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ORIGINAL RESEARCH

Mineralocorticoid Receptor Antagonism Reduces Atrial Arrhythmias Post-Cardiac Surgery and Attenuates Atrial Stress Responses to Cardioplegic Arrest

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BACKGROUND: Postoperative atrial fibrillation occurs in ~40% after cardiac surgery. Mineralocorticoid receptor antagonists (MRA) are known to reduce chronic atrial fibrillation (AF) development and burden. We evaluated the association of preoperative MRA use with postoperative atrial fibrillation and investigated atrial cell types modulated by MRAs during cold preservation.

METHODS: We studied 19042 cardiac surgery patients at Mayo Clinic. Propensity 1:3 matching identified 298 MRA users and 894 non-users. A subgroup analysis of patients on any preoperative diuretic was performed to isolate cardiac-specific effects of MRAs, matching 298 MRA users to 894 non-MRA diuretic users. AF recurrence was assessed for up to 6-years. Single-nucleus RNA sequencing (snRNA-seq) was performed on human donor atria exposed to canrenone (a water-soluble MRA) during cold preservation with ex-vivo reperfusion, and expression profiles were compared with atria from patients with AF.

RESULTS: After matching, preoperative MRA use was associated with a lower incidence of postoperative atrial fibrillation (19.8% versus 31.8%, $P<0.001$). In the diuretic-only subgroup, MRA users also had lower postoperative atrial fibrillation (19.8% versus 33.2%, $P<0.001$). MRA use was associated with a reduced incidence of paroxysmal and chronic AF at 6-years. snRNA-seq identified a cardiomyocyte subpopulation, CM2, with high mineralocorticoid receptor expression where canrenone suppressed cold preservation-induced mineralocorticoid receptor target gene expression, which was conversely elevated in chronic AF. Canrenone also attenuated stress-response in atrial macrophages and pericytes.

CONCLUSIONS: Preoperative MRAs were associated with reduced postoperative and long-term AF after cardiac surgery. Mechanistically, our ex-vivo human atrial model revealed that MRAs suppress mineralocorticoid receptor -driven atrial stress responses, particularly in conduction-relevant cardiomyocytes.

Key Words: atrial fibrillation ■ canrenone ■ cardiac preservation ■ cardiac surgery ■ mineralocorticoid receptor antagonist ■ RNA sequencing

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CLINICAL PERSPECTIVE

What Is New?

- This study shows that preoperative use of mineralocorticoid receptors antagonists (MRA) is associated with a reduced incidence of new onset postoperative atrial fibrillation after cardiac surgery using cardiopulmonary bypass.
- We show that preoperative MRA use is associated with a lower incidence of developing more chronic paroxysmal or sustained atrial fibrillation.
- Addition of canrenone, a clinically used water soluble MRA, to cardioplegia solution used during cardiac preservation can attenuate atrial inflammatory responses and reduce signaling through molecular pathways that promote atrial fibrillation.

What Are the Clinical Implications?

- Perioperative use of MRAs may be considered to reduce early postoperative atrial fibrillation as well as lowering the risk of developing more chronic atrial arrhythmias.
- These findings support pursuing a clinical trial to determine the impact of MRA use on atrial arrhythmias following cardiac surgery in the setting of cardiopulmonary bypass with cold cardiac preservation.

Nonstandard Abbreviations and Acronyms

EC	endothelial cell
HTK	Histidine-Tryptophan-Ketoglutarate
MRA	mineralocorticoid receptor antagonist
MRc	mineralocorticoid receptor
POAF	postoperative atrial fibrillation
STS	Society of Thoracic Surgeons
STS-ACSD	Society of Thoracic Surgeons Adult Cardiac Surgery Database

New onset postoperative atrial fibrillation (POAF) after cardiac surgery is associated with increased perioperative complications and early mortality.[1–3] POAF incidence ranges from 20% to 40% after coronary artery bypass grafting with or without aortic valve replacement and 64% after combined CABG and mitral surgery. POAF rates are even higher in patients with heart failure.[4–6] Importantly, patients with new POAF after cardiac surgery have a high incidence of late atrial fibrillation (AF) recurrence of 10% to 49% over 3.4 to 8.3 years.[7–10] Thus, acute POAF episodes beget more long-term occurrence of AF with implications extending far beyond the postoperative setting. POAF is recognized as multifactorial with contributions from underlying co-morbidities, operative stress, cardiac preservation and reperfusion injury during cardioplegic arrest, fluctuations in intra-cardiac pressures as well as intrinsic and extrinsic hormonal exposures (eg, catecholamines).[4,11–13]

POAF incidence remains high despite the wide use of β-blockers and amiodarone to prevent and treat this complication.[14] Mineralocorticoid receptor antagonists (MRA, eg, spironolactone, eplerenone, finerenone) are well recognized to reduce AF burden in various non-surgical settings such as heart failure and diabetes.[15–19] The role of mineralocorticoid receptor (MRc) in POAF is remains unclear although individuals with hyperaldosteronism are known to have a higher rate of cardiovascular events including a 12-fold higher incidence of AF that is independent of hypertension.[20] This is attributed to MRc signaling activating the substrates for AF such as atrial fibrosis, modulation of ion channel expression as well as myocardial inflammation and oxidative stress.[21–24] Interestingly, patients with chronic AF have elevated baseline serum aldosterone levels but these decline after reverting to sinus rhythm.[25]

Given these findings, we examined the effects of preoperative MRA use and found that it is associated with a much lower incidence of POAF after cardiac surgery. Importantly, we also found that preoperative MRA reduces the development of AF in the longer term. We also gained insights through translational science studies on the impact of MRAs on cellular signaling in human left atrial tissue at the single-nuclei level. We anticipate these results to be clinically relevant for developing peri- and intraoperative targeted therapies to reduce POAF and importantly, lower the lifetime risk of AF which may be triggered by cardiac surgery.

METHODS

Data Availability Statement

Data and methodologies supporting the findings of this study will be available from the corresponding author upon reasonable request.

Clinical Study Population

Our clinical study design with a waiver of informed consent was approved by the Mayo Clinic Institutional Review Board (IRB, 23–009355, approved October 10th, 2023). We conducted a retrospective review of 45 368 patients who underwent open cardiac surgery using cardiopulmonary bypass from January 5th, 1993, to December 29th, 2023 at the Mayo Clinic

Department of Cardiovascular Surgery in Rochester, Minnesota. The study sample included adults aged ≥18 years and <75 years undergoing a "primary sternotomy" for isolated or combined surgeries that included coronary artery bypass grafting, myectomy for hypertrophic cardiomyopathy, and/or valve surgeries (aortic, mitral, tricuspid and/or pulmonary valve). We excluded patients with preexisting atrial arrhythmias (atrial fibrillation or flutter), prior cardiac surgery using cardiopulmonary bypass, cardiac surgery for emergent indications, or surgeries not mentioned previously (eg, aortic procedures, pericardiotomy, pulmonary thromboendarterectomy, thoracic transplantation, ventricular assist device implants). We excluded patients who underwent prior transcatheter atrial ablation therapy for atrial arrhythmias, concomitant surgical atrial MAZE procedure as well as left atrial appendage excision or ligation. The final study cohort consisted of 19042 patients of which 319 patients were on MRAs preoperatively. Graphical representation of the inclusion and exclusion criteria and development of the study cohort is presented in Figure 1.

