European Heart Journal (2009) **30**, 1943–1963
doi:10.1093/eurheartj/ehp295

REVIEW

Personalized medicine and antiplatelet therapy: ready for prime time?

Céline Verstuyft[1,2], Tabassome Simon[3], and Richard B. Kim[1]*

[1]Division of Clinical Pharmacology, Department of Medicine, University of Western Ontario, 339 Windermere Road, London, Ontario, Canada N6A 5A5; [2]Pharmacology Department, Univ Paris Sud, Hôpital Bicêtre, Assistance Publique-Hôpitaux de Paris (AP-HP), 78 rue du General Leclerc, 94275 Le Kremlin Bicêtre, France; and [3]Pharmacology Department, Assistance Publique—Hopitaux de Paris (AP-HP, site St Antoine) URC-EST, Universite Pierre et Marie Curie (UPMC)-Paris 06, 27 rue de Chaligny, 75012 Paris, France

Received 5 June 2009; accepted 7 June 2009; online publish-ahead-of-print 28 July 2009

Keywords Personalized medicine • Pharmacogenetics • Antiplatelet • Aspirin • Thienopyridines • Cardiovascular disease

Introduction

The concept of personalized medicine is receiving significant attention due to the greater awareness of the influence of genes to the drug effects. Single nucleotide polymorphisms (SNPs) in the DNA are the most frequent form of sequence variations in the human genome and appear to affect the efficacy and safety of many drugs. The term 'pharmacogenetics' was coined over 40 years ago with an ultimate goal of using the genetic makeup of an individual to predict drug response and efficacy.[1–3] We are just at the beginning of a new era in personalized cardiovascular therapies. However there is little doubt that, in the near future, pharmacogenetic testing will become a valuable tool for a drug and dose selection and thus result in a more desirable benefit/risk ratio for drugs prescribed to patients.

Over the past decades, the platelet has emerged as a major pathway involved in cardiovascular diseases. The platelet as a 'drug target' has spawned a variety of new drugs that have been shown in large-scale randomized trials to improve patient outcomes in acute coronary syndromes and following percutaneous revascularization procedures.[4–6] Until recently aspirin, centred on the thromboxane pathway, was the only antiplatelet agent considered to be the gold standard for effectiveness in both primary and secondary prevention of atherothrombotic diseases.[7] Although it continues to be used as the gold standard antiplatelet therapy, adenosine diphosphate (ADP) receptor antagonists and phosphodiesterase inhibitors in combination therapy appear to exert synergistic effects and provide added benefits among high-risk patients for cardiovascular disease.[7,8]

Nevertheless an important lesson that has emerged from number of trials is that antiplatelet potency per se does not necessarily guarantee enhanced clinical benefit or tolerability for a given patient.[8–11] This may in part be due to the substantial inter-individual variation in platelet response to ADP.[9–11] The mechanism underlying such variation has recently become clearer (*Figure 1*). Specifically the wide inter-subject variabilities to antiplatelet agents such as clopidogrel, may be genetically mediated and arises from altered drug metabolism or transport.[12–15] In the current review, we will focus on the key molecular mechanisms involved in the pharmacological action of oral antiplatelet drugs, the environmental and genetic factors that may impact antiplatelet therapies. We will also provide an update on recent advances in personalized medicine of relevance to arterial thrombosis and antiplatelet drugs. Finally, we will provide our perspectives of pharmacogenetic testing for drugs used to treat cardiovascular diseases.

Mechanism of actions and clinical relevance

Current therapeutic strategies for the treatment of arterial thrombosis are based on well-known receptor systems (*Figure 2*). Collagen and/or thrombin interact with activated platelets and their receptor GPIIb–IIIa to bind fibrinogen and von Willebrand factor and initiate platelet aggregation. Stable aggregation of platelets is amplified by two autocrine factors generated upon platelet stimulation: ADP, released from platelet and Thromboxane A2 (TXA2), generated by the sequential actions of cyclooxygenase-1 (COX-1) and thromboxane synthase from the arachidonic acid released from membrane phospholipids.[5]

Aspirin

Aspirin was the first and continues to be the most widely used antiplatelet agent. In platelets, the major cyclooxygenase product

* Corresponding author. Tel: +1 519 663 3553, Fax: +1 519 663 3232, Email: richard.kim@lhsc.on.ca

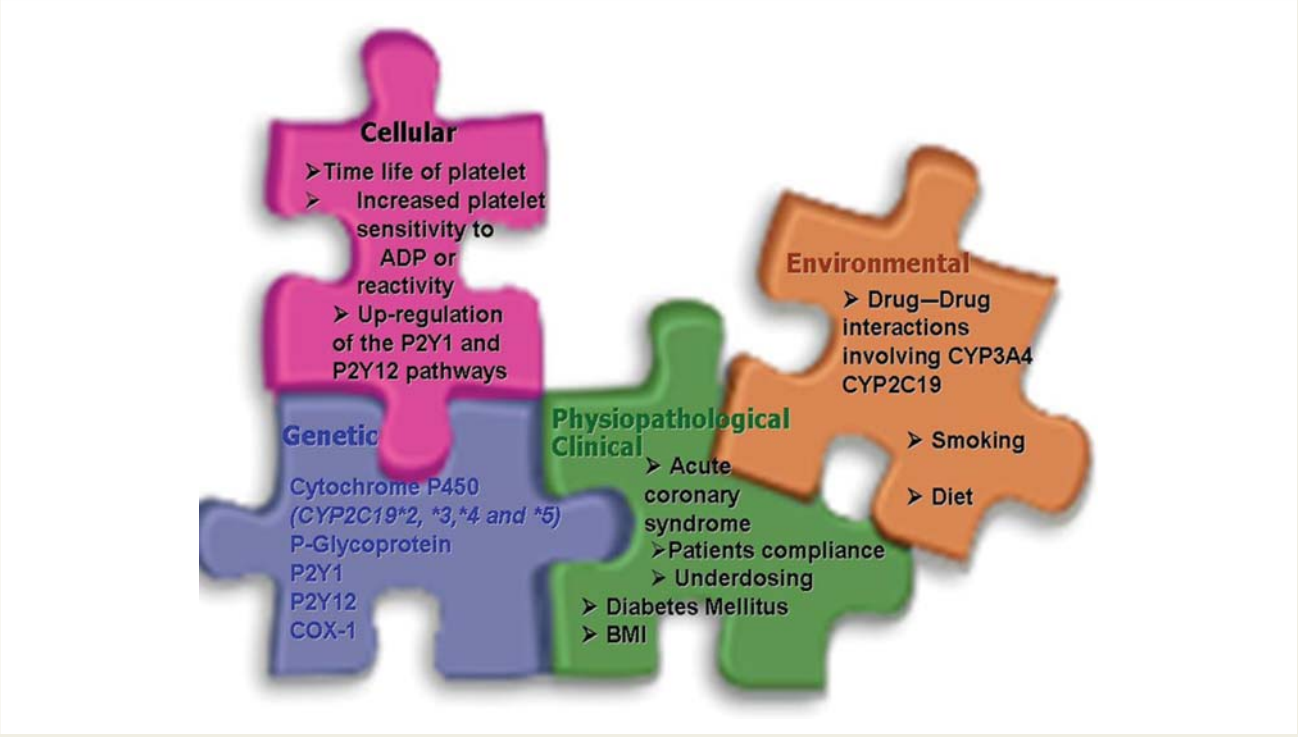


Figure 1 Factors influencing the variability of antiplatelet drug response.

is thromboxane A2. Aspirin blocks the production of TXA2 by acetylating a serine residue near the active site of platelet COX-1, the enzyme that produces the cyclic endoperoxide precursor of TXA2. Since platelets are not able to synthesize new proteins, the action of aspirin on platelet COX-1 is permanent, and persists for the life of the platelet (7–10 days). Thus, repeated doses of aspirin produce a cumulative effect on platelet function. Complete inactivation of platelet COX-1 has been shown to occur when 160 mg of aspirin is taken daily.[16]

The efficacy of aspirin has been appreciated for many years and data from the meta-analysis, the Antiplatelet Trialists' Collaboration found an ~25% relative risk reduction of vascular death, MI, or stroke for antiplatelet therapy, primarily aspirin vs. placebo.[17] This data set served as the foundation for the widespread adoption of aspirin as the standard regimen for the secondary prevention of cardiovascular events.

