Paraoxonase-1 Q192R Polymorphism and Antiplatelet Effects of Clopidogrel in Patients Undergoing Elective Coronary Stent Placement

Running title: Trenk et al.; PON1 Q192R and Antiplatelet Effects of Clopidogrel

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Abstract:

Background - Recently published data indicate that the paraoxonase-1 (PON1) Q192R genotype and not as previously shown activity of cytochrome P450 (CYP) 2C19 is the major determinant of metabolic bioactivation of clopidogrel and thereby variability of antiplatelet effect of clopidogrel. We sought to investigate whether the PON1 Q192R gene polymorphism impacts on platelet reactivity in patients undergoing elective coronary stent placement (PCI).

Methods and Results - The study included 760 consecutive patients undergoing PCI after loading with clopidogrel 600mg. Platelet function was assessed by adenosine diphosphate-induced (ADP 5 and 20 μmol/L) platelet aggregation and by flow-cytometric analysis of platelet surface protein expression before clopidogrel, at the time of PCI and pre-discharge after PCI. PON1 Q192R genotype [NM_000446.5:c.575A>G SNP (rs662)] was analyzed by TaqMan PCR. Residual platelet aggregation (RPA ADP 5 μmol/L) at pre-discharge was 8.0% (3.0% - 17.0%) [median (interquartile range)] in PON1 QQ192 patients (n=384), 8.0% (3.0% - 15.0%) in PON1 QR192 (n=304) and 11.0% (3.0% - 18.0% ) in PON1 RR192 (n=72; p=0.603). By multivariable linear regression RPA was not associated with PON1 QQ192/QR192 (partial η²: <0.001, p=0.728), but with CYP2C19*2 loss-of-function allele (partial η²: 0.045, p<0.001) as well as any CYP2C19*17 gain-of-function allele (partial η²: 0.012, p=0.004). All other platelet assays also showed no significant association between PON1 Q192R genotype and antiplatelet effect of clopidogrel. The 1-year incidence of death and myocardial infarction did not differ between PON1 Q192R genotypes.

Conclusion - On-treatment platelet reactivity in patients undergoing PCI after loading with clopidogrel 600mg was not associated with PON1 Q192R genotype.

Clinical Trial Registration – URL: http://www.ClinicalTrials.gov. Unique identifier: NCT00457236

Key words: platelets, coronary artery disease, coronary stent, genetic polymorphisms, clopidogrel
Current guidelines recommend a combination of aspirin and clopidogrel for the prevention of recurrent ischemic events in patients with acute coronary syndromes (ACS) and for patients undergoing percutaneous coronary intervention (PCI). Several studies have shown a wide inter-individual variability in the antiplatelet effects of clopidogrel, and patients with an inadequate antiplatelet response to clopidogrel are at increased risk for ischemic complications (review by 10).

Clopidogrel is an inactive pro-drug which is converted by the cytochrome P450 (CYP) system into the active metabolite that binds irreversibly to the platelet purinergic P2Y12-receptor and thereby inhibits adenosine diphosphate (ADP)-induced aggregation. Various CYP isoenzymes including CYP2C19, 3A4/5, 1A2, 2B6 and 2C9 are involved in bioactivation of clopidogrel and several pharmacodynamic and outcome studies have shown that a loss-of-function polymorphism of CYP2C19 is associated with a reduced antiplatelet effect of clopidogrel and a higher incidence of major cardiovascular events.

However, further analyses have indicated that only 5-12% of the variability seen in platelet reactivity on-clopidogrel is explained by this polymorphism.

Paraoxonase-1 (PON1) is an esterase synthesized in the liver and associated with HDL in blood. A recently published study suggests that PON1 Q192R polymorphism may have a crucial role for the rate of active metabolite formation from clopidogrel which in turn should impact on platelet reactivity and subsequently on the incidence of ischemic events in patients on clopidogrel. The common single nucleotide polymorphism in PON1 c.575A>G resulting in an amino acid exchange in p.Gln192Arg (Q192R) determines the catalytic activity of PON1. By diligent in-vitro metabolomic studies, Bouman et al. demonstrated a role of PON1 Q192R polymorphism in the conversion of 2-oxo-clopidogrel to the pharmacologically active
thiol metabolite, with the highest formation of active metabolite in homozygous mutant (RR) patients. They also suggested a clinical role of the PON1 Q192R polymorphism.\textsuperscript{20} Although they could not reproduce the well established documented relation between CYP 2C19 loss of function polymorphisms and outcome,\textsuperscript{17,21-26} they demonstrated a strong association between PON1 Q192R genotype and ischemic events. This was based on a case-cohort study as well as on a large prospective patient cohort. To establish platelet function as the link between PON1 Q192R genotype and outcome, they assessed the concentration of active metabolite as well as inhibition of platelet aggregation. They found that 73\% of the variability in platelet aggregation could be attributed to the PON1 Q192R polymorphism.\textsuperscript{20} This finding, however, was only derived from a small cohort of 112 patients.

