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ORIGINAL RESEARCH

Identification and Validation of Autophagy-Related Genes as Potential Biomarkers and Therapeutic Targets in Atrial Fibrillation

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Background: Autophagy is an evolutionary conserved important process for the turnover of intracellular substances in eukaryotes and is closely related to the development of atrial fibrillation (AF). The aim of this study is to identify and validate potential autophagy-related genes (ARGs) of AF through bioinformatics analysis and experimental validation.

Methods: We downloaded two data sets from the Gene Expression Omnibus (GEO) database, GSE14975 and GSE31821. After merging the data of the two microarrays, adjusting the batch effect, and integrating the differentially expressed genes (DEGs) with ARGs to obtain differentially expressed autophagy-related genes (DEARGs). Functional and pathway enrichment analyses were carried out based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Use the STRING database to construct a protein–protein interaction (PPI) network. Finally, mRNA expression levels of DEARGs were validated in right atrial tissue samples from AF patients and non-AF controls by qRT-PCR.

Results: Through bioinformatics analysis, we finally identified 11 DEARGs (CDKN1A, CXCR4, DIRAS3, HSP90AB1, ITGA3, PRKCD, TP53INP2, DAPK2, IFNG, PTK6, and TNFSF10) in AF using [log2 (fold change)] > 0.5 and $P < 0.05$. In the pathway enrichment analysis, the most significantly enriched pathway was the autophagy pathway. The results of validation showed that the expression levels of CXCR4, DAPK2, and TNFSF10 corroborating with our computational findings, and the results were statistically significant ($P<0.05$).

Conclusion: Our study demonstrates that these 11 potential crucial ARGs, especially CXCR4, DAPK2, and TNFSF10, may be potential biomarkers and therapeutic targets in AF, which will help the personalized treatment of AF patients.

Keywords: autophagy, AF, Gene Expression Omnibus, hub genes

Introduction

Atrial fibrillation (AF) is the most common clinically sustained cardiac rhythm disorder. There is a high prevalence of AF all over the world, the highest in North America, Europe, China, and Southeast Asia, with approximately 270–360 cases per 100,000 people.[1] Due to the lack of understanding of the pathogenesis of AF, the current therapies available can only control AF in a short period of time, but cannot cure the disease, which brings a serious burden to the lives of AF patients. The pathogenesis of AF includes atrial remodeling, electrophysiological mechanisms, and the role of the autonomic nervous system, etc. Accumulating evidence has shown that the pathogenesis of AF involves a variety of biological processes,

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including apoptosis, immunoregulation, and autophagy.[2–4] Among these biological processes, autophagy plays an important role in the development of AF.

Autophagy is the main intracellular degradation system. In the process of autophagy, the cytoplasm is encapsulated in a double-membrane structure of autophagosomes, and autophagosomes fuse with lysosomes to form autophagolysosomes, which are degraded in autophagosomes.[5] A lot of evidence shows that autophagy response is closely related to the occurrence and development of cardiovascular disease.[6–9] For example, ischemia injury activates autophagy of cardiomyocytes, enabling cardiomyocytes to cope with nutritional stress and improving cell survival rate during ischemia-reperfusion injury.[10] For coronary heart disease, enhanced autophagy can not only protect the myocardium against ischemia but also prevent the remodeling of the heart after ischemia.[11] In recent years, there are also some studies on autophagy and AF.[12–14] Observation of 170 patients in sinus rhythm who had undergone elective coronary artery bypass grafting, Garcia et al found that impaired autophagy plays an important role in the occurrence of postoperative AF.[15] Yuan et al indicated that there is AMPK-dependent autophagy in the occurrence of AF,[4] their subsequent research found that autophagy can induce atrial electrical remodeling through ubiquitin-dependent selective degradation of Cav1.2.[16] However, the understanding of the role of autophagy in the occurrence and development of AF is far from enough.

The purpose of this study is to deeply understand the potential clinical application value of autophagy-related genes (ARGs) in AF through the Gene Expression Omnibus (GEO) database using bioinformatic methods. Although Liu et al have previously conducted bioinformatics analysis of ARGs and AF, they only analyzed one dataset and did not verify the results.[17] In this study, we analyzed two datasets and verified the finally identified differential ARGs in patients with AF and non-AF individuals, which further improved the reliability of the results.

Materials and Methods

Data Collection and Preprocessing

GSE14975 and GSE31821 (10 samples in GSE14975 and 6 samples in GSE31821) were downloaded from GEO (http://www.ncbi.nlm.nih.gov/geo) and the sample platforms used were GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array.[18] The two data sets were selected because they were both derived from the patient's atrial tissue and the same sequencing platform.

The data were preprocessed as follows: to annotate the data, the probe names were converted into gene names with ActivePerl 5.28.1 software (https://www.activestate.com/products/perl/downloads/). Then we generated a synthetic dataset from GSE14975 and GSE31821 datasets. For the new dataset, eliminate probes without related gene symbols, and calculate the average expression value of those gene symbols with multiple probes. The "sva" package of R software was used to remove batch-effects for new dataset batch correction.

Screening of Differentially Expressed Genes (DEGs)

The DEGs were screened with R software limma package version 3.44.3 (http://bioconductor.org/packages/release/bioc/html/limma.html) under the criteria of [log2 (fold change)] >0.5 and P <0.05.

Protein-Protein Interaction (PPI) Network and Functional Analysis of DEGs

PPI networks of DEGs were analyzed using the STRING online tool (STRING database, version 11.0; https://string-db.org/cgi/input.pl?sessionId=1fJdSiXTVpOdandinput_page_show_search=on) to further predict protein functional associations and protein-protein interactions.[19] It might provide insights into the underlying molecular mechanism of the initiation and progression of diseases. An interaction with a confidence score >0.70 was considered statistically significant. The specific process of DEGs function and pathway enrichment analysis is consistent with that described by differentially expressed ARGs (DEARGs).

Autophagy-Related Genes

We obtained a total of 232 ARGs from the Human Autophagy-dedicated Database (HADb, http://autophagy.lu/clustering/index.html), which provides a more detailed list of human genes involved in autophagy.

Identification of DEARGs

Data were further analyzed by R software, taking the intersection of DEGs expression profile and 232 ARGs to identify DEARGs.

Functional and Pathway Enrichment Analysis of DEARGs

Through Gene Ontology (GO) enrichment analysis, we can comprehensively understand the biological process, cellular component, and molecular function of DEARGs enriched. In this study, the bohao online enrichment tool (http://enrich.shbio.com/index/ga.asp) was utilized to perform GO enrichment analysis on DEARGs. Terms of which P <0.05 were statistically significant.

The identified DEARGs were also analyzed by pathway enrichment in the Kyoto Encyclopedia of Genes and Genomes (KEGG) to find relevant important pathways. ClusterProfiler version 3.16.1 (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html) was used to perform KEGG pathway enrichment analysis in R software.[20,21] Pathways of which P <0.05 were statistically significant.

A detailed flow chart for the specific process of analysis was shown in Figure 1.

