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# Systematic Proteome-wide Mendelian Randomization to Identify Novel Therapeutic Targets for Age-related Hearing Loss

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ABSTRACT

Objectives: Age-related hearing loss is a common neurodegenerative disorder worldwide, with significant public health implications, emphasizing the urgent need for identifying novel therapeutic targets. This study seeks to uncover potential novel therapeutic targets for ARHL by employing proteome-wide Mendelian randomization analysis. Methods: This investigation employed a proteome-wide Mendelian randomization approach, utilizing publicly accessible data from GWAS and pQTL studies. Additionally, we performed phenome-wide association studies, enrichment analysis, protein network construction, single-cell RNA sequencing analysis, colocalization analysis, drug repurposing predictions, and molecular docking simulations, all aimed at identifying more precise and targeted therapeutic strategies. Results: We identified six plasma proteins with genetically predicted causal effects on ARHL risk. GO analysis highlighted their predominant roles in key biological processes, including the regulation of cytosolic calcium ion concentration, negative regulation of homotypic cell-cell adhesion, and regulation of endoplasmic reticulum unfolded protein response. Additionally, important cellular components include smooth endoplasmic reticulum, and dendritic shaft. Follow-up phenome-wide association, protein network, single-cell transcriptomic, colocalization, drug repurposing, and molecular docking analyses provided convergent evidence supporting EEF2K, C1QTNF1, ATF6, JPH4, KIR2DL3, and MICB as promising therapeutic targets for ARHL, and identified four candidate compounds (Withaferin A, Cianidanol, Danthron, and adenosine triphosphate) for further therapeutic investigation. Conclusions: Proteome-wide MR identified EEF2K, C1QTNF1, ATF6, JPH4, KIR2DL3, and MICB as candidate therapeutic targets for ARHL, offering new insights into target discovery. Focusing on these proteins may improve target prioritization and enhance the efficiency of drug development for ARHL.

KEYWORDS

ARHL, Mendelian randomization, Plasma proteome, Drug target.

## 1 Introduction

Presbycusis, or age-related hearing loss, is a prevalent condition that involves a gradual decline in auditory function as individuals age (Chester et al., 2021; Tsai et al., 2024). It typically manifests as high-frequency hearing loss, which progressively worsens over time. Globally, ARHL has become one of the most prevalent chronic conditions among the elderly, significantly affecting their quality of life (Brewster et al., 2021). According to the GBD report, approximately 1.5 billion people were impacted by hearing loss in 2019, with 62.1% of these cases affecting individuals aged over 50 (GBD 2019 Hearing Loss Collaborators, 2021). Hearing loss is expected to impact over 2.45 billion people worldwide by 2050. ARHL also places a significant burden on healthcare systems, highlighting the need for critical research to better understand its genetic mechanisms and develop effective treatment options (Wilson and Tucci, 2021).

The development of ARHL is multifactorial, encompassing genetic predispositions, environmental exposures, and intrinsic age-associated biological changes (Deng et al., 2021; Zheng et al., 2024). In recent years, researchers have utilized genome-wide association studies (GWAS) uncover genetic loci implicated in ARHL, offering new insights into its genetic basis (Cordeiro et al., 2024; Ninoyu and Friedman, 2024). These studies have highlighted multiple genetic loci related to ARHL, providing a better understanding of some of the genetic mechanisms involved in this con-

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dition. Despite these advancements, current treatments for ARHL are still limited. Existing interventions, such as hearing aids and cochlear implants, primarily serve as aids rather than offering effective solutions to reverse or slow down hearing degradation. As a result, identifying potential therapeutic targets through GWAS and developing new treatment strategies has become a major focus in this area of research (Di et al., 2020).

Human proteins are central to the pathophysiology of numerous diseases and represent primary targets for therapeutic strategies (Zheng et al., 2020). The advancement of innovative proteomics technologies has allowed for the detection of changes in protein expression, facilitating the identification of potential biomarkers and therapeutic targets in human diseases (Zhang et al., 2024). Investigating proteins related to ARHL greatly enhances our understanding of its underlying molecular mechanisms. Observational studies can identify associations between variables, but they cannot establish causality (Xiong et al., 2024). This is because the proteins identified may either be causal factors or simply consequences of the disease complicating the interpretation of these associations.

To address these limitations in drug target discovery and repurposing, Mendelian randomization (MR) has emerged as a robust analytical framework (Lin et al., 2023). MR employs genetic variants as instruments to infer causal effects, minimizing confounding and reverse causation (Burgess et al., 2015). This method offers a level of rigor comparable to that of randomized controlled trials, potentially improving the success rate of drug development. Additionally, the integration of high-throughput proteomics, especially plasma analysis, has further enhanced the application of MR in identifying promising therapeutic targets across various diseases.

