# *CYP2C19* But Not *PON1* Genetic Variants Influence Clopidogrel Pharmacokinetics, Pharmacodynamics, and Clinical Efficacy in Post–Myocardial Infarction Patients

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***Background***—Reduced concentrations of clopidogrel active metabolite have been associated with diminished platelet inhibition and higher rates of adverse cardiovascular events. Paraoxonase-1 (PON1) has recently been proposed as a key enzyme for clopidogrel metabolic activation. We tested the effects of *PON1* polymorphisms on clopidogrel pharmacokinetics and pharmacodynamics and the occurrence of cardiovascular outcomes in young post–myocardial infarction (MI) patients treated with clopidogrel.

***Methods and Results***—We genotyped *PON1* (Q192R and L55M) and *CYP2C19* variants in 106 patients enrolled in the PK/PD CLOVIS-2 trial. Patients were randomly exposed to a 300-mg or 900-mg clopidogrel loading dose in a crossover study design. Clopidogrel active metabolite isomer H4 (clopi-H4) and platelet function testing were measured serially after loading dose. There was no significant association between *PON1* Q192R or L55M and clopi-H4 formation or antiplatelet response to clopidogrel after either loading dose. Using multivariable linear regression analyses, the *CYP2C19*2* allele was the only predictor of clopi-H4 generation and platelet response irrespective of the platelet function assay. *CYP2C19* loss-of-function but not *PON1* variants were significantly associated with increased risk of major cardiovascular events (death, MI, and urgent coronary revascularization) occurring during long-term clopidogrel exposure in 371 young post-MI patients (age <45 years) enrolled in the AFIJI cohort (*CYP2C19* loss-of-function allele carrier versus noncarrier: hazard ratio, 2.26; 95% confidence interval, 1.15–4.41, $P$=0.02; *PON1* QQ192 versus QR/RR192: hazard ratio, 1.03; 95% confidence interval, 0.50–2.11, $P$=0.93; *PON1* LL55 versus LM/MM55: hazard ratio, 1.52; 95% confidence interval, 0.75–3.08, $P$=0.24).

***Conclusions***—Our study does not confirm that *PON1* Q192R or L55M can influence clopidogrel pharmacokinetics or pharmacodynamics in post-MI patients.

***Clinical Trial Registration***—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00822666.
(*Circ Cardiovasc Interv*. 2011;4:422-428.)

**Key Words:** clopidogrel ■ genetic polymorphisms ■ pharmacokinetics ■ cytochrome P450 ■ paraoxonase-1
■ coronary artery disease ■ platelets ■ coronary stenting ■ myocardial infarction

The pharmacological and clinical responses to clopidogrel are highly variable between patients.[1,2] Lesser degrees of platelet inhibition and/or persistent high on-treatment residual platelet reactivity are associated with a higher risk of acute thrombotic events.[3–5] Clopidogrel is a prodrug that requires metabolic activation to generate its active thiol metabolite.[6,7]

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Previous pharmacokinetic (PK) and pharmacodynamic (PD) analyses suggest that a significant portion of the variability in platelet response is explained by variability in plasma concentrations of the clopidogrel active metabolite.[8–10] The genetic component of such variability is important.[11] To date, the reduced-function genetic variants in the hepatic cytochrome P450–2C19 (*CYP2C19*) gene have been identified as the most prominent contributors to this variability.[12] Carriers of *CYP2C19* loss-of-function variants exhibit reduced concentrations of active drug metabolite,[9,13] diminished platelet inhibition,[9,13,14] and higher rates of adverse cardiovascular events.[15,16] However, *CYP2C19* genetic variants only account for a limited proportion of this response variability.[11]

Paraoxonase-1 (PON1) is a hepatic esterase that binds circulating high-density lipoproteins to prevent oxidative modification of low-density lipoproteins and has been recently proposed as an important factor in the transformation of the intermediate metabolite 2-oxo-clopidogrel into the active thiol metabolite.[17] Two common nonsynonymous single-nucleotide polymorphisms (Q192R and L55M) have been associated with significant changes in serum PON1 activity and level of expression.[18,19] Using in vitro liver microsomal assays, Bouman et al found that *PON1* QQ192 was associated with a lower rate of clopidogrel metabolic activation. The *PON1* Q192 allele was also associated with a decrease in both maximal plasma concentrations of clopidogrel active metabolite and platelet inhibition after a single 600-mg clopidogrel loading dose (LD). In the same study, *PON1* Q192 carriers had a higher risk for adverse cardiovascular outcomes; yet, no effect from *CYP2C19* was identified.[17]

To further replicate these important new findings, we interrogated *CYP2C19* and *PON1* (Q192R and L55M) among patients enrolled in the CLOVIS-2 randomized clinical trial (NCT 00822666) in whom clopidogrel active metabolite isomer H4 and platelet inhibition were measured in response to a 300 mg or 900 mg clopidogrel LD.[8] We also compared the PD responses to maintenance dose (MD) of clopidogrel according to *PON1* genetic variants in a larger cohort of post–myocardial infarction (MI) patients[20] and assessed the association between *PON1* and adverse cardiovascular outcomes.

