

# CYP2C19 Genotype, Clopidogrel Metabolism, Platelet Function, and Cardiovascular Events

## A Systematic Review and Meta-analysis

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**C**LOPIDOGREL IS AN ANTI-platelet drug used by approximately 40 million patients worldwide<sup>1,2</sup> to treat or prevent atherothrombotic events and after percutaneous coronary revascularization. An overview of randomized trials including 7384 cardiovascular events in 79 613 patients with acute or stable coronary heart disease (CHD) or with multiple CHD risk factors demonstrated an association of clopidogrel therapy with reduced rates of cardiovascular events (odds ratio [OR], 0.88; 95% CI, 0.83-0.93) compared with placebo. Clopidogrel is also associated with a mechanism-based increase in major bleeding (OR, 1.28; 95% CI, 1.13-1.45).<sup>3</sup> Despite the overall benefit, some individuals may be less responsive to clopidogrel than others<sup>4</sup> because clopidogrel is a prodrug activated by several enzymes, including CYP2C19,<sup>5</sup> and common genetic variation in CYP2C19 alters enzyme activity.<sup>6,7</sup>

For editorial comment see p 2727.



CME available online at [www.jamaarchivescme.com](http://www.jamaarchivescme.com) and questions on p 2736.

**Context** The US Food and Drug Administration recently recommended that CYP2C19 genotyping be considered prior to prescribing clopidogrel, but the American Heart Association and American College of Cardiologists have argued evidence is insufficient to support CYP2C19 genotype testing.

**Objective** To appraise evidence on the association of CYP2C19 genotype and clopidogrel response through systematic review and meta-analysis.

**Data Sources** PubMed and EMBASE from their inception to October 2011.

**Study Selection** Studies that reported clopidogrel metabolism, platelet reactivity or clinically relevant outcomes (cardiovascular disease [CVD] events and bleeding), and information on CYP2C19 genotype were included.

**Data Extraction** We extracted information on study design, genotyping, and disease outcomes and investigated sources of bias.

**Results** We retrieved 32 studies of 42 016 patients reporting 3545 CVD events, 579 stent thromboses, and 1413 bleeding events. Six studies were randomized trials ("effect-modification" design) and the remaining 26 reported individuals exposed to clopidogrel ("treatment-only" design). In treatment-only analysis, individuals with 1 or more CYP2C19 alleles associated with lower enzyme activity had lower levels of active clopidogrel metabolites, less platelet inhibition, lower risk of bleeding (relative risk [RR], 0.84; 95% CI, 0.75-0.94; absolute risk reduction of 5-8 events per 1000 individuals), and higher risk of CVD events (RR, 1.18; 95% CI, 1.09-1.28; absolute risk increase of 8-12 events per 1000 individuals). However, there was evidence of small-study bias (Harbord test  $P = .001$ ). When analyses were restricted to studies with 200 or more events, the point estimate was attenuated (RR, 0.97; 95% CI, 0.86-1.09). In effect-modification studies, CYP2C19 genotype was not associated with modification of the effect of clopidogrel on CVD end points or bleeding ( $P > .05$  for interaction for both). Other limitations included selective outcome reporting and potential for genotype misclassification due to problems with the \* allele nomenclature for cytochrome enzymes.

**Conclusion** Although there was an association between the CYP2C19 genotype and clopidogrel responsiveness, overall there was no significant association of genotype with cardiovascular events.

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In 2009, the US Food and Drug Administration (FDA) issued a boxed warning,<sup>8</sup> recommending "consideration of CYP2C19 genotype" prior to prescribing clopidogrel. However, the American Heart Association and American College of Cardiologists argued there was insufficient evidence for this

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warning.<sup>9</sup> Amid this uncertainty, FDA-cleared direct-to-consumer tests<sup>10</sup> are available and demand is escalating.<sup>11</sup>

We conducted a systematic review and critical appraisal to assess the strength and quality of evidence on the association of *CYP2C19* genotype with responsiveness to clopidogrel.

## METHODS

### Search Strategy

Following guidance from the Human Genome Epidemiology Network (HuGENet) on gene-disease association studies<sup>12</sup> and the reporting of systematic reviews from PRISMA,<sup>13</sup> we searched PubMed and EMBASE from their inception to October 25, 2011. The search terms comprised the following<sup>14</sup>: drug name (including the generic name, clopidogrel, and trade names, eg, Plavix, clopilet, etc); metabolic enzymes (including *CYP2C19*); and gene (see the eMethods, available at <http://www.jama.com>, for full search strings) to identify studies describing associations of clopidogrel therapy with responsiveness (platelet response or clinical outcomes) in relation to *CYP2C19* genotype (NCBI Entrez Gene 1557). Eligible articles had abstracts containing the keywords *clopidogrel* and *CYP2C19* that reported original data (editorials and reviews were omitted). We interrogated bibliographies of included articles, identified previous meta-analyses and narrative reviews, and searched articles listed in the Pharmacogenomics Knowledge Base,<sup>15</sup> the Medicines and Healthcare products Regulatory Agency (UK Department of Health), and the FDA. There was no language restriction.

Information extracted on 2 separate occasions included study design; follow-up duration; proportion of eligible individuals included in the pharmacogenetic analysis; inclusion criteria, eg, acute coronary syndrome (ACS), emergency or elective percutaneous intervention, or stable CHD; and the proportion of individuals receiving concomitant therapy with proton-pump inhibitors or aspirin. Uncertainties were resolved by contact with corresponding authors or by consensus. To evalu-

ate risk of bias, we recorded reporting of outcome ascertainment, whether studies reported blinding to case status when ascertaining genotype, genotype indices (Hardy Weinberg equilibrium and call rate), and the source of funding.

The main analysis compared individuals with 1 or more copies of any *CYP2C19* genetic variant associated with reduced enzyme function (ie, \*2, \*3, \*4, \*5, \*6, \*7, \*8) with individuals categorized as having none of these alleles (\*1/\*1) or having 1 or more \*17 gain-of-function alleles (the reference group). Separately, we conducted analyses of the association of 1 loss-of-function or 2 loss-of-function alleles with the same reference group. The primary cardiovascular outcome consisted of 1 or more of the following: all-cause mortality, fatal and nonfatal CHD, fatal and nonfatal stroke, stent thrombosis, target vessel revascularization, and hospitalization for ACS. Studies that reported stent thrombosis and no other outcome were excluded from the primary analysis. Additionally, we conducted separate analyses for the following end points: fatal and nonfatal myocardial infarction and stroke, stent thrombosis, and all-cause mortality. In studies that only reported hazard ratios, if provided we used the hazard proportion to estimate the number of events per genotype group. Otherwise, we substituted the relative risk (RR) for the summary hazards ratio. For studies that did not provide numerical values of effect estimates, we derived the point estimates and 95% confidence intervals from the published forest plots. We conducted similar analyses for the end points of any bleeding and major bleeding as defined by the contributing studies. For analysis of the association of *CYP2C19* genotype and clopidogrel metabolites, we referred to the largest study by Mega et al.<sup>16</sup> For studies of *CYP2C19* genotype and platelet function, we noted the number of individuals and the mean (or median) and standard deviation (or interquartile range) for the relevant platelet function measure in different genotype categories.

