The Architecture of Hearing

The cloning of a string of genes that, when mutated, cause deafness without any other symptoms is opening a view of the inner workings of the auditory system and how it develops

The M family of Costa Rica can trace its ancestry to Spanish explorers who settled in the Americas about 1600. They can trace another family legacy as far back as the mid-1700s: a tendency for many family members to go deaf, often beginning at age 10. Eight generations later, about half of 400 some members of this extended family—most of them still living in the same community—eagerly agreed to help Pedro León, a molecular biologist at the University of Costa Rica, track down the cause of their deafness.

Now the 20-year quest, chronicled in Costa Rican newspapers and television, is over. Geneticists Eric Lynch and Mary-Claire King of the University of Washington, Seattle, working with León, have cloned the gene responsible for the family's deafness.

The gene, described in this issue of *Science*, is only the latest harvest of an effort to track down genes for nonsyndromic deafness—deafness with no other symptoms—that accelerated in 1992, when King, Lynch, León, and their colleagues mapped the chromosomal location of the gene at fault in the Costa Rican family. King, Lynch, and León's achievement provided a big boost to the field, as it was the first to use family studies to narrow down the chromosomal location of these genes. "It was a kick in the field, the [result] we all cited in our grant applications," says Cynthia Morton, who studies the genetics of deafness at Brigham and Women's Hospital in Boston.

The King team's report, which appears on page 1315, brings to three the number of new, nonsyndromic genes pinpointed in the last year. And many more are on the way; the positions of some 30 other genes for nonsyndromic deafness have been mapped since the 1992 report. The new genes are not the first "deafness" genes. Researchers have already cloned more than two dozen genes that cause "syndromic" deafness, in which deafness comes with other symptoms, such as blindness or pigment abnormalities. But 70% of hereditary deafness is nonsyndromic, and up to 60% of the 28 million cases of hearing loss in the United States are thought to have a hereditary component. In addition, because the mutations in nonsyndromic deafness genes affect hearing alone, researchers expect these genes to offer clues to how the human auditory system works, and how it can go awry.

Indeed, all three of the new genes seem to be involved in the operation of the sound-sensitive hair cells in the inner ear, or cochlea. Two of them, including the one that is mutated in the Costa Rican family, code for proteins that apparently help organize actin, a structural protein that stiffens the microscopic projections that crown these cells; the third helps build an electrical channel that may enable hair cells to reset themselves after they are exposed to sound. All three could offer glimpses into how hair cells normally develop and function, and point to treatments to keep hearing sharp.

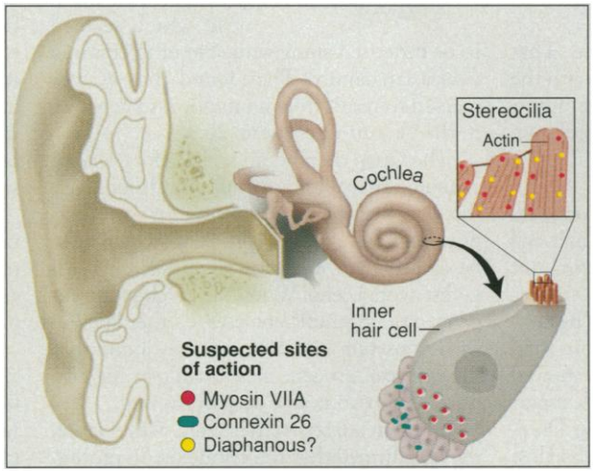


ILLUSTRATION: K. SUTLIFF

Inner sanctum. In the cochlea, the proteins of three new deafness genes probably help hair cells function.

Genetic studies of nonsyndromic deafness have been slow in coming because the symptoms of individuals who are deaf, but don't have other problems, are so similar that geneticists have had a hard time sorting out people who are likely to have the same gene defects. Until the new crop of genes, geneticists had been able to link only three other genes to hearing loss without any symptoms. All three have distinctive inheritance patterns, which made them easier to track down: Two are found in the cellular organelles called mitochondria and the third on the X chromosome. But mutations in these genes are relatively rare.

