

ORIGINAL ARTICLE

Effects of *CYP2C19* Genotype on Outcomes of Clopidogrel Treatment

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ABSTRACT

BACKGROUND

From the Population Health Research Institute, Hamilton Health Sciences (G.P., S.R.M., S.Y., S.S.A., S.J.C., J.H., J.W.E.); and the Departments of Medicine (G.P., S.R.M., S.Y., S.S.A., S.J.C., J.H., J.W.E.), Epidemiology (G.P., S.Y., S.S.A.), and Pathology (G.P.), McMaster University — both in Hamilton, ON, Canada; Bristol-Myers Squibb, New York (K.S.); Veterans Affairs Boston Healthcare System, Brigham and Women's Hospital, and Harvard Medical School — all in Boston (D.L.B.); and the Centre for Cardiovascular Science, Edinburgh University and Royal Infirmary, Edinburgh (K.A.A.F.). Address reprint requests to Dr. Paré at the Population Health Research Institute, Hamilton Health Sciences and McMaster University, David Braley Cardiac Vascular and Stroke Research Institute, 237 Barton St. E., Rm. C3-103, Hamilton, ON L8L 2X2, Canada, or at pareg@mcmaster.ca.

It has been suggested that clopidogrel may be less effective in reducing the rate of cardiovascular events among persons who are carriers of loss-of-function *CYP2C19* alleles that are associated with reduced conversion of clopidogrel to its active metabolite.

METHODS

We genotyped patients from two large, randomized trials that showed that clopidogrel, as compared with placebo, reduced the rate of cardiovascular events (the primary efficacy outcome) among patients with acute coronary syndromes and among patients with atrial fibrillation. Patients were genotyped for three single-nucleotide polymorphisms (*2, *3, *17) that define the major *CYP2C19* alleles.

RESULTS

Among 5059 genotyped patients with acute coronary syndromes, clopidogrel as compared with placebo significantly reduced the rate of the primary efficacy outcome, irrespective of the genetically determined metabolizer phenotype ($P=0.12$ for heterogeneity). The effect of clopidogrel in reducing the rate of the primary efficacy outcome was similar in patients who were heterozygous or homozygous for loss-of-function alleles and in those who were not carriers of the alleles (rate among carriers, 8.0% with clopidogrel vs. 11.6% with placebo; hazard ratio with clopidogrel, 0.69; 95% confidence interval [CI], 0.49 to 0.98; rate among noncarriers, 9.5% vs. 13.0%; hazard ratio, 0.72; 95% CI, 0.59 to 0.87). In contrast, gain-of-function carriers derived more benefit from clopidogrel treatment as compared with placebo than did noncarriers (rate of primary outcome among carriers, 7.7% vs. 13.0%; hazard ratio, 0.55; 95% CI, 0.42 to 0.73; rate among noncarriers, 10.0% vs. 12.2%; hazard ratio, 0.85; 95% CI, 0.68 to 1.05; $P=0.02$ for interaction). The effect of clopidogrel on bleeding did not vary according to genotypic subgroups. Among 1156 genotyped patients with atrial fibrillation, there was no evidence of an interaction with respect to either efficacy or bleeding between the study treatment and the metabolizer phenotype, loss-of-function carrier status, or gain-of-function carrier status.

CONCLUSIONS

Among patients with acute coronary syndromes or atrial fibrillation, the effect of clopidogrel as compared with placebo is consistent, irrespective of *CYP2C19* loss-of-function carrier status. (Funded by Sanofi-Aventis and Bristol-Myers Squibb; ClinicalTrials.gov number, NCT00249873.)

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CLOPIDOGREL, WHEN ADDED TO ASPIRIN, reduces the rate of major vascular events among patients with acute coronary syndromes and atrial fibrillation.^{1,2} Recent reports suggest that certain common genetic variants, involving the hepatic cytochrome P-450 system, that are involved in the conversion of clopidogrel to its active metabolite are associated with an increased rate of recurrent cardiovascular events, implying that the benefits of clopidogrel may be attenuated in patients with these genetic variants. Specifically, in patients who are carriers of a loss-of-function *CYP2C19* allele (including the *2 and *3 alleles), the conversion of clopidogrel to its active metabolite may be reduced, resulting in decreased inhibition of platelets. Analyses that were limited to data from clopidogrel-treated patients showed a relative risk of major cardiovascular events that was increased by a factor of 1.53 to 3.69 among carriers of loss-of-function alleles, as compared with noncarriers.³⁻⁵ On the basis of these findings and related pharmacokinetic and pharmacodynamic data (ClinicalTrials.gov number, NCT01123824), the Food and Drug Administration (FDA) has issued a black-box warning about the reduced effectiveness of clopidogrel in patients who are carriers of two loss-of-function alleles (so-called poor metabolizers) and has suggested that carriers of these alleles receive a higher dose of clopidogrel or an alternative antiplatelet agent.

Conversely, carriers of the ultrarapid enzyme activity allele *17 (so-called ultrametabolizers) have an increased platelet response to clopidogrel and an increased risk of bleeding (but not greater efficacy).⁶ However, observational analyses that do not include untreated controls may be confounded by other factors. For example, the same genetic marker may have pleiotropic effects, which influence the metabolism of drugs other than clopidogrel, or may affect outcomes through independent mechanisms such as linkage disequilibrium with other nearby *CYP* genes (e.g., *CYP2C9*, *CYP2C18*, and *CYP2C8*). To reduce the possibility of confounding, an evaluation of a potential interaction between genotype and treatment should ideally be conducted as part of a randomized, controlled trial, because any heterogeneity in event rates among carriers that is unrelated to clopidogrel will then be paralleled by a similar effect on event rates among controls who were not treated with clopidogrel.

