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Bioinformatic analysis for the identification of potential gene interactions and therapeutic targets in atrial fibrillation

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Abstract. – OBJECTIVE: Atrial fibrillation (AF) is the most common type of tachycardia. The major injury caused by AF is a systemic embolism. Although AF therapies have evolved substantially, the success rate of sinus rhythm maintenance is relatively low. The reason is the incomplete understanding of the AF mechanisms.

MATERIALS AND METHODS: A Gene Expression Omnibus (GEO) dataset (GSE79768) was downloaded. Differentially expressed genes (DEGs) were identified by bioinformatic analysis. Enriched terms and pathways were identified by gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. A protein-protein interaction (PPI) network was constructed to determine regulatory genes. CytoHubba and the Molecular Complex Detection (MCODE) algorithm were used to identify potential hub genes and important modules. The Predicting Associated Transcription Factors From Annotated Affinities (PASTAA) method was used to predict transcription factors (TFs).

RESULTS: Two hundred thirty-five upregulated DEGs and seventy-seven downregulated DEGs were identified. In the GO biological process, cellular component, and molecular function analyses, positive regulation of cell migration, anchoring junctions, and cell adhesion molecule binding were enriched significantly. The Hippo signalling pathway was the most significantly enriched pathway. In the PPI network analysis, we found that Class A/1 (rhodopsin-like receptors) may be the critical module. Ten hub genes were extracted, including 6 upregulated genes and 4 downregulated genes. CXCR2, TLR4, and CXCR4 may play critical roles in AF. In the TF prediction, we found that Irf-1 may be implicated in AF.

CONCLUSIONS: We found that the CXCR4, TLR4, CXCR2 genes, the Hippo signalling pathway and the class A/1 (rhodopsin-like receptors) module may play critical roles in AF occurrence and maintenance, which may provide novel targets for AF treatment.

Key Words:
Atrial fibrillation, Bioinformatic analysis, PPI, MOCDE.

Introduction

Atrial fibrillation (AF) is known as the most common type of cardiac tachycardia. The incidence of AF increases with aging. It is classified as paroxysmal AF, persistent AF, and permanent AF[1]. The prevalence rate is more than 10% among those older than 80 years[2]. The major harms of AF are embolism (such as stroke) and heart failure. Effective treatment of AF will improve the clinical outcome of AF through means such as reducing the disability rate caused by stroke. Although treatment strategies have evolved substantially in recent years, their efficacy is not ideal. Radiofrequency ablation of AF has evolved considerably to become safer and more effective over the past decade, while the recurrence rate is relatively high, especially for patients with persistent AF[3]. Pharmacological treatment of AF

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may induce substantial adverse side effects, such as drug-induced proarrhythmia and cardiac and noncardiac toxicity[4-6]. The reason that current treatment strategies have limited efficacy is the incomplete understanding of the mechanisms of occurrence and progression of AF. Understanding the mechanisms may help us to find novel strategies for AF treatment.

Omics has become increasingly important in investigating the mechanisms of diseases as it reveals the differential expression of genes, RNAs or proteins between patients and controls. There may be hundreds of differentially expressed genes, RNAs or proteins in a given analysis, so it is necessary to integrate them into modules and pathways through existing knowledge to understand the mechanisms of disease. Bioinformatic analysis is a powerful tool for omics dataset analysis. In this study, bioinformatic analysis was used to identify potential key genes and pathways to obtain a better understanding of AF mechanisms. GSE79768, a dataset of expression profiles of the left atrium in patients with atrial fibrillation and sinus rhythm, was used to identify DEGs. GO analysis and KEGG pathway analysis were performed to identify enriched terms and pathways. Protein-protein interaction (PPI) analysis was performed to identify key modules and hub genes involved in AF.

Materials and Methods

Data Source and Processing

The gene expression profiles associated with AF were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The search terms were "atrial fibrillation," "Homo sapiens," and "expression profiling by array". After screening, the GSE79768 dataset submitted by Tsai et al[7] was selected for obtaining gene expression profiles. In this dataset, 13 specimens from 13 patients (7 with persistent AF, 6 with sinus rhythm) were enrolled in our analysis. The specimens of atrial appendages were obtained from patients receiving surgery for mitral valve or coronary artery disease. Patients with AF presented persistent AF known for more than 6 months, and patients with SR had no clinical evidence of AF without the use of any anti-arrhythmic drugs[7]. The expression profile arrays were generated by GPL570 Affymetrix Human Genome U133 Plus 2.0 Array. The probe ID for each gene was converted to a gene symbol using hgu133a.db, org.Hs.eg.db and the annotate package in Bioconductor (*http://www.bioconductor.org*)[8].

