Identification of metabolic biomarkers in atrial fibrillation patients via the integrated application of proteomics and metabolomics

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Abstract

Atrial fibrillation (AF) is a prevalent clinical arrhythmia characterized by an irregular cardiac rhythm, increasing the risk of developing stroke and heart failure. In order to explore the potential role of serum indicators, the study employed a combination of targeted metabolomics and Tandem Mass Tag (TMT) based proteomics to examine metabolic characteristics and biomarkers in the serum of patients with AF. Furthermore, the verification of protein expressions with diagnostic significance for AF was conducted in patients of larger sample sizes by ELISA. Proteomics and metabolomics identified 174 differentially expressed proteins (DEPs) and 294 differentially metabolites (DMs) in AF patients, respectively. The clustering and functional enrichment analysis identified the complement and coagulation cascade as the primary pathway dysregulating DEPs. According to the integrated study, the most enriched proteomics and metabolomics pathways were platelet activation and cholesterol metabolism. Lactate dehydrogenase A (LDHA), lactate dehydrogenase B (LDHB), and transgelin 2 (TAGLN2) were significantly expressed in AF patients, while plasminogen (PLG) was low. In conclusion, the current study found that platelet activation, cholesterol metabolism, and the complement and coagulation cascade pathways may affect AF progression. The study also showed that LDHA, LDHB, TAGLN2, and PLG may be potential AF biomarkers.

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Integrated proteomics and metabolomics techniques facilitates target identification and understanding of the molecular regulatory mechanisms of disease. In this study, both integrated proteomics and metabolomics were used to investigate the expression levels of low-abundance proteins in the serum of patients with sinus rhythm and chronic atrial fibrillation. With these techniques, we examined the serum biological markers and intervention targets related to AF, screened the differentially expressed proteins and differentially metabolites, and then used molecular biology techniques, such as ELISA, to investigate the related pathways. Our findings showed that LDHA, LDHB, TAGLN2, and PLG were identified as potential biomarkers. In addition to these potential biomarkers, our results demonstrated that the cholesterol metabolism, the complement, and coagulation cascade pathways were prevalent in AF progression.

AAtrial fibrillation (AF) is a prevalent clinical arrhythmia characterized by an irregular cardiac rhythm, increasing the risk of developing stroke and heart failure. In order to explore the potential role of serum indicators, the study employed a combination of targeted metabolomics and Tandem Mass Tag (TMT) based proteomics to examine metabolic characteristics and biomarkers in the serum of patients with AF. Furthermore, the verification of protein expressions with diagnostic significance for AF was conducted in patients of larger sample sizes by ELISA. Proteomics and metabolomics identified 174 differentially expressed proteins (DEPs) and 294 differentially metabolites (DMs) in AF patients, respectively. The clustering and functional enrichment analysis identified the complement and coagulation cascade as the primary pathway dysregulating DEPs. According to the integrated study, the most enriched proteomics and metabolomics...
pathways were platelet activation and cholesterol metabolism. Lactate dehydrogenase A (LDHA), lactate dehydrogenase B (LDHB), and transgelin 2 (TAGLN2) were significantly expressed in AF patients, while plasminogen (PLG) was low. In conclusion, the current study found that platelet activation, cholesterol metabolism, and the complement and coagulation cascade pathways may affect AF progression. The study also showed that LDHA, LDHB, TAGLN2, and PLG may be potential AF biomarkers.

Keywords: Atrial fibrillation; Glycometabolism; Lipometabolism; Metabolomics; Proteomics

Introduction

AF is a prevalent cardiac arrhythmia that poses significant challenges worldwide and is characterized by a high morbidity and mortality rate. Investigation conducted in 2017 revealed that 37.6 million people worldwide are afflicted by atrial fibrillation and atrial flutter [1]. In China, around 20 million individuals are affected by AF, resulting in a prevalence rate of 1.6% [2]. Recent studies have suggested the likelihood of the prevalence rate of AF doubling, especially in older adults over 60; thus, aging is conceived as an important pro-AF factor [3].

