**Author's response to reviews**

**Title:** Fibrinogen beta variants confer protection against coronary artery disease in a Greek case-control study

**Authors:**

Eirini V Theodoraki (itheodoraki@gmail.com)
Tiit Nikopensius (tiitn@ebc.ee)
Julia Suhorutseko (iriss@ut.ee)
Vassileios Peppes (vpeppes@otenet.gr)
Panagiota Fili (giotafili@gmail.com)
Genolvefa Kolovou (genkolovou@gmail.com)
Vassileios Papamikos (vpapamikos@gmail.com)
Dimitrios Richter (richter@otenet.gr)
Nikolaos Zakopoulos (nzakop@med.uoa.gr)
Andres Metspalu (Andres.Metspalu@ebc.ee)
George Dedoussis (dedousi@hua.gr)

**Version:** 2  **Date:** 15 August 2009

**Author's response to reviews:** see over
Dear Editor,

Please find attached our revised manuscript. We hope that we have adequately replied to all reviewer and editor comments and we are willing to give any further information that you may need.

Sincerely Yours,

Eirini V Theodoraki

Reply to reviewer and editor comments

MS: 2123854971285867

Fibrinogen beta variants confer protection against coronary artery disease in a Greek case-control study

Eirini V Theodoraki, Tiit Nikopensius, Julia Suhorutsenko, Vassileios Peppes, Panagiota Fil, Genovefa Kolovou, Vassileios Papamikos, Dimitrios Richter, Nikolaos Zakopoulos, Andres Metspalu and George Dedoussis

Reviewer Frédéric Fumeron:

In this case control study, 13 tag SNPs in the fibrinogen gene cluster were investigated with respect to coronary artery disease or acute coronary syndrome in a Greek population. Two FGB SNPs were associated with a lower risk when analyzed in recessive models. The study has been well conducted and analyzed. The paper is correctly written.

Minor essential revisions

1) What is the definition of “family history of MI”?

The definition of family history, as well of the other risk factors, is included in the methods section in the revised manuscript (paragraph 3).

2) An association with family history of MI should have been tested for

We did not find any association with family history of MI. Nevertheless, we think that our study is not appropriate to address this issue due to the small number of subjects with positive family history (n=134).
3) In table 3, the genotype frequencies should have been shown. This table should be included in regular data (not supplementary).

In the revised manuscript we have included genotype frequencies in Table 3 and all tables in regular data.

4) There is an AOP concerning a protective effect of the G-455A (rs1800790) effect against MI in Greece. This is in accordance with the results and the meta analysis quoted in the discussion (29). This should be mentioned in the discussion.

We would like to thank the reviewer for the important comment. We have included that study in the discussion section (paragraph 5).

Reviewer Charalambos Antoniades:

In this case-control study the effect of genetic variability on fibrinogen chains’ genes on coronary artery disease (CAD) risk was investigated in a Greek population. The authors determined the genotype in a total of 13 SNPs on fibrinogen #, # and # chains genes (FGA, FGB and FGG respectively) in 305 cases and 305 controls. None of the haplotypes was associated with increased susceptibility to CAD after adjustment for risk factors. However, FGB rs1800787 and rs1800789 SNPs were associated with lower CAD risk (by about 50% in homozygotes for the minor alleles). Despite the valuable findings, the study has some major limitations regarding its design that should be addressed.

Major comments

1. A major limitation of the study is the lack of any information on the effect of genetic variability on fibrinogen levels. The adjusted OR of each haplotype / SNP should have been adjusted for fibrinogen levels.

We also think that this is an important limitation of our study that we have included in the discussion section of the revised manuscript (paragraph 8).

The enrollment of cases in the study was performed at different time points after the acute event (ranging from one day to one month) making fibrinogen levels (as well as any other APR protein’s levels) not comparable between subjects.

2. Importantly, the two groups (cases-controls) are not matched for cardiovascular risk factors (Table 1). Indeed this constitutes a major limitation of the study that makes the extrapolation of the findings debatable.

