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The Pharmacogenetics and Pharmacodynamics of Clopidogrel Response

An Analysis From the PRINC (Plavix Response in Coronary Intervention) Trial

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Objectives This study assessed the effect of pharmacogenetics on the antiplatelet effect of clopidogrel.

Background Variability in clopidogrel response might be influenced by polymorphisms in genes coding for drug metabolism enzymes (cytochrome P450 [CYP] family), transport proteins (P-glycoprotein) and/or target proteins for the drug (adenosine diphosphate–receptor P2Y12).

Methods Sixty patients undergoing elective percutaneous coronary intervention in the randomized PRINC (Plavix Response in Coronary Intervention) trial had platelet function measured using the VerifyNow P2Y12 analyzer after a 600-mg or split 1,200-mg loading dose and after a 75- or 150-mg daily maintenance dosage. Polymerase chain reaction–based genotyping evaluated polymorphisms in the *CYP2C19*, *CYP2C9*, *CYP3A4, CYP3A5*, *ABCB1, P2Y12,* and *CES* genes.

Results *CYP2C19*1*1* carriers had greater platelet inhibition 2 h after a 600-mg dose (median: 23%, range: 0% to 66%), compared with platelet inhibition in *CYP2C19*2 or *4* carriers (10%, 0% to 56%, $p = 0.029$) and *CYP2C19*17* carriers (9%, 0% to 98%, $p = 0.026$). *CYP2C19*2 or *4* carriers had greater platelet inhibition with the higher loading dose than with the lower dose at 4 h (37%, 8% to 87% vs. 14%, 0% to 22%, $p = 0.002$) and responded better with the higher maintenance dose regimen (51%, 15% to 86% vs. 14%, 0% to 67%, $p = 0.042$).

Conclusions Carriers of the *CYP2C19*2* and **4* alleles showed reduced platelet inhibition after a clopidogrel 600-mg loading dose but responded to higher loading and maintenance dose regimens. Genotyping for the relevant gene polymorphisms may help to individualize and optimize clopidogrel treatment. (Australia New Zealand Clinical Trials Registry; ACTRN12606000129583) (J Am Coll Cardiol Intv 2008;1:620–7) © 2008 by the American College of Cardiology Foundation

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Clopidogrel, an adenosine diphosphate receptor (P2Y12) blocker, is clinically used to prevent thrombotic events (1). The response to clopidogrel shows a wide interindividual variation (2), with 5% to 30% of patients not responding to initial clopidogrel therapy (3,4). Clopidogrel is a prodrug that requires conversion to its active thiol derivative catalyzed predominantly by cytochrome P450 (CYP) 3A4 and 3A5, with contributions from 2C19, 2C9, and 1A2 enzymes (5–7). Several studies (6,8–10) have evaluated the impact of polymorphisms in genes encoding enzymes involved in clopidogrel metabolism (*CYP2C19*, *CYP2C9*, *CYP3A4,* and *CYP1A2),* as well as polymorphisms of P-glycoprotein transporter protein coding gene (*ABCB1*), on clopidogrel response.

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The activity of CYP3A4, measured by radiolabeled erythromycin breath testing, correlates with the response to clopidogrel (11). CYP3A4 expression may be variable due in part to polymorphisms in the gene coding this enzyme, including *CYP3A4*1B, CYP3A4*3,* and *CYP3A4*4* (12–14). These variants are uncommon in Caucasians (15,16), with only 1 *CYP3A4* polymorphism (IVS10+12G>A) reported to date to be associated with a reduced response to clopidogrel (10,17). Expression of the CYP3A5 enzyme occurs only in subjects carrying at least 1 *CYP3A5*1* allele, which is present in up to 15% of Caucasians (18). Subjects carrying the *CYP3A5*1* allele appear to have greater platelet inhibition and fewer atherothrombotic events after percutaneous coronary intervention (PCI) than those who do not have this allele (7).

A number of well-described polymorphisms exist within the *CYP2C19* and *CYP2C9* genes. The *CYP2C19*2, *3,* and *4* alleles display a loss of function of this enzyme, and *CYP2C19*17* is associated with ultrarapid enzyme activity (19). *CYP2C19*2* carriers have a reduced antiplatelet response to a 300-mg loading dose (6) and to a 75-mg daily maintenance dosage of clopidogrel (9,10). Similarly, *CYP2C9*2* and *CYP2C9*3* code for enzymes with decreased activity compared with *CYP2C9*1* allele, and the *CYP2C9*2* allele has been associated with a reduced response to a clopidogrel 300-mg loading dose (20).

The ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) study (21) supports the idea that the apparent ceiling antiplatelet effect with clopidogrel 600 mg is due to saturable intestinal absorption of the drug, mediated by the P-glycoprotein efflux pump. A polymorphism of the *ABCB1* gene (C3435T) influences plasma levels of clopidogrel and its active metabolite; patients with the 3435T/T genotype have lower maximum concentration and area-under-the-curve values for both clopidogrel and its active metabolite compared with these values in 3435C/T and 3435C/C carriers, after 300- or 600-mg single loading doses (22). However, the polymorphism has not been related to the antiplatelet activity of clopidogrel.

A haplotype of the *P2Y12* gene, denoted H2, consists of 5 single nucleotide polymorphisms (SNPs) (intronic [i]-C139T, i-T744C, i-ins801A, C34T, and G52T) (23). As the SNPs occur in complete linkage disequilibrium, they can be tagged by the intronic SNP (i-T744C) (24). Haplotype H2 is associated with increased platelet responsiveness to adenosine diphosphate but does not appear to influence the response to clopidogrel (23,25). Plasma carboxylesterases break down the active metabolite of clopidogrel (26). Although the carboxylesterase gene (*CES*) is well conserved, a polymorphism has been described that decreases messenger ribonucleic acid transcription (27).

We evaluated the impact of polymorphisms in a number of genes to determine whether they influence response to clopidogrel therapy as part of a randomized, placebo-controlled trial comparing the antiplatelet effect of a split dose 1,200-mg clopidogrel loading dose with a standard 600-mg loading dose and comparing a 150-mg daily dose with a 75-mg daily maintenance dosage in patients undergoing PCI.

Abbreviations and Acronyms

CYP = cytochrome P450
i = intronic
PCI = percutaneous coronary intervention
SNP = single nucleotide polymorphism

Methods

The study protocol (Fig. 1), baseline patient characteristics, and additional pharmacotherapies are described in the accompanying report (28) in this issue of *JACC: Cardiovascular Interventions*.

Genotyping. Deoxyribonucleic acid was extracted from whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN N.V., Venlo, the Netherlands) and stored at −20°C. Genes of interest were identified using a candidate gene approach (Table 1). The *CYP3A4*1B* (16), *CYP3A4*3* (15), *CYP3A4*4* (12), and *CYP2C19*17* (19) polymorphisms were identified by polymerase chain reaction–restriction fragment length polymorphism methods previously described and the *CYP3A5*3*, *CYP3A5*2*, *CYP3A5*6*, *CYP2C19*2*, *CYP2C19*3*, *CYP2C19*4*, *CYP2C9*2*, *CYP2C9*3,* and *ABCB1* 1236C>T, 3435C>T, 2677G>A/T polymorphisms were investigated by real-time polymerase chain reaction using TaqMan kits (Applied Biosystems, Foster City, California) and ABI PRISM 7000 Sequence Detection System (Applied Biosystems) at the Department of Medical Science, Clinical Pharmacology, Uppsala University, Uppsala, Sweden. Genotyping for the *P2Y12* H2 haplotype and carboxylesterase intronic SNP (IVS10-88) was performed using iPlex assays on the Autoflex mass spectrometer (Sequenom, San Diego, Cali-

