Comprehensive Bioinformatics Evaluation within the Left Atrial Appendage from Individuals with Atrial Fibrillation to Enhance the Comprehension of Atrial Fibrillation-Related Thrombus Development

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Abstract: To investigate differentially expressed genes and their potential functions in left atrial appendage tissues from patients with atrial fibrillation through integrated bioinformatics analysis, providing a theoretical basis for the molecular mechanisms underlying AF-related thrombus formation. Two gene expression datasets, GSE79768 and GSE115574, were obtained from the GEO database. A total of 14 left atrial appendage samples from AF patients and 16 from sinus rhythm controls were included. Differentially expressed genes were screened using the limma package. Gene Ontology functional annotation and KEGG pathway enrichment analyses were performed using DAVID and KOBAS databases, and a protein-protein interaction network was constructed using the STRING database. A total of 96 common differentially expressed genes were identified, including 69 upregulated and 27 downregulated genes. GO analysis indicated that these genes were predominantly enriched in biological processes such as cell surface, antigen processing and presentation, and immune response. KEGG analysis suggested significant enrichment of differentially expressed genes in the phagosome pathway, as well as signaling pathways related to Staphylococcus aureus infection and tuberculosis. PPI network analysis identified 20 hub genes, including TYROBP, CD53, and CTSS. Differentially expressed genes in left atrial appendage tissues of AF patients are primarily involved in immune-inflammatory responses and phagosome-related pathways. These molecules may participate in the regulatory processes of thrombus formation under AF conditions.

Keywords: Atrial Fibrillation; Left Atrial Appendage; Thrombosis; Differentially Expressed Genes; Bioinformatics

1. Introduction

Atrial fibrillation (AF), which has emerged as a significant factor in mortality and impairment across the globe, represents a major clinical challenge. By 2010, roughly 33 million individuals had been diagnosed with AF worldwide. According to current statistics, the chronic risk of acquiring AF in certain European nations surpassed 30 percent. Individuals with AF possess a substantial probability of experiencing stroke, heart failure and hospitalization, and display varied underlying pathologies leading to major economic pressures on the healthcare system [1].

AF represents a distinct risk element for thrombotic occurrences, encompassing strokes and systemic venous thromboembolism. Research indicates that 57% of thrombi in cases of valvular atrial fibrillation (AF) and 91% in nonvalvular atrial fibrillation (NVAF) stem from the left atrial appendage (LAA) [2]. This underlying mechanism involves multiple dimensions: the LAA possesses a complex architecture and neural connection; AF can induce blood stasis via diminished peak flow velocity in the LAA because of impaired left atrial contractility and its intrinsic properties; furthermore, it is associated with endothelial injury [2-4]. The study found thatmultiple genes associated with thrombus formation are upregulated in the left atrial appendage of patients with atrial fibrillation. A combination of factors collectively contributes to the occurrence of thrombotic events in the left atrial appendage [5,6].

Lately, investigations into the cellular and molecular foundations of AF have concentrated

on atrial tissues derived from AF patients or animal models. Various patterns of electrical shifts, structural modifications and Ca^{2+} regulation deficits can produce the vulnerability to AF or sustain it [7,8]. The latest advancement in biotechnology involving gene-expression microarray techniques has permitted evaluation of numerous genes' mRNAs, which is currently frequently employed in research regarding gene expressions linked with diverse human diseases. It offers a fresh approach for the extensive investigation of disease-related genes in molecular forecasting, Molecular Diagnosis and therapy. Presently, there are numerous papers concerning the atrium gene expression landscape, and rich information has been supplied by several public databases to [9]. Nevertheless, the outcomes for the detection of significantly altered mRNAs are incongruent between different trials because of various experimental platforms, specimen preparation strategies and specimen diversity. By merging the microarray findings of several gene-expression statuses to enhance their cumulative limitations and additionally uncover extra molecular biomarkers; currently, they are extensively utilized for cancer research. Integrated bioinformatics analysis is seldom utilized in the study of tachycardia. Thus, more research should be performed on sophisticated early-warning Technologies and dependable Molecular markers to assess recurrence Risks and Prognostics; meanwhile, Innovative treatments Integrating Bioinformatics Analysis can also provide Novel viewpoints for managing Atrial Fibrillation (AF). Investigate the cellular and molecular mechanisms of thrombosis development in liver abscesses to some degree, etc., also examine protective or therapeutic strategies for LAA-associated embolism [10-12].

