Original Research

CARDIAC RESEARCH

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Histidine and the risk of incident atrial fibrillation and postablation recurrence

Evidence from 2 prospective cohorts

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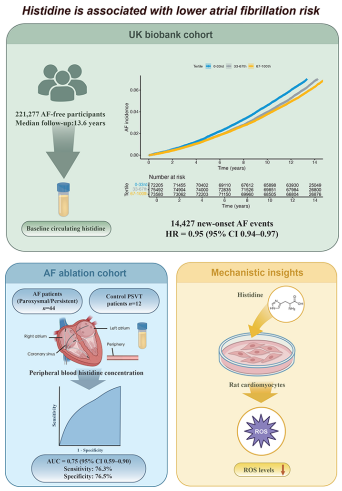
Graphical abstract



Abstract

Background: To investigate the association between circulating histidine levels and the risk of atrial fibrillation (AF) onset and recurrence, and to explore potential underlying mechanisms.

Methods: We analyzed data from 221,277 AF-free participants in the UK Biobank and from 44 patients with paroxysmal or persistent AF undergoing catheter ablation, in whom histidine was measured in both peripheral and intracardiac blood. Associations with incident and recurrent AF were evaluated using Cox proportional hazards models, subgroup analyses, and sensitivity analyses. Receiver operating characteristic analysis assessed the predictive value of histidine for AF recurrence. Mechanistic studies included a sheep AF model to examine the impact of electrical remodeling on atrial histidine metabolism, and neonatal rat cardiomyocyte experiments to assess histidine's effects on cell viability and gene expression.

Results: Over a median follow-up of 13.6 years, 14,427 participants developed new-onset AF. Higher histidine levels were associated with a lower risk of AF (hazard ratio per standard deviation: 0.95; 95% confidence interval: 0.94–0.97), with consistent findings across sensitivity analyses. In the ablation cohort, histidine predicted AF recurrence with 76% sensitivity and 77% specificity (area under the receiver operating characteristic curve = 0.75; 95% confidence interval: 0.59–0.90). In the sheep AF model, histidine levels remained stable despite increased 3-methylhistidine, indicating no effect of electrical remodeling on histidine metabolism. Histidine demonstrated a dose-dependent effect on cardiomyocyte viability, enhancing activity at nanomolar concentrations while reducing it at micromolar levels. The downregulation of oxidative phosphorylation (OXPHOS) genes at higher histidine doses, together with the reduction in relative MitoSOX intensity, suggests a dose-dependent modulation of mitochondrial function, potentially balancing energy production and oxidative stress.

Conclusion: Histidine is inversely associated with AF risk and may serve as a predictive biomarker for AF recurrence. Experimental findings suggest that changes in histidine may precede AF development, supporting its potential as a modifiable metabolic target.

Keywords: cardiomyocyte viability, catheter ablation, prognosis, recurrence

Nonstandard Abbreviations and Acronyms	
3-MEH	3-methylhistidine
AF	atrial fibrillation
CS	coronary sinus
ECGs	electrocardiograms
HR	hazard ratios
LA	left atrium
OXPHOS	oxidative phosphorylation
PaAF	paroxysmal AF
PeAF	persistent AF
PSVT	paroxysmal supraventricular tachycardia
RA	right atrium
ROC	Receiver operating characteristic
ROS	reactive oxygen species
UKB	UK Biobank

Highlights

- Higher circulating histidine is associated with a lower risk of incident atrial fibrillation.
- Histidine predicted postablation atrial fibrillation recurrence with an area under the receiver operating characteristic curve of 0.75.
- Atrial remodeling does not alter myocardial histidine abundance.
- Histidine modulates mitochondrial reactive oxygen species and oxidative phosphorylation pathways.

1. Introduction

Atrial fibrillation (AF) is a prevalent arrhythmia with a complex and multifactorial etiology, including metabolic modulation that alters atrial electrophysiology and increases susceptibility to atrial arrhythmias.[1,2] AF itself may induce changes in cardiac metabolism,[3] which may further sustain and exacerbate AF, reinforcing the concept of "AF begets AF." Evidence has demonstrated that the amino acid profile is altered in the blood of patients with AF.[4] In a mouse model of age-related AF, myocardial histidine concentrations were significantly lower compared with controls.[4] Notably, histidine is unique among the amino acids because its imidazole side chain has a pKa near physiological pH, enabling it to readily switch between protonated and deprotonated states under near-neutral conditions, thereby serving as an important biological buffer.[5] However, evidence on the relationship between histidine and cardiovascular diseases, particularly arrhythmias, remains scarce.

To address this knowledge gap, we used data from the UK Biobank (UKB), a large population-based prospective cohort, together with an independent cohort, to comprehensively investigate the association between circulating histidine levels and the risk of AF and AF recurrence after ablation. In our independent cohort, we collected peripheral and intracardiac blood samples to quantify histidine levels from patients with paroxysmal supraventricular tachycardia (PSVT), paroxysmal AF (PaAF), and persistent AF (PeAF) before catheter ablation. Our findings aim to offer new insights into the connection of histidine to AF genesis, contributing to improved AF management strategies.

2. Methods

2.1. Study population

We conducted a 2-stage analysis, using the UKB as the discovery cohort and our recurrence cohort as replication. We first analyzed the associations in the UKB, a prospective cohort of 502,187 adults aged 37–73 years.[6] Circulating histidine concentrations were measured in 273,865 UKB participants; among them, 269,206 participants were free of AF at baseline. Participants completed questionnaires and interviews, underwent physical assessments, and provided biological samples during recruitment. Demographic data, including age, sex, ethnicity, lifestyle behaviors (smoking and alcohol consumption), and medications (lipid-modifying, antidiabetic, and antihypertensive), were collected based on self-reported assessment (details of the variable definitions are shown in Supplementary Table 1, Supplemental Digital Content, https://links.lww.com/CARES/A5). The presence of baseline comorbidities was identified based on the self-reported assessment and International Classification of Diseases, 10th Revision (ICD-10) coded registry data from hospital or primary care records that were obtained

Supplemental Digital Content is available for this article.

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Cardiac Research (2026) 2:1

Received: 11 August 2025 / Received in final form: 29 January 2026 / Accepted: 13 February 2026

http://dx.doi.org/10.1097/re9.0000000000000010

before the recruitment. Participants free of AF were excluded if they were lost to follow-up or had a baseline history of valvular heart disease, myocardial infarction, stroke, rheumatic heart disease, heart failure, or cardiomyopathy. As a sensitivity analysis, we also examined a cohort, including participants with these baseline conditions to reduce selection bias and enhance generalizability. For all self-reported variables, including covariates, the values for participants who answered "do not know" or "prefer not to answer" were treated as missing. Missing data were handled using complete case analysis.[7,8] The data-cleaning process is presented in Supplementary Figure 1, Supplemental Digital Content, https://links.lww.com/CARES/A5.

