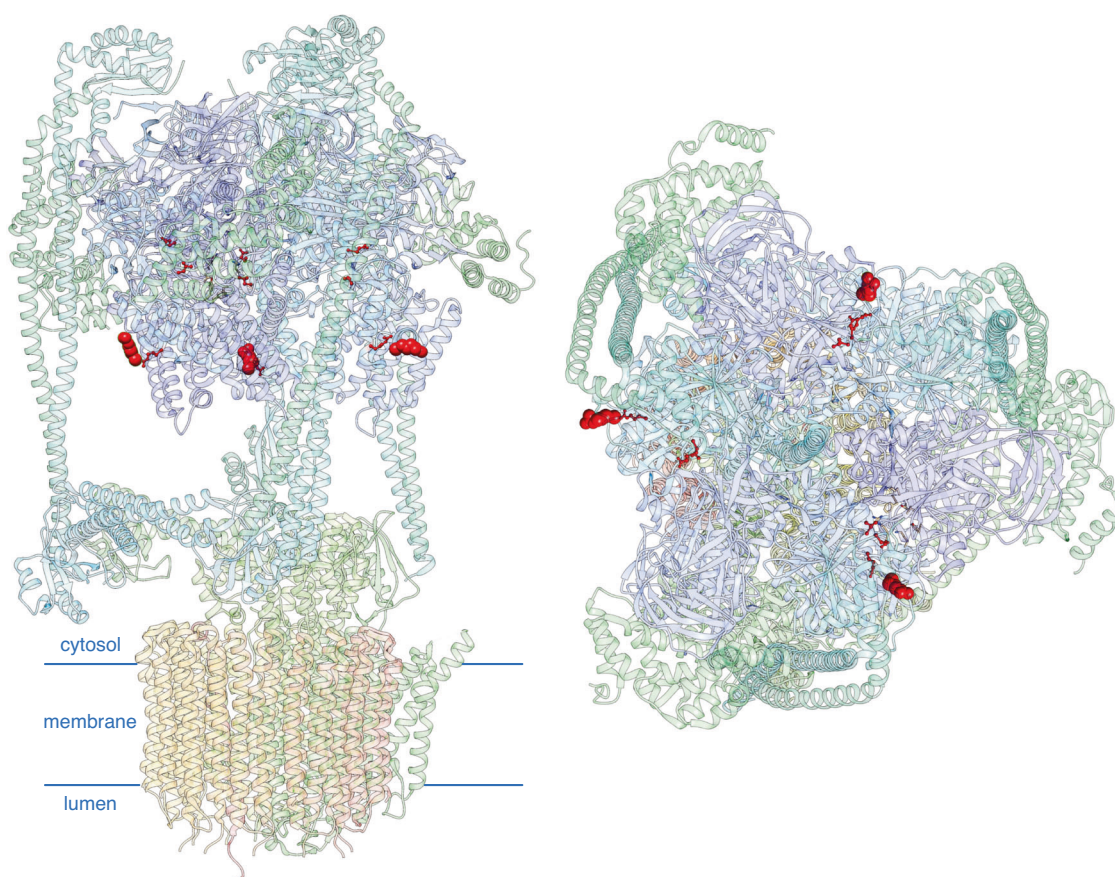
Fig. 3 Location of the variants in V-ATPase. Image made using PDBe entry 6vq8, the fitted atomic model of cryoEM structure of rat brain V-ATPase complex reported recently.[34] The large red spheres are the Arg506 amino acid affected by the nonsense variant (there are three B subunits in the V-ATPase). Smaller red ball and chain amino acids are the amino acids affected by missense variants in other conditions.

each of them. Additionally, two of them had late dentition. An individual from family 2 also had epilepsy, which was diagnosed at age 36. There was no developmental delay or intellectual disability in either family. No dysmorphisms were observed in family 1. Individuals from family 2 were described with midface under development, deep-set eyes, and hypotelorism, and one of them had two symmetrical areas of aplasia cutis on the vertex of the scalp.

Deafness was present in all individuals with DOORS syndrome, along with onychodystrophy and abnormal fingers and/or toes. Eight individuals presented absent or small nails, while another had pachyonychia and koilonychia. Developmental delay and/or intellectual disability was present in seven individuals, ranging from mild to severe. Individuals from family 3 did not have intellectual impairment. However, the father had an episode of focal seizure at age 39. Four other

individuals with DOORS syndrome had seizures. Individual 5 developed tonic–clonic seizures and infantile spasms and individual 6 developed infantile spasms at 12 months of age. Individual 9 presented recurrent tonic–clonic seizures starting at age 52 and individual 10 had tonic–clonic and absence seizures since childhood. Mild dental anomalies, namely late dentition and misalignment, were described in individuals 4 and 5. Some dysmorphic features were shared between individuals. Coarse facies, broad nasal ridge, downslanted palpebral fissures, low-set ears, and prognathism were observed most frequently. Other dysmorphisms include malformed ears, epicanthus, ptosis, high arched palate, long philtrum, prominent fingerpads, thin upper vermilion, synophris, hypertrichosis, and widely spaced teeth. Microcephaly was also present in individual 6. Level of 2-oxoglutarate acid was normal in individuals 4, 5, and 6, and was not assessed in others.