2. Methods

The gene expression datasets GSE79768 and GSE115574 used in this study were obtained from the GEO database, both of which were based on the GPL570 platform. By integrating both datasets, a total of 14 left atrial appendage specimens from atrial fibrillation individuals and 16 from sinus rhythm controls were included in the analysis. The platform and series matrix data were downloaded in TXT format. Table 1 presents the specific dataset information. Utilizing the R software package for processing retrieved files.

Table 1. Details for GEO AF data

Reference	Sample	GEO	Platform	AF	SR
Tsai F et al. (2016)[9]	LAA	GSE79768	GPL570	6	7
Peng S et al.(2024)[11]	LAA	GSE115574	GPL570	14	16
Abbreviation: LAA, left atrial appendage. GEO, Gene Expression Omnibus. AF, atrial fibrillation. SR, sinus rhythm.					

Probe names were converted into universal gene identifiers (gene symbols) and saved in TXT files. For the two microarray datasets, differentially expressed genes were screened using the criteria of |fold change| > 1.2 and adjusted P-value < 0.05 [13].

DEGs were extracted from both microarray panels via the limma package. A collection of genes showing substantial up- or down-modulation across these two panels was identified for subsequent investigation. The UpSetR and VennDiagramR libraries were utilized to determine the intersecting and unique genes accordingly.

Functional and pathway enrichment analyses of the common differentially expressed genes across the two microarray datasets were performed using the DAVID online tool [14]. Applying GO terms for categorization through DAVID web-based tools. Carrying out pathway analysis for DEG genes via the KOBAS platform using the condition that P values were below 0.05.

To ascertain the associations and pathway dynamics among proteins originating from differentially expressed genes (DEGs) within AF, the STRING database was utilized; this platform integrates experimental datasets, databases, text-mining insights, and bioinformatics prediction outcomes [15]. We established a threshold for interaction confidence scores exceeding 0.4. The resulting PPI network was then visualized using CytoScape software to facilitate additional analysis of the interactions among DEGs, as is shown in Figure 1.

3. Results

3.1 Screening of Differentially Expressed Genes

After normalization of the two microarray datasets, 4,087 DEGs were obtained from

GSE79768, including 1,740 downregulated and 2,347 upregulated genes. In the GSE115574 dataset, 574 DEGs were found, with 212 downregulated and 362 upregulated. DEGs were extracted from each sample group of the two microarrays, and a total of four microarrays were used. Figure 2 shows the cluster heatmaps of the top 100 DEGs.

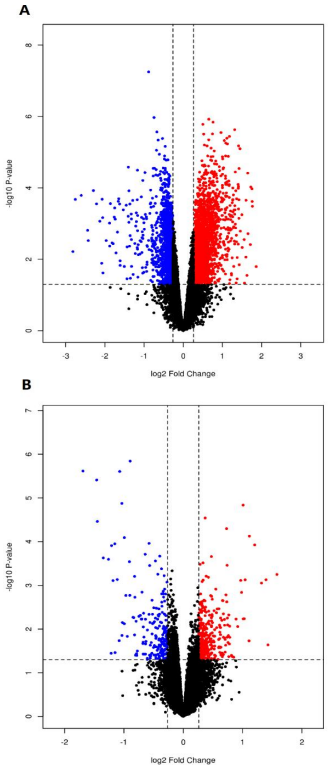
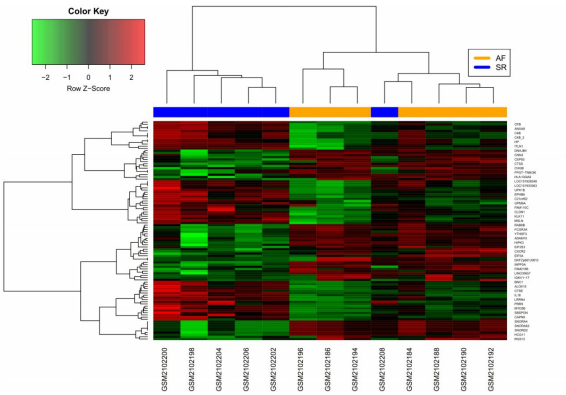


