

ORIGINAL ARTICLE

Genetic Determinants of Response to Clopidogrel and Cardiovascular Events

Tabassome Simon, M.D., Ph.D., Céline Verstuyft, Pharm.D., Ph.D., Murielle Mary-Krause, Ph.D., Lina Quteineh, M.D., Elodie Drouet, M.Sc., Nicolas Méneveau, M.D., P. Gabriel Steg, M.D., Ph.D., Jean Ferrières, M.D., Nicolas Danchin, M.D., Ph.D., and Laurent Becquemont, M.D., Ph.D., for the French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators

ABSTRACT

BACKGROUND

Pharmacogenetic determinants of the response of patients to clopidogrel contribute to variability in the biologic antiplatelet activity of the drug. The effect of these determinants on clinical outcomes after an acute myocardial infarction is unknown.

METHODS

We consecutively enrolled 2208 patients presenting with an acute myocardial infarction in a nationwide French registry and receiving clopidogrel therapy. We then assessed the relation of allelic variants of genes modulating clopidogrel absorption (*ABCB1*), metabolic activation (*CYP3A5* and *CYP2C19*), and biologic activity (*P2RY12* and *ITGB3*) to the risk of death from any cause, nonfatal stroke, or myocardial infarction during 1 year of follow-up.

RESULTS

Death occurred in 225 patients, and nonfatal myocardial infarction or stroke in 94 patients, during the follow-up period. None of the selected single-nucleotide polymorphisms (SNPs) in *CYP3A5*, *P2RY12*, or *ITGB3* were associated with a risk of an adverse outcome. Patients with two variant alleles of *ABCB1* (TT at nucleotide 3435) had a higher rate of cardiovascular events at 1 year than those with the *ABCB1* wild-type genotype (CC at nucleotide 3435) (15.5% vs. 10.7%; adjusted hazard ratio, 1.72; 95% confidence interval [CI], 1.20 to 2.47). Patients carrying any two *CYP2C19* loss-of-function alleles (*2, *3, *4, or *5), had a higher event rate than patients with none (21.5% vs. 13.3%; adjusted hazard ratio, 1.98; 95% CI, 1.10 to 3.58). Among the 1535 patients who underwent percutaneous coronary intervention during hospitalization, the rate of cardiovascular events among patients with two *CYP2C19* loss-of-function alleles was 3.58 times the rate among those with none (95% CI, 1.71 to 7.51).

CONCLUSIONS

Among patients with an acute myocardial infarction who were receiving clopidogrel, those carrying *CYP2C19* loss-of-function alleles had a higher rate of subsequent cardiovascular events than those who were not. This effect was particularly marked among the patients undergoing percutaneous coronary intervention. (ClinicalTrials.gov number, NCT00673036.)

From Assistance Publique–Hôpitaux de Paris (AP-HP), Hôpital Saint-Antoine, and Université Pierre et Marie Curie (UPMC) Paris 06 (T.S., L.Q., E.D.); AP-HP, Hôpital Bicêtre, Université Paris Sud 11 (C.V., L.B.); INSERM, Unite 720 (M.M.-K.); AP-HP, Hôpital Bichat; INSERM, Unite 698; and Université Paris 07 (P.G.S.); and AP-HP, Hôpital Européen Georges Pompidou, Université Rene Descartes Paris 05 (N.D.) — all in Paris; Centre Hospitalier Universitaire Besançon, Besançon (N.M.); and INSERM, Unite 558, Toulouse (J.F.) — all in France. Address reprint requests to Dr. Simon at the Department of Pharmacology, UPMC, 27 Rue Chaligny, 75012 Paris, France, or at tabassome.simon@sat.aphp.fr.

Drs. Danchin and Becquemont contributed equally to this article.

This article (10.1056/NEJMoa0808227) was published at NEJM.org on December 22, 2008.

N Engl J Med 2009;360:363-75.
Copyright © 2009 Massachusetts Medical Society.

DUAL ANTIPLATELET THERAPY WITH ASPIRIN and clopidogrel is currently recommended for the prevention of atherothrombotic events in patients after acute myocardial infarction.^{1,2} However, even with the use of such therapy, a substantial number of subsequent ischemic events still occur.³⁻⁶ There is interindividual variability in the response to clopidogrel.⁷⁻⁹ Some studies have suggested that hyporesponsiveness is associated with poorer clinical outcomes after an acute coronary syndrome, particularly after percutaneous coronary intervention (PCI).¹⁰ However, there is also variability in the identification of biologic hyporesponsiveness to clopidogrel, depending on the test or agonist used and the timing of the assessment.

The mechanisms leading to a poor response to clopidogrel have not yet been fully elucidated and are most likely multifactorial.^{1,2} In addition to lack of compliance, clinical factors such as obesity, insulin resistance, and the nature of the coronary event may contribute to the variability of the clopidogrel response.⁷ Clopidogrel is also a pro-drug, requiring metabolism before it can inhibit adenosine diphosphate–induced platelet aggregation. There is growing evidence that the response to clopidogrel may be influenced by pharmacokinetic variables such as intestinal absorption and metabolic activation in the liver, both of which are affected by genetic polymorphisms (Fig. 1).¹¹⁻¹⁸ The relation between known polymorphisms of relevant genes and the clinical outcome after acute myocardial infarction in patients receiving clopidogrel is unknown. To address this issue, we evaluated whether previously identified polymorphisms of genes modulating clopidogrel absorption (*ABCB1*¹⁷), metabolic activation (*CYP3A5*¹⁹ and *CYP2C19*^{16,18,20}), and biologic activity (*P2RY12*¹⁴ and *ITGB3*¹¹) were associated with death or ischemic events during a 1-year follow-up period among patients in the French Registry of Acute ST-Elevation and Non–ST-Elevation Myocardial Infarction (FAST-MI) who were receiving clopidogrel after acute myocardial infarction. A complete list of centers and investigators participating in the registry has been published elsewhere.²¹

METHODS

STUDY POPULATION

The French Registry of Acute ST-Elevation and Non–ST-Elevation Myocardial Infarction (FAST-MI)

study population and methods have been described in detail elsewhere.²¹ Briefly, the objective of the registry was to gather complete and representative data on both care and outcomes for patients with definite acute myocardial infarction who were admitted to intensive care units (ICUs), irrespective of the type of institution (i.e., university hospitals, public hospitals, or private clinics). Patients were recruited during a period of 1 month (31 days) at each center (2 months for patients with diabetes) between October 1 and December 24, 2005. Of the 374 centers in France that at that time treated patients with acute myocardial infarction, 223 (60%) participated in the study. Written informed consent for study participation was provided by each patient. In accordance with French law, the study was reviewed by the Committee for the Protection of Human Subjects in Biomedical Research of Saint Antoine University Hospital, and the data file was approved by the Commission Nationale Informatique et Liberté.

