RESEARCH ARTICLE

Web-based transcriptome analysis determines a sixteen-gene signature and associated drugs on hearing loss patients: A bioinformatics approach

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Abstract
Background: Hearing loss is becoming more and more general. It may occur at all age and affect the language learning ability of children and trigger serious social problems.
Methods: The hearing loss differentially expressed genes (HL-DEGs) were recognized through a comparison with healthy subjects. The Gene Ontology (GO) analysis was executed by DAVID. The reactome analysis of HL-DEGs was performed by ClueGO. Next, we used STRING, an online website, to identify crucial protein-protein interactions among HL-DEGs. Cytoscape software was employed to construct a protein-protein interaction network. MCODE, a plug-in of the Cytoscape software, was used for module analysis. Finally, we used DGIdb database to ascertain the targeted drugs for MCODE genes.
Results: Four hundred four HL-DEGs were identified, among which the most up-regulated 10 genes were AL008707.1, SDR42E1P5, BX005040.1, AL671883.2, MT1XP1, AC016957.1, U2AF1L5, XIST, DAAM2, and ADAMTS2, and the most down-regulated 10 genes were ALOX15, PRSS33, ILSRA, SMPD3, IGHV1-2, IGLV3-9, RHOF1P1, CACNG6, MYOM2, and RSAD2. Through STRING database and MCODE analysis, we finally got 16 MCODE genes. These genes can be regarded as hearing loss related genes. Through biological analysis, it is found that these genes are enriched in pathways related to apoptosis such as tumor necrosis factor. Among them, MMP8, LTF, ORM2, FOLR3, and TCN1 have corresponding targeted drugs. Foremost, MCODE genes should be investigated for its usefulness as a new biomarker for diagnosis and treatment.
Conclusion: In summary, our study produced a sixteen-gene signature and associated drugs that could be diagnosis and treatment of hearing loss patients.

KEYWORDS
bioinformatics, biomarker, diagnosis, hearing loss, RNA-seq

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INTRODUCTION

The most common clinical manifestation of hearing impairment is the decline in the ability to acquire external sounds, which leads to the patient’s impaired understanding of external information. Among them, age is one of the most important risk factors. Hearing impairment is closely related to people’s aging. More than 5% of the world’s population suffer from disabling hearing loss. The patients cannot communicate with the outside world normally, which seriously affects the quality of life of the patients. Another study reported that 4–11 out of every 10,000 children suffer from severe early-onset deafness, and at least 50% of cases can be attributed to genetic causes. The impact of hearing loss on patients is permanent; as time goes by, the psychological quality of patients will change. There are also reports that hearing impairment is closely related to Alzheimer’s; thus, hearing impairment is more and more worthy of attention. There are many reasons for deafness. The common cause is drug-induced deafness. The drug streptomycin used to treat tuberculosis can cause deafness. As age increases, it will cause cumulative pathophysiological changes in hearing. This is called age-related hearing loss. This type of deafness is related to demographic factors such as age, gender, and socioeconomic status; medical factors such as cardiovascular disease, cognition, diabetes, cholesterol levels, and obesity; and lifestyle, positive correlations such as noise exposure, smoking and drinking, and genetic susceptibility. At present, the clinical diagnosis of deafness is mainly through hearing tests. At this time, the patient has already developed clinical manifestations of deafness. If the discovery and diagnosis can be made early, the clinical treatment effect of patients will be improved. Apoptosis is one of the methods of programed cell death, and it seems to play a key role in the development and disease of the inner ear. When the hair cell nucleus is severely damaged, the initiation of programed cell death will lead to irreversible changes, such as metabolic arrest, structural damage, and loss of function, which can balance cell death and normal cell survival. If the balance is imbalanced, the patient will become deaf. Tumor necrosis factor will participate in the biological process (BP) of cell apoptosis and play an important role. RNA sequencing (RNA-Seq) is a powerful technology that can use the functions of next-generation sequencing technology to analyze gene expression profiles of organisms to obtain disease-related genes, thereby improving the efficiency of disease diagnosis. Some people have done research in the diagnosis and treatment of intracranial aneurysms through peripheral blood mononuclear cell sequencing. Some researchers have also sequenced and analyzed RNA in peripheral blood mononuclear cell (PBMC) cells of patients with non-ischemic cardiomyopathy, thereby providing new ideas for the diagnosis and treatment of cardiomyopathy. At present, reports on sequencing the blood of deaf patients are relatively rare. This study mainly performed PBMC-RNA sequencing on patients who were clinically diagnosed with deafness, analyzed the results of sequencing using bioinformatics technology, combined with major free databases for comprehensive analysis, and obtained novel biological markers related to deafness. Drug predictions for these markers can improve the diagnosis efficiency of deaf patients and provide new ideas for the treatment of clinical deaf patients. During the analysis, we found several very valuable deafness-related biological markers. Interestingly, they are enriched in the BP of tumor necrosis factor. Tumor necrosis factor and apoptosis are closely related, so we speculate that these biomarkers may affect the development of deafness by influencing the apoptotic pathway of hair cells.