Clinical Study End Points

We examined patient demographics, comorbidities, preoperative medication use, intraoperative parameters and postoperative occurrence of atrial arrhythmias. The primary end point of interest in the study was the occurrence of new AF after cardiac surgery. Early POAF were defined per Society of Thoracic Surgeons (STS) criteria as AF that occurs within 30 days after surgery or during index hospitalization and requires treatment or lasts longer than 1 hour.[26] This is detected during hospital EKG monitoring postoperatively. Later AF are episodes developing beyond 30 days after surgery and/or following hospital discharge. Long-term AF outcomes were defined as the time from index surgery to the first documented diagnosis of paroxysmal or chronic AF after discharge. Patients were censored at the last available clinical follow-up or death, with death treated as a competing risk. These are determined by careful medical record review. Secondary end points include cardiogenic shock, renal failure, stroke, transient ischemic attack, gastrointestinal bleeding, prolonged ventilation, length of hospital stay, and patient

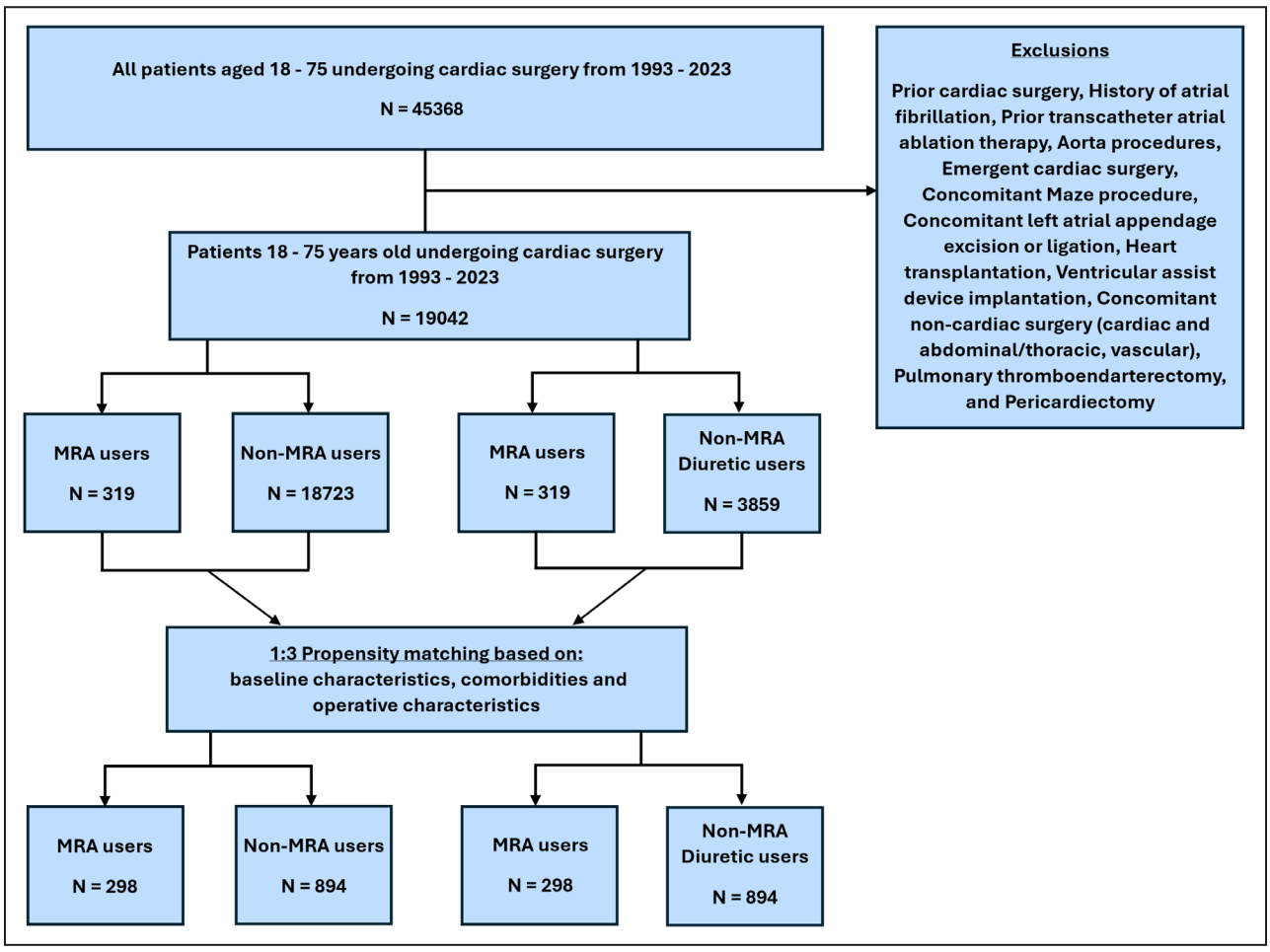

Figure 1. Consort diagram detailing the application of inclusion and exclusion criteria.

survival. Definitions for secondary end points are provided in Data S1.

Propensity Matching

Covariates were included in a logistic regression model to estimate the propensity of preoperative MRA use before undergoing cardiac surgery. We performed a 1:3 greedy matching algorithm without replacement using a caliper width of 0.02 standard deviations of the linear predictor (Figure 1). This ratio was selected after evaluating alternative schemes (1:1 and 1:2), with 1:3 providing the optimal trade-off between covariate balance and statistical power. Matched variables included patient age, sex, year of surgery, body mass index, creatinine, hypertension, diabetes, left ventricular ejection fraction, preoperative β-blocker and amiodarone use as well as history of transient ischemic attacks, cerebrovascular accidents, myocardial infarction and chronic lung disease. We also matched for intraoperative parameters including cardiopulmonary bypass time and aortic cross-clamp time as well as performance of coronary artery bypass grafting, myectomy for hypertrophic cardiomyopathy and valve surgeries (aortic, mitral, tricuspid, pulmonic valve repair or replacement). Following matching, there were 298 patients in the MRA group and 894 patients in the no MRA group. The reduction of atrial stretch and volume overload and electrolyte changes from preoperative diuretic use may impact the burden of postoperative atrial arrhythmias.[27–31] To separate the effects of diuretics drugs on volume and electrolyte status from cardiac specific effects of MRAs, we performed a subanalysis on patients using diuretics preoperatively and similarly performed propensity matching for preoperative MRA use (n = 298) or not (n=894).

Data Collection

A prospectively maintained institutional cardiovascular surgery database was used to obtain relevant demographic data, comorbidities, echocardiographic data, operative details, as well as postoperative and mid-term outcomes. The variables were defined on the basis of the criteria set forth in the (Society of Thoracic Surgeons (STS) Adult Cardiac Surgery Database). We conducted a 6-year follow-up using medical records to evaluate the severity of POAF in patients who developed the condition. Clinical details not included in the STS Adult Cardiac Surgery Database, such as development of chronic atrial arrhythmias, medication dosage and specific drug classes were extracted through retrospective chart review.

Statistical Analysis

Categorical data are presented as frequency (percentage of group), with comparisons using Pearson Chi-squared test. Continuous variables are expressed as the median (25^{th}-75^{th} percentile). Wilcoxon rank sum test was used to compare demographics, baseline characteristics, and operative parameters between patients. Patient survival was shown on a Kaplan–Meier curve and compared using log-rank statistics. The association between preoperative MRA use and new-onset POAF was assessed using logistic regression models within the matched cohorts. We also performed multivariable logistic regression in the overall cohort to identify independent predictors of POAF, adjusting for covariates chosen a priori based on clinical relevance, observed baseline imbalances, and established associations from the literature (Supplementary Table 6). Long-term AF outcomes (paroxysmal or chronic AF) were analyzed using Fine Gray subdistribution hazard models, accounting for death as a competing risk. Analyses were conducted using R software version 4.1.3 with p-values <0.05 considered statistically significant.

Research Ethics for Organ Donor and Surgical Heart Tissues

Human donor heart studies were approved by the University of Michigan Institutional Review Board (HUM00131275) and Mayo Clinic Institutional Review Board (23-006893). Atrial specimens were obtained from patients undergoing heart surgery after securing informed consent. Mayo Clinic Institutional Review Board approval (23-008400, 23-007374) was also obtained for surgical tissue collection.

Procurement and Preparation of Human Donor Atrial Tissue

Heart procurement for humans was performed as per clinical standard. A sternotomy is performed under general anesthesia. The pericardium is then opened to create a pericardial cradle for the heart. Heparin (300 U/kg) is administered in a peripheral vein (for humans). After waiting 3 minutes for adequate anticoagulation from heparin, a 9 Fr antegrade cardioplegia infusion catheter is placed in the ascending aorta. Approximately 1 liter of autologous donor arterial blood is then collected into a collection bottle containing heparin. The human donor heart is then retrieved per clinical protocol by incising the inferior vena cava to drain the right heart and then the left atrial appendage is incised to drain the left heart. Subsequently, the ascending aorta is cross-clamped distal to the cardioplegia catheter and 1 liter of cold (4 °C) Histidine-Tryptophan-Ketoglutarate solution (HTK) is infused into the coronaries at a perfusion pressure of ~80 mm Hg to induce mechanical arrest. After excising the heart completely, it is transported to the back table for infusion of 2 liters

of HTK±Canrenone (50 μmol/L) solution. Canrenone is a MRA metabolite of spironolactone. Previously, a canrenone dose–response titration showed that 50 nmol/L was most effective at optimizing human ventricular function following cold preservation.[32] Human atria in our study were therefore exposed to the same dose.