All consecutively enrolled patients 18 years of age or older were included in the registry if they had levels of serum markers of myocardial necrosis (creatinine kinase, creatine kinase MB, troponin I, or troponin T) that were more than twice the upper limit of the normal range and either symptoms consistent with acute myocardial infarction or electrocardiographic changes in at least two contiguous leads (pathologic Q waves [≥ 0.04 second in duration], persistent ST-segment elevation, or ST-segment depression >0.1 mV). The time from the onset of symptoms to admission to the ICU had to be less than 48 hours. Patients received care according to usual practice; treatment was not affected by participation in the registry. When blood was drawn at the time of admission, an additional 10 ml was taken for DNA banking.

Follow-up information was collected through contacts with the patients' physicians, the patients or their family, and registry offices at their places of birth. The primary outcome was the composite of death from any cause, nonfatal myocardial infarction, or stroke during 1 year of follow-up after admission. Events were adjudicated by a scientific committee whose members were unaware of patients' medications and genotypes. Follow-up information was available for 99.2% of patients initially enrolled.

The study was designed and conducted by the authors. Genotyping was performed by Integragen under the direction of two of the authors. Data

collection was performed by the International Clinical Trials Association and Assistance Publique–Hôpitaux de Paris, Unité de Recherche Clinique de l'Est. The authors analyzed the data, wrote the manuscript, and made the decision to submit the manuscript for publication; they vouch for the completeness and accuracy of the data. The sponsors had no role in the design or conduct of the study, in the analysis of the results, or in the decision to publish the paper.

GENOTYPING

Genomic DNA was extracted from whole-blood specimens with the use of a purifier (the MagNA Pure Compact Instrument, Roche) according to the manufacturer's recommendations. Genotyping for *CYP2C19*, *CYP3A5*, *ABCB1* and *P2RY12* was performed with the use of an oligonucleotide ligation assay (SNPlex, Applied Biosystems) after initial amplification by means of a polymerase-chain-reaction assay involving two primers for the major variant alleles *CYP2C19**2 (rs4244285), *CYP2C19**3 (rs4986893), *CYP3A5**3 (rs776746), *ABCB1* (rs1045642), and *P2RY12* (rs16846673, rs6809699, and rs6785930), as described previously²² (see the Supplementary Appendix, available with the full text of this article at NEJM.org).

Genotyping for known variants of *CYP2C19* and *ITGB3* with functional importance — *CYP2C19**4 (rs28399504), *CYP2C19**5, *CYP2C19**17 (rs12248560), and *ITGB3* (rs5918) — was performed with the use of an allelic discrimination assay (Custom TaqMan) (see the Supplementary Appendix) and a detection system (ABI prism 7900HT Sequence Detection System, Applied Biosystems). Base numbering and allele definitions follow the nomenclature of the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee (www.cypalleles.ki.se).

STATISTICAL ANALYSIS

All statistical tests, performed with the use of SAS software, version 9.1, or SPSS software, version 14.0, were two-sided. All single-nucleotide polymorphisms (SNPs) evaluated in our study were tested for deviation from Hardy–Weinberg equilibrium with the use of a chi-square test. A univariate Cox proportional-hazards model was used to compare baseline demographic and clinical characteristics and characteristics of treatment and therapeutic management during hospitalization between the group with and the group with-

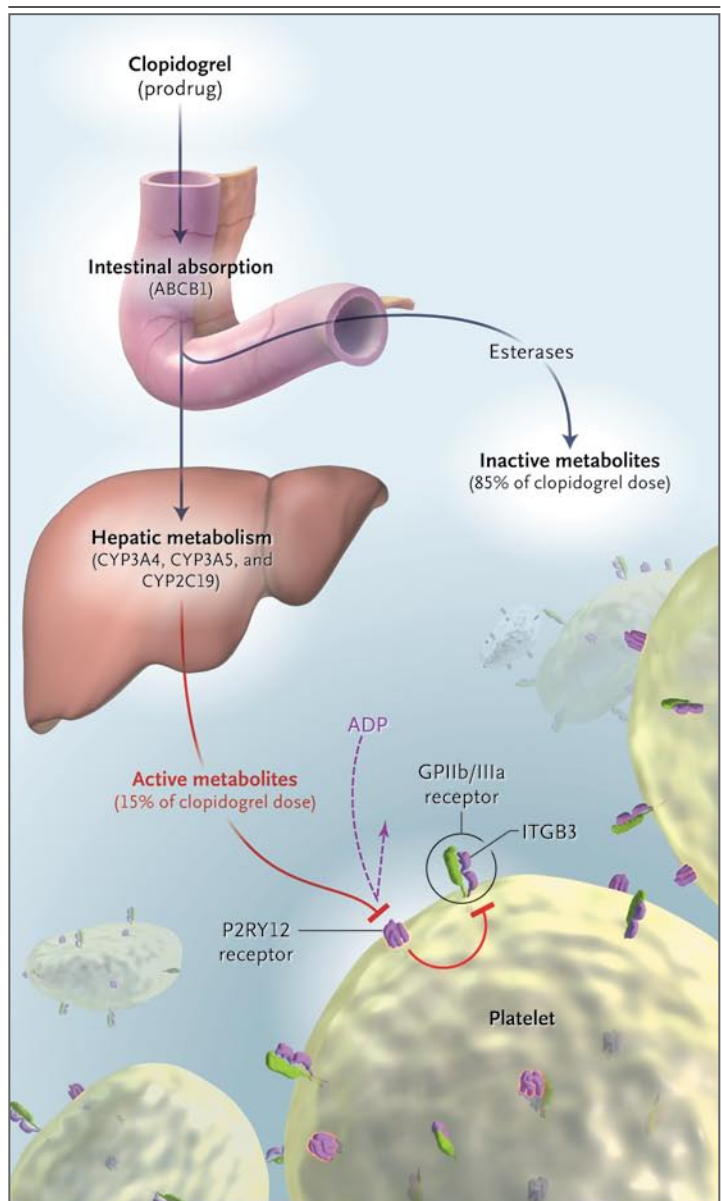


Figure 1. Roles in Clopidogrel Activity of Proteins with Known Genetic Polymorphisms.

