Analysis of the Potential Role of Key Genes in Atrial Fibrillation Based on Bioinformatics Approach

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Research Article

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Abstract

Objective: Our study aims to explore the key differentially expressed genes (DEGs) that may serve as potential biomarkers for the diagnosis and treatment of atrial fibrillation (AF) using bioinformatics tools.

Methods: Microarray datasets of GSE31821 and GSE79768 were downloaded from Gene Expression Synthesis (GEO) database. DEGs were analyzed after merging all microarray data and adjusting batch effect. The screened DEGs were further used for Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis. Protein-protein interaction (PPI) network was constructed using the STRING database and PPI nodes were counted by R software. Finally, combined with the above important bioinformatics information, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to detect some DEGs in the tissues of patients with AF.

Results:114 DEGs (|log2 FC|≥0.5) were identified in the AF group compared with the control group. Combining DEGs, enrichment analysis and PPI results, CXCL10, TLR7, DDX58, CCR2, RSAD2, KIT, LYN, and CXCL11 were identified as potential key genes. The expression of two key genes (RSAD2 and CXCL11) was also verified by qRT-PCR in the tissues of AF patients, illustrating the reliability and biomarker potential of the key genes.

Conclusion: 8 potential key genes may play an important role in the development of AF, and they may serve as potential biomarkers for the diagnosis and treatment of AF.

1. Introduction

Atrial fibrillation (AF), one of the most common arrhythmias in clinical practice has an increasing incidence with age. It is one of the main causes of heart failure (HF), stroke and sudden death in the world. With the aging of the population, the social burden is also increasing [1,2]. However, the pathophysiological mechanism of AF is still unclear, there is also no effective treatment at present [3]. Only a few patients with AF can have surgery or drugs to restore the rhythm of the heart [4]. The high prevalence and limited treatment of AF result in a huge public health and economic burden. Therefore, the pathogenesis of AF needs to be further studied.

Bioinformatics, including microarrays and sequencing for gene detection, and proteomics for protein detection, has developed very rapidly and become increasingly popular in the last decade [5]. Bioinformatics has been widely used to find new biomarkers for cancer, neurological diseases, respiratory diseases, and cardiovascular diseases [6-9]. In 2018, a preliminary genome-wide association study meta-analysis including more than 93,000 AF cases and more than 1 million reference subjects identified at least 134 genetic loci significantly associated with AF risk [10]. Zhang et al. identified 9 potential key genes of AF by bioinformatics analysis [11]. However, a large amount of bioinformatics data remains to be explored for better diagnosis and treatment of AF, meanwhile many associated genes remain to be

identified, which will help us better understand the pathogenesis of AF and contribute to the discovery of new diagnostic biomarkers or therapeutic targets.

In the present study, two sets of AF microarray data (GSE31821 and GSE79768) were included in the study. Bioinformatics methods were used to screen differentially expressed genes (DEGs) for Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis to gain insight into their expression and function. The hub genes of DEGs were further screened using Protein-protein interaction (PPI) network, and finally 8 key genes were identified, which could be used as potential biomarkers and therapeutic targets for AF.

2. Materials And Methods

2.1 Microarray Dataset

The microarray data for GSE31821 and GSE79768 were downloaded from Gene Expression Omnibus, (GEO, https://www.ncbi.nlm.nih.gov/geo/), which is the world 's largest high-throughput gene expression database. Both datasets were from GPL750 Affymetrix Human Genome U133 Plus 2.0 Array platform. In the data set, only the left atrial appendage (LAA) samples of AF and control group patients were selected, totaly 11 cases of AF and 8 cases of control group were included for subsequent analysis.

2.2. DEGs analysis

The GEO probe matrix data was annotated using ActivePerl 5.24.2 software (https://www.activestate.com/ products/perl/), converting the gene probe ids to gene names. Then the microarray data were merged and the R software "sva" and "limma" packages were used to adjust the batch effect and normalisation so that the expression values had a similar distribution across a set of arrays [12,13].

In order to evaluate the differential expression, the “Limma” package of R software was used to fit the linear model, and the simple empirical Bayesian model was used to alleviate the standard error [12]. When the adjusted P value < 0.05 and the gene expression fold change (FC)value | log2Fc | ≥ 0.5, a gene was defined as DEG between AF and control group, then the DEGs were further visualized by volcano map and heat map.

