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Original Article

Identification of mRNA expression profiles and their characterization in age-related hearing loss

Kanglun Jiang#, Tan Wang#, Xinsheng Huang, Li Gao*

Department of Otorhinolaryngology-Head and Neck Surgery, Zhongshan Hospital, Fudan University, Shanghai 200030, China

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Abstract

Age-related hearing loss (ARHL), is a pervasive health problem worldwide. ARHL seriously affects the quality of life and reportedly leads to social isolation and dementia in the elderly. ARHL is caused by the degeneration or disorders of cochlear hair cells and auditory neurons. Numerous studies have verified that genetic factors contributed to this impairment, however, the mechanism behind remains unclear. In this study, we analyzed an mRNA expression dataset (GSE49543) from the GEO database. Differentially expressed genes (DEGs) between young control mice and presbycusis mice were analyzed using limma in R and weighted gene co-expression network analysis (WGCNA) methods. Functional enrichment analyses of the DEGs were conducted with the clusterProfiler R package and the results were visualized using ggplot2 R package. The STRING database was used for the construction of the protein-protein interaction (PPI) network of the screened DEGs. Two machine learning algorithms LASSO and SVM-RFE were used to screen the hub genes. We identified 54 DEGs in presbycusis using limma and WGCNA. DEGs were associated with the synaptic vesicle cycle, distal axon, neurotransmitter transmembrane transporter activity in GO analysis, and alcoholic liver disease, pertussis, lysosome pathway according to KEGG analyses. PPI network analysis identified three significant modules. Five hub genes (CLEC4D, MS4A7, CTSS, LAPTM5, ALOX5AP) were screened by LASSO and SVM-RFE. These hub genes were highly expressed in presbycusis mice compared with young control mice. We screened DEGs and identified hub genes involved in ARHL development, which might provide novel clues to understanding the molecular basis of ARHL.

Keywords: Age-related hearing loss, Bioinformatics, Differentially expressed genes, Machine learning.

1. Introduction

Age-related hearing loss (ARHL), or presbycusis, refers to progressive, bilateral symmetrical, sensorineural hearing loss mainly at higher frequency, and is the most prevalent sensory impairment in the aging population [1]. It is estimated by the WHO that around there will be over 500 million ARHL patients by 2025 [2]. Although ARHL is not life-threatening, it can affect the communication of patients and lead to psychological and medical issues, such as depression and cognitive decline, and patient social activity and life quality are decreased [3-5]. The treatment with cochlear implants or hearing aids is the main option for ARHL patients currently. Therefore, it is essential to deepen the understanding of the molecular basis and explore promising therapeutic targets for ARHL.

Hair cell loss, spiral ganglion neuron degeneration and stria vascularis atrophy are the primary causes of ARHL [6]. Based on the sites of abnormality, the ARHL is divided into four categories including sensory presbycusis, neural presbycusis, metabolic presbycusis, and conductive cochlear loss [7]. The hearing aids can replace the amplification and compression that the outer hair cells, and the sensory-motor cells of the cochlea fail to provide. Thus, patient perception of weak sounds can be improved in quiet conditions. However, the application of hearing aids is still limited by background noise conditions, degeneration of spiral ganglion neurons in the elderly and the high costs [8]. Thus, exploration of the potential therapeutic targets might contribute to ARHL prevention and treatment.

With the development of bioinformatics and gene chips, bioinformatics analysis has attracted increasing attention in the biomedical field for the potential of exploring biomarkers in diverse diseases. Previous studies have explored the potential biomarkers and potential targets of ARHL. For example, Liu et al. have proposed that hair cell aging is related to autophagy, and oxidative stress, and Sod1, Sirt6, Jund, and Cbx3 are related targets for ARHL therapy [9]. Peng et al. have reported that four aging-related genes (Ywhag, Mapre2, Fgf1, Acss2) are differentially expressed in ARHL and can be used for the construction of the prognostic model [10]. However, the molecular mechanisms in ARHL are not fully understood, and more efforts should be made to provide basis for the therapeutic intervention.