We hypothesized that the benefits of clopidogrel as compared with placebo would be decreased in persons who carry a loss-of-function *CYP2C19* allele and increased in carriers of the gain-of-function *17 allele. To test this hypothesis, we examined the efficacy and safety of clopidogrel as compared with placebo according to genotype status among patients in two randomized trials: the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial, in which patients with acute coronary syndromes were enrolled, and the Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events (ACTIVE) A, in which patients with atrial fibrillation were enrolled.

METHODS

THE CURE STUDY

The design and results of the CURE study have been described previously.^{2,7,8} In brief, the CURE study was a randomized, double-blind, placebo-controlled trial comparing clopidogrel (at a dose of 75 mg per day) with placebo — both in combination with aspirin — among 12,562 patients with acute coronary syndromes without ST-segment elevation. For the current analyses, we used the same primary efficacy and safety outcomes as those in the CURE trial.² 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. The main safety outcome was major bleeding. Results are presented only for patients of European or Latin American ancestry. Patients with other ancestries were excluded because of small numbers (99 patients in the next largest group) and concern about the potential for population stratification (see Fig. 1, 2, and 3 in the Supplementary Appendix, available with the full text of this article at NEJM.org).

ACTIVE A

The design and results of ACTIVE A have been described previously.^{1,9} ACTIVE A was a randomized, double-blind trial comparing clopidogrel, at a dose of 75 mg per day, with placebo — both in combination with aspirin — for reducing the risk of stroke among patients with atrial fibrillation and at least one additional risk factor for stroke who were not eligible for warfarin therapy. We used the same primary efficacy and safety

outcomes as those in ACTIVE A.¹ The primary efficacy outcome was any major vascular event (stroke, systemic embolism outside the central nervous system, 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 patients who were of non-European ancestry were excluded (see Fig. 7 in the Supplementary Appendix).

STUDY OVERSIGHT

The institutional review board at each participating center approved each study, and all patients provided written informed consent. Only patients who also consented to participate in one of the two genetic studies were eligible for this analysis. The academic authors designed the study, gathered and analyzed the data, vouch for the data and the analysis, wrote all the drafts of the manuscript, and made the decision to submit the manuscript for publication.

GENOTYPING AND GENOTYPE CLASSIFICATION

Genotyping of three single-nucleotide polymorphisms (SNPs) defining the major *CYP2C19* alleles was performed with the use of TaqMan assays from stored DNA. The call rate was greater than 98% for each of rs4244285, rs4986893, and rs12248560, defining the *2, *3 and *17 allele, respectively. Hardy–Weinberg equilibrium was tested within each ethnic group and was nonsignificant for all SNPs ($P > 0.05$).

Patients were classified into categories of metabolizer phenotypes with the use of established common-consensus star allele nomenclature.^{10,11} Thus, patients without a *2, *3, or *17 allele (i.e., *1/*1) were classified as “extensive metabolizers,” those with one *2 or *3 allele (i.e., *1/*2 or *1/*3) were classified as “intermediate metabolizers,” and those with two *2 or *3 alleles (i.e., *2/*2, *2/*3 or *3/*3) were classified as “poor metabolizers.” Carriers of a single *17 allele (i.e., *1/*17) and *17 homozygotes were classified as “ultra-metabolizers,” and patients with one *17 allele and one loss-of-function allele (i.e., *2/*17 or *3/*17) were classified as having an “unknown” metabolizer phenotype. Carriers of at least one loss-of-function allele (i.e., *2 or *3) were classified as “loss-of-function allele carriers” and carriers of at least one gain-of-function allele (i.e., *17) were classified as “gain-of-function allele carriers.”

STATISTICAL ANALYSIS

We first explored the effect of *CYP2C19* genotypes on efficacy and safety outcomes among participants of European or Latin American ancestry in the CURE trial. The effect of clopidogrel as compared with placebo according to the genetically derived *CYP2C19* metabolizer phenotype was assessed with the use of Cox proportional-hazards regression. No significant effect modification according to ancestry was observed for any of the pharmacogenetic effects described (data not shown), and the results from Europeans and Latin Americans were therefore combined (with adjustment for ancestry).

We used one model to adjust for age, sex, and ancestry and another model to adjust for age, sex, ancestry, revascularization procedure (percutaneous coronary intervention [PCI] with or without stenting and coronary-artery bypass grafting [CABG]), smoking status, waist-to-hip ratio, presence or absence of diabetes, blood pressure, and country of origin. Similar results were obtained with the two models, and therefore, only results obtained with the parsimonious model are presented. Treatment effect according to loss-of-function and gain-of-function carrier status was examined with the use of analogous models. Power estimates were derived through simulations of genetic effects (5000 simulations) according to the specified models with the use of logistic regression to ensure that we had adequate power to detect the range of interactions that has been reported previously. Two-sided P values of less than 0.05 were considered to indicate statistical significance. The same approach was used for the analyses of data from the CURE and ACTIVE A trials.