Intestinal absorption of the prodrug clopidogrel is limited by an intestinal efflux pump P-glycoprotein coded by the *ABCB1* gene. The majority of the prodrug is metabolized into inactive metabolites by ubiquitous esterases. The minority is bioactivated by various cytochrome P450 (*CYP*) isoforms into active metabolites. These metabolites irreversibly antagonize the adenosine diphosphate (ADP) receptor (coded by the *P2RY12* gene), which in turn inactivates the fibrinogen receptor (the glycoprotein [GP] IIb/IIIa receptor coded by the *ITGB3* gene) involved in platelet aggregation.

out outcome events. We also compared the two groups with respect to the frequencies of the *ABCB1*, *CYP3A5*, *P2RY12*, and *ITGB3* alleles and individual variant alleles of *CYP2C19*, including

*CYP2C19**17 (associated with very rapid *CYP2C19* activity¹⁸), as well as the *CYP2C19* alleles known to result in a nonfunctional protein (*2, *3, *4, and *5), classified as the presence of zero, one, or two variant alleles.

Predictors identified through univariate analysis ($P < 0.10$) and other variables considered likely to have important prognostic value were tested in a multivariable, stepwise, forward Cox proportional-hazards model for association with the primary outcome, the composite of death from any cause, nonfatal myocardial infarction, or stroke during the 1-year follow-up period. The analysis was repeated for the subgroup of patients who underwent PCI during hospitalization. We also performed a propensity analysis for the *CYP2C19* loss-of-function alleles, using a multivariate logistic-regression model, and developed a matched cohort of five control patients for each patient with two variant alleles, on the basis of the propensity-analysis score.

CYP2C19 is involved in the metabolism of proton-pump inhibitors, including omeprazole, a commonly prescribed drug. We therefore tested the effect of the coprescription of omeprazole by forcing the variable into the model, although its P value in the univariate analysis was greater than 0.20. Similarly, we analyzed the effect of *CYP2C19**17 and the concomitant prescription of proton-pump inhibitors, including omeprazole, by forcing these variables into another multivariable Cox model.

Results are expressed as hazard ratios from the Cox models, along with the 95% confidence intervals. Survivor-function estimates for mean values of covariates in the Cox model were generated with the use of the product-limit approach (the BASELINE statement in the PHREG procedure of SAS software). We used the likelihood-ratio test for testing gene-gene interactions for the genes identified in the Cox multivariable model as having an association with outcome events. The full model, including the interactive effect (four interaction terms corresponding to the cross-products of the two genotypes for each gene), was compared with the null model, which included only marginal effects. Under the null hypothesis of no interaction, the likelihood-ratio test follows a chi-square distribution with 4 degrees of freedom, corresponding to the difference between the full model (8 degrees of freedom) and the null model (4 degrees of freedom).

RESULTS

CHARACTERISTICS OF THE PATIENTS

Of the 3670 patients enrolled in FAST-MI, 2430 patients (66%) contributed a sample to the DNA bank. Of these 2430 patients, the 2208 who received clopidogrel were included in the present analysis. The mean loading dose of clopidogrel was 300 mg per day (<300 mg per day in 36% of patients, 300 to 375 mg per day in 50%, and 450 to 900 mg per day in 15%), and the mean maintenance dose at the time of hospital discharge was 75 mg per day.

A total of 225 patients died, and 94 had a nonfatal myocardial infarction or stroke during the follow-up period. As compared with patients who did not have an outcome event, the 294 patients who had an event (13% of the study cohort) were older; more frequently had a history of hypertension, diabetes, myocardial infarction, PCI, stroke, or heart failure; and less frequently underwent reperfusion therapy consisting of primary PCI or intravenous fibrinolysis (Table 1). The use of calcium-channel blockers, aspirin, and proton-pump inhibitors was similar in the two groups, whereas patients who had an outcome event were less likely to receive statins, beta-blockers, angiotensin-converting-enzyme inhibitors, glycoprotein IIb/IIIa inhibitors, and heparin (Table 1).

ALLELIC FREQUENCIES

The observed genotype distributions did not deviate from Hardy-Weinberg equilibrium and matched those reported for white populations. The allelic frequencies of *CYP3A5*, *P2RY12*, *ITGB3*, and the individual *CYP2C19* variants did not differ significantly between the patients with and those without outcome events (Table 2). However, the frequency of the *ABCB1* variant allele and the combined *CYP2C19* loss-of-function variant alleles differed significantly between the two groups (Table 2).

PREDICTORS OF DEATH AND MAJOR EVENTS

None of the selected SNPs in the *CYP3A5*, *P2RY12*, or *ITGB3* genes were significantly associated with the risk of death, nonfatal myocardial infarction, or stroke. In contrast, there was an increase in the hazard ratio for an outcome event among patients carrying the *ABCB1* variant allele (genotype CT or TT) as compared with the wild-type allele

(genotype CC) (Fig. 2A) or any two of the *CYP2C19* loss-of-function variant alleles as compared with one or none (Fig. 2B). This increase in risk remained significant after adjustment for the risk factors and treatments listed in Table 1 (Table 3). Patients with two variant alleles of *ABCB1* (TT) had a higher event rate at 1 year than those with the *ABCB1* wild-type genotype (CC) (15.5% vs. 10.7%; adjusted hazard ratio, 1.72; 95% confidence interval [CI], 1.20 to 2.47). Similarly, patients carrying two *CYP2C19* loss-of-function variant alleles had a higher event rate than patients who did not have these alleles (21.5% vs. 13.3%; adjusted hazard ratio, 1.98; 95% CI, 1.10 to 3.58) (Table 3). After full propensity-score matching (see the Supplementary Appendix), the risk of an outcome event at 1 year among patients with two, as compared with zero, *CYP2C19* deficiency alleles was even higher (hazard ratio, 2.14; 95% CI, 1.09 to 4.17). Accounting for the presence of *CYP2C19**17 or the concomitant prescription of proton-pump inhibitors or calcium-channel blockers had no significant effect on these risks.

