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Paraoxonase-1 and clopidogrel efficacy

To the Editor:

We read with great interest the paper published in *Nature Medicine* by Bouman *et al.*[1] showing that a common paraoxonase-1 (*PON1*) polymorphism (rs662, also known as Q192R) is a major determinant of clopidogrel biological efficacy and stent thrombosis, whereas the common *CYP2C19*2* allele (rs4244285-A allele) has no effect in this population. This suggests that previous data about the effect of the rs4244285-A allele on clopidogrel efficacy[2,3] were mainly related to linkage disequilibrium with *PON1* polymorphisms.

In an attempt to replicate the results of Bouman *et al.*[1], we analyzed, in an ongoing large French registry, the association between *PON1* rs662 and *CYP2C19* rs4244285 polymorphisms and biological response to clopidogrel in 482 individuals with unstable coronary artery disease in Marseille. Subject characteristics are included in **Supplementary Table 1.** All patients were treated with 600 mg clopidogrel followed by clopidogrel 150 mg maintenance therapy. Platelet reactivity was assessed using 10 μM ADP (reported as ADP–induced platelet aggregation (ADP-Ag) values), and the specific pharmacological response to clopidogrel was assessed by detecting phosphorylation of vasodilator-stimulated phosphoprotein (VASP; reported as platelet reactivity index VASP (PRI VASP) values) between 12 and 24 h after hospital discharge and at 30 d after hospital discharge. *CYP2C19* rs4244285 and *PON1* rs662 genotyping was performed using amplification refractory mutation system PCR. *PON1* rs662 genotyping was checked with restriction fragment length polymorphism PCR and Sanger sequencing. All methods are detailed in the **Supplementary Methods**. The study protocol was approved by the University Hospital La Timone Ethics Committee, and patients gave written informed consent for their participation.

Platelet reactivity and clopidogrel response were not affected by *PON1* rs662 polymorphism status, either when measured shortly after the loading dose or after steady-state maintenance therapy (**Fig. 1** and **Supplementary Tables 2** and **3**). Conversely, a strong influence of the *CYP2C19* rs4244285 genotype on both platelet reactivity and clopidogrel response was observed at both time points (**Fig. 1** and **Supplementary Tables 2** and **3**), as previously reported by our group and others[2,3].

The lack of *PON1* rs662 genotype influence on clopidogrel efficacy observed in our cohort may have several potential explanations. First, the present study might be underpowered to find a *PON1* rs662 polymorphism effect; however, Bouman *et al.*[1] described a significant effect on clopidogrel response with a smaller sample size. Second, the selected unstable patients and high clopidogrel doses used in our study might have affected our results compared with standard clopidogrel doses for stable patients in the Bouman *et al.*[1] study. Moreover, the choice of platelet tests and timing of platelet testing was different, as this was done 12 to 24 h after loading dose and 30 d after the onset of acute coronary syndrome in our study. Bouman *et al.*[1] tested clopidogrel pharmacological response using 20 μM ADP or active metabolite. Here we used 10 μM ADP and also used the most specific pharmacological test available to assess clopidogrel response, the PRI VASP assay. Finally, confounding factors may have influenced our results, as PON1's enzymatic activity is affected by several environmental factors including chronic renal disease, lipids, smoking and inflammation. Indeed, the individuals in our study were in a Mediterranean city, which might be slightly different from the population studied by Bouman *et al.*[1]. However, the baseline characteristics of the patients according to *PON1* rs662 polymorphism were not different between genotypes in our cohort (data not shown). On the basis of our results, the necessity for *PON1* rs662 genotyping in routine clinical practice seems questionable. Further large studies are urgently needed to address the observed discordance between our study and that of Bouman *et al.*[1] and the potential relevance of *PON1* genotyping to tailor antiplatelet therapy.

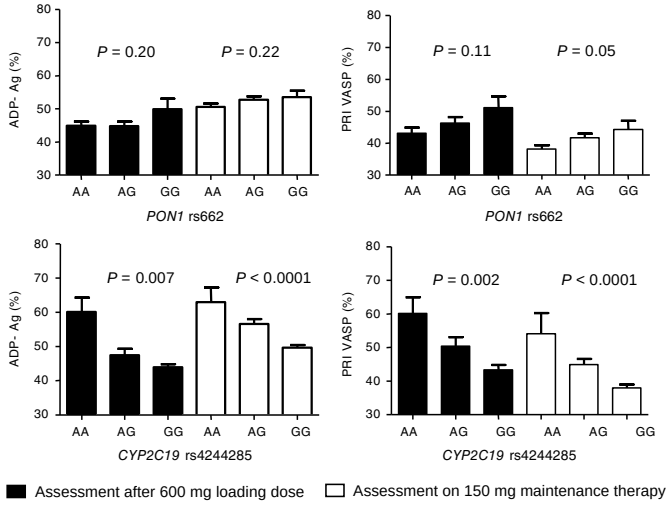

Figure 1 Platelet parameters according to *PON1* rs662 and *CYP2C19* rs4244285 genotypes. Platelet reactivity was assessed using the 10 μM ADP-Ag assay and the specific pharmacological response to clopidogrel by the PRI VASP assay.

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING FINANCIAL INTERESTS

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Thomas Cuisset[1,2], Pierre-Emmanuel Morange[2], Jacques Quilici[1], Jean Louis Bonnet[1], Christian Gachet[3] & Marie-Christine Alessi[2]

[1]Département de Cardiologie, University Hospital La Timone, Marseille, France. [2]Institut National de la Santé et de la Recherche Médicale, U626, Faculté de Médecine, Marseille, France. [3]UMR-S949 Institut National de la Santé et de la Recherche Médicale-Université de Strasbourg, Etablissement Français du Sang-Alsace, Strasbourg, France.

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