Author's response to reviews

Title: Cytochrome P450 2C19*2 polymorphism in patients with stable coronary heart disease and risk for secondary cardiovascular disease events. Results of a long-term follow-up study in routine clinical care

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Version: 2 Date: 16 March 2013

Author's response to reviews: see over
**Title:** Cytochrome P450 2C19*2 polymorphism in patients with stable coronary heart disease and risk for secondary cardiovascular disease events. Results of a long-term follow-up study in routine clinical care

**Point-to-point response to Reviewer’s report**

**Version:** 1 **Date:** 16 October 2012

**Reviewer:** Willibald Hochholzer

**Reviewer’s report:**

Overall an interesting analysis.

**Major Compulsory Revisions**

**Point 1:** The major limitation is that it remains uncertain if the described association of CYP2C19*2 and clinical outcome is really a drug independent effect or caused in fact by subsequent clopidogrel treatment. To prove their thesis, the authors would need to provide separate analyses for patients on clopidogrel (either at enrollment or during follow-up) and patients that did never receive clopidogrel.

**Response:** Thank you for addressing this important point. We now made clear that beside the issue around CYP2C19*2 related to pharmacologic response to clopidogrel with an impaired clopidogrel activation from the pro-drug to the active drug we are also interested in the question whether CYP2C19 variants itself are associated with adverse cardiovascular outcomes irrespective of clopidogrel intake (see page 4, para 1, lines 5-9 and para 2, lines 4-5; page 8, para 1, lines 5-7, page 10, para 3, lines 5-8 & figure 1b; page 11, para 1, lines 5-7 and table 4).

We now provide the number of subjects with intake of clopidogrel at baseline and during the first year of follow-up and provide a stratified analysis considering clopidogrel-intake. Although quite a large number of patients received clopidogrel in the acute care-hospital (n=257, 24.5%), prescription was not continued and at discharge from in-patients rehabilitation, (which is considered our baseline examination) only n=80 (7.6%) received it. In addition, prescription went further down at 1 year follow-up (n=50, 4.7%) (see page 9, para 2, lines 13-15 and page 10, para 1, lines 1-2). When looking at these numbers we have to consider that the baseline examination took place in the years 1999/2000, long-time before respective guidelines recommended one-year dual antiplatelet therapy to improve outcomes after ACS. At that time often only a six week prescription of clopidogrel was initiated.

**Minor Essential Revisions:**

**Point 2:** - Page 14, 1st paragraph: That the *2 polymorphism explains ~12% of the variability in response to clopidogrel might only be true in very homogenous populations (such as Amish people). In the European population, this impact appears to be smaller (J Am Coll Cardiol. 2010;55:2427).

**Response:** We changed the wording accordingly (see page 14, para 1, line 9).

**Discretionary Revisions**

**Point 3:** - Page 14, 2st paragraph: These associations are only true if patients are also treated with clopidogrel! Therefore, analyses for the whole study population might not be appropriate.

**Response:** See response to point 1.
Point 4: - Page 9, second paragraph and Table 2: Can it be that the higher proportion of CYP2C19*2 carriers in younger subjects with lower education is due to differences in race (e.g., refugees from African or Asian countries? – in these countries, there is a higher prevalence of the *2 genotype).

Response: It is very unlikely that the difference is caused by differences in race as all participants of the study were of Caucasian origin. We now included this in the description of the study population (see page 9, para 1, line 3).

Additional note: We also had to change the p-values of the exact test in the table two as due to an error the table probability values of the test were included instead of the p-values. We regret this error and apologize.
Title: Cytochrome P450 2C19*2 polymorphism in patients with stable coronary heart disease and risk for secondary cardiovascular disease events. Results of a long-term follow-up study in routine clinical care

Point-to-point response to Reviewer’s report
Version: 1 Date: 5 November 2012
Reviewer: Betti Giusti

Reviewer’s report:
This paper aimed to investigate the prognostic value for cardiovascular events at eight year follow-up of the status of carriers of the CYP2C19*2 polymorphism in a cohort of coronary heart disease (CHD) patients. The Authors studied and genotyped the CYP2C19*2 polymorphism in 1,050 patients with stable CHD.

