Original Article

AKT-mTOR signaling-mediated rescue of *PRKAG2* R302Q mutant-induced familial hypertrophic cardiomyopathy by treatment with β-adrenergic receptor (β-AR) blocker metoprolol

Jian Zhuo[1#], Haihua Geng[1#], Xiaohui Wu[1], Mengkang Fan[1], Hongzhuan Sheng[1]^, Jian Yao[2]

[1]Department of Cardiology, Affiliated Hospital of Nantong University, Nantong, China; [2]Department of Histology and Embryology, Medical School of Nantong University, Nantong, China

Contributions: (I) Conception and design: H Sheng, J Yao; (II) Administrative support: H Sheng; (III) Provision of study materials or patients: J Zhuo, H Geng; (IV) Collection and assembly of data: X Wu; (V) Data analysis and interpretation: M Fan; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Hongzhuan Sheng, MD, PhD. Department of Cardiology, Affiliated Hospital of Nantong University, 20 Xi Si Rd., Nantong 226000, China. Email: yjshz@edu.ntu.cn; Jian Yao. Department of Histology and Embryology, Medical School of Nantong University, Nantong 226001, China. Email: yao781923036@163.com.

Background: Protein kinase AMP-activated non-catalytic subunit gamma 2 gene (*PRKAG2*) cardiac syndrome, caused by mutations in *PRKAG2*, often shows myocardial hypertrophy and abnormal glycogen deposition in cardiomyocytes. However, it remains incurable due to a lack of a management guideline for treatment.

Methods: We constructed a fluorescently labeled adenovirus carrying the wild-type or R302Q mutant of the *PRKAG2* gene, infected neonatal rat cardiomyocytes (NRCMs) and H9C2 cell lines, and then analyzed changes in AMP-activated protein kinase (AMPK) activity, cell hypertrophy, glycogen storage, and cell proliferation when presence or absence of metoprolol or protein kinase A (PKA) inhibition H89, and then analyzed the changes in AKT-mTOR signal transduction activity.

Results: Overexpression of *PRKAG2* R302Q in primary cardiomyocytes increased the activity of AMPK, induced cellular hypertrophy and glycogen storage, and promoted the phosphorylation levels of AKT-mTOR signaling pathway. Application of either β1-adrenergic receptor (β1-AR) blocker metoprolol or PKA inhibitor H89 to the cardiomyocytes rescued the hypertrophic cardiomyopathy (HCM)-like phenotypes induced by *PRKAG2* R302Q, including suppression of both AKT-mTOR phosphorylation and AMPK activity.

Conclusions: The current study not only determined the mechanism regulating HCM induced by *PRKAG2* R302Q mutant, but also demonstrated a therapeutic strategy using β1-AR blocker to treat the patients with *PRKAG2* cardiac syndrome.

Keywords: *PRKAG2*; R302Q; hypertrophic cardiomyopathy (HCM); β-adrenergic receptor blocker (β-AR blocker)

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^ ORCID: 0000-0002-6005-2676.

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disorder with characteristics of increased ventricular wall thickness, cardiomyocyte hypertrophy and myocardial fibrosis (1). *PRKAG2* cardiac syndrome, as a common form of metabolic HCM caused by mutations in the *PRKAG2* gene encoding the AMPKγ2 subunit, often shows abnormal glycogen deposition in cardiomyocytes, ventricular preexcitation and progressive cardiac conduction disturbances in addition to myocardial hypertrophy (2). AMPK is a heterotrimeric protein composed of α, β, and γ subunits, acting as the key enzyme responsible for regulating cellular energy homeostasis. AMPK activation turns on the catabolic pathway to produce ATP and turns off the anabolic pathway that requires adenosine-triphosphate (ATP) consumption, maintaining the dynamic energy balance (3). Genetic mutants (MUT) in *PRKAG2* have been demonstrated to cause inappropriate activation of AMPK, leading to arrhythmias and cardiac insufficiency. For example, elevated activity of AMPK and decreased sensitivity to adenosine monophosphate (AMP) were reported in the cardiomyocytes with *PRKAG2* K475E MUT and in the myocardium of *PRKAG2* N488I MUT mice, associated with activation of mTOR/p70S6K/4EBP1 and/or Akt-mTOR-FOXO3A pathways (4,5). In consistence, *PRKAG2* MUT-induced myocardial hypertrophy can be rescued by application of the mTOR inhibitor rapamycin.

The β-adrenergic receptor (β-AR) signaling pathway is one of the pathways mediating induction of cardiac hypertrophy, which has been suggested to be associated with signaling pathways such as cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA). A phosphoproteomic analysis *in vivo* revealed that the kinases AMPK, AKT, and mTOR are also involved in β-AR signaling regulation of HCM (6). In addition, β-AR showed regulation of glucose uptake and glycogen synthesis (7). β1-AR agonists reduced insulin-induced glucose uptake and glycogen synthesis (8). Glycogen storage in cardiomyocytes has been shown to be related with myocardial hypertrophy. Reduction of glycogen was associated with improvement of cardiac function, suggesting the therapeutic values for HCM of preventing glucose uptake by cardiomyocytes. However, Kim et al found that inhibition of glycogen deposition in N488I mutation model reversed ventricular preexcitation but showed limited effect on rescuing cardiac hypertrophy, suggesting the correlation between myocardial hypertrophy and glycogen storage might be in a genetic disorder-dependent manner (4).

To date, effects of all kinds of clinic treatment to *PRKAG2* cardiac syndrome patients remain very limited yet mainly due to lacking of a well-accepted management guideline for the disease. Metoprolol, as a selective β1-AR blocker, interacts with β1-AR to inhibit β1-AR signaling, leading to improvement of myocardial remodeling, and rescue of cardiac hypertrophy caused by infarction or hypertension (9,10). Our previous study treated 5 patients in a *PRKAG2* R302Q-induced HCM family with a long-term oral administration of metoprolol, resulting in significant prevention of myocardial hypertrophy progression (11). However, the regulatory mechanism remains unclear.