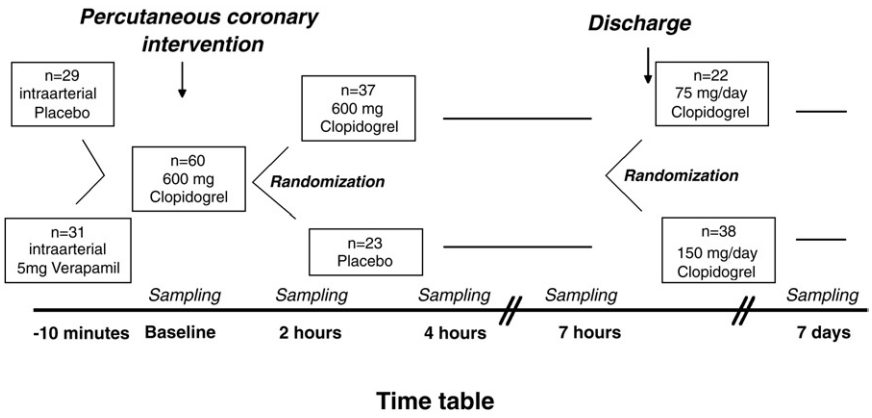

Figure 1. Timetable of the Study Protocol

A double-blind, randomized, placebo-controlled trial with 2 × 2 factorial design was undertaken in 60 patients undergoing elective percutaneous coronary intervention.

fornia). Genotyping using the Sequenom mass spectrometer was performed at the Australian Genome Research Facility (University of Queensland, St. Lucia, Australia).

Statistical analysis. The sample size was determined by the number needed to detect differences between the treatment regimens evaluated in the PRINC (Plavix Response in Coronary Intervention) study. Genotyping was undertaken as a secondary analysis. Results are reported as median and range, unless otherwise specified. A receiver operator characteristic was used to assess the predictive value of percent-

Table 1. Candidate SNPs Associated With Clopidogrel Response in the Literature

Allele	SNP	dbSNP Reference Sequence No.	Functional Effect	Effect on Clopidogrel Response	Reference #
*ABCB1*2*	1236C>T	rs1128503	Increased intestinal efflux of drugs	—	—
	3435C>T	rs1045642		Negative	Taubert et al. (22)
	2677G>T/A	rs2032582		—	—
*CYP3A4*1B*	−392A>G	rs2740574	Reduced enzyme function	None	Angiolillo et al.(17)
*CYP3A4*3*	23171T>C	rs4986910	Reduced enzyme function	None	Angiolillo et al. (17)
*CYP3A4*4*	13871A>G	—	Reduced enzyme function	—	—
*CYP3A5*1*	6986G>A 31611T>C	rs776746	Normal enzyme function	Positive	Suh et al. (7)
*CYP3A5*3*	6986A>G		Nonfunctional enzyme	Negative	Suh et al. (7)
*CYP2C19*1*	—	—	Normal enzyme function	Normal	Romkes et al. (36)
					Fontana et al. (10)
					Hulot et al. (9)
					Brandt et al. (6)
*CYP2C19*2*	19154G>A	rs4244285	Lack of enzyme activity	Negative	Fontana et al. (10)
					Hulot et al. (9)
					Brandt et al. (6)
*CYP2C19*4*	1A>G	rs28399504	Lack of enzyme activity	—	—
*CYP2C19*17*	−806C>T	rs12248560	Increased enzyme function	—	—
*CYP2C9*1*	—	—	Normal enzyme function	Normal	Brandt et al. (6)
*CYP2C9*2*	3608C>T	rs1799853	Reduced enzyme function	Negative	Brandt et al. (6)
*CYP2C9*3*	42614A>C	rs1057910	Reduced enzyme function	Negative	Brandt et al. (6)
P2Y12 H2 haplotype	i-T744C	rs2046934	Increased platelet response to ADP	None	Von Beckerath et al. (21)
CES2	g.275A>G	rs1464602	Reduced midazolam clearance	—	—
	Intronic SNP (IVS10-88)	rs3893757	Reduced mRNA gene expression	—	—

— = information not available. **1* normal variants are denoted by the absence of variant SNPs.

ABCB1 = P-glycoprotein gene; ADP = adenosine diphosphate; CES = carboxylesterase; CYP = cytochrome P450; db = database; mRNA = messenger ribonucleic acid; SNP = single nucleotide polymorphism.

