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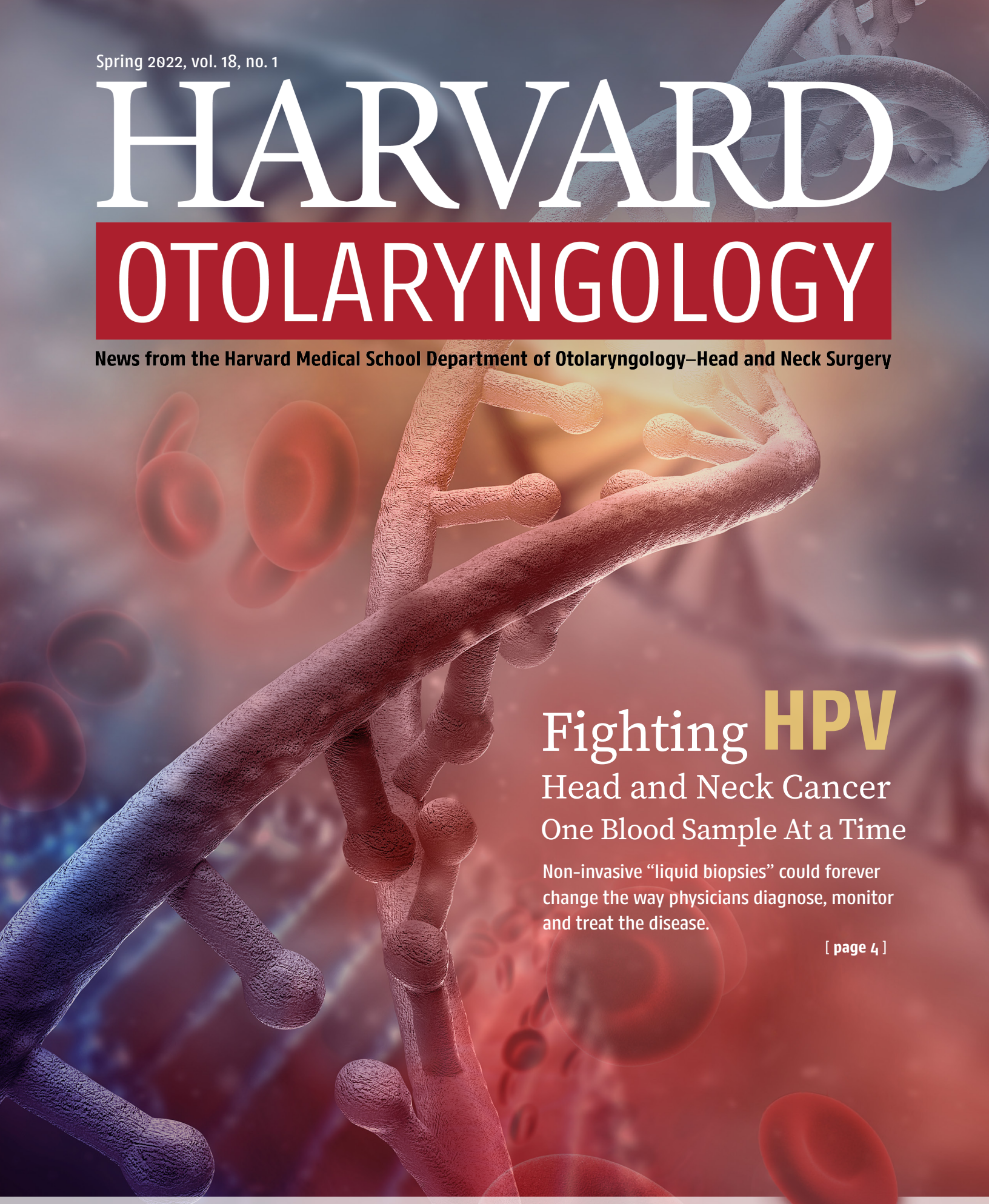
HARVARD OTOLARYNGOLOGY

News from the Harvard Medical School Department of Otolaryngology–Head and Neck Surgery



Fighting HPV Head and Neck Cancer One Blood Sample At a Time

Non-invasive "liquid biopsies" could forever change the way physicians diagnose, monitor and treat the disease.