After completion, the heart is stored in a solution identical to the perfusate at 4 °C on ice. Human hearts underwent ex-vivo perfusion after cold (4 °C) static preservation for 10 hours. We selected a prolonged cold static preservation period of 10 hours to promote robust activation of inflammation and oxidative stress, both known mediators of atrial fibrillation.[33,34] The left atrial appendage was collected and snap frozen in liquid nitrogen for later analysis. Clinical details such as demographics and comorbidities of human heart donors as well as cardiac surgery patients donating left atrial appendages are provided in "Data S1".

Human Ex-Vivo Heart Perfusion

Details of human heart perfusion were described previously.[35] Briefly, the ascending aorta is accessed using an arterial perfusion cannula and the pulmonary veins are sutured shut to close the left atrium. A catheter is placed in the left ventricle through the left atrium as a vent. The Radnoti constant perfusion pressure apparatus (ADInstruments Inc., Colorado Springs, CO) was used for these human ex-vivo heart perfusion studies. About 1 liter of autologous blood was added to ~3.5 L of Krebs buffer. This was initially infused at 24 °C with progressive warming to 37 °C. Great care is taken to remove air from the perfusion system. The ascending aorta and coronary arteries were then retrogradely perfused through the arterial cannula at ~80 mm Hg with subsequent cardiac reanimation. The perfusion pressure was kept constant at ~80 mm Hg so left ventricle contraction would need to overcome this afterload for cardiac output. Oxygen (pO_2) pressure was kept at approximately 150 to 200 mm Hg while pCO_2 was maintained at about 35 to 40 mm Hg. Arterial pH was kept relatively constant at 7.35 to 7.40 via buffering with 5% CO_2 during gas exchange. The human donor heart was ex-vivo perfused for 3 hours and then arrested with 4 °C HTK solution for left atrial appendage specimen collection with liquid nitrogen.

Single Nuclei RNA-Seq

Human heart tissues were collected from the left atrial appendage of hearts from organ donors without AF (n=3 collected immediately after procurement, n=3 cold preserved with HTK followed by ex-vivo perfusion and n=3 preserved with HTK+canrenone followed by ex-vivo perfusion) and following consent from patients with AF undergoing cardiac surgery (n=3) at the Mayo Clinic in Rochester, Minnesota. Among these 3 patients with AF, the duration of AF before surgery was approximately 77 months, 63 months, and 5 months, respectively. Collection of specimens for this study was approved by the Mayo Clinic IRB (#23-008400, approved October 3, 2023; #23-007374; #23-006893). Singleron Biotechnologies (Ann Arbor, Michigan) performed the single nuclei RNA-seq. Single nuclei were isolated from flash-frozen appendage tissue followed by GEXSCOPE® library preparation followed by library sequencing at a depth of 90gb persample. The raw sequencing results were aligned to human genome GRCh 38 and aggregated with CellRanger v7.1.03.[36] The count matrix was further analyzed with Seurat v4.4.04.[37] Cells were filtered for a RNA count <25 000, RNA count >1000, and <5% containing mitochondrial gene. This filtered library was normalized with the open-source R package SCTransform (github.com/ChristophH/sctransform).[38] The RNA integrity Number of RNA from the donor heart after 10 hours of cold storage was >8. We also examined the cDNA from snRNA to assess the quality of RNA. All samples had less than 4% of fragments with size <300 bp indicating that there was no significant amount of RNA degradation.

The expression matrix from different samples was integrated using the RPCAIntergration function in Seurat. Uniform Manifold Approximation and Projection (UMAP) was used to visualize data[39] for dimension reduction. FindClusters function[40] was used for cluster detection with the default resolution (0.8). In addition to the graphical display, we used the FindMarkers function[40] in the Seurat package to perform differential expression gene analysis among phenotype-associated subpopulations using Wilcoxon signed-rank testing. We then annotated the cell types of each cluster based on the highest positive fold changes and correlated them with relevant specific cell markers described in the literature.[41] Fgsea was used to identify the gene set enrichment in the subcluster of each cell type. The expression matrix for individual cell type was extracted for further analysis. We identified the canrenone-regulated genes using Findmarkers. The expression of canrenone-regulated genes in each cell type was then estimated using the AddModuleScore function and tested with Wilcox ranking comparison.

RESULTS

Patient Characteristics

The study cohort included 19 042 patients who met inclusion criteria (Table S1). Compared with preoperative non-MRA users, MRA users (n=319) have a higher incidence of females (43.6% versus 31.4%, $P<0.001$), hypertension (73.0% versus 62.1%, $P<0.001$), diabetes (35.4% vs 21.6%, $P<0.001$), chronic lung disease (6.6% versus 2.4%, $P<0.001$), preoperative β-blocker use

(66.1% versus 52.5%, *P*<0.001), preoperative amiodarone use (2.5% versus 0.7%, *P*<0.001) as well as higher Society of Thoracic Surgeons (STS) surgical mortality risk (1.4% versus 1.0%, *P*<0.001). MRA users also had lower GFR (61.3 versus 67.5 mL/min per 1.73 m^2, *P*<0.001). MRA users also had worse left ventricular ejection fraction, more valve pathologies and longer cardiopulmonary bypass time as shown in Table S1.

Propensity-Matched Groups and New Onset Atrial Arrhythmias

Following 1:3 matching of the entire study population, there were 298 patients in the MRA user group and 894 patients in the non-MRA group (Table S2). Propensity score distribution for the entire study population as well as jitter plot and histogram for propensity score distribution in the matched cohorts are shown in Figures S1 and S2. POAF incidence was lower in the MRA user group (19.8% versus 31.8%, *P*<0.001) compared with non-MRA users. There were no differences in other identified outcome measures (Table S3).

Volume management using diuretics may impact atrial stretch and the development of atrial arrhythmias.[27–31] In an effort to focus on cardiac specific effects of MRAs beyond renal diuresis, we subsequently limited our analysis to patients on preoperative diuretics (n=4178) where clinical demographics were more comparable between MRA (n=319) and non-MRA diuretic users (n=3859). However, MRA users still had a greater burden of valvular pathologies and related procedures (Table S4). We again performed propensity matching to obtain comparable groups of MRA (n=298) and non-MRA (n=894) users (Table 1). Propensity score distribution for the entire study population as well as jitter plot and histogram for propensity score distribution in the matched cohorts are shown in Figures S3 and S4.

In the MRA group of propensity-matched patients using preoperative diuretics, 95% used spironolactone and 5% used eplerenone. Medication history was available for 84% of MRA patients, among whom the median duration of preoperative MRA use was 63 days (IQR: 20–286). Conversely, diuretic use in the non-MRA group consisted predominantly of loop diuretics alone (60.0%) and thiazide diuretics alone (30.5%) (Table 2). Preoperative MRA dosages are shown in Table S5, with 25 mg being the most common dose for both spironolactone (60.7%) and eplerenone (53.3%).

Comparing propensity matched preoperative diuretic use patients, the MRA group had a lower incidence of postoperative AF detected during their index operative admission or within 30 days of surgery at 59/298 (19.8%) versus 297/894 (33.2%; OR, 0.43, 95% CI, 0.31–0.60, *P*<0.001, Table 3). In the propensity-matched diuretic cohort, preoperative MRA use was associated with a significantly lower cumulative incidence of AF during long-term follow-up. Follow-up data at 6 years were available for nearly half of MRA users and 60% of non-MRA diuretic users within the matched cohort. At 6 years, the incidence of combined paroxysmal or chronic AF was 9.2% in the MRA group compared with 18.9% in the non-MRA diuretic group hazard ratio (HR), 0.45, *P*<0.001; Figure 2A). When analyzed separately, paroxysmal AF occurred in 6.6% of MRA patients versus 13.3% of non-MRA patients (HR, 0.48, *P*=0.005; Figure 2B), and chronic AF developed in 2.6% versus 5.6%, respectively (HR, 0.40, *P*=0.039; Figure 2C).