In order to validate the function and mechanism of β-blocker in regulating myocardial hypertrophy, a fluorescent-labeled adenoviral virus carrying either wild type (WT) or R302Q MUT of *PRKAG2* gene was infected into neonatal rat cardiomyocytes (NRCMs) and H9C2 cell line, followed by analyses of AMPK activity, cellular hypertrophy, glycogen storage and cell proliferation with or without treatment of metoprolol or PKA inhibitor H89. The activity of AKT-mTOR signaling was assessed as well. As a result, increased AMPK activity was indicated in the *PRKAG2* R302Q-overexpressing cells, accompanied with increase of glycogen storage, cell size and expression levels of myocardial hypertrophy markers atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and β-myosin heavy chain (β-MHC). Phosphorylation of AKT, mTOR, p70S6K and 4EBP1 was suggested to mediate the *PRKAG2* R302Q-induced HCM phenotype. The current study not only determined the mechanism regulating HCM induction by *PRKAG2* R302Q MUT, but also demonstrated a therapeutic strategy using β-AR blocker to treat the patients. We present the following article in accordance with the MADR reporting checklist (available at https://cdt.amegroups.com/article/view/10.21037/cdt-22-81/rc).

Methods

Reagents

The following primary antibodies were used in our experiments: anti-β-actin (#3700), anti-AMPKα (#2535), anti-phospho-AMPKα (#5831), anti-mTOR (#2983), anti-phospho-mTOR (#2971), anti-4E-BP1 (#9644), anti-phospho-4E-BP1 (#2855), anti-P70S6K (#2708), and anti-phospho-P70S6K (#9208) were purchased from Cell Signaling Technology (USA). Primary antibodies were

diluted 1:2,000 for hybridization. 1:8,000 dilution was applied to the second antibody. BCA protein assay kit was purchased from Beyotime (Beyotime, Shanghai, China).

Cardiomyocyte cell culture and transduction

H9C2 cells (ATCC, Manassas, VA, USA) were cultured in DMEM (Hyclone, Logan, UT, USA), supplemented with 10% FBS (Gibco, Grand Island, NY, USA), and 1% penicillin-streptomycin (Gibco) at 5% CO_2 and 37 ℃. NRCMs were isolated from the heart of newborn (1 day old) rat with PBS containing 0.01% collagenase type II. The cardiomyocytes were then seeded at a density of 1×10^6 cells/well in six-well culture plates coated with fibronectin in plating medium, which consisted of F12 medium supplemented with 10% fetal calf serum and penicillin/streptomycin. Cells were grown to 70% confluence before transduction with *PRKAG2* gene γ_2WT- (Adγ_2WT), or γ_2 R302Q- (Adγ_2R302Q) overexpressing adenoviruses at a multiplicity of transduction of 36. Then, H89 (10 μM) and metoprolol (20 μM) were administered to intervene the cells respectively, and the cells were harvested after 48 h for further analysis.

AMPK activity analysis

To assess the effect of *PRKAG2* R302Q MUT on the activity of AMPK, a cell AMPK Kinase Activity Colorimetric Quantitative Detection Kit (Haring Creature, China) was used following the manufacturer's instructions. Mean values of the absorbance from triplicates were calculated for each sample.

Cellular glycogen analysis

Glycogen staining was performed using a periodic acid-Schiff kit (PAS, Beyotime, China). The amount of glycogen storage in cells was quantified using a glycogen content assay kit (Solarbio, BC0340, China) according to the manufacturer's instructions.

Immunofluorescence analysis

Cells were fixed with 3.7% formaldehyde for 15 min, permeabilized with 0.1% Triton X-100 in PBS for 40 min, blocked with a 10% BSA solution for 1 h at room temperature and then incubated with a primary antibody (1:50 dilution). Nuclei were stained with DAPI (Invitrogen, USA) for 10 min in the dark. Images were taken with a fluorescence confocal microscope. Image-Pro Plus 6.0 software was used for quantitative analysis.

Cell proliferation assay

Cell proliferation was analyzed using cell counting kit-8 (CCK-8, Beyotime, China) according to the manufacturer's instructions. For each group, mean values of the absorbance from three wells were calculated. EdU staining was performed using an EdU kit (BeyoClick™ EdU Cell Proliferation Kit with Alexa Fluor 488, Beyotime, China). Briefly, harvested cells were seeded in 24-well plates. Subsequently, cells were incubated with EdU for 3 h, fixed with 4% paraformaldehyde for 15 min, and permeated with 0.3% Triton X-100 for another 15 min. Then cells were incubated with the Click Reaction Mixture for 30 min at room temperature in a dark place and then incubated with Hoechst 33342 for 10 min.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated using Trizol Reagent (Invitrogen, USA). First Strand cDNA Synthesis Kit (Roche, USA) was used to synthesize cDNA. The mRNA levels of the indicated genes were quantified with real-time PCR using SYBR Green (Roche, USA). Sequence of primers (5' to 3') are: ANP, forward CGGAAGCTGTTGCAGCCTA, reverse GCCCTGAGCGAGCAGACCGA; BNP, forward TTTGGGCAGAAGATAGACCG, reverse TGGCAAGTTTGTGCTGGAA; β-MHC, forward GCCTACCTCATGGGACTGAA, reverse ACATTCTGCCCTTTGGTGAC; GAPDH, forward AGTGCCAGCCTCGTCTCAT, reverse AGGGGCCATCCACAGTCTTC.