Identification of Differentially Expressed Genes

DEGs in the left atrial samples of patients with AF compared with those of patients with SR were identified by the R package LIMMA[9]. The false discovery rate (FDR) was used for *p*-value correction by the Benjamin and Hochberg method[10] and for fold change (FC) calculation. $|\text{LogFC}| > 1$ and adjusted $p < 0.05$ were set as thresholds for DEGs.

Functional Enrichment Analysis

Databases for annotation, visualization, and integrated discovery (DAVID) bioinformatics resources and Metascape were used to perform GO enrichment analysis and KEGG enrichment analysis[11,12]. The cutoff for the *p*-value was 0.01. Enriched terms were selected to construct a network. Similar terms were connected with edges. The cutoff of similarity is 0.3. The network is visualized using Cytoscape, and nodes represent enriched terms colored by cluster ID and *p*-value[12].

Construction of the Differential Co-Expression Gene Network

PPI network analysis of DEGs was performed with the STRING database[13], BioGrid database[14], InWeb IM database[15], and OmniPath database[16]. Proteins that interacted with others formed a network. Key modules were identified by the Molecular Complex Detection (MCODE) algorithm. Enrichment of pathway and process was performed on MCODE modules, and the top three terms by *p*-value were extracted[17].

Analysis of Hub Genes

The cytohubba plugin of Cytoscape was used to determine key genes, also called hub genes, in the network. The top 10 hub genes were identified by the maximal clique centrality (MCC) method[17].

Analysis of TFs

Transcription factors (TFs) were predicted by using PASTAA[18]. After DEGs were established, we used both the association score and *p*-value to determine the relationship between AF and TFs by hypergeometric distribution[8].

Results

The DEGs and Functional Enrichment of DEGs

In this study, 54,675 probes corresponding to 23518 genes were identified. There were DEGs in left atrial specimens of patients with AF compared with patients with SR, including 235 upregulated DEGs and 77 downregulated DEGs. The heatmap and volcano plot are shown in Figure 1A and Figure 1B, respectively.

The top 10 GO biological process terms (Figure 2A), cell component terms (Figure 2B), molecular function terms (Figure 2C), and KEGG pathways (Figure 2D) are shown according to the *p*-value. These included positive regulation of cell migration, anchoring junction, and cell adhesion molecule binding. The Hippo signalling pathway was the most significantly enriched pathway. Enriched terms were integrated into networks by cluster ID (Figure 3A) and *p*-value (Figure 3B). In Figure 3A, nodes sharing the same cluster ID are shown in the same color. In Figure 3B, terms containing more genes tend to have lower *p*-values.

The PPI Network and Key Module of the DEGs

The PPI network is shown in Figure 4, which accounted for 79.2% of the differentially expressed genes. To investigate densely connected network components, an MCODE network was constructed (Figure 5 and Table I). The three best-scoring terms by *p*-value are shown in Figure 5 and Table I. A functional description of the corresponding components is also shown in Figure 5. The GO description of MCODE1 is Class A/1 (rhodopsin-like receptors), G alpha (i) signalling events, and peptide ligand-binding receptors. The GO description of MCODE2 is positive regulation of cellular protein localization, PI3K-Akt signalling pathway, and regulation of cellular protein localization. The GO description of MCODE3 is EPH-ephrin mediated repulsion of cells, ephrin receptor signalling pathway, and EPH-Ephrin signalling. The GO description of MCODE4 is PID HIF2 signalling, response to oxidative stress, and cellular responses to stress.

Hub Gene Selection

The top 10 hub genes were determined by cytoHubba via the MCC method. The hub genes are shown in Figure 6. The upregulated genes were CXCR2 (degree=9), TLR4 (degree=8), CXCR4 (degree=7), PTPRC (degree=7), CASP1 (degree=6), and IL33 (degree=6). The downregulated genes were IL18 (degree=6), EGFR (degree=5), NMU (degree=4), and C3 (degree=4). Detailed information on these genes is shown in Table II.

