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LABORATORY STUDY

ATP6V1B1 mutations in distal renal tubular acidosis and sensorineural hearing loss: clinical and genetic spectrum of five families

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Abstract

Distal renal tubular acidosis (DRTA) is characterized by tubular defects in urinary acidification and hyperchloremic metabolic acidosis, hypokalemia, hypercalciuria, hypocitraturia, nephrocalcinosis and nephrolithiasis. Mutations in *ATP6V1B1* cause DRTA associated with sensorineural hearing loss. The objective of this multicenter study is to screen DRTA patients with sensorineural hearing loss for *ATP6V1B1* gene mutations and present genotype/phenotype correlation. Clinical data in five unrelated consanguineous families with DRTA and hearing loss were obtained in Turkey. For mutation screening, all coding exons of *ATP6V1B1* were PCR-amplified and sequenced from genomic DNA. In our cohort of five families, there were four different homozygous *ATP6V1B1* mutations in affected individuals: c.91C>T (p.R31X), c.232G>A (p.G78R), c.497delC (p.T166RfsX9) and c.1155dupC (p.I386HfsX56). Our study shows that rare and family-specific variants in *ATP6V1B1* are responsible for DRTA and sensorineural hearing loss syndrome in Turkey. While firm genotype–phenotype correlations are not available, detailed clinical and molecular analyses provide data to be used in genetic counseling.

Keywords

ATP6V1B1 gene, distal renal tubular acidosis, sensorineural hearing loss, failure to thrive, molecular analyses

History

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Introduction

Distal renal tubular acidosis (DRTA) (MIM 267 300) is caused by defective secretion of H^+ ions by collecting tubule cells, which results in defects in urinary acidification. The disease is characterized by an elevation in urinary pH, despite the presence of systemic acidosis. Delayed growth, systemic acidosis with hyperchloremia, hypokalemia, hypocitraturia and hypercalciuria with nephrocalcinosis are also seen.[1] Both autosomal recessive and dominant patterns of transmission have been observed. Mutations in the *ATP6V1B1* gene encoding the B1 subunit of vacuolar H-ATPase result in autosomal recessive DRTA associated with sensorineural hearing loss (SNHL).[2] The aim of this study was to identify *ATP6V1B1* mutations in a cohort of Turkish patients with DRTA and SNHL. Here, we report on five unrelated families with DRTA and SNHL that showed four different *ATP6V1B1* mutations.

Materials and methods

Subjects

For each affected individual, a detailed clinical examination was obtained including otologic evaluations, renal and kidney ultrasonography, arterial blood gas with electrolytes, kidney and liver function tests and routine blood and urine tests. All patients were diagnosed with DRTA based on clinical and laboratory findings (Table 1). Diagnosis of sensorineural hearing loss was established via standard audiometry. Audiograms of the probands showed that the hearing loss was severe in all affected individuals. Anamnestic evaluation and/or serial audiogram indicated that all of the patients had congenital/early onset hearing loss. Similar histories were present in elder siblings in families 631 and 423 who passed away when they were nearly 1 year old but were not investigated. Family members of affected individuals also received regular clinical evaluations to detect any symptoms that may be potentially associated with the disease or heterozygosity for the disorder. This study was approved by Ankara University Ethics Committee and by the Institutional Review Board at the University of Miami. A signed informed consent was obtained from each participant or a parent.

Mutation analysis

The 14 coding exons of *ATP6V1B1* (NM_001692.3) were PCR amplified using primer sets designed by the primer 3.0 software (Cambridge, MA). The list of primers is available upon request. PCR reactions were run in 25 µL volume applying a touch-down protocol and annealing temperatures between 65 °C and 57 °C. PCR products were visualized on

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Table 1. Clinical and laboratory findings of the five probands with DRTA and hearing loss.