To address the question, whether the findings of Bouman et al. also apply for large cohorts undergoing elective PCI, we investigated the impact of PON1 Q192R polymorphism on the antiplatelet response to clopidogrel in 760 consecutive patients undergoing elective PCI with stent placement after loading with clopidogrel which had been enrolled in the EXCELSIOR (Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate) study.\textsuperscript{27}

**Methods**

**Study population and interventions.** The presented data represent a secondary analysis of the EXCELSIOR study that investigated the impact of on-clopidogrel variability of platelet aggregation on clinical outcome as previously published.\textsuperscript{16,27} Briefly, patients undergoing elective coronary stent placement after pretreatment with 600 mg of clopidogrel and aspirin (\(\geq 100\) mg per day for at least five days) were eligible for enrollment into this prospective single-center study conducted in a referral center setting. Key exclusion criteria
were acute myocardial infarction (MI) according to the consensus document of the European Society of Cardiology and the American College of Cardiology,\textsuperscript{28} chronic oral anticoagulation, thienopyridine treatment within the last 2 weeks before admission, contraindications to either aspirin, clopidogrel or heparin, advanced cancer, hemodialysis, and hemodynamic instability. All patients enrolled were Caucasians.

The study was performed in accordance with the ethical principles of the Declaration of Helsinki and was approved by the ethics committee of the medical faculty of the University Freiburg, Germany. All patients gave written informed consent to study participation and blood sampling for genomic assays.

**Platelet function analysis.** Baseline blood samples for platelet function assays were drawn before administration of clopidogrel using tubes containing 3.8% sodium-citrate (Sarstedt AG, Nuembrecht, Germany). We obtained the second blood sample at the time of catheterization before administration of heparin or contrast medium and a further sample at the following day after PCI before discharge 2 to 4 hours after intake of the first maintenance dose of clopidogrel 75 mg.

Platelet aggregation was assessed by light transmission aggregometry in platelet-rich plasma using a four-channel Bio/Data PAP4 aggregometer (Moelab, Langenfeld, Germany), as previously described.\textsuperscript{8,29} Platelet-rich plasma was prepared by centrifugation of citrated venous blood at 750g for 2 minutes and adjusted to 275-325×10\textsuperscript{9} thrombocytes/L by dilution with autologous platelet poor plasma. Maximum platelet aggregation was the maximal amplitude of light transmission observed while residual platelet aggregation (RPA) was determined 5 minutes after addition of adenosine diphosphate (ADP; Sigma, Munich, Germany) at final concentrations of 5 and 20 µmol/L. Percentage of light transmission was calculated using platelet poor plasma from the same patient as reference (\(= 100\%\)).
aggregation). Percent platelet inhibition was calculated as (aggregation at baseline – 
aggregation at time x)/aggregation at baseline x 100. The coefficient of variation of our 
optical aggregometry assay is 6.1%.

ADP-induced surface expression of P-selectin (CD62P) and activated GP IIb/IIIa 
(PAC-1) was determined by triple color flow cytometry as previously described. Platelets in 
whole blood were stained with an antibody mixture containing fluorescein-isothiocyanate 
tagged PAC-1 (activated GP IIb/IIIa receptors), phycoerythrin-tagged anti-CD62P (P-
selectin) and phycoerythrin-cyanin 5.1 tagged anti-CD41 (total GP IIb/IIIa receptors) 
monoclonal antibodies (PAC-1 by Becton-Dickinson, Heidelberg, Germany, all other 
anti-bodies by Beckman Coulter, Krefeld, Germany). Platelets were incubated with the 
antibodies and ADP at a final concentration of 20 μmol/L for 30 minutes. Thereafter, 300 μL 
of para-formaldehyde 4% was added for fixation. A four channel flow cytometer equipped 
with a 488 nm argon laser (FACSCalibur, Becton Dickinson, Heidelberg, Germany) was 
used. Platelets were identified in whole blood by size and a platelet-specific monoclonal 
antibody (CD41) and 10,000 events from each sample were analyzed. The mean channel of 
fluorescence intensity was taken as a measure for antibody binding, and thus antigen surface 
exposure.

Genotyping by TaqMan PCR. Blood for genomic DNA extraction was sampled using 
tubes containing 1.2 to 2 mg potassium-EDTA per mL of blood (Sarstedt AG, Nuembrecht, 
Germany). Genomic DNA was extracted from blood with the Flexigene Kit (QIAGEN, 
Hilden, Germany) according to the manufacturer's instruction. After extraction, the 
concentration of DNA was measured photometrically, and DNA was diluted to a 
concentration of 5 mg/L. Genotyping for the PON1 NM_000446.5:c.575A>G (rs662) and 
CYP2C19*17 SNPs (rs12248560) was carried out using the TaqMan Pre-Developed Assay
Reagents for Allelic Discrimination (assay ID: C_2548962_20 and C_469857_10; Applied Biosystems, Foster City, CA). Amplification was performed in a final volume of 5 μL containing 5 ng DNA, 4.5 pmol of each primer, 1.0 pmol of each probe, and 2.5 μL 2x Type-It Fast Genotyping Master Mix (contains PCR buffer, passive reference dye ROX, deoxynucleotides, and Taq DNA polymerase; Qiagen, Hilden, Germany) by use of the ABI Prism Sequence Detector 7900 (Applied Biosystems). Cycle parameters were as follows: 95°C for 5 minutes and then 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. After PCR, fluorescence yield for the 2 different dyes was measured. The SDS 2.1 software (Applied Biosystems) was used to plot and automatically call genotypes on the basis of a 2-parameter plot with fluorescence intensities of FAM and VIC. PCR genotyping of CYP2C19*2 was described previously.\(^{16}\)