Patient Survival

Preoperative MRA patients had poorer long-term survival in both the total study population (P < 0.001) and after 1:3 propensity score matching (*P*=0.004; Figure S5A and S5B). Among patients using any preoperative diuretics, MRA users also had lower survival in the unmatched analysis (*P*=0.0012; Figure S5C). However, when restricted to the propensity-matched cohort of preoperative diuretic users, there was no statistically significant difference in long-term survival between groups (*P*=0.066; Figure S5D).

MRA Suppressed MRc Target Gene Sets Associated With Atrial Fibrillation

To investigate the potential mechanisms by which MRA reduces the occurrence of AF, we performed snRNA seq on left atrial tissue from organ donors without AF (n=3), patients with AF (n=3), and donor hearts that underwent cold preservation and perfusion with or without MRA (canrenone) treatment (n=3/group). After filtering the nuclei with low-quality nuclei, doublets, and ambient RNA contamination, we obtained 135 547 nuclei that were clustered into 10 distinct clusters. We annotated these clusters into adipocytes, cardiomyocytes, endothelial cells, fibroblasts, macrophages, mesothelial cells, neuron, smooth muscle cells, T cells and pericytes based on the marker gene expression (Figure 3A and 3B and Figure S6A). The cellular composition in AF is associated with a proportional increase in mesothelial cells (Figure S6B, *P*=0.026). Moreover, we found that MRc (NR3C2) expression was significantly increased in atrial endothelial cells, fibroblasts, macrophages, and mesothelial cells. In human hearts that were cold static preserved for 10 hours followed by ex-vivo reperfusion, canrenone treatment significantly inhibited the global atrial expression of MRc target genes (Figure 3D). MRA (canrenone) suppressed MRc target genes were conversely increased in the left atrium of patients with chronic atrial fibrillation (Figure 3D).

Table 1. Baseline Demographics, Pathology, and Operative Characteristics of Diuretic Users Stratified by Using MRA After Propensity Matching

	MRA users (N=298)	Non-MRA Diuretic users (N=894)	*P* value
Age, y*	63.3 (56.1, 68.4)	63.2 (55.9, 68.9)	0.686
Female†	129 (43.3%)	373 (41.7%)	0.635
Body mass index, kg/m^2 (IQR)*	30.4 (26.4, 35.0)	30.7 (26.6, 35.3)	0.899
Year of surgery*	2012.0 (2006.0, 2017.0)	2011.5 (2006.0, 2017.0)	0.814
NYHA functional classification†			0.042
I	23 (8.3%)	63 (7.9%)	
II	66 (23.9%)	211 (26.5%)	
III	151 (54.7%)	368 (46.2%)	
IV	36 (13.0%)	154 (19.3%)	
Hypertension†	216 (72.5%)	663 (74.2%)	0.569
Diabetes mellitus†	105 (35.2%)	310 (34.7%)	0.861
Dyslipidemia†	223 (75.1%)	673 (75.3%)	0.946
Coronary artery disease†	172 (57.7%)	503 (56.3%)	0.661
Previous myocardial infarction†	58 (19.5%)	180 (20.1%)	0.802
Peripheral vascular disease†	31 (10.4%)	93 (10.4%)	1.000
Cerebrovascular accident†	19 (6.4%)	48 (5.4%)	0.513
History of TIA†	18 (6.0%)	54 (6.0%)	1.000
GFR (mL/min per 1.73 m^2)*	61.2 (48.8, 72.3)	63.2 (50.9, 75.1)	0.029
Renal failure†	11 (3.7%)	42 (4.7%)	0.465
Dialysis†	5 (1.7%)	10 (1.1%)	0.453
Chronic lung disease, moderate–severe†	21 (7.1%)	56 (6.3%)	0.964
Preoperative beta blockers†	199 (66.8%)	600 (67.1%)	0.915
Use of amiodarone†	8 (2.7%)	26 (2.9%)	0.841
STS predicted risk of mortality (%)*	1.4 (0.8, 2.5)	1.4 (0.7, 2.6)	0.834
Echocardiographic findings			
Left ventricular ejection fraction (%)*	59.5 (39.0, 66.0)	58.5 (43.0, 65.0)	0.599
Pulmonary artery systolic pressure (mmHg)*	41.0 (30.0, 56.0)	39.0 (30.0, 52.0)	0.346
Moderate/severe aortic valve regurgitation†	44 (15.7%)	119 (14.2%)	0.486
Aortic valve stenosis, severe†	80 (26.8%)	233 (26.1%)	0.798
Moderate/severe mitral valve regurgitation†	109 (36.9%)	321 (35.9%)	0.085
Mitral valve stenosis, severe†	27 (9.1%)	63 (7.0%)	0.255
Moderate/severe tricuspid valve regurgitation†	64 (21.8%)	154 (17.3%)	0.049
Moderate/severe pulmonic regurgitation†	21 (7.4%)	51 (6.0%)	0.914
Procedures			
CABG†	150 (50.3%)	464 (51.9%)	0.639
Aortic valve replacement†	86 (28.9%)	256 (28.6%)	0.941
Aortic valve repair†	2 (0.7%)	9 (1.0%)	0.600
Mitral valve replacement†	28 (9.4%)	86 (9.6%)	0.909
Mitral valve repair†	51 (17.1%)	138 (15.4%)	0.492
Tricuspid valve replacement†	22 (7.4%)	53 (5.9%)	0.371
Tricuspid valve repair†	17 (5.7%)	52 (5.8%)	0.943
Pulmonary valve replacement†	14 (4.7%)	36 (4.0%)	0.617
Pulmonary valve repair†	1 (0.3%)	2 (0.2%)	0.739
Myectomy for hypertrophic cardiomyopathy†	45 (15.1%)	85 (9.5%)	0.007
Atrial septal defect repair†	20 (6.8%)	54 (6.2%)	0.681
Intraoperative cardiopulmonary support parameters			
Cardiopulmonary bypass time, min*	83.5 (61.0, 116.0)	86.0 (60.0, 115.0)	0.949
Aortic cross-clamp time, min*	59.5 (39.5, 83.8)	60.0 (39.0, 83.0)	0.714

Values are *median (interquartile range, IQR) with comparison using the Kruskal–Wallis rank sum test, and †frequency (percentage of group) with comparison using the Pearson Chi-squared test. CABG indicates coronary artery bypass grafting; GFR, glomerular filtration rate; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; STS, Society of Thoracic Surgeons; and TIA, transient ischemic attack.

Table 2. Diuretic Types in the Mineralocorticoid Receptor Antagonist (MRA) Users and Non-MRA Diuretic Users After Propensity Matching

Diuretic groups	N (%)
MRA diuretic (n=298)	
Spironolactone	
Spironolactone alone	114 (38.2)
Spironolactone+Loop diuretic (furosemide, torsemide, or bumetanide) Spironolactone+Thiazide diuretic (hydrochlorothiazide, chlorthalidone or metolazone)	132 (44.3) 27 (8.7)
Spironolactone+Loop diuretic+Thiazide diuretic	8 (3.0)
Spironolactone+Thiazide diuretic+Potassium-sparing diuretic (Triamterene)	1 (0.3)
Spironolactone+Acetazolamide	1 (0.3)
Eplerenone	
Eplerenone alone	9 (3.0)
Eplerenone+Loop diuretic	6 (1.9)
Non-MRA diuretic (n=894)	
Loop diuretic alone	537 (60.0)
Thiazide diuretic alone	273 (30.5)
Thiazide diuretic+potassium-sparing diuretic (Triamterene or Amiloride)	61 (6.8)
Loop diuretic+thiazide diuretic	19 (2.1)
Potassium-sparing diuretic alone	2 (0.2)
Acetazolamide alone	2 (0.2)

Canrenone Inhibits Activation of Specific MRc Genes in Human Atrial Cardiomyocytes

Given the key role of cardiomyocytes in electrical signal transduction[42] and AF pathogenesis,[43–45] we performed recluster analysis on total cardiomyocytes and identified 2 groups of cardiomyocytes (Figure 4A). Patients with AF had an increase in the cardiomyocyte subtype 2 (CM2) population compared with non-AF donors (Figure 4B). Interestingly, the CM2 population was enriched for gene expression related to inflammation and cellular stress such as "apoptosis", "interferon_gamma_response" and "unfolded protein response" gene sets (Figure 4C). CM2 cardiomyocytes were also enriched for MRc downstream genes (Figure 4D, P=0.0002). Importantly, addition of canrenone to histidine-tryptophan-ketoglutarate (HTK) preservation solution in preserved-reperfused human hearts reduced atrial cardiomyocytes MRc target gene expression compared with HTK only control in the same MRc geneset[46] (Figure 4E, P=0.027). Furthermore, canrenone suppressed cardiomyocyte expression of a gene subset normally induced by cold cardiac preservation. These genes are conversely elevated in patients with chronic AF (Figure 4F). However, canrenone activated genes were also elevated in patients with chronic AF (Figure 4G). These cardiomyocyte genes suppressed by canrenone may represent mechanisms by which MRAs can prevent POAF following cardiac preservation.

