Sensorineural deafness inherited as a tissue specific mitochondrial disorder

Lutfi Jaber, Mordechai Shohat, Xiangdong Bu, Nathan Fischel-Ghodsian, Hui-Ying Yang, Sue-Jane Wang, Jerome I Rotter

Abstract
We present here a large Israeli-Arab kindred with hereditary deafness. In this family 55 deaf subjects (29M, 26F), who are otherwise healthy, have been identified and traced back five generations to one common female ancestor. The deafness is progressive in nature, usually presenting in infancy and childhood. Audiometry on six deaf and seven unaffected subjects was consistent with severe to profound sensorineural hearing loss. Based on formal family segregation analysis, the inheritance of deafness in this family closely fits the expectation of a two locus model owing to the simultaneous mutation of a mitochondrial gene and an autosomal recessive gene. Thus, this disorder appears to have the unusual features of being an inherited tissue specific mitochondrial disease and apparently requiring the homozygous presence of a nuclear gene for clinical expression. Most importantly, this disorder presents a unique opportunity to investigate the molecular basis of hereditary non-syndromic deafness and normal hearing.

Subjects and methods
The figure depicts the complete pedigree of this large kindred with deafness. All the family members live in an Israeli-Arab village 10 miles from Tel-Aviv. The proband had apparent hearing ability during the first year of life which allowed her to gain early speech skills. However, after the first year she experienced progressive hearing loss. Audiometric evaluation at 28 years showed profound deafness. Eight of her nine sibs as well as her mother were similarly affected. Subsequently, information regarding the rest of the members of this kindred was sought.

The pedigree data were collected through the various nuclear families that comprise this kindred. Information on affected subjects was always confirmed from first degree relatives. Fifty-five subjects were found to be affected. Most of them had no history of any hearing ability at any age; several, however, developed progressive hearing loss only after a few years of age, which allowed them to acquire lingual speech. Two subjects had normal hearing until adult age with no obvious cause for their late onset deafness (figure).

Six deaf and seven unaffected pedigree members underwent pure tone audiometry between 250 and 8000 Hz, and ipsilateral and contralateral acoustic reflex measurements. Eight deaf subjects were examined in detail by history, physical examination, and basic laboratory tests such as blood counts and chemistries.

The familial segregation was initially analysed for mendelian genetic inheritance patterns by standard segregation analysis. Then two locus mitochondrial and autosomal gene models were examined in detail. The large pedigree was simplified to a maternal line pedigree only. Since all the members of such a pedigree share the same mitochondrial DNA, the analysis is reduced to a one autosomal locus model, with 'A' being the normal allele and 'a' being the mutated allele. The autosomal gene frequency q was estimated from the observed segregation ratio for the pooled nuclear families with an affected mother (table 1). The estimated gene frequency from this group of families was then used to test the goodness of fit (χ²) of each model on the pooled and individual nuclear families with an unaffected mother with at least one deaf offspring (table 2). The power of this approach is that initial parameter estimation (gene frequency) and goodness of fit testing can be done in one portion of the pedigree (nuclear families with affected mothers), and then can be critically
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<table>
<thead>
<tr>
<th>Mode of inheritance</th>
<th>Mother's status</th>
<th>Sibships</th>
<th>Mating genotypes (mother × father)</th>
<th>Exp proportions of deaf offspring*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal recessive</td>
<td>Affected</td>
<td>All</td>
<td>aa × AA, aa × Aa, aa × aa</td>
<td>q</td>
</tr>
<tr>
<td></td>
<td>Unaffected</td>
<td>With at least one affected offspring</td>
<td>Aa × AA, Aa × Aa</td>
<td>1</td>
</tr>
<tr>
<td>Autosomal dominant</td>
<td>Affected</td>
<td>All</td>
<td>aa × AA, Aa × Aa, aa × aa</td>
<td>1 + q - q²</td>
</tr>
<tr>
<td></td>
<td>Unaffected</td>
<td>With at least one affected offspring</td>
<td>AA × Aa, AA × aa</td>
<td>1</td>
</tr>
</tbody>
</table>

*These proportions are calculated using classical Hardy-Weinberg segregation laws. For example, in the autosomal recessive model with mother unaffected, the expected proportion of deaf offspring is equal to P(offspring from mating Aa × Aa is deaf) × P(Aa × Aa) + P(offspring from mating Aa × aa is deaf) × P(Aa × aa) = 0.25 + 2pq(2pq - q²) + 0.5 × q²/2pq + q², which simplifies to 1/2(1-q).

Table 2A  Two locus mitochondrial and autosomal gene models: χ² test for pooled sibships with unaffected mothers.

<table>
<thead>
<tr>
<th>Mode of inheritance</th>
<th>No of affected offspring</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recessive</td>
<td>77 × 0.34 = 26.15</td>
<td>0.007</td>
<td>&gt;0.05</td>
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<tr>
<td>Expected</td>
<td>77 × 1/[2(2-0.547)] = 26.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>77 × 0.34 = 26.15</td>
<td>9.66</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Expected</td>
<td>77 × 1/[2(2-0.065)] = 39.79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2B  Two locus mitochondrial and autosomal gene models: χ² test for individual sibships with unaffected mothers.

