Author's response to reviews

Title: TGFB1 genetic polymorphisms and coronary heart disease risk: a meta-analysis

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Version: 3 Date: 3 February 2012

Author's response to reviews: see over
Referee 1:
1. Although the introduction is comprehensive with a clear description of the cellular sources and the associations between TGFB1 gene variants and CHD outcomes which have already been published, the introduction could perhaps be improved by:
   - A brief description of the physiological effects and molecular modes of action of TGFB1 within the vasculature.

As suggested by the reviewer, we included the following sentences at the beginning of the introduction at page 5. : “TGFβ1 has been demonstrated to be of fundamental importance in the development, physiology and pathology of the vascular system. Research into the mechanisms of TGFβ1 signaling over the past two decades has led to the development of a well-accepted canonical signaling cascade involving heterotetrameric complexes of type I and type II serine/threonine-kinase transmembrane receptors together with Smad transcription factors that act as intracellular signaling effectors. However, the exact mechanisms by which TGFβ1 signaling exerts its effects within the vasculature are still incompletely understood.”

   - The novelty of the analysis should be highlighted, i.e. no meta-analysis describing the association between TGFB1 variants and CHD outcomes currently exists.

As suggested by the reviewer, we added one sentence at the end of the introduction at page 7. : “No meta-analysis describing TGFB1 genetic variants in relation to CHD risk exists;”

2. I think the answer to my question is likely to be, No (as this would require the raw data probably?), but is there any possibility that data analysis could be conducted looking at the combined impact of co-inheritance of 2/3 risk allele on CHD risk. I guess if any two of the SNPs are already in high LD this analysis is irrelevant?

As the reviewer already suggested, we are not able to get the individual data (raw data) from each included study. It is therefore unfortunately not possible to apply haplotype analysis to look at the coinheritance of the SNPs included in our study. The most associated haplotype might potentially point to the location where the culprit SNP is located.

Minor points
3. Line 4 of the introduction: ‘could be secreted by several cell types’. I am not sure what this means. Are you suggesting that the literature is inconsistent as what are the main cellular sources, or the fact that the cellular source is dependent on stage of development or disease progression?

As the reviewer correctly pointed out, the cellular source of TGFB1 is somewhat inconsistent in literature at present. We tried to include all the reported cellular sources. The relevant sentence has been modified slightly at page 5: “According to the literature data [2-7], TGFβ1 could be secreted by several cell types, including peripheral blood mononuclear cells, macrophages, platelets, endothelial cells, vascular smooth muscle cells (VSMCs), myofibroblasts, and renal cells.”

4. P5, L9: ‘and this LD covered the whole 5’...’ It may be better described as ‘this DNA region covered the whole 5’.
As the reviewer suggested, we modified this sentence at page 6: “and this DNA LD block covered the whole 5′ proximal region of the TGFB1 gene in Caucasian populations”.

5. P6, L3: perhaps the word ‘validated’ may be more appropriate in the phrase ‘used proper CHD diagnostic criteria’

We changed the word “proper” into “validated” as the reviewer suggested (page 8).

6. Not clear as current written how criteria set 2 are different from criteria 4 in the ‘Selection criteria’ subsection

The criteria set 2 is to define the eligible coronary heart disease phenotypes, while the criteria set 4 is to define that eligible studies need to have investigated the association between TGFB1 gene polymorphisms and these coronary heart disease phenotypes (not with other diseases, such as stroke, or intermediate coronary heart disease phenotypes, such as carotid intima-media thickness). To clarify this, we now mention criteria 4 as the second criteria set.

Referee 2:
Major Compulsory Revisions:
Introduction: The authors should mention a few lines about the importance of genome-wide association studies in understanding the complex disorders including coronary heart disease.

As suggested by the reviewer, we added some information about genome wide association studies of coronary heart disease at the end of the introduction at page 7: “Multiple replicated loci have recently been identified from genome-wide association (GWA) studies of CHD. However, they together explain only a small part of its heritability [24]. It has been suggested that the adopted highly stringent statistical criteria and/or the imperfect coverage of our genetic variants by current GWA studies might prevent the discovery of other potential loci associated with CHD risk [25].”

Methods Section: Since various models were used in the analysis, the authors should adjust the significance for multiple testing.

As pointed out by the reviewer, we analyzed each candidate SNP association under three genetic models: the co-dominant model, the dominant model and the recessive model. With the most stringent Bonferroni correction of \( p < 0.05/3 \) (0.017) to adjust for the multiple testing, all significant associations (co-dominant and dominant models) in Figure 1 and 2 passed this correction (rs1800469: \( p = 0.0041 \) for CT vs CC and \( p = 0.0029 \) for TT+CT vs CC; and rs1982073: \( p = 0.0002 \) for TC vs TT and \( p = 0.0118 \) for CC+TC vs TT). However, for rs1800471 in Figure 3, associations were not statistically significant after Bonferroni correction (\( p = 0.0335 \) for GC vs GG and \( p = 0.0238 \) for CC+GC vs GG). Based on these results, we added the following sentence in the Quantitative synthesis result part at page 12: “After adjusting for multiple testing using Bonferroni correction, all significant associations for rs1800469 and rs1982073 under the co-dominant and dominant models remained. However, for rs1800471 associations were no longer statistically significant (data not shown).”

