Bioinformatics-Based Characterization of Key Biomarkers and Immune Infiltration in Coronary Artery Disease

LiuGang Xu (xuliugang1994@163.com)
Zhangjiagang Hospital of Traditional Chinese Medicine Affiliated to Nanjing University of Traditional Chinese Medicine https://orcid.org/0000-0002-9060-9535

JianYu Jiang
Zhangjiagang Hospital of Traditional Chinese Medicine Affiliated to Nanjing University of Traditional Chinese Medicine

YaJun Wang
Zhangjiagang Hospital of Traditional Chinese Medicine Affiliated to Nanjing University of Traditional Chinese Medicine

YiSi Shan
Zhangjiagang Hospital of Traditional Chinese Medicine Affiliated to Nanjing University of Traditional Chinese Medicine

JinHu Zhang
Zhangjiagang Hospital of Traditional Chinese Medicine Affiliated to Nanjing University of Traditional Chinese Medicine

Li Yang
The Affiliated Hospital of Nanjing University of Traditional Chinese Medicine: Traditional Chinese Medicine Hospital of Kunshan

RuiHong Cao
Zhangjiagang Hospital of Traditional Chinese Medicine Affiliated to Nanjing University of Traditional Chinese Medicine

Research Article

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Abstract

Objective: This paper aims to identify potentially related genes of coronary artery disease (CAD) and determine the relationship with immune cell infiltration.

Materials and methods: Three datasets (GSE42148, GSE98583, GSE12288) containing coronary heart disease and healthy people are downloaded from the Gene Expression Database (GEO). Use the "limma" package in the "R" software to screen the differentially expressed genes (DEGs) in the three sets of data respectively, use the "pheatmap" package to construct a heatmap of the DEGs, and draw the venn maps of the three sets of differential genes. The DAVID database is used for the analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The String platform and Cytoscape software are used to perform protein interaction analysis on differential genes, create Protein-Protein Interaction (PPI) networks on DEGs, and screen hub genes. The CIBERSORTx web version tool performs immune cell infiltration analysis on sample data sets.

Results: GSE42148 screened out 227 differential genes, of which 161 were up-regulated and 66 were down-regulated ($p<0.05$, $|logFC|>1.0$); GSE98583 screened out 254 differential genes, of which 141 were up-regulated and 113 were down-regulated ($p<0.05$, $|logFC|>1.0$). 68 differential genes were screened in GSE12288, of which 33 were up-regulated and 35 were down-regulated ($p<0.05$, $|logFC|>0.38$). There are 8 differential genes in the intersection of the three groups of DEGs, namely MAP7, RIPK4, BAALC, CA6, CXCL14, HIST1H2AE, MS4A3, GPR15. With the help of enrichment analysis and the construction of PPI networks, HIST1H2AE and CXCL14 were finally determined as the key biomarkers of CAD. Immune infiltration analysis suggests that B cells naive, macrophages M0 and T cells CD4 naïve are closely related to the pathogenesis of CAD.

Conclusion: HIST1H2AE and CXCL14 can be used as key biomarkers of CAD. Inflammation and immune cell infiltration play a key role in the occurrence and development of CAD.

Introduction

Cardiovascular disease has accounted for one third of the global death toll, and coronary heart disease is the main factor in cardiovascular disease [1]. Compared with no-CAD patients, elderly patients with CAD have higher cardiovascular recurrence events and all-cause mortality [2]. At present, coronary angiography has become the "gold standard" for diagnosing coronary heart disease, but as an invasive examination item, coronary angiography is not suitable for early coronary atherosclerosis. Early diagnosis of CAD can better help patients control risk factors [3] and reduce all-cause mortality and recurrence rates.

With the development of genomics, gene expression profile data is increasingly used to discover biomarkers related to coronary heart disease [4]. Microarray analysis of peripheral blood cells can not only reveal genetic variation, but also reflect the activity of the disease, the apparent regulation of cells, and the sensitivity of treatment. CAD is considered as a genetic disease [4, 5, 6], involving multiple

pathogenic mechanisms, manifested as coronary artery lumen involvement, which involves innate and adaptive immune inflammatory mechanisms [7, 8], Inflammation is the central stage of atherosclerosis [9], leading to plaque rupture and thrombosis. Some studies have shown [10, 11] that multiple genes are involved in the occurrence and progress of CAD. However, few bioinformatics methods have been used to study the mRNA regulation of inflammation and immune microenvironment in CAD.

In this study, three datasets (GSE42148, GSE98583, GSE12288) containing coronary heart disease and healthy people were downloaded from the Gene Expression Database (GEO). Use R to screen DEGs. Then use gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) [12] to annotate the biological processes (BP), cell components (CC), molecular functions (MF) and signal pathways involved in the three groups of DEGs. The Protein-Protein Interaction (PPI) Networks is used to visualize the interaction of DEGs and screen out important gene modules [13], and the key genes filtered out are HIST1H2AE and CXCL14. Finally, we use the deconvolution algorithm [14] to visualize the immune landscape representing the data set, reflecting the inflammation and immune microenvironment. HIST1H2AE and CXCL14 may be potential biomarkers for the prospective diagnosis and targeted therapy of coronary heart disease.

