Short Report

Homozygous and compound heterozygous mutations in the \(ATP6V1B1\) gene in patients with renal tubular acidosis and sensorineural hearing loss


Distal renal tubular acidosis (dRTA) is characterized by the inability to excrete acid in the renal collecting ducts resulting in inappropriately alkaline urine and hyperchloremic (normal anion gap) metabolic acidosis in the context of a normal (or near-normal) glomerular filtration rate. Inborn dRTA can be due to autosomal dominant or recessive gene defects. Clinical symptoms vary from mild acidosis, incidental detection of kidney stones or renal tract calcification to severe findings such as failure to thrive, severe metabolic acidosis, and nephrocalcinosis. The majority of patients with recessive dRTA present with sensorineural hearing loss (SNHL). Few cases with abnormal widening of the vestibular aqueduct have been described with dRTA. Mutations in three different genes have been identified, namely \(SLC4A1\), \(ATP6V1B1\), and \(ATP6V0A4\). Patients with mutations in the \(ATP6V1B1\) proton pump subunit develop dRTA and in most of the cases sensorineural hearing loss early in childhood. We present two patients from two different and non-consanguineous families with dRTA and SNHL. Direct sequencing of the \(ATP6V1B1\) gene revealed that one patient harbors two homozygous mutations and the other one is a compound heterozygous. To our knowledge, this is the first case in the literature describing homozygosity in the same dRTA gene on both alleles.

Conflict of interest

The authors have no conflicts of interest to disclose.

Patients

Case I

The 25-year-old woman was admitted at the age of 4 months to the Hospital because of vomiting and failure to thrive. The patients’ family originates from Kosovo and is not consanguineous (Fig. 1a). Distal RTA was diagnosed and treated with sodium bicarbonate and potassium citrate. At the age of 3.5 years, brain stem evoked response audiometry revealed bilateral SNHL and hearing aids were introduced. She showed neither episodes of kidney stones nor pyelonephritis. At the age of 14 years, she started suffering from occasional attacks of rotatory vertigo. High-resolution computed tomography (HR-CT) and magnetic resonance imaging (MRI) of the temporal bones confirmed enlarged vestibular aqueduct (EVA) syndrome on the left side (Fig. 2). She has two brothers...
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\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{pedigree.png}
\caption{Pedigree of two index patients with familial dRTA and SNHL. Males and females are represented by squares and circles, respectively; filled circles and squares indicate affected individuals; gray shading indicates individuals with no genetic or clinical information available; slash represents deceased individuals from whom DNA was not available. (a) Pedigree of family 1. (b) Pedigree of family 2.}
\end{figure}

Family 1

\begin{tabular}{|c|c|}
\hline
\textbf{I.1} & \textbf{I.2} \\
\hline
\textbf{II.1} & \textbf{II.2} \\
\hline
\textbf{L81P/E161K} & \textbf{L81P/E161K} \\
\hline
\end{tabular}

Family 2

\begin{tabular}{|c|c|}
\hline
\textbf{I.1} & \textbf{I.2} \\
\hline
\textbf{II.1} & \textbf{II.2} & \textbf{II.3} \\
\hline
\textbf{P346R} & \textbf{L81P} & \textbf{L81P} \\
\hline
\end{tabular}

Fig. 1. Pedigree of two index patients with familial dRTA and SNHL. Males and females are represented by squares and circles, respectively; filled circles and squares indicate affected individuals; gray shading indicates individuals with no genetic or clinical information available; slash represents deceased individuals from whom DNA was not available. (a) Pedigree of family 1. (b) Pedigree of family 2.

(32 and 24 years) and one sister (17 years) with no kidney problems (Fig. 1a). The younger brother suffers from insulin-dependent diabetes mellitus.

Case II

Genetic analysis of index patient II was previously published (1). The 21-year-old woman also originates from Kosovo with non-consanguineous parents. Out of six children (five ♀ and one ♂) only our index patient and two sisters are alive. The remaining siblings (one ♂ and two ♀) died at the age of two and a half years, two months, and 4 months, respectively (Fig. 1b). The cause of death is unknown in any of the deceased siblings. The deceased brother was reported to be deaf and both deceased sisters suffered from failure to thrive.

Our patient was hospitalized at the age of 12 months in Zagreb where she was diagnosed with dRTA and SNHL and subsequently treated with potassium sodium citrate. However, she developed nephrocalcinosis (Table S1, Fig. S2). Due to SNHL hearing aids were introduced and she was enrolled at the deaf school. To date, her diuresis is unremarkable, kidney function is normal, and no episodes of kidney stones or urinary tract infections were observed. Her older sister also developed kidney stones.

