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

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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 affects platelet reactivity in patients undergoing elective coronary stent placement.

Methods and Results—The study included 760 consecutive patients undergoing elective coronary stent placement after loading with clopidogrel 600 mg. Platelet function was assessed by adenosine diphosphate-induced (ADP) and by flow-cytometric analysis of platelet surface protein expression before clopidogrel, at the time of coronary stent placement, and before discharge after coronary stent placement. PON1 Q192R genotype [NM_000446.5:c.575A>G single nucleotide polymorphism (rs662)] was analyzed by TaqMan polymerase chain reaction. Residual platelet aggregation (ADP 5 μmol/L) at predischarge was 8.0% (3.0% to 17.0%) [median (interquartile range)] in PON1 QQ192 patients (n=384), 8.0% (3.0% to 15.0%) in PON1 QR192 (n=304), and 11.0% (3.0% to 18.0%) in PON1 RR192 (n=72; P=0.603). By multivariable linear regression, residual platelet aggregation was not associated with PON1 QQ192/QR192 (partial $\eta^2<0.001$, $P=0.728$) but with CYP2C19*2 loss-of-function allele (partial $\eta^2=0.045$, $P<0.001$) as well as any CYP2C19*17 gain-of-function allele (partial $\eta^2=0.012$, $P=0.004$). 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.

Conclusions—On-treatment platelet reactivity in patients undergoing coronary stent placement after loading with clopidogrel 600 mg was not associated with PON1 Q192R genotype.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00457236.

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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 and for patients undergoing percutaneous coronary intervention (PCI). Several studies have shown a wide interindividual 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 Bonello et al).

Clinical Perspective on p 436

Clopidogrel is an inactive prodrug that is converted by the cytochrome P450 (CYP) system into the active metabolite that binds reversibly to the platelet purinergic P2Y$_{12}$ receptor and thereby inhibits adenosine diphosphate (ADP)-induced platelet activation and 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% to 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 high-density lipoprotein 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 affect 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. Although they could not reproduce the well-established documented relation between CYP 2C19 loss-of-function polymorphisms and outcome,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. This finding, however, was only derived from a small cohort of 112 patients.

To address the question of 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, who had been enrolled in the EXCELSIOR (Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate) study.

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. Briefly, patients undergoing elective coronary stent placement after pretreatment with 600 mg of clopidogrel and aspirin (≥100 mg per day for at least 5 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; chronic oral anticoagulation; thienopyridine criteria were acute myocardial infarction (MI) according to the consensus document of the European Society of Cardiology and the American College of Cardiology; chronic oral anticoagulation; thienopyridine

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, Nümbrecht, Germany). We obtained the second blood sample at the time of catheterization before administration of heparin or contrast medium and a further sample 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 4-channel Bio/Data PAP4 aggregometer (Moebel, Langenfeld, Germany), as previously described. Platelet-rich plasma was prepared by centrifugation of citrated venous blood at 750g for 2 minutes and adjusted to 275 to

325×10^3 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 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×aggregation at baseline×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 antibodies 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 paraformaldehyde 1% was added for fixation. A 4-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 Polymerase Chain Reaction
Blood for genomic DNA extraction was sampled using tubes containing 1.2 to 2 mg potassium-EDTA per mL of blood (Sarstedt AG, Nümbrecht, 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 single nucleotide polymorphisms (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 of 2× Type-It Fast Genotyping Master Mix (contains polymerase chain reaction [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. 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.

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: χ² = 1.932, P = 0.165; CYP2C19*17; χ² = 0.065, P = 0.799).

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. 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 χ² test or Fisher exact test for categorical variables and with 1-way ANOVA for continuous variables. The effect of PON1 Q192R genotypes on platelet effects of clopidogrel determined by ADP-induced aggrega-
For all other statistical analyses, we used the PASW software package, version 18 (SPSS Inc, Chicago, IL). A value of $P<0.05$ in the 2-tailed test was considered as significant.

**Results**

**PON1 Q192R Genotyping Result**

PON1 Q192R genotype could be determined in 760 patients of the whole cohort of 765 patients with platelet function assessed at baseline, before PCI, and before 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 ($\chi^2=1.089; P=0.297$).

**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 for a slightly higher proportion of active smokers in the group of RR192 patients.

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

Flow cytometric analyses of surface protein expression after stimulation of the platelets with ADP $20 \mu$mol/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, body mass index, 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 nonfatal MI did not significantly differ between PON1 QQ192 (11/384; 2.9%), PON1 QR192

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 Median (Interquartile Range)</th>
<th>QR192 Median (Interquartile Range)</th>
<th>RR192 Median (Interquartile Range)</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>Platelet reactivity by light transmission aggregometry, %</td>
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<tr>
<td>RPA ADP $5 \mu$mol/L</td>
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</tr>
<tr>
<td>Baseline</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>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>Before 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>
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<tr>
<td>Max aggregation ADP $5 \mu$mol/L</td>
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<tr>
<td>Baseline</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>Before PCI</td>
<td>38.0 (27.0–50.0)</td>
<td>37.0 (27.0–47.0)</td>
<td>40.0 (30.3–48.0)</td>
<td>0.563</td>
</tr>
<tr>
<td>Before discharge</td>
<td>28.0 (20.0–37.0)</td>
<td>27.0 (20.0–35.8)</td>
<td>30.0 (20.3–37.0)</td>
<td>0.777</td>
</tr>
<tr>
<td>RPA ADP $20 \mu$mol/L</td>
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</tr>
<tr>
<td>Baseline</td>
<td>72.0 (65.0–80.0)</td>
<td>74.0 (66.0–82.0)</td>
<td>74.0 (69.3–81.0)</td>
<td>0.029</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>Before discharge</td>
<td>23.0 (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 $20 \mu$mol/L</td>
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<tr>
<td>Baseline</td>
<td>74.0 (68.0–80.0)</td>
<td>75.0 (69.0–82.0)</td>
<td>74.0 (70.0–81.0)</td>
<td>0.036</td>
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<tr>
<td>Before PCI</td>
<td>54.0 (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>Before 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>
</tbody>
</table>