<table>
<thead>
<tr>
<th>Sibship size</th>
<th>No of families</th>
<th>No of affected offspring</th>
<th>Observed</th>
<th>Expected</th>
<th>χ² = 0.11</th>
<th>p &gt; 0.73</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0.11</td>
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<tr>
<td>7</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>0.11</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>0.11</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>0.11</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>0.5</td>
<td>0.11</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>0.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>27</td>
<td>2</td>
<td>27</td>
<td>1</td>
<td>0.11</td>
</tr>
</tbody>
</table>

tested in another portion of the pedigree (nuclear families with unaffected mothers having at least one affected offspring). The observed segregation ratio of unaffected mothers with at least one deaf offspring was adjusted for ascertainment bias by the standard proband method.19

Mitochondrial restriction fragment length polymorphism analysis was performed in four deaf members of the pedigree and two normal unrelated controls. DNA was isolated from blood samples, and 5 μg aliquots were then digested with restriction enzymes, subjected to electrophoresis in 1% agarose gels, and transferred to nitran filters.20 Blots were hybridised with 32P labelled whole mitochondrial DNA (supplied by Uzi Rita, Jerusalem, Israel) at 65°C for 24 hours in buffer containing 1% bovine serum albumin, 1 mmol/l EDTA, 0.5 mol/l sodium phosphate, and 7% (w/v) sodium dodecyl sulphate. Filters were washed twice in 2% and twice in 0.1% (w/v) sodium
Results

Audiometric studies indicated severe to profound sensorineural hearing loss of at least 70 decibels for all frequencies, as well as decreased contralateral acoustic reflex thresholds, in all the deaf subjects. Normal results were obtained in all unaffected pedigree members. These results are most consistent with a cochlear lesion, although a retrocochlear lesion cannot be excluded yet. General examination of deaf subjects showed no abnormalities in general state of health, gross vestibular function, and other organ systems frequently associated with mitochondrial disorders (neurological, muscle) or with hearing deficits (kidneys, eyes).

Pedigree analysis showed that the 55 deaf subjects could all be traced back to one original couple (generation I in the figure), and transmission of the disease occurred only through the maternal line.

Transmission by mendelian autosomal or X linked inheritance alone could be summarily rejected by the following reasoning. With one or multiple autosomal locus modes of inheritance the expected number of deaf offspring of either deaf or unaffected fathers should be equal to that of the respective mothers. However, 29 deaf offspring were born to six of seven deaf mothers and 26 to 15 unaffected mothers, while not a single deaf offspring was born to either of the six deaf or 11 unaffected fathers descended from the ancestral couple. Similarly, X linked recessive inheritance was rejected because of the nearly even proportion of male and female patients affected (29 males, 26 females). X linked dominant inheritance was rejected because none of the 15 daughters of deaf males, who would be obligate carriers of the defect, was affected.

The maternal inheritance by non-mendelian genetic factors could be explained by either of two genetic mechanisms: imprinting\(^1\) and mitochondrial inheritance. Imprinting with full penetrance could be rejected because of the different segregation ratios in affected and unaffected mothers with at least one affected offspring (0.55 and 0.34, respectively; p < 0.03). Imprinting with partial penetrance could be rejected because the observed segregation ratio in affected mothers is 0.55 and the expected segregation ratio in imprinting with partial penetrance is <0.5. In addition, no transmission occurred from an affected male to any of his nine grandchildren through three daughters.

The transmission of deafness is completely compatible with mitochondrial inheritance. However, mitochondrial inheritance with full penetrance is an inadequate explanation because of the many unaffected offspring of deaf mothers. Mitochondrial inheritance requiring environmental factors for expression is also highly unlikely, given the early onset of disease, and the homogeneous environment within the village. The two remaining most parsimonious hypotheses for the genetic transmission pattern in this kindred are therefore (1) a two locus disease with the simultaneous involvement of a mitochondrial gene and an autosomal gene, and (2) mitochondrial inheritance with varying amounts of heteroplasmia.

The autosomal gene frequency estimated from pooled deaf mothers under the autosomal recessive case is \(q = 0.547\), and under the autosomal dominant case \(q = 0.065\). When tested on pooled sibships from unaffected mothers, there was remarkably close agreement between the expected and observed segregation ratios for the autosomal recessive model, while the autosomal dominant model could clearly be rejected (table 2A). Similarly, table 2B shows for individual sibships that there is good agreement with the autosomal recessive model. The autosomal dominant model could again be rejected (\(\chi^2 = 8.93\), \(p < 0.003\)).

Mitochondrial DNA analysis showed no evidence for deletions/duplications, or heteroplasmy, with the following enzymes: \(TaqI\), \(BamHI\), \(KpnI\), \(StyI\), \(Sau3AI\), \(Alul\), \(BstNI\), \(HaeIII\), \(HinTII\), and \(DdeI\).

Discussion

In the large Israeli-Arab kindred presented here, progressive sensorineural deafness was most likely to be caused by the simultaneous inheritance of a mitochondrial mutation and an autosomal recessive mutation. This is based both on the rejection of alternative models, and the excellent fit between the model and the observed family segregation in the aggregate and within individual nuclear sibships. Heteroplasmia of the mitochondrial mutation is the second most likely explanation for the deafness aggregation in this family. Although most mitochondrial deletion mutations leading to disease are heteroplasmic, several lines of evidence, in addition to the molecular studies to date, make mitochondrial inheritance with heteroplasmia unlikely in this pedigree. Most importantly, the clinical phenotype is relatively homogeneous within each family, between families, and between generations, with most subjects developing deafness in infancy or early childhood, making a dose effect less likely. In addition, there are only very few descriptions of a stable passage of heteroplasmic mitochondrial DNA from one generation to the next,\(^{25-24}\) heteroplasm is virtually absent in the general population despite a high mitochondrial mutation rate,\(^{25,27}\) and in vitro injections of mitochondria into cells lead to rapid replacement of endogenous mitochondrial DNA.\(^{28}\) Nevertheless, the final answer regarding heteroplasm must await the molecular identification of the mitochondrial mutation.

The high frequency of the autosomal gene could be explained in three possible ways. First, it could be a common polymorphism in one of the nuclear encoded oxidative phosphorylation subunits, which leads to disease only in association with the special mitochondrial genotype in this family. Second, it could
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