Author's response to reviews

Title: Association between polymorphisms in the coagulation factor VII gene and coronary heart disease risk in different ethnicities: a meta-analysis

Authors:

Xingbo Mo (xingbomo@gmail.com)
Yongchen Hao (haoyongchen123@163.com)
Xueli Yang (yangxueli1985@gmail.com)
Shufeng Chen (shufengchen2001@yahoo.com.cn)
Xiangfeng Lu (xiangfenglu@sina.com)
Dongfeng Gu (gudongfeng@vip.sina.com)

Version: 2 Date: 13 May 2011

Author's response to reviews: see over
Dear Prof Natasha Mellins-Cohen,

I am submitting the revised manuscript entitled “Polymorphisms in the coagulation factor VII gene and the risk of coronary heart disease: a meta-analysis” (manuscript ID: 1226764122521401) for your review.

We attempted to conform to the PRISMA guidelines in the report of our meta-analysis. A flow diagram for study selection process was added in the revised manuscript. All figures were cropped following the instruction of BMC medicine journals. A small table consists of the main characteristics and results of our meta-analysis was made and intended to appear within the body of the article. The previous large table which would be uploaded as an additional file has also been modified.

We have responded, point by point, to each of the suggestions and comments of the reviewers. We have highlighted the revised portions in red with underlines in the manuscript. We appreciate the thoughtful comments provided by BMC Genetics referees and believe that our manuscript has been greatly strengthened after this revision.

My co-authors and I would like to thank you for the opportunity to resubmit our work for potential publication in BMC Genetics.

Sincerely,

Dongfeng Gu, MD, PhD
Department of Evidence Based Medicine &
Division of Population Genetics,
Fu Wai Hospital & Cardiovascular Institute,
Chinese Academy of Medical Sciences &
Peking Union Medical College
No. 167 Beilishi Rd, Beijing, 100037, China

gudongfeng@vip.sina.com
Tel: 86-10-68331752 ; Fax: 86-10-88363812
Dear reviewers,
I am very grateful to your comments for our manuscript. According with your advice, we amended the relevant part in manuscript. Your questions were answered below.

**Reviewer: Jie Huang**

1. The analysis put quite a focus on the difference between Asian and European Population, but the title seems to be on a general meta-analysis without alluding to the ethnic component.

**Respond:** Thank you for your comment and we decided to change the title of this paper. The new title is:

**“Association between polymorphisms in the coagulation factor VII gene and coronary heart disease risk in different ethnicities: a meta-analysis”**

2. The manuscript aims to study the association of 3 cis-acting polymorphisms within the FVII gene with the risk of CHD, but the association of these SNPs with plasma FVII level is not well described. It is not clear either the LD structure among these SNPs in both the European and Asian population.

**Respond:** The R353Q and -323Ins10 polymorphisms were associated with a 20% to 25% reduction in plasma factor VII levels [1]. Hunault et al. reported that the R353Q polymorphism alone can decrease plasma factor VII levels [2]. Significant associations between R353Q and -323Ins10 polymorphisms and FVII coagulant activity (FVIIc) and antigen (FVIIAg) levels have also been reported in Chinese population [3]. Current GWAS identified a SNP (rs488703, in perfect LD with R353Q (rs6046)) in F7 gene significantly associated with plasma levels of coagulation factor VII (p-value=9.0*10^{-259}) [4]. We added the statements above into our revised manuscript (in the second paragraph).

We can’t identify the LD structure among these 3 SNPs in the HapMap database because data of -323Ins10 and HVR4 polymorphisms is unavailable. However, the linkage disequilibrium between R353Q and -323Ins10 polymorphisms had been reported in both Asian (Δ=0.85, P < 0.001) and European populations (no concrete data reported) [3, 5]. The R353Q and HVR4 polymorphisms were also in linkage disequilibrium, with a D’ value of 0.65 (P<0.001)[6].

**References:**


[2] Mathilde Hunault; Arnaldo A. Arbini; Stanislaw Lopaciuk; Josephine A. Carew; ; Kenneth A. Bauer. The Arg353Gln Polymorphism Reduces the Level of Coagulation Factor VII. In Vivo and in Vitro Studies.


3. For the 3 polymorphisms chosen, it would be good to have the rsID. For example, rs6046 is for R353Q. The manuscript states that "the -323Ins10, a 10-bp insertion in the promoter region in complete linkage disequilibrium with R353Q". Are these two polymorphisms really in perfect LD?

Respond: According to the NCBI dbSNP database, rs6046 is for R353Q, rs36208070 is for -323Ins10 polymorphism. These two rs numbers were added into the abstract, background section and the table of the article. We can’t identify the rsID of HVR4 polymorphism in the dbSNP database. The search of the SNP database, or the OMIM database, found nothing for HVR4. We contacted the NCBI help desk for more information. They replied and suggested us to find this variant by the links of the gene record to the SNP database. We tried but failed to find HVR4 polymorphism in the F7 gene locus. We can’t identify the \( r^2 \) for the linkage disequilibrium between these studies. The rs36208070 and HVR4 are not on any of the Illumina beadchips nor is it in the Hapmap2, 3 or 1000G imputation panels.