The concordance rate was 100% for all duplicate samples throughout the different genotype assessments. The distribution of the CYP2C19 genetic variants did not deviate significantly from Hardy-Weinberg equilibrium (CYP2C19*2: \(\chi^2=1.932;\) \(p=0.165\); CYP2C19*17: \(\chi^2=0.065;\) \(p=0.799\)).\(^{16}\)

**Statistical analysis.** The sample size of the EXCELSIOR study was based on the power calculation for the 30-day primary clinical end point as described previously.\(^{27}\) The dataset of patients with platelet function assessments available at all time points was used (\(n=765\)). Frequencies of categorical variables were given as counts (percentages) and continuous variables either as mean ± standard deviation or as median with interquartile range. Differences between groups were tested with the \(\chi^2\)-test or Fisher’s exact test for categorical variables and with one-way ANOVA for continuous variables. The effect of PON1 Q192R genotypes on antiplatelet effects of clopidogrel determined by ADP-induced
aggregation and flow-cytometry analysis of surface proteins was assessed by linear regression analysis. Cox proportional hazard models were used to evaluate the association between PON1 Q192R genotypes and the risk of major adverse cardiovascular events (composite of death and MI, stent thrombosis). The results of these models were described using the hazard ratio (HR) with the associated 95% confidence interval (95% CI).

The percentage of variability of on-clopidogrel RPA that could attribute to the variability in independent variables was derived from partial $\eta^2$ calculated by a multivariable linear model. This model comprised ADP 5 $\mu$mol/L induced RPA as dependent variable and PON1 Q192R genotype as well as all baseline clinical and demographic variables shown in Table 1 impacting on on-clopidogrel platelet reactivity in previous studies as independent variables, entering dichotomous variables as fixed factors.

PASS version 11 software (NCSS, Kaysville, UT) was used for a secondary power analysis regarding the platelet function studies and the incidence of stent thrombosis in our cohort. To get an estimate of the statistical power regarding the association of PON1 genotype and stent thrombosis in comparison to a recently published paper, we used both the lower 95% confidence interval, and the maximum likelihood estimate of the unadjusted hazard ratios of the paper by Bouman et al. We then used the observed incidence from our data, alpha = 0.05, 2-sided and 12 month follow-up time.

For all other statistical analyses, we used the PASW software package, version 18 (SPSS Inc., Chicago, Illinois). A p value < 0.05 in the 2-tailed test was considered as significant.
Results

PON1 Q192R genotyping results. PON1 Q192R genotype could be determined in 760 patients out of the whole cohort of 765 patients with platelet function assessed at baseline, before PCI and pre discharge at day 1 after PCI. Five patients refused to consent for blood sampling for genetic investigations.

Of the 760 patients, 384 (50.5%) were PON1 QQ192 homozygous individuals, 304 (40.0%) were QR192 mutant heterozygous and 72 (9.5%) were RR192 mutant homozygous. The distribution of the genetic variants did not deviate significantly from Hardy-Weinberg equilibrium (chi²=1.089; p=0.297).

Antiplatelet effect of clopidogrel and PON1 Q192R polymorphism. Baseline demographic and clinical characteristics of the study population according to their PON1 Q192R genotype are summarized in Table 1. There were no significant differences between QQ192, QR192 and RR192 patients except a slightly higher proportion of active smokers in the group of RR192 patients.

Assessment of residual platelet aggregation (RPA) determined 5 minutes after stimulation of platelet rich plasma with ADP 5 μmol/L did not reveal any significant differences in platelet aggregation between PON1 QQ192, PON1 QR192 and PON1 RR192 patients at baseline, before PCI, and pre-discharge at day 1 after PCI (Table 2 and Figure 1). Similar results were obtained for RPA after stimulation with ADP 20 μmol/L, and for maximum aggregation after stimulation with either 5 or 20 μmol/L ADP (Table 2).

Flow cytometric analyses of surface proteins expression after stimulation of the platelets with ADP 20 μmol/L did not show any significant association of PON1 Q192R genotype and on-clopidogrel platelet reactivity (Figure 2).
In a multivariable linear regression model, \( PON1 \) QQ192/QR192 did not contribute significantly to the variability in ADP (5 \( \mu \)mol/L)-induced RPA (partial \( \eta^2 \): <0.001, \( p=0.73 \), Table 3). However, carrier status of \( CYP2C19*2 \) loss-of-function allele(s) (partial \( \eta^2 \): 0.045, \( p<0.001 \)) and carrier status of \( CYP2C19*17 \) gain-of-function allele(s) (partial \( \eta^2 \): 0.012, \( p=0.004 \)) together with baseline clinical and demographic variables such as age, diabetes, BMI, platelet count, concurrent treatment with verapamil/diltiazem, and multivessel PCI were significantly related to ADP (5 \( \mu \)mol/L)-induced RPA (Table 3).