No significant interaction was found between either the *ABCB1* variant allele or the *CYP2C19* loss-of-function variant alleles and the clinical outcome (likelihood-ratio test statistic, 0.276; $P=0.99$). However, the presence of both two *CYP2C19* loss-of-function alleles and either one or two *ABCB1* variant alleles was associated with the highest risk of events (adjusted hazard ratio for the comparison with the presence of homozygous wild-type *ABCB1* and *CYP2C19* alleles, 5.31; 95% CI, 2.13 to 13.20; $P=0.009$).

Among the 1535 patients who underwent PCI during hospitalization, the adjusted risk of death, myocardial infarction, or stroke for patients with two *CYP2C19* deficiency alleles was 3.58 times the risk among patients with the wild-type genotype (95% CI, 1.71 to 7.51; $P=0.005$) (Table 3), whereas the *ABCB1* variant allele had no significant independent effect ($P=0.35$).

DISCUSSION

The present study aimed to determine whether previously identified SNPs known to alter the pharmacokinetics of clopidogrel or the ex vivo ability of platelets to aggregate were associated with clinical outcomes during the first year after acute myocardial infarction. Variant alleles of

two candidate genes involved in clopidogrel absorption (*ABCB1*) and metabolism (*CYP2C19*) were linked to an increased rate of cardiovascular events. In addition, the presence of two variant alleles of *CYP2C19*, but not *ABCB1*, were found to be associated with an increase by a factor of 3.6 in the rate of cardiovascular events among the patients who underwent PCI during hospitalization as compared with those who did not. As expected, patients with an outcome event had a worse risk profile at admission for acute myocardial infarction than did those without an event. However, there was no significant difference in the risk profile or hospital care received between patients with allelic variants for clopidogrel target genes and those without such variants.

The P2RY12¹⁴ receptor for clopidogrel and its effector, glycoprotein IIb/IIIa,¹¹ were the first pharmacogenetic targets found to explain the biologic variability in response to this antiplatelet drug. However, in subsequent studies, ex vivo antiplatelet activity after the administration of clopidogrel was not related to allelic variants in these proteins.^{15,23-25} We found no association between polymorphisms of *P2RY12* and *ITGB3* (the gene encoding the beta subunit of glycoprotein IIb/IIIa) and clinical outcomes in patients with acute myocardial infarction who were treated with clopidogrel.

Clopidogrel is a prodrug that must be metabolized in the liver by several CYP proteins, including CYP3A and CYP2C19, to become active.²⁶ Suh et al.¹⁹ reported an increased frequency of atherothrombotic events within 6 months after coronary angioplasty among patients with the CYP3A5 nonexpression genotype (*CYP3A5**3) who were receiving clopidogrel therapy. Further studies, however, showed no association between the *CYP3A5* genetic polymorphism and the antiplatelet effect of clopidogrel ex vivo, either in patients^{13,23} or in healthy subjects.¹⁴ Likewise, the results of the present study do not support a role of the *CYP3A5* genetic polymorphism in the clinical response to clopidogrel. It is unlikely that concomitant drug use blunted a putative effect of the *CYP3A5* genetic polymorphism, since most of the patients were not treated with strong or even moderate CYP3A inhibitors, as defined by current Food and Drug Administration guidelines (www.fda.gov/cder/drug/drugInteractions/default.htm).

Table 1. Baseline Characteristics and Characteristics of In-Hospital Care of the Study Patients.*

Characteristic	Patients without Outcome Event (N=1914)	Patients with Outcome Event (N=294)	P Value
Demographic and clinical characteristics			
Sex — no. (%)			0.001
Male	1375 (72)	184 (63)	
Female	539 (28)	110 (37)	
Age — yr	64.8±13.5	75.4±10.8	<0.001
Hypertension — no. (%)	1068 (56)	212 (72)	<0.001
Hypercholesterolemia — no. (%)	944 (49)	144 (49)	0.85
Diabetes mellitus — no. (%)	576 (30)	122 (41)	<0.001
Family history of CAD — no. (%)	492 (26)	35 (12)	<0.001
Previous or current smoker — no. (%)	1077 (56)	129 (44)	<0.001
Previous MI — no. (%)	299 (16)	74 (25)	<0.001
Previous PCI or CABG — no. (%)			
PCI	258 (13)	53 (18)	0.05
CABG	90 (5)	28 (10)	0.002
Previous stroke or TIA — no. (%)			
Stroke	80 (4)	23 (8)	0.005
TIA	56 (3)	16 (5)	0.03
Previous heart failure — no. (%)	60 (3)	34 (12)	<0.001
Peripheral-artery disease — no. (%)	153 (8)	56 (19)	<0.001
Cancer — no. (%)	106 (6)	29 (10)	0.01
COPD — no. (%)	75 (4)	28 (10)	<0.001
Chronic renal failure — no. (%)	74 (4)	30 (10)	<0.001
Acute MI as first cardiovascular event — no. (%)	1397 (73)	249 (85)	<0.001
STEMI — no. (%)	1050 (55)	124 (42)	<0.001
Body-mass index†	27.3±4.7	26.5±5.0	0.009
Blood pressure on admission — mm Hg			
Systolic	141±28	139±31	0.25
Diastolic	81±17	78±18	0.02
Heart rate on admission — beats/min	81±23	79±21	0.61
Killip class ≥2 — no. (%)	316 (17)	133 (45)	<0.001
GRACE risk score‡	158.2±34.9	185.9±35.5	<0.001
Leukocyte count — ×10 ³ /mm ³	10.2±3.6	10.8±3.9	0.008
Left ventricular ejection fraction — %	53.6±12.4	44.8±14.2	<0.001
Previous treatment — no. (%)			
Aspirin	414 (22)	93 (32)	<0.001
Clopidogrel	234 (12)	72 (24)	<0.001
Beta-blockers	437 (23)	88 (30)	0.01
Statins	512 (27)	100 (34)	0.02
ACE inhibitor or ARB	604 (32)	131 (45)	<0.001

Table 1. (Continued.)

