Autosomal recessive distal renal tubular acidosis (rdRTA) is characterised by severe hyperchloraemic metabolic acidosis in childhood, hypokalaemia, decreased urinary calcium solubility, and impaired bone physiology and growth. Two types of rdRTA have been differentiated by the presence or absence of sensorineural hearing loss, but appear otherwise clinically similar. Recently, we identified mutations in genes encoding two different subunits of the renal α-intercalated cell’s apical H⁺-ATPase that cause rdRTA. Defects in the B1 subunit gene ATP6V1B1, and the α4 subunit gene ATP6V0A4, cause rdRTA with deafness and with preserved hearing, respectively. We have investigated 26 new rdRTA kindreds, of which 23 are consanguineous. Linkage analysis of seven novel SNPs and five polymorphic markers in, and tightly linked to, ATP6V1B1 and ATP6V0A4 suggested that four families do not link to either locus, providing strong evidence for additional genetic heterogeneity.

In ATP6V1B1, one novel and five previously reported mutations were found in 10 kindreds. In 12 ATP6V0A4 kindreds, seven of 10 mutations were novel. A further nine novel ATP6V0A4 mutations were found in “sporadic” cases. The previously reported association between ATP6V1B1 defects and severe hearing loss in childhood was maintained. However, several patients with ATP6V0A4 mutations have developed hearing loss, usually in young adulthood. We show here that ATP6V0A4 is expressed within the human inner ear. These findings provide further evidence for genetic heterogeneity in rdRTA, extend the spectrum of disease causing mutations in ATP6V1B1 and ATP6V0A4, and show ATP6V0A4 expression within the cochlea for the first time.

Acid-base regulation by the kidney is tightly controlled through the coupled processes of acid secretion and bicarbonate reabsorption via intercalated cells of the nephron’s collecting duct segment. The result is regulated secretion into the urine of the net acid load provided by the human diet. The main proton pump responsible for urinary acidification by α-intercalated cells, the apical H⁺-ATPase, is a multi-subunit structure with a “head and stalk” configuration. The V1 (head) and V0 (membrane anchored) domains are responsible for ATP hydrolysis and transmembrane proton translocation respectively. So far, novel genes encoding two H⁺-ATPase subunits specific to intercalated cells have been identified, termed ATP6V1B1 and ATP6V0A4. In May 2002, the official symbols for these genes were altered by the HUGO Gene Nomenclature Committee from ATP6B1 and ATP6N1B respectively. They encode the B1 subunit in the catalytic head and the α4 subunit at the pump's base, which co-localise apically in α-intercalated cells and are distinct from the genes for the corresponding subunits (B2 and a1) in the ubiquitously expressed H⁺-ATPases present in intracellular organelles. Destructive mutations in both these kidney subunit genes are associated with autosomal recessive distal renal tubular acidosis (rdRTA).

rdRTA is characterised clinically by impaired urine acidification leading to severe hyperchloraemic hypokalaemic metabolic acidosis, prominent renal tract calcification, and rickets. About one third of patients with rdRTA have progressive and irreversible sensorineural hearing loss evident in childhood. Except for the hearing status, the two forms of recessive disease appear to be clinically similar. Studies to date have shown that mutations in ATP6V1B1 cause rdRTA with sensorineural hearing loss (SNHL), whereas rdRTA with preserved hearing is caused by mutations in ATP6V0A4.

A genome wide linkage screen of a set of 31 kindreds with rdRTA resulted in the identification of ATP6V1B1 as the first gene associated with this disorder. This gene is encoded in 14 exons, yielding the B1 subunit of 513 amino acids. It is expressed by interdental cells and endolymphatic sac epithelia, accounting for the associated hearing impairment. In vitro studies have previously shown that the B subunit is necessary, but not sufficient, for ATP hydrolysis.

Genome wide screening of a hearing cohort identified a linked locus at 7q33-34 that proved to contain the novel ATP6VOA4 gene. ATP6VOA4 has 23 exons, of which 20 encode the 840 amino acid transmembrane α4 subunit, whose function in the pump is currently unknown. By analogy with

**Abbreviations:** rdRTA, autosomal recessive distal renal tubular acidosis; SNP, single nucleotide polymorphism; SNHL, sensorineural hearing loss; WT, wild type; DHPLC, denaturing high performance liquid chromatography; RT, reverse transcription; SSCP, single strand conformation polymorphism
yeast, it may be involved in H+ translocation or transport
and/or assembly of the H+-ATPase.7

