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ORIGINAL RESEARCH

Metabolomics and Biomarkers for Paroxysmal and Persistent Atrial Fibrillation

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BACKGROUND: Atrial fibrillation (AF) is the most common type of arrhythmia worldwide and is associated with serious complications. This study investigated the metabolic biomarkers associated with AF and the differences in metabolomics and associated metabolic biomarkers between paroxysmal AF (AFPA) and persistent AF.

METHODS AND RESULTS: Plasma samples were prospectively collected from patients with AF and patients in sinus rhythm with negative coronary angiography. The patients were divided into 3 groups: AFPA, persistent AF, and sinus rhythm (N=54). Metabolomics (n=36) using ultra-high-performance liquid chromatography mass spectrometry was used to detect differential metabolites that were validated in a new cohort (n=18). The validated metabolites from the validation phase were further analyzed by receiver operating characteristic. Among the 36 differential metabolites detected by omics assay, 4 were successfully validated with area under the curve >0.8 (P<0.05). Bioinformatics analysis confirmed the enrichment pathways of unsaturated fatty acid biosynthesis, glyoxylate and dicarboxylate metabolism, and carbon metabolism. Arachidonic acid was a potential biomarker of AFPA, glycolic acid and L-serine were biomarkers of AFPA and persistent AF, and palmitelaidic acid was a biomarker of AFPA.

CONCLUSIONS: In this metabolomics study, we detected 36 differential metabolites in AF, and 4 were validated with high sensitivity and specificity. These differential metabolites are potential biomarkers for diagnosis and monitoring of disease course. This study therefore provides new insights into the precision diagnosis and management of AF.

Key Words: atrial fibrillation ■ metabolites ■ metabolomics

Atrial fibrillation (AF) is the most common type of arrhythmia worldwide.[1] With the progress of medical technology and the improvement of patients' awareness, the prevalence of AF is gradually increasing.[2] There are about 33.5 million patients with AF worldwide, and the incidence of AF in China is 1% to 2%.[3]

It has been demonstrated that AF can cause complications such as increased risk of thromboembolism, death, heart failure, hospitalization, and decreased anticoagulant effectiveness.[1,4,5]

Some of the risk factors for AF have been suggested, such as alcohol consumption, increased blood pressure, heart failure, and myocardial infarction.[4] According to the duration, it can be divided into paroxysmal AF (AFPA) and persistent AF (AFPE). Symptom assessment and intermittent rhythm monitoring are used in the diagnosis of AF. However, the burden is heavy for patients, and these methods are not able to fully demonstrate the pathophysiological mechanisms of AF. Further, the molecular pathogenesis of AF is not fully understood.[6] It is worth noting that more and more research efforts are being made to develop pathophysiological mechanisms of AF. There is a need to use blood-based biomarkers to predict and diagnose AF and to provide better methods for the treatment

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CLINICAL PERSPECTIVE

What Is New?

- Atrial fibrillation (AF) is the most common type of arrhythmia worldwide; it can lead to serious complications.
- This study investigated the plasma metabolic biomarkers associated with paroxysmal atrial fibrillation (AFPA) and persistent AF and the metabolomic differences between AFPA and persistent AF and their associated metabolic biomarkers.
- There were 4 validated differential metabolites with significant sensitivity and specificity: Among those, arachidonic acid is a potential metabolite of AFPA, glycolic acid and L-serine are biomarkers of AFPA and persistent AF, and palmitelaidic acid is a biomarker of AFPA compared with sinus rhythm.

What Are the Clinical Implications?

- This study provides information for specific metabolite biomarkers to be used in the diagnosis and monitoring of AF.
- The results may further improve the early diagnosis and treatment of AF to reduce the burden of AF in human health.

Nonstandard Abbreviations and Acronyms

AFPA	paroxysmal atrial fibrillation
AFPE	persistent atrial fibrillation
DMs	differential metabolites
KEGG	Kyoto Encyclopedia of Genes and Genomes
VIP	variable importance in projection

of AF.[7] Due to the lack of specific biological metabolites to diagnose and predict the progression of AF, the progress of targeted therapy for AF is delayed, and the burden of health care expenditures for AF is greatly increased. Therefore, it is important to discover new biomarkers for AF.

Previous studies have suggested metabolites associated with AF that may be potential biomarkers such as plasmatic apelin.[4,8] Other studies have found that miRNA is involved in the structure and electrical remodeling of AF and plays an important role in the signaling of the onset of AF.[7,9,10] It has been reported that inflammation and oxidative stress are related to the pathophysiology of AF.[11] In addition, metabolomics has made new discoveries in predicting outcomes of AF.[12] Over the past decade, there has been tremendous progress in multiomics studies of AF, and hundreds of potential biomarkers and targets have been identified that will lay the foundation for future research.[6] Our previous studies have suggested that the mechanism of postoperative AF is related to the peroxisome proliferator–activated receptor signaling pathway[13,14] and glutathione metabolism.[13] Additionally, complement C4A, carbonic anhydrase 1,[15] and chloride channels are involved in AF associated with heart valve disease.[16] However, a comprehensive assessment of AF by combining several biomarkers has not been fully established.[17] Further, the differential metabolites (DMs) of different types of AF are not well established.

The aim of this study was to investigate the DMs between AF and sinus rhythm and between AFPA and AFPE to discover potential biomarkers for diagnosis and pathological progress of AF.

METHODS

Data used in the current study are available upon reasonable request.

Study Design and Population

The study protocol was reviewed and approved by the institutional review board of TEDA International Cardiovascular Hospital, Tianjin University, China, and informed consent was obtained. From December 2021 to December 2022, plasma samples were prospectively collected and frozen from patients with AF. The control sample (sinus rhythm) was taken from coronary angiography with negative results. During this period, 54 subjects, including patients with AF and sinus rhythm controls, were recruited for the study. Among those, 36 patients with AF (18 AFPA and 18 AFPE) were included in the study by matching age and sex. In addition, in the patients who underwent coronary angiography with negative results and in sinus rhythm during the same duration, 18 were included by matching age and sex of the patients with AF as controls. The patients with cardiac or pulmonary diseases and diseases in other systems were excluded from controls.

Different types of AF are classified according to the duration of the episode, spontaneous termination, and presentation of the episode. According to the 2020 European Society of Cardiology Guidelines,[18] AF can be divided into first diagnosed, paroxysmal, persistent, long-term persistent, and permanent. The patients selected in our study had paroxysmal AF (duration ≤7 days) and persistent AF (duration >7 days). Therefore, patients with "long-term persistent" and "permanent" AF were included in the "persistent" AF group. Clinical diagnosis depended on the patient's symptoms and ECG record. The demographic and clinical data were collected from the patient's medical records.

For the validation phase, a new cohort of patients and controls were selected with the same inclusion and exclusion criteria as above.

Samples Collection

Blood samples were collected from all subjects after overnight fasting. All blood samples were taken during angiography. The blood sample was immediately centrifuged at 1500 *g* for 10 minutes. The plasma and blood cells were then separated and placed into a standard serum collection tube and stored at −80° until the test was carried out.

