Integrated analysis for identification of key immune related genes and patterns of immune infiltration in calcified aortic valvular disease

Li-Da Wu
Wuxi medical college, Jiangnan University, China
Feng Xiao
Wuxi People's Hospital Affiliated to Nanjing Medical University
Jin-Yu Sun
The First Affiliated Hospital of Nanjing Medical University
Feng Li
Wuxi People's Hospital Affiliated to Nanjing Medical University
Yu-Jia Chen
Wuxi People's Hospital Affiliated to Nanjing Medical University
Jia-Yi Chen
Wuxi People's Hospital Affiliated to Nanjing Medical University
Jie Zhang
Wuxi People's Hospital Affiliated to Nanjing Medical University
Ling-Ling Qian
Wuxi People's Hospital Affiliated to Nanjing Medical University
Ru-Xing Wang (ruxingw@aliyun.com)
Wuxi medical college, Jiangnan University, China

Article

Keywords: Immune-related genes, Calcific aortic valve disease, Immune cells, Immune infiltration, CIBERSORT

Posted Date: May 31st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1649950/v1

Abstract

Background: As the most prevalent valvular heart disease, calcific aortic valve disease (CAVD) has become a primary cause of aortic valve stenosis and a major health problem. We aimed to illustrate roles of immune related genes (IRGs) and immune infiltration in pathogenesis of CAVD.

Methods: We used Integrative meta-analysis of expression data (INMEX) to incorporate multiple gene expression datasets related to CAVD. By matching the differentially expressed genes (DEGs) to IRGs from ImmPort database, differentially expressed immune related genes (DEIRGs) were screened out. Biological functional enrichment analysis were conducted based on GO database, KEGG database, DisGeNET database and TRRUST database. Then, we constructed protein–protein interaction (PPI) network and identified the key DEIRGs of CAVD according to the mixed character calculation results. Moreover, CIBERSORT was performed to explore the patterns of immune cells infiltration in CAVD. Finally, using Spearman method, correlation analysis between key IRGs and infiltrating immune cells was conducted.

Results: 220 DEIRGs were identified, and the enrichment analysis of DEIRGs showed that they were significantly enriched in inflammatory responses. PPI network was constructed based on DEIRGs and *PTPN11*, *GRB2*, *SYK*, *PTPN6* and *SHC1* were identified as key IRGs. Compared with normal aortic valve tissue samples, the proportion of neutrophils, T cells CD4 memory activated and macrophages M0 was significantly elevated in calcified aortic valves tissue samples, as well as reduced infiltration of monocytes and NK cells activated. Furthermore, IRGs identified in the present study, including *PTPN11*, *GRB2*, *PTPN6*, *SYK*, and *SHC1*, was significantly correlated with the infiltration of various immune cells.

Conclusions: This study suggested that *PTPN11*, *GRB2*, *PTPN6*, *SYK*, and *SHC1* might be key IRGs associated with immune infiltration, which play a pivotal role in pathogenesis of CAVD.

Introduction

With the population aging, valvular heart disease has obtained growing attention worldwide [1]. Calcific aortic valve disease (CAVD), the most common cardiovascular valve disease, has become a major reason for aortic valve stenosis and insufficiency, especially in the elderly. It is reported that over 30% of individuals beyond the age of 65 have echocardiography evidence of CAVD [2]. With the progression of CAVD, aortic valve stenosis affects almost 3% of people over 65 years of age and in nearly 8% of people over 75 years of age. The worldwide CAVD burden is projected to be 4.5 million in 2030, considering the prolonged life expectancy [3]. Currently, surgical therapy remains the only effective therapeutic method against CAVD, which is limited in terms of high costs, perioperative complications, and the complications of life-long anticoagulation therapy [4].

CAVD is a progressive disease, including three stages. Valve endothelial cells injury and lipid deposition are the main characteristics of the first stage, which are very similar to the initial pathological characteristics of atherosclerosis; in the later phase, extracellular matrix changes are initiated, primarily for bone-matrix proteins deposition, meanwhile, valve interstitial cells differentiation and

microcalcification are also promoted; finally, with activation of numerous inflammatory and ossification signaling pathways, valvular osteogenesis occurs and CAVD eventually leads to aortic valve stenosis and incomplete closure [5]. Inflammation and immunity has been found to be important in the progression of CAVD. Indeed, nearly 15% of the cells come from hematopoietic sources in the aortic valve, with the infiltration of T lymphocytes, B lymphocytes and macrophages into the valve after inflammation, this number increases greatly, so as to promote further inflammation response [6]. It is of great value to evaluate immune cells infiltration and find key genes that regulate the infiltration of immune cells for elucidating the molecular mechanism of CAVD and searching for new immunotherapy targets.

Integrative meta-analysis of expression data (INMEX) tool has been widely used in integrating gene expression profiles [7]. In this study, INMEX was used to integrate all related gene expression microarray datasets of CAVD in GEO database (GSE12644, GSE83453, and GSE51472), and to identify differentially expressed genes (DEGs). Subsequently, we screen out differentially expressed immune related genes (DEIRGs) of CAVD via matching 2,484 immune-related genes (IRGs) in ImmPort database to DEGs [8]. CIBERSORT, a widely used algorithm, could be adopted to assess infiltrating immune cells according to gene expression patterns [9]. A large number of studies have adopted CIBERSORT to evaluate immune infiltration in various diseases [10–12]. In our analysis, for the first time, CIBERSORT was used to identify the patterns of 22 infiltrating immune cells in aortic valve tissues from CAVD and normal individuals. In addition, we constructed protein-protein interaction (PPI) network and identifed the key DEIRGs, the correlation between each key DEIRGs and infiltrating immune cells was also studied.

Materials And Methods

Inclusion of eligible microarray datasets

The CAVD microarray datasets in Gene Expression Omnibus (GEO) database [13] were downloaded via "*GEO query*" package in R 3.6.3 software [14]. As shown in Table 1, all of the microarray datasets related to CAVD were included, including GSE12644 [15], GSE51472 [16], and GSE83453 [17]. For the accuracy of the present study focusing on CAVD, stenotic aortic valve tissue samples without calcification were excluded. GSE12644, based on GPL570 platform, which includes aortic valve samples from 10 patients with CAVD and 10 normal individuals [15]. GSE51472, also performed by GPL570 platform, which includes 5 valve samples from normal individuals and 5 valve samples from patients with CAVD [16]. GSE83453, based on GPL10558, which includes 10 valve samples from patients with CAVD and 8 valve samples from normal individuals [17].

Quality assessment and removal of batch effects among different datasets

Log2-transformation and background correction were performed on the expression profiles using the"*limma*" package [18]. After the normalization process, all of the microarray probes were translated to official gene names in INMEX. Because of the gene expression profiles included in the present study were based on different platforms and carried out in different experimental conditions. We used the ComBat option and visualized the principal component analysis (PCA) results using "*ggplot2*" package.

Identification of DEIRGs in CAVD

Following the PRISMA guidelines [19], INMEX was used to integrate gene expression datasets, including GSE12644, GSE51472 and GSE83453 [7]. Considering the differences in experimental design, experimental methods, and heterogeneity among different datasets, the random effect model (REM) was selected in this study according to Cochran's Q test [7]. REM method is a statistical approach that widely used for incorporating multiple gene expression datasets and screening DEGs. For multiple probes that detected a single gene in the gene chip, we use their average expression values. Pattern extractor tool in INMEX was used to construct a heatmap of the top 100 DEGs between aortic valve samples from patients with CAVD and normal individuals. By matching 2,484 IRGs from the ImmPort database to DEGs, we screened out DEIRGs in aortic valve of patients with CAVD [20].

Assessment of immune infiltration

The present study firstly used CIBERSORT algorithm to assess 22 types of immune cells infiltration in aortic valve tissue samples of CAVD [9]. CIBERSORT algorithm has been employed to evaluate immune infiltration in various diseases, such as osteoarthritis [10], high-grade serous ovarian cancer [11], and breast ductal and lobular carcinoma [12]. For the accuracy of comparison between normal valve tissues and calcified valve tissues among different datasets, the *P* value of CIBERSORT results adopted in the present study are less than 0.05. In each sample, the proportions of immune cells were visualized using R software. A correlation heatmap was created by "*corrplot*" package in R software to describe the correlation between 22 types of immune cells. For a specific type of immune cell, the difference in immune infiltration levels between aortic valve samples from patients with CAVD and normal individuals were represented by a violin plot established by "*vioplot*" package in R software.

