Paraoxonase 1 Q192R Variant and Clopidogrel Efficacy
Fact or Fiction?

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Clopidogrel, in combination with aspirin, is the standard of care for preventing ischemic cardiovascular events in patients with coronary artery disease, especially those who undergo percutaneous coronary intervention (PCI). Despite its widespread use, significant interindividual variability in clopidogrel response is consistently observed, and recent studies have suggested that as much as 70% of this variability can be attributed to genetic factors.1,2

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Clopidogrel is a thienopyridine prodrug that once activated, exerts its antiplatelet effect by irreversibly binding to the P2Y12 receptor on the surface of platelets inhibiting ADP-stimulated platelet aggregation. Clopidogrel activation requires a 2-step conversion by hepatic enzymes, most notably CYP2C19, whereas esterases (eg, carboxylesterase 1 and carboxylesterase 2) lead to the production of biologically inactive carboxylic acid derivatives.3 Common loss-of-function variants in CYP2C19 are associated with lower active metabolite levels, higher residual on-clopidogrel ADP-stimulated platelet aggregation, and poorer cardiovascular outcomes.3 However, this variant explains only ∼12% of the variation in platelet response to clopidogrel, leaving most of the heritable variation unknown.

Recently, Bouman and colleagues4 observed through in vitro studies that paraoxonase 1 (PON1) was the major enzyme in converting 2-oxo-clopidogrel to the active thiol metabolite. Moreover, they reported that a common missense variant in PON1, Q192R, was a major determinant of clopidogrel efficacy, accounting for nearly 70% of the variability in ADP-stimulated platelet aggregation postclopidogrel treatment. 192Q PON1 had lower hydrolysis efficiency for 2-oxo-clopidogrel compared to 192R PON1 in microsomal preparations, and clopidogrel-treated PCI patients homozygous for the 192Q allele had a significantly higher rate of stent thrombosis compared with patients carrying at least 1 copy of the 192R allele. Curiously, no effect of the well-described CYP2C19*2 loss-of-function variant on clopidogrel response was observed in the same patient sample.

In the past year, our group and several others have attempted to replicate these striking results, with little success.5–12 In this issue of Circulation: Cardiovascular Genetics, Paré and colleagues13 report efficacy and safety data regarding clopidogrel among PON1 Q192R genotypes in 2 of the largest prospective placebo-controlled trials studied to date: the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial14 and the Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events (ACTIVE A).15

Consistent with the parent CURE trial report, in the subset of 5059 patients with acute coronary syndrome in this genetic substudy (2549 randomized to clopidogrel and 2510 randomized to placebo), clopidogrel-treated patients had lower cardiovascular event rates than placebo-treated patients (hazard ratio, 0.70; 95% CI, 0.59–0.84; P<0.001). There was no evidence of association between 192Q PON1 and the composite primary cardiovascular outcome in clopidogrel-treated patients (hazard ratio, 0.93 per Q allele; 95% CI, 0.76–1.13; P=0.46). Interestingly, placebo-treated patients had significantly increased incidence of the primary cardiovascular outcome (hazard ratio, 1.23 per Q allele; 95% CI, 1.03–1.47; P=0.03). These findings are consistent with a number of other (although not all) studies showing an association of PON1 variants with increased cardiovascular events, presumably as a result of their association with lower high-density lipoprotein cholesterol levels and increased underlying atherosclerotic burden.16 When comparing cardiovascular event rates in patients treated with clopidogrel to patients treated with placebo stratified by PON1 Q192R genotype to evaluate effect modification, clopidogrel-treated 192Q homozygotes had a significantly decreased risk of experiencing the primary outcome compared to placebo-treated 192Q homozygotes (8.5% versus 13.9%, respectively; P<0.001); a similar trend was apparent in 192Q heterozygotes. Clearly, this effect was primarily driven by the increased incidence of cardiovascular events in placebo-treated patients with the 192Q allele. In any event, these findings are in contrast with the results of Bouman and colleagues4 of increased risk of experiencing a cardiovascular event in clopidogrel-treated 192Q homozygotes.