Metabolomics Phase

The metabolites were quantitatively compared by liquid chromatography and mass spectrometry. Metabolite

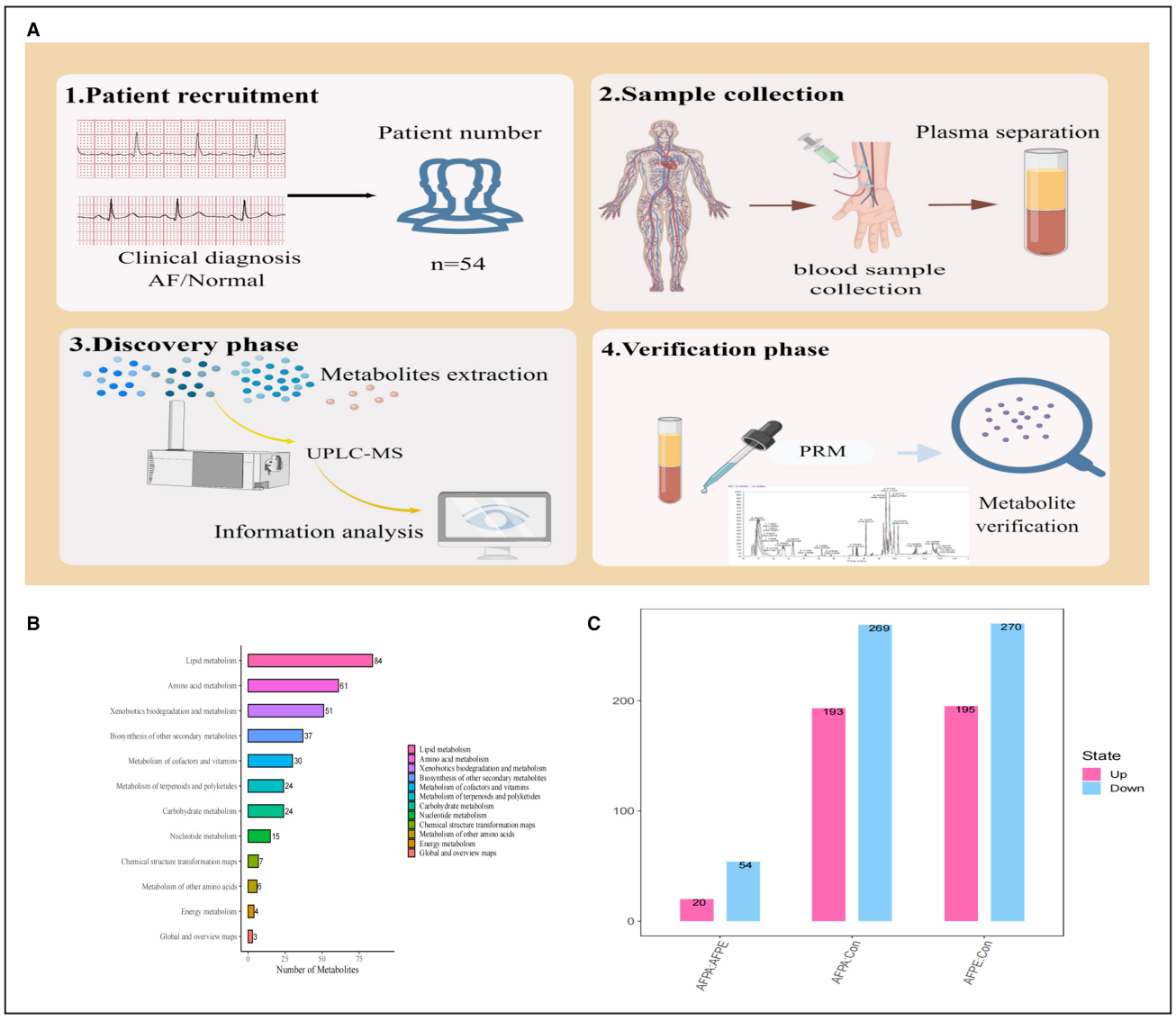

Figure 1. The flowchart shows the research procedure and omics analysis of plasma metabolites.
A, The flowchart shows the research procedure. **B**, Classification of the KEGG metabolic pathways according to which metabolites participate. The *x* axis represents the number of metabolite annotations, and the *y* axis represents the annotated KEGG Pathway. **C**, Quantitative plot of DMs in 3 comparison groups. **D**, Volcanic map of different metabolites between AFPA and control group. The abscissa is fold-change converted by $\log_2$, and the ordinate is *P* value converted by $-\log_{10}$. Blue is the downregulated significant difference metabolite, red is the upregulated significant difference metabolite, the circle shape is the metabolite with VIP ≥1, the triangle is the metabolite with VIP <1, and the nonsignificant metabolite is gray. **E**, Volcanic map of DMs between AFPE and control group. **F**, Comparison of AFPA and AFPE set of volcanic maps. AFPA indicates paroxysmal atrial fibrillation; AFPE, persistent atrial fibrillation; DMs, differential metabolities; KEGG, Kyoto Encyclopedia of Genes and Genomes; PRM, parallel reaction monitoring; UPLC-MS, ultra-high-performance liquid chromatography and mass spectrometry; and VIP, variable importance in projection.

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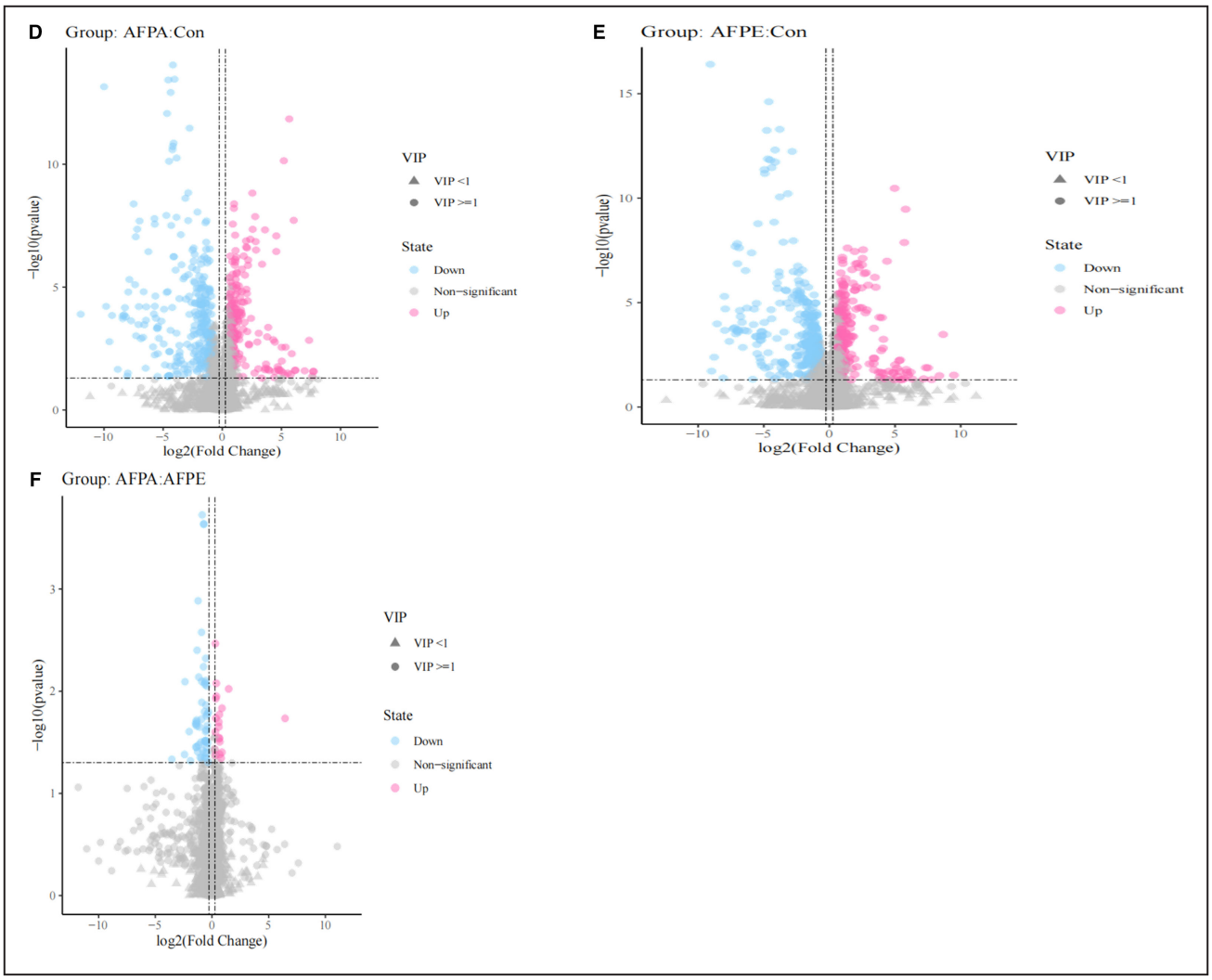


Figure 1. Continued

extraction, liquid chromatography and mass spectrometry, data preprocessing, metabolite screening, and analysis were performed. Metabolomics was conducted, and DMs were screened and analyzed. The metabolites were isolated and detected by ultra-high-performance liquid chromatography and mass spectrometry.