2.3. GO and KEGG analysis of DEGs

In order to better understand the biological functions and properties, GO functional annotation (http://geneontology.org/) [14] and KEGG pathway enrichment analysis (https://www.kegg.jp/) [15] were performed on DEGs using R software. Gene symbols were converted to Entrez IDs using the human genome annotation package "org. Hs. eg. db" prior to enrichment analysis. adjusted P-values < 0.05 and Q-values < 0.05 were considered statistically significant.

2.4. PPI network construction

To screen out the most important genes, we constructed a PPI network of 114 DEGs through the STRING database (http: //STRING -db.org/). PPI nodes were counted by R software.

2.5. Validation of DEGs using qRT-PCR

A total of 18 LAA tissue samples, AF and control groups (n=9, each), were included in this study. Informed written consent was obtained from all participants, and the study protocol was approved by the ethics committee of our hospital. Patients met the following inclusion and exclusion criteria:

Inclusion criteria: The patient's age ranges from 40 to 70 years who underwent valve replacement at the Department of Cardiothoracic Surgery, Taizhou People's Hospital.

Exclusion criteria: All patients were excluded from combined malignancy, hyperthyroidism, acute coronary syndrome, cardiomyopathy, severe infection, immune disease, pulmonary heart disease, hepatic and renal insufficiency disease, history of cardiac surgery or hypertension by history, physical examination and laboratory and ancillary tests after admission.

About 100 mg of LAA tissue was taken after the establishment of extracorporeal circulation for valve replacement in the patients, saline was flushed to remove blood, and the fatty tissue was removed with tissue scissors, frozen in liquid nitrogen, dispensed in pre-prepared lyophilized tubes, and the samples were numbered and stored in a refrigerator at- 80°C for backup.

Total RNA was extracted with Trizol reagent (Invitrogen, China).reverse transcription was performed using the AMV first-strand cDNA synthesis kit according to the manufacturer's protocol (Sangon, Shanghai). qRT-PCR was performed on ABI 7500 system using 2X SG Fast qPCR master mix (Sangon, Shanghai). Differences between samples were calculated by the $2^{-\Delta\Delta Ct}$ method. qRT-PCR primer sequences were shown in Table 1.

2.6. Statistical analysis

Grahpad Prism 8.0.1 were used for the analysis. Continuous variables were expressed as mean ± standard deviation, and t-test was used to compare the two groups. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Analysis of DEGs

The gene expression levels of the combined GEO series that had been adjusted for batch effects were normalized, and the results before and after normalization were shown in S1 File. 54675 probes corresponding to 21655 genes in the GSE31821 and GSE79768 datasets were identified to confirm DEGs in AF. A total of 114 DEGs with | log2FC | ≥ 0.5 in the LAA samples of AF patients were identified compared with the control group, including 11 up-regulated genes and 103 down-regulated genes (S2

File). The heat and volcano maps of the identified DEGs were subsequently analyzed to visualize the DEGs (Fig 1A and 1B).

3.2 GO and KEGG analysis of DEGs

To further investigate the biological functions of the DEGs, GO and KEGG enrichment analysis were performed. Biological process (BP) analysis showed that DEGs were particularly enriched in cellular calcium homeostasis, calcium homeostasis, cellular divalent inorganic cation homeostasis, divalent inorganic cation homeostasis, etc.; cellular component (CC) analysis was mainly enriched in plasma membrane outer, neuronal cytosol etc, and molecular function (MF) showed that DEGs were mainly enriched in cytokine receptor binding, cytokine binding, etc. (Fig 2A and 2B)

In addition, KEGG pathway analysis showed that DEGs were significantly enriched in cytokine-cytokine receptor interactions, viral protein interactions with cytokines and cytokine receptors, influenza A, and chemokine signaling pathways (Fig 2C and 2D).

3.3. screening of pivotal genes

To better understand the relationship among these candidate target genes, a PPI network was constructed using the STRING database and the results were shown in Figure 3A. The PPI nodes were counted by R software, and the top 12 hub genes CXCL10, TLR7, IRF8, DDX58, CCR2, IFIT2, RSAD2, KIT, LYN, CASP1, CSF2RB, CXCL11 with high significance were screened out (Figure 3B). Acording to the results of DEGs, enrichment analysis and PPI results, CXCL10, TLR7, DDX58, CCR2, RSAD2, KIT, LYN, CXCL11 were identified as potential key genes (Figure 3C).