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## WHAT IS KNOWN

- A significant portion of the variability in platelet response to clopidogrel is explained by the variable generation of its active metabolite.
- *CYP2C19* has been identified as key determinant of clopidogrel activation but explains only a limited portion of the overall variability.
- Paraoxonase-1 (*PON1*) has recently been proposed as a more crucial enzyme than *CYP2C19* for clopidogrel metabolic activation.

## WHAT THE STUDY ADDS

- In contrast to *CYP2C19*, *PON1* is not involved in clopidogrel active metabolite generation as measured in vivo in patients with coronary artery disease.
- *PON1* does not influence platelet responsiveness to clopidogrel loading and maintenance doses, nor does it modify the risk of cardiovascular outcomes in clopidogrel-treated young post–myocardial infarction patients.

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## Methods

### Patients

The present data correspond to secondary analyses of the CLOVIS-2 study (ClinicalTrials.gov number NCT00822666)[8] and the AFIJI multicenter registry (*Appraisal of risk Factors in young Ischemic patients Justifying aggressive Intervention*).[20]

The CLOVIS-2 study was designed to investigate clopidogrel PK/PD according to *CYP2C19* genetic variants. A total of 106 young post-MI patients of the AFIJI multicenter registry[20] treated with a MD of 75 mg of aspirin and/or 75 mg of clopidogrel (n=86) for at least 3 months were randomly assigned to an open label LD of 300 mg or 900 mg clopidogrel in a 2-period crossover design. An enrichment strategy was used where homozygous and heterozygous *CYP2C19*2* patients were matched according to age and sex to *CYP2C19 *1/*1* patients (noncarriers) from the same AFIJI program with a 1:1 ratio for each heterozygous and a 2:1 ratio for each homozygous patient.[8] Blood sampling was performed at sequential time points (ie, baseline, 1, 2, 4, and 6 hours after loading) during the 6 hours after drug intake for clopidogrel active metabolite isomer H4 (clopi-H4) quantification. Platelet function testing was performed before loading and 6 hours after drug loading.

The AFIJI prospective multicenter registry has been designed to investigate genetic and nongenetic factors in patients ages >18 and <45 years and who have an established coronary disease defined by an episode of MI (ST-elevation or non–ST-elevation–MI). The influence of *CYP2C19*2* carriage on clinical outcomes in 259 of these patients (included until April 1, 2008) has been previously reported.[20] The present investigation reports on 371 patients on clopidogrel MD (75 mg/d) and for whom long-term clinical follow-up was available. Furthermore, platelet function monitoring using the VerifyNow P2Y$_{12}$ assay (Accumetrics Corporation, San Diego, CA) was available for 194 of these patients. Both research programs were approved by the Pitié-Salpêtrière University Hospital Ethics Committee. A written informed consent form for study participation and genetic analyses was obtained from each patient.

### Procedures

In the CLOVIS-2 trial, platelet measurements were performed immediately after venipuncture in a single laboratory blinded to the assigned LD regimen and to the genetic profile of the study patients. Light transmission aggregometry (Model 490–4D, Chrono-Log Corporation, Kordia, The Netherlands) and the point of care (POC) VerifyNow P2Y$_{12}$ (VN-P2Y$_{12}$) assay (Accumetrics Corporation, San Diego, CA) were used to measure platelet aggregation at baseline (before LD) and 6 hours after LD during both phases. The relative change in platelet aggregation was calculated as (aggregation at baseline minus aggregation at 6 hours post-LD)/aggregation at baseline×100. The residual platelet aggregation (RPA) corresponds to the level of aggregation curve (%) measured 6 minutes after 20 μmol/L ADP-induced platelet aggregation. For the VN-P2Y$_{12}$ assay, samples were run in the same laboratory according to the device package insert and results expressed as platelet reaction units (PRU).