Bioinformatics Analysis

The peak area of metabolites was obtained by extracting and identifying the metabolite peak from the original liquid chromatography and tandem mass spectrometry data. Metax was used for pretreatment to obtain compounds and quantitative values to be formally analyzed, and then the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Metabolome Database were used to annotate the results of metabolites.

The Human Metabolomics Database contains chemical, biochemical, and clinical information about metabolites. KEGG and the KEGG PATHWAY database are at the heart of the KEGG database. The KEGG PATHWAY database was used to determine the main biochemical metabolic pathways and signal transduction pathways involved in metabolites in vivo.

Validation Phase

Metabolomics analysis identified 36 DMs that were further validated in new samples. The experiments were conducted through metabolite extraction, liquid chromatography and tandem mass spectrometry detection, global metabolite analysis, and differential metabolite validation.

Receiver Operating Characteristic Analysis

The validated metabolites from the validation phase were further analyzed by the receiver operating characteristic (ROC). The ROC analysis was performed for the 6 selected DMs: arachidonic acid, glycolic acid, L-serine,

Table. Analysis of Clinical Parameters in 3 Comparison Groups

Variable	Discovery cohort (n=36)				Validation cohort (n=18)			
	AFPE	AFPA	Con	*P* value	AFPE	AFPA	Con	*P* value
Male sex	6 (50.0)	6 (50.0)	6 (50.0)	1.0	4 (66.7)	4 (66.7)	4 (66.7)	1.0
BMI	27.2±7.1	26.2±2.8	24.1±2.1	0.08	26.6±5.1	28.4±2.3	26.4±2.0	0.6
Age, y	63.50±10.60	60.08±4.66	62.10±7.38	0.460	66.83±8.82	64.17±7.47	58.17±9.33	0.2
Hypertension	8 (66.7)	2 (16.7)	6 (50.0)	*	1 (16.7)	1 (16.7)	3 (50.0)	†
Total cholesterol, mmol/L	4.5±1.0	4.8±1.6	4.7±0.9	0.8	3.7±1.1	4.0±0.9	4.8±1.0	0.1
LDL-C, mmol/L	2.9±1.1	3.1±1.4	3.0±0.9	0.9	2.0±1.0	2.3±0.9	2.7±0.9	0.4
Fasting blood glucose, mmol/L	5.9±1.0	5.6±0.7	5.3±0.6	0.3	6.7±3.0	5.8±0.8	5.5±0.3	0.9
EF%	60.2±7.2	64.7±4.6	65.5±4.4	0.1	58.3±11.9	61.3±3.8	63.2±2.9	0.6
LVDd, mm	47.0±2.7	47.0±4.8	44.0±1.8	0.06	50.8±10.9	45.5±0.7	46.3±3.7	0.5
LADs, mm	40.8±5.4	38.2±3.1	35.1±3.9	0.03	42.7±6.3	34.5±3.5	34.5±2.6	0.03

These values are expressed as the mean±SD or the median (interquartile range). N=54. AFPA indicates paroxysmal atrial fibrillation; AFPE, persistent atrial fibrillation; BMI body mass index; EF%, ejection fraction; LADs, systolic diameter of left atrium; LDL-C, low-density lipoprotein cholesterol; and LVDd, left ventricular end-diastolic diameter.

*P=0.043 between AFPE and AFPA.

†P>0.05 among 3 groups.

palmitelaidic acid, palmitoleic acid, and γ-aminobutyric acid. There were 3 groups: AFPE, AFPA, and control. Each group had 6 patients with 6 samples (each sample had 1 concentration). A ROC curve was performed for each DM with 3 groups. The sensitivity and specificity were calculated on the basis of the above analysis.

Identification of DMs

In this study, in both the discovery cohort and the validation cohort, DMs were screened by fold change (≥1.2 or ≤0.83), *P* value (<0.05) in univariate analysis, and variable importance in projection (VIP) (≥1) value of metabolites analyzed by Orthogonal Projections to Latent Structures Discriminant Analysis in multivariate analysis.

The whole experimental process is shown in Figure 1A.

Statistical Analysis

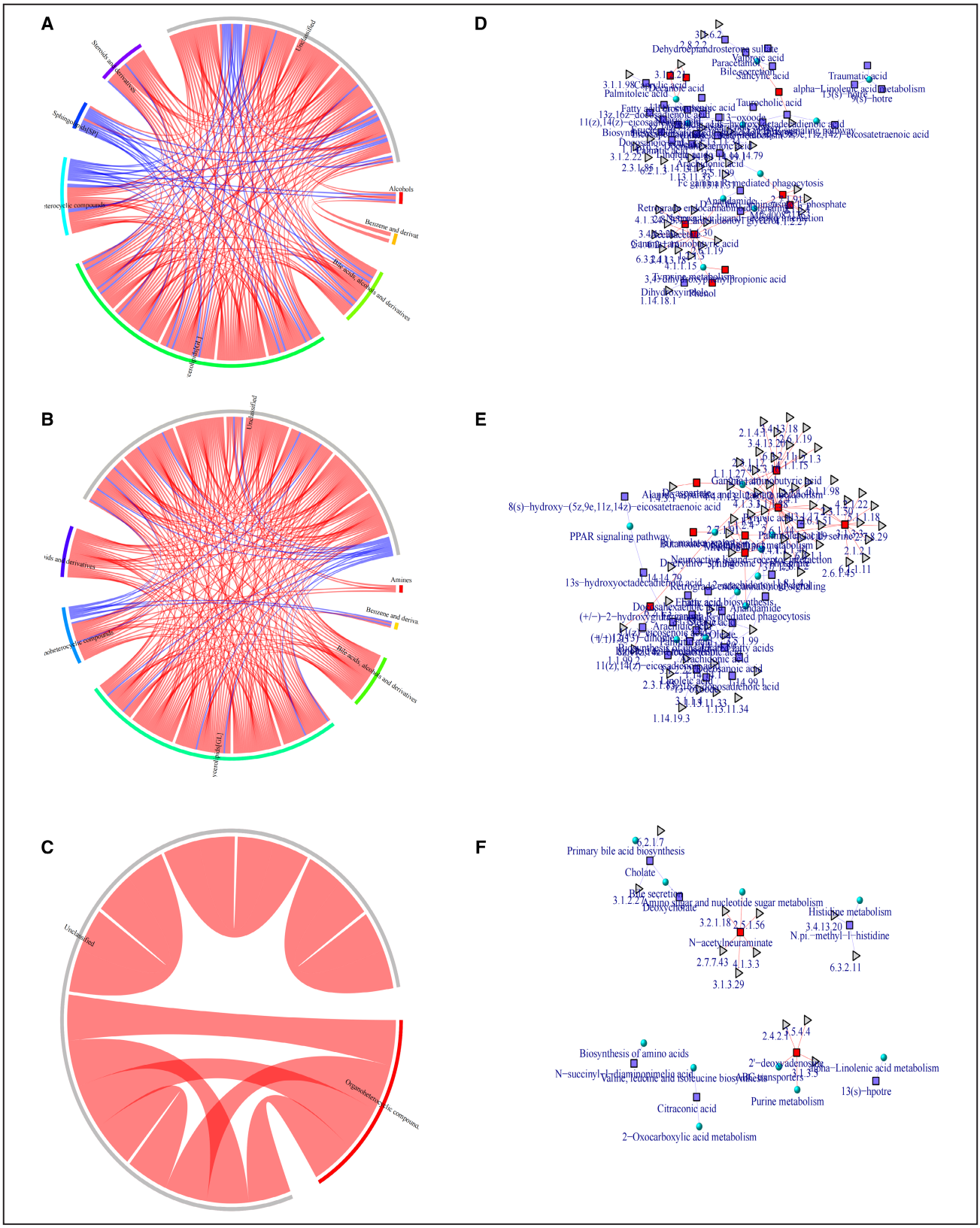
All normally distributed data were expressed as mean±SD, and nonnormally distributed data were expressed as median (interquartile range). One-way ANOVA was used for continuous numbers, and the χ^2 test or Fisher exact test was used for categorical variables. When comparison was made among 3 groups, 1-way ANOVA and the post hoc test were used. Bonferroni test (when equal variances were assumed) or Dunnett's T3 test (when equal variances were not assumed) was used as the post hoc test. Statistical significance was defined as P<0.05.

