

PKHD1L1, a gene involved in the stereocilia coat, causes autosomal recessive nonsyndromic hearing loss

The identification and characterization of new genes associated with hearing loss are crucial for achieving molecular diagnoses in children with hereditary hearing loss. Despite progress, the field of auditory genetics still faces challenges, with genetic diagnoses covering only about half of affected individuals. A molecular genetic diagnosis is vital, as it provides prognostic information, reveals associated medical conditions, and offers closure and empowerment to families. Additionally, it qualifies patients for clinical trials, exemplified by recent otoferlin clinical trials (summarized in Moser et al., 2024). Identifying all molecular targets in the inner ear is essential for a timely diagnosis, as well as advancing research aimed at restoring hearing.

Earlier this year, I co-led a study titled "*PKHD1L1*, a gene involved in the stereocilia coat, causes autosomal recessive nonsyndromic hearing loss," where we discovered and linked the gene *PKHD1L1* (polycystic kidney and hepatic disease 1-like 1) to human hearing loss for the first time (Redfield et al., 2024). Previously, in 2019, the Corey lab at Harvard Medical School identified several stereocilia coat proteins, including *PKHD1L1*, and characterized the *Pkhd1l1* conditional knockout mouse model with progressive hearing loss (Wu et al., 2019). Building on this discovery in the mouse, I hypothesized that *PKHD1L1* could also be implicated in human hearing loss.

Utilizing exome sequencing, which targets all protein-coding genes, I identified a homozygous nonsense variant in *PKHD1L1* in a patient with moderate, progressive sensorineural hearing loss with congenital onset. I then collaborated with experts at Harvard Medical School, including Prof. Artur A. Indzhykulyan, Prof. A. Eliot Shearer, and Prof. Margaret A. Kenna, who had encountered a similar case at Boston Children's Hospital. Through extensive international networking, we identified two additional cases in Pakistan and China with congenital, progressive, sensorineural hearing loss, strengthening genetic data. These four families with variants in *PKHD1L1* and hearing loss ranging from mild-moderate to severe formed strong human genetics data to continue investigations into variant function.

Functional analyses demonstrated that missense variants in *PKHD1L1* compromise protein stability and proper folding, with one variant also leading to abnormal RNA splicing from analysis using an *in vitro* splice assay, resulting in the deletion of critical amino acids. *In vitro* functional evaluation of two missense variants in expressed protein fragments using nanoscale differential scanning fluorimetry showed decreased thermodynamic stability, suggesting a detrimental effect on protein structure. *In silico* molecular modeling further supported that the patient variants reduce folding and structural stability.

Although the role of *PKHD1L1* is not fully understood, two functional hypotheses were proposed based on its expression as a coat protein in stereocilia: (1) *PKHD1L1* may be necessary for correct localization of other stereociliary proteins, or (2) *PKHD1L1* could be

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involved in the development of attachment crowns that secure the tectorial membrane to the stereocilia bundle. An immature attachment could result in a persistently relaxed tectorial membrane coupling.

Interestingly, work from Prof. Karen Steel's lab (King's College London), published while our paper was under review, associated *PKHD1L1* variants with adult-onset hearing loss (Lewis et al., 2023). This signals possible distinct roles of *PKHD1L1* throughout life, crucial for both normal hearing development and the maintenance of hearing with age and further frames the importance of our work that uncovered a role for *PKHD1L1* in recessive hearing loss.

In summary, this work emphasizes the critical role of *PKHD1L1* in hearing and highlights the importance of molecular genetic diagnosis in understanding and treating hereditary hearing loss. It asserts that genetic testing for *PKHD1L1* variants may help diagnose many more individuals around the world. Identifying genetic variants in *PKHD1L1* and understanding their effects and clinical relevance could pave the way for new therapeutic approaches for affected individuals. *PKHD1L1* has been assigned as DFNB124 and annotated in the Hereditary Hearing Loss Homepage and OMIM database as causing autosomal recessive non-syndromic sensorineural hearing loss.

This work was featured in the Spring 2024 issue of Harvard Otolaryngology Magazine, which highlighted the collaborative efforts between Mass Eye and Ear, Boston Children's Hospital and the University Medical Center Göttingen (<https://oto.hms.harvard.edu/harvard-otolaryngology>) that made this study possible.

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