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# Exploring the potential biomarkers and potential causality of Ménière disease based on bioinformatics and machine learning

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**Abstract**

Meniere disease (MD) is a common inner ear disorder closely related to immune abnormalities, but research on the characteristic genes between MD and immune responses is still insufficient. We employ bioinformatics and machine learning to predict potential biomarkers and characteristic immune cells associated with MD, investigating the Mendelian randomization causation between immune cells and MD, providing new insight for the early diagnosis, prevention, and treatment of MD. We obtained relevant data on MD from the GEO database using R, conducted differential gene analysis, and performed weighted gene co-expression network analysis (WGCNA) to identify genes associated with MD. Moreover, by integrating the selection of core genes from the PPI with machine learning techniques, we predicted potential biomarkers for MD. Simultaneously, conducted immune infiltration analysis of the core genes and identified key immune cell types. Finally, employed Mendelian randomization to comprehensively evaluate the causal relationship between immune cells and MD. Through differential gene analysis and WGCNA, we identified 550 genes associated with MD, with enrichment analysis predominantly focused on pertinent immune responses and related diseases. The protein-protein interaction (PPI) screening and machine learning techniques, we predicted 2 potential biomarkers for MD: CD5 and AJUBA, 3 core immune cell types associated with MD: T cells CD4 memory resting, T cells gamma delta and Dendritic cells activated. Mendelian randomization analysis revealed a causal relationship between 26 types of immune cells and MD. There is a causal relationship between immune cells and MD. CD5 and AJUBA are potential biomarkers of MD, while T cells CD4 memory resting, T cells gamma delta and Dendritic cells activated are core immune cells of MD. These potential biomarkers and core immune cells offer new insights for the early diagnosis, prevention, and treatment of MD.

**Abbreviations:** AUC = area under the curve, DEGs = differentially expressed genes, GEO = gene expression omnibus, GO = gene ontology, GS = gene significance, KEGG = Kyoto encyclopedia of genes and genomes, MD = Meniere disease, MM = module membership, MR = Mendelian randomization, PPI = protein–protein interaction, ROC = receiver operating characteristic, WGCN = weighted gene co-expression network analysis.

**Keywords:** bioinformatics, immune responses, machine learning, Mendelian randomization, Ménière disease

## 1. Introduction

Ménière disease (MD) is a prevalent inner ear disorder characterized by episodic vertigo, fluctuating hearing loss, tinnitus, and a sensation of ear fullness. Epidemiological studies indicate that MD predominantly affects individuals aged between 40 and 60, with an incidence rate of 190 per 100,000 people, and the trend is increasing annually.[1–3] The onset of MD typically involves 1 side and is attributed to a complex array of factors including trauma, viral infections, metabolic disorders, autoimmune elements, and allergic reactions.[4] Recent research increasingly supports the notion that autoimmune responses are linked to MD.[5–7] The immune system plays a pivotal role in maintaining human health, not only by identifying and eliminating pathogens but also by regulating inflammation and tissue repair. Abnormal immune responses can lead to allergic reactions. Endolymphatic hydrops is considered a precursor to the onset of MD, and the endolymphatic sac, serving as an organ for immune response, can initiate an immune reaction on its basal membrane.[8,9] An excessive immune response can impair the absorptive function of the endolymphatic sac, leading to hydrops and ultimately

*The authors have no funding and conflicts of interest to disclose.*

*The datasets generated during and/or analyzed during the current study are publicly available.*

*This study has been reviewed by the Ethics Committee of the Suzhou Hospital of Integrated Chinese and Western Medicine. As it utilizes publicly available databases with fully informed consent, and in accordance with the requirements of China's National Health Commission, research conducted using legally obtained public data or data generated through observation without interfering with public behavior may be exempt from ethical review. Therefore, this study does not require additional IRB approval.*

*All authors agree to the publication of this paper.*

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triggering MD.[10] Studies have shown that patients with MD often have a history of allergies, elevated levels of immune complexes, interleukins, and autoantibodies in their plasma.[7] Desensitization treatments for certain allergens have been shown to effectively control vertigo in these patients.[11,12] Furthermore, the severity of MD is associated with increased levels of a higher CD4 to CD8 ratio.[13] Although a close correlation exists between MD and immune responses, the mechanisms by which immune responses regulate the onset of MD remain elusive.

In recent years, with the advancement of big data and genetic technologies, bioinformatics, machine learning and Mendelian randomization have been extensively applied across numerous medical fields, offering potential research methodologies for unraveling the molecular mechanisms of MD and identifying novel biomarkers. Bioinformatics techniques, utilizing gene sequencing as a data source, explore the potential genetic targets of diseases, helping researchers in identifying differentially expressed genes (DEGs) and their potential pathways related to the disease.[14] Mendelian randomization, a novel design in genetic epidemiology, is employed to establish causal relationships between risk factors and diseases.[15] This approach mitigates the risk of biases inherent in traditional experimental studies. Through MR methods, it is possible to explore the causal relationships between immune cells, inflammatory factors, and the disease, as well as to identify potential biomarkers and predict targets for traditional Chinese medicine.[16,17] Combining MR allows for the prediction of potential therapeutic targets from a genetic perspective, thus employing this analysis can explore the potential relationships between MD and immune cells, aiding in the identification of high-risk populations for the disease.

This study employs bioinformatics and machine learning to identify characteristic immune cells and potential biomarkers for MD, examining the specific enriched pathways and immune infiltration mechanisms associated with core genes. Additionally, by integrating Mendelian randomization to analyze the causal relationship between immune cells and MD, this research provides innovative perspectives for the early diagnosis, prevention, and treatment of MD.

## 2. Methods

### *2.1. Data extraction and the selection of differentially expressed genes*

Using "MD" as the search term, the microarray dataset GSE202657 was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). This dataset, uploaded in August 2022 by researchers Choi KD, Oh EH, Kim HS, and others, includes peripheral blood samples from 9 healthy individuals

