Hear come more genes!

Advances in genome research are leading to the identification of new genes for non-syndromic hearing loss.

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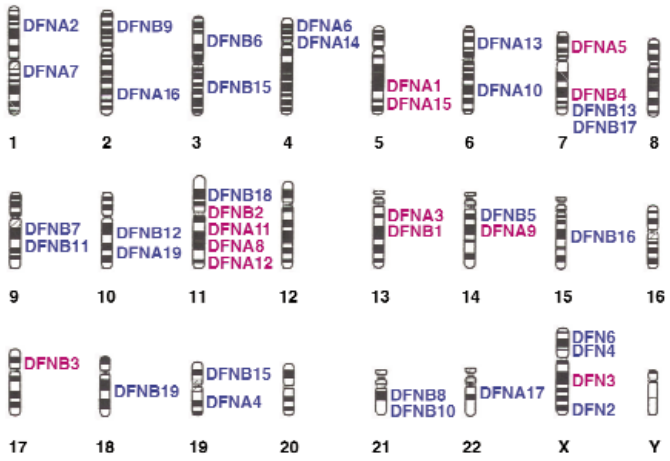
A LARGE ENSEMBLE of proteins act in concert to orchestrate the function of the sensory cells in the cochlea, through which we hear, and the vestibular apparatus of the inner ear, the organ that senses gravity and acceleration. Defects in any one of these proteins can result in deafness. Progress in the genetics of hearing loss is advancing at a dizzying speed with more than 40 loci for nonsyndromic hearing loss (NSHL; deafness with or without vestibular impairment, but with no additional symptoms) mapped so far. Estimates of the number of genes involved in NSHL stand at over 100 (ref.1). This number may seem inflated but, given the intricacy and fine precision required for the smooth operation of inner ear structures, the implication of a mere 0.1–2 percent of all genes in the genome in human NSHL actually seems quite low! Now mutations in two new genes have been reported to cause autosomal dominant hearing loss. A paper appearing in this month's *Nature Genetics*, from the Morton and Seidman groups[2], presents the discovery of a new gene involved in deafness, called coagulation factor C homology or *COCH*, which is located in the *DFNA9* linked region on chromosome 14q11.2–13. And for the second time in six months, investigators in Guy Van Camp's laboratory[3] have identified a gene responsible for autosomal dominant hearing loss, this time located in the *DFNA5* linked region on chromosome 7p15.

Genes involved in NSHL have been isolated by various means (see table). The rate-limiting step in the identification of deafness (DFN) loci has been to find one or more families harboring mutations in these genes (see Fig.1). The traditional approach has been positional cloning—following the segregation of markers in a family with hearing loss, defining the critical region, building a physical map and identifying transcripts or known genes in the region—and this has led to the isolation of three genes: *CX26*, *HDIA1*, and *PDS*. Fortunately the last (potentially tedious) step has been greatly aided by the mouse. Deaf mouse mutants, in conjunction with linkage analysis of families with inherited deafness, have made it possible to identify two human deafness genes, *MYO7A* and *MYO15*. Once the chromosomal regions for the *DFN3*, *DFNA15*, and *DFNA8/12* loci were identified by linkage analysis, examination of the homologous regions on mouse chromosomes revealed excellent candidate genes. Cloning and sequencing of the human orthologues led to the identification of mutations in the *POU3F4*, *POU4F3* and *TECTA* genes. The number of genes identified is continuing to grow: the chromosomal locations of four new autosomal dominant and recessive loci were reported at the recent Molecular Biology of Hearing and Deafness Meeting (Bethesda, Maryland, October 8–11, 1998) and a new family of proteins, the kinesins, have been implicated in hearing loss in the mouse (Y. Kikkawa and H. Yonekawa, personal communication).



Deafness loci distributed throughout the human genome. Each locus represents a family or families with nonsyndromic hearing loss. DFNA represent dominant loci, DFNB represent recessive loci, and DFN represent X-linked loci. Only the chromosomal location is known for loci shown in blue; those shown in red have been cloned.

The tissue-specific approach of Morton, Seidman and co-workers that resulted in the isolation of the *COCH* gene was facilitated by a unique resource, a human fetal cochlear cDNA library[4]. How fortuitous that the first deafness gene isolated from this library was isolated by the creators of this invaluable resource! A portion of the clones in the Morton Fetal Cochlea cDNA library are in the form of over 4,000 expressed sequence tags (ESTs, small segments of cDNAs) and these have been made accessible to the scientific community through the I.M.A.G.E. Consortium and are available on the Web at http://www.bwh.partners.org/pathology. Half of the cochlear ESTs match other ESTs already deposited in Genbank and approximately 500 of these clones represent known human genes. Another 500 have been mapped on radiation hybrid panels and a subset are candidate genes for 18 different NSHL loci.

Several findings—mapping of *COCH* to the *DFNA9* critical region and high levels of expression in human fetal cochlear and vestibular tissue—led the Morton and Seidman laboratories[2] to aggressively pursue screening of *COCH* for mutations. Furthermore, the histopathological analyses of temporal bones from DFNA9 hearing-impaired individuals revealed acidophilic deposits indicative of mucopolysaccharide ground substance, and the location of these deposits corresponded with *COCH* expression in chick inner ear. What has been more difficult to ascertain is a function for *COCH*, though some hints are provided by its homology to known proteins and the histopathology of DFNA9 temporal bones. Database searches revealed homology of the COCH protein to the type A-like domains of von Willebrand factor (vWF), which are found in secreted proteins and implicated in diverse cellular functions. In addition, homology was shown to a 100 amino-acid cysteine-rich stretch of factor C of the horseshoe crab *Limulus*, a protein that initiates coagulation after binding lipopolysaccharides. All three missense mutations in the *DFNA9* kindreds were found in the well-conserved, cysteine-rich regions of the COCH protein, implying that protein structure is compromised. The progressive nature of DFNA9 hearing loss suggests that abnormal COCH protein

(either on its own or interacting with other proteins) leads to the gradual accumulation of precipitated deposits that either contain COCH or are devoid of any protein. These deposits may 'strangle' nerve fibers innervating the sensory cells, eventually destroying the structural integrity of cochlear and vestibular organs.