However a number of clinical trials have shown that many patients receiving aspirin still sustain a thrombotic event, and therefore referred as 'aspirin resistant'. The prevalence of aspirin resistance is thought to range anywhere from 5 to 40%.[18] This phenomenon appears to be a true entity of clinical relevance since it cannot be overcome by increasing aspirin dose.[19] Despite intensive research relating to aspirin resistance, this topic remains controversial mainly because of the lack of an optimal biomarker and validated assay. A key step to understanding aspirin resistance could be the identification of the relevant genetic determinants that mediate aspirin resistance. Different target protein and genetic polymorphisms such as the PLA1/A2 polymorphism of platelet glycoprotein IIIa have been linked to the response to aspirin therapy[20–22] as well as an increased risk of thrombotic events.[23,24] Moreover increased expression of platelet COX-2 messenger RNA has been linked to aspirin resistance,[25,26] although this is controversial.[27] Further studies are needed to determine the ultimate clinical relevance of these findings.

Thienopyridines

The second most widely prescribed antiplatelet agents for chronic therapy are thienopyridines which target the $P2Y_{12}$ receptor.[28] The key mediator of platelet activation is ADP which is released from platelet dense granules by activating stimuli such as thrombin, collagen, and thromboxane A_2. Net result of ADP is the alteration of platelet conformation, intracellular calcium increase, adenylyl cyclase down-regulation, protein phosphorylations, activation of the GPIIb–IIIa complex which results in fibrinogen binding, aggregation, and release. Adenosine diphosphate is known to be the fundamental step of platelet activation via the $P2Y_1$ receptor, while binding of ADP to $P2Y_{12}$ receptor amplifies this response and allows sustained ADP-induced platelet aggregation.[29] Consequently, binding of ADP to $P2Y_{12}$ receptor not only amplifies the aggregation response but also increases granule secretion and platelet procoagulant activity.[30] Therefore ADP-mediated activation of $P2Y_{12}$ represents a critical pathway that results in arterial thrombosis and the accompanying tissue anoxia and inflammatory response. Not surprisingly pharmacological targeting of this receptor has become an important antiplatelet treatment strategy.

Clopidogrel and its predecessor ticlopidine are thienopyridine ADP receptor antagonists. These drugs function as irreversible platelet inhibitors, sustaining their activation for the life of the

platelets. Note that both are prodrugs, which undergo hepatic metabolism by cytochrome P450 enzymes (CYPs)3A4 and 2C19 before generating the active metabolite, a transient intermediate which inactivates the receptor.[28,31] Cytochrome P450 enzymes are important in the biosynthesis and degradation of endogenous compounds such as steroids, lipids, and vitamins and the metabolism of xenobiotics. They reduce or alter the pharmacological activity of most of the currently prescribed drugs and facilitate their elimination. The liver is the major site of CYP metabolism, but the small intestine is also a potentially important organ for drug metabolism and transport.[32]

Ticlopidine has been shown to be efficacious in conditions such as claudication, unstable angina, coronary artery and peripheral bypass surgery, and cerebrovascular disease.[33,34] However, ticlopidine use has been reduced because of rare, but significant, adverse side effects such as neutropenia that require regular monitoring of white blood cell count, and a potentially life-threatening thrombotic thrombocytopenic purpura.[35]

Clopidogrel requires oxidation mainly dependent on the cytochrome P450 enzymes 2C19 (CYP2C19) and to a lesser extent on isoenzymes CYP2C9, 3A4, 3A5, 2B6.[14,36–39] Only 15% of the prodrug is available as an active agent; the remaining 85% is hydrolysed into an inactive compound *(Figure 2)*. Although, its half-life is only 8 h, it has an irreversible effect on platelets that lasts 7–10 days. Inhibition of platelet aggregation appeared 2 h after the first dose, became significant after the second dose, and progressed to a steady-state value of 55–57% by day 7.[40] It was suggested that the P-glycoprotein(P-gp) transporter also limits the intestinal absorption of clopidogrel, thereby controlling its antiplatelet activity.[41,42]

The first clear evidence for the efficacy benefit from clopidogrel was shown in CAPRIE trial evaluating patients with atherosclerotic disease.[43] Subsequently, several large clinical trials have confirmed in others populations the efficacy of clopidogrel co-administration in reducing cardiovascular events (CURE,[44] CREDO,[35] PCI-CURE[45]). The use of clopidogrel has been extended to patients with non-ST-segment elevation ACS (unstable angina and non-ST-segment elevation MI) independent of coronary revascularization,[44] and patients with ST-segment elevation MI, including those undergoing PCI.[46–48]

Incidence of side effects, such as gastrointestinal disorders, neutropenia, and thrombotic thrombocytopaenic purpura,[49,50] is far lower in comparison to ticlopidine. Moreover, its second major benefit over ticlopidine was its ability to yield antiplatelet effects more rapidly through the administration of a loading dose.[51] Occasional resistance to clopidogrel and interpatient variability in drug response has spurred the development of new therapies.

Prasugrel is a third-generation oral thienopyridine that is chemically distinct from clopidogrel. Like clopidogrel, prasugrel is a specific, irreversible antagonist of the platelet $P2Y_{12}$ ADP receptor. It is rapidly hydrolysed by esterases to an inactive thiolactone, which is then metabolized by hepatic CYPs to the active metabolites. The major hepatic pathway involve CYP3A4 and CYP2B6, and to a lesser extent the CYP2C9 and CYP2C19.[52] It is rapidly absorbed and metabolized, with a median time for achieving the maximal concentration of its active metabolite in the circulation of about 30 min.[53,54] The mean elimination half-life of active metabolite is 3.7 h, and renal excretion (around 70%) is the major route for elimination.