In this study, we applied bidirectional MR to investigate the causal impact of proteins on ARHL, leveraging the largest available pQTL and ARHL GWAS datasets. To ensure the robustness of our results, multiple sensitivity analyses were performed, including MR-PRESSO, Steiger filtering, Cochran's Q test, and leave-one-out analysis. We further explored the functional relevance of key proteins through enrichment analysis, protein-protein interaction network construction, single-cell localization, drug prediction, and molecular docking. These analyses provide a foundation for the development of targeted therapeutics. Finally, phenotype-wide MR analyses were conducted to assess potential adverse effects of the identified targets. Collectively, this comprehensive framework facilitates the identification of novel therapeutic candidates and supports strategies to improve outcomes for patients with ARHL.

## 2 Materials and Methods

### 2.1 Data Sources

Plasma pQTL data were obtained from the integrative study by Ferkingstad et al. (Ferkingstad et al., 2021). This study included data on 4,907 plasma proteins from 330,759 individuals of Icelandic descent. To identify cis-pQTLs, we applied the following selection criteria: 1. Genome-wide significance ($p < 5 \times 10^{-8}$) for the pQTLs. 2. The independence of the pQTLs was assessed through LD clumping with an $r^2 < 0.001$ and a window size of 10,000 kb. 3. SNPs with a MAF < 0.01 were excluded. 4. Sufficient instrument strength for the pQTLs, indicated by F-statistics greater than 20.

### 2.2 GWAS Dataset for ARHL

Data on ARHL were obtained from the 2020 GWAS meta-analysis hosted by EMBL-EBI, comprising 330,759 subjects of European ancestry (Supplementary Table 2).

### 2.3 Mendelian randomization analysis

We employed bidirectional MR via the 'TwoSampleMR' package to investigate causal relationships between plasma proteins and ARHL, including its subtypes. SNPs exhibiting associations with potential confounding phenotypes were excluded to reduce the risk of pleiotropic bias. After harmonizing the data, MR analyses were conducted on the remaining SNPs. For analyses involving a single SNP, the Wald ratio method was used to estimate MR. For MR analyses involving multiple SNPs, we applied the IVW approach with random effects. The IVW method was adopted as the primary analytical approach. Complementary MR methods including MR-Egger, weighted median, weighted mode, simple mode and MR-RAPS were implemented for sensitivity analyses to assess the robustness of causal estimates (Supplementary Table 4).

Cochran's Q test was used to evaluate the heterogeneity of the individual causal effects of the SNPs. To assess pleiotropy, we applied the MR-Egger intercept method. P-values were adjusted using the FDR method, with a significance threshold set at 0.05.

In the reverse MR, treating ARHL as exposure and plasma proteins as outcomes, causal effects were non-significant, confirming unidirectionality. The analysis followed the same workflow and thresholds as the forward MR.

### 2.4 MR Phenome-Wide Association Study

In phenome-wide analyses, cis-pQTLs of key proteins served as exposures, with outcomes derived from the UK Biobank cohort comprising up to 408,961 individuals. During the GWAS, we employed the SAIGE method to account for imbalances in the case-control ratios (Zhou et al., 2018). To maintain sufficient statistical power, we selected 573 disease traits, each with over 500 cases, from a total of 1,403 traits. These traits were classified according to the ICD-9/-10 (Zheng et al., 2020).

### 2.5 Enrichment analysis

We used the R package 'clusterProfiler' to conduct enrichment analyses, concentrating on GO terms and KEGG pathways. The GO framework classifies annotations into three main categories: BP, MF, and CC. Additionally, KEGG pathway analysis offered insights into the involved metabolic pathways, highlighting the potential biological functions of these proteins.

### 2.6 Protein–protein interaction network construction

To visualize interactions among critical proteins, PPI networks were generated via GeneMANIA using default settings for an unbiased representation.

### 2.7 scRNA-Seq data processing

Transcriptional patterns of candidate genes in the inner ear were analyzed using scRNA-seq datasets downloaded from the GEO database (GSE137299 and GSE123241). All analytical procedures, including quality control, normalization, scaling, clustering, t-SNE dimensional reduction, and cell-type annotation, were performed via the UMGear web platform (https://umgear.org/index.html). Cell types were annotated according to well-established inner ear marker genes. The t-SNE projections were generated to visualize gene expression distribution across cell populations from mouse cochlea and zebrafish larval lateral-line neuromast. Heatmaps were constructed to present mean expression values of candidate genes across cochlear cell subtypes, as well as develop-

mental expression dynamics of these genes at multiple time points in cochlear and utricular tissues.

### 2.8 Colocalization analysis

Colocalization analysis was conducted using the R package coloc (v5.2.3) to validate the MR-derived causal relationships between candidate proteins and ARHL. We integrated pQTL and eQTL summary statistics with ARHL GWAS data to determine whether unified genetic variants jointly regulate gene expression, protein abundance, and disease susceptibility.

