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Detection of Supporting-Cell-Associated Gene Transcripts in Adult Human Cochlear Tissue from Cadaveric Temporal Bones

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ABSTRACT

Purpose: The adult human cochlea is difficult to study because viable inner-ear tissue is rarely available and postmortem samples are technically challenging to process. Supporting cells are essential for cochlear architecture, ionic and osmotic balance, synaptic stability, stress responses, and potential regenerative signaling. In this study, we aimed to evaluate the feasibility of detecting transcripts associated with cochlear supporting-cell biology in adult human cadaveric cochlear tissue.Methods: Bilateral temporal bones were obtained from six adult cadaveric donors within 24 hours of death, yielding 12 temporal bones. One temporal bone from each donor was processed for cochlear microdissection and RNA isolation. RNA suitable for downstream analysis was recovered from five of the six processed cochlear specimens. Total RNA was treated with DNase I, converted to cDNA, and analyzed by endpoint RT-PCR for AQP4, GLAST (SLC1A3/EAAT1), TAK1 (MAP3K7), and SOX21.Results: All four transcripts were detected in each of the five analyzable cochlear specimens (5/5 for AQP4, 5/5 for GLAST (SLC1A3/EAAT1), 5/5 for TAK1 (MAP3K7), and 5/5 for SOX21). These findings provide preliminary molecular evidence that AQP4, GLAST (SLC1A3/EAAT1), TAK1 (MAP3K7), and SOX21 transcripts, representing biological processes related to water and osmotic homeostasis, glutamate handling, stress-response signaling, and developmental/progenitor-associated transcriptional regulation, are detectable in adult human cadaveric cochlear tissue. Because the analysis was performed on bulk cochlear tissue rather than purified supporting cells, the detected transcripts should be interpreted as supporting-cell-associated rather than supporting-cell-specific.Conclusion: The study demonstrates the feasibility of RNA-based transcript detection from postmortem adult human cochlear tissue using endpoint RT-PCR and provides a foundation for future validation using quantitative RT-PCR, RNA scope, immunostaining, single-cell or single-nucleus RNA sequencing, and spatial transcriptomics.

KEYWORDS

human cochlea, cadaveric temporal bone, supporting cells, endpoint RT-PCR, AQP4, GLAST, TAK1, SOX21, transcript detection.

1 Introduction

Hearing depends on the coordinated activity of sensory and nonsensory cell populations within the cochlea. Sensory hair cells convert mechanical sound stimuli into neural signals, whereas cochlear supporting cells contribute to the structural organization of the sensory epithelium, maintenance of the extracellular and ionic environment, synaptic homeostasis, and cellular responses to injury. Supporting cells have also received considerable attention in hearing-restoration research because experimental studies suggest that some supporting-cell populations retain developmental and regenerative potential and may serve as targets for hair-cell regeneration (Wan et al., 2013; Kaur et al., 2023).

Direct molecular investigation of the adult human cochlea remains technically challenging because the cochlea is deeply embedded within the temporal bone, access to viable tissue from living individuals is extremely limited, and postmortem delay may compromise RNA integrity. Nevertheless, important advances have been made in the molecular characterization of the adult human inner ear. Bulk RNA-sequencing studies have provided comprehensive transcriptomic profiles of adult human cochlear and vestibular tissues (Schrauwen et al., 2016), while more recent single-cell and single-nucleus approaches have further defined the cellular and transcriptional diversity of human inner-ear tissues (van der Valk et al., 2023). In addition, transcriptional studies of adult cochlear supporting cells in experimental models, together with validation of selected markers in human temporal-bone tissue, have contributed to the characterization of supporting-cell-

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associated molecular programs (Hoa et al., 2020).

Despite these advances, comparatively limited information is available regarding the feasibility of recovering analyzable RNA from cochlear tissue obtained from adult cadaveric temporal bones during medicolegal autopsy and using such material for conventional targeted gene-expression analysis. Several molecular studies have used high-throughput sequencing platforms, specialized tissue-processing procedures, or temporal-bone material to characterize the human inner ear (Schrauwen et al., 2016; Hoa et al., 2020; van der Valk et al., 2023). A targeted endpoint RT-PCR approach may therefore provide a complementary and relatively accessible strategy for examining selected transcripts in rare postmortem human cochlear specimens.

