Modifying with mitochondria

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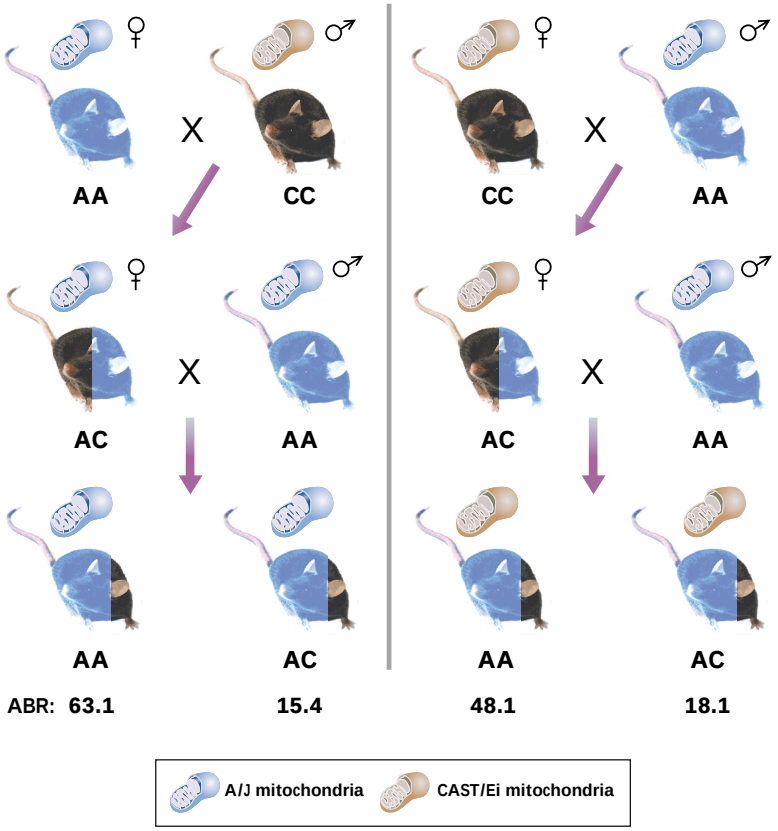
An elegant set of mouse crosses has been used to identify a mitochondrial variant that interacts with a nuclear locus on chromosome 10, *Ahl*, to modify age-related hearing loss. This discovery sets the stage for the identification of factors that modify expression levels and variability of human hearing impairments.

Genetic and environmental factors influence the severity of disease phenotypes. Indeed, our most difficult challenge in the post-sequencing era will be to understand and identify the basis of this variability—so as to better treat disease. Hearing loss, the most common form of sensory impairment, is no exception. The intricate structure and multiple cell types of the inner ear require a range of proteins with different functions, including maintenance of structural integrity, neuronal innervation and mechanoelectrical transduction (see review[1] on page 143 of this issue). Interaction of genes and proteins at different levels influence a multitude of hearing-loss parameters, which include age of onset and hearing-loss thresholds, frequency and type.

Genes that underlie approximately 20 forms of nonsyndromic hearing loss are known, but these are inherently simple forms of hearing loss, each of which is caused by defects in a specific, single gene (that is, they are monogenic forms of hearing loss). The molecular basis of more complex forms of hearing loss, such as presbyacusis (age-related hearing loss) and noise-induced hearing loss, is unknown, as are factors that influence the extent to which risk of hearing loss varies. Fortunately, just as the mouse has proved invaluable in identifying 'deafness' genes, it is leading the way in identifying genetic modifiers and new types of gene interactions that effect hearing loss. On page 191, Kenneth Johnson and colleagues[2] describe a nuclear–mitochondrial DNA interaction in a mouse model of mitochondrial hearing loss. Although the precise nature of this interaction is still unknown, the finding launches a new avenue of hearing research and helps fit together pieces of a complex puzzle.

Several mitochondrial mutations are involved in both syndromic and nonsyndromic forms of hearing loss. Heteroplasmic mitochondrial DNA (mtDNA) mutations are found in hearing loss associated with systemic neuromuscular syndromes, maternally inherited diabetes and the palmoplantar keratoderma skin disease[3]. Over six mtDNA mutations have been found in nonsyndromic hearing loss (including hearing loss induced by exposure to aminoglycosides) in both homoplasmic and heteroplasmic states. Last, but certainly not least, mtDNA mutations are speculated to be associated with presbyacusis, or age-related hearing loss, as mtDNA mutations have been observed in aging auditory tissue[1]. This is entirely feasible given the function of mitochondria in the synthesis of ATP by oxidative phosphorylation, the loss of this activity as a result of mtDNA mutations, and the association of mtDNA mutations with aging[4,5]