Materials And Methods

Data:

The gene expression profile data comes from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). We searched all data sets containing the "CAD" and "mRNA" fields, and selected three Data sets GSE42148, GSE98583 and GSE12288 comparing coronary artery disease and healthy patients. GSE42148 is composed of data from 13 patients with coronary artery disease and 11 healthy controls. GSE98583 contains data on 6 patients with normal coronary angiography and 12 patients with confirmed coronary artery disease. There are a total of 110 CAD patients (CADi>23) and 112 healthy controls (CADi=0) patients in GSE12288. In the end, there were 135 CAD cases and 129 healthy control cases. All data were standardized, and differential genes were screened separately. Table 1 shows detailed information on the three mRNA expression datasets (Table 1).

Table 1

Details of the selected data set

Dateset	Platform	Organism	Experiment type	Tissue	Samples (Case vs Control)	Year
GSE42148	GPL13607	Homo sapiens	Expression profiling by array	Asian Indians	13:11	2012
GSE98583	GPL571	Homo sapiens	Expression profiling by array	North Indian	12:6	2017
GSE12288	GPL96	Homo sapiens	Expression profiling by array	Switzerland	110:112	2008

Data Processing And Degs Determination:

GSE42148 is based on GPL13607 (Agilent-028004 SurePrint G3 Human GE 8x60K Microarray [Feature Number version]) platform, and GSE98583 is based on GPL571 (Affymetrix Human Genome U133A 2.0 Array [HG-U133A_2]) platform. GSE12288 is based on GPL96 ([HG-U133A] Affymetrix Human Genome U133A Array) platform. All data sets are standardized, and probes that do not correspond to gene symbols are deleted, and the gene symbols corresponding to multiple probes are combined with the maximum value. The "limma" package in R software was used to screen the differentially expressed genes between patients with coronary artery disease and normal controls. $P<0.05$ and $|logFC|>1.0$ in GSE42148 and GSE98583 were considered as cut-off criteria. In GSE12288, $p<0.05$ and $|logFC|>0.38$ are considered as truncation standards. Then we use the "ggplot2" and "heatmap" packages in the R software to draw volcano plots and heatmaps to visualize the differentially expressed genes in the three sets of data. We also drew a Venn diagram to visualize the intersection of the three sets of DEGs.

Go Annotation And Kegg Pathway Enrichment Analysis:

Use the DAVID website (https://david.ncifcrf.gov/) to perform GO annotation and KEGG pathway analysis on DGEs. DAVID is a biological information resource website that integrates biological information databases and analysis tools, which can perform gene ontology annotations and Kyoto genes and genome analysis of target differential genes. GO annotation analysis mainly includes biological processes (BP), cell components (CC), and molecular functions (MF). KEGG is an integrated database resource for biological interpretation of large-scale molecular data sets. We summarized and analyzed the DEGs selected by the three data sets and we visualized them with the ggplot package.

Ppi Networks Construction:

Use the online network tool STRING (https://string-db.org/) to analyze protein interactions and create PPI networks on DEGs. Cytoscape (3.8.2) is a software that is suitable for biomolecular interaction networks and visually integrates the network with expression profiles, phenotypes, and other molecular states.

Immune Infiltration:

CIBERSORTx (https://cibersortx.stanford.edu/) is a web-based tool for deconvolution analysis of standardized chip expression matrix based on linear support vector regression. We obtained 22 kinds of immune cell infiltration ratios by running on the standardized GSE12288 data set and visualized the results. The gene expression profile matrix is uploaded to CIBERSORTx, and the Permutations for significance analysis is set to 1000 times, and the result of immune cell distribution is obtained. We also evaluated the differences in the distribution of immune cells between normal specimens and CAD specimens, and the Wilcoxon test results were used to analyze differences between groups.

Result

Determination of DEGs in CAD samples

After standardizing the data sets of GSE42148, GSE98583 and GSE12288, we screened the differential genes for the three data sets. The results showed that GSE42148 screened out 227 DEGs (Figure 2(a)), of which 161 were up-regulated and 66 were down-regulated ($p<0.05$, $|logFC|>1.0$); GSE98583 screened out 254 DEGs (Figure 2(b)), of which 141 were up-regulated and 113 were down-regulated ($p<0.05$, $|logFC|>1.0$). 68 DEGs were screened in GSE12288 (Figure 2(c)), of which 33 were up-regulated and 35 were down-regulated ($p<0.05$, $|logFC|>0.38$). Draw volcano plots on the three sets of data. Three heatmaps (Figure 3) is drawn based on the top 50 DGEs with the largest change in each group. The intersection of the three groups of DEGs has a total of 8 differential genes, which are MAP7, RIPK4, BAALC, CA6, CXCL14, HIST1H2AE, MS4A3, GPR15, and the results are visualized by Venn diagrams (Figure 1(d)).