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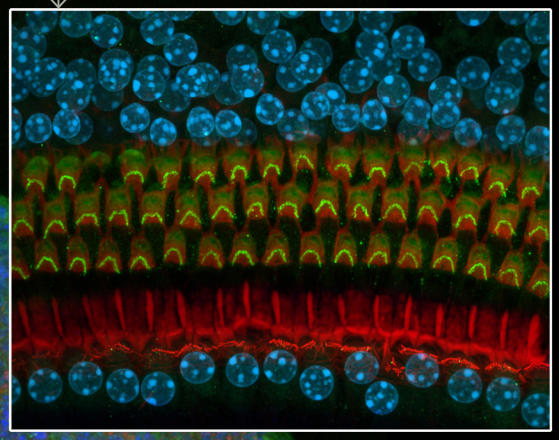
HARVARD MEDICAL SCHOOL | Department of Otolaryngology Head and Neck Surgery

Correcting Hearing Loss With the Split of a Gene

A novel dual-vector, protein recombination strategy developed at Boston Children's Hospital has galvanized the field of inner-ear therapeutics.



Blue: cell nuclei
Red: hair bundles
Green: stereocilin

Images courtesy of
Jeffrey R. Holt, PhD, and
Olga Shubina-Oleinik, PhD.

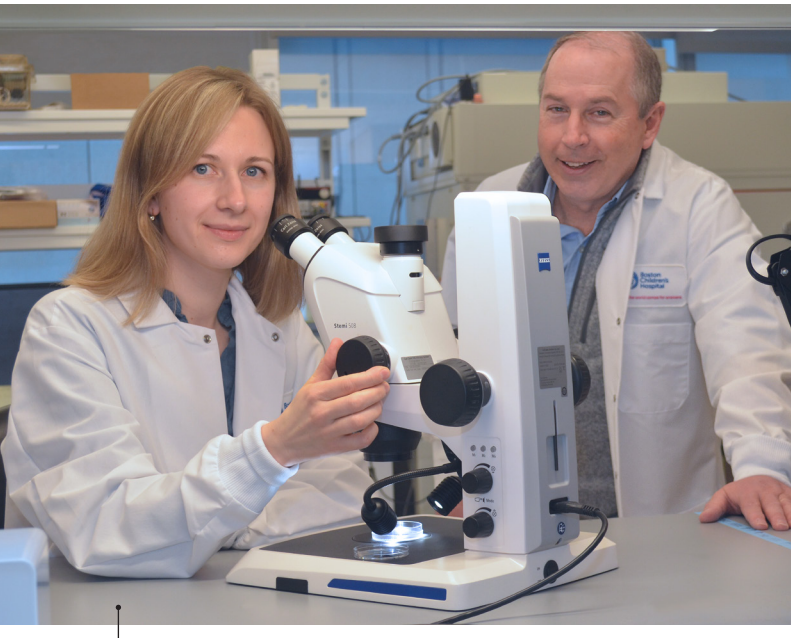
Blue is DAPI (4',6-diamidino-2-phenylindol), which stains DNA and illuminates cell nuclei. **Red** is phalloidin, which binds and stains actin in hair bundles and cell junctions. **Green** is an anti-STRC antibody conjugated to AlexaFluor488, which labels the sterocilin protein.

Over 430 million people worldwide experience some form of hearing loss, according to the World Health Organization. These cases range from mild to profound, and experts attribute more than half of all cases to genetic mutations.

In the past few decades, scientists have developed gene therapies capable of replacing mutated genes for various hereditary conditions. One common practice relies on harmless viruses called adeno-associated viruses (AAVs) to transport healthy copies of genes into cells that lack them. Once delivered to the cell, the healthy genes produce missing proteins that help restore deficits brought on by a mutation.