About two years ago, Zheng *et al.*[6] demonstrated that hearing in 19 of 80 inbred mouse strains deteriorated in an age-related fashion, similar to that of human progressive hearing loss. In mice, loss of hearing is accompanied by an elevation in auditory-evoked brainstem response (ABR), which permits a quantitative evaluation. As part of an effort to identify a mouse model of maternally inherited hearing loss, Johnson *et al.*[2] made reciprocal backcrosses between three of the inbred strains, A/J, NOD/LtJ and SKH2/J, and a wild-type inbred strain, CAST/Ei (see figure). In contrast with the laboratory strains, CAST/Ei mice maintain normal hearing beyond one year of age. The F1 hybrids resulting from reciprocal crosses



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The mighty mitochondrion. To obtain a model of deafness effected by a mitochondrial mutation, Johnson *et al.*[2] carried out reciprocal crosses between A/J ('blue') and CAST/Ei ('brown') mice. Mitochondrial DNA is transmitted through the maternal lineage, as indicated by the color of the mitochondrion above each depicted mouse. The 'original' mother for each cross was of the A/J strain, carrying two copies of the A/J *Ahl* allele (A), or the CAST/Ei strain, with two copies of the CAST/Ei *Ahl* allele (C). F1 hybrids were backcrossed to A/J mice. (Only backcrosses to males are depicted.) Mitochondrial origin, *Ahl* genotype and average ABR threshold (which indicates extent of hearing loss) at six months are shown for N2 mice.

had normal ABRs, similar to those of their CAST/Ei parents. They were then backcrossed to the hearing-impaired strains, and the hearing ability of the resultant progeny (N2) was tested. Surprisingly, the ABR thresholds were higher in descendents of 'original' A/J mothers when compared with the thresholds of descendents of 'original' CAST/Ei mothers. This pattern was not observed, however, in mice obtained through NOD/LtJ and SKH2/J crosses.

Johnson *et al.*[2] went on to compare the mtDNA sequence of all three inbred strains with published mtDNA sequence and found that most of the differences were simply sequence discrepancies. Notable, however, was their finding that A/J mice carry a stretch of ten adenine residues in the D loop of the *tRNA-Arg* gene—in contrast with the eight or nine residues in NOD/LtJ, SKH2/J and CAST/Ei strains.

A nuclear-mitochondrial interaction

The authors reasoned that the common ancestry of the inbred strains makes it likely their hearing loss is caused by the same genes. Fortunately, a good candidate, a nuclear locus, was close at hand. Last year, several of the same authors mapped the *Ahl* locus to mouse chromosome 10 (ref. 7). Johnson *et al.*[2] therefore genotyped a marker closely linked to this locus in mice derived from the (A/J × CAST/Ei)F1 × A/J backcross.

They found that mice with a double dose of the A/J *Ahl* allele have a greater degree of hearing loss than mice with a single *Ahl* allele. Further analysis revealed that maternal inheritance was only relevant in mice homozygous for the A/J *Ahl* allele—substantiating the hypothesis of a nuclear–mitochondrial DNA interaction. The nature of this interaction, however, has yet to be elucidated.

Two other genetic modifiers of hearing loss have been identified in mice through quantitative trait locus (QTL) analysis. The loci, *mdfw* and *moth1*, act on deaf waddler and tubby mice, respectively, to modify hearing levels dependent on genetic background[8,9]. As is the case for the *Ahl* locus, the genes 'behind' these loci must be identified to permit real progress. It is now known that both the *Ahl* mouse chromosomal region and the orthologous human region (on chromosome 10q) are composed of several 'deafness' loci. Mutations in a novel cadherin gene, *CDH23*, have recently been found for human nonsyndromic deafness DFNB12 and Usher syndrome type 1D on 10q21–q22 (refs. 10–12) and in its mouse homologue, *Cdh23*, for waltzer[12] (*v*). In fact, it has been proposed that the *mdfw*, *v* and *Ahl* loci are allelic, and the recent cloning of *v* allows this to be examined easily.

Despite the discovery of a large number of genes associated with hearing loss in the past four years, it is clear that hearing loss is modulated by many factors, including genetic background of the affected individual. In humans, this manifests itself in variability of hearing loss between individuals with the same mutation, which will make it difficult to design therapeutic strategies based on mutation status alone. Whereas the number of known modifier loci identified can still be counted on one hand, a worldwide effort is being made to identify modifiers of deafness caused by mutation of the connexin-26 gene. This is the most prevalent form of genetic deafness, and hearing levels range from moderate to profound in individuals harboring the same mutation[13].

The kind of genetic study carried out by Johnson *et al.*[2] will eventually lead to the identification of modifier genes, perhaps the most critical elements in presbyacusis and noise-induced hearing loss. Documenting interactions between genes expressed in the inner ear through global expression analyses[14] should accelerate progress. And, ultimately, a better understanding of how modifier genes influence hearing loss should lead to strategies that slow or reverse hearing impairment. □

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