To address this question, four genes AQP4, GLAST (SLC1A3/EAAT1), TAK1 (MAP3K7), and SOX21 were selected as a focused panel representing complementary aspects of supporting-cell-associated cochlear biology. These genes were not chosen as exclusive supporting-cell markers, but rather to represent distinct functional domains relevant to cochlear supporting cells. AQP4 was included as a representative of water and osmotic homeostasis because it has been reported in several cochlear supporting-cell populations and in human cochlear tissue (Takumi et al., 1998; Lopez et al., 2007). GLAST (SLC1A3/EAAT1) was selected to represent glutamate clearance and synaptic support because it is expressed in supporting cells surrounding inner-hair-cell afferent synapses and contributes to glutamate uptake (Furness and Lehre, 1997; Glowatzki et al., 2006). TAK1 (MAP3K7), was included as a representative of intracellular signaling associated with mature cochlear supporting cells (Parker et al., 2011), whereas SOX21 was selected to represent developmental and cell-fate-related transcriptional programs associated with supporting-cell populations (Hosoya et al., 2011; Freeman and Daudet, 2012). Collectively, the panel was designed to represent four complementary biological domains: fluid and osmotic homeostasis, glutamate handling, intracellular signaling, and developmental transcriptional regulation.

The present study therefore aimed to determine whether RNA suitable for molecular analysis could be recovered from microdissected adult human cochlear tissue obtained from cadaveric temporal bones within a controlled postmortem interval and whether AQP4, GLAST (SLC1A3/EAAT1), TAK1 (MAP3K7), and SOX21 transcripts could be detected using endpoint RT-PCR. The novelty of the present study lies in evaluating a practical postmortem temporal-bone workflow that integrates cadaveric temporal-bone acquisition, cochlear microdissection, RNA recovery, and conventional targeted RT-PCR analysis of a functionally selected gene panel.

2 Materials and Methods

2.1 Ethical approval and cadaver selection

Human temporal bones were obtained from six adult cadavers during medicolegal autopsies performed at the Department of Forensic Medicine, AIIMS, New Delhi, after ethical approval and consent from legally authorized representatives. Temporal-bone specimens were collected within 24 hours of documented death. For the present study, the postmortem interval was defined as the time from documented death to temporal-bone collection. Following temporal-bone harvesting, the specimens were transported on dry ice to the ENT laboratory, where cochlear microdissection was initiated immediately upon receipt to minimize postmortem tissue degradation. Donor details, including age and cause of death, were recorded. The mean age of donors was 39.6 years, with a range of 28-52 years and a male-to-female ratio of 4:2.

Cadavers were screened according to predefined inclusion and exclusion criteria. Cases were included if donors were older than 18 years and temporal-bone specimens were collected within 24 hours of death. Cases were excluded if there was decomposition, septicemia, poisoning, asphyxia-related death, severe head injury, genetic abnormality, or documented history of hearing loss due to inner-ear disease, trauma, or advanced age.

2.2 Temporal bone extraction and cochlear microdissection

Following craniotomy and removal of the intracranial contents, the arcuate eminence was identified as an anatomical landmark for localization of the inner-ear region. Temporal bone specimens were extracted using a modified temporal bone plug approach adapted from established autopsy-based temporal bone removal methods (Schuknecht, 1968; Nadol, 1996). A Black+Decker drill equipped with a cylindrical cutting blade was used for specimen extraction. The custom drilling device and cylindrical cutting blade are shown in Fig. 1(iA–C). The cutting device was positioned perpendicularly over the arcuate eminence to encompass the temporal bone region containing the cochlea (Fig. 1(ii D)). The tem-

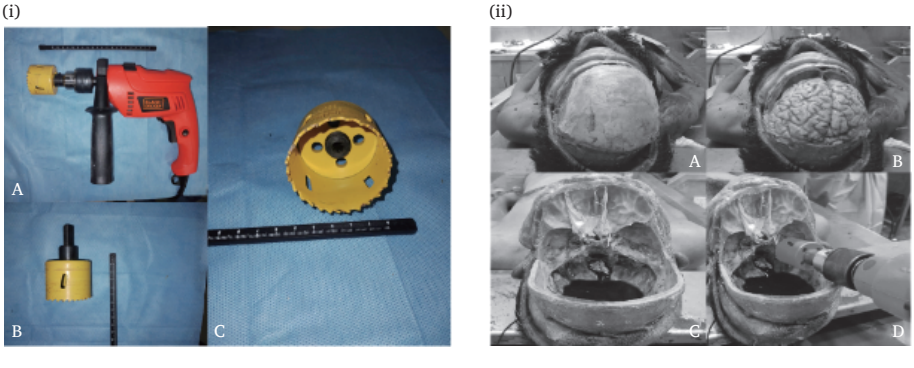


Figure 1 Device and sequential procedure for temporal bone extraction.

