MedicalXpress

Researchers identify key proteins of inner ear transduction channel

November 21 2011

National Institutes of Health-funded researchers have identified two proteins that may be the key components of the long-sought after mechanotransduction channel in the inner ear—the place where the mechanical stimulation of sound waves is transformed into electrical signals that the brain recognizes as sound. The findings are published in the Nov. 21 online issue of *The Journal of Clinical Investigation.*

The study used mice in which two genes, TMC1 and TMC2, have been deleted. The researchers revealed a specific functional deficit in the mechanotransduction channels of the mice's stereocilia (bristly projections that perch atop the sensory cells of the inner ear, called hair cells), while the rest of the hair cell's structure and function was normal.

These genes and the proteins they regulate are the strongest candidates yet in a decades-long search for the transduction channel that is at the center of the inner ear's ability to receive sound and transfer it to the brain. Andrew J. Griffith, M.D., Ph.D., chief of the molecular biology and genetics section and the otolaryngology branch at the National Institute on Deafness and Other Communication Disorders (NIDCD) at NIH, and Jeffrey R. Holt, Ph.D., an associate professor in the department of otolaryngology at Harvard Medical School's Children's Hospital in Boston, co-led the team that published the findings.

"For many years, the NIDCD has funded research using genetic approaches to discover and analyze genes underlying hereditary deafness," said James F. Battey, Jr., M.D., Ph.D., director of the

NIDCD. "We believed these studies would also help us identify genes and proteins that are critical for normal hearing. Now our efforts appear to be paying off, in this discovery of integral components in the mechanotransduction complex."

Like other sensory cells, the hair cell's transduction channel is presumed to be an ion channel—a tiny opening or pore in the cell that lets electrically charged molecules (ions) pass in and out—and which acts as a molecular mechanism for turning sound vibrations into electrical signals in the cochlea, the snail-shaped organ of the inner ear. Mechanotransduction in sensory hair cells also underlies the sense of balance in the vestibular organs of the inner ear. Researchers have theorized that the channel must be located in the tips of hair cell stereocilia, which are linked by a system of horizontal filaments (called tip links) that connect the shorter stereocilia to their taller neighbors so that the whole bundle moves as one unit when it is stimulated by sound or head movements.

Drs. Griffith and Holt and their team focused on TMC1, a gene named for its trans-membrane-channel-like amino acid sequence. Dr. Griffith and another team of NIDCD-funded collaborators had previously discovered TMC1 as a gene in which mutations cause hereditary deafness in humans and mice. Multiple regions of the protein that TMC1 encodes looked as though they would be able to span the plasma membrane (the outer membrane of a cell that controls cellular traffic) and act as a receptor or a channel. The researchers also zeroed in on TMC2, a gene that has a structure much like TMC1's and has similar membrane-spanning domains in its code.

The scientists genetically engineered mice with knocked-out versions of the two genes and then bred the mice so that some had no functional copies of TMC1 or TMC2, and some had one gene knocked out but the other present. This was to help the scientists identify redundancy in gene

function, a consequence of families of genes that can fill in for each other when one of them is deleted or mutated.

The team observed that TMC2 knockout mice had normal hearing and no balance issues (balance issues would indicate problems with the hair cells in the vestibular system), but that mice with no functional copies of TMC1 or TMC2 had the classic behaviors of dizzy mice – head bobbing, neck arching, unstable gait, and circling movements – and they were deaf. The TMC1 knockout mice were also deaf, but they had no balance issues. Looking at tissue slices of the mouse inner ears over time from birth, the researchers could see the expression of TMC1 and TMC2 in hair cells in the vestibular organs and the cochlea from birth. But a week later, TMC2 appeared to be turned off in the cochlea while it continued to be expressed in the vestibular organs. Since only TMC1 continues to be expressed in the mature cochlear hair cell, the researchers propose that TMC1 is essential for hearing, but TMC2 is not. However, in the vestibular system, TMC2 expression can substitute for TMC1 to maintain vestibular function.

To further home in on the properties of TMC1, the scientists measured the electrical activity in hair cells from the double mutant mice. Mice that had no functional TMC1 or TMC2 had no mechanotransduction currents in their cells. All other ion channels in the double mutant mice appeared to be functioning normally. The TMC1 deficit appeared to be specific to mechanotransduction -- not just a symptom of a problem that affects the whole cell.

Under a scanning electron microscope, the structure of the bundles of double mutant hair cells looked completely normal, which ruled out structural anomalies that could be interrupting transduction. Other tests probed for the presence of mechanotransduction channels by using a fluorescent dye and gentamicin (a drug that causes hearing loss by damaging hair cells), both of which are known to be freely taken up into

stereocilia. The double mutant mice did not take up either substance, while the normal mice did.

Another novel technique, adapted in labs at NIDCD for studying inner ear hair cells, used a gene gun to fire fluorescent tagged TMC1 and TMC2 genes at normal tissue to see where the genes expressed their proteins. The proteins clustered at the tips of the stereocilia, where one would expect to see them if they played a prominent role in mechanotransduction.

To further support their findings, the researchers found that by using a gene therapy technique that adds the proteins back into the cell, they could restore transduction to vestibular and cochlear hair cells of the mice missing TMC1 and TMC2. This suggests that it might be possible to reverse genetic deficits at the cellular level.

"What we see in the hair cells of these double mutant knockout mice," says Dr. Griffith, "is a unique combination of properties that one would expect to see in a hair cell that has a defective transduction channel or some defect in getting that channel to where it needs to be and functioning."

To discover exactly how the channel machinery operates, the team will continue to explore how TMC1 and TMC2 interact with each other as well as how they interact with other proteins at the stereocilia tip that are essential to transduction. These include the tip link cadherins and protocadherins, which were also identified and characterized in NIDCD-funded laboratories. If these genes encode the transduction channel, they will be useful tools to screen for drugs or molecules that bind to the channel and could be used to prevent damage to hair cells.

Provided by National Institutes of Health