	153–101	423–101	631–101	642–101	953–101
Age at last control (years)	14	16	16	12	16
Age at diagnosis (months)	1	2.5	1.5	10	2
Sex	Male	Male	Female	Male	Female
Birth weight (g)	2850	4000	2730	3500	2000
Presenting clinical manifestations	Weakness, hypotonicity, dehydration	Weakness, hypotonicity	Vomiting, dehydration	Growth and developmental delay, constipation	Weakness, hypotonicity
Weight SDS at last control	−3.664	−1.243	−1.363	−1.369	−1.746
Height SDS at last control	−3.914	−2.071	−2.170	−1.189	−2.562
Medullary nephrocalcinosis	+	+	+	+	+
Severe sensorineural deafness	+	+	+	+	+
Biochemical findings at diagnosis[1]					
Blood pH (7.35–7.45)	7.10	7.30	7.30	7.23	7.27
Urine pH (5–7)	7	8	7	7	6.5
Serum creatinine (0.5–1.4 mg/dL)	0.5	0.6	0.7	0.5	1.1
Sodium (135–147 mEq/L)	140	138	130	147	143
Potassium (3.5–5 mEq/L)	1.9	1.9	2.2	1.8	2.6
Bicarbonate (23–29 mEq/L)	8	17	15	11	15
Serum anion gap[2] (6–14 mEq/L)	10	12	11	13	10
Phosphate (2.4–4.1 mg/dL)	3	3	2.8	3.5	2.4
Alkaline phosphatase (38–126 U/L)	15	13	15	16	16
Mutation	c.91C>T (p.R31X)	c.232G>A (p.G78R)	c.497delC (p.T166RfsX9)	c.1155dupC (p.I386HfsX56)	c.1155dupC (p.I386HfsX56)

1. Normal range is given in parenthesis; 2. Serum anion gap = $Na^+ - (Cl^- + HCO^-)$; SDS, standard deviation score; N/A, not available.

agarose gels, cleaned over Sephadex columns followed by BigDye reactions performed according to the manufacturer's recommendations (Applied Biosystems Inc., Foster City, CA). A DNA Sequencer (ABI 3730xl) (Applied Biosystems Inc., Foster City, CA) was used to detect mutations. Results were visualized with the Sequencher 4.7 software (Gene Codes Corporation, Ann Arbor, MI).

Results

Details of clinical findings in five probands are shown in Table 1. Parents were first cousins in all five families. Laboratory evaluations in unaffected family members were normal. DNA sequencing showed four previously reported *ATP6V1B1* mutations (Figure 1). All mutations were homozygous in affected children and co-segregated as a fully penetrant autosomal recessive trait in each family. Identified mutations were c.91C>T (p.R31X) in Family 153, c.232G>A (p.G78R) in Family 423, c.497delC (p.T166RfsX9) in Family 631 and c.1155dupC (p.I386HfsX56) in Families 642 and 953 (Figure 1).

Discussion

Nance and Sweeney demonstrated for the first time that DRTA with SNHL was a distinct entity with autosomal recessive inheritance.[3] *ATP6V1B1* (MIM 192 132) mutations have subsequently shown to cause DRTA with SNHL syndrome.[4] Patients with *ATP6V1B1* mutations usually present at an early age (the majority was diagnosed by age 1 year) with either dehydration and vomiting, or with failure to thrive and/or delayed growth. The clinical diagnosis is based on inappropriately high urinary pH despite the presence of serum acidosis with normal anion gap, evidence of hypokalemia and no evidence of secondary causes of DRTA. While hypercalciuria with nephrocalcinosis is seen in almost all cases, renal function usually remains normal with proper and early treatment. Bilateral progressive sensorineural hearing loss ranging from mild to profound is present in the majority of patients.

ATP6V1B1 encodes the B1 subunit of the apical proton pump, mediating distal nephron acid secretion.[4] The H^+-adenosine triphosphatase (ATPase) in the apical border of the intercalated cell of the distal nephron has a multisubunit structure that plays a role in pumping H^+ into the tubular lumen.[5] To date, 26 *ATP6V1B1* mutations have been determined as the cause of the disease.[4,6–13] These include missense, nonsense, deletion, insertion and splice site changes, all of which are predicted to disrupt the structure or abrogate the production, of the normal B1 subunit protein. This leads to loss of expression of the gene in the distal tubules as well as in cochlea and endolymphatic sac. Active proton secretion is required to maintain proper endolymph pH and normal auditory function.[2] While the *ATP6V1B1* knockout mouse model has been generated and was shown to develop incomplete DRTA, it has normal ear morphology and function, making the pathophysiology of SNHL somewhat unclear.[14] The *ATP6V0A4* gene encodes a 4-subunit portion of the H^+-ATPase in which mutations may also cause DRTA. Studies to date have shown that mutations in *ATP6V1B1*

