Novel ATP6V1B1 and ATP6V0A4 mutations in autosomal recessive distal renal tubular acidosis with new evidence for hearing loss


Autosomal recessive distal renal tubular acidosis (rdRTA) is characterised by severe hyperchloremic 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 V_1 (head) and V_0 (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 α1) in the ubiquitously expressed H⁺-ATPases present in intracellular organelles. Descriptive 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 hyperchloremic 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 ATP6V0A4 gene. ATP6V0A4 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

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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 spectrum of mutations in ATP6V1B1 and ATP6V0A4 and to identify additional families unlinked to either of these loci that might be used to help discover new rdRTA genes. As a result of our finding, presented below, that some ATP6V0A4 mutations are associated with older onset SNHL, we also assessed ATP6V0A4 expression in the human inner ear.

METHODS
RdRTA kindreds and controls
RdRTA kindreds were recruited by ascertainment of affected index cases. Kindreds were defined as recessive when both parents were unaffected and were either consanguineous or had multiple affected offspring. Single affected offspring of unrelated, unaffected parents were classified as sporadic. Control subjects consisted of unrelated, unaffected subjects from similar ethnic backgrounds (13 each of Saudi Arabian, Turkish, and Spanish origin).

DNA from all subjects was isolated from whole blood by standard methods,8 and quantified using the PicoGreen® kit (Molecular Probes). Biochemical data sought from referring physicians included serum sodium, potassium, chloride, bicarbonate, pH, calcium, magnesium, phosphate, and urine pH and calcium. Differences among groups were sought using the T or Mann-Whitney test as appropriate. The presence of renal tract calcification, chronic renal impairment, and rickets was also reported. Hearing was assessed by pure tone audiometry and/or auditory evoked responses according to age. Kindreds were classified as having rdRTA associated deafness if the affected subjects had bilateral sensorineural hearing loss of >16 dB (500-2000 Hz average) and no apparent alternative causes of hearing loss. Severity of decibel loss was quantified according to standard criteria (16-25: slight; 25-40: mild; 41-55: moderate; 56-70: moderately severe; 71-90: severe; >90: profound).9

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 genotyping (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.10-12

Genotyping and linkage analysis
Genotyping was performed by PCR amplification and either RFLP analysis of SNPs (table 1) or microsatellite analysis using fluoroently end labelled primers or [32P]-dCTP incorporation, under conditions previously described13-16 (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 program17-20 under models previously described.6

Mutation detection
Individual ATP6V1B1 and ATP6V0A4 exons and flanking intronic sequences were amplified from genomic DNA, using primers and conditions previously reported,3,4 with a mixture of AmpliTaq Gold® and PfuTurbo® DNA polymerases (Applied Biosystems and Stratagene) and 38-40 PCR cycles. Double stranded DNA duplexes were formed by denaturing and slowly reannealing 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).18 All identified WAVE variants were directly sequenced on both strands using the ABI PRISM® BigDye® Terminator method (Applied Biosystems) according to standard protocols.

Table 1  Single nucleotide polymorphisms in ATP6V1B1 and ATP6V0A4 used for genotyping. Each had been identified during previous mutation screening and typed in 50 control chromosomes. All were found to be in Hardy-Weinberg equilibrium

<table>
<thead>
<tr>
<th>Exon</th>
<th>Amino acid position and change</th>
<th>Nucleotide alteration</th>
<th>% allele frequency (cut/uncut)</th>
<th>Enzyme</th>
<th>Product sizes before &amp; after digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP6V1B1</td>
<td>Exon 2</td>
<td>S46S</td>
<td>1397G&gt;C</td>
<td>36.64</td>
<td>TspRI</td>
</tr>
<tr>
<td>Intron 3</td>
<td>IVS3+117C&gt;A</td>
<td>46.54</td>
<td>HpyCH4IV</td>
<td>470 → 237 + 233</td>
<td></td>
</tr>
<tr>
<td>Exon 6</td>
<td>E161K*</td>
<td>481G&gt;A</td>
<td>90.10</td>
<td>AvaI</td>
<td>251 → 66 + 185</td>
</tr>
<tr>
<td>Exon 10</td>
<td>R334R</td>
<td>1002C&gt;T</td>
<td>28.72</td>
<td>BstUI</td>
<td>267 → 120 + 147</td>
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<tr>
<td>ATP6V0A4</td>
<td>Exon 4</td>
<td>A2V†</td>
<td>5C&gt;T</td>
<td>72.28</td>
<td>BglII</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>
<td>262 → 157 + 105</td>
</tr>
<tr>
<td>Exon 18</td>
<td>H604R</td>
<td>1812C&gt;T</td>
<td>68.32</td>
<td>BsaAI</td>
<td>323 → 174 + 149</td>
</tr>
</tbody>
</table>