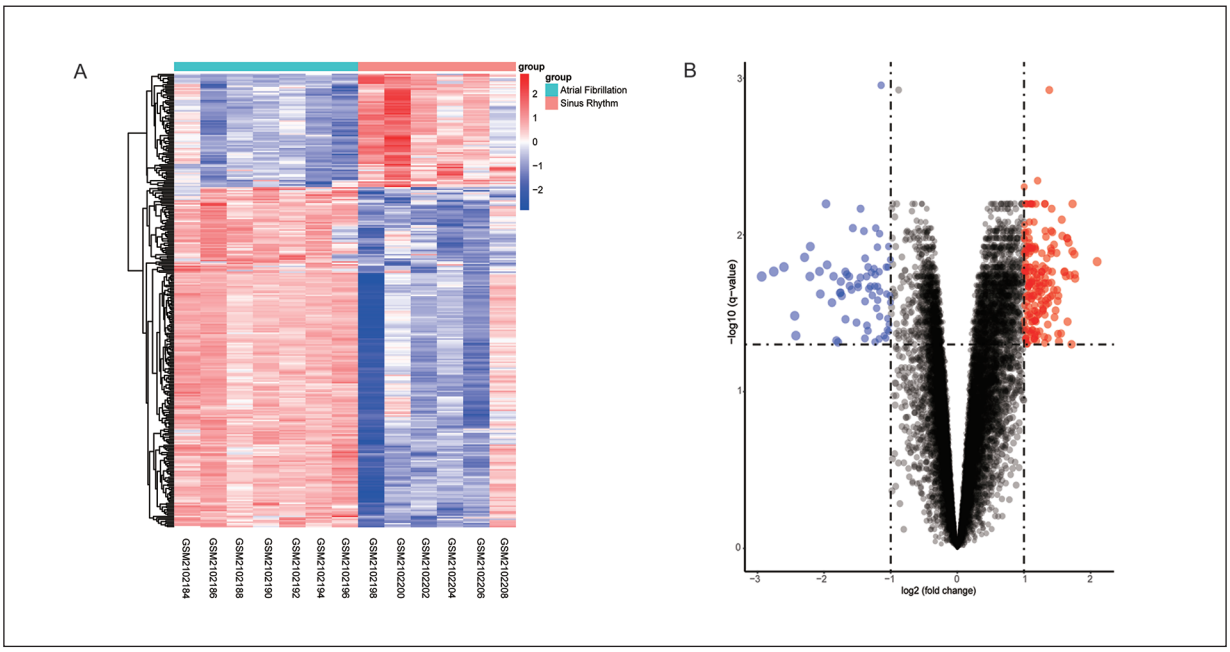

Figure 1. (**A**) Heatmap of differentially expressed genes and **(B)** volcano plot of differentially expressed genes.