Notably, AF can lead to serious side effects such as strokes and heart failure (HF). More to the point, studies speculate that AF is the main contributing factor in cardiogenic strokes for at least one in five patients with ischemic strokes [4]. It has been shown that a significant proportion, ranging from one-third to one-half of individuals diagnosed with AF, may ultimately develop HF. Once a patient diagnosed with HF develops AF, their medical status significantly deteriorates [5]. Regrettably, a considerable number of individuals afflicted with cardiac ailments may be unaware of their AF disease until it is detected during a medical evaluation or until symptoms manifest. Even worse, it is concerning to note that only 6% of patients with AF at a high risk of blood clots receive anticoagulation therapy [6]. Hence, it is imperative to prioritize the timely identification and diagnosis of AF, necessitating suitable screening measures. In this regard, the electrocardiogram (ECG) is often regarded as the most efficacious instrument for monitoring and assessing cardiac function [7]. Nevertheless, alternative tests are being developed in response to the labor-intensive nature of ECG testing. Recent studies have indicated that the identification of serum biomarkers has become a viable method for the diagnosis of many cardiovascular conditions, including coronary heart disease and HF. [8; 9]. Researchers have diligently endeavored to investigate promising biomarkers associated with AF progression in recent years. The identification of brain natriuretic peptide (BNP) and fibroblast growth factor 23 (FGF-23) as potential biomarkers for the detection of patients with AF has been reported [10].

Nevertheless, there is a dearth of biomarkers for AF compared to other cardiovascular illnesses, necessitating further research and evaluation. The etiology of AF is ascribed to the intricate interplay and modulation of numerous genes and proteins. Hence, prior examinations of genes or proteins might yield initial insights into the process that underlies the etiology of AF. Recent research indicates that the field of proteomics has the potential to facilitate the identification of biomarkers associated with different cardiovascular illnesses, hence aiding in the screening process. Furthermore, the utilization of proteomics can also provide valuable insights to inform and enhance the clinical management strategies employed for these disorders [11; 12]. The organism’s metabolic makeup undergoes alterations as a direct consequence of the influence of disease, leading to progressive metabolic disorders. Cardiovascular illnesses are distinguished by the presence of metabolic dysregulation closely linked to the course of the disease. Metabolites possess the ability to mutually control each other because of the proteome’s functionality. Therefore, researchers integrate proteomics and metabolomics methodologies to uncover potential targets and crucial metabolic pathways associated with the pathophysiology of cardiovascular illnesses. [13; 14].

In the present study, we employed a combination of proteomics and metabolomics techniques to analyze and compare the distinct metabolites and proteins in the serum of individuals diagnosed with AF and a control group of healthy individuals. The results of our study provide a foundation for future research on the pathophysiology of AF and the development of tailored drug treatments.

Material and methods
Patient studies and sample collection

The current study included 60 participants between August 2021 and December 2021 at the Department of Cardiology, Zhongshan Hospital of Traditional Chinese Medicine. These participants were separated into two groups: the AF group, consisting of 44 individuals, and the healthy group, composed of 16 individuals. The experiments were carried out in accordance with the guidelines and rules set forth by the Ethics Committee of Zhongshan Hospital of Traditional Chinese Medicine. AF was detected after a comprehensive evaluation that included a thorough patient history, a 12-lead ECG, and a dynamic ECG. The dataset excluded patients who had infective endocarditis, hyperthyroidism, sick sinus node syndrome, congenital cardiac disease, and those under 18. According to the predetermined inclusion and exclusion criteria, 16 individuals from the Healthy control group and 23 from the AF group were selected for the study. These individuals had peripheral blood collection using venipuncture, with a volume of 5 mL, following an overnight fasting period. The blood samples underwent centrifugation at a speed of 3,500 revolutions per minute (rpm) for a duration of 10 minutes. The resulting serum samples were stored at -80°C for further experiments.