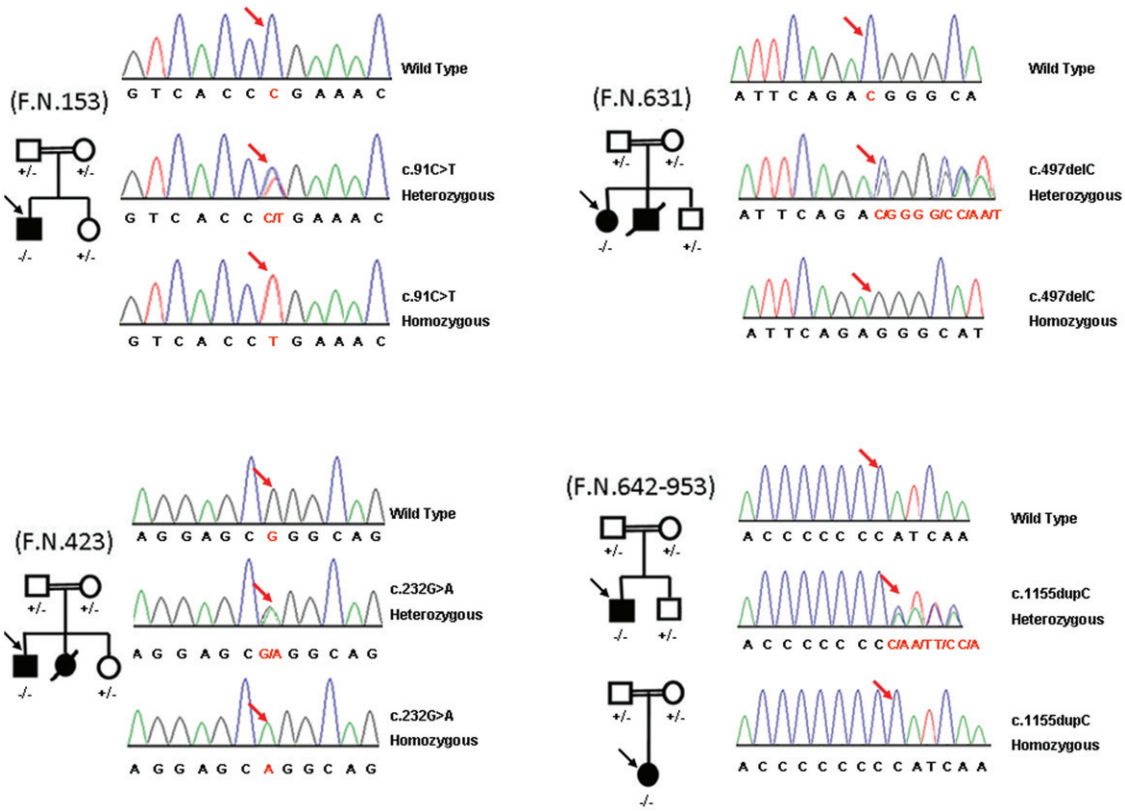

Figure 1. Pedigrees along with segregation of identified mutations. Electropherograms of each mutation is provided.

cause DRTA with SNHL, whereas mutations in *ATP6V0A4* cause DRTA with either normal hearing or later onset hearing loss.[4,12,15]

The C to T transition at nucleotide position 91 (c.91C>T) found in family 153 results in a stop codon at amino acid 31 (p.R31X). Homozygous p.R31X was originally reported in three unrelated families from Turkey and Spain, but clinical aspects of these families were not reported.[4] In a subsequent report, two children with the homozygous p.R31X mutation had features of DRTA with failure to thrive, polyuria, refractory rickets, hypokalemia and nephrocalcinosis as well as severe SNHL.[2] Clinical features in our study similarly show severe and early presentation of kidney and ear findings. And also severe growth deficiency can be due to non-compliance with the treatment.

The c.1155dupC (p.I386HfsX56) mutation was found in two unrelated families in this study. This mutation was initially reported in North Africa, Saudi Arabia and also in Sicily, suggesting a founder effect.[8] The presence of the same mutation in two unrelated families in Turkey supports the idea of a founder mutation that spread out with population movements. It also suggests that c.1155dupC is relatively common.