*Conserved in all species except Neurospora.
†Not evolutionarily conserved.
were used in nested amplification of specific primers (sequences available on request). Reactions specific PCR analysis using the appropriate restriction enzyme or allele amplification.

(forward: gctgtcctgacagtagccatc, reverse: caaagtccttcctctggcgaatctgccgag, reverse: cttctgcaccttgatgagccag), or intron 22 (forward: aggattctctccagtgattcacac), introns 9-11 (forward: gttactgtspanned intron 4 (forward: ttgtcacaactgtttctccaggtg, reverse: 497delC)

The reaction was used as template for 36 cycles of amplification of methods. One tenth of each reverse transcription (RT)

inability to acidify urine (pH >5.5) in the setting of a normal nosis of primary dRTA was made by the combination of present with failure to thrive, dehydration and vomiting, and/or growth impairment at under 9 years of age. The diagnosis of primary dRTA was made by the combination of inability to acidify urine (pH > 5.5) in the setting of a normal mutation or polymorphism. Other studies have concluded linked kindreds, variants were identified that led to detection

mutation detection

DHPLC (Transgenomic WAVE™) analysis was performed for all coding exons and flanking intronic segments in ATP6V1B1 and/or ATP6V0A4 as dictated by the linkage results. This method proved 100% sensitive in this cohort in that, in all linked kindreds, variants were identified that led to detection of a mutation or polymorphism. Other studies have concluded that DHPLC is superior in sensitivity to SSCP. Owing to parental consanguinity, most of the mutations were predicted to be homozygous, but this method detects heterozygous changes by virtue of altered HPLC mobility. Analysis was therefore carried out using a mixture of patient and known
Table 3

<table>
<thead>
<tr>
<th>Kindred/s</th>
<th>Age</th>
<th>Consang</th>
<th>Mutation</th>
<th>Protein</th>
<th>Na K</th>
<th>Cl</th>
<th>HCO₃⁻</th>
<th>Arterial pH</th>
<th>Urine pH</th>
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</thead>
<tbody>
<tr>
<td>76/F</td>
<td>3 mths</td>
<td>NA</td>
<td>1072C&gt;T</td>
<td>Q358X</td>
<td>143</td>
<td>3.5</td>
<td>125</td>
<td>7.3</td>
<td>7.12</td>
</tr>
<tr>
<td>206/M</td>
<td>6 mths</td>
<td>Y</td>
<td>2257C&gt;T</td>
<td>Q753X</td>
<td>NA</td>
<td>NA</td>
<td>16.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>217/F</td>
<td>NA</td>
<td>NA</td>
<td>2257C&gt;T</td>
<td>Q753X</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>*228/F</td>
<td>1 y</td>
<td>N</td>
<td>[221211C + 2332delG]</td>
<td>X841Q</td>
<td>141</td>
<td>3.1</td>
<td>110</td>
<td>17.8</td>
<td>7.4</td>
</tr>
<tr>
<td>*213/F</td>
<td>6 y</td>
<td>N</td>
<td>338delA</td>
<td>N1138x117</td>
<td>140</td>
<td>2.8</td>
<td>118</td>
<td>8.6</td>
<td>7.14</td>
</tr>
<tr>
<td>209/F</td>
<td>3 y</td>
<td>N</td>
<td>709–711delAA</td>
<td>K237del</td>
<td>139</td>
<td>2.1</td>
<td>118</td>
<td>7.13</td>
<td>8.0</td>
</tr>
<tr>
<td>83/F</td>
<td>15 y</td>
<td>N</td>
<td>IVS6+1G&gt;A</td>
<td>78</td>
<td>131</td>
<td>3.7</td>
<td>114</td>
<td>7.16</td>
<td>7.0</td>
</tr>
<tr>
<td>222/M</td>
<td>10 y</td>
<td>Y</td>
<td>IVS12+6A&gt;C</td>
<td>147</td>
<td>2.2</td>
<td>110</td>
<td>11.5</td>
<td>7.23</td>
<td>7.0</td>
</tr>
<tr>
<td>221/F</td>
<td>2 y</td>
<td>N</td>
<td>IVS12+5A&gt;C</td>
<td>?</td>
<td>142</td>
<td>2.8</td>
<td>115</td>
<td>16.0</td>
<td>7.30</td>
</tr>
<tr>
<td>25/F</td>
<td>18 y</td>
<td>N</td>
<td>[IV517+1G&gt;A]</td>
<td>Exon 17 skip</td>
<td>136</td>
<td>2.4</td>
<td>114</td>
<td>15.0</td>
<td>7.31</td>
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<tr>
<td>*60/M</td>
<td>12 y</td>
<td>N</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>
</tr>
<tr>
<td>225/F</td>
<td>4 y</td>
<td>Y</td>
<td>IVS12+4A</td>
<td>R449H</td>
<td>141</td>
<td>2.3</td>
<td>111</td>
<td>10.0</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Additional information:

