Gene therapy advance could reverse a common genetic cause of hearing loss

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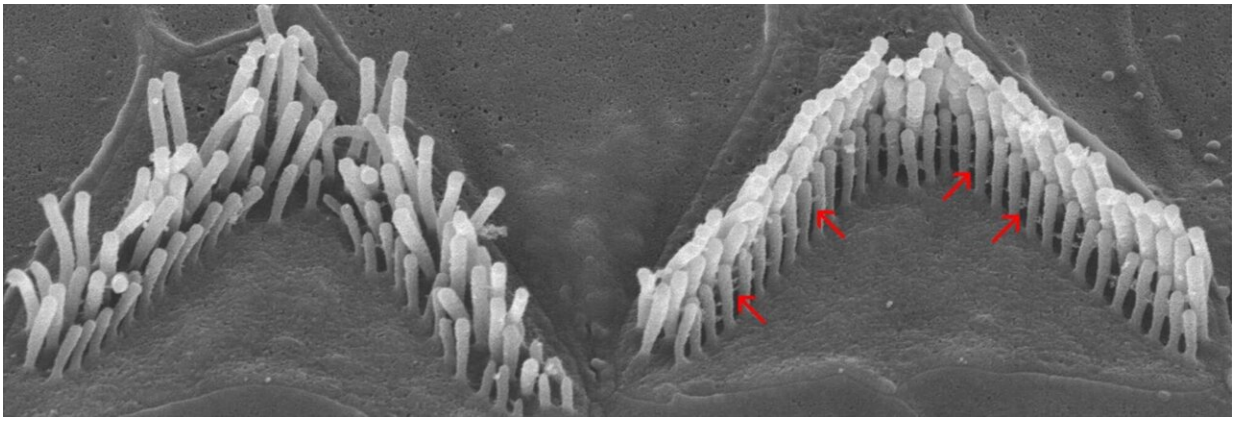

This scanning electron micrograph shows sensory outer hair cells, which are required for cochlear amplification and normal hearing. The image at left shows a disorganized sensory hair cell from the inner ear from a mouse with a mutation in the Strc gene; as a result, the cell is missing scaffolding links provided by the stereocilin protein. A neighboring hair cell, at right, took up the dual-AAV gene therapy vector and has its normal organization restored. The red arrows point out the restored stereocilin cross-links, which stand the hair cell microvilli up into organized bundles that can contact the overlying tectorial membrane and detect sound vibrations. Credit: Carl Nist-Lund, Holt/Géléoc Lab, Boston Children's Hospital

Hearing loss has been linked to mutations in at least 100 different genes, but up to 16 percent of genetic hearing loss can be traced to just one gene, *STRC*, the second most common genetic cause. A first-of-its-kind

gene therapy technique developed at Boston Children's Hospital successfully replaced the mutated protein, stereocilin, in the inner ear and reversed severe hearing loss in mice—sometimes to normal levels of hearing. Findings were published December 15 in the journal *Science Advances*.

The technique could also be used in other situations where the therapeutic gene is very large, says Jeffrey Holt, Ph.D., a scientist in the departments of Otolaryngology and Neurology at Boston Children's and the study's senior investigator.

The team will now test whether the technique works with the human stereocilin gene and test it in a human inner ear cells in a dish, derived from patients with *STRC* hearing loss. If gene therapy restores auditory function at the tissue level, Holt hopes to apply to the FDA for permission to test it in humans.

Restoring contact

For sounds to be heard, sensory hair cells in the inner ear must make contact with the ear's tectorial membrane, which vibrates in response to sound, and then convert these vibrations into signals sent to the brain. The stereocilin protein acts as a scaffold, helping the hair cell microvilli stand up together in an organized bundle, so that their tips can touch the membrane.

"If stereocilin is mutated, you don't have that contact, so the hair cells are not stimulated properly," says Holt. "But importantly, the hair cells still remain functional, so they are receptive to the gene therapy. We think this will provide a broad window of opportunity for treatment—from babies to adults with hearing loss."

Creating a new kind of gene therapy

To deliver a healthy stereocilin gene, the team used a synthetic adeno-associated virus (AAV) that is effective in targeting hair cells.

"The challenge we faced was that the gene for stereocilin is too big to fit into the gene therapy vector," says Holt. "The gene is about 6,200 DNA base pairs long, but the AAV only has a capacity of 4,700 base pairs."

Olga Shubina-Oleinik, Ph.D., the study's first author, came up with a solution: she split the mouse *Strc* gene in two, and put the two halves in two separate AAVs. She then used an existing technique called protein recombination, where two halves of a protein find each other and hook up. But in this case, it didn't work.

"We then realized that the beginning of the protein has a short stretch of amino acids that acts like an 'address,' directing the protein to its proper place in the cell," says Shubina-Oleinik. "When we split the protein in half, we realized that one half had the signal, but other half did not, so the halves might not end up in the same location."

When they added the signal to both halves of the protein, the two halves successfully came together. The researchers found robust restoration of full-length stereocilin protein in the mice and normal-appearing hair bundles that were able to contact the tectorial membrane.

Hearing restoration

The researchers used two types of hearing tests: one similar to hearing tests used in babies, and a test using electrodes on the scalp to measure auditory brainstem responses to a range of sound frequencies and intensities. On testing, the mice were found to be much more sensitive to

subtle sounds and showed improved cochlear amplification—the ability to amplify soft sounds, tamp down the response to loud sounds, and discriminate among sounds of different frequencies more precisely. In some mice, hearing was restored to normal levels.

"The results were remarkable and are the first example of hearing restoration using dual-vector gene therapy to target sensory outer hair cells," says Shubina-Oleinik.

Shubina-Oleinik and Holt have filed a patent application for the gene therapy technology. According to co-author Eliot Shearer, MD, Ph.D., a clinician-scientist in Otolaryngology at Boston Children's Hospital, approximately 100,000 patients in the U.S. and 2.3 million worldwide carry *STRC* mutations and could potentially benefit from this therapy.

"It turns out that *STRC* gene variations are more common than we thought, which makes gene therapy for this disorder so important," says Shearer, who worked with the Children's Rare Disease Cohort Initiative to screen a large genomic data set for *STRC* mutations.

More information: Olga Shubina-Oleinik et al, Dual-vector gene therapy restores cochlear amplification and auditory sensitivity in a mouse model of DFNB16 hearing loss, *Science Advances* (2021). DOI: 10.1126/sciadv.abi7629. www.science.org/doi/10.1126/sciadv.abi7629

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