Enrichment analysis of DEIRGs

For exploring biological functions of DEIRGs and its roles in immune infiltration in patients with CAVD, the "*clusterProfler*" package [21] was adopted to conduct GO and KEGG pathway enrichment analysis. For elucidating the correlation between enrichment terms, they were also rendered as a network plot and visualized by Metascape [22]. We also performed enrichment analysis based on DisGeNET [23] and TRRUST [24] database to validate the roles of DIREGs in CAVD.

PPI network and identification of key DEIRGs

To study the crosstalk among DEIRGs, the STRING plugin [25] was performed to construct PPI network (confidence value: 0.9, maximum number of connections: 3), which was visualized by Cytoscape software 3.8.1 [26]. Furthermore, using Cytoscape plugin software "*cytoHubba*", the top 5 hub DEIRGs were screened out based on mixed character numeration.

Correlation analysis between key DEIRGs and infiltrating immune cells

The correlation between the key DEIRGs and immune cells infiltration was analyzed using spearman method in R software, and then the package of "*ggplot2*" was employed to visualize the correlation analysis results.

Results

DEGs and DEIRGs screening between calcified and normal aortic valves

The workflow of this analysis is shown in **Figure 1.** We performed principal component analysis (PCA) to evaluate the batch effects among datasets included in the present study. In **Figure 2B**, PCA plot demonstrated that the batch effects among GSE12644, GSE83453 and GSE20681 were removed. In INMEX, random effect model was used to identify DEGs according to the adjusted P-value<0.05. 2465 DEGs were screened out, including 1306 upregulated and 1159 downregulated genes in aortic valve tissues from patients with CAVD. As shown in **Figure 2A**, the top 100 DEGs are shown in a heatmap. Then, after matching the IRGs from ImmPort database to the DEGs, 220 DEIRGs were finally selected (**Figure 2C**).

Immune infiltration analysis

Based on CIBERSORT, we firstly investigated 22 types of immune cells infiltration in aortic valve tissues from normal individuals and patients with CAVD. **Figure 3A** and **Figure 3B** vividly illustrate infiltrating immune cells in aortic valve tissue samples of 15 normal individuals and 25 patients with CAVD. Compared with normal aortic valve tissue samples, the proportion of neutrophils, T cells CD4 memory activated and macrophages M0 was elevated in calcified aortic valves tissue samples, as well as reduced infiltration of monocytes and NK cells activated ($P < 0.05$**; Figure 4A**). The results of correlation analysis among different immune cells demonstrated that NK cells resting and T cells CD8 had the strongest positive correlation (r = 0.75; **Figure 4B**). However, mast cells resting and NK cells resting had the most intensive negative correlation (r=-0.7). According to the proportion of infiltrating immune cells.

Enrichment analysis of DEIRGs

Based on GO and KEGG databases, biological functional enrichment analysis of DEIRGs in patients with CAVD was performed. **Figure 5A** shows that the biological processes were mainly enriched in positive regulation of leukocyte migration, T cell activation, response to external stimulus, cytokine production and cell chemotaxis. And the most enriched cellular components were mainly involved in vesicle lumen, membrane region, the external side of plasma membrane, membrane raft, and membrane microdomain. The molecular functions were mainly enriched in cytokine activity, cytokine binding, cytokine receptor binding, receptor-ligand activity and cytokine receptor activity. In **Figure 5B**, KEGG analysis shows that NK cell mediated cytotoxicity and cytokine to cytokine receptor interaction were most enriched, followed by JAK-STAT pathway, chemokine, tuberculosis. Moreover, the top 20 pathways and processes in KEGG enrichment analysis was shown in **Figure 5C**, such as leukocyte migration, cytokine signaling in the immune system, lymphocyte activation, myeloid lymphocyte activation, T cell receptor signaling pathway.

In addition, DisGeNET enrichment analysis revealed that DEIRGs were significantly associated with inflammation, periodontitis, infection, dermatitis and pneumonitis (**Figure 5D**). Then, we screened out transcription factors associated with DEIRGs based on the TRRUST database, including RELA, NFKB1, SP1, STAT3, and JUN (**Figure 5E**).

PPI network analysis

Figure 6A is the PPI network of DEIRGs, CytoHubba software was used to identify the top 5 key DEIRGs and its core network, including *PTPN11*, *GRB2*, *SYK*, *PTPN6*, and *SHC1* (**Figure 6B**). As can be seen in **Figure 6C,** *PTPN11* was down-regulated in all expression profiles included. Whereas *GRB2*, *SYK*, *PTPN6* and *SHC1* significantly up-regulated in aortic valve tissues from patients with CAVD compared with normal individuals (**Figure 6D-G**).

Correlation analysis of key DEIRGs and immune infiltration

Correlation analysis indicated that *PTPN11* was intensively correlated with mast cells resting (r=0.45, *P*=0.01) and obviously negatively correlated with mast cells activated (r=-0.37, *P*=0.04) and T cells CD4 naive (r=-0.42, *P*=0.02). *GRB2* had positive correlation with macrophages M0 (r=0.42, *P*=0.02) and plasma cells (r=0.4, P=0.03) and had negative correlation with T cells CD4 naive (r=-0.41, *P*=0.02). *SYK* had obviously positive correlation with mast cells resting (r=0.38, *P*=0.05) and T cells CD4 memory activated (r= 0.450, *P*=0.03) and had negative correlation with T cells CD4 naive (r= 0.-42, *P*=0.02). *PTPN6* was positively correlated with macrophages M0 (r=0.43, *P*=0.02) and mast cells resting (r=0.38, *P*=0.03). *SHC1* had significant positive correlation with mast cells resting (r=0.38, *P*=0.05) and T cells CD4 memory activated (r=0.4, *P*= 0.03) and had negative correlation with T cells CD4 naive (r=-0.4, *P*= 0.02) and B cells memory (r=-0.37, *P*= 0.04) (**Figure 7**). The detailed results of sperman correlation analysis is shown in **Figure S1.**

Discussion

CAVD, a chronic progressive disease, develops gradually from valvular sclerosis and valvular fibrosis to valvular calcification. CAVD eventually leads to stenosis of left ventricular outflow and severely disrupts hemodynamics [27]. As the population ages, CAVD becomes a major health problem due to its high prevalence, morbidity, and mortality rate. With a deeper understanding of CAVD, treatment measures for patients with CAVD will be more precise. Incremental proofs verified that the pathology involved in CAVD is multifactorial, including valve endothelial cells differentiation and damage, inflammation, fibrosis and calcification. In recent years, studies have demonstrated that the inflammatory response plays a pivotal role in occurance and development of CAVD [28, 29]. In the present study, we aimed to screen out important DEIRGs and identify the patterns of infiltrating immune cells in CAVD in detail. 220 DEIRGs were eventually identified in aortic valve tissue samples from patients with CAVD after a detailed integrative analysis that included all relevant gene expression profiles in the GEO database. GO analysis of DEIRGs in CAVD revealed that leukocyte migration, receptor-ligand activity, leukocyte cell-cell adhesion, myeloid leukocyte migration and membrane raft and membrane microdomain were significantly

enriched. These biological processes and molecular functions were correlated with immune cells infiltration [29]. Infiltrating immune cells could release inflammatory and fibrotic cytokines and further aggravate inflammatory response. DEIRGs are also involved in the regulation of cytokine receptor activity, cytokine activity and cytokine production in KEGG analysis. The inflammatory factors secreted by invading inflammatory cells, such as IL-1β and NF-κB, promote extracellular matrix remodeling, lipid deposition, fibrosis, ossification and calcification [30]. These findings indicate that DEIRGs in CAVD are involved in the immune and inflammatory processes In the “cytoHubba” plugin, *PTPN11*, *GRB2*, *SYK*, *PTPN6* and *SHC1* were identified as top 5 key DEIRGs according to the results of mixed character calculation.

Protein tyrosine phosphatase (PTP) is a kind of protein phosphatase, including PTPN1, PTPN2, PTPN6, PTP1 and PTPN22. PTPs function in various important biological processes, including cell circle and cell differentiation, by carrying out phosphorylation and dephosphorylation of tyrosine residues [31]. The role of PTPs in inflammatory response and immune cells infiltration was also gradually revealed [32, 33]. In the present study, *PTPN11* was significantly down-regulated, whereas *PTPN6* was up-regulated in aortic valves from patients with CAVD. PTPN11 have already been linked to inflammation response, which could inhibit STAT1 pathway and ultimately reduce the level of Th1 cytokine through preventing combination of STAT1 and IFN-γreceptor [34]. Moreover, studies have demonstrated that *PTPN11* gene variants are closely associated ulcerative colitis (UC) but not Crohn's disease (CD) [35]. PTPN11 is also an important component in growth factor pathway and closely related to Egfr signaling and formation of valve endothelial cells [36]. In addition, *PTPN11* mutation in patients present significantly higher prevalence of pulmonary valve stenosis, named Noonan syndrome (NS) [37]. Interestingly, *PTPN11* mutation has also been demonstrated to be harmful to inhibition of myocardial hypertrophy and cardiac fibrotic remodeling via crosstalk with NF-κB pathway and mTOR signaling [38, 39].