Proteomics

Sample preparation for proteomics

The high-abundance serum proteins were depleted using Thermo Scientific High Select Depletion Spin Columns (A36372), as described in a recent study [15]. The in-solution digestion process was employed to prepare serum proteins. Briefly, the serum samples were diluted to a volume of 100 μL using a lysis solution consisting of 8 M urea, 4% CHAPS, 40 mM Tris, and 65 mM DTT. Subsequently, a total volume of 600 μL of precipitation buffer, composed of a mixture of acetone, ethanol, and acetic acid in a ratio of 50:50:0.1, was employed for the purpose of protein precipitation. The proteins were reconstituted using 100 μL of a 50 mM solution of triethylammonium bicarbonate (TEAB) and subjected to enzymatic digestion using trypsin (Promega, USA; enzyme to protein ratio of 1:25) for a duration of 18 hours. Following protein digestion, the collection of peptides was carried out by utilizing a 10 kDa filter (Millipore Corporation, USA). Subsequently, the collected peptides were subjected to a drying procedure using a SpeedVac. The concentration of reconstituted peptides was assessed in a solution containing 100 mM TEAB using the BCA assay. All samples, comprising 16 control (CON) samples and 23 experimental (AF) samples, were subjected to labeling using TMT 16-plex reagents (0.1 mg each) from Thermo Fisher Scientific. Subsequently, there after, equal amounts of each TMT-labelled sample were put into new microcentrifuge tubes and dried by SpeedVac. The peptide mixture labeled with TMT was divided into two equal halves, with one half being subjected to fractionation. This process was carried out using an Agilent 1290 High-Performance Liquid Chromatography (HPLC) system equipped with a Waters XBridge BEH130 C18 3.5 μm 2.1 × 150 mm column. The fractionation was performed at a flow rate of 0.2 mL/min, as previously described [16].

Nanoflow liquid chromatography-tandem mass spectrometry

The experiments conducted in this study used a Q Exactive HF mass spectrometer with a nanoLC easy1000 from Thermo Fisher Scientific. Peptides were loaded onto a self-packed column (75 μm × 150 mm, 3 μm ReproSil-Pur C18 beads, 120 Å, Dr. Maisch GmbH, Ammerbuch, Germany) and separated using a 120-minute gradient at a flow rate of 300 nL/min. Solvent A contained 0.1% formic acid, while solvent B had acetonitrile with 0.1% formic acid. The Q Exactive HF was programmed for data-dependent acquisition mode. We obtained an MS1 scan atlas of 300-1700 m/z in the Orbitrap at a resolution of 120,000 with a maximum injection time of 50 ms. Precursor ions underwent monoisotopic precursor selection, charge state (+2 to +6), and dynamic exclusion (40 s with a ±10 ppm window). The 15 most intense precursors underwent HCD fragmentation. The instrument parameters were set: 30% normalized collision energy, 60,000 resolution, 100,000 AGC target, 120 ms maximum injection time, 110 Da first mass, 0.7 m/z isolation width.

Proteomics data analysis

Raw files were processed by the UniProt database containing 75,074 sequence entries (downloaded in August
2020) using Maxquant (1.6.17.0), with default settings for 16-plex TMT quantification. Trypsin/P was selected as the digestive enzyme, with one failed cleavage allowed. Approximately, 7 amino acids were required per protein for a peptide, >2 peptides. The false discovery rate (FDR) was set at 1% for peptide and protein identification. TMT reporter ion intensity was used for quantification.

**Enzyme-linked immunosorbent assay (ELISA).**

To further validate the proteomics results, we used human ELISA for the validation of key proteins. They were measured by the human LDHA, LDHB, PLG, TAGLN2, Fibronectin 1 (FN1) and Apolipoprotein A-I (APOA1) ELISA kit (Cusabio Biotech), respectively.