Figure 1. Differential Expression of Data between Two Sets of Samples

Notes: (A) GSE79768 data, (B) GSE115574 data. The red points represent upregulated genes screened on the basis of |fold change| > 1.2 and a corrected P-value of < 0.05. The blue points represent downregulation of the expression of genes screened on the basis of |fold change| > 1.2 and a corrected P-value of < 0.05. The black points represent genes with no significant difference. FC is the fold change.

A



B

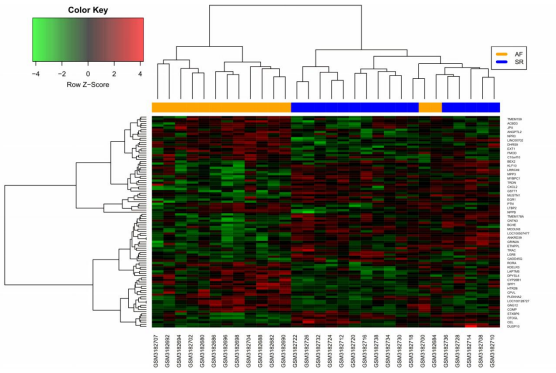


Figure 2. Hierarchical Clustering Heatmap of the Top 100 Degs in Two Sets of Sample Data of Each Microarray

Notes: (A) GSE79768 data, (B) GSE115574 data. Red indicates that the expression of genes is relatively upregulated, green indicates that the expression of genes is relatively downregulated, and black indicates no significant changes in gene expression; gray indicates that the signal strength of genes was not high enough to be detected.

Abbreviation: DEGs, differentially expressed genes.

3.2 Identification of AF-Related DEGs through Integrated Analysis

A total of 132 shared DEGs were derived from the two microarray datasets, among which 96 genes showed consistent expression trends. GO functional classification grouped these DEGs into different functional categories (Figure 3, Figure 4, Figure 5, Figure 6). In terms of molecular function, a considerable number of genes exhibited IgG-binding ability. Regarding cellular components, the DEGs were mainly localized to structures such as the cell surface, junctional complexes, and the sarcoplasmic reticulum membrane adjacent to the plasma membrane. KEGG pathway analysis indicated that these DEGs were significantly enriched in the phagosome pathway, suggesting their involvement in signaling networks related to various infectious and immune-related diseases, as is shown in Table 2, Table 3, Table 4.

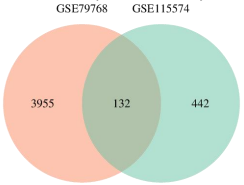


Figure 3. Venn Diagram of the Same Expression Changed DEGs Based on the Two Microarray Datasets

