Author's response to reviews

Title: T786->C polymorphism of the endothelial nitric oxide synthase gene is associated with insulin resistance in patients with ischemic or non ischemic cardiomyopathy

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Author's response to reviews: see over
Pisa, September 3, 2012

Re: MS: 1492239715714561

Title: T−786→C polymorphism of the endothelial nitric oxide synthase gene is associated with insulin resistance in patients with ischemic or non ischemic cardiomyopathy.

Dear Editor,

Please find enclosed a revised version of the above referenced manuscript. We are grateful to Reviewers for useful and constructive suggestions. In the new version we tried to resolve all the issues raised by reviewers and answer accordingly. The added sections in the manuscript according to the Reviewer’ comment are indicated in red italic.

We hope that the revised manuscript adequately addressed all criticisms.

Yours sincerely

Dr. Cecilia Vecoli

Referee 1

We thank this reviewer for the comments and recommendations. Specific responses follow to each issue raised.

• Besides T−786→C eNOS polymorphism, there is a polymorphism of eNOS I/D, why not detect the eNOS I/D polymorphism?

Authors’ replay
I apologize with the Referee but I am not able to recognize the demanded eNOS I/D polymorphism. Indeed, several polymorphisms in eNOS gene have been identified. We studied the T−786→C variant because it has been previously associated with reduced eNOS expression and has been linked to IR.

2. The sample size in this study is so small that the statistical power is limited. Additionally, the significance is marginal.

Authors’ replay

The study was powered in order to detect a 15 % difference or more in HOMA-IR in the heterozygous carriers of T−786C variant with a power of β =80% by means of a two-sided t-test with α = 5%. This was added in the section statistical analysis. However, we agree with the Referee because an undoubted limitation of the study is the modest size of the study population that make difficult a correct stratification of analysis and limits the extendibility of results to the general population. Accordingly, we specified “the modest sample size” as a study limitation in the last part of discussion.

“Assuming a mean value of HOMA of 2.0 and standard deviation of 2.5, a study with a sample size of 130 patients would be needed to detect a 15 % difference or more in HOMA-IR between the heterozygous and the TT homozygous patients for T−786→C variant, with a power of β =80% by means of a two-sided t-test with α = 5%.”

“Our study findings should be interpreted bearing in mind some limitations. Firstly, we acknowledge that our sample size may temper statistical estimations in some categories. Secondly, genetic and acquired factors able to condition the presence and the extent of cardiac damage and the development of HF in different individuals are multiple and interactions are complex. Accordingly, adequate experimental models and large longitudinal clinical studies are needed to better elucidate the pathogenetic and prognostic relevance of these observations.”

3. The data should be adjusted, at least by gender, age, smoking status.

Authors’ replay

The logistic regression analysis has been adjusted by age and sex male according with the recommendation by Referees 1 and 2 (please also see response to Referee 1).
Referee 2

We thank this reviewer for the comments and recommendations. Specific responses follow to each issue raised.

Abstract

Minor Essential Revisions
• Avoid using abbreviations. Essential abbreviations must be defined at their first mention in the abstract itself.

Authors' replay

The abstract has been revised defining the abbreviation at their first mention (red Italic)

Methods

Statistical analysis:

Major Compulsory Revisions
• Normality tests must be performed before evaluating differences in means for continuous variables by Student's t test or ANOVA. If necessary, appropriate non-parametric tests should be used.

Authors' replay

This observation is right. Because of the skewness of the distributions of biochemical values, analyses have been performed using the logarithmic transformation of data.

We clarified in the new version of the manuscript that analyses have been performed using the logarithmic transformation of data (see “Statistical analysis” in Materials and Methods”).

“Because of the skewness of the distributions of biochemical values, analyses have been performed using the logarithmic transformation of data.”

• The Fisher exact test is not appropriate to assess significant differences among pairs of means. Post hoc tests for multiple comparisons, such as Bonferroni test, should be performed for this purpose.

Authors’ replay

Thank you for your comment. We changed the data analysis as suggested.

Results

Major Compulsory Revisions
• Tables 1 and 2. Age and sex are potential confounding factors and therefore should be introduced in the conditional logistic regression models. Thus, Odds Ratio (OR) values adjusted for gender and age should be reported in the tables.

Authors’ replay

Age and sex has not been introduced in the conditional logistic regression models because we did not consider adequate to include negative statistical predictors at univariate in multivariate analysis. Anyway, we agree with the referee that age and sex are potential confounding factors. Thus, Odds Ratio values adjusted for gender and age have been reported in the tables 2 and 3 (red italic).
• Contrary to what is stated in the third paragraph of the discussion section, the p value shown in Table 2 does not confirm the association between history of hypertension and insulin resistance. This point should be clarified.

Authors’ replay
We better clarified this point in the discussion.

“Anyway, data showing a clear, exact causal order between eNOS gene expression, hypertension and insulin resistance are unavailable. Likewise, the mechanisms by which the primitive endothelial alteration can affect glucose homeostasis and systemic blood pressure are not fully known.”

References
Major Compulsory Revisions
• The style of reference is different from the requirement. Compare with one of the published papers.

Authors’ replay
The references have been styled according to the Journal instructions.