Importantly, some families with rdRTA and deafness have
been identified that exclude linkage to ATP6V1B1, and some
with rdRTA and normal hearing are not linked to ATP6V0A4,
suggesting that there may be additional genes involved in
rdRTA. The initial aims of this study were to survey the spec-
rum of mutations in ATP6V0A4 and/or assembly of the H
ATPase.8

Single nucleotide polymorphism (SNP) characterisation
Four intragenic SNPs in ATP6V1B1 and three in ATP6V0A4,
previously identified in Turkish and Saudi Arabian patients
during mutation screening, were characterised for use in
SNPing (fig 1). Using appropriate enzymes for RFLP
analysis (table 1), allele frequencies in 50 chromosomes of
unrelated Turkish and Saudi control subjects were analysed.
Primer sequences were as previously published.14

Genotyping and linkage analysis
Genotyping was performed by PCR amplification and either
RFLP analysis of SNPs (table 1) or microsatellite analysis
using fluorescetly end labelled primers or [32P]-dCTP
incorporation, under conditions previously described8 11
(fig 1). Evidence for linkage to ATP6V1B1 and ATP6V0A4 was
assessed, qualitatively by seeking homozygosity by descent in
consanguineous families or shared haplotypes in affected
members of outbred kindreds, and quantitatively using the
GENEHUNTER program2 11 under models previously
described.8

Mutation detection
Individual ATP6V1B1 and ATP6V0A4 exons and flanking
intronic sequences were amplified from genomic DNA, using
primers and conditions previously reported,14 with a mixture
of AmpliTaq Gold® and PhuTurbo® DNA polymerases (Applied
Biosystems and Stratagene) and 38-40 PCR cycles. Double
stranded DNA duplexes were formed by denaturing and
slowly renameling a 1:2 mixture of known wild type (WT)
and sample DNA. The resulting samples were analysed using
denaturing high performance liquid chromatography
(DHPLC) (WAVE™ system, Transgenomic Inc).14 All identified
WAVE variants were directly sequenced on both strands using
the ABI PRISM® BigDye® Terminator method (Applied Biosys-
tems) according to standard protocols.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Nucleotide</th>
<th>% allele frequency</th>
<th>Enzyme</th>
<th>Product sizes before &amp; after digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>position</td>
<td>alteration</td>
<td>(cut/uncut)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP6V1B1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>S46S</td>
<td>138T&gt;C</td>
<td>36-64</td>
<td>TspRI</td>
</tr>
<tr>
<td>Intron 3</td>
<td>IVS3+117C&gt;A</td>
<td>86-54</td>
<td></td>
<td>HpyCH4IV</td>
</tr>
<tr>
<td>Exon 6</td>
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<td>481G&gt;A</td>
<td>90-10</td>
<td>Aval</td>
</tr>
<tr>
<td>Exon 10</td>
<td>R334R</td>
<td>1002C&gt;T</td>
<td>28-72</td>
<td>BspUI</td>
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<tr>
<td>ATP6V0A4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 4</td>
<td>A2VT</td>
<td>5C&gt;T</td>
<td>72-28</td>
<td>BglI</td>
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<tr>
<td>Exon 17</td>
<td>F554F</td>
<td>1662T&gt;C</td>
<td>68-32</td>
<td>XcmI</td>
</tr>
<tr>
<td>Exon 18</td>
<td>H604H</td>
<td>1812C&gt;T</td>
<td>68-32</td>
<td>BsaAI</td>
</tr>
</tbody>
</table>

*Conserved in all species except Neurospora.
†Not evolutionarily conserved.

Figure 1  Polymorphic markers used for linkage analysis of the
ATP6V1B1 and ATP6V0A4 loci. Genetic distances (cM) are shown.
RT reaction was used as a template for PCR. Two primer pairs were performed with and without enzyme. One tenth of the specific primers (sequences available on request). Reactions specific PCR analysis using the appropriate restriction enzyme or allele amplification.