A total of 56 consecutive patients who underwent their first radiofrequency ablation procedure at the Department of Cardiology, Peking University First Hospital, from January 2021 to July 2022 were included in the recurrence cohort. This cohort comprised 20 patients with PaAF and 24 patients with PeAF. Additionally, 12 patients with left-sided accessory pathway PSVT were included as a control group. All participants provided informed consent for biological sample collection before the procedure. PaAF was defined as self-terminating within 7 days of onset, PeAF was defined as an episode lasting more than 7 days, which may be terminated by cardioversion (either pharmacologic or electrical), or AF that persists for more than 12 months before a rhythm control strategy. Exclusion from the study occurred if any of the following criteria were met: age < 18 years; history of prior AF radiofrequency ablation; AF secondary to rheumatic heart disease, primary cardiomyopathy, thyroid disease, congenital heart disease, or other secondary causes; concurrent interstitial lung disease, cirrhosis, chronic kidney disease, or other fibrosis-related conditions; pregnancy; and individuals with psychiatric disorders.

2.2. Follow-up and recurrence definition

Disease outcome (AF) of the UKB cohort was identified based on ICD-10 coded registry data from hospital or primary care records that were registered after the recruitment. Death information was obtained from Hospital Episode Statistics and national death registries, and the specific cause of death was identified by the ICD-10 codes (Supplementary Table 1, Supplemental Digital Content, https://links.lww.com/CARES/A5). The follow-up time was defined as the period from the baseline date to the occurrence of outcomes or the end of follow-up. Diagnostic data from England, Scotland, and Wales were censored on November 30, 2022.

AF patients who were enrolled in the recurrence cohort were followed up through scheduled outpatient visits and telephone follow-ups at 3- and 6-months postradiofrequency ablation. The follow-up included routine electrocardiograms (ECGs), 24-h Holter monitoring, and reassessment of liver and kidney function, as well as echocardiography to evaluate recurrence. For patients seen in the outpatient clinic, recurrence was defined based on ECG findings. If a rapid atrial arrhythmia lasting more than 30 s occurred 3- or 6-months after radiofrequency ablation, it was considered a recurrence of AF. For patients followed up via telephone, recurrence was defined based on ECG results indicating AF. In this study, recurrence was defined as any recurrent AF occurring in either the 3- or 6-month period.

2.3. Sample collection of the recurrence cohort

Before the radiofrequency ablation procedure, peripheral (P) blood (10 mL) was collected from the femoral vein of the enrolled patients. After successful femoral vein puncture, 10 mL of whole blood was obtained from the right atrium (RA) via a sheath. Subsequently, 10 mL of whole blood was collected from the left atrium (LA) through a transseptal puncture. Under X-ray guidance, a 10-pole electrode was carefully advanced into the coronary sinus (CS) via a long sheath. The 10-pole electrode was used to carefully guide the tip of the long sheath into the CS ostium. After removing the 10-pole electrode, at least 10 mL of blood was withdrawn and discarded from the tail end of the long sheath. Then, 10 mL of whole blood was collected from the CS through the long sheath. Respective blood samples were transferred into ethylenediaminetetraacetic acid anticoagulant tubes and immediately supplemented with 10 μL of protease inhibitor. The specimens were gently inverted several times to ensure thorough mixing. After centrifugation at 3,000 rpm for 15 min at 4°C, the upper plasma layer was collected and dispensed into EP tubes, of which 1 mL of plasma was centrifuged a second time at 12,000 g for 10 min at 4°C, and the supernatant was transferred to a new LoBind tube. All specimens were stored at –80°C.

2.4. Histidine measurement

Histidine concentration in the UKB cohort baseline was measured by Nightingale Health Ltd. using nuclear magnetic resonance spectroscopy (Nightingale Health Plc; biomarker quantification version 2020).[9] Histidine concentration in the recurrence cohort was quantified by liquid chromatography–mass spectrometry, and detailed methods of sample preparation,[10] liquid chromatography conditions, and mass spectrum conditions were previously published.[11] The LC analysis was performed on EXion LC Liquid chromatography (AB SCIEX). Mass spectrometric detection of metabolites was performed on AB6500 + (AB SCIEX). Standards including L-histidine and 3-methylhistidine (3-MEH) were obtained from Sigma-Aldrich (Shanghai, China).

2.5. Sheep model of persistent AF

A total of 14 male sheep (about 40 kg) underwent subcutaneous pacemaker (St. Jude Medical) implantation, in which an atrial lead was attached to the RA appendage as previously described.[12,13] After 2 weeks of recovery,

sheep were randomly divided into the sham group ($n = 5$) and the AF group ($n = 9$). The sheep was euthanized after exhibiting 7 days of spontaneously sustained AF without pacing (continuous intracardiac electrograms recorded through the pacemaker leads are presented in Supplementary Figure 2, Supplemental Digital Content, https://links.lww.com/CARES/A5; representative structural changes confirmed by Masson and Wheat Germ Agglutinin staining are presented in Supplementary Figure 3, Supplemental Digital Content, https://links.lww.com/CARES/A5). Sham group sheep were sacrificed at similar time points after the pacemaker was implanted without pacing. Atrial tissues were obtained and stored at −80°C.

2.6. Western blot

Cardiomyocytes cultured in 6-well plates were lysed by RIPA with a 1% protease inhibitor cocktail (PC101 and GRF101, EpiZyme). The homogenate was centrifuged at 12,000 rpm for 15 min, and the supernatant, with the addition of loading buffer (LT101, EpiZyme), was then subjected to electrophoresis separation in a 4%–15% gradient gel (LK205, EpiZyme) after transferring to polyvinylidene fluoride membranes (IPVH00010, Millipore). The membranes were blocked for 0.5–1 h (PS108P, EpiZyme) and then incubated with primary antibodies overnight at 4°C: CaMKII (MA1-048, Invitrogen), p38 MAPK, Caspase-3, and Cleaved Caspase-3 (8690 and 14220, Cell Signaling Technology) and beta Tubulin (ab6064, Abcam). After rinsing for 15 min 3 times with TBST (PS103S, EpiZyme), the membranes were incubated with a secondary antibody for 1 h at room temperature and then rinsed 3 times with TBST: Anti-rabbit IgG, HRP-linked Antibody, Anti-mouse IgG and HRP-linked Antibody (7074 and 7076, Cell Signaling Technology). The protein bands were visualized using enhanced chemiluminescence (ChemiScope S7, Clinx) and quantified by densitometry.