4. The "Key Words" should have listed FVII.

Respond: We add a key word FVII.

5. The manuscript states that both "fixed effects" and "random-effects" methods are used. But later it states that "The combined ORs along with 95% CIs were estimated using the random-effects methods, which in the presence of heterogeneity is more appropriate". So, was "fixed effects" method actually used for this study?
Respond: We are sorry for making it unclear in our article. We used fixed-effects model to analyze all the data and estimated the between-study heterogeneity at first. If significant heterogeneity existed, we analyzed the data using random-effects model instead and report the results estimated by random-effects model. If not, we report the combined OR and its 95% CI estimated by fixed-effects model. In our meta-analysis, the reported combined ORs and 95% CIs for R353Q and HVR4 polymorphisms were estimated using the random-effects method; the reported combined OR and 95% CI for -323Ins10 was estimated using fixed-effects method.

The sentence "The combined ORs along with 95% CIs were estimated using the random-effects methods, which in the presence of heterogeneity is more appropriate" was changed as below:

The combined ORs along with their 95% CIs were estimated using the fixed-effects. The random-effects method [32], which in the presence of heterogeneity, is more appropriate as it is prudent to take into account an estimate of the between-study variance ($I^2$).

6. It would not be sufficient to simply state that "further studies are needed to confirm the association and elucidate its mechanisms". The study needs to have a paragraph on the biological implication/interpretation in regard to its findings.

Respond: In the sixth and seventh paragraph of the discussion section we have discussed more the biological aspect of the underlying molecular mechanisms that confer susceptibility to CHD.

The exact biological role of the particular polymorphism remains to be determined. In theory, FVII contribute to atherosclerosis through the generation of thrombin and fibrin formation. Development of coagulation in the vessel wall may result in production of thrombin and activation of platelet, leading to the release of various cytokines and the proliferation of smooth muscle cells in the vessel wall. Thus, someone with a lower FVIIc level may have less chance of developing CAD. Moreover, thrombin can have pro-inflammatory properties and pro-angiogenic activities. Vascular tone, inflammation, blood viscosity, and angiogenesis can play roles in initiating and maintaining an elevation in BP and, therefore, are potential mechanisms contributing to the association between FACTOR VII and risk factors like smoking, diabetes, hypertension, and mental stress. The FVII polymorphisms were reported to be associated with decreased blood pressure and a decreased risk of hypertension [36], suggesting that these FVII variants might influence cardiovascular risk through mechanisms other than thrombosis.

Further studies which aim to confirm the functional variants should be needed. More
studies are needed to elucidate the complete range of the signal transduction pathways that the variant is implicated in, and thus, throw light in the underlying molecular mechanisms that confer susceptibility to CHD. The particular polymorphism associated with CHD itself may not play a functional role, but rather it may be located physically close to the actual disease-predisposing gene.

7. There are minor English writing issues such as "before the 23 November 2010". It is not clear what a "population-based case-control design" is and how "the counts of persons with different genotypes in cases and controls" could be done when genotype data is not available.

Respond:
"Before the 23 November 2010" was changed as "before November 23, 2010". "Population-based case-control design" was changed as "case-control design". We extracted data from the papers of the original studies, the counts of persons with different genotypes in cases and controls were always reported in the published papers. Some researchers did not report the genotype data. In this situation, we tried to contact the researchers for information.

8. Also, the logic is not clear in "manuscripts in languages of Chinese and English were considered for review, in order to avoid the local literature bias".

Respond: We changed it to “manuscripts in languages of Chinese and English were considered for review.” This sentence was moved to a new position after the literature searching strategy.

9. It might need a "limitations" section.

Respond: We add a limitation section in the discussion as below:

Several potential limitations of our study should be noted. First, we should realize that the results might be distorted by potential weakness and biases of genetic association studies, such as genotyping error, phenotype misclassification, population stratification, gene-gene or gene-environment interactive effect, and selective reporting biases [33, 34]. Second, although no statistically significant publication bias was found from Egger's test, exclusion of unpublished studies may affect the validity of the analysis. The eligible studies in our research were mainly from Asia and Europe, data of other populations, like African, was limited, and we look forward to
the results from those populations. Third, because we did not have access to individual data, we could not control for population stratification, nor could we adjust for variables in possible intermediate pathways.

