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Sensorineural deafness inherited as a tissue specific mitochondrial disorder

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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.

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Hereditary deafness is a heterogeneous group of disorders distinguished by their clinical manifestations and modes of inheritance.[1] Most of the hereditary forms are sensorineural rather than conductive in nature, and 60% are non-syndromic, that is, not associated with any other abnormalities.[2] It is estimated that 60 to 70% of cases with hereditary deafness are inherited in an autosomal recessive fashion, 20 to 30% are autosomal dominant, and 2% are X linked.[3] Estimates of the number of gene loci involved in autosomal recessive deafness range from five to thousands, and additional genes must be involved in autosomal dominant and X linked deafness.[4,5]

The molecular basis for several syndromic forms of deafness have recently been linked to specific chromosomal areas,[6–10] and in the case of some forms of Alport syndrome the defect has been localised to the type IV collagen α5 chain gene.[11–13]

We present here a large Israeli-Arab kindred with hereditary cochlear non-syndromic deafness, whose family aggregation is most consistent with a two locus mode of inheritance, one locus being mitochondrial and the other being autosomal recessive. This family provides for the first time the opportunity to elucidate the molecular basis leading to at least one kind of non-syndromic deafness.

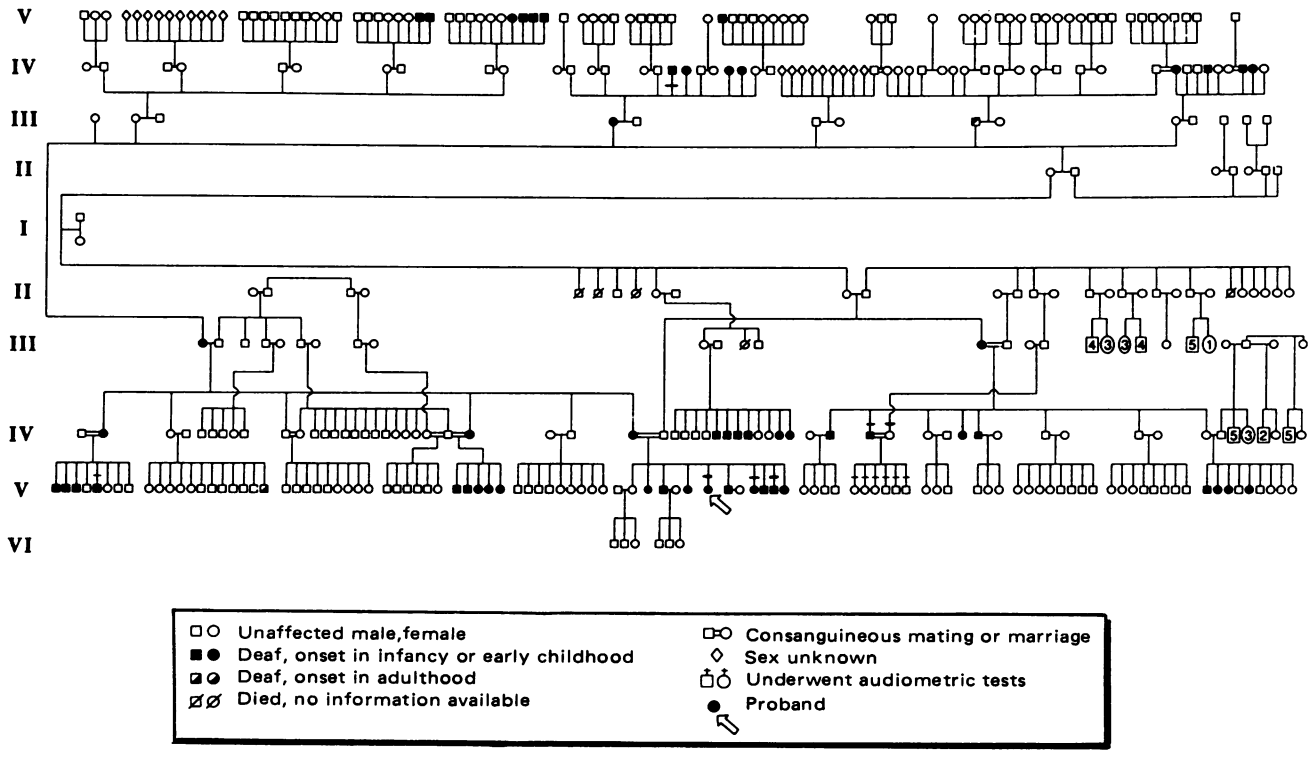
Subjects and methods

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.[14] Then two locus mitochondrial and autosomal gene models were examined in detail.[15] 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 (χ^2) of each model on the pooled and individual nuclear families with an unaffected mother with at least one deaf offspring[16,17] (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



Israeli-Arab pedigree with sensorineural deafness.