2.7. Neonatal rat cardiomyocyte isolation

To extract cardiomyocytes, ice-cold ethanol was used to anesthetize neonatal rats (0–3 days), followed by cervical dislocation for euthanasia. Dissect the chest cavity immediately to remove the heart and place the heart in a petri dish containing cold PBS buffer. Cut the heart into small pieces and isolate cardiomyocytes using a Neonatal Heart Dissociation Kit, mouse and rat (130-098-373, Miltenyi Biotec) according to protocol. After 1 h of adherence, the suspended cells were purified by the Neonatal Cardiomyocyte Isolation Kit, rat (130-105-420, Miltenyi Biotec, Germany) and considered cardiomyocytes, and were transferred to culture flasks containing dulbecco's modified eagle medium supplemented with 20% FBS for continued culture over 48 h.

2.8. Cell viability and reactive oxygen species analysis

Cell Counting Kit-8 (CCK-8, CK04, Dojindo) was used to determine cell viability. Briefly, 10 µL of reagent per well was added to neonatal rat cardiomyocytes treated for 72 h with histidine in the presence or absence of doxorubicin, cultured in 96-well plates at 37°C for 1 h. The plates were then read using a standard plate reader with a wavelength of 450 nm. Mitochondrial reactive oxygen species (ROS) production was assessed using MitoSOX Red (HY-D1055, Medchemexpress). Cardiomyocytes were incubated with 5 µM MitoSOX Red for 10 min at 37°C, washed, and immediately analyzed by fluorescence microscopy and plate reader. To account for differences in mitochondrial mass, fluorescence intensity was normalized to MitoTracker Green staining (100 nM, 30 min at 37°C, HY-135056, Medchemexpress). Relative MitoSOX intensity was expressed as the ratio of MitoSOX to MitoTracker fluorescence and presented as values relative to the untreated control.

2.9. Bulk RNA-seq and bioinformatics analysis

Cell pellets from 5 biological replicates of rat primary cardiomyocytes treated with 100 nM histidine or vehicle in the presence or absence of doxorubicin for 72 h were sent to Biomedi (Beijing, China) for RNA isolation, library preparation, and sequencing. HISAT2 was used to align reads to an RN7.2 index. DESeq2 was used to perform differential expression testing. Differentially expressed genes of an adjusted p value threshold of 0.05 were then annotated using clusterProfiler.

2.10. Statistical analysis

The Cox proportional hazards model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) between histidine (per standard deviation [SD]) and outcomes. For incident AF, Fine–Gray subdistribution hazard models as sensitivity analyses accounted for the competing risk of all-cause mortality. Models were adjusted for age, sex, ethnicity, body mass index (BMI), systolic blood pressure, antihypertensive medication use, and history of hypertension, smoking status, drinking status, antidiabetic medication use, and history of diabetes, thyroid diseases, sleep disorders, chronic kidney disease, C-reactive protein (CRP), and dyslipidemia. Restricted cubic spline models with 3 knots were fitted to evaluate the dose-response relationship between circulating histidine levels and AF risk. The median histidine value was used as the reference point (HR = 1.0).

For normally distributed data, the mean ± SD was used. For non-normally distributed data, the median and the interquartile range (Q1–Q3) were used. Descriptive statistics for continuous data were presented as frequency (percentage). Comparisons of means between two groups were conducted by t tests, while comparisons of means among 3 or more groups were performed using one-way ANOVA. Nonparametric tests were employed for comparing other continuous data that did not follow a normal distribution. Count data comparisons were carried out using the chi-square test or Fisher exact test. Receiver operating characteristic (ROC) curves were used to

Table 1

Baseline Characteristics of the UKB Cohort

Variables	Total (n = 221,277)	Q1 (lowest) (n = 72,205)	Q2 (n = 75,492)	Q3 (highest) (n = 73,580)	p
Age	57 (50, 63)	58 (51, 63)	57 (50, 63)	56 (49, 62)	< 0.001
Sex					< 0.001
Female	122,289 (55)	44,174 (61)	41,737 (55)	36,378 (49)	
Male	98,988 (45)	28,031 (39)	33,755 (45)	37,202 (51)	
Ethnicity					0.005
White	209,504 (95)	68,206 (94)	71,527 (95)	69,771 (95)	
Other	11,773 (5)	3,999 (6)	3,965 (5)	3,809 (5)	
Smoking status					< 0.001
Current	22,998 (10)	7,912 (11)	7,756 (10)	7,330 (10)	
Never	122,862 (56)	39,717 (55)	42,050 (56)	41,095 (56)	
Previous	75,417 (34)	24,576 (34)	25,686 (34)	25,155 (34)	
Drinking status					< 0.001
Non-to-light drinker	66,596 (30)	23,026 (32)	22,618 (30)	20,952 (28)	
Moderate-to-heavy drinker	154,681 (70)	49,179 (68)	52,874 (70)	52,628 (72)	
Body mass index	26.66 (24.09, 29.76)	26.64 (23.96, 29.98)	26.64 (24.06, 29.73)	26.69 (24.24, 29.58)	0.305
SBP (mmHg)	136 (124.5, 149.5)	137 (125, 150.5)	136.5 (124.88, 149.5)	135.5 (124, 148)	< 0.001
DBP (mmHg)	82 (75.5, 89)	82 (75.5, 89)	82 (75.5, 89)	81.5 (75, 88.5)	< 0.001
LDL-C (mmol/L)	1.74 (1.47, 2.04)	1.7 (1.43, 2)	1.74 (1.47, 2.04)	1.78 (1.5, 2.08)	< 0.001
HDL-C (mmol/L)	1.29 (1.09, 1.53)	1.29 (1.09, 1.53)	1.29 (1.09, 1.53)	1.29 (1.1, 1.53)	0.19
TC (mmol/L)	4.65 (4.05, 5.27)	4.58 (3.98, 5.21)	4.65 (4.06, 5.26)	4.71 (4.12, 5.33)	< 0.001
TG (mmol/L)	1.48 (1.04, 2.14)	1.43 (1.02, 2.06)	1.46 (1.03, 2.11)	1.54 (1.08, 2.23)	< 0.001
CRP (mg/L)	1.31 (0.65, 2.72)	1.53 (0.73, 3.3)	1.31 (0.65, 2.67)	1.16 (0.59, 2.29)	< 0.001
Diabetes mellitus	12,256 (6)	4,385 (6)	4,046 (5)	3,825 (5)	< 0.001
Hypertension	120,196 (54)	40,862 (57)	41,178 (55)	38,156 (52)	< 0.001
Thyroid diseases	14,330 (6)	5,249 (7)	4,787 (6)	4,294 (6)	< 0.001
Sleep disorders	3,963 (2)	1,341 (2)	1,359 (2)	1,263 (2)	0.125
CKD	2,518 (1)	837 (1)	829 (1)	852 (1)	0.446
Medications					
Antidiabetic	7,176 (3)	2,454 (3)	2,335 (3)	2,387 (3)	0.004
Antithrombotic	24,557 (11)	8,468 (12)	8,345 (11)	7,744 (11)	< 0.001
Antihypertensive	40,770 (18)	14,785 (20)	13,966 (18)	12,019 (16)	< 0.001
Lipid modifying	33,333 (15)	11,742 (16)	11,396 (15)	10,195 (14)	< 0.001
Histidine (mmol/L)	0.07 (0.06, 0.07)	0.06 (0.05, 0.06)	0.06 (0.06, 0.07)	0.08 (0.07, 0.08)	< 0.001

