Unraveling the Pharmacogenetics of Clopidogrel
The Paraoxonase-1 Controversy

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Clopidogrel forms a cornerstone in the treatment of patients undergoing coronary stenting. Although the clinical effectiveness of clopidogrel has been shown repeatedly in large clinical trials, there appears to be a large interindividual response variability that influences the risk of atherothrombotic events. Studies have shown that differences in the plasma concentration of the active metabolite of clopidogrel are an important determinant of its antiplatelet effect. Although several environmental factors influence the formation of the active metabolite, most variation appears to be genetic. Numerous studies have shown that polymorphisms in genes encoding the cytochrome (CYP) p450 system, especially CYP2C19, influence bioactivation of clopidogrel and, hence, the risk of cardiovascular events, especially stent thrombosis. Still, polymorphic variation in CYP2C19 seems to explain only 5% to 11% of the response variability to clopidogrel, leaving variation in bioactivation of clopidogrel largely unexplained.

A new player in the pharmacogenetics of clopidogrel was introduced by Bouman et al, who identified paraoxonase-1 (PON1) as a major determinant of the bioactivation and clinical efficacy of clopidogrel in a series of elegant experiments and clinical studies among subjects from European descent. This hepatic esterase is associated with high-density lipoprotein and has antioxidative effects on low-density lipoprotein and macrophages. Bouman et al identified PON1 as the crucial enzyme for the bioactivation of clopidogrel, with its common Q192R polymorphism determining the rate of active thiol metabolite formation. In a case-cohort study among 41 cases with stent thrombosis and 71 control subjects, the Q allele was dose-dependently associated with lower PON1 activity and active metabolite concentrations in plasma, reduced platelet inhibition, and a higher risk of stent thrombosis. Q192R explained >70% of the response variability to clopidogrel. The authors corroborated their findings in a cohort of 1982 subjects with acute coronary syndrome, in whom the Q allele was also associated with an increased risk of stent thrombosis and, to a lesser extent, other atherothrombotic end points. Remarkably, there was no clear effect of CYP2C19 on the various end points, although, on close scrutiny, all point estimates indicate decreased effects of clopidogrel in subjects with the loss-of-function variant. Furthermore, although the Q192R polymorphism is common and had large effects in the study of Bouman et al, this polymorphism was not associated with platelet inhibition by clopidogrel in a recent genome-wide quantitative trait analysis among Amish persons.

After this landmark publication, several studies, also mainly among subjects of European descent have rapidly been published. In these studies, the PON1 polymorphism was not associated with platelet inhibition or on-clopidogrel platelet reactivity and clinical end points, whereas in all of those studies, the effects of CYP2C19 were confirmed. Those studies are corroborated and further extended by the work of Hulot et al, published in this issue of Circulation: Cardiovascular Interventions. In 2 well-designed studies, they assessed the effects of 2 PON1 polymorphisms, Q192R and L55M. The effects of L55M on clopidogrel efficacy were not previously studied. In the study of Bouman et al, this genotype was not associated with bioactivation of clopidogrel and, therefore, was not evaluated in further detail. First, Hulot et al studied the pharmacokinetic and pharmacodynamic effects of clopidogrel in the CLOpidogrel and response Variability Investigation Study 2 (CLOVIS-2), a randomized crossover trial among 106 young patients after myocardial infarction; these patients received a loading dose of either 300 or 900 mg of clopidogrel. The authors meticulously assessed associations between PON1 polymorphisms and clopidogrel active metabolite formation over 6 hours, platelet inhibition with ADP-induced light transmission aggregometry, and the VerifyNow P2Y12 point-of-care assay. Second, in the Appraisal of risk Factors in young Ischemic patients Justifying aggressive Intervention registry (AFIJII) registry (a cohort of 371 stable young patients who survived a myocardial infarction and were treated with a clopidogrel maintenance dose of 75 mg/d), they evaluated the relation between these polymorphisms and on-clopidogrel platelet reactivity (VerifyNow P2Y12 assay) and major adverse cardiac events. The Q192R polymorphism was not associated with active metabolite formation or platelet inhibition. There was a trend for a reduction of active metabolite formation after loading with 300 mg in PON1 55MM individuals (which is associated with less PON1 activity), but this did not translate into a reduction of platelet inhibition. Furthermore, in their second study, Q192R was not associated with platelet function and clinical events. If anything, subjects with the QQ genotype had an even lower risk of cardiovascular events (QQ, 8.3%; QR,
clinical end points. Except for the study by Bouman et al,9 metabolite isomer H4 formation, platelet inhibition, and study of the effects of PON1 Q192R and L55M on active CYP2C19 were corroborated.

