Received: 24 September 2021 | Revised: 7 October 2021 | Accepted: 8 October 2021

DOI: 10.1002/jcla.24065

RESEARCH ARTICLE

WILEY

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

Min Lei[1] | **Dongdong Zhang**[1] | **Yixin Sun**[2] | **Cong Zou**[1] | **Yue Wang**[1] | **Yongjun Hong**[1] | **Yanchao Jiao**[1] | **Chengfu Cai**[1,3,4]

[1]Department of Otorhinolaryngology - Head and Neck Surgery, School of Medicine, Zhongshan Hospital, Xiamen University, Xiamen, China

[2]School of Medicine, Xiamen University, Xiamen, China

[3]Department of Otorhinolaryngology Head and Neck Surgery, Teaching Hospital of Fujian Medical University, Xiamen, China

[4]Department of Otorhinolaryngology - Head and Neck Surgery, Xiamen University, Xiamen, China

Correspondence
Chengfu Cai, Department of Otorhinolaryngology - Head and Neck Surgery, Zhongshan Hospital, School of Medicine, Xiamen University, Xiamen 361003, China.
Email: yscc96@126.com

Funding information
This work was supported by Natural Science Fund of Fujian Province (NO. 2018J01375), the Natural Science Foundation of Fujian Science and Technology Department (grant number 2020J02060), and the Key Medical and Health Project of Xiamen Science and Technology Bureau (grant number 3502Z20204009)

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 Clue-GO. 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, IL5RA, SMPD3, IGHV1-2, IGLV3-9, RHOXF1P1, 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

Min Lei, Dongdong Zhang, and Yixin Sun contribute equally to this article.

1 | 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.[1] 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.[2] The impact of hearing loss on patients is permanent; as time goes by, the psychological quality of patients will change.[3] There are also reports that hearing impairment is closely related to Alzheimer's; thus, hearing impairment is more and more worthy of attention.[4] There are many reasons for deafness. The common cause is drug-induced deafness. The drug streptomycin used to treat tuberculosis can cause deafness.[5] 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.[6,7] 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.[8] 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.[9–11] Tumor necrosis factor will participate in the biological process (BP) of cell apoptosis and play an important role.[12] 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.[13] Some people have done research in the diagnosis and treatment of intracranial aneurysms through peripheral blood mononuclear cell sequencing.[14] 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.[15] 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.

2 | MATERIALS AND METHODS

2.1 | 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.

2.2 | 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.

2.3 | RNA-seq and bioinformatics

After the RNA extraction test is qualified, RNA sequencing (RNA-Seq) is performed. Select Illumina NovaSeq 6000 for sequencing platform.[16] 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 $p < 0.05$, |LogFC| ≥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.[17–19] 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.[20] 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.[21,22]

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.[23] 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.[24] We upload the results from the STRING database to the Cytoscape database for analysis. We choose the MCODE[25] 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.[26–28] 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".[29] 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.

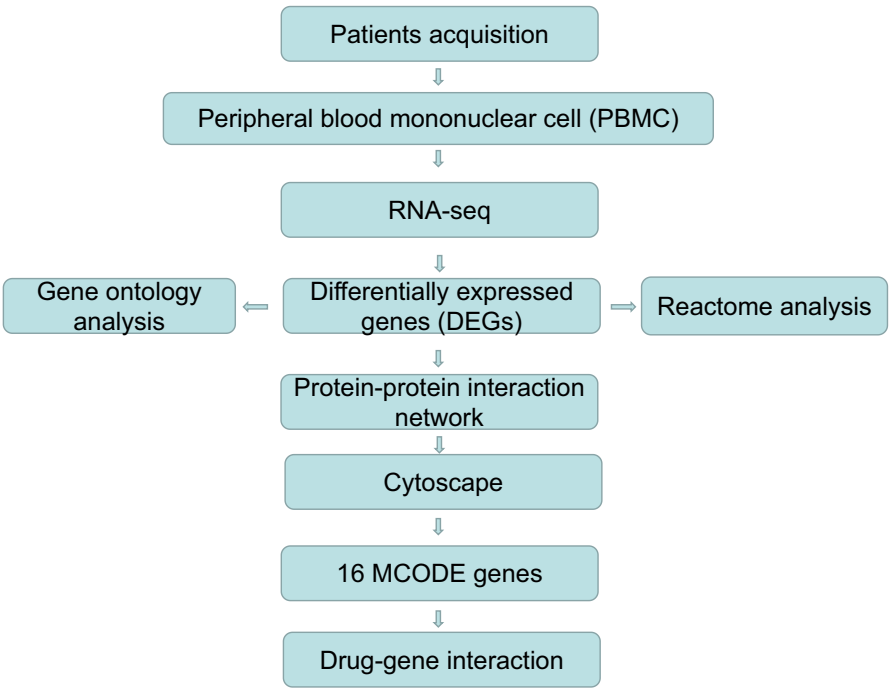

FIGURE 1 Flow chart of this article

3 | 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 ($p < 0.05$,and $|\log2FC| \geq 0.5$), 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