**Metabolomics**

**Sample preparation for metabolomics**

The samples were collected from the -80 °C freezer and thawed in preparation for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Subsequently, qualitative analysis was performed based on retention time (RT), daughter ion pairs information, and the detected substances’ secondary spectral data. Extraction methods for hydrophilic substances: After thawing, the samples were vortexed for 10 s and mixed well. Then 50 μL of the samples were transferred to an appropriate numbered centrifuge tube, and 300 μL of methanolic extract of 20 % acetonitrile was added, vortexed for 3 min, and centrifuged for 10 min at 12000 rpm/min at 4 °C. After centrifugation, 200 μL of the supernatant was placed in the corresponding numbered tube and left for 30 min at -20 °C in the refrigerator. The supernatant was then centrifuged at 12000 rpm/min for 3 min at 4 °C. 180 μL of the supernatant was transferred to the tube of the corresponding injection vial and used for analysis. The preceding steps were the same as for hydrophilic substances. Then, 1 mL of lipid extract containing the internal standard (methyl tert-butyl ether: methanol = 3:1, V/V) was added and vortexed for 15 min. 200 μL of water was added, vortexed for 1 min and centrifuged at 12,000 rpm/min for 10 min at 4 °C. After centrifugation, 200 μL of supernatant was transferred to the corresponding numbered centrifuge tube and concentrated until completely dry. Finally, 200 μL of mobile phase B was added, vortexed for 3 min, centrifuged at 12000 rpm/min for 3 min, and the supernatant was removed for LC-MS/MS analysis.

**Metabolomics data analysis**

The software Analyst (1.6.3) was utilized to process data for qualitative analysis based on the detected substances retention time (RT), precursors and ion pair products, and secondary spectra data. The data was processed using Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA). Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Metabolome Database (HMDB) (https://hmdb.ca) were used to identify relevant functional pathways. Pathways with significantly regulated metabolites mapped were then fed into metabolite sets enrichment analysis (MSEA). Significantly regulated metabolites between groups were determined by projection value (VIP > 1 and |Log2FC| > 1.0). VIP values were extracted from the OPLS-DA result, which also contains score plots and permutation plots, and were generated using the R package MetaboAnalystR. The reproducibility of metabolite extraction and detection can be determined by overlapping display analysis of total ion flow maps from mass spectrometric detection analysis of different QC samples. The high stability of the instrument provides an important guarantee for the reproducibility and reliability of the data. The metabolite content data were processed using unit variance scaling, and a heatmap was generated using the ComplexHeatmap package in R software. Hierarchical Cluster Analysis (HCA) was performed to analyze the accumulation patterns of metabolites across different samples.

**Bioinformatics analysis**

Principal component and cluster analyses were performed to further explore the associated metabolic changes and pathways, including the Gene Ontology (GO) function and KEGG pathway enrichment analysis. To complement the limitations of KEGG and GO analysis, we utilized the Molecular Signatures Database
(MSigDB) in the GSEA software (version 4.3.2) to explore the hallmark functions of all proteins. Thereafter, the Search Tool was employed for the Retrieval of Interacting Genes database (STRING) (https://string-db.org/) and Cytoscape software (version 3.9.1) to construct a protein-protein interaction network (PPI) and the “protein-metabolite” network. The R software (version 4.1.0) was used to generate the diagrams. The ROC curves were performed to predict the diagnostic value of DEPs and DMs. Furthermore, the potential therapeutic compounds were explored using Connectivity Map (cMAP) database [17].

Results

Baseline characteristics of participants

The baseline characteristics of proteomics and metabolomics samples are shown in Table 1.

AF-induced differential expression of proteins in serum samples of AF patients

In the obtained findings, a total of 786 proteins were identified. Among them, proteins with missing values exceeding 50% were discarded. Approximately, 580 proteins with more than 50% missing values also had quantitative information. In total, 174 DEPs were identified based on a \( p \)-value threshold < 0.05, of which 91 were up-regulated with an FC value > 1.2 and 83 down-regulated with an FC value < 0.833 (Figure 1).