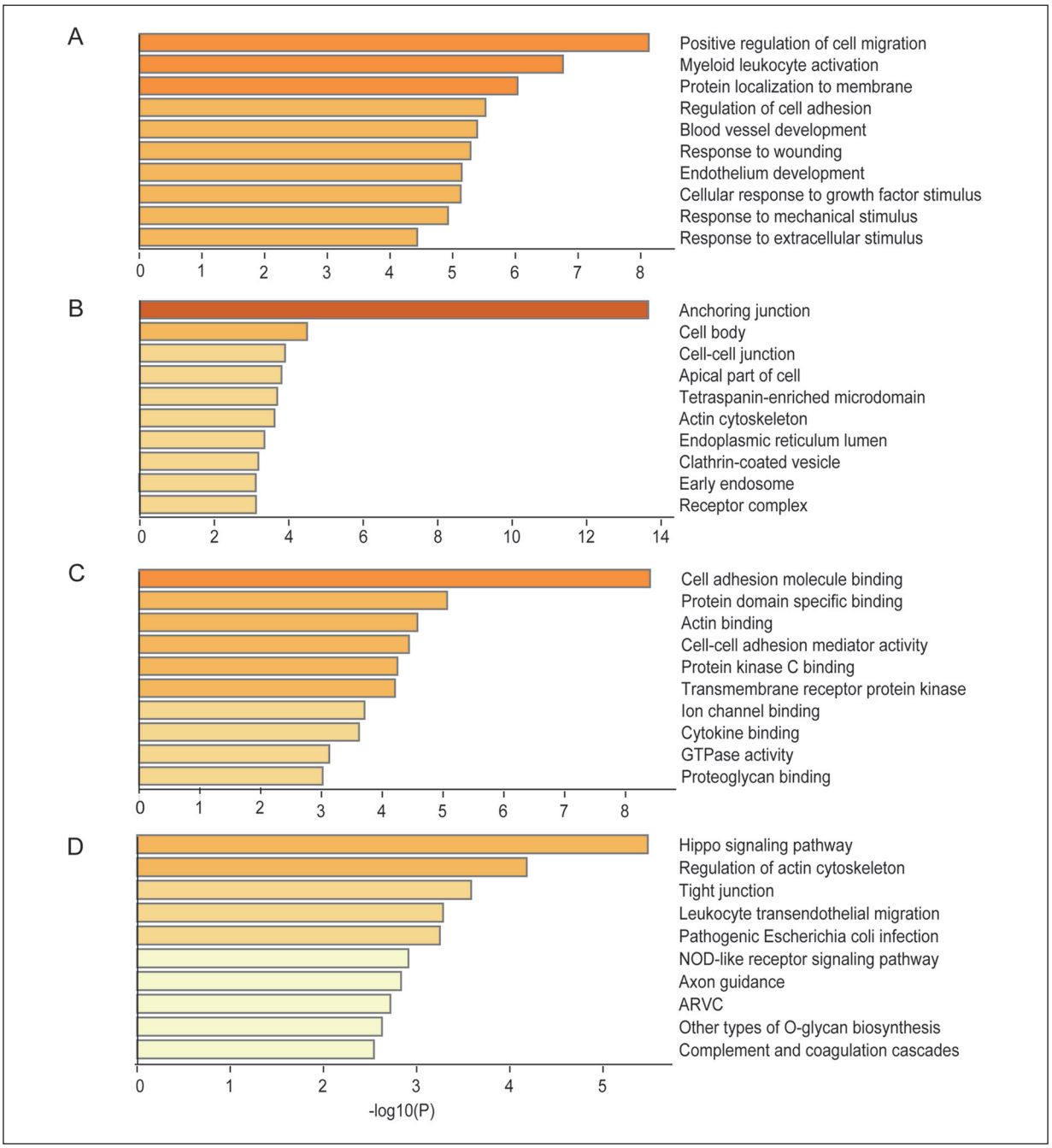

Figure 2. Enriched terms of **(A)** biological process **(B)** Cell component **(C)** molecular function and **(D)** KEGG pathway. The most significant 10 terms are shown in this figure.

Analysis of TFs

We used PASTAA to predict transcription factors of hub genes. These transcription factors are shown in Table III and Table IV. The Irf-1 and Irf-10 families were the top transcription factors of the upregulated hub genes by *p*-value. For1 and For2 families were the top transcription factors of downregulated hub genes by *p*-value.

Discussion

AF is the most common sustained arrhythmia in the world. Current therapies for AF are imperfect due to an incomplete understanding of the mechanism. Revealing the mechanism may help us treat AF better. Bioinformatic analysis of omics data is a powerful tool for mechanis-