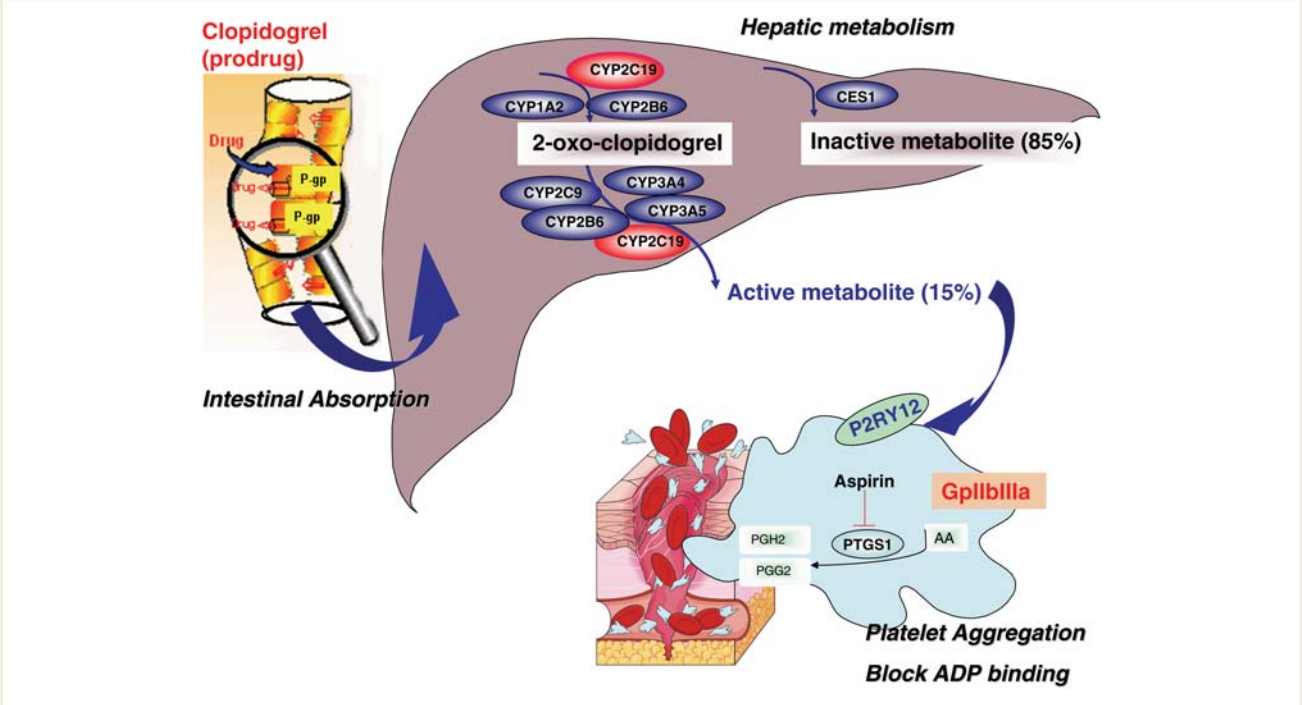


Figure 2 Clopidogrel absorption, metabolism, and aspirin target.

The major difference between clopidogrel and prasugrel is their bioavailability; in fact a significant portion of the administered dose of clopidogrel is activated rapidly through metabolism, resulting in lower apparent bioavailability of the active metabolite. As a consequence, preclinical studies have shown that prasugrel is an orally active antiplatelet agent that is a more potent inhibitor of platelet aggregation on a milligram per kilogram basis, with a faster onset of action.[54,55]

Limitations of current antiplatelet therapies

Dual antiplatelet pathway inhibition appears to offer synergistic benefit in preventing thrombus formation,[56,57] but all patients do not benefit to the same extent. Up to 15% of the high-risk patients with acute coronary syndrome continue to suffer from ischaemic events, and up to one-third of patients have a marked interindividual variability in the extent of platelet inhibition.[58]

The prevalence of this phenomenon, referred to a clopidogrel non-responsiveness or resistance, varies widely according to the literature.[10,58,59] *Table 1* summarizes the studies in which various measures of clopidogrel responsiveness, mainly post-treatment platelet reactivity, have been studied. A recent meta-analysis found an overall prevalence of 21% (95% CI, 17–25%) of laboratory-defined clopidogrel non-responsiveness. The differences in reported prevalences partly depend on the loading dose of clopidogrel and the methods of determining non-responsiveness.[60] Interestingly patients labelled as clopidogrel resistant using *ex-vivo* assays have an increased risk of stent thrombosis and other cardiovascular outcomes,[60] but the use of 600 mg clopidogrel loading dose appears to reduce such risks.

Contemporary basic and clinical pharmacology have evolved to embrace an increasingly sophisticated molecular view of the mechanisms underlying drug action. Variability in drug action may be the result of pharmacokinetic or pharmacodynamic differences. Pharmacokinetic variability refers to variability in delivery of drugs to, or removal from, key molecular sites of action that mediate efficacy and/or toxicity. Pharmacodynamic variability refers to variable drugs effects despite equivalent drug delivery to molecular sites of action. In fact, although the best method of assessing antiplatelet drug response has not been established yet, there is sufficient evidence to support that persistence of enhanced platelet reactivity plays a key role in atherothrombotic complications.[8] The mechanisms leading to poor response to clopidogrel have not been fully elucidated and are probably multi-factorial.[61] Compliance, cellular, environmental, genetic, and clinical factors such as obesity, diabetes mellitus, nature of coronary injury, and inflammation are known to contribute to variable antiplatelet drug response (*Figure 1*).[62,63]

Furthermore, another major limitation inherent to the thienopyridines is attributed to the irreversible antiplatelet effects. Indeed, bleeding events are one of the well-known major side effects for all antithrombotic agents, particularly with antiplatelet therapies. The development of new antiplatelet agents with a reversible mechanism of action, allowing platelet function to return more rapidly to baseline status will likely reduce the risk of bleeding in patients undergoing surgery.[28,61]

Determinants of antiplatelet therapy: non-genetic factors of variability

The environmental factors, such as diet, drug–drug interaction with drug transporter, protein target function, and CYPs are known to be involved as key determinants of intersubject variation in drug responsiveness[32] (*Figure 1*). In fact, it was described that the level of clopidogrel active metabolite concentration needed to inhibit $P2Y_{12}$ receptor is suboptimal in some patients.[63] The limited efficacy of aspirin and clopidogrel suggests the existence of alternative pathways for platelet activation and/or possible drug interactions such as proton-pump inhibitors (PPIs). Indeed similar to clopidogrel, PPIs are sharing the same metabolic pathway extensively metabolizing in the liver.[64] The increase in the loading dose, a pharmacokinetic solution that takes into consideration elimination pathways such as certain intestinal transporters, has been suggested as a way for decreasing the risk of drug non-responsiveness.[42]

Drug transporters are increasingly recognized to be important to drug disposition and response. The oral bioavailability of various drugs is limited by active luminal secretion via adenosine triphosphate binding cassette (ABC) efflux transporters in the intestine—in particular P-gp encoded by the multidrug resistance gene *ABCB1* (*MDR1*). Many substrates of drug metabolizing enzymes, particularly CYP3A4, are also substrates of P-gp; the overlap between CYP3A4 and P-gp substrates may have resulted in part from the coordinated regulation and tissue expression of CYP3A4 and ABCB1 organs such as the liver and the intestine.[65] P-gp was found to be a key factor for intestinal absorption of clopidogrel, limiting its bioavailability.[41] Moreover, a linear correlation of Cmax values has been shown between clopidogrel and its active metabolite, suggesting that interindividual differences in the activity of metabolizing enzymes (CYP3A4 or 3A5) are not the rate-limiting step for generation of the active metabolite.[42]

This is interesting with regard to ongoing and future clinical trials. Some studies and a recent meta-analysis support the hypothesis that an increase of clopidogrel loading dose (600 mg/day) could lead to a lower prevalence of clopidogrel non-responsivenes with a more potent and rapid antiplatelet effects than 300 mg dose.[60,66,67] Three other studies have confirmed this finding.[68–70]

The results of the large ongoing CURRENT-OASIS 7 trial may help to better define optimal dosing regimens for clopidogrel in acute coronary syndrome patients.[71] However, recently in a small number of NSTEACS patients ($n = 256$), clopidogrel 600 mg LD compared with 300 mg LD was associated with significantly reduced ADP-induced platelet aggregation (49.7 vs. 55.7% with ADP 20 μmol/L) but did not reduce post-PCI myonecrosis or adverse clinical outcomes to 6 months.[72] Moreover, the ISAR-CHOICE trial[69] showed that an increase of clopidogrel loading dose from 600 to 900 mg was not associated with an additional suppression of platelet function because of limited clopidogrel absorption.