(\text{forward: gctgtcctgacagtagccatc, reverse: caaagtccttcctctggcgaatctgccgag, reverse: cttctgcaccttgatgagccag}), or intron 22 (aggattctctccagtgattcac), introns 9-11 (forward: gttactgt\text{spanned intron 4 (forward: ttgtcacaactgtttctccaggtg, reverse:}

\text{reverse transcription performed with oligo-dT}_{12-18} by standard solution (GibcoBRL) as per the manufacturer's protocol, and were snap frozen in liquid nitrogen at the time of surgery for four kindreds respectively), as shown in fig 2. Kindreds were considered to be neutral biallelic polymorphisms. GENE-HUNTER analysis (data not shown) and inspection of haplotypes were used to classify kindreds as probably linked to \(\text{ATP6V1B1}\), \(\text{ATP6V0A4}\), both, or neither (seven, eight, seven, and four kindreds respectively), as shown in fig 2. Kindreds were defined as unlinked where lod scores were \(< -2\), and/or there was heterozygosity in affected offspring of a consanguineous union and/or non-identical haplotypes were observed among affected sibs.

The data followed the expected pattern of hearing impaired kindreds showing evidence for linkage to \(\text{ATP6V1B1}\) and normal hearing kindreds being probably linked to \(\text{ATP6V0A4}\). Notably, four families (two with normal audiometry, one with mal hearing kindreds being probably linked to \(\text{ATP6V0A4}\) and nor-

\text{nor}
wild type PCR products to ensure the creation of heteroduplexes. The correspondence among linkage results, gene mutation, and hearing status for all kindreds is shown in fig 2. No significant differences in means (or medians where data were not normally distributed) for biochemical parameters were found among the groups of patients harbouring mutations in each gene (tables 2 and 3), nor the group of four kindreds unrelated to either gene (data not shown).

**ATP6V1B1**

ATP6V1B1 mutations were identified in the affected members of 10 kindreds, nine of them deaf and one with indeterminate hearing loss, as shown in table 2 and fig 3. All of these kindreds had shown evidence of consanguinity, and hearing status was evident in childhood in all. The mutations were homozygous in all cases, even where there was no previous evidence of consanguinity. Identified sequence alterations to glutamine, extending the encoded protein by 52 residues. In a further 11 sporadic cases we identified another nine different mutations. Four sporadic cases were compound heterozygotes and the remaining homozygotes, again without previous evidence of consanguinity. In contrast to ATP6V1B1 mutations, the great majority observed in ATP6V0A4 were novel. There was geographical evidence for a founder effect for several of these, such as Y502X in subjects from northern Spain and loss of the intron 12 splice acceptor site in patients from Turkey.

Also clustered among subjects from northern Spain was the intron 17 donor splice site change inserting a T at position +3. This mutation was present both homozygously and in compound heterozygosity with a second severe mutation in a total of four kindreds. It was absent from controls, including an extended panel of 30 unrelated unaffected Spaniards.

**Hearing status among ATP6V0A4 mutants**

Of the 23 rdRTA cases reported here where ATP6V0A4 mutations were found, information about hearing status is available for 20. Notably, most of these were recorded to

### Table 3 Diagnostic clinical, biochemical, and sequencing features of index cases where mutations in ATP6V0A4 were found. Residues numbered according to the sequence in Genbank NM_020632