A Bayesian framework was applied to evaluate five competing locus-level hypotheses: H0 (no association), H1 (only gene/protein association), H2 (only ARHL association), H3 (two independent causal variants), and H4 (one shared causal variant for both molecular trait and ARHL). Prior probabilities were set to 1×10e4 for trait-specific and shared variant associations. A robust colocalization was defined as PPH4 ≥ 0.75, supporting a shared genetic basis underlying gene-protein expression and ARHL risk. Regional colocalization patterns were visualized using the R package locuscomparer (LocusCompareR, v1.0.0) to illustrate variant association profiles and linkage disequilibrium structures.

### 2.9 Candidate drug prediction

The DSigDB database, encompassing 22,527 gene sets, 17,389 compounds, and 19,531 proteins, was used to prioritize candidate proteins. After preprocessing, the list of proteins was uploaded to the DSigDB platform. This database integrates information on the known mechanisms of action, safety profiles, and pharmacokinetics to facilitate identification of promising therapeutic targets.

### 2.10 Molecular docking

To assess the binding affinities and interaction patterns between potential drugs and their target proteins, molecular docking simulations were conducted. This approach facilitated the optimization of drug design and the selection of therapeutic targets for further experimental validation, focusing on ligands that exhibited strong binding affinities and favorable interaction profiles. Structural data for the drugs were obtained from the PubChem Database, while protein structures were sourced from the Protein Data Bank. The molecular docking was performed using CB-Dock2, analyzing the six most significant drugs along with their corresponding target proteins. The final structures of six proteins and four drugs were successfully identified and visualized using PyMOL 3.0.3.

## 3 Results

### 3.1 Candidate Druggable Proteins and Instrumental Variant Selection

We extracted data on 4,907 druggable plasma proteins from the deCODE Health database, which includes 330,759 individuals from Iceland. In this study, we identified 3,453 cis-pQTLs and 31,315 SNPs as instrumental variables, based on the selection criteria for instrumental variants (Supplementary Table 1). A schematic representation of the Mendelian randomization study design, following the outlined protocol, is provided in Figure 1.

### 3.2 Screening the proteome for ARHL causal proteins

MR analysis identified a total of 133 plasma proteins associated with ARHL from 4,907 pQTLs of plasma proteins targeting drug-related proteins identified by deCODE (Supplementary Table 3). After FDR correction, we identified six plasma proteins that show a significant potential causal association with the risk of ARHL. These proteins include JPH4, EEF2K, C1QTNF1, KIR2DL3, ATF6, and MICB. Specifically, JPH4 (OR = 1.067; 95% CI 1.042–1.093; P = 1.58e-07), EEF2K (OR =1.046; 95% CI 1.022–1.071; P = 1.316e-04), C1QTNF1 (OR = 1.033; 95% CI 1.017–1.049; P = 6.015e-05), and KIR2DL3 (OR = 1.030; 95% CI 1.017–1.042; P = 1.838e-06), and ATF6 (OR = 1.026; 95% CI 1.013–1.040; P = 1.432e-04) were associated with an increased risk of ARHL, while MICB (OR = 0.983; 95% CI 0.974–0.991; P =7.521e-05) was associated with a decreased risk of ARHL (Figure

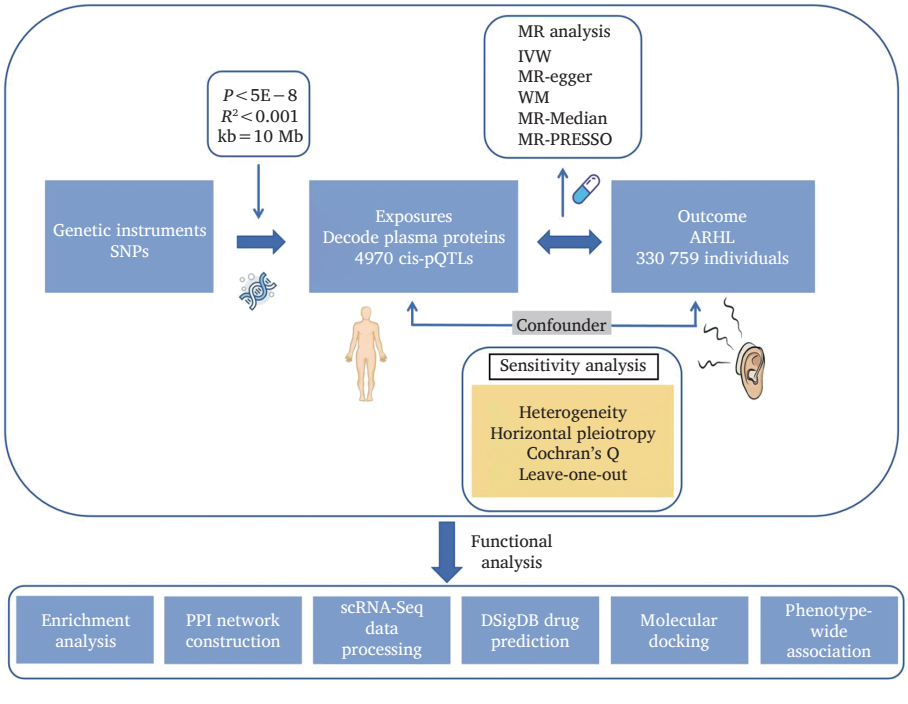



**Figure 1** Overview of the study design.

2; Supplementary Figure 1,2).

