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Effect of *PON1* Q192R Genetic Polymorphism on Clopidogrel Efficacy and Cardiovascular Events in the Clopidogrel in the Unstable Angina to Prevent Recurrent Events Trial and the Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events

Guillaume Paré, MD, MSc, FRCPC; Stephanie Ross, MSc; Shamir R. Mehta, MD, FRCPC; Salim Yusuf, DPhil, FRCPC; Sonia S. Anand, MD, PhD; Stuart J. Connolly, MD; Keith A.A. Fox, MBChB, FRCP; John W. Eikelboom, MBBS, MSc

Background—A recent report suggested that carriers of the Q allele of the *PON1* Q192R polymorphism had decreased biotransformation of clopidogrel into its active metabolite and decreased efficacy of clopidogrel in preventing cardiovascular events. Furthermore, *PON1* has been reported to have a central role in the antioxidant function of high-density lipoprotein, and the Q192R polymorphism has been previously associated with cardiovascular risk in patients not treated with clopidogrel.

Methods and Results—Patients (n=5059) from the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) randomized trial that demonstrated benefits of clopidogrel versus placebo in preventing cardiovascular events in acute coronary syndromes were genotyped for the *PON1* Q192R polymorphism. Clopidogrel compared with placebo significantly reduced the first primary efficacy outcome, irrespective of *PON1* Q192R genotype ($P=0.07$ for heterogeneity). No association was observed between the Q192R polymorphism and cardiovascular events in the overall sample (hazard ratio [HR], 1.09 per allele; 95% confidence interval [CI], 0.95–1.24; $P=0.23$). However, an association was observed between the Q allele and increased cardiovascular events in the placebo group (HR, 1.23 per allele; 95% CI, 1.03–1.47; $P=0.03$) but not in the clopidogrel group (HR, 0.93 per allele; 95% CI, 0.76–1.13; $P=0.46$). In 1156 atrial fibrillation patients from the Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events, there was no evidence of interaction between *PON1* genotype and clopidogrel for any outcome or for an association between genotype and cardiovascular events.

Conclusions—In conclusion, our study shows that *PON1* Q192R genotype does not modify the efficacy and safety of clopidogrel in patients with acute coronary syndromes. Further studies are needed to confirm or refute the association of the Q allele with adverse cardiovascular events independent of clopidogrel in secondary prevention patients. (*Circ Cardiovasc Genet.* 2012;5:250-256.)

Key Words: antiplatelet therapy ■ atrial fibrillation ■ clopidogrel ■ paraoxonase 1 gene ■ pharmacogenetics

Clopidogrel when added to aspirin reduces major vascular events in patients with acute coronary syndromes (ACS).¹ Clopidogrel is a prodrug that requires biotransformation into the active metabolite to inhibit the platelet P2Y₁₂ receptor. Differences in the extent of clopidogrel biotransformation are believed to account for interindividual variability in platelet response to clopidogrel.² Most of the variability appears to be genetically determined, but common cytochrome P450 2C19 polymorphisms account for only a mi-

nority of this variability. The *PON1* enzyme has been reported to play a crucial role in clopidogrel biotransformation, and a common nonsynonymous genetic variant, Q192R, has recently been associated with decreased conversion of clopidogrel to its active metabolite, implying that the benefits of clopidogrel may be attenuated in affected patients.³ Specifically, carriers of the Q allele were found to have a significantly higher risk of stent thrombosis after stent percutaneous coronary intervention (PCI), with an odds ratio

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From the Population Health Research Institute, Hamilton Health Sciences and Departments of Medicine, Epidemiology, and Pathology, McMaster University, Hamilton, Ontario, Canada; and the Center for Cardiovascular Science, Edinburgh University and Royal Infirmary, Edinburgh, United Kingdom.

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Correspondence to Guillaume Paré, MD, Hamilton Health Sciences—General Site, Population Health Research Institute (DBCVSRI); C3-103, 237 Barton St E, Hamilton, ON, Canada L8L 2X2. E-mail pareg@mcmaster.ca

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(OR) of 3.6 (95% confidence interval [CI], 1.6–7.9; $P=0.003$) for QQ 192 homozygous individuals as compared with individuals with either the RR or QR genotype.³

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Clinical Perspective on p 256

