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MAIN ARTICLE

Investigation of *ATP6V1B1* and *ATP6V0A4* genes causing hereditary hearing loss associated with distal renal tubular acidosis in Iranian families

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Abstract

Background: Hearing defects are the most common sensory disorders, affecting 1 out of every 500 newborns. *ATP6V1B* mutations are associated with early sensorineural hearing loss, whereas *ATP6V0A4* mutations are classically associated with either late-onset sensorineural hearing loss or normal hearing. *ATP6V1B1* and *ATP6V0A4* genetic mutations cause recessive forms of distal renal tubular acidosis.

Method: Ten unrelated deaf Iranian families with distal renal tubular acidosis were referred to the Genetics Research Centre, University of Social Welfare and Rehabilitation Sciences, Tehran. All exons of the *ATP6V1B1* and *ATP6V0A4* genes were sequenced in affected family members.

Results: We identified a previously reported *ATP6V1B1* frameshift mutation (P385fsX441) in two families and a nucleotide substitution in exon 10 (P346R) in three families. In addition, one patient was homozygous for a novel nucleotide substitution in exon 3.

Conclusion: *ATP6V1B1* genetic mutations were detected in more than half of the families studied. Mutations in this gene therefore seem to be the most common causative factors in hearing loss associated with distal renal tubular acidosis in these families.

Key words: Distal Renal Tubular Acidosis; Hearing Loss, Sensorineural; DNA Sequence; Mutation

Introduction

Hearing loss, one of the most common sensorineural disorders (affecting 1 in 500 births), is a highly heterogeneous trait. It can be caused by genetic (approximately 50 per cent), environmental or idiopathic factors. In most cases, hereditary hearing loss is monogenic.[1] It can display autosomal recessive, autosomal dominant, X-linked or mitochondrial inheritance. Autosomal recessive non-syndromic hearing loss is the most frequent type of hearing loss (approximately 80 per cent of cases).[1,2] Seventy per cent of hearing loss cases have no other features (non-syndromic). Of the remaining 30 per cent, hearing loss is accompanied by other physical features (syndromic). Approximately 400 types of syndromic hearing loss are now recognised.[3] Syndromic and autosomal dominant hearing loss may cause conductive, sensorineural or mixed hearing loss, whereas prelingual non-syndromic and autosomal recessive hearing loss are almost always sensorineural.[4] In the Iranian population, where there is a high rate of consanguineous marriage,[5] autosomal recessive disorders such as hearing loss are some of the most common disabilities, affecting 1 in every 166 persons.[2]

Syndromic hearing impairment associated with distal renal tubular acidosis is caused by a defect in acid–base homeostasis in kidney α-intercalated cells and inner-ear endolymphatic sacs. In the kidney, pH abnormalities lead to low serum potassium (K^+), elevated urinary calcium (Ca^{2+}), nephrocalcinosis (renal deposition of calcium salts), and a normal or near-normal anion gap resulting from hyperchloraemic acidosis. Impaired growth, rickets or osteomalacia are also seen in some patients.[6,7]

Vacuolar H^+-adenosine triphosphatase (ATPase) is a multisubunit adenosine triphosphate-dependent proton pump with two domains: a cytoplasmic V1 domain that hydrolyses ATP and a transmembrane V0 domain that translocates protons. This proton pump acidifies intracellular compartments or transports protons (H^+) across the plasma membrane. It is also responsible for the acidification of several

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intracellular organelles.[8] In 1999, Karet and coworkers showed that different mutations in the B1 and a4 subunits encoded by the *ATP6V1B1* gene on chromosome 2p13 and the *ATP6V0A4* gene on chromosome 7q33–34, respectively, are responsible for distal renal tubular acidosis with sensorineural hearing loss (SNHL).[9–11] For some subunits, such as B1 and a4, expression is cell type specific.[12] We present a case study in which 6 out of 10 unrelated Iranian consanguineous families carry different types of mutation in the *ATP6V1B1* gene.

Materials and methods

We investigated 10 unrelated consanguineous Iranian families with prelingual hearing loss varying in severity from moderate to profound. All families were referred to us by the Ali Asghar Children's Hospital, a referral centre for paediatric nephrological disorders in Tehran. Audiometric tests demonstrated impaired hearing in all patients. Distal renal tubular acidosis diagnosis was based on metabolic acidosis with a normal anion gap and minimum urinary pH greater than 5.5, nephrocalcinosis and/or nephrolithiasis associated with hypercalciuria, low K^+ and low urinary ammonium.

Written informed consent was received from all patients and the study was approved by the ethics committee at the University of Social Welfare and Rehabilitation Sciences. First, DNA from 10 probands from 10 families was subjected to Sanger sequencing (ABI 3130 Automated Capillary DNA Sequencer) to investigate possible *ATP6V1B1* mutations. If no mutation was identified in the *ATP6V1B1* gene, then the *ATP6V0A4* gene was sequenced. All sequencing data were analysed using CodonCode Aligner version 4.0.4 software (CodonCode, Centerville, Massachusetts, USA).