Notes: GO term enrichment analysis and KEGG pathway analysis of DEGs

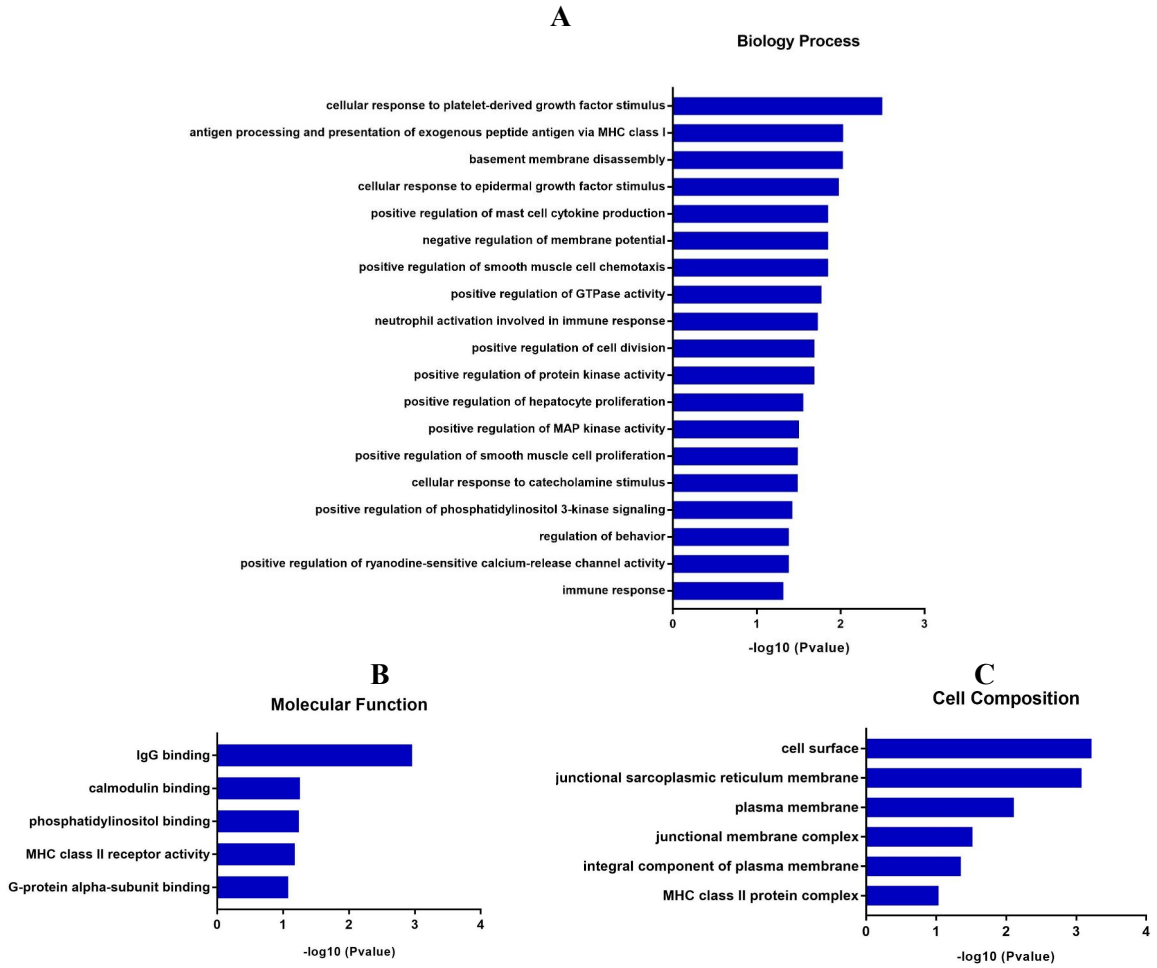


Figure 4. GO Enrichment Analysis of Degs in AF

Notes: GO analysis divided DEGs into three functional groups: (A) biological processes, (B) cell composition, and (C) molecular function.

Abbreviations: DEGs, differentially expressed genes; GO, gene ontology.

