No association of paraoxonase-1 Q192R genotypes with platelet response to clopidogrel and risk of stent thrombosis after coronary stenting

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Aims
In clopidogrel-treated patients undergoing coronary stenting, high on-treatment platelet reactivity was linked to a higher risk of stent thrombosis (ST). Platelet response to clopidogrel is significantly influenced by genetic factors. Recently published findings showed a highly significant impact of a common polymorphism (Q192R) within the paraoxonase-1 (PON1) gene on clopidogrel treatment efficacy but no influence of the CYP2C19*2 genetic variant as previously demonstrated. The aim of this study was to assess the impact of the PON1 Q192R genotype in parallel to that of CYP2C19*2 on the antiplatelet effect of clopidogrel and the risk of ST in clopidogrel-treated patients.

Methods and results
In 1524 patients undergoing percutaneous coronary intervention, ADP-induced platelet aggregation was assessed in relation to PON1 Q192R and CYP2C19*2 genotypes. The clinical impact of genetic variants was investigated by comparing genotype frequencies of both genetic variants in a registry of 127 cases with early ST vs. an early ST-free control cohort (n = 1439). For PON1 Q192R genotypes, platelet aggregation values were similar across all genotype groups (P = 0.65). For CYP2C19*2 genotypes, significantly higher aggregation values were found in CYP2C19 wt/*2 and *2/*2 patients when compared with wt/wt allele carriers (P < 0.0001). Comparing genotype frequencies between ST cases and controls, no differences were observed for PON1 Q192R genotype distributions (P = 0.23), whereas the genotype distribution differed for CYP2C19*2 genotypes (P = 0.019).

Conclusion
The PON1 Q192R genotype did not influence platelet response to clopidogrel or the risk of ST in clopidogrel-treated patients, whereas the CYP2C19*2 genotype impacted on both antiplatelet effect of clopidogrel and risk of coronary ST.

Keywords
Clopidogrel ● Genetics ● Stent thrombosis

Introduction
Simultaneous dual inhibition of blood platelets by the cyclooxygenase-1 inhibitor aspirin as well as by P2Y12 receptor inhibitors such as clopidogrel is the treatment of choice in patients with acute coronary syndromes or those undergoing coronary stenting.1 Major shortcomings of clopidogrel therapy however include its delayed onset of action,2 its large inter-individual response variability,3,4 and the fact that despite adequate treatment with the drug, a significant proportion of patients exhibit a status of high (on-treatment) platelet reactivity (HPR).3,6 Across numerous clinical studies, HPR to clopidogrel was found to be a strong predictor for a higher risk of ischaemic events including the occurrence of stent thrombosis (ST).3,5
The second-generation thienopyridine clopidogrel is a pro-drug that requires enzymatic bioactivation into its active thiol metabolite before interacting with the P2Y12 receptor on blood platelets. Pharmacokinetic, pharmacodynamic as well as genetic studies provided abundant evidence that in vivo bioactivation of clopidogrel is a two-step process that is closely linked to the cytochrome P450 (CYP) system. The isoenzyme CYP2C19 was found to play a key role in this setting by contributing to both clopidogrel bioactivation steps. A common genetic variant within the CYP2C19 gene, the CYP2C19*2 loss-of-function polymorphism, was found to be associated with an attenuated response to clopidogrel and a worse clinical outcome in patients undergoing coronary stenting. The latter association was demonstrated and confirmed across numerous observational studies in a genome-wide association study (GWAS), and in analyses of randomized clinical trials. A recent collaborative meta-analysis (n = 9685 patients) demonstrated a significant association of CYP2C19*2 allele carriage and ischaemic events. This association was uniformly reported across all studies included, and it was most pronounced for the risk of ST with 84 ST cases included in the meta-analysis.

Recently, a different genetic variant (Q192) within the gene encoding for the paraoxonase-1 (PON1) enzyme was described by Bouman et al. to be linked to clopidogrel bioactivation, to the response to clopidogrel treatment, and to the clinical outcome of clopidogrel-treated patients. Against prior observations, the authors identified PON1 as the single key factor for the second step of clopidogrel bioactivation and found no evidence for the involvement of CYP2C19 in any of the steps of clopidogrel metabolism. While showing a significant impact of the PON1 Q192R genotype on the clinical outcome of clopidogrel-treated patients, the authors refuted the established association of CYP2C19*2 allele carriage and ST in clopidogrel-treated patients. Hence, the findings of the latter study question the current concept of clopidogrel bioactivation and cast doubts on a large body of previous genetic association studies.