### Analyses

For binary outcomes, we used RR as a measure of effect. We pooled studies using random- and fixed-effects models. Results reported in all figures and tables are from fixed-effects models unless otherwise stated. Sources of heterogeneity were investigated using meta-regression and quantified using the  $I^2$  statistic.<sup>17</sup> Tests for interaction using summary estimates were performed using the method described by Altman and Bland.<sup>18</sup> For studies reporting platelet reactivity, we restricted the analysis to those with more than 500 participants and calculated standardized mean differences using fixed-effects (inverse variance) models. Small-study bias was assessed through the following: comparison of effect estimates in studies stratified by number of cardiovascular disease (CVD) events (1-99, 100-199,  $\geq 200$ ), the Harbord test, visual inspection of funnel plots, and trim-and-fill analysis. To evaluate the change in effect estimates with the addition of new evidence, we created cumulative meta-analysis plots. We estimated absolute risk differences between genotype categories based on the control group event rate from the CURE<sup>19</sup> (ACS) and CHARISMA<sup>20</sup> (stable CHD) trials and the trim-and-fill summary RR (to minimize small-study bias), comparing carriers of any loss-of-function *CYP2C19* allele vs the reference category, and assuming consistency of RRs across a range of baseline absolute risks of cardiovascular and bleeding events. We used a *P* value of  $<.05$  to indicate statistical significance. All statistical tests were 2-sided. We used Stata version 11.2 for statistical analyses.

## RESULTS

### Studies, Genotyping, and Outcomes

We identified 32 studies<sup>16,21-50</sup> including 42 016 participants (29% female), with a weighted mean age of 63 years (TABLE, eTable 1, and eFigure 1). Twenty-one studies included patients with ACS at the time of recruitment and 8 included patients with stable CHD,

**Table.** Characteristics of Eligible Studies

Source	Outcomes Reported	ACS at Study Entry, % <sup>a</sup>	Women, %	Age, Mean (SD), y	Participants in PGx Substudy, No. (%) <sup>b</sup>	Duration of Follow-up, mo (Range)	Clopidogrel Dose, mg		CYP2C19* Alleles Genotyped and Reported (NR)
							Loading	Maintenance	
<b>Treatment-Only Studies (All Individuals Were Treated With Clopidogrel)</b>									
Anderson et al, <sup>21</sup> 2009	CVD-c, MI, death	NR	27	63 (NR)	1250 (100)	12 (NR-24)	NR	NR	*2
Bouman et al, <sup>23</sup> 2011	STTH	50.9	21	61.3 (7.7)	112 (1.5)	18 (NR)	600/300/0	75	*2, *17 (*3, *4, *5, *6, *7, *8)
Campo et al, <sup>24</sup> 2011	CVD-c, death, STTH, bleeding	61	23	66 (13)	300 (59.2)	12 (NR)	600	75	*2, *17
Collet et al, <sup>25</sup> 2009	CVD-c, MI, STTH, death	100	7.7	40.1 (5.1)	259 (68.5)	12 (NR-96)	NR	75	*2 (*3, *4, *5, *6)
Giusti et al, <sup>26</sup> 2009	CVD-c, STTH, death	65.67 <sup>c</sup>	25.4 <sup>c</sup>	69 (11) <sup>c</sup>	772 (96)	6 (NR)	600	75	*2
Harmsze et al, <sup>28</sup> 2010	STTH	24.6 <sup>d</sup>	20.5 <sup>d</sup>	62.1 (9.4) <sup>d</sup>	596 (NA)	NA	NR	NR	*2, *3
Harmsze et al, <sup>27</sup> 2011	CVD-c	0	24	63.2 (10.2)	725 (68)	12 (NR)	600/300	75	*2, *17
Jeong et al, <sup>29</sup> 2011	CVD-c	100	30.9	63.0 (12.4)	266 (48)	>12 (NR)	NR	NR	*2, *3, *17
Komarov et al, <sup>30</sup> 2011	CVD-c	0	NR	59.4 (NR)	399 (NR)	18 (NR)	NR	75-150	*2
Malek et al, <sup>31</sup> 2008	MI, STTH	100	30	60 (11.4)	105 (100)	12 (NR)	600/300	75	*2
Malek et al, <sup>32</sup> 2010	Death	100	33	60 (11)	261 (94.6)	48 (NR)	600/300	75	*2
Oh et al, <sup>34</sup> 2011	CVD-c, MI, death, STTH	20.6	34.3	60.8 (9.8)	2146 (64.8)	12 (NR)	600/300	75	*2
Ono et al, <sup>35</sup> 2011	CVD-c	0	24.3	68.8 (9.8)	202 (100)	≤12 (NR)	300	75	*2, *3
Sawada et al, <sup>37</sup> 2010	CVD-c, MI, death, STTH	9	15	69.6 (9.9)	100 (27.3)	8 (3)	300	75	*2
Shuldiner et al, <sup>38</sup> 2009	CVD-c	0	40.1	64.3 (11.2)	227 (100)	12 (NR)	600/300	75	*2
Sibbing et al, <sup>41</sup> 2009	CVD-c, death, MI, stroke, STTH	34	22	66.5 (10.2)	2485 (46.2)	1 (0.8-1.2)	600	75	*2
Sibbing et al, <sup>39</sup> 2010	CVD-c, death, MI, STTH, bleeding	11	22.6	67.4 (10.7)	1524 (94.8)	1 (NR)	600	75	*17
Sibbing et al, <sup>40</sup> 2011	STTH	NR	22.4 <sup>d</sup>	67.5 (10.4) <sup>d</sup>	1566 (95)	NA	600	75	*2
Simon et al, <sup>42</sup> 2009	CVD-c	100	29.4	66.2 (12.2)	2208 (60.2)	12 (NR)	<300 to 900	75	*2, *3, *4, *5, *17
Tello-Montoliu et al, <sup>43</sup> 2011	CVD-c	100	NR	NR	471 (95.5)	6 (NR)	NR	NR	*2, *17
Tiroch et al, <sup>44</sup> 2010	CVD-c, death, MI, STTH, stroke	100	25	64.8 (12.7)	928 (100)	12 (NR)	600	75	*2, *17
Trenk et al, <sup>45</sup> 2008	CVD-c	0	21.8	66.4 (9.1)	797 (99.4)	12 (NR)	600	75	*2
Worrall et al, <sup>47</sup> 2009	CVD-c	100	NR	NR	104 (40.2)	12 (NR)	NR	75	*2
Yamamoto et al, <sup>48</sup> 2011	MI, death, stroke	0	33	68.6 (10.0)	123 (100)	12 (NR)	300	75	*2, *3
Yan et al, <sup>49</sup> 2011	CVD-c	100	17.3	65.2 (10.7)	497 (100)	20 (NR)	NR	NR	*2
Yuan et al, <sup>50</sup> 2011	CVD-c	NR	NR	NR	267 (NR)	12 (NR)	NR	NR	*2
<b>Studies Nested Within Randomized Trials Without Effect-Modification Analysis<sup>e</sup></b>									
Mega et al, <sup>16</sup> 2009	CVD-c, MI, stroke, STTH, bleeding	100	29.3	60.1 (11.1)	1477 (10.9)	NR (6-15) <sup>f</sup>	300	75	*2, *3, *4, *5, *8 (*6, *7, *9, *10, *12, *13, *14)
Wallentin et al, <sup>46</sup> 2010	CVD-c, bleeding, STTH	100	31	62.5 (11.0)	10285 (55.2)	NR (NR-12)	600/300	75	*2, *3, *4, *5, *6, *7, *8, *17
<b>Studies Nested Within Randomized Trials With Effect-Modification Analysis</b>									
Bhatt et al, <sup>22</sup> 2009	CVD-c, bleeding	0	29.7 <sup>g</sup>	64 (9) <sup>g</sup>	4862 (31.2)	28 (NR)	0	75	*2, *3, *17
Mega et al, <sup>33</sup> 2008	CVD-c	100	19.7 <sup>h</sup>	57.5 (10.3) <sup>h</sup>	465 (13.3)	1 (NR)	300	75	*2
Pare et al, <sup>36</sup> 2010 <sup>i</sup>	CVD-c, bleeding	0 <sup>j</sup>	45.4	71.0 (9.9)	1156 (15.3)	43.2 (NR) <sup>j</sup>	0 <sup>j</sup>	75	*2, *3, *17
Pare et al, <sup>36</sup> 2010 <sup>i</sup>	CVD-c, bleeding	100	41.0	63.8 (11.0)	5059 (40.3)	NR (3-12)	300 <sup>k</sup>	75	*2, *3, *17