**Clinical outcome and \( PON1 \) Q192R polymorphism.** Follow-up was complete in >99% of patients. The composite 12-month incidence of death and non-fatal myocardial infarction did not significantly differ between \( PON1 \) QQ192 (11/384; 2.9%), \( PON1 \) QR192 (9/304; 3.0%) and \( PON1 \) RR192 (4/72; 5.6%) genotype (\( p=0.47 \)). In particular, the risk for the combined endpoint of death and non-fatal myocardial infarction did not differ between either the \( PON1 \) QQ192 (hazard ratio [HR]: 0.61; 95% confidence interval [95% CI]: 0.20 – 1.88; \( p=0.390 \)) or the QR192 genotype (HR: 0.53; 95% CI: 0.16 – 1.73; \( p=0.295 \)) compared with RR192 homozygous patients.

The incidence of stent thrombosis (definite, probable or possible according to the ARC criteria\(^{30} \)) was determined for the various \( PON1 \) Q192R genotypes (QQ192: 6/384, 1.6%; QR192: 6/304, 2.0%; RR192: 4/72, 5.6%) with the highest incidence being observed in \( PON1 \) RR192 patients. There was no significant genotype effect of the \( PON1 \) Q192R polymorphism on the incidence of stent thrombosis (\( p=0.094 \)). The risk for any stent thrombosis did not differ between \( PON1 \) QQ192 (HR: 0.28; 95% CI: 0.08 – 1.01; \( p=0.051 \)) or QR192 carriers (HR: 0.36; 95% CI: 0.10 – 1.28; \( p=0.116 \)) compared with RR192 patients.

No sex-specific effects regarding antiplatelet effect of clopidogrel were found.
Discussion

This study aimed to analyze the association between PON1 Q192R genotype and the antiplatelet effects of clopidogrel in patients undergoing elective coronary intervention. The main finding of this analysis was that no significant association between PON1 Q192R genotype and platelet reactivity before administration of clopidogrel and on clopidogrel was found – irrespective of the type of platelet function assay (optical aggregometry and flow cytometry), and the dose of ADP used for stimulation. Assuming a similar effect size for mean platelet inhibition between the three genotypes as described by Bouman et al., the power to detect this effect in the EXCELSIOR cohort (n=760) would have been >0.999.

These findings were confirmed by a multivariable linear model that did not show any significant association of PON1 QQ192QR192 genetic status with platelet function after clopidogrel. This model confirmed that CYP2C19*2 loss-of-function allele(s) carrier status is the strongest variable significantly contributing to the variability in ADP (5 μmol/L)-induced RPA on clopidogrel, together with CYP2C19*17 gain-of-function allele(s) carrier status and further baseline demographic and clinical variables such as age, diabetes, BMI and platelet count, concomitant treatment with verapamil/diltiazem, and multivessel PCI.

Our results are therefore in contrast to data published recently by Bouman et al. These authors performed in vitro metabolomic experiments using cytochrome P450 oxidoreductase isoenzymes and esterases overexpressed in a human embryonic kidney cell line and determined conversion of clopidogrel and potential intermediate metabolites in microsomal preparations. They confirmed that conversion of clopidogrel to the first metabolite 2-oxo-clopidogrel is catalyzed by previously identified CYP450 isoenzymes. However, they identified that the conversion of 2-oxo-clopidogrel to the pharmacologically active thiol metabolite is catalyzed by the esterases PON1 and PON3 with PON1 being
approximately 12-fold more efficient than PON3. The rate of formation of the active metabolite of clopidogrel was determined by the Q192R polymorphism in PON1 with the highest systemic exposure to the active metabolite observed in homozygous mutant patients. The increased availability of the active metabolite was associated with superior inhibition of platelet aggregation induced by ADP 20 μmol/L.

The impact of PON1 Q192R genotype on pharmacokinetics and antiplatelet effect of clopidogrel was investigated in a case-cohort study comprising 112 patients with and without stent thrombosis. In this selected cohort, Bouman and co-workers observed close correlations between PON1 Q192R genotype and maximal plasma concentration of the active metabolite of clopidogrel and inhibition of platelet aggregation. Unfortunately, the absolute level of platelet reactivity during treatment (i.e., on-treatment platelet reactivity) within the three PON1 Q192R genotypes was not reported, although, in a recent consensus paper, this was proposed to be a better measure of thrombotic risk than responsiveness to clopidogrel.10 Due to the known wide interindividual variability in antiplatelet effect of clopidogrel that can lead to arbitrary findings in small cohorts, sample size of their cohort is a major limitation regarding the association between PON1 Q192R genotype and antiplatelet effect. A further limitation of these findings is that a substantial period of time had elapsed between the initial clinical event and the latter assessment because the patients investigated were already off regular-prescribed clopidogrel at this time.