AF Patients and Non-AF Controls

A total of 10 AF patients and 10 non-AF controls were obtained from the Fuwai Yunnan Cardiovascular Hospital between April 2021 and July 2021. This study included patients who underwent thoracotomy due to mitral valve replacement and/or atrial septal defect. Patients with AF (defined as persistent atrial fibrillation lasting more than 7 days) were assigned to AF, while those without AF were assigned to the control group. Patients were excluded if they had thyroid dysfunction and active rheumatism. This study was approved by the Ethics Committee of Fuwai Yunnan Cardiovascular Hospital (Ethical Application Ref: IRB2021-BG-006). Written informed consent was obtained from all individual participants included in the study. Right atrial tissue was collected from patients who participated in the study.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from right atrial tissue with TRIzol Reagent (Life Technologies, CA, USA). Reverse transcription was conducted using SureScript-First-strand-cDNA-synthesis-kit (GeneCopoeia, Guangzhou, China). qPCR was conducted using a BlazeTaq™ SYBR® Green qPCR Mix 2.0 kit (GeneCopoeia, Guangzhou, China) following the instructions. The thermocycling conditions were as follows: initial activation at 95°C for 30 s, followed by 40 cycles at 95°C for 10 s, 60°C for 20 s and 72°C for 30 s. GAPDH was used as the internal reference for the mRNA for data normalization. The relative

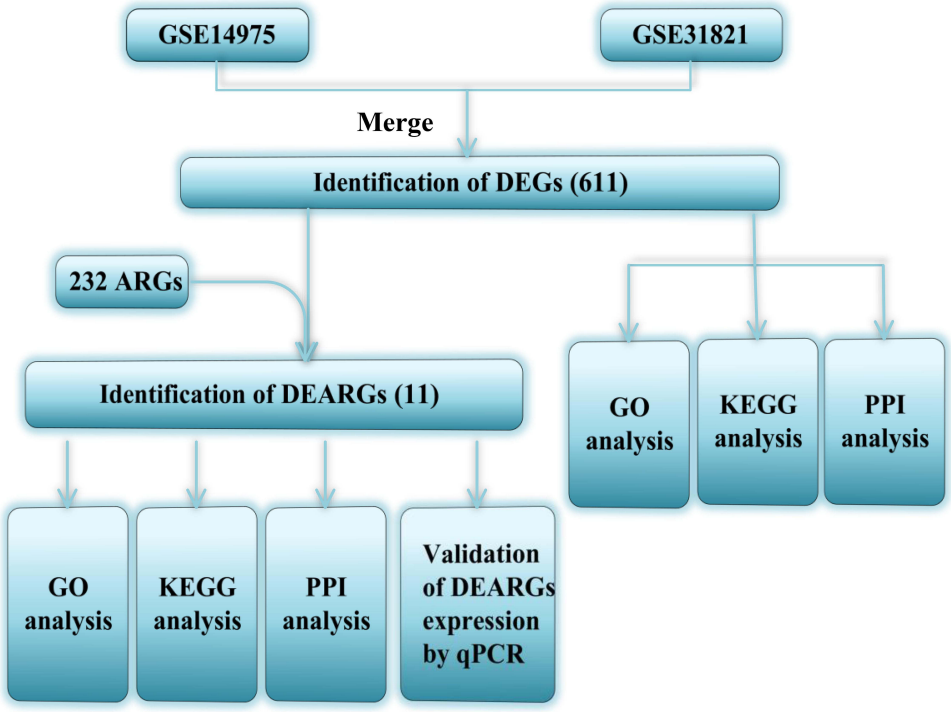

Figure 1 The flow chart shows the design of the present study.
Abbreviations: DEGs, differentially expressed genes; ARGs, autophagy-related genes; DEARGs, differentially expressed autophagy-related genes; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 1 Primer Sequences for qRT- PCR

Gene Names	Forward (5′-3′)	Reverse (3′-5′)
CDKN1A	TGAGTTGGGAGGAGGCAG	GAGCGAGGCACAAGGGTA
DAPK2	GAGAGGAGCTGGGGAGTGG	GCTTGATGTGTGGAATGGG
DIRAS3	ACGCCTTCGTCCTGGTCTAC	GGGCATCTGGGATTTCTTCT
HSP90AB1	GAACTAAACAAGACCAAGCC	TCCTCAGAGTCAACCACACC
IFNG	CATCGTTTTGGGTTCTCTTG	TTTTTCGCTTCCCTGTTTTA
ITGA3	GTAGGAAGCCCCCTCAAG	GGGTAGCCCAGCCATTTA
PRKCD	TACGAGATGCTCATTGGC	GTCTTGAAGAAGGGGTGG
PTK6	CGTCTGGTCCTTTGGGATTCTC	GTCGGGTTCTCGTAGCTGGTGA
TNFSF10	AGCAACACATTGTCTTCTCCA	TAAGCTCAAATATTCCCCCTT
TP53INP2	AAAGAAAACACAAAGAACGACAA	ACTAAAAAGGCCCCAAAAAAACT
CXCR4	AGCAAGGGTGTGAGTTTGAGA	GAAAGCATAGAGGATGGGGTT
GAPDH	CGCTGAGTACGTCGTGGAGTC	GCTGATGATCTTGAGGCTGTTGTC

Abbreviation: qRT-PCR, quantitative reverse transcription-quantitative polymerase chain reaction.

expression was calculated by $2^{-\Delta\Delta Ct}$ method. Primers are available in Table 1.

Statistical Analysis

The statistical analyses were performed using GraphPad Prism 9.0 software (San Diego, CA, USA). Real-time gene expression levels of our tissue samples were compared using Student's *t*-test. $P < 0.05$ was considered to indicate statistical significance.

Results

Data Preprocessing and Differential Expression Analysis

A total of 54,675 probes were obtained from GSE14975 and GSE31821. After preprocessing, 21,644 genes were identified. Considering the criteria for [log2 (fold change)] >0.5 and P <0.05, we finally obtained a total of 611 significant DEGs, of which 309 were up-regulated and 302 were down-regulated. The clustering heatmap is shown in Figure 2.

PPI Network and Functional GO Terms and Pathway Enrichment Analyses of DEGs

Altogether, 202 nodes and 389 interaction pairs were identified in the PPI network (Figure 3). According to the view that highly connected genes were noted to possibly play important roles in diseases, we calculated the connectivity between the nodes through R software and the results are displayed in Figure 4. Here, the first 6 nodes are all members of the collagen family, including collagen type I alpha 1 chain (COL1A1, degree =21), collagen type III alpha 1 chain (COL3A1, degree =17), collagen type IV alpha 1 chain (COL4A1, degree =16), collagen type IV alpha 2 chain (COL4A2, degree =16), collagen type V alpha 1 chain (COL5A1, degree =15) and collagen type IX alpha 3 chain (COL9A3, degree =15) are considering as hub genes in related to AF maintaining.

To investigate the biological effects of DEGs, we performed GO and KEGG functional enrichment analyses, the top 3 GO terms related biological processes were collagen catabolic process (enrich factor: 6.62; P-value: 3.148e-07), collagen fibril organization (enrich factor: 7.92; P-value: 1.713e-05) and negative regulation of cell-cell adhesion (enrich factor: 7.00; P-value: 3.780e-07), the results are shown in the Figure 5A.

KEGG pathway analysis data appear in Figures 5B and C. The results suggest that DEGs were significantly enriched in pathways of protein digestion and absorption (P-value: 4.24e-08), amoebiasis (P-value: 4.72e-06), and IL-17 signaling pathway (P-value: 0.00037).