Go Annotation And Kegg Analysis:

The top five GO terms are shown in Table 2, and the top five GO terms are shown in Figure4(a)-4(c) according to the gene count. The GO annotation results of DEGs: the biological processes of DEGs are mainly enriched in signal transduction, positive regulation of transcription from RNA polymerase II promoter, positive regulation of cell proliferation, negative regulation of transcription from RNA polymerase II promoter, cell adhesion. The cellular components of DEGs are enriched in nucleus, integral component of plasma membrane, extracellular space, extracellular region, cell surface. The molecular functions of DEGs are enriched in transcription factor activity, sequence–specific DNA binding, sequence–specific DNA binding, RNA polymerase II core promoter proximal region sequence–specific DNA binding, protein homodimerization activity, and metal ion binding. The top five KEGG terms of DEGs based on the gene count are shown in Table 3 and Figure 4(d). The results of KEGG enrichment analysis of DEGs are Serotonergic synapse, Neuroactive ligand–receptor interaction, Glycine, serine and threonine metabolism, Fat digestion and absorption, and Cocaine addiction.

Table 2

Significantly enriched GO terms and KEGG pathways of DEGs.

Category	Term	Description	Count	P value
BP	GO:0045944	positive regulation of transcription from RNA polymerase II promoter	41	7.07E-04
BP	GO:0007165	signal transduction	40	2.06E-02
BP	GO:0008284	positive regulation of cell proliferation	27	6.05E-05
BP	GO:0000122	negative regulation of transcription from RNA polymerase II promoter	27	2.47E-02
BP	GO:0007155	cell adhesion	21	7.46E-03
CC	GO:0005634	nucleus	144	3.37E-02
CC	GO:0005615	extracellular space	63	1.46E-07
CC	GO:0005576	extracellular region	59	4.83E-04
CC	GO:0005887	integral component of plasma membrane	56	1.04E-04
CC	GO:0009986	cell surface	21	2.79E-02
MF	GO:0046872	metal ion binding	60	7.16E-02
MF	GO:0003700	transcription factor activity, sequence-specific DNA binding	35	1.25E-02
MF	GO:0042803	protein homodimerization activity	30	4.56E-03
MF	GO:0043565	sequence-specific DNA binding	21	2.22E-02
MF	GO:0000978	RNA polymerase II core promoter proximal region sequence-specific DNA binding	19	2.06E-03

Table 3
Significantly enriched KEGG pathways of DEGs.

Category	Term	Description	Count	P value
Pathway	hsa04080	Neuroactive ligand-receptor interaction	11	9.17E-02
Pathway	hsa04726	Serotonergic synapse	7	3.78E-02
Pathway	hsa00260	Glycine, serine and threonine metabolism	4	5.59E-02
Pathway	hsa04975	Fat digestion and absorption	4	5.59E-02
Pathway	hsa05030	Cocaine addiction	4	9.62E-02

Ppi Networks Construction And Analysis Of Important Modules

We constructed a PPI network to identify the central gene module and used Cytoscape (3.9.0) software to visualize the screening results. The PPI network constructed by all DEGs contains a total of 357 nodes and 774 edges. The MCODE plug-in is used to identify significant gene modules. Set Degree Cutoff=2, Node Score Cutoff=0.2, K-Core=2, Max Depth=100. We screened out the gene modules with a score of 4.0 from the PPI networks. There are four groups, namely 9.556 (Figure 5(a)), 9.00 (Figure 5(b)), 4.993 (Figure 5(c)) and 4.50 (Figure 5(d)). The cytoHubba application in Cytoscape was used to find the hub gene. According to the intersection results of the PPI network and the three groups of DEGs, we noticed that HIST1H2AE and CXCL14 were intersected many times.

Immune Infiltration Analysis

We obtained the distribution ratio of immune cells in 112 normal samples and 110 CAD samples, and compared the difference in the abundance of immune cells between the two groups. The proportion of B cells naive (p=0.023) and Macrophages M0 (p=0.004) in CAD samples was significantly higher than that in normal samples, while the proportion of T cells CD4 naive (p=0.024) in CAD samples was lower than that in normal samples. These results suggest that the immune inflammatory microenvironment is involved in the pathological process of CAD.

Discussion

Coronary artery disease is a chronic disease with high mortality and high disability [15]. Hypertension, dysglycemia and dyslipidemia, smoking, obesity, gender, and stress are all independent risk factors [7, 16,

17, 18, 19], and these risk factors are important reasons for the early occurrence and development of CAD. In this study, we specially selected the data of patients with early CAD lesions, used microarray method to analyze the differences in gene expression profiles in the peripheral blood of patients with coronary artery stenosis, and determined the difference genes in the CAD population compared with healthy people. Three groups of up-regulated and down-regulated differential genes were enriched by GO annotation and KEGG pathway analysis. We use the PPI networks to find key modules and identify key genes. GSE12288 contains 112 normal samples and 110 coronary artery stenosis samples. The immune infiltration analysis of these samples revealed that inflammation and immune mechanisms are involved in the development of coronary artery disease.