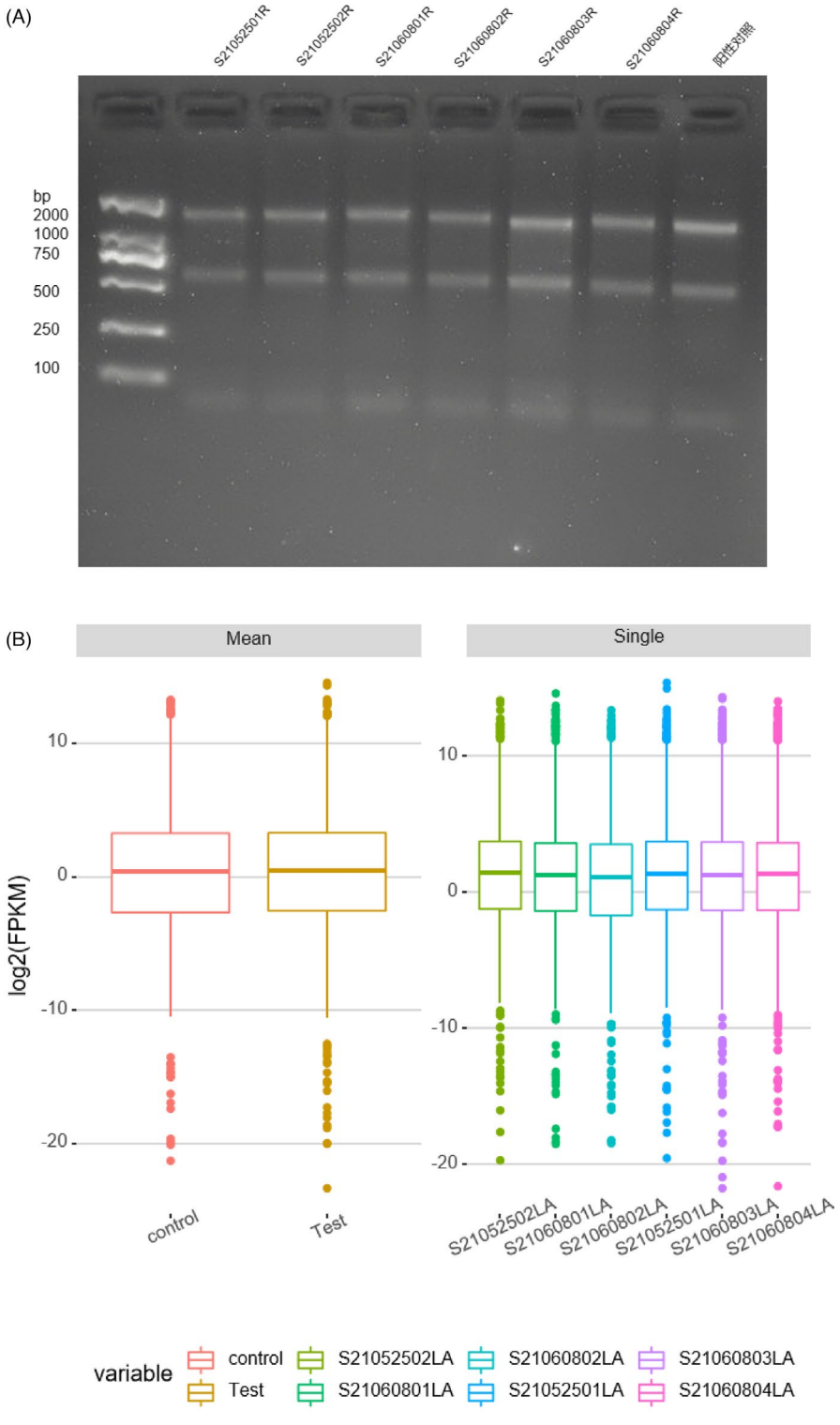

FIGURE 2 RNA sample detection and sequencing data standardization. (A) Agarose gel electrophoresis of RNA in PBMC cells. (B) RNA-Seq data reads count FPKM standardization

