Author's response to reviews

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

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

Dear Dr. Tim Sands

Executive Editor,

BMC Medical Genetics

Please find attached the revised version of manuscript MS6144397646368746. We appreciated the assistance of the reviewers, and we have made all suggested changes providing a point-by-point description.

We hope this new 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: Vicky Cameron

The Revised manuscript is very much improved and the authors have addressed all of the major comments I raised. The style of English writing has greatly improved, although is still non-colloquial in parts and could be improved further. The journal editors may need to provide some guidance here if the manuscript is accepted.

Answer: Grammar was reviewed throughout the manuscript.
Reviewer 2's comments to author:

Reviewer: Gilberto Velho

1. Methods / Statistical analysis. The authors should adjust the threshold of statistical significance to take into account the stratification of the comparisons by the glycaemic status (analyses performed separately in dysglycaemic subjects and in subjects with normal glucose tolerance). Applying a simple Bonferroni correction, authors should consider significant p<0.025.

**Answer:** We inserted in the revised Statistical analysis section the following sentence: “Multiple testing correction was not performed”. Unfortunately, our sample size does not preclude adjustment for multiple testing. This limitation is expressed in the revised manuscript. In addition, it should be pointed-out that even with a conservative correction (i.e. Bonferroni), our results would still be considered significant.

2. Results. Text and tables 2, 3 and 4: It is not clear which statistical test was used and when it was used. P-values in tables 2, 3 and 4 seem to be derived from chi-square tests. This test is not appropriate for incidence studies. In the methods section the authors state that “Survival curves were calculated with the Kaplan–Meier method and differences between the curves were evaluated with the log-rank statistic. We assessed the relationship between baseline variables and composite end-point events using a Cox proportional hazards survival model. Hazard ratios (relative risks) with 95% confidence intervals (CI) demonstrate the risk for combined events.” In the results section, which comparison was tested by log-rank statistics and which by Cox? In the last paragraph of results, some odds ratios are given. How were they computed? If they are derived from Cox analyses, they should be given as hazard ratios.

**Answer:** We indicated in the statistical tests performed in tables 2-4 (now, table 2) in the Statistical analysis section. We agree this information was not clear in the last version of
our manuscript. Indeed, we provide the reader with 3 different analyses showing the same results: a frequency comparison using a Chi-square test (used in Table 2). The same comparison using Kaplan-Meier curves (Figure 1) and a multivariate adjusted model provided in the results section. We also changed the OR for HR (this was wrongly typed in the last version).

3. The genotype frequencies and number of subjects (AA, AV and VV) at baseline, and at 2 and 5 years of follow-up should be given for subjects with or without CAD, with stratification by glycaemic status. A proper analysis of the data might help to understand what happens between 2 and 5 years of follow-up. It is important to describe what's going on with the data (AA/AV incidence of CAD truly catching up, different rates of lost to follow-up for different genotypes including mortality bias) before considering other possible causes for the lack of association (impact of duration of diabetes). Tables 2-4 are difficult to understand because the total number of subjects at each time point is not given. For instance, if we consider table 3, 32.5% of VV carriers had a CAD event after 2 years of follow-up, but only 8.9% of them had a CAD event after 5 years of follow-up. This presentation does not make much sense. Moreover, if the number of subjects was greatly reduced at year 5 of follow-up your comparisons might have lacked power to detect an allelic association at this time point.

Answer: We inserted number of patients (AA, AV, VV) according to glycemia status at baseline, and at 2 and 5 years of follow-up in the Result section: “From 611 eligible patients, 559 individuals were genotyped and followed-up. There were 260 dysglycemic patients (148 diabetic and 112 glucose intolerants) and 299 patients with normal glucose tolerance as shown in table 1.”

We included the number of subjects in the Tables according reviewer’s suggestion.
We corrected some numbers and frequencies on tables; in addition, we also checked the other tables.

Thank you very much for your assistance.

Best regards,

Alexandre Pereira, MD, PhD.

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