The intersection of the three sets of differential genes has a total of 8 genes, namely MAP7, RIPK4, BAALC, CA6, CXCL14, HIST1H2AE, MS4A3, GPR15. These genes have become candidates for key biomarkers of coronary heart disease. The construction of the PPI networks further refined the differential genes, and finally two hub genes (HIST1H2AE, CXCL14) were obtained. HIST1H2AE belongs to histone cluster and is the core component of nucleosomes. Nucleosomes wrap and compress DNA into chromatin, limiting DNA's accessibility to cellular machinery that requires DNA as a template. Therefore, histones play a central role in transcriptional regulation, DNA repair, DNA replication, and chromosome stability. The interaction between histone post-translational modifications and dynamic nucleosomes is related to the aging of the cardiovascular system [20], and the occurrence of atherosclerosis is often also related to this [21]. Another study showed that HIST1H2AE may be related to coronary artery disease in different populations [22]. CXCL14 is a relatively new chemokine that helps regulate immune cell migration [23]. CXC14 has pleiotropic functions in mammals [24]. CXCL14 has chemotactic activity on activated macrophages, immature dendritic cells and natural killer cells [25, 26]. In addition, some studies have shown that CXCL14 is involved in feeding. Behavior-related neuronal circuits, glucose metabolism, and antimicrobial defenses. Although few studies have shown that CXCL14 is directly related to coronary artery disease, there is still some evidence that there is a potential link between CXCL14 and coronary atherosclerosis. CXCL14 has a natural antagonistic effect on the CXCL12/CXCR4 axis through competitive binding [27], and the latter has been confirmed to be related to the pathogenesis of atherosclerosis [28]. In addition, CXCL14 can participate in thrombosis and platelet migration through its direct interaction with CXCR4 [29], which may be related to the formation of coronary thrombosis.

According to our pathway enrichment results, Serotonergic synapse, Neuroactive ligand–receptor interaction, Glycine, serine and threonine metabolism, Fat digestion and absorption, Cocaine addiction, etc. were significantly enriched. Although the Serotonergic synapse pathway has not been reported to have an impact on the cardiovascular system, studies have pointed out that Serotonergic synapse can cause the activation of the NF-κB pathway [30]. The increase in endothelial inflammation and atherosclerosis is closely related to the activation of the NF-κB pathway. [31]. Glycine and threonine metabolism levels are closely related to oxidative stress and inflammation [32]. A study [33] showed that the increased level of fat digestion and absorption would aggravate the inflammation of adipose tissue and significantly increase the area of atherosclerosis. The above studies provide evidence for the role of inflammation and immune response in the physiological process of coronary heart disease.

New anti-inflammatory pathways linking lipids to inflammatory immunology can reveal the mutations involved in coronary heart disease. The immune infiltration analysis we selected showed a significant increase in B cells naive and Macrophages M0 in CAD samples, and a significant decrease in T cells CD4 naïve. The polarized M1 and M2 phenotypes of monocyte-derived macrophages M0 have pro-inflammatory and anti-inflammatory effects, respectively, and maintain the inflammatory mechanism in the pathological process of coronary heart disease. In addition, the increased macrophage M0 through arterial macrophage accumulation and lipoprotein deposition produces foam cells [34], which is conducive to lipid precipitation and plaque growth [34, 35]. In the process of atherosclerosis, it activates the adaptive T cell process, which makes T cells CD4 naïve differentiate into effector cells and/or memory cells of specialized T cell subsets. Studies have shown that people who usually suffer from atherosclerosis T cells CD4 naïve tend to decrease, on the contrary, effector T cells or memory cells will increase [36, 37]. These studies show that inflammation and immune mechanisms play a key role in the occurrence and development of coronary heart disease. In addition, B cell naive is also increased in CAD samples, but there is no clear research evidence that B cell naive is related to atherosclerosis. It is still necessary to further explore the mechanism between B cells naive and CAD. B cells naive, macrophages M0 and T cells CD4 naïve may have a complicated relationship with HIST1H2AE and CXCL14. More studies are needed to reveal the potential role of these genes on the immune system in coronary atherosclerosis.

Conclusion

In summary, we screened out 541 DEGs and 8 candidate key genes, and further identified HIST1H2AE and CXCL14 as key genes and diagnostic markers of CAD. Pathway enrichment analysis provides more ideas for understanding the pathophysiological mechanism of CAD. Immune infiltration analysis suggests that B cells naive, macrophages M0 and T cells CD4 naïve are closely related to the pathogenesis of CAD. The role of key genes in CAD on the immune system should be further studied.

Abbreviations

CAD
Coronary Artery Disease
GEO
Gene Expression Database
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
PPI
Protein-Protein Interaction
BP

Biological Processes
CC
Cell Components
MF
Molecular Functions
DEGs
Differentially Expressed Genes

Declarations

Ethics approval and consent to participate

Data were obtained from the GEO public dataset. Ethics committee approval and informed consent statement were not required for this study.

Consent for publication

Not applicable.

Availability of data and materials

The gene expression profiles of GSE42148 were downloaded from Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42148).

The gene expression profiles of GSE98583 were downloaded from Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE98583). The gene expression profiles of GSE98583 were downloaded from Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE12288).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceived and designed the experiments: LGX, JYJ, RHC. Data collection and/or processing: LGX, YJW, YSS. Writing: LGX, RHC. Critical review: YSS, JHZ, LY.