Western blotting

Whole cell lysates were obtained by homogenizing cells in RIPA buffer. Thirty μg of protein lysate was separated via running SDS-PAGE. PVDF membrane (Millipore, Massachusetts, USA) was used for transfer. Ten-percent non-fat milk was used for blocking. Primary antibodies were applied for incubation overnight at 4 ℃, followed by an incubation with secondary antibody for 1.5 h at room temperature. ECL reagents (170-5061, Bio-Rad, Shanghai, China) were applied for bands staining. Fluor Chem E

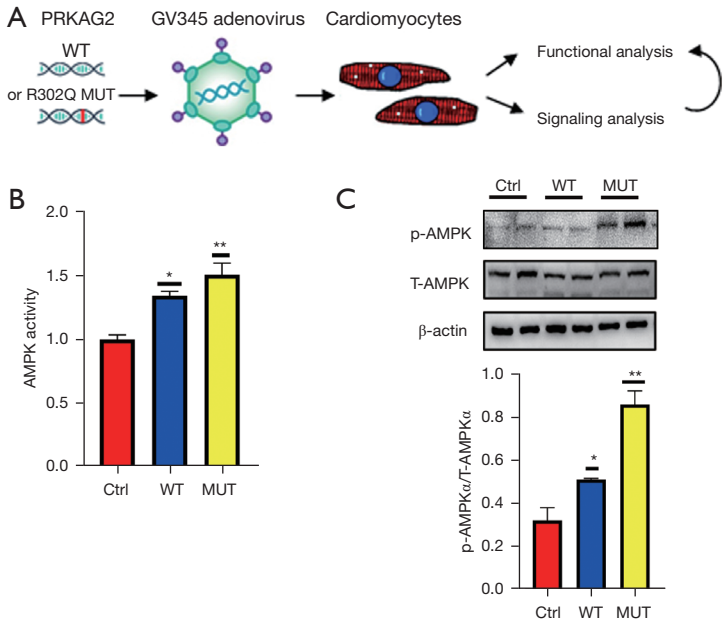

Figure 1 Overexpression of *PRKAG2* R302Q in cardiomyocytes increased the AMPK activity. (A) Schematic representation of the work flow to determine the function of WT and R302Q MUT in cardiomyocytes. (B) Increased AMPK activity in cardiomyocytes overexpressing either WT or MUT. (C) Western blot showing increased phosphorylation of AMPK by both WT and MUT in cardiomyocytes. Bottom panel showed the quantitative analysis results of the bands in the top panel. Data are presented as the mean ± SD (n=3). *, P<0.05, **, P<0.01. Ctrl, control; WT, wild type; MUT, mutant; SD, standard deviation.

imager (Cell Biosciences, Santa Clara,USA) was used to capture images.

Statistical analysis

The data are represented as the mean ± standard deviation (SD). Student's two-tailed *t*-test was used for statistical analysis using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). P<0.05 was considered a statistically significant difference.

Results

Treatment of a PRKAG2 gene cardiac syndrome family line with the β1-AR blocker metoprolol

In our previous published article, a *PRKAG2* (R302Q) cardiac syndrome family line was followed for a long period and five family members were diagnosed with *PRKAG2* cardiac syndrome presenting with complete atrioventricular (AV) block and asymmetric septal hypertrophy (11) (Figure S1). The five patients were treated with metoprolol, during which the patients were regularly reviewed. By comparing cardiac ultrasound before and after medication in the five patients, we found that the progression of myocardial hypertrophy was significantly delayed in all patients after metoprolol treatment (Table S1) and that the mean annual rate of increase in septal thickness decreased, accompanied by an increase in left ventricular end-diastolic dimension (LVEDD).

Activation of AMPK in cardiomyocytes by overexpression of PRKAG2 gene R302Q

In order to determine the regulation of AMPK activity by *PRKAG2* R302Q MUT in cardiomyocytes, WT or R302Q MUT *PRKAG2* gene were cloned into an adenovirus vector, and then transduced into primary NRCMs for further analysis (*Figure 1A*, Figure S2). AMPK activity showed upregulation by both WT and MUT *PRKAG2* (*Figure 1B*). In consistence, phosphorylation of AMPK was promoted by both WT and MUT *PRKAG2* (*Figure 1C*). Notably, MUT *PRKAG2* promoted phosphorylation of AMPK and activity of AMPK with a higher level than WT *PRKAG2* (*Figure 1B,1C*).

PRKAG2 gene R302Q induced myocardial hypertrophy and glycogen storage in cardiomyocytes

In order to determine the mechanism regulating *PRKAG2* R302Q MUT-induced myocardial hypertrophy, NRCMs and H9C2 cells overexpressing WT or MUT *PRKAG2* were analyzed with myocardial hypertrophy, cell proliferation and glycogen storage. Cell hypertrophy showed induction by MUT *PRKAG2*, which was measured through α-SMA staining (*Figure 2A,2B*). Glycogen storage in cardiomyocytes was increased by WT or MUT *PRKAG2* (*Figure 2C,2D*). In consistence, increased expression levels of myocardial hypertrophy markers, such as ANP, BNP and β-MHC, were observed in those cells (*Figure 2E*). To determine the regulation of cell proliferation by *PRKAG2*, both EdU staining and CCK8 assay were applied to the cardiomyocytes. As shown in *Figure 2F-2H*, *PRKAG2* overexpression significantly promoted the proliferation of cardiomyocytes. Notably, MUT *PRKAG2* promoted cellular hypertrophy, glycogen deposition and cell proliferation at higher levels than WT *PRKAG2*.

Rescue of PRKAG2 gene R302Q-induced myocardial hypertrophy by β1-AR blocker and PKA inhibitor

In order to validate the therapeutic effects and explore the mechanisms of β1-AR blockers to cure the patients with *PRKAG2* cardiac syndrome, either β1-AR blocker metoprolol or PKA inhibitor H89 was applied to the *PRKAG2* R302Q MUT-expressing cardiomyocytes, followed by analysis of cellular hypertrophy, glycogen storage and cell proliferation. As shown in *Figure 3*, both metoprolol or H89 treatment can partly or completely rescue the *PRKAG2* R302Q MUT-induced phenotypes including AMPK activity (*Figure 3A*), cellular size (*Figure 3B,3C*), glycogen storage (*Figure 3D,3E*), expression levels of ANP, BNP and β-MHC (*Figure 3F*), and cell proliferation (*Figure 3G-3I*). In order to exclude the effect of drugs on the cell status, we also detected the changes in cell viability, the results showed that the two drugs did not affect the cell viability significantly decreased (Figure S3).

