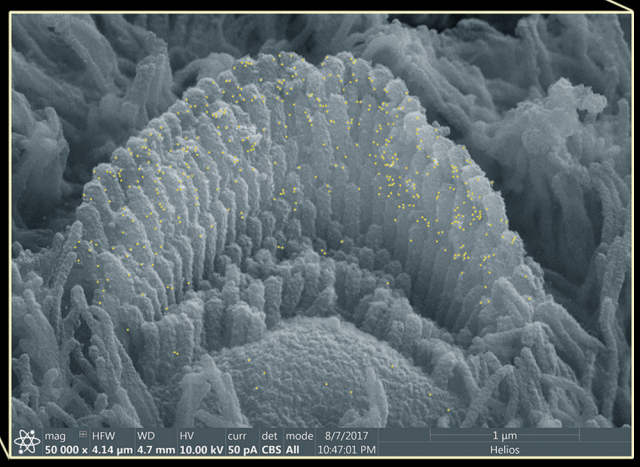
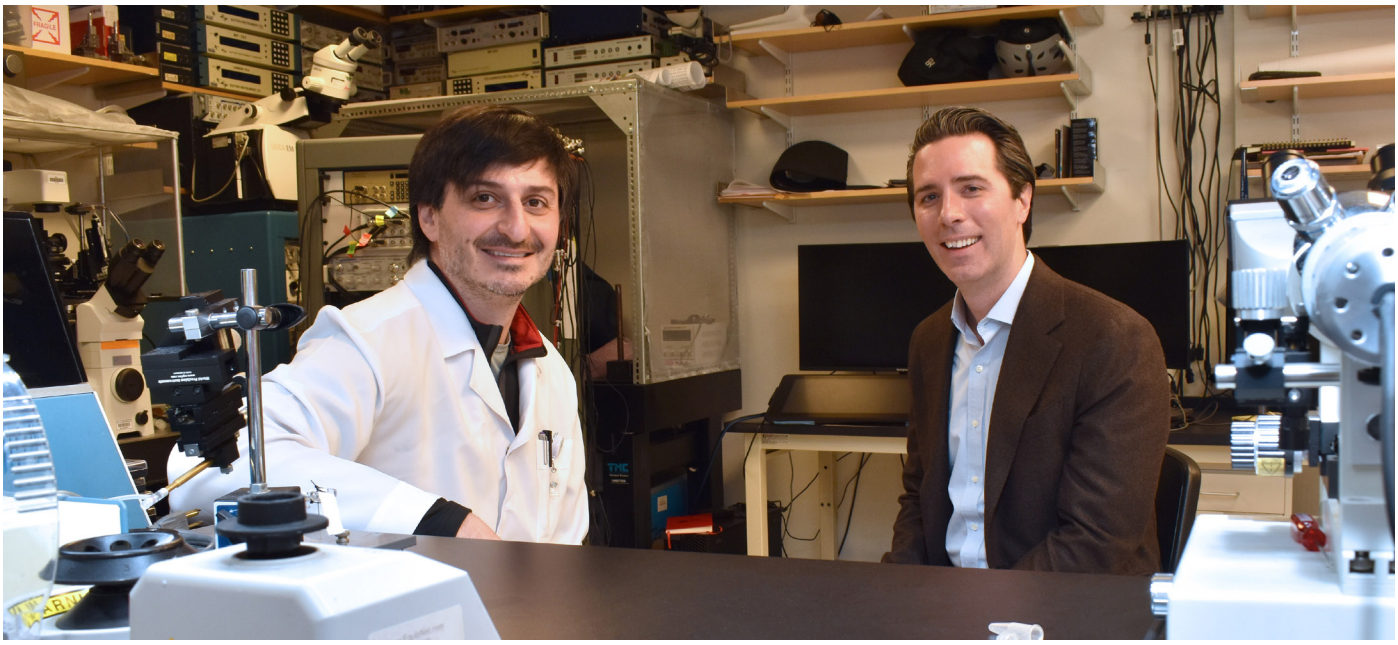
# Establishing a Nonsyndromic Hearing Loss Gene

**A robust collaboration between Boston Children's Hospital and Mass Eye and Ear resulted in the discovery of a novel gene for nonsyndromic hearing loss called *PKHD1L1*.**




Scanning Electron Microscopy photomicrographs of early postnatal mouse outer hair cell stereocilia bundle, labeled with an antibody against PKHD1L1. Multiple 12-nm gold beads (pseudo-colored as yellow dots) are detected on the surface of stereocilia, localizing the PKHD1L1 protein to the bundle.



From left to right: Artur A. Indzhykulian, MD, PhD, and Eliot Shearer, MD, PhD, FACS, collaborating in the Indzhykulian lab at Mass Eye and Ear.

Conventionally, children who have hearing loss are treated with hearing aids or cochlear implants, depending on the severity. However, understanding the underlying cause of hearing loss is critical for patients and families as well as their treating physicians. Only about a decade ago, most children with hearing loss had no concrete diagnosis. Thanks to new advances including genetic testing and the discoveries of more hearing loss genes, many more children with hearing loss can receive a diagnosis today.