Therefore, the aim of the present study was to investigate the impact of PON1 Q192R and CYP2C19*2 genotypes on the anti-platelet efficacy of clopidogrel and on the risk of ST in clopidogrel-treated patients undergoing coronary stent placement.

Methods

Study cohorts and study principle

Two study cohorts of patients with percutaneous coronary intervention (PCI) and dual antiplatelet therapy with aspirin and clopidogrel were included in the present analyses. First, a consecutive, prospectively recruited PCI cohort of 1524 patients enabled us to assess the impact of PON1 Q192R and CYP2C19*2 genotypes on platelet aggregation after clopidogrel treatment; second, the addition of a registry of 127 cases of early ST enabled us to assess the impact of PON1 Q192R and CYP2C19*2 genotypes on the risk for this complication. Genotyping for and assessment of the role of CYP2C19*2 parallel to PON1 Q192R were performed with the intention of using it as a control bearing in mind available evidence on the association of CYP2C19*2 allele carriage with clopidogrel treatment efficacy.

All platelet aggregation measurements were undertaken by laboratory personnel who were unaware of patients’ outcome and genotyping results. Concordantly, genotyping was performed by laboratory personnel who were unaware of patients’ outcome and platelet aggregation results. The study complies with the Declaration of Helsinki and was approved by the local Ethics Committee. All patients gave written informed consent prior to study inclusion.

Prospective percutaneous coronary intervention cohort

This cohort was recruited between February 2007 through April 2008 at the Deutsches Herzzentrum München (Munich, Germany) in the setting of a prospective trial including 1608 patients with platelet function testing during the coronary intervention that aimed to assess the association of HPR and ST risk. For the present study, blood for genotyping was available in 1524 patients (95%) of this cohort. A sensitivity analysis showed that patients without available DNA (n = 84) did not differ from those with available DNA (n = 1524) with respect to age, clinical presentation, and platelet aggregation measurements (P > 0.16). The design of the primary trial and details of the study population under investigation here have been described in detail previously. Both genotyping for PON1/CYP2C19*2 and inclusion of the group with ST were primarily done for this specific study. All patients included in this study were pre-treated with a loading dose of 600 mg of clopidogrel prior to the procedure. The recommended pre-treatment interval was ≥ 2 h. Percutaneous coronary intervention was performed according to current standard guidelines. Exclusion criteria were contraindications to aspirin or clopidogrel treatment and prior treatment with GP IIb/IIIa inhibitors during the 10 days before the PCI.

Stent thrombosis registry and control group

A registry of cases with early definite ST was also included in this study. A total of 127 definite ST occurring within 30 days after stenting were part of this registry and cases included were without apparent discontinuation of clopidogrel prior to the event. All of them were recruited consecutively between January 1999 and April 2008. During this time period, a total of 130 ST cases were screened by means of clinical follow-up and in 127 of them, DNA for genotyping was available. Definite ST was defined according to the academic research consortium criteria. For comparing genotype distributions between ST cases and event-free controls, the respective control group was formed from the prospective PCI cohort mentioned above (n = 1524) after excluding patients who only received plain balloon angioplasty without stent (n = 75) and those who incurred early definite ST (n = 10). Thus, a total of 1439 patients were included in this control cohort.

Blood sampling and genotyping

Blood for genomic DNA extraction and genotyping was taken from the arterial sheath of all patients directly prior to PCI. DNA was extracted from 200 μL of blood using commercially available kits (NucleoSpin Blood Quick Pure, Macherey-Nagel, Germany) according to the manufacturer’s instructions. Genotypes were determined with a TaqMan assay using an ABI Prism Sequence Detector 7000 (Applied Biosystems) according to standard protocols. Primers 5′-ACCCTGACACTTTTTTA GGCCACAA-3′ and 5′-ACCCACTGAACTTACCTGACATATG-3′ were used to amplify the sequence of the PON1 gene containing the Q192R polymorphism (rs662) in exon 6 of the gene. The sequence of the A allele-specific probe was 5′-FAM-CCTACTTACATCCTG-3′ and the sequence of the G allele-specific probe was 5′-CTACTTACC GTTCCTG-3′. Primers 5′-GATATGGCATTATTTTCCCACTAT CATTG-3′ and 5′-GGTGTTCCTTACTTCTCCCAAATATCAC-3′.

