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Original Article

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# Increased Cyclic Guanosine Monophosphate and Interleukin-1Beta Is Activated by Mitochondrial Dysfunction and Associated With Heart Failure in Atrial Fibrillation Patients

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## Abstract

**Background:** This study aimed to identify the association of cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase-stimulator interferon genes (cGAS-STING) pathway with heart failure (HF) in atrial fibrillation (AF) patients.

**Methods:** We prospectively enrolled 106 AF patients without evidence of HF. The serum levels of 2'3'-cyclic GMP-AMP (2'3'-cGAMP) and interleukin (IL)-1β were measured by enzyme-linked immunoassay (ELISA). To determine the underlying mechanism, we supplemented the complex I inhibitor rotenone and the specific cGAS inhibitor RU.521 in neonatal rat ventricular cardiomyocytes.

**Results:** During 18-month follow-up, serum concentrations of 2'3'-cGAMP (baseline 51.82 ± 11.34 pg/mL vs. follow-up 124.50 ± 75.83 pg/mL, $P_{paired}$ $t < 0.01$) and IL-1β (baseline 436.07 ± 165.82 vs. follow-up 632.48 ± 119.25 ng/mL, $P_{paired}$ $t < 0.01$) were substantially upregulated in AF patients with HF as compared with those without HF. Furthermore, serum 2'3'-cGAMP and IL-1β levels at 18-month follow-up were independently associated with the occurrence of HF in AF patients. Inhibition of cGAS by RU.521 effectively reversed the upregulation of 2'3'-cGAMP and STING phosphorylation induced by mitochondrial dysfunction, accompanied with inhibition of nod-like receptor protein 3 (NLRP3) inflammasome, IL-1β and IL-18 secretion.

**Conclusions:** Induction of mitochondrial dysfunction causes an upregulation of 2'3'-cGAMP and activation of NLRP3 inflammasome through cGAS-STING pathway in cardiomyocytes.

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**Keywords:** Atrial fibrillation; Heart failure; Mitochondria; cGAS-STING pathway; NLRP3 inflammasome

## Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia that causes ischemic stroke, heart failure (HF) and even mortality [1]. Although the current body of evidence has indicated that catheter ablation is superior to antiarrhythmic medication for management of AF and HF, nonparoxysmal AF such as persistent and long-standing persistent AF is prone to develop HF due to complex mechanism and limited therapeutic strategies [2, 3]. It is widely accepted that AF is initiated by electrical remodeling of various ion channels and exacerbated by structural remodeling [4]. During this pathological process, calcium overload in sarcoplasmic reticulum and elevated cytoplasmic calcium levels play an important bridging role, both of which lead to mitochondrial dysfunction [5, 6].

Recent studies suggest that mitochondrial dysfunction releases the mitochondrial DNA into cytosols, binding to cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS), producing a secondary messenger called 2'3'-cyclic GMP-AMP (2'3'-cGAMP) and activating stimulator interferon genes (STING) in failing cardiomyocytes upon metabolic abnormality and myocardial ischemia [7, 8]. Active STING traffics to lysosomes, promotes the efflux of potassium and membrane perturbation. Decreased cytosolic potassium turns on the classical model of nod-like receptor protein 3 (NLRP3) inflammasome activation and subsequently facilitates the secretion of interleukin (IL)-1β and IL-18 to accelerate sterile inflammation and pyroptosis [9, 10]. Therefore, mitochondria-cGAS-STING axis has recently emerged as a crucial mediator of cardiac remodeling and inflammation [11, 12]. Targeting mitochondrial DNA release and downstream cGAS-STING axis has been proved to alleviate cardiac hypertrophy and preserve myocardial function in murine model [13, 14]. However, it remains unclear that whether mitochondria-cGAS-STING axis is an early and independent signal for new-

onset HF in patients with nonparoxysmal AF.

In this study, we sought to determine the association of 2'3'-cGAMP and IL-1β with the occurrence of HF in patients with persistent and long-standing persistent AF. In addition, we investigated whether mitochondrial dysfunction induced by oxidative stress turned on cGAS-STING axis and promoted IL-1β secretion in cardiomyocytes.