PTPN6, another phosphatase of PTPs, specially expressed in the cytoplasm, and prevented excessive autoimmunity in IL-1 dependent inflammatory diseases and pyroptosis dependent inflammatory diseases [40]. Studies have also demonstrated that PTPN6 significantly ameliorates inflammatory disease by decreasing TNF-α, TGF-β and IL-6 [41]. In addition, PTPN6 is important in preventing the harmful effects of pathogens on the host, which is crucial for successful defense mechanisms against invading microorganisms [42]. PTPN6 is known as an important negative regulator of inflammatory response and significantly down regulated in aortic valve tissues from patients with CAVD.

GRB2 is a 25-kDa adaptor protein that functions in modulating and integrating signals from cell membrane surface receptors to intracellular effector proteins [43]. In CAVD, *GRB2* was up regulated in aortic valve tissues form patients with CAVD compared with normal individuals [44]. GRB2 is best known in the cardiovascular field for activating Egfr tyrosine kinase and its downstream renin-angiotensin system [45]. Recent studies also demonstrated that GRB2 was involved in the process of development of T cells and Th cells. *GRB2*-knockout animals have reduced T cells and more prone to inflammatory diseases [46].

Spleen-associated tyrosine kinase (SYK), a member of the none receptor type tyrosine kinase family [47]. SYK was involved in numerous biological functions, including cellular adhesion, vascular development, platelet activation and relaying adaptive immune receptor signaling related to immune cells infiltration [48–50]. SYK, as a proinflammatory molecule, has receiving increasing attention in a variety of diseases. Liang *et al.* demonstrated that SYK was a crucial biomarker and closely related to the occurrence of coronary heart disease (CHD) as an proinflammatory factor [51]. Researches on the specific role of SYK in CAVD is helpful to better understand the role of inflammatory response and immune infiltration in patients with CAVD.

SHC1, a member of SHC family of adaptor proteins, roles of SHC1 in reactive oxygen species (ROS) production has been widely studied. It is well known that overexpression of SHC1-induce ROS production is related to development of atherosclerosis and CHD [52–53]. Recent evidence suggests that ROS also plays a role in the pathophysiology of CAVD by inducing the differentiation of valvular stromal cells into myofibroblasts, which then become osteoblasts. In addition, ROS directly causes valve endothelial cell damage and lipid deposition in a variety of chronic diseases, including diabetes, and plays an important role in the early stage of CAVD [54]. More studies are needed to determine the involvement of *PTPN1*, *GRB2*, *PTPN6*, *SYK*, and *SHC1* in CAVD.

CIBERSORT algorithm was performed to evaluate immune cells infiltration in aortic valve tissues of patients with CAVD. We found reduced monocytes and NK cells activated, as well as increased neutrophils, T cells CD4 memory activated and macrophages M0, which may be related to the pathogenesis of CAVD. Imbalance of M1 and M2 polarization in macrophages is known to be critical in regulating the intensity of inflammatory responses. Our results are the same as studies have reported before that compared with aortic valves from normal individuals, there were fewer M2 macrophages in calcified aortic valves [55]. In addition, our study has also shown that the macrophages M0 population were significantly elevated in CAVD. Monocytes are the largest white blood cells in volume, which is an important part of the defense system of human bodys. The decrease of monocytes and NK cells in patients with CAVD may be one of reasons for the intensification of inflammatory process and calcification of valve interstitial cells. Neutrophils and C-reactive protein (CRP) are indirect blood markers that roughly reflect the level of inflammation in our bodies, along with some pathological changes in aortic valve tissue [56]. We found that neutrophils was also significantly elevated in valve tissues from patients with CAVD. These results are consistent with previous studies suggesting that calcified aortic stenosis is characterized by chronic inflammation with infiltration of phagocytes, lymphocytes and mammary cells [57].

We also studied the correlation between key DEIRGs and infiltrating immune cells. Based on these results, *PTPN1*, *GRB2*, *PTPN6*, *SYK* and *SHC1* may play a key role in CAVD by modulating immune infiltration. However, roles of various infiltrating immune cells and key DEIRGs in development and progression of CAVD still need to be studied further.

Conclusion

Above all, we found that the *PTPN11*, *GRB2*, *SYK*, *PTPN6*, and *SHC1* are key DEIRGs of CAVD. Compared with normal aortic valve tissue samples, the proportion of neutrophils, T cells CD4 memory activated and macrophages M0 was significantly elevated in calcified aortic valves tissue samples, as well as reduced infiltration of monocytes and NK cells activated. Moreover, the regulation of *PTPN11*, *GRB2*, *SYK*, *PTPN6*, and *SHC1* on immune cells infiltration may play an important role in the pathogenesis of CAVD. Further researches are needed to explore whether it might be a new molecular targeted therapic method for patients with CAVD.

Declarations

Data availability statement

Publicly available datasets were analyzed in this study. All the raw data used in this study are derived from the public GEO data portal (https://www.ncbi.nlm.nih.gov/geo/).

Acknowledgments

We acknowledge GEO database for providing their platforms and contributors for uploading their meaningful datasets. And we thank all participants involved in studies included in our present study.

Funding

This work was supported by the Natural Science Foundation of China (81770331) and Research Foundation from Wuxi Health Commission for the Youth（Q202034）.

Author Contributions: Ru-Xing Wang was involved in the experiment design. Li-Da Wu, Feng Xiao, Jin-Yu Sun and Feng Li performed the experiments. Ling-Ling Qian, Yu-Jia Chen and Jia-Yi Chen analyzed the data. Ru-Xing Wang, Li-Da Wu and Jie Zhang wrote the manuscript. Ling-Ling Qian, Yu-Jia Chen and Jia-Yi Chen edited the manuscript. All authors declare no conflicts of interest. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

1. Tsimikas S, Fazio S, Ferdinand KC, Ginsberg HN, Koschinsky ML, Marcovina SM, et al. NHLBI working group recommendations to reduce lipoprotein(a)-mediated risk of cardiovascular disease and aortic stenosis. J Am Coll Cardiol, 2018;71(2):177–192. doi: 10.1016/j.jacc.2017.11.014.
2. Otto CM, Prendergast B. Aortic-valve stenosis–from patients at risk to severe valve obstruction. N Engl J Med, 2014;371(8):744–756. doi: 10.1056/NEJMra1313875.
3. Vahanian A, Alfieri O, Andreotti F, Antunes MJ, Barón-Esquivias G, Baumgartner H, et al. Guidelines on the management of valvular heart disease (version 2012): the joint task force on the management of valvular heart disease of the European society of cardiology (ESC) and the European association for cardio-thoracic surgery (EACTS). Eur J Cardiothorac Surg, 2012;42(4):S1-44. doi: 10.1093/ejcts/ezs455.
4. Myasoedova VA, Ravani AL, Frigerio B, Valerio V, Moschetta D, Songia P, et al. Novel pharmacological targets for calcific aortic valve disease: prevention and treatments. Pharmacol Res, 2018;136:74–82. doi: 10.1016/j.phrs.2018.08.020.
5. Liu X, Xu Z. Osteogenesis in calcified aortic valve disease: From histopathological observation towards molecular understanding. Prog Biophys Mol Biol, 2016;122(2):156–161. doi: 10.1016/j.pbiomolbio.2016.02.002.
6. Bartoli-Leonard F, Zimmer J, Aikawa E. Innate and adaptative immunity: the understudied driving force of heart valve disease. Cardiovasc Res, 2021, Online ahead of print. doi: 10.1093/cvr/cvab273.
7. Xia J, Fjell CD, Mayer ML, Pena OM, Wishart DS, Hancock REW, et al. INMEX–a web-based tool for integrative meta-analysis of expression data. Nucleic Acids Res, 2013;41 (Web Server issue):63–70. doi: 10.1093/nar/gkt338.
8. Fu M, Wang Q, Wang H, Dai Y, Wang J, Kang W, et al. Immune-related genes are prognostic markers for prostate cancer recurrence. Front Genet, 2021;12:639642. doi: 10.3389/fgene.2021.639642.
9. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods, 2015;12(5):453–457. doi: 10.1038/nmeth.3337.
10. Deng YJ, Ren EH, Yuan WH, Zhang GZ, Wu ZL, Xie QQ, et al. GRB10 and E2F3 as diagnostic markers of osteoarthritis and their correlation with immune infiltration. Diagnostics (Basel), 2020;10(3):171. doi: 10.3390/diagnostics10030171.
11. Liu R, Hu R, Zeng Y, Zhang W, Zhou H-H. Tumour immune cell infiltration and survival after platinum-based chemotherapy in high-grade serous ovarian cancer subtypes: a gene expression-based computational study. EBioMedicine, 2020;51:102602. doi: 10.1016/j.ebiom.2019.102602.
12. Zhang B, Wang Q, Fu C, Jiang C, Ma S. Exploration of the immune-related signature and immune infiltration analysis for breast ductal and lobular carcinoma. Ann Transl Med, 2019;7(23):730. doi: 10.21037/atm.2019.11.117.
13. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: Archive for functional genomics data sets–update. Nucleic Acids Res, 2013;41:991–995. doi: 10.1093/nar/gks1193.