MATERIALS AND METHODS

Ethics approval and patients data acquisition

This study complies with the ethical guidelines of the Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association and was approved by the Institutional Research Committee of the Zhongshan Hospital, Xiamen University, China. Each patient signed informed consent. All specimens are handled in accordance with ethical and legal standards (Approval number: 2021-077). Three hearing loss patients and three healthy subjects were recruited from the Department of Otorhinolaryngology - Head and Neck Surgery, Zhongshan Hospital, Xiamen University. Each patient collected 5 ml of blood into a heparin anticoagulation tube (No. 31, Fuzhou Chang Gung Medical Device Company) and then extracted PBMC cells from the blood for the next experiment. The choice of patients tries to control potential confounding variables.

PBMC isolation and RNA preparation

We take 3 ml of fresh blood from the blood collection tube into a 15 ml centrifuge tube, add 3 times the volume of red blood cell lysate, place on ice for 15 min, centrifuge at 4°C for 10 min, remove the supernatant, add 1 ml Trizol to the cell pellet, and mix well. We add 270 ul chloroform, vortex until the solution is emulsified and appear milky white, let it stand for 5 min, after centrifugation, take the supernatant into a new tube, add an equal volume of isopropanol, mix and let stand for 10 min, remove the supernatant after centrifugation, and precipitate. We add 50 ul of nucleic acid-free plum water and measure the concentration after fully dissolving. We take 300 ng RNA and run on 2% agarose gel.

RNA-seq and bioinformatics

After the RNA extraction test is qualified, RNA sequencing (RNA-Seq) is performed. Select Illumina NovaSeq 6000 for sequencing platform. After the platform sequencing was completed, we obtained the RNA-Seq transcriptome dataset of PBMC of deaf patients. The raw data handing process is completed by Xiamen Aimo
Gene Biotechnology Co., Ltd. We further filter the hearing loss differentially expressed genes (HL-DEGs), the threshold value is set to $p < 0.05$, $|\text{LogFC}| \geq 1.5$, and the HL-DEGs obtained according to this standard are included in our research scope.

2.4 | Gene ontology analysis

Gene ontology (GO) analysis is a useful method for annotating genes and gene products, and identifying the biological significance of features of the genome and transcriptome. A variety of online tools are used to analyze the functional level enrichment of candidate HL-DEGs. Among them, DAVID is an online website with gene annotation, visualization, and providing gene attributes. The $p$ value $< 0.05$ is considered as statistically significant cutoff criterion.

2.5 | Reactome analysis

ClueGO is a plug-in of Cytoscape software, which can be used for Reactome enrichment analysis, and the threshold is set to $p < 0.05$. ClueGO has two main features: it can be used to visualize the terms corresponding to the gene list, and it can also be used to compare the functional annotations of two clusters.

