Original Research *Featured Article*

AMPKα2 deficiency enhanced susceptibility to ventricular arrhythmias in mice by the role of β-adrenoceptor signaling

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Impact statement

As far as we know, this is the first time the role of AMP-activated protein kinase-α2 (AMPKα2) on the cardiac electrophysiology is explored, and we found that the β-adrenoceptor activation resulting from catecholamine release was mainly responsible for the changes of electrophysiology related to the absence of AMPKα2. This has great clinical significance, as in patients who have problems with AMPKα2 gene, we may use β-adrenoceptor antagonists for the prevention of arrhythmias in future.

Abstract

AMP-activated protein kinase-α2 is the main catalytic subunit of the heart, which is mainly located in cardiac myocytes. The effect of AMP-activated protein kinase-α2 on the cardiac electrophysiology is barely studied. From the previous study, it is possible that AMP-activated protein kinase-α2 may have some effect on the electrophysiology of the heart. To prove the hypothesis, we used the AMP-activated protein kinase-α2 knockout (AMPK$\alpha2^{-/-}$) mice to estimate the electrophysiological characteristics of AMPK$\alpha2^{-/-}$ mice and try to find the mechanism between them. We used AMP-activated protein kinase-α2 gene knockout (AMPK$\alpha2^{-/-}$) mice and control wild-type mice as the experimental animals. In the experiment, we measured the monophasic action potential duration and test the inducibility to ventricular arrhythmia in isolated mice heart with and without β-adrenoceptor antagonist metoprolol. Meanwhile, plasma concentration of catecholamine was collected. We found that AMPK$\alpha2^{-/-}$ significantly shortened 90% repolarization of monophasic action potential (MAP) ($MAPD_{90}$) than wild-type (47.4 ± 2.6 ms vs. 55.5 ± 2.4 ms, $n = 10$, $P < 0.05$) and were more vulnerable to be induced to ventricular arrhythmias (70% (7/10) vs. 10% (1/10), $P < 0.05$), accompanied by the higher concentration of catecholamine (epinephrine: 1.75 ± 0.18 nmol/L vs. 0.68 ± 0.10 nmol/L $n = 10$, $P < 0.05$; norepinephrine: 9.56 ± 0.71 nmol/L vs. 2.52 ± 0.31 nmol/L $n = 10$, $P < 0.05$). The shortening of $MAPD_{90}$ and increased inducibility to ventricular arrhythmias of AMPK$\alpha2^{-/-}$ could almost be abolished when perfusion with β-adrenoceptor antagonist metoprolol. It indicated that the β-adrenoceptor activation resulting from catecholamine release was mainly responsible for the relating changes of electrophysiology of AMPK$\alpha2^{-/-}$. It had great clinical significance, as in patients who had problem with AMP-activated protein kinase-α2 gene, we might use β-adrenoceptor antagonists as the prevention of arrhythmias in future.

Keywords: AMP-activated protein kinase-α2, arrhythmias, β-adrenoceptor antagonist, catecholamine, mice

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Introduction

Arrhythmias is a common cause of morbidity and mortality and is a great burden to world. Although there are several methods used to treat arrhythmias, some treatment are ineffective or with limitations.[1–3] To some extent, our knowledge about arrhythmia is still limited. We should explore more about the potential mechanism of arrhythmias and look for the novel target for the treatment of arrhythmias. Mutations that affect gene expression or the normal function of the codified proteins involved in cell electrophysiology and calcium handling underlie arrhythmogenic syndromes and some gene mutations have been proven related with cardiac arrhythmias.[4–7] In our study, we used gene knockout mice to study the relationship between the absence of AMP-activated protein kinase-α2 (AMPKα2) and cardiac electrophysiology, hoping to provide experimental foundation to the treatment of arrhythmias.

AMPK is a heterologous trimer protein which has a catalytic subunit (either α1 or α2) and two regulatory subunits (β and γ).[8] AMPKα2 is the main catalytic subunit of the heart, which is mainly located in cardiac myocytes.[9,10] It was reported that AMPKα2 deficiency (AMPKα2$^{-/-}$) could exacerbate ventricular hypertrophy in mice.[11,12] But the effect of AMPKα2$^{-/-}$ on the cardiac electrophysiology is barely studied. In the previous study, it is reported that in AMPKα2 knockout mice, there is an increased daily urinary catecholamine excretion,[13] suggesting changed function of the sympathetic nervous system. The increased catecholamine could activate β-adrenoceptors and result in the change of the cardiac electrophysiology in mice.[14,15]

To prove the hypothesis, we used AMPKα2$^{-/-}$ mice to estimate the electrophysiological characteristics of AMPKα2$^{-/-}$ mice and try to find the mechanism between them.

