CASE REPORT

# Genetic Studies in a Family with Distal Renal Tubular Acidosis and Sensorineural Deafness

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Distal renal tubular acidosis (RTA) with sensorineural deafness is a rare entity, inherited in an autosomal recessive manner. It is caused by mutations in the *ATP6V1B1* gene, leading to defective function of $H^+$-ATPase pump in the distal nephron, cochlea and endolymphatic sac. We report two siblings with distal RTA and sensorineural deafness having mutation C>T in the first coding exon of the gene, resulting in a non functional protein. The parents were found to be carriers for the mutation.

**Key words:** *ATP6V1B1* gene, *R*enal tubular acidosis, Sensorineural deafness.

Distal renal tubular acidosis (RTA) is characterized by impaired urine acidification leading to severe hyperchloremic metabolic acidosis, hypokalemia, hypercalciuria, hypocitraturia, nephrocalcinosis and nephrolithiasis. The disease is characterized by failure to thrive, nephrocalcinosis, polyuria and urolithiasis. In untreated cases, the progression of nephrocalcinosis may lead to chronic renal failure(1-3). Mutations in the *ATP6V1B1* gene, encoding the B1 subunit of vacuolar $H^+$-ATPase, result in autosomal recessive distal RTA associated with nerve deafness (OMIM #267300). Genetic screening in multiple kindreds show different mutations in the gene and almost all affected individuals have sensorineural hearing loss. The majority of these mutations are likely 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 human cochlea and in endolymphatic sac epithelium, in addition to the renal tubular defect(1,4).

We report two siblings with distal RTA and sensorineural hearing loss having mutation in the first coding exon of the *ATP6V1B1* gene. Their parents showed the same mutation in a heterozygous state.

## CASE REPORT

***Patient* 1.** This 3-yr-old girl, born of non-consanguineous marriage to a north-Indian Hindu family, presented with complaints of polyuria, polydipsia, failure to thrive and bony deformities. Similar history was present in an elder sibling who passed away at 1-yr of age, but was not investigated. The parents were apparently normal. Investigations showed metabolic acidosis (pH 7.08, bicarbonate 7.8 mEq/L) with plasma anion gap of 10 mEq/L; serum potassium levels ranged between 2.7-3.2 mEq/L ***(Table* I)**. Urine pH was 6.5, urine anion gap was positive and there was evidence of hypercalciuria (urine calcium to creatinine ratio between 0.7-0.9 on multiple occasions). Following bicarbonate loading, the fractional excretion of bicarbonate was 7.2% and difference between urinary to plasma $CO_2$ (U-P $CO_2$) was 1.7 mm Hg. Ultrasonography of abdomen showed bilateral medullary nephrocalcinosis. Pure tone audiometry showed severe sensorineural nerve deafness.

**TABLE I** BIOCHEMICAL AND GENETIC FEATURES*

| | Patient 1 | Patient 2 |
|---|---|---|
| Serum creatinine (mg/dL) | 0.4 | 0.7 |
| Sodium; potassium (mEq/L) | 138; 3.1 | 136; 3.1 |
| pH; bicarbonate (mEq/L) | 7.08; 7.8 | 7.16; 8.0 |
| Anion gap (mEq/L) | 10.0 | 11.4 |
| Calcium; phosphate (mg/dL) | 9.4; 3.2 | 9.5; 3.0 |
| Alkaline phosphatase (U/L) | 680 | 800 |
| Urine pH; anion gap (mEq/L) | 6.50; 11.2 | 6.12; 12.8 |
| Calcium/creatinine ratio (mg/mg) | 0.9 | 2.2 |
| Fractional excretion of bicarbonate | 7.2% | 6.8% |
| Urine - plasma $pCO_2$ difference, (mm Hg) | 1.7 | -4.6 |
| Mutation in exon 1: C>T (R31X) | Both siblings were homozygous, parents heterozygous | |

** Both patients showed a severe growth retardation with height and weight z-scores (WHO 2007)[8] less than -3, severe sensorineural deafness and nephrocalcinosis.*

***Patient* 2.** This 1-year old younger sibling of the first patient also presented with similar history of polyuria, polydipsia, failure to thrive and bony deformities. Investigations showed metabolic acidosis with plasma anion gap of 11.4 mEq/L; serum potassium levels ranged between 3.0-3.2 mEq/L ***(Table* I)**. Urine pH was 6.12, urine anion gap was positive and there was evidence of hypercalciuria (calcium to creatinine ratio ranging between 1.8-2.2 on multiple occasions). Following bicarbonate loading, the fractional excretion of bicarbonate was 6.8% and U-P $CO_2$ was -4.6 mm Hg. Ultrasonography of abdomen also showed bilateral medullary nephrocalcinosis and audiometry showed severe sensorineural deafness.

A diagnosis of familial distal RTA with sensorineural deafness was made. Both patients were treated with Polycitra-K to provide 8 mEq/kg/day bicarbonate, and were provided aural rehabilitation with hearing aids and speech therapy.

***ATP6VIBI* gene sequencing:** After informed consent, DNA was extracted from the peripheral blood from the patients and their parents. The 14 coding exons of *ATP6V1B1* gene were amplified by polymerase chain reaction using the primers described previously(1). The amplified fragments were purified and sequenced on an automated ABI310 system, using BigDye chemistry (Applied Biosystems-Applera Corporaton, Drive Foster City, CA). Sequencing of the coding exons and the intronic flanking region showed DNA mutation at exon 1: C>T (R31X). Both siblings were homozygous, while parents were heterozygous for the mutation ***(Fig.*1)**.