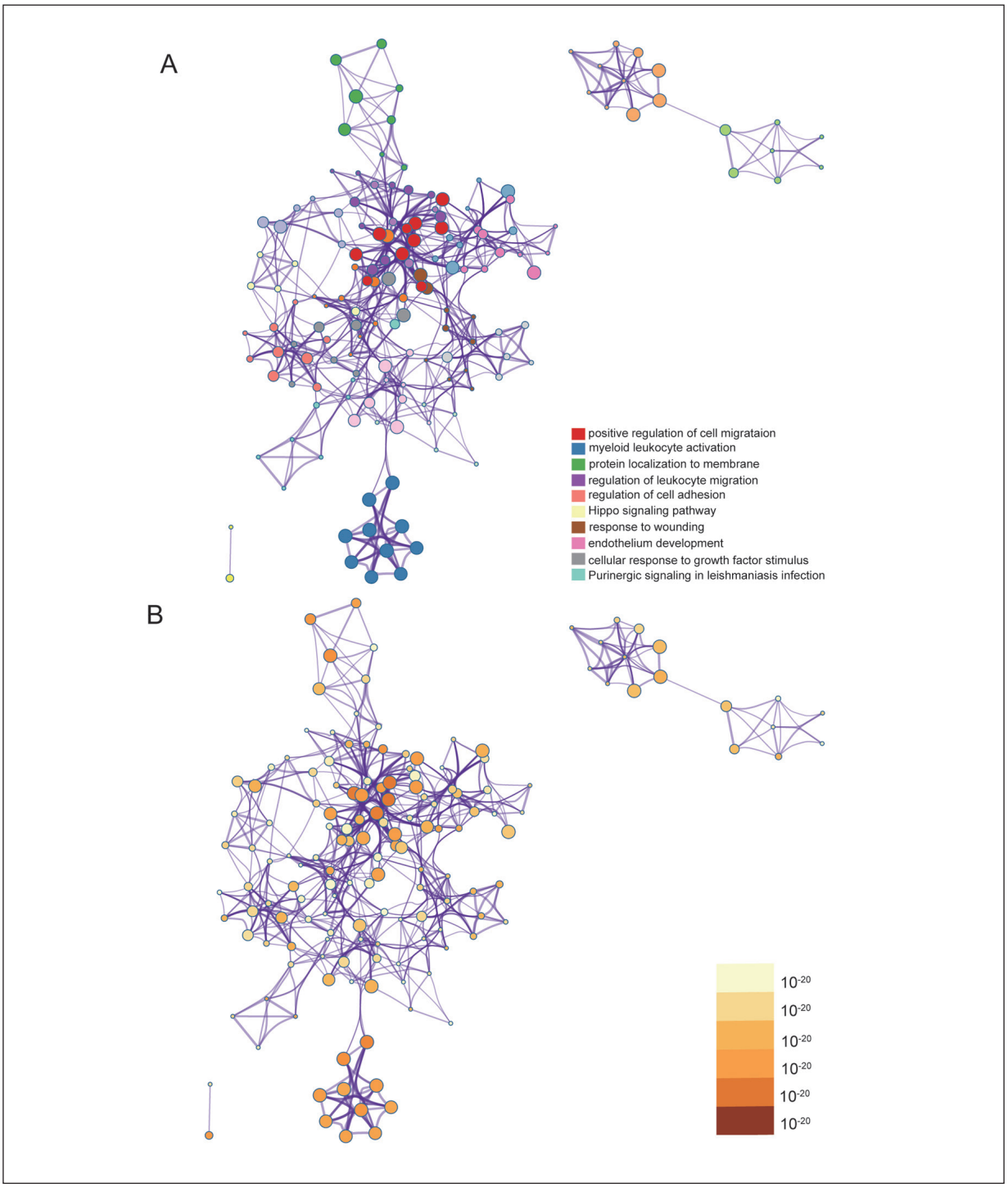

Figure 3. Network of enriched terms is shown in cluster ID **(A)** and *p*-value **(B)** form. In network shown in *p*-value form, the more significant *p*-value was, the deeper color was painted.

tic investigation. In our study, 235 upregulated and 77 downregulated genes were identified in patients with AF compared with patients with SR. The GO biological process enrichment analysis showed that positive cell migration was the most significantly enriched biological process. The regulation of cell migration has been proven to be related to atrial fibrillation occurrence and maintenance[19]. It has been reported that inhibition of fibroblast migration may attenuate atrial