Materials and methods

Experiment animals

Institutional animal care and use committee in China approved the animals used in this study. The protocols were conforming to the Guide for the Care and Use of Laboratory Animals, which is published by US National Institutes of Health (NIH Publication No.85–23, revised 1996). The experimental animals we used were wild-type (WT) mice and AMPKα2$^{-/-}$ mice (C57 background). All the animals were male and the ages were about eight weeks.

Echocardiography

Isoflurane (1.5%) was used to anesthetize the mice, and the machine used in the echocardiography study was MyLab 30CV ultrasound (Biosound Esaote, Inc.). We measured the left ventricular septum, diastolic (IVSD), left ventricular end diastolic diameter (LVDD), left ventricular posterior wall diameter (LVPWD), left ventricular end systolic diameter (LVSD), ejection fraction (EF) and fractional shortening (FS) of mice.

Telemetry ECG recording

Pentobarbital sodium was used to anaesthetize the mice (60mg/kg i.p.), then placed the mice on a custom-made ECG recording platform. The temperature of the body was kept to 37°C. Leads were tunneled subcutaneously. Recordings did not begin until mice recovered for more than 24 h and recordings lasted for 24 h. The data acquisition system (DSI, US) was used to record the signals. The modification of Bazett's formula was used to correct QT interval (QTI), $QTc = QT/(RR/100)^{1/2}$.[16]

Langendorff perfused hearts

The mice were heparinized by heparin sodium (100U, i.p.) for 10 min. Then they were anesthetized using pentobarbital sodium. The isolated heart was quickly excised and perfused with Tyrode's solution (mmol/L): NaCl 130; KCl 5.4; $MgCl_2$ 1; $CaCl_2$ 1.8; Na_2HPO_4 0.3; glucose 10; HEPES 10; pH 7.4. Then the heart was transferred to the Langendorff perfusion system (AD instruments, Australia). The speed of the perfusion was 2–2.5 ml/min.

Monophasic action potential recording

The pacing electrode was placed on the surface of the basal ventricular. Epicardial MAPs were recorded from left ventricle of the heart. Chrat7.0 software was used to analyze the MAP waveforms.

Electrical stimulated protocol

S1 pacing

The S_1S_1 pacing protocol was used to record MAP, and the pacing cycle length (PCL) was 125 ms.

S1-S2 pacing

The programmed electrical stimulation (PES) comprised eight stimuli (S1) (CL = 125 ms) and an extra stimulus (S2). The S1 S2 interval was gradually decreased until arrhythmia was induced or no more ventricular deflection was evoked. S1–S2 pacing was used as a method to determine the susceptibility of cardiac arrhythmia.

Burst pacing

Burst pacing was another method used to determine the susceptibility of cardiac arrhythmia. Burst pacing was consisted of 2 ms pulses at 50 Hz and the duration was 2 s.[17]

Measurement of serum catecholamine concentrations

Animals were lightly anesthetized with 1.5% isoflurane. The 200 μl blood was taken into heparinized capillaries by puncturing the retro-orbital plexus. ELISA kit was used to test the concentrations of epinephrine and norepinephrine (Nanjing Senbeijia Biological Science and Technology Co, Nanjing, China).

Role of β-adrenoceptor activation

To test the role of β-adrenoceptor on the AMPKα2$^{-/-}$ mice, the MAP and the susceptibility to ventricular arrhythmia were compared with and without of 1.8 μmol/L metoprolol (the β-adrenoceptor antagonist, Sigma).

Statistical analysis

All data reported were expressed in the form of mean ± SD. Statistical methods used were Fisher exact test and Student's *t* test, which were completed by SPSS 17.0. $P < 0.05$ was considered as significant.