Identification of DEARGs

Using the DEGs identified in the previous step and extracted the expression values of 232 ARGs. A total of 11 DEARGs were identified (see Table 2 and Figure 6A for details), including 7 up-regulated genes (CDKN1A, CXCR4, DIRAS3, HSP90AB1, ITGA3, PRKCD, TP53INP2) and 4 down-regulated genes (DAPK2, IFNG, PTK6, TNFSF10). Besides, a heat map was visualized to show the relative expression pattern of 11 DEARGs between control samples and AF samples (Figure 6B).

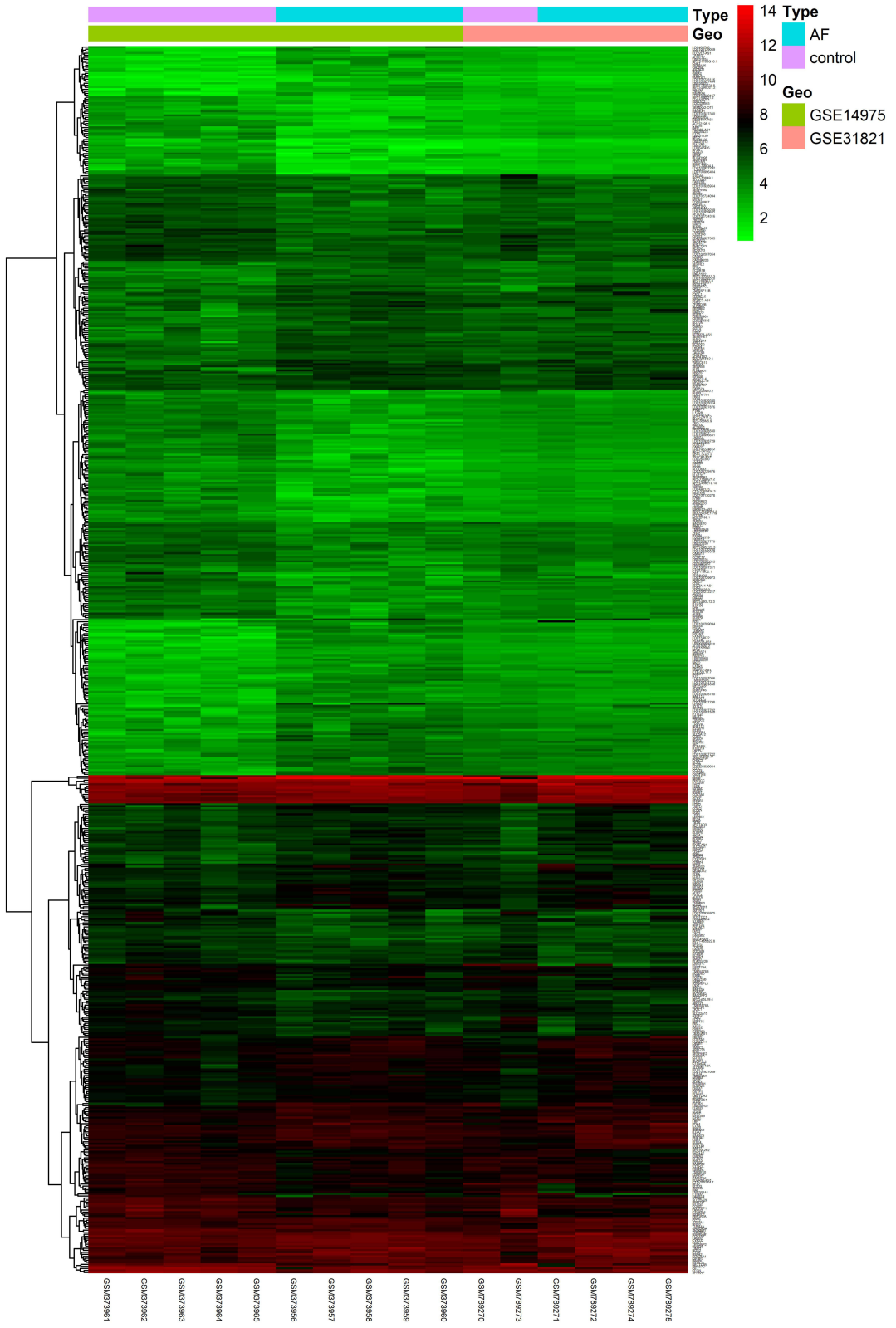

Figure 2 Clustering heatmap of DEGs in GSE14975 and GSE31821 datasets. n = 611 DEGs. The red signifies upregulation, whereas the green indicates the downregulation of genes.

Abbreviation: DEGs, differentially expressed genes.