## Materials and Methods

### Study population

A total of 106 persistent or long-standing persistent AF patients without evidence of HF were consecutively enrolled in our hospital from February 2018 to September 2019. Persistent AF was defined according to 2017 Heart Rhythm Society Expert consensus [15]. Long-standing persistent AF was determined if AF episode lasted more than 1 year without the detection of sinus rhythm. During the 18-month follow-up, 32 patients in New York Heart Association functional class II to IV with left ventricular ejection fraction (LVEF) ≤ 40% were documented as HF with reduced ejection fraction (HFrEF). We also calculated the number of patients with HFmrEF (HF with mid-range ejection fraction) and HFpEF (HF with preserved ejection fraction) defined as LVEF > 40% according to the 2021 European Society of Cardiology (ESC) guidelines on HF [16]. These HFrEF patients were also required to have a plasma level of N-terminal fragment B-type natriuretic peptide (NT-proBNP) ≥ 400 pg/mL. Exclusion criteria were 1) combination with other types of arrhythmias; 2) age ≤ 18 years; 3) a life expectancy < 1 year; 4) valvular heart disease. Simultaneously, we documented the comorbidities and medication of enrolled patients at baseline. Coronary artery disease (CAD) was diagnosed as lumen diameter of coronary narrowing over 50% by coronary angiography or coronary computed tomography angiography at baseline.

The study protocol adhered to the Declaration of Helsinki and was approved by the Institutional Review Board of our hospital. Proper written informed consent was obtained from all patients.

### Induction of mitochondrial dysfunction in primary cardiomyocytes

Neonatal rat ventricular cardiomyocytes (NRVMs) were isolated from 3-day-old Sprague Dawley rats. In brief, pups were placed on ice for 5 - 10 min for light anesthesia. The ventricles were minced and digested in 1 mg/mL collagenases type II solution in a 37 °C water bath pot, shaking gently. Every 10 min the solution containing the digested cells was transferred to a new tube containing fetal bovine serum (FBS) (Gibco, USA). The remaining not dissociated tissue residue was supplemented with fresh enzymatic solution for additional five times. The supernatant was centrifuged for 5 min (400 × g) and resuspended in Hank's balanced salt solution (HBSS) supplemented with 20% FBS. To remove any contaminating fibroblasts, collected cells were seeded onto uncoated 100-mm plastic dishes for 90 min. Unattached NRVMs were seed to new culture dishes containing Dulbecco's modified Eagle medium/nutrient mixture F-12 (DMEM/F12) medium (HyClone, USA) supplemented with 20% FBS and 1% penicillin/streptomycin.

We then applied the complex I inhibitor rotenone (0.1 μM, catalog R8875, Sigma Aldrich, USA), dissolved in dimethyl sulfoxide (DMSO), for 24 h to induce reactive oxygen species and mitochondrial dysfunction in NRVMs. RU.521 (catalog HY-114180, MedChemExpress, China), the specific cGAS inhibitor, was dissolved in DMSO to generate 10 nM solution. NRVMs were pretreated with RU.521 (10 μM) for 24 h to block cGAS-STING pathway.

### Measurement of serum 2'3'-cGAMP and IL-1β

Venous blood was obtained at admission with patients fasting from midnight onward. To determine the change of serum 2'3'-cGAMP and IL-1β, we also collected the venous blood of all enrollments at 18-month follow-up. The serum levels of 2'3'-cGAMP were measured by 2'3'-cGAMP enzyme-linked immunoassay (ELISA) kit (catalog 501700, Cayman, USA). The serum levels of IL-1β were determined by IL-1β ELISA kit (catalog CB10347-Hu, COIBO BIO, China). The supernatant of NRVMs was collected to quantitatively analyze the secretion of IL-1β (catalog CB10205-Ra, COIBO BIO, China) and IL-18 (catalog CB10203-Ra, COIBO BIO, China) by using commercially available ELISA kits.

### Measurement of mitochondrial dysfunction

To evaluate intracellular calcium, NRVMs were loaded with 1 μM of Rhod-2 probe (Invitrogen, USA) for 30 min. Rhod-2 AM was excited using 545-nm laser and the fluorescence emission was documented at 578 nm (PMID 28669047). Adenosine triphosphate (ATP) production in NRVMs was determined using colorimetric tests by ATP assay kit (catalog MAK190, Sigma-Aldrich, USA).

### Western blots

After treatment, NRVMs were lysed in 20 μL of 1 × cell lysis buffer (Cell Signaling Technology, USA). Protein samples were separated in 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF, Millipore, USA) membranes. Membranes were blocked for 1 h in 5% non-fat milk in tris-buffered saline and Tween 20 (TBST) and incubated with the following primary antibodies against cGAS (catalog sc-515802, SantaCruz, USA), phosphorylated STING (catalog 72971, Cell Signaling Technology, USA), STING (catalog ab288157, Abcam, USA), NLRP3 (catalog ab264468, Abcam, USA), gasdermin D (GSDMD) (catalog 39754, Cell Signaling Technology, USA), IL-1β (catalog sc-12742, SantaCruz, USA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