Binary regression analysis assessed the association between the clinical parameters and AF, and the results were represented as odds ratios with 95% CIs. A P<0.1 was the selection criterion to enter the model. The model fitting for the final models was checked. A final risk factor from the regression model had P<0.05.

ROC analysis was undertaken to evaluate the predictive power of DMs for AF. For the validation cohort, we calculated the sample size on the basis of the difference of the 6 quantitative values of metabolite concentration among the AFPE, AFPA, and control groups to detect the difference of the candidate metabolites among the 3 groups.

VIP values of metabolites in Orthogonal Partial Least Squares Discriminant Analysis and fold change with *P* value <0.05 in univariate analysis were used to screen

Figure 2. Different metabolite correlation volcano map and network map of single comparison group.
A, Chords of differential metabolite correlation between paroxysmal atrial fibrillation and control group. The starting point of the inner circle link in the figure represents each significantly different metabolite, and the arc on the outer circle represents the classification of the significantly different metabolite. Colored lines represent correlations within various metabolites, red lines represent positive correlations, and blue lines represent negative correlations. When the number of DMs is ≥20, the chord diagram is drawn with the first 20 DMs (<20, use all data) with the smallest *P* by default. **B**, Chords of differential metabolite correlation between persistent atrial fibrillation and control group. **C**, Chords of differential metabolite correlation between paroxysmal atrial fibrillation and persistent atrial fibrillation. **D**, Comparison between paroxysmal atrial fibrillation and control group differential metabolite correlation network diagram. Correlation network diagram: The color of the line represents the correlation, the red line represents the positive correlation, and the blue line represents the negative correlation. **E**, Network diagram of differential metabolite correlation between persistent atrial fibrillation and control group. **F**, Correlation network diagram of DMs between paroxysmal atrial fibrillation and persistent atrial fibrillation. DMs indicates differential metabolities.



DMs. Metabolic pathways with *P* value <0.05 were defined as significantly enriched metabolic pathways based on KEGG database. Differential abundance scores were used to analyze pathway metabolic changes, and ROC curve analysis was used to screen potential biomarkers.

The area under the curve value in receiver ROC analysis was used as an indicator of whether

metabolites of AFPA and AFPE could be used as biomarkers.

Statistical analysis was performed using SPSS 21.0 software. Compound Discoverer 3.3 software (Thermo Fisher, Waltham, MA) combined with the BGI Metabolome database, McCloud database, and Chem Spider database were used for mass spectrum data analysis, and Metax was used for data preprocessing and subsequent analysis. The flowchart and the schema to describe possible mechanism were drawn by Figdraw.

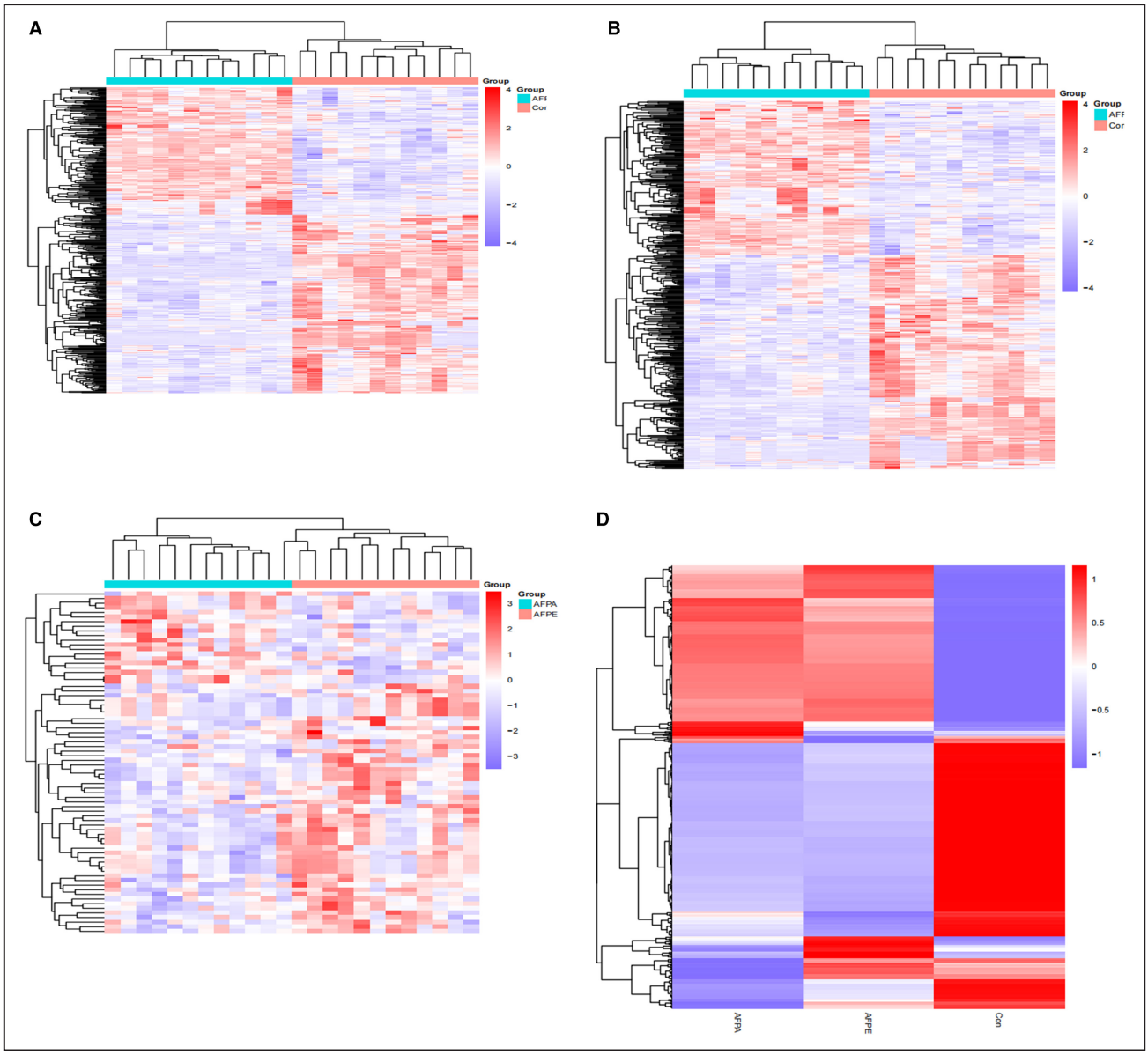


Figure 3. DM cluster diagram of individual comparison and all comparisons of groups and enrichment maps of metabolite pathways in 3 different comparison groups.

A, DM cluster diagram of comparison between paroxysmal atrial fibrillation and control group. In the figure, each row represents a DM; each column represents a sample. The color represents the expression level, and blue to red correspond to the expression level from low to high. **B**, Cluster map of DMs between persistent atrial fibrillation and control group. **C**, Cluster map of DMs between paroxysmal atrial fibrillation and persistent atrial fibrillation. **D**, All the DM cluster maps were compared between groups. One metabolite per row and one comparison group per column. Metabolites are derived from the union of DMs across all groups, and the color indicates the fold change of that metabolite in the comparison group. The expression was $\log_2$ transformed. **E**, Metabolic pathway enrichment map of DMs in paroxysmal atrial fibrillation and control group. The metabolic pathways with $P<0.05$ were defined as the metabolic pathways with significant enrichment of DMs, and the top 10 metabolic pathways (<10, use all data) with the smallest P value were drawn as column charts. **F**, Metabolic pathway enrichment map of DMs in persistent atrial fibrillation and control group. **G**, Metabolic pathway enrichment map of DMs between paroxysmal and persistent atrial fibrillation group. DM indicates differential metabolite; and TCA, tricarboxylic acid.