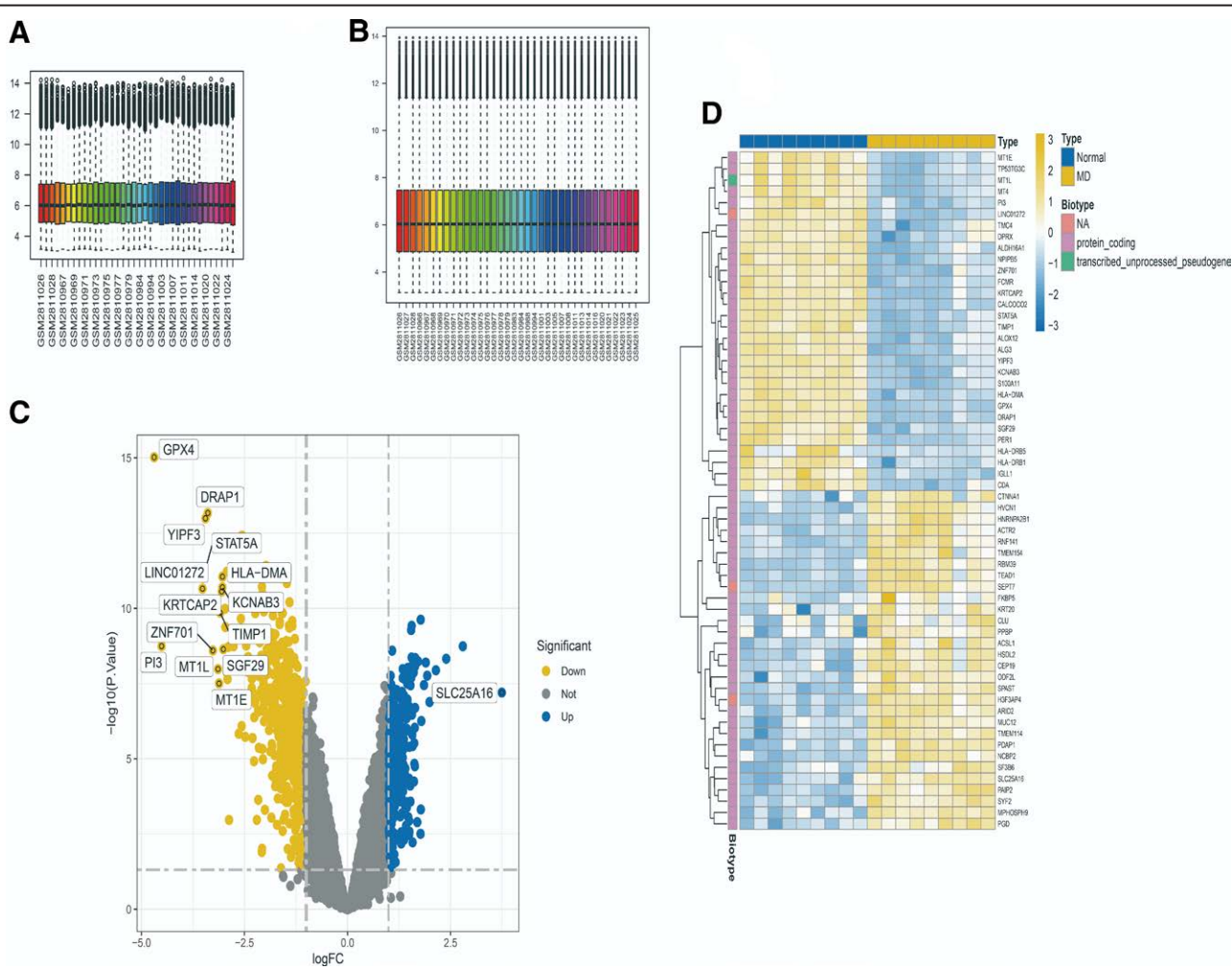


**Figure 1.** (A) represents the dataset GSE20265 prior to normalization; (B) illustrates the matrix post-normalization; (C) depicts the volcano plot of differentially expressed genes in MD, with blue indicating upregulation and yellow indicating downregulation; (D) shows the heatmap of differentially expressed genes, where yellow signifies high expression, blue signifies low expression, "normal" denotes the control group, and "MD" represents the Ménière disease group. MD = Ménière disease.

and 9 MD patients. We utilized the "normalize Between Arrays" function from the "limma" package in R to standardize the GSE202657 dataset and visualized the normalized results with box plots. The Limma package in R was employed to study differentially expressed genes (DEGs). In GEO, the p-values were adjusted to correct for false positives. We used an absolute log2 fold change > 1 and an adjusted *P*-value < .05 as criteria for selecting DEGs, where negative values indicate downregulation and positive values indicate upregulation. Volcano plots and heatmaps of the differential genes were created using the "ggpubr" and "pheatmap" packages in R.

### 2.2. Weighted gene co-expression network analysis and the selection of associated genes

We utilized R software to construct a weighted gene co-expression network for MD. The "good Samples Genes" function was employed to filter the differential gene expression matrix, removing unsuitable genes and samples. An appropriate soft-thresholding power was selected to approximate a scale-free network distribution. A dendrogram was plotted to facilitate module selection, and module data was correlated with clinical feature data to identify MD co-expressed gene modules. module membership and gene significance within the module to ascertain the significance of each gene. To comprehensively consider the MD genes selected by WGCNA and the DEGs, a Venn diagram was used to identify the intersecting genes, which are relevant to MD disease.

### 2.3. Functional and pathway enrichment analysis

We employed the tools within R software, utilizing the Bioconductor, ClusterProfiler, and ggplot packages along with associated add-ons, to conduct Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses on key genes. GO circle plots and KEGG bubble charts were generated. We filtered for biological processes, cellular components, molecular functions, and signaling pathways with a significance level of $P < .05$.

### 2.4. Construction of a protein interaction network and identification of hub genes

The relevant genes from section 2.2 were imported into the STRING database (https://string-db.org/) and R software was utilized to construct a protein-protein interaction (PPI) network. The results were imported into Cytoscape and hub genes were identified using 3 distinct algorithms available in Cytoscape.

### 2.5. Employ machine learning techniques to identify key genes associated with MD

We utilized the LASSO logistic regression algorithm and the random forest algorithm, employing cross-validation to select key genes associated with MD. A diagnostic model was constructed based on these key genes using the logistic regression algorithm. The diagnostic performance of the genes was evaluated by plotting the receiver operating characteristic (ROC) curve and calculating the area under the curve (AUC). A 2-tailed test was performed, considering a *P*-value < .05 as statistically significant.

### 2.6. Conduct an immune infiltration analysis and select core immune cells

The CIBERSORT (https://cibersortx.stanford.edu) bioinformatics algorithm was utilized to quantitatively assess the relative

