Author's response to reviews

Title: ACTN3 R577X polymorphism and long-term survival in patients with chronic heart failure

Authors:

Sabrina C Bernardez-Pereira (s.bernardez@globo.com)
Paulo CJL Santos (pacaleb@usp.br)
Jose E Krieger (krieger@usp.br)
Alfredo J Mansur (alfredojm@incor.br)
Alexandre C Pereira (alexandre.pereira@incor.usp.br)

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"ACTN3 R577X polymorphism and long-term survival in patients with chronic heart failure"

Dear Dr. Timothy Shipley
BioMed Central
Executive Editor

Please find attached the revised version of manuscript MS1598270842126551. We appreciated the assistance of the reviewers. We are sending a revised version marked in blue plus a letter with a point-by-point explanation of all changes made in the previous version of the manuscript. Also, English grammar was revised in the manuscript.

We hope this revised version has reached the high standards of BMC Cardiovascular Disorders and that it will be interesting to the readers.

Sabrina Bernardes-Pereira, Paulo Caleb Junior Lima Santos, Jose Eduardo Krieger, Alfredo Jose Mansur, Alexandre Costa Pereira.
Reviewer’s report
Reviewer: Isabel Rodriguez

1. In the “Abstract” it is not correct to say that polymorphism “was detected by polymerase chain reaction”. The technique used was “high resolution melting” as it is stated in “Methods”.

Answer: We provided the correction in the abstract.

2. The description of inclusion criteria for patients in “Methods” is not clear (second paragraph).

Answer: Inclusion criteria for this study were very broad. We reference a previous article that already has a description of eligibility for the study and, furthermore, we included some extra information according to reviewer’s suggestion (page 4, lines 6, 8).

3. It is not appropriate to refer to the less-frequent allele (X) as “mutant” (“Genotyping” in “Methods” and second paragraph in “Results”).

Answer: We corrected this (page 5, line 14 and page 6, line 13).

4. Authors set statistical significance at P≠0.05 when usually P=0.05 is not consider statistically significant.

Answer: The level of significance was set at p<0.05 according to reviewer’s suggestion (page 6, line 4).

5. Check typographic errors: “carries” in penultimate paragraph of “Discussion” should be “carriers”, “R577R” in “Conclusion” should be “R577X”.

Answer: We corrected these words and we also revised the grammar of the full manuscript.
Reviewer’s report
Reviewer: Kátia G. Santos

1) As the ACTN3 R577X polymorphism was not previously investigated in heart failure, additional information about it would be interesting, such as the chromosomal location and change at the DNA level (i.e. C/T).

Answer: We included a new sentence in the Introduction section.

2) a) The authors refer to the 577X allele as it would be the polymorphism itself (Abstract section, third paragraph and Results section, second paragraph). The sentence “The frequency of the ACTN3 R577X variant allele was 39.0%” does not state which allele is the variant. By the context, one can infer that the X allele is the variant, but this is not always the case.
b) Patients do not carry polymorphisms. Polymorphism is a genetic concept applied to the population. The population has or does not have certain polymorphisms. Subjects carry alleles, genotypes or haplotypes.
c) Generic sentences as “R577X polymorphism in the ACTN3 gene was independently associated with worse survival in patients with chronic heart failure” (Abstract section, last paragraph) are acceptable in the title and conclusion, but some specification is necessary in the text. Only in the third paragraph of the Discussion section it is specified that the R allele is the wild-type allele.

Answer: a) We corrected the sentence: “ACTN3 577X variant allele was 39.0%”.
b) We provided the correction in throughout the full manuscript.
c) We included in the Introduction section a sentence indicating the wild-type allele. We also added information with specific sentences in the Genotyping and Discussion sections.

3) Authors state in the Abstract section (third paragraph) and in the Results section (first paragraph) that “after mean follow-up of five years, 239 (51.6%) patients met the pre-defined study endpoint”. However, the survival curves (Figure 1) end shortly after the 60th month. So, it seems that 60 months do not correspond to the mean.