Functional enrichment analysis of DEPs

The results of the GO enrichment study indicated that the down-regulated DEPs were primarily associated with neutrophil degranulation in biological processes (BP) (Figure 2A). On the other hand, the up-regulated DEPs were mainly enriched in the cellular protein metabolic process in BP. For cell components (CC) (Figure 2B), the most enriched function was an extracellular region for both types of DEPs; for molecular function (MF) (Figure 2C), actin binding and antigen binding were the most enriched categories for down-regulated DEPs and down-regulated DEPs respectively. In addition, KEGG pathway analysis identified some signaling pathways related to the progression of AF, including cholesterol metabolism, glycolysis/gluconeogenesis, the HIF-1\( \alpha \) signaling pathway, the PI3K-Akt signaling pathway, etc. (Figure 2D). The interaction between the core DEPs was then constructed, and the PPI network showed that DEPs such as APOA1, FGG, PLG, and FN1 might be the hub proteins mediating the interactions between all the DEPs (Figure 2E). GSEA analysis was used to determine the hallmark functions of the proteins between AF and sinus rhythm (SR) (Figure 2F). GSEA enrichment showed that glycolysis was significantly upregulated in AF samples (\( p < 0.05, \text{NES} > 1 \)). These proteomic enrichment analyses indicate that several metabolic pathways are altered in AF patients. A metabolomic analysis was performed to further understand the metabolic changes in AF. Moreover, we employed the cMAP analysis to find the potential small molecular drugs against the AF. According to the ranking by cMAP connective score, the top 10 small molecular compounds are shown in Figure 2G. Three medications have been launched, and four small molecular compounds are in the clinical phase.

3.3 Validation of Candidate Proteins by ELISA

To further validate the reliability of biomarkers, we utilized the PPI network to perform ELISA verification on several key DEPs (Figure 3A). Among them, LDHA, LDHB, TAGLN2, and PLG were successfully validated and showed consistent results with the proteomics. APOA1 and FN1 exhibited trends consistent with the proteomic results, although the \( p > 0.05 \) (Figure 3B). LDHA, LDHB, and TAGLN2 were higher in the AF group than in the SR group (\( p < 0.05 \)). PLG was lower in the AF group than in the SR group (\( p < 0.05 \)).

3.4 AF-induced differential production of metabolites in serum samples of AF patients

The application of metabolomics analysis revealed the presence of 294 DMs. Among these DMs, 173 exhibited a rise in abundance, while 121 exhibited a decrease in abundance in AF samples when compared to a group of healthy controls. It is worth noting that all these healthy controls were diagnosed with SR, as depicted in Figure 4A. The HCA results of samples and metabolites were presented as heatmaps with dendrograms, while Pearson correlation coefficients (PCC) between samples were calculated by the cor function in R and presented as only heatmaps. Both HCA and PCC were carried out by the R package ComplexHeatmap. For
HCA, normalized signal intensities of metabolites (unit variance scaling) are visualized as a color spectrum (Figure 4B and C).

### 3.5 Functional enrichment analysis of DMs

The results of the top 20 KEGG enrichment analyses are depicted in (Figure 4). The thermogenesis critical route exhibits a differential abundance score (DA Score) below zero, suggesting a downregulation in the expression pattern of the identified metabolites within this pathway. In addition, changes in metabolic pathways were observed, mainly related to cholesterol metabolism, glycerolipid metabolism, vitamin digestion, and absorption (Figure 4D). Conventional enrichment analysis relies on DMs, which may miss some metabolites that are not significantly differentially expressed but hold crucial biological significance. MSEA does not require specifying explicit thresholds for differentially expressed metabolites. The methodology entails the establishment of a collection of metabolite sets, each delineating a distinct biological function. Subsequently, the metabolomic data is subjected to enrichment analysis using these pre-established sets, enabling significantly perturbed metabolite sets to be statistically identified. The metabolite database used for MSEA analysis was derived from MebaboAnalyst. As shown in Figure 4E, we selected the top 50 metabolite sets based on $p$-value for MSEA enrichment analysis. The top two ranked pathways were phenylalanine metabolism and phenylalanine, tyrosine, and tryptophan biosynthesis. This may complement the findings from KEGG enrichment analysis and shed light on the role of amino acid synthesis and metabolism in AF.