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were used to amplify a sequence of the CYP2C19 gene containing the single nucleotide polymorphism 681G>A (rs4244285, *2) in exon 5. The sequence of the G allele-specific probe was 5’-FAM-TTATTTCCGGGAAACC-3’ and the sequence of the A allele-specific probe was 5’-VIC-ATTATTTCCGGGAAACC-3’. To control for correct sample handling, genotyping was repeated in 20% of the patients for both variants investigated. Repeated genotyping revealed identical results, and the call rate for both PON1 Q192R and CYP2C19*2 SNPs was 100%.

**Platelet function testing**

For platelet function testing on the Multiplate analyser using multiple electrode aggregometry (MEA), whole blood was obtained from the arterial sheath of all patients directly before PCI and prior to the administration of any anticoagulant/antiplatelet treatment in the cath lab. Blood was placed in 4.5 mL plastic tubes containing the anticoagulant lepirudin (25 μg/mL, Refludan, Dynabyte, Munich, Germany). The ADP(6.4 μM)-induced platelet aggregation was assessed. Details of this method have been reported previously.6,24

Aggregation measured with MEA is quantified as area under the curve (AUC = AU × min) of aggregation units (AU). All material used for platelet function testing was obtained from the manufacturer.

**Statistical analysis**

Variables are presented as mean ± standard deviation (SD), counts (percentages), or median with interquartile range (IQR). Categorical variables were compared using the χ² test. The Kolmogorov–Smirnov test was used to check for normal distribution of continuous data. Normally distributed continuous data were compared across two groups with the two-sided unpaired t-test and for genotype group comparisons with the one-way analysis of variance test. Non-normally distributed continuous data such as those related to platelet function across genotype groups were compared using the Wilcoxon test and genotype group comparisons with the Kruskal–Wallis test. In the prospective PCI cohort, we tested for a possible deviation of PON1 Q192R and CYP2C19*2 genotype distribution from Hardy–Weinberg equilibrium. The Smirnov test was used to check for normal distribution of continuous variables displayed in Table 1 in addition to PON1 Q192R and CYP2C19*2 genotypes, CYP2C19*2 (P < 0.0001) significantly correlated with ADP-induced platelet aggregation was significantly different across genotype groups (P < 0.0001). In the 377 patients, who were carriers of at least one *2 allele (wt/*2 or *2/*2), ADP-induced platelet aggregation was significantly higher when compared with the remaining patients (n = 1147): [286 (186–460) AU × min vs. 208 (134–329) AU × min, respectively; P < 0.0001].

In the multivariable linear regression analysis including all variables displayed in Table 1 in addition to PON1 Q192R and CYP2C19*2 genotypes, CYP2C19*2 (P < 0.001) but not PON1 Q192R (P = 0.76) significantly correlated with ADP-induced platelet aggregation.

**PON1 Q1922R and CYP2C19*2 genotypes and stent thrombosis**

PON1 Q1922R and CYP2C19*2 genotypes were determined in 127 ST cases and 1439 ST-free controls. Table 2 contains main characteristics of these two groups. Figure 3 shows the PON1 Q192R genotype distribution in cases vs. controls. The genotype distribution did not differ between cases and controls (P = 0.23). Among the 127 ST cases, 121 patients (95%) were carriers of at least one Q192R allele (45 ST cases in QR192 patients and 76 ST cases in QQ192 patients), whereas six patients (5%) were RR192 homozygous carriers. This was not significantly different from the distribution observed in the control group (92 vs. 8%, respectively; P = 0.20). Figure 4 shows the CYP2C19*2 genotype distribution in cases vs. controls. The genotype distribution was significantly different between cases and controls (P = 0.019). Among the 127 ST cases, 46 patients (36%) were carriers of at