Results

The basic character between WT and AMPKα2$^{-/-}$mice

No significant difference was seen in body weight, ventricular mass, and lung mass between the two groups. In addition, echocardiography data (with 1.5% isoflurane) showed there were no difference in IVSD, LVDD, LVPWD, LVSD,

EF and FS between AMPKα2$^{-/-}$ and WT mice (Table 1). Telemetry ECG was used to assess the basic electrophysiological character of the mice in non-anesthetized and unrestricted conditions. From the telemetry ECG data, it showed that AMPKα2$^{-/-}$ significantly increased the heart rate (HR), shorted QRS wave, QTI and QTc of the heart (Table 2).

Table 1. The basic character between WT and AMPKα2$^{-/-}$ mice.

	WT ($n = 9$)	AMPKα-2$^{-/-}$ ($n = 6$)
Body weight, g	25.1 ± 0.5	24.7 ± 0.7
Ventricular mass, mg	108.2 ± 1.8	107.1 ± 2.3
Lung mass, mg	134.1 ± 3.2	133.7 ± 4.3
IVSD, mm	0.65 ± 0.04	0.66 ± 0.04
LVDD, mm	3.60 ± 0.13	3.62 ± 0.22
LVPWD, mm	0.64 ± 0.05	0.65 ± 0.05
LVSD, mm	2.01 ± 0.11	2.05 ± 0.23
EF, %	81.50 ± 2.43	80.50 ± 3.62
FS, %	43.67 ± 2.25	42.83 ± 3.43

WT: wild type; IVSD: left ventricular septum, diastolic; LVDD: left ventricular end-diastolic diameter; LVPWD: left ventricular posterior wall diameter; LVSD: left ventricular end-systolic diameter; EF: ejection fraction; FS: fractional shortening.

Table 2. Telemetry recordings from WT and AMPKα2$^{-/-}$ mice.

	WT ($n = 9$)	AMPKα-2$^{-/-}$ ($n = 6$)
HR, bpm	604.9 ± 19.5	656.7 ± 26.0*
RRI, ms	100.5 ± 8.5	92.3 ± 8.9
PRI, ms	34.4 ± 4.8	33.7 ± 5.3
QRS, ms	8.1 ± 0.2	7.8 ± 0.3*
QTI, ms	47.1 ± 2.1	42.8 ± 1.7*
QTc, ms	47.0 ± 2.1	44.6 ± 1.8*

Note: QTc = QT/(RR/100)$^{1/2}$.
*$P < 0.05$.
WT: wild type; HR: heart rate; RRI: RR interval; PRI: PR interval; QTI: QT interval.

Effect of AMPKα2$^{-/-}$ on MAP

In order to test the effect of AMPKα2$^{-/-}$ on MAPD of the heart, MAPs were recorded at left ventricle during pacing at PCL 125 ms. The range of the amplitudes of MAP was from 10.2 to 23.5 mV. MAPD was measured at 90% repolarization ($MAPD_{90}$). The $MAPD_{90}$ of AMPKα2$^{-/-}$ (47.4 ± 2.6 ms, $n = 10$) was significantly shortened than WT (55.5 ± 2.4 ms, $n = 10$, $P < 0.05$) (Figure 1).

AMPKα2$^{-/-}$ enhanced susceptibility of cardiac arrhythmia

In order to test the susceptibility of cardiac arrhythmia, we use PES and burst pacing to induce ventricular arrhythmias in isolated heart from AMPKα2$^{-/-}$ and WT mice. PES was consisted of an eight S1 and a single premature pulse (S2), which elicited triggered activity, mimicking the physiological initiation of cardiac arrhythmias, and burst pacing is a robust arrhythmogenic test. In AMPKα2$^{-/-}$ heart, 7 out 10 were induced to ventricular arrhythmias, while in WT, only 1 out of 10 was induced to ventricular arrhythmias. It showed that AMPKα2$^{-/-}$ enhanced the susceptibility to ventricular arrhythmias than WT (70% (7/10) vs. 10% (1/10), $P < 0.05$) (Figure 2). What's more, spontaneous premature ventricular contractions (PVCs) (>15 events/min) were more frequency in AMPKα2$^{-/-}$ mice than C57 (40%, 4/10 vs. 0%, 0/10) in telemetry ECG (Figure 3).