Table 1 Details of included studies on prevalence of laboratory clopidogrel non-responsiveness

Study	Design	*n*	Clopidogrel dose (mg)	Aspirine dose (mg)	Functional parameter and/or outcome	Definition of non-responsiveness aggregation assay	Determination of platelet aggregation	End-point	Follow-up	Non-responsiveness *n* (%)
Stent thrombosis										
Muller *et al.* 2003[10]	Prospective cohort	105	LD 600; MD 75	100	Decrease inhibition of platelet aggregation	LTA (5 or 20 μmol/L ADP): <10% reduction /baseline	4 h after LD	Stent thrombosi (ST)	14 days	5 (5), 12 (11)
Barragan *et al.* 2003[93]	Prospective cohort	1684	MD 75×2	250	Increase P2Y12 reactivity ratio; increase platelet aggregation	VASP-P (sodium citrate 0.129 mol/L) monoclonal antibody	0, 2, and 4.8 days after PCI (controls)	ST	30 days	17 (1.03)
Gurbel *et al.* 2005[131]	Case–control	20 cases; 100 controls	LD 300; MD 75	81–325	Increase P2Y12 reactivity ratio, increase platelet aggregation	LTA (5 or 20 μmol/L ADP): after treatment PR > 75th percentile in controls	Cases: 218 ± 204 days after LD; Controls: 5–14 days after LD	ST	Cases: 218 ± 204 days after LD; Controls: 5–14 days	NA
Ajzenberg *et al.* 2005[132]	Case–control	10 cases; 22 controls; 17 healthy volunteers	LD 300; MD 75	75–250	Increase shear-induced platelet aggregation (SIPA) increase P2Y12 reactivity ratio	SIPA and LTA monoclonal antibody	Cases: within 4.6 ± 3.4 days of SAT; Controls within 3 days after clopidogrel	ST	NA	19 (1.2)
Buonamici *et al.* 2007[133]	Prospective cohort	804	LD 600; MD 75	325	Increase platelet aggregation	LTA (10 μmol/L ADP) 90th percentile of controls (70%)	12–108 h from dose 6 days after PCI	ST	6 months	25 (3.1)
POST-PCI myonecrosis and ischaemic events										
Matetzky *et al.* 2004[134]	Prospective cohort	60	LD 300; MD 75	200	Increase platelet aggregation	LTA (5 μmol/L ADP): first quartile of reductions compared with baseline	6 days after LD	STEMI, ACS, PAD ischaemic stroke	6 months	15 (25.0)
Gurbel *et al.* 2005[135]	Prospective cohort	192	LD 300; MD 75	81–325	Increase platelet aggregation	LTA (20 μmol/L ADP) 4th quartile of aggregation	24 h after LD	CV death, MI, ACS stroke	6 months	NA
Cuisset *et al.* 2006[136]	Randomized controlled trial	292	LD 600 ($n = 146$); LD 300 ($n = 146$); MD 75	160	Increase platelet aggregation	LTA (10 μmol/L ADP) aggregation >70%	12 h after LD	CV death, SAT, ischaemic stroke, ACS	1 month	58 (20); 15% LD 600 mg; vs. 25% LD 300 mg

Continued

Table 1 *Continued*

Study	Design	*n*	Clopidogrel dose (mg)	Aspirine dose (mg)	Functional parameter and/or outcome	Definition of non-responsiveness aggregation assay	Determination of platelet aggregation	End-point	Follow-up	Non-responsiveness *n* (%)
Lev *et al.* 2006[137]	Prospective cohort	150	LD 300; MD 75	81–325	Increase clopido/aspirin-resistant patients	LTA (5 or 20 μmol/L ADP): <10% reduction/baseline	20–24 h after LD	CK-MB >5 ng/mL	20–24 h	36 (24)
Cuisset el al. 2006[138]	Prospective cohort	106	LD 300; MD 75	160	Increase platelet aggregation	LTA (10 μmol/L ADP) aggregation 4th quartile of aggregation	12 h after LD	CV death, ST, stroke, ACS	1 month	23 (22)
Hochholzer *et al.* 2006[139]	Prospective cohort	802	LD 600; MD 75	>100	Increase platelet aggregation	LTA (20 μmol/L ADP): no definition	At least 2 h after LD	Death, MI, revascularization	1 month	NA
Geisler *et al.* 2006[140]	Prospective cohort	379	LD 600; MD 75	100	Decrease platelet inhibition	LTA (20 μmol/L ADP) >70%	34.8 ± 25.9 h after LD	CV death, MI, stroke	3 months	22 (6)
Bliden *et al.* 2007[141]	Prospective cohort	100	MD 75	81 (7 days); 325	Augm platelet aggregation	LTA (5 μmol/L ADP); thromboelastograph; haemostasis	Before, 3, 18, and 24 h afterwards	CV death, MI, stroke, ischaemia	1 year	2/22 (9.0)
Cuisset *et al.* 2007[142]	Prospective cohort	190	LD 600; MD 75	250	Increase platelet aggregation	LTA (10 μmol/L ADP) aggregation >70%	Before, 12 h, 24 h	AMI	NA	54
Bonello *et al.* 2007[143]	Prospective cohort	144	LD 300; MD 75	160	Increase P2Y12 reactivity ratio	VASP (monoclonal antibody 16C2 (2nd through 5th quintiles)	After LD 25 ± 3 h	MACE	6 months	21
Angiolillo *et al.* 2007[144]	Prospective cohort	173	MD 75	100	Increase platelet aggregation	LTA (10 μmol/L ADP) aggregation (4th quartile)	3–6 and 24 months	MACE	2 years	(19.7)
Frere *et al.* 2007[145]	Prospective cohort	195	LD 600; MD 75	LD 250; MD 75	Increase platelet aggregation; increase P2Y12 reactivity ratio,	VASP (monoclonal antibody 16C2 LTA (10 μmol/L ADP) aggregation >70%	Before and 18.2 ± 2.2 h	CV, death, acute SAT, ACS, and stroke	30 days	14
Price *et al.* 2008[146]	Prospective cohort	380	LD 600; MD 75	LD 325; MD 325	Increase P2Y12 reactivity units	VerifyNow NA $P2Y_{12}$ (ADP20 μmol/L), PRU	12 h afterwards	CV, death, MI, stent	6 months	10 (2.6)
Bonello *et al.* 2008[147]	Prospective control randomized	162	LD 600 control; LD 600; MD 75	MD 160	Increase platelet aggregation; increase P2Y12 reactivity ratio	VASP (monoclonal antibody 16C2); LTA (ADP+PGE1)	24 h after 1 LD; 12 h after 2 LD	MACE	1 month	3/84 (3.6); 0% in VASP-P guided group
Patti *et al.* 2008[148]	Prospective cohort	160	LD 600; MD 75	NA	Increase P2Y12	VerifyNow NA $P2Y_{12}$ PRU assay	Before and 8, 24 h afterwards	MACE	6 months	NA
Marcussi *et al.* 2009[127]	Prospective cohort	683	LD 600; MD 75	100–325	Increase P2Y12	VerifyNow $P2Y_{12}$ assay (ADP10 μmol/L), PRU assay	24 h after LD	CV death, MI	12 months	NA

Von Beckerath *et al.* 2009[149]	Randomized; study	66	LD 600; MD 75 or 150	200	% inhibition platelet aggregation; increase P2Y12	VerifyNow $P2Y_{12}$ assay (ADP 5 μmol/L), PRU assay	30 days after PCI	Platelet aggregation	30 days	NA	
Sibbing *et al.* 2009[150]	Prospective cohort	1608	LD 600; MD 150 (3 days); MD 75	200	Increase platelet aggregation	MEA Multiplate Analyser (ADP 6.4 μmol/L)	Before and after aspirin dose	SAT, death, TIMI major bleeding	30 days	323 (20)	

LTA, light transmittance aggregometry; PCI, percutaneous coronary intervention; PRU, platelt reactivity unit; SIPA, shear-induced platelet aggregation; VASP-P, vasodilator-stimulate phosphoprotein phosphorylation; ST, stent thrombosis; LD, loading dose; MD, maintenance dose; NA, not available; MACE, major cardiovascular events; TIMI, Thrombolysis In Myocardial Infarction.

