

## Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel

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### ABSTRACT

**Background:** Clopidogrel is a prodrug requiring metabolism by cytochrome P450 3A (CYP3A) isoenzymes, including CYP3A5, in order to be active. It is controversial whether clopidogrel interacts with CYP3A inhibitors. We investigated the influence of CYP3A5 polymorphism on the drug interaction of clopidogrel.

**Methods:** In phase 1 of the study, we administered clopidogrel to 16 healthy volunteers who had the CYP3A5 non-expressor genotype (\*3 allele) and 16 who had the CYP3A5 expressor genotype (\*1 allele) with and without pretreatment with itraconazole, a potent CYP3A inhibitor. A platelet aggregation test was performed at baseline, 4 hours, 24 hours and 6 days after clopidogrel administration. In phase 2, we compared clinical outcomes of 348 patients treated with clopidogrel after successful coronary angioplasty with bare-metal stent implantation according to their CYP3A5 genotype; the primary end point was a composite of atherothrombotic events (cardiovascular death, myocardial infarction and non-hemorrhagic stroke) within 1 and 6 months after stent implantation.

**Results:** In phase 1, the change in platelet aggregation after clopidogrel administration and pretreatment with itraconazole was greater among the subjects with the CYP3A5 expressor genotype than among those with the non-expressor genotype: 24.9% (standard deviation [SD] 13.9%) v. 6.2% (SD 13.5%) at 4 hours ( $p < 0.001$ ); 27.7% (SD 16.5%) v. 2.5% (SD 8.3%) at 24 hours ( $p < 0.001$ ); and 33.5% (SD 18.6%) v. 17.8% (SD 13.8%) at day 7 ( $p < 0.01$ ). In phase 2, atherothrombotic events occurred more frequently within 6 months after stent implantation among the patients with the non-expressor genotype than among those with the expressor genotype (14/193 v. 3/155;  $p = 0.023$ ). Multivariable analysis showed that the CYP3A5 polymorphism was a predictor of atherothrombotic events in clopidogrel users.

**Interpretation:** People with the CYP3A5 non-expressor genotype are vulnerable to drug interactions between clopidogrel and CYP3A inhibitors. This phenomenon may be associated with worse outcomes in patients with the non-expressor genotype who are given clopidogrel after coronary angioplasty and implantation of bare-metal stents.

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Clopidogrel is an antiplatelet drug widely used in the management of coronary artery disease.<sup>1</sup> It decreases the incidence of coronary artery stent thrombosis<sup>2,3</sup> and has been approved for the management of non-ST-segment elevation myocardial infarction.<sup>4</sup>

Recent reports have indicated a lack of platelet inhibitory activity by clopidogrel (clopidogrel resistance) in some patients.<sup>5-8</sup> The drug interaction of clopidogrel with CYP3A inhibitors has been suggested as a mechanism of this resistance.<sup>9-11</sup>

Clopidogrel is a prodrug that needs to be metabolized before it can inhibit adenosine diphosphate (ADP)-induced platelet aggregation (Fig. 1). Metabolism is predominantly by the cytochrome P450 (CYP) 3A system, which consists of the 3A4 and 3A5 isoenzymes.<sup>9</sup> CYP3A4 is generally thought to be the predominant form expressed in liver cells. However, CYP3A5, which is expressed polymorphically in the liver,<sup>12</sup> may contribute as much as 50% to hepatic CYP3A activity in one-third of white people and in half of black people.<sup>13</sup>

The CYP3A5 gene has a functional polymorphism that distinguishes expressor (\*1) and non-expressor (\*3) alleles.<sup>14</sup> This polymorphism has been reported to influence total CYP3A activity and shows racial differences in its frequency.<sup>13</sup>

For drugs equally metabolized by CYP3A4 and CYP3A5, the net rate of metabolism is the sum of CYP3A4 and CYP3A5 metabolism rates.<sup>13</sup> Thus, a substantial change in CYP3A5 activity may influence the pharmacokinetics of CYP3A substrates, such as midazolam, triazolam, nifedipine,<sup>15</sup> tacrolimus<sup>16</sup> and lipophilic statins<sup>17</sup> (Fig. 2).

We postulated that the drug interaction between clopidogrel and a CYP3A inhibitor would differ according to a person's *CYP3A5* genotype. To test this hypothesis, we first investigated, in volunteers, the association between *CYP3A5* genotype and platelet aggregation after clopidogrel administration with or without CYP3A inhibition (phase 1). We then investigated the influence of this genotype on risk of atherothrombotic events among patients taking clopidogrel after coronary angioplasty with stent implantation (phase 2).