For the PK analyses, whole blood was immediately stabilized after venipuncture with 500 mmol/L MPBr (3-methoxyphenacyl bromide) and processed to obtain platelet-poor plasma aliquots and stored at −80°C. Plasma concentration of active metabolite isomers stabilized as 3′methoxyacetophenone derivates was determined by a stereoselective assay using a validated electrospray liquid chromatography tandem mass spectrometry (LC/MS/MS) method.[21] Data on the active metabolite isomer H4 (clopi-H4) were reported since isomer H3 was inactive and isomers H1 and H2 were non quantifiable. The area under the plasma concentration (AUC)–time curve from the time of administration of the LD to 6 hours (AUC$_{0-6}$) of active metabolite and maximal plasma concentration (Cmax) were computed by noncompartmental methods of analysis with the use of the log-linear trapezoidal method (Winnonlin software).

In the AFIJI cohort, the primary end point was a composite of cardiovascular death, nonfatal MI, and urgent revascularization as previously described.[20] Definite stent thrombosis was additionally assessed on the basis of definitions from the Academic Research Consortium.[22] Follow-up was every 6 months, with a detailed report of drug treatment. Clinical events occurring under clopidogrel exposure were retained for this analysis.

*PON1* Q192R (rs662) and L55M (rs 854560) were interrogated using commercially available genotyping assays (TaqMan-validated SNP assays, Applied Biosystems, Foster City, CA, USA) and the

**Table 1.    Median (interquartile range) Pharmacokinetic Parameters After 300-mg and 900-mg Loading Doses of Clopidogrel According to *PON1* Q192R, L55M, and *CYP2C19* Genetic Variants**

| | Clopidogrel, 300 mg LD | | | | Clopidogrel, 900 mg LD | | | |
|---|---|---|---|---|---|---|---|---|
| | Cmax, ng/mL | P Value | AUC$_{0-6}$, ng · h/mL | P Value | Cmax, ng/mL | P Value | AUC$_{0-6}$, ng · h/mL | P Value |
| *PON1* A672T (Q192R) | | | | | | | | |
| AA (QQ) n=44/46 | 6.8 [3.4–11.6] | 0.08* | 12.1 [8.5–19.5] | 0.41* | 14.6 [8.7–24.1] | 0.91* | 25.9 [16.9–48.7] | 0.97* |
| AG (QR) n=44/45 | 9.3 [6.6–14.2] | | 15.1 [10.5–22.0] | | 15.0 [9.5–26.9] | | 30.5 [20.0–42.9] | |
| GG (RR) n=15/15 | 7.3 [3.7–11.4] | | 14.5 [8.6–23.7] | | 15.2 [8.2–32.8] | | 30.7 [16.9–49.1] | |
| *PON1* T260A (L55M) | | | | | | | | |
| TT (LL) n=55/56 | 9.2 [4.9–12.9] | 0.69* | 15.3 [9.3–22.3] | 0.45* | 19.7 [8.9–29.6] | 0.45* | 33.5 [20.1–53.7] | 0.24* |
| TA (LM) n=41/42 | 8.0 [3.6–11.6] | | 13.7 [9.0–21.5] | | 12.9 [8.4–19.5] | | 23.5 [17.4–36.9] | |
| AA (MM) n=6/8 | 7.4 [6.5–8.4] | | 11.2 [11.1–12.9] | | 13.9 [5.7–24.0] | | 28.6 [16.4–48.7] | |
| *CYP2C19* G681A (CYP2C19*2) | | | | | | | | |
| GG (*1/*1) n=55/58 | 9.0 [5.7–13.9] | 0.11 | 16.5 [11.2–26.1] | 0.01 | 17.3 [10.9–32.1] | 0.002 | 33.8 [22.2–55.8] | 0.001 |
| GA (*1/*2) n=41/41 | 7.9 [4.2–12.1] | | 11.5 [8.9–17.7] | | 12.7 [8.4–23.8] | | 25.0 [16.9–38.1] | |
| AA (*2/*2) n=7/7 | 5.1 [3.4–6.7] | | 9.3 [7.6–11.2] | | 6.3 [5.2–10.0] | | 16.1 [11.8–18.5] | |

*PON1* indicates Paraoxonase-1.

*Overall P value, Kruskall-Wallis test. All pairwise comparisons are nonsignificant; n, number of patients with data.

7900HT sequence detection system (Applied Biosystems). The *CYP2C19 *2* to *6* loss-of-function variants were assessed as previously described.[20]

### Statistical Analysis

Analyses were done with SAS (version 9.1). Continuous variables were expressed as mean±SD and categorical variables as frequencies and percentages. Baseline characteristics of patients by genotypes were compared using the $\chi^2$ test for categorical variables and Student $t$ test or 1-way ANOVA for continuous variables as appropriate.