Results

Blood and urine parameters

Laboratory data are summarized in Tables S1 and S2.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{mri.png}
\caption{(a) Axial magnetic resonance image (0.4 mm) of the temporal bone and labyrinth of index patient I: bilateral enlarged endolymphatic duct (arrow) is depicted with a diameter in the midthird that exceeds the size of the corresponding posterior semicircular canal (short arrow). (b) Three-dimensional reconstruction of the labyrinth showing the enlarged endolymphatic duct and sac (arrow) and bulbous dysplasia of the apical turn of the cochlea (short arrow). Axial high-resolution bone algorithm computed tomography images (0.6 mm) of the right (c) and left (d) temporal bone with enlargement of the bony vestibular aqueducts (long arrow) in comparison to the diameter of the posterior semicircular canal (short arrow).}
\end{figure}
both patients showed a normal anion gap hyperchloric metabolic acidosis (Table S1). Urinary pH was alkaline and urinary-specific gravity was diminished. Urinary calcium to creatinine ratio was elevated in index patient II (Table S1).

Both parents showed no abnormalities in blood pH, bicarbonate, serum electrolyte values, or calcium and phosphate excretion (Table S2). Urinary pH values were normal (Table S2).

Genetic findings

The result of patients’ gene analysis and previously published disease-causing human mutations are shown in Table S3 and Fig. 1.

Family 1

Direct sequencing of the ATP6V1B1 gene detected a homozygous missense mutation in exon 3 c.242T>C (p.Leu81Pro) (Tables S1 and S3, Figs 2a and S1a). A second homozygous variant previously thought to represent a single nucleotide polymorphism (SNP) c.481G>A (p.Glu161Lys) in exon 6 was also found in index patient I (Tables S1 and S3, Figs 2a and S1a). This variant has been shown to impair proton pump assembly and activity in vitro and may therefore represent a pathogenic mutation (2, 3). Additionally, direct sequencing of exons 3 and 6 of the ATP6V1B1 gene showed that both parents are heterozygous for these two mutations (Table S2, Figs 2a and S1a).

Family 2

Genetic analysis of index patient II has been previously published (1) (Tables S1 and S3, Figs 2a and S1b). This patient is compound heterozygous for two missense mutations c.242T>C (p.Leu81Pro) (Tables S1 and S3, Figs 2a and S1a). A second homozygous variant previously thought to represent a single nucleotide polymorphism (SNP) c.481G>A (p.Glu161Lys) in exon 6 was also found in index patient I (Tables S1 and S3, Figs 2a and S1a). This variant has been shown to impair proton pump assembly and activity in vitro and may therefore represent a pathogenic mutation (2, 3). Additionally, direct sequencing of exons 3 and 6 of the ATP6V1B1 gene showed that both parents are heterozygous for these two mutations (Table S2, Figs 2b and S1b).

Audiovestibular findings

Family 1

An audiogram from August 2009 showed a bilateral severe sensorineural hearing loss with complete deafness at 8 and 12 kHz bilaterally and also at 6 kHz on the left ear (Fig. 3a).

The patient has a vestibular hypofunction of 56% of the right lateral semicircular canal in the bilateral bithermal calorific vestibular stimulation test (calorics) but normal gains for the vestibulo-ocular reflex at head impulse testing of all semicircular canals with magnetoculography (search coil technique). Subjective visual vertical and fundus photography calorics indicated a left vestibular hypofunction but all other tests suggested normal vestibular function.

MRI of the temporal bones showed a markedly enlarged endolymphatic duct (Fig. 2a). Bulbous dysplasia of the apical turn of the cochlea is an additional finding characteristic of EVA syndrome (Fig. 2b). CT to better advantage depicts the enlarged bony canal of the vestibular aqueduct (Fig. 2c,d) which is commonly affected bilaterally (90%).

Discussion

We present two patients from two different families with dRTA and SNHL with interesting and uncommon features.

Mutations in ATP6V1B1 gene cause dRTA with or without SNHL (Table S3) (4–14). The majority of these mutations are missense or nonsense mutations. Mutations occur relatively rare in Western populations and are more prevalent in countries with higher rates of parental consanguinity (1, 4, 5). Both patients’ families originated from Kosovo and were not consanguineous and shared one mutation, the p.Leu81Pro on exon 3, suggesting that this mutation may be more common in this region. The same mutation was also previously found in a patient from Macedonia (4) and patients from Cyprus (11, 13). The second mutation in case I, p.Glu161Lys on exon 6, was described before as a SNP (4). The p.Pro346Arg mutation was previously detected in one patient among 62 studied kindreds of Turkish, Spanish, Saudi Arabian, North American, Pakistani, and European origin (5). A recent study found this mutation in patients from various ethnic groups suggesting to be a common pan-ethnic mutation (14).