14. Davis S, Meltzer PS. GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor. Bioinformatics, 2007;23(14):1846–1847. doi: 10.1093/bioinformatics/btm254.
15. Bossé Y, Miqdad A, Fournier D, Pépin A, Pibarot P, Mathieu P. Refining molecular pathways leading to calcific aortic valve stenosis by studying gene expression profile of normal and calcified stenotic human aortic valves. Circ Cardiovasc Genet, 2009;2(5):489–498. doi: 10.1161/CIRCGENETICS.108.820795.
16. Ohukainen P, Syväranta S, Näpänkangas J, Rajamäki K, Taskinen P, Peltonen T, et al. MicroRNA-125b and chemokine CCL4 expression are associated with calcific aortic valve disease. Ann Med, 2015;47(5):423–429. doi: 10.3109/07853890.2015.1059955.
17. Guauque-Olarte S, Droit A, Tremblay-Marchand J, Gaudreault N, Kalavrouziotis D, Dagenais F, et al. RNA expression profile of calcified bicuspid, tricuspid, and normal human aortic valves by RNA sequencing. Physiol Genomics, 2016;48(10):749–761. doi: 10.1152/physiolgenomics.00041.2016.
18. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res, 2015;43(7):e47. doi: 10.1093/nar/gkv007.
19. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med, 2009;6(7):e1000097. doi: 10.1371/journal.pmed.1000097.
20. Li S, Rouphael N, Duraisingham S, Romero-Steiner S, Presnell S, Davis C, et al. Molecular signatures of antibody responses derived from a systems biology study of five human vaccines. Nat Immunol, 2014;15(2):195–204. doi: 10.1038/ni.2789.
21. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. Innovation (N Y), 2021, 2(3):100141. doi: 10.1016/j.xinn.2021.100141.
22. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun, 2019;10(1):1523. doi: 10.1038/s41467-019-09234-6.
23. Pinero J, Bravo A, Queralt-Rosinach N, Gutierrez-Sacristan A, Deu-Pons J, Centeno E, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res, 2017;45(D1):D833-D839. doi: 10.1093/nar/gkw943.
24. Han H, Cho JW, Lee S, Yun A, Kim H, Bae D, et al. TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions. Nucleic Acids Res, 2018;46(D1):D380-D386. doi: 10.1093/nar/gkx1013.
25. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019;47(D1):D607–613. doi: 10.1093/nar/gky1131.
26. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res, 2003;13(11):2498–2504. doi: 10.1101/gr.1239303.

27. Büttner P, Feistner L, Lurz P, Thiele H, Hutcheson JD, Schlotter F. Dissecting calcific aortic valve disease-the role, etiology, and drivers of valvular fibrosis. Front Cardiovasc Med, 2021;8:660797. doi: 10.3389/fcvm.2021.660797.
28. Sikura KE, Combi Z, Potor L, Szerafin T, Hendrik Z, Méhes G, et al. Hydrogen sulfide inhibits aortic valve calcification in heart via regulating RUNX2 by NF-κB, a link between inflammation and mineralization. J Adv Res, 2020;27:165–176. doi: 10.1016/j.jare.2020.07.005.
29. Cho KI, Sakuma I, Sohn IS, Jo SH, Koh KK. Inflammatory and metabolic mechanisms underlying the calcific aortic valve disease. Atherosclerosis, 2018;277:60–65. doi: 10.1016/j.atherosclerosis.2018.08.029.
30. Liu RM, Pravia KAG. Oxidative stress and glutathione in TGF-beta-mediated fibrogenesis. Free Radic Biol Med, 2010;48(1):1–15. doi: 10.1016/j.freeradbiomed.2009.09.026.
31. Pulido R, Stoker AW, Hendriks WJ. PTPs emerge as PIPs: protein tyrosine phosphatases with lipid-phosphatase activities in human disease. Hum Mol Genet, 2013;22(R1):R66-76. doi: 10.1093/hmg/ddt347.
32. Xiao P, Zhang H, Zhang Y, Zheng M, Liu R, Zhao Y, et al. Phosphatase Shp2 exacerbates intestinal inflammation by disrupting macrophage responsiveness to interleukin-10. J Exp Med, 2019;216(2):337–349. doi: 10.1084/jem.20181198.
33. Zhao L, Xia J, Li T, Zhou H, Ouyang W, Hong Z, et al. Shp2 deficiency impairs the inflammatory response against haemophilus influenzae by regulating macrophage polarization. J Infect Dis, 2016;214(4):625–633. doi: 10.1093/infdis/jiw205.
34. Tseng PC, Huang WC, Chen CL, Sheu BS, Shan YS, Tsai CC, et al. Regulation of SHP2 by PTEN/AKT/GSK-3β signaling facilitates IFN-γ resistance in hyperproliferating gastric cancer. Immunobiology, 2012;217(9):926–934. doi: 10.1016/j.imbio.2012.01.001.
35. Spalinger MR, McCole DF, Rogler G, Scharl M. Role of protein tyrosine phosphatases in regulating the immune system: implications for chronic intestinal inflammation. Inflamm Bowel Dis, 2015;21(3):645–655. doi: 10.1097/MIB.0000000000000297.
36. Chen B, Bronson RT, Klaman LD, Hampton TG, Wang JF, Green PJ, et al. Mice mutant for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. Nat Genet, 2000;24(3):296–299. doi: 10.1038/73528.
37. Brasil AS, Pereira AC, Wanderley LT, Kim CA, Malaquias AC, Jorge AA, et al. PTPN11 and KRAS gene analysis in patients with Noonan and Noonan-like syndromes. Genet Test Mol Biomarkers, 2010;14(3):425–432. doi: 10.1089/gtmb.2009.0192.
38. Schramm C, Fine DM, Edwards MA, Reeb AN, Krenz M. The PTPN11 loss-of-function mutation Q510E-Shp2 causes hypertrophic cardiomyopathy by dysregulating mTOR signaling. Am J Physiol Heart Circ Physiol, 2012;302(1):H231-243. doi: 10.1152/ajpheart.00665.2011.
39. Zhou H, Li N, Yuan Y, Jin YG, Wu Q, Yan L, et al. Leukocyte immunoglobulin-like receptor B4 protects against cardiac hypertrophy via SHP-2-dependent inhibition of the NF-κB pathway. J Mol Med (Berl), 2020;98(5):691–705. doi: 10.1007/s00109-020-01896-w.