The reviewer is right to observe that the two groups are not matched for cardiovascular risk factors. In order to account for the potential residual confounding of risk factors we have adjusted all our models for age, sex, hypercholesterolemia, diabetes, hypertension and smoking. The fact that the effect of rs1800787 and rs1800789 SNPs on disease did not differ much before and after adjustment enhances our belief that the associations observed in our study are independent of those risk factors.
3. More demographic data should have been reported. For example, the selected SNPs/haplotypes associated in bivariate analysis with increased OR for CAD, should have been adjusted not just for presence of dyslipidemia but for LDL, HDL and total cholesterol levels. Besides, all participants obviously undergone a routine laboratory screening before entry to exclude renal and/or hepatic disease and subsequently this data should exist. Please present this data in Table 1 and present the relative adjusted OR and p-value in Table 3.

We agree with the comment of the reviewer. In the revised manuscript we have included lipid values in Table 2 and we have mentioned in the Methods section that subjects with renal or hepatic disease were excluded from both study groups. Nevertheless, specific values concerning tests for renal or hepatic function are not available at the moment and were not included in Table 2. Moreover, the adjustment for lipid levels i.e. total cholesterol, LDL cholesterol and HDL cholesterol did not significantly affect our results. This information is now included in the results section (paragraph 8).

4. Please provide power calculations for the study.

We have included power calculations in the discussion section (paragraph 7). We have performed a posterior power analysis that revealed that the power of our sample to detect an odds ratio from 0.4-0.5 was 0.5-0.9, with type I error level 0.05.

5. It would be interesting to present the linkage disequilibrium measurements performed by Haploview. Table 4 can be replaced by the relative Haplotype maps.

We agree with the reviewer’s suggestion and we have included the linkage disequilibrium structure between SNPs in Figure 1. Nevertheless, we also kept Table 4 as we think that it is important for the understanding of haplotypes.

6. Furthermore, as it has been previously demonstrated (Jacquemin et al. JACC 2008) some fibrinogen SNPs exhibit high sensitivity to pro-inflammatory stimulation, inflicting greater changes on fibrinogen plasma levels under pro-inflammatory states. Therefore acute phase response status has a critical impact on the role of selected SNPs. In the present study the proinflammatory background (e.g determination of proinflammatory cytokines levels) or acute phase response status (e.g determination of CRP or even fibrinogen per se) was not determined in any of the participants. Subsequently the role of each SNP/haplotype may have been over- or underestimated. A multivariate regression analysis that included an inflammatory marker (e.g CRP, IL-6) as a possible confounder will significantly enhance study’s findings.

7. The abovementioned limitation is important since almost 2/3 of the cases were acute coronary syndromes (ACS) and only 1/3 stable coronary patients. It would be interesting to examine whether ACS-induced changes in inflammatory status have the ability to affect fibrinogen levels in this population.

We agree with the reviewer regarding the importance of the findings of Jacquemin et al. Although it would be interesting to examine the effect of SNPs after adjustment for an inflammatory marker, we think that our study’s design is not appropriate to investigate this hypothesis. As we have already mentioned in comment 1, the enrollment of cases in the study was performed at different time points after the acute
event (ranging from one day to one month) making fibrinogen levels (as well as any other APR protein’s levels) not comparable between subjects. In the revised version of our manuscript we have included this limitation of our study in the Discussion section (paragraph 8). Nevertheless, the strength of our study is that in the multivariate analysis we have taken into account the presence of obesity, hypertension, hypercholesterolemia and diabetes that represent in a way the proinflammatory status and may partially compensate for the lack of information for the levels of specific inflammatory markers. After those adjustments, the association of rs1800787 and rs1800789 SNPs with disease remained significant (Results section, paragraph 5).

Minor comments
Abstract: p=0,026 replace comma (",") with "."
The same applies for:
Page 7 last line: p=0,081
Page 8, first two lines: OR=1,51 (p=0,013) and OR=1,39 (p=0,077)
Page 10, line 6 same with r2 values

We thank the reviewer for the comments. In the revised version of our manuscript we have made the appropriate corrections.

Editor’s comment:

In addition to the points raised by the two reviewers, the authors should discuss the issue of multiple testing. The association observed for rs1800787 and rs1800789 would probably not remain significant after correcting for the number of tested SNPs (or haplotypes). The discussion and the abstract should then be modified accordingly.

The issue of multiple testing is discussed in the revised version of the manuscript (Discussion section, paragraph 9).
The results of our study should be interpreted with caution, taking into account the multiple tests performed. If we applied the conservative Bonferroni’s correction then the level of statistical significance should be 0.001 and none of our associations would remain significant. Nevertheless, the fact that rs1800787 and rs1800789 are highly correlated with rs1800790 that has been previously associated with disease increases our confidence for our results.