**Table 1.** Baseline Characteristics of Enrollments

| | No HF during follow-up (n = 61) | HFpEF/HFmrEF during follow-up (n = 13) | HFrEF during follow-up (n = 32) | P value |
|---|---|---|---|---|
| Age (years) | 64.46 ± 10.94 | 67.23 ± 11.43 | 64.88 ± 9.71 | 0.70 |
| Male, n (%) | 16 (26.2) | 4 (30.8) | 16 (50.0) | 0.07 |
| Persistent/long-standing persistent AF, n (%) | 37 (60.7)/24 (39.3) | 6 (46.2)/7 (53.8) | 13 (40.6)/19 (59.4) | 0.16 |
| Diabetes, n (%) | 11 (18.0) | 2 (15.4) | 6 (18.8) | 0.07 |
| Smoking, n (%) | 16 (26.2) | 4 (30.8) | 16 (50.0) | 0.07 |
| Hypertension, n (%) | 40 (65.6) | 9 (69.2) | 20 (62.5) | 0.91 |
| CAD, n (%) | 15 (24.6) | 6 (46.2) | 11 (34.4) | 0.25 |
| Stroke, n (%) | 15 (24.6) | 5 (38.5) | 9 (28.1) | 0.59 |
| Anticoagulant, n (%) | 29 (47.5) | 12 (92.3) | 24 (75.0) | < 0.01* |
| ACEI/ARB/ARNI, n (%) | 37 (60.7) | 8 (61.5) | 13 (40.6) | 0.16 |
| β-blocker, n (%) | 29 (47.5) | 6 (46.2) | 17 (50.0) | 0.86 |
| MRA, n (%) | 5 (8.2) | 0 (0.0) | 2 (6.3) | 0.56 |
| Antiarrhythmic drugs, n (%) | 24 (39.3) | 8 (61.5) | 10 (31.3) | 0.17 |
| LAD (mm) | 45.93 ± 5.29 | 46.23 ± 3.86 | 48.44 ± 6.04 | 0.10 |
| LVEF (%) | 57.03 ± 9.36 | 50.31 ± 10.56 | 33.78 ± 2.97 | < 0.01* |
| NT-proBNP (pg/mL) | 143.72 ± 90.10 | 662.87 ± 98.61 | 1,099.46 ± 107.67 | < 0.01* |

*P < 0.05. Data are expressed as mean ± SD or n (%). Antiarrhythmic drugs include amiodarone and propafenone. ACEI/ARB/ARNI: angiotensin-converting enzyme inhibitor/angiotensin receptor II blockade/angiotensin receptor neprilysin inhibitor; AF: atrial fibrillation; CAD: coronary artery disease; LAD: left atrial diameter; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonist; NT-proBNP: N-terminal fragment B-type natriuretic peptide; HF: heart failure; HFrEF: HF with reduced ejection fraction; HFmrEF: HF with mid-range ejection fraction; HFpEF: HF with preserved ejection fraction.

(catalog ab9485, Abcam, USA) at 4 °C overnight. GAPDH served as a house keeping gene. Afterward, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (SantaCruz, USA) and visualized by chemiluminescence. The band intensity was measured with ImageJ software (version V1.8.0.112, National Institutes of Health, USA).

### Statistical analysis

Statistical analysis was performed by SPSS software (version 20.0, SPSS Inc., USA). Continuous data with normal distribution were shown as mean ± standard deviation (SD). Unpaired Student's *t*-test was conducted to analyze the difference between two groups. Paired *t*-test was applied to compare the change of biomarker concentration within each group at baseline and 18-month follow-up. Differences among multiple groups were evaluated with one-way analysis of variance (ANOVA) followed by Bonferroni *post-hoc* test. To identify independent risk factors for developing HF with mid-range ejection fraction (HFmrEF)/HFpEF and HFrEF, statistically significant risk factors on univariable analysis were further incorporated in the multivariable logistic regression analysis. A P value < 0.05 was considered as statistical significance.

The sample size was determined a priori and powered to test the hypothesis. A power calculation was performed to detect a treatment difference with a two-tailed alpha 0.05, power 80%, minimum allowable difference in mean = 0.5 and conservative estimate of within-population variation = 3. A sample size of n = 87 was calculated [2, 17].