Canrenone Suppresses Macrophage Expression of Genes Associated With Atrial Fibrillation

Given the key role of macrophages in chronic atrial fibrillation,[47] we also reclustered the macrophage population from combined donor and AF samples thus revealing 3 different subclusters (Figure 5A). There were no significant differences in the relative composition of macrophage subsets between the donor and AF atrial samples (Figure 5B). We then examined the differential expression genes between the preserved-reperfused donor hearts with and without canrenone. Interestingly, macrophage expression of genes that were repressed by canrenone are significantly higher in patients with chronic AF (Figure 5C). However, genes that were activated by canrenone following preservation-reperfusion were also activated in AF atria (Figure 5D). In the human preserved-reperfused hearts for example, canrenone suppressed macrophage associated FKBP5[48,49] and ZBTB16[50,51] expression which are elevated in the setting of chronic AF (Figure 5E). Both are known key genes associated with cellular stress, cell death, inflammation as well as AF. However, we did not see any difference in MRc geneset expression in AF versus donor atria (Figure 5F) nor in cold preserved-reperfused donor heart with or without canrenone treatment (Figure 5G).

Canrenone Suppresses Pericyte Expression of Genes Associated With Atrial Fibrillation

Atrial endothelial cells (ECs) from combined donor and AF atrial samples were reclustered into 8 different subsets (Figure S7A). The atrial EC1 population was significantly enriched in AF patients (Figure S7B). EC1 cells had enriched gene sets related to adipogenesis, and interferon alpha/gamma responses (Figure S7C). EC expression of canrenone-repressed (Figure S7D) gene were increased in atria of AF patients. However canrenone-activated genes (Figure S7E) were also activated in chronic AF atria. There was no significant enrichment of MRc signals in the EC1 population compared with other EC subclusters (Figure S7F, P=0.38). The addition of canrenone did not impact endothelial expression of MRc gene targets compared with HTK-only treated hearts (Figure S7G, P=0.81).

In atrial smooth muscle cells, we identified MRA (canrenone) repressed genes in smooth muscle cells that were also similarly decreased in AF patients compared with donor atria (Figure S8A). Similarly, MRA (canrenone)

Table 3. Postoperative Outcomes and Complications of Diuretic Users Stratified by Using MRA After Propensity Matching

Variable	MRA users (N=298)	Non-MRA Diuretic users (N=894)	*P* value
Postoperative atrial fibrillation*	59 (19.8%)	297 (33.2%)	<0.001
Length of hospital stay, admit to discharge (d)†	7.0 (5.0, 9.0)	7.0 (5.0, 10.0)	0.652
Length of ICU stay at index admission (h)†	26.0 (21.0, 50.5)	25.0 (21.0, 47.0)	0.197
Operative mortality within 30 d*	8 (2.7%)	13 (1.5%)	0.164
Readmission within 30 d*	26 (9.4%)	91 (10.8%)	0.492
Stroke*	5 (1.7%)	12 (1.3%)	0.672
TIA*	3 (1.1%)	7 (0.9%)	0.697
Superficial/deep sternal infection*	6 (2.0%)	16 (1.8%)	0.804
Permanent pacemaker*	14 (4.7%)	44 (4.9%)	0.876
Intra-aortic ballon pump use*	20 (6.7%)	68 (7.6%)	0.609
Extracorporeal membrane oxygenation use*	1 (0.3%)	4 (0.4%)	0.800
Cardiogenic shock*	5 (1.7%)	11 (1.2%)	0.561
Tamponade requiring pericardiocentesis*	6 (2.0%)	15 (1.7%)	0.703
Postoperative blood products used†			
Postoperative red blood cells (units)**	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	0.933
Postoperative fresh frozen plasma (units)**	2.0 (2.0, 4.0)	2.0 (2.0, 3.0)	0.268
Postoperative platelet (units)**	1.0 (1.0, 3.0)	1.0 (1.0, 2.0)	0.236
Prolonged ventilation*	29 (9.7%)	82 (9.2%)	0.774
Pneumonia*	8 (2.7%)	25 (2.8%)	0.919
Sepsis*	6 (2.0%)	9 (1.0%)	0.177
Renal failure*	13 (4.4%)	37 (4.1%)	0.867
New dialysis*	7 (2.3%)	24 (2.7%)	0.753
Gastrointestinal bleeding*	6 (2.0%)	25 (2.8%)	0.462

Values are *frequency (percentage of group) with comparison using the Pearson Chi-squared test, †median (interquartile range, IQR) with comparison using the Kruskal–Wallis rank sum test, **frequency (percentage of group) with comparison using the Chi-squared test for given probabilities. ICU indicates intensive care unit; MRA, mineralocorticoid receptor antagonist; and TIA, transient ischemic attack.

activated genes in smooth muscle cells were also increased in atria of AF patients compared with donors (Figure S8B). Smooth muscle cell expression of MRc target genes were also not different between atria of AF patients (Figure S8C) and donors nor did canrenone treatment impact their expression in cold preserved-reperfused donor hearts (Figure S8D).

We observed that MRA treatment with canrenone attenuated atrial stress responses by suppressing MRc target gene expression in cardiomyocytes, macrophages, and pericytes under conditions simulating cardiac preservation and reperfusion. In atrial pericytes, MRA (canrenone) repressed and activated genes that are conversely up (*P*=0.036) and down-regulated (*P*=0.00067) in atrial pericytes from patients with AF (Figure S8E and S8F). While atria of patients with AF had higher expression of MRc target genes compared with donors (Figure S8G), canrenone treatment did not impact pericyte expression of MRc target genes in cold preserved-reperfused hearts. Findings from the vascular cell populations suggest that the EC and smooth muscle cells may not be the target of canrenone for preventing POAF. However, MRA treatment of cold preserved hearts did impact pericyte expression of genes that conversely demonstrate mirrored expression in the atria of patients with AF.

DISCUSSION

While MRAs are known to reduce the development and burden of AF in chronic medical settings,[15–19] initiating MRA in the acute setting for reducing POAF is less clear. Importantly, MRA is mostly prescribed for patient population with heart failure and hypertension thus skewing the MRA group to have sicker patients.[52,53] Using propensity matching to obtain more comparable patient populations, we show that preoperative MRA use in the total propensity-matched population reduced POAF from 31.8% to 19.8%. Even for propensity-matched populations who used preoperative diuretics, POAF was reduced from 33.2% to 19.8% suggesting that MRA has effects on reducing POAF that is independent of volume reduction. Furthermore, to improve cohort homogeneity and minimize age-related confounding, we excluded patients aged older than 75 years, as extremes of biological age potentially introduce substantial variability in atrial substrate, comorbidities, and