Reviewer: Jeanette Erdmann
Reviewer’s report:
Major Compulsory Revisions
I don’t understand why R353Q is only associated with disease in Asians but -323Ins10 polymorphism is associated in both Asians and Europeans although both variants are in complete LD. Please explain this.
I would suggest that the authors contact either the CARDIoGRAM or the C4D consortium to look-up these variants in these very large data sets. This might give a more definite answer whether Factor VII variants are indeed associated with CHD risk.
Respond: We contacted the CARDIoGRAM and the C4D consortium. Only R353Q (rs6046) is available in the CARDIoGRAM data set. The results are: p=0.62, OR = 0.98 (0.94-1.03) (fixed effect model), n=75,359 cases and controls (Prof Jeanette Erdmann’s reply). The results are consistent with our meta-analysis. The C4D consortium did not have measures of the two variants in their dataset (Prof Rory Collins’ reply).
The strong linkage disequilibrium (but not perfect LD) between R353Q and -323Ins10 polymorphisms was observed in both Asian (Δ =0.85, P < 0.001) and European populations (no concrete data reported), reported by Liu et al. and Livers et al. respectively.
Whether two polymorphisms have the same association or not is determined by the r² between the polymorphisms. If two polymorphisms are in perfect LD (r²=1), the associations should be consistent. We can’t identify the r² for the linkage disequilibrium. The rs36208070 SNP is not on any of the Illumina beadchips nor is it in the Hapmap2, 3 or 1000G imputation panels (Prof Hugh Watkins’ reply). We can’t say that these SNPs were in perfect LD based on the reports of previous studies, so the associations can be inconsistent.

Reviewer: Caroline Hayward
Reviewer’s report:
Major Compulsory Revisions
This paper is an "updated" meta-analysis of literature findings of FVII polymorphisms associated with CHD. Literature only surveys can be difficult to perform.
1. I am confused e.g. as in the present study of R353Q there are fewer controls for than in the earlier one(10,789 compared with 12,110) Were the studies out of HWE excluded? An explanation is required, as the crux of the paper is to assess which of
the 2 previous meta-analyses is correct.

**Respond:** The earlier meta-analysis was published in 2006 by Ye et al. We included another five studies published after 2006 in our meta-analysis. The previous meta-analysis included the studies of myocardial infarction and coronary stenosis. The number of cases/controls for studies of myocardial infarction and coronary stenosis combined was 7444/12110, the number of cases/controls for studies of myocardial infarction was 5286/8578, the number of cases/controls for studies of coronary stenosis was 2158/6754 (data from table 2 in Ye’s paper).

Ye’s meta-analysis included unpublished studies (Yokota and Inbal), and data of those studies were unavailable for us. Besides, we can’t identify the genotype data in four original studies which were included in previous meta-analysis. We have included those studies into our meta-analysis in the revised manuscript; the number of cases/controls of our study would be 9151/14099. The result indeed does not change much. The table below shows the comparison of the results before and after including the data of the four studies into our meta-analysis:

<table>
<thead>
<tr>
<th>Populations</th>
<th>Before</th>
<th></th>
<th>After</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR(59%CI)</td>
<td>I² (%)</td>
<td>OR(59%CI)</td>
<td>I² (%)</td>
</tr>
<tr>
<td>Europeans</td>
<td>1.00 (0.90-1.10)</td>
<td>22.3</td>
<td>1.02(0.94-1.11)</td>
<td>11.4</td>
</tr>
<tr>
<td>East Asians</td>
<td>0.70 (0.55-0.90)</td>
<td>55.5</td>
<td>0.70(0.55-0.90)</td>
<td>55.5</td>
</tr>
<tr>
<td>Other</td>
<td>0.92(0.78-1.07)</td>
<td>0.0</td>
<td>0.92(0.78-1.07)</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>0.88(0.79-0.98)</td>
<td>53.2</td>
<td>0.91(0.83-1.01)</td>
<td>51.2</td>
</tr>
</tbody>
</table>

I²: The inconsistency index for between-studies heterogeneity, where higher values of the index (I²>50%) indicate the existence of heterogeneity.

2. Although the data seems to have been appropriately analyzed and checks appear to have been completed for study bias etc I found the interpretation of these in the discussion difficult to follow. It may be more appropriate to emphasise the difference between Asian and European cohorts.

**Respond:** Thanks for your helpful comment. We performed subgroup analysis according to ethnicities. We found that the associations between F7 polymorphisms and CHD were different between Asian and European populations. We revised our manuscript and discussed the different association between Asian and European cohorts as below:

According to our results, the association between F7 polymorphisms and CHD risk may be different in different ethnicities. Significant association was only found in East Asian population for the R353Q polymorphism in our meta-analysis. We found no significant associations in the Europeans, this result was consistent with the study of CARDIoGRAM consortium (by corresponding to authors). The -323Ins10 polymorphism was significantly associated with CHD risk in both European and East Asian populations. Lack of association was found for HVR4
polymorphism both in European and East Asian populations.

Minor Essential Revisions

Table 1 should have rows with totals for Europeans, Asians and others for each category as well as the total for all. This would make it easier to follow the data.

Respond: Table 1 has been modified, we classified and rearranged the studies into groups by ethnicity and added rows with totals for European, Asian and other populations as well as the total for all. This table will be uploaded as an additional file. A small table consists of the main characteristics and results of our meta-analysis was added in the revised version.