Author's response to reviews

Title: Variation in the human soluble epoxide hydrolase gene and risk of restenosis after percutaneous coronary intervention

Authors:

Silke Kullmann (kullmann@herz-kreislaufforschung.de)
Priska Binner (binner@herz-kreislaufforschung.de)
Kirsten Rackebrandt (rackebrandt@herz-kreislaufforschung.de)
Andreas Huge (a.huge@uni-muenster.de)
Georg Haltern (georg.haltern@helios-kliniken.de)
Mark Lankisch (mark.lankisch@helios-kliniken.de)
Reiner Fueth (reiner.fueth@helios-kliniken.de)
Eberhard von Hodenberg (eberhard.hodenberg@heart-lahr.com)
Hans-Peter Bestehorn (hans-peter.bestehorn@herzzentrum.de)
Thomas Scheffold (scheffold@herz-kreislaufforschung.de)

Version: 2 Date: 18 August 2009

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

We appreciate your interest in our work and thank both Reviewers for carefully reviewing our manuscript. We have attempted to address both of the two Reviewer`s comments in detail.

Please do not hesitate to contact us with any questions.

Silke Kullmann, PhD
Kullmann@herz-kreislaufforschung.de

Comments of Reviewer 1

1. “The observed and expected frequencies of the polymorphism are in Hardy-Weinberg equilibrium?. Please include this information and the test used for the analysis. If the data are not in H.-W equilibrium, the authors should discuss this information.“

Authors Reply: We agree that the information on the Hardy-Weinberg equilibrium is missing in the first draft of the manuscript. This has now been rectified and the calculation included in the manuscript.

Manuscript Change(s): The information on the Hardy-Weinberg equilibrium has been inserted into the manuscript sections as follows:

Materials and Methods, P. 8:
Genotypes were tested for Hardy-Weinberg equilibrium among MI cases and controls using a chi-square test with one degree of freedom.

Results, P. 10:
The genotype distribution did not significantly (P > 0.1) deviate from the Hardy-Weinberg equilibrium (Table 3).

Table 3, P. 23:
The data of calculated Hardy-Weinberg equilibrium was included in the table.

2. “All the presented data are not agree with previous studies, so more information about the differences with previous studies should be included in the discussion section (ethnic, diagnostic differences, etc.). “

Authors Reply: First, we would like to point out that - to our knowledge - the cited publication is the only comparable study investigating the association of the EPHX2 K55R sequence variant with CHD.
However, especially with respect to the diagnostic differences we agree that we have to discuss the differences between our study population and the previously reported one in more detail.

**Manuscript Change(s):**

**Discussion, P. 11:**
Based on the previously reported association of the \textit{EPHX2 K55R} polymorphism with incident CHD in an American study population of Caucasian origin [1], we investigated whether the \textit{K55R} variant allele was associated with restenosis in patients who had undergone primary successful PCI.

**Discussion, P. 12:**
However, due to the inclusion of patients who were exclusively from Central Europe and the resulting well defined demography it is assumed that the ethnical background of our study cohort is more homogenous than in the previously reported study.

**Discussion, P. 12:**
The fundamental difference of our study population compared to the previous study was the diagnostic inclusion criteria of the patients. In the present study all CHD patients had undergone a PCI with exclusion of cases suffering from acute coronary syndrome, whereas in the previously cited study more than half of all the patients suffered from acute myocardial infarction. The difference in patient characteristics may be the cause for the contrary findings.

In addition, we have described the main diagnostic characteristics of the underlying patients collective in the Materials and Methods section in more detail.

**Materials and Methods, P. 6-7**
Patients aged 35-80 years from Central Europe with symptomatic coronary heart disease who had undergone primary successful PCI of a native coronary artery were included into the study as described previously [2]. This multicenter, placebo-controlled study was designed to assess the effect of the calcium channel blocker Verapamil on restenosis after intervention. \textit{Successful intervention was defined by residual stenosis < 30\% on visual estimation or desired position of stent.}

**Discretionary revisions**
1. “In my opinion the figure 1 is not necessary and should be deleted.”

**Authors Reply:** We appreciate that the figure gives no essential or further information on the subject the manuscript deals with. Therefore, we elected to remove the figure from the manuscript.
Manuscript Change(s): Figure 1 has been deleted.

Comments of Reviewer 2

1. “What is the specific theory behind the study? Why should this gene relate to restenosis?”

