Integration of Gene Expression Profile Data of Human Epicardial Adipose Tissue From Postoperative Atrial Fibrillation to Verification of Hub Genes

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Research article

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Abstract

Background: The occurrence of post-operative atrial fibrillation (POAF) can significantly reduce the prognosis of patients and increase the mortality of cardiovascular diseases. Meanwhile, epicardial adipose tissue (EAT), plays a crucial role in the occurrence and development of a variety of cardiovascular diseases.

Methods: To link EAT with POAF and explore biomarkers with predictive value or therapeutic intervention significance for POAF in EAT.

Through the Gene Expression Omnibus (GEO) database, gene expression profiles were collected while GSE 143924 contained EAT from POAF patients and sinus rhythm (SR) patients. And the R package "ClusterProfiler" as well as genetic ontology (GO) and The Kyoto Genomic Encyclopedia (KEGG) were utilized to annotate differential genes and to assess relevant functional categories. Cytoscape was used for protein interaction network (PPI) analysis of the differentially expressed genes. In the end, specimens from clinical patients were collected to further verify the selected genes.

Results: The screened differentially expressed genes includes 53 up-regulated genes and 95 down-regulated genes. According to the results obtained by analyzing KEGG, GO enrichment analysis and PPI were made to identify the five genes with the highest degree of connectivity, which includes TNF-α, TLR2, CCL4, TIMP1 and CDH2. Therefore, in peripheral blood samples of clinical POAF patients, CCL4 showed significantly high expression while TIMP1 and CDH2 showed low expression, which was consistent with the results of data screening. But the expression of TLR2 is higher in the POAF group, which is inconsistent with the results of data screening.

Conclusion: TNF-α and CCL4 were up-regulated and TIMP1 and CDH2 were down-regulated in EAT with POAF through data analysis and clinical experiments, which features great clinical value for the prevention, diagnosis and treatment of POAF.

Introduction

As one of the most common cardiac arrhythmia, atrial fibrillation(AF)may increase mortality and lead to poor prognosis in patients with coronary artery diseases (CAD), hypertension, heart failure（HF）or cardiac valve diseases (1). According to clinical statistics, the incidence of post-operative atrial fibrillation (POAF) can reach 10-40%(2). And POAF is multi-mechanism, which may mainly relate to inflammatory response, oxidative stress and sympathetic activation (Monti and others 2020; Viviano and others 2018) In addition, studies have shown that the occurrence of POAF may be closely related to the structural and functional changes of the atrial myocardium which compose its substrate. In recent years, studies on the role of obesity in the pathogenesis of POAF,epicardial adipose tissue (EAT) has attracted more public attention. Visceral heterotopic fat deposition affects both the local and systemic aspects of the body, in the same way that EAT may contribute to POAF through a variety of mechanisms (Nomani and others 2020)(Zhou and others 2020)(1).

Epicardial adipose tissue (EAT) is a kind of metabolically activated beige adipose tissue(3) located between the myocardium and the cardiac membrane and mainly seen on the surface of the right ventricle and the anterior wall of the left ventricle. The EAT also surrounds the atrioventricular grooves and the large coronary vessels, reaching the main thickness of the anterior and outer walls of the right atrium. Due to its elasticity and compressibility, the tissue performs mechanical functions to protect coronary arteries from excessive warps caused by arterial pulses and myocardial contractions(4). EAT can regulate the role of pro-inflammatory cytokines in cardiomyocytes and promote the secretion of inflammatory cytokines such as IL-6, IL-1 and TNF-α(5). Moreover, studies have shown that the expression of YKL40 (CHI3L1) in EAT is highly expressed in patients with POAF and is closely associated with atrial myocyte fibrosis (6). However, no relevant research at the present is on the specific role of EAT in the pathogenesis of POAF.

Materials And Methods

2.1. Analysis of Data from GEO

On a basis of gene expression omnibus (GEO) database, the gene profile was searched(http://www.ncbi.nlm.nih.gov/geo). And all the data were provided from GPL25483 platform. GSE 143924 contained 15 EAT from POAF patients and 15 SR patients. The collected data were analyzed with GEO2R online tool with two classifications including POAF and SR. The data of GSE 143924 were then screened by the following criteria: fold change >1.2 or < −1.2 and p-value <0.05. Use the R package "ClusterProfiler" to annotate the differential genes in order to fully explore the functional relevance of these differential genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to assess related functional categories. The GO and KEGG enrichment pathways with p-value and q-value both less than 0.05 are regarded as significant categories. In order to analyze the protein interaction network of the differential gene and calculate the degree of connection of the differential gene, cytoscape was applied. As for the evaluation of the interactions among the co-expression genes, the STRING (version 10.5) database was utilized and a combined interaction score of >0.4 was set as significant observation.

2.2 Sample Collection

The study conducts a sample of 50 patients who underwent first time coronary artery bypass grafting (CABG) surgery between January 2018 and March 2019 at our hospital. And the written informed consent was obtained from all patients. Persistent AF was defined as AF lasting >7 days. A total of 25 consecutive patients with persistent AF were recruited as the AF group. Exclusion criteria included moderate to severe valvular heart disease, active inflammation (such as infection or systemic autoimmune disease), acute heart failure, electrolyte imbalance, tumor, cardiomyopathy, acute coronary syndrome, history of AF ablation therapy, and receiving treatment with corticosteroids or nonsteroidal anti-inflammatory drugs instead of aspirin. A total of 25patients who had no recorded history of AF and

matched for age, gender, and body mass index (BMI) with the AF group were enrolled as the control group. Moreover, the study was approved by the ethics committee of Jinan Fourth People's Hospital.