- 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.
- Hearing impairment not definitely attributable to dRTA.
- Standard criteria for hearing loss.
- Effect of splice acceptor loss unknown.

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 remainder 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.

Hearing status among ATP6V0A4 mutants

Of the 23 dRTA 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.

Expression of ATP6V0A4 in inner ear epithelium
We therefore examined the expression of ATP6V0A4 in the human inner ear. Suitable tissue is difficult to obtain in quantity from adults, and we have to date been limited to RT-PCR amplification of mRNA from tiny fragments of epithelia from the vestibular system, together with fetal cochlear cDNA, as we have previously reported for ATP6V1B1. There was clear evidence for ATP6V0A4 expression in both adult and fetal material, with the appearance of specific bands following amplification of exons 4-5, 9-12, and 22-23. Control amplification of ATP6V1B1 was also positive in both. Representative results are displayed in fig 4.

DISCUSSION
In this study, linkage analysis results correlated well with the subsequently identified mutations. All kindreds that showed linkage to ATP6V1B1 and could be excluded from linkage to ATP6V0A4 had an ATP6V1B1 mutation, and vice versa, and all kindreds showing evidence of potential linkage to both genes had a mutation in either ATP6V1B1 or ATP6V0A4. These data
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 the 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 ATP6V1B1.

Our previous separate studies of ATP6V1B1 or 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 excluded also had the same clinical profile as those with mutations in either gene. Therefore, the search for novel candidate genes can be focused by attempting to identify proteins that would produce the same physiological effects if their functions were abolished.

We have extended the spectrum of coding alterations in both of 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 mutations suggestive of a founder effect (for example, Y502X and the intron 17 splice site mutation in subjects from Spain). There is also some evidence for geographical drift, for example, among the subgroup harbouring the insertion frameshift at position 1386 in ATP6V1B1. These originate from North Africa, Saudi Arabia, and also Sicily, where there was Arab domination several centuries ago. Both insertion and deletion frameshifts in 1386 were observed in three kindreds in our earlier study (one Swedish, two Spanish). It is also possible that 1386 appears as a relative “mutation hotspot” owing to a string of seven cytosine residues at this position, suggesting that slippage may occur during replication. Similarly, we found a deletion at P395 in ATP6V0A4, where there is a row of five cytosines and an A insertion into a row of five at L103.

Although the third intronic base at the donor splice site is not fixed like the first and second, it is almost invariably A or G. We predict that splicing will therefore be disrupted by the T insertion in intron 17 of ATP6V0A4, which also changes the relatively well conserved G at position +5 to A. Calculation of consensus values for similar mutations in previous studies suggests that exon skipping is highly likely, rather than cryptic splice site usage. Indeed, identical intronic changes are reported to cause exon skipping in chronic granulomatous disease (+5 position) and osteogenesis imperfecta (+5 position). However, because of the tissue specificity of 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.