RESULTS

THE CURE STUDY

The characteristics of the genotyped patients are presented in Table 1. A total of 5059 participants of self-reported European or Latin American ancestry were successfully genotyped, of whom 2549 had been randomly assigned to clopidogrel and 2510 to placebo. The benefit of clopidogrel treatment as compared with placebo with respect to the first primary composite efficacy outcome was similar between participants who were genotyped for our analysis and the total cohort in the parent study²: among the genotyped participants, the primary outcome occurred in 9.1% of the pa-

Table 1. Baseline Characteristics of Genotyped Patients in the CURE and ACTIVE A Trials.*

Characteristic	CURE Trial			ACTIVE A		
	Placebo (N=2510)	Clopidogrel (N=2549)	Total (N=5059)	Placebo (N=586)	Clopidogrel (N=570)	Total (N=1156)
Ancestry (%)†						
European	85.7	86.2	85.9	100	100	100
Latin American	14.3	13.8	14.1	0	0	0
Female sex (%)	40.9	41.2	41.0	45.2	45.6	45.4
Age (yr)	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‡	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
Current smoker (%)	21.6	23.1	22.4	6.8	8.4	7.6
Blood pressure (mm Hg)						
Systolic	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	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	NA	NA	NA
With stent	13.5	15.5	14.5	NA	NA	NA
CABG (%)	10.4	9.8	10.1	NA	NA	NA
Metabolizer phenotype (%)§						
Poor	2.2	2.4	2.3	2.1	1.6	1.9
Intermediate	17.8	17.3	17.5	16.0	16.6	16.3
Extensive	39.7	40.8	40.3	40.9	35.5	38.3
Ultra	33.2	33.5	33.4	34.8	39.6	37.2
Unknown	7.1	6.0	6.5	6.1	6.6	6.3

* Plus-minus values are means ±SD. ACTIVE denotes Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events, CABG coronary-artery bypass grafting, CURE Clopidogrel in Unstable Angina to Prevent Recurrent Events, NA not applicable, and PCI percutaneous coronary intervention.

† Ancestry was self-reported.

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

§ Patients with two *2 or *3 alleles (i.e., *2/*2, *2/*3, or *3/*3) were classified as having the poor-metabolizer phenotype, those with one *2 or *3 allele (i.e., *1/*2 or *1/*3) were classified as having the intermediate-metabolizer phenotype, those without a *2, *3, or *17 allele (i.e., *1/*1) were classified as having the extensive-metabolizer phenotype, those with a single *17 allele (i.e., *1/*17) and *17 homozygotes were classified as having the ultrametabolizer phenotype, and those with one *17 allele and one loss-of-function allele (i.e., *2/*17 or *3/*17) were classified as having an unknown metabolizer phenotype.

tients receiving clopidogrel (231 of 2549 patients) versus 12.6% of those receiving placebo (316 of 2510) (hazard ratio with clopidogrel, 0.71; 95% confidence interval [CI], 0.60 to 0.84; $P < 0.001$), and in the parent study, the primary outcome occurred in 9.3% of the patients receiving clopidogrel (582 of 6259) versus 11.4% of those receiving placebo (719 of 6303) (hazard ratio, 0.80; 95% CI, 0.72 to 0.90; $P < 0.001$).

Figure 1 shows the estimates of the relative risk of the first and second primary composite efficacy outcomes among patients treated with clopidogrel as compared with those who received placebo, stratified according to metabo-

lizer phenotype. The effects of clopidogrel were consistent in subgroups defined according to metabolizer status for both the first and second primary composite efficacy outcomes ($P = 0.12$ and $P = 0.29$ for heterogeneity, respectively). The effect of clopidogrel as compared with placebo in reducing the first (and second) primary composite efficacy outcome was similar between carriers of loss-of-function alleles and noncarriers (hazard ratio with clopidogrel among carriers, 0.69; 95% CI, 0.49 to 0.98; hazard ratio among noncarriers, 0.72; 95% CI, 0.59 to 0.87; $P = 0.84$ for the interaction). In contrast, a significant interaction was observed between gain-of-function