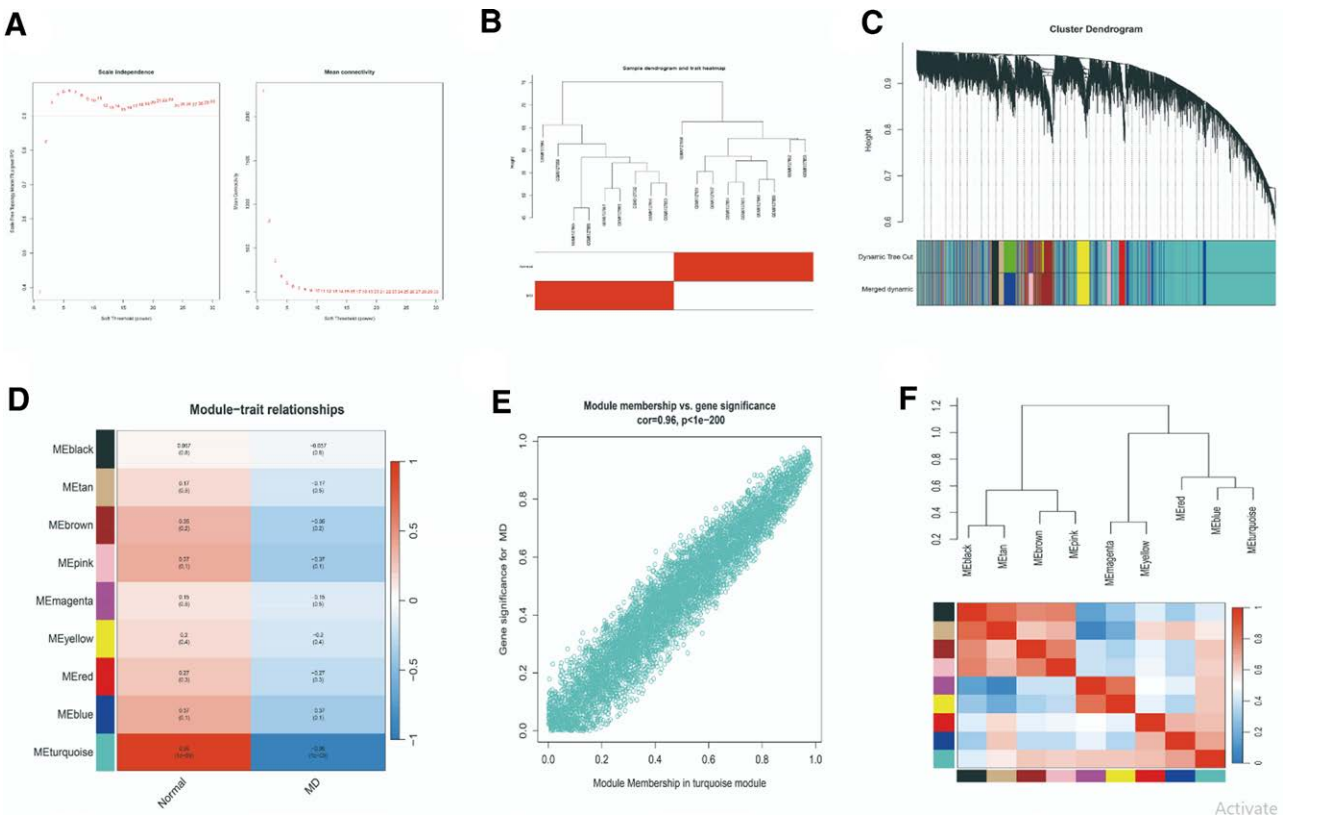


**Figure 2.** (A) Selection of an appropriate threshold; (B) dendrogram of sample clustering; (C) merging of similar modules; (D) relationship between different modules and groups; (E) significance of MD-related genes in turquoise; (F) dendrogram of module characteristic gene clustering. MD = Ménière disease.

proportions of immune cell infiltration in MD patients compared to a control group and to evaluate differences in immune infiltration between the 2 groups. The ssGSEA algorithm was employed to calculate enrichment scores for immune-related gene sets in each sample, thereby gauging the overall activity of immune cells within the samples. After completing the CIBERSORT analysis, we explored the correlation between key genes and immune cells and compared the differences in immune cells between the MD group and the control group to identify divergent immune cells. Finally, we used Lasso regression to select MD-related immune cells. The intersection of these analyses will identify the core immune cells associated with MD.

### *2.7. Mendelian randomization analysis of the causal relationship between MD and immune cells*

We utilized the GWAS database (https://gwas.mrcieu.ac.uk/) to obtain genetic data related to MD (ebi-a-GCST90018880), encompassing a sample size of 482,774 individuals, all of European descent, which includes 24,194,682 single nucleotide polymorphisms (SNPs). Additionally, genetic data on 731 immune cells, foundational to autoimmune characteristics, were derived from a 2020 study.[18] We conducted an association analysis by selecting SNPs based on a significance level of $P < 1 \times 10^{-5}$. Subsequently, we eliminated SNPs in linkage disequilibrium using $R^2 < 0.001$ and Kb = 10,000 criteria, calculated the *F*-statistic for the selected SNPs to remove weak instrumental variables, and considered *F* values >10 as indicative of non-weak instrumental variable. A total of 13,318 SNPs associated with immune cells were included in the analysis. The primary method employed was the inverse variance weighted approach to assess the causal relationship with MD, supplemented by MR-Egger, weighted median, simple mode and weighted mode methods. We employed 5 methods to assess their causal relationships, with inverse variance weighted as the primary method and 4 others as supplementary methods. We conducted sensitivity analysis using the "leave-one-out" approach and tested for pleiotropy and heterogeneity using Cochran *Q* test, MR-Egger intercept test, and model residual permutation test for outliers and small samples, ensuring the accuracy of our MR analysis results. All analyses were conducted using the R language (version 4.3.3). The specific package employed was TwoSampleMR (version 0.6.0).