The EXCELSIOR study enrolled consecutive patients undergoing elective PCI which represents a cohort with lower clinical risk. However, no association between the PON1 Q192R genotype and the primary combined clinical endpoint of all cause mortality and non-fatal myocardial infarction was observed. We observed a trend for an association between the incidence of stent thrombosis and PON1 Q192R genotype with the highest incidence in
*PON1* RR92 patients which is in contrast to the association suggested by Bouman et al.\(^\text{20}\)

For the comparison between *PON1* QQ192 vs. RR192, the power to detect an effect size comparable to the study of Bouman et al.\(^\text{20}\) was >0.999 if the analysis was based on the maximum likelihood estimate, or 0.730, if the analysis was based on the lower 95%-confidence limit of the study of Bouman et al.\(^\text{20}\) For comparison between QR182 vs. RR192, we calculated a power of 0.221 and 0.958, respectively.

**Study limitations.** Several *in vitro* studies with a number of compounds consistently demonstrated that the non-synonymous *PON1* rs662 SNP significantly affects paraoxonase 1 catalytic activity.\(^\text{20,31-33}\) For this reason we and other groups focused on the rs662 SNP. We cannot rule out that other SNPs in linkage disequilibrium (LD) may affect, in addition to rs662, paraoxonase 1 activity. Of note, HapMap Caucasian group data indicate that – within the 150 kbp region on chromosome 7q21, where *PON1*, *PON2* and *PON3* are clustered – no other coding SNP is in LD (\(r^2>0.4\)) with rs662. We cannot rule out that other SNPs in LD with rs662 may affect paraoxonase 1 activity or expression.

Since plasma concentrations of the active metabolite of clopidogrel (R130,964) were not determined in the analyzed cohort, an analysis of the association between metabolite levels and *PON1* Q192R genotype could not be performed. The chemical instability of the metabolite requiring extensive pre-analytical precautions and rapid chemical derivatization after blood draw could not be implemented in this study enrolling a large patient cohort under clinical conditions.

The number of clinical endpoint events in our current analysis is limited. Thus, the study was not powered to detect an association of *PON1* Q192R genotype and major cardiovascular events observed during the 12-month follow-up after PCI.
Clinical implications. When given in addition to aspirin, clopidogrel has been demonstrated to have an incremental benefit in patients with acute coronary syndromes especially in those undergoing PCI. However, clinical efficacy of clopidogrel is hampered by substantial variability in antiplatelet response. The combination of CYP2C19 reduced function status with known clinical risk factors such as diabetes, BMI or age contributes no more than 12 % to variability in antiplatelet response to clopidogrel. Therefore, a significant proportion of the excessive risk in patients with high-on clopidogrel platelet reactivity might be due to other mechanism(s). The study by Bouman and coworkers suggested that PON1 Q192R polymorphism might close this gap. If their finding that 73 % of the variability in response to clopidogrel attributes to this polymorphism could have been confirmed, this might have allowed to obviate platelet function testing. Allocation of patients to clopidogrel or other P2Y₁₂-receptor antagonists could have been based only on genetic testing for the PON Q192R polymorphism.

In this large consecutive clinical cohort, however, the impact of PON1 Q192R polymorphism on platelet function in patients treated with clopidogrel could not be confirmed. Based on our study, we cannot exclude a link between PON1 Q192R polymorphism and clinical outcome after stenting, but we have no evidence that on-clopidogrel platelet reactivity constitutes this link. Thus, independent of PON1 Q192R carrier status, high-on clopidogrel platelet reactivity remains a risk factor for ischemic events that based on current evidence is best assessed by phenotyping.

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Conflict of Interest Disclosures: Dr. Trenk reports receiving a research grant from Eli Lilly and Company; receiving speaking and advisory board fees from AstraZeneca, Eli Lilly and Company, and Daiichi Sankyo, Inc. Dr. Hochholzer reports receiving consulting fees from Sanofi-Aventis. All other authors have no conflicts of interest to disclose.

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major demographic characteristics on residual platelet function after loading and 
maintenance treatment with clopidogrel in patients undergoing elective coronary stent 


Table 1. Baseline Demographic and Clinical Characteristics of the Study Cohort According to PONI Q192R Genotype