The *PON1* gene encodes an esterase that hydrolyzes endogenous and exogenous esters, most notably organophosphate pesticides and nerve gas.⁴ *PON1* is also believed to enhance the antioxidant properties of high-density lipoproteins by breaking down biologically active oxidized phospholipids and oxidized cholesteryl esters,^{5–8} thereby preventing oxidation of low-density lipoproteins. Because of the importance of oxidized low-density lipoproteins in the initiation and progression of atherosclerotic plaque, *PON1* has also been proposed as an atherosclerotic susceptibility gene.^{9,10} The *PON1* Q192R polymorphism results in the substitution of glutamine (Q) for arginine (R) at position 192, and the Q allele has been associated with lower enzymatic activity^{11,12} and an increased risk of cardiovascular (CV) disease independent of clopidogrel action, although this remains controversial.^{13–15}

We hypothesized that the *PON1* polymorphism Q192R is associated with both a decreased efficacy of clopidogrel and an increased risk of CV events. To test this hypothesis, we investigated the efficacy and safety of clopidogrel among carriers of the Q192R polymorphism compared with noncarriers and the association between genotype and risk of CV disease in the placebo-controlled Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial that enrolled patients with ACS and the placebo-controlled Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events (ACTIVE A) that enrolled patients with atrial fibrillation.

Methods

The CURE Study

The design and results of the CURE trial have been described previously.^{16,17} In brief, CURE was a randomized, double-blind, placebo-controlled trial comparing clopidogrel (75 mg/d) with placebo on a background of aspirin (75–325 mg per day) in 12 562 patients with ACS without ST-segment elevation. For the current analyses, we used the same primary efficacy and safety outcomes as in the CURE trial.¹⁶ The first primary outcome was the composite of death from CV causes, nonfatal myocardial infarction, or stroke, and the second primary outcome was the composite of the first primary outcome, recurrent ischemia, or hospitalization for unstable angina. The main safety outcome was major bleeding. Results are presented only for individuals of European and Latin American ancestry. Individuals from other ethnic groups were excluded because of small numbers ($n=99$ for the next largest group) and concerns about the potential for population stratification.

The ACTIVE Study

The design and results of the Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events (ACTIVE A) study have been described previously.^{18,19} ACTIVE A was a randomized, double-blind trial comparing 75 mg/d clopidogrel with placebo for stroke prevention on a background of aspirin therapy (75–100 mg/d) in patients with atrial fibrillation and at least 1 additional risk factor for stroke who were not eligible for warfarin therapy. We adopted the primary efficacy and safety outcomes used in the ACTIVE trial.¹⁸ The primary efficacy outcome was any major

vascular event (stroke, non-central nervous system systemic embolism, myocardial infarction, or death from vascular causes). Major hemorrhage was defined as any overt bleeding requiring transfusion of at least 2 units of blood or any overt bleeding meeting the criteria for severe hemorrhage. The 58 individuals of non-European ancestry were excluded.

The institutional review board at each center approved each study, and all patients provided written informed consent. Only those patients who also consented to participate in either of the 2 genetic studies were eligible for this analysis (without any further selection criteria). Baseline characteristics of both genetic groups were similar to those of the CURE and ACTIVE study population, as previously reported.²⁰

Genotyping

Genotyping of the *PON1* Q192R polymorphism (rs662) was performed using TaqMan assays from stored DNA. The call rate was >98%, and Hardy-Weinberg equilibrium was tested within each ethnic group ($P>0.05$ in all groups).

Statistical Analysis

We first explored the effect of *PON1* Q192R genotypes on efficacy and safety of clopidogrel, and we then looked at the association between genotype and outcome. These analyses were restricted to CURE trial participants of European and Latin American ancestry. The effect of clopidogrel compared with placebo according to genotype was assessed using Cox proportional hazard regression under additive and dominant genetic models. No statistically significant ($P>0.05$) effect modification by ethnicity was observed for any of the pharmacogenetic effects described (data not shown), and results from Europeans and Latin Americans were therefore combined (with adjustment for ancestry).

We used separate models to adjust for (1) age, sex, and ethnicity and (2) age, sex, ethnicity, revascularization (PCI with or without stent, coronary artery bypass surgery), smoking, waist-to-hip ratio, diabetes, blood pressure, and country of origin. Similar results were obtained with both models and therefore only results obtained with the parsimonious model are presented. A 2-sided $P<0.05$ was considered significant throughout.

The same analytic approach that was used for CURE was used for ACTIVE A.