Characteristic	Patients without Outcome Event (N=1914)	Patients with Outcome Event (N=294)	P Value
In-hospital care			
PCI — no. (%)	1397 (73)	138 (47)	<0.001
Thrombolysis — no. (%)	327 (17)	27 (9)	<0.001
CABG — no. (%)	72 (4)	7 (2)	0.20
Statin — no. (%)	1755 (92)	234 (80)	<0.001
Beta-blocker — no. (%)	1670 (87)	194 (66)	<0.001
Calcium-channel blocker — no. (%)	446 (23)	74 (25)	0.57
ACE inhibitor or ARB — no. (%)	1437 (75)	206 (70)	0.05
Nitrated derivative — no. (%)	1297 (68)	211 (72)	0.86
Aspirin — no. (%)	1885 (98)	287 (98)	0.11
Heparin — no. (%)	1719 (89)	248 (84)	0.005
Proton-pump inhibitor — no. (%)			
Any	1397 (73)	209 (71)	0.75
Omeprazole	1006 (53)	141 (48)	0.12
Diuretic — no. (%)			
Any	606 (32)	191 (65)	<0.001
Aldosterone antagonist	131 (7)	39 (13)	<0.001
Glycoprotein IIb/IIIa inhibitor — no. (%)	746 (39)	93 (32)	0.02
Digitalis glycoside — no. (%)	31 (2)	19 (6)	<0.001

* Plus-minus values are means \pm SD. P values were calculated with the use of univariate Cox analysis. ACE denotes angiotensin-converting enzyme, ARB angiotensin-receptor blocker, CABG coronary-artery bypass grafting, CAD coronary artery disease, COPD chronic obstructive pulmonary disease, MI myocardial infarction, PCI percutaneous coronary intervention, STEMI ST-elevation MI, and TIA transient ischemic attack.

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

‡ The global registry of acute coronary events (GRACE) score for the risk of in-hospital death can range from 1 to 319, with higher scores indicating a greater risk.

Genetic polymorphisms of *CYP2C19* modulate clopidogrel pharmacokinetics¹³ and pharmacodynamics in healthy volunteers,^{13,16} as well as in patients.^{15,18,27} As compared with subjects with no *CYP2C19* variant allele, subjects carrying one or two *CYP2C19* loss-of-function alleles have been shown to have lower plasma concentrations of the active metabolite of clopidogrel and a decrease in the antiplatelet effect of clopidogrel in ex vivo aggregation tests.¹³ Our results support and extend these findings from previous studies by showing a worse clinical outcome in patients carrying two *CYP2C19* loss-of-function alleles who were treated with clopidogrel after acute myocardial infarction. This effect was particularly marked in the subgroup of patients who underwent PCI. In contrast, patients with one *CYP2C19*

variant allele did not have an increased risk (and actually had a slightly lower risk in the overall population), as compared with those who had no *CYP2C19* variant alleles.

The antiplatelet activity of clopidogrel has been shown to be reduced in patients receiving omeprazole, a *CYP2C19* inhibitor.²⁸ In the present study, the use of omeprazole, or any other proton-pump inhibitor, had no effect on the clinical response to clopidogrel. This is an important clinical observation, considering the high frequency of coprescription of proton-pump inhibitors and dual antiplatelet therapy. It is possible that biologic differences detected by platelet-function tests are not large enough to have clinical relevance. This possibility would account for the absence of a discernible effect of omeprazole

or the *CYP2C19**17 allele, which slightly increases *CYP2C19* expression, on clinical outcomes after clopidogrel therapy.

The drug-efflux transporter, P-glycoprotein (encoded by the *ABCB1* gene), is a physiologic intestinal barrier against the absorption of several drugs, including clopidogrel.¹⁷ The relation

between the noncoding *ABCB1* C3435T SNP and P-glycoprotein expression or activity remains controversial.²⁹⁻³⁴ Discrepancies in the reported effect of C3435T may reflect differences in *ABCB1* SNP frequencies among ethnic groups,³⁵ complex effects of various polymorphisms along the same gene within a haplotype,³⁶ or confounding

Table 2. Allelic Frequencies of SNPs among the Study Patients, According to Gene.

Nucleotide Change and Genotype*	Patients without Outcome Event (N=1914)	Patients with Outcome Event (N=294)	P Value†
P2RY12			
G→A (rs16846673)			0.96
No. of patients with data	1899	290	
GA — no. (%)	3 (<1)	0	
AA — no. (%)	1886 (>99)	290 (100)	
G52T (rs6809699)			0.42
No. of patients with data	1861	283	
TT — no. (%)	47 (3)	6 (2)	
GT — no. (%)	471 (25)	83 (29)	
GG — no. (%)	1343 (72)	194 (69)	
C34T (rs6785930)			0.36
No. of patients with data	1897	288	
TT — no. (%)	192 (10)	22 (8)	
CT — no. (%)	777 (41)	116 (40)	
CC — no. (%)	928 (49)	150 (52)	
ITGB3			
T→C (rs5918)			0.86
No. of patients with data	1878	288	
CC — no. (%)	43 (2)	6 (2)	
CT — no. (%)	501 (27)	80 (28)	
TT — no. (%)	1334 (71)	202 (70)	
ABCB1			
C3435T (rs1045642)			0.04
No. of patients with data	1898	290	
TT — no. (%)	489 (26)	85 (29)	
CC — no. (%)	507 (27)	57 (20)	
CT — no. (%)	902 (48)	148 (51)	
CYP3A5			
A6986G, CYP3A5*3 (rs776746)			0.69
No. of patients with data	1886	285	
GG — no. (%)	1573 (83)	243 (85)	
AA — no. (%)	20 (1)	3 (1)	
AG — no. (%)	293 (16)	39 (14)	