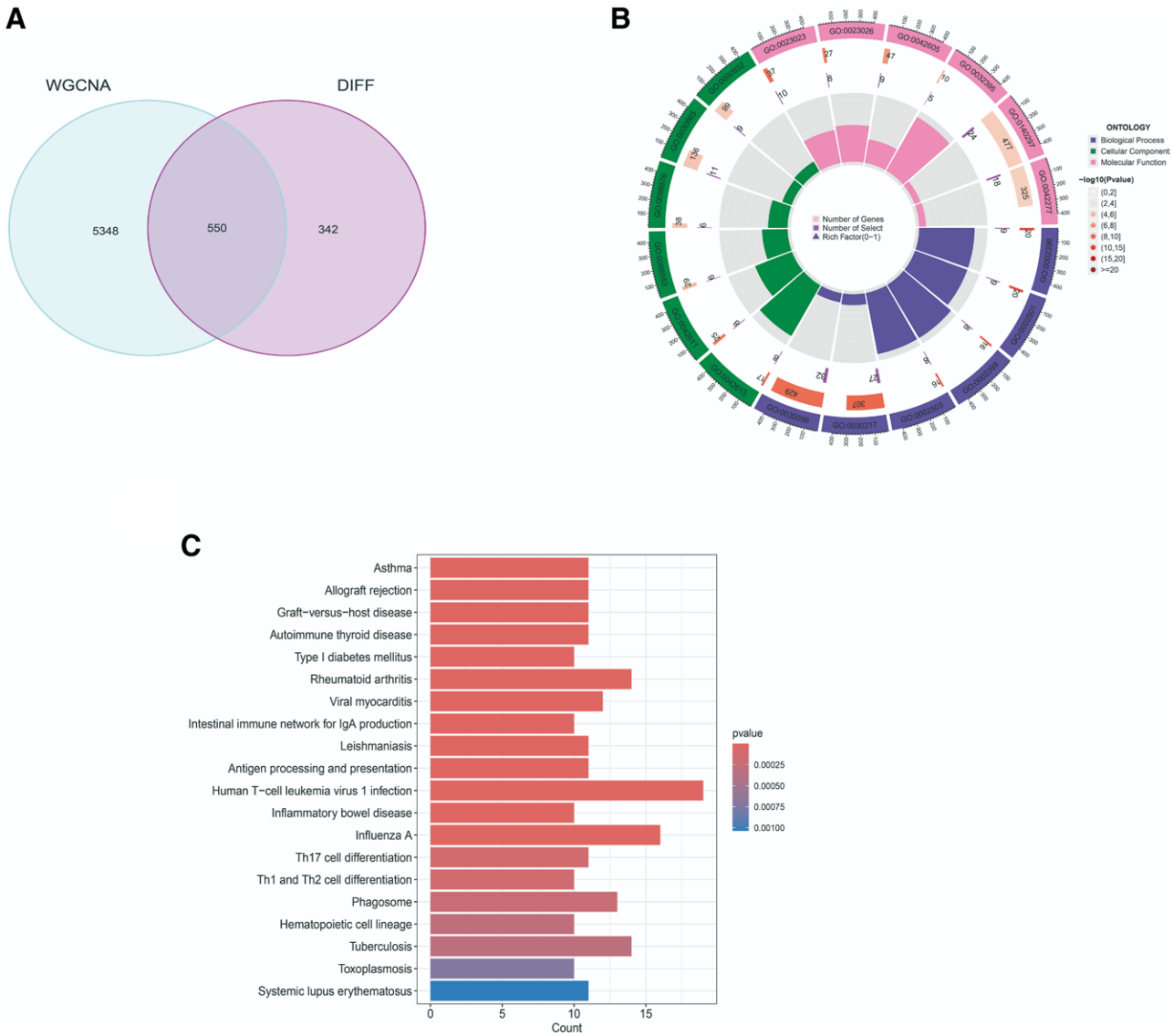


**Figure 3.** (A) depicts the genes associated with MD; (B) presents the GO enrichment analysis of MD-related genes; (C) shows the bar chart of the KEGG enrichment analysis. (D) shows the network diagram of KEGG enrichment analysis. GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, MD = Meniere disease.

## 3. Results

### 3.1. Screening MD differentially expressed genes

Using R software, we extracted the relevant dataset GSE202657 from the GEO database and performed normalization on the expression matrix, which included 9 MD patients and 9 healthy controls in the study. After standardization via principal component analysis, the data distribution showed a tendency toward consistency, as illustrated in Figure 1A and B. According to the selection criteria, we identified a total of 892 differential genes compared to healthy controls, comprising 320 upregulated and 572 downregulated genes. Volcano plots and heatmaps were created using ggplot2 and pheatmap, respectively, as shown in Figure 1C and D.

### 3.2. Weighted gene co-expression network analysis network analysis and associated gene selection

Weighted gene co-expression network analysis analysis was performed on 9 MD patients and 9 healthy controls. When $R^2$ was >0.9 and the mean connectivity was high (Fig. 2A), the authors cluster the samples and remove the samples with obvious abnormalities (Fig. 2B). By using the clustering height limit of 0.6 to combine the strongly correlated modules, 9 modules were selected for further analysis. It is displayed below the cluster tree (Fig. 2C). Looking at the correlation between ME values and the clinic, the turquoise module was positively correlated with the healthy control group ($R = 0.95$, $P = 1\times10^{-09}$) and negatively correlated with the MD group ($r = -0.95$, $P = 1\times10^{-09}$) (Fig. 2D). The turquoise module and MD-related genes were further studied (Fig. 2E). The correlation between modules indicates that there is no significant relationship (Fig. 2F).

### 3.3. Acquisition and functional enrichment analysis of genes associated with MD disease

The intersection of the 5898 genes obtained from the turquoise module in WGCNA and the 892 DEGs identified in section 3.1 revealed 550 common targets, associated with MD disease (Fig. 3A). An enrichment analysis of these 550 disease genes indicated that the GO biological processes predominantly focused on T cell differentiation, lymphocyte differentiation, activation of T cells involved in immune responses, and monocyte differentiation. In terms of GO cellular components, the primary concentration was on MHC class II protein complexes, the lumen side of the endoplasmic reticulum membrane, coated vesicle membranes, and membranes of late endosomes. The GO molecular functions were primarily oriented towards peptide binding, antigen binding, histone binding, and interaction with MHC class II protein complexes (Fig. 3B). The KEGG enrichment pathways included asthma,

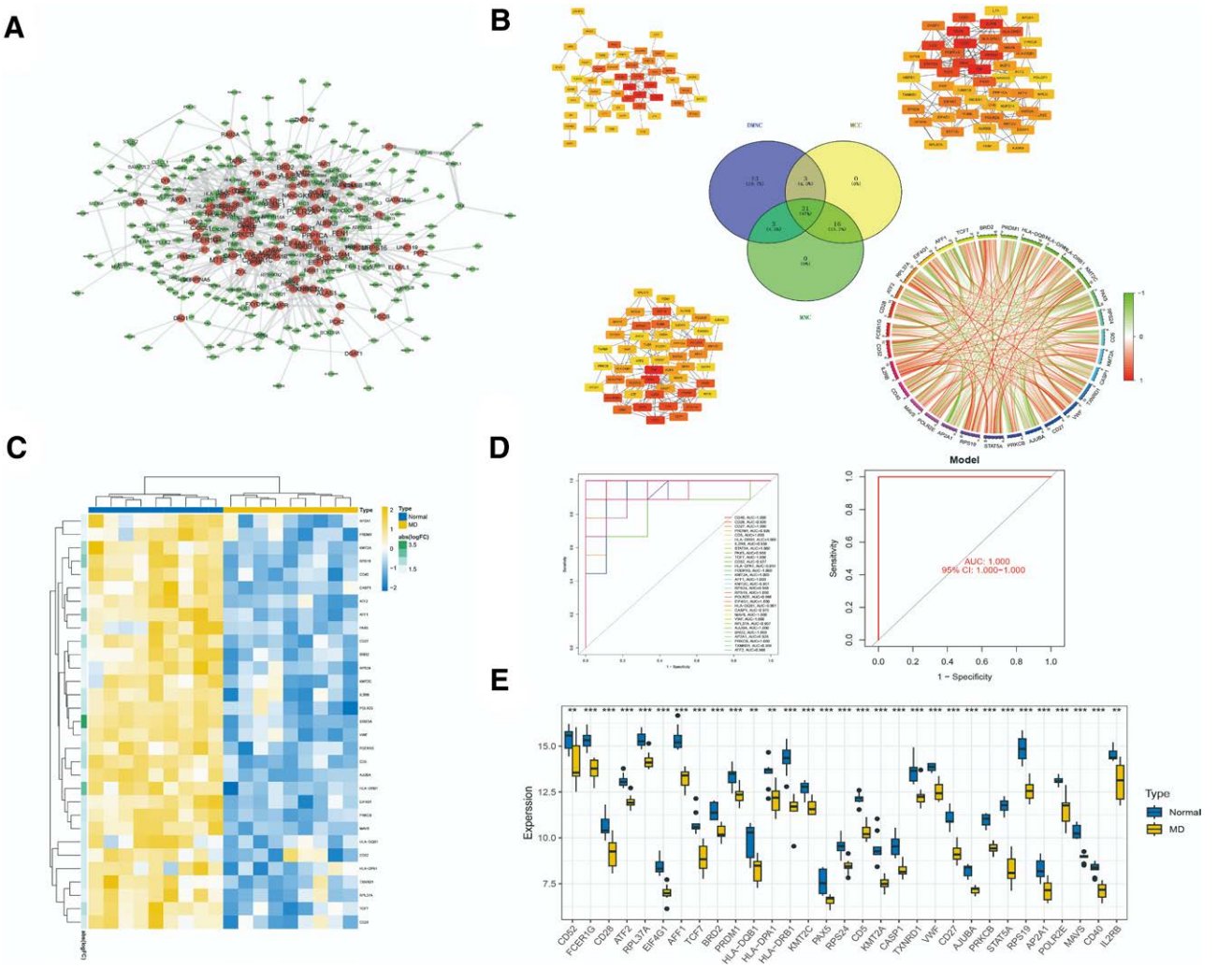


**Figure 4.** (A) Selection of PPI genes; (B) identification of core genes through different algorithms; (C) expression heatmap of core genes; (D) ROC analysis of core genes; (E) expression differences of core genes between the 2 groups. PPI = protein–protein interaction, ROC = receiver operating characteristic.

differentiation of Th17 cells, differentiation of Th1 and Th2 cells, phagosome formation, the intestinal immune network responsible for IgA production, and inflammatory bowel diseases (Fig. 3C and D).