40. Speir M, Nowell CJ, Chen AA, O'Donnell JA, Shamie IS, Lakin PR, et al. Ptpn6 inhibits caspase-8- and Ripk3/Mlkl-dependent inflammation. Nat Immunol, 2020;21(1):54–64. doi: 10.1038/s41590-019-0550-7.
41. Lin L, Jian J, Song CY, Chen F, Ding K, Xie WF, et al. SHP-1 ameliorates nonalcoholic steatohepatitis by inhibiting proinflammatory cytokine production. FEBS Lett, 2020;594(18):2965–2974. doi: 10.1002/1873-3468.13879.
42. Kanwal Z, Zakrzewska A, Hertog J, Spaink HP, Schaaf MJM, Meijer AH. Deficiency in hematopoietic phosphatase ptpn6/Shp1 hyperactivates the innate immune system and impairs control of bacterial infections in zebrafish embryos. J Immunol, 2013;190(4):1631–1645. doi: 10.4049/jimmunol.1200551.
43. Dharmawardana PG, Peruzzi B, Giubellino A, Burke TR Jr, Bottaro DP. Molecular targeting of growth factor receptor-bound 2 (Grb2) as an anti-cancer strategy. Anticancer Drugs, 2006;17(1):13–20. doi: 10.1097/01.cad.0000185180.72604.ac.
44. Abazyan B, Nomura J, Kannan G, Ishizuka K, Tamashiro KL, Nucifora F, et al. Prenatal interaction of mutant DISC1 and immune activation produces adult psychopathology. Biol Psychiatry, 2010;68(12):1172–1181. doi: 10.1016/j.biopsych.2010.09.022.
45. Tari AM, Lopez-Berestein G. GRB2: a pivotal protein in signal transduction. Semin Oncol, 2001;28(5 Suppl 16):142–147. doi: 10.1016/s0093-7754(01)90291-x.
46. Radtke D, Lacher SM, Szumilas N, Sandrock L, Ackermann J, Nitschke L, et al. Grb2 is important for T cell development, Th cell differentiation, and induction of experimental autoimmune encephalomyelitis. J Immunol, 2016;196(7):2995–3005. doi: 10.4049/jimmunol.1501764.
47. Alhazmi A. Spleen tyrosine kinase as a target therapy for pseudomonas aeruginosa infection. J Innate Immun, 2018;10(4):255–263. doi: 10.1159/000489863.
48. Mocsai A, Ruland J, Tybulewicz VL. The SYK tyrosine kinase: A crucial player in diverse biological functions. Nat Rev Immunol, 2010;10:387–402. doi: 10.1038/nri2765.
49. Correll PH, Paulson RF, Wei X. Molecular regulation of receptor tyrosine kinases in hematopoietic malignancies. Gene, 2006;374:26–38. doi: 10.1016/j.gene.2006.01.023.
50. Kurosaki T. Functional dissection of BCR signaling pathways. Curr Opin Immunol. 2000;12:276–281. doi: 10.1016/s0952-7915(00)00087-x.
51. Liang K, Ma N, Luo S, Niu W, Wang Y, Bu P. The expression and clinical significance of spleen tyrosine kinase in patients with coronary heart disease. Med Sci Monit, 2019;25:2112–2121. doi: 10.12659/MSM.913543.
52. Tomilov AA, Bicocca V, Schoenfeld RA, Giorgio M, Migliaccio E, Ramsey JJ, et al. Decreased superoxide production in macrophages of long-lived p66Shc knock-out mice. J Biol Chem, 2010;285(2):1153–1165. doi: 10.1074/jbc.M109.017491.
53. Miao Q, Wang Q, Dong L, Wang Y, Tan Y, Zhang X. The expression of p66shc in peripheral blood monocytes is increased in patients with coronary heart disease and correlated with endothelium-dependent vasodilatation. Heart Vessels, 2015;30(4):451–457. doi: 10.1007/s00380-014-0497-4.

54. Liu H, Wang L, Pan Y, Wang X, Ding Y, Zhou C, et al. Celastrol alleviates aortic valve calcification via inhibition of NADPH oxidase 2 in valvular interstitial cells. JACC Basic Transl Sci, 2019;5(1):35–49. doi: 10.1016/j.jacbts.2019.10.004.
55. Zhou P, Li Q, Su S, Dong W, Zong S, Ma Q, et al. Interleukin 37 suppresses M1 macrophage polarization through inhibition of the Notch1 and nuclear factor kappa B pathways. Front Cell Dev Biol, 2020;8:56. doi: 10.3389/fcell.2020.00056.
56. Song J, Zheng Q, Ma X, Zhang Q, Xu Z, Zou C, et al. Predictive roles of neutrophil-to-lymphocyte ratio and c-reactive protein in patients with calcific aortic valve disease. Int Heart J, 2019;60(2):345–351. doi: 10.1536/ihj.18-196.
57. Steiner I, Krbal L, Rozkoš T, Harrer J, Laco J. Calcific aortic valve stenosis: Immunohistochemical analysis of inflammatory infiltrate. Pathol Res Pract, 2012;208(4):231–234. doi: 10.1016/j.prp.2012.02.009.

Tables

Table 1 Characteristics of the datasets included in the integrated analysis.

GEO ID	Platform	Citation	Region	Normal	CAVD
GSE12644	GPL570; Affymetrix Human Genome U133 Plus 2.0 Array	Bossé Y, et al. Circ Cardiovasc Genet, 2009;2(5):489-498. PMID: 20031625 Derbali H, et al. Am J Pathol, 2010;176(6):2638-2645. PMID: 20382708	Quebec, Canada	10	10
GSE51472	GPL570; Affymetrix Human Genome U133 Plus 2.0 Array	Ohukainen P, et al. Ann Med, 2015;47(5):423-429. PMID: 26203686 Rysä J, et al. Genom Data, 2016;7:107-108. PMID: 26981379	Oulu, Finland	5	5
GSE83453	GPL10558; Illumina HumanHT-12 V4.0 expression beadchip	Guauque-Olarte S, et al. Physiol Genomics, 2016;48(10):749-761. PMID: 27495158	Quebec, Canada	8	10

GEO: gene expression omnibus; CAVD: calcific aortic valve disease.

Figures

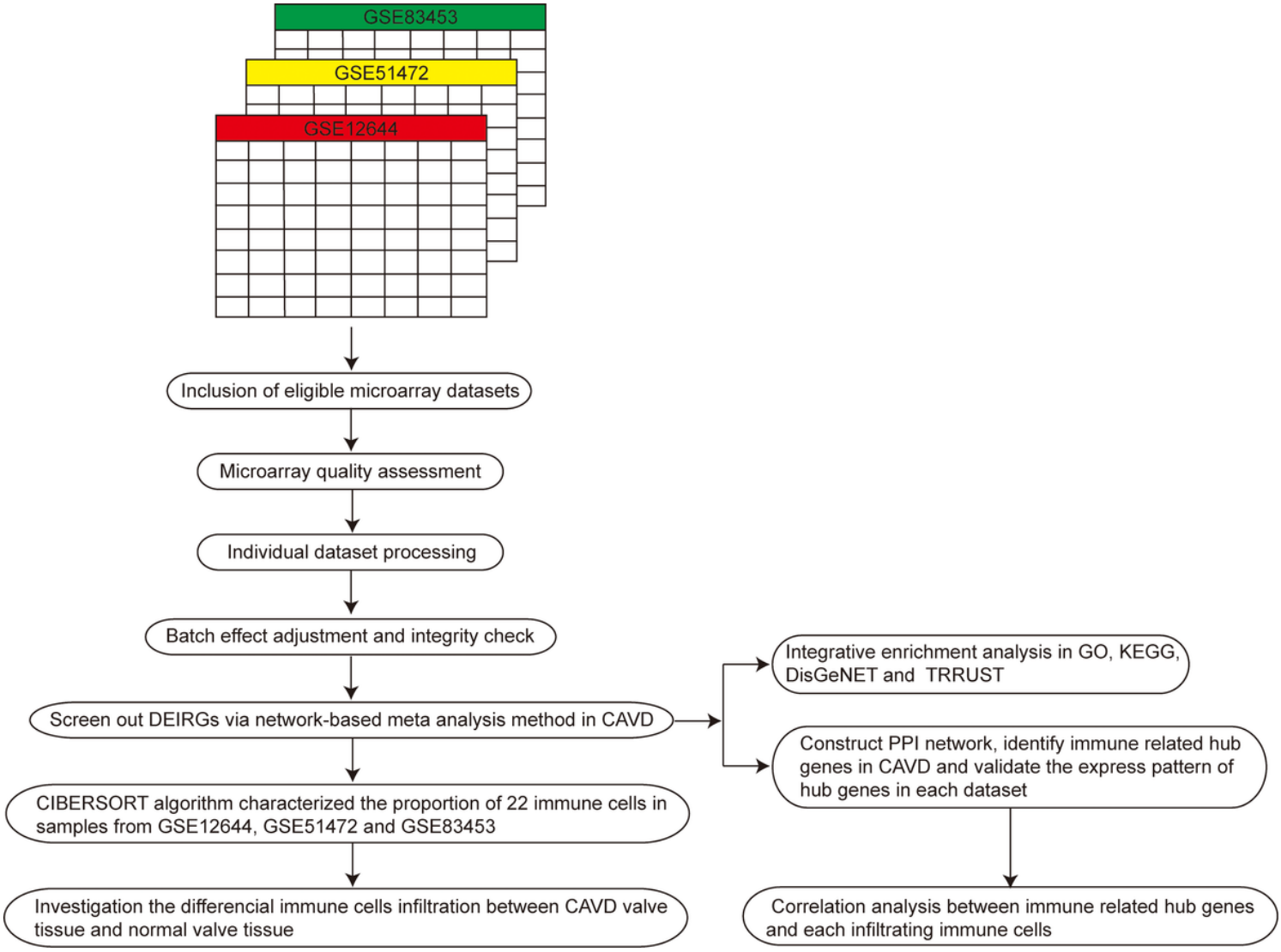

Figure 1

Workflow of the present study. PPI, protein–protein interaction; GO, gene ontology; CAVD, calcific aortic valve disease; DEIRGs, differentially expressed immune related genes; KEGG, kyoto encyclopedia of genes and genomes.