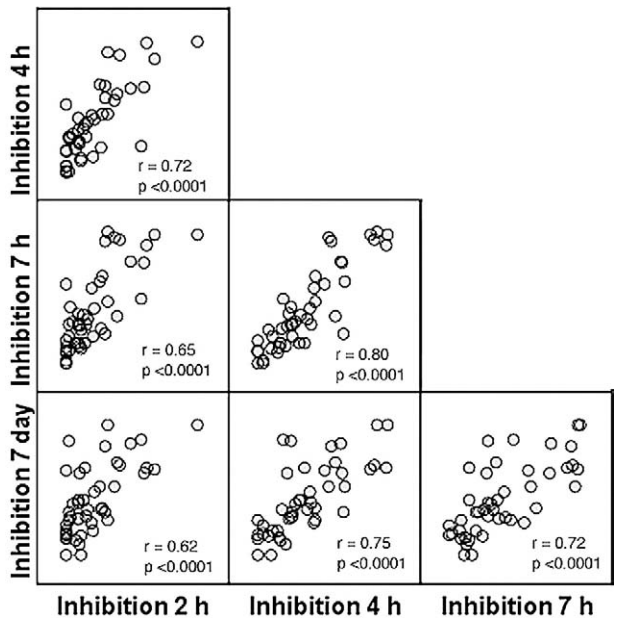

Figure 2. Prediction of Late Platelet Inhibition Based on Platelet Inhibition at Earlier Time Intervals After Clopidogrel Dosing

Pearson correlation matrix showing the relationship between platelet function response at different time intervals. Platelet inhibition of <2% at 2 h predicted nonresponse at 7 h (inhibition <10%), with a sensitivity of 100% and a specificity of 88%.

age platelet inhibition at 2 h, compared with the percentage at 7 h. The Kruskal-Wallis test was used to compare 3 or more genotype groups and the Mann-Whitney *U* test was used to compare 2 genotype groups for platelet inhibition as a percentage of the baseline platelet activity using GraphPad Prism 4 (GraphPad Software, San Diego, California). Analysis of covariance was used to assess the influence of genotyping for *CYP2C19* and *CYP2C9* polymorphisms and clopidogrel dose on the variance in maximal platelet inhibition using SAS (version 9.1) and R (version 2.1.1) software (SAS Institute, Cary, North Carolina). Statistical adjustment for multiple hypothesis testing was not used.

Results

The effect of different clopidogrel loading and maintenance dose regimens on platelet inhibition is reported in the accompanying report (28).

Prediction of response with phenotyping. A receiver-operator characteristic analysis found that lack of platelet inhibition at 2 h predicted clopidogrel resistance at 7 h ($p = 0.02$) (Fig. 2). There were fewer nonresponders in the 1,200-mg group than in the 600-mg group; 2 of 37 in the high-dose group (5%) compared with 6 of 26 in the low-dose group (26%) had 7-h platelet inhibition <10%. The area under the curve for these receiver-operator characteristic curves was 0.90. Platelet inhibition of less than 2% at 2 h was the best predictor of nonresponse (inhibition <10%) in all patients, regardless of dose, at 7 h (sensitivity 100% and specificity 88%).

Prediction of response with genotyping. The frequency of the genotypes tested in our study population and previously reported frequencies in Caucasians are outlined in Table 2.

***CYP3A4/5* POLYMORPHISMS.** None of the patients carried the *CYP3A4*1B*, **3*, **4*, or *CYP3A5*2* and **6* variant alleles, but 3 patients had the *CYP3A5*1*1* genotype, and 3 patients had the *CYP3A5*1*3* genotype. However, no significant difference was observed in platelet inhibition in those with the *CYP3A5* genotype (data not shown).

Table 2. Frequency of Detectable Variant Alleles in Our Study Population and Those Reported in Hapmap Database

Allele	SNP	dbSNP Reference Sequence No.	SNP Frequency Cohort Versus Hapmap (CEU)			
			Homozygotes		Heterozygotes	
			Cohort	Hapmap	Cohort	Hapmap
*ABCB1*2*	1236C>T	rs1128503	0.13	0.13	0.62	0.52
	3435C>T	rs1045642	0.22	0.24	0.63	0.60
	2677G>T/A	rs2032582	0.12	0.11	0.6	0.56
*CYP3A5*3*	6986A>G	—	0.92	0.88	0.05	0.12
*CYP2C19*2*	19154G>A	rs4244285	0	0.05	0.27	0.21
*CYP2C19*4*	1A>G	rs28399504	0	—	0.05	—
*CYP2C19*17*	–806C>T	rs12248560	0.07	0.02	0.27	0.40
*CYP2C9*2*	3608C>T	rs1799853	0.02	0	0.25	0.21
*CYP2C9*3*	42614A>C	rs1057910	0	0	0.05	0.12
P2Y12 H2 haplotype	i-T744C	rs2046934	0.03	0.03	0.25	0.38

Dash denotes information not available.
CEU = Caucasian; other abbreviations as in Table 1.

