Author's response to reviews

Title: Association between UCP2 polymorphism A55V and risk of cardiovascular events in patients with multi-vessel coronary arterial disease.

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Author's response to reviews: see over
"Association between UCP2 polymorphism A55V and risk of cardiovascular events in patients with multi-vessel coronary arterial disease."

Dear Dr. Tim Sands
Executive Editor,
BMC-series Journals

Please find attached the revised version of manuscript MS: 6144397645368746. We appreciated the assistance of the reviewers, and we have made all suggested changes providing a point-by-point description. We hope this revised version has reached the high standards of BMC Medical Genetics and that it will be interesting to its readers.

Reviewer 1's comments to author:
Reviewer: Gilberto Velho

1. Methods / Statistical analysis. The authors have not adjusted the threshold of statistical significance to take into account stratification by glycaemic status. This point deserves justification and discussion.

Answer: Thank you for the review of this manuscript and suggestions sent. We would like to emphasize that the results were adjusted for different variables, including blood glucose as the following sentence below (last paragraph of the Results section):
"Results were adjusted for different variables (hypertension, blood glucose, age, BMI, type of treatment, smoking status and coronary anatomy) and maintain significant incidence of
AMI p=0.0070 (OR=6.83 [1.70 – 27.54]), new revascularization intervention p= 0.034 (OR=3.77 [1.29 – 11.03]) and combined-events p=0.034 (OR=1.91 [1.05 – 3.48])”.

2. Methods / Statistical analysis. The sample size of this investigation is small for an association study, especially considering the low incidence of cardiovascular events during the follow-up. Authors should present a power calculation.

Answer: We now provide power calculations and inserted a limitation paragraph in the Discussion section with the following sentences:

“There are some limitations in our study. First, we were not able to perform replication studies in a similar sample because there are very few studies similar to MASSII in the literature. Second, our sample size only provide 80% power to detect an association with the death, AMI, revascularization intervention, events variables with an effect size of 1.7-2.4 or 1.8-3.6 for the A55V polymorphism in the total group and in the dysglicemic patients, respectively.”

3. The introduction section could be more concise. There is too much general information that is not necessarily needed to introduce the present work.

Answer: We reorganized the Introduction section and excluded irrelevant paragraphs according to reviewer’s suggestions.

4. Methods / Data collection. The criteria (and methods) used for classifying the glycaemic status should be given.
Answer: We inserted a paragraph in Methods section describing the criteria used for classifying the glycaemic status of the patients. We used the definition of the WHO (World Health Organization) effective at that time of the randomization.

5. Methods / A55V genotyping. This is another section that could be more concise. There is a long paragraph that could be replaced by something much more simple such as “Genotyping was performed with an Array Tape technology (Douglas Scientific, Alexandria MN) (reference or web site).” The manufacturer of the allele-specific PCR assay that was used could be given.

Answer: We modified the Methods section according to reviewer’s suggestions and completed the missing information.

6. Methods / A55V genotyping it would be important to present the rational for selecting the A55V polymorphism of UCP2 (as opposed to others that have been reported).

Answer: In respect to this point, we opted to analyze the A55V polymorphism despite of others polymorphisms in UCP2 have already been identified, once recent reports described that UCP2 might play an important role in cardiovascular diseases and that A55V polymorphism can cause UCP2 dysfunction.

7. Results. What is the rational for grouping Ala/Ala and the Ala/Val genotypes? When considering the frequency of cardiovascular events according to genotype, is there any evidence for a dominant Ala effect?
Answer: We include a paragraph in Discussion section with the following: “It was described that in humans, the extent of uncoupling is partially regulated by the genetic polymorphism Ala55Val, in which the VV genotype uncouples at a lower rate than the AA genotype. In addition, it was reported that A allele was related to the presence of higher oxidative stress markers and it represented a significant bigger risk when associated to other risk factors (obesity, hypertension and diabetes). At that rate, we opted to group the Ala/Ala (AA) and Ala/Val (AV) genotypes in order to observe some effect but there was no significant difference in baseline values.”

8. Results. Tables 2, 3 and 4 could be combined into a single table. Moreover, those p-values are redundant with those of Cox analysis shown in the text and figures. I would suggest keeping the genotype frequencies and giving the hazard ratios (95% C.I.) and p-values of the Cox regression analyses. Cox is more appropriate than chi-square for incidence studies.

Answer: We improved the formats and contents of the tables but we did not combine them why this would cause difficulty in the reading, once we would have to reduce the size of the font text. This point was observed by the second referee.

9. Discussion. Authors could include a paragraph with strengths / weaknesses of the study.

Answer: We inserted a limitation paragraph in the Discussion section according to reviewer’s suggestions (mentioned above, item 7).
10. Discussion. Please discuss clearly the reasons you believe may underline the lack of association after 5 years of follow-up. Is there any experimental evidence that the duration of diabetes plays a role as you suggested? What about mortality related bias?