Table 1 Expected proportions of deaf offspring under different mating types in maternally derived line of families.

Mode of inheritance	Mother's status	Sibships	Mating genotypes (mother × father)	Exp proportions of deaf offspring*
Autosomal recessive	Affected	All	aa × AA, aa × Aa, aa × aa	q
	Unaffected	With at least one affected offspring	Aa × Aa, Aa × aa	$\frac{1}{2(2-q)}$
Autosomal dominant	Affected	All	Aa × AA, Aa × Aa, Aa × aa aa × AA, aa × Aa, aa × aa	$\frac{1+q-q^2}{2-q}$
	Unaffected	With at least one affected offspring	AA × Aa, AA × aa	$\frac{1}{2-q}$

*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) = $0{\cdot}25 \times 2pq/(2pq+q^2) + 0{\cdot}5 \times q^2/(2pq+q^2)$, which simplifies to 1/2(2-q).

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

Mode of inheritance	No of affected offspring	χ^2	p
Recessive			
Observed	$77 \times 0{\cdot}34 = 26{\cdot}15$	0·007	>0·95
Expected	$77 \times 1/[2(2-0{\cdot}547)] = 26{\cdot}50$		
Dominant			
Observed	$77 \times 0{\cdot}34 = 26{\cdot}15$	9·66	<0·002
Expected	$77 \times 1/(2-0{\cdot}065) = 39{\cdot}79$		

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

Sibship size	No of families	No of affected offspring		
		Observed	Expected	
2	1	1	1·2	
7	1	3	2·5	
8	2	6	5·6	
9	2	5	6·3	
10	1	4	3·5	
12	2	7	8·2	
Total		26	27·3	$\chi^2 = 0{\cdot}11$ p>0·73

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.[18 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 nytran filters.[20] Blots were hybridised with ^{32}P 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

dodecyl sulphate, each time for 15 minutes at 65°C. The filters were exposed to Kodak XAR film at −70°C.

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 threshold, 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[21] 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 heteroplasmy.

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: *Taq*I, *Bam*HI, *Kpn*I, *Sty*I, *Sau*3AI, *Alu*I, *Bst*NI, *Hae*III, *Hin*fI, and *Dde*I.

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. Heteroplasmy 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 heteroplasmy 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,[22–24] heteroplasmy 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 heteroplasmy 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

be the result of a high frequency of consanguineous matings, and, third, because of a founder effect in our study population. It is common among Israeli-Arabs who live in the villages to prefer consanguineous marriages, particularly among first cousins. In addition, such villages are often populated by a few (<20) original families. However, consanguineous marriages in the Israeli-Arab community are usually through the paternal side, with the goal of conserving the lands within the family, and thus cannot explain the maternal transmission observed in this family.

The tissue specificity of this disease is intriguing. The mitochondrial genome codes for 13 of the approximately 70 subunits engaged in oxidative phosphorylation, and oxidative phosphorylation is ubiquitous in all nucleated human cells. Most inherited mitochondrial disorders affect a number of tissues, and in particular those with the highest energy requirements such as striated muscle and nerves.[29 30] However, LHON is mainly limited to the optic nerve, and has recently been shown to be a two locus disease with one mitochondrial point mutation and one locus linked to *DXS7* on the X chromosome.[31-33] It is possible to speculate, both for LHON and our deafness pedigree, that a mutation affects a tissue specific isoform of a nuclear encoded oxidative phosphorylation subunit, which interacts with the mutated mitochondrial encoded subunit, and leads to tissue specific disease. Although such isoforms have not been reported in humans, they have been described in other species.[34] Alternatively, it is possible that the mutated mitochondrial subunit functions normally in oxidative phosphorylation in our pedigree, but interacts abnormally with a hearing specific protein encoded by the nuclear locus.