<table>
<thead>
<tr>
<th>Kindred/sex</th>
<th>Age</th>
<th>Origin</th>
<th>Sex</th>
<th>Age at dx</th>
<th>Consang</th>
<th>Control chromosomes</th>
<th>Mutation</th>
<th>Protein</th>
<th>Na (mEq/L)</th>
<th>K (mEq/L)</th>
<th>Cl (mEq/L)</th>
<th>HCO₃⁻ (mEq/L)</th>
<th>Arterial pH</th>
<th>Urine pH</th>
<th>pH</th>
</tr>
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<tbody>
<tr>
<td>76/F</td>
<td>6 mth</td>
<td>Turkey</td>
<td>Female</td>
<td>3 mth</td>
<td>N</td>
<td>NA</td>
<td>1072C&gt;T</td>
<td>G358X</td>
<td>143</td>
<td>3.5</td>
<td>125</td>
<td>7.3</td>
<td>7.12</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>206/M</td>
<td>10 y</td>
<td>Saudi</td>
<td>Male</td>
<td>2 mth</td>
<td>Y</td>
<td>NA</td>
<td>2257C&gt;T</td>
<td>G753X</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>7.6</td>
</tr>
<tr>
<td>217/F</td>
<td>2 mth</td>
<td>NA</td>
<td>Female</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2257C&gt;T</td>
<td>G753X</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>7.6</td>
</tr>
<tr>
<td>*228/F</td>
<td>1 y</td>
<td>Sicily</td>
<td>Male</td>
<td>1 y</td>
<td>N</td>
<td>?</td>
<td>[2521Tc+c &gt; 2332delG]</td>
<td>Exon 17 skip</td>
<td>141</td>
<td>3.1</td>
<td>110</td>
<td>17.8</td>
<td>7.4</td>
<td>7.0</td>
<td></td>
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<tr>
<td>213/F</td>
<td>6 y</td>
<td>Iran</td>
<td>Male</td>
<td>1 mth</td>
<td>Y</td>
<td>N</td>
<td>338delA</td>
<td>N113X117</td>
<td>110</td>
<td>2.8</td>
<td>118</td>
<td>8.6</td>
<td>7.14</td>
<td>5.6</td>
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<td>209/F</td>
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<td>Lebanon</td>
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<td>11 mth</td>
<td>Y</td>
<td>N</td>
<td>709–711delAAG</td>
<td>K237del</td>
<td>139</td>
<td>2.1</td>
<td>118</td>
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<td>7.13</td>
<td>8.0</td>
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<tr>
<td>83/F</td>
<td>15 y</td>
<td>Canada</td>
<td>Male</td>
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<td>Y</td>
<td>N</td>
<td>IVS6+1G&gt;A</td>
<td>?</td>
<td>131</td>
<td>3.7</td>
<td>114</td>
<td>7.8</td>
<td>7.16</td>
<td>7.0</td>
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<tr>
<td>220/M</td>
<td>10 y</td>
<td>Turkey</td>
<td>Male</td>
<td>1 mth</td>
<td>Y</td>
<td>Mild†</td>
<td>IVS12+2&gt;c-G&gt;CT</td>
<td>?</td>
<td>147</td>
<td>2.2</td>
<td>110</td>
<td>11.5</td>
<td>7.23</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>221/F</td>
<td>2 y</td>
<td>Turkey</td>
<td>Female</td>
<td>4 mth</td>
<td>Y</td>
<td>N</td>
<td>IVS12+2&gt;c-G&gt;CT</td>
<td>?</td>
<td>142</td>
<td>2.8</td>
<td>115</td>
<td>16.0</td>
<td>7.30</td>
<td>7.5</td>
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<tr>
<td>25/F</td>
<td>18 y</td>
<td>Pakistan</td>
<td>Male</td>
<td>2 mth</td>
<td>Y</td>
<td>Moderate</td>
<td>[IVS17&gt;1g&gt;A]</td>
<td>Exon 17 skip</td>
<td>136</td>
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<td>114</td>
<td>15.0</td>
<td>7.31</td>
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<tr>
<td>*60/M</td>
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<td>Male</td>
<td>3 mth</td>
<td>Y</td>
<td>NA</td>
<td>1346G&gt;A</td>
<td>R449H</td>
<td>145</td>
<td>2.5</td>
<td>128</td>
<td>NA</td>
<td>NA</td>
<td>7.5</td>
<td></td>
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<tr>
<td>225/F</td>
<td>4 y</td>
<td>Turkey</td>
<td>Male</td>
<td>Birth</td>
<td>Y</td>
<td>N</td>
<td>1346G&gt;A</td>
<td>R449H</td>
<td>141</td>
<td>2.3</td>
<td>111</td>
<td>10.0</td>
<td>7.2</td>
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<table>
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<tr>
<th>Sporadics</th>
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<td>73/M</td>
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<td>3/M</td>
</tr>
<tr>
<td>41/F</td>
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<tr>
<td>2/F</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SEM</td>
</tr>
</tbody>
</table>

Consang = consanguineous parental union; dx = diagnosis; * = additional affected with mutation; NA = not available or not tested; fs = frameshift; del = deleted; ins = inserted; SEM = standard error of mean.

§Effect of splice acceptor loss unknown.

†[2521Tc+c > 2332delG] X841Q + Y778X788

A result we designated it a novel neutral polymorphism (table 4).

**ATP6V0A4**

ATP6V0A4 mutations were identified in affected members of 12 kindreds, as listed in table 3 and fig 3. All of these kindreds had shown evidence of linkage to ATP6V0A4. These mutations comprised two different nonsense, two frameshift, three consensus splice site, one amino acid deletion, and two missense mutations. In one of these, the normal termination codon was altered to glutamine, extending the encoded protein by 52 residues. In a further 11 sporadic cases we identified another nine different mutations. Four sporadic cases were compound heterozygotes and the remaining homozygotes, again without previous evidence of consanguinity. In contrast to ATP6V1B1 mutations, the great majority observed in ATP6V0A4 were novel. There was geographical evidence for a founder effect for several of these, such as Y502X in subjects from northern Spain and loss of the intron 12 splice acceptor site in patients from Turkey.