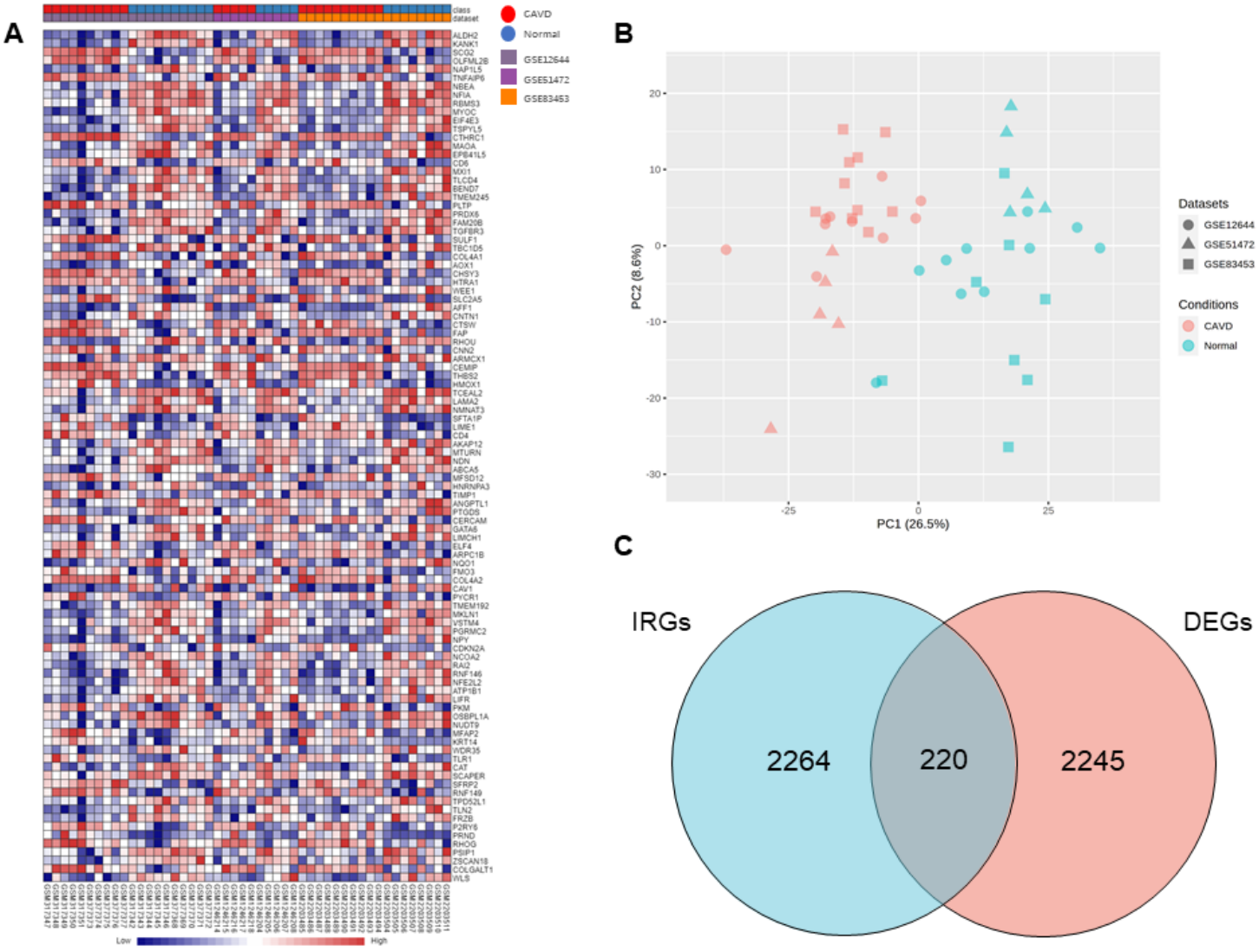

Figure 2

Identification of DEIRGs between aortic valve samples from patients with CAVD and normal individuals. A Heatmap of top 100 DEIRGs across different datasets. B PCA plot after removing batch effect between GSE12644, GSE51472 and GSE83453. C Venn plot of screening DEIRGs by matching the 2484 IRGs from ImmPort database to the 2465 DEGs. CAVD, calcific aortic valve disease; IRGs, immune related genes; DEGs, differentially expressed genes; DEIRGs, differentially expressed immune related genes.

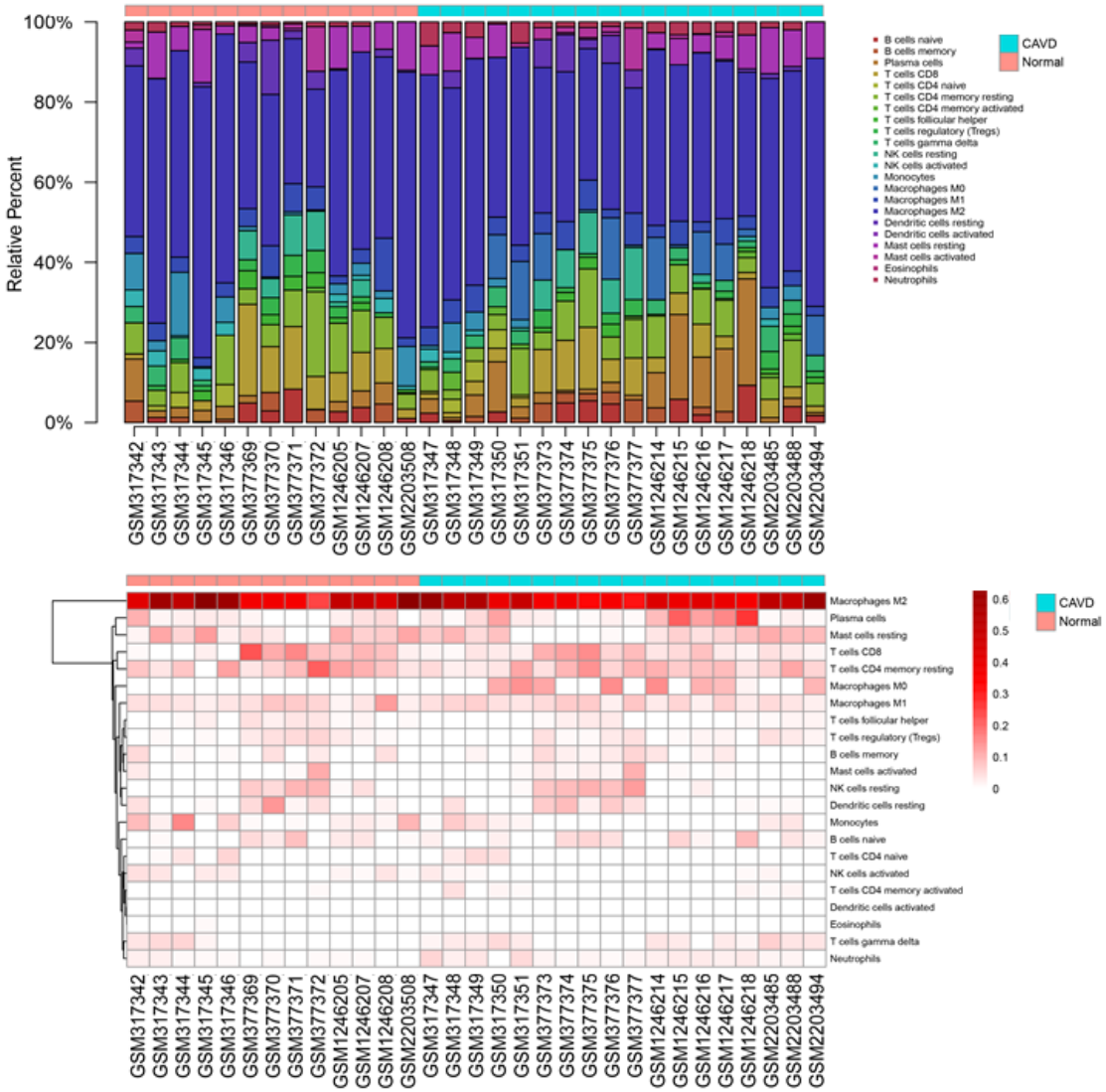

Figure 3

Summary of immune infiltration in calcified and normal aortic valve samples. A Barplot shows the proportion of 22 types of immune cells in each sample. B Heatmap of the 22 subpopulations of infiltrating immune cells in aortic valve samples from patients with CAVD and normal individuals. CAVD, calcific aortic valve disease.