Results

Effect of *PON1* Genotype on Clopidogrel Efficacy in CURE

Characteristics of study participants are presented in the Table. A total of 5059 participants of European and Latin American self-defined ancestry were successfully genotyped, of whom 2510 were randomly assigned to placebo and 2549 to clopidogrel. The benefit of clopidogrel treatment on the first primary composite efficacy outcome (231 events, 9.1% versus 316 events, 12.6%; HR, 0.71; 95% CI, 0.60–0.84; $P<0.001$) was similar to the parent study¹⁶ (582 events, 9.3% versus 719 events, 11.4%; HR, 0.80; 95% CI, 0.72–0.90; $P<0.001$).

Figure 1 presents estimates of the hazard ratios of the first and second primary composite efficacy outcomes in patients treated with clopidogrel compared with placebo stratified by *PON1* Q192R genotype. A trend was observed for the first primary outcome whereby carriage of the Q allele was associated with greater efficacy of clopidogrel as compared with placebo, albeit this apparent heterogeneity was not statistically significant ($P=0.07$). Individuals carrying 2 Q alleles derived an increased benefit of clopidogrel (HR, 0.60; 95% CI, 0.47–0.77; $P<0.001$) as compared with individuals

Table. Baseline Characteristics of CURE-Genetics and ACTIVE-Genetics Participants

Characteristic	CURE-Genetics Study			ACTIVE-Genetics Study		
	Placebo	Clopidogrel	Total	Placebo	Clopidogrel	Total
No.	2510	2549	5059	586	570	1156
Ethnicity, %						
European	85.7	86.2	85.9	100	100	100
Latin American	14.3	13.8	14.1	0	0	0
Female, %	40.9	41.2	41.0	45.2	45.6	45.4
Age, y, mean (SD)	63.9 (11.1)	63.8 (11.0)	63.8 (11.0)	71.1 (9.7)	70.8 (10.1)	71.0 (9.9)
Body mass index, mean (SD)	27.6 (4.1)	27.7 (4.2)	27.6 (4.2)	29.1 (5.5)	28.9 (5.7)	29.0 (5.6)
Diabetes, %	21.5	20.7	21.1	20.6	21.8	21.2
Smoking, %	21.6	23.1	22.4	6.8	8.4	7.6
Systolic blood pressure, mm Hg, mean (SD)	134.6 (22.0)	135.5 (22.3)	135.0 (22.1)	136.3 (18.9)	136.6 (19.0)	136.4 (18.9)
Diastolic blood pressure, mm Hg, mean (SD)	78.3 (13.8)	78.6 (13.6)	78.4 (13.7)	80.3 (11.4)	80.9 (11.5)	80.6 (11.5)
PCI without stent, %	3.9	3.2	3.5	N/A	N/A	N/A
PCI with stent, %	13.5	15.5	14.5	N/A	N/A	N/A
CABG, %	10.4	9.8	10.1	N/A	N/A	N/A
Follow-up time, d, mean (SD)	277.8 (101.1)	279.7 (99.9)	278.8 (100.5)	1243.9 (429.8)	1248.3 (404.3)	1246.4 (416.3)
<i>PON1</i> Q192R genotype, %						
QQ	48.5	49.2	48.8	50.3	52.3	51.3
QR	42.1	40.8	41.5	41.7	39.3	40.5
RR	9.4	10.0	9.7	7.9	8.4	8.1

CURE indicates Clopidogrel in Unstable Angina to Prevent Recurrent Events trial; ACTIVE, Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft.

No variable was significantly different between treated and untreated subjects ($P > 0.05$) for both the CURE and ACTIVE datasets.

with either the QR (HR, 0.80; 95% CI, 0.62–1.05; $P = 0.10$) or RR (HR, 0.90; 95% CI, 0.50–1.64; $P = 0.74$) genotypes. This trend was less pronounced for the second primary end point or for major bleeds. The corresponding Kaplan-Meier survival curves are shown in Figure 2. For survival analysis, individuals of the QR and RR genotypes were grouped and compared with individuals of the QQ genotype, corresponding to a dominant genetic model. Results were consistent, with individuals of the QR and RR genotypes collectively deriving a similar benefit of clopidogrel (HR, 0.82; 95% CI, 0.65–1.05; $P = 0.12$). Likewise, no effect was observed in the

smaller CURE-PCI datasets (734 individuals with genotype; online-only Data Supplement Figure I). Power to detect an effect size similar as the one described by Bouman et al³ was estimated at $>99\%$ in clopidogrel-treated participants from the CURE dataset for the first and second primary efficacy outcomes.