Table 2. (Continued.)			
Nucleotide Change and Genotype*	Patients without Outcome Event (N=1914)	Patients with Outcome Event (N=294)	P Value†
CYP2C19			
G681A, CYP2C19*2 (rs4244285)			0.17
No. of patients with data	1890	288	
AA — no. (%)	43 (2)	10 (3)	
AG — no. (%)	500 (26)	64 (22)	
GG — no. (%)	1347 (71)	214 (74)	
G636A, CYP2C19*3 (rs4986893)			0.97
No. of patients with data	1896	291	
AG — no. (%)	1 (<1)	0	
GG — no. (%)	1895 (>99)	291 (100)	
A1G, CYP2C19*4 (rs28399504)			0.31
No. of patients with data	1899	290	
GG — no. (%)	1882 (99)	286 (99)	
GA — no. (%)	17 (1)	4 (1)	
C1297T, CYP2C19*5			0.97
No. of patients with data	1887	289	
CT — no. (%)	1 (<1)	0	
CC — no. (%)	1886 (>99)	289 (100)	
Any CYP2C19 loss-of-function SNP (*2, *3, *4, or *5)			0.045
No. of patients with data	1914	294	
No variant allele — no. (%)	1355 (71)	218 (74)	
1 Variant allele — no. (%)	513 (27)	64 (22)	
2 Variant alleles — no. (%)	46 (2)	12 (4)	
C806T CYP2C19*17 (rs12248560)			0.18
No. of patients with data	1877	287	
TT — no. (%)	89 (5)	11 (4)	
CT — no. (%)	597 (32)	77 (27)	
CC — no. (%)	1191 (63)	199 (69)	

* The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology Information single-nucleotide polymorphism (SNP) database, dbSNP.

† P values were calculated with the use of univariate Cox analysis.

by environmental factors.^{31,32} In the present study, patients with the *ABCB1* TT and CT genotypes had worse clinical outcomes than those with a CC genotype. There was no interaction between the *ABCB1* polymorphism and *CYP2C19* loss-of-function variant alleles and the clinical outcome, but the association of two *CYP2C19*-deficient alleles and either one or two *ABCB1* variant alleles was associated with a rate of events that was more than five times the rate among patients with the

wild-type genotypes. Regardless of the exact link between the *ABCB1* C3435T polymorphism and P-glycoprotein expression, the results of our study are consistent with the finding in a previous study that plasma concentrations of clopidogrel and its active metabolite were reduced in patients carrying the TT genotype.¹⁷ However, since the *ABCB1* polymorphism was not an independent predictor of the outcome in the subgroup of patients undergoing PCI in our study,

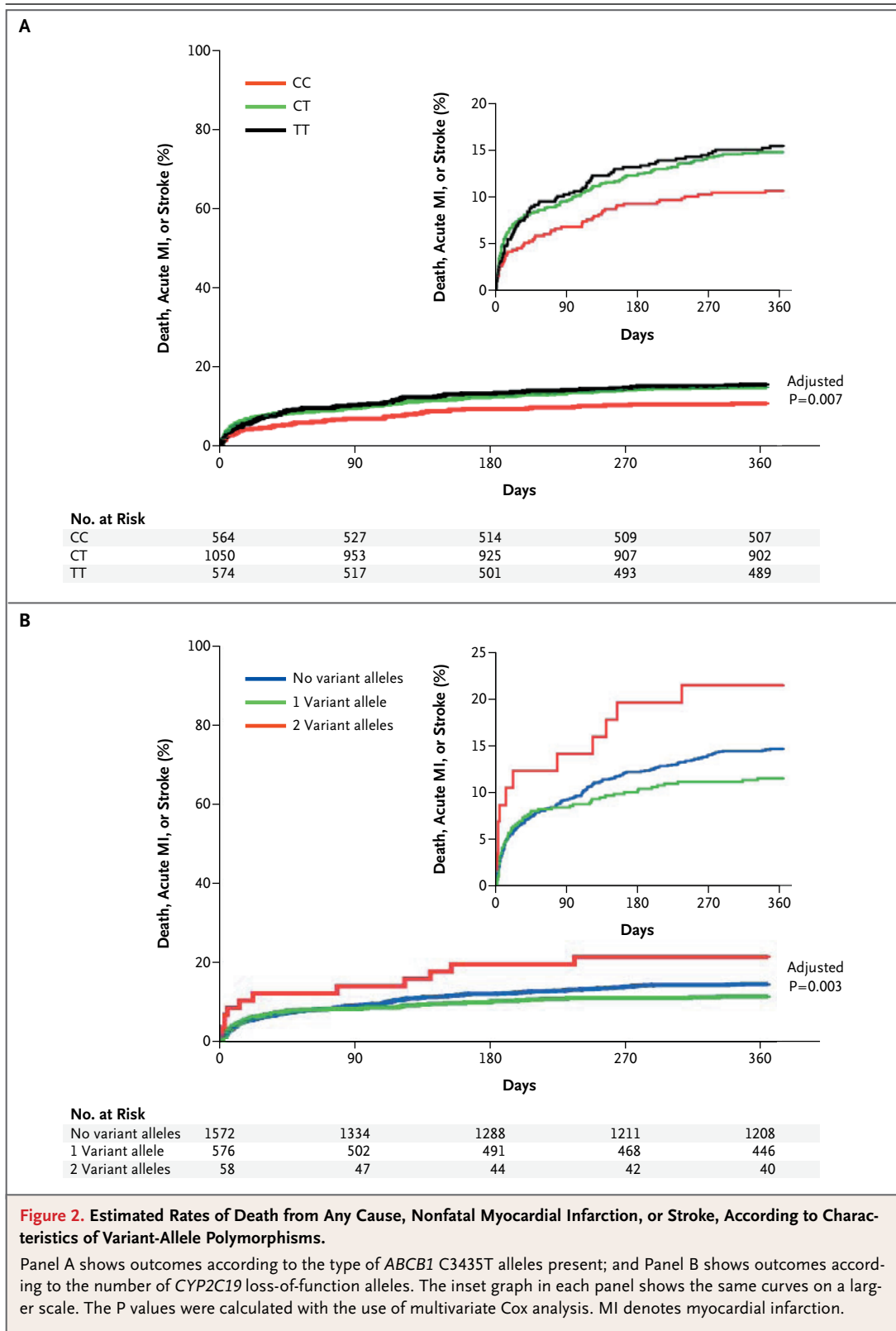


Table 3. Predictors of Death from Any Cause, Nonfatal Myocardial Infarction, or Stroke among the Study Patients.