## Methods

### Phase 1

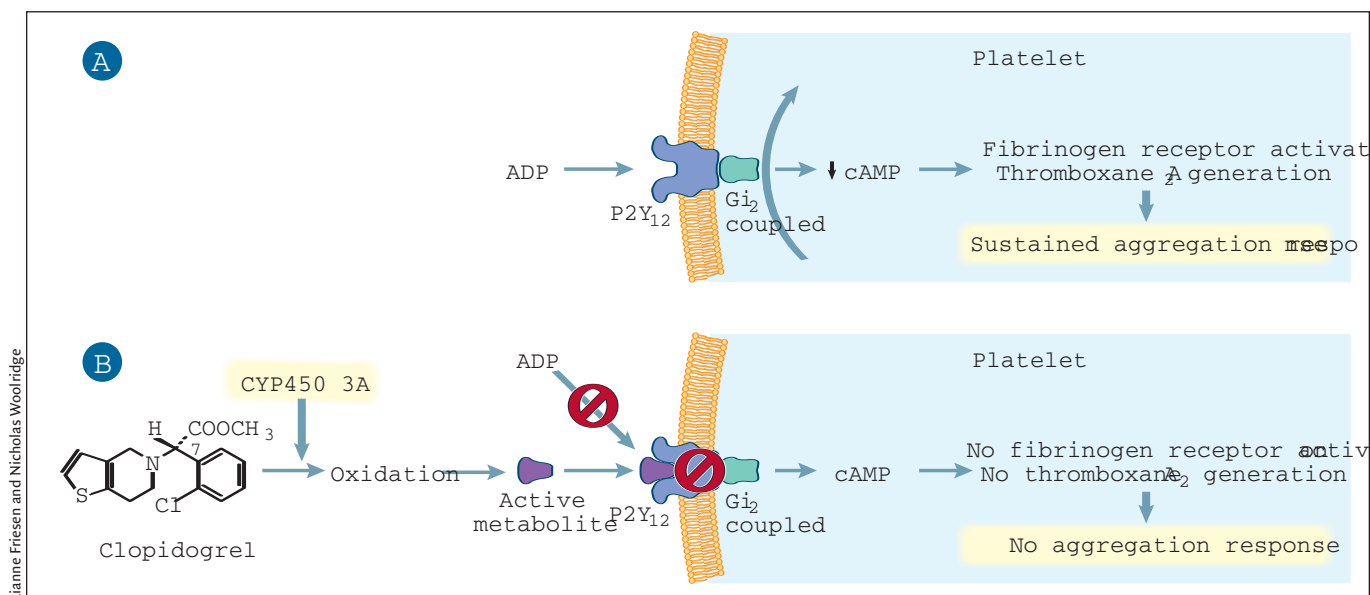
The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (SNUH), and the study was conducted at the SNUH Clinical Trial Center. All subjects provided written informed consent before enrolment.

Among 486 unrelated people who had undergone genotyping for the *CYP3A5* \*3 allele to establish baseline data for a DNA bank for the study of pharmacogenetics in Koreans (data not published), 16 people with expressor alleles (\*1\*1 or \*1\*3 alleles) and 16 with non-expressor alleles (\*3\*3 alleles) volunteered to participate in our study. The 2 groups did not differ significantly in terms of age, height or weight. The participants were ascertained to be healthy by medical history, physical examination, vital signs and routine clinical laboratory tests performed within 3 weeks before the start of the study. None was a regular heavy drinker or a smoker, or

had a body weight more than 120% of his or her ideal weight.

Patients were given a loading oral dose of 300 mg of clopidogrel (Plavix; Sanofi-Synthelabo Korea) followed by daily oral doses of 75 mg for 6 days. A platelet aggregation test was performed at baseline and at 4 hours, 24 hours and 6 days after the loading dose. After a washout period of 28 days, we performed the same experiment but with a pre-treatment of 200 mg of itraconazole (Sporanox cap; Janssen Korea) for 4 days to assess the effect of *CYP3A5* genotype on the antiplatelet efficacy of clopidogrel. Itraconazole is a well-known CYP3A inhibitor and has been frequently used in pharmacokinetic and pharmacodynamic experiments of CYP3A4 substrates.<sup>20–22</sup> Therefore, we chose itraconazole for CYP3A4 inhibition. (The 2 dosing regimens for phase 1 of the study are appear in Appendix 1, available online at [www.cmaj.ca/cgi/content/full/174/12/1715/DC1](http://www.cmaj.ca/cgi/content/full/174/12/1715/DC1).) Participants were instructed to refrain from taking other medications, herbal drugs, alcohol, caffeinated beverages and grapefruit products for the 7 days before and the 7 days during the clopidogrel-only regimen and the 10 days before and the 7 days during the clopidogrel plus itraconazole regimen.

Platelet aggregation was measured using platelet-rich plasma stimulated with 5  $\mu\text{mol/L}$  ADP and was assessed with a Chronolog Lumi-Aggregometer (model 560-Ca; Chronolog Corporation) equipped with an AggroLink software package.<sup>23</sup> After inverting the vacutainer tube 3–5 times, the blood-citrate mixture was centrifuged at 1200 g for 2.5 minutes. The platelet count was determined in the platelet-rich plasma and adjusted to  $3.0 \times 10^5/\text{mL}$  with homologous platelet-poor plasma. Aggregation was expressed as the max-



**Fig. 1:** **A:** Adenine diphosphate (ADP) is an important physiologic agonist that plays a vital role in normal hemostasis and thrombosis. The P2Y<sub>12</sub> receptor of platelets signals through a G<sub>i2</sub> coupled G-protein receptor (G<sub>i2</sub> coupled) and is important for potentiation of platelet activation mediated by other physiologic agonists, including collagen, von Willebrand factor and thromboxane A<sub>2</sub>.<sup>18</sup> **B:** Inhibition of ADP-induced platelet aggregation by clopidogrel. Clopidogrel is a prodrug requiring metabolism by cytochrome P450 (CYP) 3A isoforms in order to be active. The active metabolite of clopidogrel blocks the platelet's P2Y<sub>12</sub> receptor.<sup>19</sup> Note: cAMP = cyclic adenosine monophosphate.

imal percentage change in light transmittance from the baseline value, using platelet-poor plasma as a reference. The change in platelet aggregation after clopidogrel treatment was expressed as the absolute reduction in aggregation achieved by 5  $\mu\text{mol/L}$  of ADP after clopidogrel administration compared with the baseline aggregation values before clopidogrel administration.<sup>6</sup>

## Phase II

The study protocol was approved by the SNUH Institutional Review Board, and the study was conducted at the SNUH cardiovascular centre. All subjects provided written informed consent before enrolment.