Although viable in some cases, AAV vectors are limited in practice. Genes larger than 4.7 kilobases cannot fit inside standard AAVs. This renders the technique ineffective for correcting larger genes, such as the 6.2 kilobase *STRC* gene. Humans rely on functional *STRC* genes to produce stereocilin proteins, which enables the ear to amplify and distinguish sounds. Without a healthy *STRC* gene, a mild-to-moderate form of hearing loss referred to as DFNB16 occurs.

According to Jeffrey R. Holt, PhD, Professor of Otolaryngology–Head and Neck Surgery and Neurology at Harvard Medical School and a Principal Investigator of the Holt/Géléoc Laboratory at Boston Children's Hospital, there are no biological treatments for DFNB16 patients. Hearing aids and cochlear implants can help amplify sound volume but are less effective in noisy environments.



Olga Shubina-Oleinik, PhD, (left) working alongside Jeffrey R. Holt, PhD, (right) in the Holt/Géléoc Laboratory at Boston Children's Hospital.

"To fully restore auditory function in these patients, we would need to make a giant leap in replacing genes once deemed too large to fit in single AAVs," Dr. Holt said. "This problem isn't exclusive to otolaryngology; it's a hurdle for many forms of inherited human disease across medical disciplines."

In a study published in *Science Advances,* a team of scientists led by Dr. Holt tested a novel gene therapy technique capable of overriding the size limitations of standard AAVs. The new technique, developed by Olga Shubina-Oleinik, PhD, a Research Fellow in Dr. Holt's lab, used two AAV vectors—not just one—to deliver healthy *Strc* genes into mice, and employed a protein recombination strategy to ensure the successful creation of full-length stereocilin proteins.

"If our dual-vector approach can replace full-length stereocilin protein, then perhaps it can also work for other human genetic disorders," Dr. Holt said. "A new pathway for gene therapy research is being pioneered and it's hard not to imagine its immense potential."

A swing set inside the ear

Mutations to the *STRC* gene, which were first discovered in 2008, are the second most common cause of genetic hearing loss in the world. The gene's role in controlling auditory function begins deep inside the inner ear, where sensory cells, called outer hair cells, control the ear's internal volume.

The microscopic outer hair cells detect the movement of sound waves entering the inner ear. When loud sounds enter, the outer hair cells turn down the volume, sending quieter signals to the brain. The opposite occurs when soft sounds enter the ear; outer hair cells can amplify the softest sounds by a millionfold.

This mechanism, referred to as cochlear amplification, can only occur if tiny microvilli on top of outer hair cells stay organized in bundles. Stereocilin proteins, produced by the *STRC* gene, are what keep the microvilli on top of the outer hair cell glued together. The absence of a functional *STRC* gene, however, results in a faulty protein and disorganized microvilli.

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Dr. Holt compares the phenomenon to a child swinging their legs on a swing set.

"Every child knows that, if they kick their legs together on a swing, they can control how high the swing goes," Dr. Holt said. "Flailing legs cause them to completely lose control of how high or low the swing goes. If outer hair cells don't have their microvilli swinging in sync, they can no longer control the sound waves coming into the inner ear, stripping them of their ability to amplify sounds."

To better understand the prevalence of *STRC* mutations in the global human population, Eliot Shearer, MD, PhD, Assistant Professor of Otolaryngology–Head and Neck Surgery and an Attending Surgeon at Boston Children's Hospital, examined a large genomic database at Boston Children's Hospital called the Children's Rare Disease Cohort Initiative. According to results published in *Science Advances,* approximately two of every 100 people carry a mutation in the *STRC* gene, suggesting that an estimated 2.3 million people worldwide suffer from DFNB16 hearing loss.