PRKAG2 R302Q activated AKT-mTOR signaling in cardiomyocytes

In order to explore the molecular mechanisms through which *PRKAG2* R302Q induces myocardial hypertrophy, we firstly analyzed AKT-mTOR signaling in cardiomyocytes overexpressing WT or MUT *PRKAG2*. As shown in *Figure 4A-4D*, MUT *PRKAG2* significantly activated AKT-mTOR signaling by promoting the phosphorylation levels of AKT, mTOR and downstream target genes *p70S6K* and *4EBP1*. In consistence with the cellular phenotypes in *Figure 4*, both β1-AR blocker metoprolol and PKA inhibitor H89 treatment reversed the MUT *PRKAG2*-induced phosphorylation of AKT, mTOR, p70S6K and 4EBP1 (*Figure 4E,4F,4H*).

Discussion

As a highly genetically heterogeneous disease, 50–60% of HCM are caused by mutations in myosin genes (12). Recent evidence indicated that metabolism-related MUT can also result in myocardial hypertrophy, called “metabolic HCM”. *PRKAG2* cardiac syndrome is a typical form of metabolic HCM caused by mutations in the PRKAG2 gene, which encodes the γ2 subunit of AMPK (13). *PRKAG2* cardiac syndrome has a high incidence of sudden cardiac death (14,15). However, there is still no official guidelines available for management and treatment of *PRKAG2* cardiac syndrome (16). As described in our recent publication, a family with *PRKAG2* R302Q MUT were diagnosed as *PRKAG2* cardiac syndrome, showing complete AV block and asymmetric ventricular septal hypertrophy (11) (Table S1, Figure S1). Metoprolol, as a selective β1-AR blocker, was applied to these patients for a long-term treatment. As a result, progression of the cardiac hypertrophy was significantly postponed in all the patients upon metoprolol treatment, including decreased ventricular hypertrophy rate measured by the thickness of the intraventricular septum (IVSth) and increased size of LVEDD. In the present study, we are the first to apply β1-AR blocker metoprolol to five patients with *PRKAG2* cardiac syndrome, resulting in great therapeutic effects. In vitro studies using NRCMs and H9C2 cell line demonstrated rescue of the *PRKAG2* R302Q-induced cardiomyocyte hypertrophy and glycogen storage by treatment with β-AR inhibitors. Furthermore, AKT-mTOR signaling pathway was demonstrated to involve in the regulation of *PRKAG2* cardiac syndrome. AKT is a serine/threonine kinase involved in the regulation of multiple cellular functions, including metabolism, glucose uptake, proliferation and protein synthesis (17,18). In the heart, mTOR is a large-molecule serine-threonine protein kinase that is activated by phosphorylation of TSC2 by AKT (19). Activation of mTOR leads to increased proliferation and elevated size of cardiomyocytes, which