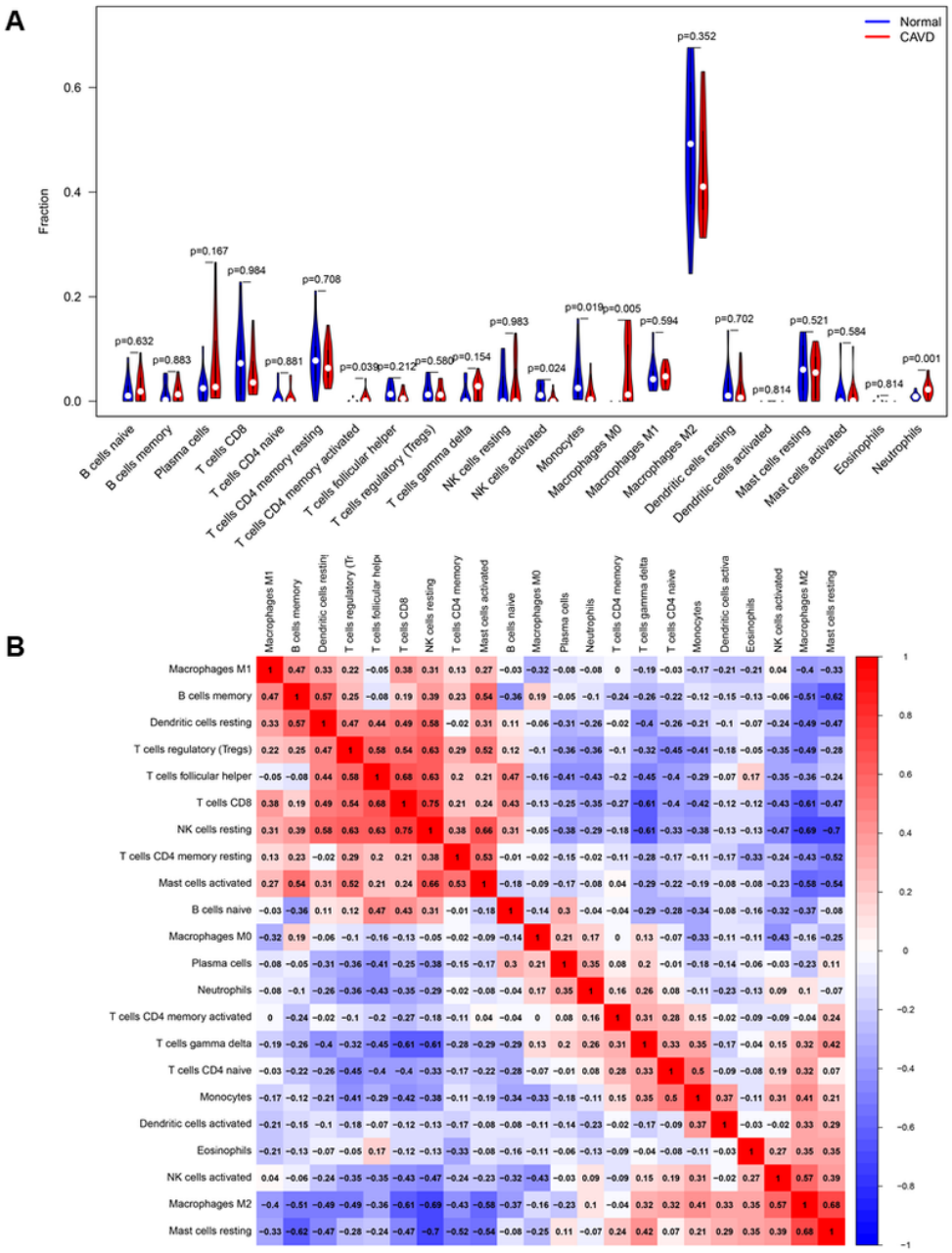

Figure 4

Evaluation of the difference in immune cells infiltration between aortic valve samples from patients with CAVD and normal individuals. A The difference of 22 subpopulations of immune cells between calcified and normal aortic valve samples. B Correlation heatmap shows the correlation between 22 immune cell subpopulations. CAVD, calcific aortic valve disease.

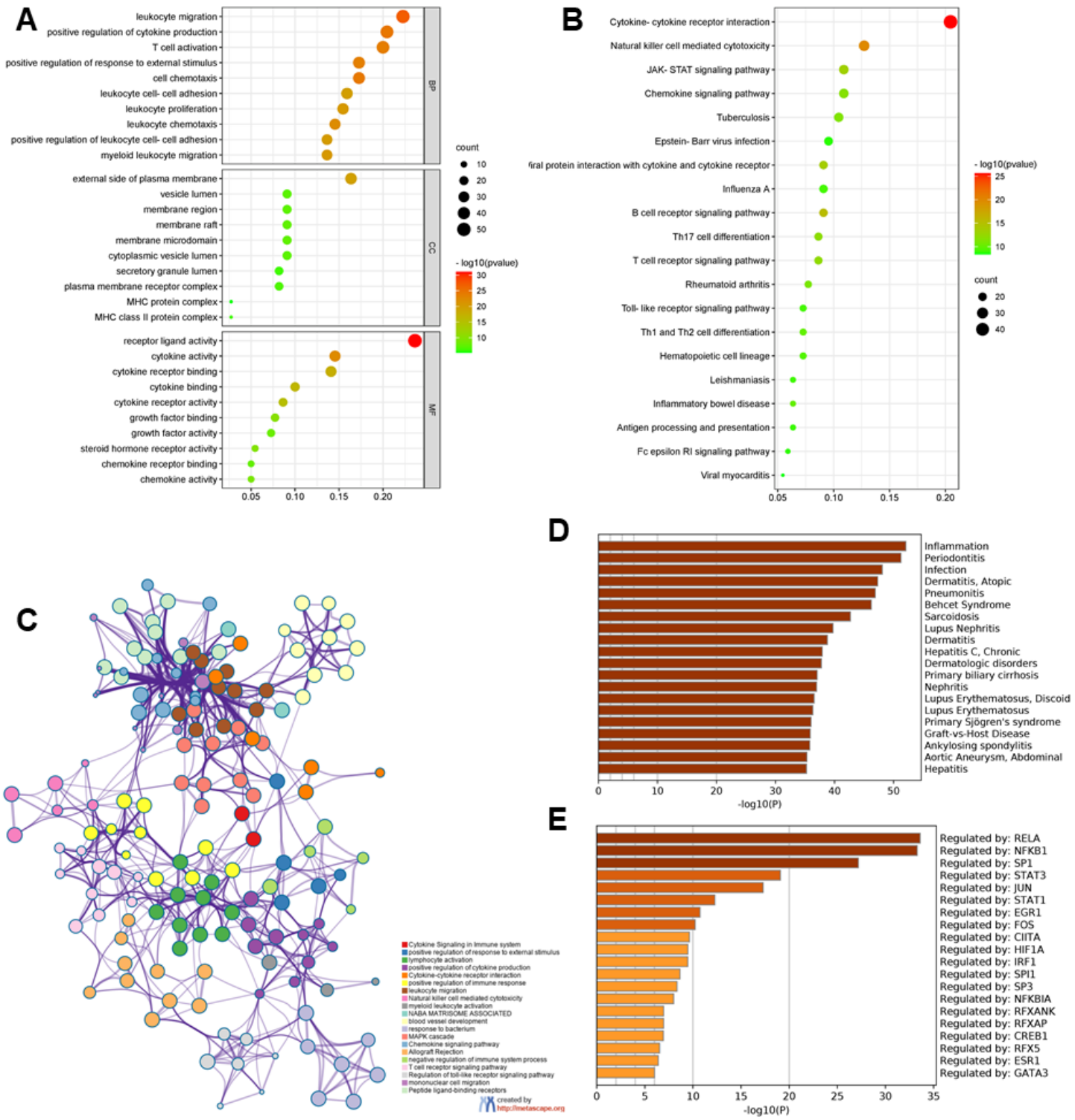

Figure 5

Enrichment analysis of DEIRGs in CAVD. A GO enrichment analysis. B KEGG pathway enrichment analysis. C The network of enriched terms and each node represents an enriched term. D Summary of enrichment analysis in DisGeNET. E Summary of enrichment analysis in TRRUST. DEIRGs, differentially expressed immune related genes; GO: gene ontology; KEGG: kyoto encyclopedia of genes and genomes.