According to the Hereditary Hearing Loss Homepage, 152 genes are known to cause nonsyndromic hearing loss, and within those genes, there are thousands of genetic variants. Unfortunately, there are still many deafness-causing genes not confirmed, making final diagnoses challenging.

"In my practice, I primarily see pediatric patients with hearing loss, and for most of my patients—but not all—we are able to determine a diagnosis," said Eliot Shearer, MD, PhD, FACS, Assistant Professor of Otolaryngology–Head and Neck Surgery at Harvard Medical School and Pediatric Otolaryngologist at Boston Children's Hospital. "Identifying the underlying cause of hearing loss, the diagnosis, is critical as it provides prognostic information, reveals associated medical conditions and provides a sense of closure and empowerment to families. My goal is to use the best clinical and research tools so that one day all children with hearing loss have a diagnosis."

Dr. Shearer and his mentor, Margaret A. Kenna MD, MPH, FACS, FAAP, Professor of Otolaryngology–Head and Neck Surgery at Harvard Medical School and Director of Clinical Research in the Department of Otolaryngology and Communication Enhancement at Boston Children's Hospital, manage an extensive hearing loss research program at Boston Children's Hospital. When they work with a pediatric patient presenting with hearing loss, they initially conduct a standard diagnostic workup, which often includes imaging and genetic testing. If results are inconclusive, and the family is interested, the team enrolls the patient in their research study. During the study, advanced genetic diagnostic tools, such as exome sequencing and genome sequencing, are used to discover new hearing loss genes.

In 2020, Dr. Kenna evaluated a 10-year-old female patient presenting with moderate hearing loss and found no cause of genetic hearing loss within the known hearing loss genes. The patient then enrolled in the large genetic hearing loss study run by Dr. Kenna at Boston Children's Hospital, supported by the Children's Rare Disease Research Cohort Initiative.

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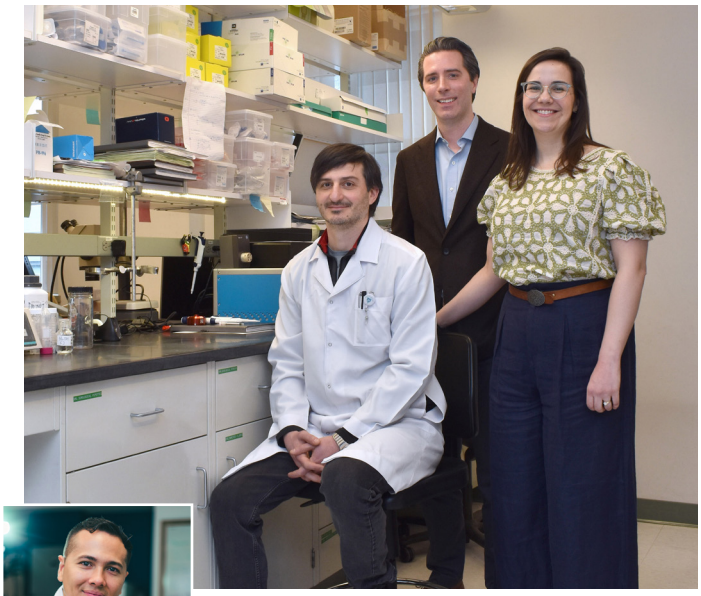

From left to right: Artur A. Indzhykulian, MD, PhD, Eliot Shearer, MD, PhD, FACS, and Shelby Redfield, MS, CGC.
Inset photo: Pedro De-la-Torre, PhD.

Dr. Shearer, a co-investigator on the study, then looked outside the already established hearing loss genes and discovered some interesting genetic variants in a gene called polycystic kidney and hepatic disease 1-like 1 (*PKHD1L1*).

"*PKHD1L1* rang a bell for me, but I couldn't pinpoint where, until I came across the Harvard Medical School publication in *Nature Communications* titled 'PKHD1L1 is a coat protein of hair-cell stereocilia and is required for normal hearing,'" said Dr. Shearer. "My colleague Artur Indzhykulian, MD, PhD, was one of the corresponding authors, and we quickly connected."

## Discovery of PKHD1L1 in mouse cochlea

Over a decade ago, Artur A. Indzhykulian, MD, PhD, Assistant Professor of Otolaryngology–Head and Neck Surgery at Harvard Medical School and Assistant Scientist in the Eaton-Peabody Laboratories (EPL) at Mass Eye and Ear, was a postdoctoral researcher in the Harvard Medical School Department of Neurobiology, conducting research under the direction of David Corey, PhD, Bertarelli Professor of Translational Medical Science at Harvard Medical School.

Scientists in the Corey Laboratory investigated different proteins that might be at the surface of stereocilia carried by the hair cells—the sensory cells of our inner ear. Using a database of proteins found in hair cells, they identified PKHD1L1, which is highly enriched in the inner ear sensory cells, particularly in the high-frequency region of the cochlea.

Following this observation, Dr. Corey's team created a mouse model lacking the PKHD1L1 protein specifically within the sensory cells of the inner ear. This enabled the team to investigate how the absence of PKHD1L1 affected hearing in mice.