Results

Based on family history and clinical presentation, all 10 consanguineous families were classified as having autosomal recessive distal renal tubular acidosis associated with hearing impairment. *ATP6V1B1* gene mutations were identified in a total of 10 affected individuals from 6 of the 10 families (Table I): 5 girls and 5 boys, aged from 5 to 10 years. The P385fsX441 frameshift mutation was identified in two families (Table 1, Figure 1); this mutation modifies the encoded protein. A 1037C > G (P346R) substitution in exon 10 was identified in three families (Table 1, Figure 2). A novel nucleotide substitution (233G > C in exon 3), leading to a known amino acid substitution (G78R) was identified in a homozygous individual in family 6 (Table 1, Figure 3). Parental DNA samples were sequenced for identified variants and showed that each parent was heterozygous for the mutation. No mutations in subunit a4 of the H^+-ATPase pump (*ATP6V0A4* gene) were identified in the remaining four families.

Discussion

Distal renal tubular acidosis is a clinical condition characterised by a renal tubular defect that causes abnormal urinary H^+ excretion and failure to maintain a normal plasma HCO_3^- concentration.[9] Autosomal recessive forms are associated with *ATP6V1B1* and *ATP6V0A4* gene mutations in patients with early SNHL and absent or late SNHL, respectively. The H^+-ATPase pump plays a key role in maintaining the acid–base balance in the kidney. An active acidification process also operates in the inner ear to maintain an endolymph pH of 7.5.[8] Patients with distal renal tubular acidosis are often affected by growth retardation caused by chronic metabolic acidosis unless alkali therapy is initiated early in life. Thus, treatment with alkali, such as potassium citrate, can reverse most biochemical abnormalities and restore normal growth. Although this treatment corrects many of the problems associated with distal renal tubular acidosis, it cannot prevent SNHL onset or progression, probably because of the anatomical isolation of the cochlea.[13,14] However, early diagnosis of deafness enables hearing aids to be fitted to assist in speech acquisition and provide sufficient auditory nerve stimulation to ensure that affected infants remain candidates for cochlear implantation.[15] A large proportion of autosomal recessive distal renal tubular acidosis cases are associated with mutations in the *ATP6V1B1* gene, which encodes the B1 subunit of the H^+-ATPase. Consistent with its autosomal recessive inheritance pattern, this disease is more prevalent in populations with a high rate of

TABLE I
MUTATION TYPE IN SIX UNRELATED DEAF FAMILIES WITH DISTAL RENAL TUBULAR ACIDOSIS AND NUMBER OF AFFECTED FAMILY MEMBERS

ID	Symptoms	Affected family members (*n*)	cDNA mutation	Encoded protein
Family 1	Prelingual moderately severe HL, dRTA	1	1155–1157 ins C	P385fsX441
Family 2	Prelingual profound HL, dRTA	2	1155–1157 ins C	P385fsX441
Family 3	Prelingual mild/profound HL, dRTA	2	1037C > G	P346R
Family 4	Prelingual moderate to severe HL, dRTA	3	1037C > G	P346R
Family 5	Prelingual profound HL, dRTA	1	1037C > G	P346R
Family 6	Prelingual profound HL, dRTA	1	233G > C	G78R

cDNA = complementary DNA; HL = hearing loss; dRTA = distal renal tubular acidosis

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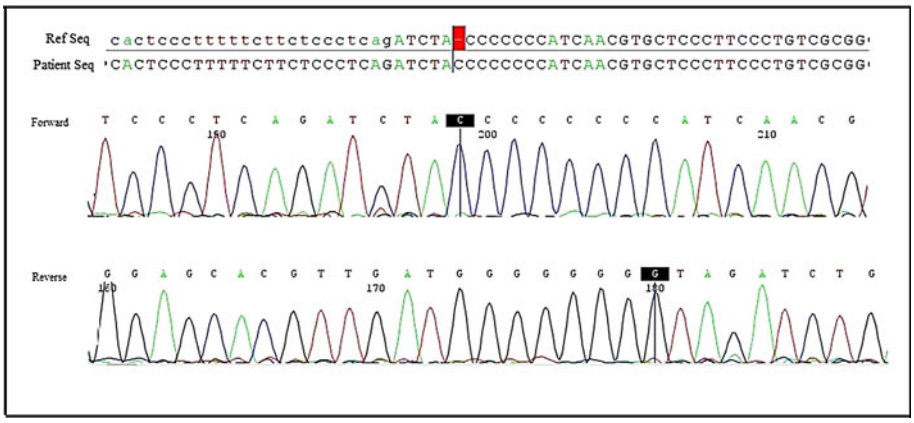

FIG. 1

Chromatogram showing the P385fsX441 mutation in one of the two affected families. The box indicates the position of the cytosine (C) insertion. Ref = reference; seq = sequence