### 3.6 Integrated proteomics and metabolomics analysis revealed

The data from the two omics was integrated to offer a more thorough understanding of the molecular regulation of AF. Initially, we used two omics of co-enriched KEGG pathways to perform a bubble plot, sorted by $p$ values as shown in Figure 5A. The top three ranked pathways were cholesterol metabolism, platelet activation, and Glycolysis / Gluconeogenesis. To better understand the correlation between DEPs and DMs, we constructed a “protein-metabolite” integrative network. This network contained co-enriched KEGG pathways, DEPs, and DMs (Figure 5B). These several pathways, including cholesterol and glycolysis metabolism, HIF-1α signaling pathway, and platelet activation, may be crucial pathways through which metabolites influence proteins in AF. In addition, we performed a nine-quadrant plot showing the variety of differences between compounds with Pearson correlation coefficients $>0.8$ and a $p$-value $<0.05$ in each group (Figure 5C). The nine-quadrant plot, from top to bottom, from left to right, is divided into quadrants one to nine. Among them, the third and seventh quadrants represented proteins and metabolites with consistent differential expression patterns and were positively correlated. The consistent differential expression patterns of DEPs and DMs in these quadrants suggested that these proteins might be involved in the positive regulation of metabolites. Conversely, DEPs in the 1st and 9th quadrants exhibited a reverse differential expression pattern compared to DMs, indicating that these quadrants of proteins might be involved in the negative regulation of metabolites.

Furthermore, we used ROC curves showing the predicted (diagnostic) value of the 4 DEPs verified by ELISA, including LDHA, LDHB, TAGLN2, and PLG. The combinations of these four proteins resulted in a high AUC value (0.951, 95% CI (0.890,1.000), and each protein was as follows: LDHB [0.764, 95%CI (0.613, 0.914)], LDHA [0.766, 95%CI (0.613, 0.920)], TAGLN2 [0.899, 95%CI (0.800, 0.999)], PLG [0.793, 95%CI (0.627, 0.960)] (Figure 5D). Then we showed the predicted values of the DMs associated with these four proteins by ROC analysis, including L-Glutamic Acid, a-ketoglutaric Acid(a-KG), D-piperidine acid, Leu-Ala, L-threo-3-Methylaspartate, SPH(d18:1), (±)12-HETE. The summary AUC of these seven metabolite was AUC (0.940, 95% CI (0.872,1.000), and each metabolite was as follows: L-Glutamic Acid [0.870, 95%CI (0.761, 0.978)], a-Ketoglutaric Acid(a-KG) [0.878, 95%CI (0.771, 0.984)], D-piperidine acid [0.889, 95%CI (0.783, 0.994)], Leu-Ala [0.870, 95%CI (0.760, 0.979)], L-threo-3-Methylaspartate [0.870, 95%CI (0.761, 0.978)], SPH(d18:1) [0.777, 95%CI (0.627, 0.927)], (±)12-HETE [0.812, 95%CI (0.663, 0.962)] (Figure 5E).

### Discussion

The covert initiation of AF may result in many patients failing to identify the most opportune moment for addressing the ailment when they present themselves for medical care. Timely detection has the potential to
greatly enhance treatment efficacy and minimize the occurrence of complications. One of the most crucial and early detection of AF is highlighted by the need to identify reliable biomarkers for assessing the illness. This phenomenon stems from metabolic remodeling, which plays a substantial role in the development of aberrant atrial remodeling and AF. Therefore, it is suggested that identifying biomarkers and investigating associated metabolic alterations can yield significant insights for the timely detection and prognosis of AF [18; 19]. According to this theoretical framework, a wide array of research has demonstrated that the comprehensive examination of proteomics and metabolomics can contribute to a more systematic comprehension of the pathophysiology of cardiovascular disorders [20].

In the present study, 174 DEPs and 294 DMs were acquired, thereby augmenting the enrichment of numerous pathways. The complement and coagulation cascade pathways exhibited the highest level of significance. The deregulation of these pathways is purportedly implicated in the onset of many cardiovascular illnesses, including strokes. Furthermore, it is worth noting that an elevated coagulation status is associated with an increased likelihood of experiencing a stroke, which is a prevalent clinical presentation observed in individuals diagnosed with atrial fibrillation [21].