Abbreviations: ACS, acute coronary syndrome, comprising ST-segment elevation myocardial infarction, non-ST-segment elevation myocardial infarction, and unstable angina; CVD-c, cardiovascular disease composite; MI, myocardial infarction; NA, not applicable; NR, not reported; PCI, percutaneous coronary intervention; PGx, pharmacogenetic; STTH, stent thrombosis.

<sup>a</sup>Patients with non-ACS coronary heart disease (ie, stable coronary heart disease) were recruited at the time of PCI and stent insertion with the exception of CHARISMA trial,<sup>20</sup> which evaluated the addition of clopidogrel to aspirin in patients with stable CVD (or risk factors for CVD) not undergoing PCI.

<sup>b</sup>Number and percentage of total possible study participants who were included in the pharmacogenetic analysis.

<sup>c</sup>Derived from RECLOSE original study.<sup>51</sup>

<sup>d</sup>Values for controls only.

<sup>e</sup>Effect-modification analysis was not permitted because either the study did not genotype the comparator group (Mega et al<sup>16</sup>), or the comparator group was not placebo (Wallentin et al<sup>46</sup>).

<sup>f</sup>Derived from original TRITON-TIMI 38 trial.<sup>52</sup>

<sup>g</sup>Derived from original CHARISMA trial.<sup>20</sup>

<sup>h</sup>Obtained from original CLARITY-TIMI 28 trial.<sup>53</sup>

<sup>i</sup>Published in same article.

<sup>j</sup>Obtained from original ACTIVE-A trial.<sup>54</sup>

<sup>k</sup>Obtained from original CURE trial.<sup>19</sup>

mainly recruited at the time of coronary stent placement. (The remaining 3 studies did not report CHD status.) Of 32 studies, only 6 were nested within a randomized trial<sup>16,22,33,36,46</sup>; in 5 of these, *CYP2C19* genotyping was conducted in participants from both the clopidogrel and comparator groups. In 4 (referred to as “effect-modification” studies), the comparator was placebo (CURE, ACTIVE-A, CHARISMA, and CLARITY-TIMI 28<sup>22,33,36</sup>). In the PLATO trial,<sup>46</sup> the comparator was ticagrelor. In the pharmacogenetic substudy of the TRITON-TIMI 38 trial,<sup>16</sup> only the clopidogrel group was genotyped. All but 2 of the remaining 26 studies were prospective patient cohorts<sup>‡</sup> including participants who were all receiving clopidogrel (referred to as “treatment-only” studies). The remaining 2 were retrospective case-control studies.<sup>28,40</sup>

‡References 21, 23-27, 29-32, 34, 35, 37-39, 41-45, 47-50.

Of the 28 *CYP2C19* variant (\*) alleles identified to date,<sup>55</sup> 13 were evaluated in the pharmacogenetic studies of clopidogrel (the loss-of-function \*2 through \*10 and \*12 through \*14 inclusive, as well as the gain-of-function \*17) (Table). Individuals without a *CYP2C19* variant associated with slow or fast metabolism were assumed to possess the \*1 allele, indicating normal enzyme function. The \*2 allele was typed by 31 of 32 studies (97%); both \*3 and \*17 alleles by 12 studies (38%), and the remaining 10 \* alleles by 5 or fewer studies. Only 6 of the 28 single-nucleotide polymorphisms (SNPs) that uniquely identify the known *CYP2C19* \* alleles were listed in the Human HapMap (builds 21, 22, and 3[2]).<sup>56</sup> Only 2 studies reported detailed linkage disequilibrium between *CYP2C19* SNPs,<sup>28,38</sup> and 1 study imputed an untyped SNP.<sup>33</sup>