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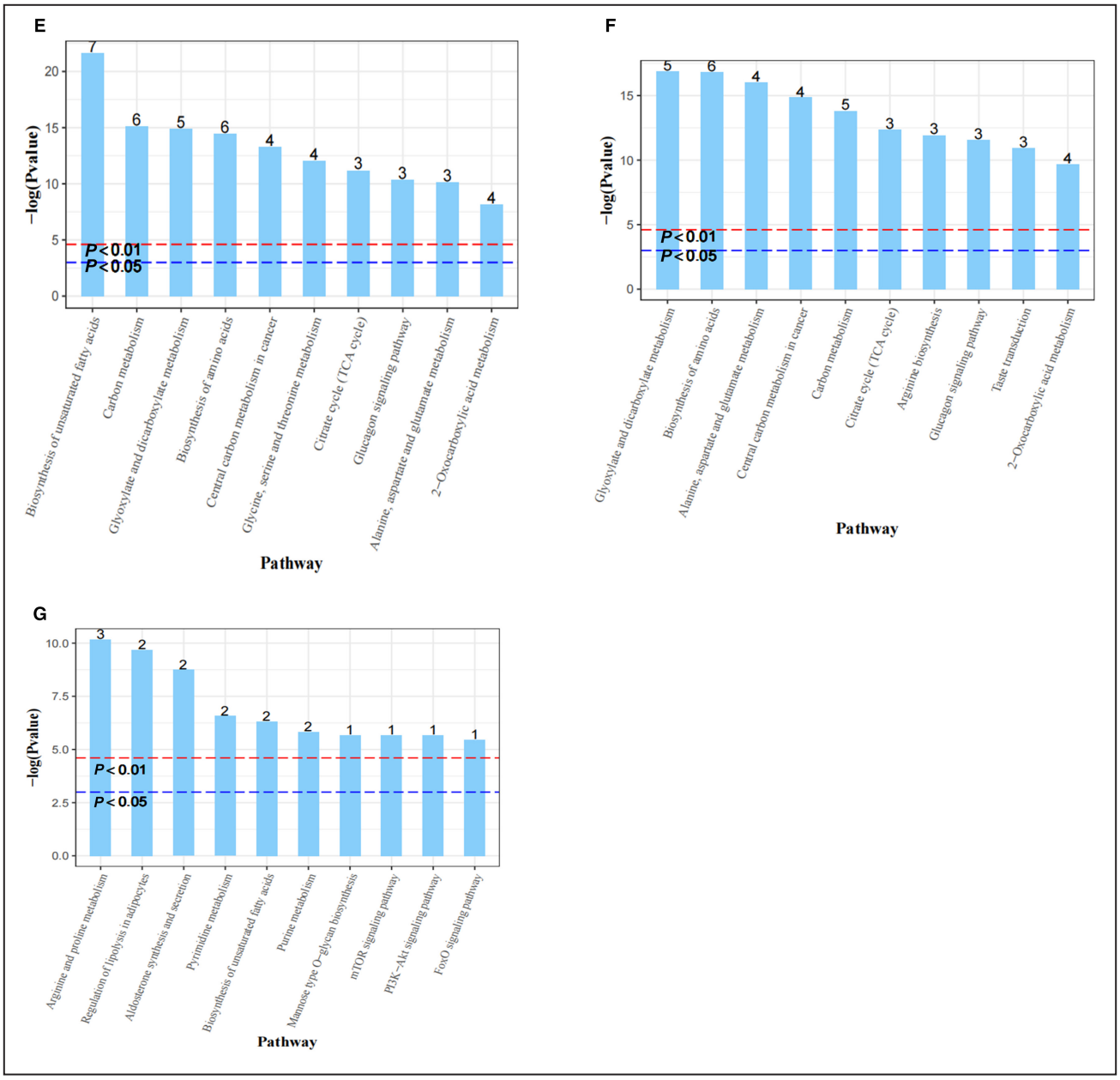

Figure 3. **Continued**

RESULTS

Demographic and Clinical Characteristics

The geographic characteristic and clinical features of the discovery and validation cohort in metabolomics are shown in Table.

Metabolomics Analysis

A total of 2170 metabolites were identified in the 3 study groups: AFPA, AFPE, and control (sinus rhythm). The number of metabolites was counted according to the major categories of KEGG metabolic pathways in which metabolites participated, and it was found that metabolites involved in lipid metabolism were the most abundant, followed by amino acid metabolism (Figure 1B). There were 462 metabolites significantly upregulated or downregulated in the AFPA group compared with the sinus rhythm group. Compared with the sinus rhythm group, we identified 465 metabolites that were significantly upregulated or downregulated in the AFPE group. A total of 74 metabolites were upregulated or downregulated in the AFPA group compared with the AFPE group (Figure 1C). The volcano map shows the difference of metabolites up and down in different groups (Figure 1D through 1F). In addition, R2Y and Q2 values in Partial Least Squares Discriminant Analysis were used to evaluate the model,

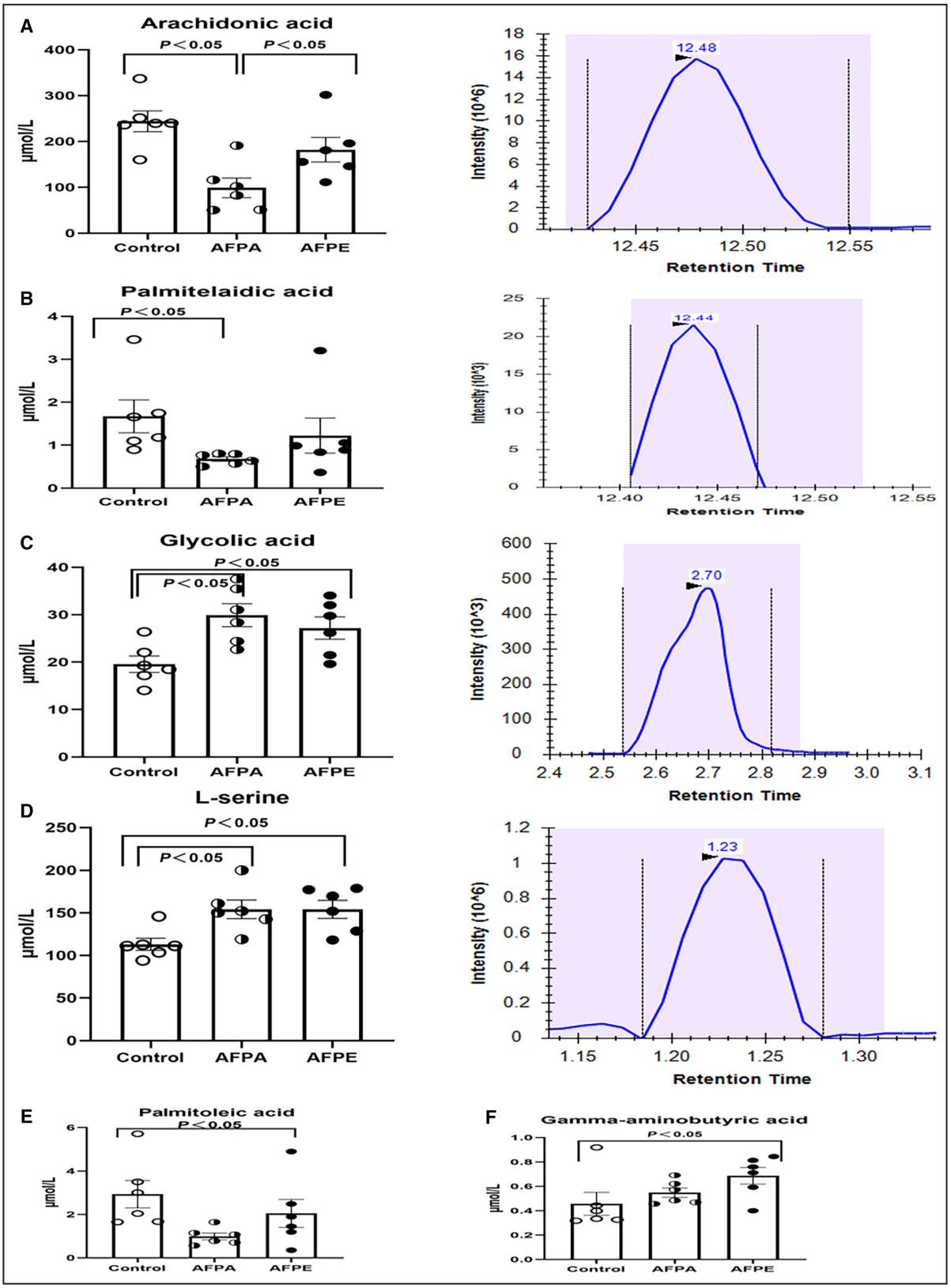

Figure 4. Dot plot analysis of 6 different metabolites in different comparison groups (A through F, n=6 in each group) and the receiver operating characteristic analysis of 6 differential metabolites in different comparisons (G through L).