2.6 | Protein-protein interaction network analysis

We upload the HL-DEGs filtered by the threshold to the STRING database for protein-protein interaction network analysis. Species choose people. We hide disconnected nodes in the network. Minimum required interaction score: 0.700. We download the analysis results in TSV format.

2.7 | Cytoscape and MCODE

Cytoscape is an open source software project used to integrate biomolecular interaction networks with high-throughput expression data and other molecular states into a unified conceptual framework. We upload the results from the STRING database to the Cytoscape database for analysis. We choose the MCODE plug-in to analyze the entire network and set the parameters: Node score cutoff: 0.2, K-core = 15. The MCODE gene is the hub gene. We further analyzed MCODE genes to explore the biological processes involved in these genes.

2.8 | Drug-gene interaction

The DGIdb database is used to search for hub genes for known and potential small drug-like molecules. We upload the genes obtained in MCODE to the DGIdb database. We set the parameter to preset filters: approved and advanced filters: source databases (22 of 22), gene categories (43 of 43), and interaction types (31 of 31).

2.9 | Statistical analysis

R packages in this article include “edge” and “limma”. GO and Reactome analyses are based on the HL-DEGs with $p$ value $< 0.05$. The $p$ value $< 0.05$ was considered statistically significant. The flow chart of this manuscript is shown in Figure 1.

FIGURE 1 Flow chart of this article
RESULT

3.1 PBMC

We fulfilled RNA-Seq to discern genes that are differentially expressed in deaf patients and normal patients. The RNA standard and sequencing quality indicators of all samples are shown in Figure 2A, B.

3.2 Bioinformatics

In our research, we first use RStudio to correct and standardize the most primitive data; a total of 849 DEGs were identified \((p < 0.05, \text{and} \ |\log_{2}\text{FC}| \geq 0.5)\), including 436 up-regulated genes and 413 down-regulated genes. We use RStudio software to visualize the results with volcano map and heatmap (Figure 3A, B). The differential expressed genes were further screened according to

![Figure 2: RNA sample detection and sequencing data standardization. (A) Agarose gel electrophoresis of RNA in PBMC cells. (B) RNA-Seq data reads count FPKM standardization](image-url)
the threshold set by ourselves, and 176 up-regulated differential expressed genes and 228 down-regulated differential expressed genes were obtained. The screening threshold was \( p < 0.05 \) and \(|\log_{2}FC| \geq 1.5\) (Table 1).

### 3.3 Gene ontology analysis

In order to outline the GO of DEG, we used DAVID website to visualize functional annotation. As shown in Figure 4 and Table 2, GO analysis shows that DEGs are most significantly enriched in GO:0006958–complement activation, classical pathway; GO:0006956–complement activation; GO:0034987–immunoglobulin receptor binding, GO:0001730–2′-5′-oligoadenylate synthetase activity, GO:004908–interleukin-1 receptor activity, and GO:0003824–catalytic activity in molecular function process. In addition, up-regulated DEGs are significantly enriched in GO:0070062–extracellular exosome, GO:0005576–extracellular region, and GO:0005615–extracellular space; down regulated DEGs were enriched in GO:0003823–antigen binding; GO:0006958–complement activation, classical pathway; and GO:0006956–complement activation (Supplement 1).