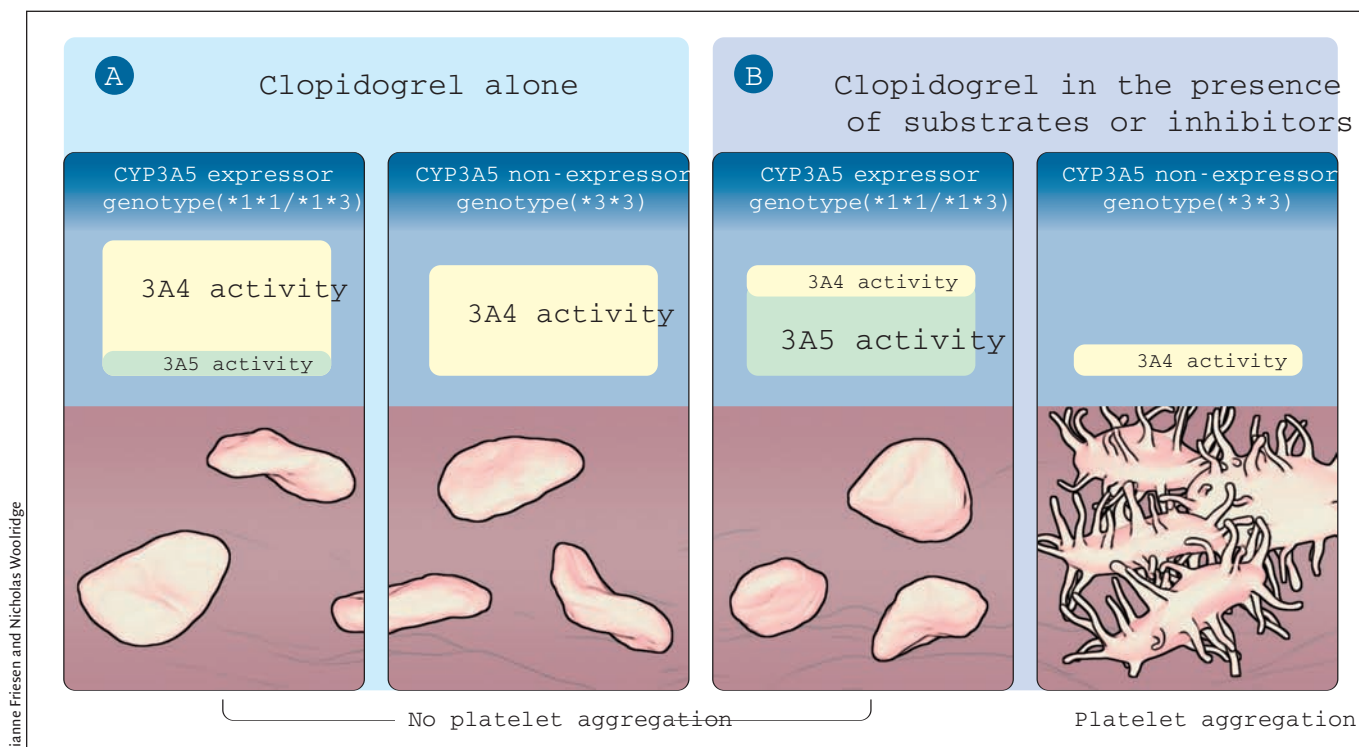
We enrolled consecutive patients at the Seoul National University Cardiovascular Center who had undergone successful coronary angioplasty with implantation of bare-metal stents after pretreatment with clopidogrel for at least 24 hours from November 2001 to August 2003. We excluded patients who underwent primary percutaneous coronary intervention, received drug-eluting stents, were treated with ticlopidine or were in cardiogenic shock. DNA analysis was performed in all cases to determine the patients' *CYP3A5* genotypes. All patients gave informed consent to participate in the study.

Coronary angioplasty and stent implantation were per-

formed using conventional techniques.<sup>24</sup> Antiplatelet therapy consisted of ASA (100–300 mg/d, prescribed indefinitely) and clopidogrel (75 mg/d after a 300-mg loading dose), administered for at least 4 weeks after the procedure. Other anti-anginal and antihypertensive medications were given at the treating physician's discretion. All treating physicians were blind to the results of the DNA analysis.

Genomic DNA was prepared from the whole blood of each subject by means of standard methods.<sup>25</sup> The presence of the *CYP3A5*\*3 allele was determined by mismatch polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis, as previously reported.<sup>20</sup> In brief, genomic DNA was amplified using *CYP3A5* 6956Fm (5'-CTT TAA AGA GCT CTT TTG TCT CTC A-3') as the forward primer and *CYP3A5* 7155R (5'-CCA GGA AGC CAG ACT TTG AT-3') as the reverse primer. After amplification, the 200-base pair (bp) PCR product was digested using the enzyme DdeI. Digestion of the *CYP3A5*\*3 allele yielded fragments of 22, 71 and 107 bp, and digestion of the *CYP3A5*\*1 allele yielded fragments of 71 and 129 bp.

The end point of phase 2 was a composite of atherothrombotic events (cardiac death, myocardial infarction and nonhemorrhagic cerebral infarction) within 1 and 6 months after stent implantation. The cause of all deaths was regarded as cardiovascular unless there was documented evidence of a clear noncardiovascular cause. Myocardial infarc-



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**Fig. 2: A:** Cytochrome P450 (CYP) 3A4 and CYP3A5 are major isoforms of the CYP3A system. Total CYP3A activity accounts for 20% of all phase I reactions in the liver and metabolizes more than 50% of drugs. Under usual conditions, where both CYP3A4 and CYP3A5 contribute to total CYP3A activity, CYP3A4 is probably the main contributor. Therefore, the antiplatelet activity of clopidogrel may not differ substantially between patients with the *CYP3A5* expressor genotype and those with the non-expressor genotype. **B:** In the presence of multiple substrates or inhibitors, CYP3A4 is more easily inhibited than CYP3A5, and therefore CYP3A5 becomes the main contributor to total CYP3A activity. In this condition, total CYP3A activity would differ depending on the patient's *CYP3A5* genotype.

tion was defined as the presence of at least 2 of the following findings: ischemic symptoms; cardiac enzyme (CK MB) concentration at least twice the upper limit of normal; or new electrocardiographic changes compatible with myocardial infarction. Nonhemorrhagic cerebral infarction was defined as a new focal neurologic deficit of vascular origin lasting at least 24 hours that was proven to be nonhemorrhagic by either CT or MRI scanning. The follow-up protocol included a medical visit at the outpatient clinic at 1 month after stent placement and every 2 or 3 months thereafter. Clinical events were assessed on the basis of the information provided by hospital readmission records, the referring physician or a phone interview with the patient. The investigators who evaluated the clinical end points were blinded to the results of the DNA analysis.

