Peer Review File

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Reviewer A:

1: How do the authors confirm or judge the level of over expression? Does the AAV construct have a fluorescent labelled tag and if not is there a way that the authors could confirm the AAV transfection in the cells?

Reply: Thank you for the important question. Yes, the AAV construct carries RFP, and was labelled with a flag tag. In order to show the transfection efficiency, the RFP red fluorescence in cells after transfection and western blots against flag antibody were shown in the revised Supplemental Figure 2. The text was accordingly updated in the revised manuscript at page 8 line 188.

2. It is very confusing if the patient data is from this study or an extract from a previous study. While in line 243 it says present study, the patient data is not discussed in the results section.

Reply: Thank you for the important question. The patient data was derived from our previous publication (reference [11]). The current manuscript is the mechanism study to the observations from the patients in the previous study. The text has been updated to make it clearer. Further discussion has been added to the section of Results at page 8, line174-183.

3. Concentration of H-89 and metoprolol used must be clearly given in method section and in figure legends. From Fig 4A, it seems that the cells are unhealthy in the presence of H-89/metoprolol and hence viability of cells should be provided along with these treatment. Because high concentration and cell death could be the reason for suppression of all pathways described in Fig 5.

Reply: The concentrations of H-89 and metoprolol have been added in both Methods and Figure legends at page 6, line 123 and page 19, line 418. At the same time, the cell viability after treatment with H-89 or metoprolol was further measured by MTT method, and there were no significant changes of cell viability observed after drug intervention (as shown in the revised Supplemental Figure 3). The representative images of the cells in Figure 3B were updated too.

4. Fig 5 E-H must have a WT sample in the blots

Reply: Following the reviewer's comment, data from WT samples were added in the revised Figures 4E-4H, which is consistent with the results in Figures 4A-4D. The quantitative analyses were accordingly updated.Actually, the original blots of Figures 4E-4H included WT samples. In order to avoid showing similar results with Figures 4A-4B, we did not show the WT results in the previous version. Following the reviewer's suggestion, the full blots including WT samples were used in the revised version.

5. The representative image is sometimes not matching with the data shown. Eg: Fig 4 G-H (seems H89 treatment suppresses cell proliferation below control), Fig 5A (there seems to be no increase pmTOR levels in the representative blot)

Reply: Thank you for the important comments. The cell proliferation and other data in Figure 3H and 3I have been statistically reanalyzed between H-89 or metoprolol group vs MUT group, and vs WT group. As shown in the revised Figure 3, after treatment with H-89 or metoprolol in the MUT cells, the AMPK activity, cellular glycogen, ANP/BNP expression and cell proliferation showed significant decrease to the levels of that in the WT cells.

For the western blot of p-mTOR in Figure 4, we repeated the experiment and updated the representative images in the revised manuscript.

6.Please give the full form of PRKAG2 the first time you mention. Please mention all abbreviation in full form the first time they appear on manuscript.

Reply: Following the reviewer's comment, full names of all abbreviations have been provided whenever they appear for the first time in the manuscript.

7. The figure number label has to correlate with the results section

Reply: We have carefully checked though the text to correct all the figure numbers to match the figure labels.

Reviewer B:

1. Although there is a lack of uniform guidelines, I would like to see positive controls and negative controls based on the results of the current experiments.

Reply: Thank you for the important comments. In this experiment, we set the empty vector and WT PRKAG2 in parallel as control groups to determine the MUT-PRKAG2 induced phenotypes. In addition, H-89 and metoprolol were used to treat MUT-PRKAG2 cells in comparison with the WT or MUT cells without any treatment. For positive control,due to the lack of uniform guidelines and the fact that we did not find a suitable positive control on Pubmed, at the same time, due to the impact of COVID-19, the purchase and delivery of reagents are greatly restricted, we are difficult to find a suitable positive control in the short term, We will look further in the next experiments, and thank you again for your valuable comments!

2. In the discussion, the authors do not discuss enough the flaws of the article and simply mention a sentence that future clinical trials are still needed to validate. I think it is not enough. The authors should have faced the shortcomings of this article. For example, no transgenic animals were done to achieve animal validation at the tissue and function levels. Table 1 provided is only observational data, not an interventional RCT.

Reply: Thank you for the important comments. In the section of Discussion, we have added a paragraph of discussion to describe the flaws and shortages of the current study, which was also shown below:

"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 an RCT design. Second, the myocardial 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-blocker.