The PK (AUC$_{0-6}$ and Cmax) and PD(relative change in platelet aggregation) response parameters were compared between genotype groups and the 2 LDs tested (300 mg versus 900 mg-LD) by the Kruskall-Wallis test. The 2-sided unpaired Wilcoxon test was used to compare parameters between 2 genotypes. Linear regression analyses were performed to evaluate the association between the tested genotypes and PK and PD parameters.

We tested for associations between the tested genetic variants in *PON1* and *CYP2C19* and major cardiovascular adverse events with the use of Cox proportional-hazards regression. We used both general and recessive genetic models. Two-sided probability values of <0.05 were considered statistically significant.

## Results

### Pharmacokinetic and Pharmacodynamic Responses According to *PON1* Genetic Variants in the CLOVIS-2 Study

A total of 106 male patients (mean age, 40.1±4.8 years; body mass index, 26.1±3.8 kg/m$^2$) completed the 2 periods of the randomized CLOVIS-2 study. There were no significant differences in baseline characteristics according to *PON1* and *CYP2C19* genetic variants (data not shown). Primary results of the CLOVIS-2 study have been previously reported.[8] Briefly, we found that *CYP2C19*2* carriers had significantly reduced responses to clopidogrel with a gene dose-effect and that clopidogrel resistance could be overcome by increasing the LD to 900 mg in heterozygotes but not in homozygous patients.

In the present study, there was no significant association between *PON1* Q192R or L55M and either the maximal

clopi-H4 concentrations or the AUC$_{0-6}$ (in ng · h/mL). As shown in Table 1, both clopi-H4 Cmax and AUC$_{0-6}$ were similar among *PON1* Q192R genotype groups after administration of either the 300-mg or the 900-mg clopidogrel LD. After administration of the 300-mg LD, there was a nonsignificant trend for a reduction by ≈25% in clopi-H4 Cmax and AUC$_{0-6}$ in *PON1* MM55 individuals as compared with LL55 individuals ($P$=0.35 for Cmax; $P$=0.18 for AUC$_{0-6}$); however, this pattern was not observed after the 900-mg LD. In contrast, carriers of the *CYP2C19*2* loss-of-function allele had a significant reduction in both clopi-H4 Cmax and AUC$_{0-6}$ compared with noncarriers (Table 1), which was observed after both 300- and 900-mg LDs. After adjustment for *CYP2C19*2*, both *PON1* variants were still not significantly associated with clopidogrel PK in this population ($P$=nonsignificant for all analyses on Cmax and AUC$_{0-6}$ after both LDs). No significant interaction between the tested *CYP2C19* and *PON1* genetic variants was observed for either LDs.

In multivariable linear regression analyses containing the tested *PON1* and *CYP2C19* polymorphisms, the *CYP2C19*2* allele was the only predictor of clopi-H4 Cmax and AUC$_{0-6}$ after both LDs (AUC$_{0-6}$ 300 mg: $P$<0.005; AUC$_{0-6}$ 900 mg: $P$<0.01). Adjustment for potential confounders (weight, diabetes and use of proton pump inhibitor) did not alter this result. Of note, *CYP2C19*2* genotype accounted for 8% of the variation in clopidogrel PK after both LDs.

Consistent with the PK analyses, platelet inhibition was not significantly associated with the tested *PON1* genetic variants among these patients. Figure 1A and 1B show the relative change in platelet aggregation as assessed by the VerifyNow-P2Y$_{12}$ assay. Similar results were found for residual and maximal platelet aggregation after stimulation with ADP 20 $\mu$mol/L (data not shown). The proportion of patients with high on-treatment platelet reactivity (as defined by maximal platelet aggregation >59%) on maintenance therapy was not significantly influenced by *PON1* genetic variants: QQ192:
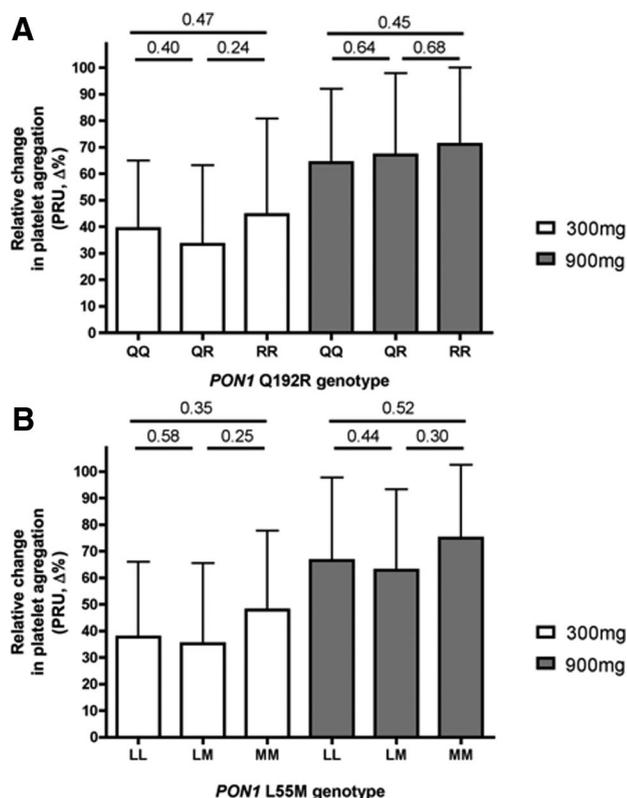
**Figure 1.** Relative change in platelet response to 300- and 900-mg clopidogrel loading doses according to Paraoxonase-1 (*PON1)* Q192R (**A**) and L55M (**B**).