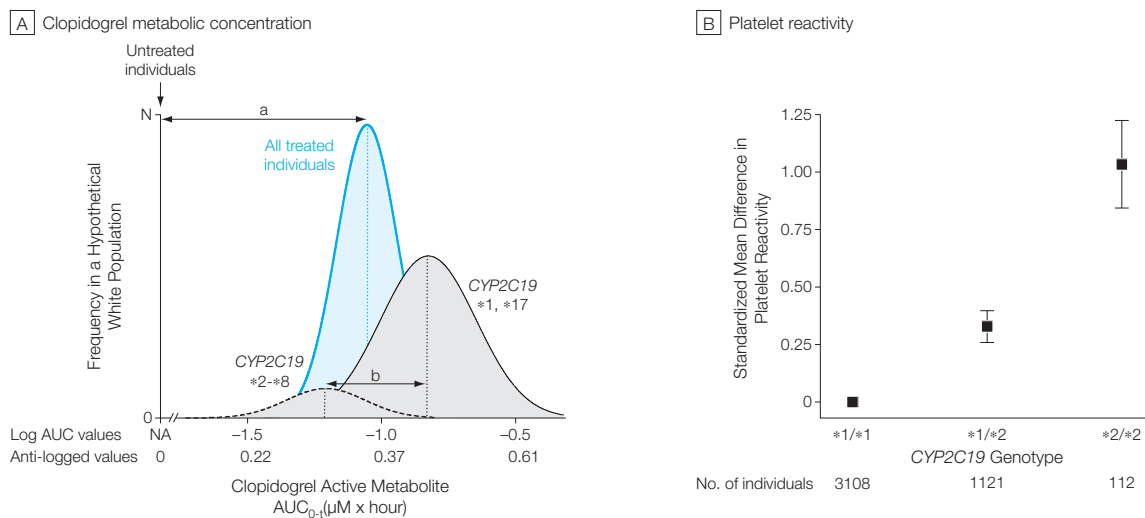
Twenty-six of 32 studies (81%) evaluated a composite outcome, but the individual components varied substan-

tially between studies and included both hard clinical end points (eg, ST-segment elevation myocardial infarction) and softer end points (eg, hospitalization for ACS). A considerable proportion of disease outcomes were ascertained but not reported either individually or as part of the combined end point (eTable 2A and B).

### CYP2C19 Genotype and Clopidogrel Metabolites and Platelet Reactivity

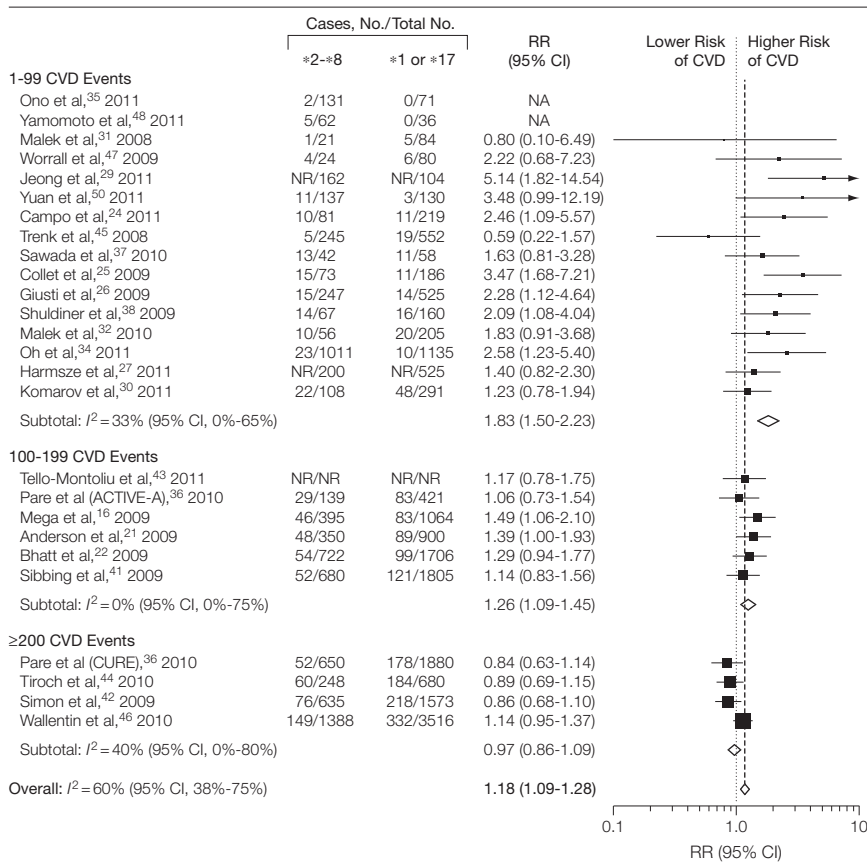
Among individuals with the loss-of-function alleles \*2 through \*8 receiving a dose of 75 mg of clopidogrel (the usual maintenance dose), the mean area under the plasma concentration-time curve from the time of administration to the last measurable concentration ( $AUC_{0-t}$ ) for the active clopidogrel metabolite was lower by 0.14  $\mu\text{M} \times \text{hour}$  compared with individuals carrying \*1 or \*17 alleles (FIGURE 1A). The difference in the active metabolite concen-

**Figure 1.** Relationship Between *CYP2C19* Genotype, Active Drug Metabolite, and Platelet Reactivity



A, The expected mean active clopidogrel metabolite concentration in a white population for all individuals treated with 75 mg and for individuals with loss-of-function and normal/increased-function *CYP2C19* alleles. <sup>a</sup> indicates mean active clopidogrel metabolite concentration regardless of genotype, area under the plasma concentration-time curve from the time of administration to the last measurable concentration ( $AUC_{0-t}$ ) = 0.35  $\mu\text{M} \times \text{hr}$ ; <sup>b</sup> indicates difference in clopidogrel active metabolite concentration between \*2 through \*8 and \*1 or \*17,  $AUC_{0-t}$  = 0.14  $\mu\text{M} \times \text{hr}$ . The central tendency and measure of dispersion are obtained from Mega et al<sup>16</sup>: *CYP2C19*\*1 or \*17 summary estimates were pooled from ultra and extensive metabolizer groups and \*2 through\*8 from intermediate and poor metabolizer groups. The heights of the plots are proportional to the allele frequency of \*2 (the most common loss-of-function \* allele; rs4244285 mean allele frequency = 0.13; European ancestry [NCBI Single Nucleotide Polymorphism database, <http://www.ncbi.nlm.nih.gov/projects/SNP/>]); ie, as \*1 is more common (87%) than \*2 (13%), the height of the plot for the \*1 or \*17 group is higher than that for \*2, reflecting the number within the population that will harbor this genotype. B, Meta-analysis of 4 treatment-only studies (4341 individuals) reporting *CYP2C19* genotype and platelet reactivity after 600 mg of clopidogrel (using various assays). Error bars indicate 95% CIs.

**Figure 2.** Meta-analysis of CYP2C19 Genotype and Risk of Composite Cardiovascular Outcome in Individuals Treated With Clopidogrel: "Treatment-Only" Analysis



Comparison of any copy of CYP2C19 \*2 through \*8 to wild-type (\*1) or \*17 (reference) is stratified according to the number of events per study (1-99, 100-199, ≥200). Data-marker sizes indicate the weight applied to each study using fixed-effects meta-analysis. CVD indicates cardiovascular disease; NR, not reported; RR, relative risk.

tration between the 2 genotype categories was approximately half the geometric mean value seen in clopidogrel-treated individuals overall ( $AUC_{0-t} = 0.35 \mu M \times \text{hour}$ ). In 4 studies using the treatment-only approach (4341 individuals)<sup>40,45,57,58</sup> in which the intermediate phenotype of platelet reactivity was assessed following 600 mg of clopidogrel, there was a per-allele association of the \*2 variant on platelet aggregation when compared with subjects with \*1\*1 genotype (Figure 1B).