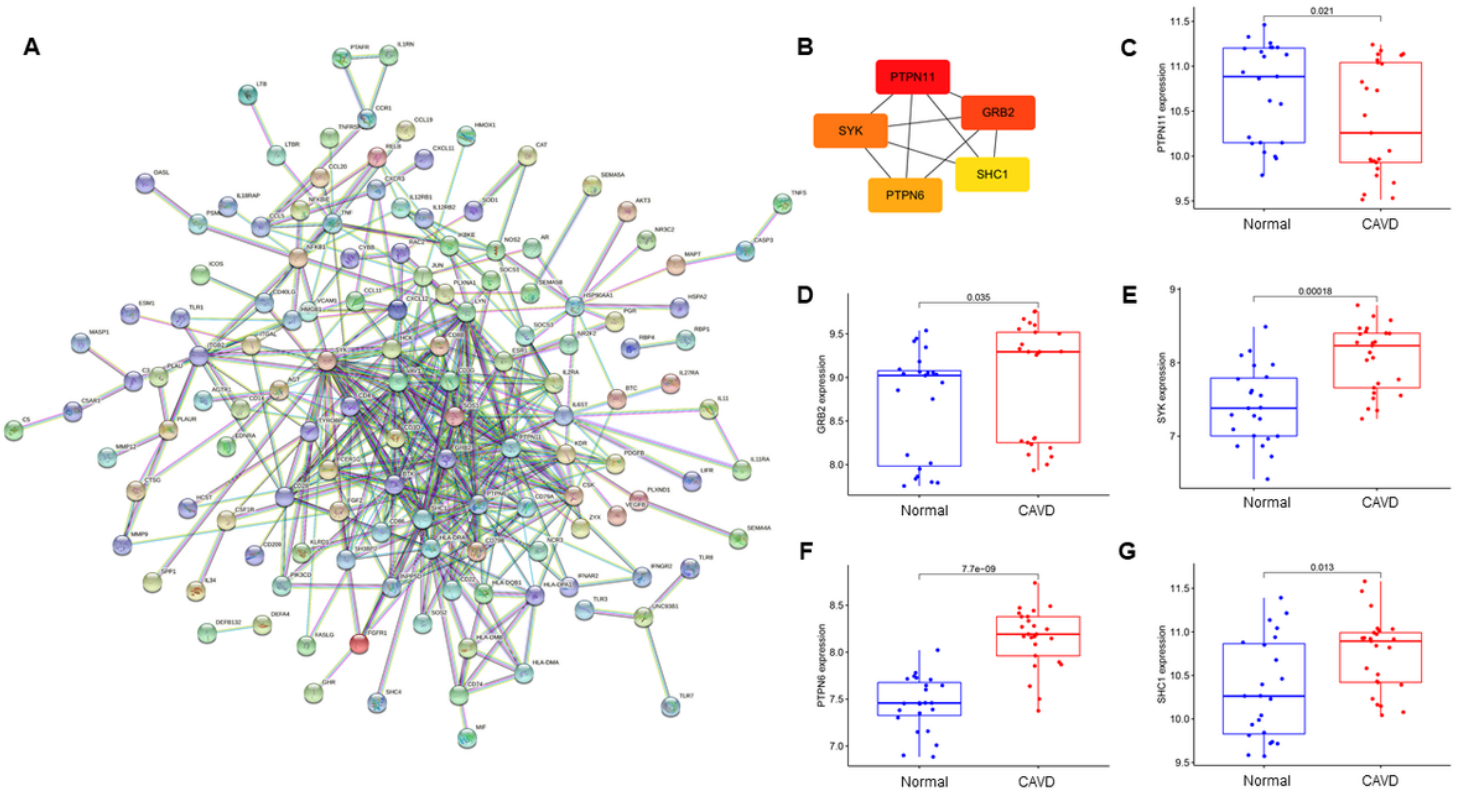

Figure 6

PPI network and identification of hub genes. A PPI network of DEIRGs in CAVD. B Top 5 hub genes identified by 'cytoHubba' according to mixed character calculation and its core network. The significance of hub genes gradually increases from yellow to red. C-G The expression pattern of *PTPN11, GRB2, SYK, PTPN6* and *SHC1* in calcified or normal aortic valve tissues. PPI: protein-protein interaction; DEIRGs: differentially expressed immune related genes; CAVD, calcific aortic valve disease.

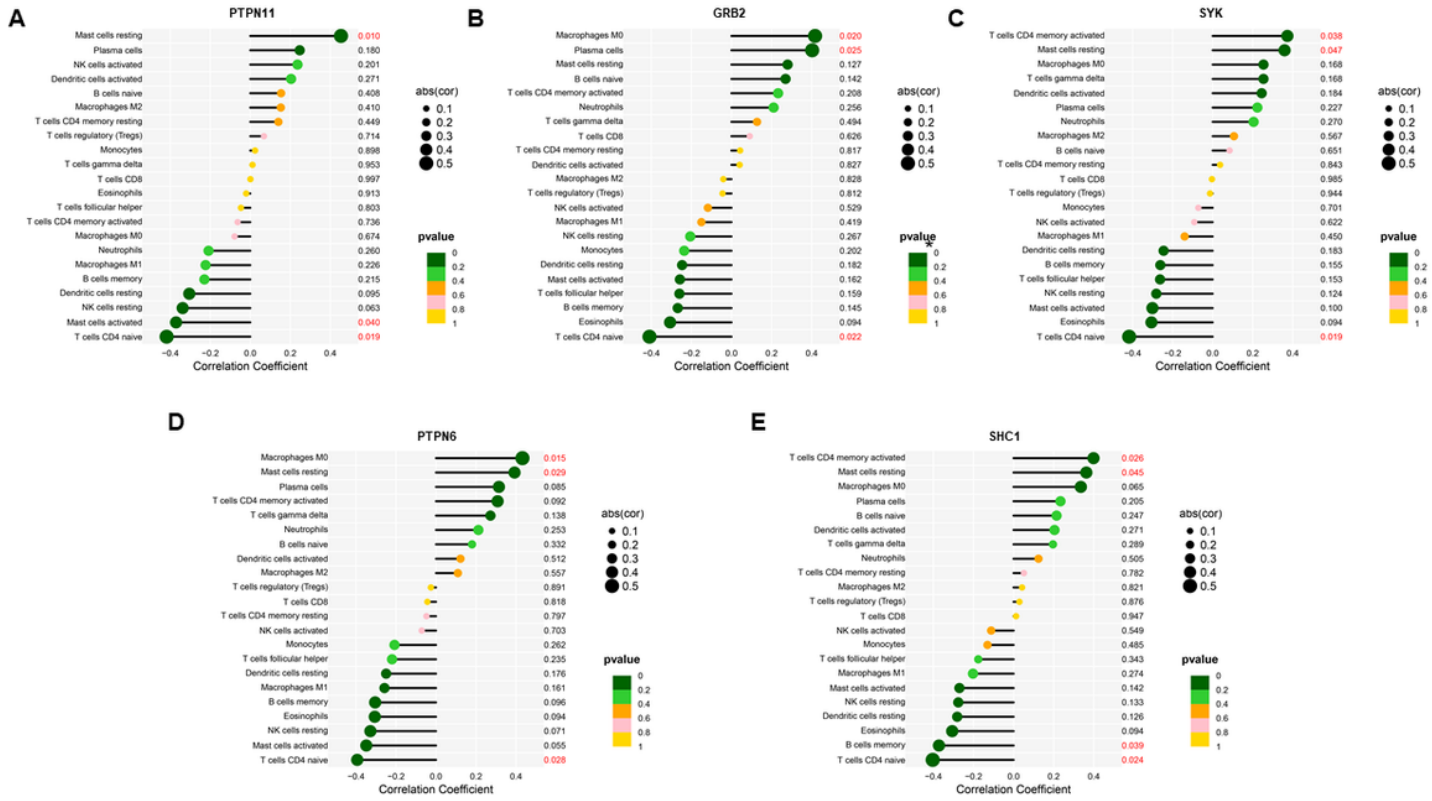

Figure 7

Correlations between DEIRGs in CAVD and infiltrating immune cells. A-E Correlation between *PTPN11, GRB2, SYK, PTPN6* and *SHC1* and infiltrating immune cells.

Supplementary Files

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

- FigS1.tif
- FiguretitleandlegendofFigureS1.docx