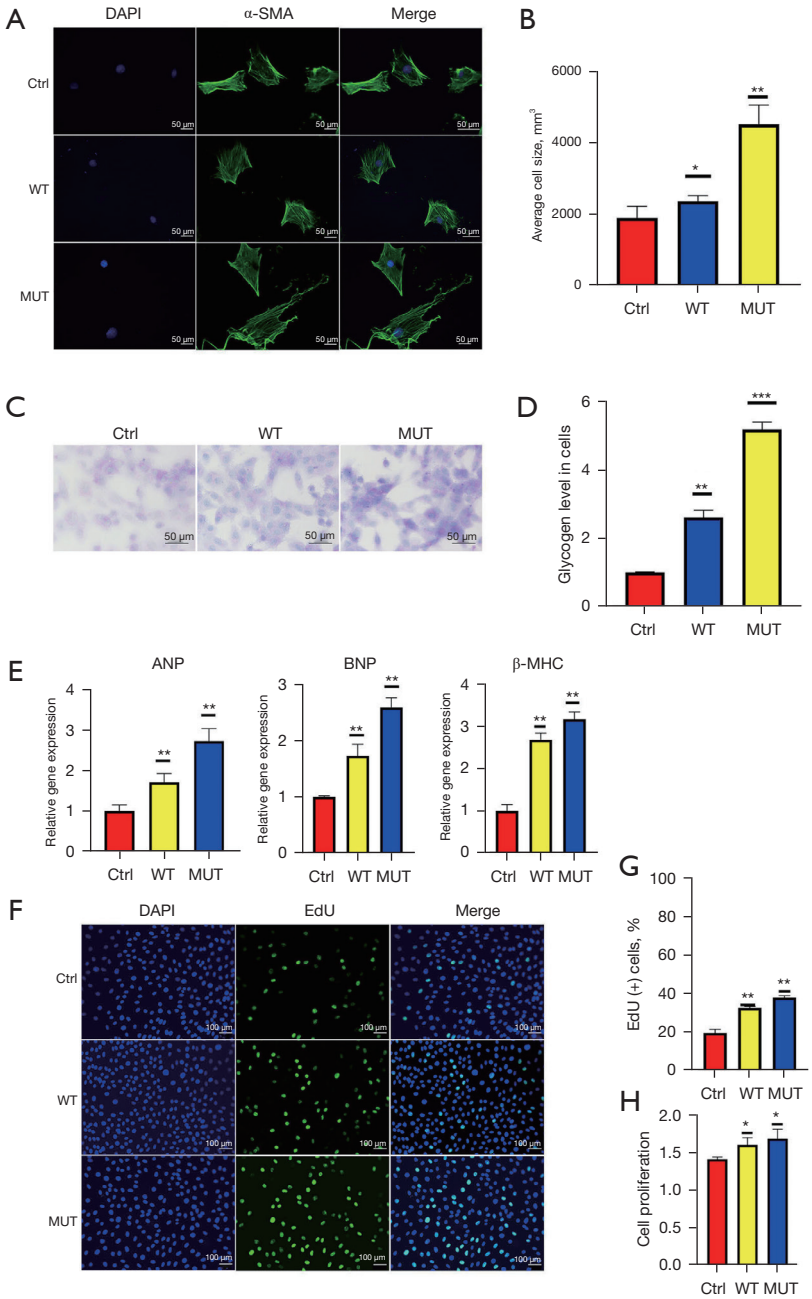

Figure 2 *PRKAG2* R302Q induced myocardial hypertrophy, glycogen storage and proliferation in cardiomyocytes. (A,B) α-SMA immunofluorescent staining of neonatal rat cardiomyocytes showing induced cell hypertrophy by WT and MUT (scale bar: 50 μm). (C,D) PAS staining and quantitative detection of glycogen showed that increased glycogen storage in cardiomyocytes by WT and MUT (scale bar: 50 μm). (E) Increased expression of myocardial hypertrophy markers ANP, BNP and β-MHC in cardiomyocytes by WT and MUT. (F,G) EdU staining showing increased cell proliferation in cardiomyocytes by WT and MUT (scale bar: 100 μm). (H) CCK8 assay further validated the increased cell proliferation in cardiomyocytes by WT and MUT. All assays were performed in neonatal rat cardiomyocytes. The microscopic images were taken with the 40× objective; data are presented as the mean ± SD (n=3). *, P<0.05; **, P<0.01; ***, P<0.001. p, phosphorylation; T, total; Ctrl, control; WT, wild type; MUT, mutant; PAS, periodic acid-Schiff; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; β-MHC, β-myosin heavy chain; SD, standard deviation.