### Statistical analysis

Continuous variables are presented as means and standard deviations (SDs). In phase 1, a repeated-measures analysis of variance was used to compare platelet aggregation test results at baseline, 4 hours, 24 hours and 6 days after clopidogrel administration, and the Student's *t* test was used to compare the degree of platelet inhibition between the 2 genotype groups.

In phase 2, the  $\chi^2$  test was used to compare the incidence of clinical end points between the genotype groups. A logistic regression analysis, which included age, sex, diabetes mellitus, hypertension, hypercholesterolemia, previous myocardial infarction, clinical diagnosis, *CYP3A5* genotype, left ventricular ejection fraction, stent size, multivessel disease,

multivessel intervention and the number of co-administered CYP3A substrates or inhibitors (lipophilic statins, metoprolol, diltiazem, nifedipine, cimetidine and losartan) as covariates, was performed to identify determinants of atherothrombotic events (cardiovascular death, myocardial infarction and nonhemorrhagic stroke) after coronary artery stenting within 6 months. A *p* value below 0.05 was considered statistically significant.

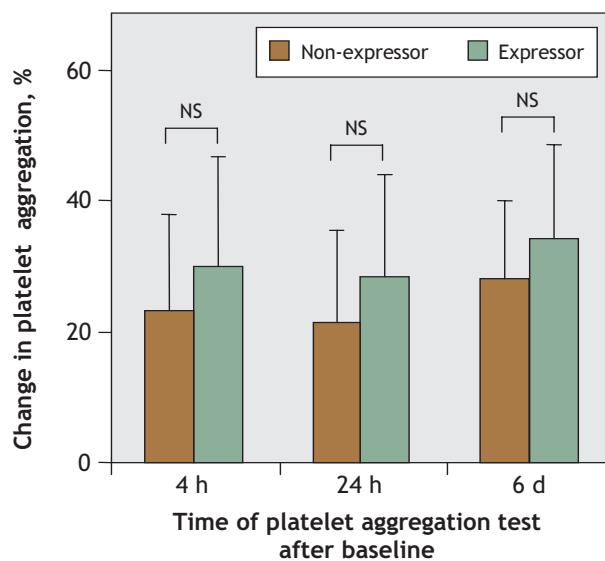


Fig. 4: Change in platelet aggregation from baseline after administration of clopidogrel, by *CYP3A5* genotype.

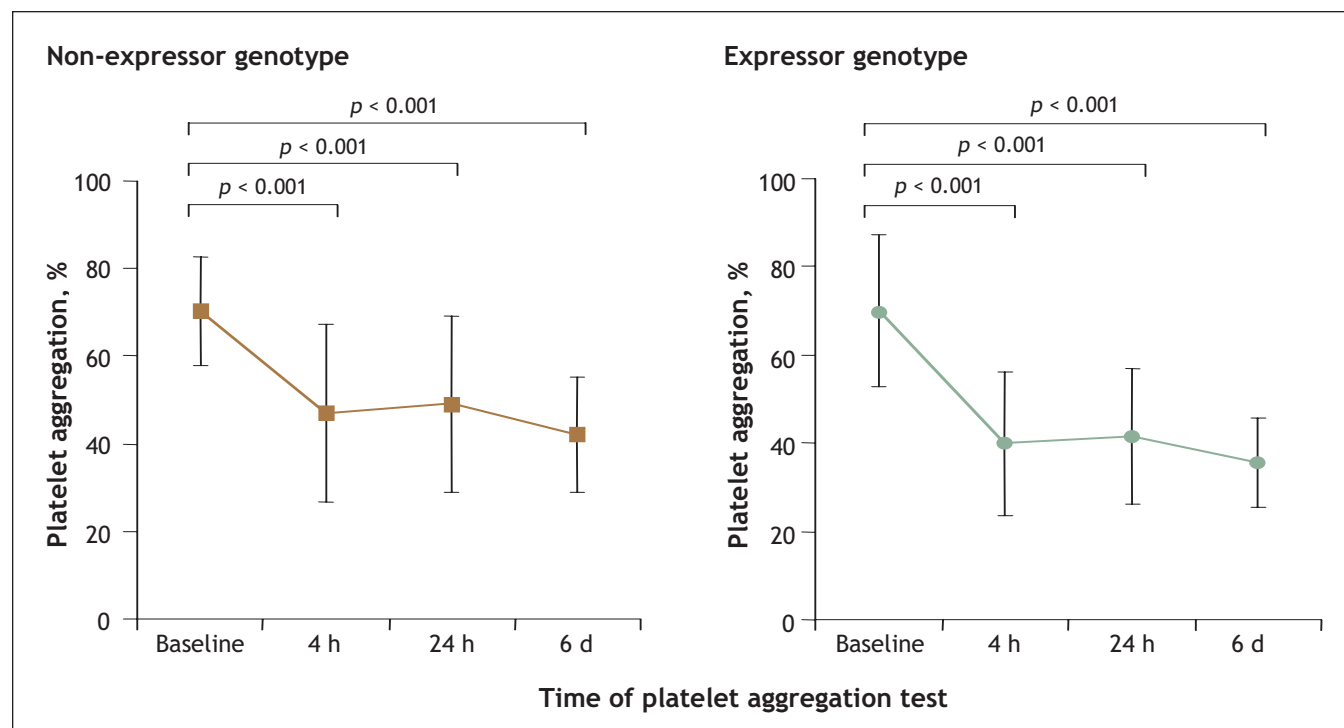


Fig. 3: Platelet aggregation in healthy volunteers taking clopidogrel, by *CYP3A5* genotype (non-expressor v. expressor).