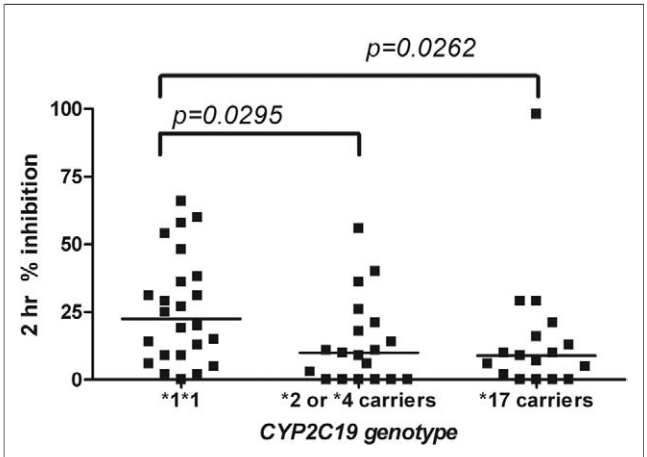

Figure 3. Platelet Inhibition (% of Baseline) at 2 h After a 600-mg Clopidogrel Loading Dose in *CYP2C19* Genotype Groups

*CYP2C19*1*1* carriers have greater platelet inhibition 2 h after a 600-mg dose compared with platelet inhibition in *CYP2C19*2* or **4* carriers and *CYP2C19*17* carriers.

***CYP2C19* AND *CYP2C9* POLYMORPHISMS.** None of the patients carried the *CYP2C19*3* allele. The frequency of the *CYP2C19*17* allele was 20% in our study population, which is similar to that reported in Swedish and Ethiopian subjects (18%) and higher than in a Chinese population (4%) (19). Platelet inhibition 2 h after a 600-mg dose was greater in *CYP2C19*1*1* carriers (median: 23%, range: 0% to 66%) than in *CYP2C19*2* or **4* carriers (10%, 0% to 56%, p = 0.029) and *CYP2C19*17* carriers (9%, 0% to 98%, p = 0.026) (Fig. 3). The platelet inhibition did not differ significantly in *CYP2C19*1*1* carriers after a 1,200- and 600-mg loading dose at 4 h (43%, 13% to 97% and 35%, 0% to 65%, respectively, p = 0.3) (Fig. 4) and 7 h (63%, 15% to 98% and 29%, 0% to 75%, respectively, p = 0.05) and after a 150- and 75-mg daily maintenance dosage (46%, 18% to 97% and 32%, 24% to 64%, respectively, p = 0.2) (Fig. 4). In contrast, *CYP2C19*2* or **4* carriers had greater platelet inhibition with the higher 1,200-mg loading dose than with 600 mg at 4 h (37%, 8% to 87% and 14%, 0% to 22%, respectively, p = 0.002) (Fig. 5) and tended to show a similar trend at 7 h (42%, 7% to 94% and 22%, 0% to 51%, respectively, p = 0.09). Similarly, platelet inhibition was significantly greater with the 150-mg dose than the 75-mg daily maintenance dosage regimen (51%, 15% to 86% and 14%, 0% to 67%, respectively, p = 0.042) (Fig. 5).

The *CYP2C9* genotype did not significantly influence the platelet response, except that *CYP2C9*1*3* carriers had reduced platelet inhibition compared with platelet inhibition in *CYP2C9*1*1* carriers at 7 h with the 600-mg clopidogrel dose (9%, 8% to 11% and 31%, 0% to 96%, respectively, p = 0.045).