CKD = chronic kidney disease; CRP = C-reactive protein; DBP = diastolic blood pressure; HDL-C = high-density lipoprotein cholesterol; IDL-C = intermediate-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SBP = systolic blood pressure; TC = total cholesterol; TG = triglycerides.

assess the predictive value regarding the AF recurrence of histidine. The optimal cutoff value was determined by identifying the point on the ROC curve that maximized the Youden Index (sensitivity + specificity − 1). Potential associations were analyzed for their independent predictive value using univariable and multivariable logistic modeling. A p value < 0.05 was regarded as a significant difference. All analyses were performed in R 4.2.3 and GraphPad Prism 10.

3. Results

3.1. Histidine is associated with a lower incidence of AF

The baseline characteristics of 221,277 participants free of AF in the UKB cohort are presented in Table 1. After a median follow-up period of 13.7 years, a total of 14,427 participants experienced new-onset AF, corresponding to an incidence of 5.0 events per 1000 person-years. The proportion of males increased across histidine quartiles, whereas CRP concentrations, the prevalence of hypertension, and the use of antihypertensive and lipid-modifying medications significantly decreased. The multivariable Cox model showed that histidine was associated with a lower risk of AF (HR per SD: 0.95, 95% CI: 0.94–0.97, $p < 0.001$). Subgroup analyses revealed consistent directions of association across all subgroups, in line with the overall association (Fig. 1). Fine–Gray model adjusted for the competing risk of death showed similar results (HR per SD: 0.95, 95% CI: 0.94–0.97, $p < 0.001$). Kaplan–Meier curves showed significant differences in the cumulative incidence of AF across histidine quartiles, with lower histidine quartiles consistently exhibiting higher AF incidence (Fig. 2). Restricted cubic spline analysis (Supplementary Figure 4, Supplemental Digital Content, https://links.lww.com/CARES/A5) demonstrated a significant overall association between histidine levels and AF risk ($p_{overall} < 0.001$), with evidence of nonlinearity ($p_{nonlinearity} < 0.001$). Although the association was statistically nonlinear, the risk curve showed a monotonic decreasing trend across the histidine distribution without reversal, indicating that higher histidine levels were consistently associated with lower AF risk (Supplementary Figure 5, Supplemental Digital Content, https://links.lww.com/CARES/A5). Sensitivity analyses were conducted,