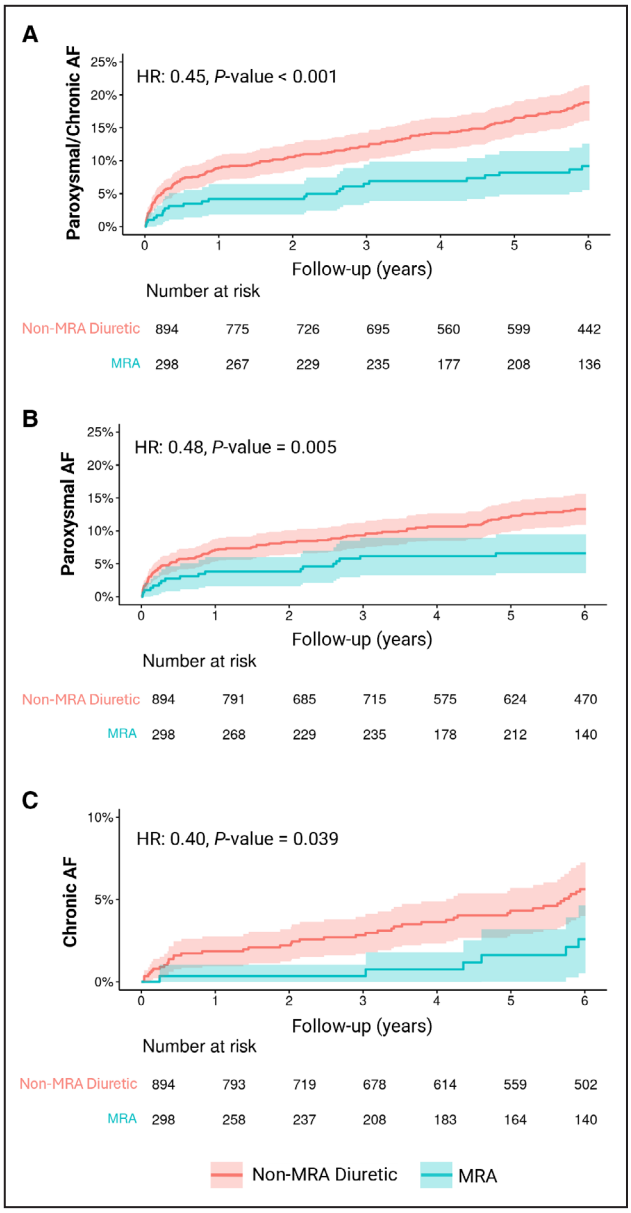

Figure 2. Long-term atrial fibrillation outcomes in propensity-matched diuretic users stratified by preoperative mineralocorticoid receptor antagonist use.
A, Cumulative incidence of combined paroxysmal or chronic atrial fibrillation (AF) after cardiac surgery. **B**, Cumulative incidence of paroxysmal AF. **C**, Cumulative incidence of chronic AF. Hazard ratios (HR) and *P* values were derived from Fine-Gray subdistribution hazard models, accounting for death as a competing risk. AF indicates atrial fibrillation; HR, hazard ratio; and MRA, mineralocorticoid receptor antagonist.

competing risks that could obscure the independent effects of preoperative MRA therapy.

Importantly, patients with POAF are more prone to develop chronic atrial fibrillation long-term. Bianco et al reported that the POAF cohort had a significantly higher incidence of atrial fibrillation on follow-up for up to 5 years (11.74% vs 4.75%; P < 0.001).[54] In the current study, preoperative MRA use was associated with a significantly lower incidence of paroxysmal or chronic AF at 6 years. This suggests that preoperative MRA use should be further studied as a strategy to not only reduce POAF short term but also reduce the longer-term morbidity of chronic AF.

This study integrates clinical outcomes with mechanistic transcriptomic insights to strengthen the biological plausibility of our findings. The observed reduction in POAF and long-term AF with preoperative MRA use is supported by gene expression analyses showing that canrenone attenuates atrial stress responses by suppressing MRc target pathways in key atrial cell types. This dual approach provides a comprehensive rationale for MRA use in AF prevention.

MR activation as a mechanism for POAF have been implicated in the ALDO-POAF study (ALDOsterone for prediction of Post-Operative Atrial Fibrillation, NCT 02814903) where higher preoperative aldosterone levels were shown to be a biomarker for identifying patients susceptible to developing POAF after coronary artery bypass grafting (CABG) with or without aortic valve replacement.[55] This implies a key pathogenic role of MRc activation in POAF.[55,56] Based on this rationale, Pretorius et al[57] randomized 445 patients to preoperative oral ramipril (2.5–5 mg daily), spironolactone (25 mg daily) or placebo for 4–7 days before elective CABG or valve surgery. Although preoperative MRA was associated with less postoperative renal failure and quicker extubation, there was unfortunately no difference in POAF rates between the groups.[57]

However, limitations in the trial by Pretorius et al[57] include being inadequately powered since it recruited 147 to 151 patients per group for a relatively low incidence of POAF of 27% in their study population. Furthermore, this study included patients who had paroxysmal or chronic AF as long as it did not occur within 6 months of the enrollment. It is also likely that in this baseline MRA naïve population, there was a failure to reach therapeutic circulating MRA threshold levels for inhibition of cardiac inflammation and oxidative stress during cardioplegic arrest. We previously showed that optimized cardiac preservation quality can be achieved with intracardiac dosing of canrenone at a concentration of 50 μM given along with cardioplegia solution during cardiac surgery.[32] The Aldosterone Targeted Neurohormonal Combined with Natriuresis Therapy in Heart Failure (ATHENA-HF) trial for determining the efficacy of oral MRA (spironolactone) for treating acute heart failure gave important insight into challenges of oral spironolactone treatment duration and dose for reaching therapeutic systemic level of spironolactone active metabolites. It was shown that even at spironolactone 100 mg oral daily, many of its active metabolites