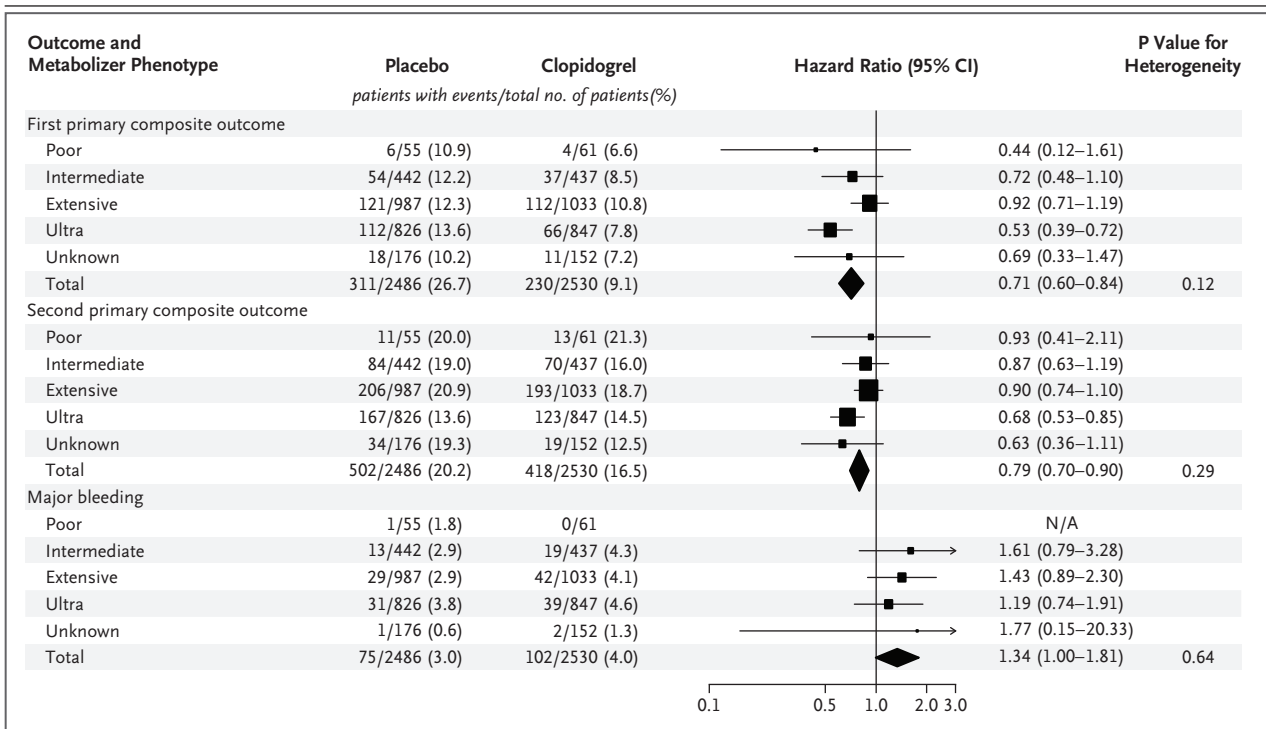


Figure 1. Effect of Clopidogrel as Compared with Placebo on Clinical Outcomes among Patients with Acute Coronary Syndromes in the CURE trial, Stratified According to Metabolizer Phenotype.

Hazard ratios for clopidogrel as compared with placebo are shown for efficacy and bleeding outcomes according to metabolizer phenotype. The size of each symbol is in inverse proportion to the standard deviation of the effect-size estimates. Analyses were performed on data from patients of European or Latin American ancestry, with adjustment for age, sex, and ancestry. Patients with two *2 or *3 alleles (i.e., *2/*2, *2/*3, or *3/*3) were classified as having the poor-metabolizer phenotype, those with one *2 or *3 allele (i.e., *1/*2 or *1/*3) were classified as having the intermediate-metabolizer phenotype, those without a *2, *3, or *17 allele (i.e., *1/*1) were classified as having the extensive-metabolizer phenotype, those with a single *17 allele (i.e., *1/*17) and *17 homozygotes were classified as having the ultrametabolizer phenotype, and those with one *17 allele and one loss-of-function allele (i.e., *2/*17 or *3/*17) were classified as having an unknown metabolizer phenotype. Only patients who were successfully genotyped for all three single-nucleotide polymorphisms were included in these analyses.

allele carrier status (i.e., *1/*17, *17/*17, *2/*17, or *3/*17) and study-group assignment with respect to the first primary composite efficacy outcome, such that carriers had a more pronounced reduction in cardiovascular events with clopidogrel treatment as compared with placebo than did noncarriers (hazard ratio with clopidogrel among carriers, 0.55; 95% CI, 0.42 to 0.73; hazard ratio among noncarriers, 0.85; 95% CI, 0.68 to 1.05; P=0.02 for the interaction). A similar interaction was observed with respect to the second composite primary outcome (hazard ratio with clopidogrel among carriers, 0.66; 95% CI, 0.54 to 0.82; hazard ratio among noncarriers, 0.90; 95% CI, 0.76 to 1.06; P=0.03 for heterogeneity). The corresponding Kaplan–Meier survival curves are shown in Figure 2 (see also

Fig. 4 in the Supplementary Appendix). The benefits of clopidogrel as compared with placebo were similar between patients with the poor-metabolizer phenotype and patients with all other metabolizer phenotypes, irrespective of whether the patients with the intermediate metabolizer phenotype were included (data not shown).

The rate of major bleeding with clopidogrel as compared with placebo was similar between genotyped patients and the total cohort in the parent study: 4.0% (102 of 2549 patients) versus 3.0% (76 of 2510) among genotyped patients (hazard ratio, 1.33; 95% CI, 0.99 to 1.79), and 3.7% (231 of 6259 patients) versus 2.7% (169 of 6303) in the total cohort in the parent study (hazard ratio, 1.38; 95% CI, 1.13 to 1.67). The effects of clopidogrel, as compared with placebo, on major bleeding were

consistent in subgroups defined according to metabolizer phenotype (Fig. 1) or functional allele carrier status (Fig. 2).

ACTIVE A

The characteristics of the genotyped patients are summarized in Table 1. A total of 1156 participants of self-reported European ancestry were successfully genotyped, of whom 570 had been randomly assigned to clopidogrel and 586 to placebo. The effect of clopidogrel in reducing the primary composite efficacy outcome was similar between the genotyped patients and the total cohort in the parent study⁴; the rate of the primary outcome among the genotyped patients was 20.0% (114 of 570 patients) in the clopidogrel group versus 26.3% (154 of 586) in the placebo group (hazard ratio with clopidogrel, 0.74; 95% CI, 0.58 to 0.94; $P=0.01$), and the rate in the total cohort in the parent study was 22.1% (832 of 3772 patients) versus 24.4% (924 of 3782) (hazard ratio, 0.89; 95% CI, 0.81 to 0.98; $P=0.01$). The results were consistent in subgroups defined according to metabolizer phenotype (Fig. 3) or functional allele carrier status (Fig. 4, and Fig. 8 in the Supplementary Appendix). The effect of clopidogrel as compared with placebo in reducing the rate of cardiovascular events was similar between carriers of loss-of-function alleles and noncarriers (hazard ratio among carriers, 0.78; 95% CI, 0.48 to 1.28; hazard ratio among noncarriers, 0.72; 95% CI, 0.54 to 0.95; $P=0.73$ for heterogeneity). The benefits of clopidogrel as compared with placebo were similar in patients with the poor-metabolizer phenotype and in patients with all other metabolizer phenotypes, irrespective of whether the patients with the intermediate metabolizer phenotype were included (data not shown).