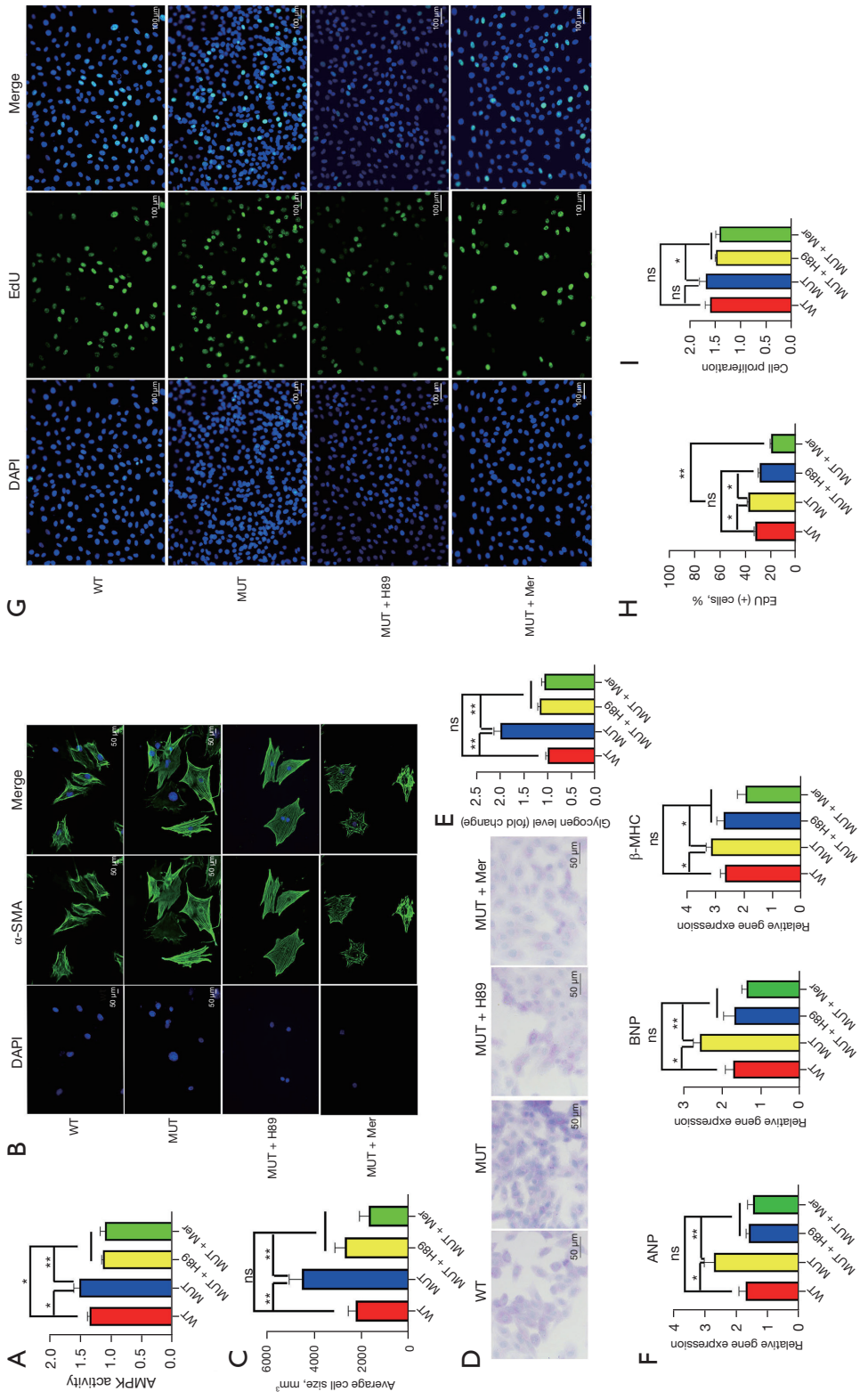

Figure 3 Rescue of *PRKAG2* R302Q-induced myocardial hypertrophy by β1-AR blocker or PKA inhibitor. (A) Application of β1-AR blocker metoprolol (20 μM) or PKA inhibitor H89 (10 μM) rescued the *PRKAG2* R302Q-induced AMPK activity in cardiomyocytes. (B,C) Immunofluorescent staining indicated that application of metoprolol or H89 rescued the *PRKAG2* R302Q-induced cell hypertrophy in cardiomyocytes (scale bar: 50 μm). (D,E) PAS staining and quantitative detection of glycogen showed that application of metoprolol or H89 rescued the *PRKAG2* R302Q-induced glycogen storage in cardiomyocytes (scale bar: 50 μm). (F) Application of metoprolol or H89 rescued the *PRKAG2* R302Q-induced expression of ANP, BNP and β-MHC in cardiomyocytes. (G-I) Application of metoprolol or H89 rescued the *PRKAG2* R302Q-induced cell proliferation in cardiomyocytes. Both EdU staining and CCK8 assays were performed (scale bar: 100 μm). The microscopic images were taken with the 40× objective; Data are presented as the mean ± SD (n=3). *, P<0.05; **, P<0.01. ns, non-significant; WT, wild type; MUT, mutant; PAS, periodic acid-Schiff; Mer, metoprolol; PKA, protein kinase A; β1-AR, β1-adrenergic receptor; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; β-MHC, β-myosin heavy chain; SD, standard deviation.

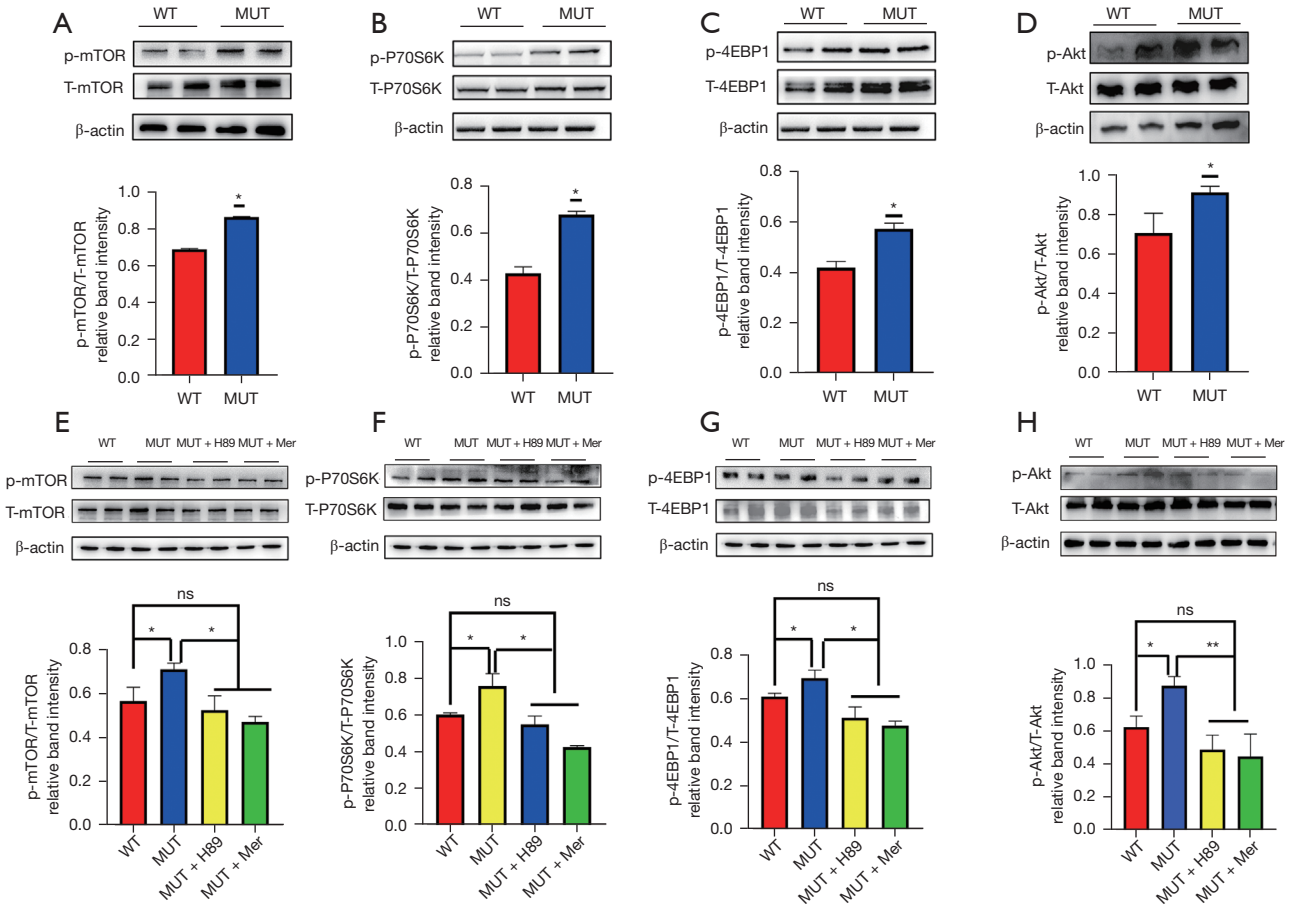

Figure 4 *PRKAG2* R302Q activated AKT-mTOR signaling in cardiomyocytes. (A-D) *PRKAG2* R302Q MUT significantly activated AKT-mTOR signaling in cardiomyocytes by promoting the phosphorylation levels of mTOR (A), p70S6K (B), 4EBP1 (C) and AKT (D). (E-H) Application of β1-AR blocker metoprolol or PKA inhibitor H89 treatment rescued the *PRKAG2* R302Q-induced phosphorylation of mTOR (E), p70S6K (F), 4EBP1 (G) and AKT (H). Western blot analyses were performed. Data are presented as the mean ± SD (n=3). *, P<0.05; **, P<0.01. p, phosphorylation; T, total; WT, wild type; MUT, mutant; Mer, metoprolol; PKA, protein kinase A; β1-AR, β1-adrenergic receptor; SD, standard deviation.

are associated with cardiac hypertrophy (20). *p70S6K* and *4EBP1*, as two of the main target genes downstream of mTOR, are important regulators of protein synthesis in the heart (21,22). In the current study, overexpression of *PRKAG2* R302Q in cardiomyocytes activated AKT-mTOR-p70S6K/4EBP1 signaling, leading to increased cell size and promoted cell proliferation (*Figure 4*).

