Genes of Silence

Scientists track down a slew of mutated genes that cause deafness

By JOHN TRAVIS

On the north shore of the island of Bali sits Bengkala, a 700-year-old village that has earned a measure of fame for its remarkably large number of deaf people—currently 48 of the nearly 2,200 inhabitants. These deaf residents have had a profound influence on Bengkala's culture.

"This is a very unusual community. The village has created a unique sign language, and most people in the village have some facility with it," says John T. Hinnant of Michigan State University in East Lansing, an anthropologist who has recently spent time documenting the village's culture on videotape.

Signed names have replaced spoken names. Villagers refer to Hinnant by making the sign for a videocamera or, he ruefully admits, the sign for a big nose.

Bengkala isn't fertile scientific ground just for anthropologists. Hinnant learned of the village from geneticists who are working to isolate the mutated gene responsible for the widespread hearing loss. So far, they've localized the gene to a small region of chromosome 17.

"We're quite close to having it," says Thomas B. Friedman of the National Institute on Deafness and Other Communication Disorders in Bethesda, Md.

Once found, the Bengkala gene will join the numerous other genes that investigators have recently tied to hearing loss. Last year was "quite dizzy with deafness genes," remarks Karen P. Steel of the Medical Research Council's Institute of Hearing Research in Nottingham, England.

These new genes are providing some of the first molecular insights into the workings of the auditory system and into the reasons why about 1 child in 1,000 is born deaf. Mutations in some of the genes produce hearing loss alone. In other cases, the gene mutations cause a range of other problems as well, such as blindness.

While the identification of these deafness genes may not help people born with profound, almost always permanent hearing loss, it may benefit the many whose deafness strikes later in life. "If you know at a biological level what's going wrong in the ear, you at least have a chance of intervening, preventing the hearing loss from getting any worse, or maybe even reversing it," says Steel.

Discerning the origins of deafness, genetic or otherwise, is no easy task. Indeed, physicians often cannot diagnose the flaw that eliminates a person's perception of sound.

A person's ability to hear depends on the spiral-shaped cochlea, a pea-size organ in the inner ear. In each cochlea, a mere 16,000 hair cells, each bearing a cap of bristles called stereocilia, detect noises. Sound-induced movement of the stereocilia stimulates the hair cells to generate electric impulses that convey auditory information to the brain. Physicians suspect that most cases of deafness stem from problems with the hair cells—but that's tough to confirm, since the cochlea is hidden within a bony labyrinth.

Resolving the genetics of deafness is no less challenging. Genetic flaws account for about half the incidence of congenital deafness, but researchers estimate that mutations in more than a hundred genes may trigger such hearing loss. This genetic diversity shows up in the striking fact that the majority of children born to two deaf parents can hear.

Scientists have been able to find many genes responsible for the syndromic forms of deafness, in which hearing loss is accompanied by other symptoms. In 1992, for example, investigators found the mutated gene behind Waardenburg's syndrome, a condition characterized by deafness, widely spaced eyes that are sometimes mismatched in color, and a white forelock (SN: 5/2/92, p. 296). Last year, several research groups examining people with deafness, frequent loss of consciousness, and a dangerous heart arrhythmicity attributed the syndrome to mutations in various genes that encode components of a protein complex that allows potassium ions to enter cells.

In contrast, investigators have struggled to find genes behind nonsyndromic deafness, which is the far more common type, accounting for the majority of congenital hearing loss.

The hunt has been complicated by a phenomenon to which scientists have given the unromantic name of assortative mating. "Deaf individuals tend to intermarry, at least in developed countries," explains Christine Petit of the Pasteur Institute in Paris.

Since the two members of a deaf couple generally owe their hearing loss to different genes, assortative mating creates a nightmare for geneticists trying to track a single deafness gene through a family's history, a useful step in finding its chromosomal location.

For a long time, investigators considered mapping of nonsyndromic deafness genes an impossible task, notes Richard Smith of the University of Iowa in Iowa City, who maintains a World Wide Web site that tracks the field's progress (the hereditary hearing loss home page at http://dnalab-www.uia.ac.be/dnalab/hhh).

The first major break in the hunt for nonsyndromic deafness genes came when Pedro E. León of the University of Costa Rica in San José learned of a large, local family with many deaf members. The affected members of the family can hear at birth, but around age 10 they start to go deaf. By age 30, all hearing is lost.