## Results

### Baseline characteristics of enrolled patients

The baseline characteristics of 56 persistent and 50 long-standing persistent AF patients are summarized in Table 1. Among the 32 patients who developed HFrEF during follow-up, 16 (47.1%) were male and 20 (58.8%) were long-standing persistent AF. Thirteen persistent/long-standing persistent AF patients developed HFmrEF/HFpEF during follow-up. The rate of anticoagulant therapy was markedly higher in HFmrEF/HFpEF and HFrEF groups than that in AF patients without HF (HFrEF 75.0%, HFmrEF/HFpEF 92.3%, normal 47.5%, P < 0.01). The proportion of administration of anticoagulants was markedly higher in AF patients with HFmrEF/HFpEF (92.3%) and HFrEF (75.0%) than those without HF (47.5%, P < 0.01). The usages of angiotensin-converting enzyme inhibitor/angiotensin receptor II blockade/angiotensin receptor neprilysin inhibitor (ACEI/ARB/ARNI), β-blocker, mineralocorticoid receptor antagonist (MRA) and antiarrhythmic drugs were comparable among the three groups. Likewise, we did not find significant differences in the incidences of comorbidities such

 

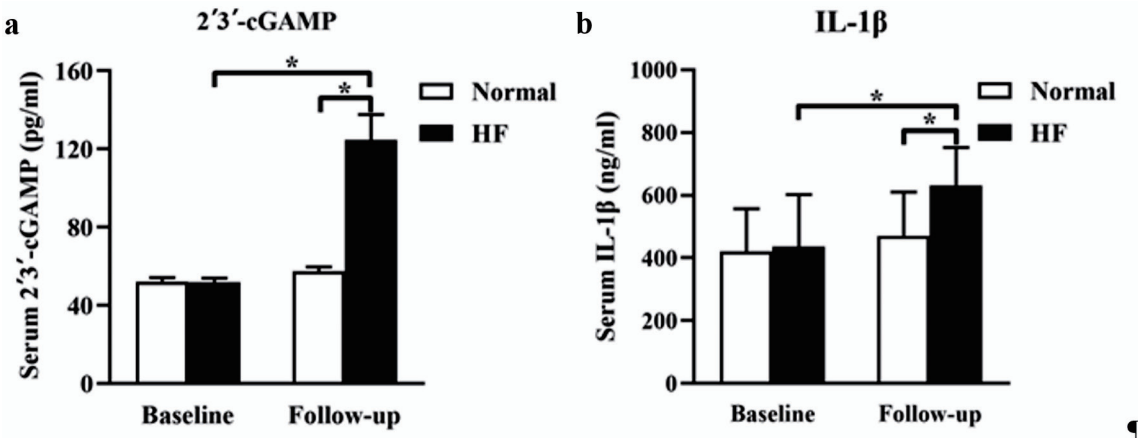


**Figure 1.** Comparison of serum 2'3'-cGAMP and interleukin (IL)-1β in AF patients with and without HF. Differences in serum levels of 2'3'-cGAMP (a) and IL-1β (b) between two groups were performed with Student's *t*-test. *P < 0.05. 2'3'-cGAMP: 2'3'-cyclic GMP-AMP; GMP: guanosine monophosphate; AMP: adenosine monophosphate; HF: heart failure; AF: atrial fibrillation.

smoking, hypertension, diabetes, CAD and stroke among the three groups. Echocardiographic data showed that left atrium tended to be larger in patients with HFmrEF/HFpEF and HFrEF than those without HFrEF.

### Increased serum 2'3'-cGAMP and IL-1β in AF patients with HF

The comparisons of serum 2'3'-cGAMP and IL-1β in AF patients with and without developing HF are shown in Figure 1. There were no significant differences in the baseline levels of serum 2'3'-cGAMP and IL-1β among the three groups. While serum concentration of 2'3'-cGAMP (baseline 51.82 ± 11.34 pg/mL vs. follow-up 124.50 ± 75.83 pg/mL, $P_{paired}$ $t$ < 0.01) and IL-1β (baseline 436.07 ± 165.82 vs. follow-up 632.48 ± 119.25 ng/mL, $P_{paired}$ $t$ < 0.01) was substantially upregulated in HF group during 18-month follow-up, their levels remained unchanged in AF patients with preserved heart function. Since NT-proBNP is referred to as an established index for severity and prognosis of HF, we found that serum 2'3'-cGAMP (r = 0.438, P < 0.01) and IL-1β (r = 0.378, P < 0.01) was positively associated with the levels of NT-proBNP in AF patients, respectively.

According to the multivariate logistic analysis, we found that administration of anticoagulants (odds ratio (OR): 2.86, 95% confidence interval (CI): 1.54 - 4.21, P = 0.02), elevation of serum 2'3'-cGAMP (OR: 1.74, 95% CI: 1.34 - 2.85, P = 0.02) and IL-1β (OR: 2.59, 95% CI: 1.83 - 4.25, P < 0.01) at 18-month follow-up were the independent predictors for the incidence of HF in AF patients (Table 2).