Also clustered among subjects from northern Spain was the intron 17 donor splice site change inserting a T at position +3. This mutation was present both homozygously and in compound heterozygosity with a second severe mutation in a total of four kindreds. It was absent from controls, including an extended panel of 30 unrelated unaffected Spaniards.

**Hearing status among ATP6V0A4 mutants**

Of the 23 rdRTA cases reported here where ATP6V0A4 mutations were found, information about hearing status is available for 20. Notably, most of these were recorded to
have normal audiograms when they were referred for study, in contrast to the ATP6V1B1 cohort. Currently, patients 83-1, 228-1, 25-1, 53-1, 70-1, 72-1, and 220-1 from this cohort have hearing impairment. Patient 83-1 has a deaf mother and therefore may have a different, non-syndromic dominant cause for her hearing loss. Hearing loss in 228-1 has been attributed to ototoxic antimicrobial exposure in infancy. Thus, in neither case can SNHL definitely be associated with the dRTA. In four of the remaining five, hearing loss is either mild to moderate, or became evident only in the second to fourth decade, or both. For example, we have recently learned that 72-1 has been found to have SNHL for the first time at the age of 33 years. He and 25-1 have had previous audiograms that were within normal limits. By contrast, 70-1 has the most marked hearing loss and now requires hearing aids. We have consequently reviewed the hearing status of those in our original ATP6V0A4 cohort and found that one (17-1) has developed mild SNHL at the age of 22.

**DISCUSSION**

In this study, linkage analysis results correlated well with the subsequently identified mutations. All kindreds that showed linkage to ATP6V1B1 alone were deaf, and all where audiometry was normal linked to ATP6VOA4. The lower panel illustrates the distribution of mutations (tables 2 and 3). Notably, the association of deafness with ATP6V1B1 was strongly maintained, while hearing loss has recently been found in cases 25-1 and 220-1 who harbour ATP6VOA4 mutations (asterisked).
show that analysis of linkage to these two loci is a good predictor of mutation status in these genes, which may be helpful for future screening and molecular diagnostic efforts, particularly since both genes are large. Moreover, in these kindreds linked to both loci, the presence of hearing loss at a young age provided good predictive evidence for the subsequent finding of a mutation in \( \text{ATP6V1B1} \).

Our previous separate studies of \( \text{ATP6V1B1} \) or \( \text{ATP6V0A4} \) have each excluded some kindreds from linkage, but in neither case were they formally tested for linkage to the alternative locus. Thus, analysis of linkage under models of genetic heterogeneity in this study provides the first definite evidence for the existence of at least one additional rdRTA locus. Notably, the group of kindreds in which linkage to both loci was studied had a high prevalence of hearing loss at a young age (94.4%), which is now recognized as a helpful predictor of mutation status in these genes, which may be helpful for future screening and molecular diagnostic efforts, particularly since both genes are large. Moreover, the presence of hearing loss at a young age provided good predictive evidence for the subsequent finding of a mutation in \( \text{ATP6V1B1} \).

We have extended the spectrum of coding alterations in both the kidney specific H-ATPase genes associated with rdRTA in this large cohort of both familial and sporadic cases, in the process identifying a number of novel mutations. Subjects from some geographical regions appear to share certain alterations suggestive of a founder effect (for example, \( \text{Y502X} \) and the intron 17 splice site mutation in subjects from Spain). However, because of the tissue specificity of \( \text{ATP6V0A4} \) expression, we are unable to assess this effect directly in the absence of available kidney tissue from affected patients. A similar limitation applies to the identified intronic splice acceptor alterations, where aberrant splicing effects are less predictable.

Table 4 Novel missense alterations in \( \text{ATP6V1B1} \) and \( \text{ATP6V0A4} \). Each was typed in 78 control chromosomes; all except T30I were conserved across numerous species including yeast

<table>
<thead>
<tr>
<th>Amino acid position and change</th>
<th>Nucleotide alteration</th>
<th>% allele frequency in controls</th>
<th>Enzyme</th>
<th>Product sizes before &amp; after digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1 T30I</td>
<td>90C&gt;T</td>
<td>77:23</td>
<td>BstEIi</td>
<td>240 → 177+63</td>
</tr>
<tr>
<td>Exon 5 G123V</td>
<td>368G&gt;T</td>
<td>100:0</td>
<td>Bsa36I</td>
<td>205 → 50+155</td>
</tr>
<tr>
<td>Exon 6 R137C</td>
<td>469C&gt;T</td>
<td>100:0</td>
<td>HpyCH4V</td>
<td>251 → 124+68+59 ('cut') or 183+68 ('uncut')</td>
</tr>
</tbody>
</table>

