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INVITED EDITORIAL
Mitochondrial Mutations and Hearing Loss: Paradigm for Mitochondrial Genetics

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mtDNA mutations have been implicated in a great variety of diseases, ranging from rare neuromuscular syndromes, with acronyms such as KSS, MELAS, MERRF, and NARP, to such common conditions as diabetes, Parkinson disease, and Alzheimer disease (Wallace et al. 1995). While the study of the role of mitochondrial mutations in each of these diseases has helped to describe and catalogue the spectrum and frequency of oxidative phosphorylation disorders, it has not led to an understanding of the factors contributing to the two major clinical and biological issues: penetrance and tissue specificity. These two issues are frequently lumped together under the genotype-phenotype correlation heading, but, since different molecular pathways may account for each of the two mechanisms in question, keeping them separate may be warranted. Hearing loss due to mitochondrial mutations has, somewhat surprisingly, emerged as the mitochondrial disease that is providing some of the answers.

Hearing Loss Due to Mitochondrial Mutations

Hearing loss can be due to inherited, acquired, heteroplasmic, and homoplasmic mitochondrial mutations. The mostly acquired mitochondrial mutations associated with the systemic neuromuscular syndromes noted above, which frequently have hearing loss as one of their clinical signs, are both the easiest to understand and the least instructive. In these cases, the heteroplasmic mutation(s) can generally be found at highest levels in nerves and in muscle. Because of the higher energy requirements of muscle and nerve tissue, and because small numbers of dysfunctional muscle and nerve cells can interrupt the function of many neighboring normal cells, mitochondrial DNA mutations in those tissues are thought to be particularly harmful. It is not unexpected that generalized neuronal dysfunction is also expressed in the auditory system.

The first surprise in this area of research came with the description of several families with both diabetes mellitus and sensorineural hearing loss. They were found to have inherited the heteroplasmic A3243G mutation in the gene for $tRNA^{leu(UUR)}$, the very same mutation associated with the systemic mitochondrial encephalomyopathy, lactic acidosis and stroke-like symptoms (MELAS) syndrome (Reardon et al. 1992; van den Ouweland et al. 1992). In none of these cases were other neurological symptoms present. Instead of the 3243 mutation, one family had a heteroplasmic large deletion/insertion event (Ballinger et al. 1992). This association between diabetes mellitus, hearing loss, and mitochondrial mutations has been confirmed in population studies of diabetic patients (Oka et al. 1993; Alcolado et al. 1994; Kadowaki et al. 1994; Katagiri et al. 1994). For example, Kadowaki et al. found the 3243 mutation in 2%–6% of diabetic patients in Japan, and 61% of their patients with diabetes and the 3243 mutation had hearing loss (Kadowaki et al. 1994). The hearing loss is sensorineural, and it usually develops only after the onset of diabetes. While the 3243 mutation raises the questions of penetrance and tissue specificity, the confounding feature of heteroplasmy makes an experimental approach to these questions difficult. Percentages of mutated mitochondrial chromosomes, even when associated with quantitative estimates of their absolute number, do not take into consideration the distribution differences either between cells in the same area or between mitochondrial organelles within the same cell. Unfortunately, these variables are impossible to control, and they are probably impossible to measure accurately.

Another condition associated with acquired heteroplasmic mutations and hearing loss is presbycusis, the hearing loss that occurs with age in a significant proportion of individuals. Since mtDNA mutations and the resulting loss of oxidative phosphorylation activity seem

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to play an important role in the aging process (for a review, see Nagley et al. 1993), it is not unlikely that mitochondrial mutations in the auditory system can also lead to presbycusis. We examined recently the spiral ganglion and membranous labyrinth from archival temporal bones of five patients with presbycusis for mutations within the mitochondrially encoded cytochrome oxidase II gene (Fischel-Ghodsian et al. 1997*a*). Results indicated that, when compared with controls, at least a portion of people with presbycusis have a significant load of mtDNA mutations in auditory tissue. Results also indicated great individual variability in both quantity and cellular location of these mutations. The greatest advantage of studying acquired mutations in the ear relates to the availability of temporal bone tissue banks; these banks have functional audiological data available, and thus they allow correlation of measurable functional status, histology, immunohistochemistry of oxidative phosphorylation complexes, and mtDNA analysis.