Effect of *PON1* Genotype on CV Events in CURE

To further explore the association of *PON1* Q192R polymorphism with CV events, we performed similar analysis but stratifying individuals by treatment allocation and testing for

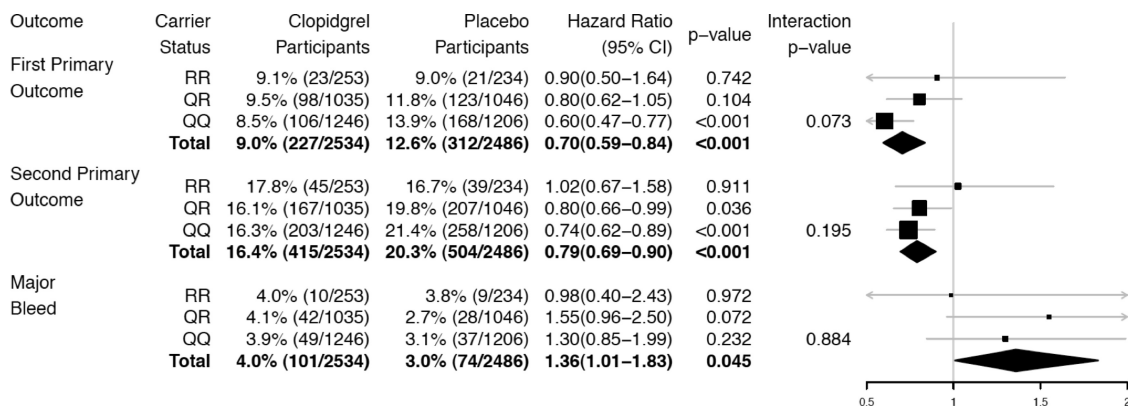


Figure 1. Effect of clopidogrel compared with placebo on clinical outcomes stratified by *PON1* Q192R genotype in the Clopidogrel in Unstable Angina to Prevent Recurrent Events trial. The first primary outcome was the composite of death from cardiovascular causes, nonfatal myocardial infarction, or stroke, and the second primary outcome was the composite of the first primary outcome, recurrent ischemia, or hospitalization for unstable angina. P value refers to the effect of clopidogrel versus placebo for each genotype subgroup. Interaction P value refers to heterogeneity of effect of clopidogrel versus placebo between genotype subgroups.