**Results**

**PON1 Q1922R and CYP2C19*2 genotypes and platelet function**

Baseline characteristics of the cohort of 1524 PCI patients are summarized in Table 1 according to the PON1 Q192R and CYP2C19*2 genotypes. Clinical variables were well balanced between the three groups of genotypes for both allelic variants studied. Of the 1524 patients included in this study, 812 (53.3%) were carriers of the QQ192 genotype, 593 (38.9%) were heterozygous allele carriers (QR192), and 119 patients (7.8%) were homozygous RR192 genotype carriers. For the CYP2C19*2 allele, 1147 (75.3%) were wild-type homozygous for the *2 allele variant (wt/wt), 345 (22.6%) were heterozygous *2 allele carriers (wt/*2), and 32 patients (2.1%) were homozygous *2 allele carriers (*2/*2). For both genotype distributions, no significant deviations from Hardy–Weinberg equilibrium were observed (P = 0.46 for PON1 Q192R and P = 0.31 for CYP2C19*2 genotypes).

The median (IQR) value of ADP-induced platelet aggregation in the study population was 226 (141–364) AU × min. The ADP-induced platelet aggregation values across PON1 Q192R genotypes were as follows: 230 (141–346) AU × min for PON1 RR192 patients, 218 (142–346) AU × min for PON1 QR192 patients, and 233 (141–381) AU × min for PON1 QQ192 patients. As demonstrated in Figure 1, the ADP-induced platelet aggregation did not differ across genotype groups (P = 0.65). In the 1405 patients, who were carriers of at least one Q allele (QR192 or QQ192), ADP-induced platelet aggregation was similar and numerically lower when compared with the remaining patients (n = 119): [225 (141–367) AU × min vs. 230 (141–364) AU × min, respectively; P = 0.94].

The ADP-induced platelet aggregation values across CYP2C19*2 genotypes were as follows: 208 (134–329) AU × min for CYP2C19 wt/wt patients, 267 (175–428) AU × min for CYP2C19 wt/*2 patients, and 494 (341–732) AU × min for CYP2C19 *2/*2 patients. As demonstrated in Figure 2, the ADP-induced platelet aggregation was significantly different across genotype groups (P < 0.0001). Among the 127 patients, who were carriers of at least one *2 allele (wt/*2 or *2/*2), ADP-induced platelet aggregation was significantly higher when compared with the remaining patients (n = 1147): [286 (186–460) AU × min vs. 208 (134–329) AU × min, respectively; P < 0.0001].
### Table 1  Baseline characteristics of the PCI cohort according to PON1 Q192R and CYP2C19*2 genotypes