Subgroup	All Patients (N=2208)		Patients Who Underwent PCI (N=1535)	
	Adjusted Hazard Ratio (95% CI)	P Value	Adjusted Hazard Ratio (95% CI)	P Value
Age		<0.001		<0.001
<60 yr	1.00		1.00	
60–74 yr	2.29 (1.44–3.64)		2.36 (1.34–4.17)	
≥75 yr	4.81 (3.11–7.45)		3.97 (2.26–6.98)	
Left ventricular ejection fraction		<0.001		0.009
>40%	1.00		1.00	
Unknown	1.40 (1.02–1.92)		1.25 (0.77–2.02)	
≤40%	2.29 (1.70–3.09)		2.18 (1.22–3.91)	
In-hospital care				
PCI during hospitalization (yes vs. no)	0.50 (0.39–0.64)	<0.001		
Beta-blocker (yes vs. no)	0.67 (0.52–0.86)	0.02	0.63 (0.43–0.92)	0.02
Low-molecular-weight heparin (yes vs. no)	0.73 (0.57–0.93)	0.01		
Digitalis glycoside (yes vs. no)	2.10 (1.25–3.53)	0.005		
Diuretic (yes vs. no)	1.86 (1.41–2.45)	<0.001	1.78 (1.19–2.66)	0.005
Previous use of clopidogrel (yes vs. no)			1.72 (1.10–2.69)	0.02
<i>ABCB1</i> 3435C→T (rs1045642)		0.007		
CC	1.00			
CT	1.51 (1.09–2.10)			
TT	1.72 (1.20–2.47)			
Any <i>CYP2C19</i> loss-of-function allele (*2, *3, *4, or *5)		0.003		0.005
0 Variant alleles	1.00		1.00	
1 Variant allele	0.69 (0.51–0.93)		0.78 (0.50–1.21)	
2 Variant alleles	1.98 (1.10–3.58)		3.58 (1.71–7.51)	

these results should be interpreted with caution and considered exploratory findings that need to be replicated.

The use of clopidogrel with aspirin is recommended for reducing recurrent atherothrombotic events after acute myocardial infarction and is deemed mandatory after stent placement.^{1,2} Although the optimal duration of clopidogrel therapy is uncertain, a duration of 1 year is common in patients with myocardial infarction, particularly those who undergo PCI.^{1,2} As a consequence, the prevalence of clopidogrel use in this population is substantial and increasing.³⁷ Among patients for whom clopidogrel therapy is indicated, genotyping rather than repeated platelet monitoring could be an affordable and suitable strat-

egy to identify patients at high risk for atherothrombotic events.

The observational nature of our study does not allow us to investigate cause-and-effect relationships. We cannot rule out the possibility that both *ABCB1* and *CYP2C19* polymorphisms affect atherothrombosis directly rather than acting as modulators of the clopidogrel response. However, no such effect was seen in the subgroup of 222 patients who contributed a blood sample to the DNA bank but who did not receive clopidogrel (event rate at 1 year for patients with the *CYP2C19* wild-type genotype, those with one deficient allele, and those with two deficient alleles, 33%, 46%, and 25%, respectively; $P=0.17$). In addition, the patients in our study simultane-

ously received drugs other than clopidogrel that are known to prevent the recurrence of atherothrombotic events, including aspirin, statins, angiotensin-converting-enzyme inhibitors, and beta-blockers, and we cannot exclude the possibility that the influence of genetic factors on the response to clopidogrel would have been different in the absence of these other medications. However, current management of acute myocardial infarction includes the concomitant prescription of these drugs. Therefore, the results of this nationwide observational study reflect that which can be expected from the pharmacogenetics of clopidogrel in clinical practice.

In summary, in a study of 2208 patients with acute myocardial infarction who were treated with clopidogrel, we evaluated the relationship between genetic determinants of the response to clopidogrel and subsequent cardiovascular events. Genetic variants in *CYP2C19* that result in loss of function were associated with an increase in the risk of death, myocardial infarction, or stroke, especially among patients undergoing PCI.

Supported by unrestricted grants from Pfizer and Servier for FAST-MI, a registry of the French Society of Cardiology, and a research grant from the French Caisse Nationale d'Assurance Maladie.

Dr. Simon reports receiving consulting fees from Bayer-Schering, Pfizer and Eli Lilly, lecture fees from Bayer-Schering, and grant support from Pfizer and Servier; Dr. Steg, consulting fees from Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Endotis, GlaxoSmithKline, Medtronic, Merck Sharp & Dohme, Nycomed, Sanofi-Aventis, Servier, and the Medicines Company, lecture fees from Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Medtronic, Nycomed, Sanofi-Aventis, Servier, and the Medicines Company, and grant support from Sanofi-Aventis; Dr. Danchin, consulting fees from Servier, Sanofi-Aventis, Eli Lilly, and AstraZeneca, lecture fees from Servier, Sanofi-Aventis, and Bristol-Myers Squibb, and grant support from Pfizer and Servier; and Dr. Bequemont, consulting fees from Sanofi-Aventis, Pfizer, and Servier and lecture fees from GlaxoSmithKline. No other potential conflict of interest relevant to this article was reported.

We thank the physicians who cared for the patients at the participating institutions, E. Martin and F. Rousseau (IntegraGene, Paris), the International Clinical Trials Association Contract Research Organization (Fontaine-lès-Dijon, France), Liliane Dubert (Department of Pharmacology, Université Pierre et Marie Curie [UPMC] Paris 06), the Clinical Research Assistant team of Unité de Recherche Clinique de l'Est Parisien (Assistance Publique-Hôpitaux de Paris and UPMC Paris 06), and Benoît Pace and Geneviève Mulak (French Society of Cardiology) for their assistance in designing the electronic case-record form and data management during the follow-up period.

