Author’s response to reviews

Title: The impact of Cytochrome 450 and Paraoxonase polymorphisms on clopidogrel resistance and major adverse cardiac events in Coronary Heart Disease patients after Percutaneous Coronary Intervention

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Dear Prof. Giovanni Tarantino

We are extremely thankful for the invaluable comments we have received from editors and reviewers who have generously shared their precious time and professional expertise to help us improve this paper. We are even more grateful to the two reviewers for their insightful scientific suggestions to help make the manuscript more suitable for publication. We have therefore revised the manuscript in keeping with the editor and reviewers’ comments below. Taking all your comments, we have revised the manuscript accordingly and labeled them in red color. Our responses immediately follow each comment.

We greatly appreciate the editor and reviewers’ time and comments.

Sincerely,
Zhaowei Zhang,

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For Reviewer 1: The CYP 450 activity is crucial in determining activity or toxicity of the drugs. The paper deals an important issue. It is well organized and the data are clearly presented. I suggest to discuss the evaluation of CYP450 activity by using the probe drug approach (see Analytical and

Response: Thanks very much for the suggestion. In the revised manuscript, We have discussed the evaluation of CYP450 activity and quoted the passage Phenotyping of CYP 4501A2 Activity by Total Overnight Salivary Caffeine Assessment (TOSCA) in Patients on Warfarin Treatment: A Cross-Sectional Study in the discussion section (line227-232, page8).

For Reviewer 2

The manuscript of Zhang and colleagues describes the genetic analyses performed in Chinese Han patients to establish every possible influence of polymorphisms in genes coding for drug metabolism on clopidogrel pharmacodynamics. The study is interesting and achieves important results, but some issue may limit the reliability of the study and final findings. Therefore, the Authors are kindly asked to reply to the following comments and queries.

1.Genotype analyses in all of the enrolled patients were addressed to the CYP2C19 and PON-1 isoforms, but the metabolism of clopidogrel is more complex, because the drug is substrate of the 3A4 isoform among the others, as well as ABCB1 transmembrane transporter. Saying this means that a wide panel of genes/polymorphisms could add further interest, even the significant differences observed by the authors among the different genotype subgroups. For example, the variant allele CYP2C19*17 is associated with an altered enzyme activity, therefore it could be a rationale target for pharmacogenetics analyses.

Response: Thanks very much for the suggestion. the metabolism of clopidogrel is complex, It is not only metabolized by CYP2C19 and PON-1 but also CYP3A4, ABCB1 and so on. In the revised manuscript, We pointed out the role of CYP3A4, ABCB1 in the metabolism of clopidogrel in the background section(line66-73, page3),we also add the limitation of our study in the discussion section (line277-278, page10).

2.From a methodological point of view some further explanations are needed. Indeed, the Authors stated that the study was registered as a retrospective protocol, hence the reader may conclude that the platelet aggregation assays are part of the clinical and laboratory routine. Is this correct? If not, the study should be considered a prospective protocol. Moreover, the manuscript does not present nor discuss additional information regarding the clinical condition of patients, the site of arterial occlusion that required a PCI, etc.

Response: Thanks very much for the suggestion. Our study is a prospective study, In the revised manuscript, we correct the method of our study in the Trial registration section (line 54-55, page 2).We added the clinical condition of patients, the site of arterial occlusion, the intervention method in Table 2 and the results section (line 159-164.page 6).
3. Did the Authors calculate the sample size a priori?
Response: Thanks very much for the suggestion. We have calculated the sample size before the study by quanto software. Power calculation was performed by Quanto software. Assuming a allele frequency of 0.33 and disease prevalence of 5–26%, we had 80% power to detect genetic effects at an OR of 2.25 under an additive model in our samples (line 87-90, page4).

4. Which is the need to re-analyse patients by a RFLP-PCR when the Authors adopt a Sanger sequencing method to investigate patients' genotypes for the selected loci?
Response: Thanks very much for the suggestion. In the revised manuscript, We removed the results of RFLP-PCR from the passage.

5. CYP2C19 haplotype analyses for linkage disequilibrium were not done, but some of these combination had a significant effect on clopidogrel effect as the Authors demonstrated.
Response: Thanks very much for the suggestion. In the revised manuscript, We analysised the linkage disequilibrium and the haplotype analysis was done in the methods section (line139-141,page5), in the results section (line 209-215, page 8) and in the discussion section (line255-256, page9).

6. No information about concomitant drugs (with the exception of CCB, ACEI/ARB, statins and PPI), herbs, nutraceuticals etc. are included within the text despite their possible influence on liver metabolism (i.e., inducers or inhibitors) and, consequently, on clopidogrel biotransformation.
Response: Thanks very much for the suggestion. In the revised manuscript, We supplemented the information about concomitant drugs as Diuretics, Rivaroxaban, β-receptor blocker, Nitrates, Panax Notoginseng in table2.

7. The Authors should present the non-MACE events.
Response: Thanks very much for the suggestion. In the revised manuscript, We have listed the non-MACE events in table 6.

8. Conclusions. The PON polymorphism affected platelet response to clopidogrel but not the incidence of MACE. This point should be better explained, especially in the presence of a dual step for drug biotransformation into an active metabolite.
Response: Thanks very much for the suggestion. In the revised manuscript, We have added the explanation on why the PON polymorphism affected platelet response to clopidogrel but not the incidence of MACE in the discussion section (line268-274, page9-10).