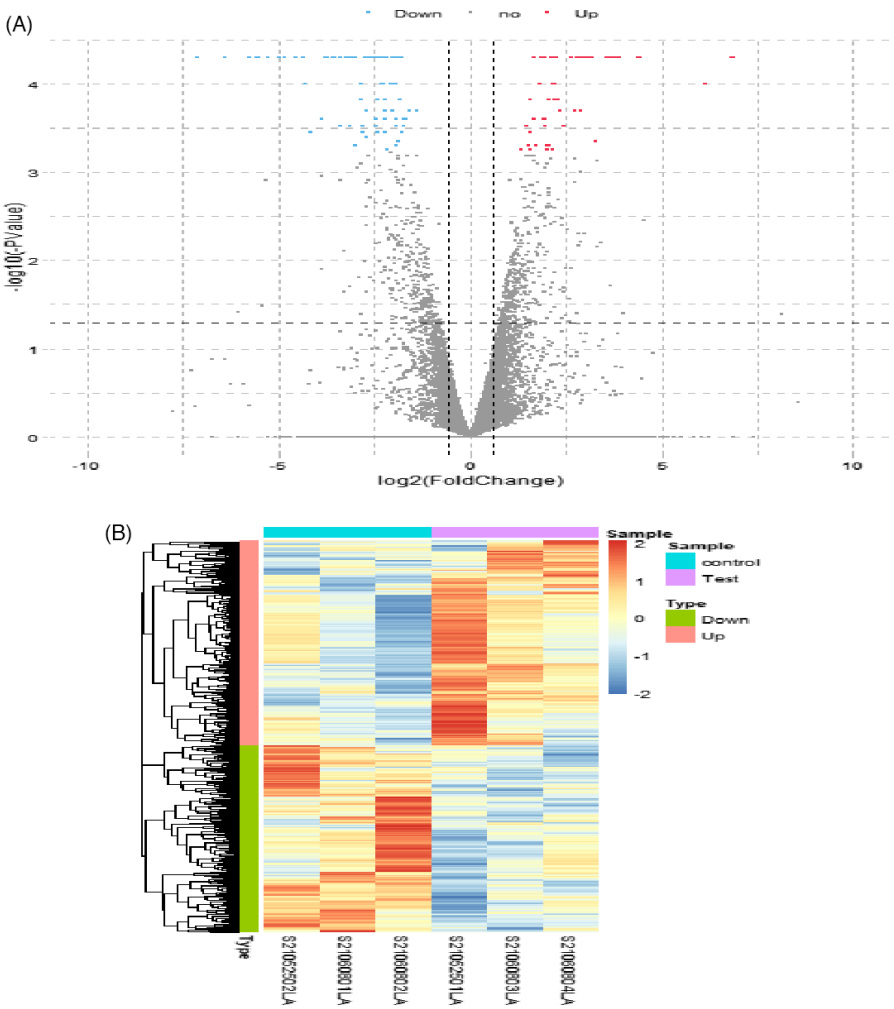

FIGURE 3 Volcano plot and heatmap of the hearing loss differentially expressed genes (HL-DEGs), between hearing loss patients and healthy subjects. (A) Volcano plot of HL-DEGs. Red means up-regulated HL-DEGs; Blue means down-regulated HL-_DEGs; Gray means no different. (B) Heatmap of 436 up-regulated HL-DEGs and 413 down-regulated HL-DEGs between healthy subjects and hearing loss patients. Green means up-regulated HL-DEGs; Pink means down-regulated HL-_DEGs

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 |log2FC| ≥1.5 (Table 1).

3.3 | Gene ontology analysis

In order to outline the GO of DEG, we used DAVID website to visualize functionalannotation.AsshowninFigure4andTable2,GOanalysisshows that DEGs are most significantly enriched in GO:0006958~complement activation, classical pathway; GO:0006956~complement activation; GO:0038096~Fc-gamma receptor signaling pathway involved in phagocytosis; GO:0006898~receptor-mediated endocytosis; GO:0006955~immune response; and GO:0050776~regulation of immune response in BP process. DEGs are most significantly enriched in GO:0072562~blood microparticle; GO:0005576~extracellular region; GO:0005886~plasma membrane; GO:0042571~immunoglobulin complex, circulating; GO:0009897~external side of plasma membrane; and GO:0005615~extracellular space in cellular component process. DEGs are most significantly enriched in GO:0003823~antigen binding, GO:0004252~serine-type endopeptidase activity, GO:0034987~immunoglobulin receptor binding, GO:0001730~2′-5′-oligoadenylate synthetase activity, GO:0004908~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).

TABLE 1 The differentially expressed genes in hearing loss patients and normal healthy subjects

DEGs	Gene names									
Up-genes	AL008707.1	DDX11L10	OLFM4	ABCA13	PXYLP1	CPM	CYBRD1	ORM1	KIF27	GRB10
	SDR42E1P5	FOLR3	AC007271.1	MMP9	DEFA4	HECW2	CHI3L1	EFHC2	LGALSL	PFKFB2
	BX005040.1	FGF13	ORM2	LINC02207	SIK1	SLC38A11	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	CCNJL	CNTNAP3	FLT3	NEDD4	B3GNT8
	AC016957.1	TMTC1	COL9A2	LINC02289	GPR141	AP006222.1	KIAA1324	RASGEF1A	PADI4	CEACAM6
	U2AF1L5	MMP8	LTF	AL163541.1	DUSP13	PHF24	AL512306.3	SULT1B1	TCN1	ATP2C2
	XIST	VSIG4	IL1R2	BMX	ERICH6-AS1	TPST1	PCBP3	CLEC12B	NQO2	TSC22D3
	DAAM2	CRISP3	LCN2	ST6GALNAC3	AOC4P	LRRC6	RAB36	CD177	TCL1A	CD163
	ADAMTS2	ARG1	ARMC12	IL18R1	SEPTIN7P8	ETV5	MGAM	MEF2C-AS1	MRVI1	ZNF66
	ACSS3	FXYD1	EXOG	TOB1	RPGRIP1	TP53I11	PHC2	NLRP12	MKNK1	MACIR
	LINC01093	HMOX1	DMRTC2	AC005392.2	KBTBD7	MFN2	CSF3	LINC01220	KATNBL1	IRS2
	GIHCG	CES1	ARL11	NSUN7	ARHGEF40	FRAT1	TSPAN2	CATSPERG	CD300LD	CDC42BPG
	ARHGEF17	BPI	PGLYRP1	BEND7	TNS1	KIF3C	SORT1	IL1R1	LINC00672	SDHAF3
	MIR223HG	LINC00671	LINC02649	SAP30	SELENOP	GAS7	FAM71F2	SLC37A3	ABHD6	APMAP
	HBA1	MINCR	FKBP5	PI3	AL035661.1	OSBPL1A	SCNN1D	SLPI	CALCRL	AMPD3
	ZNF429	HROB	BMP2	AL391807.1	GAREM2	SCN9A	PTGES	CCDC30	C4A	LINC00659
	ZNF282	FHIT	C20orf197	HBA2	CCIN	AP002495.1				
Down-genes	ALOX15	GCSAML	ADORA3	MX1	RAB44	CHPF	SERPING1	NBPF1	IGLC2	IGHV2-5
	PRSS33	ISG15	BIRC5	IGLV1-47	FFAR3	IGKV1-8	EPSTI1	ZNF823	PLK1	IGHV3-43
	IL5RA	PMP22	AC139495.2	STK33	GRHL1	USP18	IGHV3-72	PIK3R6	NEURL1	IGKV1-5
	SMPD3	AC111000.4	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	EIF4BP6	IGHG4	BUB1B	PKD1P5	ALDH8A1	TOP2A
	RHOXF1P1	CMPK2	NEU4	LIN7B	MICA	IGLL5	GIPR	DDX60	ITM2C	IGKV2-28
	CACNG6	IFIT3	HDC	SCAMP5	OAS1	TROAP	IGHV3-21	IGHV4-39	CNR2	UBE2C
	MYOM2	OAS3	IGLV2-18	XAF1	IGKV1-39	ADGRG5	PKMYT1	IGHV3-11	IFIT5	GALNT6
	RSAD2	TNNT1	TNFRSF17	IGLV9-49	GBP1	MGAT3	CDKN3	ZFP1	AGRN	CD36
	ADGRE4P	KCNH2	IGHV2-70	KNL1	IGHV1-69D	IGLV3-25	PLD4	IGLV2-8	WASF1	CIT
	IGLV3-1	IGLV1-36	IGKV1-6	PLAAT5	IGKV1-12	BACE2	MZB1	CDT1	HRK	ANLN
	CLC	IGHV3-49	TMEM176B	IGKV1-17	SOCS2	TPPP3	SEPTIN10	EPN2	CLEC10A	TRDC
	IDO1	IGHV3-48	HERC5	TMEM176A	AC073332.1	IL1RL1	IGKV2D-30	IGHV6-1	IGKV3-15	IGKV1-16

