Author's response to reviews

Title: Common Genetic Variants on Chromosome 9p21 Are Associated with Myocardial Infarction and Type 2 Diabetes in an Italian Population.

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Author's response to reviews: see over
To Scott Edmunds, PhD

Milan, 22nd December 2009

Dear Dr Edmunds,

we wish to submit our revised manuscript entitled “Common genetic variants on Chromosome 9p21 are associated with myocardial infarction and type 2 diabetes in an Italian population” for publication as research article in BMC Medical Genetics.

The manuscript has not already been published in any journal and all authors have read and agreed to its revised content.

We thank reviewers for their stimulating comments on our manuscript. The revised version of the manuscript attempts to address these comments.

Reviewer Harald Goring

Major points:

Based on Figure 2, it appears that the joint results from this investigation and the PROCARDIS dataset are quite convincing with regard to the association for these 2 SNPs, but no p-values are provided.

P-values for the pooled data were provided and added to the text in the results section:

“SNP rs2891168 was confirmed as associated with CAD (p<0.0001) with a G-allele OR of 1.28 (95% CI 1.20-1.36), and SNP rs10811661 was associated with T2D (p=0.04) with a T-allele OR of 1.19 (95%CI 1.01-1.41).”

The authors should explicitly summarize the findings regarding these 2 specific SNPs from other studies, such as various genome-wide association studies.

According to the reviewer’s suggestion, a table has been added to the text:

Table 1

The authors should state explicitly where the samples come from. Simply referring to earlier manuscript is not sufficient.

Details on the origin of samples have been added to the text in the study population section:

“Both GISSI-P and IGLOO study are multicentre Italian studies approved by the local ethics committees of the participating hospitals (see references 16,17 for the complete list). Written informed consent to participate in the study including blood sampling was obtained for each subject.

The characteristics of the studied subjects have been described previously [16,17]. In brief, GISSI-P patients were selected on the basis of clinical diagnosis of recent (<=3 months) MI, without limits of age
and distinction of sex. The IGLOO population was represented by men and women aged between 55 and 75 years, without a history of cardiovascular events as angina and myocardial infarction and with one or more cardiovascular risk factors. All patients were referred to a diabetes outpatient clinic to perform an oral glucose tolerance test, with determination of venous plasma glucose, fasting and 2 hours after the ingestion of 75 g glucose. Those recruited in the present study have been identified to have T2D.

Minor points:

“a [...] power calculation indicated [...] 80% power to highlight at least a 30% genetic risk for diabetes ...”: 30% genetic risk does not appear to be the appropriate terminology.

The sentence in the results section:

“Given these allele frequencies and assuming a prevalence of diabetes of 5% [21], a post-hoc power calculation indicated that the Italian T2D cases (602) pooled with the PROCARDIS diabetics (156) had 80% power to highlight at least a 30% genetic risk for diabetes associated with the rs10811661 T variant (alpha = 0.05).”

has been changed into

“Given these allele frequencies and assuming a prevalence of diabetes of 5% [21], and a type 1 error rate of 0.05, a post-hoc power calculation indicated that our sample of Italian T2D cases (602) pooled with the PROCARDIS diabetics (156) had a 80% power to detect association in terms of an odds ratio equal to 1.30 between the rs10811661 T variant and diabetes.”

“The susceptibility effects of rs2891168 on MI [...] in the Italian population is less strong, partially because the sample is smaller”: This makes no sense. The variance of the estimate is influenced by sample size, but the expected value is not.

According to the reviewer’s suggestion, we have removed from the sentence the statement “partially because the sample is smaller”.

Regarding Figures 1 and 2: “Solid squares centered on the OR [are] scaled in proportion to sample size”: Why are the sample sizes different for the 2 SNPs? The number of individuals in the 3 case groups are essentially the same.

We agree with the reviewer. In both figures 1 and 2 solid squares represent the odds ratios and their size is proportional to the inverse variance of the estimates. We corrected both legends accordingly.

Reviewer Rector Arya

Major Compulsory Revisions

It is possible to have some impact of population (group/sex) stratification on the observed associations. Therefore, it would be useful, if authors can comment on potential stratification effects, if any, in this study.
In order to avoid spurious association as a result of population subdivision, the Italian population of the present study can be considered ethnically homogenous. Each member has been asked for his ethnicity in the previous studies from which patients have been selected and all controls are Italians with Italian extraction. Frequency distributions by genotype and by minor allele were compared across groups and there were no differences. Moreover risk allele frequencies in our population agree with those of the PROCARDIS European population.