The primary objective in clinical practice is to ascertain individuals with AF at a heightened risk and implement measures to reduce the likelihood of stroke and thromboembolism. The present study PLG as a central protein associated with the complement and coagulation cascade pathway. Notably, using ELISA detection, a substantial decrease in PLG expression was observed in patients with AF. In cardiovascular diseases, PLG has been associated with thrombosis, restenosis, and atherosclerosis [22; 23]. Moreover, tissue PLG activator (tPA) or urokinase PLG activator can convert inactive PLG into active fibrin (uPA). In the PLG−/− model, Gong et al. demonstrated the crucial role of PLG in stem cell-mediated heart healing after myocardial infarction. Their findings suggest that targeting PLG could potentially serve as a novel therapeutic strategy for facilitating stem cell-mediated cardiac repair after myocardial infarction [24]. The irregular contractions of the atria in AF increase the likelihood of thrombosis, a significant contributing factor to ischemic stroke. Moreover, it has been observed that in patients with AF, there is a correlation between hypercoagulability and a heightened susceptibility to stroke [25]. Our findings also led to a similar conclusion, indicating that PLG has the potential to serve as a biomarker for AF and could be a significant target for the development of novel anticoagulant medications for AF.

Furthermore, our findings demonstrated metabolic remodeling in AF patients. The functional enrichment analysis showed significant cholesterol, glucose, and amino acid metabolism dysregulation. Similarly, the study by van et al. indicated a substantial shift from fatty acid to glucose metabolism which was detected in the hypertrophied heart [26]. Changes in energy metabolism may promote electrical and structural remodeling in AF, thereby prolonging the course of this prevalent arrhythmia [27]. In a state of physiological health, the heart tends to rely more heavily on fatty acids as a source of energy. Conversely, when the heart is in a state of physiological unhealthiness, fatty acid oxidation is diminished [28; 29]. When the high atrial rate in AF fails to adequately compensate for glucose metabolism, atrial lipid metabolism has a consequential impairment. This impairment subsequently leads to the deposition of lipids and the buildup of glycogen in the atrial tissue, ultimately resulting in atrial remodeling. Furthermore, it is essential to note that this phenomenon could manifest without significant cardiac performance alterations [30]. In a recent study conducted by Nicoline et al., the researchers examined the occurrence of AF in patients who underwent left atrial resection. The findings of this study suggest that a potential downregulation of energy metabolism may have occurred prior to the onset of AF [31], consistent with our results.

Additionally, our study has provided evidence indicating potential alterations in amino acid metabolism among patients with AF as determined using MSEA. Specifically, our findings suggest perturbations in the metabolic pathways associated with phenylalanine, tryptophan, and tyrosine. The findings of a comprehensive prospective cohort study indicate a positive correlation between heightened phenylalanine levels and an augmented risk of cardiovascular complications [32]. Through targeted metabolomics analysis, Rusnak revealed changes in essential amino acid levels in patients with interventional closure of the left atrial appendage [33]. Therefore, metabolic remodeling plays a significant role in the onset and sustenance of AF.
Our results also showed the compromised functionality of the thermogenesis pathway. Similarly, Pérez-Belmonte et al. showed that the loss of brown-like characteristics in epicardial adipose tissue (EAT) may be detrimental to cardiac metabolism. Consequently, thermogenesis-related genes could be potential therapeutic targets for HF [33]. In recent times, there has been a growing focus on the significance of EAT in the context of AF. For instance, Nalliah et al. found that EAT could cause atrial conduction abnormalities by influencing gap junctions [34]. The existing body of research on this issue indicates a dearth of the thorough influence of EAT on atrial remodeling via its thermogenic action. For example, Gaborit et al. showed that the thermogenic function of EAT might decrease under pathological conditions, and gene expression could increase encoding fibrosis-promoting factors [35]. He et al. also found that the reduction of the thermogenesis gene unique uncoupling protein-1 (UCP-1) might be accompanied by the transformation of white-like adipocytes in EAT, which could be involved in the occurrence and maintenance of AF [36]. The findings align with our research outcomes, and this finding necessitates additional investigation.