### Mitochondrial dysfunction activates proinflammatory cytokine release through cGAS-STING pathway

Mitochondrial dysfunction is a prevalent subcellular alteration underlying atrial and ventricular remodeling, predisposing to the development of HF in AF patients [18]. Moreover, previous studies reported that DNA released from mitochondria boosted cGAMP production, activating cGAS-STING pathway [11, 19]. To evaluate the potential effect of mitochondrial dysfunction on cGAS-STING and NLRP3 pathway, we applied complex I inhibitor rotenone to mimic mitochondrial dysfunction and used RU.521 to mitigate the activation of cGAS-STING pathway in NRVMs. First, we confirmed that administration of rotenone (0.1 μM) disrupted ATP production (Fig. 2a) and

**Table 2.** Multivariate Logistic Analysis for the Incidence of HF in AF Patients

| Variables | OR (95% CI) | P value |
|---|---|---|
| Male | 0.93 (0.17, 8.14) | 0.92 |
| Type of AF | 7.01 (0.85, 56.37) | 0.47 |
| Smoking | 0.92 (0.17, 3.80) | 0.54 |
| Hypertension | 1.85 (0.58, 3.92) | 0.64 |
| Diabetes | 1.94 (0.42, 7.23) | 0.83 |
| CAD | 1.72 (0.26, 3.47) | 0.91 |
| Stroke | 1.88 (0.77, 4.28) | 0.28 |
| Anticoagulant | 2.86 (1.54, 4.21) | 0.02* |
| ACEI/ARB/ARNI | 0.49 (0.13, 3.42) | 0.27 |
| β-blocker | 1.37 (0.54, 2.09) | 0.53 |
| MRA | 1.41 (0.75, 3.03) | 0.42 |
| Antiarrhythmic drugs | 1.55 (0.71, 4.08) | 0.37 |
| LAD | 1.32 (0.56, 3.93) | 0.68 |
| 2'3'-cGAMP | 1.74 (1.34, 2.85) | 0.02* |
| IL-1β | 2.59 (1.83, 4.25) | < 0.01* |

*P < 0.05. HF: heart failure; ACEI/ARB/ARNI: angiotensin-converting enzyme inhibitor/angiotensin receptor II blockade/angiotensin receptor neprilysin inhibitor; AF: atrial fibrillation; CAD: coronary artery disease; LAD: left atrial diameter; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonist; OR: odds ratio; CI: confidence interval; IL: interleukin; 2'3'-cGAMP: 2'3'-cyclic GMP-AMP; GMP: guanosine monophosphate; AMP: adenosine monophosphate.

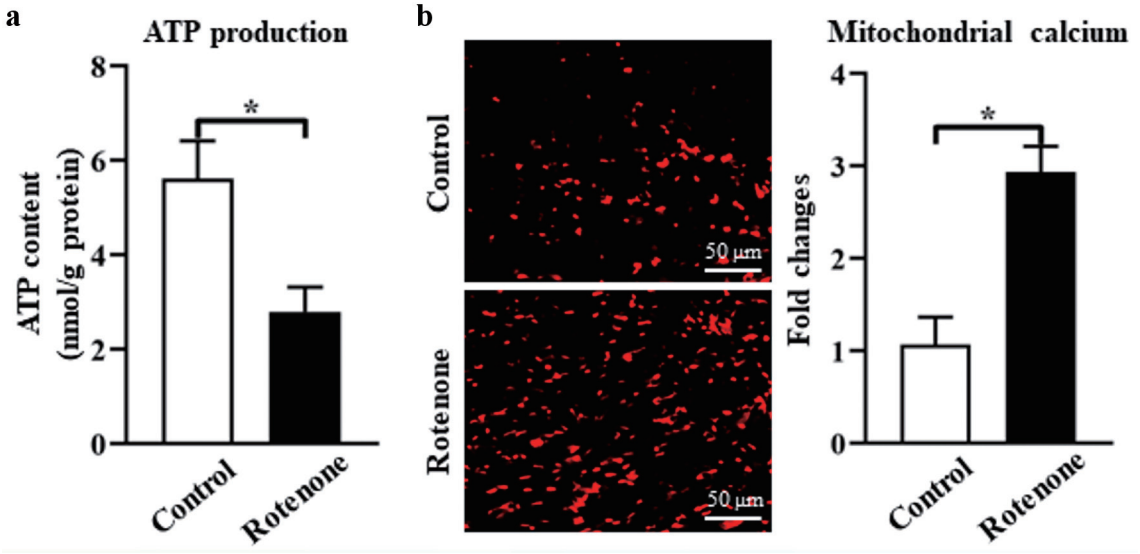


**Figure 2.** Complex I inhibitor rotenone induced mitochondrial dysfunction in neonatal rat ventricular cardiomyocytes (NRVMs). (a) NRVMs were treated with GAS antagonist RU.521 (10 μM) for 24 h in the presence of rotenone (0.1 μM). ATP content was measured using colorimetric tests. (b) Using immunofluorescence, cytoplasmic calcium in NRVMs was detected by Rhod-2 probe (labeled in red). Scale bar = 50 μm. * $P < 0.05$. GAS: guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase; ATP: adenosine triphosphate.