Our study intended to elucidate the expression pattern of DEGs and identify hub genes involved in ARHL based on bioinformatics analysis. We explored the gene expression pattern in ARHL in GSE49543 dataset using

* Corresponding author.
E-mail address: drgaoli@126.com (L. Gao).
# These authors contributed equally
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the R limma package combined with WGCNA. The PPI network of the DEGs was constructed and the machine learning algorithms were applied for the selection of the hub genes. The findings of our work might provide clues for understanding the molecular basis of ARHL.

2. Materials and Methods

2.1. Data collection and processing

GeneChip data were downloaded from the GSE49543 dataset [11] from GEO database. The GSE49543 dataset contains 41 samples, with 9 young control CBA mouse cochlea samples and 32 ARHL mouse cochlea samples, and the annotation platform was GPL339 [MOE430A] Affymetrix Mouse Expression 430A Array.

2.2. Identification of differentially expressed genes (DEGs)

The DEGs between ARHL and young mice were screened using the limma package in R (version 4.2.1) in GSE49543 dataset and with |logFold Change (FC)| > 0.7, and P value < 0.05 as the threshold value. The statistical significance was calculated using Wilcoxon rank sum test. The results were visualized as heatmaps and volcano plots using the ggplot2 package.

2.3. Weighted gene co-expression network analysis (WGCNA)

WGCNA was used for the exploration of gene expression correlation [12]. The co-expression network of all genes in the GSE49543 dataset was constructed, and for the estimation of the network connection, adjacency was evaluated using the "soft" thresholding power (β) derived from co-expression similarity. Then the weighted adjacency matrix was converted into a topological overlap matrix, and its clustering tree structure is built using a hierarchical clustering method. Gene modules were identified by classifying genes with same expression pattern based on weighted correlation coefficient. The gene modules were represented by different clustering tree branches, and indicated by different colors. The modules with significant differences between the young-control and presbycusis group were then confirmed and genes in the differential modules were obtained.

The genes in the MEyellowgreen and MElightcyan modules and DEGs identified using limma in GSE49543 dataset were intersected and visilized by Venn diagram, and finally, the DEGs between presbycusis and young mice were screened for further analysis.

2.4. Functional enrichment analyses

Gene Ontology (GO) and Kyoto Genome Encyclopedia (KEGG) enrichment analyses of the screened DEGs were performed for the exploration of the biological functions and signalings implicated in ARHL progression using the clusterProfiler R package and ggplot2 R package.

2.5. Hub gene identification by machine learning

For the analyses of screened DEGs in presbycusis, two machine learning algorithms, Least Absolute Shrinkage and Selection Operator (LASSO) [13] using glmnet package and Support Vector Machine-Recursive Feature Elimination (SVM-RFE) [14] using e1071 package were performed. Finally, the results of the two machine learning algorithms were intersected and shown by Venn diagram.

2.6. Construction of the PPI networks

The STRING database (https://string-db.org/) was applied for the exploration of the interaction between target proteins and constructing the PPI networks. Cytoscape (version 3.7.2) was used for the evaluation and visualization of the networks. The clustering analysis was conducted using the MCODE function.

3. Results

3.1. Identification of DEGs in ARHL

The RMA-normalized microarray data in aging CBA mice were downloaded from the GSE49543 dataset. Totally 129 DEGs were in presbycusis mice compared with young control mice. The top 100 DEGs between ARHL mouse cochlea samples and young control samples were identified and shown in the heatmap (Fig. 1A). Fig. 1B presented the volcano plot of the DEGs screened by limma. For WGCNA network construction, totally 41 samples in the GSE49543 dataset were included, and we identified 17 modules on the thresholding power of β = 6 (Fig. 1C). We found that the yellow-green (coefficient=0.83, p=1e-04) and light-cyan modules (coefficient=-0.61, p=0.02) showed the highest correlation with ARHL, and were used as the pivotal modules for further analysis (Fig. 1D). We then evaluated the correlation between module membership and gene significance for age in the two selected modules. The results showed a positive correlation between the module membership in yellow-green modules and gene significance for age (Cor=0.7, p=3.9e-09) (Fig. 1E), and also between the module membership in light-