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Figures

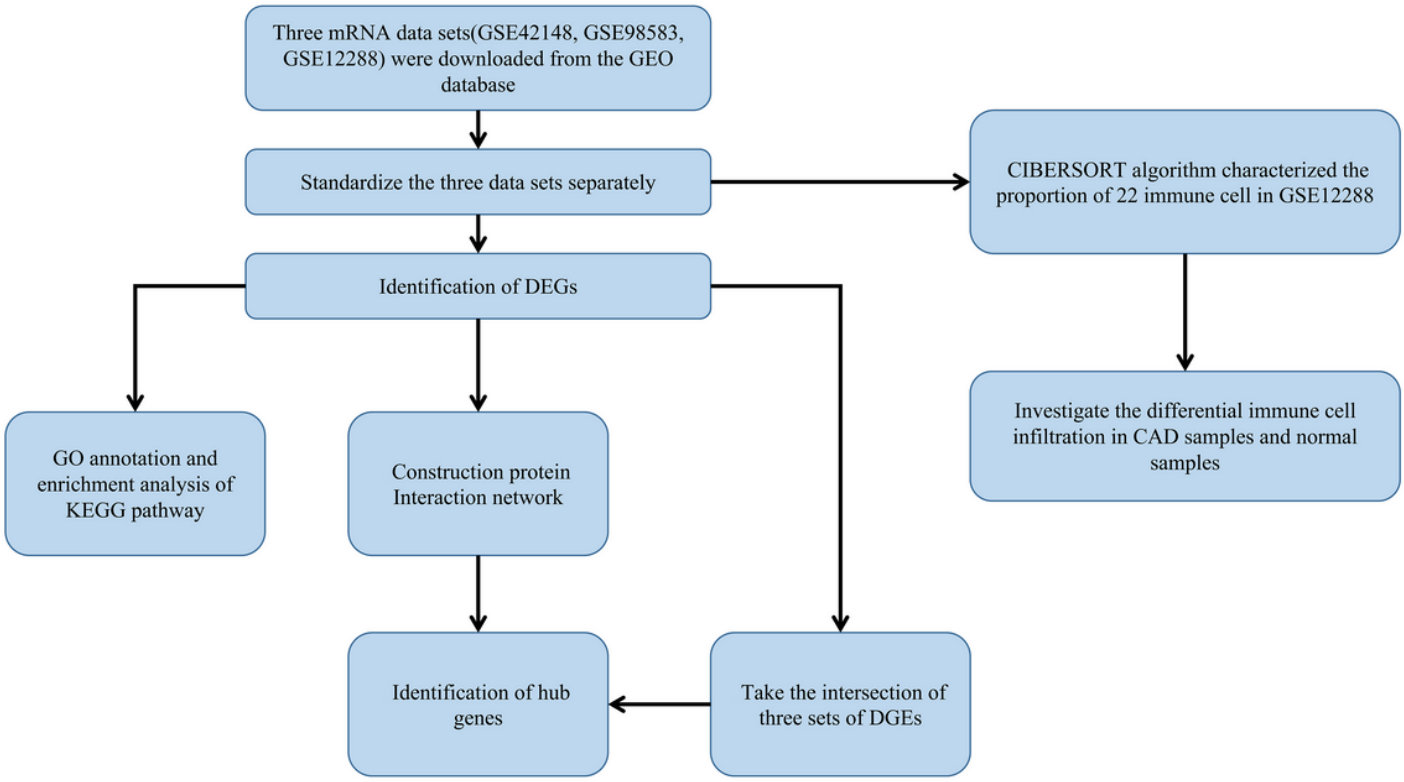

Figure 1

The workflow of this study

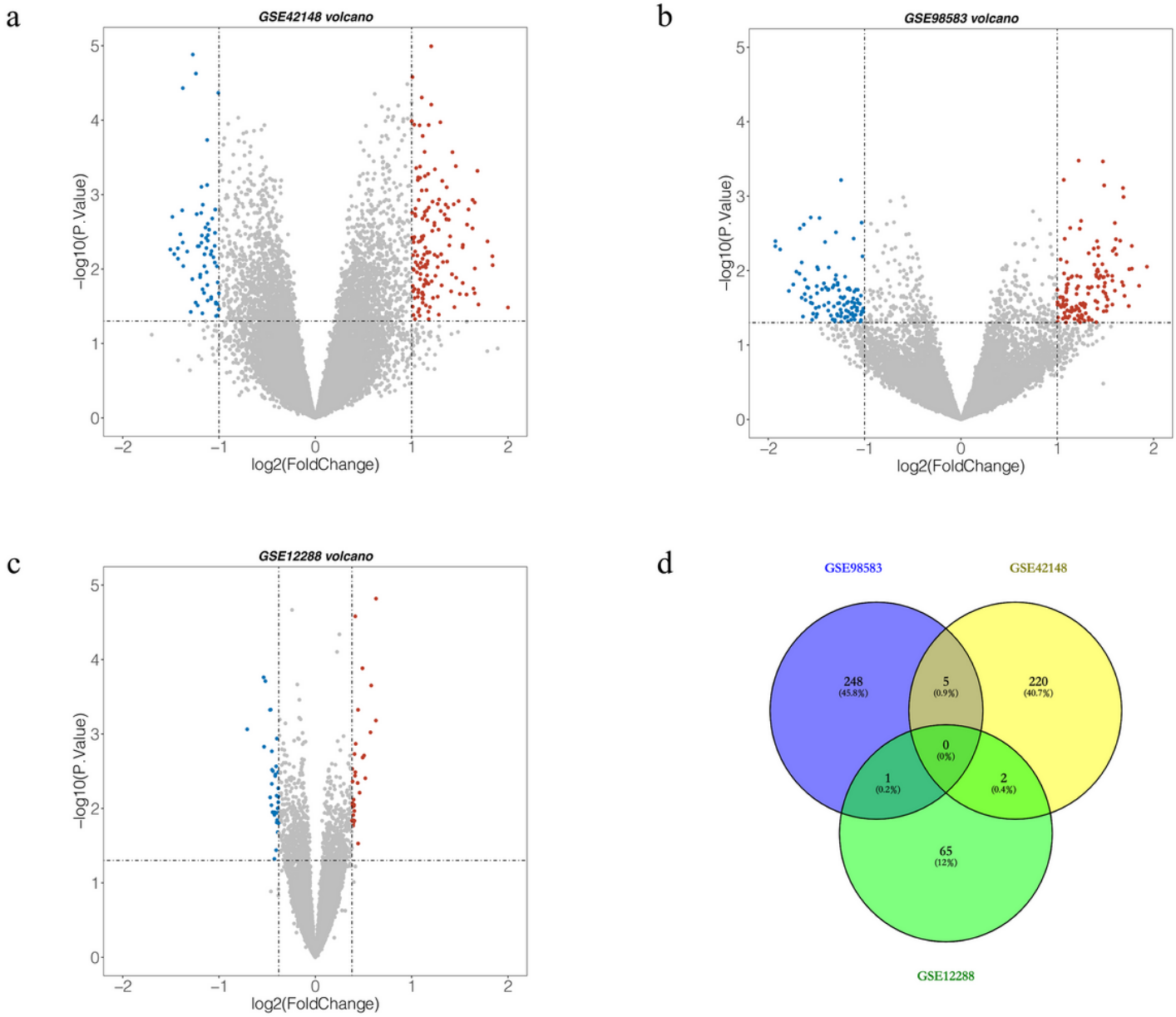

Figure 2

Identify DEGs from three human data sets. (a-c) red represents up-regulated, blue represents down-regulated. (a) Volcano plot of 227 DEGs in GSE42148, of which 161 were up-regulated and 66 were down-regulated. (b) Volcano plot of 254 DEGs in GSE98583, of which 141 were up-regulated and 113 were down-regulated. (c) Volcano plot of 68 DEGs in GSE42148, of which 33 were up-regulated and 35 were down-regulated. (d) The Venn diagram of three sets of DEGs.