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=760)</th>
<th>PONI Q192R</th>
<th>PONI QR192</th>
<th>PONI RR192</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.3 ± 9.2</td>
<td>66.1 ± 9.7</td>
<td>66.4 ± 8.7</td>
<td>67.4 ± 8.7</td>
<td>0.549</td>
</tr>
<tr>
<td>Women</td>
<td>165 (21.7)</td>
<td>79 (20.6)</td>
<td>68 (22.4)</td>
<td>18 (25)</td>
<td>0.661</td>
</tr>
<tr>
<td>CYP2C19*2 LoF</td>
<td>233 (30.7)</td>
<td>122 (31.8)</td>
<td>80 (26.3)</td>
<td>21 (29.2)</td>
<td>0.796</td>
</tr>
<tr>
<td>CYP2C19*17 GoF</td>
<td>296 (38.9)</td>
<td>132 (39.8)</td>
<td>111 (36.5)</td>
<td>32 (44.4)</td>
<td>0.406</td>
</tr>
<tr>
<td>Time from loading dose to PCI (h)</td>
<td>6.2 ± 11.1</td>
<td>5.9 ± 9.6</td>
<td>6.9 ± 13.2</td>
<td>5.3 ± 7.3</td>
<td>0.378</td>
</tr>
<tr>
<td>Active smoker</td>
<td>82 (10.8)</td>
<td>44 (11.5)</td>
<td>25 (8.2)</td>
<td>3 (4.1)</td>
<td>0.045</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>624 (82.1)</td>
<td>318 (82.8)</td>
<td>247 (81.3)</td>
<td>59 (81.9)</td>
<td>0.868</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>185 (24.3)</td>
<td>94 (24.5)</td>
<td>69 (22.7)</td>
<td>22 (30.6)</td>
<td>0.375</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>198 ± 46</td>
<td>199 ± 45</td>
<td>198 ± 47</td>
<td>198 ± 42</td>
<td>0.948</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.7 ± 3.9</td>
<td>27.8 ± 4.0</td>
<td>27.8 ± 3.7</td>
<td>27.2 ± 3.5</td>
<td>0.424</td>
</tr>
<tr>
<td>Platelets (x10³/L)</td>
<td>217 ± 54</td>
<td>219 ± 54</td>
<td>215 ± 53</td>
<td>216 ± 63</td>
<td>0.630</td>
</tr>
<tr>
<td>Serum creatinine (mmol/L)</td>
<td>88.3 ± 31.4</td>
<td>87.7 ± 26.7</td>
<td>89.6 ± 37.4</td>
<td>86.4 ± 26.8</td>
<td>0.643</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>524 (68.9)</td>
<td>270 (70.3)</td>
<td>205 (67.4)</td>
<td>49 (68.1)</td>
<td>0.710</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>320 (42.1)</td>
<td>157 (40.9)</td>
<td>127 (41.8)</td>
<td>36 (50.0)</td>
<td>0.352</td>
</tr>
<tr>
<td>AT1-antagonists</td>
<td>118 (15.5)</td>
<td>52 (13.5)</td>
<td>55 (18.1)</td>
<td>11 (15.3)</td>
<td>0.262</td>
</tr>
<tr>
<td>Diuretics</td>
<td>254 (33.4)</td>
<td>121 (31.5)</td>
<td>115 (37.8)</td>
<td>18 (25.0)</td>
<td>0.061</td>
</tr>
<tr>
<td>Nitrates</td>
<td>229 (30.1)</td>
<td>111 (28.9)</td>
<td>99 (32.6)</td>
<td>19 (26.4)</td>
<td>0.447</td>
</tr>
<tr>
<td>Statins</td>
<td>430 (56.6)</td>
<td>211 (54.9)</td>
<td>169 (55.6)</td>
<td>50 (69.4)</td>
<td>0.068</td>
</tr>
<tr>
<td>Verapamil/Diltiazem</td>
<td>31 (4.1)</td>
<td>16 (4.2)</td>
<td>13 (4.3)</td>
<td>2 (2.8)</td>
<td>0.840</td>
</tr>
<tr>
<td>Previous balloon angioplasty</td>
<td>263 (34.6)</td>
<td>134 (34.9)</td>
<td>101 (33.2)</td>
<td>28 (38.9)</td>
<td>0.652</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>105 (13.8)</td>
<td>53 (13.8)</td>
<td>41 (13.5)</td>
<td>11 (15.3)</td>
<td>0.925</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>175 (23.0)</td>
<td>98 (25.5)</td>
<td>60 (19.7)</td>
<td>17 (23.6)</td>
<td>0.200</td>
</tr>
<tr>
<td>Impaired LV function</td>
<td>267 (35.1)</td>
<td>132 (34.4)</td>
<td>108 (35.5)</td>
<td>27 (37.5)</td>
<td>0.863</td>
</tr>
<tr>
<td>CCS Angina class III or IV</td>
<td>185 (24.3)</td>
<td>98 (25.5)</td>
<td>70 (23.0)</td>
<td>17 (23.6)</td>
<td>0.742</td>
</tr>
<tr>
<td>Multivessel PCI</td>
<td>174 (22.9)</td>
<td>90 (23.4)</td>
<td>74 (24.3)</td>
<td>10 (13.9)</td>
<td>0.155</td>
</tr>
</tbody>
</table>

CYP2C19*2 LoF; carrier of cytochrome P450 2C19*2 loss-of-function allele; CYP2C19*17 GoF; carrier of cytochrome P450 2C19*17 gain-of-function allele; CABG, coronary artery bypass grafting; Impaired LV function, Left ventricular ejection fraction <55%; CCS, Canadian Cardiovascular Society; PCI, percutaneous coronary intervention; Data are expressed as mean value ± SD or number of patients (percentage). P, one-way ANOVA, or chi² between PONI Q192R genotypes.
Table 2. ADP-induced Platelet Reactivity and Inhibition of Platelet Aggregation According to PON1 Q192R Genotype