AFPA indicates paroxysmal atrial fibrillation; AFPE, persistent atrial fibrillation; and CON, control.

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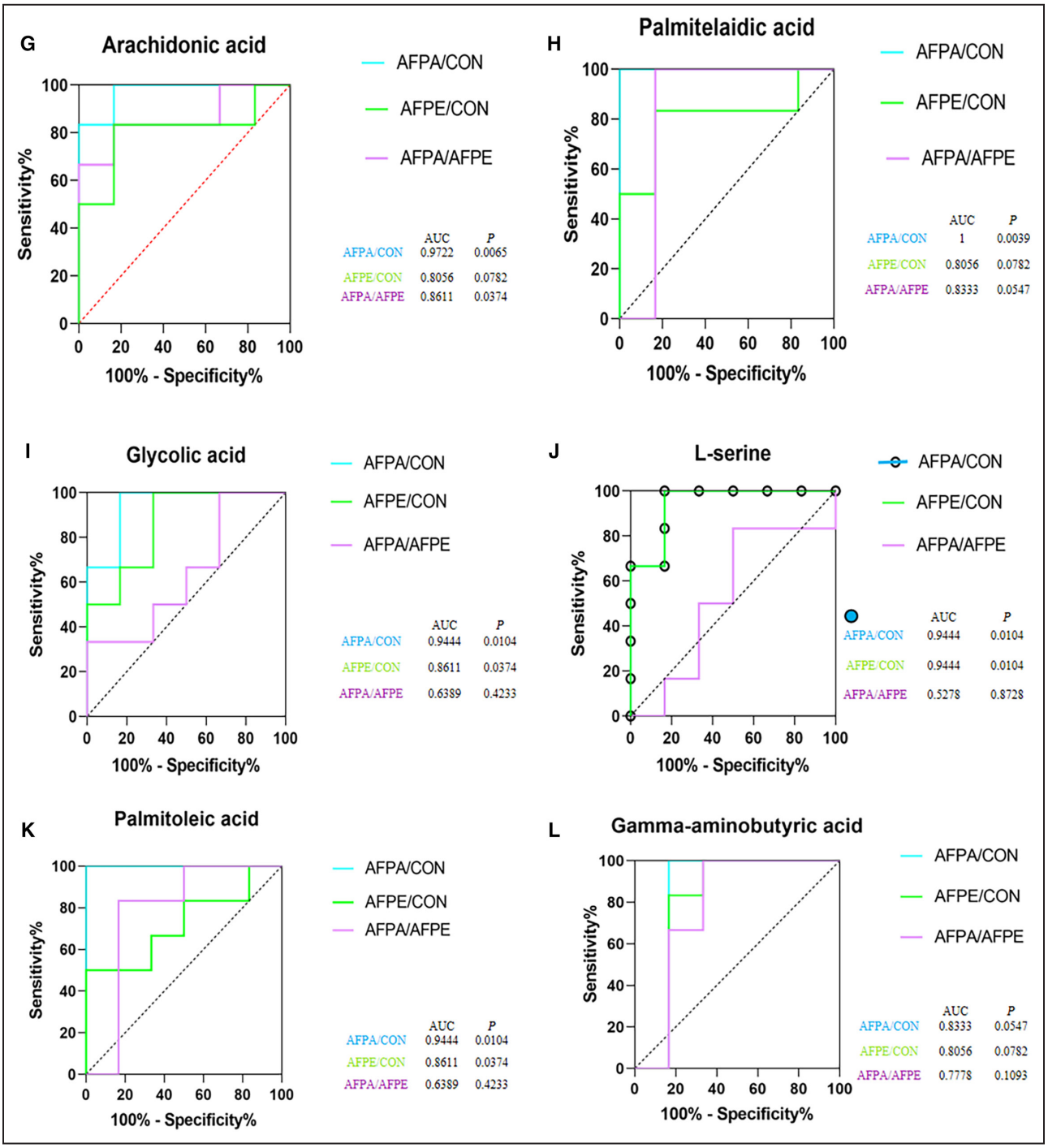

Figure 4. **Continued**

which well distinguished the AFPA, AFPE, and control groups. These metabolites are involved in a variety of metabolic pathways. For the detected metabolites, to more directly observe the strength of correlation between various metabolites in different comparison groups, a correlation chord diagram was drawn for the metabolite–metabolite relationship of Spearman correlation $P<0.05$ (Figure 2A through 2C). Similarly, we drew the correlation network diagram to clearly see which metabolite nodes were in the core position in the 3 comparisons among the groups (Figure 2D through 2F). Figures S1 through S3 give an enlarged and clearer demonstration.

Clustering of DMs

The DMs screened from each comparison among the group and a correlation in the DMs between the

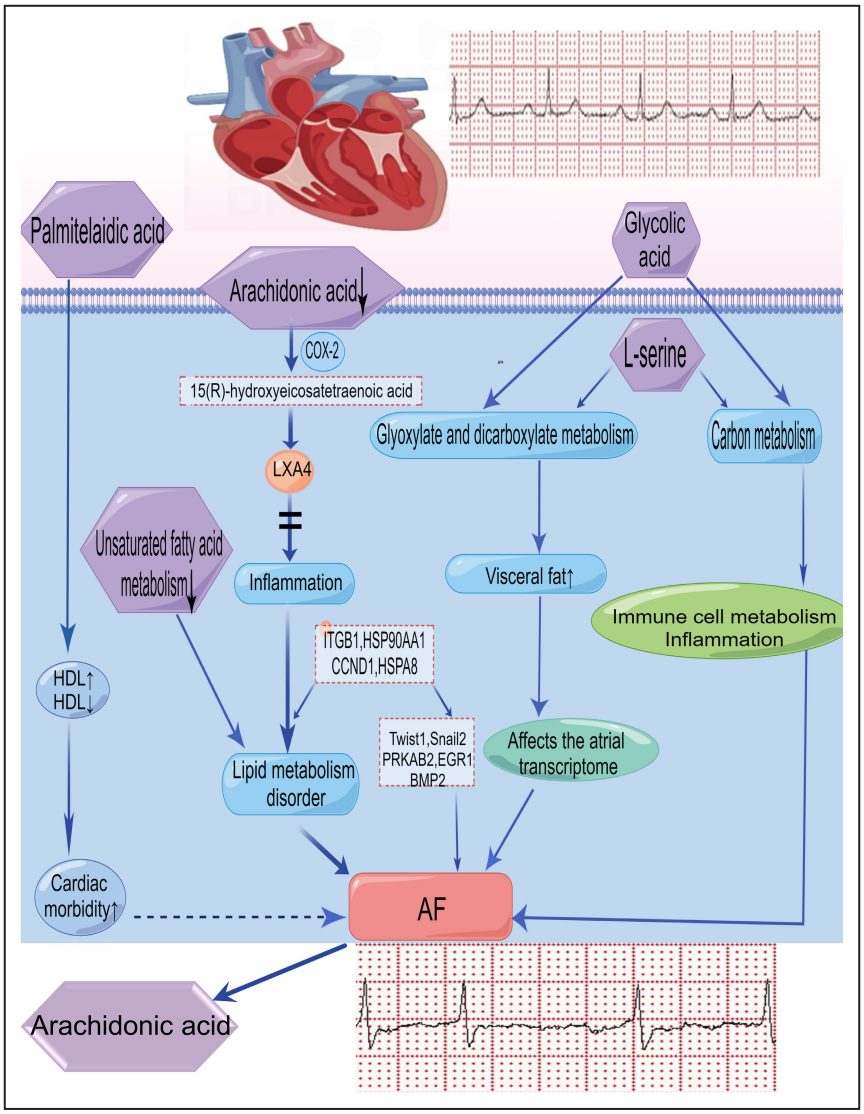

Figure 5. Schema for the possible mechanisms of involvement of 4 differential metabolites DMs in atrial fibrillation.
See "Discussion" for the detailed explanation of this schema. AF indicates atrial fibrillation; COX-2, cyclooxygenase 2; DM, differential metabolite; and HDL, high-density lipoprotein.

comparison of AFPA/control and AFPE/control were analyzed (Figure 3A through 3C). The DMs of the 3 comparisons are grouped as follows (Figure 3D). The up- or downregulation situation of all comparisons can be visually seen, and the DMs with the same up- or downregulation pattern can be clustered.