### 3.4 Reactome analysis

ClueGo is a plug-in of Cytoscape, which is used for reactome pathway analysis of differential expressed genes. We set the screening threshold to \( p < 0.05 \). DEGs were enriched in OAS antiviral response, scavenging of heme from plasma, G1/S-specific transcription, interferon alpha/beta signaling, antiviral mechanism by IFN-stimulated genes, binding and uptake of ligands by scavenger receptors, interferon signaling, interleukin-4 and interleukin-13 signaling, neutrophil degranulation, and cytokine signaling in immune system (Figure 5 and Table 3).
| DEGs  | Gene names                      |
|-------|---------------------------------|
| Up-genes | AL008707.1 DDX11L10 OLFM4 ABCA13 PXYLP1 CPM CYBRD1 ORM1 KIF27 GRB10 |
|        | SDR42E1P5 FOLR3 AC007271.1 MMP9 DEFA4 HECW2 CHIL11 EFHC2 LGAL5L PFKFB2 |
|        | BXO00540.1 FG13 OR2 LINC02207 SIK1 SL38A11 FN1 PRUNE2 ATP9A TMIGD3 |
|        | AL671883.2 AL669831.4 FZD3 LINC01127 COL17A1 AC092746.1 AL590560.2 CEACAM4 ECHDC3 HLA-DRB1 |
|        | MT1XP1 LAMTOR5-AS1 SDK1 ZNF608 CAMP CNJL CNTAPT3 FLT3 NEDD4 B3GNT8 |
|        | AC016957.1 TMTC1 COL9A2 LINC02289 GPR141 AP006222.1 KIAA1324 RASGEF1A PADI4 CEACAM6 |
|        | U2AF1L5 MPP8 LTF AL163541.1 DUSP13 PHF24 AL512306.3 SULT1B1 TCN1 ATP2C2 |
|        | XIST VSIG4 IL1R2 BMX ERIC6H-A51 TPS1 PCBP3 CLEC12B NQO2 TSC22D3 |
|        | DAAAM2 CRISP3 LCN2 ST6GALNAC3 AOC4P LRRC6 RAB36 CD177 TCL1A CD163 |
|        | ADAMTS2 ARG1 ARMC12 IL18R1 SEPTIN7P8 ETV5 MGAM MEF2C-AS1 MRV1 ZNF66 |
|        | ACSS3 FXYD1 EXOG TOB1 RPRGIP1 TP5311 TPH2 NLRP12 MKNK1 MACIR |
|        | LINC01093 HMOX1 DMRTC2 AC005392.2 KBTBD7 MFN2 CSF3 LINCO1220 KATNB1 IR52 |
|        | GIHCG CES1 ARMC12 IL18R1 SEPTIN7P8 ETV5 MGAM MEF2C-AS1 MRV1 ZNF66 |
|        | ARHGEF17 BPI PGLYRP1 BEND7 TNS1 KIF3C SORT1 IL1R1 LINCO0672 SDHAF3 |
|        | MIR223HG LINC00671 LINC02649 SAP30 SELENOP GA57 FAM71F2 SL3CA7A3 ABHD6 APMAP |
|        | HBA1 MNCR FBKSP PI3 AL035661.1 OSBPL1A SCNN1D SLPI CALCRL APM3D |
|        | ZNF429 HROB BMP2 AL391807.1 GAREM2 SCN9A PTGES CCDC30 C4A LINCO0659 |
|        | ZNF282 FHT C20orf197 HBA2 CCIN AP002495.1 |
| Down-genes | ALOX15 GCSAML ADORA3 MX1 RAB44 CHPF SERPING1 NBPF1 IGLC2 IGHSV2-5 |
|           | PRSS33 ISG15 BIRC5 IGLV1-47 FFA3 IGKV1-8 EPTST1 ZNF823 PLK1 IGHSV3-43 |
|           | IL5RA PMP22 AC139495.2 STK33 GRHL1 USP18 IGHV3-72 PIK3R6 NEURL1 IGKV1-5 |
|           | SMPD3 AC110000.4 CYSLTR2 IGLV3-19 MYBL2 RHEX HES4 PPP1R17 PSEN2 IGHA1 |
|           | IGHV1-2 TREML4 MT1G TMEM135 E2F2 IGLC1 KNDC1 IGLV1-40 TPX2 ZWINT |
|           | IGLV3-9 IFI44L IL6 GINS3 E1F4B6 IGHG4 BUB1B PKD1P5 ALDH8A1 TOP2A |
|           | RHOFX1P1 CMPK2 NEU4 LIN7B MICA IGL5 GIPR DDX10 ITM2C IGKV2-28 |
|           | CACNG6 IFIT3 HDC SCAMP5 OAS1 TROAP IGHV3-21 IGHSV4-39 CNR2 UBE2C |
|           | MYOM2 OAS3 IGLV2-18 XAF1 IGKV1-39 ADGRG5 PKMYT1 IGHSV3-11 IFIT5 GALNT6 |
|           | RSD2 TNNT1 TNRFSF17 IGLV9-49 GBP1 MGAT3 CDKN3 ZFPL1 AGRN CD36 |
|           | ADGRE4P KCNH2 IGHV2-70 KN1 IGKV1-69D IGLV3-25 PLD4 IGLV2-8 WASF1 CIT |
|           | IGLV3-1 IGLV1-36 IGKV1-6 PLAT5 IGKV1-12 BACE2 MB1 CD1 CDT1 HRK ANLN |
|           | CLC IGHV3-49 TMEM176B IGHV1-17 SOCS2 TPP3 SEPTIN10 EPN2 CLEC10A TRDC |
|           | IDO1 IGHV3-48 HERC5 TMEM176A AC073322.1 IL1RL1 IGKV2D-30 IGHV6-1 IGKV3-15 IGKV1-16 |