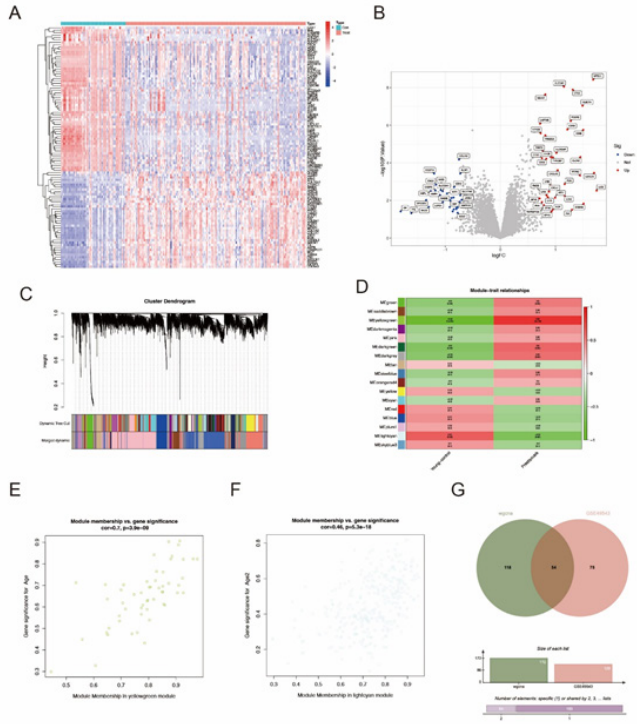

Fig. 1. Identification of DEGs in presbycusis. (A) Heatmap and (B) volcano plot of the differentially expressed genes in presbycusis in GSE49543 dataset. (C) WGCNA cluster dendrogram. Different colors represented gene co-expression modules under the gene tree. (D) Heatmap of the relationships between modules and presbycusis. Gene correlation scatter plots in the (E) yellow-green module or (F) light-cyan module. (G) Venn of the intersecting genes between DEGs selected by WGCNA and DEGs selected by limma in the GSE49543 dataset.

cyan module and gene significance for age (Cor=0.46, p=5.3e-18) (Fig. 1F). As shown in Figure 1G, totally 172 DEGs in the two modules were selected by WGCNA, and 129 DEGs were selected by limma in GSE49543 dataset, and 54 genes were identified in the intersection.

3.2. Functional enrichment analysis of the DEGs in presbycusis

GO and KEGG enrichment analyses of the DEGs between presbycusis and young mice were conducted, and the results indicated that these selected DEGs were closely related to transmission of neural signals such as synapse organization, synaptic transmission as well as neurotransmitter transport in biological processes (Fig. 2A); neuron, axon, and collagen in cellular component (Fig. 2B); glycosaminoglycan binding and in molecular function (Fig. 2C). KEGG analysis showed that the age-related DEGs were associated with the infectious diseases, IL-17 signaling pathway as well as synaptic vesicle cycle (Fig. 2D).

3.3. Protein-protein interaction (PPI) network of the DEGs

The PPI network analysis of the 54 DEGs was constructed on the STRING database. (Fig. 3A). The Cytoscape software was used to calculate the PPI network via the degree and the isolated nodes were removed. Furthermore, clustering analysis was conducted using the MCODE, and three significant gene clusters were obtained (Fig. 3B-D).