The rate of major bleeding with clopidogrel as compared with placebo was similar between genotyped patients and the total cohort in the parent study: 5.8% (33 of 570 patients) versus 3.9% (23 of 586) among genotyped patients (hazard ratio, 1.50; 95% CI, 0.88 to 2.55), and 6.7% (251 of 3772 patients) versus 4.3% (162 of 3782) among patients in the total cohort (hazard ratio, 1.57; 95% CI, 1.29 to 1.92). No interaction between metabolizer phenotype (Fig. 3) and functional allele carrier status (Fig. 4) was observed. Nevertheless, in an analysis of clopidogrel-treated patients only, carriers of loss-of-function alleles, as compared with noncarriers, had an increased

risk of bleeding ($P=0.01$ by log-rank test) (hazard ratio, 2.47; 95% CI, 1.23 to 4.97; $P=0.01$) (Fig. 4). However, when patients who received placebo were included in the comparison, there was no evidence of an interaction between study treatment and loss-of-function carrier status ($P=0.16$ for heterogeneity), since a similar trend among carriers as compared with noncarriers was also noted for the patients who received placebo (hazard ratio, 1.13; 95% CI, 0.45 to 2.89; $P=0.79$).

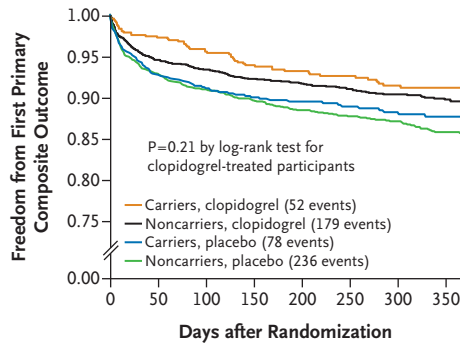
SECONDARY ANALYSIS

Previous studies of clopidogrel have shown a deleterious effect of loss-of-function alleles when carriers are compared with noncarriers in an analysis that includes clopidogrel-treated patients only.³⁻⁵ Therefore, we tested whether loss-of-function carrier status was associated with the outcome in an analysis that was restricted to data from clopidogrel-treated patients. Among these patients, there were no significant increases in the rates of the first primary composite outcome in the CURE trial (hazard ratio, 0.86; 95% CI, 0.63 to 1.17), the second primary composite outcome in the CURE trial (hazard ratio, 0.93; 95% CI, 0.74 to 1.16), or the primary outcome in ACTIVE A (hazard ratio, 1.07; 95% CI, 0.70 to 1.63). When we compared the poor-metabolizer phenotype with all other metabolizer phenotypes, there was no evidence of an increased risk of the first primary composite outcome in the CURE trial (hazard ratio, 0.69; 95% CI, 0.26 to 1.87), the second primary composite outcome in the CURE trial (hazard ratio, 1.29; 95% CI, 0.74 to 2.25), or the primary outcome in ACTIVE A (hazard ratio, 0.59; 95% CI, 0.08 to 4.24).

STUDY POWER

A hazard ratio of 1.53 is the smallest increase in the risk of cardiovascular events that has been reported to date in analyses of data from clopidogrel-treated patients who are carriers of a loss-of-function allele.⁴ Among the participants in the CURE trial who had an acute coronary syndrome, our study had more than 85% power to detect an interaction of treatment with carrier status with respect to the primary efficacy outcome, assuming such an effect size, and greater than 95% power to detect an interaction with respect to the second primary composite outcome. Among patients with atrial fibrillation in ACTIVE A, our study had much lower power (45%) to detect a similar

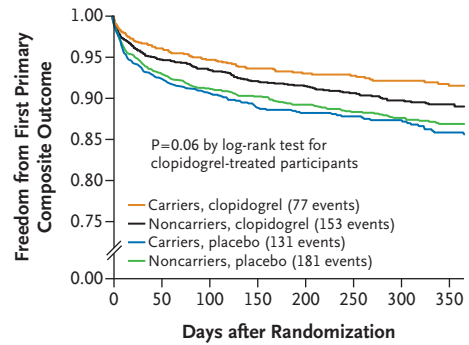
A First Primary Composite Outcome According to Loss-of-Function Allele Carrier Status



No. at Risk

Carriers, clopidogrel	651	632	608	545	484	425	358	297
Noncarriers, clopidogrel	1886	1778	1723	1541	1352	1191	960	804
Carriers, placebo	674	626	609	551	483	423	356	281
Noncarriers, placebo	1819	1686	1634	1456	1259	1103	922	774

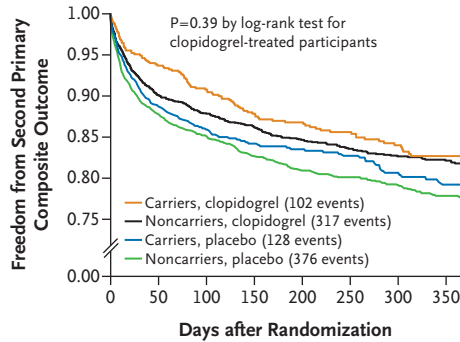
B First Primary Composite Outcome According to Gain-of-Function Allele Carrier Status



No. at Risk

Carriers, clopidogrel	1001	960	932	828	715	640	513	428
Noncarriers, clopidogrel	1536	1451	1400	1258	1122	977	805	673
Carriers, placebo	1004	926	899	789	678	596	494	407
Noncarriers, placebo	1489	1386	1343	1218	1066	931	783	647

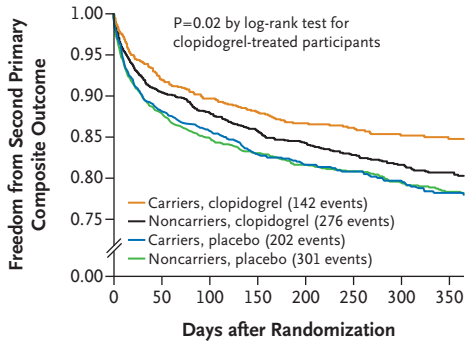
C Second Primary Composite Outcome According to Loss-of-Function Allele Carrier Status