3.4. Validation DEGs by qRT-PCR

Two DEGs (RSAD2 and CXCL11) were randomly selected for further clinical validation. Differential expression between 9 AF and 9 control patients was verified by qRT-PCR. The results (Figure 4) showed that the mean $2^{-\Delta\Delta CT}$ values of RSAD2 and CXCL11 in the AF group were 0.292 (p=0.0167) and 0.325 (p=0.0003), respectively, compared to the control group. Both genes were significantly downregulated in AF compared to the control group, indicating the reliability of the expression of DEGs identified by using bioinformatics tools.

4. Discussion

In this study, we integrated the gene expression profiles of AF and control groups (11 and 8 samples, respectively) from GSE31821 and GSE79768 datasets, then used bioinformatics tools to analyze the data. Compared with control groups, there were 114 DEGs with $|\log2\ FC| \geq 0.5$ in AF. In addition, 8 potential key genes (CXCL10, TLR7, DDX58, CCR2, RSAD2, KIT, LYN, CXCL11) and several important pathways were found to be associated with AF risk, suggesting that they may play an important role in the pathogenesis of AF. Two DEGs (RSAD2 and CXCL11) were randomly selected for further verification

in AF. The results showed that the expression of RSAD2 and CXCL11 was significantly lower in the tissues of patients with AF compared to controls, indicating the reliability of DEGs, which can be used as potential biomarkers for the diagnosis and treatment of AF.

CXCL10 and CXCL11 are both inflammatory chemokines with effects on vascular dysfunction, remodeling, oxidative stress and fibrosis [16]. CXCL10, also known as IP-10, is involved in biological processes by binding to CXCR3. After activation by interferon (IFN) or lipopolysaccharide (LPS), CXCL10 has chemotactic effects in monocytes, T cells and smooth muscle cells [17]. The CXCL10/CXCR3 axis is involved in cardiac inflammatory or non-inflammatory infections playing a role in cardiac remodeling [18]. CXCL10 may be a predictor of cardiovascular disease in the elderly, as elevated CXCL10 levels were observed in the aorta of elderly rats [19]. Furthermore, CXCL10 is found to be a marker of circulating inflammation in patients with advanced HF,which is involved in cardiac remodeling in a study in a rat HF mode [20]. Chen et al. found that MIAT-containing serum extracellular vesicles in AF patients promoted atrial remodeling and exacerbated AF by eliminating miR-485-5p-mediated CXCL10 inhibition [21].

CXCL11, also known as interferon-inducible T cell alpha chemoattractant(I-TAC), is another chemokine that binds to CXCR3 and also to Ackr3 [22]. Elevated CXCL11 levels have been observed in patients with nonalcoholic cirrhotic portal hypertension as well as in patients with severe graft coronary artery disease [23]. CXCL11 and CXCL10 receptor antagonists attenuate phenylephrine-dependent cardiac remodeling [24], they also involve in the pathogenesis of hypertension [25]. Gang et al. have identified CXCL11 as a key AF gene [26].

DDX58 is a member of the retinoic acid-induced receptor , which can recognize exogenous RNA from many viruses, including coronavirus, Zika virus, and rubella [27].DDX58 signaling downstream through the mitochondrial activation complex culminates in activation of TANK-binding kinase (TBK1), leading to transcriptional activation of interferon-responsive genes and release of pro-inflammatory cytokines [28].Mutations in the DDX58 gene may be associated with features typical of Singleton-Merten syndrome, including dental hypoplasia, tendon rupture and severe cardiac sequelae [29].In patients with acute respiratory distress syndrome (ARDS), the DDX58 is found to be highly expressed after infection and significantly enriched in ARDS-related pathways, scoring high in PPI analysis, suggesting that they may be associated with ARDS, providing a new biomarker for the diagnosis, treatment and monitoring of ARDS [30].The role of DDX58 in AF is not reported at present and needs to be further investigated.