In 570 patients treated with clopidogrel and 586 patients who received placebo in the ACTIVE A genetic substudy, clopidogrel-treated patients had lower cardiovascular event rates than placebo-treated controls. Like in the CURE trial, no association was observed between PON1 Q192R genotype...
and cardiovascular events in clopidogrel-treated patients, and there tended to be higher cardiovascular event rates in 192Q allele carriers in the placebo-treated group. Thus, there was a nonsignificant trend of decreased cardiovascular events in the clopidogrel-treated group with the \textit{PON1} 192Q-allele relative to placebo-treated patients with the 192Q allele, consistent with results of the CURE trial, and again in the opposite direction of the initial report of Bouman.

In the present study by Paré and colleagues, none of the treatment group × \textit{PON1} genotype interaction terms were statistically significant. Clearly, this investigation does not support a role for the 192Q allele of \textit{PON1} in clopidogrel therapy response and is consistent with a number of recent studies showing no effect of this allele on clopidogrel active metabolite levels, on-clopidogrel ADP-stimulated platelet aggregation, or cardiovascular outcomes. The reasons for discrepant findings between the initial report by Bouman and colleagues and subsequent investigations are unclear.

The population investigated in the study by Bouman and colleagues consisted of white patients with coronary artery disease who underwent PCI, with stent thrombosis being the primary outcome evaluated. Indeed, it is this patient population that benefits most from clopidogrel therapy. Although most of the participants in the current investigation by Paré et al were white, a limitation of this study, as pointed out by the authors, is the limited number of clopidogrel-treated PCI patients (≈400 in the CURE trial population). Furthermore, the ACTIVE A trial enrolled patients with atrial fibrillation, an indication for clopidogrel in which we know has limited benefit. In other words, the lower-risk patients of the current investigation derived less benefit from clopidogrel compared to the higher-risk PCI patients examined by Bouman and colleagues and, therefore, may be less susceptible to the potential detrimental effects of the \textit{PON1} Q192R variant on clopidogrel response. If indeed the 192Q allele is associated with greater underlying atherosclerotic burden (see next paragraph), the results of Paré et al showing that clopidogrel-treated patients with the 192Q allele have lower cardiovascular event rates than placebo-treated patients with the 192Q allele is internally consistent with the hypothesis that clopidogrel has its greatest effect in decreasing cardiovascular events in higher-risk patients. Thus, although the current investigation supports a lack of effect of the Q192R variant on clopidogrel response in lower-risk patients, it adds limited new information regarding the effect of this variant in higher-risk PCI patients. However, it should be noted that other replication efforts that focused primarily or exclusively on clopidogrel-treated PCI patients also did not observe an effect of the Q192R variant on stent thrombosis or other cardiovascular events (Table).

The relationship between \textit{PON1} and cardiovascular disease, irrespective of clopidogrel treatment, has been the focus of multiple investigations for >10 years. \textit{PON1} is a 45-kDa high-density lipoprotein-associated arylesterase that is believed to have antiatherosclerotic properties through its ability to metabolize oxidized lipids of low-density lipoproteins. Furthermore, previous studies have shown that paraoxonase activity is significantly reduced in patients with ST-segment-elevation myocardial infarction, non–ST-segment-elevation myocardial infarction, or unstable angina compared with healthy controls. Consistent with these findings, the current investigation by Paré and colleagues shows a significant association between the decreased function \textit{PON1} 192Q allele and increased cardiovascular events in placebo-treated patients. However, this association was not observed in patients treated with clopidogrel. This novel finding suggests that somehow clopidogrel can ameliorate the increased risk of cardiovascular events in 192Q allele carriers. As mentioned previously, the explanation for this observation may be that clopidogrel has its greatest effect in decreasing cardiovascular events in higher-risk patients. It is intriguing to speculate that this effect may be independent of clopidogrel inhibition of platelet function because we and others have shown that the 192Q allele is not associated with on-clopidogrel ADP-stimulated platelet aggregation.