(Continues)
3.5 | Module analysis, key candidate genes from PPI network, and functional analysis

We upload the list of all DEGs to the STRING database and construct the protein-protein interaction (PPI) network. The results of the STRING database are the following: number of nodes: 294, number of edges: 595, average node degree: 4.05, average local clustering coefficient: 0.392, expected number of edges: 192, PPI enrichment p value: <1.0e−16, and output files in TSV format. We long transfer the TSV format file to the Cytoscape software for analysis, set K = 15, and get the MCODE module (Figure 6A, B). The gene in the module is MMP8, LTF, OLFM4, LCN2, PGLYRP1, ARG1, ORM2, CRISP3, FOLR3, SLPI, DEFA4, CAMP, BPI, ORM1, TCN1, and CHI3L1. Interestingly, All MCODE genes are up-regulated in deaf patients. MCODE genes are used as potential targets, and functional enrichment analysis is performed in the DAVID database to obtain biological information corresponding to the potential targets (Figure 6C).

3.6 | Drug-gene interaction

We upload the MCODE gene to the DGIdb database, and all drug options have been approved. A total of eight kinds of drugs were obtained, among which there are three kinds of drugs that target the MMP8 target, namely: Doxycycline, Collagenase clostridium histolyticum, and Doxycycline calcium. There are two kinds of drugs that target the LTF target, namely: Parecoxib and Bacitracin. The drug targeting the ORM2 target is Docetaxel. The drug that targets the FOLR3 target is Pemetrexed. The drug targeting the TCN1 target is Cyanocobalamin (Table 4).

4 | DISCUSSION

Studies have reported that hearing loss affects one in six people in the UK and is a major disease burden. In addition to communication problems, it is also related to depression and dementia. In this study, we comprehensively analyzed differentially expressed genes in patients with hearing impairment. Functional analysis and reactome analysis revealed important biological functions and signal pathways involved in hearing impaired patients. Furthermore, the targeted drugs of the MCODE genes were obtained, which provided new ideas for the diagnosis and treatment of clinical patients.

Apoptosis is an important physiological process, which is cell death mediated by intracellular BPs. Normally, healthy cells maintain a delicate balance between pro-apoptotic and anti-apoptotic factors, allowing them to survive and proliferate. As we all know, apoptosis can also cause several acquired forms of hearing impairment. Although previous studies have reported that several genes may be related to hearing loss, the specific mechanism is still unclear, and new hearing impairment related genes
**Figure 4** Gene ontology (GO) analysis of HL-DEGs. The enriched GO terms in the biological process (BP), cellular component (CC), molecular function (MF).