**CYP2C19 Genotype and Clinical Outcomes: Treatment-Only Analysis**

A pooled analysis of 22 studies using the treatment-only approach,<sup>†</sup> supple-

mented by a treatment-only analysis using data from the clopidogrel-treatment group of 4 randomized trials<sup>22,36,46</sup> (with a total 2465 clinical events among 26 251 individuals), indicated that individuals with any copy of CYP2C19 alleles \*2 through \*8 when compared with individuals with \*1 or \*17 alleles had a higher risk of CVD events (RR, 1.18; 95% CI, 1.09-1.28;  $I^2 = 60%$ ; 95% CI, 38%-75%, using fixed-effects models and RR, 1.34; 95% CI, 1.15-1.56, using random-effects models) (FIGURE 2). Meta-cumulative analysis, with the genotype group reversed to make the comparison akin to a trial of a higher vs lower dose of active clopidogrel metabolite, showed that at the time of FDA approval, individuals with

\*1 or \*17 had an RR of CVD events of 0.82 (95% CI, 0.72-0.93) for fixed-effects and an RR of 0.72 (95% CI, 0.57-0.92) for random-effects modeling compared with individuals with \*2 through \*8, with some attenuation of the summary estimate with addition of new studies (most recent estimate: RR, 0.86; 95% CI, 0.79-0.94, for fixed-effects and RR, 0.76; 95% CI, 0.65-0.89, for random-effects modeling) (FIGURE 3).

When studies were stratified by number of outcome events, there was a clear trend toward the null in larger studies (Figure 2), consistent with small-study bias ( $\chi^2 = 30$ ;  $P = 3.2 \times 10^{-7}$ ). The Harbord test for small-study bias was positive ( $P = .001$ ), and the funnel plot was asymmetric (eFigure 2). When we quantified the potential effect of small-study bias using trim-and-fill analysis, addition of the 8 hypothetical missing studies reduced the summary RR to 1.10 (95% CI, 1.02-1.19) for fixed-effects and an RR of 1.13 (95% CI, 0.96-1.33) for random-effects models. In the 4 largest studies reporting 200 or more CVD events (51% of all reported events), the summary RR was 0.97 (95% CI, 0.86-1.09) for fixed-effects and 0.95 (95% CI, 0.81-1.11) for random-effects modeling. Assuming a control event rate of 73 and 114 CV events per 1000 individuals in the setting of stable CHD and ACS, respectively, the estimated excess number of CV events for subjects carrying \*2 to \*8 alleles ranged from 8 to 12 events per 1000 individuals (eTable 3).

Eleven of 32 studies involving 10 291 individuals provided data that allowed us to obtain the effect of 1 loss-of-function allele (>238 CVD events) or 2 loss-of-function alleles (>37 CVD events) compared with the \*1 or \*17 reference group. The RR of CVD events among carriers of 1 loss-of-function allele was 1.77 (95% CI, 1.27-2.47;  $I^2 = 45%$ ; 95% CI, 0%-78%) for fixed-effects and 2.01 (95% CI, 1.21-3.34) for random-effects modeling in studies with fewer than 100 cases. In studies with 100 or more events, the values for the same exposure were an RR of 0.94 (95% CI, 0.80-1.10;  $I^2 = 31%$ ; 95% CI, 0%-

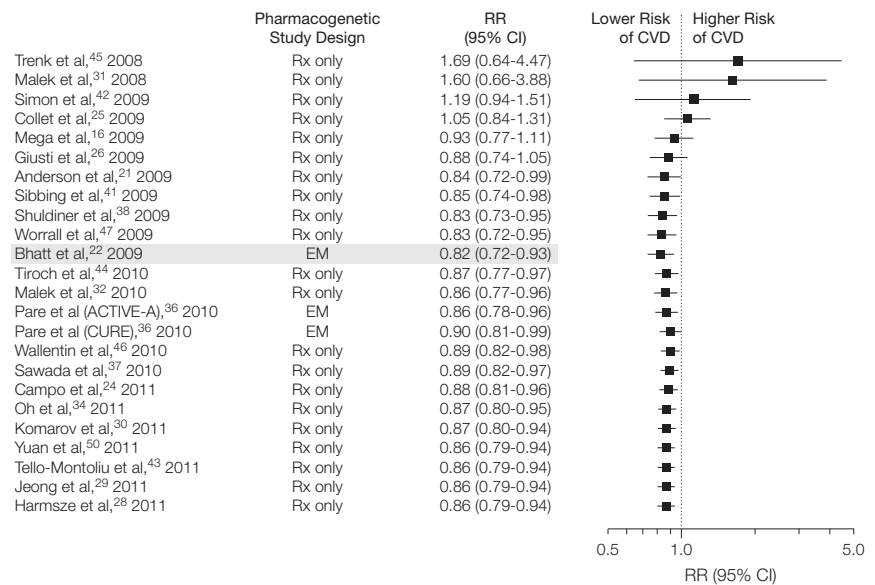
<sup>†</sup>References 16, 21, 24-27, 29-32, 34, 35, 37, 38,

41-45, 47, 48, 50.

75%) for fixed-effects and an RR of 0.95 (95% CI, 0.78-1.15) for random-effects modeling. Using the estimate from studies reporting 100 or more cases, this corresponded to 4 to 7 fewer CVD events per 1000 individuals in the setting of stable CHD and ACS, respectively. By comparison, the RR of CVD events among carriers of 2 loss-of-function alleles was 3.75 (95% CI, 2.40-5.86;  $I^2=8\%$ ; 95% CI, 0%-73%) for fixed effects and 3.76 (95% CI, 2.34-6.06) for random-effects modeling in studies with fewer than 100 cases and 1.52 (95% CI, 1.04-2.21;  $I^2=51\%$ ; 95% CI, 0%-86%) for fixed effects and 1.45 (95% CI, 0.82-2.56) for random-effects modeling in studies with 100 or more cases (eFigure 3). Using the estimate from studies reporting 100 or more cases, this corresponded to an absolute increase of 38 and 59 CVD events per 1000 individuals in the setting of stable CHD and ACS, respectively.