Some mutations identified in this study may in the future provide insight into a4 structure and function, which are presently unknown in mammals. For example, one patient (209-1) has a homozygous deletion of K236; this lysine is conserved across numerous species including yeast, suggesting that it may be a critical residue for the function, folding, or trafficking of this protein. In a similar vein, the R807Q substitution may be important, as yeast mutagenesis studies implicate the C-terminus in assembly and stability of the pump.

This study confirms the association between \( \text{ATP6V1B1} \) mutations and rdRTA with deafness in childhood, and \( \text{ATP6V0A4} \) mutations with normal hearing (at least until young adulthood). Where hearing status was recorded in childhood in this cohort, none of the subjects with \( \text{ATP6V0A4} \) mutations had rdRTA associated SNHL, whereas all affected patients with \( \text{ATP6V1B1} \) mutations manifest SNHL in early life. With more recent clinical follow up, we have become aware that hearing loss may develop at an older age in a number of those with \( \text{ATP6V0A4} \) mutations, though it appears less severe in most cases. This led us to begin exploring the expression of \( \text{ATP6V0A4} \) in the human inner ear. Addressing this question is problematical as appropriate tissue samples are rarely available, and animal studies could be limited by species differences. However, while the results obtained from the small amounts of ex vivo human tissue we could assess must be regarded as preliminary, they clearly show that this gene is expressed by epithelia within the inner ear. It will require long term follow up to determine whether all those with \( \text{ATP6V0A4} \) mutations will eventually develop clinically significant hearing impairment.

![Figure 4](http://jmg.bmj.com/)

**Figure 4** ATP6V0A4 is expressed in human inner ear. PCR analysis of human cDNA from (A) fetal cochlea and (B) three samples of adult vestibular epithelium is shown. Positive controls were provided by amplification of the same four exons (9-12) of ATP6V0A4 from kidney cDNA in the adult, and concomitant amplification of ATP6V1B1 from the same fetal cDNA. Water was used as template for negative controls. Product sizes (in bp) are shown, with 100 bp ladder on the right. See text for primer details.
These new findings raise the question of why mutations in different subunits of the same proton pump apparently have different effects on the severity of hearing loss. The lack of a severe auditory phenotype in patients with \textit{ATP6V0A4} mutations could be simply explained if the ubiquitous \(\alpha_1\) isoform encoded by \textit{ATP6V0A1} (or another, as yet undiscovered, tissue specific isoform) were able largely to compensate for a4 functions. However, as the role of a4 within the proton pump complex has yet to be elucidated at either site, further studies will be required to understand the physiological basis for the differential effects of \textit{ATP6V1B1} and \textit{ATP6V0A4} mutations on the auditory system.

Such studies might have important clinical implications. Currently, understanding of the molecular mechanism of hearing loss in rdtRNA may lead to new preventative or therapeutic options for patients with this disease. With the larger cohorts of patients with \textit{ATP6V1B1} and \textit{ATP6V0A4} mutations available from this study, we were able to revisit the question of whether any differences in biochemical parameters exist between these two groups. We failed to find any significant differences in electrolytes, arterial pH, or urine pH at diagnosis, either in comparing the earlier studies of \textit{ATP6V1B1} and \textit{ATP6V0A4} or in the new cohort reported here. This indicates that, in contrast to their apparently distinct roles in the ear, both proteins probably influence intercalated cell function in a similar manner.

A comparison of age at diagnosis suggests that those with \textit{ATP6V0A4} mutations were diagnosed at younger ages, which could reflect a more severe phenotype. However, this could also reflect ascertainment bias, differences in contact with the medical profession, or awareness of rdtRNA among health care professionals in different societies. In summary, these findings provide further evidence for genetic heterogeneity in rdtRNA, extending the spectrum of disease causing mutations in \textit{ATP6V1B1} and \textit{ATP6V0A4}, confirm the association of \textit{ATP6V1B1} with severe deafness in childhood, identify hearing loss as a feature associated with \textit{ATP6V0A4} mutations, and show \textit{ATP6V0A4} expression within the cochlea for the first time. The results will provide a focus for future structure-function correlations, prompt further assessment of proton pumps in the inner ear, and facilitate screens for new rdtRNA genes.

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