Our pedigree presents a unique opportunity to investigate for the first time the molecular basis of non-syndromic deafness. The mitochondrial mutation can be identified because of the small size of the mitochondrial chromosome,[35] and identification of the autosomal locus is greatly simplified by the power of linkage analysis in this large kindred and the likely interaction between the mitochondrially encoded and autosomally encoded proteins.

Other mitochondrial DNA defects can lead to deafness, as evidenced by the recent description of 22 pedigrees with deafness caused by mitochondrial transmitted susceptibility to aminoglycosides.[36] It is possible that the mitochondrial DNA mutation in these cases is similar to the one in our pedigree, and that the phenotypic expression can be induced either by an autosomal recessive mutation or by the aminoglycosides. It can also be speculated that one of the common forms of acquired hearing loss, strial presbyacusis, in which decreased oxidative phosphorylation activity in the stria vascularis has been documented,[37] is related to mitochondrial DNA degeneration with age.[38 39] These data suggest that the molecular study of mitochondrial organelles will lead to an elucidation of biochemical pathways involved in hearing and hearing deficits.

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1 McKusick VA. *Mendelian inheritance in man: catalogs of autosomal dominant, autosomal recessive, and X-linked phenotypes.* Baltimore: The Johns Hopkins University Press, 1990: 252–4, 1132–5, 1583–5.
2 Beighton P. Hereditary deafness. In: Emery AEH, Rimoin DL, eds. *Principles and practice of medical genetics.* 2nd ed. Edinburgh: Churchill Livingstone, 1990.
3 Konigsmark BW, Gorlin RJ. *Genetic and metabolic deafness.* Philadelphia: Saunders, 1976.
4 Chung CS, Brown KS. Family studies of early childhood deafness ascertained through the Clarke school for the deaf. *Am J Hum Genet* 1970;**22**:630–44.
5 Sank D. Genetic aspects of early total deafness. In: Rainer JD, Altshuler KZ, Kallman FJ, eds. *Family and mental health problems in a deaf population.* New York: New York Psychiatric Institute, 1963:28–81.
6 Kimberling WJ, Weston MD, Moeller C, *et al.* Localization of Usher syndrome type II to chromosome 1q. *Genomics* 1990;7:245–9.
7 Lewis RA, Otterud B, Stauffer D, Lalouel J-M, Leppert M. Mapping recessive ophthalmic diseases: linkage of the locus for Usher syndrome type II to a DNA marker on chromosome 1q. *Genomics* 1990;7:250–6.
8 Foy C, Newton V, Wellesley D, Harris R, Read AP. Assignment of the locus for Waardenburg syndrome type I to human chromosome 2q37 and possible homology to the splotch mouse. *Am J Hum Genet* 1990;**46**:1017–23.
9 Shiloh Y, Litvak G, Ziv Y, *et al.* Genetic mapping of X-linked albinism-deafness syndrome (ADFN) to Xq26.3–q27.1. *Am J Hum Genet* 1990;**47**:20–7.
10 Ngo JT, Bateman JB, Spence MA, *et al.* Ornithine aminotransferase (OAT): recombination between an X-linked OAT sequence (7·5 kb) and the Norrie disease locus. *Genomics* 1990;**6**:123–8.
11 Barker D, Hostikka SL, Zhou J, *et al.* Identification of mutations in the COL4A5 collagen gene in Alport syndrome. *Science* 1990;**248**:1224–7.
12 Zhou J, Hostikka SL, Chow LT, Tryggvason K. Characterization of the 3' half of the human type IV collagen α5 gene that is affected in the Alport syndrome. *Genomics* 1991;**9**:1–9.
13 Zhou J, Barker DF, Hostikka SL, Gregory MC, Atkin CL, Tryggvason K. Single base mutation in a5(IV) collagen chain gene converting a conserved cysteine to serine in Alport syndrome. *Genomics* 1991;**9**:10–8.
14 Emery AEH. Segregation analysis. In: *Methodology in medical genetics. An introduction to statistical methods.* 2nd ed. Edinburgh: Churchill Livingstone, 1986:37–54.
15 Bu X, Yang H-Y, Shohat M, Rotter JI. Two-locus mitochondrial and nuclear gene models for mitochondrial disorders. *Am J Hum Genet* 1990;**47**:128A.
16 Smith CAB. A test for segregation ratios in family data. *Ann Hum Genet* 1956;**20**:257–65.
17 Crow JF. Problems of ascertainment in the analysis of family data. In: Neel JV, Shaw MW, Schull WJ, eds. *Genetics and the epidemiology of chronic diseases.* Washington, DC: US Department of Health, Education, and Welfare, Public Health Service, 1965:23–60.
18 Weinberg W. Methoden und Fehlerquellen der Untersuchung auf Mendleschen Zahlen beim Menschen. *Arch Rass u Ges Biol* 1912;**9**:165–74.
19 Fisher RA. The effects of methods of ascertainment upon the estimates of frequencies. *Ann Eugen* 1934;**6**:13–25.
20 Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975;**98**:503–17.
21 Hall JG. Genomic imprinting: review and relevance to human diseases. *Am J Hum Genet* 1990;**46**:857–73.
22 Lott MT, Voljavec AS, Wallace DC. Variable genotype of Leber's hereditary optic neuropathy patients. *Am J Ophthalmol* 1990;**109**:625–31.
23 Vilkki J, Savontaus M-L, Nikoskelainen EK. Segregation of mitochondrial genomes in a heteroplasmic lineage with Leber's hereditary optic neuroretinopathy. *Am J Hum Genet* 1990;**47**:95–100.
24 Shoffner JM, Lott MT, Lezza AMS, Seibel P, Ballinger SW, Wallace DC. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA $tRNA^{Lys}$ mutation. *Cell* 1990;**61**:931–7.
25 Brown WM, George M, Wilson AC. Rapid evolution of animal mitochondrial DNA. *Proc Natl Acad Sci USA* 1979;**76**:1967–71.
26 Monnat RJ, Loeb LA. Nucleotide sequence preservation of human mitochondrial DNA. *Proc Natl Acad Sci USA* 1985;**82**:2895–9.
27 Cann RL, Stoneking M, Wilson AC. Mitochondrial DNA and human evolution. *Nature* 1987;**325**:31–6.
28 King MP, Attardi G. Injection of mitochondria into human cells leads to a rapid replacement of the endogenous mitochondrial DNA. *Cell* 1988;**52**:811–9.
29 Shoffner JM, Wallace DC. Oxidative phosphorylation diseases: disorders of two genomes. *Adv Hum Genet* 1990;**19**:267–330.
30 Menkes JH. Genetic disorders of mitochondrial function. *J Pediatr* 1987;**110**:255–9.
31 Wallace DC, Singh G, Lott MT, *et al.* Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 1988;**242**:1427–30.