2.3 Human EAT collection

Samples of EAT (average 0.5–1.0 g) were collected from all enrolled patients undergoing routine CABG surgery. The EAT samples were obtained near the proximal tract of the right coronary artery within 30 min of beginning surgery. In additionally, the specimens were shock-frozen and immediately stored at -80℃ for total RNA extraction.

2.4 ELISA for TNF-α, TLR2, CCL4, TIMP1 and CDH2

On the morning of surgery, venous peripheral blood samples were collected for measurement of hematological variables. For plasma, the ethylene diamine tetra-acetic acid (EDTA) tubes were centrifuged at 2,000 g for 15 min at 4℃. Then the plasma was stored at -80℃ until further analysis. Serum TNF-α, TLR2, CCL4, TIMP1 and CDH2 were measured by an ELISA kit (R&D Systems). In addition, assays were conducted according to the manufacturer's recommended procedures.

2.5 Statistical analysis

The data were analyzed by using the program SPSS 11.5 for Windows. Quantitative data were presented as mean ± SD. For comparison between multiple groups while the data was analyzed by ANOVA with the Student-Newman-Keuls post hoc analysis. Values of $P<0.05$ were considered significant.

Results

3.1Deferentially Expressed Genes and Networks

The up- and downregulated genes under POAF were analyzed and 53 upregulated genes and 95 downregulated genes (figure1) were observed. The results of GO enrichment analysis showed that differential genes were mainly enriched in humoral immune response, positive regulation of cell adhesion, positive regulation of cell-cell adhesion, positive regulation of ERK1 and ERK2 cascade, and acute inflammatory response (figure2). According to the results of KEGG enrichment analysis, differential genes were mainly enriched in NF−kappa B signaling pathway and Complement and coagulation cascades signaling pathway (figure3). And the results of PPI showed that the top 5 genes with the highest degree of connectivity were TNF-α, TLR2, CCL4, TIMP1, CDH2 (figure4).

3.2 Baseline Characteristics

Table 1. Patient Clinical Characteristics

	Atrial fibrillation(n =30)	Sinus rhythm (n =20)	P
Age, years	64.6±4	66.6±5	0.562
Current smoking (%)	27.5	27.6	1
BMI(kg /m2)	24.34±1.11	24.14±1.52	0.818
SBP	137.8	135.6	0.298
Diastolic blood pressure	65.4	65.6	0.848
Heart rate (beats/min)	89	72.2	0.016
Diabetes (%)	24.3	24.5	1
Total cholesterol	4.05±0.63	4.16±0.43	0.76
HDL cholesterol	1.22±0.21	1.40±0.36	0.38
LDL cholesterol	5.34±0.21	5.32±0.16	0.72
HbA1C (%)	2.75±0.43	2.85±0.43	0.38
LA diameter, mm	48.8±2.59	39.8±3.3	0.87
Ejection fraction (%)	47.2±5.63	52±5.43	0.207
EAT thickness (mm)	5.6±0.16	3.52±0.19	<0.01
β-blockers (%)	45.3	45.2	1
Statins (%)	78	78	1
ACEI/ARB (%)	43	43	1
MRA	5.9	5.8	1

ACEI/ARB, angiotension converting enzyme inhibitor; BMI, body mass index; EAT, epicardial adipose tissue; HbA1C, hemoglobin A1C; HDL, high-density lipoprotein; LA, left atria; LDL, low-density lipoprotein; MRA, mineralocorticoid receptors antagonist; SBP, systolic blood pressure

3.3 TNF-α, TLR2、CCL4, TIMP1 and CDH2 in the serum

In the POAF group, TNF-α, TLR2 and CCL4 were higher than what observed in the SR group (P<0.01). In the POAF group, TNF-α was 1.72-fold compared to the SR group. And the level of TLR2 was 1.74 times in the POAF group. In the POAF group, CCL4 was 2.31-fold compared with the group of SR. However, the levels of TIMP1 and CDH2 decreased to a level between POAF group and SR group (P<0.01) (figure5).

3.4 Histological changes

Hematoxylin-eosin staining (HE) to observe EAT tissue while EAT adipocytes present a typical vacuole-like structure. Hardly no inflammatory cell infiltration between EAT fat cells in SR patients is observed, while inflammatory cell infiltration in EAT is obvious in patients with POAF patients (Figure 6).

Discussion

POAF is defined as Postoperative atrial fibrillation that occurs when it does not exist before surgery and not occur persistently and paroxysmally after surgery(7). The fibrillation is the most common postoperative complication of heart surgery and will lead to longer hospital stays, increasing treatment costs and the mortality (8). Meanwhile, effective postoperative monitoring and over attention to risk factors are effective methods to reduce POAF. In this study, 148 genes were firstly filtrated out. Then PPI results showed that the top 5 genes with the highest degree of connectivity are TNF-α(9), TLR2, CCL4, TIMP1 and CDH2. After identifying the differentially expressed genes, peripheral blood and EAT samples from 15 POAF and 15 SR groups were clinically collected to detect the expression of differentially expressed genes and thus verify the expression of the genes. Except TLR2, genes of TNF-α, CCL4, TIMP1 and CDH2 are in line with data statistics. Therefore, TNF-α and CCL4 increases TIMP1 as CDH2 decreases, which makes independent risk factors for POAF. A growing interest is to find reliable POAF biomarkers to better identify patients with high-risk diseases and finally benefit those patients from the preventive treatment.