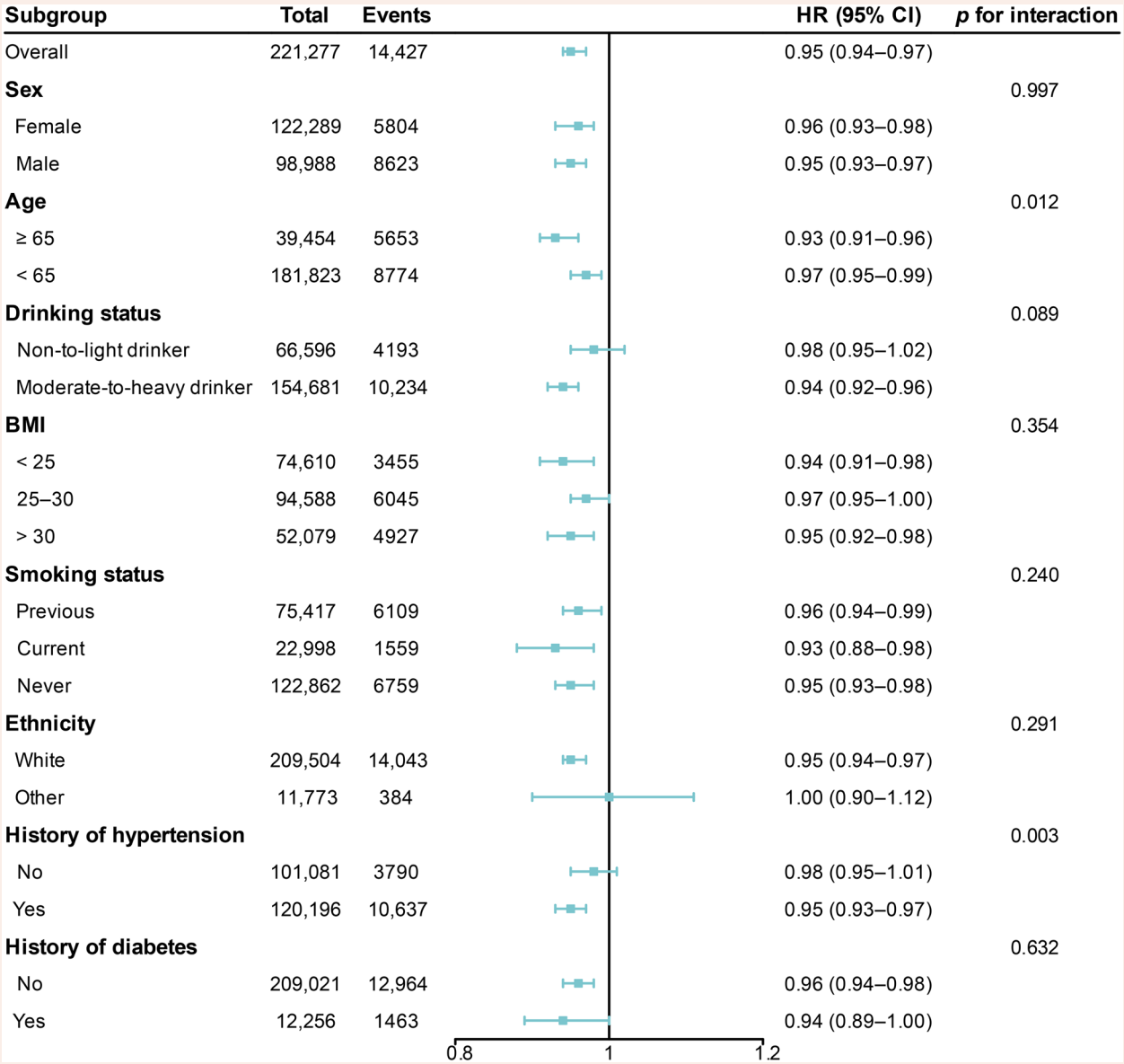

Figure 1. Overall and subgroup analyses of histidine with AF risk in UK Biobank. The Cox regression model was adjusted for age, sex, ethnicity, body mass index, antihypertensive medication and history of hypertension, systolic blood pressure, drinking status, smoking status, antidiabetic medication and history of diabetes, thyroid diseases, sleep disorders, and C-reactive protein. AF, atrial fibrillation.

including all participants regardless of baseline comorbidities. The results were highly consistent with those of the primary analysis, supporting the robustness and validity of our findings (HR per SD: 0.96, 95% CI: 0.95–0.98, $p < 0.001$).

3.2. Characteristics of the recurrence cohort

Patients with AF had significantly higher age, BMI, prevalence of hypertension, and levels of B-type natriuretic peptide compared with those with PSVT (Supplementary Table 2, Supplemental Digital Content, https://links.lww.com/CARES/A5). In this cohort of 44 patients undergoing AF ablation (12 with recurrence and 32 without), there were no statistically significant differences between groups in sex, age, BMI, blood pressure, smoking/drinking status, most comorbidities, medications, or laboratory parameters. However, patients with recurrence had a higher prevalence of coronary heart disease (50% vs. 16%, $p = 0.05$), a larger left atrial transverse diameter (4.82 ± 0.54 cm vs. 4.28 ± 0.59 cm, $p = 0.02$), and a lower rate of propafenone use (33% vs. 69%, $p = 0.05$). Echocardiographic indices (e.g., LVEF, LAEF), lipid levels, and inflammatory markers (hs-CRP) showed no significant differences (Supplementary Table 3, Supplemental Digital Content, https://links.lww.com/CARES/A5). Histidine levels did not differ significantly among sampling sites within the same patient group (Supplementary

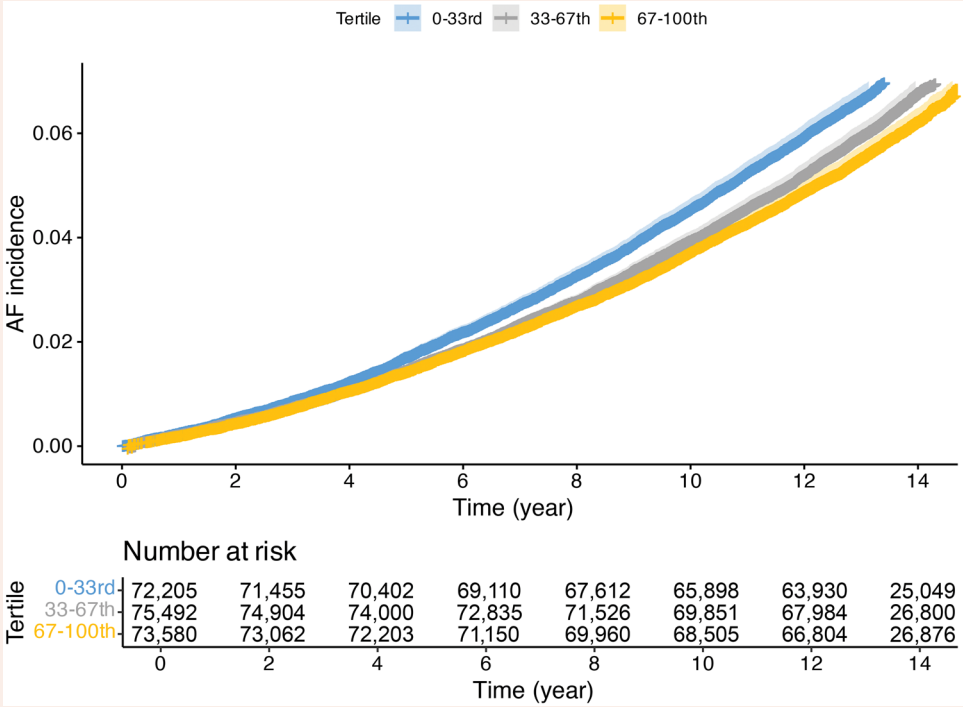

Figure 2. Kaplan–Meier estimates of the cumulative incidence of AF by histidine tertiles. Cumulative incidence curves are statistically different (log-rank, $p < 0.001$). AF, atrial fibrillation.

Figure 4, Supplemental Digital Content, https://links.lww.com/CARES/A5). In the analysis of histidine gradients across anatomical sites, no significant differences were observed between patients with and without postablation AF recurrence (Supplementary Table 4, Supplemental Digital Content, https://links.lww.com/CARES/A5). Specifically, the periphery-to-RA gradient was comparable between the 2 groups (–4.06 vs. –3.61 mmol/mL, $p = 0.82$). Similarly, the RA-to-LA gradient (5.13 vs. 2.81 mmol/mL, $p = 0.33$) and the LA-to-CS gradient