The expression levels of other DEPs, including LDHA, LDHB, and TAGLN2, were assessed in clinical serum samples using the ELISA technique to validate their status. LDHA and LDHB are two subunits of LDH, which are crucial catalytic enzymes for anaerobic glycolysis. In cardiovascular diseases, LDH is involved in diagnosing myocardial infarction [37]. Moreover, prior studies have demonstrated the elevated expression of LDHA and the high production of lactate in a canine model of AF [38]. Additionally, a recent study has proposed a unique perspective indicating that aerobic glycolysis is elevated in AF and demonstrates a Warburg effect akin to that observed in tumors [39]. In our study, the ELISA assay revealed that LDHA and LDHB were increased in AF patients, bolstering proteomics findings and previous studies. The data demonstrated that LDHA and LDHB may contribute to the development of AF by affecting glucose metabolism levels. Interestingly, these glucose levels may serve as biomarkers for AF diagnosis. In addition, TAGLN2 is a member of the calponin family of actin-bundling proteins, which has been less studied in cardiovascular diseases. In the present study, we observed an up-regulation of TAGLN2 in AF patients, suggesting a potential biomarker for AF diagnosis.

The treatment of AF mainly includes medication therapy and catheter ablation. Catheter ablation is only effective for some patients, while medication therapy remains integral throughout AF treatment. Thus, the pursuit of new pharmaceutical agents for the treatment of AF holds similar importance to the exploration of biomarkers associated with this condition. In this study, mTOR inhibitor QL-X-138 exhibited the highest ranking among prospective therapeutic medications for AF. mTOR inhibitors have been widely used in research to treat diseases such as renal cell carcinoma [40]. For instance, Andrea L. Marat et al. showed that inhibiting the mTOR signaling pathway can treat diabetes through cellular starvation experiments [41]. Recently, Hu et al. also discovered that inhibiting the mTOR signaling pathway in microglial cells could effectively suppress the elevation of glycolysis induced by LPS and ATP [42]. According to the present research results, proteins related to glucose metabolism and metabolic pathways exhibited increased expression. Thus, it is reasonable to hypothesize that the mTOR inhibitor QL-X-138 may also potentially treat AF by modulating glucose metabolism remodeling. This could hold the potential for future AF treatment.

The present investigation employed a combination of proteomics and metabolomics to investigate biomarkers and metabolic pathways. The primary objective is to enhance our comprehension of the molecular processes and regulatory mechanisms behind AF. This work offers valuable insights that can serve as a solid foundation for this condition’s clinical diagnosis and treatment. According to our findings, AF pathophysiology can be notably impacted by the dysregulation of pathways related to thrombosis, platelet activation, complement, and metabolic remodeling of glycolipids. Biomarkers, including LDHA, LDHB, TAGLN2, and PLG, have the potential to be utilized as novel targets for prospective pharmacological interventions in the treatment of AF. Furthermore, the chemical QL-X-138 exhibited favorable characteristics that suggest its potential as a therapeutic agent for the treatment of AF.

Ethical Statement

Ethics Committee approval was obtained from the Institutional Ethics Committee of Zhongshan Hospital of Traditional Chinese Medicine to the commencement of the study. Informed consent was obtained from the
subjects when the samples were collected.

Authors Contributions

The manuscript was conceptualized by Xiaoying Chao and Chi Shu; Yunjing Xu, Francis Chanda, Na Xing and Abdallah Iddy Chaurembo carried out the manuscript reviewing and editing process; Jianyuan Huang, Huijuan Zhang and Lidan Fu were responsible for the manuscript’s graphical illustrations; Kaixuan Lin, Guoqiang Zhong, and Hanbin Lin were responsible for the supervision and validation of the whole process.

Conflict of Interest

The authors declare no potential conflict of interest.

Acknowledgements

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