<table>
<thead>
<tr>
<th>Variable</th>
<th>PON1 QQ192 (n = 812)</th>
<th>PON1 QR192 (n = 593)</th>
<th>PON1 RR192 (n = 119)</th>
<th>PON1 RR192 (n = 1119)</th>
<th>PON1 RR192 (n = 1147)</th>
<th>PON1 RR192 (n = 345)</th>
<th>PON1 RR192 (n = 32)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.9 ± 10.5</td>
<td>66.7 ± 10.7</td>
<td>67.7 ± 9.9</td>
<td>0.13</td>
<td>67.2 ± 10.7</td>
<td>68.4 ± 10.1</td>
<td>64.8 ± 10.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Woman, n (%)</td>
<td>186 (22.9)</td>
<td>130 (21.9)</td>
<td>28 (23.5)</td>
<td>0.88</td>
<td>261 (22.8)</td>
<td>71 (20.6)</td>
<td>12 (37.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.0 (24.8–29.9)</td>
<td>26.8 (24.6–29.8)</td>
<td>27.5 (25.5–30.3)</td>
<td>0.20</td>
<td>26.9 (24.7–30.0)</td>
<td>27.0 (24.8–29.8)</td>
<td>27.7 (24.1–31.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.6</td>
<td>1.1 ± 0.5</td>
<td>0.47</td>
<td>1.0 ± 0.5</td>
<td>1.1 ± 0.4</td>
<td>1.0 ± 0.4</td>
<td>0.41</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>222 (27.3)</td>
<td>173 (29.2)</td>
<td>35 (29.4)</td>
<td>0.72</td>
<td>326 (28.4)</td>
<td>95 (27.5)</td>
<td>9 (28.1)</td>
<td>0.95</td>
</tr>
<tr>
<td>Active smokers, n (%)</td>
<td>95 (11.7)</td>
<td>94 (15.9)</td>
<td>18 (15.1)</td>
<td>0.07</td>
<td>163 (14.2)</td>
<td>43 (12.5)</td>
<td>1 (3.1)</td>
<td>0.15</td>
</tr>
<tr>
<td>Arterial hypertension, n (%)</td>
<td>734 (90.4)</td>
<td>548 (92.4)</td>
<td>110 (92.4)</td>
<td>0.38</td>
<td>1049 (91.5)</td>
<td>315 (91.3)</td>
<td>28 (87.5)</td>
<td>0.74</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>552 (68.0)</td>
<td>425 (71.7)</td>
<td>91 (76.5)</td>
<td>0.09</td>
<td>805 (70.2)</td>
<td>241 (69.9)</td>
<td>22 (68.8)</td>
<td>0.98</td>
</tr>
<tr>
<td>Multisvessel disease, n (%)</td>
<td>694 (85.5)</td>
<td>503 (84.8)</td>
<td>95 (79.8)</td>
<td>0.28</td>
<td>977 (85.2)</td>
<td>288 (83.5)</td>
<td>27 (84.4)</td>
<td>0.74</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>269 (33.1)</td>
<td>187 (31.5)</td>
<td>30 (25.2)</td>
<td>0.22</td>
<td>355 (31.0)</td>
<td>121 (35.1)</td>
<td>10 (31.3)</td>
<td>0.35</td>
</tr>
<tr>
<td>Prior bypass surgery, n (%)</td>
<td>109 (13.4)</td>
<td>96 (16.2)</td>
<td>18 (15.1)</td>
<td>0.35</td>
<td>173 (15.1)</td>
<td>49 (14.2)</td>
<td>1 (3.1)</td>
<td>0.16</td>
</tr>
<tr>
<td>Platelet count, × 10^3/µL</td>
<td>218.3 ± 65.2</td>
<td>220.4 ± 70.0</td>
<td>206.4 ± 45.4</td>
<td>0.19</td>
<td>216.8 ± 62.1</td>
<td>221.1 ± 77.4</td>
<td>235.5 ± 66.6</td>
<td>0.40</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>54.2 ± 11.4</td>
<td>55.1 ± 10.9</td>
<td>56.0 ± 10.0</td>
<td>0.15</td>
<td>54.9 ± 10.9</td>
<td>54.2 ± 11.8</td>
<td>54.8 ± 10.0</td>
<td>0.81</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>50.9 ± 15.2</td>
<td>51.7 ± 15.0</td>
<td>51.1 ± 15.2</td>
<td>0.62</td>
<td>51.1 ± 15.1</td>
<td>52.1 ± 15.0</td>
<td>46.5 ± 14.3</td>
<td>0.14</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>100.0 ± 37.0</td>
<td>100.3 ± 39.7</td>
<td>101.7 ± 35.6</td>
<td>0.96</td>
<td>99.6 ± 37.6</td>
<td>102.9 ± 39.6</td>
<td>93.6 ± 27.9</td>
<td>0.25</td>
</tr>
<tr>
<td>PPI at admission</td>
<td>136 (16.7)</td>
<td>112 (18.9)</td>
<td>28 (23.5)</td>
<td>0.16</td>
<td>213 (18.6)</td>
<td>56 (16.2)</td>
<td>7 (21.8)</td>
<td>0.52</td>
</tr>
<tr>
<td>CCB at admission</td>
<td>111 (13.7)</td>
<td>90 (15.2)</td>
<td>17 (14.3)</td>
<td>0.72</td>
<td>170 (14.8)</td>
<td>43 (12.5)</td>
<td>5 (15.6)</td>
<td>0.53</td>
</tr>
<tr>
<td>Statin at admission</td>
<td>559 (68.8)</td>
<td>418 (70.5)</td>
<td>85 (71.4)</td>
<td>0.73</td>
<td>806 (70.3)</td>
<td>236 (68.4)</td>
<td>20 (62.5)</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Baseline characteristics in relation to PON1 QR192 and CYP2C19*2 genotypes are shown. CCB, calcium channel blocker; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; PPI, proton pump inhibitor.