Since protein-protein Interaction (PPI) algorithm can identify key proteins in both dense and sparse areas in the protein network, the accuracy of key proteins identification degree is effectively improved. Identifying protein complexes through biological experiments can be costly, highly affected by the environment, low efficiency, and can lead to high false positives in experimental results(10). The method of excavating protein complexes in the protein interaction network with the help of bioinformatics is not only low cost and high efficiency, but also can dig out transient and dynamic protein complexes. Using protein interaction networks, this article conducts a more in-depth study on the popular issues, such as protein complex mining, key protein identification, and disease-causing gene prediction.

In clinical practice, the incidence of POAF shows a trend of daily increase, which makes the prevention of POAF a hot spot in clinical research in recent years. In this study, GEO data set was analyzed to screen for dysregulated genes in POAF and SR, and collected EAT from 15 POAF patients and 15 SR patients to identify new biomarkers. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to assess related functional categories (11, 12). The GO and KEGG enrichment pathways with p-value and q-value both less than 0.05 are regarded as important categories. The results of GO enrichment analysis showed that differential genes are mainly enriched in humoral immune response, positive regulation of cell adhesion, positive regulation of cell-cell adhesion, positive regulation of ERK1 and ERK2 cascade, and acute inflammation. The results of KEGG enrichment analysis showed that differential genes are mainly enriched in NF-κB signaling pathway and complement and coagulation cascade signaling pathways. Studies have shown that thrombin mainly promotes hemostasis and thrombosis, which is the main effect protease of the coagulation cascade and the main target of

anticoagulation therapy in thromboembolism. Thrombin mainly activates the signal transduction mediated by the protease activated receptor (PAR), thereby activating the MAP kinase pathway (ERK1/2) and the NF-κB pathway(13–15). However, postoperative atrial fibrillation is related to the activation of coagulation factors and protease-activated receptors in the local and circulation. Atrial fibrillation-related hypercoagulability significantly increases the risk of thrombosis and stroke in patients with atrial fibrillation. Therefore, by inhibiting the thrombin signaling pathway, the occurrence of postoperative atrial fibrillation can be effectively reduced. In the research, the KEGG enrichment analysis showed that the differential genes were mainly enriched in the NF-κB signaling pathway and related to the complement and coagulation cascade signaling pathway. NF-κB is a pleiotropic inducing transcription factor. In addition to regulating the expression of multiple genes and producing cytokines, it can also regulate the expression of multiple inflammatory factors and cells Apoptosis. Many previous studies have shown that the inflammatory factor NF-κB has important significance in the occurrence of cardiovascular diseases (16). NF-κB has a regulatory effect on a variety of cytokines and adhesion factors necessary for immune response, inflammatory response, cell differentiation and growth, cell adhesion and cell apoptosis. A number of previous studies have shown that NF-κB is an important transcription factor that regulates the expression of inflammatory genes (17), and the NF-κB signaling pathway is of great significance in the occurrence of cardiovascular diseases, and it may become an important target for the treatment of atrial fibrillation and the prediction of POAF in the future.

Existing studies have found that the expression of TNF-α in the myocardium of atrial fibrillation rats is up-regulated (18, 19), and both artesunate and captopril can reduce the expression of TNF-α. Similar studies have confirmed that Artemisia annua extract can reduce TNF-α by interfering with the expression of NF-κB(20), indicating that NF-κB is an upstream regulator of TNF-α. And studies have shown that patients with rheumatic valvular disease and atrial fibrillation have higher expression of NF-κB protein and higher levels of TNF-α while a positive correlation builds between NF-κB and TNF-α(21). This suggests that the NF-κB levels may affect atrial fibrosis by interfering with the expression of TNF-α to promote collagen deposition. In our study, we found that compared with the SR group, the AF group had higher levels of TNF-α, CCL4 mRNA and protein in atrial tissue. Therefore, CCL4 may be an independent risk factor for POAF and participate in the up-regulation mechanism of TNF-α. Existing research evidence shows that there is inflammation in the local atrial muscle of patients with atrial fibrillation, which plays an important role in atrial remodeling and the occurrence and maintenance of atrial fibrillation.

Atrial fibrillation can also be a postoperative complication during cardiopulmonary bypass of cardiac surgery, human blood, contact with the extracorporeal circulation channel, can induce systemic inflammatory response syndrome. In addition to systemic inflammation, local inflammation (such as pericarditis) during cardiac surgery is also an important factor in causing POAF. Detecting the level of inflammatory markers can be used to study the relationship between inflammation and POAF. The study also found that TNF-α and CCL4 were significant increases after heart surgery. And TNF-α and CCL4 are considered as the basic pathological mechanisms of inflammation leading to POAF. The analysis also showed that the AF group had higher levels of TNF-α and CCL4 mRNA and protein in atrial tissue compared with the SR group, which indicates that the elevated expression levels of TNF-α and CCL4 may

affect atrial fibrosis by promoting inflammation. Meanwhile, the increased expression levels of TNF-α and CCL4 can be the independent predictors of POAF. Therefore, the risk of postoperative atrial fibrillation can be assessed by simply measuring the expression levels of TNF-α and CCL4 after CABG and high-risk patients can be screened, and corresponding intensive interventions can be taken to reduce the incidence of postoperative atrial fibrillation.