This conclusion, this article does not support a major role of PON1 inhibition, and a higher risk of cardiovascular events. In contrast, the CYP2C19*2 loss-of-function variant was clearly associated with less active metabolite formation, less platelet reactivity, strictly at 6 hours after loading with clopidogrel. Although on-treatment platelet reactivity might be more helpful in clinical practice, given the large pretreatment variability,15 platelet inhibition is more informative in cases than in controls (17 versus 5), although the causal effect may be underestimated. Also, the association between PON1 activity and cardiovascular events and 18 stent thromboses occurred.

The authors should be applauded for their comprehensive study of the effects of PON1 Q192R and L55M on active metabolite isomer H4 formation, platelet inhibition, and clinical end points. Except for the study by Bouman et al,9 other studies10–13 did not assess active metabolite formation. Another strong feature of the CLOVIS-2 trial is that the authors quantified platelet inhibition rather than on-treatment platelet reactivity, strictly at 6 hours after loading with clopidogrel. Although on-treatment platelet reactivity might be more helpful in clinical practice, given the large pretreatment variability,15 platelet inhibition is more informative in pharmacodynamic studies. The most important limitation of the present studies is their limited sample size, especially regarding clinical end points: only 35 major adverse cardiac events and 18 stent thromboses occurred.

Given the study of Hulot et al14 and other studies10–13 that could not replicate the findings of Bouman et al,9 the crucial question is whether PON1 and polymorphic variation in its coding gene play a role in clopidogrel bioactivation.

There are several differences between the various studies previously published (Table 1 and Table 2), some of which will be mentioned. First, the used study designs are different. Bouman et al9 studied pharmacokinetics, pharmacodynamics, and the relation with stent thrombosis in a case-cohort study. Especially with rare outcomes, such as stent thrombosis, this elegant study design prevents measurements in many unnecessary control subjects without losing much power. Because genetic polymorphisms are allocated randomly at conception, there is no confounding. However, because blood samples were acquired after 18 months of follow-up in the present study, the investigators selected surviving cases and control subjects, which could introduce a selection bias. Of 92 subjects with stent thrombosis and 140 control subjects, eventually only 40 and 72 subjects, respectively, could participate in the case-cohort study. It is not likely that selection bias influenced the pharmacokinetic and pharmacodynamic studies: although the genotype frequencies are influenced (Table 1), the effect of the polymorphism on in vitro experiments is not likely affected. However, it could have introduced a spurious association with stent thrombosis because nonparticipation as the result of mortality was more frequent in cases than in controls (17 versus 5), although the causal effect may be underestimated.