## Results

### Phase 1

The inhibition of platelet aggregation after clopidogrel treatment was significant at all points compared with baseline in both of the genotype groups (Fig. 3). When we compared the efficacy of clopidogrel in terms of the change in aggregation from baseline, there were no significant differences between the 2 groups, although we observed a slight tendency for those with the *CYP3A5* expressor genotype to achieve greater inhibition of platelet aggregation (Fig. 4).

After the washout period and treatment with the *CYP3A4* inhibitor itraconazole, clopidogrel significantly inhibited platelet aggregation at all time points only among the subjects with the *CYP3A5* expressor genotype (Fig. 5). In contrast, clopidogrel had a significant effect only on day 7 among those with the non-expressor genotype (Fig. 5, right panel). In terms of the change in aggregation from baseline, the change was significantly greater among those with the expressor genotype than among those with the non-expressor genotype at 4 hours, 24 hours and 6 days after baseline (Fig. 6).

### Phase 2

A total of 348 patients were enrolled in phase 2. In these patients, 453 lesions were treated with angioplasty and implantation of bare-metal stents. Genotype analysis revealed the following frequency among the patients: 33 (9%) had the *CYP3A5* \*1\*1 allele, 122 (35%) had the \*1\*3 allele, and 193 (55%) had the \*3\*3 allele; which gave a frequency of non-expressor (\*3) allele of 73%. These findings were consistent

with the Hardy–Weinberg expectations. The patients were divided into 2 groups: those with the *CYP3A5* expressor alleles ( $n = 155$ ) and those with the non-expressor allele ( $n = 193$ ). The clinical and angiographic characteristics of the patients did not differ significantly between the 2 groups (Table 1), nor was there a significant difference in the duration of clopidogrel treatment (4.1 [SD 2.3] and 3.9 [SD 2.3] months in the non-expressor and expressor groups respectively) or co-administered drugs (Table 2).

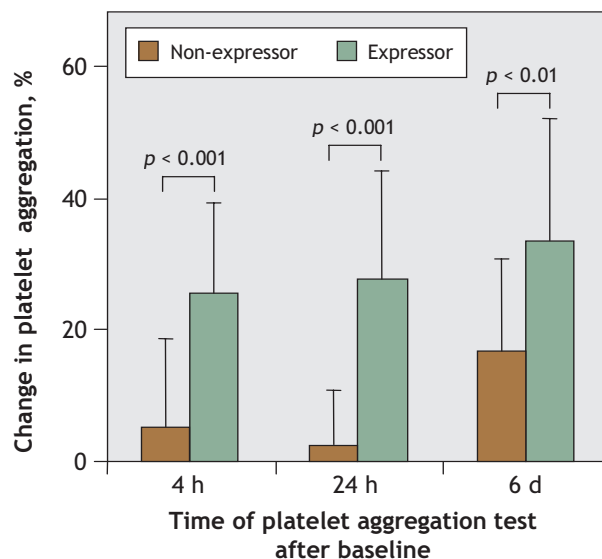


Fig. 6: Change in platelet aggregation from baseline after administration of clopidogrel and pretreatment with itraconazole.