Therefore, it is probable that there is a threshold, likely attributable to the absorption and clopidogrel metabolite formation rate, which limits additional enhancement of the platelet inhibitory effects beyond a certain dose.

Metabolism and interindividual variability

Differences in drug metabolism are common, often marked and are frequently major contributors to differences in drug response among patients. CYP3A4, CYP2C9, CYP2C19, and CYP1A2 are involved in the formation of the active clopidodrel metabolites.[73] CYP3A isoenzymes (CYP3A4 and CYP3A5), which are per se heterogenous, appear to be the primary oxidative pathway for clopidogrel.[39,74] CYP3A5, which is polymorphically expressed, may contribute as much as 50% of hepatic CYP3A activity in certain ethnic populations.[75,76]

Drug–drug interactions resulting in either inhibition or induction of the involved enzymes, especially those in the intestine and liver, can markedly alter oral bioavailability.[32] The metabolism of clopidogrel is inhibited by the CYP3A4 inhibitor, ketoconazole and induced by rifampicin.[37] Moreover, drug–drug interactions with lipophilic statins and PPIs are thought to alter the pharmacodynamic effects of clopidogrel.

Statins

Some studies,[77–79] but not all,[80–86] have shown that atorvastatin and simvastatin, which are metabolized by CYP3A4 appear to reduce clopidogrel-induced antiplatelet effects. The discrepancies between the pharmacological findings can be explained at least in part by the study designs. Several studies[80,83] have considered all statins instead of evaluating those inhibiting CYP3A. Although many statins (atorvastatin, simvastatin, lovastatin, cerivastatin) are substrate of CYP3A4, the attained therapeutic plasma levels are not sufficient to inhibit CYP3A4. Moreover, the frequent concomitant administration of other CYP3A substrates and inhibitors, modulating clopidogrel activity, were not taken into account in the control groups.[87]

Finally, these findings were not replicated in larger studies which did not show a clinical or biological interaction between lipophilic statins and clopidogrel.[85,86]

Proton-pump inhibitors

Recent guidelines published by the American Heart Association, the American College of Gastroenterology, and the American College of Cardiology advocate PPI therapy for patients receiving ASA after myocardial infarction, especially those 60 years or older.[88] Proton-pump inhibitors are thus often prescribed prophylactically at the initiation of clopidogrel therapy although the rationale for this co-prescription is not fully validated.

Proton-pump inhibitors can alter the extent of drug absorption through modifying intragastric pH.[89] Similar to clopidogrel, they share the same metabolic pathway in terms of hepatic metabolism.[64,90]

As shown in *Table 2*, PPIs are not only substrates,[90] but also inhibitors of CYP2C19;[64] therefore, those poor metabolizer (PM) patients with *CYP2C19* loss-of-function alleles may not only have impaired formation of clopidogrel active metabolite but also the highest concentrations of omeprazole, a potential double hit.

Table 2 Common drug substrates and clinically important inhibitors of CYP2C19

CYP2C19 substrates	CYP2C19 inhibitors	CYP2C19 inducers
Proton-pump inhibitors: omeprazole, esomeprazole, lansoprazole, rabeprazole, and pantoprazole	Omeprazole, esomeprazole, lansoprazole, rabeprazole	Rifampicin
Antiprotease: Nelfinavir		
Antiplatelet: clopidogrel, ticlopidine	Ticlopidine, clopidogrel	
Antifungal	Voriconazole	
Anticonvulsivant: phenytoin, diazepam		Carbamazepine
Anticancer: cyclophosphamide, tamoxifene		
Antidepressants: amitriptyline, citalopram, clomipramine, sertraline	Fluvoxamine	

Recent mechanistic studies have shown that omeprazole, the most potent CYP2C19 inhibitors in clinical use, reduced the inhibitory effect of clopidogrel on platelet aggregation.[91,92] Gilard *et al.* used the vasodilator-stimulated phosphoprotein phosphorylation (VASP) test as the index of platelet reactivity to clopidogrel and defined poor responders according to Barragan *et al.*[93] criteria, in patients receiving omeprazole, 60.9% of patients were considered as poor clopidogrel responders compared with 26.7% in the placebo group (odds ratio 4.31, 95% CI 2.0–9.2). However, this interesting finding might be biased since the authors did not evaluate the percentage of clopidogrel non-responders before inclusion and did not exclude them. Because the primary hypothesis of the study was that omeprazole–clopidogrel drug–drug interaction is via a CYP2C19 competitive or non-competitive inhibitory mechanism, patient carriers of *CYP2C19* loss-of-function alleles should have been excluded. Nevertheless, this study remains to date the only randomized placebo-controlled trial evaluating this important drug–drug interaction. Interestingly, some small studies have suggested that the PPI–clopidogrel interaction is not a class effect. Concomitant treatment with lansoprazole, pantoprazole, and esomeprazole did not alter the pharmacokinetic or pharmacodynamics of clopidogrel while omeprazole and rabeprazole appeared to interact[94,95] (*Table 3*).

Five recent studies in large populations addressed the issue of clopidogrel–PPI interactions by examining their impact on the incidence of clinical events.[12,96–99] In a retrospective claims-based analysis, Pezalla *et al.*[98] found a link between the PPIs use and the incidence of MI among patients aged below 65 years receiving clopidogrel. In the French FAST-MI registry, the use of PPIs had no impact on the clinical response of clopidogrel among the subgroup of 2208 AMI genotyped patients receiving clopidogrel.[12] In contrast, a significant association was found between incidence of recurrent myocardial infarction within 90 days after discharge and current use of PPI (adjusted OR 1.27, 95% CI 1.03–1.57) in a Canadian nested case–control study.[96] Treatment with pantoprazole, which does not potently inhibit CYP2C19, was not associated with recurrent infarction, whereas treatment with other PPI (omeprazole, lansoprazole and rabeprazole) was associated with reinfarction. However, neither major cardiac risk factors nor the use of over-the-counter medications, particularly aspirin, were taken into account in the multivariate analysis. The use of PPI was also associated with a higher risk for recurrent ACS (OR, 1.86; 95% CI 1.57–2.20) in a retrospective study of 8205 ACS patients receiving clopidogrel.[97] The association was observed with both omeprazole (OR, 1.24, 95% CI 1.08–1.41) and rabeprazole (OR, 2.8, 95% CI 1.96–4.09). Unfortunately, the interaction with other PPIs (i.e. lansoprazole and pantoprazole) was not explored given the small numbers of patients. Finally, a possible 'class effect' for PPIs was outlined recently by Stanek[100] who reported the findings, as a late-breaking clinical trial at the SCAI 2009 Scientific Sessions (unpublished data). They evaluated major cardiovascular events (MACE) among 16 700 patients, members of the Medco Health Solutions pharmacy, who received clopidogrel after a PCI. All PPIs were associated with a higher risk of MACE in clopidogrel users [hazard ratio: 1.51 (95% CI 1.39–1.64); $P < 0.0001$] (MACE rate: 25.1% for omeprazole, 24.9% for esomeprazole, 29.2% for pantoprazole, and 24.3%. lansoprazole) when compared with non-PPI users (17.9%). Further studies are needed to replicate these findings and determine the precise clinical impact of the drug–drug interaction in terms of benefit/risk considering the high rate of the co-prescription in North America[96,97] and European countries.[12,99] Moreover, it is noteworthy to underline that the clinical relevance for this co-prescription effect should be viewed with caution as the findings are from a single randomized clinical trial.[101] In the latter, 123 patients with *Helicobacter pylori* infection and ulcer complications after using low-dose aspirin continuously for more than 1 month were randomized. The recurrence of ulcer complications during the 1 year follow-up was 14.8% compared with 1.6% in the placebo and lansoprazole groups, respectively (adjusted hazard ratio, 9.6; 95% CI 1.2–76.1). Therefore, prospective larger-scale studies are needed for evaluating the effectiveness of PPIs used concomitantly with clopidogrel and their potential class effects in terms of clinical outcomes Their design should include proper pharmacokinetics/pharmacodynamics investigations of different PPIs with clopidogrel and exclude or analyse separately those patients with *CYP2C19* loss-of-function polymorphisms.

