Author's response to reviews

Title: The interactive role of diabetes mellitus type 2 and E-selectin S128R mutation on coronary heart disease manifestation

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Author's response to reviews: see over
Response to reviewers’ comments

We have completely revamped our manuscript putting into consideration the suggestions and queries of the reviewers. We have adopted virtually all the suggestions of the reviewers, and provided an explanation to those queries we might not have been able to comply with.

Further to the Editorial queries, we have now explained in greater detail the issue of ethical approval to read: “Full informed consent was obtained from all patients or family members before participating in the study. This study was performed in accordance with the Declaration of Helsinki as adopted and promulgated by the US National Institutes of Health, as well as rules and regulations laid down by the Hospital’s Ethics Committee”.

Attached is the (detailed) point-by-point response to the reviewers’ comments on the manuscript.

We hope that the manuscript is now suitable for publication.
The interactive role of diabetes mellitus type 2 and E-selectin S128R mutation on coronary heart disease manifestation

Reviewer: Carmine Gazzaruso

Major Compulsory Revisions

The study groups should be well characterized. Clinical and demographic features of patients and controls should be given. Indeed traditional cardiovascular risk factors (such as lipids, hypertension, obesity in the whole populations, and diabetes control in diabetic patients) can affect the results. Some medications, such as statins and some antihypertensive drugs, can reduce the cardiovascular risk. So other variables should to be considered in univariate analysis.

We have now characterized the study groups as much as we could. A table has been included on the clinical and demographic features of the control and patient groups (Table 1, page 15). The variables have also been included in the univariate analysis and described in the Results section.

In addition multivariate analysis is needed to explore the impact of other variables on the association between E-selectin S128R mutation and CAD.

Multivariate analysis has been performed and the results presented in Table 3 page 17.

The authors should clarify how type 1 diabetic patients were excluded from the study.

Exclusion of type 1 diabetic was based on the patient demographic data and the guidelines for characterizing of patients as having type 2 diabetes followed by our Institution.

The exact meaning of “no significant CAD” should be clarified. Indeed patients with a coronary stenosis <70% but near to 70% cannot be considered as a control.

“No significant CAD” is a modest way of classifying angiographed individuals who were defined as having no coronary heart disease under the Institutional guidelines. The reason for choosing 70% as cut-off was simply to make sure that the patients have positive evidence of disease, but no individual with any evidence of coronary insult was included as a control. We have now omitted the word “severe” in order to avoid possible misinterpretation of the criteria.

In any case, CAD extent should be considered. The number of vessels diseased or a scoring system, such as the Gensini one, could be used.

It is true that correlating the number of diseased vessels would have been of interest. Our initial attempt to do this kind of analysis indicated that there was no significant difference among the groups with single, double or triple diseased vessels, but this might be due to the inequality of the sample sizes in the three groups. We feel that this might constitute a separate study and we intend to eventually assess this idea is a much larger population, which would give us greater statistical power. However, we feel the present data suffices for the assessment of the possible implication of this interaction on CAD as a whole.

Minor Essential Revisions

In the title and in the text the term “manifestation of CAD” should be changed to “CAD” or coronary atherosclerosis, since the study does not refer to clinical presentations of angiographic CAD. “Diabetes mellitus type 2” should be changed to “type 2 diabetes mellitus”

We have now changed the title accordingly, and the phrase “diabetes mellitus type 2” to read “type 2 diabetes mellitus”
The interactive role of diabetes mellitus type 2 and E-selectin S128R mutation on coronary heart disease manifestation

Reviewer: Maria Grazia Andreassi

We have revised our manuscript paying attention to the suggestions of the reviewer. The following is our response to her queries and suggestions.

General

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The study has some limitations that need to be addressed.

1) The paper is extremely unclear in terms of the multivariable analysis of data. My main comment, therefore, is to reanalyze the data by using a multivariate logistic regression analysis in order to adjust for potentially confounding variables.

   We did multivariate logistic regression. Accordingly, we have now included cholesterol, triglyceride, gender, age, hypertension, family history of CAD and smoking in addition to the variable representing the interaction between diabetes and E-selection. The interaction was 6.41 (95% CI 3.61-11.37) adjusted for all these variables, which is similar to the results from the univariate analysis. This has been explained further in the manuscript.

2) CAD was defined as severe stenosis (at least 70%). If they set the threshold of coronary CAD at mild stenosis was the obtained result identical?