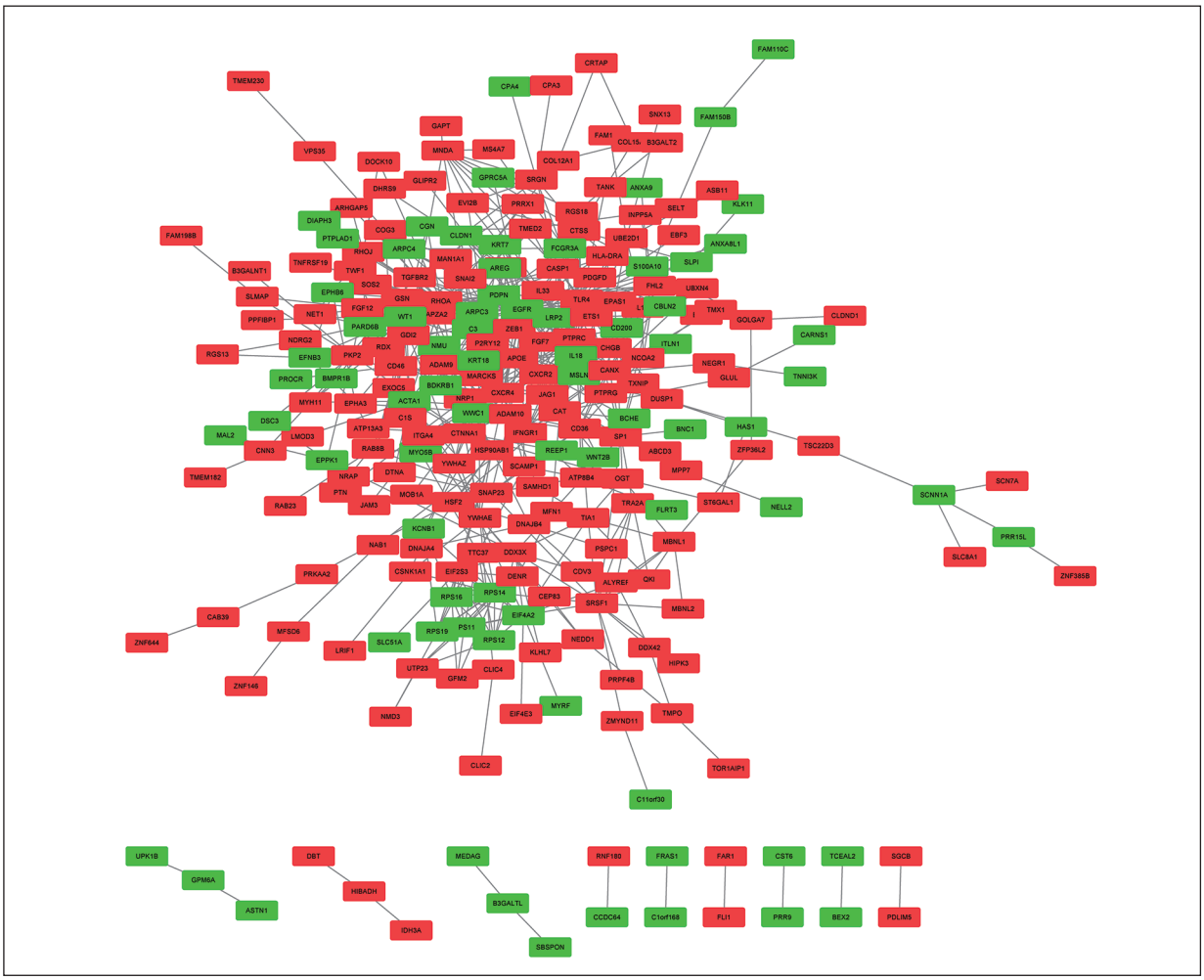

Figure 4. Protein-protein interaction network of DEGs. Upregulated genes were colored red, downregulated genes were colored green.

fibrosis and reduce AF vulnerability[20]. It has also been reported that inhibition of cell migration may prevent postinfarct atrial fibrillation in rats[21]. The anchoring junction was the most significantly enriched cellular component in the GO analysis. Growing evidence suggests that anchoring junctions play an important role in maintaining normal conduction. Disturbance of the anchoring junction has been proven to be related to arrhythmia, especially arrhythmogenic cardiomyopathy and Brugada syndrome[22,23]. Abnormal propagation of action potential has been shown to contribute to AF[24]. Anchoring junctions may be a potential target of AF treatment. In the GO molecular function analysis, cell adhesion molecule binding was the most significantly enriched molecular function. Similar to cell migration, cell adhesion has also been reported to be related to atrial fibrillation[19].

In the KEGG pathway analysis, the Hippo signalling pathway was the most enriched pathway. Studies have shown that Hippo signalling is related to arrhythmia. Xu et al[25] reported that Hippo signalling is likely to play a substantial role in the preventive effects of mechanical ventricular arrhythmia in response to left ventricle afterload increase. Chen et al[26] and Schlegelmilch et al[27] have reported that Hippo signalling is implicated in arrhythmogenic cardiomyopathy pathogenesis and adipogenesis by regulating cell-cell contact. Changes in cell-cell contact may affect the excitation and conduction of atrial cardiomyocytes, which may cause atrial arrhythmia, including atrial fibrillation. Therefore, we hypothesize that Hippo signalling may be involved in atrial fibrillation. Among the identified hub genes, CXCR2, TLR4, and CXCR4 were the top 3 genes. Wang et al[28] have

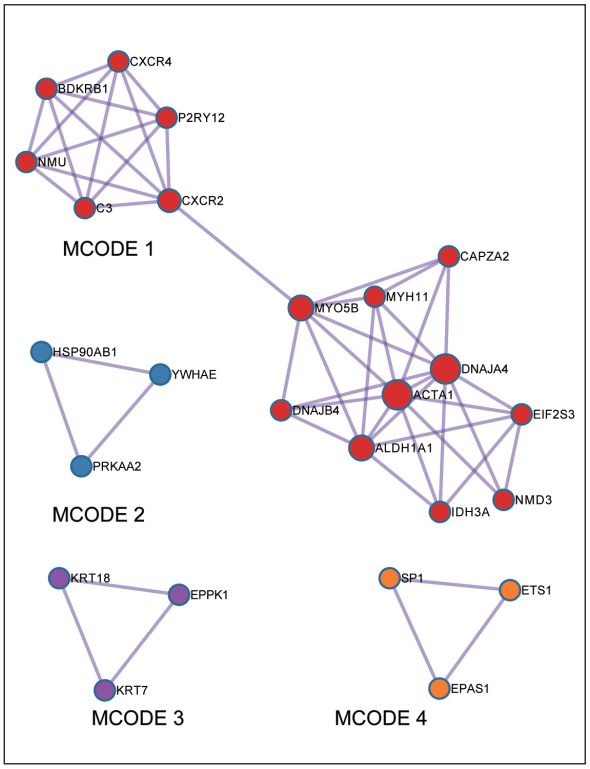

Figure 5. MCODE identified in the PPI network.

demonstrated that CXCR2 is implicated in the pathogenesis of Ang-II-induced cardiac remodelling. Atrial remodelling, such as atrial fibrosis, contributes to the pathogenesis of atrial fibrillation. TLR4 has been reported to be related to new-onset atrial fibrillation in acute myocardial infarction[29]. Soppert et al[30] showed that CXCR4 is involved in myofibroblast necroptosis, which may modulate cardiac remodelling in heart failure. Based on these studies, we speculate that these 3 genes may be a novel target for atrial fibrillation treatment.