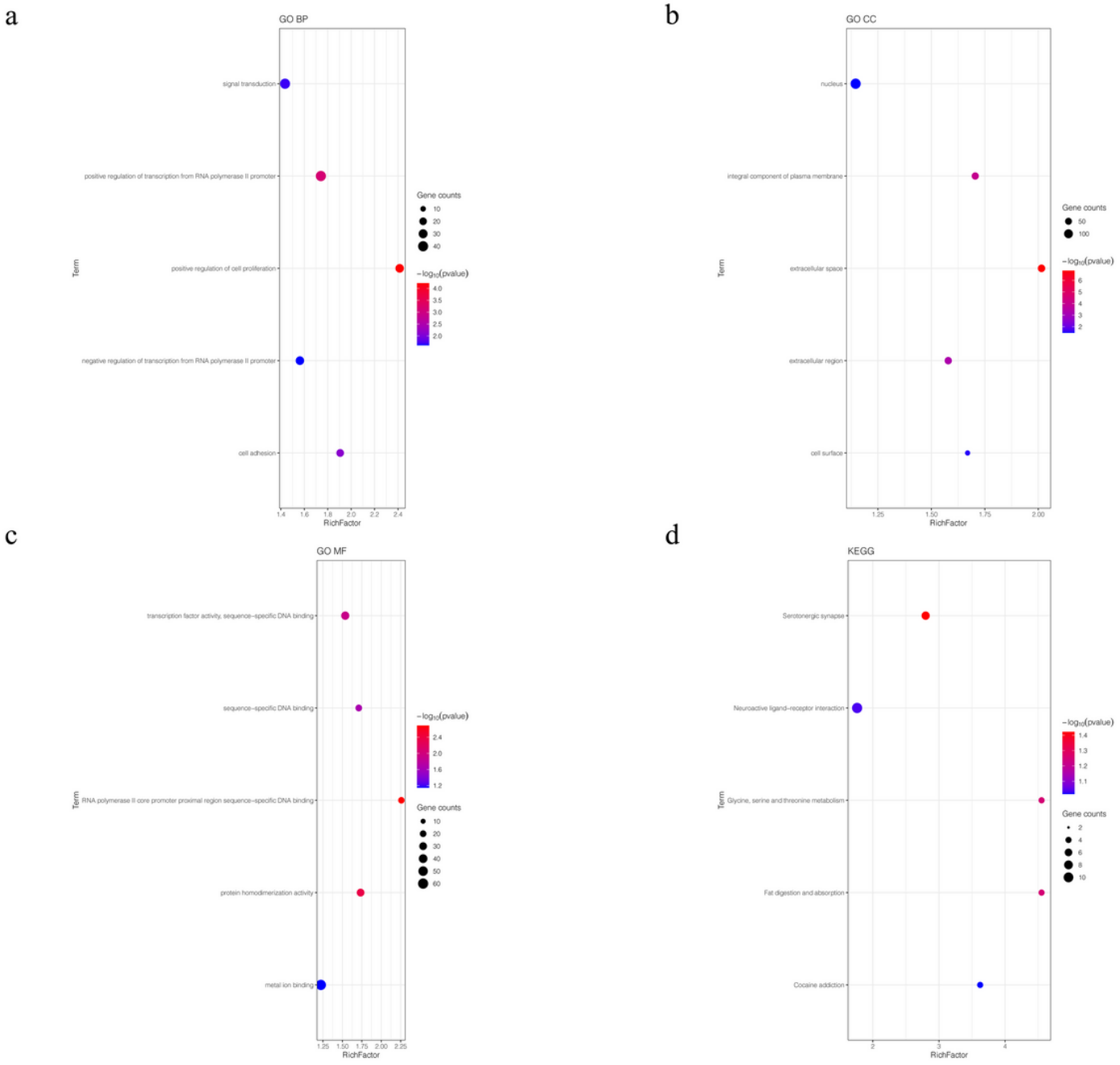

Figure 4

Top 5 enriched GO terms and top 5 KEGG pathways of differentially expressed genes. (A-C) GO term enrichment analysis for (a) biological process, (b) cellular component, (c) molecular function. (d) KEGG pathway analysis. Node size represents gene count; node color represents P-value. GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes