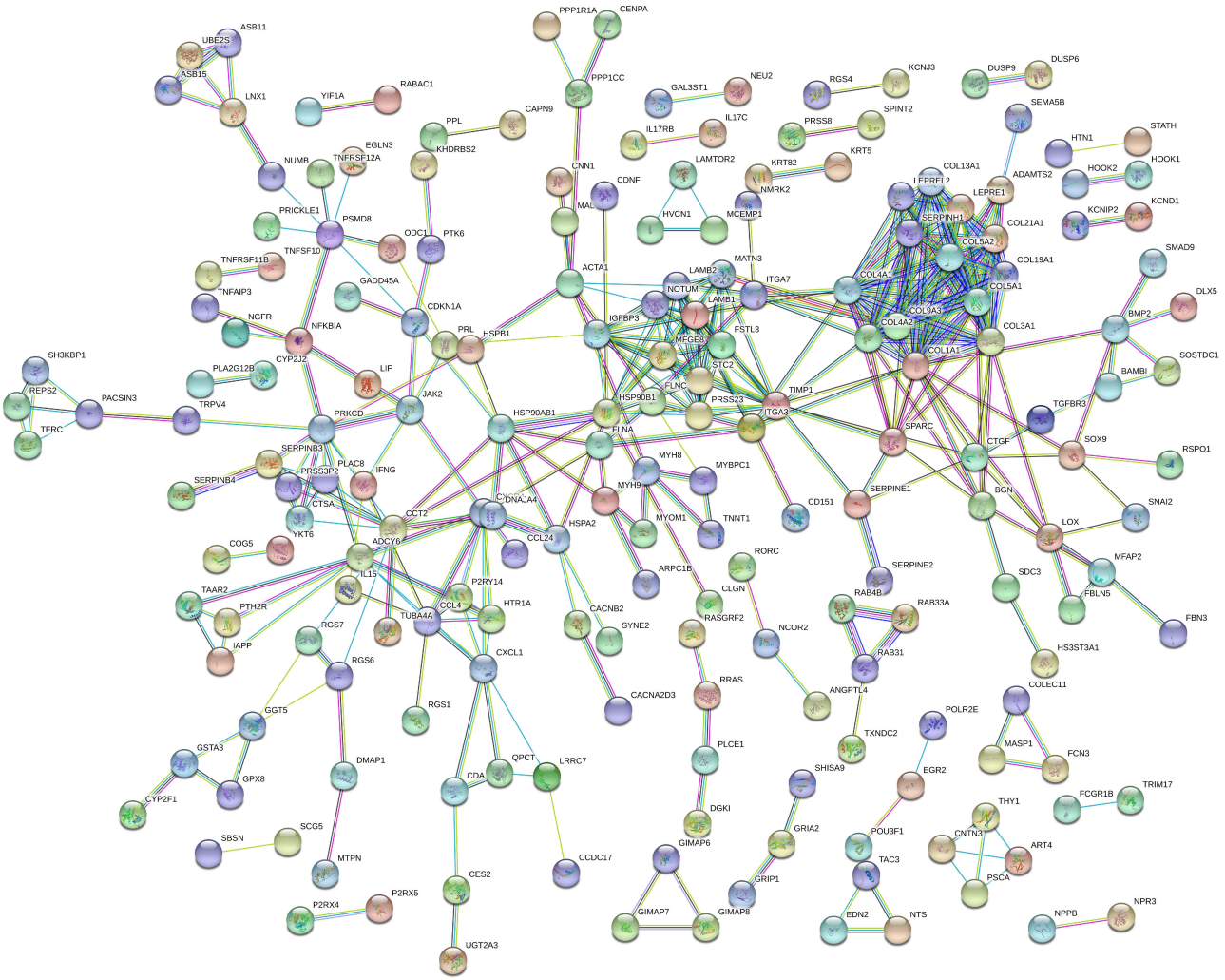

Figure 3 PPI network of DEGs between control samples and atrial fibrillation samples.
Abbreviations: PPI, protein-protein interaction; DEGs, differentially expressed genes.

PPI Network and Functional Enrichment of the DEARGs

The PPI network based on DEARGs consisted of 17 nodes and 90 interaction pairs (Figures 7 and 8). The top 8 nodes are mostly proteins related to cell cycle regulation, respectively TP53, CDK4, CDK6, CDKN1A, CCND1, CDK2, CCNA2, and CCNE1.

To further explore the biological functions of the DEARGs, functional enrichment and pathway analyses were performed and the results are presented in Figure 9A–C.

GO enrichment showed that changes in the biological process (BP) of DEARGs were mainly enriched in the protein phosphorylation, protein kinase activation, and apoptotic signaling pathway. (Figure 9A, Table 3).

The results of KEGG enrichment analysis showed that pathways of DEARGs mainly involve pathways in autophagy, necroptosis, and bladder cancer (Figures 9B and C). It is worth mentioning that it is directly enriched into autophagy, which proves the correctness of the analysis results.

Validation of DEARGs Expression in AF Patients and Non-AF Controls

To confirm the reliability of the bioinformatics analysis, we validated DEARGs in patients with AF and control by qRT- PCR. The clinicopathological variables of cases and controls are summarized in Table 4. Similar to the results of mRNA microarray in AF tissue samples, the expression level of CXCR4 was found to be significantly up-regulated (Figure 10B), the expression levels of DAPK2 and

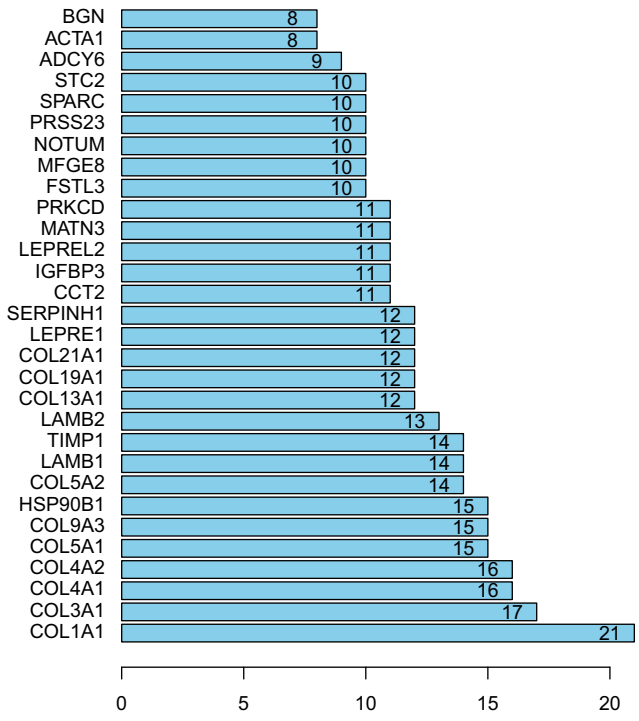

Figure 4 Top 30 nodes of PPI networks of DEGs between control samples and AF samples.

Abbreviations: PPI, protein-protein interaction; DEGs, differentially expressed genes; AF, atrial fibrillation.

TNFSF10 were found to be down-regulated (Figures 10F and G). However, the expression levels of CDKN1A, HSP90AB1, and TP53INP2 showed no significant difference between AF patients and non-AF controls (Figure 10A–D). Furthermore, we found that DIRAS3 was significantly down-regulated in AF patients (Figure 10E), which did not correlate with our bioinformatics analysis. IFNG, ITGA3, PRKCD, and PTK6 were not detected by qRT- PCR due to their low expression levels.

Discussion

The pathogenesis of AF includes atrial remodeling, electrophysiological mechanisms, and the role of the autonomic nervous system, etc. Among all of them, atrial remodeling is most closely associated with AF. It is well-known that myocardial fibrosis is the most important part of atrial remodeling. When the content of fiber components (collagen fiber) in myocardial tissue is higher, AF is more likely to persist. In this study, PPI network and GO analysis of DEGs also showed that collagen family and collagen catabolic process play a key role in the pathogenesis of AF, especially type I and III collagen, have been confirmed that their metabolic changes are significantly related to AF.[22] With the in-depth research in recent years, increasing evidence has demonstrated that autophagy may be involved in the regulation of myocardial fibrosis.[23–25] Chikusetsu saponin IVa can activate autophagy through AMPK/mTOR/ULK1 pathway to reduce isoproterenol-induced myocardial fibrosis.[23] Calhex231 may ameliorate myocardial fibrosis by inhibiting autophagy-mediated activation of NLRP3 inflammasome.[26] However, extensive validation is needed to improve our understanding of autophagy in the pathogenesis of AF.

Autophagy is strongly linked to the development of AF, but the mechanisms involved are complex. The study of Hu et al has directly pointed out that, through in vivo and in vitro studies, they found that quercetin can inhibit the expression of miR-223-3p, while enhancing the expression of FOXO3 and activating the autophagy pathway, which significantly inhibits Myocardial fibrosis in AF.[27] But, there have been no studies using bioinformatics to explore the role of ARGs in AF and to conduct experimental verification. In the present study, we used bioinformatics tools to analyze the integrated data of gene expression profiles from two GEO datasets to identify key ARGs related to the therapeutic targets of AF patients. We found that 11 ARGs (CDKN1A, CXCR4, DIRAS3, HSP90AB1, ITGA3, PRKCD, TP53INP2, DAPK2, IFNG, PTK6, TNFSF10) under the criteria of [log2 (fold change)] >0.5 and P <0.05 were differentially expressed in AF patient myocardial tissue samples. Some of these ARGs of AF have been previously studied. For example, the expression of CXCR4 is upregulated in patients with chronic AF, leading to atrial remodeling.[28] This result is consistent with the results of our bioinformatics analysis and experimental verification. We intend to explore more potential ARGs of AF in the future.