**Figure 1** PON1 Q192R genotypes and platelet aggregation. Box-plot analyses showing the ADP-induced platelet aggregation (in AU × min) in relation to Q192R genotypes (QQ192, QR192, RR192). Boxes indicate 25th and 75th percentiles and whiskers denote 10th and 90th percentiles. ADP denotes adenosine diphosphate.
least one CYP2C19*2 allele (43 ST cases in wt/*2 patients and three ST cases in *2/*2 patients), whereas 81 patients (64%) were CYP2C19 wt/wt allele carriers. This was significantly different from the distribution observed in the control group (25 vs. 75%, respectively; \( P = 0.006 \)).

The results of a multivariable logistic regression model that assumed a co-dominant allele effect and that adjusted for all variables (Table 2) that differed between ST cases and controls \( (P < 0.10) \) demonstrated that the PON1 Q192R genotype did not significantly correlate with ST \( (P = 0.22) \). In contrast, the CYP2C19*2 genotype correlated significantly \( (P = 0.03) \) with the risk of this complication with an adjusted odds ratio of 2.27 (95% CI, 1.08–4.74) for CYP2C19 *2/*2 vs. CYP2C19 wt/wt. In this model, the odds ratio per one allele carriage was 1.51 (95%
CI, 1.04–2.18) for CYP2C19*2 and 1.24 (95% CI, 0.88–1.75) for PON1 Q192R. Detailed results of the multivariable logistic regression analysis are shown in Table 3.

**Discussion**

In the present study, we assessed in parallel a possible influence of PON1 Q192R and CYP2C19*2 genotypes on the clopidogrel antiplatelet effect as well as the risk for ST in patients undergoing PCI.

Key results of our study can be summarized as follows: First, only CYP2C19*2 influences the ADP-induced platelet aggregation in clopidogrel-treated patients, whereas no influence on aggregation measurements was observed for PON1 Q192R genotypes. Indeed, median aggregation values were virtually identical across PON1 genotype groups and even numerically lower in Q192 allele carriers. In contrast, we found a strong gene-dose effect for CYP2C19*2 allele carriage on platelet aggregation values. Second, only CYP2C19*2 but not the PON1 Q192R genotype was found to be associated with a higher risk for ST. Thus, on both a mechanistic level of aggregation measurements and also in terms of clinical outcome results, our observations are in contrast to recently published data. With the results reported here, we can confirm the previously indicated key role of CYP2C19*2 on clopidogrel treatment efficacy, and refute a significant impact of the PON1 QR192 genotype in this regard.

The strengths of the present study include the comparatively large cohort of patients with platelet function measurements available as well as the approach of testing the influence of two SNPs on platelet aggregation in parallel. Bearing in mind that numerous studies reported on an association of CYP2C19*2 allele carriage with higher ADP-induced platelet aggregation values, CYP2C19*2—in the present setting—may be thought to serve as a ‘positive control’ to confirm or refute the impact of other variants under investigation. On the level of platelet aggregation measurements, an influence of PON1 Q192R seems highly unlikely as we saw no signal of a trend towards higher values in QR192 or QQ192 patients, while we did find a strong gene-dose effect for CYP2C19*2 allele carriage with the highest aggregation values observed in homozygous allele carriers. Importantly, the platelet function assay used for testing (Multiplate analyser) was shown to be reliable and reproducible.

![Figure 3](https://example.com/f3.png)  **Figure 3** PON1 Q192R genotype frequencies in cases vs. controls. The PON1 Q192R genotypes are shown for ST cases (n = 127) and event-free controls (n = 1439). Genotype distribution was similar between cases vs. controls (P-value calculated with χ² test). PON1 denotes paraoxonase-1 and ST denotes stent thrombosis.