3.4. Screening of the hub genes based on machine learning

Two machine learning algorithms LASSO and SVM-RFE were used to screen the hub genes. Firstly, 5 genes were selected by LASSO regression algorithm from the DEGs (Fig. 4A). Next, 16 genes were identified by the SVM-RFE algorithm (Fig. 4B). Moreover, as shown in the Venn diagram, totally 5 intersecting genes were selected and confirmed as the hub genes, namely CLEC4D, MS4A7, CTSS, LAPTM5, and ALOX5AP. Moreover, the hub gene expression pattern in presbycusis was explored, and all these five hub genes were significantly upregulated in presbycusis mice in comparison with the young control mice in the GSE49543 dataset, which might be associated with the presbycusis progression (Fig. 4D-H).

4. Discussion

ARHL, also known as presbycusis, is a prevalent neurodegenerative disorder as well as a common chronic medical condition in the elderly [4]. Increasing evidence has revealed that the risk of cognitive impairment or decline increases in ARHL patients, which considerably affects public health and quality of life of patients. In the current study, we explored the potential genomic changes induced by age-related hearing loss based on the bioinformatics analysis. Totally 54 DEGs were screened based on the WGCNA and limma in the GSE49543 dataset. Five

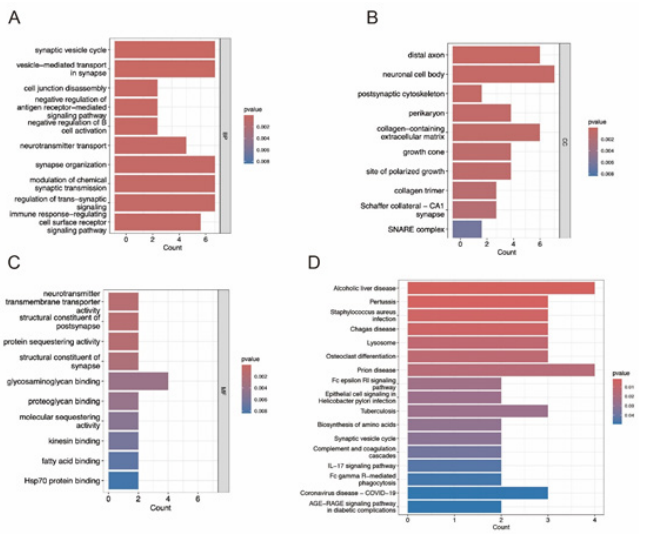

Fig. 2. Functional enrichment analysis of the DEGs in presbycusis. GO enrichment analysis regarding (A) biological process, (B) cellular component, (C) molecular function, and (D) KEGG enrichment analysis of the age-related DEGs in presbycusis.

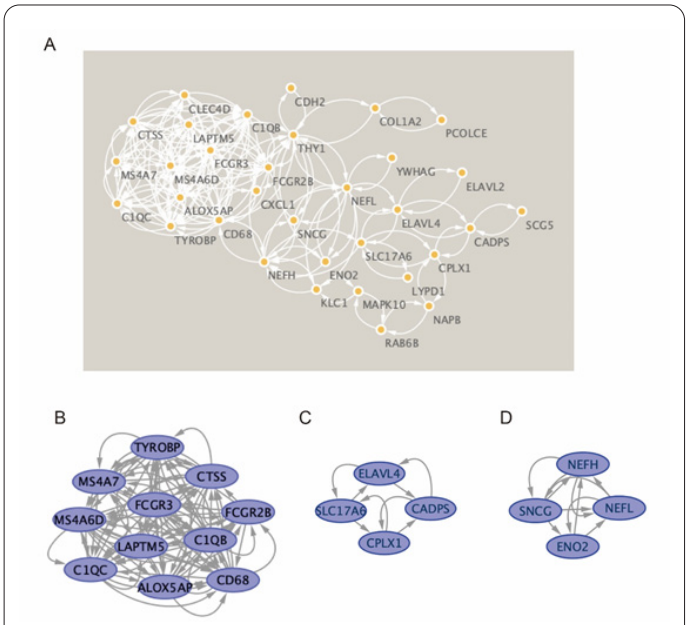

Fig. 3. Construction of the PPI network of the DEGs. (A) The PPI networks of the DEGs presbycusis were constructed using String database and Cytoscape software. (B-D) Three significant modules were identified in the PPT network.