Genetic analysis of index patient I revealed an uncommon result. She had two homozygous mutations which she had from each parent. Thus segregation analysis confirmed that both mutations are on the same allele. To our knowledge, this is the first reported case in the literature showing two homozygous mutations in the ATP6V1B1 gene. Index patient II presented with a compound mutation in the ATP6V1B1 gene. Only few dRTA patients with compound heterozygous mutations have been reported (7, 9, 11–13). Elia et al. detected compound heterozygous mutations in the ATP6V1B1 gene in six members of three families from Cyprus and one Greek family. All affected individuals suffered from SNHL, dRTA, and bilateral nephrocalcinosis (13). Other compound heterozygous mutation were found in a Spanish child, and a patient from Japan with dRTA, SNHL and EVA (9, 12). EVA was present in one of our patients, however, none of the mutations detected in the Japanese patient were found in our patient. To date, only four patients with dRTA and EVA have been reported (6, 12, 15, 16). These patients showed either homozygous or compound heterozygous mutations (6, 12). None of the heterozygous relatives showed any signs of vestibular dysfunction such as vertiginous attacks. Thus, homozygous or compound heterozygous mutations in ATP6V1B1 may be responsible for development of EVA.

ATP6V1B1 encodes the B1-subunit of the vacuolar H+-ATPase and is expressed only in few organs and cells. A mouse model deficient for this gene has been generated and shown to develop incomplete distal renal
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Fig. 3. (a) Audiogram of index patient family 1. A combined hearing loss with air bone gaps in the lower to medium frequencies is seen bilaterally. The air bone gap on the right at 0.25, 0.5 and 1 kHz is 55, 30 and 10 db, respectively, and on the left of 55 db at 250 Hz and of 15 db at 1 kHz. At 500 Hz, bone conducted sound is not heard at the maximal frequency of 65 db (indicated by the downwards directed arrow) so the air bone gap is below 20 db. At 2 and 4 kHz, no air bone gap is detectable. (b) Audiogram of index patient family 2. A symmetric combined hearing loss is seen ascending from 55/60 db right/left at 125 Hz to 110 db bilaterally at 4 kHz. At all frequencies, the air bone gap is between 40 and 50 db bilaterally. Circle or × indicate unmasked examination of air conduction, triangle or square indicate masked examination of air conduction, < or > indicate unmasked bone conduction, [or] indicate masked bone conduction.

In conclusion, we present the first patient with two homozygous mutations in the ATP6V1B1 gene and dRTA, SNHL and vertiginous attacks, caused by EVA. Patients with dRTA and vertigo should undergo appropriate examination for EVA. We also describe a patient with a compound heterozygous mutation in the ATP6V1B1 gene where three family members deceased at early childhood and all other tested family members have been identified as heterozygous carriers with no phenotype for dRTA. The detection of the p.Leu81Pro mutation in both patients from Kosovo, Macedonia, and a patient from Greece suggests that this mutation may be of higher prevalence in this region.

Supporting Information

The following Supporting information is available for this article: Fig. S1. Mutations in ATP6V1B1 in patients and their relatives. Control sequence shows an unrelated unaffected individual. (a) Sequencing data showed one homozygous ATP6V1B1 missense mutation in exon 3 (c.242T>C, p.Leu81Pro) and a second homozygous variant in exon 6 (c.481G>A, p.Glu161Lys) in index patient family 1. Direct Sequencing of exons 3 and 6 of the ATP6V1B1 gene showed that both parents are heterozygous for the mutation c.242T>C (p.Leu81Pro) in exon 3 and c.481G>A (p.Glu161Lys) in exon 6, respectively. (b) Heterozygous ATP6V1B1 missense mutations in exon 3 (c.242T>C, p.Leu81Pro) were detected in one sister and the mother of the index patient family 2. The father and another sister showed a heterozygous mutation in exon 10 (c.1037C>G, p.Pro346Arg).

Fig. S2. Ultrasound examination of the right (a) and left (b) kidney of index patient II. Typical signs of nephrocalcinosis (hyperechoic renal medullary pyramids) are shown by ultrasound.

Table S1. Biochemical and clinical data of index patients.

Table S2. Biochemical and clinical data of patients’ family members.

Table S3. Published disease-causing human mutations in ATP6V1B1

Appendix S1. Methods.

Additional Supporting information may be found in the online version of this article.

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