(Continues)

TABLE 1 (Continued)

DEGs	Gene names										
	IGHG1	IGLV1-44	LY6E	FGFR2	TK1	OASL	ANKRD36BP2	IGHV3-33	PTPRS	HJURP	
	IGHV1-24	SYNGAP1	IGHV3-73	IGHV4-34	FCER1A	CENPV	IGHV4-59	IGHV3-66	ZNF287	PCDH1	
	IGHV4-61	SPNS3	IGKV3D-15	IFI44	PTGER3	GBP5	ZMAT4	IGKV4-1	ATP2A1	GOLM1	
	IGKV2-24	IFI6	IGKV1-33	LGALS12	OAS2	IGHV3-13	IGKV1-9	ORC1	TXNDC5	SLC7A8	
	SIGLEC1	PDK4	SEMA7A	ADTRP	FRRS1	XCL1	VSTM1	IGLC3	LTC4S	ZIK1	
	IGLV3-21	NDFIP2	CPA3	GATA2	NPIPB11	MS4A2	PARP14	ACSM3	UBE2L6	BCAR3	
	HRH4	CCR3	CENPM	NRARP	SLC16A14	KLK1	CDC20	NRG1	JCHAIN	CD38	
	SLC29A1	IGKV3-7	GATA1	IGLV1-51	SPATS2L	AC020910.5	IGLV2-23	DHCR24	ACOT11	PI4KAP1	
	TTC7B	SLC45A3	MS4A4A	IGKV1D-39	RRM2	CDC45	KIF11	UHRF1			

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.[30] 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.[31] 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.[32] 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