Role of catecholamine

To test whether higher catecholamine was responsible for the effect of AMPKα2$^{-/-}$ on the cardiac electrophysiology, we test the concentration of catecholamine in the blood. It showed that AMPKα2$^{-/-}$ had a higher concentration of plasma catecholamine. The concentration of plasma epinephrine of AMPKα2$^{-/-}$ was 1.75 ± 0.18 nmol/l, compared with 0.68 ± 0.10 nmol/l in WT ($n = 10$, $P < 0.05$), and the

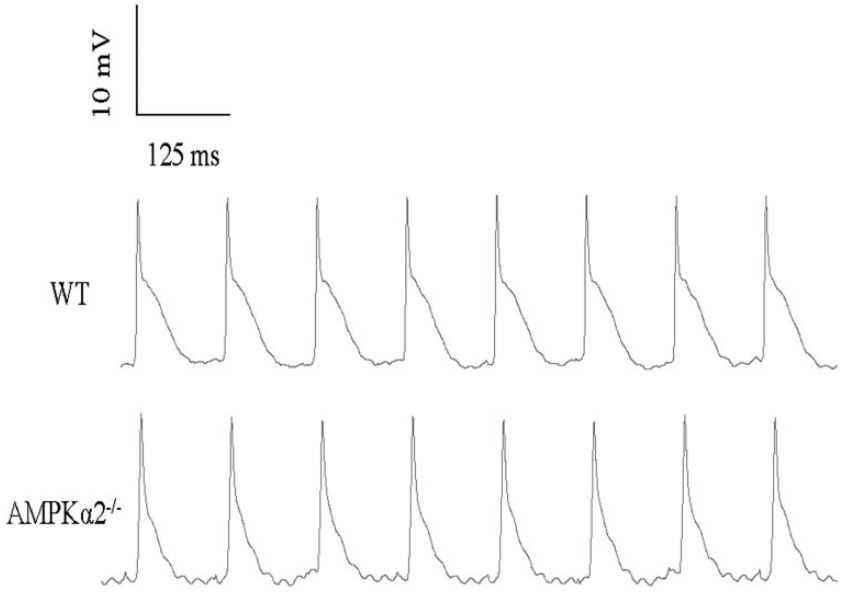

Figure 1. Representative action potential waveforms from epicardium between AMPKα2$^{-/-}$ and wild-type (WT) in isolated heart. It showed that AMPKα2$^{-/-}$ shortened the action potential duration than WT.