### 3.4. Construction of the PPI network, selection of hub genes, and identification of characteristic genes for MD using machine learning

PPI network was constructed for 550 MD genes using R software, and core genes were screened at the same time, a total of 94 core genes were screened (Fig. 4A). Based on cytoHubba plug-in in Cytoscape, 3 algorithms were used to select the top 50 related genes and intersection them to obtain 31 Hub genes (Fig. 4B). Heat maps were used to further demonstrate the expression and differences of characteristic genes in MD. There were differences in the expression of characteristic genes between MD and healthy control group ($P < .05$) (Fig. 4C and E). ROC evaluation of 31 characteristic cardiac genes showed that AUC values of 31 core genes were all >0.9, and AUC of the overall training set was 1, indicating that core genes had a good diagnostic ability for MD (Fig. 4D).

Subsequently, we employed machine learning algorithms to identify core feature genes among the 31 hub genes. The SVM-REF algorithm identified 9 core feature genes: CD27, FCER1G, CACNA1C, CD5, MAVS, NEK9, FEN1, AJUBA, and RPS19. Meanwhile, the LASSO algorithm unveiled 6 core feature genes: CD5, HLA-DRB1, STAT5A, AFF1, AJUBA, and PRKCB (Fig. 5A). The intersection of these results yielded 2 core feature genes, CD5 and AJUBA. In the GSE202657 dataset, we analyzed the expression level differences of these 2 core feature genes between MD patients and healthy controls. The results revealed that both genes exhibited significantly reduced expression in MD compared to the healthy control group, with statistical significance (Fig. 5B and C).

### 3.5. Immune infiltration of Hub gene and screening of core immune cells

By analyzing the correlation between Hub genes and immune cells using the CIBERSORT method, we found that 31 Hub genes were correlated with 24 types of immune cells. The Hub genes may influence the progression of MD by affecting immune cells such as B cells memory, T cells CD8, T cells gamma delta and Dendritic cells activated (Fig. 6A). Further analysis of the immune cell characteristics in the MD group compared to the healthy control group identified differential expression in 5 types of immune cells: B cells memory, T cells CD8, T cells gamma delta and Dendritic cells activated ($P < .05$) (Fig. 6B). Using Lasso regression, 5 immune cells were selected: plasma cells, T cells CD4 memory resting, T cells gamma delta, NK cells activated and dendritic cells activated. The intersection of these 2 sets yielded 3 immune cells: T cells CD4 memory resting, T cells gamma delta and dendritic cells activated, further suggesting a potential causal relationship between these 3 immune cells and MD (Fig. 6C).

### 3.6. Mendelian randomization study between immune cells and MD

Through the study of Mendelian randomization, it was discovered that 26 out of 731 immune cell types have a causal relationship with MD, we identified 10 cells that the risk factors of MD, namely CD28 + CD45RA-CD8dim%CD8dim, CD25 on CD45RA-CD4 not Treg, EM CD4+%T cell, CD33-HLADR-AC, CD3 on EMCD8br, BAFF-R on CD20-, CD45RA-CD4+%CD4+, IgD- CD24- %lymphocyte, HLADR on HLADR + CD8br and CD27 on IgD- CD38br. As the levels of these immune cells increase, the risk of developing MD rises. Conversely, the remaining immune cells, such as CD20 on IgD+, IgD + CD24 + AC, CD127 on CD28- CD8br, CD45 on CD33- HLA DR-, CD8dim %leukocyte, CD62L- DC %DC,

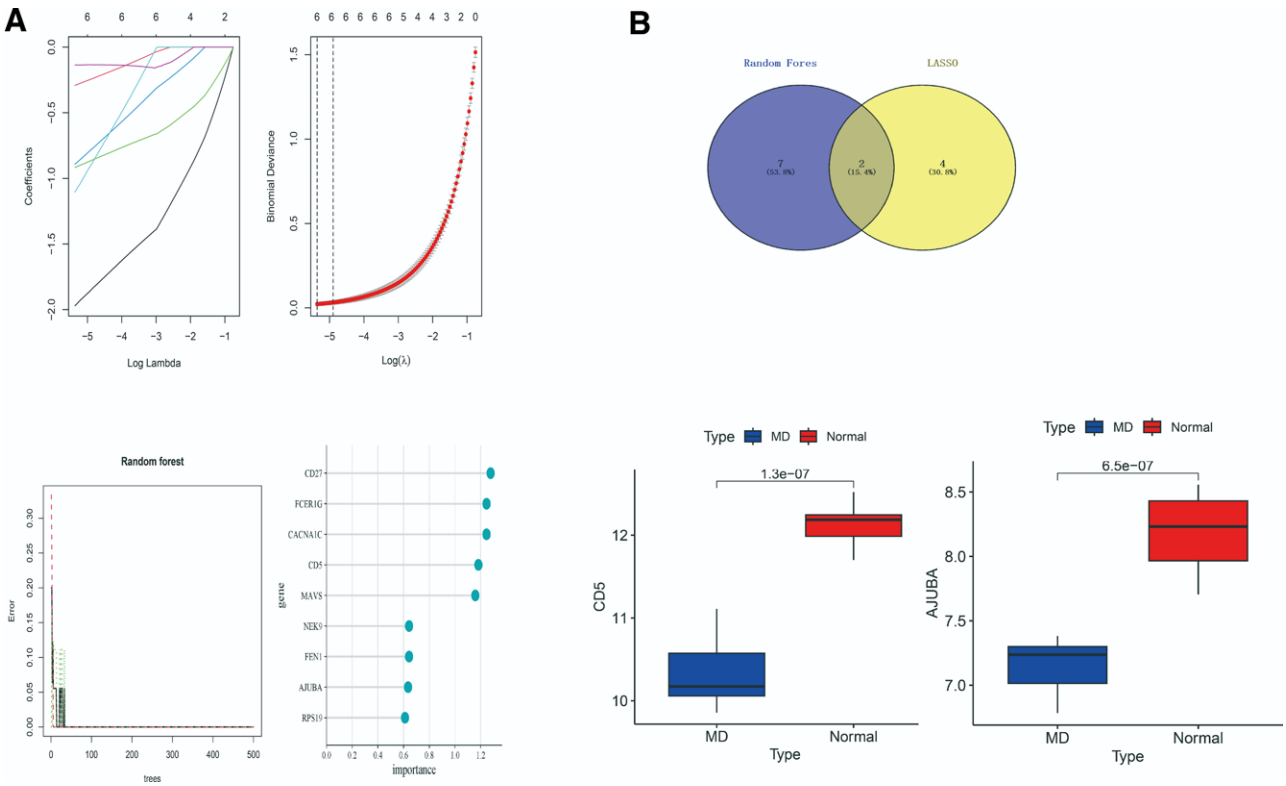


**Figure 5.** (A) Two kinds of machine learning screening feature genes; (B) intersectional screening core feature gene (C) expression level difference of 2 feature genes between MD and healthy control group in GSE20265 data set. MD = Meniere disease.