The STRING database was used to identify the eight nodes with the greatest degree of network connection (TP53, CDK4, CDK6, CDKN1A, CCND1, CDK2, CCNA2, and CCNE1). These targets are mainly regulatory factors related to inflammation, DNA replication and repair, cellular senescence, cell cycle, and so forth.[29–32] Myocardial fibrosis is one of the important mechanisms for the occurrence of AF, and these proteins may indirectly participate in the regulation of AF through myocardial fibrosis. The greatest number

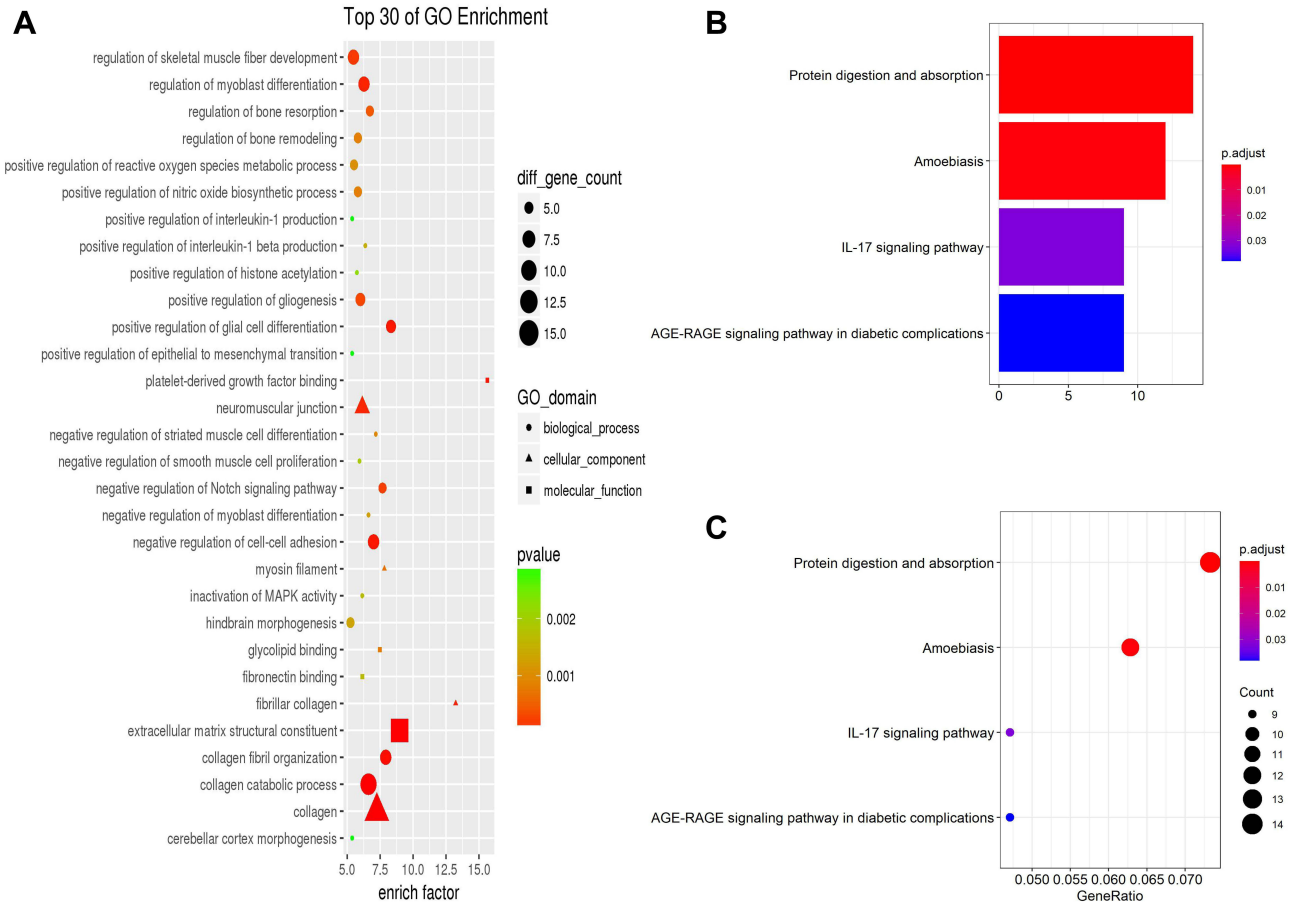

Figure 5 GO (**A**) analysis shows the biological processes, cellular components, and molecular functions involved in DEGs. Bar plot (**B**) and dot plot (**C**) show KEGG pathway enrichment of DEGs.
Abbreviations: GO, Gene Ontology; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

of node networks (n=20) was TP53. Tumor protein p53 (TP53) is a critical protein involved in the process of cell cycle, apoptosis, senescence, and DNA repair. Overactivation of TP53 pathway in cardiomyocytes induces myocardial fibrosis.[33] Similar to the studies conducted by Chen et al, the TP53 signaling pathway

Table 2 The DEARGs of Merged Data of 611 DEGs and 232 ARGs with the Use of Criteria of Log2 (Fold Change) >0.5 and P <0.05

Gene Names	Log2 FC	AveExpr	t	P-value	Adj. P. Val	B
CDKN1A	0.6755	8.0689	2.7433	0.0136	0.7170	−2.8357
CXCR4	0.8774	6.3564	2.8709	0.0104	0.6877	−2.6383
DAPK2	−0.6586	7.1420	−4.1998	0.0006	0.4001	−0.5576
DIRAS3	0.8147	6.0380	2.3157	0.0330	0.7937	−3.4772
HSP90AB1	0.6056	9.8253	2.3393	0.0314	0.7870	−3.4427
IFNG	−0.9761	3.2009	−2.4571	0.0247	0.7607	−3.2693
ITGA3	0.8263	5.0535	2.3481	0.0309	0.7870	−3.4300
PRKCD	0.9270	5.1578	2.2153	0.0403	0.8028	−3.6217
PTK6	−0.8182	3.8179	−3.7636	0.0015	0.5276	−1.2334
TNFSF10	−0.7106	8.0994	−2.5080	0.0223	0.7607	−3.1933
TP53INP2	0.5637	9.7921	3.7557	0.0015	0.5276	−1.2457

Abbreviations: DEARGs, differentially expressed autophagy-related genes; DEGs, differentially expressed genes; ARGs, autophagy-related genes; log2 FC, log2 (fold change); AveExpr, average expression; adj. P. Val, adjust P-value.