REFERENCES

1. Antman EM, Hand M, Armstrong PW, et al. 2007 Focused update of the ACC/AHA 2004 guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2008;51:210-47. [Erratum, *J Am Coll Cardiol* 2008;51:977.]
2. Anderson JL, Adams CD, Antman EM, et al. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non ST-Elevation Myocardial Infarction): developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons: endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine. *Circulation* 2007;116(7):e148-e304. [Erratum, *Circulation* 2008;117(9):e180.]
3. Chen ZM, Jiang LX, Chen YP, et al. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet* 2005;366:1607-21.
4. Sabatine MS, Cannon CP, Gibson CM, et al. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med* 2005;352:1179-89.
5. Yusuf S, Zhao F, Mehta SR, et al. 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. [Errata, *N Engl J Med* 2001;345:1506, 1716.]
6. Steinhubl SR, Berger PB, Mann JT III, et al. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized trial. *JAMA* 2002;288:2411-20. [Erratum, *JAMA* 2003;289:987.]
7. Angiolillo DJ, Alfonso F. Platelet function testing and cardiovascular outcomes: steps forward in identifying the best predictive measure. *Thromb Haemost* 2007;98:707-9.
8. Gurbel PA, Becker RC, Mann KG, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. *J Am Coll Cardiol* 2007;50:1822-34.
9. Wang TH, Bhatt DL, Topol EJ. Aspirin and clopidogrel resistance: an emerging clinical entity. *Eur Heart J* 2006;27:647-54.
10. Bonello L, Camoin-Jau L, Arques S, et al. Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: a multicenter randomized prospective study. *J Am Coll Cardiol* 2008;51:1404-11.
11. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. PIA polymorphism and platelet reactivity following clopidogrel loading dose in patients undergoing coronary stent implantation. *Blood Coagul Fibrinolysis* 2004;15:89-93.
12. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Contribution of gene sequence variations of the hepatic cytochrome P450 3A4 enzyme to variability in individual responsiveness to clopidogrel. *Arterioscler Thromb Vasc Biol* 2006;26:1895-900.
13. Brandt JT, Close SL, Iturria SJ, et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. *J Thromb Haemost* 2007;7:2429-36.
14. Fontana P, Dupont A, Gandrille S, et al. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. *Circulation* 2003;108:989-95.
15. Giusti B, Gori AM, Marcucci R, et al. Cytochrome P450 2C19 loss-of-function polymorphism, but not CYP3A4 IVS10 +12G/A and P2Y12 T744C polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. *Pharmacogenet Genomics* 2007;17:1057-64.
16. Hulot JS, Bura A, Villard E, et al. Cyto-

- chrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood* 2006;108:2244-7.
17. Taubert D, von Beckerath N, Grimb G, et al. Impact of P-glycoprotein on clopidogrel absorption. *Clin Pharmacol Ther* 2006;80:486-501.
18. Trenk D, Hochholzer W, Fromm MF, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol* 2008;51:1925-34.
19. Suh JW, Koo BK, Zhang SY, et al. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. *CMAJ* 2006;174:1715-22. [Erratum, *CMAJ* 2006;175:64.]
20. Sim SC, Risinger C, Dahl ML, et al. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 2006;79:103-13.
21. Cambou JP, Simon T, Mulak G, Bataille V, Danchin N. The French registry of Acute ST elevation or non-ST-elevation Myocardial Infarction (FAST-MI): study design and baseline characteristics. *Arch Mal Coeur Vaiss* 2007;100:524-34.
22. Philippi A, Roschmann E, Tores F, et al. Haplotypes in the gene encoding protein kinase c-beta (PRKCB1) on chromosome 16 are associated with autism. *Mol Psychiatry* 2005;10:950-60.
23. Smith SM, Judge HM, Peters G, et al. Common sequence variations in the P2Y12 and CYP3A5 genes do not explain the variability in the inhibitory effects of clopidogrel therapy. *Platelets* 2006;17:250-8.
24. Lev EI, Patel RT, Guthikonda S, Lopez D, Bray PE, Kleiman NS. Genetic polymorphisms of the platelet receptors P2Y(12), P2Y(1) and GP IIIa and response to aspirin and clopidogrel. *Thromb Res* 2007;119:355-60.
25. Cuisset T, Frere C, Quilici J, et al. Role of the T744C polymorphism of the P2Y12 gene on platelet response to a 600-mg loading dose of clopidogrel in 597 patients with non-ST-segment elevation acute coronary syndrome. *Thromb Res* 2007;120:893-9.
26. Clarke TA, Waskell LA. The metabolism of clopidogrel is catalyzed by human cytochrome P450 3A and is inhibited by atorvastatin. *Drug Metab Dispos* 2003;31:53-9.
27. Fontana P, Senouf D, Mach F. Biological effect of increased maintenance dose of clopidogrel in cardiovascular outpatients and influence of the cytochrome P450 2C19*2 allele on clopidogrel responsiveness. *Thromb Res* 2008;121:463-8.
28. Gilard M, Arnaud B, Cornily JC, et al. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J Am Coll Cardiol* 2008;51:256-60.
29. Gerloff T, Schaefer M, John A, et al. MDR1 genotypes do not influence the absorption of a single oral dose of 1 mg digoxin in healthy white males. *Br J Clin Pharmacol* 2002;54:610-6.
30. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U S A* 2000;97:3473-8.
31. Leschziner GD, Andrew T, Pirmohamed M, Johnson MR. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J* 2007;7:154-79.
32. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* 2004;75:13-33.
33. Moriya Y, Nakamura T, Horinouchi M, et al. Effects of polymorphisms of MDR1, MRP1, and MRP2 genes on their mRNA expression levels in duodenal enterocytes of healthy Japanese subjects. *Biol Pharm Bull* 2002;25:1356-9.
34. Nakamura T, Sakaeda T, Horinouchi M, et al. Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther* 2002;71:297-303.
35. Schaeffeler E, Eichelbaum M, Brinkmann U, et al. Frequency of C3435T polymorphism of MDR1 gene in African people. *Lancet* 2001;358:383-4.
36. Kroetz DL, Pauli-Magnus C, Hodges LM, et al. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. *Pharmacogenetics* 2003;13:481-94. [Erratum, *Pharmacogenetics* 2003;13:701.]
37. Mehta RH, Roe MT, Chen AY, et al. Recent trends in the care of patients with non-ST-segment elevation acute coronary syndromes: insights from the CRUSADE initiative. *Arch Intern Med* 2006;166:2027-34.

Copyright © 2009 Massachusetts Medical Society.

ELECTRONIC ACCESS TO THE JOURNAL'S CUMULATIVE INDEX

At the *Journal's* site on the World Wide Web (NEJM.org), you can search an index of all articles published since January 1975 (abstracts 1975–1992, full text 1993–present). You can search by author, key word, title, type of article, and date. The results will include the citations for the articles plus links to the full text of articles published since 1993. For nonsubscribers, time-limited access to single articles and 24-hour site access can also be ordered for a fee through the Internet (NEJM.org).