León traced this trait back through eight generations of the family and obtained blood samples from nearly 150 living members. He then teamed up with a research group headed by Mary-Claire King of the University of Washington in Seattle. In 1992, after tracking genetic markers known to be inherited along with the deafness, the investigators announced that they had mapped the gene responsible to a part of chromosome 5.

With that first success in hand, says Smith, many other scientists began to search around the world for large, inbred populations in which hearing loss is common. Meanwhile, León and his colleagues continued their quest until, in

the Nov. 14, 1997 SCIENCE, they identified the mutated gene behind the Costa Rican family's deafness.

By examining a library of genes active in the cochlea of a developing fetus, León's team has shown that the normal version of this gene is turned on in the inner ear. Yet the brain, heart, lungs, kidney, and many other tissues also seem to use the gene. That finding poses a difficult question about those with mutations in the gene.

"Why are these people deaf and otherwise normal, when this gene's product is used by a vast array of cell types?" asks the University of Washington's Eric D. Lynch, who led the search for the gene.

The deafness gene resembles a fruit fly gene called *diaphanous*, which has offered some clues to the human gene's possible role in hearing. Studies of *diaphanous* and a similar mouse gene indicate that their proteins help a molecule called profilin assemble filaments of another protein, actin.

Such filaments provide a dynamic skeleton for cells. They may be especially important to the cochlea's hair cells because the stereocilia owe their stiffness to bundles of actin filaments that are continuously broken apart and rebuilt.

"The hair cells are possibly so reliant on the actin cytoskeleton to perform their hearing function that we see a [consequence of the gene's mutation] there and not elsewhere," says Lynch.

To study further the cause of the Costa Rican family's deafness, the researchers plan to genetically engineer mice to suffer a similar form of hearing loss. "We're trying to mimic the mutation as closely as possible," says Lynch.

The scientists are also investigating whether some cases of hearing loss stem from mutations in a second human gene that they have found resembles *diaphanous*. "We think it makes a good candidate, considering its sibling's role in deafness," says Lynch.

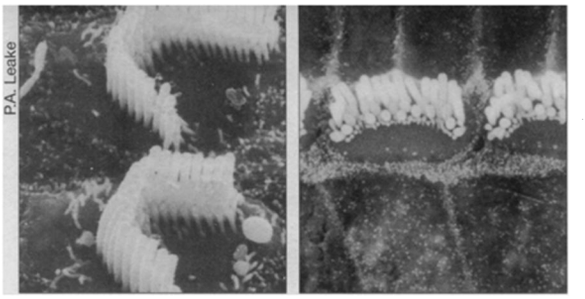
The Costa Rican gene wasn't actually the first nonsyndromic deafness gene isolated. That honor went several months earlier to the gene encoding a protein called connexin 26. Ironically, this gene came to light during the search for the cause of an apparently syndromic form of hearing loss.

Investigators were studying a family that had both deafness and a skin disorder. The researchers focused on a group of channel-forming proteins, or connexins, that play an important role in the skin. Connexins allow small molecules to pass between cells.

As suspected, some deaf family members had mutations in the gene for a connexin. The scientists realized, however, that the family's symptoms had been mistakenly linked. "Not everyone who had skin disorders was deaf and vice versa," notes Robert F. Mueller of St. James' University Hospital in Leeds, England.

In the May 1, 1997 NATURE, Mueller and his colleagues reported that mutations in the gene for connexin 26 produce deafness, but not the family's dermatological problems.

The investigators also used antibodies that bind to connexin 26 to show that the protein is present in various regions of the inner ear, but they're still unsure what role it plays in hearing. Some scientists speculate that flawed connexins may disrupt the ability of hair cells to take in or expel potassium ions. Both activities are crucial to the cell's generation of electric impulses.



These scanning electron micrographs show surface views of outer hair cells (left, magnified 7,500 times) and inner hair cells (right, magnified 4,000 times) within the mammalian cochlea. The white rods, or stereocilia, enable the cells to detect sound waves.

The gene for connexin 26 illustrates one emerging theme of deafness genetics. In several cases, different mutations in a single gene can cause either recessive or dominant forms of inherited hearing loss. In the recessive forms, a deaf child has inherited a mutant copy of the gene from both parents, and deafness is usually present at birth. If the gene has a dominant mutation, only one flawed gene is needed to produce deafness, and the hearing loss often occurs gradually and later in life.