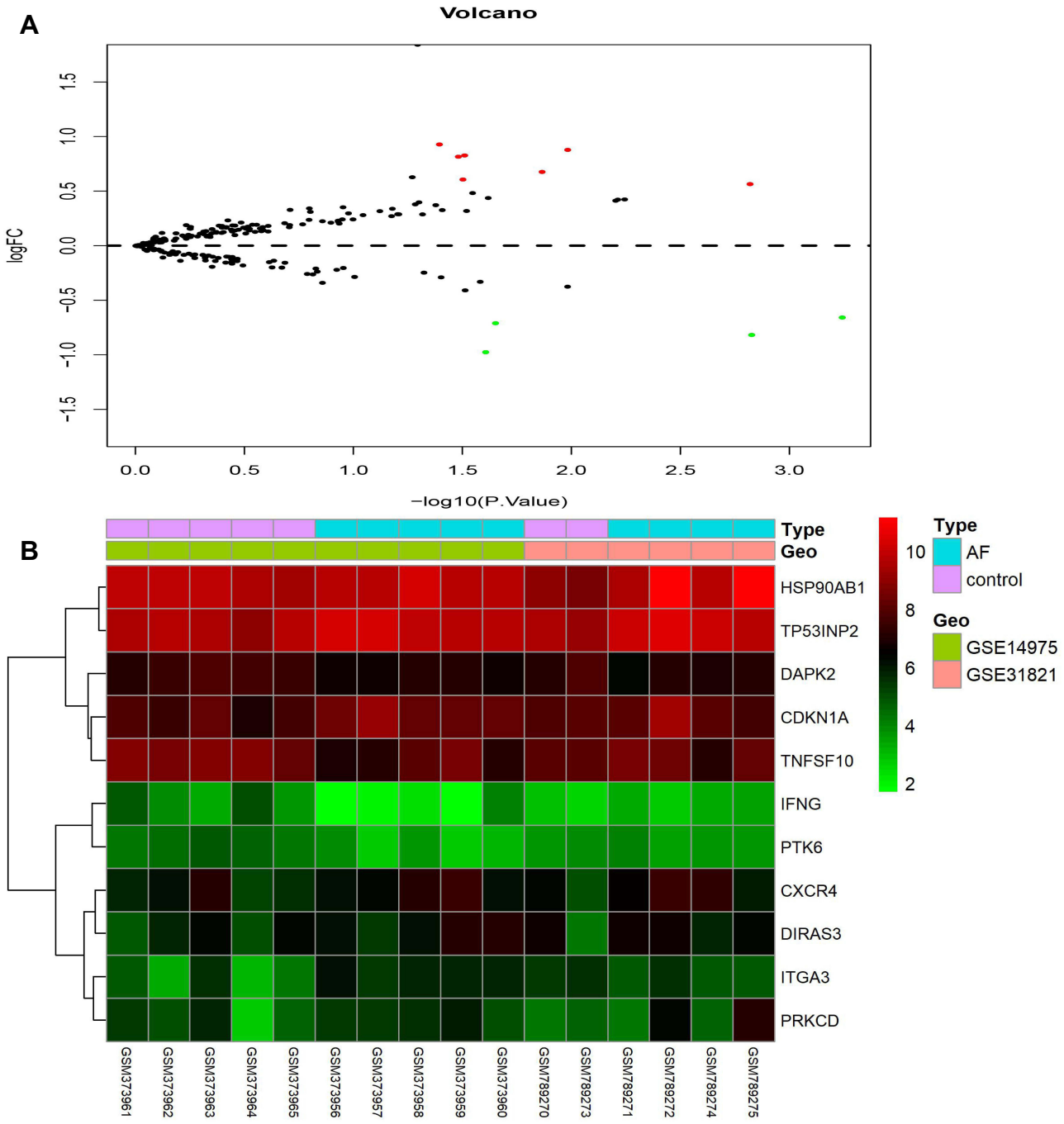

Figure 6 Volcano map (**A**) shows DEARGs, with red dots representing up-regulated genes, green dots representing down-regulated genes, and the remaining black dots representing no differences gene. Heat map (**B**) of 11 DEARGs in AF samples and control samples.
Abbreviations: DEARGs, differentially expressed autophagy-related genes; AF, atrial fibrillation.

is activated in ventricular arrhythmias in dilated cardiomyopathy.[34] Therefore, TP53 may be an important target gene of AF. CDK4, CDK6, CDKN1A, CDK2, and CCNA2 are important proteins involved in the regulation of the mammalian cell cycle. The present study shows that CDK4/6 inhibitors could delay the progression of bleomycin-induced pulmonary fibrosis.[35] MicroRNA-1 can inhibit the proliferation of cardiac fibroblasts by directly acting on CDK6.[36] Moreover, activation of AMPK further inhibited CDK2 and cyclin E complexes by up-regulating CDKN1A expression, ultimately suppressing the

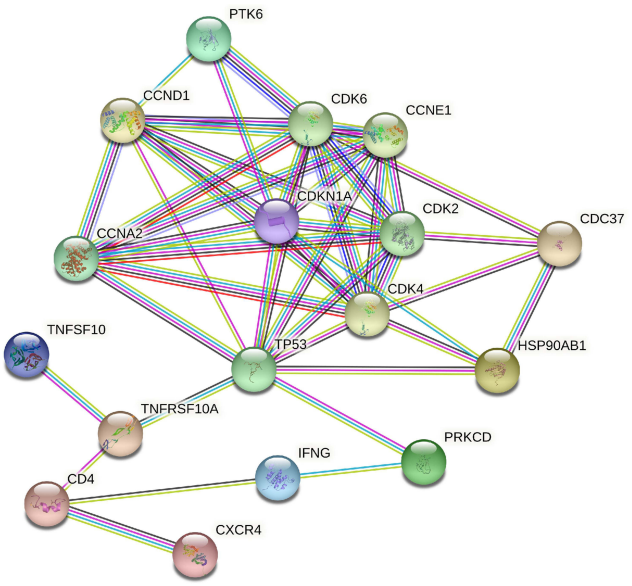

Figure 7 PPI network of DEARGs between control samples and atrial fibrillation samples.
Abbreviations: PPI, protein-protein interaction; DEARGs, differentially expressed autophagy-related genes.

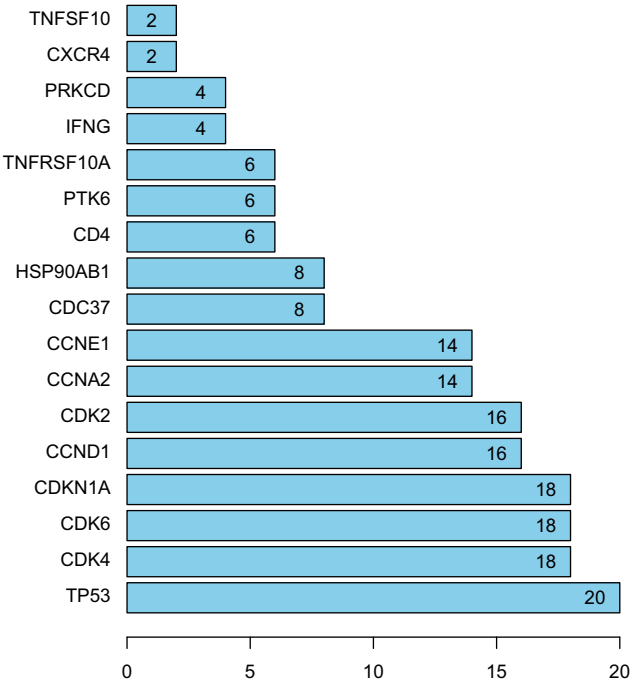

Figure 8 Top 17 nodes of PPI networks of DEARGs between control samples and AF samples.
Abbreviations: PPI, protein-protein interaction; DEARGs, differentially expressed autophagy-related genes; AF, atrial fibrillation.

progression of cardiac fibrosis.[37] Overexpression of CCNA2 also alleviates myocardial fibrosis in a porcine model of myocardial infarction.[38] These studies provide a convincing framework for the development of therapies to alleviate myocardial fibrosis based on cardiomyocyte cycle regulation. However, there exists no study to investigate the relationships between CCND1, CCNE1 and myocardial fibrosis.