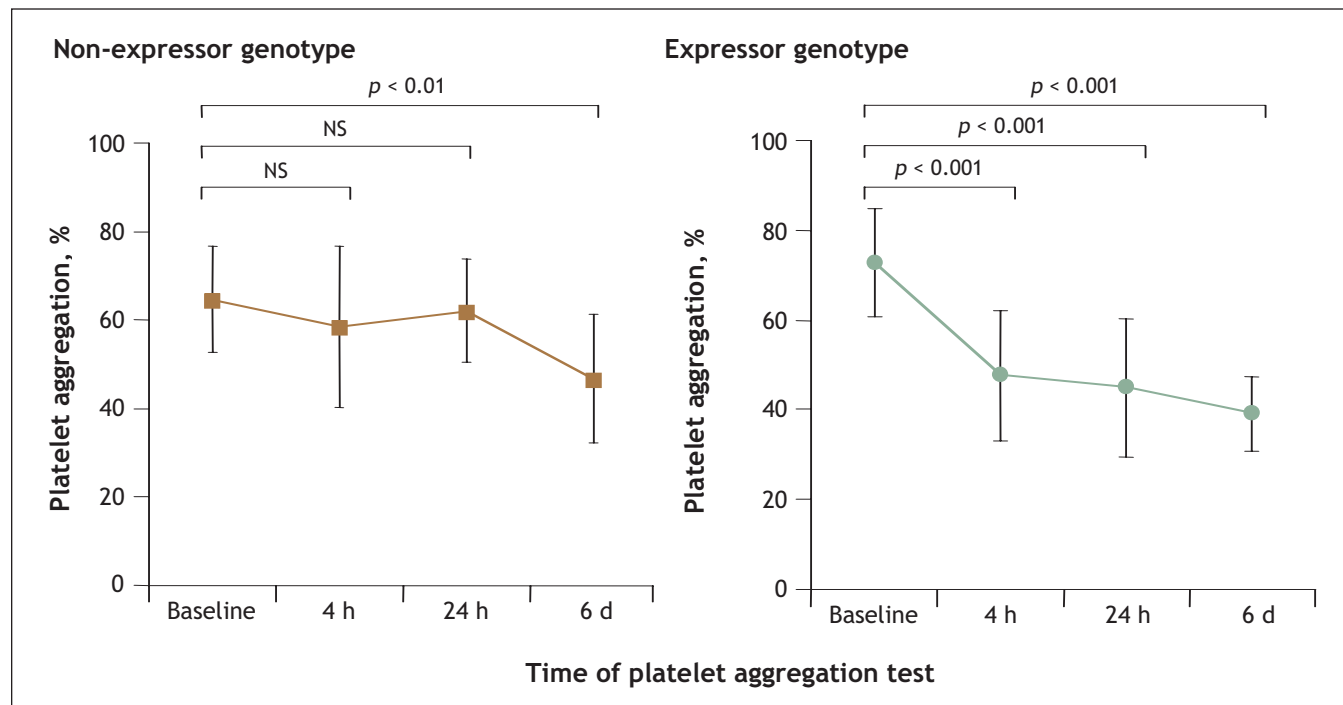


Fig. 5: Platelet aggregation in healthy volunteers taking clopidogrel after pretreatment with the *CYP3A* inhibitor itraconazole. NS = not significant.

**Table 1:** Baseline characteristics of patients undergoing coronary angioplasty and bare-metal stent implantation, by CYP3A5 genotype

Characteristic	CYP3A5 genotype; no. (%) of patients*		p value
	Non-expressor n = 193	Expressor n = 155	
Age, yr, mean (SD)	60.8 (11.0)	62.6 (8.7)	0.10
Sex, female	55 (29)	47 (30)	0.73
Diabetes mellitus	68 (35)	62 (40)	0.51
Hypertension	115 (60)	86 (56)	0.45
Hypercholesterolemia	57 (30)	48 (31)	0.74
LVEF < 45%	26 (14)	19 (12)	0.87
Previous MI	45 (23)	28 (18)	0.29
Previous PCI	30 (16)	18 (12)	0.28
Previous CABG	9 (5)	6 (4)	0.80
Unstable angina or non-STEMI	96 (50)	80 (52)	0.75
Multivessel disease	95 (49)	79 (51)	0.78
Stents per lesion, mean no. (SD)	1.3 (0.6)	1.3 (0.6)	0.94
Stent diameter, mm, mean (SD)	3.3 (0.5)	3.2 (0.6)	0.17
Stent length, mm, mean (SD)	22.0 (7.4)	21.2 (6.6)	0.29
Use of GP IIb/IIIa inhibitors	4 (2)	2 (1)	0.70

Note: SD = standard deviation, LVEF = left ventricular ejection fraction, MI = myocardial infarction, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft surgery, STEMI = ST-segment elevation MI, GP = glycoprotein.

\*Unless stated otherwise.

Six-month clinical follow-up data were available for all patients (Table 3). During this period, atherothrombotic events occurred in 17 patients (14 in the non-expressor group and 3 in the expressor group). One myocardial infarction in the non-expressor group was due to subacute stent thrombosis; all cases of stroke were confirmed as nonhemorrhagic by means of imaging studies. All of the patients in the non-expressor group who experienced an atherothrombotic event were taking both clopidogrel and ASA at the time.

Multivariable analysis revealed the following as independent predictors of atherothrombotic events within 6 months after coronary angioplasty and bare-metal stent implantation: CYP3A5 non-expressor genotype, every increase in the number of co-administered CYP3A substrates or inhibitors, and a cholesterol level of 5.18 mmol/L (200 mg/dL) or higher (Table 4).

In the subgroup analysis according to genotype, the number of CYP3A metabolizers was an independent predictor of atherothrombotic events only in patients with the CYP3A5 non-expressor genotype but not in those with the expressor genotype (Table 5).

**Table 2:** Concurrent medication use among patients undergoing coronary angioplasty and bare-metal stent implantation

Medication	CYP3A5 genotype; no. (%) of patients*		p value
	Non-expressor n = 193	Expressor n = 155	
Statin	92 (47.4)	64 (41.3)	0.33
Atorvastatin	17 (8.8)	19 (12.3)	
Simvastatin	64 (33.2)	36 (23.2)	
Lovastatin	9 (4.6)	9 (5.8)	
Pravastatin	2 (1.0)	0	
β-Blocker	123 (63.4)	111 (71.6)	0.11
Metoprolol	32 (16.5)	29 (18.7)	
Other	91 (46.9)	82 (52.9)	
Calcium-channel blocker	40 (20.6)	40 (25.8)	0.33
Diltiazem	26 (13.4)	20 (12.9)	
Nifedipine	3 (1.9)	2 (1.0)	
Other	14 (7.6)	17 (11.0)	
Nitrate	111 (57.2)	93 (60.0)	0.66
ACE inhibitor	79 (40.7)	50 (32.3)	0.11
Angiotensin II receptor blocker	18 (9.3)	17 (11.0)	0.72
Co-administered CYP3A metabolizer,† mean no. (SD)	0.97 (0.74)	0.87 (0.74)	0.22

Note: ACE = angiotensin-converting enzyme, SD = standard deviation.

\*Unless stated otherwise.

†Includes lipophilic statins, metoprolol, diltiazem, nifedipine, cimetidine and losartan.

## Interpretation

The results of our study demonstrate that the drug interaction between clopidogrel and the CYP3A inhibitor itraconazole differed according to CYP3A5 genotype and that the genotype may lead to different clinical outcomes after coronary angioplasty with bare-metal stent implantation in clopidogrel users.