CD20- CD38- %lymphocyte, CD8dim AC and others serve as protective factors against MD; their increase correlates with a reduced risk of the disease. 26 immune cell types are associated with T cells, suggesting a potential link between T cells and the risk factors for MD. However, reverse Mendelian randomization validates that there is no causal relationship between MD and immune cells (Table 1 and Fig. 7).

## 4. Discussion

MD is a disabling disease of the inner ear, which is mainly caused by the disturbance of the inner ear microcirculatory system leading to the inner ear effusion. The elevation of inner lymphatic fluid adversely impacts both balance and auditory functions.[19,20] As the disease progresses, patients gradually lose their balance function, resulting in persistent dizziness, while auditory capabilities are completely lost.[21] This imposes significant negative effects on both the physical and mental well-being of the patients, severely diminishing their quality of life. At present, the diagnosis of MD mainly depends on clinical symptoms. However, not all disease characteristics can appear in the early stage of MD, and there is a lack of specific diagnostic tests, which is extremely challenging for the diagnosis of MD,[22] resulting in many patients being unable to receive timely diagnosis and treatment and delaying the disease. Research indicates the presence of immune cells, including lymphocytes, macrophages, B lymphocytes, and T lymphocytes, within the human inner ear.[23,24] The endolymphatic sac acts as a potential target organ for immune responses, allowing antigens to enter from its surrounding tissues and blood vessels, thereby producing inflammatory mediators that affect the filtering function of the endolymphatic sac.[25] Additionally, immune cells may infiltrate cochlear tissue from the periphery in noisy environments, which could be a contributing factor to the symptoms of tinnitus and vertigo observed in patients with MD.[26] Clinical studies have confirmed that there are local and systemic immune reactions in patients with MD, and anti-allergy treatment has a positive effect on patients with MD,[11] suggesting that its pathogenesis may be related to immune factors. Therefore, the study of MD disease from immune response has become a research hotspot.

In this study, 550 MD disease genes were obtained through the intersection of DEGs, WGCNA and machine learning results, and the correlation enrichment analysis was conducted to explore the potential mechanism. Additionally, through PPI network analysis and machine learning, 2 potential therapeutic genes for MD (CD5 and AJUBA) and key immune cells (T cells CD4 memory resting, T cells gamma delta and Dendritic cells activated) were selected, which were subsequently validated through ROC and MR analyses.

GO and KEGG results suggested that MD disease genes were correlated with immune or inflammatory response pathways such as T cell differentiation, antigen binding, Asthma, Th17 cell differentiation, Th1 and Th2 cell differentiation, phagosome, Intestinal immune network for IgA production and Inflammatory bowel disease. Approximately one-third of MD patients have immune dysfunction,[22] and there is a notable correlation between immune responses and the pathology of MD.[27] The dizziness experienced by MD patients may stem from abnormal immune reactions.[28] Furthermore, there is a

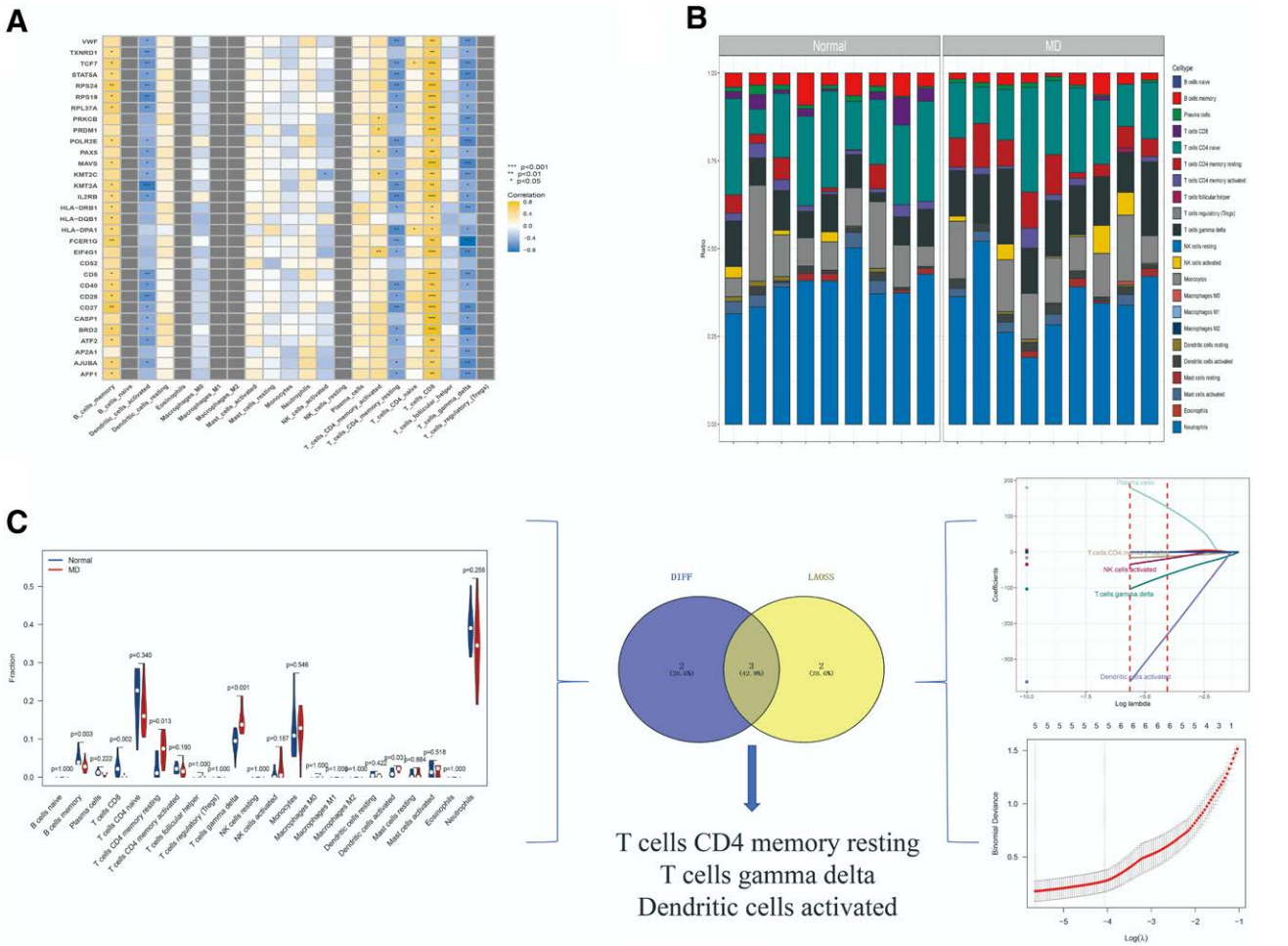


**Figure 6.** (A) Correlation between hub genes and immune infiltration; (b) immune infiltration of MD; (C) screening of core immune cells. MD = Meniere disease.