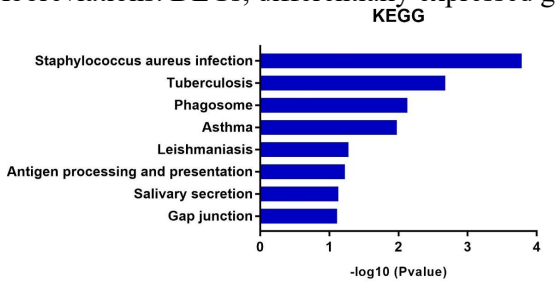


Figure 5. KEGG Pathway Enrichment Analyses of Degs in AF

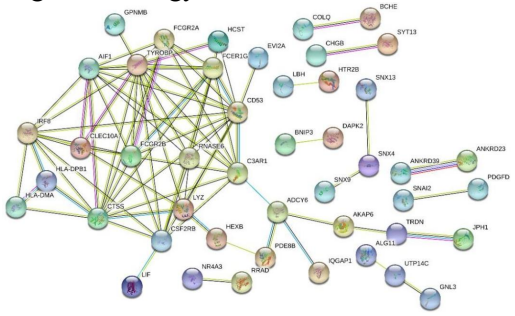


Figure 6. PPI Network of Degs in AF

Table 2. Screening Degs in AF by Integrated Microarray

DEGs	Gene names
Downregulated	JPH1 FAM212B LOC101928718 ANKRD23 HPN MARCH11 USP54 BEX2 HOOK1 ANKRD39 MUSTN1 LIF LRRC49 PCP4 SLC26A9 LOC100129129 TADA2B CPNE5 NR4A3 SYT13 AAMDC DAPK2 TRDN SMTNL2 C1orf105 PSMG3-AS1 BCHE
Upregulated	HLA-DMA RELN PDE8B DPYSL4 FCGR2B FCGR2A VILL GPNMB TMEM159 MTURN NDUFAF7 SRPX HCST PTN FCER1G ZFP36L2 LPXN EVI2A DIS3 SMARCC1 AKAP6 C3AR1 DHRS9 SNAI2 COLQ MRPS18B OIP5-AS1 CTSS UAP1L1 LYZ RNASE6 TYW3 BNIP3 LOC101928304 MBOAT1 ALG11 RRAD MPHOSPH8 IQGAP1 SLC6A6 LOC101927990 ADCY6 COL21A1 TRIP11 SNX9 HTR2B TYROBP BRCC3 SNORD19B POPDC3 LBH ATP1B4 UTP14C SNX4 IRF8 CLEC10A CSF2RB HLA-DPB1 GATA6-AS1 NIN SNX13 CHGB KMT2E HEXB DENND5A AIF1 RGS10 CD53 PDGFD
Abbreviation: DEGs, differentially expressed genes.	

Table 3. GO Analysis of Genes Associated with AF

Term	Description	Gene count	P-value
GO:0009986	cell surface	10	6.05E-04
GO:0014701	junctional sarcoplasmic reticulum membrane	3	8.37E-04
GO:0019864	IgG binding	3	0.0011
GO:0036120	cellular response to platelet-derived growth factor stimulus	3	0.003184
GO:0005886	plasma membrane	29	0.007781
GO:0019886	antigen processing and presentation of exogenous peptide antigen via MHC class II	4	0.009322
GO:0034769	basement membrane disassembly	2	0.009387
GO:0071364	cellular response to epidermal growth factor stimulus	3	0.010499
GO:0032765	positive regulation of mast cell cytokine production	2	0.014048
GO:0045837	negative regulation of membrane potential	2	0.014048
GO:0071673	positive regulation of smooth muscle cell chemotaxis	2	0.014048
GO:0043547	positive regulation of GTPase activity	8	0.016918
GO:0002283	neutrophil activation involved in immune response	2	0.018688
GO:0051781	positive regulation of cell division	3	0.020605
GO:0045860	positive regulation of protein kinase activity	3	0.020605
GO:2000347	positive regulation of hepatocyte proliferation	2	0.027902
GO:0030314	junctional membrane complex	2	0.030332
GO:0043406	positive regulation of MAP kinase activity	3	0.031458
GO:0048661	positive regulation of smooth muscle cell proliferation	3	0.032445
GO:0071870	cellular response to catecholamine stimulus	2	0.032477
GO:0014068	positive regulation of phosphatidylinositol 3-kinase signaling	3	0.03756
GO:0050795	regulation of behavior	2	0.041563
GO:0060316	positive regulation of ryanodine-sensitive calcium-release channel activity	2	0.041563
GO:0005887	integral component of plasma membrane	12	0.044512
GO:0006955	immune response	6	0.048242