Moreover, the remodeling of atrial extracellular matrix (ECM) during POAF involves changes in the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs)(22). Relevant studies have proved that in terms of qualitative and quantitative, analyzing the dynamic remodeling of atrial ECM is more likely to occur in patients with atrial fibrillation than the elderly (23), and the increased activity of MMPs, TIMPs and their interaction may be one of the reasons for atrial ECM remodeling. Many heart diseases and other diseases can eventually lead to myocardial fibrosis. As a characteristic of myocardial fibrosis, accumulation of ECM can reduce the compliance of the heart, change the biochemical properties, electrical conduction of the heart, ultimately damage the function of myocardial cells and cause arrhythmia. The integrity of ECM and its network structure is mainly maintained by the balance between the activities of MMPs and its inhibitor TIMPs. Related studies have found that the balance of MMPs and TIMPs is closely related to the atrial remodeling of atrial fibrillation(24). Matrix metalloproteinase inhibitors (TIMPs) affect the degradation of extracellular matrix (ECM) during the occurrence and development of hypertension and atrial fibrillation, which plays an important role in the research. Compared with the SR group, the AF group showed a lower level of TIMP1 mRNA and protein in atrial tissue. Identically, EAT thickness was significantly higher than those of the SR group in the study, which suggests that the decreased TIMP1 levels may affect atrial fibrosis by promoting collagen deposition. Therefore, the reduced TIMP1 expression level may be an independent risk factor for POAF. In addition, other studies showing that TIMPs can regulate various biological processes by inhibiting MMPs, including ECM remodeling, growth factors and their receptor activities(25). The TIMP family consists of four members of TIMP-1~TIMP-4, can also inhibit other mechanisms besides MMP to regulate other important processes, such as proliferation and apoptosis (26).

In addition, N-Cadherin (CDH2) has a significant tendency to regulate patients with POAF, and may be closely related to POAF. N-cadherin is a type of transmembrane protein that is expressed in various tissues and plays a role in mediating cell-cell adhesion. Studies have shown that, compared with patients with sinus rhythm, the expression of connective tissue growth factor (CTGF) in the left atrium of patients with atrial fibrillation is significantly up-regulated(27). CTGF is an important mediator of atrial remodeling during atrial fibrillation, and Rac1 is a key regulator of CTGF in vivo. Angiotensin II activates CTGF by activating Rac1 and nicotinamide adenine dinucleotide phosphate oxidase, leading to the up-regulation of CDH2 to participate in the signal transduction of atrial remodeling(28). In the study, the AF group has lower levels of CDH2 mRNA and protein in atrial tissue compared with the SR group. This suggests that the reduction of CDH2 levels may affect atrial fibrosis by promoting collagen deposition. Therefore, the reduction in CDH2 levels can be used as a predictor of POAF.

The study also found that the EAT thickness, LDL cholesterol level, and LA diameter of POAF patients were significantly higher than those of the SR group, while the HDL cholesterol level and Ejection fraction of POAF patients were significantly lower than that of the SR group. By monitoring the index of TNF-α, CCL4,TIMP1 and CDH2, the thickness of EAT thickness, LDL cholesterol and LA diameter can be reduced in the long-term prognosis, and HDL cholesterol and Ejection fraction can be improved to obtain better quality of life and increase life expectancy. Nevertheless, this research may still have some deficiencies such as the lack of further verification of the pathway.

The occurrence of postoperative atrial fibrillation (POAF) significantly reduces the patient's prognostic quality of life and increases the risk of death from cardiovascular disease. Therefore, the Clinical postoperative patients can greatly benefit from improving the predictive ability of POAF and reducing the incidence of postoperative atrial fibrillation. Based on the data analysis and clinical experiments, TNF-α and CCL4 were up-regulated while TIMP1 and CDH2 were down-regulated in EAT, which has important clinical value for the prevention, diagnosis and treatment of POAF.

Declarations

Ethics approval and consent to participate

This investigation conforms to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The animals were afforded by the laboratory animal center, the Shandong University of Traditional Chinese Medicine (NO.SCXX2015-0006) and were approved by the Ethical Committee of the Fourth People's Hospital of Jinan (Jinan, China).

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the GEO database repository, https://www.ncbi.nlm.nih.gov/.The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WG and BD designed the study. CYJ completed the samples collection and wrote the manuscript. XW was involved in the statistical analyses. All authors read and approved the final manuscript.

Acknowledgments

Not applicable.