Toll-like receptors (TLRs) are congenital pathogen recognition receptors that recognize exogenous microorganisms, including bacteria and viruses, through pathogen associated molecular patterns (PAMPs) to protect the host from infection. The activation of TLRs activation leads to an inflammatory response, resulting in the release of cytokines and chemokines and an influx of inflammatory cells [31]. TLR7 belongs to the TLRs within the cytosol. The TLR7 ligands identified so far are single-stranded RNA and imidazoquinoline derivatives [32]. Interestingly, TLR7 has been shown to have a protective effect against atherosclerosis [33]. In a mouse model of acute myocardial infarction TLR7 is found to mediate

the response to acute myocardial injury and chronic remodeling, possibly by regulating post-infarction scar formation and myocardial inflammatory infiltration [34]. In terms of AF, the use of gene therapy TLR2and TLR7 / TLR8 involved in CD14 + monocytes, blocking the proteasome proteolysis, reducing pro-inflammatory cytokine response, thereby reducing the heart-specific immune response in autoimmune myocarditis animal model [35].

As a chemokine receptor, C-C chemokine receptor 2 (CCR2) regulates the immune response by inducing the recruitment of macrophages and monocytes to sites of inflammation [36], and studies have demonstrated that CCR2 knockdown or treatment with CCR2 inhibitors can protect cardiac function in diabetic patients [37]. In a mouse study, the expression of chemokines or cytokines in CCR2 + macrophages increased after myocardial injury, indicating that monocytes are recruited to the injured sit [38]. A recent study found that CCR2 is a key gene associated with AF progression [39]. The high number of CCR2-positive monocytes/macrophages in the LAA of patients with progressive atrial remodeling in AF may suggest enhanced monocyte/macrophage infiltration. Infiltrating monocytes may differentiate into macrophages and then activate pro-inflammatory processes by releasing cytokines or chemokines to further recruit monocytes/macrophages or induce repair function by promoting tissue fibrosis [40].

Lyn is a non-receptor tyrosine kinase of the Src family, mainly found in myeloid cells and B lymphocytes, and the lack of Lyn and p110δ (an isoform of PI3K) resulte in a significant decrease in autoimmune-mediated renal pathology and improved survival [41].Wang et al. have identified the Lyn gene as a potential diagnostic marker for coronary artery disease (CAD) by bioinformatic methods and therapeutic targets [42], and Lyn gene is identified as a biomarker associated with hypertrophic cardiomyopathy using co-expression analysis [43].Therefore, we speculate that Lyn may also play a role in AF and needs to be further investigated.

The protein encoded by the KIT gene is the stem cell factor receptor (SCFR), also known as the proto-oncogene c-KIT or tyrosine protein kinase KIT or CD117, a receptor tyrosine kinase. c-KIT positivity is a general marker for the identification of cardiac stem cells (CSC), and the protein plays a central role in the ability to self-renew and differentiate in cardiac myocytes [44]. Importantly, c-KIT expression is increased in damaged myocardial tissue such as in myocardial infarction or end-stage HF [45]. It is found that a decrease in c-KIT positive cardiac stem cells in cell cultures derived from left ventricular (LA) tissue of AF patients may be involved in LA remodeling [46].

RSAD2 is commonly considered an antiviral protein, also known as Viperin or Cig5, that plays a key role in the innate immune response system. RSAD2 can be induced by interferon to resist viral infection [47]. The miR-200 family are found to promote podocyte differentiation and discover its novel role as a regulator of cell differentiation possibly by suppressing RSAD2 expression[48]. STAT1-mediated epigenetic control of RSAD2 is found to be a key mechanism for promoting NK cell survival and death during viral infection[49]. It is found that in the presence of IFNc and LPS (or any other exogenous or endogenous TLR4 ligand), RSAD2 can be overproduced in endothelial cells (ECs) and vascular smooth

muscle cells (VSMCs). This may in turn regulate leukocyte attraction, adhesion, and the proliferation and migration of VSMCs, which are important features of vascular dysfunction and early triggers of atherosclerosis [50]. We had observed the differential expression of RSAD2 in AF patients and need to further understand its mechanism.

Although the results based on bioinformatics methods are enlightening, the present study still has limitations. Firstly, We only randomly selected two genes for qRT-PCR validation in a small sample of tissues, which may bias the results, and a larger, multicenter study is needed to complete experimental validation. Secondly, we currently do not know how these genes contribute to atrial fibrillation, and more evidence is needed to identify their biological basis.