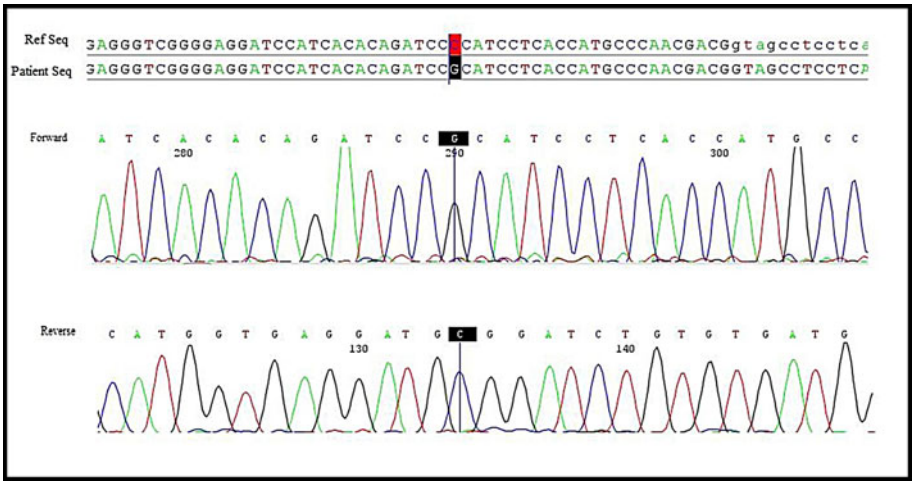

FIG. 2

Chromatogram showing the P346R mutation in one of the three affected families. The box indicates the position of the 1037C > G substitution in exon 10. Ref = reference; seq = sequence

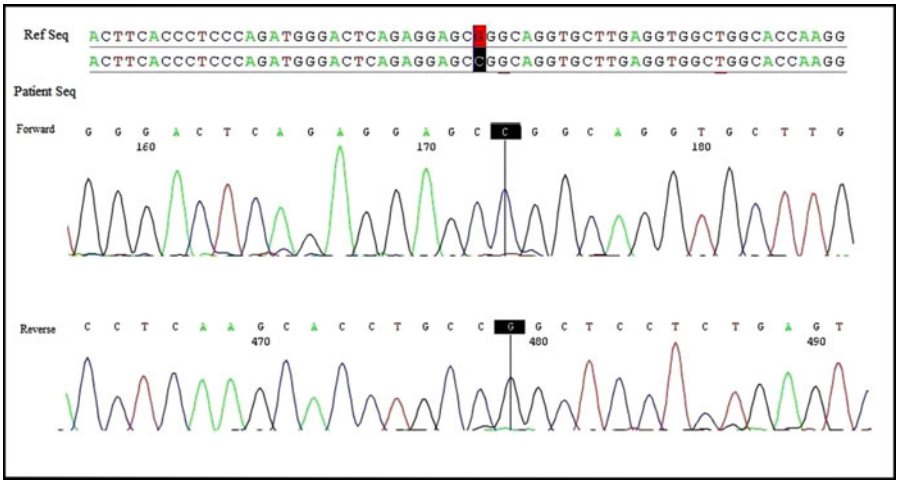

FIG. 3

Chromatogram showing the G78R mutation in one affected family. The box indicates a novel G > C substitution at position 232 leading to an amino acid change. Ref = reference; seq = sequence

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consanguinity, such as in Iran. This is the first study to investigate *ATP6V1B1* and *ATP6V0A4* mutations in Iranian families with both distal renal tubular acidosis and hearing loss. We evaluated two subunits of the H^+-ATPase pump (*ATP6V1B1* and *ATP6V0A4*) implicated in recessive distal renal tubular acidosis in 10 unrelated Iranian families. Six family members harboured mutations in the *ATP6V1B1* gene and the remaining four did not have mutations in either the *ATP6V1B1* or *ATP6V0A4* gene.

- **This is the first Iranian study to investigate *ATP6V1B1* and *ATP6V0A4* mutations in families with distal renal tubular acidosis and hearing loss**
- **The most common genetic causes of hearing loss associated with distal renal tubular acidosis in our families are *ATP6V1B1* mutations**
- **A correct clinical diagnosis is essential to identify the genetic cause of distal renal tubular acidosis**
- **Early diagnosis of deafness enables hearing aids to be used to assist speech acquisition and provide sufficient auditory nerve stimulation for affected infants to remain candidates for cochlear implantation**

We identified a novel G > C missense mutation at position 232 (leading to a G78R amino acid change) in a patient with profound SNHL (Table 1, Figure 3), although a G > A substitution at this site was first reported by Borthwick *et al*.[16] After clinical characterisation, we identified three different mutation types in *ATP6V1B1*, the gene most frequently responsible for hearing loss associated with distal renal tubular acidosis, in our families. We detected *ATP6V1B1* mutations in more than half of the families studied (six out of 10 families containing 10 affected individuals). Our results confirm previous reports that the *ATP6V1B1* gene is mutated more frequently than the *ATP6V0A4* gene.[9]

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