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Figures

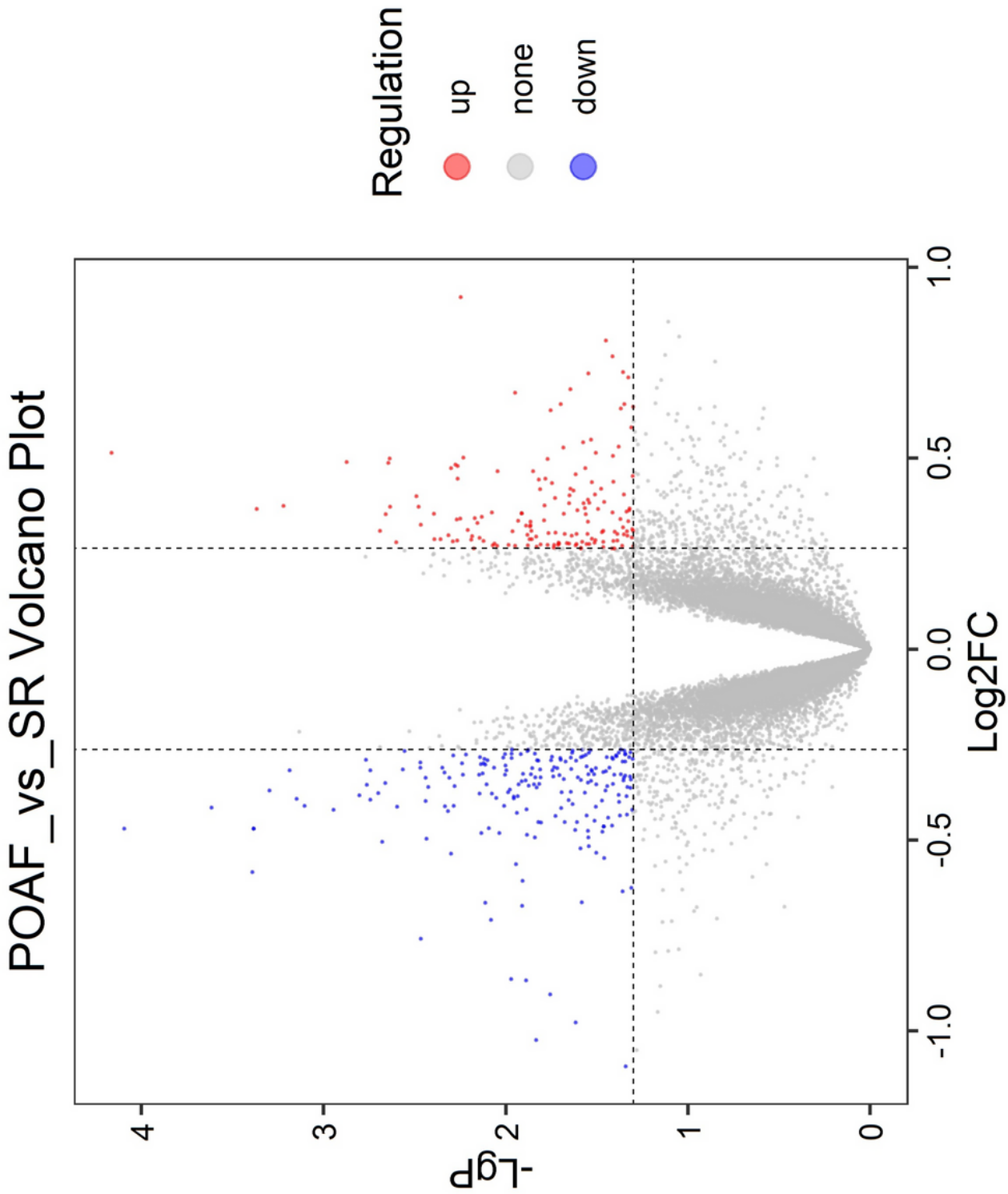

Figure 1

Analysis of dysregulated genes between POAF and SR groups. The volcano plot graph of genes was constructed according to fold change values and p values. The X axis represents the log2 (fold change) value of differential expression, and the Y axis represents the -log10 (padj) value of differential expression.