5. Conclusion

We identified a total of eight key DEGs (CXCL10, TLR7, DDX58, CCR2, RSAD2, KIT, LYN, CXCL11) by integrating data from the GSE31821 and GSE79768 datasets. The expression of two DEGs (RSAD2 and CXCL11) was also validated by qRT-PCR in tissues from patients with AF, illustrating the reliability and biomarker potential of DEGs screened by bioinformatic methods in the diagnosis and treatment of AF.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of Taizhou People's Hospital (approval number: KY202010901), All the LAA tissue samples were collected with the consent of the patients and their family members, and the informed consents were signed.

Consent for publication

All authors of the article Consent for publication.

Availability of data and materials

The data and materials used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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Author's contribution

Zhong-bao Ruan, Dan He and Li Zhu designed the study, Zhong-bao Ruan, Dan He and Gui-xian Song analyzed the data and wrote the paper. Dan He performed the experiments and wrote the first draft of the paper. Zhong-bao Ruan and Gui-xian Song revised the paper. Ge-cai Chen, Fei Wang and Mei-xiang Wang analyzed the data and contributed to the paper. All authors read and approved the final manuscript.

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Tables

Table 1 List of primer sequences for qRT-PCR

Gene	Forward primer	Reverse primer
RSAD2	TGGGTGCTTACACCTGCTG	GAAGTGATAGTTGACGCTGGTT
CXCL11	GTGGCATTCAAGGAGTACCTC	TGATGGCCTTCGATTCTGGATT
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

