Title: Expanding the phenotype of a truncating *ATP6V1B2* variant to include DOORS syndrome

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABSTRACT

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 no *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 individuals with DOORS syndrome. This variant was already reported in individuals with DDOD (dominant deafness onychodystrophy) 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 described two additional families with DDOD syndrome in which the same variant was found.

Conclusion

We expand the phenotype associated with *ATP6V1B2* and propose another causal gene for DOORS syndrome. This finding might suggest that DDOD and DOORS syndromes are on a spectrum instead of being two distinct conditions.

Key words: DOORS syndrome, DDOD syndrome, ATP6V1B2 gene, TBC1D24 gene, exome sequencing

INTRODUCTION

Biallelic variants in *TBC1D24* (MIM 613577), which encodes a protein that regulates vesicular transport, are identified in half of the patients with DOORS syndrome (MIM 220500); while to date, no causal gene was 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. DDOD (dominant deafness onychodystrophy; 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, nail 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 nails 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.

PATIENTS 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 for each participant to the study or their legal representant. Consents for publication of photos that are included in this article were also obtained.

Participants

Families were identified in a cohort which includes 46 families with DOORS syndrome (based on the presence of 3 of the 5 main features making the syndrome acronym) and 2 with DDOD syndrome recruited in a study led by Dr. Campeau. Families were excluded from this report no variant in *ATP6V1B2* was detected.

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 figure 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 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 both families. No dysmorphisms were observed in family 1. Individuals from family 2 were described with midface hypoplasia, 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 hypoplastic 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 the most frequently. Other dysmorphisms include dysplastic ears, epicanthus, ptosis, high arched palate, long philtrum, prominent fingerpads, thin upper lip, 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, predominately broad nose. Five out of nine had seizures, which is 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 pre-synaptic 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 behavioural 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 the case also 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 (figure 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 might suggest that DDOD and DOORS syndromes are on a spectrum instead of being two distinct conditions. Our data provides 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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FIGURE LEGENDS

Figure 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.

Figure 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 Zimmerman-Laband syndrome,[4] the p.Glu374Gln was reported by Popp et al.,[5] and the p.Leu398Val variant by Shaw et al.[6]

Figure 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.