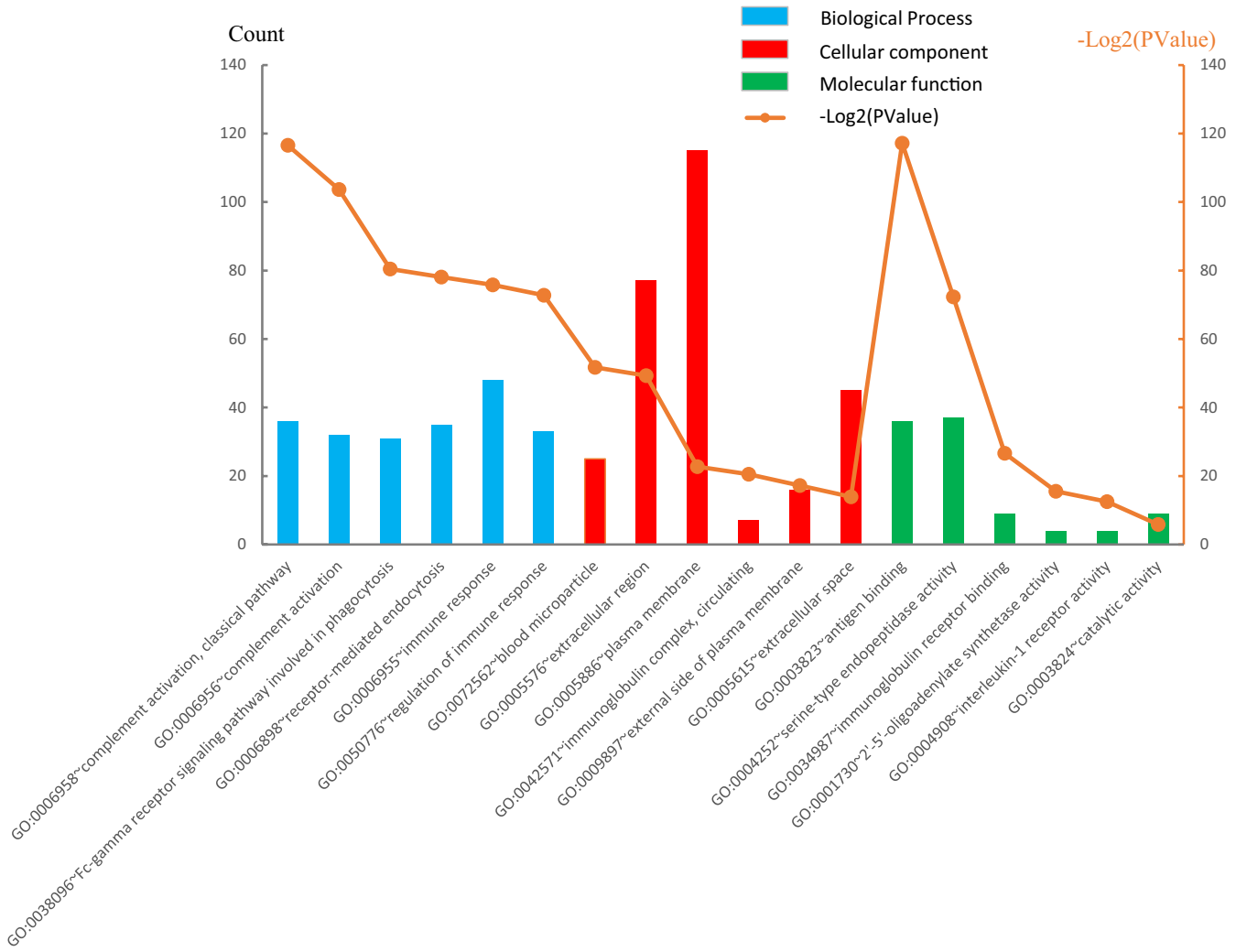

FIGURE 4 Gene ontology (GO) analysis of HL-DEGs. The enriched GO terms in the biological process (BP), cellular component (CC), molecular function (MF)

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

Category	Term	Count	*p* value
GOTERM_BP_DIRECT	GO:0006958~complement activation, classical pathway	36	8.13E−36
GOTERM_BP_DIRECT	GO:0006956~complement activation	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:0006898~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_CC_DIRECT	GO:0072562~blood microparticle	25	2.73E−16
GOTERM_CC_DIRECT	GO:0005576~extracellular region	77	1.41E−15
GOTERM_CC_DIRECT	GO:0005886~plasma membrane	115	1.43E−07
GOTERM_CC_DIRECT	GO:0042571~immunoglobulin complex, circulating	7	6.73E−07
GOTERM_CC_DIRECT	GO:0009897~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:0004252~serine-type endopeptidase activity	37	1.70E−22
GOTERM_MF_DIRECT	GO:0034987~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:0004908~interleukin−1 receptor activity	4	1.76E−04
GOTERM_MF_DIRECT	GO:0003824~catalytic activity	9	0.017706181