Inhibition of platelet reactivity by light transmission aggregometry, %

<table>
<thead>
<tr>
<th>PON1 Q192R Genotype</th>
<th>QQ192 Median (Interquartile Range)</th>
<th>QR192 Median (Interquartile Range)</th>
<th>RR192 Median (Interquartile Range)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of RPA ADP $5 \mu$mol/L</td>
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<tr>
<td>Before PCI</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 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 $5 \mu$mol/L</td>
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<tr>
<td>Before PCI</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 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 $20 \mu$mol/L</td>
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</tr>
<tr>
<td>Before PCI</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 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 $20 \mu$mol/L</td>
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</tr>
<tr>
<td>Before PCI</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 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 indicates residual platelet aggregation; ADP, adenosine diphosphate; and PCI, percutaneous coronary intervention. 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.
Compared with RR192 homozygous patients.

95% CI, 0.10 to 1.28; 95% CI, 0.08 to 1.01; thrombosis did not differ between
clopidogrel were found.

death and nonfatal MI did not differ between either the
QQ192 (HR, 0.61; 95% CI, 0.20 to 1.88; RR192 (4/72; 5.6%) genotype
PON1 Q192R polymorphism on the
PON3. The rate of formation of the active metabolite of
clopidogrel was determined by the Q192R polymorphism in
PON1 being approximately 12-fold more efficient than

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charged according to
ADP 5 μmol/L. Values are
Median with interquartile range; probability values by linear
regression analysis.

QR192 (9/304; 3.0%), and PON1 RR192 (4/72; 5.6%) genotype
(P=0.47). In particular, the risk for the combined end point of
death and nonfatal MI did not differ between either the PON1
QQ192 (HR, 0.61; 95% CI, 0.20 to 1.88; P=0.390) or the
QR192 genotype (HR, 0.53; 95% CI, 0.16 to 1.73; P=0.295)
compared with RR192 homozygous patients.

The incidence of stent thrombosis (definite, probable, or
possible according to the ARC criteria) 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 to 1.01; P=0.051) or QR192 carriers (HR, 0.36;
95% CI, 0.10 to 1.28; P=0.116) compared with RR192 patients.

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

Discussions

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
QQ192/QR192 genetic status with platelet function after clopi-
dogrel. 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, body mass index and
platelet count, concomitant treatment with verapamil/diltiazem,
and multisvessel PCI.

Our results are therefore in contrast to data published recently
by Bouman et al. These authors performed in vitro metabolo-
ic 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 identi-
fied 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 metab-
olite 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 et al 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 (ie, on-treatment
platelet reactivity) within the 3 PON1 Q192R genotypes was not
reported, although in a recent consensus report, this was pro-
posed to be a better measure of thrombotic risk than responsive-
ness to clopidogrel. Because of 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

Figure 1. Residual platelet aggregation (RPA) at baseline,
before percutaneous coronary intervention, and before
discharge according to PON1 Q192R genotype assessed by light
transmission aggregometry, using ADP 5 μmol/L. Values are
median with interquartile range; probability values by linear
regression analysis.

Table 1. Platelet Reactivity Before and After Percutaneous
Coronary Intervention.
between the initial clinical event and the latter assessment because the patients investigated were already off regularly 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 end point of all-cause mortality and nonfatal MI 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.\(^{20}\) For the comparison between \( PON1 \) \( QQ192 \) versus RR192, the power to detect an effect size comparable to the study of Bouman et al.\(^{20}\) 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.\(^{20}\). For comparison between QR182 versus RR192, we calculated a power of 0.958 and 0.221, respectively.

**Study Limitations**

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

Because 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 preanalytical 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 end point 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.\(^{34}\) However, clinical efficacy of clopidogrel is hampered by substantial variability in antiplatelet response.\(^{8,9,16,27}\) The combination of \( CYP2C19 \) reduced function status with known clinical risk factors such as diabetes, body mass index, or age contributes no more than 12% to variability in antiplatelet response to
Table 3. Multivariable Regression Model for RPA After Stimulation With 5 μmol/L ADP at Discharge

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial η²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PON1 Q192R/QR192</strong></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, y</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/m²</td>
<td>0.009</td>
<td>0.01</td>
</tr>
<tr>
<td>Platelets, ×10⁹/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>β-blockers</td>
<td>&lt;0.001</td>
<td>0.963</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>0.001</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 indicates 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; PCI, percutaneous coronary intervention; ACE, angiotensin-converting enzyme; AT, angiotensin; CABG, coronary artery bypass grafting; impaired LV function, left ventricular ejection fraction <55%; and CCS, Canadian Cardiovascular Society.

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 et al 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 PON1 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. On the basis of 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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Disclosures
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References
There is strong evidence that the variability in antplatelet response to clopidogrel is associated with an increased risk for ischemic events. A diminished pharmacodynamic response to the inactive prodrug clopidogrel is related to a decreased in vivo formation of the pharmacologically active metabolite. This study reported that the CYP2C19 *17 gain-of-function allele together as well as baseline clinical and demographic variables. Thus, our study does not provide evidence that PON1 genotyping may be useful in tailoring antplatelet treatment with clopidogrel.
Paraoxonase-1 Q192R Polymorphism and Antiplatelet Effects of Clopidogrel in Patients Undergoing Elective Coronary Stent Placement

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