The first homoplasmic mutation associated with nonsyndromic deafness was identified in an Arab Israeli pedigree, when the striking pattern of transmission only through mothers was noted (Jaber et al. 1992; Prezant et al. 1993). Most of the deaf family members had onset of severe to profound sensorineural hearing loss during infancy, but a minority of family members had onset during childhood or even adulthood. The homoplasmic A1555G mutation in the mitochondrial 12S rRNA gene was identified as the pathogenic mutation, and the same mutation was also found to predispose individuals to aminoglycoside-induced hearing loss (Prezant et al. 1993). The postulated mechanism of aminoglycoside susceptibility was that the 1555 mutation makes the rRNA more similar to the bacterial rRNA, which is involved in aminoglycoside-induced bactericidal activity (Prezant et al. 1993). Subsequent binding experiments have proven that point (Hamasaki and Rando 1997). More generally, this family demonstrated that, even in a homoplasmic model, the two basic questions of mitochondrial genetics, penetrance and tissue specificity, remain unanswered. Why does the same mutation cause severe hearing loss in some family members but not in others, and why is the ear the only organ affected?

While many pedigrees and individual patients with the same A1555G mutation have been described subsequently, hearing loss in all of these cases occurred only after aminoglycoside exposure (Fischel-Ghodsian et al. 1993, 1997*b;* Hutchin et al. 1993; Pandya et al. 1997). However, three pedigrees in Zaire and Spain were described recently, with family members who went deaf with and without aminoglycosides (Matthijs et al. 1996; El-Schahawi et al. 1997). The study by Estivill et al. (1998) in this issue of the *Journal* is remarkable for two reasons, both of which indicate a higher-than-expected frequency of this mutation. First, of 70 families with sensorineural hearing loss, the study describes 19 families with the 1555 mutation. Even if the selection process led to a bias toward families with multiple affected individuals, and even when only the individuals without aminoglycoside exposure are considered, this represents an unexpectedly high frequency of familial sensorineural hearing loss due to the 1555 mutation. Second, the fact that the mutation arose on different haplotypes indicates both that it is likely that this mutation exists in other populations and that it may not be rare. It is also interesting to note that the onset of hearing loss in the Spanish families was rarely congenital, in contrast to the age at onset in the Arab Israeli pedigree.

Another close-to-homoplasmic inherited mutation leading to hearing loss is the 7445 mutation. It was first described in a family from Scotland, and it was confirmed and established in two unrelated pedigrees from New Zealand and Japan (Reid et al. 1994; Fischel-Ghodsian et al. 1995; Sevior et al., in press). In two of these pedigrees, such skin conditions as palmoplantar keratoderma also segregate in the maternal line (Sevior et al., in press). Interestingly, in the Scottish pedigree the penetrance of this mutation for hearing loss is quite low, while in the New Zealand and Japanese pedigrees the penetrance is very high. Thus, as in the Arab Israeli pedigree, the mitochondrial mutation does not appear to be sufficient by itself to cause hearing loss in these pedigrees. It requires additional genetic or environmental factors that seem to be rare in the Scottish pedigree and common in the New Zealand and Japanese pedigrees. The 7445 mutation is a silent change of both the last nucleotide of the cytochrome oxidase I gene on the heavy strand and the nucleotide immediately adjacent to the 3′ end of the $tRNA^{Ser(UCN)}$ gene on the light strand. Mechanistically, the mutation appears to interfere with normal processing of the light-strand polycistronic mRNA, and significant decreases both in the amount of $tRNA^{Ser(UCN)}$ and in rates of mitochondrial protein synthesis have been observed (Guan et al. 1997). In this situation, the difference in penetrance appears to be due to a difference in mitochondrial haplotype. In the New Zealand pedigree, complete sequencing of the mtDNA revealed three additional sequence changes in complex I protein genes, two of which have also been labeled as secondary Leber hereditary optic neuroretinopathy mutations (Fischel-Ghodsian et al. 1995). Since these or similar sequence changes are not present in the Scottish pedigree, mitochondrial haplotype appears to account for the differences in penetrance in this case.

Clinical Relevance

The major clinical relevance of mitochondrial mutations to hearing loss remains the prevention of aminoglycoside-induced hearing loss. In countries where ami-