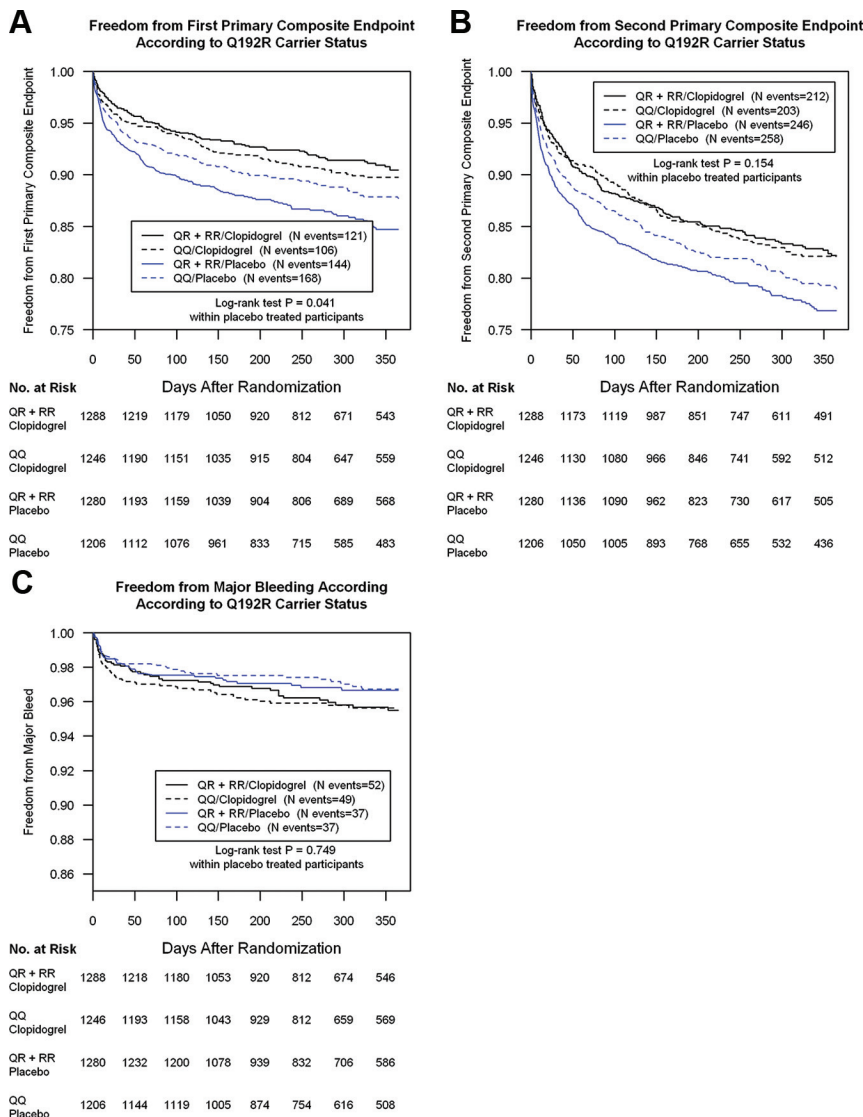


Figure 2. Kaplan-Meier event-free survival according to *PON1* Q192R carrier status in Clopidogrel in Unstable Angina to Prevent Recurrent Events trial. The first primary outcome was the composite of death from cardiovascular causes, nonfatal myocardial infarction, or stroke, and the second primary outcome was the composite of the first primary outcome, recurrent ischemia, or hospitalization for unstable angina. **A** represents the survival curves for the first primary composite endpoint; **B** represents the survival curves for the second primary composite endpoint; and **C** represents the survival curves for major bleeding.

association with Q192R genotype, using an additive genetic model (Figure 3). Overall, there was no association between the Q allele and the first primary outcome when combining clopidogrel and placebo-treated participants (HR, 1.09 per allele; 95% CI, 0.95–1.24; $P=0.23$) and no evidence of

heterogeneity ($P=0.07$). The Q allele was significantly associated with incidence of the first primary outcome in placebo-treated participants (HR, 1.23 per allele; 95% CI, 1.03–1.47; $P=0.03$) but not in clopidogrel-treated individuals (HR, 0.93 per allele; 95% CI, 0.76–1.13; $P=0.46$). A similar trend was

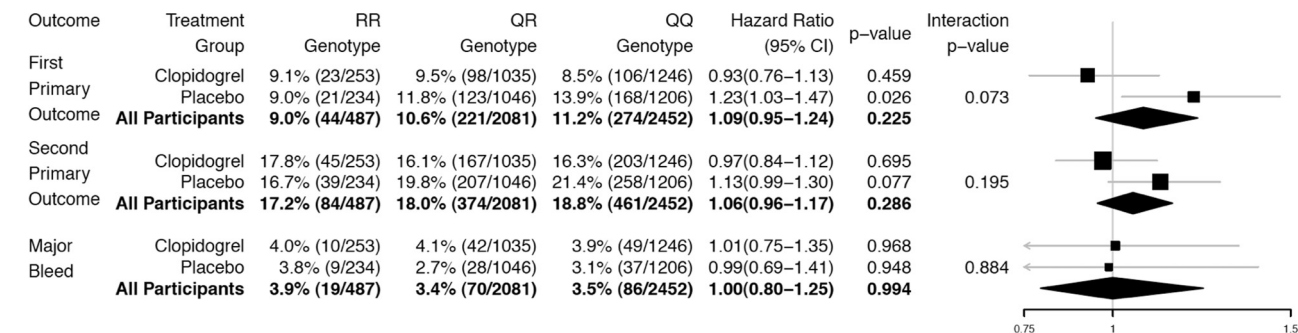


Figure 3. Effect of *PON1* Q192R genotype clopidogrel on clinical outcomes stratified by treatment allocation in Clopidogrel in Unstable Angina to Prevent Recurrent Events trial. The first primary outcome was the composite of death from cardiovascular causes, nonfatal myocardial infarction, or stroke, and the second primary outcome was the composite of the first primary outcome, recurrent ischemia, or hospitalization for unstable angina. P value refers to the additive (per allele) genetic effect of the R allele for each treatment group. Interaction P value refers to heterogeneity of additive genetic effects of the R allele between treatment groups.