### Table 1. Characteristics of Pharmacokinetic and Pharmacodynamic Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Clopidogrel Use</th>
<th>No. of Participants</th>
<th>RAF, %</th>
<th>Main Outcomes Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bouman et al9</td>
<td>Stable patients with a history of PCI with stenting for ACS or stable angina, 18 mo after initial PCI</td>
<td>Naïve; loading dose, 600 mg</td>
<td>112</td>
<td>67</td>
<td>PON1 activity; active thiol metabolite; platelet inhibition (LTA, 20 μmol/L ADP, baseline and 6 h)</td>
</tr>
<tr>
<td>Sibbing et al10</td>
<td>Patients with CAD undergoing elective PCI with stenting</td>
<td>Naïve; loading dose, 600 mg</td>
<td>1524</td>
<td>73</td>
<td>On-treatment platelet reactivity (Multiple, 6.4 μmol/L, ≥2 h after loading)</td>
</tr>
<tr>
<td>Fontana et al11</td>
<td>Patients with CAD, ≥1 mo after an incident event</td>
<td>Long-term; maintenance dose, 75 mg</td>
<td>538</td>
<td>71</td>
<td>PON1 activity; on-treatment platelet reactivity (VASP assay and LTA, 20 μmol/L ADP)</td>
</tr>
<tr>
<td>Trenk et al12</td>
<td>Patients with CAD undergoing elective PCI with stenting</td>
<td>Naïve; loading dose, 600 mg</td>
<td>760</td>
<td>71</td>
<td>Platelet inhibition and on-treatment platelet reactivity (LTA, 5 and 20 μmol/L ADP and P-selectin expression, 20 μmol/L ADP; baseline, PCI, discharge)</td>
</tr>
<tr>
<td>Rideg et al13</td>
<td>Patients with CAD undergoing elective PCI with stenting</td>
<td>Naïve; loading dose, 600 mg</td>
<td>189</td>
<td>71</td>
<td>On-treatment platelet reactivity (VASP assay and LTA, 5 μmol/L ADP, 12–24 h after loading)</td>
</tr>
<tr>
<td>Hulot et al14</td>
<td>CLOVIS-2 trial</td>
<td>Stable patients with a history of MI, at least 3 mo taking clopidogrel</td>
<td>106</td>
<td>65</td>
<td>Active metabolite isomer H4; platelet inhibition (LTA, 20 μmol/L ADP and VerifyNow P2Y12, baseline and 6 h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stable patients with a history of MI, included 3 mo after the event</td>
<td>194</td>
<td>65</td>
<td>On-treatment platelet reactivity (VerifyNow P2Y12)</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndrome; AFUFI, Appraisal of risk Factors in young Ischemic patients Justifying aggressive Intervention registry; CAD, coronary artery disease; CLOVIS-2, CLOpidogrel and response Variability Investigation Study 2; LTA, light transmission aggregometry; MI, myocardial infarction; PCI, percutaneous coronary intervention; RAF, risk allele frequency; VASP, vasodilator-stimulated phosphoprotein phosphorylation.

*The frequency of the Q192 allele.
with stent thrombosis was nearly similar in their replication cohort, in which such a selection was not applied.9 Thus, selection bias is not likely to explain the differences between the findings of Bouman et al and the other studies.

Second, there are differences in the laboratory methods. Hulot et al14 measured the active metabolite isomer H4 in contrast to the thiol metabolite that was measured by Bouman et al.9 Although this difference may have influenced the results, it is not likely to explain such a large difference between their studies. Moreover, only Bouman, Trenk,12 and Hulot and colleagues studied platelet inhibition in subjects who received new clopidogrel loading, whereas others10,11,13,14 studied on-treatment platelet reactivity, which is less sensitive for measuring pharmacodynamic effects. However, because Trenk and Hulot and colleagues also did not find effects of Q192R on platelet inhibition, this could also not explain the disparity. Furthermore, different platelet function tests were used.

Third, the clinical end points used differ. Bouman et al9 found the largest association with definite stent thrombosis, whereas the association with more causative diverse outcomes was smaller. The CYP2C19*2 loss-of-function variant also seems to affect mainly the risk of stent thrombosis.7 As a primary end point, Hulot et al14 report hazard ratios for the effect of PON1 on major adverse cardiac events. However, PON1 polymorphisms were also not associated with definite stent thrombosis in this study. Table 2 presents the association of Q192R with definite stent thrombosis for all current studies, with the exception of the study by Trenk et al,12 who also included probable and possible stent thromboses.