**Table 1**

**Mendelian randomization and sensitivity analysis of immune cells and MD.**

| | | | | | | Pleiotropy test | | Heterogeneity test | |
|---|---|---|---|---|---|---|---|---|---|
| **Exposure** | **Method** | **SNP** | **Beta** | **SE** | ***P*** | **MR-PRESSO** | **MR-Egger intercept** | **IVW Q** | **MR-Egger Q** |
| CD20 on IgD+ | IVW | 21 | –0.071 | 0.027 | .010 | 0.958 | 0.002 (*P* = .851) | 10.582 (*P* = .956) | 10.546 (*P* = .938) |
| CD27 on IgD- CD38br | IVW | 15 | 0.221 | 0.066 | .001 | 0.982 | –0.044 (*P* = .315) | 4.933 (*P* = .986) | 3.844 (*P* = .992) |
| EM CD4+ %T cell | IVW | 23 | 0.076 | 0.036 | .039 | 0.872 | –0.005 (*P* = .745) | 14.984 (*P* = .862) | 14.875 (*P* = .829) |
| CD3 on EM CD8br | IVW | 20 | 0.093 | 0.039 | .018 | 0.533 | –0.001 (*P* = .944) | 18.238 (*P* = .506) | 18.233 (*P* = .440) |
| CD62L- DC %DC | IVW | 24 | –0.097 | 0.032 | .003 | 0.585 | –0.003 (*P* = .839) | 21.993 (*P* = .520) | 21.951 (*P* = .462) |
| IgD + CD24 + AC | IVW | 23 | –0.136 | 0.050 | .007 | 0.997 | –0.012 (*P* = .534) | 9.671 (*P* = .989) | 9.272 (*P* = .986) |
| HLA DR on HLA DR + CD8br | IVW | 20 | 0.125 | 0.053 | .018 | 0.254 | –0.007 (*P* = .708) | 22.937 (*P* = .240) | 22.754 (*P* = .200) |
| CD45 on CD33- HLA DR- | IVW | 16 | –0.119 | 0.048 | .013 | 0.834 | 0.051 (*P* = .125) | 10.292 (*P* = .800) | 7.640 (*P* = .907) |
| CD20 on naive-mature B cell | IVW | 25 | –0.058 | 0.023 | .014 | 0.729 | 0.008 (*P* = .408) | 20.213 (*P* = .684) | 19.503 (*P* = .671) |
| CD24 on memory B cell | IVW | 32 | –0.069 | 0.028 | .014 | 0.880 | –0.003 (*P* = .801) | 22.664 (*P* = .861) | 22.599 (*P* = .831) |
| CD25 on CD45RA- CD4 not Treg | IVW | 26 | 0.069 | 0.031 | .024 | 0.235 | –0.003 (*P* = .822) | 31.433 (*P* = .175) | 31.366 (*P* = .143) |
| IgD- CD24- %lymphocyte | IVW | 24 | 0.116 | 0.045 | .011 | 0.160 | –0.025 (*P* = .132) | 31.515 (*P* = .110) | 28.360 (*P* = .164) |
| CD28 + CD45RA- CD8dim %CD8dim | IVW | 30 | 0.053 | 0.023 | .023 | 0.957 | 0.011 (*P* = .294) | 18.440 (*P* = .934) | 17.296 (*P* = .942) |
| CD80 on plasmacytoid DC | IVW | 21 | –0.061 | 0.030 | .042 | 0.669 | –0.004 (*P* = .749) | 16.633 (*P* = .676) | 16.528 (*P* = .621) |
| CD45RA- CD4+ %CD4+ | IVW | 30 | 0.104 | 0.034 | .002 | 0.821 | –0.002 (*P* = .867) | 22.902 (*P* = .781) | 22.873 (*P* = .739) |
| CD20 on IgD + CD24- | IVW | 25 | –0.053 | 0.024 | .025 | 0.886 | 0.006 (*P* = .550) | 15.514 (*P* = .904) | 15.147 (*P* = .889) |
| PB/PC %lymphocyte | IVW | 19 | –0.069 | 0.025 | .007 | 0.990 | –0.007 (*P* = .519) | 7.096 (*P* = .989) | 6.664 (*P* = .987) |
| CD33- HLA DR- AC | IVW | 21 | 0.081 | 0.033 | .016 | 0.573 | –0.029 (*P* = .181) | 19.520 (*P* = .488) | 17.597 (*P* = .549) |
| CD8dim AC | IVW | 28 | –0.070 | 0.0331 | .034 | 0.943 | 0.012 (*P* = .454) | 15.827 (*P* = .956) | 15.251 (*P* = .952) |
| CD25 on IgD + CD38- | IVW | 27 | –0.049 | 0.024 | .042 | 0.240 | –0.006 (*P* = .662) | 32.518 (*P* = .176) | 32.267 (*P* = .150) |
| CD39 + CD4 + AC | IVW | 29 | –0.061 | 0.027 | .024 | 0.781 | –0.010 (*P* = .447) | 20.797 (*P* = .833) | 20.204 (*P* = .822) |
| CD8dim %leukocyte | IVW | 18 | –0.098 | 0.042 | .021 | 0.954 | 0.001 (*P* = .929) | 8.964 (*P* = .941) | 8.956 (*P* = .915) |
| CD127 on CD28- CD8br | IVW | 20 | –0.132 | 0.045 | .0035 | 0.203 | –0.027 (*P* = .106) | 24.589 (*P* = .174) | 21.181 (*P* = .270) |
| BAFF-R on CD20- | IVW | 14 | 0.097 | 0.047 | .040 | 0.897 | –0.005 (*P* = .834) | 7.631 (*P* = .866) | 7.585 (*P* = .816) |
| CCR7 on naive CD4+ | IVW | 24 | –0.055 | 0.027 | .043 | 0.494 | –0.003 (*P* = .776) | 22.334 (*P* = .500) | 22.250 (*P* = .445) |
| CD20- CD38- %lymphocyte | IVW | 25 | –0.094 | 0.035 | .007 | 0.570 | 0.008 (*P* = .563) | 22.594 (*P* = .543) | 22.250 (*P* = .505) |

IVW = inverse variance weighted, MD = Meniere disease, MR = Mendelian randomization, MR-PRESSO = model residual permutation test for outliers and small samples, SE = standard error, SNP = single nucleotide polymorphism.