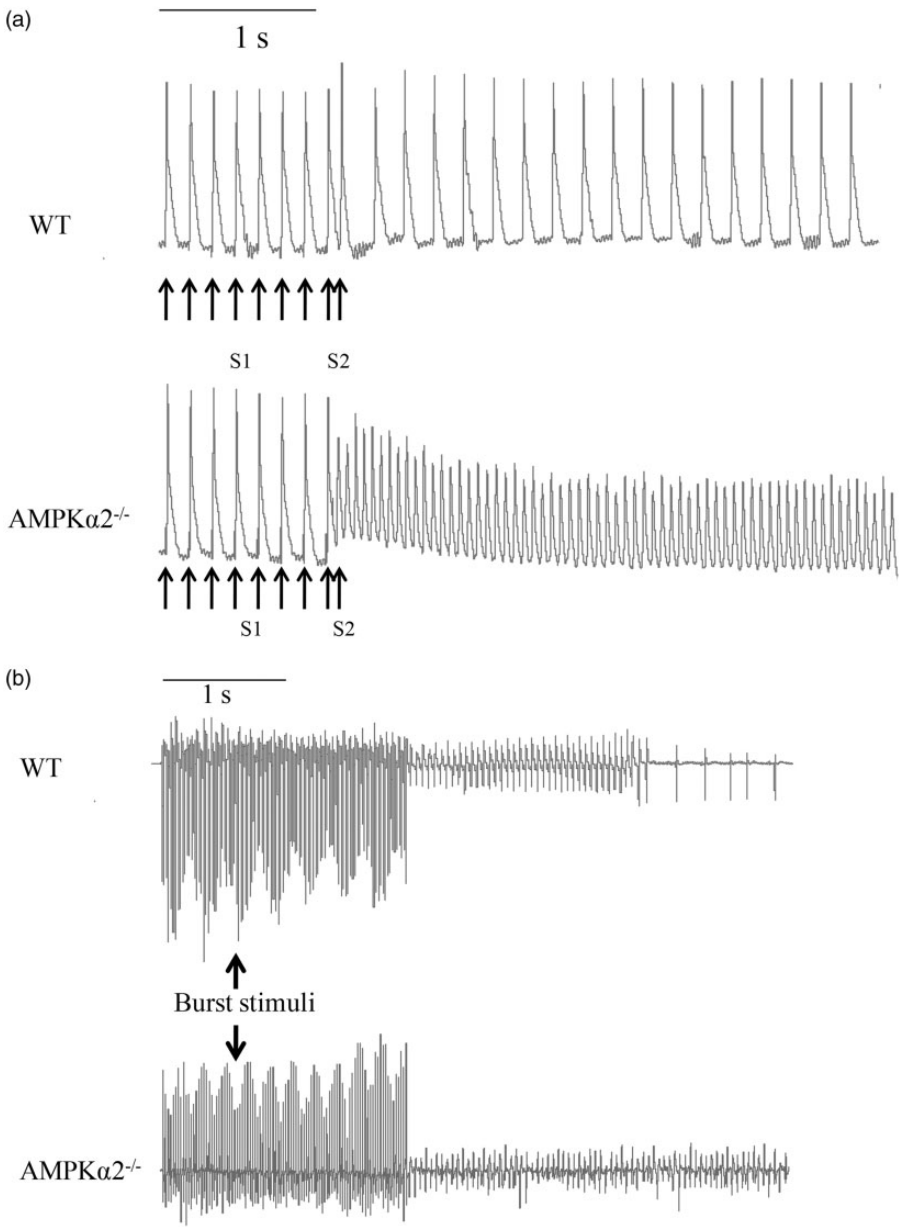

Figure 2. PES and burst stimuli were applied to induce ventricular arrhythmias. During PES pacing (a) and burst pacing (b), the sustained rapid ventricular activities were occurred in 7 out of 10 mice in AMPK$\alpha 2^{-/-}$ hearts, while in wild-type mice only 1 out of 10 hearts showed short time rapid ventricular activities.

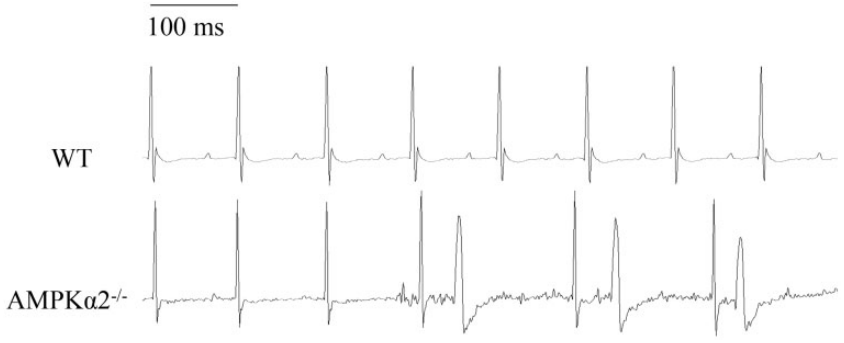

Figure 3. Representative telemetry recordings from AMPK$\alpha 2^{-/-}$ and wild-type (WT) mice. More premature ventricular contractions can be seen in AMPK$\alpha 2^{-/-}$ mice than WT mice.

concentration of plasma norepinephrine was 9.56 ± 0.71 nmol/l, while the WT was 2.52 ± 0.31 nmol/l ($n = 10$, $P < 0.05$) (Figure 4).

Role of β-adrenoceptors

After release of catecholamine, to test whether the electrophysiological change of AMPK$\alpha 2^{-/-}$ was mediated via the activating of β-adrenoceptors, we compared the effect of AMPK$\alpha 2^{-/-}$ on cardiac electrophysiology with and without β-adrenoceptor antagonist metoprolol. It showed that the shortening of MAPD90 and increase in ventricular arrhythmias inducibility can almost be abolished when perfusion with β-adrenoceptor antagonist metoprolol (Figure 5).

Discussions

In the present study, we found that AMPK$\alpha 2^{-/-}$ significantly shortened MAPD of the heart and were more vulnerable to ventricular arrhythmias. Perfusion with β-adrenoceptor antagonist metoprolol almost abolished the effect of absence of AMPKα2. It indicated that the β-adrenoceptor activation resulting from catecholamine release is mainly responsible for the electrophysiological changes associated with AMPK$\alpha 2^{-/-}$.