<table>
<thead>
<tr>
<th>PON1 Q192R Genotype</th>
<th>QQ192 (Interquartile range)</th>
<th>QR192 (Interquartile range)</th>
<th>RR192 (Interquartile range)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet reactivity by light transmission aggregometry (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPA ADP 5 μmol/L</td>
<td>46.0 (35.0 - 57.8)</td>
<td>46.0 (37.0 - 59.0)</td>
<td>49.0 (40.0 - 55.8)</td>
<td>0.571</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before PCI</td>
<td>14.0 (4.0 - 33.8)</td>
<td>13.0 (4.0 - 28.8)</td>
<td>16.0 (3.0 - 36.0)</td>
<td>0.353</td>
</tr>
<tr>
<td>Pre-discharge</td>
<td>8.0 (3.0 - 17.0)</td>
<td>8.0 (3.0 - 15.0)</td>
<td>11.0 (3.0 - 18.0)</td>
<td>0.603</td>
</tr>
<tr>
<td>Max. aggregation ADP 5 μmol/L</td>
<td>56.0 (47.0 - 64.0)</td>
<td>56.0 (49.0 - 65.0)</td>
<td>58.5 (51.5 - 62.0)</td>
<td>0.203</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before PCI</td>
<td>38.0 (27.0 - 50.0)</td>
<td>37.0 (27.0 - 47.0)</td>
<td>40.0 (30.0 - 48.0)</td>
<td>0.563</td>
</tr>
<tr>
<td>Pre-discharge</td>
<td>28.0 (20.0 - 37.0)</td>
<td>27.0 (20.0 - 35.8)</td>
<td>30.0 (22.0 - 37.0)</td>
<td>0.777</td>
</tr>
<tr>
<td>RPA ADP 20 μmol/L</td>
<td>72.0 (62.0 - 80.0)</td>
<td>74.0 (66.0 - 84.0)</td>
<td>74.0 (69.3 - 81.0)</td>
<td>0.029</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before PCI</td>
<td>40.5 (15.5 - 60.8)</td>
<td>38.0 (14.0 - 55.8)</td>
<td>42.5 (20.0 - 61.0)</td>
<td>0.506</td>
</tr>
<tr>
<td>Pre-discharge</td>
<td>27.0 (8.0 - 42.0)</td>
<td>21.0 (9.0 - 41.0)</td>
<td>25.0 (11.3 - 44.3)</td>
<td>0.573</td>
</tr>
<tr>
<td>Max. aggregation ADP 20 μmol/L</td>
<td>74.0 (68.0 - 80.0)</td>
<td>78.0 (69.0 - 82.0)</td>
<td>74.0 (70.0 - 81.0)</td>
<td>0.036</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before PCI</td>
<td>54.5 (42.0 - 66.0)</td>
<td>54.0 (43.3 - 64.8)</td>
<td>56.0 (44.0 - 63.8)</td>
<td>0.728</td>
</tr>
<tr>
<td>Pre-discharge</td>
<td>44.0 (34.0 - 54.0)</td>
<td>43.0 (33.0 - 54.0)</td>
<td>46.5 (36.0 - 55.8)</td>
<td>0.788</td>
</tr>
<tr>
<td>Inhibition of platelet reactivity by light transmission aggregometry (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of RPA ADP 5 μmol/L</td>
<td>65.4 (28.6 - 90.1)</td>
<td>71.3 (35.8 - 90.3)</td>
<td>64.4 (25.5 - 92.2)</td>
<td>0.178</td>
</tr>
<tr>
<td>Before PCI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-discharge</td>
<td>81.5 (62.9 - 92.5)</td>
<td>82.7 (64.3 - 93.5)</td>
<td>78.9 (60.1 - 91.2)</td>
<td>0.457</td>
</tr>
<tr>
<td>Inhibition of maximal aggregation ADP 5 μmol/L</td>
<td>29.8 (10.4 - 50.8)</td>
<td>34.7 (16.0 - 50.8)</td>
<td>34.2 (12.2 - 45.1)</td>
<td>0.173</td>
</tr>
<tr>
<td>Before PCI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-discharge</td>
<td>47.7 (32.1 - 64.4)</td>
<td>50.7 (35.0 - 65.5)</td>
<td>49.7 (32.7 - 65.3)</td>
<td>0.337</td>
</tr>
<tr>
<td>Inhibition of RPA ADP 20 μmol/L</td>
<td>41.8 (17.8 - 80.1)</td>
<td>46.7 (26.6 - 80.7)</td>
<td>39.8 (23.3 - 73.2)</td>
<td>0.326</td>
</tr>
<tr>
<td>Before PCI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-discharge</td>
<td>67.7 (43.1 - 88.9)</td>
<td>71.6 (45.1 - 87.4)</td>
<td>64.8 (42.8 - 86.1)</td>
<td>0.522</td>
</tr>
<tr>
<td>Inhibition of maximal aggregation ADP 20 μmol/L</td>
<td>25.0 (9.7 - 42.2)</td>
<td>28.9 (13.9 - 43.4)</td>
<td>28.1 (17.4 - 39.3)</td>
<td>0.142</td>
</tr>
<tr>
<td>Before PCI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-discharge</td>
<td>40.0 (26.0 - 53.2)</td>
<td>43.0 (28.4 - 56.7)</td>
<td>38.0 (26.0 - 55.4)</td>
<td>0.382</td>
</tr>
</tbody>
</table>

RPA, residual platelet aggregation; ADP, adenosine diphosphate; Data are expressed as median (interquartile range). P by linear regression analysis between PON1 QQ192 [n=384], QR192 [n=304] and RR192 [n=72] genotype.
Table 3. Multivariable Regression Model for RPA After Stimulation With 5 μmol/L ADP at discharge.