Individuals were aggregated based upon their *CYP2C19* and *CYP2C9* genotype status as either predictive of response (*CYP2C19*1*1* or *CYP2C9*1*1* or **2* carriers) or predictive of poor response *(CYP2C19*2* or **4* or **17* or *CYP2C9*3)*, as has been described previously (6). Despite the different dosing regimens of clopidogrel, platelet inhibition at 7 h was

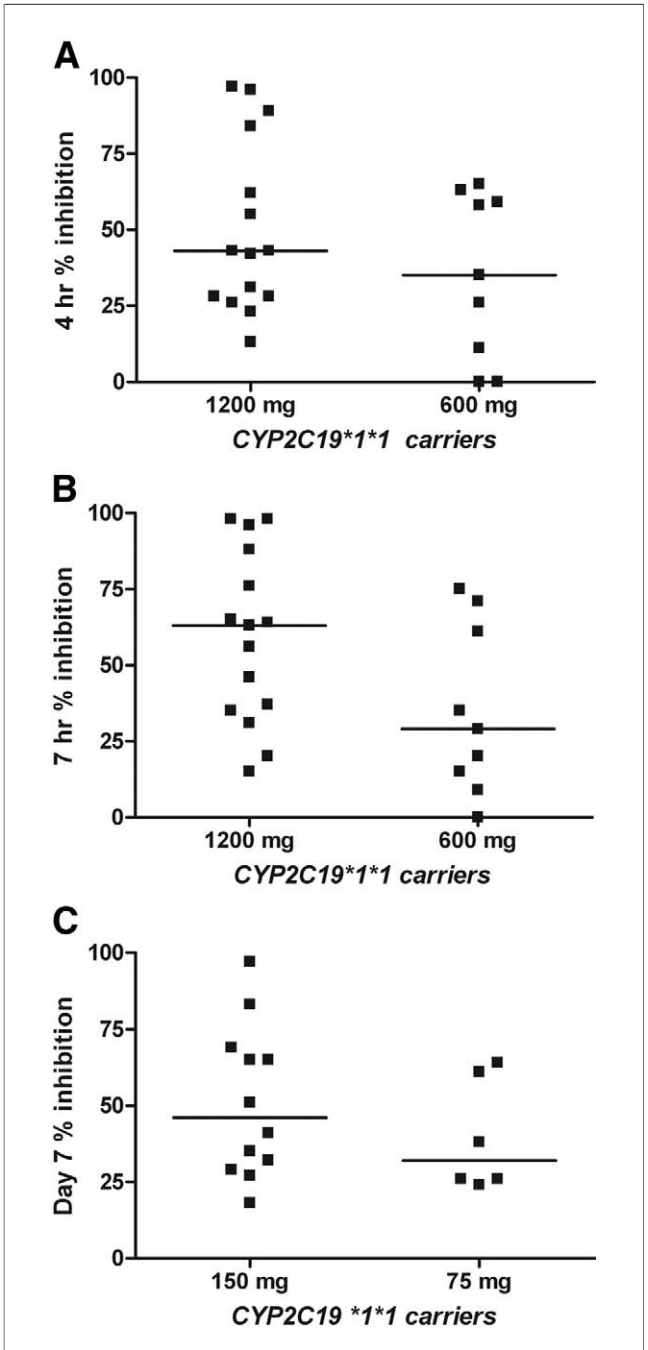

Figure 4. Platelet Inhibition (% of Baseline) at 4 h, 7 h, and 7 Days After Clopidogrel Loading in *CYP2C19*1*1* Carriers

*CYP2C19*1*1* carriers had similar platelet inhibition after a 1,200- and 600-mg loading dose at 4 h **(A)**, 7 h **(B)**, and after a 150 and 75 mg daily maintenance dosage **(C)**.