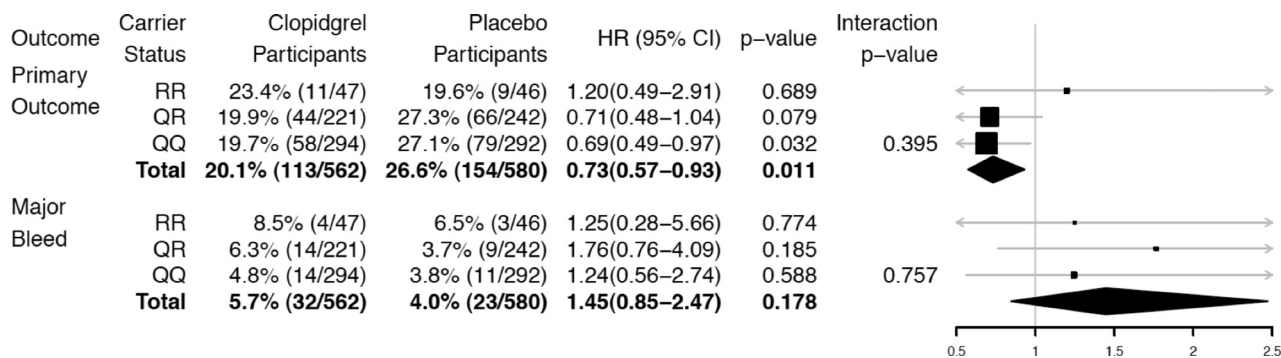


Figure 4. Effect of clopidogrel compared with placebo on clinical outcomes stratified by *PON1* Q192R genotype in Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events. The primary efficacy outcome was any major vascular event (stroke, non-central nervous system systemic embolism, myocardial infarction, or death from vascular causes). *P* value refers to the effect of clopidogrel versus placebo for each genotype subgroup. Interaction *P* value refers to heterogeneity of effect of clopidogrel versus placebo between genotype subgroups.

observed for the second primary outcome in placebo-treated individuals (HR, 1.13 per allele; 95% CI, 0.99–1.30); $P=0.08$) but not in clopidogrel-treated individuals (HR, 0.97 per allele; 95% CI, 0.84–1.12; $P=0.70$).

Effect of *PON1* Genotype on Clopidogrel Efficacy and CV Events in ACTIVE

Characteristics of study participants are summarized in the Table. A total of 1156 participants of European self-defined ancestry were successfully genotyped, of whom 586 were randomly assigned to placebo and 570 to clopidogrel. The benefit of clopidogrel on the primary composite efficacy outcome (114 events, 20.0% versus 154 events, 26.3%; HR, 0.74; 95% CI, 0.58–0.94; $P=0.01$) was comparable with the benefit reported in the parent study¹⁸ (832 events, 22.1% versus 924 events, 24.4%; HR, 0.89; 95% CI, 0.81–0.98; $P=0.01$). The results were consistent in subgroups based on *PON1* genotype (Figure 4). No effect of *PON1* on CV events was observed in either clopidogrel-treated or placebo-treated participants.

Discussion

Our results suggest that *PON1* Q192R genotype does not modify the effect of clopidogrel. We also found that contrary to a previous report, the QQ genotype was not associated with an increased hazard of major CV events in individuals treated with clopidogrel. In fact, a trend was observed toward greater benefit of clopidogrel in individuals carrying 2 Q alleles in CURE, mainly because the Q allele was associated with adverse events in placebo-treated individuals. Our results are consistent with the recently published null association of *PON1* Q192R polymorphism with stent thrombosis²¹ and CV events.^{22,23} They also support the lack of association between Q192R genotype and platelet aggregation.^{22,23}

Several reports have investigated the association of Q192R with coronary heart disease, with mixed results. A large meta-analysis of 39 studies comprising 10 738 cases and 17 068 control subjects reported a pooled odds ratio of 1.10 (95% CI, 1.06–1.13) per R allele for coronary heart disease.¹³ The prospective REGRESS study of 793 secondary prevention patients reported a hazard ratio of 1.71 (95% CI, 1.0–2.8; $P=0.03$) per Q allele for death due to

ischemic disease.²⁴ Finally, the GeneBank study of 1399 sequential patients undergoing diagnostic coronary angiography reported that the Q allele was associated with lower *PON1* activity, increased levels of systemic indices of oxidative stress, and an increased risk of major adverse CV events (HR, 1.48; 95% CI, 1.09–2.03 for QQ versus QR and RR).²⁵ These latter 2 reports are particularly relevant to our study because they included a large fraction of individuals with established CV disease (100% and 80%, respectively). Although the use of clopidogrel was not reported, neither study is likely to have included a substantial number of patients on clopidogrel therapy because the REGRESS study was conducted between 1989 and 1993 (ie, before the approval of clopidogrel) and the GeneBank study recruited patients undergoing elective diagnostic coronary angiography in 2002 and 2003.