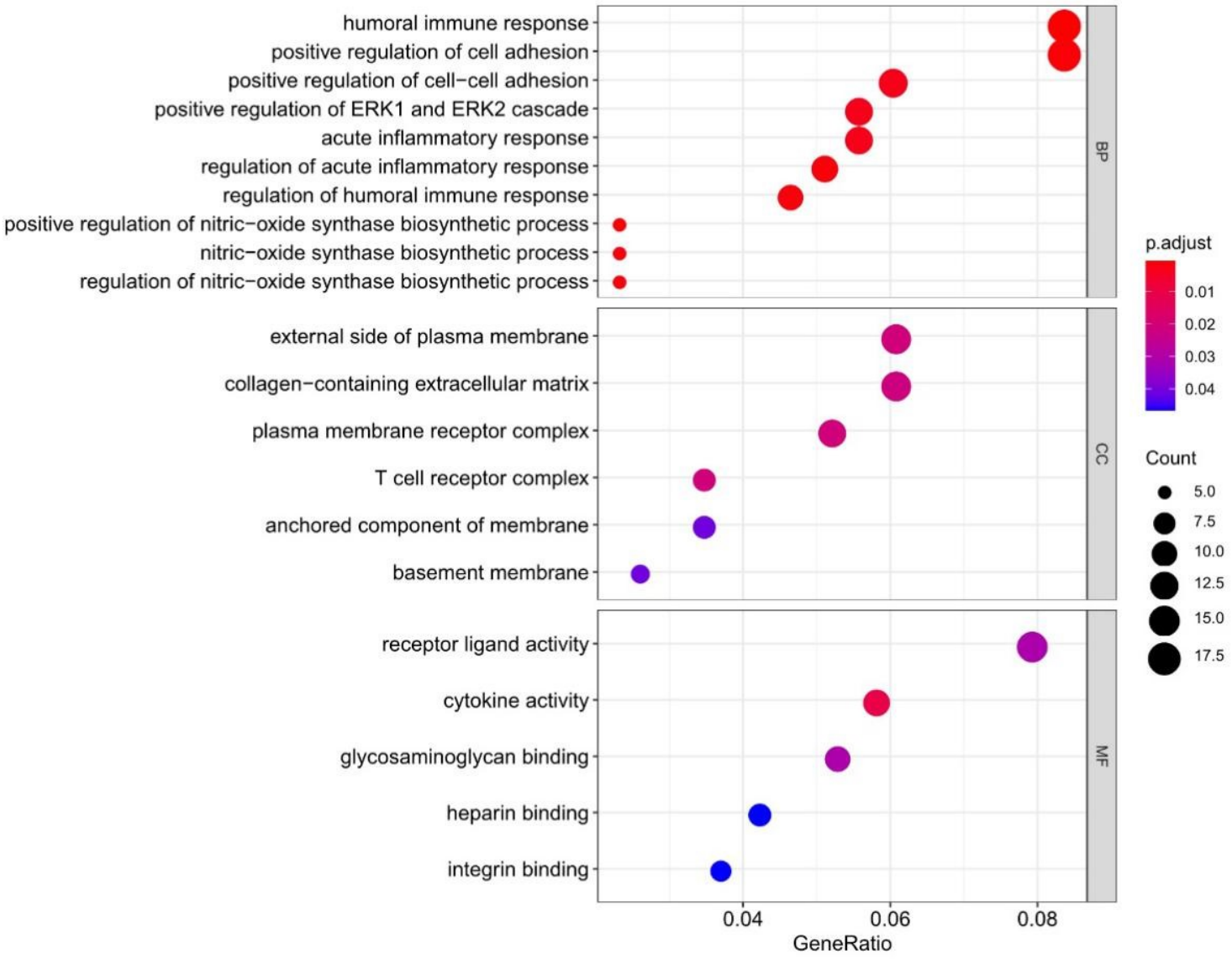

Figure 2

Gene set enrichment analysis of POAF. GO enrichment results.

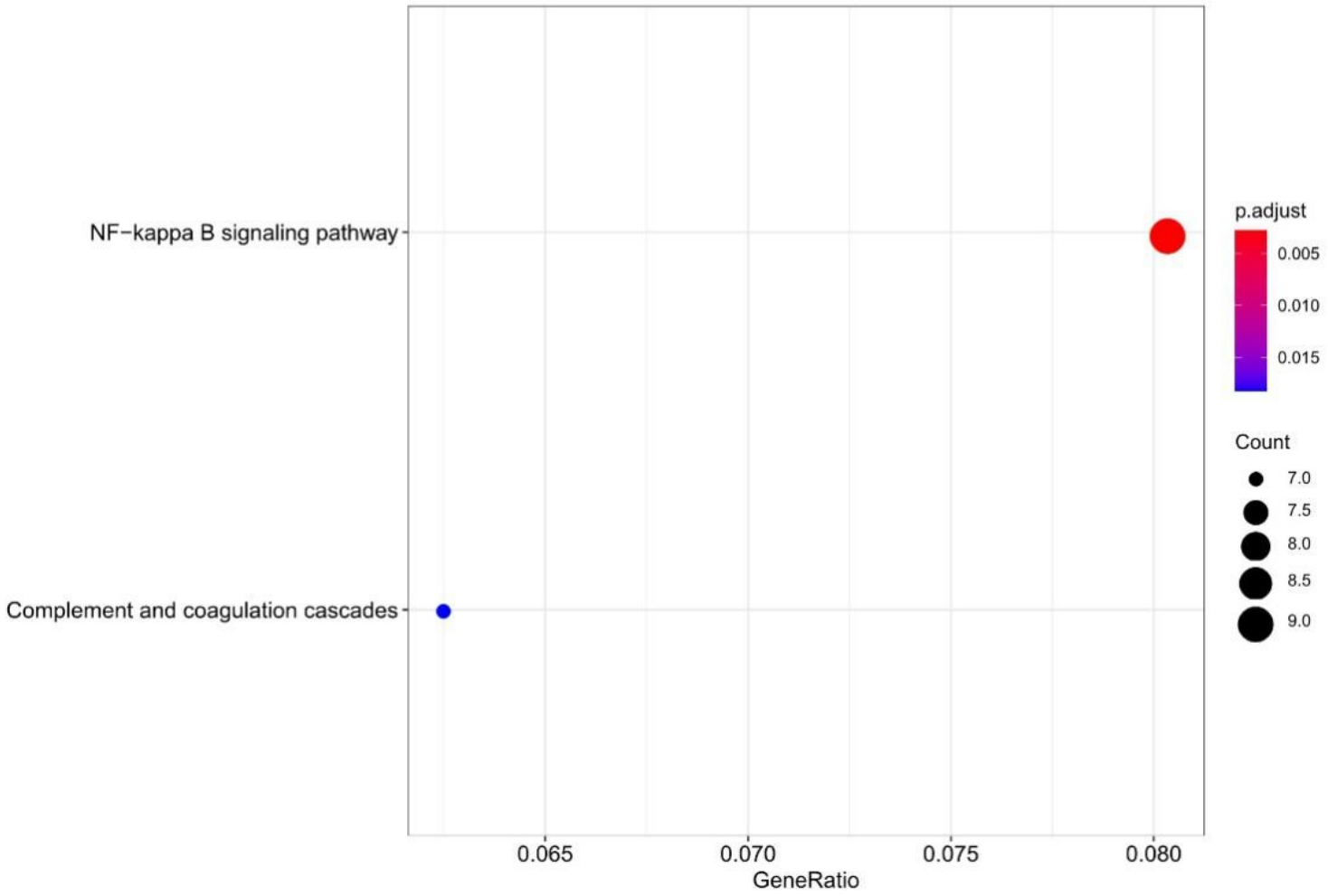

Figure 3

Gene set enrichment analysis of POAF. KEGG enrichment results.