Mice to men

Because of the difficulty of human family studies, some researchers took a different tack entirely. For example, mouse geneticist Karen Steel of the Medical Research Council (MRC) Institute of Hearing Research in Nottingham, United Kingdom, and her colleagues turned to mutant mice, hoping to ferret out deafness genes that would lead them to comparable genes in humans. She began with a mouse called *Shaker1*, which can't hear or keep its balance. With Steve Brown from the MRC Mouse Genome Centre in Harwell, she then interbred these mice, keeping track of how often various genetic markers were associated with hearing loss.

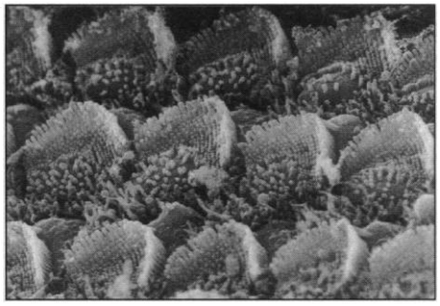
This analysis showed that the animals' deafness is caused by a mutation in one of their genes for myosin, a protein that interacts with actin in many types of cells. The human counterpart of the gene, called *myosin VIIA*, resides at a site on the long arm of human chromosome 11 that had already been linked to a condition called Usher syndrome 1b, whose victims are both blind and deaf. In work completed about 2 years ago, Brown and Steel went on to show that the syndrome is indeed caused by a mutant *myosin VIIA* gene.

But the researchers suspected that the gene might be responsible for other cases of deafness as well. Because *Shaker1* mice are not blind, it seemed that a still less severe *myosin VIIA* mutation might produce hearing loss alone—a hunch Brown and Steel subsequently confirmed.

The researchers first studied eight Chinese families in which deafness was a recessive trait. In two of these the condition could be traced to a defective *myosin VIIA* gene. At the same time, Christine Petit at the Pasteur Institute in Paris found the same recessive defect in the deaf members of a large Tunisian family. (Both sets of results appeared in the June issue of *Nature Genetics*.) And now, in this month's *Nature Genetics*, Brown and Steel show that this same gene, mutated yet another way, causes a progressive form of nonsyndromic deafness. The recessive deafness seen in both the Chinese and Tunisian families is present at birth. But the new mutation identified by Brown and Steel is dominant—only one copy of the gene needs to be mutated to cause the condition—and the deafness develops after birth and exposure to language. "*myosin VIIA* is involved in a spectrum of deafness," Steel concludes.

The key to its involvement, further work by Steel and her colleagues suggested, may be the

effect that *myosin VIIA* mutations have on the structure of the hair cells. Normal hair cells are crowned with a V-shaped array of projections called stereocilia, which consist of an actin core and a myosin outer cover. The arrangement of stereocilia is important because they bend when sound vibrates the fluid surrounding the hair cells, causing those cells to fire a signal at the auditory nerve. But in mice with a mutated *myosin VIIA* gene, Steel says, "instead of a nice V shape, you get little clumps of stereocilia."



TIM SELF/MRC

Precise alignment. As these hair cells mature, the projections will form a distinct V pattern.

She and Brown think that the myosin may carry or anchor other molecules that are important for forming the precise arrangement of the stereocilia, and also for maintaining them over time. That double role may be why some people with the mutations are deaf from birth, while others develop the condition later in life, Steel says.

Family history

The new deafness gene described by Lynch and King in this issue may also lead to hair-cell disruptions. This group was able to surmount the difficulties of genetic linkage studies thanks to León's Costa Rican family. The family tree León worked out covered more than 190 people over eight generations and showed that about half the children born to deaf parents also became deaf. By analyzing DNA from 147 family members, 78 of whom had lost their hearing, the researchers were able to narrow the location of the faulty gene to an 800,000–base pair region on chromosome 5.

Next, Lynch sequenced that portion of the chromosome and checked the resulting sequence against genes or partial genes on file in the public database called GenBank. The computer searches turned up more than 15 candidate genes. One of them—the human equivalent of a gene called *diaphanous*, previously identified in fruit flies and mice—is consistently mutated in the deaf members of the Costa Rican family, but not in unaffected members or in unrelated controls.

Evidence that these mutations might also impair hair-cell function came when Lynch, with help from Morton's lab, showed that the *diaphanous* gene is active in the human cochlea. In addition, work in mice and fruit flies indicates that the gene's protein product serves as temporary scaffolding for actin as it rearranges to help a cell divide or form projections such as the stereocilia. "We've identified a gene that encodes a protein that when normal and healthy is probably critical to the maintenance of normal hearing," King says.