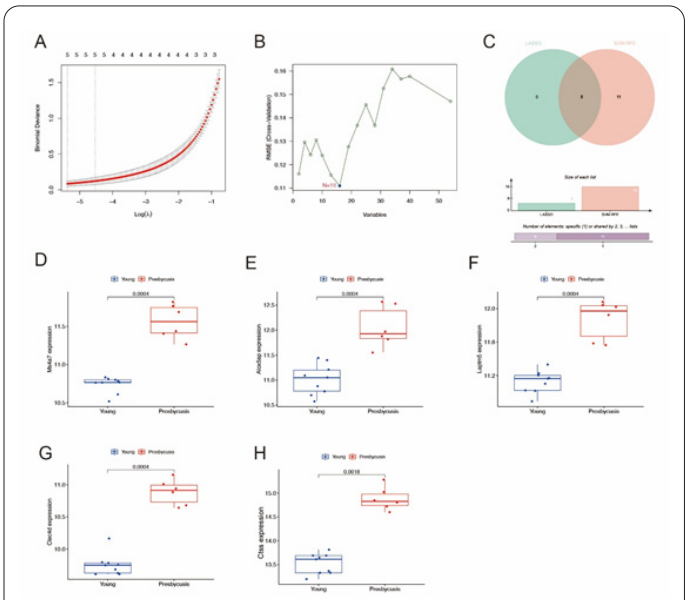

Fig. 4. Selection of the hub genes in presbycusis. (A) LASSO regression algorithm. (B) SVM-RFE algorithm. (C) Venn diagrams showed the intersecting genes in the two algorithms. Relative expression of (D) Ms4a7, (E) Alox5ap, (F) Laptm5, (G) Clec4d, and (H) Ctss.

hub genes were identified via machine learning, namely Clec4d, Ms4a7, Ctss, Laptm5, and Alox5ap, and the upregulation of the 5 hub genes was further demonstrated in presbycusis mice based on the GSE49543.

Cochlea is the auditory portion of the inner ear, and changes with aging process, which is considered as the main reason for presbycusis [15]. The process of cochlea aging is a complex process, and structures and genes related to sensory transduction and neurotransmission were involved [16]. Thus, analyzing the genomic alterations in presbycusis could deepen the understanding of the physiopathological mechanisms of this disease [17]. Zeng et al. have reported that the ferroptosis-related gene lactotransferrin (LTF) is a hub gene involved in cochlear ferroptosis and demonstrated that LTF is donw-regulated in the aging HEI-OC1 auditory cells and in the aging cochleae in C57BL/6J mice [18]. Schubert et al. have found that the genes related to aging, senescence, and deafness were differentially expressed in the cochlear substructures according to transcriptomic analyses [19]. In our study, we explored the DEGs in the cochlea of aging CBA mice based on the GSE49543 dataset. The CBA mouse strain is commonly used for the establishment of mouse models for presbycusis and are valuable for understanding the behavioral, neural and molecular bases of presbycusis at both cochlear and brain levels [9, 20, 21]. Based on WGCNA and analysis with limma, we found 54 DEGs in the cochlea of aging CBA mice. Moreover, enrichment analysis was conducted to explore the biological functions and related signaling pathways of the selected DEGs. We identified that these DEGs were primarily enriched in the synapse organization and transmission of neural signal as well as the immune response, axon growth and activity of neurotransmitter transmembrane transporter according to GO enrichment analysis. Degeneration of stria vascularis, hair cells, and neurons are closely related to the pathological changes of presbycusis, and can be induced by both noise exposure and aging [6]. According to KEGG enrichment analysis, these DEGs were associated with alcoholic liver disease, pertussis, lysosome pathway and inflammation-related pathways such as IL-17 signaling pathway, which is consistent with the previous findings that chronic inflammation occurs in response to aging of immune system, and the expression of pro-inflammatory cytokines such as IL-6 and TNF-α is closely associated with the hearing loss process [22].