### 3.3 Sensitivity analysis

The sensitivity analyses further confirmed the robustness of our primary Mendelian randomization findings. We did not observe any evidence of heterogeneity in the associations involving the six proteins, as indicated by Cochran's Q statistic ($p > 0.05$). Additionally, no evidence of horizontal pleiotropy was observed among the IVs ($p > 0.05$; Supplementary Table 5). The funnel plot demonstrated a symmetrical distribution of the SNPs. A leave-one-out analysis was performed to evaluate the potential impact of individual SNPs on the results, which revealed that no single SNP had a substantial effect (Supplementary Figures 3-5). Furthermore, scatter plots were created to illustrate the causal relationships between the proteins and ARHL, offering a clearer visualization of the data. Bidirectional MR analysis showed no evidence of reverse causality, and Steiger filtering confirmed the correct directionality of the causal inference (Supplementary Table 6).

### 3.4 Enrichment analysis

GO enrichment analysis indicated that the six candidate targets are mainly involved in biological processes such as positive regulation of cytosolic calcium ion concentration (GO:0007204), negative regulation of homotypic cell-cell adhesion (GO:0034111), and positive regulation of the unfolded protein response (GO:1900103). The main cellular components include smooth endoplasmic reticulum (GO:0005790), and dendritic shaft (GO:0043198). The main molecular functions include calcium/calmodulin-dependent protein kinase activity (GO: 0004683), and translation factor activity (GO:0008135). KEGG enrichment analysis demonstrated that ARHL-related druggable genes are mainly enriched in pathways such as Antigen processing and presentation, AMPK signaling pathway, Oxytocin signaling pathway, and Protein processing in endoplasmic reticulum (Figures 3A,B).

### 3.5 Protein–protein interaction network construction

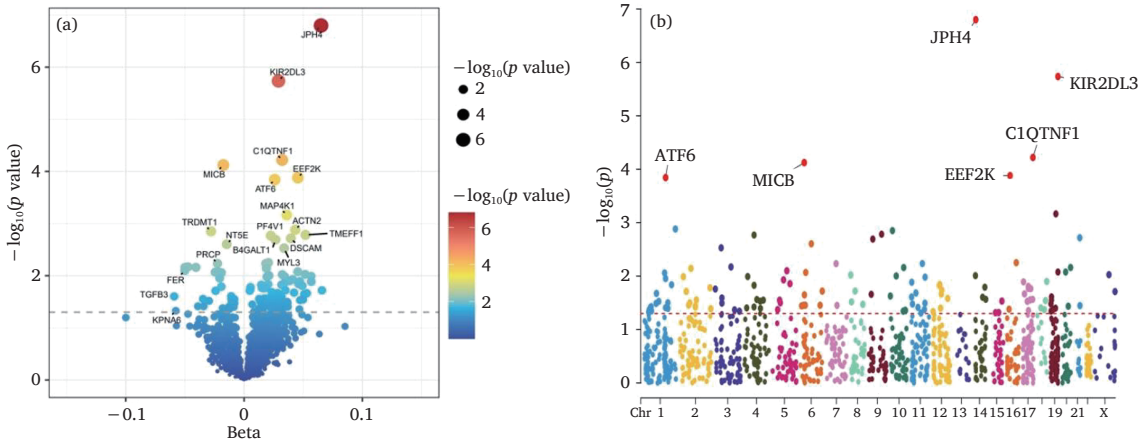
A PPI network based on six druggable proteins was generated using GeneMANIA. The network, consisting of six nodes and 20 edges (Figure 3C), demonstrated a high degree of connectivity, implying close functional relationships. These interactions were supported by evidence of co-expression, shared pathways, and genetic associations, suggesting that the identified proteins may act in concert to modulate key biological processes underlying ARHL.

### 3.6 Phenotype-wide association study

Phenome-wide MR analyses were conducted to assess potential off-target effects of interventions targeting JPH4, EEF2K, C1QTNF1, KIR2DL3, ATF6, and MICB. A total of 573 traits from the UK Biobank were included in the screening, and no significant causal associations were identified between these proteins and other diseases. The corresponding Manhattan plots are shown in Supplementary Figure 6.