Answer: After 2 years of follow-up, patients with glycemic disturbance harboring the VV genotype had higher occurrence of AMI, Death+AMI, new revascularization intervention and combined events as compared with dysglycemic patients with another genotype. We were not able in detect these differences between the groups after 5 years of follow-up. We speculate that the predictive effect of UCP2 genotype in dysglycemic patients could be related to the time since the glycemic status has been acquired. On the other hand, there is not experimental evidence that the duration of diabetes could play a role in this result. Further studies are necessary to establish the role of VV genotype as an independent genetic marker of cardiovascular events in this specific subgroup.

11. General comment: The manuscript needs a writing revision. There are several awkward and wordy sentences and many typos.

Answer: We have made all suggested changes regarding to spelling mistakes, missing or wrong data in the manuscript.

12. Methods / Patients selection. One sentence summarizing the main results of MASS II would be welcome.

Answer: We have provided more details about MASSII study in Methods section and the summary sentence as suggested.
Reviewer2's Comments to Author:

Reviewer: Vicky Cameron

1. There is no definition of ?dysglicemia? or ?glycemic disturbance.? Do the authors mean type 2 diabetes, glucose intolerance or insulin resistance? Please define these terms in the Methods. What the total number of patients with dysglicemia? Please state in Results how many in this subgroup.

Answer: Thank you for the review of this manuscript and suggestions sent. We inserted a paragraph in Methods section describing the criteria used for classifying the glycaemic status and the total number of patients with dysglicemia in Results section as the following sentences below:

"Glycemic status was defined using effective WHO classifications at that time of the randomization. Briefly, diabetic patients should present classic symptoms of hyperglycemia and an abnormal blood test [plasma glucose concentration >=7 mmol/L (or 126 mg/dL) or >=11.1 mmol/L (or 200mg/dL) 2 hours after a 75g glucose drink]. Impaired glucose intolerance were diagnosed in patients with fasting plasma glucose <7.0 mmol/L (or 126 mg/dL) and 2 hour post 75g glucose drink of >= 7.8 mmol/L or 140 mg/dL and <11.1 mmol/L (or 200 mg/dL). Patients with diabetes and impaired glucose intolerance composed the dysglycemic group."

2. The Introduction is too long and contains irrelevant information. In paragraph 2 it would be possible to keep only the first sentence and delete the rest of the paragraph. Also delete paragraph 7.
Answer: We reorganized the Introduction section according suggestions and deleted the irrelevant information.

3. In the Methods chapter, more details are needed and the methods need to be more rigorously described. Please give details the ethnicites that constitute the patient group. On page 6 the authors state that events were tracked; how were they tracked? Under genotyping, what is the reference for the salting?out procedure of DNA extraction? The allele-specific PCR assay was modified from the published method in what way? The confirmation of genotyping results was performed on 32 samples; how were these repeat samples selected? The statistics section mentions haplotype analysis, but no haplotyping data are provided.

Answer: We provided the information requested in Methods section.

4. In the Results section, what was the justification for combining the major allele homozygotes and heterozygotes? Is there any previous evidence that the polymorphism effects fit a recessive model? Please explain the rationale for including the variables (hypertension, blood glucose, age, BMI, type of treatment, smoking status, coronary anatomy) in the multiple regression model.

Answer: We answered the first question in item 7 (to Reviewer 1).

In respect of the variables used in the multiple regression, we would like to emphasize that they were selected in the beginning of the MASSII study. In our manuscript, we investigated the association of A55V polymorphism with cardiovascular events in this group of patients and we maintained the same analyses.
5. The meaning of last paragraph of the Results section is unclear. At 5 years the authors observed no differences between the groups in what? The suggestion that the predictive effect of UPC2 genotype in dysglycemic patients could be related to the time since the glycemic status has been acquired does not make sense. Surely the time of follow up started from the index admission for angina, not the time when the patients became dysglycemic?

Answer: In fact, the follow up did not start depending on glycemic status, but when patient were diagnosed with coronary artery disease and a treatment was proposed. At this way, previous diseases like diabetes or impaired glucose intolerance could increase the cardiovascular risk but we were not able to explain it yet. Here, we speculate that the predictive effect of UCP2 genotype in dysglycemic patients could be related to the time since the glycemic status has been acquired. On the other hand, there is not experimental evidence that the duration of diabetes could play a role in this result. Further studies are necessary to establish the role of VV genotype as an independent genetic marker of cardiovascular events in this specific subgroup.

6. The Kaplan-Meier survival curve was missing in my copy of the manuscript.

Answer: We submitted the tables and figure of the manuscript according to instructions of the journal.

The Discussion needs to be rewritten. The first paragraph could be deleted as it adds nothing to the manuscript. The remainder of the Discussion needs to be re-formatted so
that it follows a logical sequence. The English language needs to be corrected as many passages are unintelligible.

Answer: Discussion section was reformulated according to reviewer’s suggestion. We corrected and reviewed manuscript grammar.

8. The tables are very difficult to read, with 8 font text, columns that do not line up and the text squashed up against the horizontal lines.

Answer: We improved the formats and contents of the tables according to reviewer’s suggestions.

Minor Essential Revisions

Please note that there are numerous typographical errors and uses of non-colloquial English throughout the manuscript.

Answer: We improved all the manuscript with special attention to the orthographic and grammatical mistakes according to reviewer’s observation.

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

Best regards,

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