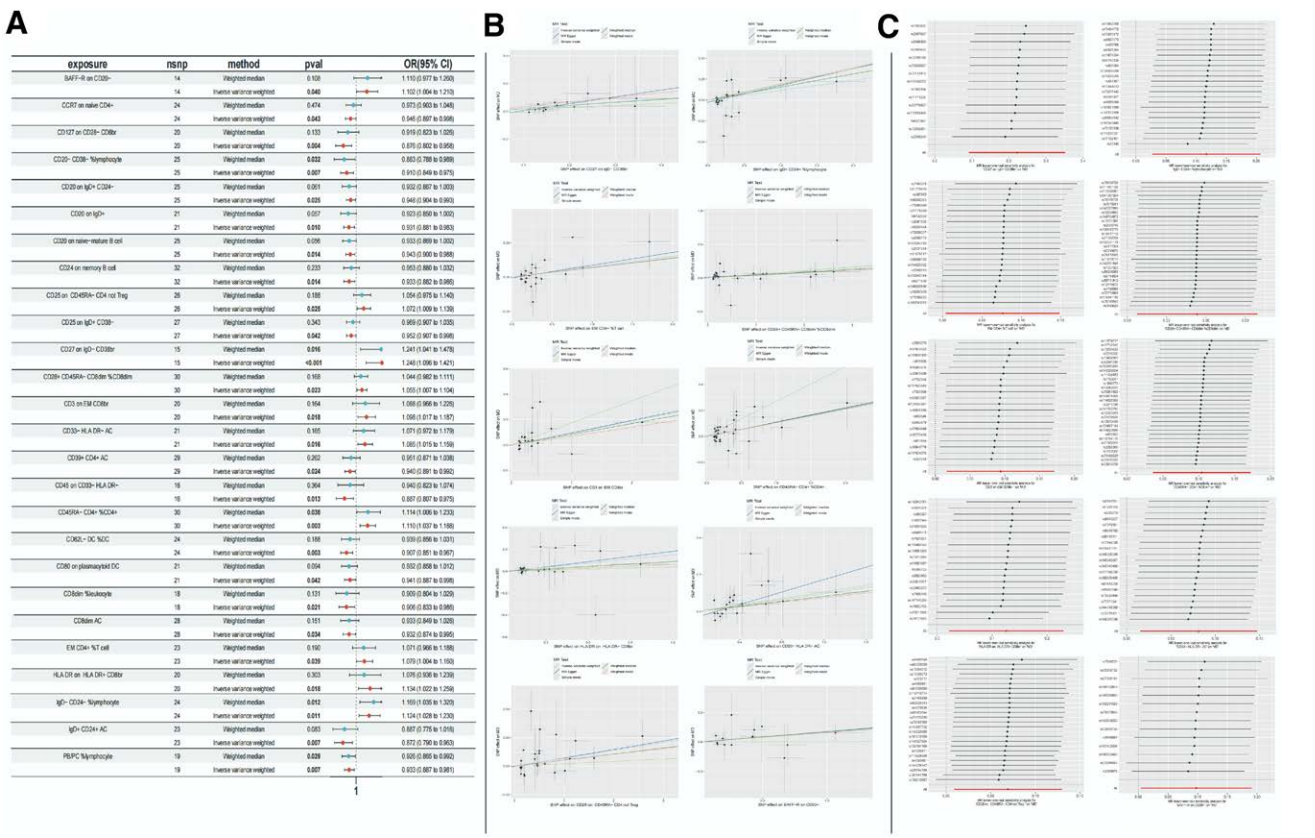


**Figure 7.** Mendelian randomized forest map of immune cells and MD (A), 10 types of immune cells with positive correlation MR scatter plot (B), results of leave-one-out sensitivity analysis of 10 types of immune cells (C). MD = Meniere disease, MR = Mendelian randomization.

relationship between vestibular function in MD and immune inflammatory responses; localized inflammatory reactions may serve as the underlying cause of MD.[29] Additionally, inflammatory factors have a causal relationship with MD, and compared to healthy individuals, MD patients demonstrate elevated levels of inflammatory cytokines.[5,30,31] This aligns with our enrichment analysis, as the balanced differentiation of T cells is crucial for both the immune response and host protection in patients.[32] Th17 cells are implicated in the pathogenesis of autoimmune diseases,[33,34] and modulation of Th17 cell activity can alleviate various associated inflammatory conditions.[35,36] Antigen binding serves as a fundamental mechanism underpinning adaptive immune responses.[37] Numerous clinical observations and systematic reviews have established a link between MD and conditions such as allergies, asthma, and intestinal inflammation,[38,39] which is consistent with our findings.

In summary, CD5 and AJUBA may be important pathogenic genes in the pathogenesis of MD. T cells CD4 memory resting, T cells gamma delta and Dendritic cells activated may be the key immune cells in the development of MD.

By screening, we identified the core genes CD5 and AJUBA, which play a pivotal role in the development and progression of MD. These 2 essential genes are significantly involved in the maturation of the immune system, T cell differentiation, and the regulation of immune tolerance.[40,41] CD5 is a cell surface protein expressed predominantly on T cells and a subset of B cells (specifically the B1a subgroup). It plays a significant role in the production of reactive antibodies and the regulation of immune responses.[42] CD5 not only guides T cells to maintain immunotherapy responses,[43] but is also associated with B cell-mediated immunosuppression[44] and involved in inflammatory responses.[45] AJUBA is a protein-coding gene involved in a variety of cellular processes, gene expression regulation, and chromatin remodeling, including cell adhesion, migration, and proliferation. There are few studies on AJUBA, which can regulate apoptosis,[46] has a certain correlation with Hippo signaling pathway,[47] and can regulate inflammatory factors.[48] The Hippo signaling pathway has a notable correlation with hearing, serving as a critical regulatory mechanism for the growth, differentiation, and regenerative capacity of cochlear sensory epithelial cells.[49] Modulating the Hippo signaling pathway can enhance cell proliferation and restore hearing, while also preventing damage to cochlear hair cells.[49,50] Thus, the core genes, CD5 and AJUBA, may play significant roles in the onset and progression of MD.

We screened 3 types of core immune cells that predicted MD by CIBERSORT combined with Lasso regression. It was found that T cells CD4 memory resting, T cells gamma delta and Dendritic cells activated may have a certain correlation with MD. T cell activation can be used to diagnose and evaluate the therapeutic response of various inflammatory and immunomedulatory diseases,[51] is associated with chronic inflammation,[52,53] and may affect the central nervous system of the brain through immune aging and inflammation.[54,55] Dendritic cells can induce inflammatory cell response through ligands,[56] regulate allergic inflammation,[57] and have a certain correlation with hearing.[58] We further explored the causal relationship between the 2 from the perspective of genetics. MR Analysis found that 26 kinds of immune cells had causal relationship with MD, 5 kinds had positive correlation and 9 kinds had negative correlation. Unfortunately, reverse MR Analysis showed that there was no causal relationship between the 2.

Although this study further elucidates the potential connection between immune responses and the pathogenesis and progression of MD, providing a reference for exploring immunotherapeutic targets to delay or prevent the progression of MD. However, this study has certain limitations. First, the population is restricted to individuals of European descent, making it impossible to further analyze factors such as gender and age, which may introduce some bias and limit the generalizability of the findings. Second the study lack of protein level verification for characteristic gene screening. All of these may have contributed to the inaccuracy of the results of this study. In the future, based on our conclusions, we will apply for relevant funding to conduct validations through clinical trials, and at the animal and cellular levels, to further investigate the underlying mechanisms of MD.

## 5. Conclusion

This study explores potential biomarkers and potential causal relationships of MD through bioinformatics, machine learning, and Mendelian randomization analysis. CD5 and AJUBA may serve as potential biomarkers of MD, while T cells CD4 memory resting, T cells gamma delta and Dendritic cells activated may represent the core immune cells involved in MD. Mendelian randomization analysis has identified causal relationships between 26 types of immune cells and MD. These findings further elucidate the potential mechanisms of interaction immune cells and MD, offering new insights into the complex mechanisms underlying the occurrence and development of MD. This research provides a novel perspective for MD treatment, aiding in early diagnosis, screening, and prognostic decision-making.

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