   We actually recruited only patients with at least 70% narrowing of vessels simply as a way of being positively sure that individuals included in the study were all having CAD. We do not believe that the result would have been different with CAD at mild stenosis. We have now omitted the word “severe” in order to avoid possible misinterpretation of the criteria.

3) The genetic architecture of atherosclerosis and/or diabetic macrovascular vascular complications is likely to result from the contribution of many genes interacting with different environmental factors. An undoubted limitation of our study is the lack of a more comprehensive genetic analysis of attractive candidate genes.

   Recently developed assays capable of simultaneously genotyping multiple loci are in common use in association studies. Therefore, screening for genotype combinations of candidate genes might offer a better opportunity to identify diabetic patients at a high risk for macro-complications. Briefly, discuss this issue as a potential study limitation.

   We find this suggestion appropriate, and have integrated it into the Discussion section virtually as suggested by the reviewer.
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Reviewer: Oswald Friedrich F Wagner

Below is a detailed descriptions of actions taken in response to the reviewer’s queries and comments

General Comments

The population is well selected, however this is a purely descriptive paper and thus might be better published as a letter or short communication. Additionally, the paper should be critically read for grammar and wording.

We have completely revamped the paper and double-checked for grammatical errors. We hope that we have now reduced the typos to a minimum.

Major Compulsory Revisions

Results

The Result section is very short and difficult to understand – could the authors please describe their findings in more detail.

The results have now been described in greater detail as requested. We hope that the changes meet the requirement of the reviewer.

What does: The E-selectin – diabetes mellitus interaction was studied by creating a variable which was used as a factor to predict CAD by logistic regression. confer?

The statement has been re-written for clarity.

the presence of DM2 was consider as the environmental factor – considered ?

We have now re-phrased the paragraph to read “In assessing the interaction of E-selectin genotypes with diabetes (environment), we followed the approach of Yang and Khoury (1997). In this model the genotype is dichotomous (carriers versus non-carriers of E-selectin) and the environmental exposure (diabetes) is dichotomous (diabetic versus non-diabetic). In the present case, the four possible combinations of genotype and exposure can be displayed in a 2 x 4 table, and three categories of joint exposure can be compared with a reference category (for which the relative risk is by definition, 1.0). The relative risk of developing CAD in individuals who are both genetically susceptible to the condition and have been exposed to the environmental variable compared to the reference group will be the measure of interaction. ……” This information has been incorporated into the Results Section (page 6)

The selected statistical method may be applied in addition. However descriptive and non-parametric tests should not be omitted entirely.

We have improved the statistical analysis accordingly.

Why did the authors not measure plasma selectin levels in addition? – this might also help speculate on the underlying mechanism.

It is true this plasma selectin levels could have enhanced our findings. We did not include this simply because we unfortunately did not plan to look at this point at the time of initial sample collection.

Was the Hardy Weinberg equilibrium reached?

We do not think the hardy-Weinberger equilibrium was necessarily reached, since it is difficult to completely exclude the issue of consanguinity among the studied community.

Discussion

It suggests also that role of this gene in CAD is not specific for a certain ethnic group, but rather linked to the prevalence of DM2. – do the authors have any reference or data to support their statement?
We base our argument on the conflicting observations related to whether the importance of the gene is related to gender, ethnic groups or there is any direct association with CAD.

Could the authors speculate on any underlying effects or regulatory effect of the polymorphism?

We chose to make some speculation on possible underlying effect along the lines of some recent findings suggesting that this mutation may be involved in prothrombotic activity (page 9 of the revised manuscript).

References

As this is not a short report – unless not stated - the current work - especially the Discussion section - should be more extensively referenced.

We have referenced the Discussion more extensively as required

Minor Essential Revisions

Abstract

Line 2: is not fully understood.

The results show that in the absence of DM2, the presence of R mutant allele does not have a significant effect on the development of CAD (p = .431, OR 1.28). – please rephrase – does the author mean carriers?

We have rephrased the statement to read “In the absence of DM2, the presence of the R mutant allele alone is not significantly associated with CAD (p = 0.431, OR 1.28). In contrast, in the presence of DM2 and the S allele, the likelihood of an individual acquiring CAD is significant (odds ratio = 5.44; p = < 0.001). “

What does the author mean with deleterious?

By “Deleterious” we meant “detrimental”. Anyhow, we have now changed the statement as a result of the overhauling of the manuscript in general.

Methods

among others - please describe

rs Number of the selected polymorphism is missing

The rs number of the S128R polymorphism is rs5361. It has been included into the text on page 3.