![Figure 4](https://example.com/f4.png)  **Figure 4** CYP2C19*2 genotype frequencies in cases vs. controls. The CYP2C19*2 genotypes are shown for ST cases (n = 127) and event-free controls (n = 1439). Genotype distribution was significantly different between cases vs. controls (P-value calculated with χ² test). ST denotes stent thrombosis.

| Table 3 | Results of a multivariable logistic regression model on predictors of stent thrombosis |
|---|---|---|
| Variable | Odds ratio (95% CI) | P |
| PON1 QQ192 vs. PON1 RR192 | 1.53 (0.77–3.05) | 0.22 |
| CYP2C19 *2/*2 vs. CYP2C19 wt/wt | 2.27 (1.08–4.74) | 0.03 |
| Diabetes mellitus | 2.38 (1.55–3.65) | < 0.001 |
| Smoking | 1.87 (1.15–3.04) | 0.01 |
| Arterial hypertension | 0.39 (0.23–0.68) | < 0.001 |
| Hypercholesterolaemia | 0.88 (0.57–1.37) | 0.58 |
| Previous myocardial infarction | 1.93 (1.25–2.98) | 0.003 |
| STEMI at admission | 12.69 (6.97–23.10) | < 0.001 |
| Platelet count (per 10 × 10³/μL increase) | 1.06 (1.03–1.08) | < 0.001 |
| Serum creatinine (per 0.1 mg/dL increase) | 1.03 (1.00–1.07) | 0.02 |
| Ejection fraction (per 10% decrease) | 1.23 (1.05–1.44) | 0.01 |
| Stent location in LAD | 1.72 (1.14–2.59) | 0.01 |
| AHA/ACC type B2/C lesion | 1.74 (0.98–3.10) | 0.06 |
| Number of stents per lesion | 1.32 (0.93–1.86) | 0.12 |

LAD, left anterior descending coronary artery; MI, myocardial infarction; STEMI, ST-elevation MI. Unadjusted odds ratios (OR) for CYP2C19*2/*2 vs. CYP2C19 wt/wt: OR 2.25, 95% CI 1.17–4.32, P = 0.015; for PON1 QQ192 vs. PON1 RR192: OR 1.69, 95% CI 0.92–3.11, P = 0.09.
previously to be highly capable of detecting the impact of genetic and non-genetic markers on platelet aggregation in clopidogrel-treated patients.10,25,26

Concerning the clinical outcome of clopidogrel-treated patients undergoing coronary stenting, our results reported here are in line with a number of prior studies9–17,19–22 and confirm the central role of CYP2C19*2 as a genetic risk marker of ST. Correspondingly, the CYP2C19 locus located on chromosome 10 was found to be the only locus that was associated with clopidogrel treatment efficacy in a GWAS.9 Importantly, the same GWAS on clopidogrel did not identify any SNPs surrounding or including the PON1 locus to be associated with clopidogrel treatment efficacy at all. Moreover, results of a recently published collaborative meta-analysis17 with data from more than 9000 PCI-treated patients clearly confirmed a significant impact of *2 allele carriage on the clinical outcome of patients. Interestingly, this meta-analysis found the strongest associations of clinical events with *2 allele carriage for the endpoint of ST (based on 84 ST events included in the entire meta-analysis) and for early events after the stenting procedure. This underscores the value of choosing early ST as a clinical endpoint, as it was the case in the present study (including 127 ST events), when it comes to exploring predictors of clopidogrel treatment failure. Results reported here add and extend the knowledge on the role of CYP2C19 in the setting of clopidogrel treatment in patients undergoing coronary stenting. A bulk of evidence exists in support of a relevant role of *2 allele carriage in patients undergoing coronary stenting.9–17,19–22 In contrast, additional information on the risk associated with the *2 allelic variant from post hoc analyses of the CURE and ACTIVE trials has provided conflicting results, showing no impact of *2 on the clinical outcome of patients.27 However, the rate of PCI-treated patients in CURE was low, and it may well be that the negative impact of CYP2C19*2 is confined only to patients treated with coronary stenting.