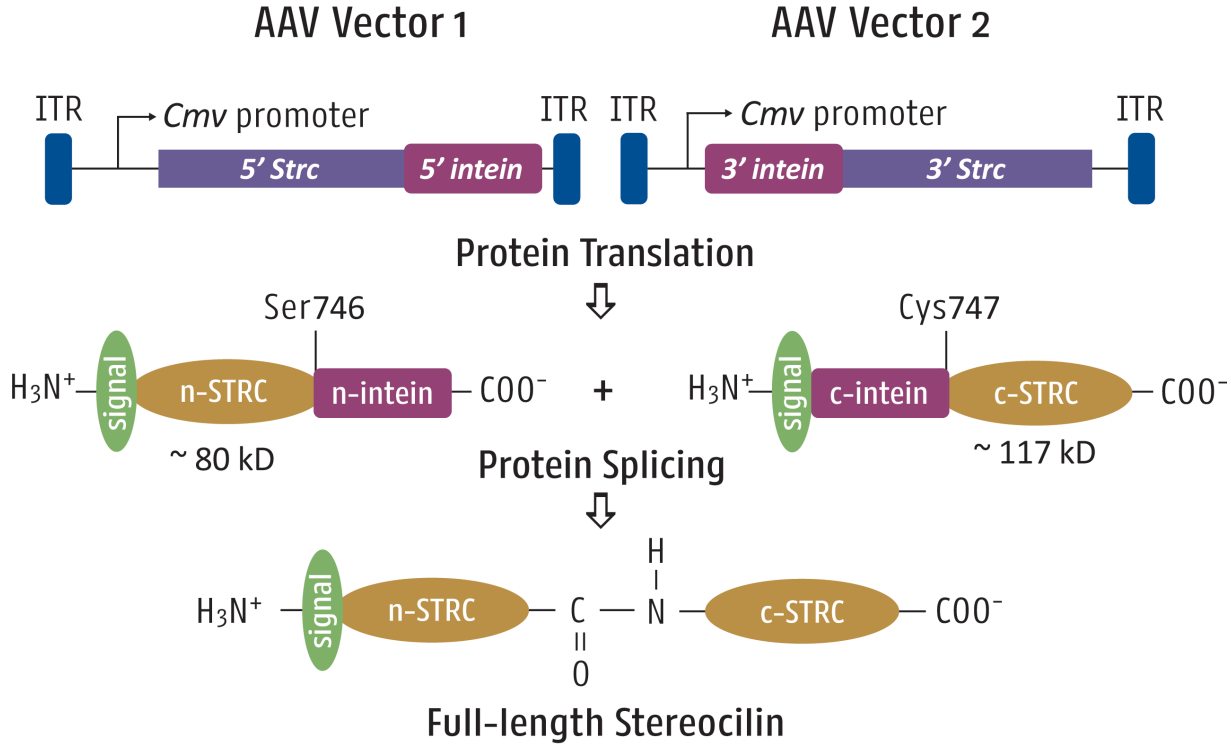
"Having a sense of how widespread the mutation really is helps underscore the urgency for a treatment," said Dr. Shearer, a co-author of the study. "A gene therapy solution that could restore cochlear amplification would drastically improve the lives of millions of patients worldwide."

Correcting the mailing address

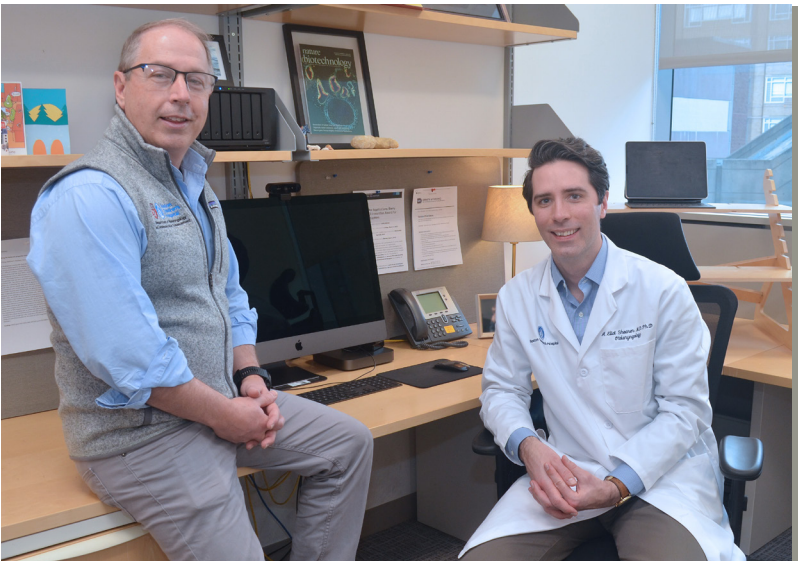
Ever since the discovery of the *STRC* gene, scientists have contemplated gene therapies capable of accommodating its large size. Despite their efforts, none of these techniques have succeeded in generating stereocilin in an animal model.