Furthermore, the PPI network of the selected DEGs was built with three significant modules. Genes such as MS4A7, MS4A6D, CTS5, ALOX5AP and some others are closely associated with other proteins with high degree on the interaction network. Additionally, the hub genes were screened by two machine learning algorithms LASSO and SVM-RFE. Finally, 5 hub genes including Clec4d, Ms4a7, Ctss, Laptm5, and Alox5ap were identified. C-type lectin receptor 4D (CLEC4D) is a member of the C-type lectin receptor family and is mainly expressed in the surface of dendritic cells and macrophages, playing a critical role in the immune response [23]. Previous literature has revealed that CLEC4D is involved in cancer development and might be deregulated in the context of aging [24]. Membrane spanning 4-domains A7 (MS4A7) belongs to the MS4A family, and is potentially involved in the mature cell functions in the monocyte lineage, and participates in signal transduction [25]. Studies have also shown that Ms4a7 is an immune signature related to cancer prognosis [26-28]. Cathepsin S (CTSS) is aberrantly expressed in various diseases, including neurological pathologies. The secretion of CTSS is revealed to affect the neuron activation as well as synaptic responses in normal conditions, and CTSS expression is reported to be upregulated in neurodegenerative diseases [29]. Additionally, Ctss is related to microglia inflammatory response in the stroke, and inhibition of the Ctss expression can attenuate the inflammation and protect brain from damage, improving the neurological functions of mice with stroke [30]. Lysosomal protein transmembrane 5 (LAPTM5) is a protein preferentially expressed in immune cells and is reported to negatively regulate the T and B cell receptor signaling by interacting with the T and B cell receptors [31]. Arachidonate 5-lipoxygenase activating protein (Alox5ap) is a critical enzyme related to leukotriene production, and which is an important immunomodulating and proinflammatory lipid mediator in diseases [32]. Overall, the direct involvement of the hub genes in age-related hearing loss is rarely reported. In this study, we revealed that these 5 hub genes were at higher expression levels in the presbycusis mice, which suggested the potential of the five genes for the therapeutic intervention of presbycusis. However, future studies are needed to further validate the biological roles in presbycusis development using gene KO mouse models.

5. Conclusion

In conclusion, 54 DEGs between ARHL and young control mice were identified, and 5 5 hub genes including Clec4d, Ms4a7, Ctss, Laptm5, and Alox5ap upregulated in ARHL were screened, which might provide clues for the anti-ARHL treatment.

Conflict of interests

The authors declare no competing interests.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Informed consent

Not applicable.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

GL contributed to the study conception and design. Software, data collection and analysis were performed by JK, WT and HX. The first draft of the manuscript was written by JK. All authors commented on previous versions of the manuscript.