**Table 2** The gene ontology analysis of HL-DEGs associated with hearing loss

| Category       | Term                                                                 | Count | p value       |
|----------------|----------------------------------------------------------------------|-------|---------------|
| GOTERM_BP_DIRECT | GO:0006956–complement activation, classical pathway                  | 36    | 8.13E–36      |
| GOTERM_BP_DIRECT | GO:0006958–complement activation, classical pathway                  | 32    | 6.09E–32      |
| GOTERM_BP_DIRECT | GO:0038096–Fc-gamma receptor signaling pathway involved in phagocytosis | 31    | 5.91E–25      |
| GOTERM_BP_DIRECT | GO:0068998–receptor-mediated endocytosis                            | 35    | 3.11E–24      |
| GOTERM_BP_DIRECT | GO:0006955–immune response                                          | 48    | 1.50E–23      |
| GOTERM_BP_DIRECT | GO:0050776–regulation of immune response                             | 33    | 1.25E–22      |
| GOTERM_BP_DIRECT | GO:002562–blood microparticle                                        | 25    | 2.73E–16      |
| GOTERM_CC_DIRECT | GO:0005576–extracellular region                                      | 77    | 1.41E–15      |
| GOTERM_CC_DIRECT | GO:0005806–plasma membrane                                           | 115   | 1.43E–07      |
| GOTERM_CC_DIRECT | GO:0042571–immunoglobulin complex, circulating                       | 7     | 6.73E–07      |
| GOTERM_CC_DIRECT | GO:0098997–external side of plasma membrane                          | 16    | 6.64E–06      |
| GOTERM_CC_DIRECT | GO:0005615–extracellular space                                       | 45    | 6.46E–05      |
| GOTERM_MF_DIRECT | GO:0003823–antigen binding                                           | 36    | 5.18E–36      |
| GOTERM_MF_DIRECT | GO:004252–serine-type endopeptidase activity                          | 37    | 1.70E–22      |
| GOTERM_MF_DIRECT | GO:003987–immunoglobulin receptor binding                            | 9     | 9.40E–09      |
| GOTERM_MF_DIRECT | GO:0001730–2′-5′-oligoadenylate synthetase activity                   | 4     | 2.09E–05      |
| GOTERM_MF_DIRECT | GO:004908–interleukin-1 receptor activity                             | 4     | 1.76E–04      |
| GOTERM_MF_DIRECT | GO:0003824–catalytic activity                                        | 9     | 0.017706181    |
FIGURE 5 Reactome analysis of HL-DEGs

TABLE 3 Reactome analysis of hearing loss differentially expressed genes

| GOID                | GO term                                           | p value | Nr. genes |
|---------------------|--------------------------------------------------|---------|-----------|
| R-HSA:1280215       | Cytokine signaling in immune system              | 0.00    | 40.00     |
| R-HSA:6785807       | Interleukin-4 and interleukin-13 signaling       | 0.04    | 8.00      |
| R-HSA:6798695       | Neutrophil degranulation                         | 0.00    | 25.00     |
| R-HSA:69205         | G1/S-specific transcription                      | 0.01    | 5.00      |
| R-HSA:2168880       | Scavenging of heme from plasma                   | 0.00    | 4.00      |
| R-HSA:2173782       | Binding and uptake of ligands by scavenger receptors | 0.05    | 5.00      |
| R-HSA:1169410       | Antiviral mechanism by IFN-stimulated genes      | 0.00    | 10.00     |
| R-HSA:8983711       | OAS antiviral response                            | 0.00    | 4.00      |
| R-HSA:909733        | Interferon alpha/beta signaling                  | 0.00    | 11.00     |
| R-HSA:913531        | Interferon signaling                             | 0.00    | 17.00     |
still need to be explored. There are human immune system-related cells in the cochlea, and these cells reflect noise stimulation. The number of immune cells increases with the increase of noise intensity. Compared with the control cochlea, the number of B cells, T cells, macrophages, NK cells and neutrophils in the noise-exposed cochlea increased significantly. This indicates that the immune mechanism plays a role in cochlear cells and is related to human hearing.33