No. at Risk

Carriers, clopidogrel	651	609	578	510	450	397	332	270
Noncarriers, clopidogrel	1886	1695	1622	1444	1248	1091	871	732
Carriers, placebo	674	599	574	514	446	389	323	248
Noncarriers, placebo	1819	1595	1530	1349	1152	1004	832	699

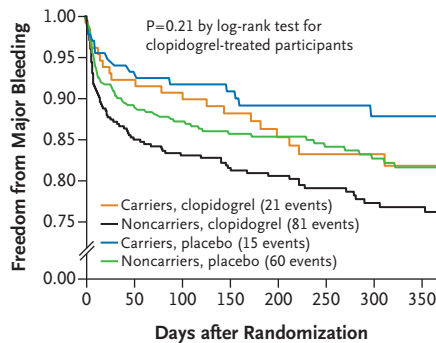
D Second Primary Composite Outcome According to Gain-of-Function Allele Carrier Status



No. at Risk

Carriers, clopidogrel	1001	919	882	780	664	592	467	390
Noncarriers, clopidogrel	1536	1386	1319	1174	1035	897	736	612
Carriers, placebo	1004	883	851	740	629	551	453	374
Noncarriers, placebo	1489	1310	1251	1122	970	842	701	572

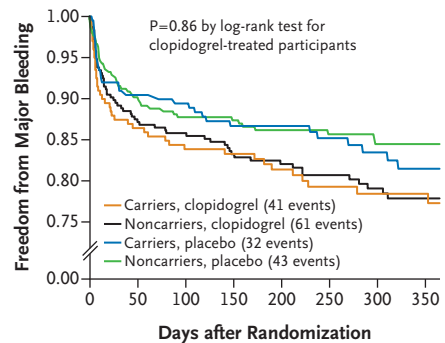
E Freedom from Major Bleeding According to Loss-of-Function Allele Carrier Status



No. at Risk

Carriers, clopidogrel	651	630	613	548	485	426	362	297
Noncarriers, clopidogrel	1886	1782	1726	1549	1365	1198	971	817
Carriers, placebo	674	647	633	577	504	444	371	294
Noncarriers, placebo	1819	1737	1693	1513	1314	1146	954	803

F Freedom from Major Bleeding According to Gain-of-Function Allele Carrier Status



No. at Risk

Carriers, clopidogrel	1001	957	929	828	718	637	512	427
Noncarriers, clopidogrel	1536	1456	1411	1269	1133	988	821	687
Carriers, placebo	1004	957	938	827	715	625	517	429
Noncarriers, placebo	1489	1427	1388	1264	1106	967	807	667

Figure 2 (facing page). Kaplan–Meier Curves for Event-free Survival According to CYP2C19 Loss-of-Function and Gain-of-Function Allele Carrier Status among European and Latin American Patients with Acute Coronary Syndromes in the CURE Trial.

Loss-of-function allele carriers were defined as patients with at least one loss-of-function allele (i.e., *2 or *3): *1/*2, *1/*3, *2/*2, *2/*3, *3/*3, *2/*17, or *3/*17; loss-of-function noncarriers were defined as patients with no loss-of-function allele: *1/*1, *1/*17, or *17/*17. Gain-of-function carriers were defined as carriers of at least one gain-of-function allele (i.e., *17): *1/*17, *17/*17, *2/*17, or *3/*17; gain-of-function noncarriers were defined as patients with no gain-of-function allele: *1/*1, *1/*2, *1/*3, *2/*2, *2/*3, or *3/*3.

interaction. However, the consistent effects in our two trials reinforce our results and suggest that there was no difference in outcomes among clopidogrel-treated patients in subgroups defined according to loss-of-function carrier status.

SUBGROUP ANALYSES

Among the genotyped participants in the CURE trial, the results of our analyses of the effects of clopidogrel as compared with placebo were consistent across subgroups defined according to loss-of-function carrier status, whether or not participants underwent PCI (see Fig. 5 and 6 in the Supplementary Appendix). Even though only 736 patients who received stents were included in the genetic analysis, there was a significant treatment effect of clopidogrel on the first composite primary outcome among loss-of-function allele carriers (hazard ratio, 0.37; 95% CI, 0.14 to 0.96; $P=0.04$). A similar treatment effect was observed among noncarriers (hazard ratio, 0.62; 95% CI, 0.37 to 1.02; $P=0.06$), and there was no evidence of an interaction between carrier status and study treatment with respect to either the first or the second composite primary outcome ($P=0.37$ and $P=0.28$, respectively). We also tested for an interaction between loss-of-function carrier status and both current smoking status^{12,13} and the time to the first event after the initiation of clopidogrel treatment (<24 hours, <7 days, or <30 days).¹⁴ No significant interactions were observed (data not shown).

DISCUSSION

Our results suggest that the efficacy and safety of clopidogrel as compared with placebo are not

modified by CYP2C19 loss-of-function alleles. No significant difference in the effects of clopidogrel treatment on clinical outcomes was observed when patients were stratified according to metabolizer phenotype. In contrast to findings in previous studies,^{3-5,15} the presence of loss-of-function alleles in patients with acute coronary syndromes or atrial fibrillation was not associated with an increased risk of major cardiovascular events, even when the analyses were restricted to data on homozygous patients (poor-metabolizer phenotype). However, clopidogrel showed enhanced efficacy for the reduction of ischemic events in patients with acute coronary syndromes who were carriers of the gain-of-function allele, although this effect was not observed in patients with atrial fibrillation who were carriers of the gain-of-function allele.