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References

1. Bowl MR, Dawson SJ (2019) Age-Related Hearing Loss. Cold Spring Harb Perspect Med 9 (8). doi: 10.1101/cshperspect.a033217
2. Organization WH Addressing the rising prevalence of hearing loss. World Health Organization: Geneva. (Ed.^, Eds.), 2018.
3. Golub JS, Brickman AM, Ciarleglio AJ, Schupf N, Luchsinger JA (2020) Association of Subclinical Hearing Loss With Cognitive Performance. JAMA Otolaryngol Head Neck Surg 146 (1): 57-67. doi: 10.1001/jamaoto.2019.3375
4. Rutherford BR, Brewster K, Golub JS, Kim AH, Roose SP (2018) Sensation and Psychiatry: Linking Age-Related Hearing Loss to Late-Life Depression and Cognitive Decline. Am J Psychiatry 175 (3): 215-224. doi: 10.1176/appi.ajp.2017.17040423
5. Slade K, Plack CJ, Nuttall HE (2020) The Effects of Age-Related Hearing Loss on the Brain and Cognitive Function. Trends Neurosci 43 (10): 810-821. doi: 10.1016/j.tins.2020.07.005
6. Keithley EM (2020) Pathology and mechanisms of cochlear agingJ Neurosci Res 98 (9): 1674-1684. doi: 10.1002/jnr.24439
7. Rizk HG, Linthicum FH, Jr. (2012) Histopathologic categorization of presbycusis. Otol Neurotol 33 (3): e23-24. doi: 10.1097/MAO.0b013e31821f84ee
8. Shibata SB, Budenz CL, Bowling SA, Pfingst BE, Raphael Y (2011) Nerve maintenance and regeneration in the damaged cochlea. Hear Res 281 (1-2): 56-64. doi: 10.1016/j.heares.2011.04.019
9. Liu H, Giffen KP, Chen L, Henderson HJ, Cao TA, Kozeny GA, Beisel KW, Li Y, He DZ (2022) Molecular and cytological profiling of biological aging of mouse cochlear inner and outer hair cells. Cell Rep 39 (2): 110665. doi: 10.1016/j.celrep.2022.110665
10. Peng L, Li N, Huang Z, Qiu C, Yin S (2022) Prognostic Gene Expression Signature for Age-Related Hearing Loss. Front Med 9: 814851. doi: 10.3389/fmed.2022.814851
11. D'Souza M, Zhu X, Frisina RD (2008) Novel approach to select genes from RMA normalized microarray data using functional hearing tests in aging mice. J Neurosci Methods 171 (2): 279-287. doi: 10.1016/j.jneumeth.2008.02.022
12. Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9: 559. doi: 10.1186/1471-2105-9-559
13. Pierre JF, Akbilgic O, Smallwood H, Cao X, Fitzpatrick EA, Pena S, Furmanek SP, Ramirez JA, Jonsson CB (2020) Discovery and predictive modeling of urine microbiome, metabolite and cytokine biomarkers in hospitalized patients with community acquired pneumonia. Sci Rep 10 (1): 13418. doi: 10.1038/s41598-020-70461-9
14. Sundermann B, Bode J, Lueken U, Westphal D, Gerlach AL, Straube B, Wittchen HU, Ströhle A, Wittmann A, Konrad C, Kircher T, Arolt V, Pfleiderer B (2017) Support Vector Machine Analysis of Functional Magnetic Resonance Imaging of Interoception Does Not Reliably Predict Individual Outcomes of Cognitive Behavioral Therapy in Panic Disorder with Agoraphobia. Front Psychiatry 8: 99. doi: 10.3389/fpsyt.2017.00099
15. Fujioka M, Okano H, Edge AS (2015) Manipulating cell fate in the cochlea: a feasible therapy for hearing loss. Trends Neurosci 38 (3): 139-144. doi: 10.1016/j.tins.2014.12.004
16. Gourévitch B, Edeline JM, Occelli F, Eggermont JJ (2014) Is the din really harmless? Long-term effects of non-traumatic noise on the adult auditory system. Nature reviews. Neurosci 15 (7): 483-491. doi: 10.1038/nrn3744