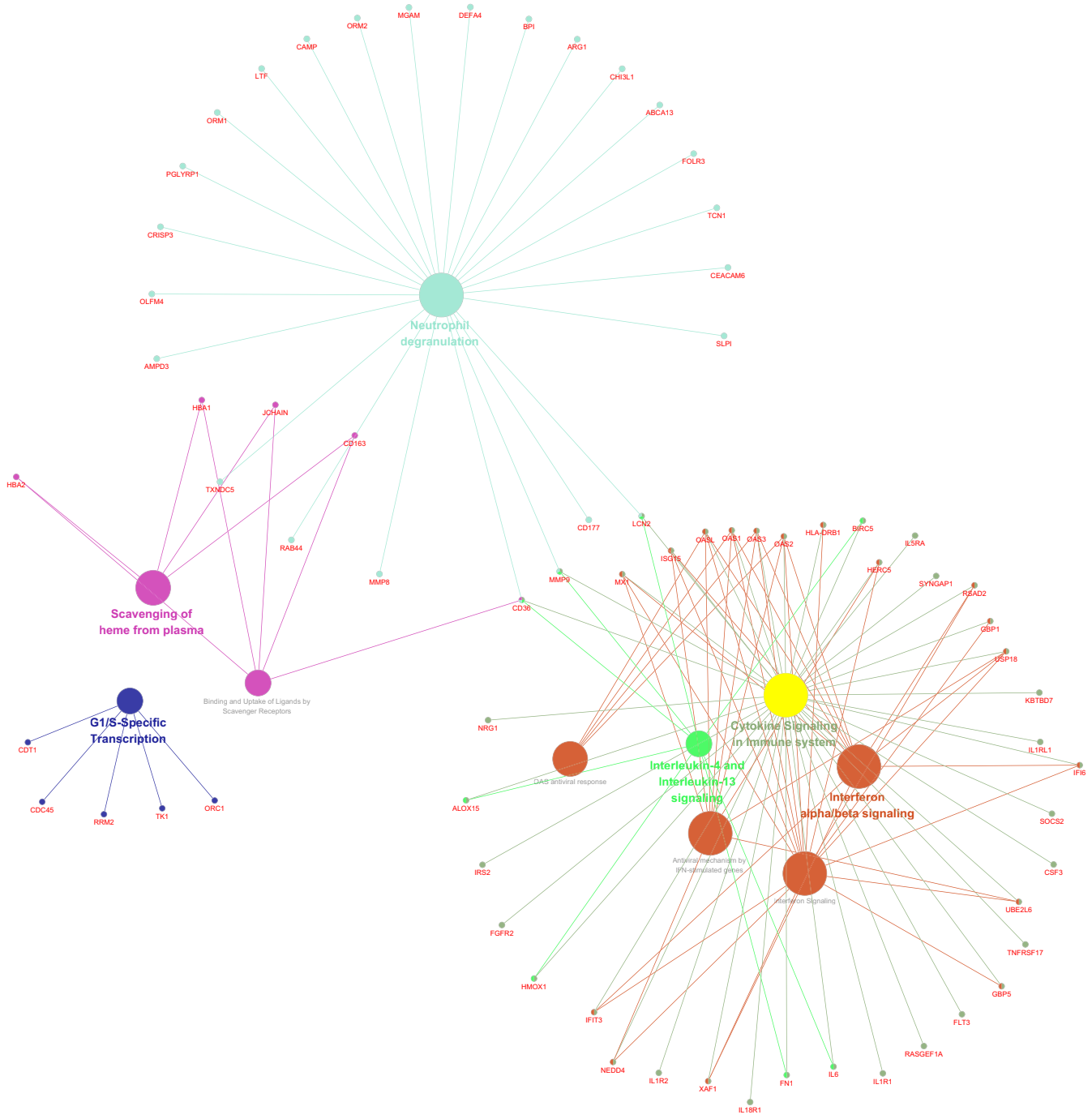

FIGURE 5 Reactome analysis of HL-DEGs

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

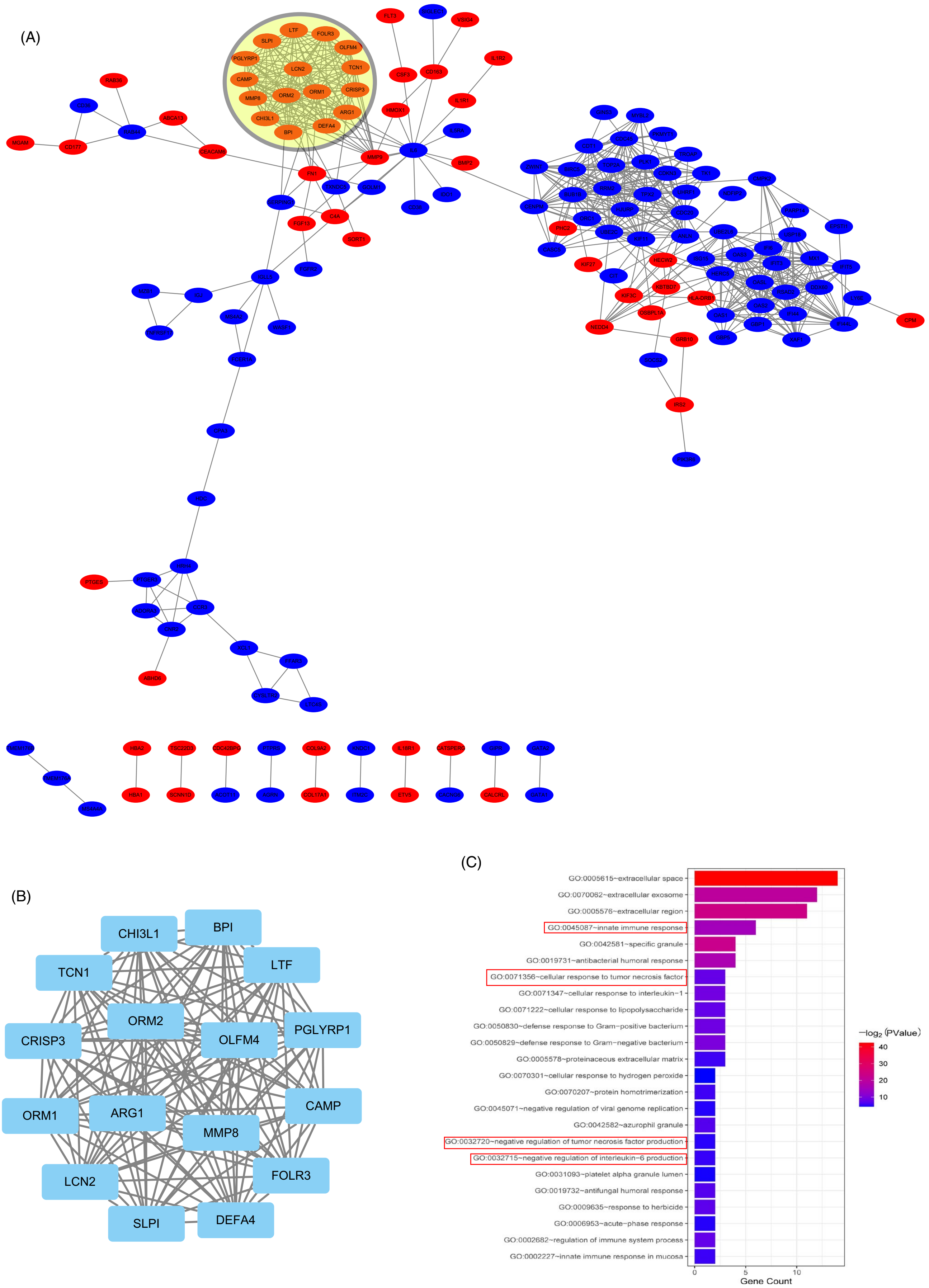
(A)
(B)
CHI3L1
BPI
TCN1
LTF
ORM2
PGLYRP1
CRISP3
OLFM4
CAMP
ORM1
ARG1
MMP8
FOLR3
LCN2
SLPI
DEFA4
(C)
GO:0005615~extracellular space
GO:0070062~extracellular exosome
GO:0005576~extracellular region
GO:0045087~innate immune response
GO:0042581~specific granule
GO:0019731~antibacterial humoral response
GO:0071356~cellular response to tumor necrosis factor
GO:0071347~cellular response to interleukin−1
GO:0071222~cellular response to lipopolysaccharide
GO:0050830~defense response to Gram−positive bacterium
GO:0050829~defense response to Gram−negative bacterium
GO:0005578~proteinaceous extracellular matrix
GO:0070301~cellular response to hydrogen peroxide
GO:0070207~protein homotrimerization
GO:0045071~negative regulation of viral genome replication
GO:0042582~azurophil granule
GO:0032720~negative regulation of tumor necrosis factor production
GO:0032715~negative regulation of interleukin−6 production
GO:0031093~platelet alpha granule lumen
GO:0019732~antifungal humoral response
GO:0009635~response to herbicide
GO:0006953~acute−phase response
GO:0002682~regulation of immune system process
GO:0002227~innate immune response in mucosa
0
5
10
Gene Count
−log2 (PValue)
40
30
20
10