(i) Custom drilling device: (A) Modified Black + Decker, 550 W variable-speed hammer drill equipped with a custom cylindrical cutting blade used for temporal bone extraction. (B, C) Different views of the cylindrical cutting blade used to core the temporal bone region containing the cochlea; the blade had an internal depth of 4.5 cm and a diameter of 5 cm. (ii) Sequential steps of temporal bone extraction: (A) Reflection of the scalp to expose the calvarium. (B) Removal of the calvarium, exposing the cerebrum. (C) Removal of the cerebrum and intracranial contents, exposing the anterior and middle cranial fossae. (D) Positioning of the modified cylindrical cutting device perpendicular to the arcuate eminence for extraction of the temporal bone region containing the cochlea.

poral bone plug was then carefully removed while minimizing damage to the surrounding structures. Extracted specimens were examined externally and transported on dry ice to the ENT laboratory for further processing.

Microdissection was performed under surgical microscopy. The external auditory canal, tympanic membrane, and ossicles were sequentially removed to expose the middle-ear cavity, promontory, round window, and oval window. Representative extracted temporal bone specimens are shown in Fig. 2(i). The promontory was carefully drilled to expose the basal turn of the cochlea (Fig. 2 (ii A–D)). The scala media and associated inner-ear tissue were harvested using fine microsurgical instruments (Fig. 2 (iii A–D)) and immediately processed for RNA extraction. All procedures were performed under sterile conditions to minimize contamination and RNA degradation.

2.3 RNA isolation and cDNA synthesis

Total RNA was isolated from microdissected cochlear tissue using TRIzol reagent according to the manufacturer's protocol. RNase contamination was minimized by using DEPC-treated solutions and RNase-free plasticware. RNA concentration was assessed spectrophotometrically. RNA integrity was evaluated by electrophoresis on a 1.2% agarose gel, and samples showing discernible 28S and 18S rRNA bands without marked degradation were considered suitable for downstream analysis.

Before cDNA synthesis, RNA samples were treated with RNase-free DNase I to reduce genomic DNA contamination. Complementary DNA was synthesized from 1.0 μg of total RNA in a 25 μL reaction using oligo(dT) primers, M-MLV reverse transcriptase, RNasin, dNTPs, and the recommended reaction buffer.

2.4 Endpoint RT-PCR and amplicon assessment

Endpoint RT-PCR was performed using gene-specific primers for AQP4 (aquaporin-4), GLAST (SLC1A3/EAAT1), TAK1 (MAP3K7), and SOX21. The AQP4 primers were 5′-ACATGGAGGTGGAGGACAAC-3′ (forward) and 5′-GTCAATCACATGCACCACTCC-3′ (reverse); GLAST (SLC1A3/EAAT1) primers were 5′-AGCAGGGAGTCCGTAAACG-3′ (forward) and 5′-AGCATTCCGAAACAGGTAACTTT-3′ (reverse); TAK1 (MAP3K7) primers were 5′-CAACCACAGCCTATTCCAAG-3′ (forward) and 5′-GATCTCAGGGACATCCAGAA-3′ (reverse); and SOX21 primers were 5′-GCTCGCCAATCCCGAGAAG-3′ (forward) and 5′-ATCTCTGCCATTTTGGAGCCC-3′ (reverse). The expected amplicon sizes were approximately 80 bp for AQP4, 92 bp for GLAST/SLC1A3, 80 bp for TAK1 (MAP3K7), and 156 bp for SOX21.

PCR amplification was carried out with an initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 45 s, followed by a final extension at 72°C for 10 min. No-reverse-transcriptase (no-RT) controls and no-template controls (NTCs) were included to assess potential genomic DNA contamination and reagent contamination, respectively.

PCR products were resolved on a 1.2% agarose gel containing ethidium bromide and visualized under UV transillumination. Discrete amplification products were observed for the selected targets and were considered consistent with target amplification. Primer specificity was assessed in silico using NCBI Primer-BLAST against human reference sequences to confirm amplification of the targets. Because endpoint RT-PCR was performed using bulk postmortem cochlear tissue, the results were interpreted as qualitative detection of the selected transcripts rather than quantitative or cell-type-specific expression.