According to Dr. Indzhykulian, the team quickly discovered that the absence of PKHD1L1 in sensory cells led to progressive hearing loss in the mice. They also found that this protein was located on the tips of stereocilia, which respond to sound stimulation.

The results of this study, published in 2019 in *Nature Communications,* indicate that the PKHD1L1-deficient mice lack the surface coat of stereocilia. This deficiency resulted in progressive hearing loss in mice, demonstrating the critical role of PKHD1L1 for normal hearing in mice.

"At the time our study was published, I had already established my own laboratory in the EPL and was awarded a major grant through the National Institutes of Health to further investigate the role of PKHD1L1 in this mouse model," said Dr. Indzhykulian. "However, I had not heard of any patients with this protein deficit until Dr. Shearer connected with me, which was a game-changer for the next phase of this research."

## A cross-institutional effort

After the genetic screening was conducted for the 10-year-old patient from Boston Children's Hospital, it was determined that she had two different amino acid changes within the PKHD1L1 protein. Based on the previous study implicating the gene, there was strong reason to believe that her hearing loss could be attributed to this protein deficit.

Dr. Indzhykulian's team studied PKHD1L1's protein structure, aiming to understand how a single amino acid change could affect the protein's overall folding architecture, stability and its function.

Pedro De-la-Torre, PhD, a research scientist in the Indzhykulian laboratory, designed experiments to examine, at the atomic level, both the normal fragment of the protein and the fragment carrying this pathogenic variant. His goal was to understand

whether this protein, with the single point pathogenic variant, could be deleterious.

The hypothesis was that the pathogenic variant protein fragment was less stable compared to the normal protein sequence. To test it, the team cloned and purified both proteins, and gradually increased the temperature to determine the protein melt temperature, at which the protein undergoes structural failure and collapses, rendering non-functional proteins.

These findings, complemented by computer simulations, led the team to conclude that the pathogenic variant carried by the patient yields a protein of lower stability, which could be the cause of her hearing loss.

To further bolster evidence that a mutation in *PKHD1L1* is a cause of human hearing loss, Drs. Shearer and Indzhykulian had reached out to human genetics groups world-wide to learn if any additional groups had patients with the PKHD1L1 deficit.

Barbara Vona, PhD, Group Leader at University Medical Center Göttingen-Institute of Human Genetics and Institute of Auditory Neuroscience and InnerEarLab in Göttingen, Germany, was immediately interested in this collaboration and used her connections to spread the word about the study. Through collaborations of her own, Dr. Vona helped identify three additional pediatric patients—from Iran, Pakistan and China—who have symptoms of hearing loss and carry plausible variants in the gene *PKHD1L1*.

"A lot of these pathogenic variants are extremely rare, so it's critical to be connected with as many groups as possible to stay on top of which genes are being discovered in different parts of the world," said Dr. Indzhykulian. "Dr. Vona played a significant role in this study, because without confirming additional pediatric patients with deleterious *PKHD1L1* variants who present symptoms of hearing loss, this wouldn't have been nearly the study it is today."

Clinicians from Iran, Pakistan and China examined these patients and conducted genetic testing on each, analyzed that information and compared it to the findings the Boston Children's Hospital team reported. The results indicated that each pediatric patient has pathogenic variants within the *PKHD1L1* gene, and each patient demonstrates hearing loss. Therefore, there is enough evidence between mouse models, in vitro experimental data in the lab, computational models and observations in patients to indicate that *PKHD1L1* is a nonsyndromic hearing loss gene.

On March 9, 2024, these results were published in *Human Genetics* in a paper titled, "*PKHD1L1*, a gene involved in the stereocilia coat, causes autosomal recessive nonsyndromic hearing loss." Drs. Shearer, Indzhykulian and Vona were co-senior authors and Dr. De-la-Torre and Shelby Redfield, MS, CGC, genetic counselor and clinical research coordinator in the Shearer Lab, were co-first authors.

## Looking into the future

This research suggests that going forward, clinicians should test hearing loss patients for the *PKHD1L1* gene in addition to the other genes that are known to cause hearing loss.

As a next step, Dr. Shearer plans to study the prevalence of pathogenic variants in *PKHD1L1* worldwide, starting by combing through the database of approximately 450 patients who are enrolled in his study with Dr. Kenna.

Dr. Indzhykulian will take a deeper dive into understanding how the disease manifests in humans—considering environmental factors such as exposure to loud noise— as the hearing loss phenotype varies among patients. Their most recent findings, reported in a preprint, suggest that noise exposure may contribute to hearing loss presenting differently across the patient population, as mice lacking PKHD1L1 were more susceptible to noise insult.

Knowing the number of patients and the timeframe to address this deficit to either maintain or potentially reverse hearing loss will enable the team to explore different gene therapeutic options.

"Establishing *PKHD1L1* as a nonsyndromic hearing loss gene is a remarkable first step in directly helping patients who have this protein deficit. The goal is to provide all patients with a concrete diagnosis, to truly improve their quality of life," said Dr. Shearer. "And now with clinical trials for gene therapy for hearing loss beginning, new avenues of treatment may soon be available for these children." ■