Pathway Enrichment Using the KEGG Database of the DMs

According to the KEGG database, the enrichment of metabolic pathways of DMs could be analyzed. Significant changes of metabolic pathways could be observed in the 3 comparisons. In addition, the first 10 metabolic pathways between AFPA and control group, AFPE and control group, and AFPA and AFPE were plotted, respectively (Figure 3E through 3G).

Identification of Candidate DMs

Metabolomics eventually screened out the following 36 DMs (Table S1). Through parallel reaction monitoring validation analysis, we finally validated 6 metabolites. Significant differences in metabolites between different comparisons and the related mass spectrometry result are shown in Figure 4A through 4F. To verify that candidate DMs can distinguish different comparisons and determine whether they can be used as candidate biomarkers, we demonstrated this correlation by ROC analysis, as shown in Figure 4G through 4L. Notably, our parallel reaction monitoring validation showed

significant differences in arachidonic acid between the AFPA and control groups and between the AFPA and AFPE groups. There were significant differences in palmitelaidic acid between the AFPA and control groups and significant differences in glycolic acid and L-serine between each of the 2 different AF groups and the control group.

DISCUSSION

In this study, we compared plasma metabolites between patients with AFPA and patients with AFPE in addition to the comparison between the patients with AF and subjects with sinus rhythm. We found that (1) there are a large number of DMs in the patients with AF compared with sinus rhythm and between patients with AFPA and patients with AFPE; (2) 6 DMs were found between patients with AF and those with sinus rhythm: palmitoleic acid, arachidonic acid, γ-aminobutyric acid, palmitelaidic acid, glycolic acid, and L-serine; (3) arachidonic acid was found as a DM between AFPA and AFPE; (4) ROC analysis found that among a total of 6 DMs based on sensitivity and specificity, 4 were sensitive and specific enough to be developed as biomarkers. Among those, palmitelaidic acid, glycolic acid, and L-serine may be used as biomarkers for AF and arachidonic acid may be used as a biomarker for AFPA; (5) the above 4 DMs are related to the signaling pathways of biosynthesis of unsaturated fatty acids, glyoxylate and dicarboxylate metabolism, and carbon metabolism.

Role of Arachidonic Acid in AF

In this study, the expression of arachidonic acid and palmitelaidic acid was downregulated. These 2 metabolites are fatty acids and arachidonic acid is an unsaturated fatty acid. Unsaturated fatty acids can esterify cholesterol, reduce blood cholesterol and triglyceride as well as blood viscosity, and improve blood microcirculation. In this study, through KEGG metabolic pathway analysis, it was found that the unsaturated fatty acid biosynthesis pathway and regulation of lipolysis were also downregulated.

Previous studies have suggested that n-6 polyunsaturated fatty acids have cardiovascular benefits.[19] In addition, inhibition of epoxide hydrolase in arachidonic acid metabolism with small-molecule inhibitors reduces electrical and structural remodeling in the atria.[19] It is well known that the pathogenesis of AF is related to the anatomy of the heart.[19] Arachidonic acid can produce lipoxin a4 through cyclooxygenase-2 and lipoxygenase, which has antioxidant stress effects.[20] Further, in the process of inflammation, the lipid metabolism of the heart is disturbed, and 4 genes, *ITGB1*, *HSP90AA1*, *CCND1*, and *HSPA8*, are involved in the occurrence of AF by regulating the metabolic reprogramming of *Twist1*, *Snail2*, *PRKAB2*, *EGR1*, and *BMP2*[3] (Figure 5). The results from the present study regarding arachidonic acid are in accordance with these findings.

This study also demonstrated that by the analysis of KEGG, arachidonic acid was involved in the regulation of lipolysis in adipocytes in the pathway of adipose cells. It was reported that fat accumulation increases the risk of obesity that has an effect on the atrial transcriptome and that there is close link between obesity and AF.[21] In addition, AF increases inflammation and leads to the release of arachidonic acid.[22]

Role of Glycolic Acid and L-Serine in AF

In this study, the expression of glycolic acid and L-serine was upregulated.

Previous studies have linked inflammation and oxidative stress to the pathophysiology of AF.[23] In the KEGG analyses, both glycolic acid and L-serine are linked with the carbon metabolism and glyoxylate and dicarboxylate metabolic pathways that are associated with AF (Figure 5). Glycolic acid is highly associated with xanthine, which is related to oxidative stress.[24] As mentioned above, oxidative stress is correlated to the pathophysiology of AF.[23]

A recent study has shown that L-serine can reduce oxidative stress.[25] In this study, L-serine expression is upregulated and the carbon metabolic pathway involved is also upregulated. Carbon metabolism is involved in immune cell metabolism, inflammation, endothelial cell function changes, and other activities, and therefore the alteration of this pathway as seen in this study may be related to the occurrence of AF.[26] Figure 5 shows the possible pathway that links the alteration of L-serine and glyoxylate and dicarboxylate metabolism and carbon metabolism to the development of AF. As reported, the metabolism of glyoxylate and dicarboxylate has strong correlation with visceral fat[27] and obesity,[28] which is associated with AF, as mentioned before.[21]

Clinical Data and AF

In this study, we also looked at the clinical data with both univariate and multivariate analyses. The results show that the systolic diameter of the left atrium is a risk factor for AF (Table S2). This is in accordance with clinical studies of AF.[29] However, although the above 4 metabolites are significantly associated with AF, the clinical application of these metabolites regarding the correlation to other clinical characteristics remains to be further investigated.

Limitations

The present study is a metabolomics study with validation in a new cohort of patients. As usual, the omics study has a limited number of patients involved. The results from this study will be further validated in a large

cohort of patients with AF. Nevertheless, the results from this study reveal useful metabolites that could be further studied as biomarkers for AF.

CONCLUSIONS

In summary, this study used metabolomics techniques to identify metabolites associated with AF and further compared metabolites between AFPA and AFPE. Our study suggests that arachidonic acid may be further developed as a biomarker specific to AFPA and that glycolic acid and L-serine may be common potential biomarkers for both AFPA and AFPE. Further, palmitelaidic acid may be a potential biomarker for patients with AFPA. These findings provide new insights into the mechanism of AF and diagnostic biomarkers.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S2
Figures S1–S3