## DISCUSSION

Both patients in the present report had features of distal RTA with failure to thrive, polyuria, refractory rickets, hypokalemia and nephrocalcinosis. They also showed severe sensorineural deafness requiring rehabilitation. Investigations confirmed the diagnosis of secretory distal RTA and homozygous mutations were found in the *ATP6V1B1* gene in both cases.

Most cases of primary distal RTA in children result from defective function of the proton pump vacuolar $H^+$-ATPase, located at the apical surface of the $\alpha$-intercalated cells. The vacuolar $H^+$-ATPase is

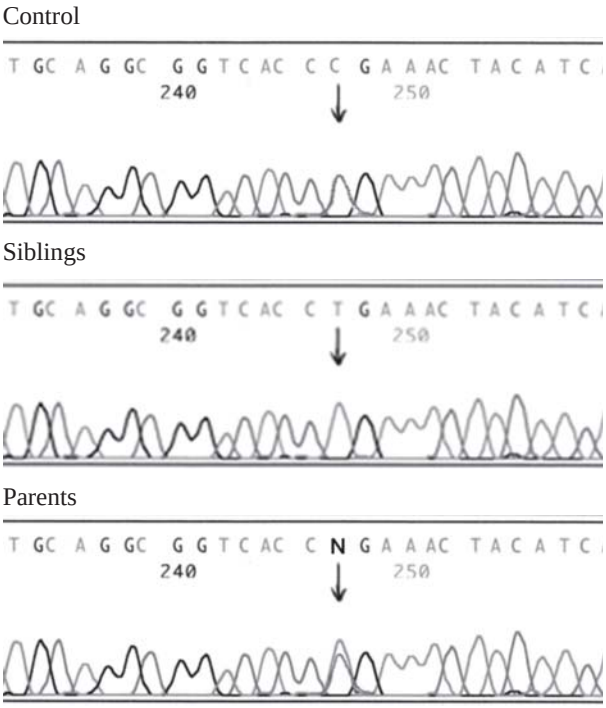


**FIG.*1*** *The electrophoregrams show the exon 1 sequences of the ATP6V1B1 gene of a normal control and the family. Both siblings were homozygous for the mutation exon 1: 91 C>T, and the both parents were carriers for the same mutation.*

formed by several subunits. Mutations in the *ATP6V0A4* gene, which encodes for the a4 subunit, cause autosomal recessive dRTA (OMIM # 602722)(3-5). *ATP6V1B1,* a gene on chromosome 2, encodes for the B1-subunit of the vacuolar $H^+$-ATPase. *ATP6V1B1* is also expressed in the human cochlea and in endolymphatic sac epithelium(4). Endolymph is a unique extracellular fluid having low sodium and high potassium concentrations, which maximizes the sensitivity of hair cells. To preserve its pH at 7.4, there is presumably a requirement for proton pumping into endolymph. It is assumed that $H^+$-ATPase contributes to maintenance of endolymph pH and defects in its B1 subunit cause irreversible hair cell damage because of abnormalities in electrolyte and pH(6).

Apart from the presence or absence of hearing loss, there do not appear to be major phenotypic differences at diagnosis between patients with homozygous *ATP6V1B1* and *ATP6V0A4* mutations. Long term follow-up of a cohort of affected individuals with *ATP6V0A4* mutations shows mild and/or older-onset hearing impairment in some patients who were initially considered to have normal hearing by audiometry(3).

Both siblings in this report were homozygous for a known mutation in exon 1:C>T (R31X) in the *ATP6V1B1* gene, as previously reported by Karet, *et al.*(4). This change introduces a termination codon at 31 position, resulting in a non functional protein(4). Systemic alkali therapy, although correcting systemic pH, fails to prevent progressive hearing loss in patients. It is important to note that after 3 years of follow-up of our kindred, the sensorineural hearing deficits still persist. Failure of alkali treatment to address the abnormal endolymphatic physiology is believed to account for the progressive hearing loss(4,7). Genetic evaluation of the affected family has important implications for genetic counseling and understanding the pathogenesis of the rare association.

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## REFERENCES

1. Gil H, Santos F, García E, Alvarez MV, Ordóñez FA, Málaga S, *et al*. Distal RTA with nerve deafness: clinical spectrum and mutational analysis in five children. Pediatr Nephrol 2007; 22: 825-828.
2. Laing CM, Toye AM, Capasso G, Unwin RJ. Renal tubular acidosis: developments in our understanding of the molecular basis. Int J Biochem Cell Biol 2005; 37: 1151-1161.
3. Karet FE. Inherited distal renal tubular acidosis. J Am Soc Nephrol 2002; 13: 2178-2184.
4. Karet FE, Finberg KE, Nelson RD, Nayir A, Mocan H, Sanjad SA, *et al*. Mutations in the gene encoding B1 subunit of *$H^+$-ATPase* cause renal tubular acidosis with sensorineural deafness. Nat Genet 1999; 21: 84-90.
5. Stover EH, Borthwick KJ, Bavalia C, Eady N, Fritz DM, Rungroj N, *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.
6. Sterkers O, Saumon G, Tran Ba Huy P, Ferrary E, Amiel C. Electrochemical heterogeneity of the cochlear endolymph: Effect of acetazolamide. Am J Physiol 1984; 246: F47–53.
7. Rodriguez-Soriano J, Vallo A, Castillo G, Oliveros R. Natural history of primary distal renal tubular acidosis treated since infancy. J Pediatr 1982; 101: 669-676.
8. World Health Organisation. Child growth standards. Available from: URL: http://www.who.int/nutrition/media_page/en/. Accessed on 30 April 2008.