FIGURE 6 Protein-protein interaction (PPI) network of HL-DEGs and MCODE analysis. (A) PPI network analysis of differential genes. (B) The result of MCODE analysis is 16 hub genes. (C) Biological analysis of MCODE gene

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 CLOSTRIDIUM HISTOLYTICUM	-	Tdg clinical trial
MMP8	MMP8	Definite	DOXYCYCLINE	inhibitor	Guide to pharmacology
MMP8	MMP8	Definite	DOXYCYCLINE CALCIUM	inhibitor	Chembl interactions
LTF	LTF	Definite	PARECOXIB	-	TTD
LTF	LTF	Definite	BACITRACIN	-	NCI
ORM2	ORM2	Definite	DOCETAXEL	-	PharmGKB
FOLR3	FOLR3	Definite	PEMETREXED	-	PharmGKB
TCN1	TCN1	Definite	CYANOCOBALAMIN	-	NCI

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 TNC1 may play a role in the apoptotic pathway of colorectal cancer. Analysis of our sequencing results found that TNC1 is highly expressed in patients with deafness. We speculate that TNC1 may be involved in hair cell apoptosis and affect deafness.[38] MMP8 is a member of the matrix metalloproteinase family. It is involved in the breakdown of extracellular matrix in embryonic development, tissue remodeling, and certain diseases. 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.

ACKNOWLEDGMENTS

Thanks to Amo Gene (Xiamen) Biotechnology Co., Ltd. for supporting the sequencing of this manuscript sample.

CONFLICT OF INTEREST

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

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.