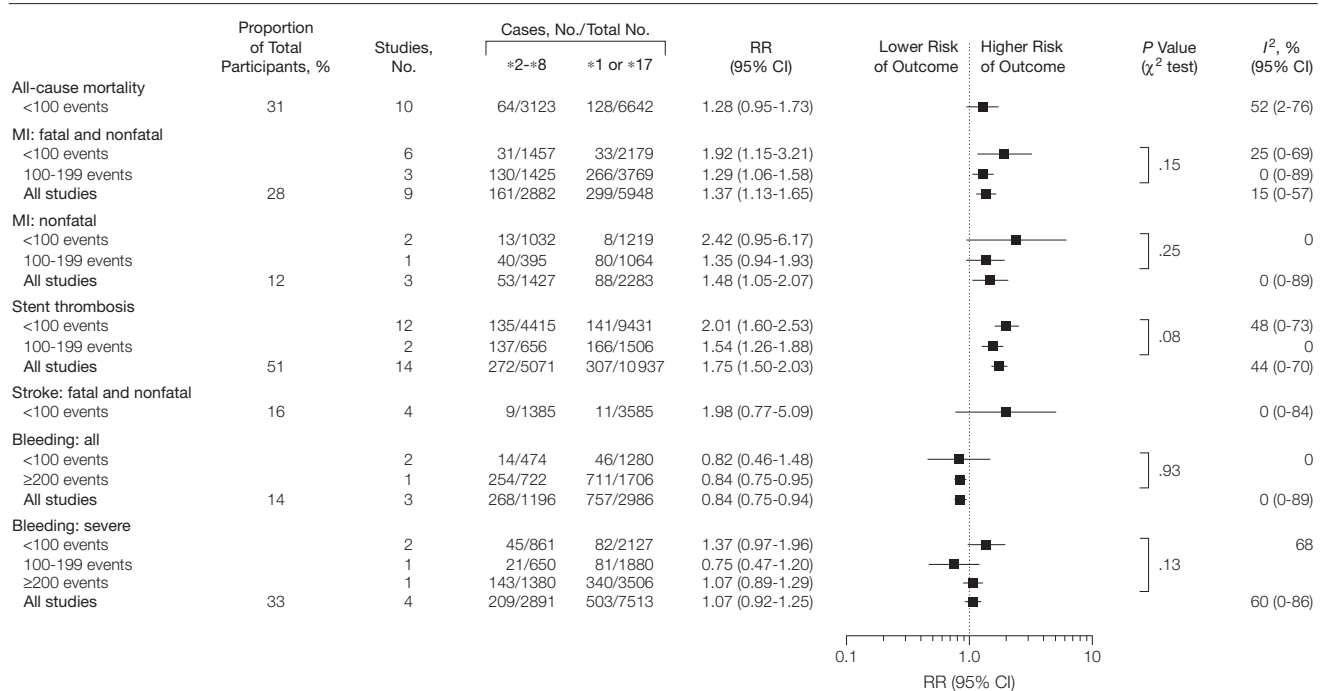
Stent thrombosis was the clinical outcome with the strongest association

**Figure 3.** Meta-cumulative Plot Comparing CYP2C19 Genotype in Individuals Treated With Clopidogrel: "Treatment-Only" Analysis



Comparing wild-type (\*1) or \*17 to any copy of CYP2C19 \*2 through \*8 makes the relative risk (RR) directionally consistent with a more-vs-less clopidogrel trial. Shading indicates the level of evidence at the time of Food and Drug Administration approval and attenuation of the summary estimate with subsequent studies. CVD indicates cardiovascular disease; EM, effect modification; RR, relative risk; Rx, treatment. Although Mega et al<sup>16</sup> and Wallentin et al<sup>46</sup> were set in a randomized clinical trial, effect-modification analysis was not permitted.

**Figure 4.** Association Between CYP2C19 Genotype (Any Copy of \*2 Through \*8 vs \*1 or \*17) and Risk of Individual Outcomes in the Treatment-Only Analysis



Each outcome is stratified by number of events per study. Proportion of total participants was calculated by dividing the number of individuals contributing to each individual outcome by the total number of individuals contributing toward the treatment-only analysis (n=31 076). MI indicates myocardial infarction; RR, relative risk.

with CYP2C19 loss-of-function alleles (579 cases in 16 008 individuals: RR, 1.75; 95% CI, 1.50-2.03;  $I^2=44\%$ ; 95% CI, 0%-70% for fixed-effects and RR, 1.88; 95% CI, 1.46-2.41 for random-effects modeling), but a trend toward the null was observed in larger studies (FIGURE 4). Assuming an event rate of

18 per 1000 individuals in the control group,<sup>59</sup> this corresponded to an absolute increase of 14 stent thromboses per 1000 individuals.

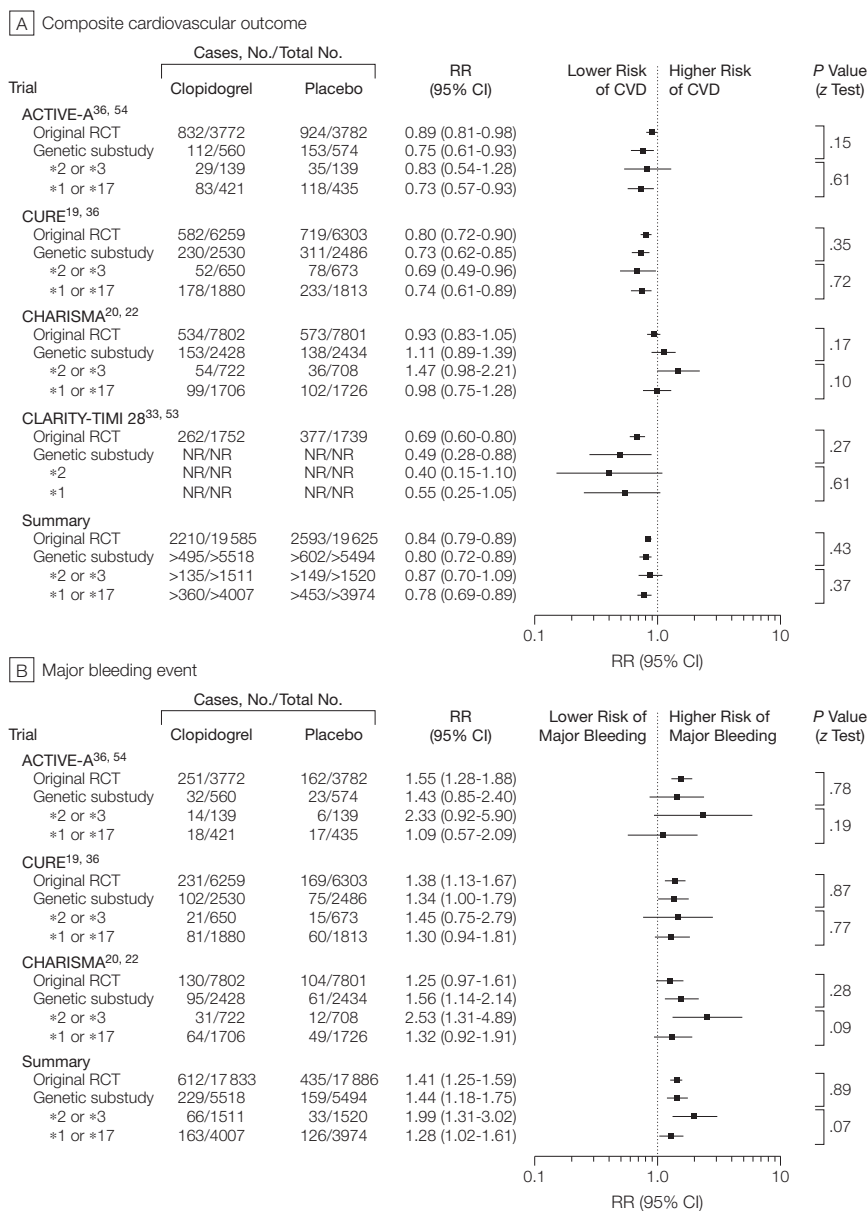
Individuals carrying any copy of \*2 through \*8 alleles had a lower risk of any bleeding when compared with individuals carrying the \*1 or \*17 allele (1025 events in 4182 individuals; RR, 0.84; 95% CI, 0.75-0.94;  $I^2=0\%$ ; 95% CI, 0%-89% for both fixed- and random-effects modeling) (Figure 4), corresponding to an absolute reduction in the risk of bleeding that ranged from 5 to 8 events per 1000 individuals depending on clinical setting (and assuming a control event rate of 31 and 50 bleeding events per 1000 individuals for stable CHD and ACS, respectively) (eTable 3). Other clinical outcomes (including all-cause mortality, myocardial infarction, and stroke) with respective fixed- and random-effects estimates are presented in Figure 4 and eTable 4A.