enhanced the levels of cytoplasmic calcium (Fig. 2b), resulting in mitochondrial dysfunction and escape of mitochondrial DNA into the cytosol. Second, we investigated the predominant cytosolic double-stranded (ds)DNA sensor and found a considerable upregulation of 2'3'-cGAMP content in NRVMs after rotenone treatment, accompanied with the increased expression of cGAS and phosphorylation of STING (Fig. 3a-c). By contrary, pretreatment of RU.521 significantly suppressed the content of 2'3'-cGAMP, which in turn reduced the expression of cGAS and phosphorylated STING (Fig. 3a-c).

As shown in Figure 4a, the protein level of NLRP3 was augmented in NRVMs which was induced by rotenone and returned to the normal range after RU.521 treatment. In parallel, inhibition of cGAS-STING pathway by RU.521 reversed the excessive cleavage of GSDMD induced by rotenone in NRVMs (Fig. 4a). Lastly, ELISA showed that RU.521 abrogated rotenone-induced accumulation of IL-1β and IL-18 in the supernatant of NRVMs (Fig. 4b). These data suggest the importance of cGAS-STING pathway and NLRP3 inflammasome in the connection of mitochondrial dysfunction and inflammation during the progression of HF.

## Discussion

In this study, we have shown that higher levels of serum 2'3'-cGAMP and IL-1β could independently predict the incidence of HFrEF in AF patients. We have also explored the underlying interaction among 2'3'-cGAMP, IL-1β and HF in NRVMs, finding that mitochondrial dysfunction induced by the complex I inhibitor results in the activation of cGAS-STING pathway and secretion of IL-1β, which could be partially reversed by inhibition of cGAS.

AF is considered as a predisposing risk factor for HF. Furthermore, compared with those with paroxysmal AF, a two-fold increase in either new-onset or worsening HF was observed in patients with nonparoxysmal AF [20]. It is widely accepted that catheter ablation in patients with nonparoxysmal AF achieves significant improvement of LVEF and retards the development of HF as compared to optimal medical treatment comprising antiarrhythmic drugs [21]. Unfortunately, hybrid ablation procedures do not yield a high rate of success in long-standing persistent AF patient. By contrast, complex ablation strategies may create additional costs and expose patients to increased risk of complications [22]. Thus, early prediction and prevention of HF in persistent and long-standing persistent AF patients warrant extensive attention. Oyama et al [23] indicated that higher baseline values of high-sensitivity cardiac troponin T (hs-cTnT), NT-proBNP and growth differentiation factor-15 (GDF-15) were closely associated with increased risk of HF outcomes in AF patients. Similarly, our study demonstrates the predictive value of circulating 2'3'-cGAMP and IL-1β for new-onset HF in patients with persistent and long-standing persistent AF without a history of HF. This also implies the potential benefits of blockage of cGAS-STING pathway and NLRP3 inflammasome in the prevention of HF.

While AF and HF are interlinked, mitochondrial dysfunction and redox imbalance are crucial indicators in the pathogenesis of cardiac remodeling and HF [24]. In the atrium of AF patients, reduction of complex I and II activity, augmentation of reactive oxygen species and diminished ATP production, which are paralleled by excessive translocation of mitochondrial DNA to cytosols, are found to facilitate mitochondrial dysfunction [25]. To mimic this pathogenic process, we applied complex I inhibitor to induce mitochondrial