*Although they provided power calculations for the combined sample, it is not clear if there is adequate statistical power for just the present sample not pooling with PROCARDIS data.*

In the PROCARDIS study (reference 15 of the manuscript), the numbers of non-CAD diabetics were relatively low which inevitably limited the power of the study to reject the null hypothesis of non association between diabetes and the SNP rs10811661. Therefore the aim of the present investigation was to give strength to the results previously obtained in the PROCARDIS study, allowing for a larger number of diabetics collected in our case-control study. For this reason, in the manuscript we have reported power calculation for the association of the SNP rs10811661 with diabetes with regard to the pooled data only. However, also in the Italian sample, with a slightly reduced number of subjects, SNP rs10811661 results to be significantly associated to diabetes as shown in figure 1.

*It would also be helpful to see if there is any impact of multiple comparisons on the observed associations.*

The reviewer noted that different comparisons have been evaluated in this paper, and that the p-values and confidence intervals have not been adjusted for multiple comparisons. However, we think that multiplicity is not an issue in our confirmatory study, since we focused primarily only on two candidate SNPs (rs2891168 and rs10811661) and three pre-specified outcomes (T2D only, MI only or both diseases ), in order to confirm results observed in the PROCARDIS sample.

*It is not clear if authors have tested for any residual association in Italians with diabetes after correcting for MI and vice versa.*

Association analyses have been performed using unconditional multinomial logistic regression where case groups (MI, T2D, MI&T2D and controls) were the outcome and the presence of the SNP rs2891168 G-allele and the SNP rs10811661 T-allele were the independent variables. We verified that the two susceptibility variants are independently associated with MI and T2D respectively by comparing the homogeneity of genetic risks in the diagnostic groups. In particular we obtained that the rs2891168 G-allele OR of the MI&T2D group was significantly different from the one of the T2D group (p=0.04), while the rs10811661 T-allele OR of the MI&T2D group was significantly different from the one of the MI group (p=0.005).

According to the reviewer suggestions, we have performed a different analysis, in order to confirm that the MI effect was not confounded by T2D, and vice versa. For example, we fitted a simple logistic regression model considering the group (MI only + MI & T2D) as cases and (T2D only + Controls) as controls. We found that the OR estimate for the SNP rs2891168 did not materially change after adjustment for the presence of T2D (OR from 1.18 to 1.19), leading to the conclusion that T2D did not confound the association. Similarly, OR estimate for the SNP rs10811661 did not change after adjustment for MI, in a simple logistic model considering the group (T2D only + MI & T2D) as cases and (MI only + Controls) as controls (OR equal to 1.30 in both models).

We did not include this further analysis to the manuscript.
Minor essential revisions

In the abstract: last sentence in the background section “to verify whether their effects... with these two diseases” may be rephrased for clarity.

According to the reviewer’s suggestion we rephrased the sentence as follow:

“Our aim was to verify the independence of their susceptibility effects: rs2891168 associated with MI but not with T2D and rs10811661 associated with T2D but not with MI.”

Second sentence in abstract conclusions and in the discussion, “We were also able to integrate and complete the PROCARDIS group analysis...” is not clear and it may be rephrased.

According to the reviewer’s suggestion we rephrased the sentence, both in the abstract conclusion and in the discussion, as follow:

Abstract conclusions: “Combining our results with those reported by the PROCARDIS group, we were also able to obtain a significant result of association with diabetes for rs10811661 in the European population.”

Discussion: “Pooling our data and the PROCARDIS data, thus increasing the sample size, we confirmed the association of rs2891168 with CAD but, most importantly, we were able to obtain a significant result of association with diabetes for rs10811661 in the European population.”

In the discussion, paragraph 4, second sentence “We considered that this approach...” is not clear, please clarify.

As suggested by the reviewer we clarify as follow:

“.....adjusted for confounders. Actually they tested if the susceptibility between CAD and rs2891168 might change significantly in CAD patients divided into clinical and risk factor subgroups (regular smoker, sex, age, obesity, diabetes and hypertension) obtaining that none of these potential confounders varied the association results. In order to pool our data with those of the PROCARDIS study we repeated their statistical approach.”

In the discussion, paragraph 5, on line 1, the phrase “are unexpectedly unable” may be replaced by “fail”

As suggested we replaced “are unexpectedly unable” with “fail” in the text.

In the discussion, paragraph 6, first sentence, it is not clear what authors are referring to “so far nobody knows the correlations between genotype and phenotype”. Please elaborate or clarify.

As requested by the reviewer to clarify the mining of sentence “so far nobody ...”, we elaborate paragraph 6 as follow:
“Despite many GWAS have been done in the last few years, and many genetic factors have been identified, the precise genetic background to complex human diseases such as CAD and T2D is still not clear. Strong evidence of associations between common variants within chromosome 9p21 and the risk of CAD and T2D have been demonstrated, but so far nobody knows which is the biological function of most of them and how they are linked with the clinical phenotype.”

Sincerely yours

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