33.3% versus QR192: 34.3% versus RR192: 33.3%, *P*=0.98; LL55: 38.3% versus LM55: 31.2% versus MM55: 14.3%, *P*=0.50. In multivariate linear regression analyses using residual platelet aggregation after stimulation with ADP 20

$\mu$Mol/L or P2Y12 PRU values, *CYP2C19*2* carriage remained the only significant predictor of platelet function response to clopidogrel LD irrespective of the platelet function assay (*P*<0.001 for both loading doses). Multivariate analyses using maximal platelet aggregation or high on-treatment platelet reactivity as dependent variables lead to similar results. Again, no significant interactions between the tested *CYP2C19* and *PON1* genetic variants were observed.

## Pharmacodynamic and Clinical Outcomes in the AFIJI Patients

The characteristics of the 371 genotyped patients are presented in Table 2. Interestingly, we found a significant association between the *PON1* L55M loss-of-function variant and diabetes. *PON1* LL55 patients were more frequently diabetics than *PON1* LM55 or MM55 genotype carriers (LL55 18.1% versus LM/MM55 5.6%; *P*<0.0001). There were no other significant differences identified according to *PON1* L55M or Q192R carriage.

Most of our study population did not carry *CYP2C19*2* (wt/wt homozygotes, n=262; 71.0%). The vast majority of carriers were heterozygotes (wt/*2, n=94, 25.5%), with a small proportion being homozygous (*2/*2, n=13, 3.5%). Four patients were carriers of the *4 loss-of-function allele and two of the *3 loss-of-function allele. All of them were also carriers of one copy of the *2 allele. Baseline characteristics according to *CYP2C19*2* carriage were well balanced[20] (data not shown). The median clopidogrel exposure time was 2.6 years (interquartile range, 1.0–4.8) and did not differ according to *PON1* or *CYP2C19* genotypes.

Thirty-five patients presented with a major adverse cardiac event during clopidogrel exposure. Eighteen patients presented with a stent thrombosis. The rate of major adverse cardiac events (MACE) did not differ according to *PON1* Q192R

**Table 2.    Baseline Characteristics of Genotyped Patients in the AFIJI Cohort**

| | Overall (n=371) | *PON1* Q192R | | | | *PON1* L55M | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | QQ192 (n=168) | QR192 (n=148) | RR192 (n=55) | P Value | LL55 (n=138) | LM55 (n=163) | MM55 (n=68) | P Value |
| Age, y | 40.3±5.5 | 39.6±5.4 | 41.0±5.5 | 40.3±5.6 | 0.10 | 40.7±4.9 | 40.1±5.3 | 40.0±7.1 | 0.52 |
| Male sex, % | 84.6 | 83.3 | 86.5 | 83.6 | 0.72 | 88.4 | 81.6 | 83.8 | 0.26 |
| BMI, kg/m$^2$ | 25.9±4.0 | 25.9±4.5 | 26.1±3.6 | 25.2±3.7 | 0.37 | 25.7±3.9 | 26.0±4.2 | 26.0±3.8 | 0.87 |
| Ancestry, % | | | | | 0.64 | | | | 0.69 |
| European | 94.8 | 96.4 | 94.6 | 90.9 | | 92.8 | 96.9 | 94.1 | |
| Black | 2.2 | 1.2 | 2.7 | 3.6 | | 3.6 | 0.6 | 2.9 | |
| Asian | 3.0 | 2.4 | 2.7 | 5.4 | | 3.6 | 2.4 | 2.9 | |
| Risk factors, % | | | | | | | | | |
| Diabetes | 10.5 | 8.3 | 10.1 | 18.2 | 0.12 | 18.1 | 5.5 | 5.9 | 0.0007 |
| Familial CAD | 41.2 | 41.7 | 41.9 | 38.2 | 0.88 | 43.5 | 39.9 | 41.2 | 0.82 |
| Current smoker | 51.5 | 54.8 | 50.7 | 43.6 | 0.35 | 50.7 | 56.4 | 39.7 | 0.07 |
| Hypertension | 23.2 | 23.2 | 20.9 | 29.1 | 0.47 | 23.9 | 23.9 | 20.6 | 0.84 |
| Dyslipidemia | 56.1 | 53.6 | 56.8 | 61.8 | 0.55 | 61.6 | 51.5 | 54.4 | 0.21 |
| PCI, % | 79.8 | 78.0 | 80.4 | 83.6 | 0.64 | 81.2 | 80.4 | 75.0 | 0.56 |
| CABG, % | 8.1 | 8.3 | 8.1 | 7.3 | 0.97 | 8.7 | 8.6 | 4.4 | 0.50 |