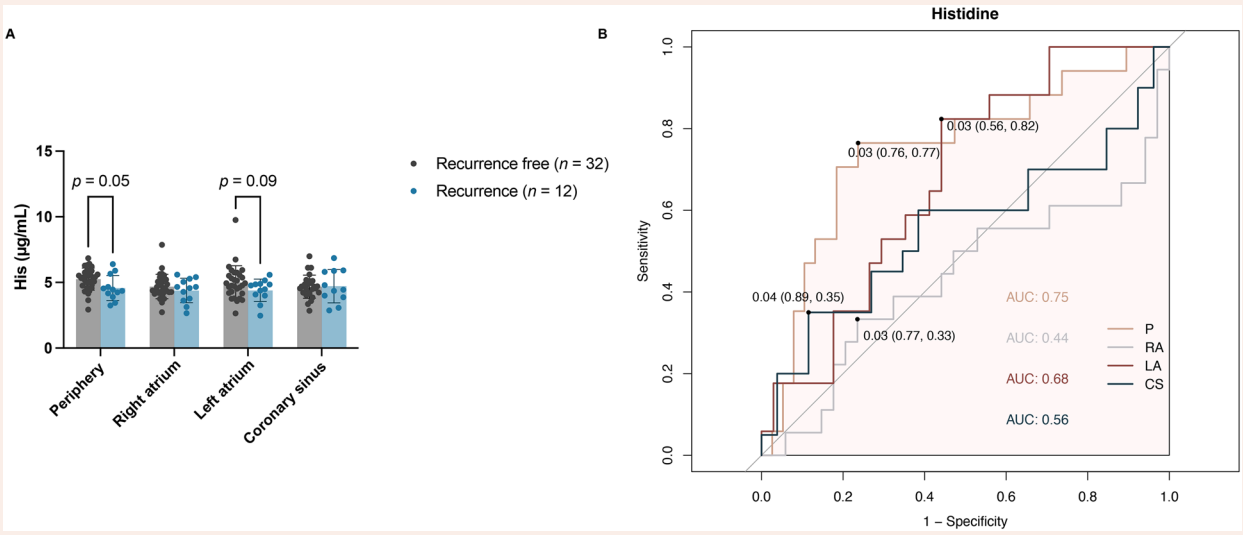

Figure 3. Comparison of concentrations and ROC curve of histidine levels between recurrence and recurrence-free AF patients. (A) Concentrations of histidine between recurrence and recurrence-free AF patients, although the nominal p value indicated significantly lower histidine levels in the recurrence group in peripheral blood samples ($p = 0.04$), this association was no longer significant after Šidák correction; (B) ROC curve of histidine predicting AF recurrence obtained from the peripheral (P) circulation, right atrium (RA), left atrium (LA), and coronary sinus (CS). AF, atrial fibrillation, ROC, receiver operating characteristic.

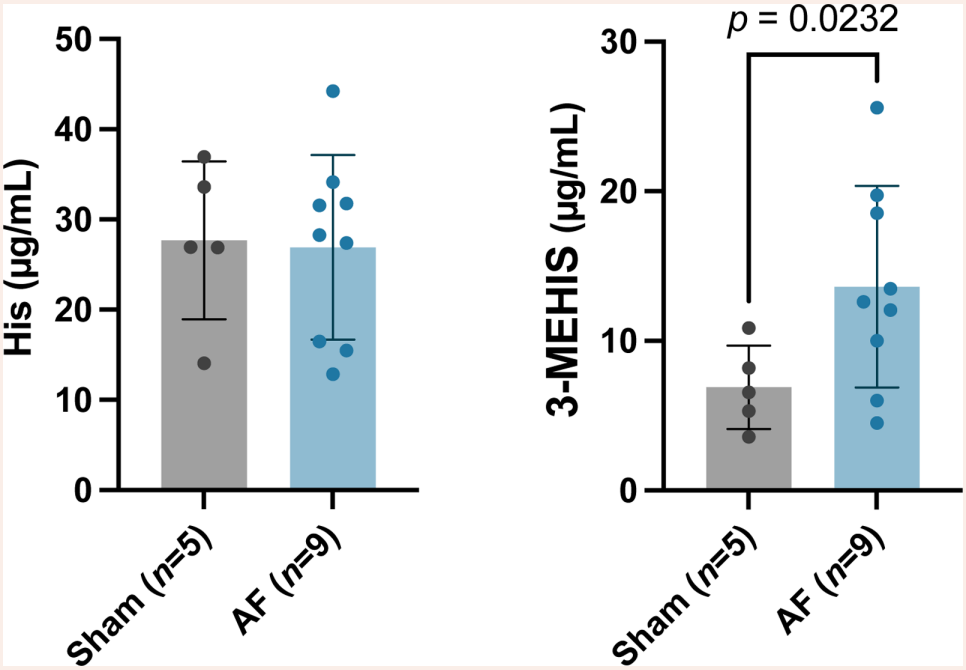

Figure 4. Histidine and 3-methylhistidine abundance in AF sheep. A comparison of left atrial histidine and 3-methylhistidine from sham and self-sustained persistent AF sheep (unpaired *t* test, *n* = 6).

(–0.90 vs. –2.16 mmol/mL, $p = 0.69$) did not differ significantly between recurrence and recurrence-free patients.

3.3. Predictive value of the histidine for AF recurrence

Peripheral histidine levels were nominally significantly lower in recurrent AF patients compared with recurrence-free patients (0.025 vs. 0.030 mmol/L, $p = 0.04$) (Fig. 3A). ROC curve analysis indicated that peripheral histidine was predictive of AF recurrence, with an optimal cutoff value of ≤ 0.030 mmol/L, yielding a sensitivity of 76% and a specificity of 77%. The area under the ROC curve was 0.75 (95% CI: 0.59–0.90) (Fig. 3B). In addition, peripheral histidine was robustly associated with a lower incidence of AF recurrence after adjusting for age, sex, blood pressure, BMI, and drinking and smoking status (OR 0.33 per SD, 95% CI: 0.14–0.71, $p = 0.008$).

3.4. Atrial remodeling may not change histidine metabolism

The levels of histidine and 3-MEH were measured in left atrial tissues obtained from the AF sheep model, which is considered predominantly electrical remodeling. The results demonstrated that although 3-MEH, which is typically recognized as a biomarker of muscular degradation, showed significant elevation in atrial tissues of the AF group, no statistically significant alterations were observed regarding histidine (Fig. 4).