The potential biological functions of these DEARGs were also analyzed by GO and KEGG enrichment analysis. Functional enrichment analysis in the present study indicated that the GO terms (biological process) were mainly enriched in protein phosphorylation regulation, protein kinase activity, and kinase activity. KEGG pathway enrichment analysis showed that PRKCD, TP53INP2, and DAPK2 were enriched in the autophagy pathway. Some published articles confirmed that autophagy can affect the progress of AF. One paper mentioned that autophagy can promote the occurrence and persistence of AF through the degradation of L-type calcium channels.[16] One other study found osteopontin induces the activation of AKT/mTOR, inhibits autophagy, aggravates the AF.[13] These findings suggest that the study of ARGs and AF may reveal the pathogenesis of AF.

Based on the bioinformatics analysis results, we further identified the expression level of DEARGs in our clinical samples by qRT-PCR. According to the results of qRT- PCR, the expression levels of CXCR4, DAPK2, and TNFSF10 were in accordance with the bioinformatics analysis results from mRNA microarray. Among them, CXCR4 has been confirmed to be closely associated with the occurrence and maintenance of AF. It was found that CXCR4 expression is up-regulated in patients with chronic AF with mitral valve disease.[28] Inhibition of CXCL12/CXCR4 axis can reduce the recruitment of inflammatory cells and suppress the hyperactivation of atrial ERK1/2 and AKT/mTOR signaling pathways, thereby alleviating AF.[39] As previously mentioned, myocardial fibrosis is one of the important pathogenesis of AF, and CXCR4 also plays a key regulatory role in myocardial fibrosis. It was found that CXCR4 antagonist significantly reduced the expression of collagen I mRNA and alleviated myocardial fibrosis in experimental dilated cardiomyopathy mice.[40] In addition, study on DAPK2, TNFSF10 and AF is not reported. It should be noted that due to the limited sample volume, we did not conduct experimental verification of differential protein abundance by analyzing gene expression. However, the differential protein identified in this study has also

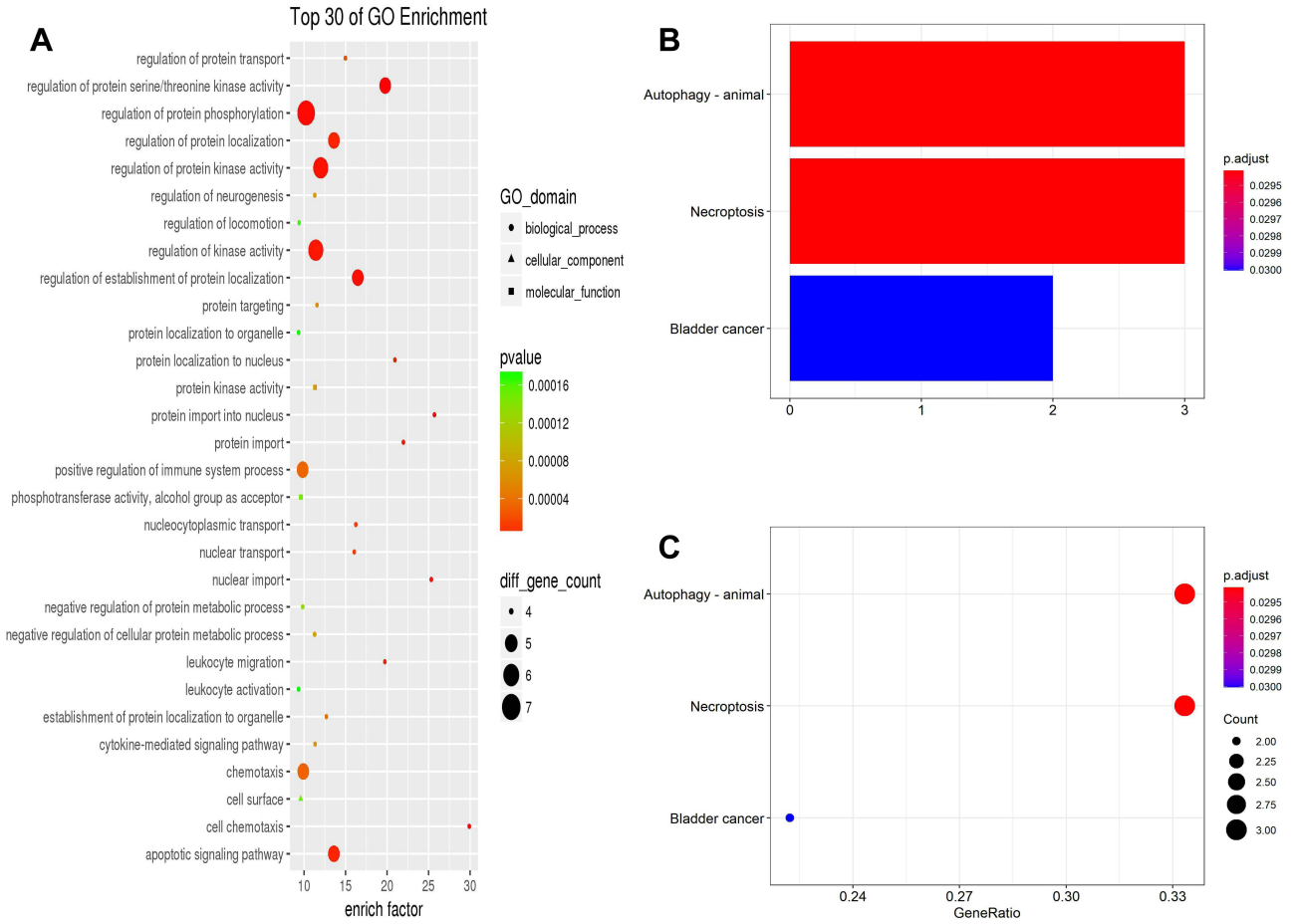

Figure 9 GO (**A**) analysis shows the biological processes, cellular components, and molecular functions involved in DEARGs. Bar plot (**B**) and dot plot (**C**) show KEGG pathway enrichment of DEARGs.
Abbreviations: GO, Gene Ontology; DEARGs, differentially expressed autophagy-related genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

been found in other studies, as demonstrated by Liu et al, the protein expression level of CXCR4 in AF patients was significantly higher than that in patients with sinus rhythm.[39] Nevertheless, the current research on ARGs and AF is far from enough and needs further exploration.