Determinants of antiplatelet therapy: genetic factors of variability

Aspirin

Aspirin covalently modifies both COX-1 and COX-2, although its affinity for COX-1 is 50 to 100 times greater than for COX-2. Importantly up to 40% of patients with cardiovascular disease do not comply with aspirin therapy.[102] Incomplete platelet response to aspirin, likely reflects a composite of multiple processes. However, the mechanisms of aspirin resistance remain uncertain.[103,104]

From a pharmacological perspective, COX-1 is the key target for aspirin and non-selective non-steroidal anti-inflammatory drugs (NSAIDs). Genetic polymorphisms in enzymes involved in arachidonic acid metabolism (including COX-1), platelet glycoprotein, and collagen receptors have been identified. A clinical study in healthy volunteers showed that COX-1 genetic polymorphism (A682-G), which might affect enzyme expression, is present in 10% of the population.[105] In patients taking aspirin for secondary prevention of CAD, genetic variability in COX-1 appears to have some impact on AA-induced platelet aggregation and thromboxane generation.[106]

Clopidogrel

As outlined earlier, there is growing evidence that a subtherapeutic response to clopidogrel may relate to altered pharmacokinetic parameters such as intestinal absorption and liver metabolic activation, both of which are affected by genetic polymorphisms. The impact of *ABCB1* genetic polymorphism on clopidogrel clinical response was found recently in FAST-MI study.[12] Patients with the *ABCB1 3435TT* genotype had a higher rate of cardiovascular events at 1 year than those with the *ABCB1* wild-type genotype (adjusted HR, 1.72; 95% CI 1.20–2.47).[12] Regardless of the exact link between the *ABCB1 C3435T* genetic polymorphism and P-glycoprotein expression, these results are consistent with a prior study showing lower plasma concentrations of clopidogrel and its active metabolite in patients carrying the *ABCB1 3435TT* genotype.[41] However, as *ABCB1* genetic polymorphism was not an independent predictor of outcomes in the large population of patients undergoing PCI, these results should be considered with caution until additional studies replicate the findings.

To become active, clopidogrel requires oxidation dependent on CYP as described previously. Although *in vitro* studies have shown that CYP3A4 was the major oxidative pathway for clopidogrel, CYP2C19 is now believed to be the major pathway in the bioactivation of clopidogrel as confirmed recently with pharmacodynamic or/and pharmacokinetic studies in healthy volunteers.[14,73,107]

CYP3A4 and *CYP3A5* genetic polymorphisms

Most *CYP3A4* variants are SNPs of low allelic frequencies, and many are population specific.[108] However, because of their low allelic frequencies, their contribution to the interindividual variability of CYP3A4 expression is limited,[109] although they may play a role in the atypical response to drugs such as clopidogrel.[32] The impact of *CYP3A5* genetic polymorphism on clopidogrel metabolism was controversial until recently. Suh *et al.*[110] reported a higher frequency of atherothrombotic events within 6 months of coronary angioplasty in patients with the *CYP3A5* non-expressor genotype (*CYP3A5*3*) receiving clopidogrel therapy. While others studies found no association between *CYP3A5* genetic polymorphism and the antiplatelet effect of clopidogrel *ex vivo* both in patients[111,112] and in healthy subjects.[14,107,113] We confirmed the lack of association between *CYP3A5* genetic polymorphism and major clinical outcomes at 1 year follow-up in the large-scale FAST-MI cohort[12] (*Table 4*).

CYP2C19 genetic polymorphisms

Almost 25 genetic variants in *CYP2C19* has been found www.cypalleles.ki.se, although only two (*CYP2C19*2* and **3*) account for more than 95% of cases of PM phenotypes. There are substantial differences in the prevalence of *CYP2C19* polymorphisms among various population groups, as described in *Table 3*. Two to 3% of Caucasians and 4% of Africans have the PM phenotype, whereas 10–25% of Southeast Asians exhibit the PM phenotype.[114] Recently, a new allele (*CYP2C19*17*) was described, and noted to be associated with an increased activity *in vivo* as measured by omeprazole and mephenytoin as probe drugs. The variant is fairly common among Caucasians and Ethiopians (18%)[115] (*Table 5*).

In healthy subjects, carriers of the defective *CYP2C19* allele, are more likely to have an impaired antiplatelet activity.[14,107] Moreover, they have significantly lower levels of the active clopidogrel metabolite and diminished platelet inhibition.[14] The impact of *CYP2C19* loss-of-function alleles on clinical outcomes has been recently evaluated in several studies.[12–14,116–118] In the FAST-MI study,[12] we found that patients carrying any two *CYP2C19* loss-of-function alleles (**2, *3, *4, or *5*) had a higher rate of death, recurrent MI or stroke, than patients with none (21.5 vs. 13.3%; adjusted hazard ratio, 1.98; 95% CI 1.10–3.58). Among the 1535 patients who underwent PCI 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 1.71–7.51). In contrast, patients with one *CYP2C19* loss-of-function allele did not have an increased risk when compared with those who had no *CYP2C19* variant alleles. Accounting for the presence of *CYP2C19*17* had no significant effect on these risks. In FAST-MI registry, the loading dose of clopidogrel was 300 mg and the mean daily dose was 75 mg/day. In a German cohort of patients undergoing coronary stent placement after pre-treatment with 600 mg of clopidogrel, the risk of stent thrombosis at 30 days was increased in *CYP2C19*2* allele carriers (**1/*2* or **2/*2*) with the highest risk in patients with the *CYP2C19 *2/*2* genotype.[118] Among clopidogrel-treated subjects in TRITON–TIMI 38,[14] carriers of one or both *CYP2C19* loss-of-function alleles had increased risk of cardiovascular events when compared with non-carriers (HR = 1.53; 95% CI = 1.07–2.19) and stent thrombosis (2.6 vs. 0.8%; HR = 3.09; 95% CI 1.19–8.00). Unfortunately, the authors did not evaluate separately the impact of one or two variants alleles on outcome. This is an important issue considering the percentage of patients involved. Further studies are needed before drawing a definite conclusion of the range of patients at high risk of events.

Clopidogrel targets: GIIbIIa, P2Y12

Marked variations reported in the concentration of ADP required to produce irreversible aggregation have been reported suggesting a possible genetic determinant of the ADP effect on aggregation. The effect of ADP on platelets is mediated by two P2Y receptors, designated $P2Y_1$ and $P2Y_{12}$. Both are heterotrimeric G-protein coupled receptors: $P2Y_1$ to Gq and $P2Y_{12}$ to Gi. Stimulation at $P2Y_1$ leads to intracellular calcium mobilization and change in platelet shape,[119] whereas stimulation at $P2Y_{12}$ leads to inhibition