Figures

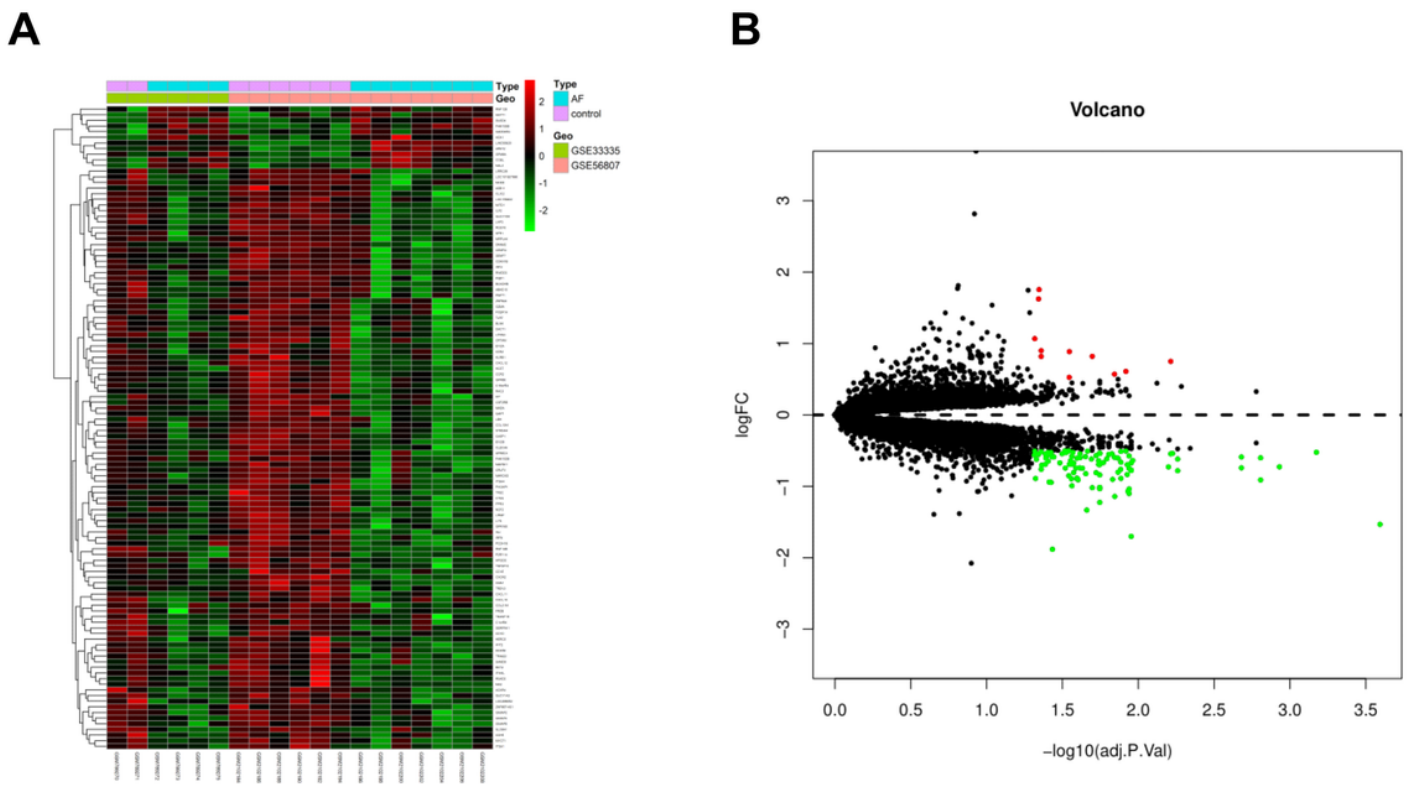

Figure 1

Heat and volcano maps of DEGs identified from the combined dataset.