### 3.7 Single-Cell RNA Sequencing Analysis and Genetic Colocalization Analysis

Single-cell transcriptomic analysis was performed on inner ear scRNA-seq datasets using the UMGear platform to evaluate cell-type-specific expression and developmental dynamics of candidate genes in mouse cochlea and mechanosensory cell populations from zebrafish larval lateral-line neuromasts. Key candidate genes, including Eef2k, Atf6, Jph4, and C1qtnf, showed enriched expression in principal functional inner ear cell subtypes such as hair




(c)

| Exposure | nsnp | Method | *p* val | OR (95% CI) |
|---|---|---|---|---|
| JPH4 | 4 | Inverse variance weighted | <0.001 | 1.067 (1.042 to 1.093) |
| EEF2K | 3 | Inverse variance weighted | <0.001 | 1.046 (1.022 to 1.071) |
| C1QTNF1 | 12 | Inverse variance weighted | <0.001 | 1.033 (1.017 to 1.049) |
| KIR2DL3 | 12 | Inverse variance weighted | <0.001 | 1.030 (1.017 to 1.042) |
| ATF6 | 17 | Inverse variance weighted | <0.001 | 1.026 (1.013 to 1.040) |
| MICB | 13 | Inverse variance weighted | <0.001 | 0.983 (0.974 to 0.991) |

0.9 1.0 1.1

**Figure 2** Summary of Mendelian randomization analyses for associations between plasma proteins and age-related hearing loss.

(A). Volcano plot of the MR results for plasma proteins on age-related hearing loss. (B). Manhattan plot for chromosomal localization of MR results for plasma proteins on age-related hearing loss. The x-axis denotes chromosome position. The red dashed line indicates the significance threshold. Top significant protein-coding genes are annotated. (C).Forest plot of six significant genes associated with age-related hearing loss.

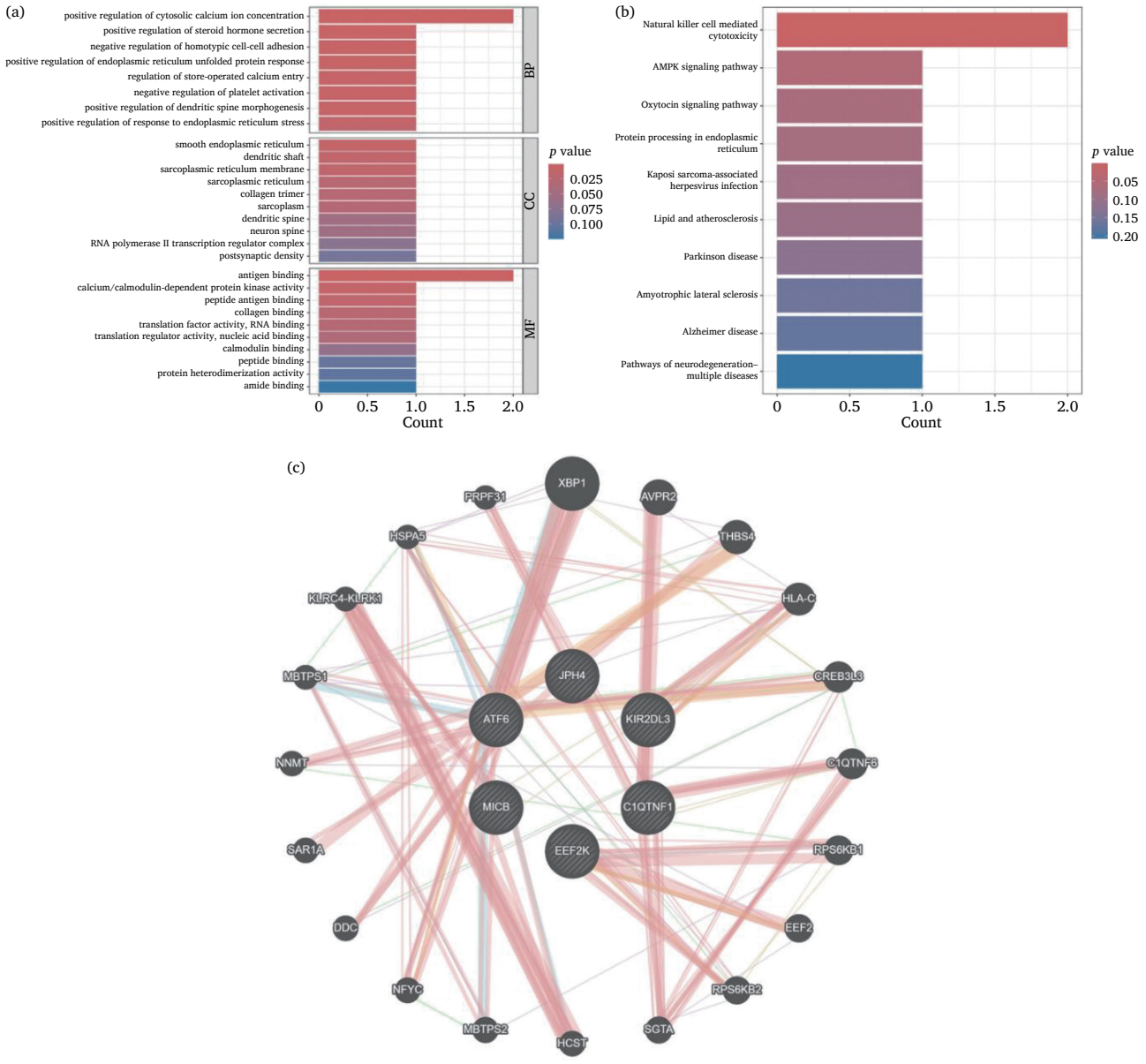



**Figure 3** Functional enrichment analysis and protein-protein interaction network of candidate proteins associated with age-related hearing loss.