Table 3 Proton-pump inhibitors–antiplatelet agents drug–drug interaction studies

Study	Design	Subjects	*n*	Proton-pump inhibitor	Antiplatelet	Functional parameter and/or outcome	Follow-up	Result
Small *et al.* 2008[94]	Prospective study	Healthy volunteers	26	Lansoprazole 30 mg (6 days)	Clopidogrel 300 mg; prasugrel 60 mg	Inhibition platelet aggregation IPA	7 days	Lansoprazole + clopido: decrease IPA. Lansoprazole + prasugrel: no decrease
Gilard *et al.* 2006[92]	Observational study	Patients at high-risk coronary angioplasty.	105	Omeprazole	Clopidogrel (dose NA); + aspirin	VASP phosphorylation test Day 2	2 days	Higher VASP values in PPI users when compared with PPIs non-users
Gilard *et al.* 2008[91]	Prospective double blind, placebo, controlled, randomized OCLA study	Undergoing artery stent implantation	124	Omeprazole 20 mg/day or placebo	Clopidogrel (LD: 300 mg+MD: 75 mg/day) + aspirin	VASP phosphorylation test; Day 1; Day 7	7 days	Omeprazole decrease clopido inhibitory affect on platelet P2Y12
Pezalla *et al.* 2008[98]	Case–control study	Acute coronary syndrome	1010	All PPIs	N.A	Incidence of Acute MI	1 year	Acute MI rates higher in the high PPI exposure group
Sibbing *et al.* 2009[99]	Cross-sectional observational study previous coronary sten	CAD with previous PCI (median 7 months)	1000	PPI group $n = 268$; omeprazole $n = 64$; pantoprazole $n = 162$; esomeprazole $n = 42$	Clopidogrel (MD: 75 mg/day) + aspirin	Aggregometry test (Multiplate analyser)	7 months	Omeprazole associated with an attenuated platelet response; no effect with pantoprazole and esomeprazole
Siller *et al.* 2009[95]	Non-randomized study	CAD undergoing PCI	300	No PPI $n = 74$; PPI group $n = 226$; pantoprazole $n = 152$; esomeprazole $n = 74$	Clopidogrel (LD: 600 mg+MD: 75 mg/day) + aspirin (100 mg/day)	VASP; Aggregometry test (Multiplate analyser) MI	3 months	No effect
Juurlink *et al.* 2009[96]	Case–control retrospective study	Following acute myocardial infarction	13636; controls 2057	All PPIs	Clopidogrel (dose NA)	Risk of reinfarction	1 year	PPIs, other than pantoprazole, were associated with reduced beneficial effects of clopidogrel and an increased risk of reinfarction
Simon *et al.* 2009[12]	Cohort prospective	Following acute myocardial infarction	2208	PPI group $n = 1606$; omeprazole $n = 1147$	Clopidogrel (LD: 300 mg, MD: 75 mg/day) ± aspirin	Recurrence of events	1 year	No effect
Chen *et al.* 2009[151]	Randomized cross over trial	Healthy volunteers CYP2C19 genotype	12	Omeprazole 40 mg	Clopidogrel [LD: 300 mg, MD: 75 mg/day (3d)]	Pharmacokinetic	4 days	AUC of omeprazole increased by 30.02% in EMs. No change in PMs.
Ho *et al.*, 2009[97]	Cohort observational	Acute coronary syndrome	8205; PPI group 5244	All PPIs	Clopidogrel (dose NA)	All-cause mortality, rehospitalization for ACS	Median 521 days	Use of PPIs associated with an attenuation of the clopidogrel efficacy

LD, loading dose; MD, maintenance dose; NA, not available; PPI, proton-pump inhibitor.

Table 4 Genetic polymorphisms associated with platelet or antiplatelet drug responsiveness

Study	Design	Subjects or patients	*n*	Antiplatelet	Gene or allelic variants	Functional parameter and/or outcome	Effect outcome	Follow-up	Results
Fontana *et al.* 2003[152]	Prospective study	Healthy	98	No drug	*P2Y12*; *GPIIb/IIIa*	ADP-induced platelet aggregation	Pharmacodynamic	7 days	ADP-induced platelet aggregation is associated with a haplotype of P2Y12 receptor
Fontana *et al.* 2003[124]	Case–control	PAD	184	No drug	*P2Y12* $\alpha_{IIIb}\beta_3$PL$^{A1/A2}$ $\alpha_2\beta_1$	NA	Risk of PAD	ND	Role of H2 haplotype in atherosclerosis
Lau *et al.* 2004[153]	Prospective study	Healthy	25	Clopidogrel; LD: 450 mg		ADP-induced platelet aggregation (before and 5 days after stent)	Pharmacodynamic	5 days	Interindividual variability in platelet inhibition which correlates with CYP3A4 activity: contribution to the clopido resistance
		Healthy	10	Clopidogrel; MD: 75 mg (6 days) + rifampicin 300 mg × 2/day (4 days)		CYP3A4 activity measured by Erythromycin Breath TestADP induced platelet aggregation (before and 4 h after LD)		4 h	
		CAD	32	Clopidogrel LD: 300 mg; MD: 75 mg/day		ADP-induced platelet aggregation.		30 days	
Hetherington *et al.* 2005[123]	Prospective study	Subject with no history of CAD	200	No drug	*P2Y1*; *P2Y12*	ADP-induced platelet aggregation	Pharmacodynamic	NA	P2Y1 variant associated with platelet reactivity to ADP
Angiolillo *et al.* 2006[109]	Prospective study	Patients stable CAD	82	Aspirin + clopidogrel; MD: 75 mg/day); clopidogrel; LD: 300 mg	*CYP3A4*	ADP induced platelet aggregation; 2 h, 4 h after intake; ADP-induced platelet aggregation before 4 h, 24 h after LD	Pharmacodynamic		CYP3A4 IVS10+12G>A modulates platelet activation
		Naive patients scheduled coronary stenting	45						

Continued

Table 4 *Continued*

Study	Design	Subjects or patients	*n*	Antiplatelet	Gene or allelic variants	Functional parameter and/or outcome	Effect outcome	Follow-up	Results
Suh *et al.* 2006[110]	Prospective cohort	Healthy volunteers Koreans	32	Clopidogrel; LD: 300 mg; MD: 75 mg (6 days)	*CYP3A5*3*	ADP-induced platelet aggregation	Itraconazole interaction	6 day	CYP3A5 expressor: change in platelet aggregation greater
		Patients coronary angioplasty with stent	348				Pharmacodynamic atherothrombotic events	6 months	Atherotrombotic events occurred more frequently within 6 months after stent among CYP3A5 non-expressor
Hulot *et al.* 2006[107]	Prospective study	Healthy volunteers	28	Clopidogrel; MD: 75 mg/day (7 day)	*CYP2C19*; *CYP2B6*5*; *CYP1A2*1F*; *CYP3A5*3*	Platelet aggregation (5, 10 μmol/L ADP); VASP phosphorylation test	Pharmacodynamic	14 day	CYP2C19*2 is associated with a decrease in platelet responsiveness
Fontana *et al.* 2007[154]	Prospective study	Healthy volunteers	94	Clopidogrel; LD: 300 mg; MD: 75 mg/day (7 day)	*CY2C19*; *CYP3A4 (IVS10+12G>A)*	ADP-induced platelet aggregation (20 μmol/L ADP)	Pharmacodynamic	8 day	No association between CYP3A4 (IVS10+12G>A) and responsiveness; Association with CYP2C19*2
Giusti *et al.* 2007[155]	Prospective study	Patients acute coronary syndrome	1419	Clopidogrel; LD 600 mg+500 mg aspirin IV followed by 75 mg clopido +100 mg aspirin /day	*CYP2C19*; *CYP3A4/5*; *P2Y12*; *GpIa*; *GpIIIa*; *GpIb-alpha*; *GpVI*; *P-selectin*; *COX1/2*	Platelet aggregation (PRP: 2, 10 μmol/L ADP and AA); residual platelet; reactivity	Pharmacodynamic	24 h after PCI	CYP2C19*2 associated with a higher platelet aggregability and RPR in high-risk vascular
Brandt *et al.* 2007[112]	Prospective study	Healthy volunteers	74	Clopidogrel 300 mg	*CYP2C19*; *CYP1A2*; *CYP2B6*; *CYP3A4/5*	LTA (20 μmol/L ADP) 4 h after dose	Pharmacodynamic; pharmacokinetic	1 day	Loss-of-function alleles CYP2C19 and CYP2C9 decreased metabolite of Clopidogrel but not prasugrel. Decrease pharmacodynamics response for Clopidogrel