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 implicating the C-terminus in assembly and stability of the pump.

This study confirms the association between ATP6V1B1 mutations and rdRTA with deafness in childhood, and 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 ATP6V0A4 mutations had rdRTA associated SNHL, whereas all affected patients with 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 ATP6V0A4 mutations, though it appears less severe in most cases. This led us to begin exploring the expression of 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 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 implicating the C-terminus in assembly and stability of the pump.

Table 4 Novel missense alterations in ATP6V1B1 and ATP6V0A4. Each was typed in 78 control chromosomes; all except T301 were conserved across numerous species including yeast.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Mutation</th>
<th>% allele frequency in controls</th>
<th>Enzyme</th>
<th>Product sizes before &amp; after digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP6V1B1</td>
<td>Exon 1</td>
<td>T301C 90C&gt;T</td>
<td>77.23</td>
<td>BstEII</td>
<td>240 → 177 +63</td>
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<tr>
<td></td>
<td>Exon 5</td>
<td>G123V 368G&gt;T</td>
<td>100.0</td>
<td>Bsp36I</td>
<td>205 → 50 +155</td>
</tr>
<tr>
<td></td>
<td>Exon 6</td>
<td>R137C 495C&gt;T</td>
<td>100.0</td>
<td>HpyCH4V</td>
<td>251 → 124 +68 +59 (cut)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>or 183 +68 (uncut)</td>
</tr>
<tr>
<td>ATP6V0A4</td>
<td>Exon 9</td>
<td>G175D 524G&gt;A</td>
<td>100.0</td>
<td>MspI</td>
<td>234 → 171 +63</td>
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<td>Exon 15</td>
<td>R449H 1346G&gt;A</td>
<td>100.0</td>
<td>HfI</td>
<td>295 → 190 +105</td>
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<tr>
<td></td>
<td>Exon 22</td>
<td>R807Q 2420G&gt;A*</td>
<td>100.0</td>
<td>MseI</td>
<td>2420G&gt;A*</td>
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<tr>
<td></td>
<td>Intron 17</td>
<td>I234+68+59</td>
<td>100.0</td>
<td></td>
<td>240 → 190 +50</td>
</tr>
</tbody>
</table>

*Detected by allele specific PCR.
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 ATP6V0A4 mutations could be simply explained if the ubiquitous a1 isoform encoded by ATP6V0A1 (or another, as yet undiscovered, tissue specific isoform) were able largely to compensate for a4 function in the inner ear. Alternatively, α-intercalated cells may require an a4 function that is not as important in the ear. In terms of the polarisation of acid-base transporters and their functional roles, epithelial cells in the endolymphatic sac and cochlea have similar organisation to renal α-intercalated cells, but molecular trafficking may differ at these sites. 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 ATP6V1B1 and ATP6V0A4 mutations on the auditory system.

Such studies might have important clinical implications. Currently, the understanding of molecular hearing loss in rdtRAs may lead to new preventative or therapeutic options for patients with this disease.

With the larger cohorts of patients with ATP6V1B1 and 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 ATP6V1B1 and 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 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 rdtRAs among health care professionals in different societies.

In summary, these findings provide further evidence for genetic heterogeneity in rdtRAs, extend the spectrum of disease causing mutations in ATP6V1B1 and ATP6V0A4, confirm the association of ATP6V1B1 with severe deafness in childhood, identify hearing loss as a feature associated with ATP6V0A4 mutations, and show ATP6V0A4 expression within the cochlea for the first time. The results will provide evidence for future structure-function correlations, prompt further assessment of proton pumps in the inner ear, and facilitate screens for new rdtRA genes.

ACKNOWLEDGEMENTS

This work was supported by the Wellcome Trust (Senior Fellowship to FEK, Prize Studentship to KJB) and NIH (DC03402 to CCM). EWS was a Henry Fellow of Harvard University. We thank Sumit Bhattacharya and Bryan Barratt for technical assistance with the Transgenomic WAVE® system; Denise Schofield for administrative assistance; and patients and their families, without whom this study would not have been possible.

REFERENCES