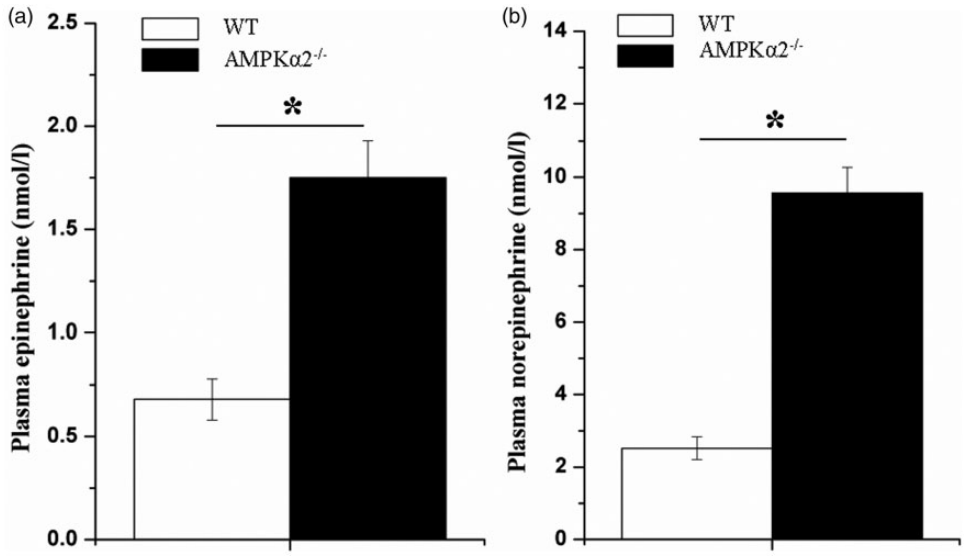

Figure 4. The plasma concentration of catecholamine between AMPK$\alpha 2^{-/-}$ and wild-type (WT) mice. (a) The concentration of epinephrine, (b) the concentration of norepinephrine. *$P < 0.05$.

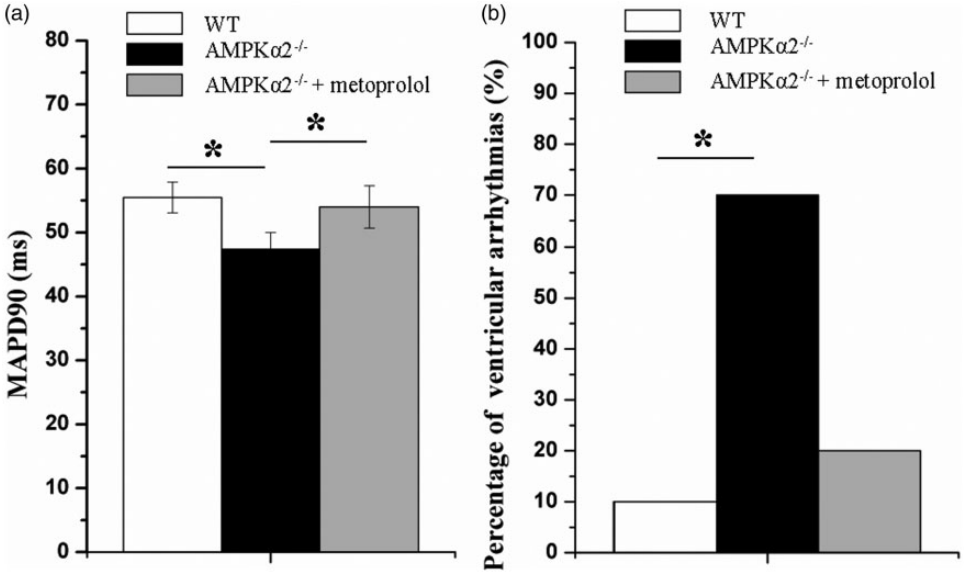

Figure 5. The role of β-adrenoceptor antagonist metoprolol on the monophasic action potential (MAP) (a) and the susceptibility to ventricular arrhythmia (b). In the presence of metoprolol, the effect of absence of AMPKα2 on shortening of the MAP and increased susceptibility to ventricular arrhythmia were almost abolished. *$P < 0.05$.