<table>
<thead>
<tr>
<th></th>
<th>Partial $\eta^2$</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 QQ192/QR192</td>
<td>&lt;0.001</td>
<td>0.728</td>
</tr>
<tr>
<td>CYP2C19*2 LoF carrier status</td>
<td>0.045</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CYP2C19*17 GoF carrier status</td>
<td>0.012</td>
<td>0.004</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.007</td>
<td>0.024</td>
</tr>
<tr>
<td>Women</td>
<td>0.001</td>
<td>0.544</td>
</tr>
<tr>
<td>Time from loading dose to PCI (h)</td>
<td>&lt;0.001</td>
<td>0.946</td>
</tr>
<tr>
<td>Active smoker</td>
<td>&lt;0.001</td>
<td>0.781</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>0.002</td>
<td>0.207</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.010</td>
<td>0.008</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>&lt;0.001</td>
<td>0.872</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>0.009</td>
<td>0.01</td>
</tr>
<tr>
<td>Platelets (x10$^9$/L)</td>
<td>0.017</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum creatinine (mmol/L)</td>
<td>&lt;0.001</td>
<td>0.728</td>
</tr>
<tr>
<td>$\beta$-Blockers</td>
<td>&lt;0.001</td>
<td>0.963</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>0.004</td>
<td>0.455</td>
</tr>
<tr>
<td>AT1-antagonists</td>
<td>0.005</td>
<td>0.057</td>
</tr>
<tr>
<td>Diuretics</td>
<td>0.001</td>
<td>0.343</td>
</tr>
<tr>
<td>Nitrates</td>
<td>&lt;0.001</td>
<td>0.742</td>
</tr>
<tr>
<td>Statins</td>
<td>&lt;0.001</td>
<td>0.706</td>
</tr>
<tr>
<td>Verapamil/Diltiazem</td>
<td>0.015</td>
<td>0.001</td>
</tr>
<tr>
<td>Previous balloon angioplasty</td>
<td>0.004</td>
<td>0.090</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>0.001</td>
<td>0.439</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>&lt;0.001</td>
<td>0.648</td>
</tr>
<tr>
<td>Impaired LV function</td>
<td>&lt;0.001</td>
<td>0.629</td>
</tr>
<tr>
<td>CCS Angina class III or IV</td>
<td>0.002</td>
<td>0.286</td>
</tr>
<tr>
<td>Multivessel PCI</td>
<td>0.012</td>
<td>0.004</td>
</tr>
</tbody>
</table>

RPA, Residual platelet reactivity; CYP2C19*2 LoF; cytochrome P450 2C19*2 loss-of-function allele carriage; CYP2C19*17 GoF; cytochrome P450 2C19*17 gain-of-function allele carriage; CABG, coronary artery bypass grafting; Impaired LV function, Left ventricular ejection fraction <55%; CCS, Canadian Cardiovascular Society; PCI, percutaneous coronary intervention.
Figure Legends:

**Figure 1.** Residual platelet aggregation (RPA) at baseline, before PCI and before discharge according to *PON1* Q192R genotype assessed by light transmission aggregometry using ADP 5 μmol/L.

Median with interquartile range; P values by linear regression analysis.

**Figure 2.** Expression of P-selectin and activated GP IIb/IIIa after stimulation with ADP 20 μmol/L according to *PON1* Q192R genotype.

Median with interquartile range; P values by linear regression analysis.
Surface expression of P-selectin

**Baseline**

- QQ192
- QR192
- RR192

Expression, au

- Baseline p=0.267

**At PCI**

- QQ192
- QR192
- RR192

Expression, au

- At PCI p=0.654

**Before Discharge**

- QQ192
- QR192
- RR192

Expression, au

- Before Discharge p=0.621

Surface expression of PAC-1

**Baseline**

- QQ192
- QR192
- RR192

Expression, au

- Baseline p=0.510

**At PCI**

- QQ192
- QR192
- RR192

Expression, au

- At PCI p=0.626

**Before Discharge**

- QQ192
- QR192
- RR192

Expression, au

- Before Discharge p=0.483

*PON-1 Genotype*
Paraoxonase-1 Q192R Polymorphism and Antiplatelet Effects of Clopidogrel in Patients Undergoing Elective Coronary Stent Placement
Dietmar Trenk, Willibald Hochholzer, Martin F. Fromm, Oliver Zolk, Christian M. Valina, Christian Stratz and Franz-Josef Neumann

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