(A) GO enrichment bar plot showing significantly enriched Gene Ontology biological process, cellular component and molecular function terms for candidate genes. The color gradient represents the P value, and the X-axis shows the gene count enriched in each term. (B) KEGG pathway enrichment bar plot displaying enriched signaling pathways. Color gradient corresponds to P value and the X-axis represents gene count. (C) PPI network of ARHL candidate proteins. Nodes represent proteins encoded by candidate genes, and edges indicate protein-protein interactions; edge color/transparency reflects the strength of interaction evidence.

cells and supporting cells, and exhibited temporally dynamic expression patterns during inner ear development (Figure 4, Supplementary Figure 7). Genetic colocalization analysis was conducted by integrating pQTL, eQTL, and ARHL GWAS summary statistics. Strong colocalization signals were identified at the EEF2K locus. The shared variants modulated both gene expression and protein abundance, coinciding with ARHL-associated genetic signals (Figure 4).

### 3.8 *Candidate drug prediction*

DSigDB analysis identified 33 candidate compounds with potential therapeutic relevance. We then screened four candidate drugs for ARHL: Withaferin A, Cianidanol, Danthron, and Adenosine triphosphate. These drugs have been employed in the treatment of neurodegenerative diseases, cardiovascular and cerebrovascular disorders, as well as certain aspects of cancer, or have been investigated in preclinical therapeutic studies. However, none have been specifically evaluated for the treatment of ARHL.

### 3.9 *Molecular docking*

Molecular docking was performed using CB-Dock2 to characterize binding sites and interactions between four candidate compounds and their corresponding target proteins (EEF2K, C1QTNF1, ATF6, and MICB). Binding affinities were calculated for each protein–ligand pair, yielding 16 valid docking results. The top four interactions with the lowest binding energies were subsequently selected for further analysis. Detailed information on interacting residues and hydrogen bond distances is presented in Figure 5.