Answer: We agree with the reviewer. Indeed, follow-up was conducted until 5-years, and not the mean follow-up was 5-years. The follow-up made and used in this analysis was 5 years. Thus, we corrected these sentences for “follow-up of 5 years”.

4) The last sentence of the last paragraph of the Abstract section states that “Further studies are necessary to ensure its use as a marker of risk for this syndrome”. As the susceptibility to the heart failure was not addressed in the manuscript, I think that the word “prognosis” would be more appropriate rather than “risk”.

Answer: Thank you for this comment. The sentence was changed.

5) Some reference is missing in the first paragraph of the Background section.

Answer: We checked all references.

6) The association of the R577X polymorphism with all-cause death was evaluated by Cox proportional hazard model adjusting for age, gender, body mass index,
ethnicity, LVEF, etiology, hemoglobin, and creatinine. Question: what were the criteria to include these covariates in the multivariate analysis? Were they chosen based on the univariate analysis or were they chosen based on their clinical relevance?

**Answer:** They were chosen based on their clinical relevance regarding to heart failure. The built and added model in the manuscript was the most complete using our patient records. We also included information about clinical criteria in the Statistical section.

7) **How many deaths occurred in both groups of patients (RR and RX + XX)? This information could be included in the Table 1.**

**Answer:** We included this information in the Table 1 according to the suggestion.

8) **Question: Why the chagasic etiology entered the Cox regression model (Table 2) as the reference category? Was it because it has the worst prognosis?**

**Answer:** Yes, we included Chagas Disease as the reference category in the Cox Regression Model because it has the worse prognosis. The following references provide empirical data to this analytical approach. We also included this information in the Methods section.


9) **Typo errors: ‘carries’ instead of ‘carriers’ in Discussion section, fifth paragraph, ‘R577R’ instead of ‘R577X’ in Conclusion section, first paragraph, ‘Kg’ instead of ‘kg’ in Table 1, and ‘Chagastic’ instead of ‘Chagasic’ in Table 2.**

**Answer:** We provided the corrections.
Reviewer’s report
Reviewer: Daowen Wang

1. Please indicate the study have sufficient samples size to get all the robust results. It would be appropriate to give a description of the power calculation with the QUANTO program or other software in the statistical analysis section.

Answer: We added the following information in the Statistical section according to the reviewer’s suggestion.

“Our sample size provides 80% power to detect an association with the endpoint event with an effect size of 1.4 for the ACTN3 R577X polymorphism.”

2. The author intended to present this polymorphism as a biomarker to predict the prognosis of heart failure. To confirm this result, a validation in a second population is needed. Additionally, it will be better if the author could evaluate the sensitivity and specificity of the biomarker by some way, for example, the Received Operator Curve (ROC).

Answer: We inserted the context above in the limitation paragraph of the Discussion section.

“…In addition, we suggested that the R577X polymorphism might be used as a prognostic marker in heart failure. However, validation in a second population and analysis of analytical parameters of the biomarker are needed.”

Minor Essential Revisions:
1. In this prospective cohort study, the Cox proportional-hazards model was used to investigate the risk factors of all-cause mortality for patients with heart failure including the R577X polymorphism. The author showed X allele carriers had a higher risk of mortality (HR 1.72, 95% CI 1.14- 2.62, p=0.01). It should be elucidated more clearly whether the hazard ratio has been adjusted for the confounders, especially hemoglobin, creatinine, LVEF, and the etiology of hypertension that have been approved to affect the mortality risk of heart failure.

Answer: We clarified in the manuscript that the HR modeled was adjusted for all cited confounders from model (Table 2). Variables were based on clinical relevance regarding heart failure, even without a significant p value in the model. The model using only significant variables were not different from the current one described.

2. The median follow-up time and dropout rate should been explicitly given in the manuscript in order to evaluate the integrity and reliability of the follow-up information.

Answer: We included follow-up data and dropout rate in the Results section.

Thank you very much for your assistance. We are certain that the revised manuscript is much improved thanks to the help of the three reviewers.
Best regards,

Alexandre Pereira, MD, PhD.
Laboratory of Genetics and Molecular Cardiology,
Heart Institute, University of Sao Paulo Medical School, Sao Paulo, Brazil.