noglycosides are used commonly, aminoglycoside-induced ototoxicity is the major cause of hearing loss (presbycusis excluded). For example, in a study that reviewed all deaf-mutes in a district of Shanghai, 21.9% had aminoglycoside-induced hearing loss, representing 167 individuals from a population of nearly half a million (Hu et al. 1991). The 1555 mutation accounted for at least 30% of these. In the United States, the 1555 mutation accounts for ~15% of all cases of aminoglycoside-induced deafness (Hu et al. 1991; Fischel-Ghodsian et al. 1997*b*). The difference in frequency may reflect use of aminoglycosides in the United States only for severe in-hospital infection. These patients receive significantly higher levels for more prolonged periods, and they are more likely to have other medical conditions that exacerbate or cause the hearing loss. Physicians should inquire about a family history of aminoglycoside-induced hearing loss prior to the administration of systemic aminoglycosides and prior to local administration of aminoglycosides into the cochlea as treatment for Meniere disease. In addition, every individual with aminoglycoside-induced hearing loss should be screened for at least the presence of the mitochondrial 1555 mutation, since presence of the mutation will allow counseling of all maternally related relatives to avoid aminoglycosides. Similarly, the description by Estivill et al. (1998) indicates that it might not be unreasonable to screen every individual with nonsyndromic hearing loss for the mutation, unless maternal inheritance can clearly be excluded. Since the test is easily done, and since prevention of hearing loss in maternal relatives can easily be accomplished, this may be cost-effective medical practice. The tragic and avoidable hearing loss of at least 40 patients in the report by Estivill et al. (1998) is a case in point. With the exception of aminoglycosides and mitochondrial mutations in the 12S rRNA gene, there are no proven preventive or therapeutic interventions for mitochondria-related hearing impairments. Diagnosis of such defects is useful for genetic counseling, however, and is indicated in all families with an inheritance pattern of hearing loss consistent with maternal transmission. Diagnosis of such defects may also be indicated in all patients who have both diabetes mellitus and adult-onset hearing loss.

Penetrance and Tissue Specificity

Why are some individuals in families with homoplasmic or near-homoplasmic 1555 or 7445 mutations affected with severe hearing loss, while others have completely normal hearing? Study of the mitochondrial mutations has identified three factors that could modulate phenotypic expression. These factors all affect the level of oxidative-phosphorylation capacity in the cell. The first such factor is environmental agents, of which administration of aminoglycosides, as a triggering event in the case of the 1555 mutation, is the prime example. It is likely that other as-yet-unrecognized environmental factors could play similar, but perhaps less dramatic, roles. Both diet and drugs that affect oxygen-radical formation and breakdown come to mind. The second factor is the rest of the mitochondrial genome; as noted above, the 7445 mutation provides a dramatic example of that effect. The third factor is nuclear genes, of which the Arab Israeli pedigree is a good example. The entire family lives in the similar environmental surroundings of a small Arab village in Israel, and all maternal relatives share the same mitochondrial haplotype. The difference between family members with normal hearing and those with congenital profound hearing loss has been postulated to be due to nuclear genes, and biochemical differences between lymphoblastoid cell lines of hearing and of deaf family members provide support for this hypothesis (Guan et al. 1996). A genomewide search led to the conclusion that this effect is due to several or many genetic loci and not to one major nuclear locus (author's unpublished data). Thus, the model that emerges to explain penetrance is a threshold model—that is, a combination of environmental, mitochondrial, and nuclear factors can push a cell over a threshold, with dramatic clinical differences on either side of the threshold.

The second major biological question relates to tissue specificity: If a homoplasmic mutation affects oxidative phosphorylation (the only known function of the human mitochondrial chromosome and an essential process in every nucleated cell of the human body), it is unclear how the clinical defect remains confined to the cochlea rather than affecting every tissue in the body. We have proposed two different mechanisms in which the tissue specificity in these as well as in some heteroplasmic mitochondrial disorders can be explained (Bernes et al. 1993; Fischel-Ghodsian 1996). First, it is possible that tissue-specific subunits of mitochondrial ribosomes or oxidative-phosphorylation complexes interact specifically with the mitochondrial defect only in tissues in which they are expressed, leading to insufficient oxidative phosphorylation. Tissue-specific subunits for general cellular processes, including oxidative phosphorylation, have been described (Arnaudo et al. 1992). Second, it cannot be excluded that human mitochondrial genes have functions in addition to their functions in oxidative phosphorylation. In this model the mitochondrial mutation would interfere with a tissue-specific secondary function of the mitochondrial gene, which also has to make the cell more sensitive to changes in oxidative-phosphorylation capacity. Precedent for part of this hypothesis can be found in studies of mice and of *Drosophila melanogaster* (Wang et al. 1991; Kobayashi et al. 1993). For example, in addition to being involved

in mitochondrial translation, the mitochondrial large-rRNA gene in *Drosophila melanogaster* has also been identified in the cytoplasm, where it induces pole cell formation in embryos, a key event in the determination of the germ line (Kobayashi et al. 1993).

The experimental approach to the problem of tissue specificity in Leber hereditary optic neuroretinopathy and in the Arab Israeli pedigree has in the past been the search for nuclear factors based on penetrance. The hope was that a single nuclear factor that would explain both the penetrance and tissue specificity would emerge. With the conclusion that such a single factor for penetrance does not exist, even in the simplified model of the Arab Israeli pedigree, the limits of the experimental approaches in humans are close. Major efforts over the next years should focus on the identification or the development of animal models of mitochondrial disorders, followed by the use of established genetic methods to identify the multiple factors contributing to both penetrance and tissue specificity.

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