Another lesson hearing researchers have learned is that syndromic and nonsyndromic deafness can represent two sides of the same coin. They have found that certain mutations in a gene produce both hearing loss and other symptoms, while other mutations seem to cause just deafness.

"For clinical geneticists, who have spent their lives asking, 'Is this syndromic or nonsyndromic?' it's mind-boggling to find out it's just an artifact of where the mutation is," says Mueller.

Take Pendred's syndrome, perhaps the most common syndromic cause of hereditary hearing loss. Usually born deaf, though the hearing loss sometimes occurs during childhood, people with Pendred's syndrome also develop goiter, an enlarged thyroid gland, around puberty.

In 1996, two research groups that had studied large, inbred families exhibiting Pendred's syndrome reported that they had mapped the responsible gene to chromosome 7. One group, led by Benjamin Glaser of Hadassah University Hospital in Jerusalem and Val C. Sheffield of the Howard Hughes Medical Institute at the University of Iowa in Iowa City, then contacted Eric D. Green of the National Human Genome Research Institute in Bethesda, Md.

"My lab's passion is chromosome 7," says Green, who has developed a detailed map of genetic markers on the chromosome.

After narrowing the chromosomal region under suspicion, Green and his colleagues began sifting through genes there, testing whether deaf family members had mutations in any of them. As reported in the December 1997 NATURE GENETICS, the investigators finally struck gold with a gene that appears to encode a protein, which they call pendrin, that ferries sulfate molecules across cellular membranes.

Cells attach sulfates to proteins and many other molecules for myriad reasons, and scientists have recently discovered that mutations in other sulfate transporter genes can cause dwarfism or constant diarrhea, notes Green.

As expected, the gene encoding pendrin turned out to be active in the adult thyroid gland. Preliminary experiments suggest that it is also active in the developing fetal cochlea, which may explain the deafness associated with Pendred's syndrome.

"We think there's a direct role this protein plays in the development of the inner ear," says Green. "The cochlea is like a spiral staircase, and [in people with Pendred's] it just doesn't have the right number of turns in it."

Blurring the definition of Pendred's syndrome, however, the investigators have also found that certain mutations in the pendrin gene can cause deafness without thyroid problems. "Not all individuals develop goiters," says Green.

This finding, he adds, may mean that mutations in the pendrin gene account for many more cases of deafness than researchers had previously suspected.

A similar conclusion now seems likely for a gene tied a few years ago to Usher's syndrome. In this condition, people born deaf slowly lose their sight. The gene encodes a protein called myosin VIIa, and in several reports this year, investigators have described mutations in the gene among deaf people with no obvious visual problems.

The gene first came to light through studies of mice. Over the years, scientists have identified many deaf mouse strains. As names like *shaker* and *waltzer* indicate, these strains were often first identi-

fied by their head-tossing, odd circling behavior, or other abnormal movements that seem to reflect balance difficulties stemming from inner ear problems.

Several years ago, Steel and her colleagues discovered that a mouse strain called *shaker-1* owes its hearing loss to mutations in the gene for myosin VIIa. This protein and many similar ones can bind to actin filaments and, through the effort of a built-in motor, move along them, often ferrying some form of molecular cargo.

Steel and her colleagues then realized that the human version of the gene for myosin VIIa resides in a part of chromosome 11 already implicated in one form of Usher's syndrome. Further research by Petit and other investigators established that mutations in this gene indeed cause the syndrome's visual and auditory problems.

Several cell types in the eye produce myosin VIIa, and according to studies conducted by Tama Hasson of Yale University School of Medicine, the protein is abundant in stereocilia and other specific regions of hair cells. "We know where the protein is, so now the question is, what does the protein do," says Hasson.

While the search for the protein's natural roles continues, Steel and her colleagues report in the November 1997 NEURON that myosin VIIa appears to play a role in the accumulation of a certain antibiotic inside hair cells. Consequently, the presence of myosin VIIa in the inner ear may explain why antibiotic-induced deafness is one of the major nongenetic causes of hearing loss.

As investigators continue to catalog new deafness genes, they need to firm up their estimates of how many cases of hearing loss can be attributed to each gene. At the moment, most of the excitement centers around connexin 26. The most dramatic evidence concerning its gene comes from a study by Petit and her colleagues in the November 1997 HUMAN MOLECULAR GENETICS. Among 65 families with histories of hearing loss, most of them from Tunisia, France, New Zealand, and the United Kingdom, the researchers found that about half had mutations in the gene for connexin 26.