There was no evidence that CHD status at study recruitment (ACS vs stable CHD), blinding to genotype, source of funding, and concomitant use of proton-pump inhibitors or aspirin modified the association of CYP2C19 genotype with CVD events ( $P>.09$  for all meta-regressions) (eFigure 4).

**CYP2C19 Genotype and Clinical Outcomes: Effect-Modification Analysis**

In the 4 placebo-controlled randomized trials<sup>22,33,36</sup> (11 477 subjects and >1097 major CVD events), the RR of major CVD events in patients treated with clopidogrel compared with placebo was 0.78 (95% CI, 0.69-0.89) in genotype category \*1 or \*17 and 0.87 (95% CI, 0.70-1.09) in genotype category \*2 or \*3 ( $z=0.89$ ;  $P=.37$  for interaction) (FIGURE 5A). We found weak evidence for a treatment  $\times$  genotype interaction for major bleeding; the RR for major bleeding comparing clopidogrel with placebo was 1.99 (95% CI, 1.31-3.02) in genotype category \*2 or \*3 and 1.28 (95% CI, 1.02-1.61) in genotype category \*1 or \*17 ( $z=1.83$ ;  $P=.07$  for interaction) (Figure 5B), but

**Figure 5.** Analysis of CYP2C19 Genotype on Composite Cardiovascular End Points and Major Bleeding in Randomized Trials Where Both Clopidogrel and Placebo Groups Were Genotyped: "Effect-Modification" Analysis



Meta-analysis of risk of (A) composite cardiovascular outcome and (B) major bleeding event, comparing clopidogrel with placebo, stratified by the following: findings from original randomized clinical trials (RCTs), genetic substudy, and CYP2C19\* allele status into any copy of \*2 or \*3 and \*1 or \*17. The P value reflects the z test for interaction between subgroups, comparing original RCT and genetic substudy, which assesses the representativeness of the genetic substudy to the original cohort, and \*2 or \*3 compared with \*1 or \*17, which tests for effect modification of the effect of clopidogrel vs placebo by CYP2C19 genotype. CVD indicates cardiovascular disease; RR, relative risk.

the pattern was inconsistent with that predicted from the relationship between genotype and active clopidogrel metabolite concentration or platelet function (Figure 1). The evidence remained similar when using random-effects modeling (eTable 4B).

## COMMENT

Despite associations between *CYP2C19* genotype, clopidogrel metabolism, and platelet aggregation, this systematic review and meta-analysis does not demonstrate a clinically important association of genotype with cardiovascular outcomes with the possible exception of stent thrombosis. Treatment-only studies were affected by small-study bias while the effect-modification studies provided no evidence for a genotype  $\times$  treatment interaction. Our appraisal has identified issues pertaining to the design and analysis of pharmacogenetic studies that are important in the understanding of the significance of the *CYP2C19* genotype.

Only 5 studies of clopidogrel treatment response were nested within a randomized trial in which both treatment and comparator group were genotyped, a design that allows evaluation of the clopidogrel treatment effect overall (through comparison of event rates in treatment and control groups) and an assessment of differential treatment response by *CYP2C19* genotype (assessed as a statistical test for genotype  $\times$  treatment interaction). Randomized trials are routinely registered in advance of reporting, and trial protocols and study outcomes are usually prespecified, reducing the risk of publication and selective reporting bias. Among the 4 effect-modification placebo-controlled trials included in this meta-analysis, there was no evidence of a clopidogrel treatment  $\times$  *CYP2C19* genotype interaction.

By contrast, most studies examining the relationship between *CYP2C19* genotype and cardiovascular outcomes were performed among patients treated with clopidogrel, usually in an observational setting. In these treatment-only studies, subjects clas-

sified as poor metabolizers (ie, carrying any \*2 to \*8 *CYP2C19* allele) had a higher risk of CVD when compared with normal or fast metabolizers (\*1 or \*17). However, there was strong evidence of small-study bias. We also identified evidence for selective outcome reporting bias. Not all ascertained outcomes were reported either individually or as part of the CVD composite (eTable 2A and B). Moreover, if a particular \* allele was not typed, the participant was presumed to lack the variant allele in that position. Thus, some individuals assigned to the \*1 category (lacking all alternative alleles) may have been misclassified, simply because genotyping was not attempted for some \* alleles.<sup>60</sup> Taken together, these observations cast doubt on the association of *CYP2C19* genotype with clinical cardiovascular end points.