AFIJI indicates Appraisal of risk Factors in young Ischemic patients Justifying aggressive Intervention; *PON1*, Paraoxonase-1; BMI, body mass index; CAD, coronary artery disease; PCI, percutaneous coronary intervention; and CABG, coronary artery bypass graft.

**A** *PON1 Q192R*

— QQ192
— QR/RR 192

p=0.62

Number at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| QQ | 168 | 126 | 96 | 74 | 44 | 40 |
| QR/RR | 203 | 146 | 115 | 89 | 67 | 48 |

**B** *PON1 L55M*

— LL55
— LM/MM 55

p=0.13

Number at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| LL | 138 | 108 | 88 | 71 | 52 | 38 |
| LM/MM | 231 | 163 | 123 | 92 | 69 | 50 |

**C** *CYP2C19*2*

— Non carriers
— Carriers

p=0.01

Number at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| Non carriers | 262 | 194 | 151 | 118 | 91 | 65 |
| Carriers | 107 | 77 | 60 | 45 | 30 | 23 |
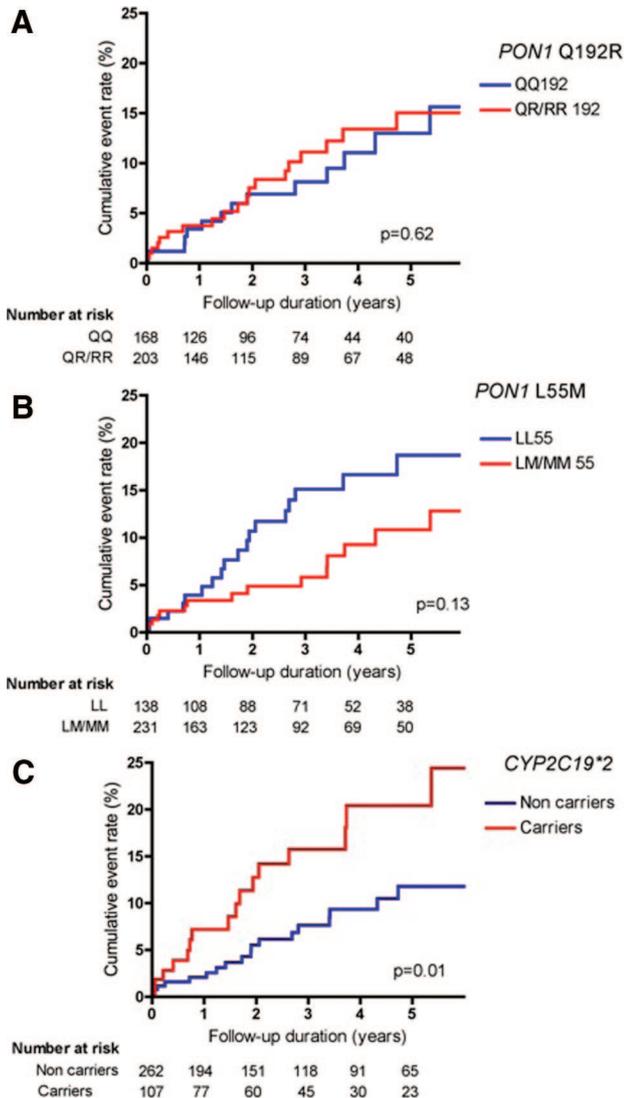
**Figure 2.** Cumulative Kaplan-Meier estimates of the rates of cardiovascular events (death, nonfatal myocardial infarction, or urgent revascularization) under clopidogrel according to Paraoxonase-1 (*PON1*) Q192R (**A**), *PON1* L55M (**B**), and *CYP2C19*2* (**C**).