The absence of an effect of the CYP2C19 loss-of-function alleles on cardiovascular risk among patients treated with clopidogrel is in contrast to findings from previous studies. One possible explanation for the divergence between our findings and those of previous studies involving patients with acute coronary syndromes is the difference in the rates of PCI with stenting. Only 18.0% of patients in the CURE population included in our study underwent PCI, and only 14.5% underwent PCI with placement of a stent, as compared with more than 70% in previous studies.^{3-5,15} Results of randomized, controlled trials have consistently shown that the greatest benefit of clopidogrel, including its use in high doses (as in the Clopidogrel and Aspirin Optimal Dose Usage to Reduce Recurrent Events [CURRENT] study, NCT00335452), and of newer generation P2Y12 inhibitors such as prasugrel and ticagrelor is the reduction in the rate of stent thrombosis. We found no evidence of an interaction between study treatment and genotype with respect to cardiovascular events among patients who underwent PCI with or without stenting, but we cannot definitely exclude the possibility of an interaction in the subgroup of patients who receive stents, particularly those who receive drug-eluting stents, which were not in use at the time of the CURE trial. In the study by Collet et al.³ and in the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel (TRITON; NCT00097591),⁴ the greatest effect of loss-of-function alleles on the outcome in clopidogrel-

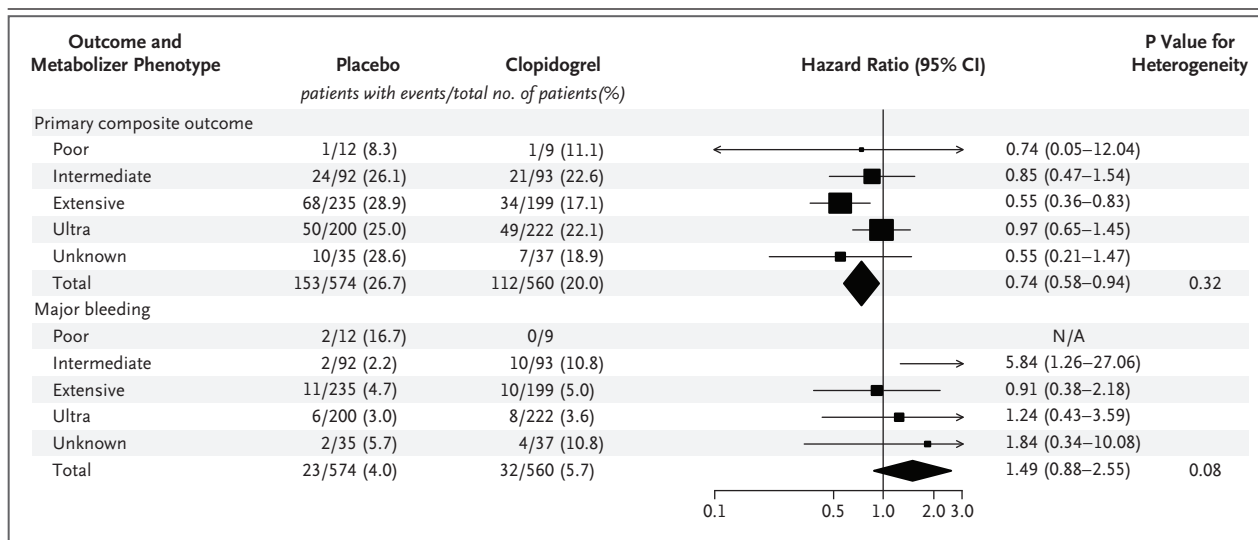


Figure 3. Effect of Clopidogrel as Compared with Placebo on Clinical Outcomes among Patients with Atrial Fibrillation in ACTIVE A, Stratified According to Metabolizer Phenotype.

Hazard ratios for clopidogrel as compared with placebo are shown for efficacy and bleeding outcomes according to metabolizer phenotype. The size of each symbol is in inverse proportion to the standard deviation of the effect-size estimates. Analyses were performed on data from patients of European ancestry, with adjustment for age and sex. Patients with two *2 or *3 alleles (i.e., *2/*2, *2/*3, or *3/*3) were classified as having the poor-metabolizer phenotype, those with one *2 or *3 allele (i.e., *1/*2 or *1/*3) were classified as having the intermediate-metabolizer phenotype, those without a *2, *3, or *17 allele (i.e., *1/*1) were classified as having the extensive-metabolizer phenotype, those with a single *17 allele (i.e., *1/*17) and *17 homozygotes were classified as having the ultrametabolizer phenotype, and those with one *17 allele and one loss-of-function allele (i.e., *2/*17 or *3/*17) were classified as having an unknown metabolizer phenotype. Only patients who were successfully genotyped for all three single-nucleotide polymorphisms were included in these analyses.