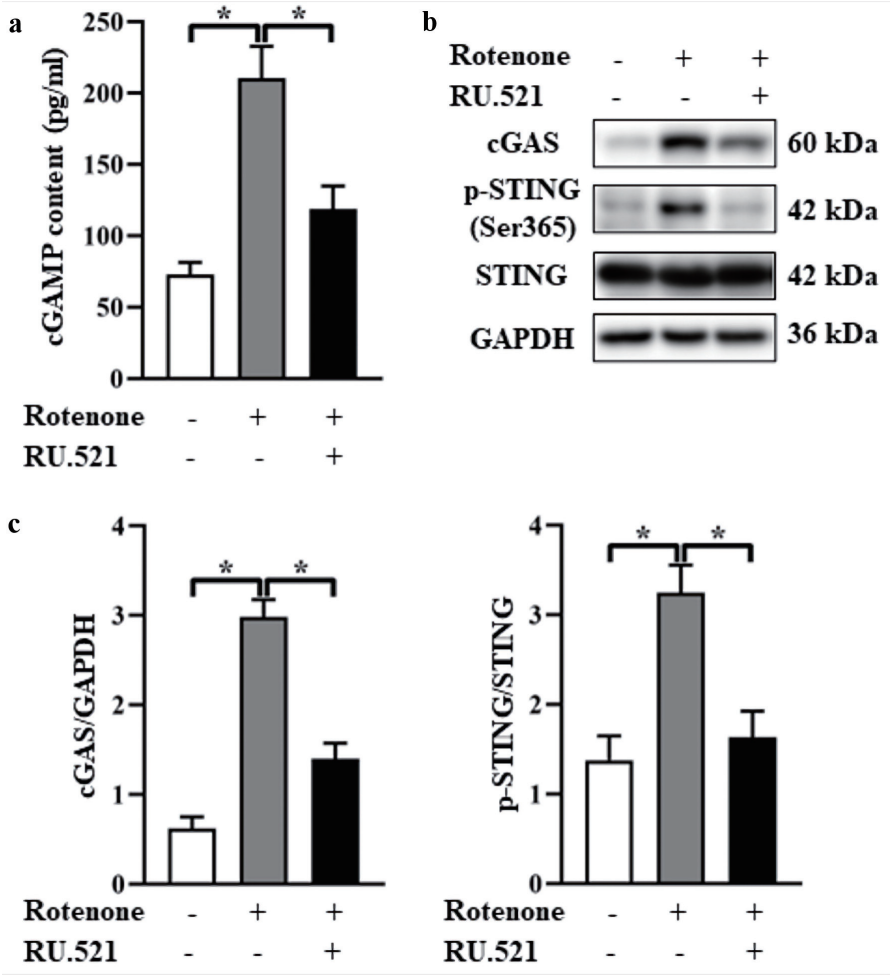


**Figure 3.** Rotenone inhibited mitochondrial dysfunction-induced cGAS-STING pathway in neonatal rat ventricular cardiomyocytes (NRVMs). (a) NRVMs were treated with GAS antagonist RU.521 (10 µM) for 24 h in the presence of rotenone (0.1 µM). The content of 2'3'-cGAMP in NRVMs was measured using ELISA kit. (b) Western blot analysis showed the expression of cGAS and STING phosphorylation. (c) Quantification of the expression of cGAS and STING phosphorylation evaluated by western blots. *P < 0.05. ELISA: enzyme-linked immunoassay; cGAS-STING: cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase-stimulator interferon genes; 2'3'-cGAMP: 2'3'-cyclic GMP-AMP.

dysfunction in primary cardiomyocytes, finding a reduction of ATP production and elevated cytoplasmic calcium levels. Previous studies have shown that pathogen-derived DNA activated the type I interferon response by stimulating cGAS-STING pathway and synthesizing the secondary messenger 2'3'-cGAMP in response to viral and microbial infections [26, 27]. Mitochondrial damage coupled with cytosolic accumulation of mitochondrial DNA also serves as an intrinsic trigger for cGAS-STING activation during the pathogenesis of many cardiovascular diseases and metabolic disorders [28]. Pharmacological inhibition of cGAS-STING pathway was proved to alleviate myocardial infarction and doxorubicin-induced cardiotoxicity [13, 29]. In this regard, we report that induction of mitochondrial dysfunction promotes intracellular cGAMP content and cGAS-STING pathway.

Recent evidence reveals a pivotal role of NLRP3 inflammasome in mitochondrial dysfunction-induced cardiac abnormalities. The NLPR3 inflammasome is a multi-protein complex including NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase-1. Activation of NLRP3 inflammasome provokes the cleavage of GSDMD and pro-IL-1β, subsequently forming mature IL-1β [30]. When a consistent upregulation of serum 2'3'-cGAMP and IL-1β in AF patients with developing HF is observed, we then attempt to dissect the connection between cGAS-STING pathway and NLRP3 inflammasome. We indicate that inhibition of cGAS by rotenone substantially reduces STING phosphorylation, the activation of NLRP3 inflammasome and IL-1β secretion stimulated by impaired mitochondria in cardiomyocytes. In diabetic cardiomyopathy, alteration of cGAS-STING pathway led to activation of NLRP3 inflammasome and proinflammatory cytokine release into serum [11]. Consistently, Kim et al [31] proposed that the release of oxidized mitochondrial DNA triggered the activation of NLRP3 inflammasome through cGAS-STING pathway in cardiomyocytes. Thus, future studies are required to validate the performance of 2'3'-cGAMP in