AMPKα2 is the main catalytic subunit in the heart, mainly expressed in cardiac myocytes.[9,10] In the previous studies, it was shown that AMPKα2 played a negative role in cardiac hypertrophy.[11,12] AMPKα2$^{-/-}$ could increase ventricular hypertrophy induced by transverse aortic constriction in mice,[11] and cardiac hypertrophy that is induced by isoproterenol was significantly higher in AMPKα2$^{-/-}$ mice.[12] However, the effect of AMPKα2$^{-/-}$ on the cardiac electrophysiology is barely studied. It is known before that the main role of AMPK is regulating energy metabolism, which include striated skeletal, nerve cells, fat tissue, liver and heart.[10,18] And even recently, the roles of AMPK in electrophysiology were somewhat neglected.[19] So we need to explore more about the role of AMPK in electrophysiology. It is known that the ion channels are very important in the electrophysiology of the heart and some ion channels are regulated by AMPK.[20,21] Since AMPKα2 is the catalytic subunit of the AMPK, our hypothesis is that AMPKα2 is likely to be associated with the electrophysiology of the heart. And from the result of our study, AMPKα2 did have some effect on the cardiac electrophysiology in mice. But what is the possible mechanism between them?

In our study, AMPKα2$^{-/-}$ mice were more vulnerable to ventricular arrhythmias. We thought that the high level of catecholamine might contribute to it. In our study, we tested the concentration of catecholamine in the blood and found that AMPKα2$^{-/-}$ had a higher concentration of catecholamine. It is consistent with the research of Viollet *et al*.[13] In their research, they found that AMPKα2$^{-/-}$ mice had highly excretion of urinary catecholamine. Catecholamine is consisted of norepinephrine, epinephrine, and dopamine and could activate through the β-adrenoceptor and change the electrophysiology of the heart.[14,15] In the previous study, it showed that increased catecholamine was associated with ventricular arrhythmias not only in animals but also in people.[22–24] For example, in animals, it was found that catecholamine could facilitate burst pacing-induced ventricular arrhythmias.[22] While in patients with right ventricular tachycardia (idiopathic right ventricular outflow tract tachycardia (RVO-VT)), they were easy to be induced to ventricular tachycardia by catecholamine infusion during electrophysiological studies[23] and the efficacy of antiarrhythmic with antiadrenergic drug suggested abnormalities of cardiac sympathetic innervation.[24] So the increased concentration of catecholamine might be the cause of increased inducibility to cardiac arrhythmias in AMPKα2$^{-/-}$ mice.

To examine whether the effect of increased catecholamine in the AMPKα2$^{-/-}$ was mediated via the activating of β-adrenoceptors, we compared the effect of AMPKα2$^{-/-}$ on cardiac electrophysiology with and without β-adrenoceptor antagonist metoprolol, and it showed that the shortening of MAPD and increase in ventricular arrhythmias inducibility of AMPKα2$^{-/-}$ could almost be abolished by perfusion with metoprolol. When β-adrenoceptor was stimulated, it could activate the slow delayed rectifier outward potassium current, which contributed to the shortening of ventricular APD and QT interval.[14,15] On the contrary, it was found that in mouse with the truncated delayed rectifier potassium current, a significant prolongation of APD and QT intervals can be observed.[25,26] In the result of our experiment, the APD and QT interval of AMPKα2$^{-/-}$ mice was shortened. When the ventricular APD and QT interval were shortened, then it would be easy to be induced to ventricular arrhythmias.[27,28] Shortening of APD was related with increased probability of ventricular fibrillation induction and decreased the probability of spontaneous defibrillation by allowing a greater number of fibrillation waves in the ventricle in the presence of catecholamine.[28] In the short QT syndrome, transmural heterogeneity of repolarization was increased and then created the substrate for reentry which as easy to develop to malignant ventricular arrhythmia.[27] So we could conclude that in AMPKα2$^{-/-}$ mice, the increased catecholamine activated β-adrenoceptors and resulted in the change of the cardiac electrophysiology.

In conclusion, AMPKα2$^{-/-}$ shortened the MAPD of the heart and facilitated the incidence of ventricular arrhythmias. It indicated that β1-adrenoceptor activation is mainly responsible for the relating changes of electrophysiology of AMPKα2$^{-/-}$. It had great clinical significance, as in patients who had problem with AMPKα2 gene, we might use β-adrenoceptor antagonists as the prevention of arrhythmias. What's more, we can conclude that AMPKα2 acted as a protected role in ventricular arrhythmogenesis and it might also be a good way to prevent some arrhythmias by up-regulating or activating expression of AMPKα2.

Authors' contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; Hong Cao, Xin Wang and Shaozhen Ying conducted the experiments, Hong Cao wrote the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

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