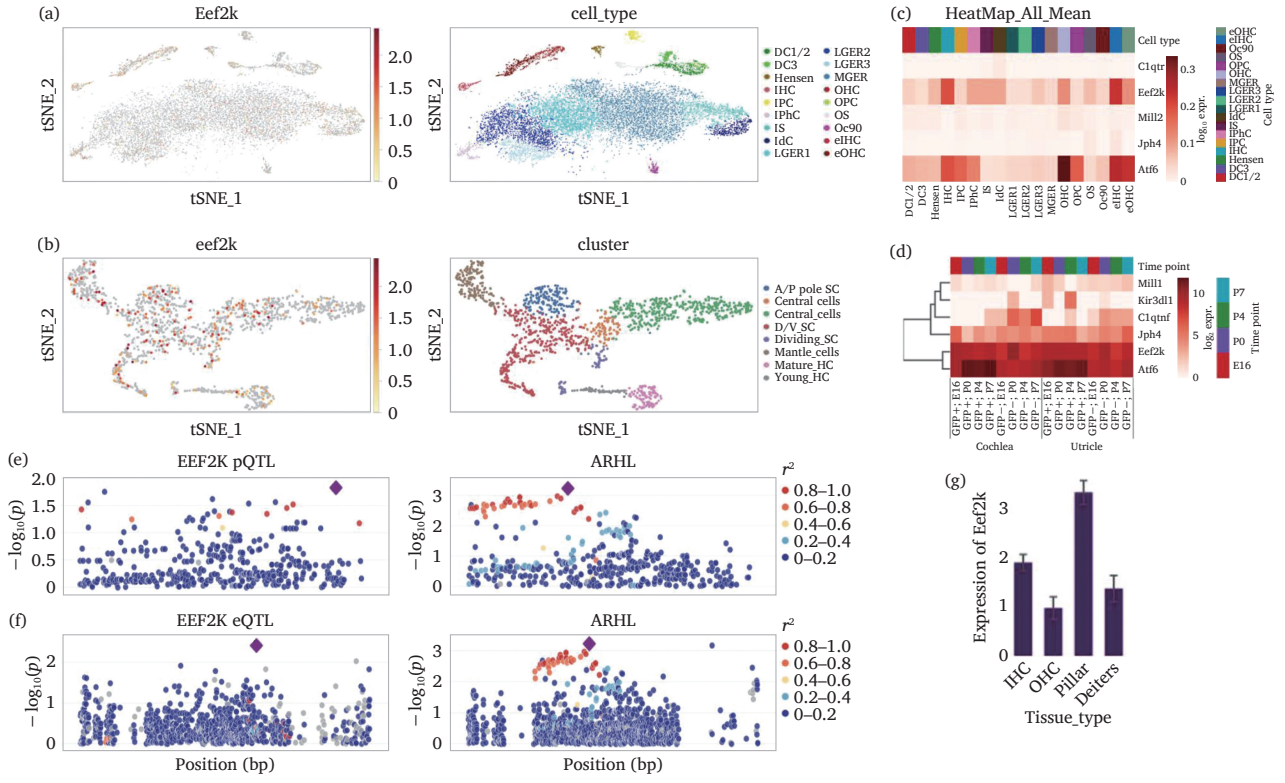



**Figure 4** Cell-type-specific expression, developmental dynamics and genetic colocalization analysis of candidate genes.

(A, B) t-SNE plots showing the expression distribution of Eef2k. (A) Mouse cochlear single-cell t-SNE, annotated to label distinct cochlear cell subtypes. (B) Single-cell t-SNE from zebrafish larval lateral-line neuromast, annotated for major mechanosensory cell clusters including hair cells and supporting cells. (C) Heatmap displaying the mean expression levels of candidate genes across different cochlear cell types. (D) Heatmap showing developmental expression dynamics of candidate genes across multiple time points in cochlea and utricle tissues. (E, F) Colocalization Manhattan plots for EEF2K pQTL and eQTL with ARHL GWAS signals. The shared colocalized variant is highlighted as purple diamond. The colour of each dot represents the linkage disequilibrium ($r^2$) relative to the lead variant. (G) Bar plot summarizing aggregated expression of Eef2k in major cochlear cell populations (IHC, OHC, Pillar and Deiters cells).

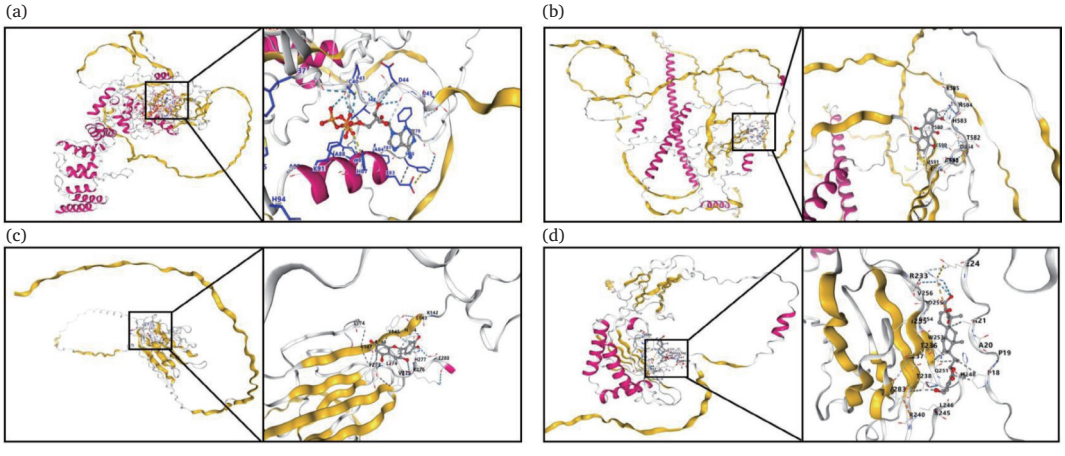



**Figure 5** Molecular docking results of available proteins and drugs.

A. EEF2K docking Adenosine triphosphate, B. ATF6 docking Danthron, C. C1QTNF1 docking Cianidanol, D. MICB docking withaferin A.

## 4 Discussion

In this study, we used MR to identify and evaluate potential causal proteins associated with ARHL from a comprehensive database of druggable plasma proteins. Our MR analysis identified six plasma proteins that show a significant causal relationship with the risk of ARHL. These proteins are JPH4, EEF2K, C1QTNF1, KIR2DL3, ATF6, and MICB. These findings provide strong evidence regarding the molecular pathways involved in ARHL and highlight new targets for potential therapeutic interventions. We conducted an enrichment analysis and constructed PPI networks as well as KEGG pathways for the six significant proteins to explore the biological relevance and interaction mechanisms of these drug targets. A detailed analysis of single-cell RNA sequencing data from the inner ear revealed distinct expression patterns of the target proteins across various cell subpopulations. To evaluate the potential side effects of targeting these proteins, we performed MR

phenome-wide association studies. Lastly, we employed drug prediction models and molecular docking simulations to further assess the pharmaceutical potential of these identified druggable genes.