Table 4. KEGG Pathway Analysis of DEGs Associated with AF

Pathway	ID	Database	Gene count	P-value	Genes
Staphylococcus aureus infection	hsa05150	KEGG Pathway	5	1.66E-04	C3AR1, FCGR2B, FCGR2A, HLA-DPB1, HLA-DMA
Tuberculosis	hsa05152	KEGG Pathway	6	0.002104	FCGR2B, FCER1G, FCGR2A, CTSS, HLA-DPB1, HLA-DMA
Phagosome	hsa04145	KEGG Pathway	5	0.007439	FCGR2B, FCGR2A, CTSS, HLA-DPB1, HLA-DMA
Asthma	hsa05310	KEGG Pathway	3	0.010566	FCER1G, HLA-DPB1, HLA-DMA

Abbreviations: DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Notes: Circles represent genes, lines represent the interaction of proteins between genes, and the results within the circle represent the structure of proteins. Line color represents evidence of the interaction between the proteins.
Abbreviation: PPI, protein–protein interaction.

3.3 Construction and Analysis of the PPI Network

To further explore the biological functions of the differentially expressed genes, a protein-protein interaction network was constructed using the STRING database. After removing isolated and weakly connected nodes, the final network consisted of 46 nodes and 84 interaction edges. Through module analysis, several key genes were identified as important nodes in the PPI network, including adcyc6, hcastle, hexb, snx4, and tyrop.

4. Discussion

In this study, 96 differentially expressed genes were identified through bioinformatics analysis of left atrial appendage tissue from patients with atrial fibrillation. GO analysis showed that these genes are primarily enriched in functions related to cytokine response and antigen presentation. KEGG analysis indicated their involvement in

pathways associated with the phagosome pathway, as well as infections such as Staphylococcus aureus and tuberculosis. By constructing a PPI network, 20 hub genes, including TYROBP and CD53, were further screened.

AF represents the major cause for mortality due to cardiovascular events or various cardiac complications globally. Furthermore, numerous studies indicate that the prevalence of AF among adults older than 20 years reaches approximately 3%, with roughly 20%-30% of cerebrovascular accidents linked to it [16,17]. Via ECG, AF can be diagnosed; its characteristic presentation appears as follows. Numerous AF sufferers are asymptomatic and intermittent, and are frequently overlooked during clinical visits [18]. Its emergence and progression are intricate biological mechanisms. Consequently, utilizing R programming software and combined bioinformatics analytical approaches within this research to investigate the molecular foundation of AF.

Multiple studies have shown that inflammatory-mediated immunological reactions facilitate the onset and progression of AF by modifying atrial electrophysiological properties or morphology via tissue injury. Furthermore, the AF process itself stimulates inflammation and aggravates atrial fibrotic changes [19,20,21]. The primary inflammatory origins in AF patients are derived from systemic conditions, atrial myocardial trauma, and immunological abnormalities involving autoantibodies [22,23]. AF likewise serves as a consequence of ongoing inflammatory stimulation, which consequently intensifies atrial structural remodeling. Collectively, data has revealed that inflammatory and immunological biomarkers such as CRP, HSP27, and TNF are heightened during AF episodes relative to individuals with prior AF in sinus rhythm [24-27].

AF-mediated enhancement of pro-inflammatory signaling; nevertheless, multiple factors are associated with thrombotic complications. Soluble CD40 ligand, which is vital for the progression of cardiovascular disorders and has been demonstrated to be pivotal in stimulating platelets among various others. Scd-40L concentrations have increased in individuals with AF; consequently, a correlation exists between inflammatory mechanisms and thrombosis [28,29].

Phagosomes participate in the degradation of pathogens and cellular debris by utilizing enzymes; they furthermore contribute to inflammasome assembly and modulation once it is established as essential. Upon the surface of phagocytes, numerous receptors identify particulate or pathogenic entities like bacteria and apoptotic, as well as necrotic cells, which are ubiquitously spread. Particles or microorganisms following opsonization by antibodies to serve as targets for Fc receptor coupling. Cross-linking of FcγR triggers a sequence of signal transduction regulated by tyrosine phosphorylation of various proteins, leading to modifications in actin cytoskeletal patterns and membrane-level shifts which facilitate phagosome development [30,31,32]. Through our investigation, it was identified that low-affinity immunoglobulin gamma FC-RC2B along with other genes in the PPI network created by drugs and host cells. FCGR2B and FCGR2A, possessing reduced affinity for immunoglobulin Fc fragments, were recognized as DEGs within this research and enriched in the pathways of phagosome maturation.