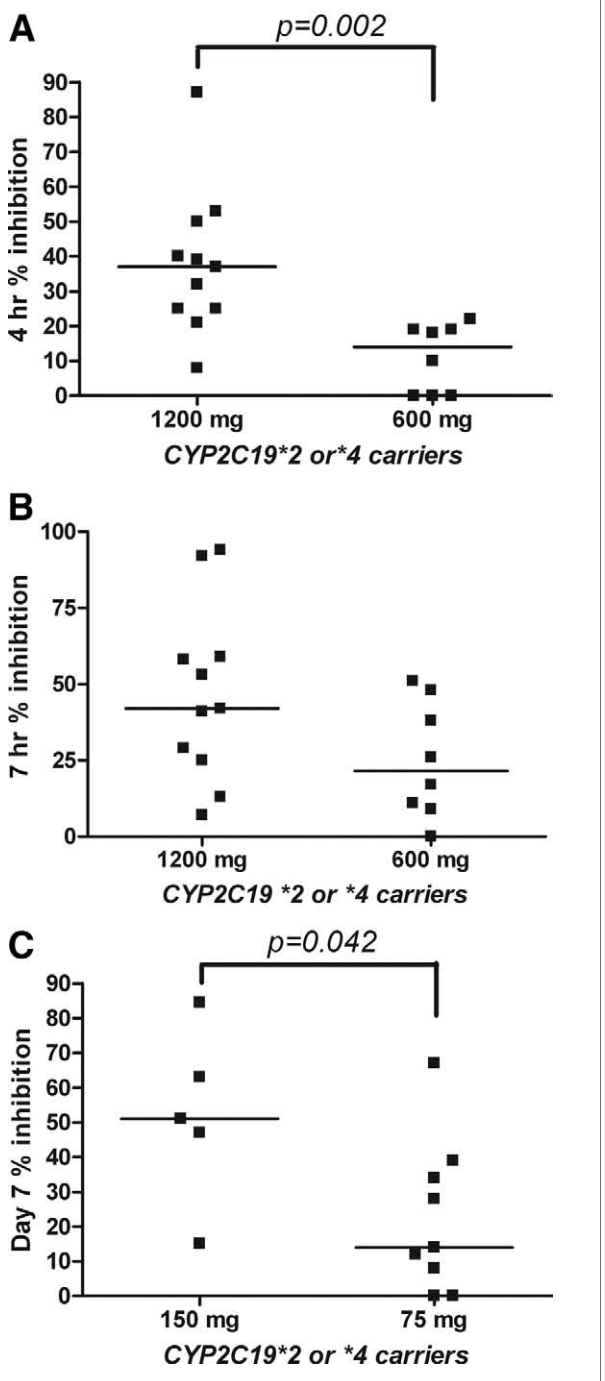

Figure 5. Platelet Inhibition (% of Baseline) at 4 h, 7 h, and 7 Days After Clopidogrel Loading in *CYP2C192 or *4 Carriers**

*CYP2C19*2* or **4* carriers had greater platelet inhibition, with the higher loading dose than with the lower dose at 4 h **(A)** and responded better with the higher maintenance dosage regimen **(B)** 7 h; **(C)** after 150 and 75 mg daily maintenance dose.

significantly influenced by having a poor-response genotype (p = 0.04, Mann-Whitney *U* test). Two individuals who were compound heterozygotes for both *CYP2C19* *2 and *CYP2C9 *3* genotypes had platelet inhibition of 8% and 11% at 7 h, respectively.

Analysis of covariance was used to compare the influence of genetics, which expressed as number of variants, with that of clopidogrel dose on platelet inhibition at 7 h. The F values of 8.3 for clopidogrel dose (p = 0.006) and 5.9 for genetics (p = 0.02) indicate that both the clopidogrel dose and genotype influence the variance in maximal platelet inhibition.

***ABCB1* POLYMORPHISMS.** No significant influence of *ABCB1* polymorphisms on platelet response was observed at any time interval.

***P2Y12* H2 HAPLOTYPE.** Patients homozygous for the P2Y12 H2 haplotype denoted by the insertion variation i-T744C were uncommon; only 2 subjects were CC homozygotes. Although neither had a high baseline peripheral resistance unit value, platelet inhibition 2 h after 600-mg clopidogrel was <2% in both individuals.

***CES* POLYMORPHISMS.** The carboxylesterase intronic SNP (IVS10-88) was not present in this cohort.

Discussion

Patients carrying the *CYP2C19*2, CYP2C19*4,* and *CYP2C19*17* alleles have a decreased antiplatelet response to clopidogrel compared with the response of *CYP2C19*1*1* carriers. Patients carrying any of these alleles displayed both a reduced early response to clopidogrel loading and a reduced sustained response after 1 week of maintenance therapy. CYP2C19 appears to be important in the first step in biotransformation of the parent drug into the active metabolite, and a loss of function in this enzyme system leads to a reduction in the plasma levels of the active metabolite (6).

The *CYP3A4* variant alleles are uncommon in a predominantly Caucasian population. As few patients carried the variants, the potential influence of *CYP3A4* polymorphisms on clopidogrel response could not be assessed. Moreover, in the 6 patients who carried the *CYP3A5*1* allele, no significant impact of CYP3A5 expression was observed on clopidogrel response.