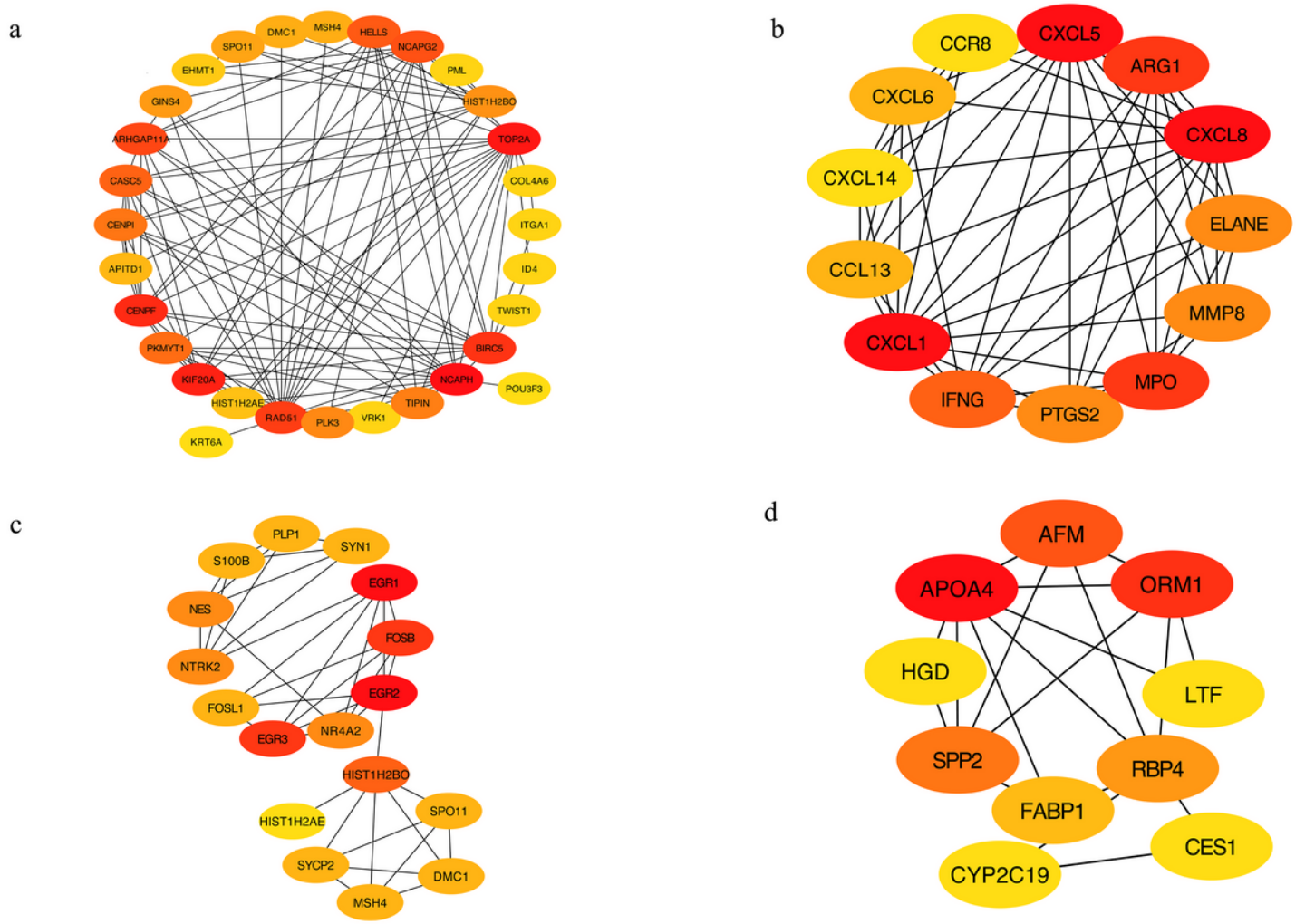

Figure 5

Construction of the PPI networks with four different scores. The nodes represent proteins, and the edges represent the interaction of proteins. The vividness of the color represents the score. (a) PPI network with a score of 9.556. (b) PPI network with a score of 9.00. (c) PPI network with a score of 4.993. (d) PPI network with a score of 4.50.

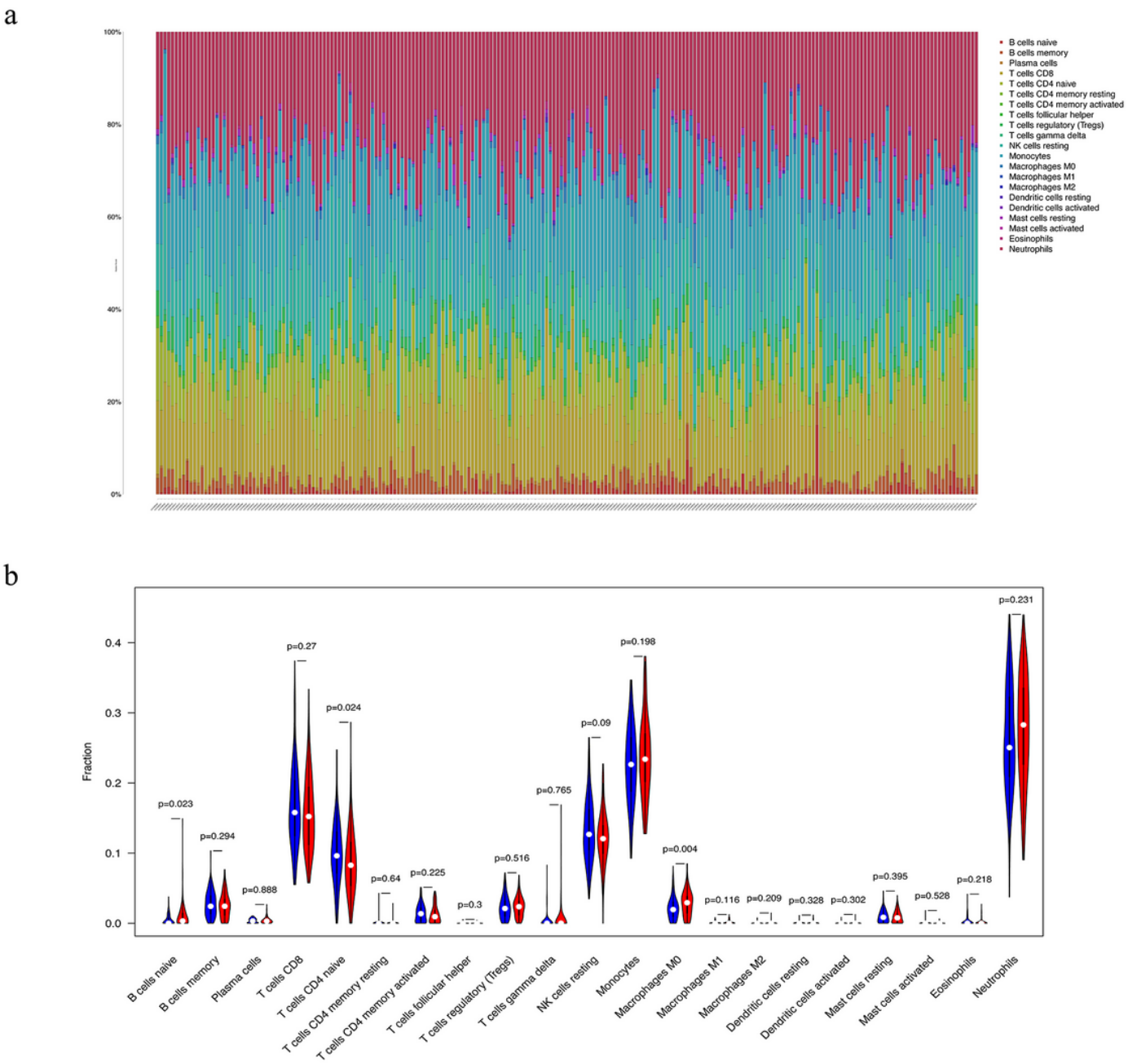

Figure 6

Immune Infiltration Analysis of GSE12288. (a) The bar plot visualizing the relative percent of 22 immune cell in each sample. Different colors represent different types of immune cells. (b) The difference of immune infiltration between CAD samples and normal control samples. Blue, normal controls group; Red, CAD group. CAD, Coronary Artery Disease

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GSE12288nrDEG.fc.csv
- GSE42148nrDEG.fc.csv
- GSE98583nrDEG.fc.csv