LTF is considered to be a gene closely related to the prognosis of cancer patients and plays an important role in tumors. LTF can promote the apoptosis of intestinal cancer cells. In our research, we found that the expression of LTF in patients with deafness increases, and LTF is enriched in apoptosis-related BPs. Therefore, we predict that LTF may affect hair cell apoptosis and promote the occurrence and development of deafness.34 Orosomucoid 2 (ORM2) is an important glycoprotein, mainly biosynthesized and secreted by liver cells. Zhang's research team found that ORM2 can significantly increase the apoptosis of intestinal cancer cells.35 Based on our research results, we predict that ORM2 may affect hearing loss by affecting hair cell apoptosis. Transcobalamin I (TCN1) is a vitamin B12 (cobalamin) binding protein that transports cobalamin from the stomach to the intestine. It plays various roles in maintaining the basic functions of cell proliferation and metabolism, especially in hematopoiesis and nervous system.36,37 Liu's research team found through bioinformatics that high expression of TCN1 may play a role in the apoptotic pathway of colorectal cancer. Analysis of our sequencing results found that TCN1 is highly expressed in patients with deafness. We speculate that TCN1 may be involved in hair cell apoptosis and affect deafness.38 MMP8 has cancer-promoting and anti-cancer properties. Our analysis found that the expression of MMP8 increased in patients with deafness, and we predicted that MMP8 may affect the deafness by affecting the development process.39 FOLR3 is believed to play an important role in the transport of folic acid from the maternal intestinal lumen to the developing embryo. Abnormal expression of these genes may affect the availability of folic acid and lead to the risk of neural tube defects. Our analysis found that the expression of FOLR3 is increased in deaf patients, and we predict that FOLR3 may affect embryonic development and thus affect deafness.40 Among the drugs selected, we found a common drug, vitamin B12. Previous studies have shown that there is a relationship between vitamin B12 and cochlear health. Through bioinformatics analysis, we found that the drug corresponding to TCN1 is vitamin B12. Therefore, we predict that vitamin B12 may affect TCN1 and then affect hearing.41 The role of other drugs in deafness needs to be further explored by researchers.

In short, we obtained genomic information based on RNA-Seq of clinical samples, looking for biomarkers that may be involved in the process of hearing loss, and then predicting drugs for these notable biomarkers. These notable biomarkers and drugs can be used as new indicators for the diagnosis and treatment of hearing loss patients.

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CONFLICT OF INTEREST
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

TABLE 4 Drug-gene interaction analysis of MCODE genes

| Search term | Match_term | Match_type | Drug                  | Interaction_types | Sources              |
|-------------|------------|------------|-----------------------|-------------------|----------------------|
| MMP8        | MMP8       | Definite   | DOXYCYCLINE           | inhibitor         | Chembl interactions  |
| MMP8        | MMP8       | Definite   | COLLAGENASE           |                   |                      |
| MMP8        | MMP8       | Definite   | HISTOLYTICUM          |                   |                      |
| MMP8        | MMP8       | Definite   | DOXYCYCLINE CALCIUM   | inhibitor         | Chembl interactions  |
| LTF          | LTF        | Definite   | PARECOXIB             |                   | TTD                  |
| LTF          | LTF        | Definite   | BACITRACIN            |                   | NCI                  |
| ORM2         | ORM2       | Definite   | DOCETAXEL             |                   | PharmGKB             |
| ORM2         | ORM2       | Definite   | PEMETREXED            |                   | PharmGKB             |
| TCN1         | TCN1       | Definite   | CYANOCOBALAMIN        |                   | NCI                  |
AUTHORS CONTRIBUTIONS
All authors performed data analysis and manuscript writing. Min Lei designed this article and analyzed the data. Zhang Dongdong investigated and studied the background knowledge of this article, and participated in the experiment of collecting patient specimens. Yixin Sun is responsible for analyzing the data obtained. Cong Zou, Yue Wang, Yongjun Hong, and Yanchao Jiao were responsible for visualizing sequencing data. Chengfu Cai made the final review and correction of this article. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The data used to support the findings of this study are included within the article. All raw data can be obtained from the author.

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