17. Ciorba A, Hatzopoulos S, Bianchini C, Aimoni C, Skarzynski H, Skarzynski PH (2015) Genetics of presbycusis and presbystasis. Int J Immunopathol Pharmacol 28 (1): 29-35. doi: 10.1177/0394632015570819
18. Zeng C, Gu X, Chen Y, Lin Y, Chen J, Chen Z, Chen C, Yao G, Lin C (2024) Identification and experimental validation of ferroptosis-related gene lactotransferrin in age-related hearing loss. Front Aging Neurosci 16: 1309115. doi: 10.3389/fnagi.2024.1309115
19. Schubert NMA, van Tuinen M, Pyott SJ (2022) Transcriptome-Guided Identification of Drugs for Repurposing to Treat Age-Related Hearing Loss. Biomolecules 12 (4). doi: 10.3390/biom12040498
20. Bowl MR, Dawson SJ (2015) The mouse as a model for age-related hearing loss - a mini-review. Gerontol 61 (2): 149-157. doi: 10.1159/000368399
21. Xie R, Manis PB (2017) Synaptic transmission at the endbulb of Held deteriorates during age-related hearing loss. J Physiol 595 (3): 919-934. doi: 10.1113/jp272683
22. Watson N, Ding B, Zhu X, Frisina RD (2017) Chronic inflammation - inflammaging - in the ageing cochlea: A novel target for future presbycusis therapy. Ageing Res Rev 40: 142-148. doi: 10.1016/j.arr.2017.10.002
23. Drouin M, Saenz J, Chiffoleau E (2020) C-Type Lectin-Like Receptors: Head or Tail in Cell Death Immunity. Front Immunol 11: 251. doi: 10.3389/fimmu.2020.00251
24. Fang X, Liu D, Zhao J, Li X, He T, Liu B (2023) Using proteomics and metabolomics to identify therapeutic targets for senescence mediated cancer: genetic complementarity method. Front Endocrinol 14: 1255889. doi: 10.3389/fendo.2023.1255889
25. Gingras MC, Lapillonne H, Margolin JF (2001) CFFM4: a new member of the CD20/FcepsilonRIbeta family. Immunogenetics 53 (6): 468-476. doi: 10.1007/s002510100345
26. Wu H, Feng J, Zhong W, Zouxu X, Xiong Z, Huang W, Zhang C, Wang X, Yi J (2023) Model for predicting immunotherapy based on M2 macrophage infiltration in TNBC. Front Immunol 14: 1151800. doi: 10.3389/fimmu.2023.1151800
27. Tan Q, Liu H, Xu J, Mo Y, Dai F (2021) Integrated analysis of tumor-associated macrophage infiltration and prognosis in ovarian cancer. Aging 13 (19): 23210-23232. doi: 10.18632/aging.203613
28. Ni B, Huang G, Yang R, Wang Z, Song H, Li K, Zhang Y, Wu K, Shi G, Wang X, Shen J, Liu Y (2023) The short isoform of MS4A7 is a novel player in glioblastoma microenvironment, M2 macrophage polarization, and tumor progression. J Neuroinflammation 20 (1): 80. doi: 10.1186/s12974-023-02766-1
29. Smyth P, Sasiwachirangkul J, Williams R, Scott CJ (2022) Cathepsin S (CTSS) activity in health and disease - A treasure trove of untapped clinical potential. Mol Aspects Med 88: 101106. doi: 10.1016/j.mam.2022.101106
30. Wu J, Wu Z, He A, Zhang T, Zhang P, Jin J, Li S, Li G, Li X, Liang S, Pei L, Liu R, Tian Q, He X, Lu Y, Tang Z, Li H (2021) Genome-Wide Screen and Validation of Microglia Pro-Inflammatory Mediators in Stroke. Aging Dis 12 (3): 786-800. doi: 10.14336/ad.2020.0926
31. Glowacka WK, Alberts P, Ouchida R, Wang JY, Rotin D (2012) LAPTM5 protein is a positive regulator of proinflammatory signaling pathways in macrophages. J Biol Chem 287 (33): 27691-27702. doi: 10.1074/jbc.M112.355917
32. Ye X, An L, Wang X, Zhang C, Huang W, Sun C, Li R, Ma H, Wang H, Gao M (2021) ALOX5AP Predicts Poor Prognosis by Enhancing M2 Macrophages Polarization and Immunosuppression in Serous Ovarian Cancer Microenvironment. Front Oncol 11: 675104. doi: 10.3389/fonc.2021.675104