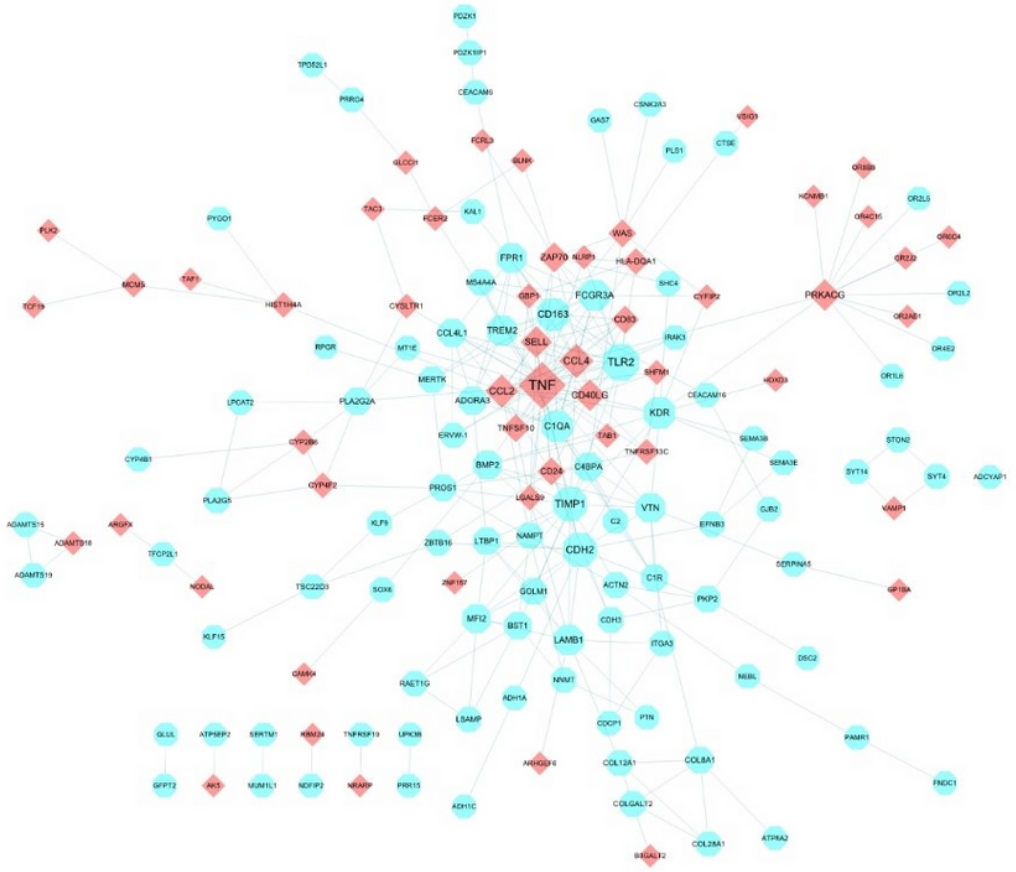

Figure 4

Protein-protein interaction network of up and downregulated genes. Nodes are colored by the trend of regulation: upregulated (red) and downregulated (green). Node size is proportional to the p value: the lower the value, the larger the node.