The p.G78R mutation was first described in two Turkish families.[7] Clinical findings in the original report included four affected children with DRTA and SNHL. Although all four cases showed clinical findings of DRTA, three patients manifested radiographic changes of osteopetrosis. Subsequently osteopetrosis was found to be the result of a homozygous deletion in *TCIRG1*, which encodes an osteoclast specific isoform of subunit a of the H^+-ATPase, while the DRTA was associated with a homozygous mutation in *ATP6V1B1*.[7]

The c.497delC (p.T166RfsX9) mutation that was found in family 631 is a single base-pair deletion at codon 166 causing a frame shift and resulting in premature termination at codon 174. It was first reported in a 1-month-old boy who had hearing loss and laboratory findings compatible with DRTA.[4] This mutation was also later identified in a consanguineous family from Saudi Arabia.[8]

We conclude that while genotype–phenotype correlations for *ATP6V1B1* mutations are not clearly established, detailed clinical and molecular analyses help in clinical setting for predicting outcome of patients with the same mutation. The aims of the treatment of DRTA must be to correct biochemical abnormalities, improve growth and prevent nephrocalcinosis that can lead to chronic renal failure and skeletal abnormalities. While early treatment corrects the biochemical abnormalities and helps to foster normal growth, deafness appears not to be prevented. Thus, early diagnosis of SNHL is important to improve speech, intellectual development and social communication skills of the affected children.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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References

1. Sharifian M, Esfandiar N, Mazaheri S, et al. Distal renal tubular acidosis and its relationship with hearing loss in children: preliminary report. *Iran J Kidney Dis.* 2010;4:202–206.
2. Sethi SK, Singh N, Gil H, Bagga A. Genetic studies in a family with distal renal tubular acidosis and sensorineural deafness. *Indian Pediatr.* 2009;46:425–427.
3. Nance WE, Sweeney A. Evidence for autosomal recessive inheritance of the syndrome of renal tubular acidosis with deafness. *Birth Defects Orig Art Ser.* 1971;7:70–72.
4. Karet FE, Finberg KE, Nelson RD, et al. Mutations in the gene encoding B1 subunit of H(+)-ATPase cause renal tubular acidosis with sensorineural deafness. *Nature Genet.* 1999;21:84–90.
5. Andreucci E, Bianchi B, Carboni I, et al. Inner ear abnormalities in four patients with dRTA and SNHL: clinical and genetic heterogeneity. *Pediatr Nephrol.* 2009;24:2147–2153.
6. Hahn H, Kang HG, Ha IS, Cheong HI, Choi Y. ATP6B1 gene mutations associated with distal renal tubular acidosis and deafness in a child. *Am J Kidney Dis.* 2003;41:238–243.
7. Borthwick KJ, Kandemir N, Topaloglu R, et al. A phenocopy of CAII deficiency: a novel genetic explanation for inherited infantile osteopetrosis with distal renal tubular acidosis. *J Med Genet.* 2003; 40:115–121.
8. Stover EH, Borthwick KJ, Bavalia C, et al. Novel ATP6V1B1 and ATP6V0A4 mutations in autosomal recessive distal renal tubular acidosis with new evidence for hearing loss. *J Med Genet.* 2002;39: 796–803.
9. Gil H, Santos F, García E, et al. Distal RTA with nerve deafness: clinical spectrum and mutational analysis in five children. *Pediatr Nephrol.* 2007;22:825–828.
10. Vargas-Poussou R, Houillier P, Le Pottier N, et al. Genetic investigation of autosomal recessive distal renal tubular acidosis: evidence for early sensorineural hearing loss associated with mutations in the ATP6V0A4 gene. *J Am Soc Nephrol.* 2006;17: 1437–1443.
11. Ruf R, Rensing C, Topaloglu R, et al. Confirmation of the ATP6B1 gene as responsible for distal renal tubular acidosis. *Pediatr Nephrol.* 2003;18:105–109.
12. Yashima T, Noguchi Y, Kawashima Y, Rai T, Ito T, Kitamura K. Novel ATP6V1B1 mutations in distal renal tubular acidosis and hearing loss. *Acta Otolaryngol.* 2010;130:1002–1008.
13. Elia A, Voskarides K, Demosthenous P, et al. Founder mutations in the ATP6V1B1 gene explain most Cypriot cases of distal renal tubular acidosis: first prenatal diagnosis. *Nephron Clin Pract.* 2011; 117:206–212.
14. Dou H, Finberg K, Cardell EL, Lifton R, Choo D. Mice lacking the b1 subunit of h+-atpase have normal hearing. *Hear Res.* 2003;180: 76–84.
15. Nikali K, Vanegas JJ, Burley MW, et al. Extensive founder effect for distal renal tubular acidosis (dRTA) with sensorineural deafness in an isolated South American population. *Am J Med Genet A.* 2008;146:2709–2712.