REFERENCES

1. Kalarus Z, Sredniawa B, Mitrega K, Wierucki L, Sokal A, Lip G, Bandosz P, Stokwiszewski J, Boidol J, Zieleniewicz P, et al. Prevalence of atrial fibrillation in the 65 or over Polish population. Report of cross-sectional NOMED-AF study. *Kardiol Pol*. 2023;81:14–21. doi: 10.33963/KP.a2022.0202
2. Zulkifly H, Lip GYH, Lane DA. Epidemiology of atrial fibrillation. *Int J Clin Pract*. 2018;72:e13070. doi: 10.1111/ijcp.13070
3. Fang Y, Wu Y, Liu L, Wang H. The four key genes participated in and maintained atrial fibrillation process via reprogramming lipid metabolism in AF patients. *Front Genet*. 2022;13:821754. doi: 10.3389/fgene.2022.821754
4. Lai S, Hua X, Gao R, Zeng L, Song J, Liu J, Zhang J. Combinational biomarkers for atrial fibrillation derived from atrial appendage and plasma metabolomics analysis. *Sci Rep*. 2018;8:16930. doi: 10.1038/s41598-018-34930-6
5. She J, Guo M, Li H, Liu J, Liang X, Liu P, Zhou B, Liu S, Deng Y, Lou B, et al. Targeting amino acids metabolic profile to identify novel metabolic characteristics in atrial fibrillation. *Clin Sci (Lond)*. 2018;132:2135–2146. doi: 10.1042/CS20180247
6. Kornej J, Hanger VA, Trinquart L, Ko D, Preis SR, Benjamin EJ, Lin H. New biomarkers from multiomics approaches: improving risk prediction of atrial fibrillation. *Cardiovasc Res*. 2021;117:1632–1644. doi: 10.1093/cvr/cvab073
7. Kang JY, Mun D, Kim H, Yun N, Joung B. Serum exosomal long non-coding RNAs as a diagnostic biomarker for atrial fibrillation. *Heart Rhythm*. 2022;19:1450–1458. doi: 10.1016/j.hrthm.2022.05.033
8. Bezak B, Snopek P, Tothova L, Jajcay N, Mojto V, Farsky S, Gmitterova K, Krivosik M, Stankova S, Duranikova O, et al. Plasmatic apelin shows a promising potential as a screening biomarker for atrial fibrillation. *Bratisl Lek Listy*. 2023;124:368–372. doi: 10.4149/BLL_2023_056
9. Menezes Junior ADS, Ferreira LC, Barbosa LJV, Silva DME, Saddi VA, Silva A. Circulating MicroRNAs as specific biomarkers in atrial fibrillation: a meta-analysis. *Noncoding RNA*. 2023;9:13. doi: 10.3390/ncrna9010013
10. Liu H, Wang Q, Liu D, Li Z, Fu Y, Tse G, Li G, Liu T, Xu G. Manganese superoxide dismutase as a novel oxidative stress biomarker for predicting paroxysmal atrial fibrillation. *J Clin Med*. 2022;11:5131. doi: 10.3390/jcm11175131
11. Pauklin P, Zilmer M, Eha J, Tootsi K, Kals M, Kampus P. Markers of inflammation, oxidative stress, and fibrosis in patients with atrial fibrillation. *Oxid Med Cell Longev*. 2022;2022:4556671. doi: 10.1155/2022/4556671
12. Hennings E, Blum S, Aeschbacher S, Coslovsky M, Knecht S, Eken C, Lischer M, Paladini RE, Krisai P, Reichlin T, et al. Bone morphogenetic protein 10-a novel biomarker to predict adverse outcomes in patients with atrial fibrillation. *J Am Heart Assoc*. 2023;12:e028255. doi: 10.1161/JAHA.122.028255
13. Li XY, Hou HT, Chen HX, Liu XC, Wang J, Yang Q, He GW. Preoperative plasma biomarkers associated with atrial fibrillation after coronary artery bypass surgery. *J Thorac Cardiovasc Surg*. 2021;162:851–863. doi: 10.1016/j.jtcvs.2020.01.079
14. Li MY, Chen HX, Hou HT, Wang J, Liu XC, Yang Q, He GW. Biomarkers and key pathways in atrial fibrillation associated with mitral valve disease identified by multi-omics study. *Ann Transl Med*. 2021;9:393. doi: 10.21037/atm-20-3767
15. Gao G, Xuan C, Yang Q, Liu XC, Liu ZG, He GW. Identification of altered plasma proteins by proteomic study in valvular heart diseases and the potential clinical significance. *PLoS One*. 2013;8:e72111. doi: 10.1371/journal.pone.0072111
16. Jiang YY, Hou HT, Yang Q, Liu XC, He GW. Chloride channels are involved in the development of atrial fibrillation—a transcriptomic and proteomic study. *Sci Rep*. 2017;7:10215. doi: 10.1038/s41598-017-10590-w
17. Merino-Merino A, Saez-Maleta R, Salgado-Aranda R, AlKassam-Martinez D, Pascual-Tejerina V, Martin-Gonzalez J, Garcia-Fernandez J, Perez-Rivera JA. A differential profile of biomarkers between patients with atrial fibrillation and healthy controls. *J Pers Med*. 2022;12:1406. doi: 10.3390/jpm12091406
18. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomstrom-Lundqvist C, Boriani G, Castella M, Dan GA, Dilaveris PE, et al. 2020 ESC guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): the task force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. *Eur Heart J*. 2021;42:373–498. doi: 10.1093/eurheartj/ehaa612
19. Tajik B, Tuomainen TP, Isanejad M, Salonen JT, Virtanen JK. Serum n-6 polyunsaturated fatty acids and risk of atrial fibrillation: the Kuopio

Ischaemic heart disease risk factor study. *Eur J Nutr*. 2022;61:1981–1989. doi: 10.1007/s00394-021-02780-0
20. Edwards JM, McCarthy CG, Wenceslau CF. The obligatory role of the acetylcholine-induced endothelium-dependent contraction in hypertension: can arachidonic acid resolve this inflammation? *Curr Pharm Des*. 2020;26:3723–3732. doi: 10.2174/1381612826666200417150121
21. Bapat A, Li G, Xiao L, Yeri A, Hulsmans M, Grune J, Yamazoe M, Schloss MJ, Iwamoto Y, Tedeschi J, et al. Genetic inhibition of serum glucocorticoid kinase 1 prevents obesity-related atrial fibrillation. *JCI Insight*. 2022;7:e160885. doi: 10.1172/jci.insight.160885
22. Sirish P, Diloretto DA, Thai PN, Chiamvimonvat N. The critical roles of proteostasis and endoplasmic reticulum stress in atrial fibrillation. *Front Physiol*. 2021;12:793171. doi: 10.3389/fphys.2021.793171
23. Aragon-Herrera A, Couselo-Seijas M, Feijoo-Bandin S, Anido-Varela L, Morana-Fernandez S, Tarazon E, Rosello-Lleti E, Portoles M, Martinez-Sande JL, Garcia-Seara J, et al. Relaxin-2 plasma levels in atrial fibrillation are linked to inflammation and oxidative stress markers. *Sci Rep*. 2022;12:22287. doi: 10.1038/s41598-022-26836-1
24. Weber R, Perkins N, Bruderer T, Micic S, Moeller A. Identification of exhaled metabolites in children with cystic fibrosis. *Metabolites*. 2022;12:980. doi: 10.3390/metabo12100980
25. Langroudi FE, Narani MS, Kheirollahi A, Vatannejad A, Shokrpoor S, Alizadeh S. Effect of L-serine on oxidative stress markers in the kidney of streptozotocin-induced diabetic mice. *Amino Acids*. 2023;55:799–806. doi: 10.1007/s00726-023-03270-9
26. Lu Y, Zhao N, Du Y. Comprehensive bioinformatics analysis reveals common potential mechanisms, progression markers, and immune cells of corona virus disease 2019 and atrial fibrillation. *Front Cardiovasc Med*. 2022;9:1027026. doi: 10.3389/fcvm.2022.1027026
27. Beaumont M, Goodrich JK, Jackson MA, Yet I, Davenport ER, Vieira-Silva S, Debelius J, Pallister T, Mangino M, Raes J, et al. Heritable components of the human fecal microbiome are associated with visceral fat. *Genome Biol*. 2016;17:189. doi: 10.1186/s13059-016-1052-7
28. Zhang C, Jia J, Zhang P, Zheng W, Guo X, Ai C, Song S. Fucoidan from Laminaria japonica ameliorates type 2 diabetes mellitus in association with modulation of gut microbiota and metabolites in Streptozocin-treated mice. *Foods*. 2022;12:33. doi: 10.3390/foods12010033
29. Celik E, Hokamp NG, Goertz L, Fehske W, Nguyen DQ, Lichtenberg L, Reimer RP, Maintz D, Duber C, Achenbach T. Is there an association between left atrial outpouching structures and recurrence of atrial fibrillation after catheter ablation? *PLoS One*. 2022;17:e0276369. doi: 10.1371/journal.pone.0276369