REFERENCES

1. Bowl MR, Dawson SJ. Age-related hearing loss. *Cold Spring Harb Perspect Med*. 2019;9(8):a033217.
2. Marazita ML, Ploughman LM, Rawlings B, Remington E, Arnos KS, Nance WE. Genetic epidemiological studies of early-onset deafness in the U.S. school-age population. *Am J Med Genet*. 1993;46(5):486-491.
3. Michels TC, Duffy MT, Rogers DJ. Hearing loss in adults: differential diagnosis and treatment. *Am Fam Physician*. 2019;100(2):98-108.
4. Ralli M, Gilardi A, Stadio AD, et al. Hearing loss and Alzheimer's disease: a review. *Int Tinnitus J*. 2019;23(2):79-85.
5. Davies RA. Audiometry and other hearing tests. *Handb Clin Neurol*. 2016;137:157-176.
6. Sarin R, Behera D, Khanna A, Singh V, Narang P, Deepak TS. Second-line injectable induced ototoxicity in drug resistant tuberculosis: a systematic review of Indian studies. *Indian J Tuberc*. 2019;66(2):279-287.
7. Huang Q, Tang J. Age-related hearing loss or presbycusis. *Eur Arch Otorhinolaryngol*. 2010;267(8):1179-1191.
8. Hoffmann TJ, Keats BJ, Yoshikawa N, Schaefer C, Risch N, Lustig LR. A large genome-wide association study of age-related hearing impairment using electronic health records. *PLoS Genet*. 2016;12(10):e1006371.
9. Overholtzer M, Mailleux AA, Mouneimne G, et al. A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion. *Cell*. 2007;131(5):966-979.
10. Wu J, Ye J, Kong W, Zhang S, Zheng Y. Programmed cell death pathways in hearing loss: a review of apoptosis, autophagy and programmed necrosis. *Cell Prolif*. 2020;53(11):e12915.
11. Someya S, Prolla TA. Mitochondrial oxidative damage and apoptosis in age-related hearing loss. *Mech Ageing Dev*. 2010;131(7–8):480-486.
12. Chen NN, Wei F, Wang L, Cui S, Wan Y, Liu S. Tumor necrosis factor alpha induces neural stem cell apoptosis through activating p38 MAPK pathway. *Neurochem Res*. 2016;41(11):3052-3062.
13. Zararsiz G, Goksuluk D, Korkmaz S, et al. A comprehensive simulation study on classification of RNA-Seq data. *PLoS One*. 2017;12(8):e0182507.
14. Tutino VM, Zebraski HR, Rajabzadeh-Oghaz H, et al. Identification of circulating gene expression signatures of intracranial aneurysm in peripheral blood mononuclear cells. *Diagnostics (Basel)*. 2021;11(6):1092.
15. Li N, Zhou JN. Screening and validating the immune-related gene expression signatures in peripheral blood mononuclear cells of nonischaemic cardiomyopathy. *Math Biosci Eng*. 2020;17(3):2330-2347.
16. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics*. 2007;8(1):118-127.
17. Gene Ontology C. The gene ontology project in 2008. *Nucleic Acids Res*. 2008;36:D440-D444.
18. Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. The gene ontology consortium. *Nat Genet*. 2000;25(1):25-29.
19. Gene Ontology C. Gene ontology consortium: going forward. *Nucleic Acids Res*. 2015;43:D1049-D1056.
20. Dennis G Jr, Sherman BT, Hosack DA, et al. DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol*. 2003;4(5):P3.
21. Bindea G, Mlecnik B, Hackl H, et al. ClueGO: a cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics*. 2009;25(8):1091-1093.
22. Jassal B, Matthews L, Viteri G, et al. The reactome pathway knowledgebase. *Nucleic Acids Res*. 2020;48(D1):D498-D503.
23. von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P, Snel B. STRING: a database of predicted functional associations between proteins. *Nucleic Acids Res*. 2003;31(1):258-261.
24. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498-2504.
25. Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics*. 2003;4:2.
26. Cotto KC, Wagner AH, Feng YY, et al. DGIdb 3.0: a redesign and expansion of the drug-gene interaction database. *Nucleic Acids Res*. 2018;46(D1):D1068-D1073.
27. Griffith M, Griffith OL, Coffman AC, et al. DGIdb: mining the druggable genome. *Nat Methods*. 2013;10(12):1209-1210.
28. Wagner AH, Coffman AC, Ainscough BJ, et al. DGIdb 2.0: mining clinically relevant drug-gene interactions. *Nucleic Acids Res*. 2016;44(D1):D1036-D1044.
29. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47.
30. Lee JW, Bance ML. Hearing loss. *Pract Neurol*. 2019;19(1):28-35.
31. Cheng X, Ferrell JE Jr. Apoptosis propagates through the cytoplasm as trigger waves. *Science*. 2018;361(6402):607-612.
32. Op de Beeck K, Schacht J, Van Camp G. Apoptosis in acquired and genetic hearing impairment: the programmed death of the hair cell. *Hear Res*. 2011;281(1–2):18-27.
33. Rai V, Wood WB, Feng H, Schabla NM, Tu S, Zuo J. The immune response after noise damage in the cochlea is characterized by a heterogeneous mix of adaptive and innate immune celll. *Sci Rep*. 2020;10(1):15167.
34. Ni L, Yuan C, Zhang C, et al. Co-expression network analysis identified LTF in association with metastasis risk and prognosis in clear cell renal cell carcinoma. *Onco Targets Ther*. 2020;13:6975-6986.
35. Zhang X, Xiao Z, Liu X, et al. The potential role of ORM2 in the development of colorectal cancer. *PLoS One*. 2012;7(2):e31868.
36. Ray JG, Blom HJ. Vitamin B12 insufficiency and the risk of fetal neural tube defects. *QJM*. 2003;96(4):289-295.
37. Magnus EM. Cobalamin and unsaturated transcobalamin values in pernicious anaemia: relation to treatment. *Scand J Haematol*. 1986;36(5):457-465.
38. Liu GJ, Wang YJ, Yue M, et al. High expression of TCN1 is a negative prognostic biomarker and can predict neoadjuvant chemosensitivity of colon cancer. *Sci Rep*. 2020;10(1):11951.

39. Insodaite R, Liutkevicius V, Uloza V, Solovejute R, Smalinskiene A. Association between MMP8 gene polymorphisms and laryngeal squamous cell carcinoma. *Anticancer Res*. 2020;40(4):2003-2009.
40. Findley TO, Tenpenny JC, O'Byrne MR, et al. Mutations in folate transporter genes and risk for human myelomeningocele. *Am J Med Genet A*. 2017;173(11):2973-2984.
41. Karaer I, Akalin Y. Low vitamin B12 level and vitamin D level adversely affect on cochlear health in women. *Int J Vitam Nutr Res*. 2020;90(3–4):333-338.

How to cite this article: Lei M, Zhang D, Sun Y, et al. Web-based transcriptome analysis determines a sixteen-gene signature and associated drugs on hearing loss patients: A bioinformatics approach. *J Clin Lab Anal*. 2021;35:e24065. https://doi.org/10.1002/jcla.24065