Author's response to reviews

Title: A role for CETP TaqIB polymorphism in determining susceptibility to atrial fibrillation: a nested case control study

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

Thank you for reconsidering our manuscript entitled “A role for CETP TaqIB polymorphism in determining susceptibility to atrial fibrillation: a nested case control study” for publication in BMC Medical Genetics.

Besides the point-by-point response to the comments of the reviewers, we also went through the manuscript formatting list and formatted our revised manuscript accordingly. We deleted the figures in our previous submission and uploaded them separately.

Comments reviewer 1:
We want to thank dr. van Belle for reviewing our work and his positive comments. Dr van Belle did not ask for any changes and accepted the manuscript in the submitted format.

Comments reviewer 2:
We also want to thank dr. Kuivenhoven for his critical review and providing us with useful comments. We will address his comments point-by-point and changed the manuscript accordingly:

Major compulsory revisions:

1. The main concerns raised by dr. Kuivenhoven is the choice of candidate genes in our study and the biological explanation of the findings. The rationale of this study was to investigate common genetic variations and environmental risk factors, which might be associated to the presence of atrial fibrillation. In addition, we further explored the interactions between those factors using a non-traditional approach. The focus of this paper was not to detect a new genetic mutation causing atrial fibrillation, but to find combinations of factors predisposing to atrial fibrillation, which might be indirectly related through other pathways such as atherosclerosis or inflammation. With this approach in mind, we chose several interesting candidate genes, which might be (indirectly) involved in the development of atrial fibrillation. This selected choice is arbitrary and restricted by logistic and financial factors. As suggested by dr. Kuivenhoven, we changed the background section to explain the choice of candidate genes more thoroughly. Furthermore, we added a limitation paragraph to the discussion section to emphasize the explorative and hypothesis generating nature of this analysis.

2. The study design limits us to draw any conclusion about the cause-effect relationship between the CETP Taq IB polymorphism and the presence of atrial fibrillation and the data are purely hypothesis generating. We agree with dr. Kuivenhoven that the possibility exists that the CETP TaqIB may be a marker of a genetic variation somewhere else in the CETP gene or even outside the CETP gene locus. However, several mechanisms explaining the observed association can be postulated such as underlying atherosclerosis, inflammation, oxidative stress, or alcohol intake. We extended the discussion section to describe the potential mechanisms linking the CETP TaqIB polymorphism with the presence of atrial fibrillation. This study conforms with most of the mandatory ‘rules’ for associating SNP’s to multifactorial disease: presence of
a logical biological explanation for genotype-phenotype interaction and a low p-value,
which we obtained by permutation testing. The main limitation is the small sample size.
As described in the methods, we started with 8592 subjects to obtain 97 cases from the
general population. It would take a vast effort to undertake a study with a substantially
higher number of cases. Therefore, we chose to use the MDR method for collapsing high-
dimensional genetic data into a single dimension, which permitted us to investigate
interactions in a relatively small sample.

3. We created an interaction graph using entropy (measurement of randomness)
estimates to confirm and visualize the observed interactions and to make it more easy to
interpret the results obtained by logistic regression analysis and MDR. This interaction
graph will allow the reader to compare the independent main effects of each factor to the
interaction effects and whether the interactions are additive or non-additive. These graphs
showed that the interaction between albuminuria and CETP TaqIB was (partly)
synergistic. On the other hand, an additive interaction was observed between the CETP
TaqIB polymorphism and elevated C-reactive protein, presence of renal dysfunction or
ischemic heart disease. Hopefully, this will clarify the results. Furthermore, we will
discuss the relevance of the found interactions more profoundly in the discussion section.

4. As suggested, we added alcohol intake and history of ischemic heart disease in the
analyses. The use of medication was already included in the definition of hypertension
and hypercholesterolemia as mentioned in the method section. Alcohol intake was
obtained by questionnaire and no difference could be demonstrated between intake and
presence of atrial fibrillation. We defined the presence of ischemic heart disease as prior
myocardial infarction with hospitalization reported by questionnaire and/or an infarct
and/or major ischemia patterns on the electrocardiogram. Ischemic heart disease was
significantly associated with the presence of atrial fibrillation and MDR analyses showed
a significant interaction between the CETP TaqIB polymorphism and ischemic heart
disease. We added the MDR plot to the results and added the interaction between CETP
TaqIB and ischemic heart disease in the interaction graphs.

5. We used a new method in this paper to determine statistical interactions and in our
view, more extensive explanation of this method is necessary to interpret the results. The
large description of the statistical methods is one of the reasons why we chose to submit
our paper to the online journal BMC Medical Genetics. In the revision, we extended the
background and the discussion of the results to balance the proportion between methods
and results as dr. Kuivenhoven advised.

6. All subjects participating in the PREVEND study visited the outpatient clinic for
screening. During this visit, several measurements were done including the recording of a
standard 12-lead electrocardiogram during two minutes, which was stored digitally, and
classified according to Minnesota codes. This recording is a snapshot at one time point
and therefore we cannot distinguish between permanent, persistent and paroxysmal atrial
fibrillation. We added this limitation to the discussion section of the manuscript.

Minor Essential Revisions:

1. We specified the odds ratio’s in the statistical method section and the table
   legends: Odds ratio’s for the presence of atrial fibrillation on the electrocardiogram.
2. We added the description of technology used to measure lipids in the laboratory measurements section.

3. Unfortunately, we did not measure LDL-c directly. We estimated the LDL cholesterol values using the Friedewald formula and added the values in table 1.

4. We corrected the reference to the tables.

5. We added the reference of Tsai et al. to the text on page 11, second paragraph, second sentence.

6. We added the number of controls in table 2.

We would like to thank the reviewers again for taking time to review our work and for providing us with useful comments. We hope that with the current changes, the manuscript will be suitable for publication in BMC Medical Genetics.

On behalf of all co-authors,

Yours sincerely,

Folkert W. Asselbergs