Enrichment analyses of the identified proteins demonstrated that they are primarily involved in biological processes such as the regulation of cytosolic calcium ion concentration and the endoplasmic reticulum (ER) unfolded protein response. These processes are implicated in various forms of cellular stress, which may contribute to the degeneration of cochlear cells in ARHL. EEF2K is a serine/threonine kinase that plays a crucial role in protein synthesis. It is important for cellular stress responses, metabolic regulation, and the development of various neurodegenerative diseases, including Alzheimer's and Parkinson's disease (Jan et al., 2018; Beckelman et al., 2019). ATF6 is a transcription factor involved in the response to endoplasmic reticulum stress. Its main function is to regulate processes related to protein folding, repair, and degradation. When ATF6 is abnormally activated, it can contribute to the onset of several diseases, including neurodegenerative disorders like Alzheimer's and Parkinson's disease, as well as type 2 diabetes and cancer (Zhang et al., 2024; Cheng et al., 2025). The JPH4 protein is mainly located at the junctions between the cell membrane and the endoplasmic reticulum. It regulates the transport and release of intracellular calcium ions, playing a vital role in several physiological processes (Hogea et al., 2021; Vidal et al., 2021). These include neurotransmission, the stabilization of intracellular membrane structures, and the regulation of stress responses. The involvement of proteins like EEF2K and ATF6, both of which regulate stress responses, further supports the hypothesis that cellular stress pathways play a critical role in the pathophysiology of ARHL. Additionally, the KEGG pathway analysis identified several key pathways, including AMPK signaling, and protein processing in the endoplasmic reticulum, which are consistent with known mechanisms of hearing loss and ARHL.

The PPI network created from six druggable proteins revealed a complex molecular framework that may offer insights into the biological mechanisms underlying ARHL. The interactions within this network could serve as potential therapeutic targets or biomarkers for the disease. Notably, the expression patterns of these proteins, as observed in scRNA sequencing data from the inner ear, further underscore their potential as therapeutic targets. For instance, the significant expression of ATF6 and EEF2K in specific inner ear cell subpopulations suggests that these proteins may play central roles in cochlear cell function and survival, warranting further investigation into their involvement in ARHL.

In addition to identifying potential causal proteins, we conducted phenome-wide MR association studies to examine possible side effects of therapeutic interventions targeting the identified proteins. Our findings indicated that none of the target proteins were significantly associated with other diseases in the UK Biobank cohort, suggesting that targeting these proteins for ARHL may carry a relatively low risk of adverse effects. This consideration is crucial in developing new treatments for ARHL, as off-target effects could compromise the safety and efficacy of potential therapies.

Moreover, we employed molecular docking analysis to predict potential drug candidates for ARHL, identifying 33 candidate drugs that could interact with the target proteins. Noteworthy compounds such as adenosine triphosphate, Withaferin A, Danthron, and Cianidanol demonstrated promising binding interactions with the identified proteins. Adenosine triphosphate is a coenzyme involved in energy metabolism. It is widely used in clinical practice to treat myocardial dysfunction, cerebrovascular diseases, and other conditions (Fu et al., 2025). Withaferin A is a natural compound that exhibits various pharmacological effects, including antitumor, anti-inflammatory, antioxidant, and immunomodulatory properties (Zhang et al., 2025). Danthron, an anthraquinone derived from the rhubarb plant, possesses antioxidant properties that help reduce cellular damage caused by free radicals (Chou et al., 2018). Cianidanol is a phenolic compound derived from plants, exhibiting antioxidant, antitumor, and antibacterial properties (Zeng et al., 2025). They demonstrate significant promise in treating cancer, inflammation-related diseases, neurodegenerative diseases, diabetes, and cardiovascular disorders (Li et al., 2019; Nisar et al., 2025). These drugs could serve as potential candidates for further experimental validation and clinical testing.

Overall, our findings provide strong evidence for proteins that could serve as druggable targets for the treatment of ARHL, highlighting their potential as promising therapeutic candidates. While our study provides new insights into the molecular basis of ARHL and identifies several promising drug targets, it is not without limitations. First, although Mendelian randomization offers strong evidence for causality, it relies on genetic instruments and may not fully account for environmental or other non-genetic factors contributing to ARHL. Second, the generalizability of our findings to diverse populations remains to be established. Future studies, including replication in independent cohorts and functional validation of the identified proteins in animal models, will be crucial to confirm the causal relationships and explore the therapeutic potential of these targets in ARHL.

## 5 Conclusions

In conclusion, our study identifies six plasma proteins with a significant causal association with ARHL and provides a comprehensive framework for elucidating its molecular mechanisms. The identification of these proteins highlight promising targets for therapeutic development, with potential to streamline drug discovery. Future studies should investigate the functional roles of these proteins in cochlear degeneration and explore their potential as therapeutic targets in treating ARHL.

## Funding

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## Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Ethical approval

Ethical approval was waived because this study used the data from publicly available databases.

## Informed consent

All patients provided informed consent.

## Conflicts of interest

The authors declare that they have no competing interests.

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