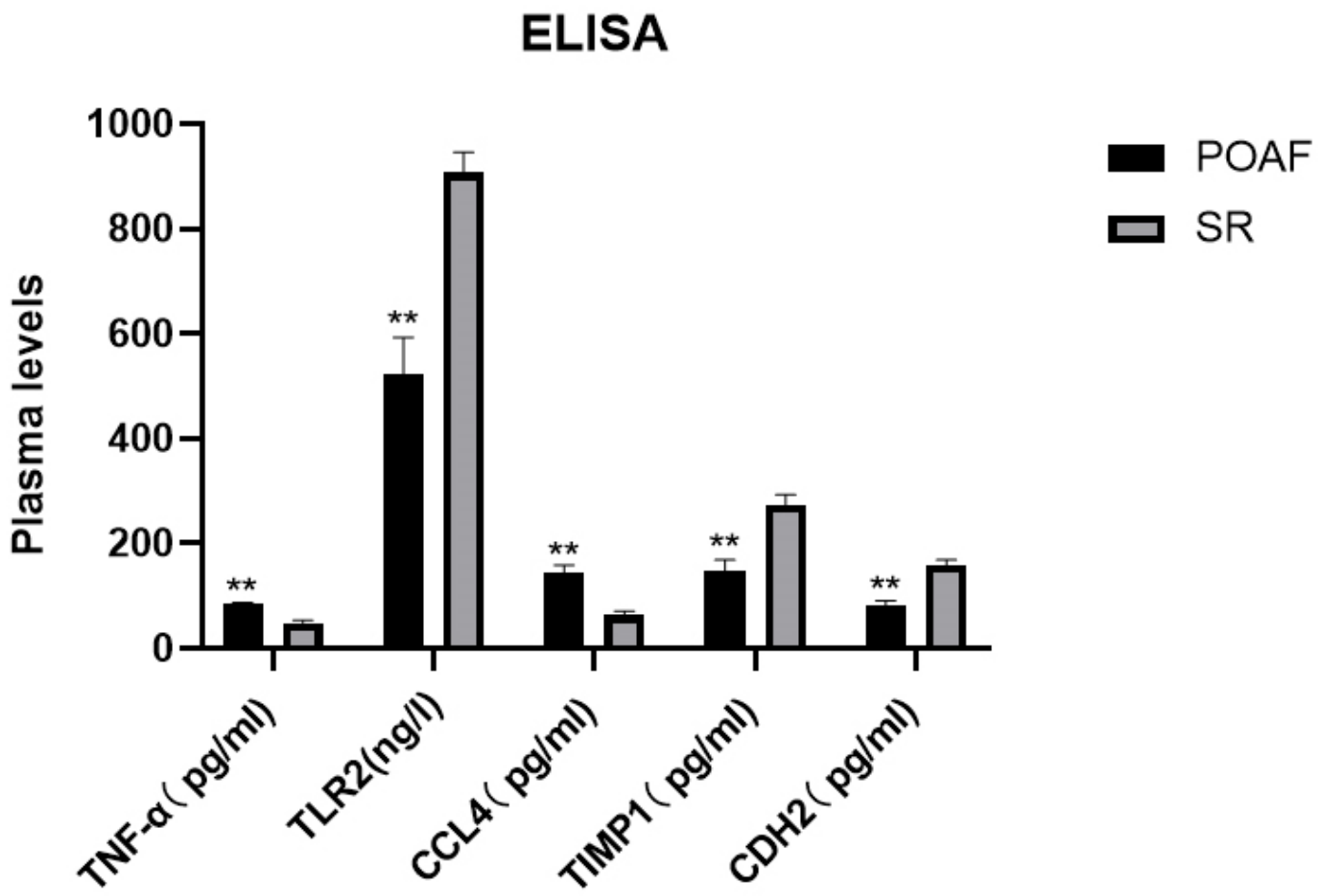

Figure 5

Serum levels of TNF-α, TLR2、CCL4, TIMP1 and CDH2. **P<0.01, vs. SR group. SR, Sinus rhythm; POAF, vehicle.

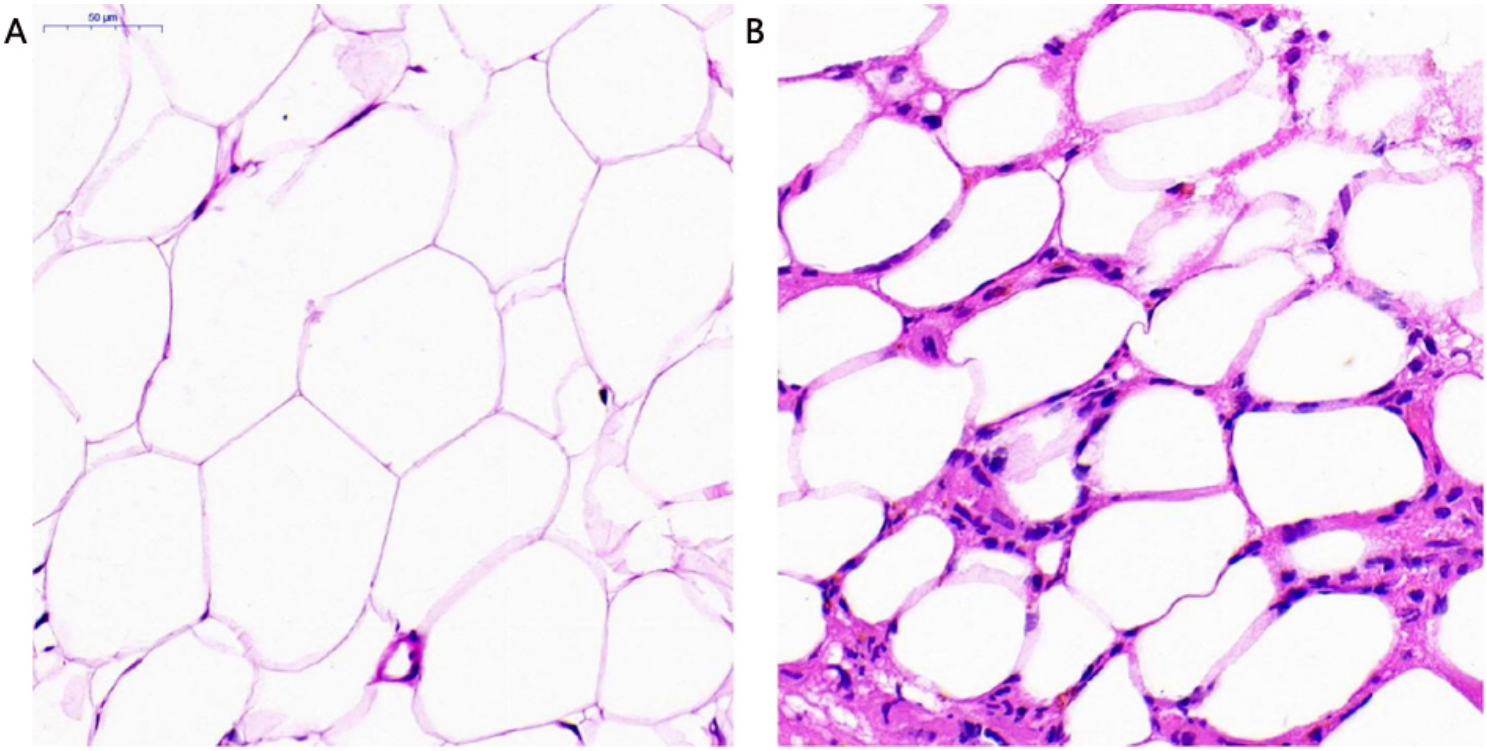

Figure 6

HE staining of epicardial adipose tissue (×40). Significant inflammatory cell infiltration in patient with persistent atrial fibrillation. A: SR group; B: POAF group.