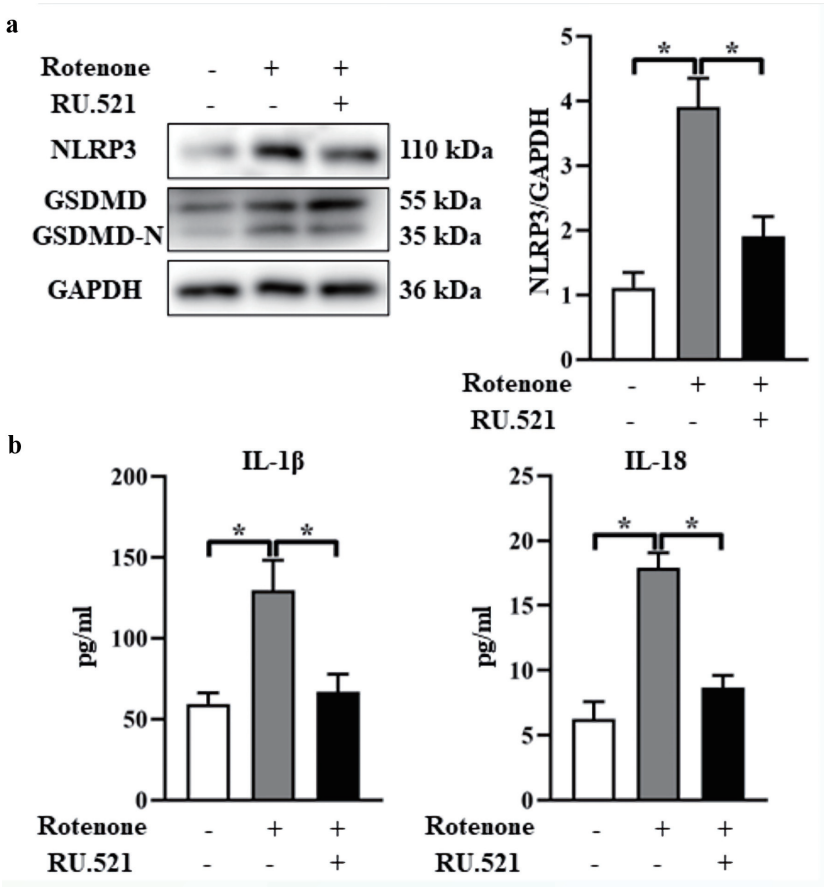


**Figure 4.** Inhibition of cGAS-STING attenuated the activation of NLRP3 inflammasome in neonatal rat ventricular cardiomyocytes (NRVMs). (a) NRVMs were treated with GAS antagonist RU.521 (10 μM) for 24 h in the presence of rotenone (0.1 μM). Western blot analysis showed the expression of NLRP3 and the cleavage of GSDMD. (b) The supernatant was collected and the concentration of interleukin (IL)-1β and IL-18 was measured using ELISA kit. *P < 0.05. NLRP3: nod-like receptor protein 3; ELISA: enzyme-linked immunoassay; cGAS-STING: cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase-stimulator interferon genes; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GSDMD: gasdermin D.

predicting the outcomes of AF patients and the perspectives of regulating cGAS-STING pathway in the prevention of HF in AF patients.

However, we must acknowledge the limitations of our study. Neonatal rat cardiomyocytes are the cell type of choice for imitating cardiac remodeling *in vitro* [32]. With the development of stem cell biology, recent studies also focus on the use of cardiomyocytes derived from human induced pluripotent stem cells, but it requires higher levels of technology platform.

In conclusion, our study provides evidence that elevated serum 2'3'-cGAMP and IL-1β are independently associated with the occurrence of HF in AF patients without a history of HF. Induction of mitochondrial dysfunction causes an upregulation of 2'3'-cGAMP and activation of NLRP3 inflammasome through cGAS-STING pathway in cardiomyocytes.

## Acknowledgments

None to declare.

## Financial Disclosure

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## Conflict of Interest

None of the authors declared any potential conflict of interest with respect to the research, authorship, and/or publication of this article.

## Informed Consent

Proper written informed consent was obtained from all patients.

## Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Juledezi Hailati, Zhi Qiang Liu, Yun Fei Zhang and Lei Zhang. The first draft of the manuscript was written by Juledezi Hailati and Muhuyati Wulasihan, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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