The cardio-protective effects of β-blockers have been well defined in treatment of patients with coronary heart disease, heart failure, and hypertension, showing clinical outcomes including anti-myocardial ischemia, anti-hypertension, anti-arrhythmias, and increased left ventricular ejection fraction (23,24). In the model of pressure overload-induced myocardial hypertrophy, β1-AR was highly activated and enriched in the heart, accounting for about 70% of the total cardiac β-ARs (25). Continuous activation of β1-AR led to myocardial remodeling, myocardial hypertrophy and even heart failure (26). In consistence, metoprolol showed therapeutic effects here in our study to *PRKAG2* R302Q-induced HCM, adding a node to the regulatory network between β-blockers and heart diseases.

Abnormal activation of AMPK has been considered as a main reason causing myocardial hypertrophy, cardiac conduction disturbances, arrhythmias and even sudden death (27,28). Contradictory results about the effect of *PRKAG2* MUT on the AMPK activity were reported. Introduction of *PRKAG2* MUT into CCL13 cell line decreased the AMPK activity (29). However, *PRKAG2*

cardiac syndrome was shown in the *PRKAG2* MUT-transgenic mice, associated with increased AMPK activity in the heart of young mice and decreased sensitivity to AMP (30). In consistence, here we demonstrated activation of AMPK by *PRKAG2* R302Q in cardiomyocytes.

This is the mechanism study to our previous publication of clinical observations in 5 patients from a *PRKAG2* R302Q-induced HCM family (11). There are still quite a few limitations in the current study. First, this study was based on a small sample size of clinical patients, which did not meet the condition to carry out a randomized controlled trial (RCT) design. Second, the myocardial magnetic resonance imaging (MRI) and myocardial biopsy from the patients were not obtained. Third, the follow-up validation experiments such as transgenic animal models and intervention study *in vivo* were lacking. In order to overcome these shortages, we are planning to establish the induced pluripotent stem cells from the blood cells of the patients, and establish *PRKAG2* R302Q transgenic mice to perform further study. In addition, we will continue to follow up the family patients to validate the therapeutic effects of β-blockers.

In conclusion, the current study demonstrated that application of β1-AR blocker metoprolol is able to ameliorate or even reverse myocardial hypertrophy and glycogen storage induced by *PAKAG2* R302Q MUT via PKA inhibiting activation of the AKT-mTOR signaling pathway, suggesting the potential of metoprolol to be developed as a clinical first-line drug in treatment of *PRKAG2* cardiac syndrome. Nevertheless, there is no doubt that further study through clinical trials will be still required to confirm the therapeutic effects and administrative strategy.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://cdt.amegroups.com/article/view/10.21037/cdt-22-81/rc

Data Sharing Statement: Available at https://cdt.amegroups.com/article/view/10.21037/cdt-22-81/dss

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Conflicts of interest: All authors have completed the ICMJE uniform disclosure form (available at https://cdt.amegroups.com/article/view/10.21037/cdt-22-81/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supplementary

Table S1 The clinical information of PRKAG2 cardiac syndrome patients in a Chinese Han family

Patient No.	Sex	Age at gene sequencing	Preexcitation	AV-block	Hypertrophy	Dose (mg) of metoprolol maintenance
II-3	F	73	–	+	+	47.5
II-7	M	63	–	+	+	47.5
II-9	M	57	–	+	+	47.5
III-2	F	42	–	+	+	47.5
III-7	F	34	+	–	–	–
IV-1	M	23	–	+	+	47.5

F, female; M, male.