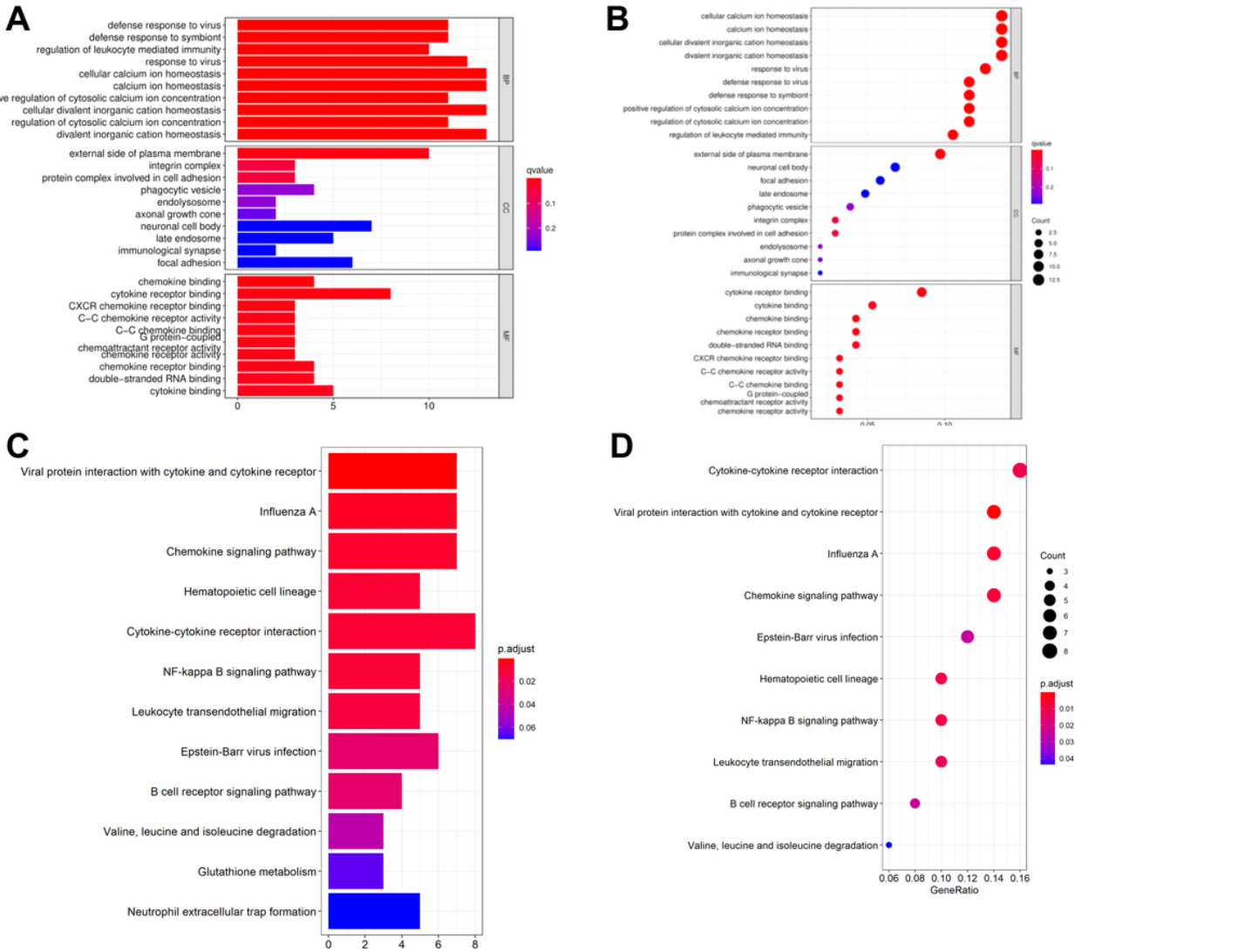

Fig 2 .DEGs function enrichment top 10 analysis results.A, B : GO annotation histogram and bubble diagram, including biological processes, cellular components and molecular functions. C, B:KEGG pathway histogram and bubble diagram.

Figure 2

DEGs function enrichment top 10 analysis results.

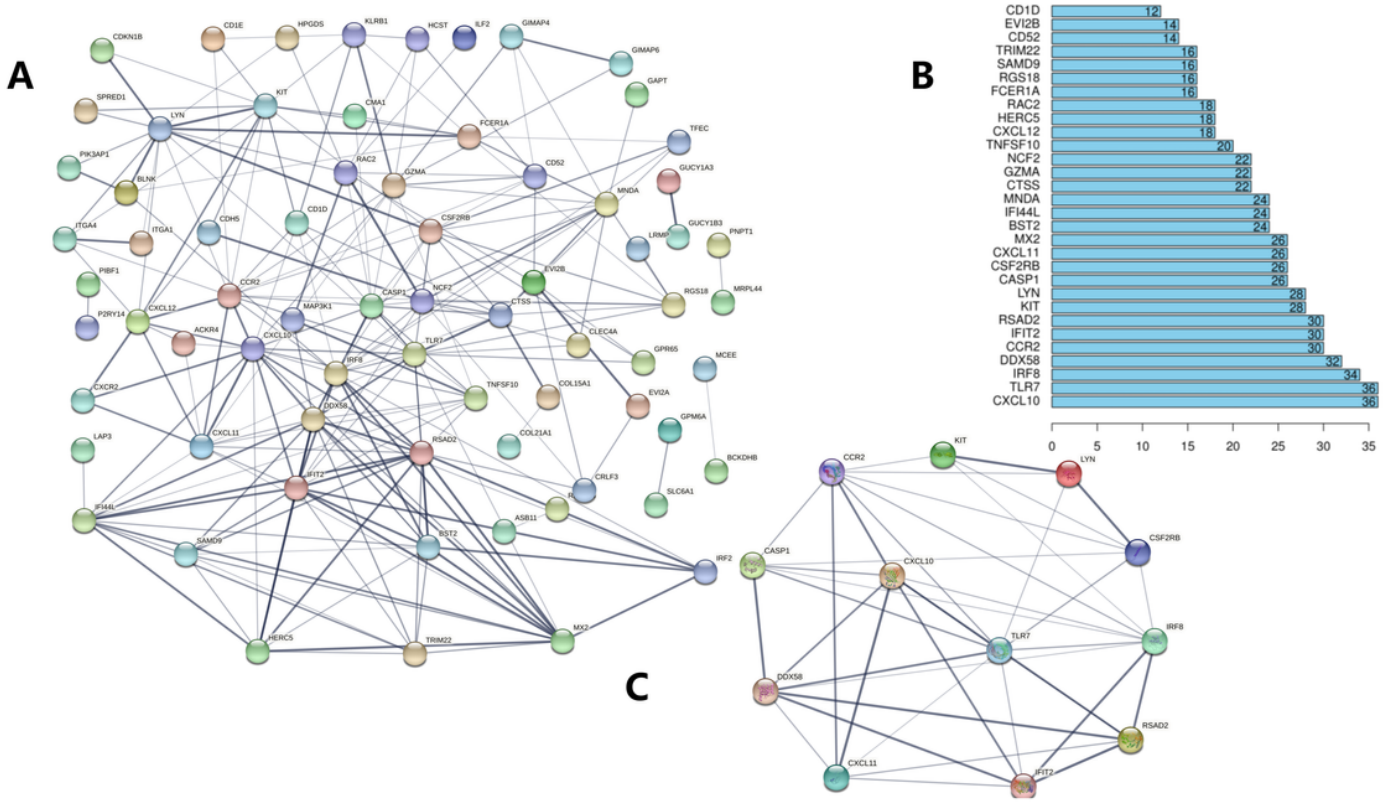

Figure 3

Protein-protein interaction network of DEGs.