3 Results

3.1 Cadaveric temporal bone extraction and cochlear tissue recovery

Temporal bone extraction was successfully performed from adult cadaveric specimens using the modified temporal bone plug method. The arcuate eminence was identified as an anatomical landmark for localization of the inner-ear region, and the cylindrical cutting approach enabled access to the cochlear region (Fig. 1(ii D) and Fig. 2(i)). Subsequent microdissection exposed the middle-ear structures, promontory, round window, oval window, basal cochlear turn, and scala region (Fig. 2(ii A–D)). Cochlear tissue was subsequently harvested for downstream molecular analysis (Fig. 2(iii A–D); Fig. 3A).

3.2 RNA recovery from adult human cochlear tissue

RNA extraction was performed from microdissected cochlear

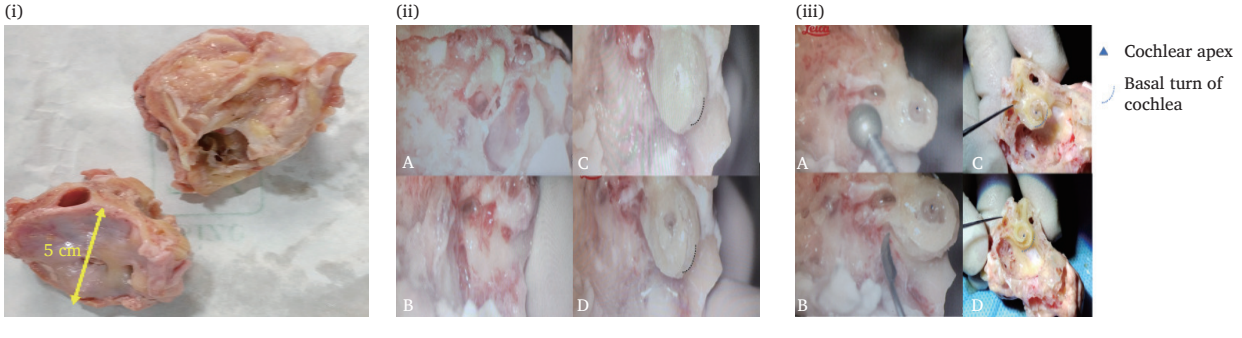


Figure 2 Temporal bone specimens, cochlear access, and stepwise microdissection of the scala media.

(i) Temporal bone specimens obtained during cadaveric extraction: Bilateral temporal bone specimens obtained during cadaveric extraction, showing preservation of the inner-ear region for subsequent cochlear microdissection and molecular analysis. The yellow double-headed arrow indicates an approximate specimen dimension of 5 cm. (ii) Sequential dissection of the middle ear to expose the basal turn of the cochlea: (A) Drilling of the attic region to expose the tympanic membrane (TM) with the ossicular chain intact. (B) Removal of the tympanic membrane and ossicles, exposing the promontory, round window, and oval window. (C, D) Progressive drilling of the promontory to expose the basal turn of the cochlea; dotted lines indicate the approximate course of the cochlear turns. (iii) Stepwise microdissection of the basal cochlear turn and isolation of tissue from the scala media: (A, B) Progressive drilling and microdissection of the basal cochlear region to expose the cochlear duct and underlying cochlear structures. (C, D) Further exposure of the cochlear turns during microdissection and tissue harvesting. The arrow indicates the cochlear apex, and the dotted annotation indicates the approximate basal turn of the cochlea.