Aside from biases that may have influenced treatment-only studies, some other design limitations are noteworthy. Inferring effect modification through a treatment-only analysis would be considered invalid for a non-genetic variable (age or sex), but there are 3 reasons for its widespread use (~95%)<sup>61</sup> in pharmacogenetic studies. First, ethical, logistic, or financial considerations may preclude DNA collection from trial participants. Second, based on Mendel's second law of independent assortment, random allocation of genetic variants from parent to offspring should mean that groups categorized by genotype for a drug metabolizing enzyme do not differ systematically (except in their response to drug treatment).<sup>62</sup> Third, it is implicitly assumed that genetic variation in a drug metabolizing enzyme should have no disease association in the absence of exposure to medication.<sup>63</sup> Although the *CYP2C19* genotype has not been associated with CHD events in individuals not treated with clopidogrel, *CYP2C19* genotype was associated with inflammation markers (linked to CVD risk) in a candidate gene study,<sup>64</sup> and recent genome-wide association studies have implicated other *CYP* gene variants in the susceptibility

to elevated blood pressure (*CYP1A1*,<sup>65</sup> *CYP1A2*,<sup>65</sup> and *CYP17A1*<sup>65,66</sup>) and CHD (*CYP17A1*<sup>67</sup>).

If Mendel's second law and Motulsky's assumptions<sup>63</sup> apply, a treatment-only study of a drug-metabolizing gene variant can be considered analogous to a randomized trial of a high vs low dose of a drug with the association with clinical outcomes being related to the difference in concentration of the active metabolite between the genotype categories for a given administered dose. The summary RR in CVD events seen in placebo-controlled trials of 75-mg clopidogrel, irrespective of genotype, was 0.88 (95% CI, 0.83-0.93). In the study by Mega et al,<sup>16</sup> the ratio of the difference in mean AUC values for the active clopidogrel metabolite for the 2 major *CYP2C19* genotype categories (\*2 to \*8 vs \*1 or \*17) to the mean AUC regardless of genotype was 0.14/0.35 (Figure 1A). Assuming a log-concentration response relationship between the active clopidogrel metabolite and CVD events, the RR in CVD events comparing individuals with 1 or more *CYP2C19* gain-of-function alleles with the reference category should be 0.95 (ie,  $0.88^{0.14/0.35}$ ), with the genotype comparison switched as compared with most of the genotype comparisons in the "Results" section, to make it akin to a trial of a higher vs lower dose of active clopidogrel metabolite. However, the observed summary estimate for the association of the gain-of-function genotype with CVD events in the treatment-only studies available at the time of the FDA boxed warning suggested greater benefit from *CYP2C19* than predicted (RR, 0.82; 95% CI, 0.72-0.93) (Figure 3). With the addition of 13 studies, the benefit associated with the gain-of-function genotype is slightly less (RR, 0.86; 95% CI, 0.79-0.94) yet still suggests greater benefit than the predicted effect (RR, 0.95) and is similar in magnitude to the RR observed in the meta-analysis of trials of 75-mg clopidogrel vs placebo, regardless of genotype.

Two other observations raise questions regarding the utility of *CYP2C19*

genotyping in evaluation of clopidogrel response. Where the major harms of a drug treatment are mechanism-based, as is the case for clopidogrel where the major adverse effect is bleeding, any attempt to individualize dose to optimize treatment response (ie, to reduce CVD risk) is likely to be offset by an opposing effect on the rate of harm (ie, risk of bleeding), and this was observed in the current analysis (eTable 3). The net potential clinical benefit of genotyping to adjust dose may therefore not be as great as initially assumed. When genetic variants under investigation lie in drug metabolizing enzymes, the interaction will likely be quantitative (ie, differences in the magnitude of effect) rather than qualitative (differences in the direction of effect)<sup>68</sup>; thus, even if a “real” difference between subgroups is detected, the net clinical utility could be limited (because the drug could still be beneficial in individuals with the “poor” responder genotype), and a careful cost-effectiveness analysis is necessary to justify a change in clinical practice.

Our interpretation is consistent with the findings of a recent meta-analysis,<sup>69</sup> but the current review differs from that one in several important ways. First, the current review includes 17 more studies than the recent meta-analysis. Second, the current review includes analysis of the association between the *CYP2C19* genotype and clopidogrel metabolites and platelet reactivity. Third, the current review includes analysis of individual outcomes as well as composite cardiovascular outcomes. Fourth, the current review compares and contrasts findings from treatment-only and effect-modification study designs. Fifth, we placed the observed risk of cardiovascular outcomes in the context of the expected risk based on the observed effect of *CYP2C19* genotype on active clopidogrel metabolites.

Our study has several limitations. We used aggregate (not participant-level) data and our power to detect differences in subgroups (eg, based on use of proton-pump inhibitors or

aspirin) was consequently limited. The components of the composite CVD end points differed across studies, which may have influenced the meta-analysis summary RR.<sup>70,71</sup> However, on sensitivity analysis, the summary RR was not overly influenced by the exclusion of individual studies (eFigure 5). Moreover, we also examined the main components of the composite end point. The inclusion of studies with both stable CHD and ACS might dilute an association if the magnitude of effect of clopidogrel was greater in ACS than in patients with stable CHD; however, when we stratified studies according to recruitment at the time of ACS, we found no heterogeneity in the association between *CYP2C19* genotype and risk of CVD events (eFigure 4). We report estimates from both fixed- and random-effects meta-analysis modeling because of considerable between-study heterogeneity in the effect of *CYP2C19* on risk of CVD events in treatment-only analysis ( $I^2=60\%$ ). However, when adjusting for number of events per study, the  $I^2$  attenuated to 20%, a value considered to reflect low heterogeneity.<sup>72</sup> Therefore, by assigning more weight to smaller studies,<sup>73,74</sup> the random-effects model may exacerbate the small-study bias identified in our analysis.

## CONCLUSION

In conclusion, this study identified no clinically significant interaction of *CYP2C19* genotype with the association of clopidogrel therapy and cardiovascular events.

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**Author Contributions:** Dr Holmes had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Holmes, Hingorani, Casas.

**Acquisition of data:** Holmes, Shah.

**Analysis and interpretation of data:** Holmes, Perel, Shah, Hingorani, Casas.

**Drafting of the manuscript:** Holmes, Casas, Hingorani. **Critical revision of the manuscript for important intellectual content:** Holmes, Perel, Shah, Hingorani, Casas.

**Statistical analysis:** Holmes, Casas.

**Obtained funding:** Casas, Hingorani.

**Administrative, technical, or material support:** Shah.

**Study supervision:** Hingorani, Casas.

**Conflict of Interest Disclosures:** All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Hingorani was previously the principal investigator receipt of a Biomarker Award from the Medical Research Council with Pfizer as an Industrial partner. This award was to investigate complement factor H in the prediction or pathogenesis of age-related macular disease or coronary heart disease. The award is now closed. Dr Hingorani has provided free advice to colleagues at University College London on the design and analysis of pharmacogenetic studies, including an analysis funded in part by Celera through a contract with University College London and Medical Research Council. All other coauthors reported no conflicts of interest.

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