carriage: QQ192: 14/168 (8.3%), QR192: 14/148 (9.5%) and RR192: 7/55 (12.7%), log-rank test across genotypes $P=0.86$. The hazard ratio (HR) for QQ192 versus QR/RR192 was 0.84 (95% confidence interval [CI], 0.43–1.67, $P=0.62$). There was a nonsignificant trend for a higher rate of MACE according to *PON1* L55M carriage (LL55: 19/138, 13.8%; LM55: 12/163, 7.4%; and MM55: 4/68, 5.9%; log-rank test across genotypes: $P=0.30$). The HR for LL55 versus LM/MM55 was 1.68 (95% CI, 0.86–3.27, $P=0.13$). However, this trend was attenuated after adjustment for diabetes status, and the association between the *PON1* L55M polymorphism and the risk of cardiovascular events remained nonsignificant (adjusted HR, 1.32; 95% CI, 0.66–2.64], $P=0.43$).

As previously reported, there was a significant increase in the occurrence of MACE in *CYP2C19*2* carriers as compared with noncarriers (log-rank test across genotypes, $P=0.03$; HR for carriers versus noncarriers, 2.37; 95% CI,

1.22–4.59, $P=0.01$). The corresponding Kaplan-Meier events curves are shown in Figure 2.

In a Cox regression model containing *PON1* R192, M55, and *CYP2C19* loss-of-function allele carrier status, the presence of *CYP2C19* loss-of-function variants was the only significant predictor of the primary composite clinical end point (*PON1* QQ192 versus QR/RR192: HR, 1.03; 95% CI, 0.50–2.11, $P=0.93$; *PON1* LL55 versus LM/MM55: HR, 1.52; 95% CI, 0.75–3.08, $P=0.24$; and *CYP2C19* loss-of-function allele carrier versus noncarrier: HR, 2.26; 95% CI, 1.15–4.41, $P=0.02$). Similar results were found when considering the occurrence of stent thrombosis (*PON1* QQ192 versus QR/RR192: HR, 1.07; 95% CI, 0.39–2.89, $P=0.89$; *PON1* LL55 versus LM/MM55: HR, 1.94; 95% CI, 0.71–5.27, $P=0.19$; and *CYP2C19* loss-of-function allele carrier versus noncarrier: HR, 2.79; 95% CI, 1.09–7.16, $P=0.03$).

Finally, PD response to a clopidogrel 75 mg/d MD was assessed using the VerifyNow $P2Y_{12}$ assay in 194 of the 371 patients. *PON1* allelic frequencies were similar between this subgroup of patients and the overall cohort of patients. In line with our findings on the effect of 300/900 mg clopidogrel LDs, there was no detectable effect of *PON1* Q192R (PRU: $166\pm85$ in QQ192 versus $166\pm86$ in QR192 versus $143\pm88$ in RR192 patients; $P=0.45$) or *PON1* L55M carriage (PRU: $167\pm87$ in LL55, $164\pm85$ in LM55 and $146\pm86$ in MM55 patients; $P=0.46$) on the PD response to a 75 mg clopidogrel MD regimen. However, there was a strong and significant effect of *CYP2C19*2* carriage (PRU: $154\pm91$ in *1/*1 versus $173\pm72$ in *1/*2 versus $242\pm69$ in *2/*2 homozygous patients; overall probability value, 0.01; noncarriers versus carriers, $P=0.04$). In a linear regression model containing *PON1* R192, M55 and *CYP2C19* loss-of-function allele carrier status, carriage of *CYP2C19* loss-of-function variants was the only significant predictor of platelet reactivity under clopidogrel MD ($P=0.04$).

## Discussion

The major finding of the present investigation is that *PON1* polymorphisms (Q192R and L55M) are unlikely to be major determinants of the pharmacokinetic and pharmacodynamic responses to clopidogrel, and clinical efficacy of clopidogrel in young post-MI patients. This evidence comes from (1) the lack of difference in clopidogrel active metabolite isomer H4 plasma levels (measured with a stereoselective assay[21]) according to *PON1* genotype, (2) the concordant lack of influence of *PON1* on the PD responsiveness to clopidogrel loading and maintenance doses in 2 separate studies, and (3) the absence of significant association between *PON1* and the risk of cardiovascular outcomes in a large cohort of clopidogrel-treated young post-MI patients followed for up to 6 years. Conversely, this study did confirm the influence of *CYP2C19* genetic variants on clopidogrel PK, PD, and clinical efficacy.