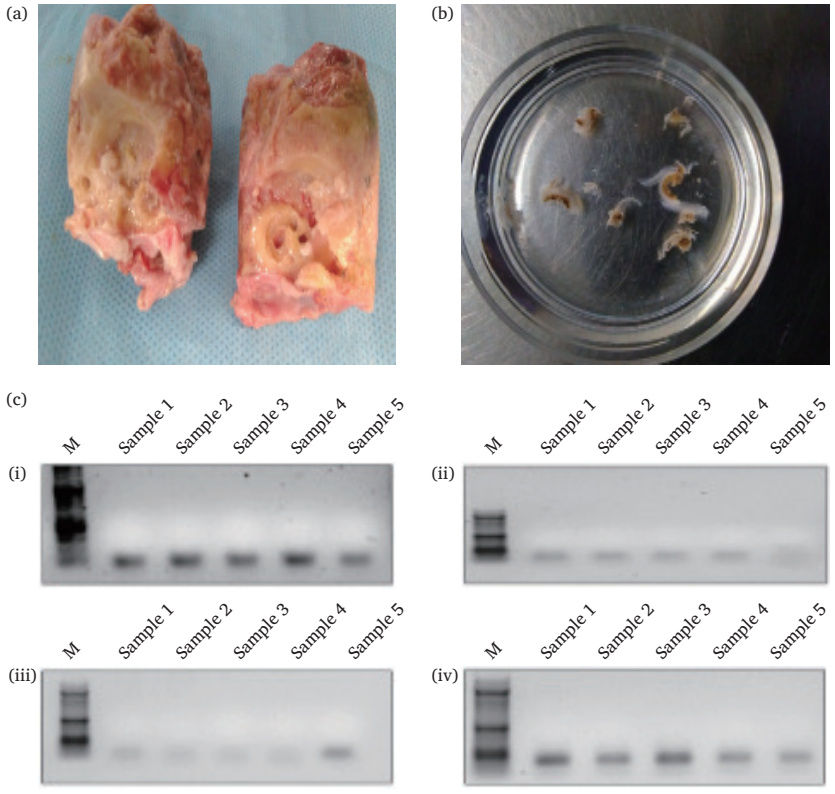


Figure 3 Cochlear tissue isolation and endpoint RT-PCR detection of selected transcripts in adult human cadaveric cochlear tissue.

(A) Gross temporal-bone specimens following dissection to access the cochlear region. (B) Harvested cochlear tissue prepared for RNA extraction and subsequent molecular analysis. (C) Endpoint RT-PCR detection of selected transcripts in five analyzable adult human cochlear specimens. The four gel panels show amplification of (i) AQP4, (ii) GLAST (SLC1A3/EAAT1), (iii) TAK1 (MAP3K7), and (iv) SOX21. Discrete amplification products were detected for each target in all five specimens. M, DNA molecular-weight marker.

tissue obtained from all six cadaveric specimens. Representative harvested cochlear tissue prepared for RNA extraction is shown in Fig. 3B. Five specimens met the predefined RNA integrity criterion and were taken forward for reverse transcription and endpoint RT-PCR. One specimen did not meet the RNA integrity criterion and was therefore excluded from subsequent transcript analysis. Accordingly, molecular analysis was performed on five cochlear specimens.

3.3 Detection of selected cochlear transcripts

Endpoint RT-PCR detected transcripts corresponding to AQP4, GLAST (SLC1A3/EAAT1), TAK1 (MAP3K7), and SOX21 in each of the five analyzable adult human cochlear specimens (5/5 for each target) (Fig. 3C (i–iv)). These targets represent biological processes associated with fluid and osmotic homeostasis, glutamate handling and synaptic support, intracellular signaling, and developmental/cell-fate-related transcriptional regulation, respectively.

The consistent detection of these transcripts across the analyzable cochlear specimens supports the feasibility of targeted transcript detection in postmortem human cochlear tissue. Figure 3C presents endpoint RT-PCR results from the five specimens that met the RNA integrity criterion and were taken forward for transcript analysis; the sixth specimen was excluded from subsequent analysis because it did not meet the RNA integrity criterion.

Because the analysis was performed on bulk cochlear tissue rather than isolated supporting-cell populations, the detected transcripts cannot be assigned exclusively to supporting cells. The findings should therefore be interpreted as supporting-cell-associated rather than supporting-cell-specific transcript detection in adult human cochlear tissue

4 Discussion

The detection of AQP4, GLAST (SLC1A3/EAAT1), TAK1 (MAP3K7), and SOX21 transcripts in all five analyzable cochlear specimens indicates that molecular signatures associated with distinct cochlear biological functions remain detectable in appropriately processed cadaveric tissue. The exclusion of one specimen due to inadequate RNA integrity highlights the importance of assessing sample quality before downstream molecular analysis. Although these findings establish the presence of the selected transcripts, they do not provide quantitative information regarding gene expression or identify the specific cellular populations responsible for the observed signals. Further investigations using cell-type-resolved molecular approaches are required to establish their anatomical localization and biological significance within the adult human cochlea.