3.5. Histidine increases the viability of cardiomyocytes

Despite the presence of 0.2 mM histidine hydrochloride monohydrate already in dulbecco's modified eagle medium, supplemental histidine administration still exerted a significant impact on the viability of neonatal rat cardiomyocytes (Fig. 5A). Intriguingly, histidine induced a dose-dependent response in cellular viability: lower concentrations (1–100 nM) of histidine supplementation markedly enhanced cell viability, whereas at 10 μM, viability exhibited a decline. This phenomenon was further amplified under the influence of doxorubicin, a cardiotoxic agent. Subsequently, cardiomyocytes were stimulated with 100 nM histidine for 72 h in the presence or absence of doxorubicin, followed by bulk RNA-seq analysis. Transcriptomic analysis further demonstrated that histidine treatment altered multiple biological processes, including mitochondrial inner membrane organization, oxygen transport, and ribosomal function (Fig. 5B). Among these enriched pathways, OXPHOS emerged as a key target, with GSEA confirming significant enrichment of the KEGG OXPHOS gene set in histidine-treated cardiomyocytes (Fig. 5C). MitoSOX analysis showed that doxorubicin treatment increased mitochondrial ROS production in neonatal rat cardiomyocytes. Histidine supplementation attenuated this effect in a concentration-dependent manner, as reflected by the decrease in relative MitoSOX/MitoTracker ratios compared with baseline (Fig. 5C). The reduction became more evident at higher histidine concentrations, with the lowest ROS

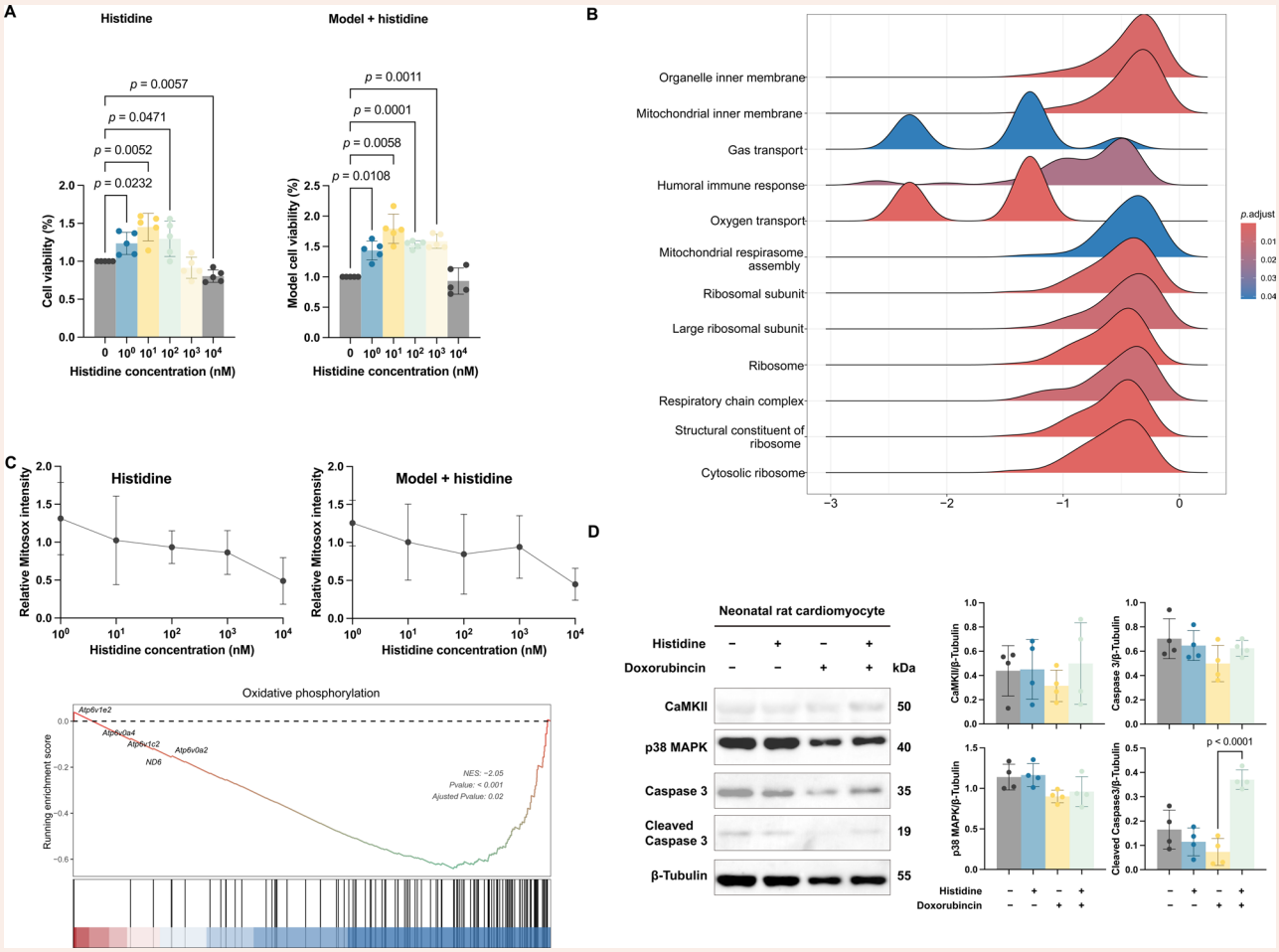

Figure 5. Cell viability and bulk RNA-seq analysis of histidine on neonatal rat cardiomyocytes. (A) Cardiomyocytes were stimulated with varying concentrations of histidine for 72 h, and cell viability was subsequently quantified using the CCK-8 assay. Multiple comparison of 2-tailed Student *t* tests was corrected by the Dunnett method, with a minimum of 5 independent biological replicates per concentration. The model represents 1 μM doxorubicin. (B) GSEA analysis of differentially expressed genes of cardiomyocytes treated with 100 nM histidine using GO enrichment gene sets under 1 μM doxorubicin. The *x*-axis represents the normalized enrichment score across samples. (C) Top, neonatal rat cardiomyocytes were stained with MitoSOX Red to detect mitochondrial ROS, and fluorescence signals were normalized to mitochondrial content assessed by MitoTracker Green. Relative MitoSOX intensity is expressed as the ratio to baseline (untreated control). The model represents 1 μM doxorubicin. Bottom panel, GSEA analysis of differentially expressed genes using KEGG oxidative phosphorylation gene set. The model represents 1μM doxorubicin. (D) Western blot of CaMKII, p38 MAPK, and Caspase-3 of cardiomyocytes stimulated with 100 nM histidine and 1 μM doxorubicin. Multiple comparison of 2-tailed *t* tests was corrected by the Dunnett method, with a minimum of 4 independent biological replicates per concentration. ROS, reactive oxygen species.

level observed at 10 μM. We also investigated the effects of histidine on the protein abundance of p38 MAPK, CaMKII, and caspase-3 via Western blotting. Notably, the abundance of cleaved caspase-3 was significantly elevated under histidine treatment in the presence of doxorubicin (Fig. 5D).