Working in collaboration with Dr. Holt, Dr. Shubina-Oleinik innovated her own gene therapy technique. She began by splitting a mouse *Strc* gene in half and packaging each half into separate AAVs. The dual vector approach would ensure both pieces of the gene arrived at a targeted cell. Once inside the cell, each piece of the gene would create a fragment of the stereocilin protein. Both fragments would then recombine to form a complete protein.

Or so she thought.



Gene therapy strategy for restoring hearing with full-length stereocilin. The *STRC* gene is split in two fragments and packaged into AAV vectors (top line). When introduced into outer hair cells, the split *STRC* gene is translated into two protein fragments (middle line). The signal sequences direct the protein fragments to the same intracellular site where the inteins mediate protein recombination to yield the full-length sterocilin protein (bottom line).



"If outer hair cells don't have their microvilli swinging in sync, they can no longer control the sound waves coming into the inner ear, stripping them of their ability to amplify sounds."

—Jeffrey R. Holt, PhD (left)

"A gene therapy solution that could restore cochlear amplification would drastically improve the lives of millions of patients worldwide."

—Eliot Shearer, MD, PhD (right)

Her hypothesis, she found out, had overlooked a complication in the split *Strc* gene that had prevented recombination from occurring. According to Dr. Shubina-Oleinik, some genes carry a signal sequence that instructs cells where to deliver a protein. The instructions are transcribed as a signal peptide in a protein made of thousands of other amino acids. Without the signal peptide, the protein floats around the cell without a target destination. When Dr. Shubina-Oleinik first examined her results in *Strc*-mutant mice, she noticed that only one piece of the split gene contained the necessary signal sequence. The other did not, which meant there was no way for the two protein fragments to arrive at the same location in the cell.

"Imagine ordering a computer for work, but you only assign a mailing address for the desktop," Dr. Shubina-Oleinik said. "Instead of the desktop and monitor both arriving at your home, only the desktop arrives, and the monitor is lost in the mail because there was no delivery address. What good does that do?"

Dr. Shubina-Oleinik hypothesized that both protein strands would arrive at the same location if she copied the signal peptide from one strand and added it to the other. After adjusting her methodology and retesting the mice, a drastic change occurred. According to the new study, the mice recovered expression of full-length stereocilin protein in their outer hair cells, and approximately 60 percent of the hair cell bundles demonstrated proper organization. Perhaps even more encouraging: The mice demonstrated normal cochlear amplification and enhanced auditory sensitivity, signaling the presence of newly generated, functional stereocilin protein.

Limitless possibilities

Many other genetic disorders brought on by mutations to large genes could also benefit from Dr. Shubina-Oleinik's novel technique. Several genes responsible for Usher syndrome, a rare disease that causes blindness and deafness, are larger than *STRC*. Even larger is the gene responsible for Huntington's disease, a neurodegenerative disorder in the brain. None of these genes could fit inside a single, standard AAV vector.

The new gene therapy technique must undergo further testing before it can reach clinics. Dr. Holt's team will first need to test whether the technique can work on the human *STRC* gene *ex vitro*. Human inner-ear organoids developed in the lab of Karl Koehler, PhD, Assistant Professor of Otolaryngology–Head and Neck Surgery at Harvard Medical School, will provide Dr. Holt and his team with the proper environment needed to test the effectiveness of the technique on human cells.

"Our proof-of-concept study is the first step in tackling hereditary conditions across multiple disciplines," said Dr. Shubina-Oleinik, who, together with Dr. Holt, has filed a patent application for the technique. "I'm confident that, with the world-class resources at our disposal across the Harvard Medical School Department of Otolaryngology–Head and Neck Surgery, our team can turn a treatment that seemed impossible a few years ago into a reality." ■

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