The finding of reduced platelet inhibition in patients with the *CYP2C19*17* allele is unexpected given that **17* codes for an enzyme with higher activity than **1* does (19). To our knowledge, this is the first study to evaluate the impact of *CYP2C19*4* and **17* on clopidogrel response.

Several previous studies (6,9,10) have shown that the *CYP2C19*2* allele is associated with a reduced antiplatelet response to clopidogrel therapy. Hulot et al. (9) found that heterozygote *CYP2C19*2* subjects had no significant change in platelet function after 7 days of clopidogrel 75 mg daily, as assessed by both 10-μmol/l adenosine diphosphate light transmittance aggregometry and vasodilator-stimulated phosphorylation. In another normal subject cohort, plate-

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let inhibition after clopidogrel 75 mg daily for 1 week, measured by 20 μmol/l adenosine diphosphate aggregometry, was significantly reduced in subjects carrying the *CYP2C19*2* allele (10). Brandt et al. (20) found that the *CYP2C9*2* and **3* alleles, in addition to the *CYP2C19*2* allele, influence the plasma levels of clopidogrel active metabolite as well as the response to clopidogrel. These variants are not in linkage disequilibrium and, when considered together, reportedly accounted for two-thirds of poor responders. The third-generation thienopyridine, prasugrel, is not as dependent on either the CYP2C19 or CYP2C9 enzymes for metabolic activation (6).

Patients given a split 1,200-mg clopidogrel loading dose and a 150-mg daily maintenance dosage had greater platelet inhibition than 600 mg and 75 mg, respectively. Interestingly, the higher clopidogrel loading and maintenance dose regimens achieved greater platelet inhibition in *CYP2C19*2* or **4* carriers compared with inhibition in lower dose regimens, whereas a dose-dependent response was not observed in *CYP2C19*1*1* carriers. In other words, those with the poor-response genotype may specifically benefit from a higher dose of clopidogrel. However, the numbers in this study are small and this finding requires confirmation in a dose escalation study with larger patient numbers.

The *CYP2C19*2* allele is present in 13% of Caucasians, 18% of African Americans, and 29% of East Asians (29). The higher frequency of the *CYP2C19*2* allele in Chinese suggests that there may be ethnic differences in clopidogrel response. Although an ethnicity-based treatment approach, such as targeting the antihypertensive drug BiDil to African Americans, has yet to be widely accepted (30), potential differences among ethnic groups in drug response should be considered when evaluating trial data.

Drugs that are competitively metabolized or inhibit the activity of CYP2C19 also have the potential to reduce the activity of clopidogrel. This drug interaction has recently been shown with omeprazole (10,31,32) and may theoretically also occur with phenytoin and fluoxetine. The clinical significance of this interaction remains uncertain.

Study limitations. The major limitation of this study is the population size, which particularly affects assessment of the influence of the less common genotypes. As there were only 2 subjects with compound heterozygosity for *CYP2C19* and *CYP2C9* with a poor response to clopidogrel, the suggestion that a combination of genotypes may be more predictive of clopidogrel response than 1 genotype remains a hypothesis with initial supportive data. A recent study has suggested a gene-gene interaction between the *P2Y12* haplotype and the *CYP2C19*2* variant (33). Although a poor response to clopidogrel has been associated with an increase in periprocedural myonecrosis during PCI (34), it remains to be shown whether these genotypes will predict those at increased risk for vascular atherothrombotic complications while on clopidogrel and whether some of those complications will be prevented by an individualized, higher dose treatment regimen targeting carriers of *CYP2C19*2* and **4* genotypes. A recent study of 797 patients undergoing PCI failed to show a relationship between the *CYP2C19*2* genotype and cardiovascular events (35). Larger clinical trials are also needed to confirm the merits of a genotype-focused dosing approach over individualized treatment based on either laboratory or point-of-care platelet function analysis.

Conclusions

This study provides evidence for the impact of *CYP2C19* genotype on the platelet response to clopidogrel and shows that higher-dose clopidogrel regimens can increase the degree of platelet inhibition in patients with genotypes predictive of poor response. Personalized therapy targeting patients who carry the *CYP2C19* variants predictive of poor response may help to improve clinical outcomes and the cost–benefit of treatment.

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