Results Section:
1. Page 9, para 2 is already mentioned in the table and doesn’t need to be mentioned in the text.
As suggested by the reviewer, we deleted this paragraph.

2. It is mentioned that Iranian study (Ref 11) was dropped from the meta-analysis due to the HWE issue, data for SNP rs1982073 was still utilized in the meta-analysis. This needs to be clarified.
   To be more clear, we added one sentence about the utilization of data for rs1982073 at the beginning of Statistical analysis (page 9): “In an Iranian study [11], only data on rs1982073 was utilized in the meta-analysis because other SNPs deviated from HWE”. The corresponding data for rs1800469, rs1800471 and rs1800472 were also deleted from table 1.

3. In the quantitative synthesis, the text has already been explained in the figures and can be shortened in one or two sentences.
   As suggested by the reviewer, the quantitative synthesis part has been shortened. In our opinion, we cannot shorten it further in order to give the reader a “complete” result.

4. The format for additional files is not understandable and could not be opened using the routine programmes available.
   To overcome this unfortunate problem pointed out by the reviewer, we changed the formats for all three additional files in order to give the reader the easy access to our additional data.

5. All the studies included for the meta-analysis were from Caucasian population except for one SNP (rs1982073) where Japanese population is included. The heterogeneity issue therefore may be ignored.
   When leaving the Japanese study out of the meta-analysis in order to get a more homogeneous Caucasian population, we observed slightly stronger evidence for an association between rs1982073 and CHD risk (Additional file 1). Since there are not too many data on associations between TGFB1 genetic polymorphisms and CHD risk, we prefer to include as many legitimate studies as possible in our meta-analysis. We therefore decided to include the Japanese study and presented the meta-analysis results for rs1982073 with both fixed-effect and random-effect models (Additional file 1). By doing so, we provide the reader a relatively comprehensive overview of the latest status of studies on this topic.

Discussion:

6. No mention of the studied polymorphisms was made in the discussion part. Authors are expected to throw some light on the similarities or differences among the results for studied polymorphisms in the original studies with the current meta-analysis and what are their biological implications.
   As suggested by the reviewer, we now mention all the studied genetic polymorphisms at the beginning of the discussion: “Several studies have been carried out to test the hypothesis that genetic polymorphisms in the TGFB1 gene including rs1800468, rs1800469, rs1982073, rs1800471 and rs1800472 might be associated with CHD risk”. Furthermore, we added some explanations for the differences among the published results for rs1800469 and rs1982073 at the end of the 1st paragraph of the discussion at page 14. : “The inconsistency between the previously reported results for these SNPs might be due to the small sample sizes in most of the studies, especially when combining their potential modest effects on CHD risk.”
At this stage, we cannot explain why there were no positive associations between rs1800468, rs1800471 and rs1800472 and CHD risk. It might be due to the lack of a genuine effect on CHD risk, insufficient power or slightly weaker linkage disequilibrium between them and the underlying causal SNPs compared with rs1800469 and rs1982073 in this gene region. Therefore, we only speculated on the possible potential biological implications for rs1800469 and rs1982073 in our discussion based on available data from published molecular cell biological studies in the 2nd paragraph of discussion at page 14.

Minor Revision:
Expand SMAD3 gene in the discussion.

As suggested by the first reviewer, we added a brief description on molecular models of action of TGFβ1 at the beginning of the introduction: “Research into the mechanisms of TGFβ1 signaling over the past two decades has led to the development of a well-accepted canonical signaling cascade involving heterotetrameric complexes of type I and type II serine/threonine-kinase transmembrane receptors together with Smad transcription factors that act as intracellular signaling effectors.”

Smad3 encoded by the SMAD3 gene is only one of the Smads activating TGFβ1 signaling that forms complexes with the common mediator Smad4. These complexes translocate into the nucleus, where they can regulate, together with other partner proteins, the transcription of specific target genes. Therefore, we modified the relevant sentence at page 15 only slightly to indicate that Smad3 is involved in the activation of the TGFβ1 signaling pathway: “A genetic variant in the SMAD3 gene that encodes one of the downstream activating transcriptional mediators (Smad3) of TGFβ1 signalling [1, 2] was associated with CHD risk in a genome-wide association study [43],”.

Editorial Requirement:
Structure: Please check the instructions for authors on the journal website to ensure that your manuscript follows the correct structure for this journal and article type.
We made the necessary changes according to the requirements of BMC Medical Genetics.

Although no statistically significant publication bias was detected, it is nonetheless possible that studies with null findings were not published and are missing from the systematic review. In an attempt to overcome this limitation, could the authors consider to include in their work published GWAS data on CHD. Two studies encompassing almost all GWAS on CAD/MI/CHD through meta-analysis have been published, the CardioGRAM and CardioGRAMplusC4D consortia. Dr Inke König and Dr Martin Farrall are the corresponding contacts where genotype counts could be requested.

As requested by the Editors, we added the relevant data. The individual allele effects for rs1800468, rs1800469, rs1982073 and rs1800471 from the PROCARDIS study were included in the co-dominant model analyses. The results did not change substantially (Additional file 1). Additional data for rs1800469 from the CARDioGRAM study were also included (Additional file 1). Due to the low-quality imputed data for other SNPs, they were not included in this meta-analysis.