AQP4 (aquaporin-4) contributes to water transport and fluid balance in the inner ear. Prior studies have reported AQP4 expression in cochlear nonsensory and supporting cell population, supporting its role in inner-ear fluid and osmotic homeostasis (Takumi et al., 1998; Beitz et al., 2003; Lopez et al., 2007). The detection of AQP4 transcripts in the present study is consistent with these functions. However, because the analysis was performed on mixed cochlear tissue, the signal may arise from more than one cochlear cell type.

GLAST (EAAT1/SLC1A3) is involved in glutamate uptake and

synaptic protection in the cochlea. Previous work has localized GLAST to supporting cells surrounding inner-hair-cell afferent synapses, where it contributes to glutamate clearance (Furness and Lehre, 1997; Glowatzki et al., 2006). Its detection in adult human cochlear tissue supports the detectability of transcripts associated with glutamate handling in postmortem cochlear tissue.

TAK1 is a stress-activated kinase implicated in inflammatory signaling, apoptosis, and cochlear injury responses. Parker et al. (2011) demonstrated TAK1 expression in adult cochlear supporting-cell populations and proposed a potential role for TAK1 in cochlear inflammatory responses following injury. The detection of TAK1 transcripts in adult human cochlear tissue suggests that stress-response signaling components remain transcriptionally detectable in these samples. Nevertheless, transcript detection alone does not establish pathway activity or protein expression.

SOX21 is associated with developmental and progenitor-related transcriptional programs in the inner ear. Reports from animal models have described SOX21 expression in supporting-cell populations and have implicated SOX-family transcription factors in sensory-cell fate decisions (Hosoya et al., 2011; Freeman and Daudet, 2012). Detection of SOX21 transcripts in adult human cochlear tissue indicates that this transcript is detectable in the adult cochlea. However, transcript detection alone does not establish progenitor activity or regenerative potential.

A major strength of the study is the use of human cadaveric temporal bone tissue, which provides rare access to adult cochlear material. The standardized screening of cadavers, collection of specimens within the defined postmortem interval, careful temporal bone extraction, microdissection, RNA integrity assessment, DNase treatment, and inclusion of no-reverse-transcriptase and no-template controls strengthen the reliability of transcript detection. The detection of the selected transcripts across five independent cadaveric specimens indicates consistency across biological samples.

4.1 Limitations

This study has important limitations. First, the analysis was performed on bulk cochlear tissue rather than purified cell populations; therefore, the results cannot demonstrate supporting-cell-specific expression. Second, endpoint RT-PCR confirms transcript detection but does not provide quantitative expression levels. Third, postmortem interval, tissue heterogeneity, RNA degradation, and dissection variability may influence transcript detection.

4.2 Future directions

Future studies should validate these observations using quantitative RT-PCR with larger numbers of biological specimens and sequencing of representative PCR products. Cell-type localization should be addressed using RNA scope, immunohistochemistry, spatial transcriptomics, or single-cell/nucleus RNA sequencing. These approaches will help distinguish supporting-cell expression from signals derived from sensory cells, neurons, fibrocytes, vascular cells, and other cochlear populations. Additional studies may also explore epigenetic and injury-response pathways relevant to inner-ear repair and regeneration.

5 Conclusion

The detection of AQP4, GLAST (SLC1A3/EAAT1), TAK1 (MAP3K7), and SOX21 transcripts demonstrates the feasibility of recovering and detecting selected molecular targets from postmortem adult human cochlear tissue using endpoint RT-PCR. Because the analysis was performed on bulk cochlear tissue, these findings do not establish cell-type-specific expression, protein abundance, or functional pathway activity. Cell-type-resolved validation will be required to determine the precise localization and biological significance of these transcripts in the adult human cochlea.

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Data availability statement

Not applicable

Ethical approval

Informed consent was obtained from all subjects mentioned in manuscript.

Informed consent

Informed consent for the collection and research use of cadaveric temporal bone specimens was obtained from the legally authorized representatives of the deceased donors.

Conflicts of interest

The authors declare that they have no competing interests.

Artificial intelligence

AI was used for writing/grammar and sentences formation.

Authors' contributions

All authors contributed to the study. The conceptualization, experimental design, data analysis and interpretation, as well as the writing of the manuscript, were carried out by Pankaj Keshari and Riyaz Ahmad Mir. Ayesha collected the cadaveric specimens and contributed to the review and editing of the manuscript. Arvind and Arun were involved in the extraction of temporal bones and cochlear tissues All authors read and approved the final manuscript.

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