Major Compulsory Revisions. I have the following concerns:

Point 1: Abstract Background:
“CYP2C19 polymorphisms are related to metabolizer phenotypes resulting in reduced effectiveness ……..” The sentence is not correct. In fact, the CYP2C19*17 polymorphism results in an increased effectiveness…..
Response: Thank you for this hint. We changed the wording to limit this fact to the CYP2C19*2 polymorphism (see page 2, para 1, line 1).

Point 2: - “An additional role of the genotype itself is discussed”, The Authors should clarify the meaning.
Response: We changed the wording to clarify the meaning. (see page 2, para 1, line 3).

Abstract Methods:
Point 3: -“Genotyping of one single………follow-up. The Authors should improve the two sentences.
Response: We changed the wording of the two sentences to clarify the meaning (see page 2, para 2, lines 1-5).

Abstract Results:
Point 4: The very few CYP2C19*2 homozygous patients are 27 subjects.
Response: Indeed, only n=27 patients were homozygous for CYP2C19*2. As a consequence of this very limited group size, we have phrased our discussion section cautiously throughout, in order to avoid any over-interpretation of results. Furthermore, to exclude the risk of small sample bias we no also abstain from the calculation of an Hazard ratio in the homozygous for CYP2C19*2 who also took clopidogrel or ticlopidine at baseline as only two subjects were in this group, of whom one got indeed a cardiovascular event. We now just mention the numbers in the respective paragraph in the results section (see page 10, para 4, lines 6-8).

Abstract Conclusions:
Point 5: -With respect to available literature data on this issue this cohort is a medium group not a large group of patients.
Response: We changed the wording as suggested (see page 2, para 4, line 1 and page 12, para 1, line 1).
Introduction

Point 6: - The introduction poorly addresses the needed background. Even if in brief, the Authors should better define the reference data on this topics.

Response: We now clarified the objectives of the study and added additional information into the introduction section of the paper. We are aware that the main issue in clinical medicine is the fact that mutant CYP2C19*2 polymorphisms interfere with the necessary activation of clopidogrel and there reduced efficacy of this treatment. Beside this well investigated fact we are also interested in the question whether the CYP2C19 polymorphism is associated with adverse clinical outcomes in subjects who did never take clopidogrel. We now state this more explicitly in the introduction and objectives of the paper (see page 3, para 3 and page 4, para 1, lines 1-2 and lines 5-10 and para 2, lines 4-5).

Point 7: - The Authors should clarify the meaning “The role of the genotype itself in patients with coronary heart disease (CHD) is not well described.”

Response: We changed the sentences to clarify the meaning – see response to point 6 above.

Methods

Point 8: - Study population: - Clarify ….. initial response 58%

Response: The number describes the response at baseline. We deleted the word “initial” to avoid any misunderstanding (see page 5, para 1, lines 4-5).

Point 9: - What is the statistical power of the study?

Response: We had a power of 80% to detect a relative risk of 3.66 ($\alpha = 0.05$) or larger for the homozygous CYP2C19*2 loss-of-function carriers. In general, we were now more cautious in the interpretation of our data.

Results

Point 10: - How many patients were on clopidogrel maintaining dose? The Authors should take in mind that the pharmacogenetic effect of the CYP2C19*2 polymorphism is possible whether the patients/subjects are on clopidogrel treatment.

Response: (See also response to the point one reviewer one)

Response: Thank you for addressing this important point. We now made clear that beside the issue around CYP2C19*2 related to pharmacologic response to clopidogrel with an impaired clopidogrel activation from the pro-drug to the active drug we are also interested in the question whether CYP2C19 variants itself are associated with adverse cardiovascular outcomes irrespective of clopidogrel intake (see page 4, para 1, lines 5-9 and para 2, lines 4-5; page 8, para 1, lines 5-7, page 10, para 3, lines 5-8 & figure 1b; page 11, para 1, lines 5-7 and table 4).