32 Vilkki J, Ott J, Savontaus M-L, Aula P, Nikoskelainen EK. Optic atrophy in Leber hereditary optic neuroretinopathy is probably determined by an X-chromosomal gene closely linked to DXS7. *Am J Hum Genet* 1991;**48**:486–91.
33 Bu X, Rotter JI. X-chromosome linked and mitochondrial gene control of Leber hereditary optic neuropathy: evidence from segregation analysis for dependence on X-chromosome inactivation. *Proc Natl Acad Sci USA* (1991;**88**:8198–202).
34 Capaldi RA, Gonzalez-Halphen D, Zhang YZ, Yanamura W. Complexity and tissue specificity of the mitochondrial respiratory chain. *J Bioenerg Biomembr* 1988;**20**:291–311.
35 Anderson S, Bankier AT, Barrell BG, *et al.* Sequence and organization of the human mitochondrial genome. *Nature* 1981;**290**:457–65.
36 Hu D-N, Qiu W-Q, Wu B-T, *et al.* Genetic aspects of antibiotic induced deafness: mitochondrial inheritance. *J Med Genet* 1991;**28**:79–83.
37 Schuknecht HF. *Pathology of the ear*. Cambridge, Mass: Harvard University Press, 1974:388–408.
38 Linnane AW, Marzuki S, Ozawa T, Tanaka M. Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *Lancet* 1989;**i**:642–5.
39 Cortopassi GA, Arnheim N. Mitochondrial DNA deletions in normal adult humans detected by PCR. *Am J Hum Genet* 1990;**47**(suppl.):395A.