Table 3 Significant Enriched GO Terms and Pathways of DEARGs

	Term	Count	Genes	P-value	Adj. P. Val	Q value
GO terms						
GO:0001932 (BP)	Regulation of protein phosphorylation	7	HSP90AB1/DIRAS3/PTK6/CDKN1A/IFNG/CXCR4/PRKCD	0.000001317		0.0001825
GO:0045859 (BP)	Regulation of protein kinase activity	6	HSP90AB1/DIRAS3/PTK6/CDKN1A/CXCR4/PRKCD	0.000002055		0.0001708
GO:0043549 (BP)	Regulation of kinase activity	6	HSP90AB1/DIRAS3/PTK6/CDKN1A/CXCR4/PRKCD	0.000002858		0.0001485
GO:0097190 (BP)	Apoptotic signaling pathway	5	DAPK2/TNFSF10/CDKN1A/IFNG/PRKCD	0.000005167		0.0001464
KEGG Pathway						
hsa04140	Autophagy - animal	3	DAPK2/ PRKCD/ TP53INP2	0.000376076	0.029412614	0.0220710
hsa04217	Necroptosis	3	HSP90AB1/ IFNG/ TNFSF10	0.000582428	0.029412614	0.0220710
hsa05219	Bladder cancer	2	CDKN1A/ DAPK2	0.000891239	0.030005034	0.0225155

Abbreviations: GO, Gene Ontology; DEARGs, differentially expressed autophagy-related genes; BP, biological process; adj. P. Val, adjust P-value.

Table 4 Summary of Clinicopathological Variables of Cases and Controls

Variables	AF (N=10)	Controls (N=10)	P-value
Age (years)	50±9.73	45.5±12.73	0.386
Gender (male/female)	6/4	7/3	0.549
Height (cm)	165.1±9.267	165±8.524	0.980
Weight (kg)	61.55±12.61	65.2±12.51	0.524
Body mass index (kg/m^2)	22.34±2.409	23.76±2.901	0.249
Hypertension	2	3	0.227
Hyperlipidemia	3	7	0.179
Smokers	3	5	0.774
NYHA functional class			0.631
I	0	1	
II	4	4	
III	5	4	
IV	1	1	

Notes: Data are presented as mean ± SD. P-values were calculated using chi-square test, rank sum test, or Student's *t*-test.
Abbreviations: N, number; SD, standard deviation.

In the current study, we discussed 11 potential crucial DEARGs involved in the occurrence and development of AF, suggesting that these genes may serve as potential biomarkers and therapeutic targets for AF. However, there are still some limitations in this study. Firstly, due to the small sample size of this study and the heterogeneity of AF, the interpretation of the study results needs to be cautious. In addition, the specific pathophysiological mechanisms of ARGs regulating the initiation and progression of AF need to be further studied. Finally, the working mechanism of these genes is not yet fully understood, so more evidence is needed to discover its biological basis.

Conclusion

In summary, we conducted the gene differential expression analysis, functional enrichment analysis, and protein-protein-interaction analysis of autophagy genes in AF, these results show that these DEARGs have great potential as biomarkers and therapeutic targets in AF. Future studies need to further validate the protein interactions identified by RT-PCR. In addition, the crucial genes CXCR4, DAPK2, and TNFSF10 may provide new possibilities for further identifying the susceptibility of AF and finding useful therapeutic targets.

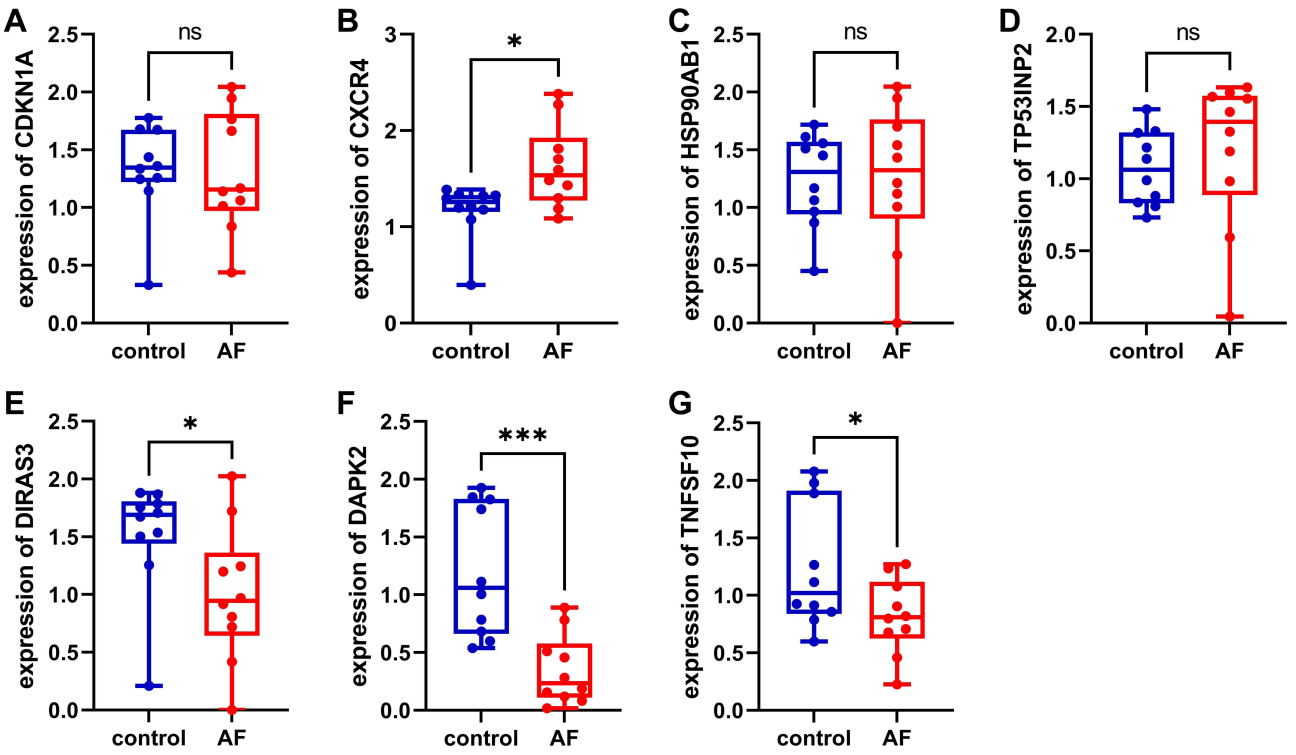

Figure 10 The mRNA expression levels of 11 DEARGs were measured in AF and non-AF samples. (**A**) CDKN1A, (**B**) CXCR4, (**C**) HSP90AB1, (**D**) TP53INP2, (**E**) DIRAS3, (**F**) DAPK2, (**G**) TNFSF10. *P<0.05; ***P<0.001.
Abbreviation: ns, non significant.

Abbreviations

AF, atrial fibrillation; ARGs, autophagy-related genes; GEO, Gene Expression Omnibus; DEGs, differentially expressed genes; PPI, protein-protein interaction; DEARGs, differentially expressed ARGs; HADb, Human Autophagy-dedicated Database; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; qRT-PCR, quantitative real-time PCR; BP, biological process; BMI, body mass index; log2 FC, log2 (fold change); AveExpr, average expression; adj. P. Val, adjust P-value.

Data Sharing Statement

The data analyzed in the present study can be accessed on the GEO (https://www.ncbi.nlm.nih.gov/geo/) website.

Ethics Approval and Informed Consent

The study protocol was approved by the Ethics Committee of Fuwai Yunnan Cardiovascular Hospital (Ethical Application Ref: IRB2021-BG-006), Kunming, Yunnan, China. Informed consent forms were signed voluntarily by all individual participants included in the study.

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Disclosure

The authors report no conflicts of interest in this work.

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