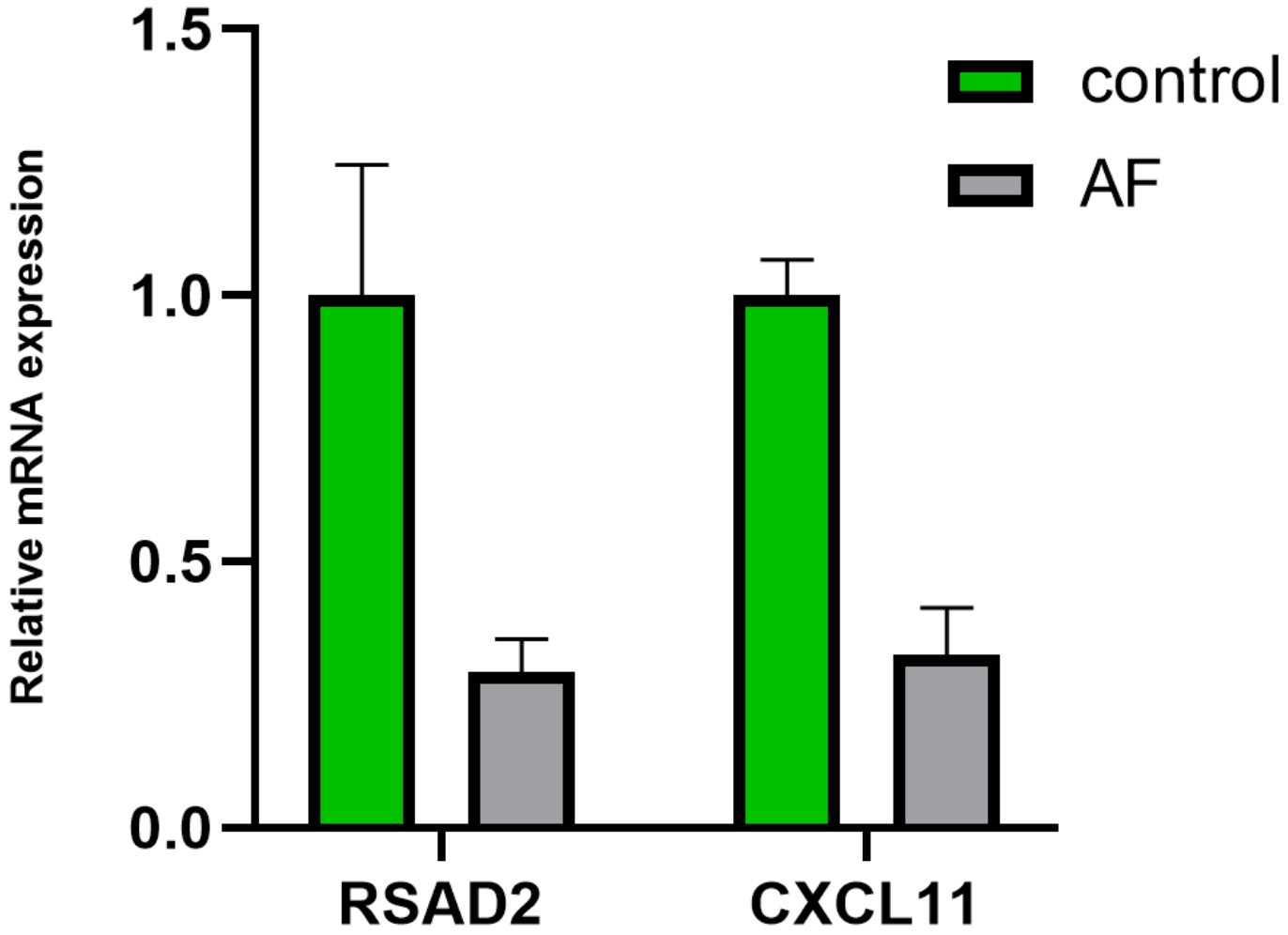

Figure 4

Validation DEGs by qRT-PCR.

Supplementary Files

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

- S1file.rar
- S2file.rar