In line with platelet aggregation measurements that lack an impact of Q192R genotypes, we found no influence of the Q192R genotype with ST risk as well. This is in contrast to the results reported by Bouman et al.18 The precise reason for this discrepancy remains unclear. Human PON1 polymorphisms and especially the PON1 Q192R genotype have attracted considerable attention in recent years as a possible candidate gene for the development and risk of coronary artery disease in numerous genetic association studies.22,28,29 The study by Bouman et al.18 expands the areas of research associated with this variant to clopidogrel bioactivation and drug treatment efficacy. In their study, Bouman et al. compared PON1 Q192R genotype frequencies in ST cases (n = 41) and controls (n = 71) and reported a highly significant difference in genotype distributions between the two groups. Of note, PON1 Q192R genotype distributions markedly differed between the control group in the study of Bouman et al.18 and the control group in our study (35 vs. 53% for QQ192 patients, 47 vs. 39% for QR192 patients, and 18 vs. 8% for RR192 patients, respectively). Thus, differences were most pronounced for the RR192 genotype. However, the genotype distribution in our large control group (n = 1439) is in line with that reported in other studies assessing PON1 Q192R genotype distributions in large cohorts of patients with coronary artery disease.22,28,29

Bearing in mind the poor prognosis of ST following coronary stenting with mortality rates exceeding 40%,20 great efforts are warranted to identify patients with a higher genetic risk that may benefit from more potent P2Y12 receptor inhibitors such as prasugrel or ticagrelor.11,32 Notwithstanding, it seems unlikely that all patients will benefit from more potent antiplatelet drugs as the value of antithrombotic treatment is determined by the balance between the prevention of ischaemic complications and the induction of bleeding33,34 with the latter not surprisingly being a major shortcoming of the more potent agents in general.11,32 The potential risk and poor prognosis34 of bleeding complications in the setting of coronary stenting procedures also underscore the importance of thoroughly testing certain genetic variants for their impact on antiplatelet drug response. Specifically for the results reported here, it seems unlikely that the PON1 Q192R genotype might be a useful risk marker for guiding antiplatelet therapy after stenting. For CYP2C19*2, however, this question can now only be addressed in dedicated randomized trials that randomize *2 allele carriers to an intensified treatment regimen. The Thromboocyte Activity Reassessment and GenoTyping for PCI (TARGET-PCI) trial (ClinicalTrials.gov Identifier: NCT01177592) is currently addressing this issue with a combined approach of both platelet function testing and genotyping. Trials such as GRAVITAS15 and TRIGGER-PCI with the approach of intensifying antiplatelet treatment based on platelet function testing only have been conducted in the past. Whereas the TRIGGER-PCI trial was stopped prematurely due to low event rate occurrence at interim analysis, the GRAVITAS trial failed to show a benefit of an intensified clopidogrel treatment in patients with HPR.

Besides, prior studies have suggested a possible link between positive smoking status and a better response to clopidogrel treatment.36 Here, we observed a higher proportion of smokers in ST cases vs. controls, which argues against a clinical relevance of this presumed association.

Limitations
There are some limitations of this study that merit being mentioned. Here, we only assessed the impact of two genetic variants on clopidogrel treatment efficacy. Further GWASs and candidate gene studies in large cohorts of patients are necessary to search for other new and as-yet unexplored genetic variants that significantly impact on clopidogrel responsiveness. However, the results reported here are only relevant to clopidogrel-treated patients and the introduction of newer antiplatelet agents such as prasugrel or ticagrelor may reduce the overall clinical impact of the present study in the future. In addition, for the mechanistic part of our study, we recorded only platelet aggregation measurements, and we did not assess plasma levels of the active metabolite of clopidogrel, which would have provided more mechanistic insights into the observed platelet response. A further limitation of the present study is that in addition to PON1 genotyping, PON1 enzyme activity was not assessed specifically. Here, platelet function testing was done with only one single device (Multiplate analyser), and we cannot exclude that results may differ with other devices. Finally, analyses reported here are post hoc analyses of study populations that stem from a prospective trial and a
registry of consecutively recruited ST cases; therefore, it is subject to the limitations inherent to all such analyses.

Conclusions

The PON1 Q192R genotype did not influence platelet response to clopidogrel or the risk of ST in clopidogrel-treated patients. In contrast, CYP2C19*2 genotype impacted on both antiplatelet effect of clopidogrel and risk of coronary ST.

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Conflict of interest:

D.S. reported receiving speaker fees from Dynabase and The Medicines Company and fees for advisory board activities from Eli Lilly and Astra Zeneca. A.K. reported receiving speaker fees from Eli Lilly, Daiichi Sankyo, Brystol-Myers Squibb, and Astra Zeneca.

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