CYP3A activity accounts for 20% of all phase I reactions in the liver and metabolizes more than 50% of drugs.<sup>12</sup> Since CYP3A can easily be induced or inhibited by several drugs, the variability of CYP3A activity may be a common cause of drug–drug interactions.<sup>26</sup> In the setting of CYP3A4 inhibition, the activity of CYP3A5 may be the key determinant of CYP3A activity.<sup>20</sup> Under usual conditions, where both CYP3A4 and CYP3A5 contribute to CYP3A activity, the antiplatelet activity of clopidogrel did not differ significantly between the 2 genotype groups in phase I of our study. However, when CYP3A4 activity was inhibited preferentially by pretreatment with itraconazole, the antiplatelet activity of clopidogrel was significantly greater among those with the CYP3A5 expressor genotype than among those with the non-expressor genotype.

**Table 3:** Clinical outcomes after coronary angioplasty and bare-metal stent implantation, by *CYP3A5* genotype

Outcome after stent implantation	<i>CYP3A5</i> genotype; no. of patients		<i>p</i> value
	Non-expressor <i>n</i> = 193	Expressor <i>n</i> = 155	
<b>At 1 mo</b>			
Sudden death	1	0	
MI (subacute thrombosis)	6 (1)	3	
Nonhemorrhagic stroke	3	0	
Total	10	3	0.16
<b>1-6 mo</b>			
Sudden death	0	0	—
MI	4	0	
Nonhemorrhagic stroke	0	0	
Total	4	0	0.10
<b>6-mo cumulative</b>	14	3	0.023

Note: MI = myocardial infarction.

Azoles are inhibitors of the CYP3A system and are reported to inhibit CYP3A4 more potently than CYP3A5, *in vitro*<sup>15</sup> and *in vivo*.<sup>20</sup> When CYP3A is inhibited by multiple substrates or inhibitors, CYP3A4 is more easily inhibited than CYP3A5, and in this condition, CYP3A5 may be the main contributor to total CYP3A activity.<sup>20</sup> These previous reports along with our results suggest that people with the *CYP3A5* non-expressor genotype rely more on CYP3A4 activity, which is more susceptible to drug interactions.

We also examined the clinical significance of the *CYP3A5* genotype in determining the risk of atherothrombotic events among patients using clopidogrel after coronary angioplasty

and bare-metal stent implantation. The clinical events in our study (cardiac death, myocardial infarction and nonhemorrhagic stroke) were the same as those used in previous studies to represent atherothrombotic events.<sup>27</sup> In our study, atherothrombotic events occurred more frequently among patients with the *CYP3A5* non-expressor genotype. Vulnerability to drug interaction of clopidogrel and the resulting insufficient conversion to the active metabolite in these patients may have contributed to their worse clinical outcomes, which is supported by the findings that the number of co-administered CYP3A metabolizers was an independent risk factor for atherothrombotic events only among patients with the non-expressor genotype.

Our study has some limitations. First, in phase 1, we did not assay the active metabolite of clopidogrel directly but, rather, performed a platelet aggregation test with ADP as an indirect marker of the active clopidogrel metabolite, as done by previous investigators. Therefore, we cannot rule out the involvement of other factors between the interaction of the active metabolite and the platelet ADP receptor.<sup>28</sup> Second, we did not co-assay other *CYP3A5* alleles or *CYP3A4* polymorphism. Because other alleles in the *CYP3A5* gene are rare (\*2, \*6 and \*7),<sup>29-32</sup> we speculated that the *CYP3A5*\*3 allele is responsible for most, if not all, of the polymorphic expression of *CYP3A5*. Another limitation was the small number of patients enrolled in phase 2 of the study.

In summary, the degree of drug interaction between clopidogrel and the CYP3A inhibitor itraconazole differed according to *CYP3A5* genotype. People with the *CYP3A5* non-expressor genotype were more vulnerable than those with the expressor genotype to drug interaction between clopidogrel and the CYP3A metabolizer, and patients taking clopidogrel may be at increased risk of atherothrombotic events after coronary angioplasty with bare-metal stent implantation. Further studies in a large population are warranted to confirm these findings.

**Table 4:** Risk factors for atherothrombotic events after coronary angioplasty and bare-metal stent implantation among patients taking clopidogrel

Risk factor	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
<i>CYP3A5</i> non-expression (v. expression)	3.96 (1.12-14.0)	4.89 (1.28-18.7)
Co-administered CYP3A metabolizers† (every increase in no.)	2.15 (1.15-4.03)	2.22 (1.10-4.47)
Total cholesterol level ≥ 5.18 mmol/L (v. < 5.18 mmol/L)	1.01 (0.99-1.02)	1.02 (1.01-1.03)
Age ≥ 65 yr (v. < 65 yr)	0.98 (0.94-1.03)	0.98 (0.93-1.03)
Male sex (v. female)	1.74 (0.64-4.70)	2.08 (0.65-6.61)
Previous MI (v. no previous MI)	0.80 (0.22-2.86)	0.72 (0.17-3.10)
Diabetes mellitus (v. no diabetes)	1.52 (0.57-4.05)	1.15 (0.39-3.40)
LV systolic ejection fraction < 45% (v. > 45%)	1.12 (0.25-5.07)	1.13 (0.20-6.34)
Stent diameter ≥ 2.75 mm (v. < 2.75 mm)	0.89 (0.36-2.23)	0.66 (0.24-1.85)
Stent length ≥ 20 mm (v. < 20 mm)	0.99 (0.92-1.06)	0.98 (0.91-1.06)

Note: OR = odds ratio, CI = confidence interval, LV = left ventricle, MI = myocardial infarction.

\*Adjusted for *CYP3A5* genotype, number of co-administered CYP3A metabolizers, cholesterol level, age, sex, previous MI, diabetes mellitus, LV systolic dysfunction (ejection fraction < 45%), stent diameter and stent length.

†Includes lipophilic statins, metoprolol, diltiazem, nifedipine, cimetidine and losartan.