Our results offer a further caution against the interpretation of pharmacogenetic data in the absence of a nontreated control group. Such analyses should ideally be performed in the context of a randomized, controlled trial to minimize the potential for confounding. Prior reports have provided evidence for a role of *PON1* genotype in atherosclerosis irrespective of clopidogrel treatment. Thus, any pharmacogenetic analysis performed exclusively in clopidogrel-treated individuals could reflect a genetic effect that is independent of clopidogrel treatment, as opposed to a therapeutic failure of clopidogrel. Our data support an effect of *PON1* genotype on CV risk in patients not receiving clopidogrel, although we cannot explain the lack of association between *PON1* Q192R genotype and outcome in individuals treated with clopidogrel. Our results could reflect a hitherto unknown pharmacological interaction but might also be explained by lack of statistical power, given the smaller number of events in individuals treated with clopidogrel. Nevertheless, our data argue against a detrimental effect of the Q allele on clopidogrel metabolism.

Our study has a few potential limitations. First, despite the large number of participants and events in the CURE genetic datasets, our study cannot exclude smaller interactions, especially for the PCI subgroup. Furthermore, stent thrombosis was not adjudicated in CURE, precluding direct comparison with the study by Bouman et al. Nev-

ertheless, our data provide randomized confirmation of a lack of deleterious effect of the Q allele on clopidogrel efficacy on spontaneous CV events not induced by PCI. The ACTIVE genetic dataset contained fewer participants and outcome events than the CURE dataset and therefore had less statistical power. Second, our results might be specific to our patient population and how they were treated. Third, only participants of European and Latin American ancestries could be adequately analyzed. Even though there is no reason to suspect different results in other populations a priori, further studies in diverse populations will be needed. It should be noted that inclusion of randomized groups lessens the risk of confounding by population stratification because individuals in any strata were equally likely to receive clopidogrel or placebo.

In conclusion, our study shows that *PON1* Q192R genotype does not modify the efficacy and safety of clopidogrel in ACS or atrial fibrillation patients. Further studies will be needed to confirm the association of the Q allele with adverse CV events independent of clopidogrel in secondary prevention patients. Taken together, these results emphasize the need for randomized comparison groups in pharmacogenetic studies.

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References

1. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med.* 2001;345:494–502.
2. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, et al. Association of cytochrome p450 2c19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA.* 2009;302:849–857.
3. Bouman HJ, Schomig E, van Werkum JW, Velder J, Hackeng CM, Hirschhauser C, et al. Paraoxonase-1 is a major determinant of clopidogrel efficacy. *Nat Med.* 2011;17:110–116.
4. Camps J, Marsillach J, Joven J. The paraoxonases: role in human diseases and methodological difficulties in measurement. *Crit Rev Clin Lab Sci.* 2009;46:83–106.
5. Mackness MI, Arrol S, Mackness B, Durrington PN. Alloenzymes of paraoxonase and effectiveness of high-density lipoproteins in protecting low-density lipoprotein against lipid peroxidation. *Lancet.* 1997;349:851–852.
6. Antikainen M, Murtomaki S, Syvanne M, Pahlman R, Tahvanainen E, Jauhainen M, et al. The gln-arg191 polymorphism of the human paraoxonase gene (humpona) is not associated with the risk of coronary artery disease in Finns. *J Clin Invest.* 1996;98:883–885.
7. Herrmann SM, Blanc H, Poirier O, Arveiler D, Luc G, Evans A, et al. The gln/arg polymorphism of human paraoxonase (pon 192) is not related to myocardial infarction in the ECTIM study. *Atherosclerosis.* 1996;126:299–303.
8. Suehiro T, Nakauchi Y, Yamamoto M, Arai K, Itoh H, Hamashige N, Hashimoto K. Paraoxonase gene polymorphism in Japanese subjects with coronary heart disease. *Int J Cardiol.* 1996;57:69–73.
9. Mackness B, Durrington P, McElduff P, Yarnell J, Azam N, Watt M, Mackness M. Low paraoxonase activity predicts coronary events in the Caerphilly prospective study. *Circulation.* 2003;107:2775–2779.
10. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2001;21:473–480.
11. Adkins S, Gan KN, Mody M, La Du BN. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective a or b allozymes. *Am J Hum Genet.* 1993;52:598–608.
12. Humbert R, Adler DA, Distechi CM, Hassett C, Omiecinski CJ, Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat Genet.* 1993;3:73–76.
13. Lawlor DA, Day IN, Gaunt TR, Hinks LJ, Briggs PJ, Kiessling M, et al. The association of the pon1 q192r polymorphism with coronary heart disease: findings from the British Women's Heart and Health Cohort Study and a meta-analysis. *BMC Genet.* 2004;5:17.
14. Wheeler JG, Keavney BD, Watkins H, Collins R, Danesh J. Four paraoxonase gene polymorphisms in 11212 cases of coronary heart disease and 12786 controls: meta-analysis of 43 studies. *Lancet.* 2004;363:689–695.
15. Wang M, Lang X, Zou L, Huang S, Xu Z. Four genetic polymorphisms of paraoxonase gene and risk of coronary heart disease: a meta-analysis based on 88 case-control studies. *Atherosclerosis.* 2011;214:377–385.
16. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet.* 2001;358:527–533.
17. Mehta SR, Yusuf S; Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) Study Investigators. The Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial programme: rationale, design and baseline characteristics including a meta-analysis of the effects of thienopyridines in vascular disease. *Eur Heart J.* 2000;21:2033–2041.
18. Connolly SJ, Pogue J, Hart RG, Hohnloser SH, Pfeffer M, Chrolavicius S, Yusuf S. Effect of clopidogrel added to aspirin in patients with atrial fibrillation. *N Engl J Med.* 2009;360:2066–2078.
19. Connolly S, Yusuf S, Budaj A, Camm J, Chrolavicius S, Commerford PJ, et al. Rationale and design of active: the atrial fibrillation clopidogrel trial with irbesartan for prevention of vascular events. *Am Heart J.* 2006;151:1187–1193.
20. Pare G, Mehta SR, Yusuf S, Anand SS, Connolly SJ, Hirsh J, et al. Effects of cyp2c19 genotype on outcomes of clopidogrel treatment. *N Engl J Med.* 2010;363:1704–1714.
21. Sibbing D, Koch W, Massberg S, Byrne RA, Mehilli J, Schulz S, et al. No association of paraoxonase-1 Q192R genotypes with platelet response to