DISCUSSION

We identified a variant in *ATP6V1B2* in families with DOORS syndrome. This variant creates a premature stop codon removing five amino acids and results in a truncated protein. The same variant causes DDOD syndrome and has been shown to impair lysosome acidification.[2]

Clinical findings in our cohort were mostly consistent with previously published patients. Individuals with DDOD syndrome shared sensorineural deafness and onychodystrophy. Regarding digital anomalies, bulbous fingertips of digits, and finger-like thumbs were found in both families, but there was no triphalangeal thumb. Dental anomalies, namely oligodontia and conical teeth, have already been reported,[14,15] but those specific features were not observed among the two families we described. However, late dentition was reported in one.

Individuals with DOORS syndrome exhibited typical findings of sensorineural hearing loss, onychodystrophy, osteodystrophy, and different degrees of cognitive impairment. Both triphalangeal thumb and hypoplastic or absent distal phalanges, which are characteristically found in DOORS syndrome,[1,16] were observed. Facial dysmorphisms in our cohort also overlapped those previously described, predominantly broad nose. Five of nine had seizures, which are reported in most but not all patients with DOORS syndrome and *TBC1D24* variants.[1]

All previously reported patients with DOORS syndrome had an autosomal recessive inheritance while the *ATP6V1B2* variant is associated with an autosomal dominant inheritance, as in DDOD syndrome. Nonetheless, because all families fulfilled clinical criteria for DOORS syndrome instead of DDOD syndrome—with developmental delay or intellectual disability, and seizures as the distinctive features—we considered that they should be designated as having a form of DOORS syndrome.

Variants in *TBC1D24* were previously identified in about half of the individuals with DOORS syndrome. *TBC1D24* encodes a member of the Tre2-Bub2-Cdc16 (TBC) domain-containing RAB-specific GTPase-activating proteins, which regulate Rab proteins and other GTPases playing a role in the transport of intracellular vesicles.[1] TBC1D24 deficiency leads to a defect in neuronal presynaptic endocytosis and an enlarged endosomal compartment resulting in a decreased spontaneous neurotransmission.[17] Similarly, TBC1D24 was shown to be critical for removal and degradation of damaged synaptic vesicle proteins from the synapses.[18,19] By solving the crystal structure of Sky (fly homolog of TBC1D24), a region of the TBC domain was identified that directly associates with phosphoinositides (PI) in the membrane, especially PI(4,5)P_2.[20] In addition, certain *TBC1D24* variants cause an increased sensitivity to oxidative stress.[21] *ATP6V1B2*, in turn, encodes a component of the V-ATPase, which mediates acidification of eukaryotic intracellular organelles and regulates exocytosis of vesicles. Vacuolar ATPases pump protons in cellular vesicles and have key roles in sensing and regulating cellular pH, vesicular transport, endocytosis, and synaptic vesicle loading.[22,23]

Previous studies found that cochlear-specific *Atp6v1b2*-knockdown mice displayed severe sensorineural hearing loss and that *Atp6v1b2* c.1516C>T knock-in mice exhibited behavioral defects without hearing loss, suggesting possible hearing compensation mechanisms in the mice and potential cognitive consequences in individuals with DDOD syndrome,[24] although intellectual disability has not been associated with DDOD syndrome. Interestingly, *TBC1D24*-related syndromes also show a wide clinical spectrum, with findings such as intellectual disability not consistent among individuals.[25] As such, our new clinical description associating the recurrent *ATP6V1B2* variant with DOORS syndrome provides a potentially important missing link between genes known to be associated with overlapping syndromes. TBC1D24 was shown to interact with V-ATPase subunits,[26] and it was also the case for other TLDc domain-containing proteins, OXR1 and NCOA7.[26–29] The recurrent variant identified (p. Arg506*) affects an amino acid located in periphery of the protein (Fig. 3), which could suggest a possible impact on protein–protein interactions.

In summary, we described nine individuals with a clinical diagnosis of DOORS syndrome in whom we identified a heterozygous truncating variant in *ATP6V1B2*. Therefore, we expand the phenotype associated with *ATP6V1B2* and propose another causal gene for DOORS syndrome. This finding suggests that DDOD and DOORS syndromes might be on a spectrum instead of being two distinct conditions. Our data provide new clues to help understand the clinical heterogeneity in DOORS syndrome since both proteins are involved in membrane trafficking events, including synaptic vesicle cycling.[17,30] Importantly, other members of the V-ATPase complex have been associated with neurodevelopmental disorders causing epilepsy.[31–33] Further studies are needed to unravel the functional relationship between TBC1D24 and V-ATPase and the impact of their relationship on neurons and cerebral development.

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