Of the modules extracted from the PPI network, Class A/1 (rhodopsin-like receptors) may be the critical module. It has high sequence similarity to the angiotensin receptor AT1 and plays an important role in the occurrence and development of cardiovascular and metabolic diseases, including atherosclerosis (AS), coronary heart disease (CAD), heart failure (HF), pulmonary arterial hypertension (PAH), myocardial hypertrophy and atrial fibrillation[31]. The hub genes CXCR2 and CXCR4 were included in this module, so the critical role of this module was further confirmed.

Transcription factors (TFs) may play important roles in gene expression. Of the predicted TFs of upregulated genes, Irf-1 has been reported to be required for cardiac remodelling in response to pressure overload[32].

Atrial fibrillation is a complex disease with both environmental and genetic risk factors that contribute to arrhythmia. In recent years, genetic analysis has identified several genes associated with AF. The novelty of our research is the identification of several key genes and modules in the left atrium. Since the left atrium is known to be highly related to AF occurrence and maintenance, these genes and modules may ultimately facilitate the identification of new therapeutic targets and enable more precise risk stratification for this common arrhythmia. Our study may increase the understanding of atrial fibrillation mechanisms. Based on GO analysis, KEGG pathway analysis, PPI network analysis, hub gene prediction, key module prediction and transcription factor prediction, we found that the CXCR4, TLR4, and

Table I. Pathway and process enrichment analysis.

MCODE	GO	Description	Log10 (*p*)
MCODE_1	R-HSA-373076	Class A/1 (Rhodopsin-like receptors)	-7.3
MCODE_1	R-HSA-418594	G alpha (i) signalling events	-6.8
MCODE_1	R-HSA-375276	Peptide ligand-binding receptors	-6.8
MCODE_2	GO:1903829	positive regulation of cellular protein localization	-5.6
MCODE_2	hsa04151	PI3K-Akt signalling pathway	-5.6
MCODE_2	GO:1903827	Regulation of cellular protein localization	-5
MCODE_3	R-HSA-3928665	EPH-ephrin mediated repulsion of cells	-8.1
MCODE_3	GO:0048013	ephrin receptor signalling pathway	-7.4
MCODE_3	R-HSA-2682334	EPH-Ephrin signalling	-7.3
MCODE_4	M44	PID HIF2PATHWAY	-8.6
MCODE_4	GO:0006979	Response to oxidative stress	-5.2
MCODE_4	R-HSA-2262752	Cellular responses to stress	-4.8

GO: Gene ontology,

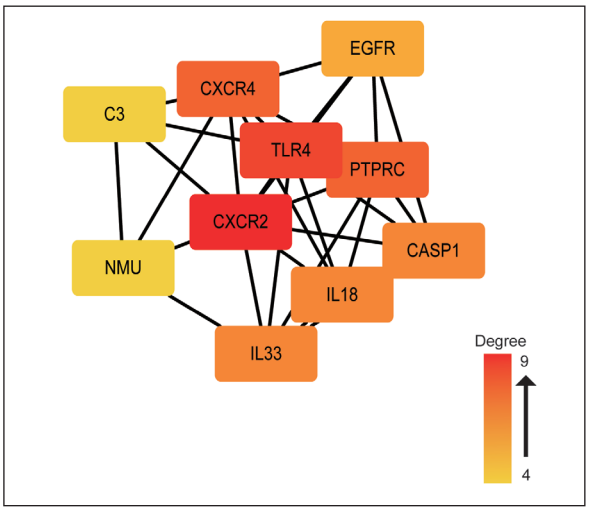

Figure 6. PPI network of 10 hub genes.

Table II. Description of 10 hub genes.

Gene	LogFC	*p*	Degree
CXCR2	1.50	0.022	9
TLR4	1.36	0.027	8
CXCR4	1.30	0.030	7
PTPRC	1.26	0.017	7
CASP1	1.10	0.015	6
IL33	1.07	0.020	6
IL18	-1.39	0.014	6
EGFR	-1.15	0.001	5
NMU	-1.79	0.048	4
C3	-1.20	0.026	4

CXCR2 genes and the Class A/1 (rhodopsin-like receptors) module may play critical roles in atrial fibrosis. Hippo signalling may also contribute to atrial fibrillation occurrence.