4. Discussion

In this study, we identified a significant inverse association between circulating histidine levels and both the risk of AF and postablation AF recurrence across 2 prospective cohorts. In the UKB cohort, we applied strict inclusion and exclusion criteria to eliminate diseases that could influence AF risk. Multivariable Cox and Fine–Gray models were constructed with extensive adjustment for potential confounders. In our independent recurrence cohort, although the sample size was relatively small, similar analytical steps were undertaken. Consistent results observed across these ethnically distinct cohorts support the robustness of the association between histidine levels and AF risk. To explore causality, we demonstrated that electrical remodeling in an AF model did not alter atrial histidine concentrations, whereas histidine supplementation influenced cardiomyocyte viability in an *in vitro* doxorubicin-induced cardiotoxicity model. These findings suggest that changes in histidine levels may precede the development of AF.

There are few studies that have evaluated histidine in cardiovascular diseases. An amino acid profile study

found lower histidine levels in aged AF mice induced by acetylcholine injections compared with adult AF mice.[4] Our data showed that histidine levels across all blood sampling sites were consistently lower in the recurrent AF group, with peripheral histidine showing the highest predictive value among the potential predictors evaluated.

Histidine is an essential amino acid involved in pH buffering,[14] metal ion chelation,[15] antioxidant activity,[16] and the histaminergic system. Histidine administration in rats ameliorates isoproterenol- and doxorubicin-induced cardiotoxicity.[17] A double-blind, placebo-controlled study showed that histidine supplementation (4 g/day) improved insulin sensitivity and decreased BMI, waist circumference, body fat, and markers of systemic inflammation in obese women with metabolic syndrome.[18]

Whether histidine supplementation could reduce the recurrence of AF is an avenue worth exploring in future research. Histidine exhibited a dose-dependent effect on cardiomyocyte viability, enhancing cell activity at nanomolar concentrations but reducing it at micromolar levels. This concentration-dependent response may reflect distinct metabolic and signaling pathways engaged at different doses. At lower concentrations, histidine may act as a metabolic substrate and antioxidant precursor, supporting mitochondrial function and cellular energetics. In contrast, higher concentrations were associated with the downregulation of OXPHOS-associated genes, which could limit mitochondrial ATP production and reduce ROS generation. This suppression of OXPHOS gene expression at micromolar levels may represent an adaptive mechanism to mitigate oxidative stress but could also impair high-energy-demand tissues such as the myocardium. Together, these findings suggest that histidine's influence on cardiomyocyte function is dose-dependent, with potential implications for its therapeutic modulation in AF. The observed elevation of cleaved caspase-3 in histidine-treated cardiomyocytes without a corresponding decline in viability may reflect a nonapoptotic role of cleaved caspase-3 or pro-survival mechanisms might counteract full apoptotic execution, preserving cellular viability. The caspase-3 activity is required for skeletal muscle differentiation,[19] and cleaved caspase-3 can be upregulated in such contexts, contributing to cellular processes without leading to cell death.

In the ablation cohort, patients who remained free of AF recurrence exhibited higher histidine concentrations in peripheral plasma, whereas chamber-specific levels (LA, RA, and CS) did not differ significantly. This pattern suggests that histidine's association with favorable rhythm outcomes primarily reflects systemic metabolic homeostasis rather than localized myocardial release or uptake. Given histidine's physicochemical properties as a small, rapidly equilibrating amino acid with key roles in acid–base buffering and oxidative stress modulation, the lack of chamber gradients is physiologically consistent. These findings imply that circulating histidine acts as a systemic metabolic biomarker—potentially indicating enhanced antioxidant or anti-inflammatory capacity—rather than a chamber-specific cardiac metabolite. Clinically, this interpretation strengthens the translational relevance of peripheral histidine measurement as a noninvasive indicator of metabolic resilience and arrhythmia susceptibility. Given that plasma histidine can be quantified rapidly and inexpensively, its integration into clinical practice could be feasible.

While this study offers valuable insights into a potential biomarker for AF, we recognize several limitations. First, only baseline histidine was assessed in both cohorts, and the potential impact of longitudinal variability in histidine levels on AF risk was not evaluated. The in-house cohort is a small cohort, and the need to collect blood from four different sites for each patient reduced its statistical power. Additionally, because intracardiac blood sampling was necessary, we included patients with PSVT undergoing radiofrequency ablation as the control group for ethical considerations. This resulted in discrepancies, such as age differences, between the PSVT and AF groups, potentially introducing confounding factors. It should also be noted that circulating metabolites in the UKB were quantified using nuclear magnetic resonance spectroscopy, whereas metabolites in our recurrence cohort were measured using liquid chromatography–mass spectrometry. Although both techniques are well established, they differ in sensitivity, specificity, and the range of metabolites detected. In addition, differences in ethnic composition between the predominantly White UKB cohort and our local cohort may influence metabolomic profiles and AF risk. Finally, the absence of *in vivo* validation experiments testing whether histidine supplementation can directly modulate atrial remodeling or AF susceptibility represents a key limitation of this study.

5. Conclusions

Histidine may represent a potential biomarker for AF risk and a modifiable metabolic target. Future studies with postablation analyses and careful matching of patient characteristics are required to validate our findings and better understand the value of histidine.

Acknowledgements

The authors would like to thank all the participants included in this study.

Ethical statement

The UK Biobank study was approved by the North West Multicentre Research Ethics Committee (reference 11/NW/0382) and conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent to participate in the UK Biobank study. The study is reported in accordance with ARRIVE guidelines. The use of human samples in the recurrence cohort was approved by the Institutional Review Board of the Peking University First Hospital (Beijing, China, 2020-451, approved on April 3, 2023) and was

conducted following the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. All participants provided informed consent. The animal research protocols were approved by the Institutional Animal Care and Use Committees at Peking University (Beijing, China, IRB00001052-14045, approved in August 2014). All methods were performed in accordance with the relevant guidelines and regulations.

Conflicts of interest

This research has been conducted using the UK Biobank resource under Application Number 217390. The authors have no conflicts of interest to disclose.

Funding source

This work was funded by the National Natural Science Foundation of China (Grant Nos. 81930105 and 82370312).

Data availability statement

The Bulk RNA-seq datasets generated and analyzed during the current study are available in the GEO repository (GSE289284).

Author contributions

Hongxuan Xu, Pinchao Lv, and Lin Wu conceived the idea of this study and performed animal experiments. Hongxuan Xu performed most *in vitro* experiments; analyzed and interpreted the results; and was a major contributor to writing the manuscript. Panhui Tian curated and analyzed the UKB data. Panliang Zhong contributed substantially to the statistical method. Bingxun Li collected the clinical characteristics and plasma of the participants and performed the ELISA tests. All authors read and approved the final manuscript.

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