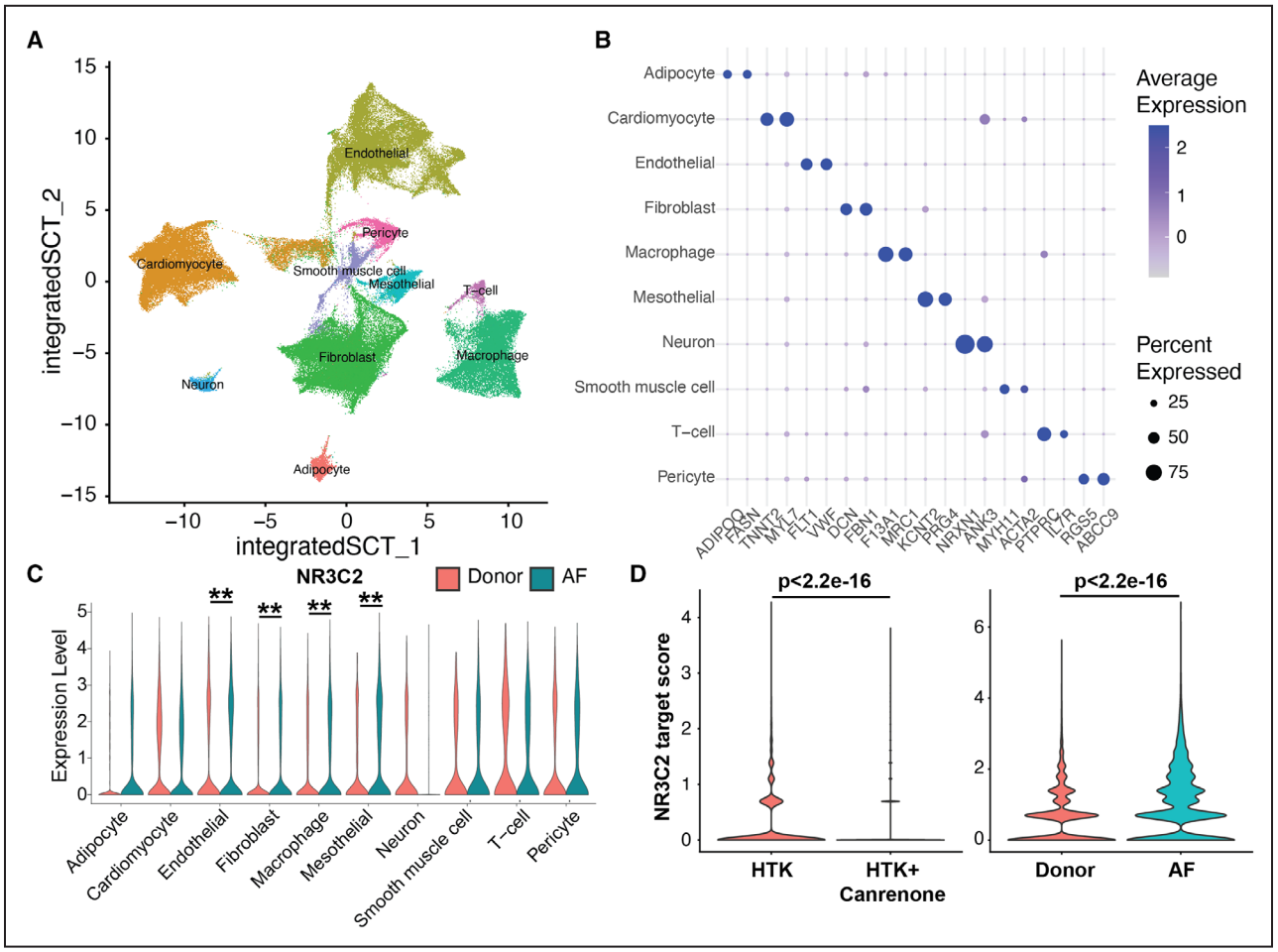

Figure 3. Single-nucleus RNA sequencing of human atria.
A, Representative uniform manifold approximation and projection (UMAP) plot of snRNA-seq of LA tissues from different donors with integrated SCT reduction. 10 different cell types were clustered and identified. **B**, Dot plots of the cell-specific marker gene expression. **C**, MRc (NR3C2) expression in each cell type in donor and AF patients was shown in the violin plot. ** and cell types in red letters indicate $P<0.001$. **D**, Violin plot of MRc target expression in the left atrium of reperfused donor heart with or without canrenone treatment as well as donor and patients with AF. AF indicates atrial fibrillation; LA, left atrium; MRc, mineralocorticoid receptor; NR3C2, Nuclear Receptor Subfamily 3 Group C Member 2; snRNA-seq, single-nucleus RNA sequencing; SCT, SCTransform normalization method; and UMAP, uniform manifold approximation and projection.

(including canrenone) remained at very low circulating concentrations after 96 hours in MRA naïve patients.[58] Patients who were on spironolactone previously had significantly higher concentrations[58] suggesting that oral spironolactone administration would take much longer than 1 week to reach therapeutic steady state concentration at a lower 25 mg dosage in MRA naïve patients in the study by Pretorius et al.[57] Direct intracardiac MRA (canrenone) administration by addition to cardioplegic solution during cardiac surgery is expected to overcome the limitations of patient compliance and achieving therapeutic MRA levels in cardiac tissue.

To further characterize the effects of MRA on gene expression in the left atrial cell population in the setting of cold cardioplegic arrest and cardiac preservation during cardiac surgery, we performed single-nuclei RNA sequencing (snRNA-Seq) analysis. Using canrenone, a water soluble MRA, we determine the cell populations that affected by MRc inhibition in terms of AF associated gene expression obtained by comparing gene expression between the atria of patients with AF and donors without AF. Canrenone, a water-soluble active metabolite of spironolactone, was able to suppress the upregulation of MRc target genes in cold preserved donor hearts and these MRc gene sets are conversely upregulated in the left atrium of patients with chronic AF in our study. These transcriptomic findings provide direct evidence that MRA therapy, particularly canrenone, attenuates atrial stress responses by suppressing key gene programs across multiple atrial cell types, supporting the biological plausibility of its

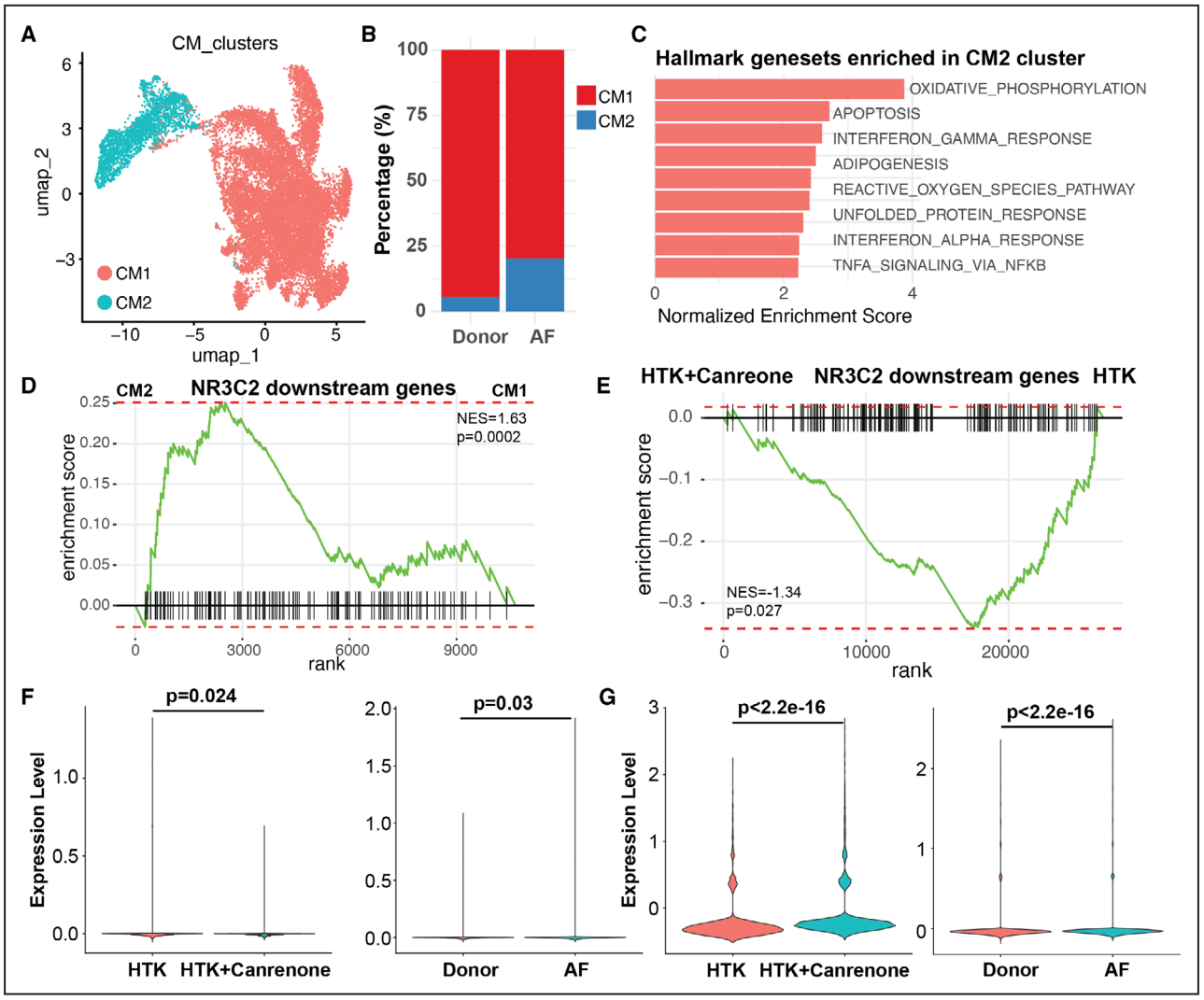

Figure 4. Effects of canrenone on atrial cardiomyocytes.
A, Recluster of cardiomyocytes showed in UMAP plot. **B**, Percentage of cardiomyocyte subclusters in donor (n=3) vs AF (n=3) patients. **C**, GSEA analysis showed enriched gene sets in the cardiomyocyte, CM2, compared with CM1. **D**, GSEA of NR3C2 downstream gene set expression in CM2 compared with CM1. **E**, GSEA showing effects from canrenone treatment of cold preserved-reperfused hearts on NR3C2 downstream genes in cardiomyocytes. **F**, Violin plot of canrenone repressed expression of global gene expression in the LA of reperfused donor heart with or without canrenone treatment as well as donor and AF patients. **G**, Violin plot of canrenone activated expression of global gene expression in the left atrium of reperfused donor heart with or without canrenone treatment as well as donor and patients with AF. AF indicates atrial fibrillation; LA, left atrium; GSEA, gene set enrichment analysis; NR3C2, Nuclear Receptor Subfamily 3 Group C Member 2; and UMAP, uniform manifold approximation and projection.

observed clinical effects in reducing both POAF and long-term AF burden.

Cardiomyocytes are known to serve an important role in cardiac electrical signal transduction,[42] and atrial fibrillation pathogenesis, including in the post-operative setting.[43–45] Therefore, we focused on the human atrial cardiomyocyte population showing the human CM2 cardiomyocytes are enriched for MRc expression consistent with their preferred activation of signaling pathways relevant for inflammation, cellular stress and cell death. Canrenone was able to suppress preservation associated MRc activation in atrial cardiomyocytes suggesting intraoperative canrenone added to cardioplegic solution used for cardiac preservation during cardiac surgery may reduce POAF burden.[59] As indicated in our global gene expression analysis in cardiomocytes, canrenone is also able to reduce the expression of other non-MR target genes that may be relevant for AF pathogenesis.