Serendipity aided the discovery of the third member of this year's trilogy of nonsyndromic deafness genes. The gene is *connexin 26*, which was already known to code for a protein that helps make gap junctions—electrical channels between adjacent cells—and genetic linkage studies by several research teams had already suggested that both dominant and recessive forms of nonsyndromic deafness might be due to mutations in the same region of chromosome 13 that contains the gene. But Irene Leigh, David Kelsell, and Howard Stevens of the University of London and their colleagues were pursuing a third form of deafness, which seemed to be inherited along with skin problems in a Caucasian family. They found that it, too, seemed to result from a mutation somewhere in the vicinity of *connexin 26*.

The group then began looking for *connexin 26* mutations in family members and found that this gene was indeed responsible for their deafness, but not for the skin problems, which must be caused by a mutation in a different, still undiscovered gene. When the Leigh group expanded their genetic analyses in other families with nonsyndromic deafness, they found that the *connexin 26* gene was at fault in the two types of nonsyndromic deafness as well.

As the team reported in the 1 May *Nature*, they confirmed that the connexin 26 protein is made in the human cochlea by treating tissue from the inner ear with mouse antibodies to the protein. She and her colleagues suspect that the gap junction it helps build normally creates a channel that pumps out the potassium ions that flood the hair cells when they are stimulated. Without the gap junctions, Kelsell speculates, the potassium levels in the cells may stay high, and the mechanism that enables the cells to respond quickly to sounds may not be reset.

Researchers do not yet know what percentage of hereditary deafness is caused by *myosin VIIA* and *diaphanous* mutations, but *connexin 26* mutations are turning out to be surprisingly common in families with a history of deafness. In this month's issue of *Human Molecular Genetics*, Petit's team at Pasteur reports that the gene was mutated in 39 of the 65 families tested. "It's clearly a major contributor to deafness," says Thomas Friedman, a human molecular geneticist at the National Institute on Deafness and Other Communication Disorders (NIDCD) in Rockville, Maryland.

What's more, the gene apparently has a mutational "hot spot." Although the families studied by Petit come from all over the world—Tunisia, France, New Zealand, and the United Kingdom—half had the same mutation: the loss of one of a string of six guanine bases located near the beginning of the gene. Other teams made similar observations in deaf families in an isolated Israeli-Arab village and in the Mediterranean area. That finding, combined with the fact that *connexin 26* is a simple gene, containing only one coding region, means it should be relatively simple to develop a genetic test for *connexin 26* mutations. Such a test would allow rapid diagnosis of deafness in newborns so that they can be taught sign language from a very early age, keeping them from falling behind in their language acquisition skills.

Other hearing researchers are optimistic that these gene finds will lead to treatments, especially for people who are born able to hear and then become deaf later. In addition, researchers speculate that subtle mutations in the genes might also cause the very common type of hearing loss associated with aging. If so, "you stand a much better chance of being able to do something about [progressive deafness]," says Steel.

Indeed, researchers predict that the deafness genes found this year are just the beginning. In Boston, Morton has created the first cDNA library of genes expressed in the human cochlea, and Petit has created a mouse cochlea cDNA library. Each of these genes could be a candidate deafness gene. "The field is really just bursting at the seams," says James Battey, scientific director of NIDCD. He predicts that a half dozen more will be cloned in the next year.*

NONSYNDROMIC DEAFNESS GENES

Gene	Chromosome	Dominant	Recessive
diaphanous (p140mDia)	5q	x	
myosin VIIA	11q	x	x
connexin 26	13q	x	x
POU3F4	Xq		x
12S rRNA	mitochondrial*		
tRNA$^{Ser(UCN)}$	mitochondrial*		

* These mutations are not inherited as either dominant or recessive genes. With the 12S rRNA mutation, hearing loss results only after treatment with an aminoglycoside antibiotic.

These new additions should help piece together the mystery of hearing as well as the puzzle of deafness. "We're on the brink of a whole new understanding of the molecular dynamics of hearing," says Lynch.

–Elizabeth Pennisi

* The Hereditary Hearing Loss home page (http://dnalab-www.uia.ac.be/dnalab/hhh) tracks new gene finds.