The effort to sequence the human genome has contributed to the discovery of the *DFNA5* gene[3]. The investigators mapped *DFNA5* to chromosome 7p15 and were then able to identify genes within the candidate region from sequences on line, made available daily through the Washington University Genome Sequencing Center (World Wide Web URL http://genome.wustl.edu/gsc). The availability of sequence saved the authors from the very costly venture of sequencing a large section of the candidate region themselves, or from performing other tedious gene identification experiments. This is of course the very intention of this resource and the investigators were indeed fortunate that their gene lay in the six percent of human sequence already available. The *DFNA5* gene appears to encode a completely novel protein, with no ancestral homology to previously identified protein families. Elucidating the function of *DFNA5* will be especially important because, uniquely, mutations in this gene are associated with progressive high frequency hearing loss, which resembles the much more common age-related deafness (presbyacusis).

What is the point of isolating all of these NSHL genes? First, from a biological point of view, the relationship between the proteins these genes encode and how they interact with one another to regulate hearing and balance is fascinating. From a clinical point of view, this information will benefit investigators interested in establishing genotype-phenotype correlations for deafness and will enable them to make 'educated guesses' about genetic screening for mutations. The high proportion of connexin 26 mutations appearing in prelingual autosomal recessive deafness[5] has changed the face of genetic counseling, emphasizing the need for geneticists and physicians to discuss the implications of screening. Our knowledge of the molecular basis of deafness will continue to accelerate rapidly and should lead to a new generation of treatments, such as sensory hair cell regeneration and gene therapy, for audiovestibular disorders induced by both genetic and environmental factors

Genes and the proteins they encode involved in hearing loss[1]

Protein	*Locus*	*Human deafness*	*Mouse deafness*
Transcription factors			
POU3F4	*POU3F4*	DFN3	Brn-4.0 knockout
POU4F3	*POU4F3*	DFNA15	Brn-3.1 knockout
Motor proteins			
Unconventional myosins			
Myosin VIIA	*MYO7A*[2]	DFNB2, DFNA11	shaker-1 (*sh1*)
Myosin VI	*MYO6*	-	Snell's waltzer (*sv*)
Myosin XV	*MYO15*	DFNB3	shaker-2 (*sh2*)
Kinesins			
Deafness-associated-kinase	*DAK*[3]	-	jackson shaker (*js*)
Gap junction proteins			
Connexin 26	*GJB2*	DFNA3, DFNB1	-
Formins			
Diaphanous	*HDIA1*	DFNA1	-
Transporters			
Pendrin	*PDS*[2]	DFNB4	-
Extracellular matrix proteins			
α-tectorin	*TECTA*	DFNA8, DFNA12	-
Ion pumps			
plasma membrane calcium-ATPase type 2	*Atp2b2*	-	deaf waddler (*dfw*)
Novel			
COCH	*COCH*	DFNA9	-
DFNA5	*DFNA5*	DFNA5	-

[1]References and additional information can be obtained on the Hereditary Hearing Loss Homepage, World Wide Web URL: http://dnalab-www.uia.ac.be/dnalab/hhh.html.
[2]Also involved in syndromic hearing loss.
[3]Strong positional candidate.

1. Morton, N.E. Genetic epidemiology of hearing impairment. *Ann. NY Acad. Sci.* **630**, 16–31 (1991).
2. Robertson, N.G. *et al.* Mutations in a novel cochlear gene *COCH* cause DFNA9, a human nonsyndromic sensorineural deafness with vestibular dysfunction. *Nature Genet.* **20**, 299–303 (1998).
3. Laer, L.V. *et al.* Nonsyndromic hearing impairment is associated with a mutation in *DFNA5*. *Nature Genet.* **20**, 194–197 (1998).
4. Robertson, N. G., Khetarpal, U., Gutierrez-Espeleta, G. A., Bieber, F. R. & Morton, C. C. Isolation of novel and known genes from a human fetal cochlear cDNA library using subtractive hybridization and differential screening. *Genomics* **23**, 42–50 (1994).
5. Denoyelle, F. *et al.* Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene. *Hum. Mol. Genet.* **6**, 2173–2177 (1997).

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Giving DNA vaccines a helping hand

Pathogen-derived 'helper' sequences promote anti-tumor immune responses induced by a naked DNA vaccine (pages 1281–1286).

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DNA VACCINE TECHNOLOGIES offer considerable promise for the improvement of existing immunization strategies (reviewed in refs. 1–3). Since the first observation that the simple injection of plasmid DNA vectors results in the induction of an antibody response, many preclinical studies have suggested that DNA-based immunization can induce protective and therapeutic immune responses against infectious and neoplastic diseases that were not possible using conventional immunization strategies. The efficacy of DNA-based immunization against viral disease and cancer is now being evaluated in human clinical trials. On page 1281 of this issue, King *et al.*[4] present a new strategy for enhancing the immune response induced by a DNA vaccine against B-cell lymphoma and