Recent reports have also indicated macrophages as playing a key role in AF.[47] Although there was no difference in the proportion of macrophage subsets, cardiac preservation using canrenone did suppress

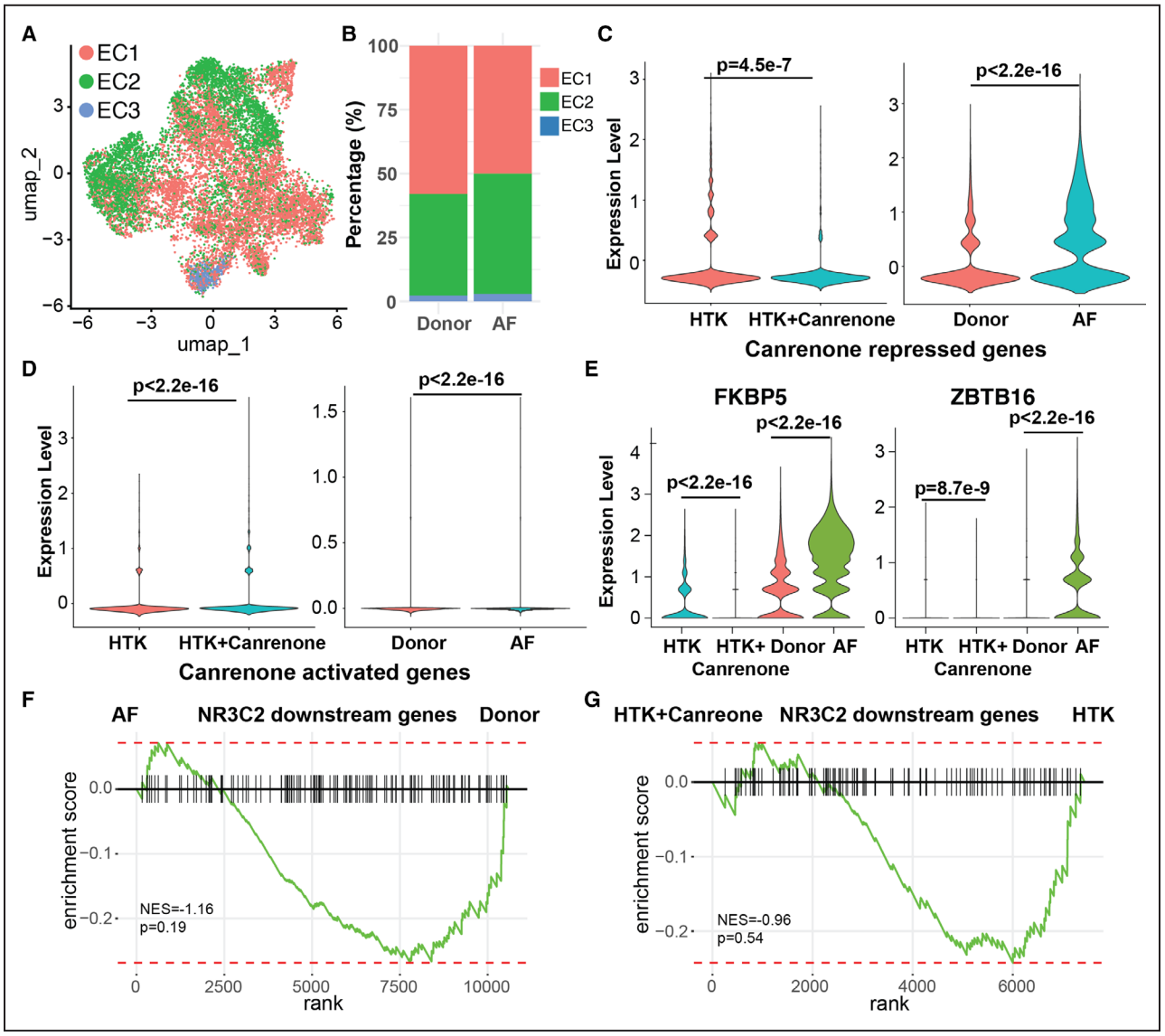

Figure 5. Effects of canrenone on atrial macrophages.
A, Recluster of macrophages showed in UMAP plot. **B**, Percentage of macrophage subclusters in donor vs AF patients. Violin plot of canrenone (**C**) repressed and (**D**) activated genes in macrophage of reperfused donor atria with or without canrenone treatment as well as in atria of donor heart (n=3) and AF (n=3) patients. **E**, Violin plot shows FKBP5 and ZBTB16 expression. **F,** GSEA showing macrophage expression of NR3C2 downstream gene set in AF vs donor atria. **G**, GSEA showing the effects of canrenone treatment on NR3C2 downstream gene expression in macrophages. AF indicates atrial fibrillation; FKBP5, FK506-binding protein 5; GSEA, gene set enrichment analysis; NR3C2, Nuclear Receptor Subfamily 3 Group C Member 2; UMAP, uniform manifold approximation and projection; and ZBTB16, Zinc Finger and BTB Domain Containing 16.

macrophage expression of genes that are elevated in atrial macrophages of patients with chronic AF. Examples include suppression of FKBP5 and ZBTB16 which have key role in cellular survival, stress responses, inflammation as well as specially in AF pathogenesis.[50,60] However, we did not detect an impact by canrenone on MRc geneset expression in macrophages. Canrenone also suppressed expression of a subset of cell specific genes in endothelial smooth muscle cells and pericytes, that were elevated in the respective cells in chronic AF. However, canrenone activated genes in these vascular cell types were also activated in the atria of patients with chronic AF. This suggests that cardiomyocytes are likely key targets for canrenone suppression of AF through MRc antagonism. However, it is possible that canrenone may also act on macrophages and vascular cells to reduce AF although this is more likely through a MRc independent or nongenomic mechanism[61] since it did not impact MRc geneset expression.

Study Limitations

Our study remains limited by its single institution retrospective design and associated biases, despite the use of propensity matching to obtain more comparable groups. Nonetheless, residual imbalances may remain despite propensity matching and covariate adjustment. Furthermore, POAF may also occur after patient discharge but might not be detected due to a lack of symptoms or patients not presenting for evaluation will be missing from our data set. Longer-term follow-up for the development of AF and its severity was based on medical documentation of AF noted during chart review. This may have underestimated true total burden of AF although we do not expect differences in documentation based on preoperative MR use. It is also possible that the effect of MRA reducing the incidence of POAF results from chronic effects of antagonizing structural remodeling[21,62,63] leading to a less dilated atrium and/or attenuating electrical remodeling of atrial tissue[21,62] rather than inhibiting the acute effects of cold preservation on the atria. Moreover, a systemic proinflammatory milieu from the effects of cardiopulmonary bypass[64,65] and/or general physiological stress from surgical trauma[65] may impact propensity for POAF independent of hyperactivation of MRc within the heart from cold preservation injury. Indeed, the sources of stimulus for POAF in the cardiac surgery setting likely differ from those in nonsurgical paroxysmal or chronic AF. Our snRNA-seq data are suggestive of the role of MRc signaling in POAF by correlating with gene expression signature of patient with chronic AF. We have not taken atrial tissue from patients who experience new POAF versus those who did not since atrial tissue is generally not removed unless there is pre-existing AF. Furthermore, it remains uncertain whether the observed benefits of MRA arise from chronic atrial remodeling effects of therapy or from the acute presence of circulating drug at the time of cardiac surgery. Removal of the left atrial appendage in the setting of pre-existing AF is done to reduce stroke risk.[66]

CONCLUSIONS

We present propensity matched retrospective data that show an important association between preoperative use of oral MRA with significantly reduced POAF and development of persistent AF after cardiac surgery using cardiopulmonary bypass. Furthermore, single-nuclei RNA sequencing studies suggests that canrenone added to cardiac preservation solution can inhibit signaling pathways related to chronic AF including MRc activation. Future mechanistics studies leveraging genetic MRc deficiency or knockdown in an AF prone model to specifically determine the role of MRc activation in POAF. Furthermore, these findings support the conduct of future clinical trials to determine the role of preoperative MRA therapy and/or canrenone added to cardioplegia solution for reducing early POAF as well as later development of persistent AF.

ARTICLE INFORMATION

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Supplemental Material

Data S1
Tables S1–S6
Figures S1–S8

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