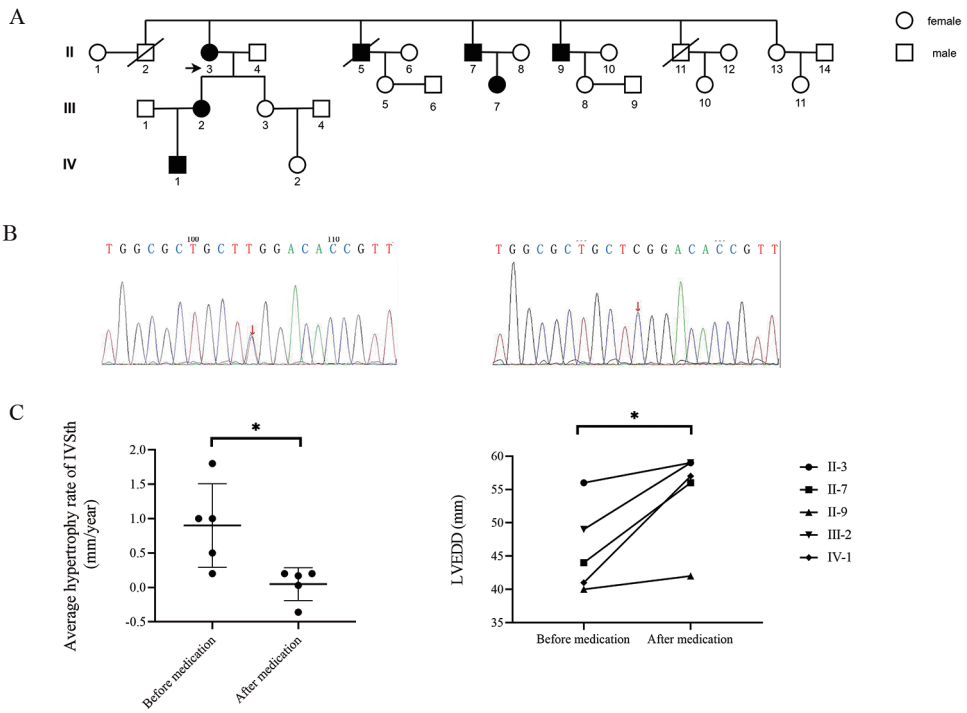

Figure S1 β1-AR blocker metoprolol suppressed the cardiac hypertrophy in patients with *PRKAG2* cardiac syndrome. (A) The pedigree of a Chinese Han family. Squares and circles indicate males and females respectively. An arrow indicates the index patient. (B) Gene sequencing peak map showing the *PRKAG2* R302Q mutation. (C) Annual thickness change of the intraventricular septum in the 5 patients before and after treatment with metoprolol. Change of LVEDD in the 5 patients before and after treatment with metoprolol. Data are presented as the mean ± SD. *, P<0.05 (n=5). IVSth, thickness of the intraventricular septum; LVEDD, left ventricular end-diastolic dimension; β1-AR, β1-adrenergic receptor; SD, standard deviation.

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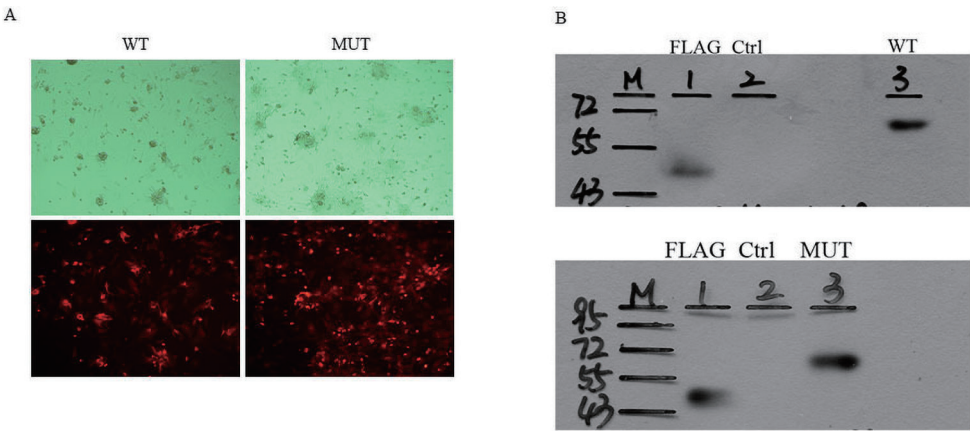

Figure S2 Expression of red fluorescent protein and FLAG after transfection with wild-type and mutant viruses. (A) Red fluorescent expression after virus transfection (magnification, ×5). (B) Identification of viral FLAG tag protein expression, significantly higher in WT and MUT groups compared to FLAG protein standard group. M, marker; Ctrl, control; WT, wild type; MUT, mutant.

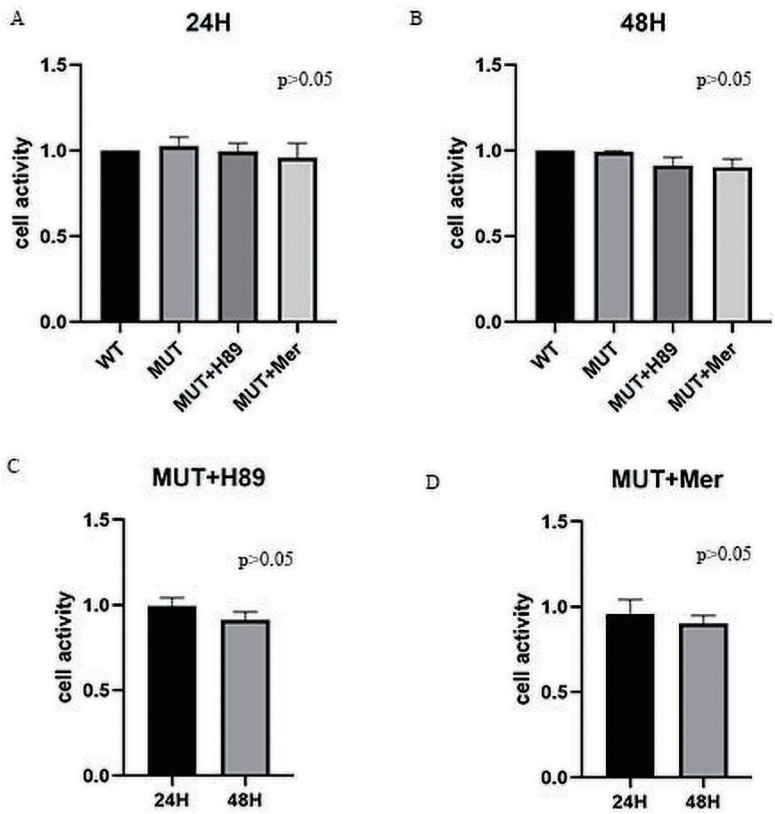

Figure S3 Effect of H89 or metoprolol on cellular activity. (A) Changes in cell activity after 24 h of H89 or metoprolol intervention. (B) Changes in cell activity after H89 or metoprolol intervention for 48 h. (C) Changes in cell activity in the MUT group at 24 and 48 h after the addition of H89. (D) Changes in cell activity in the MUT group at 24 and 48 h after the addition of metoprolol. Data are presented as the mean ± SD (n=6). P>0.05. WT, wild type; MUT, mutant; Mer, metoprolol; SD, standard deviation.

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