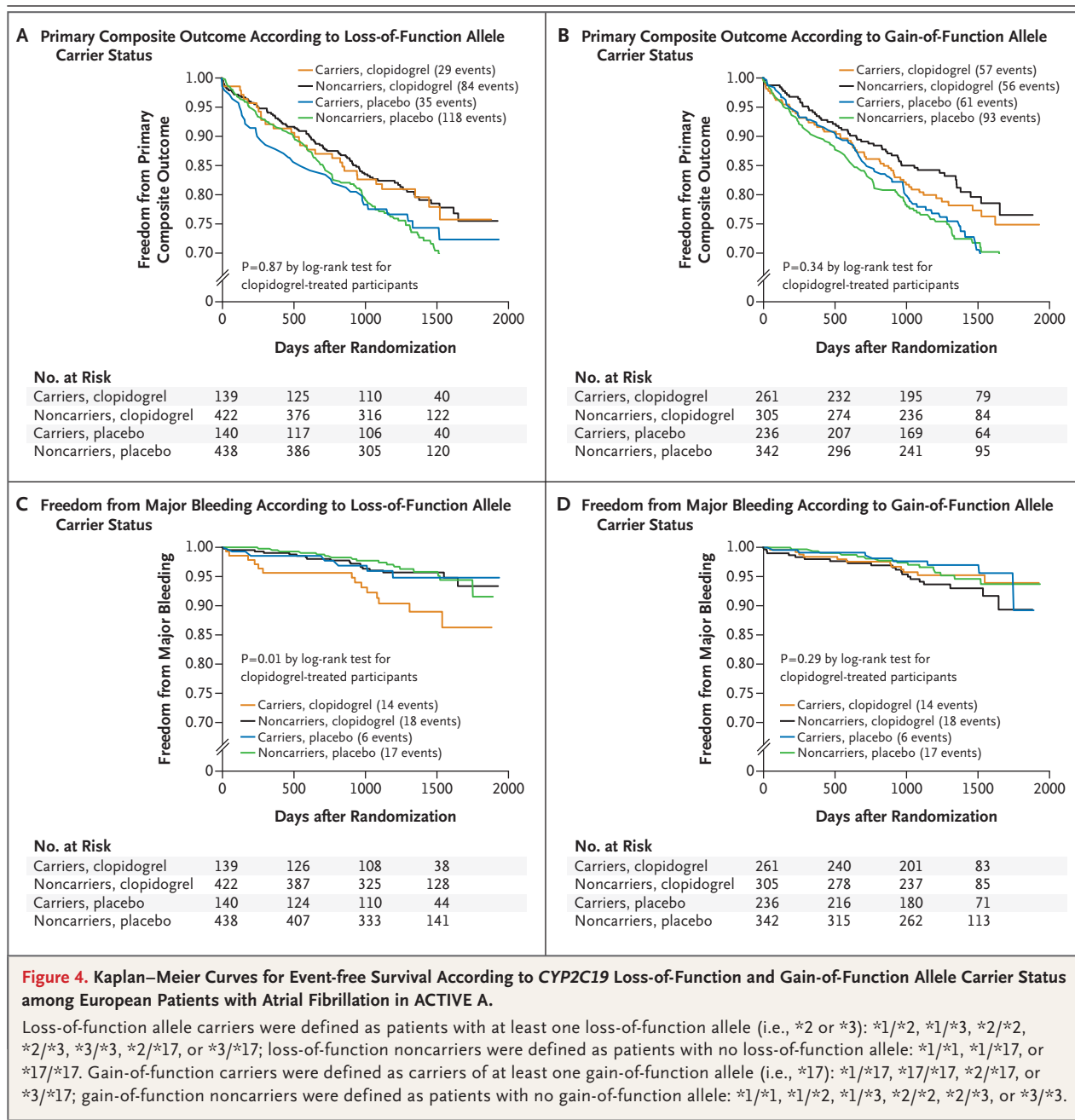
treated patients was the effect on stent thrombosis, a finding that is consistent with such an interaction.

Previous studies that have shown an attenuated benefit of clopidogrel among carriers of loss-of-function alleles did not include a randomized control group, and therefore potential pleiotropic effects of loss-of-function alleles (i.e., independent of their effects on active metabolite levels of clopidogrel) could not be excluded. For instance, in analyses of the ACTIVE A database that were confined to patients treated with clopidogrel, we observed an increased risk of major bleeding among carriers of loss-of-function alleles as compared with noncarriers ($P=0.01$). However, a similar pattern of increased bleeding was also observed among those who received placebo, such that there was no evidence of an interaction between study drug and loss-of-function carrier status ($P=0.16$ for heterogeneity). In other words, the association would have been considered significant if we had not included the placebo group in the analysis. This illustrates the potential pitfalls of interpreting

subgroup data and highlights the importance of including a placebo group to control for potential confounding in analyses of pharmacogenetic data.

The increased benefit of clopidogrel in gain-of-function allele carriers in the CURE trial is consistent with pharmacodynamic data associating this genetic variant with increased enzymatic activity, an enhanced platelet response to clopidogrel, and an increased risk of bleeding.^{6,16,17} Although this is a new finding, the lack of validation of this observation among patients in ACTIVE A highlights the need for replication in larger studies. Apart from the modest sample size in ACTIVE A, population-specific pharmacogenetic effects could also explain this observation.

Our study has some limitations. First, despite the large number of participants and events in the CURE genetic data sets, we cannot exclude the possibility that there are smaller interactions. Second, the ACTIVE A genetic data set contained fewer participants and outcome events than did the CURE data set and therefore had



less statistical power. Third, our results might be specific to our patient population and the way their cases were managed. Fourth, only participants of European or Latin American ancestries could be adequately analyzed. Even though there is no reason to suspect, a priori, that there would be different results in other populations, further studies in diverse populations will be needed. Finally, we studied only the three most

common functional alleles. The low allele frequency of other known alleles should not materially alter our conclusions but may be relevant for individual patients.

Our study also has several strengths. First, the inclusion of a randomized placebo group in our analyses reduces various sources of confounding, such as potential pleiotropic genetic effects or population stratification. Second, the large

numbers of patients and events in our study ensure adequate statistical power and robust estimates of genetic effect sizes. Third, we observed consistent benefits of clopidogrel, irrespective of *CYP2C19* genotype, in two different patient populations, which validates our findings and suggests that they could be generalizable to other populations.

In conclusion, our study shows that *CYP2C19* loss-of-function variants do not modify the efficacy and safety of clopidogrel. Therefore, loss-of-function allele carrier status should not preclude the use of clopidogrel at currently recommended doses in patients with acute coronary syndromes whose condition is being managed conservatively. Although similar results were observed in patients with atrial fibrillation, larger studies will be needed to definitively rule out a genetic effect of the loss-of-function alleles in this patient population.

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