"It looks like connexin 26 accounts for a lot [of deafness] in certain areas of the world, but it's not clear how much it accounts for hereditary deafness in the United States," says Friedman.

As the genetic data accumulate, physicians may begin to screen for mutations that might cause late-onset hearing loss or counsel people about potential outcomes of future pregnancies.

The latter issue is particularly important, since only half of congenital hearing loss stems from inherited mutations. "The main question of a family with a deaf child is whether they will have another one," notes Petit.

Outside the clinic, scientists will continue to examine how the proteins encoded by these deafness genes make hearing possible. They will investigate primarily the mouse inner ear, especially in mice bred to have mutant copies of the various deafness genes.

Mice will also serve as the initial testing ground for any treatments that might emerge from the discovery of deafness genes. Last year, for example, Anil K. Lalwani of the University of California, San Francisco reported at a meeting that he and his colleagues had for the first time successfully introduced a foreign gene into the mouse cochlea. With the growing number of identified deafness genes, physicians should soon know which ones will be useful in gene therapy for preventing or reversing hearing loss. □

Behavior

To dream, perchance to scan

Brain scans obtained from sleeping men have helped illuminate the neural system that makes dreaming possible, reports a team of investigators.

During rapid eye movement (REM) sleep, the brain's visual system retains its power to generate images but cannot process external sensations, the group notes in the Jan. 2 SCIENCE. Several inner-brain structures involved in memory and emotion exhibit heightened activity during REM sleep. In contrast, frontal lobe areas that integrate visual information with other sensations and make possible temporary recall of related items display sharp drops in neural effort, contends neuroscientist and report coauthor Allen R. Braun of the National Institutes of Health in Bethesda, Md.

These physiological features of REM sleep lay the groundwork for characteristic elements of dreams, such as intense emotions, bizarre happenings that seem unquestionably real, fractured time sequences, and a surreal lack of reflection about all the strange goings-on, the researchers theorize.

Braun and his colleagues obtained positron emission tomography (PET) scans of blood flow in the brains of 10 men during REM sleep and compared the images to scans taken during another stage of sleep—slow wave sleep—and to scans taken while the men were awake. Dreams occur most often and most vividly during REM sleep, the investigators assert.

REM sleep was characterized by low blood flow in the frontal lobes and in the tissue at the back of the brain that receives initial visual information from the eyes. A drop in flow reflects lessened neural activity. Blood flow surged in areas that orchestrate visual scenes and that contribute to emotion and long-term memory.

In an earlier PET scan study, researchers had found that the amygdala—a brain structure involved in processing fear and other emotions—becomes particularly active during REM sleep (SN: 9/21/96, p. 184).

Long-time sleep researcher David Foulkes, formerly of the Georgia Mental Health Institute and now working independently in Atlanta, doubts that these new brain studies shed light on the anatomy of dreaming—precisely because they focus solely on REM sleep.

Much evidence suggests that dreams of the same kind and of comparable intensity occur in nonREM sleep, Foulkes argues. Thus, he says, no physiological property unique to REM sleep can explain dreaming.

In the absence of environmental input or voluntary self-control, consciousness—a cognitive system for producing awareness of sensations and memories—creates dreams, Foulkes theorizes. Under some circumstances, ranging from relaxed wakefulness to REM sleep, this mechanism results in dreaming.

People develop the capacity to dream during childhood, says Foulkes, who notes that dreams do not assume a story-like form until age 7 or 8. Maturation of the frontal lobes may be required for dreams with a narrative structure, in his view.

The new PET findings offer valuable insights into the anatomy of dreaming, counters psychiatrist J. Allan Hobson of Harvard Medical School in Boston. Optimal brain conditions for dreaming exist only during REM sleep, he holds.

Braun's data support the notion that dreams reflect the activation of an individual's "emotional memory bank," with little direction or interpretation from the frontal lobe, he proposes.

"The PET results are consistent with Freud's idea that dreams have meaning," Hobson says, because they draw on neural repositories of memories. "But they challenge his theory that an unconscious dream censor [which would be located in the frontal lobes] screens out and masks particularly disturbing wishes."

Whatever the anatomy of dreaming, notes Braun, the core functions of REM sleep remain poorly understood. —*B.B.*