Authors Reply: Soluble epoxide hydrolase (sEH) encoded by the EPHX2 gene metabolises epoxyeicosatrienoic acids (EETs). EETs are known for their cardio-protective properties i.e. due to the regulation of the endothelial function. The K55R polymorphism within EPHX2 was linked to accelerated reduction of plasma EET levels and reported to be significantly more common in CHD cases versus non CHD controls. In the revised manuscript, we describe the background in more detail so that our hypothesis will become clearer to the readership.

Manuscript Change(s): In the revised manuscript the background reads as follows

Background, P. 6
The K55R variant within the exon 2 of the EPHX2 gene was shown to be significantly more common among CHD cases than in the controls [1]. Additionally, carriers of the variant allele showed a higher epoxide hydrolase activity in vivo, indicated by lower plasma EET levels. No statistical differences were observed in the comparison of the genotype distribution in CHD cases and controls in the other analysed polymorphisms. Therefore, the findings of this study implicate the EPHX2K55R variant to be a genetic factor which increases the risk of CHD, even though this association has not been confirmed by further studies to date. Due to the evident effects of EPHX2 in the regulation of vascular function and the association to CHD in the reported data, we assumed that the K55R variant may also be involved into the process of restenosis after percutaneous coronary intervention (PCI).

2. “The particular gene variant should be better described including rs-numbering. Is the SNP in a coding region? Does it affect expression or protein structure? Are there other SNPs in this gene that would be of importance? Why was this particular gene variant chosen?”

Authors Reply: We appreciate that we have to give some more information on the chosen SNP. As you can see in the revised manuscript, we provide a more comprehensive description of the gene region and why this particular variant was chosen for our association study. We would like to point out that the answer to this question partly overlaps with comment 1.
Manuscript Change(s): This section of the background now reads as follows:

Background, P. 5
The degradation of endogenous EETs to their corresponding diols, dihydroxyeicosatrienoic acids (DHETs), is catalyzed by soluble epoxide hydrolase (sEH) encoded by the \textit{EPHX2} gene located on chromosome 8p21-p12 [3, 4].

Background, P. 5
Previously, a study reported the correlation of the \textit{K55R} single nucleotide polymorphism (rs41507953) within the \textit{EPHX2} gene with CHD in Caucasians [1]. The authors analysed a total of ten polymorphisms in coding and non-coding regions of \textit{EPHX2}, which were selected based on their previously published functional relevance in vitro [5, 6] and/or haplotype-tagging properties.

3. “Restenosis is expressed as a binary variable that is the value of choice. However, degree of late loss in mm could be tried to get more insight? No association in such an analysis would give more strength to the negative results.”

Authors Reply: In fact, we employed both methods, the morphological measurement (lumen re-narrowing ≥ 50%) and the estimation of late lumen loss for the classification of the restenosis patients, which was not explicitly described in the paper. Therefore we changed the manuscript to read as follows:

Manuscript Change(s):

Abstract, Results, P. 3
In CHD patients 6 month follow-up coronary angiography revealed a restenosis rate of 29.4%, classified as late lumen loss as well as lumen re-narrowing ≥ 50%.

Materials and Methods, Study Population, P. 7
Restenosis was determined through the measurement of the late lumen loss as well as the re-narrowing of the lumen ≥ 50%.

Results, P. 9
CHD cases were subdivided into two groups according to the occurrence or lack of restenosis at the 6 month follow up. For the further analysis, no differentiation was made between patients with late lumen loss and angiographic restenosis.

4. “What were the the causes of restenosis in this patient cohort? This might have been published in another publication but should be mentioned also in this manuscript.“

Authors Reply: The cause for the development of restenosis in 29.4 % of the patients could not be identified. We agree with one of the co-authors of the present manuscript who
argued previously that late lumen loss in the studied collective is mainly caused by hyperplasia of the vascular endothelium [2].

**Manuscript Change(s):**

Discussion, P. 11

None of the known risk factors for restenosis, such as diabetes mellitus or hypertension, could be identified as the cause for the occurrence of restenosis in 29.4% of our patient cohort. We assume that late lumen loss in the studied patient cohort is mainly caused by hyperplasia of the vascular endothelium [7, 8]. The findings in the present study indicate that an involvement of the EPHX2 K55R variant on this mechanism can largely been excluded.

**References**