Although our study may provide novel targets for atrial fibrillation treatment, there are still limitations. The number of samples is relatively small, so there may be confounding factors. More samples are needed to confirm our findings. Although the sample size is small, this is the only dataset comparing the expression profiles of persistent AF patients and sinus rhythm patients. In addition to nuclear DNA, mitochondrial DNA (mtDNA) also carries genes. Mutation of mtDNA may also cause atrial fibrillation because elevated ROS levels have been proven to promote atrial fibrillation[33]. We did not analyze mtDNA data, and further investigations on mtDNA need to determine the effect of mtDNA mutations on atrial fibrillation.

Conclusions

Our study found that the CXCR4, TLR4, and CXCR2 genes and the class A/1 (rhodopsin-like receptors) module may play critical roles in atrial fibrosis. Hippo signalling may also contribute to atrial fibrillation occurrence. These genes and modules may ultimately facilitate the identification of new therapeutic targets and enable more precise risk stratification for this common arrhythmia.

Table III. Top 20 transcription factors of up-regulated genes.

Rank	Matrix	Transcription factor	Association score	*p*-value
1	IRF_Q6	Irf-1, Irf-10	4.212	2.04E-04
2	IRF_Q6_01	Irf-1, Irf-10	3.366	9.41E-04
3	PU1_Q6	Pu.1	3.123	1.68E-03
4	ETS_Q6	Elf-1, Elfr	3.106	1.68E-03
5	ICSBP_Q6	Irf-8	3.015	2.88E-03
6	CEBP_Q2_01	C/ebpalpha, C/ebpalpha(p30)	2.72	5.95E-03
7	PEA3_Q6	Pea3	2.646	6.45E-03
8	TATA_C	Tbp	2.574	6.83E-03
9	SEF1_C	N/A	2.522	9.05E-03
10	INR_HAND100	N/A	2.473	9.47E-03
11	SRF_Q5_01	Srf	2.346	1.31E-02
12	CEBP_Q3	C/ebp, C/ebpalpha	2.282	1.44E-02
13	BLIMP1_Q6	Blimp-1	2.278	1.44E-02
14	OCT4_01	N/A	2.221	1.70E-02
15	POLY_C	N/A	2.221	1.70E-02
16	COUPTF_Q6	Coup, Coup-tf1	2.221	1.70E-02
17	HNF4_Q6	Hnf-4, Hnf-4alpha	2.123	2.10E-02
18	MEIS1AHOXA9_01	Hoxa9, Hoxa9b	2.046	2.25E-02
19	MEIS1BHOXA9_02	Hoxa9, Hoxa9b	2.046	2.25E-02
20	OCT4_02	N/A	2.046	2.25E-02

Table IV. Top 20 transcription factors of up-regulated genes.

Rank	Matrix	Transcription factor	Association score	*p*-value
1	FXR_IR1_Q6	For1, For2	2.965	3.15E-03
2	AP4_01	Ap-4	2.876	3.52E-03
3	AP2ALPHA_03	N/A	2.346	1.31E-02
4	R_01	N/A	2.346	1.31E-02
5	CEBPB_01	C/ebpbeta(lap), C/ebpbeta(p35)	2.221	1.70E-02
6	DPE_HAND	N/A	2.221	1.70E-02
7	PAX4_01	Pax-4a	1.976	2.50E-02
8	BARBIE_01	N/A	1.919	2.91E-02
9	DR3_Q4	Car, Pxr-1	1.87	3.16E-02
10	E2F1_Q4	E2f-1	1.868	3.16E-02
11	AP2ALPHA_02	Ap-2alphaa	1.824	3.41E-02
12	MTATA_B	N/A	1.743	3.84E-02
13	AP2_Q3	Ap-2alpha, Ap-2alphaa	1.618	5.01E-02
14	SP1_01	Sp1	1.617	5.01E-02
15	SF1_Q6_01	N/A	1.606	5.01E-02
16	E2_Q6_01	N/A	1.57	5.58E-02
17	TFIII_Q6	Tfii-i	1.57	5.58E-02
18	TTF1_Q6	Nkx2-1	1.567	5.58E-02
19	ETF_Q6	N/A	1.446	6.98E-02
20	SPZ1_01	Spz1	1.446	6.98E-02

Conflict of Interest

The Authors declare that they have no conflict of interests.

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