Author's response to reviews

Title: Genetic Variation in Fcgamma Receptor Ila and Risk of Coronary Heart Disease: Negative Results From Two Large Independent Populations.

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Author's response to reviews: see over
Dear Dr. Edmunds,

Thank you very much for your kind letter of November 24, 2008 giving us the opportunity to revise the above mentioned manuscript.

We would like to thank the editors and the reviewers for their constructive criticisms and especially for their insightful recommendations concerning changes of several aspects of the content of this manuscript. We have considerably shortened the introduction and the discussion section by one third. Furthermore we also provide a detailed point-by-point response to each comment of the reviewers.

In case you are not satisfied with the changes, I would of course be prepared to consider other suggestions.

I hope that the revised version will now be acceptable for publication in BMC Medical Genetics.

Looking forward to hearing from you.

Sincerely,

Wolfgang Koenig, MD, FRCP, FACC, FESC, FAHA
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Point-by-point response

First, we would like to thank the reviewers for their constructive criticisms and we are grateful for the opportunity to improve this paper.

**Response to Reviewer #1:**

**Point 1:** A reference should be given for the LURIC study (on page 6).

→ See Reference No 27; page 21.

**Point 2:** Table 1 and 2 are missing some information. Mean values are most likely given together with SD’s, but this should be clarified also for alcohol consumption and cholesterol levels.

→ See table 1 and 2; page 23 and 24.

**Point 3:** If cohort size allows for sufficient power, a subgroup analysis of fatal versus non-fatal AMI would be of interest.

→ Unfortunately, cohort sizes does not allow for this subgroup analysis.

**Response to Reviewer #2:**

**Point 1:** Please reduce the introduction section by 30%, it is really long and wordy.

→ We considerably shortened the introduction by one third.

**Point 2:** Explain in the Methods section ethnic background of the subjects. If you have a mixed racial population, please include this in the logistic regression analysis, since it is known that various races have different allelic frequencies of Fc#RIIa-R/H131. If needed recalculate results for subjects of German background as a subgroup.

→ In both study populations all subjects were of Caucasian background.

**Point 3:** All background variables between patients and controls in both study groups (Tables 1 and 2) should be statistically tested. Only if you see statistical differences you can make statements in the Results section that the groups are different for certain characteristics.
We are reluctant in providing p-values for table 1 in order to show a statistical difference between cases and control population. The main purpose of table 1 is to characterize patients and controls, and to show the distribution of socioeconomic variables and traditional risk factors for CAD. As confounding is not a statistical issue, providing of p-values in table one does not assure that the final results do not suffer some form of confounding bias. As confounding is a very complex issue, especially in the context of a case-control study, it is essential to take a multivariate analysis strategy and take potential confounding factors in consideration. We have taken this into account and the main risk factors were controlled carefully in the final analysis.

**Point 4:** Add in Table 3 a footnote on the results of Hardy Weinberg testing.

The genotype distribution in LURIC as well as in the MONICA study was in Hardy-Weinberg equilibrium among both patients and controls. See page 25.

**Point 5:** Add also in Table 3 allelic frequencies and OR and P-values.

The allelic frequencies are added in table 3; See Page 25. The odds ratios are around 1 with wide confidence intervals, thus no formal statistical testing seems to be needed.

**Point 6:** Add in the top of Table 3 under “cases” and “controls” the n (number of Subjects).

See table 3; Page 25

**Point 7:** Combine Tables 4 and 5, similar presentation as Table 3.

See table 4; Page 26

**Point 8 + 9:** Could you please reconsider your Discussion; it is quite long/extensive, yet on the other hand several aspects are missing.

I think a discussion on Bovine EC is not so relevant in the current context. Omit this completely. Instead include discussion on the role of bacteria in relation to cardiovascular disease. In certain conditions gram-negative bacteria may get into the circulation on a daily basis. This may stimulate IgG2 production and the Fc#RIIa-H131 variant can strongly bind to the bacteria IgG2 complex. In fact, several papers have suggested for example neutrophil and monocytic hyperreactivity, due to this strong binding. Therefore in your Discussion include not only CRP but also IgG2, and view the hypotheses from 2 angles: both in case of strong binding and in case of weak binding. In this respect it is also not brought forward that CRP can opsonise bacteria and that CRP helps to clear bacteria in the circulation.
We carefully shortened and reconsidered the discussion section, according to the suggestions of the reviewer. Furthermore we delineate the role of bacteria and opsonophagocytosis related to CVD in detail.

**Point 10:** An important aspect missing in the Results section is the statistical power of the various results. Please perform power calculations and state the power of the current results.

Although the present study did not have the power to detect a very weak association between FcγRIIa genotypes (i.e. HH131 vs. others) and CHD, it had a power of 80% to detect an OR of 1.46 ($\alpha = 0.05$) or larger in the MONICA study and an OR of 1.26 or larger in the LURIC study; see Page 15