Phagocytes deliver antigens to activate specific T/B-cell populations, which act as vital mediators in subsequent inflammatory and immune reaction processes. Cytotoxic lymphocytes have been identified as participating in the fibrotic response triggered by inflammation within subepicardial fatty infiltration, which contributes to atriofibrillation (AF). Antigen processing and presentation trigger the onset of immune reactions. APCs display antigens via MHC-II molecules following phagocytosis; consequently, this stimulates CD4 T cell activation. Cathepsin S plays a pivotal function in MHC II-mediated antigen presentation. MHC-II is synthesized via diverse gene products, including HBPD2, HLA6, HLA5 and HLA7, etc. Our study validated that cathepsin S (CTSS), HLA-DPB1, and HLA-DMA are essential genes identified through the evaluation of the PPI network, which were found to be enriched in phagosome pathways as determined by KEGG pathway analysis.

Among the scores derived from module analysis, TYROBP (tyrosinase-binding protein) was identified as the primary candidate. TYROBP represents cytotoxicity-associated genes that could function as novel diagnostic biomarkers or prognostic tools for atrial fibrillation (AF). Cell-mediated immunity throughout the development of AF has been confirmed by various studies

[33,34]. TYROBP possesses a crucial role in the cytotoxic activity of NK cells [35,36]. Based on GO functional enrichment analysis, it was discovered that TYROBP primarily involves neutrophil activation within the immune system. Increased NLR (neutrophils/lymphocytes ratio) has been observed to be more strongly associated with the onset of AF [37,38]. According to our findings, there might be certain participation of NK cells and neutrophils in the AF pathophysiology that necessitates additional exploration via more comprehensive investigations.

Numerous cardiomyopathic disorders can trigger apoptosis, involving diverse mechanisms. Investigations demonstrate that cardiac myocytes undergo distinct apoptotic alterations following exposure to chronic AF-[39-42]. CSF2RB (cytokine receptor common subunit beta) displayed a substantial differential expression at the molecular level via the comparative protein-protein interaction datasets previously discussed in this manuscript. CSF2RB primarily functions to facilitate apoptosis and the engagement of JAK/STAT signaling cascades; consequently, it is anticipated to exert a crucial function in AF. Thus, further validation must be conducted through additional research.

Furthermore, the KEGG analysis indicated that these differentially expressed genes (DEGs) played a role in signaling pathways associated with infections caused by Staphylococcus aureus, as well as Tuberculosis and Asthma. The subjects within this research do not receive a diagnosis for the diseases mentioned. We therefore observed that, owing to their involvement in inflammatory reactions and immune activation, they were notably abundant in several signaling pathways [43].

Contrary to previous studies regarding AF, this work introduced an original approach by employing a Venn diagram specifically to investigate the distinctions in DEGs within LAA regions for ASF analysis. This research possesses significant implications for clinical practices concerning the treatment and secondary effects of AF [44]. Based on these findings, a causal link may be identified between inflammatory responses and immunological processes as well as thrombotic events in individuals with atrial fibrillation characterized by large left atrial appendages (LAAA) [45]. It enhances our understanding of AF pathogenesis by incorporating these correlations with specific molecular signaling networks. Subsequent molecular biological research will verify the actual modifications in the biological roles of those genes involved.

5. Conclusions

Consequently, we perform a unified bioinformatics evaluation of DEGs within LAA tissues derived from AF subjects. Collectively, we identified 96 DEGs; specifically, 69 were upregulated while 27 were downregulated. Through GO and KEGG pathway investigations, it was observed that a significant majority of these DEGs participate in inflammatory processes and associated phagocytic activities.

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