\begin{table}
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\begin{tabular}{llll}
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\textbf{Study} & \textbf{Primary Outcome} & \textbf{No. of Clopidogrel-Treated Participants} & \textbf{Effect of PON1 Q192R on CV End Point} \\
\hline
Bouman et al\textsuperscript{a} & Stent thrombosis & 41 cases, 71 controls & Yes \\
Sibbing et al\textsuperscript{b} & Stent thrombosis & 127 cases, 1439 controls & No \\
Trenk et al\textsuperscript{c} & Composite CV end point & 24 events, 760 patients & No \\
 & Stent thrombosis* & 18 events, 760 patients & No \\
Lewis et al\textsuperscript{d} & Composite CV end point & 27 events, 227 patients & No \\
Simon et al\textsuperscript{e} & Composite CV end point & 296 events, 2210 patients & No \\
Hulot et al\textsuperscript{f} & Composite CV end point & 35 events, 371 patients & No \\
 & Stent thrombosis & 18 events, 371 patients & No \\
Delaney et al\textsuperscript{g} & Composite CV end point & 225 cases, 468 controls† & No \\
Paré et al\textsuperscript{h} & Composite CV end point & 227 events, 2534 patients & No \\
 & Composite CV end point & 114 events, 570 patients & No \\
\hline
\end{tabular}
\caption{Effect of PON1 Q192R Variant on Cardiovascular Events in Clopidogrel-Treated Patients}
\end{table}

\textit{PON1} indicates paraoxonase 1; CV, cardiovascular.

*Cases were included that met Academic Research Consortium criteria for definite, possible, or probable stent thrombosis.

†Cases and controls were identified by algorithm using an electronic medical record-linked DNA repository.

Paré and colleagues conclude with a caution regarding interpretation of pharmacogenetic analyses in the absence of nontreated controls. Indeed, without such a control group, it is often difficult to determine whether observed associations are the result of drug–genotype interactions or represent an underlying effect that is independent of drug treatment. Although randomized placebo-controlled trials have and continue to be the gold standard in evidence-based medicine, these investigations are costly and inefficient in studies of variable drug response. Therefore, we believe that it is
important to also consider complementary study designs and analytic approaches. For example, phenotypic characterization of surrogate end points before and after drug treatment (eg, ex vivo ADP-stimulated platelet aggregation in the case of clopidogrel pharmacogenetics) allows for patients to serve as their own controls.

Given the current evidence base, should patients with an indication for antiplatelet therapy undergo PON1 genetic testing? Probably not. By contrast, there is a large body of converging evidence (pharmacokinetic, pharmacodynamic, clinical outcomes) supporting a role for CYP2C19 genetic testing to guide antiplatelet therapy in PCI patients. Despite this evidence, many cardiologists have resisted changing practice in lieu of a prospective randomized clinical trial of CYP2C19 genotype-directed antiplatelet therapy to demonstrate efficacy and cost-effectiveness. One such trial, the PAPI-2 (Pharmacogenomics of Anti-Platelet Intervention-2) study (http://clinicaltrials.gov/ct2/show/NCT01452152) is ongoing. Such studies as well as large meta analyses will offer the opportunity to further evaluate PON1 and other candidate gene variants. In addition, larger genome-wide studies will provide the opportunity to discover novel variants that influence clopidogrel response. Ultimately, with the discovery of additional genetic determinants of clopidogrel response, a panel of genetic tests may be used along with clinical data to guide more-effective personalized antiplatelet therapy. Currently, the evidence base does not support PON1 for inclusion in such a panel.

Disclosures
Dr Shuldiner receives grant support from the National Institutes of Health to study the pharmacogenomics of antiplatelet therapy. He is also a consultant for United States Diagnostic Standards, Inc. Dr Lewis reports no conflicts.

References
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