We now provide the number of subjects with intake of clopidogrel at baseline and during the first year of follow-up and provide a stratified analysis considering clopidogrel-intake. Although quite a large number of patients received clopidogrel in the acute care-hospital (n=257, 24.5%), prescription was not continued and at discharge from in-patients rehabilitation, (which is considered our baseline examination) only n=80 (7.6%) received it. In addition, prescription went further down at 1 year follow-up (n=50, 4.7%) (see page 9, para 2, lines 13-15 and page 10, para 1, lines 1-2). When looking at these numbers we have to consider that the
baseline examination took place in the years 1999/2000, long-time before respective guidelines recommended one-year dual antiplatelet therapy to improve outcomes after ACS. At that time often only a six week prescription of clopidogrel was initiated.

**Point 11:** - Table 2: Clopidogrel/ticlopidine: Is it possible that only 87 patients out of 1050 were on clopidogrel?
Response: **See response to point 10 above.**

**Point 12:** - The Authors should better describe the duration of the clopidogrel treatment.
Response: **(See also response to the point 10 above)**

**Point 13:** - Did the Authors have information on the use of clopidogrel during the follow-up? This is a crucial information.
Response: **See response to point 10 above.**

**Point 14:** - The Authors should explain the rationale, the hypothesis, the aim to evaluate some parameters measured (e.g. NT-proBNP, Cystatin C, IL-6, etc) according to CYP2C19*2 genotype.
Response: The various biomarkers represent different pathophysiological concepts which are directly related to prognosis in patients with CHD (NT-proBNP = cardiac wall stress, Cystatin C = renal function, IL-6 and hs-CRP, WBC = inflammation, etc.). The aim of table 3 is mainly to assess potential relationship between these cardiovascular risk factors and our main exposure variable (CYP2C19C-alleles) to assess the potential for confounding. The latter can only be present if there is a clear association between these factors. However, these factors seem not to qualify as important confounders as this pre-requisite is not met (see results in table 3). The interpretation of these parameters in this context is also provided in the discussion section of the paper (see page 13, para 2, lines 4-7).

Discussion

**Point 15:** - The very few CYP2C19*2 homozygous patients are 27 subjects.
Response: Indeed, only n=27 patients were homozygous for CYP2C19*2. As a consequence of this very limited group size, we have phrased our discussion section cautiously throughout, in order to avoid any over-interpretation of results. Furthermore, to exclude the risk of small sample bias we no also abstain from the calculation of an Hazard ratio in the homozygous for CYP2C19*2 who also took clopidogrel or ticlopidine at baseline as only two subjects were in this group, of whom one got indeed a cardiovascular event. We now just mention the numbers in the respective paragraph in the results section (see page 10, para 4, lines 6-8).

**Point 16:** - How do the Authors explain the risk of homozygous carriers without clopidogrel? Also in consideration of the large trials results evaluating the effect of the CYP2C19*2 polymorphism in patients on antiaggregant therapy different from clopidogrel.
Response: Please find the following explanations in the manuscript: “Our data add to the evidence from clinical trial populations indicating an increased risk in carriers of the CYP2C19*2 loss-of-function allele compared to non-carriers taking clopidogrel” (page 12, para 2, lines 1-3). [...] “As the CYP2C19*2 genotype may only account for about up to 12% of the clopidogrel response, the higher percentages may only be found in homogeneous
populations, other relevant factors such as diabetes or age may play a role, too. Although the bedside-genotyping may be of additional value to gather prognostic information, especially for the few homozygous CYP2C19*2 loss-of-function carriers, as the association with an adverse cardiac outcome seems evident for these patients, the consequences for a modified treatment schedule are not clear yet”.

(page 14, para 1, lines 8-14).

**Point 17:** The paper is poorly written and suffer from poor organization.  
**Response:** We checked spelling and grammar. In addition we now provide more information and re-wrote certain statements. This way we hope the paper is now more clearly written. The article is written according to the guidelines of the STROBE-statement (details see www.strobe-statement.org).

Additional note: We also had to change the p-values of the exact test in the table two as due to an error the table probability values of the test were included instead of the p-values. We regret this error and apologize.

**Level of interest:** An article whose findings are important to those with closely related research interests  
**Quality of written English:** Needs some language corrections before being published  
**Statistical review:** No, the manuscript does not need to be seen by a statistician.