Fourth, the sample sizes of the different studies warrant comment. The original study of Bouman et al9 is among the smallest. Because active metabolite formation and platelet inhibition are heterogeneous and all subsequent studies did not find an effect, their finding may be false positive. Furthermore, the number of stent thromboses is limited in all available studies, thus spurious (positive and negative) findings could easily arise. When all available evidence is pooled using a random-effects model, the odds ratio for the effect on stent thrombosis per Q allele is 1.40 (95% CI, 0.77–2.53), assuming an additive model, which seems most appropriate. Although the pooled odds ratio is close to 1, the CI is still compatible with a 27% decreased to a 153% increased risk per Q allele. This, however, is not necessarily the result of effects on clopidogrel bioactivation but could also reflect antiatherogenic effects of PON1.

In conclusion, the study of Bouman et al9 suggested that PON1 Q192R is an important determinant of clopidogrel bioactivation. This introduced hope that predicting clopidogrel efficacy in clinical practice might become feasible.

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Table 2. Characteristics of Clinical End Point Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bouman et al9</td>
<td>Case-cohort</td>
<td>Cases, definite ST; controls, random sample of the same cohort of patients with a history of PCI with stenting for ACS or stable angina</td>
</tr>
<tr>
<td></td>
<td>Cohort</td>
<td>Patients with ACS undergoing PCI with stenting; outcome, definite ST</td>
</tr>
<tr>
<td>Sibbing et al10</td>
<td>Case-control</td>
<td>Cases, early definite ST after stenting (&lt;30 d); controls, external cohort of patients with CAD undergoing elective PCI with stenting and without early ST</td>
</tr>
<tr>
<td>Trenk et al12</td>
<td>Cohort</td>
<td>Patients with CAD undergoing elective PCI with stenting; outcome, definite, probable, or possible ST</td>
</tr>
<tr>
<td>Hulot et al14</td>
<td>Cohort</td>
<td>Stable patients with a history of MI, included 3 mo after the event; outcome, definite ST</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of Clinical End Point Studies (continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pooled‡</td>
<td>...</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndrome; CAD, coronary artery disease; MI, myocardial infarction; OR, odds ratio; PCI, percutaneous coronary intervention; RAF, risk allele frequency.

*The RAF is the frequency of the Q192 allele. The frequency is determined in the study base (ie, the whole cohort in cohort studies and the control subjects in case-control/case-cohort studies). The RAF in the case-cohort study of Bouman et al9 differs from that in Table 1 (pharmacokinetics and dynamics were studied in the combined population of cases and controls, which changes the RAF).

†Per-allele OR, assuming an additive model. When recessive, instead of additive, models were used, the results were largely comparable.

‡Pooled per-allele OR using a random-effects model.
soon. However, although promising, accounting for the results of the present study of Hulot et al.14 and other recent studies10–13 with negative results, variation in PON1 seems a less important factor than originally thought. Currently, awaiting the results of other studies, the available evidence suggests that, although the studies of Bouman et al were comprehensive and consistent, the identification of PON1 may be a spurious finding. In contrast, the role of CYP2C19 in clopidogrel bioactivation was corroborated by subsequent studies.10–14 Trials, such as Thrombocyte Activity Reassessment and GENoTyping for Percutaneous Coronary Intervention (TARGET-PCI) are addressing the value of the measurement of CYP2C19 in tailoring antiplatelet therapy. Nevertheless, because CYP2C19 seems to explain only a minor part of clopidogrel bioactivation,5,8 future studies to unravel the pharmacogenetics of clopidogrel are highly warranted.

Disclosures
Dr Gilles Montalescot, MD, PhD, provided per-allele frequencies of definite stent thrombosis in the AFIJI registry. Dr Gilles Montalescot, MD, PhD, was not involved in the writing of this editorial.

References

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