- clopidogrel and risk of stent thrombosis after coronary stenting. *Eur Heart J*. 2011;32:1605–1613.
22. Lewis JP, Fisch AS, Ryan K, O'Connell JR, Gibson Q, Mitchell BD, et al. Paraoxonase 1 (pon1) gene variants are not associated with clopidogrel response. *Clin Pharmacol Ther*. 2011;90:568–574.
 23. Trenk D, Hochholzer W, Fromm MF, Zolk O, Valina CM, Stratz C, Neumann FJ. Paraoxonase-1 q192r polymorphism and antiplatelet effects of clopidogrel in patients undergoing elective coronary stent placement. *Circ Cardiovasc Genet*. 2011;4:429–436.
 24. Regieli JJ, Jukema JW, Doevendans PA, Zwinderman AH, Kastelein JJ, Grobbee DE, van der Graaf Y. Paraoxonase variants relate to 10-year risk in coronary artery disease: impact of a high-density lipoprotein-bound antioxidant in secondary prevention. *J Am Coll Cardiol*. 2009;54:1238–1245.
 25. Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X, Schmitt D, et al. Relationship of paraoxonase 1 (pon1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA*. 2008;299:1265–1276.

CLINICAL PERSPECTIVE

It has recently been suggested that efficacy of the antiplatelet agent clopidogrel depends on biotransformation by the PON1 enzyme. The *PON1* gene carries a common genetic variant—Q192R—which has been shown to be associated with stent thrombosis in patients treated with clopidogrel. In this latter study, carriers of the Q allele had decreased biotransformation of clopidogrel and increased cardiovascular events. In contrast to previous results, we show that the *PON1* genetic variant Q192R does not influence efficacy and safety of clopidogrel in 2 large, randomized studies. In the Clopidogrel in Unstable Angina to Prevent Recurrent Events trial of clopidogrel versus placebo in non-ST-elevation–myocardial infarction (NSTEMI) patients, clopidogrel was equally effective at reducing cardiovascular events irrespective of the presence of the Q allele. Similarly, in the Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events of clopidogrel versus placebo in high-risk atrial fibrillation patients, no effect of the *PON1* genetic variant was observed. In conclusion, our study shows that the Q192R genetic variant does not modify the efficacy and safety of clopidogrel in NSTEMI and atrial fibrillation patients.

SUPPLEMENTAL MATERIALS

Supplemental Figure Legend

Supplementary Figure 1. Effect of clopidogrel compared with placebo on clinical outcomes stratified by PON1 Q192R genotype in stented ACS patients from the CURE trial

Supplementary Figure 1.