**Table 5:** Risk factors for atherothrombotic events after coronary angioplasty and bare-metal stent implantation in patients taking clopidogrel, by CYP3A5 genotype

Risk factor	CYP3A5 genotype; adjusted OR (95% CI)	
	Non-expressor	Expressor
Co-administered CYP3A metabolizers (every increase in no.)	3.07 (1.36-6.93)	0.29 (0.03-2.96)
Total cholesterol level $\geq 5.18$ mmol/L (v. $< 5.18$ mmol/L)	1.00 (0.99-1.02)	1.02 (0.98-1.05)
Age $\geq 65$ yr (v. $< 65$ yr)	0.97 (0.91-1.02)	1.10 (0.92-1.33)
Stent length $\geq 20$ mm (v. $< 20$ mm)	0.98 (0.90-1.07)	1.02 (0.82-1.26)

This article has been peer reviewed.

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**Competing interests:** None declared.

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## REFERENCES

- Quinn MJ, Fitzgerald DJ. Ticlopidine and clopidogrel. *Circulation* 1999;100:1667-72.
- 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.
- Mehta SR, Yusuf S, Peters RJ, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet* 2001;358:527-33.
- Braunwald E, Antman EM, Beasley JW, et al. ACC/AHA 2002 guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients with Unstable Angina). *Circulation* 2002;106:1893-900.
- Müller I, Besta F, Schulz C, et al. Prevalence of clopidogrel non-responder among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost* 2003;89:783-7.
- Gurbel PA, Bliden KP, Hiatt BL, et al. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003;107:2908-13.
- Jaremo P, Lindahl TL, Fransson SG, et al. Individual variations of platelet inhibition after loading doses of clopidogrel. *J Intern Med* 2002;252:233-8.
- Serebruany VL, Steinhubl SR, Berger PB, et al. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J Am Coll Cardiol* 2005;45:246-51.
- 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.
- Lau WC, Waskell LA, Watkins PB, et al. Atorvastatin reduced the ability of clopidogrel to inhibit platelet aggregation. *Circulation* 2003;107:32-7.
- Lau WC, Gurbel PA, Watkins PB, et al. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of clopidogrel resistance. *Circulation* 2004;109:166-71.
- Wrighton SA, Schuetz EG, Thummel KE, et al. The human CYP3A subfamily: practical considerations. *Drug Metab Rev* 2000;32:339-61.
- Evans WE, McLeod HL. Pharmacogenomics: Drug disposition, drug targets, and side effects. *N Engl J Med* 2003;348:538-49.
- Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001; 27:383-91.
- Patki KC, Von Moltke LL, Greenblatt DJ. In vitro metabolism of midazolam, triazolam, nifedipine, and testosterone by human liver microsomes and recombinant cytochromes P450: role of CYP 3A4 and CYP3A5. *Drug Metab Dispos* 2003;31:938-44.
- Thervet E, Anglicheau D, King B, et al. Impact of cytochrome P450 3A5 genetic polymorphism on tacrolimus doses and concentration-to-dose ratio in renal transplant recipients. *Transplantation* 2003;76:1233-5.
- Kivisto KT, Niemi M, Schaeffeler E, et al. Lipid-lowering response to statins is affected by CYP3A5 polymorphism. *Pharmacogenetics* 2004;14:523-5.
- Savi P, Labouret C, Delesque N, et al. P2y(12), a new platelet ADP receptor, target of clopidogrel. *Biochem Biophys Res Commun* 2001;283:379-83.
- Murugappa S, Kunapuli SP. The role of ADP receptors in platelet function. *Front Biosci* 2006;11:1977-86.
- Yu KS, Cho JY, Jang JJ, et al. Effect of the CYP3A5 genotype on the pharmacokinetics of intravenous midazolam during inhibited and induced metabolic states. *Clin Pharmacol Ther* 2004;76:104-12.
- Jung SM, Kim KA, Cho HK, et al. Cytochrome P450 3A inhibitor itraconazole affects plasma concentrations of risperidone and 9-hydroxyrisperidone in schizophrenic patients. *Clin Pharmacol Ther* 2005;78:520-8.
- Osani T, Ohkubo T, Yasui N, et al. Effect of itraconazole on the pharmacokinetics and pharmacodynamics of a single oral dose of brotizolam. *Br J Clin Pharmacol* 2004;58:476-81.
- Gawaz M, Ruf A, Pogatsa-Murray G, et al. Incomplete inhibition of platelet aggregation and glycoprotein IIb/IIIa receptor blockade by abciximab: Importance of internal pool of glycoprotein IIb/IIIa receptors. *Thromb Haemost* 2000;83:915-22.
- Schomig A, Kastrati A, Mudra H, et al. Four-year experience with Palmaz-Schatz stenting in coronary angioplasty complicated by dissection with threatened or present vessel closure. *Circulation* 1994;90:2716-24.
- Miller SA, Dykes DD, Polesky HF. A simple salting out method for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- Flockhart DA, Tanus-Santos JE. Implication of cytochrome P450 interactions when prescribing medication for hypertension. *Arch Intern Med* 2002;162:405-12.
- Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* 2004;109:3171-5.
- 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.
- Fukuen S, Fukuda T, Maune H, et al. Novel detection assay by PCR-RFLP and frequency of the CYP3A5 SNPs, CYP3A5\*3 and \*6, in a Japanese population. *Pharmacogenetics* 2002;12:331-4.
- Hiratsuka M, Takekuma Y, Endo N, et al. Allele and genotype frequencies of CYP2B6 and CYP3A5 in the Japanese population. *Eur J Clin Pharmacol* 2002;58:417-21.
- Shih PS, Huang JD. Pharmacokinetics of midazolam and 1'-hydroxymidazolam in Chinese with different CYP3A5 genotypes. *Drug Metab Dispos* 2002;30:1491-6.
- Balram C, Zhou Q, Cheung YB, et al. CYP3A5\*3 and \*6 single nucleotide polymorphisms in three distinct Asian populations. *Eur J Clin Pharmacol* 2003;59:123-6.

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