Our results are thus in contrast with the previous report from Bouman et al,[17] who found that (1) PON1 is a crucial enzyme for transformation of the intermediate 2-oxo-clopidogrel metabolite to the active thiol metabolite using an in vitro metabolizing enzyme microsomal expression system, and (2) *PON1* QQ192 genotype was associated with a 5-fold

reduction of active thiol metabolite concentration compared with RR192 patients. This discrepancy may be linked to differences in the methodology of active metabolite measurements between studies. Due to its stereochemical structure, the clopidogrel active metabolite belongs to a mixture of 4 different isomers.[6,7] Among these, only the inactive H3 and active H4 isomers are detectable in human plasma.[21] The existence of several active metabolite isomers and their chemical instability in blood might lead to approximation when measuring active metabolite concentrations with non-selective techniques.[21] In our study, the clopidogrel active metabolite isomer H4 was specifically quantified with a recently developed stereoselective assay using chemically synthesized H4 isomer as a calibration standard.[21] As such, we cannot exclude the possibility that PON1 participates in the generation of other inactive metabolites.

Differences in the study design are another potential explanation. In the CLOVIS-2 study, the PK/PD measurements were performed prospectively in stable coronary patients who were randomly assigned to an open-label 300-mg or 900-mg clopidogrel LD in a 2-period crossover fashion. This design should have limited the introduction of confounding factors as opposed to a post hoc evaluation of clopidogrel PK/PD in a selected case/control cohort.[23] Notably, *CYP2C19* was a significant predictor of clopidogrel PK and PD in the CLOVIS-2 study; however, it had no impact in the study from Bouman et al.[17] The critical role of the *CYP2C19* loss-of-function allele on the generation of clopidogrel active metabolite,[9,24] platelet inhibition,[9,14] and clinical outcome in clopidogrel-treated patients has been replicated in many independent studies and 2 recent meta-analyses[15,16] and is further confirmed in the present investigation. However, we failed to observe such a clear continuum with the tested *PON1* genetic variants.

The significant association between *PON1* L55M carriage and diabetes is of importance given the established correlation between PON1 activity and the occurrence of diabetes,[25,26] glucose intolerance,[27] and vascular complications in diabetics.[28] Moreover, *PON1* genetic polymorphisms have also been associated with an overall increase in cardiovascular risk.[18] Consistent with these findings, we report a trend for higher rates of cardiovascular events among *PON1* LL55 patients, a genotype previously associated with glucose intolerance and impaired insulin secretion.[27] Of note, this effect was attenuated after adjustment for diabetes status. These results further suggest that *PON1* might influence clinical outcomes in clopidogrel-treated patients irrespective of a direct impact on clopidogrel PK or PD. This hypothesis warrants further investigation, however, because *PON1* Q192R has been suggested to be the principal determinant of unexplained clopidogrel response variability[17] while no data have been reported for *PON1* L55M and clopidogrel PK or PD before our current study.

Our study has some limitations. Pharmacological and clinical responses to clopidogrel vary widely between patients. This interindividual variability can lead to spurious findings when analyzing cohorts of limited sample size, thus explaining the crucial need for replication of genetic association studies.[23] Our study was conducted on a limited sample

of young coronary patients and could have been underpowered to detect the influence of *PON1* polymorphisms. Despite this limitation, our results do not support the Bouman et al finding that 73% of clopidogrel response variability is linked to *PON1* Q192R whereas *CYP2C19* genetic variants have no detectable effect.[17] In addition, PON1 activity is also highly variable among patients. It is possible that such variability has not been fully captured by the current *PON1* genotyping, further accounting for our overall negative results. However, many studies have reported a consistent link between the *PON1* Q192R and L55M polymorphisms and PON1 activity. MM55 and QQ192 homozygotes have the lowest PON1 activity.[18,19,25] In particular, Bouman et al reported that *PON1* Q192R genotype and paraoxonase plasma activity have similar capacities to predict cardiovascular outcome.[17] With respect to additional *PON1* polymorphisms, a common promoter allele (eg, −108C/T) has been reported[29] but this polymorphism was not specifically tested in the present study. However, a specific effect from this *PON1* promoter polymorphism is unlikely, given its strong linkage disequilibrium with the currently tested L55M polymorphism. Finally, we cannot exclude the possibility that other ethnic-specific rare *PON1* variants that were not genotyped in our study or the Bouman et al[17] study participate in variable clopidogrel responsiveness.

Taken together, these results do not support an important contribution of *PON1* Q192R and L55M as major determinants of clopidogrel PK and PD responsiveness, including the clinical efficacy of clopidogrel in young post-MI patients. Rather, these *PON1* polymorphisms might be associated with metabolic disorders and/or cardiovascular risk independent of clopidogrel pharmacogenetics.

## Disclosures

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