			71	Prasugrel 60 mg					
Kim *et al.* 2008[113]	Prospective study	Healthy volunteers	35	Clopidogrel; LD 300 mg; MD 75 mg (6 day); metabolite SR26334	*CYP3A5*	ADP induced platelet aggregation (8 day just before the daily MD); pharmacokinetic (24 h after LD)	Pharmacodynamic; pharmacokinetic	8 day	CYP3A5 did not substantialially affect pharmacokinetic and pharmacodynamics effect of clopidogrel
Trenk *et al.* 2008[118]	Prospective cohort	PCI	797	Clopidogrel; LD 600 mg; MD 75 mg; aspirin 100 mg/day for at least 5 days	*CYP2C19*2*	RPA (5 μmol/L ADP)	Clinical outcome: death, non-fatal MI	1 year	Carriers of at least one CYP2C19*2 allele are more prone to high RPA on poor clinical outcome after PCI
Geisler *et al.* 2008[15]	Prospective cohort	CAD	237	Clopidogrel; LD 600 mg	*CYP2C19*2*; *CYP2C19*3*; *CYP3A4*; *CYP3A5*	RPA (20 μmol/L ADP) 6 h after LD	Pharmacodynamic	6 h	Risk for higher RPA increased with one CYP2C9*2 allele (OR: 3.71) and 2 variant (OR:10.72)
Taubert *et al.* 2008[41]	Prospective	Patients CAD percutaneous coronary intervention	60	Clopidogrel 300 mg and 600 mg	*MDR1 C3435T*		Pharmacokinetic		Clopido absorption and thereby active metabolite formation are diminished by Pgp influenced by MDR1 genotype
Mega *et al.* 2009[14]	Prospective study	Healthy volunteers	162	Clopidogrel; LD 300 mg or 600 mg; MD 75 mg	*CYP2C19*; *CYP1A2*; *CYP2B6*; *CYP3A4/5*	LTA (20 μmol/L ADP) 4 h after dose	Pharmacodynamic pharmacokinetic	15 months	Reduced function CYP2C19 allele: lower levels of active metabolite; diminished platelet inhibition; higher rate of CV events, including stent thrombosis
		ACS with PCI	1477	Clopidogrel; LD 300 mg; MD 75 mg		CV events TIMI major and minor bleeding	Clinical outcome		
Simon *et al.* 2009[12]	Prospective cohort	Patients after AMI	2208	Clopidogrel; LD 300 mg; MD 75 mg	*CYP2C19*; *CYP3A5*; *P2Y12*; *ITGB3*; *MDR1 C3435T*	CV events	Clinical outcome	1 year	Carriers of at least one *CYP2C19*2* allele are higher risk bad outcome; TT 3435 bad outcome

Continued

Table 4 *Continued*

Study	Design	Subjects or patients	*n*	Antiplatelet	Gene or allelic variants	Functional parameter and/or outcome	Effect outcome	Follow-up	Results
Sibbing *et al.* 2009[116]	Prospective	Patients CAD undergoing coronary stent	2485	Clopidogrel; LD 600 mg; MD 75 mg	*CYP2C19**2	Stent thrombosis (ST)	Clinical outcome: cumulative incidence of definite ST	30 days	*CYP2C19**2 associated with an increased risk of ST following coronary stent placement
Collet *et al.*, 2009[13]	Prospective study	Patients (<45y) after AMI	259	Clopidogrel MD 75 mg	*CYP2C19*	CV events	Clinical outcome	1.07 year	*CYP2C19**2 major determinant in young patients
Mega *et al.* 2009[125]	Prospective study	Healthy volunteers	238	Prasugrel; LD 60 mg; MD 10 mg	*CYP2C19*; *CYP1A2*; *CYP2B6*; *CYP3A4/5*	LTA (20 μmol/L ADP) 4 h after dose	Pharmacodynamic; Pharmacokinetic	15 months	No effect on Pharmacodynamic pharmacokinetic response or clinical CV events rates in carriers vs. non-carriers of at least one loss function allele for any CYP
		Patients; acute coronay syndrome; TRITON TIMI 38	1466			CV events; TIMI major, and minor bleeding	Clinical outcome		

LTA, light transmittance aggregometry; PCI, percutaneous coronary intervention; RPR, residual platelet reactivity; VASP-P, vasodilator-stimulate phosphoprotein phosphorylation; LD, loading dose; MD, maintenance dose; NA, not available; MACE, major cardiovascular events; CV, cardiovascular events; TIMI, Thrombolysis In Myocardial Infarction.

Table 5 Allele frequencies of *CYP2C192 and *3 polymorphisms in various ethnic populations**

Population	Subject, *n*	*CYP2C19* *1	*2	*3	Study
Caucasians					
Caucasians, Germany	328	84	15.9	0.3	Aynacioglu *et al.*[156]
Caucasians, Italy	360	88.9	11.1	0	Scordo *et al.*[157]
Caucasians, Turkey	404	84	15.9	0.15	Aynacioglu *et al.*[156]
Caucasians, European-American	210	87	13	0	Ozawa *et al.*;[158] Goldstein *et al.*[159]
Caucasians, European-American	546	86.4	12.7	0.9	Luo *et al.*[160]
Non-oriental					
African American	216	75	25	0	Goldstein *et al.*[159]
African American	472	81	18.2	0.8	Luo *et al.*[160]
Bolivian	778	92.2	7.8	0.1	Bravo-Villalta *et al.*[161]
Ethiopian	114	86.4	13.6	0	Persson *et al.*[162]
Mexican Americans	692	90.2	9.7	0.1	Luo *et al.*[160]
Palestinian	200	91.3	5.8	3	Sameer *et al.*[163]
Saudi Arabian	194	85	15	0	Ozawa *et al.*[158]
Native Canadian Indians	115	80.9	19.1	0	Nowak *et al.*[164]
Asians					
Burmese	127	66	30	4	Tassaneeyakul *et al.*[165]
Chinese	27	50.0	45.5	4.5	Yamada *et al.*[166]
Chinese Han	400	69.73	24.67	3.27	Chen *et al.*[167]
Filipinos	104	54	39	7	Goldstein *et al.*[159]
Iranian	400	86	14	0	Zand *et al.*[168]
Indian-North	200	70	30	0	Lamba *et al.*[169]
Indian-Tamilian	112	60	38	2	Adithan *et al.*[170]
Japanese	30	61.8	27.4	10.8	Takakubo *et al.*[171]
Japanese	106	67	23	10	Ozawa *et al.*[158]
Korean	206	67.5	20.9	11.6	Herrlin *et al.*[173]
Korean	377	64.2	28.3	7.6	Lee *et al.*[172]
Thai	774	68	29	3	Tassaneeyakul *et al.*[165]
Southeast Asians	160	63.1	31.2	5.7	Luo *et al.*[160]
Vietnamese	165	68.8	26.4	4.9	Lee *et al.*[172]
Vietnamese	90	62	24	14	Yamada SJ *et al.*[166]

of adenyl cyclase[120] and activation of phosphoinositide 3 kinase.[121] The net effect is the modulation affinity of the glycoprotein Iib–IIIa (GPIIb–IIIa).[122]

Among different genetic polymorphisms observed in Caucasians with no history of coronary heart disease and no antiplatelet medication, *$P2Y_1A1622G$* polymorphism was found to have a significant association with platelet response to ADP, as defined by the binding of fibrinogen to activate GPIIb–IIa.[123] For $P2Y_{12,}$ some genetic polymorphisms defined as the haplotype H2 has been found to be strongly associated with increased ADP-induced platelet aggregation in healthy volunteers.[124] Most studies have evaluated the impact of the pharmacological parameters of clopidogrel on biological platelet function. Their clinical impact was not confirmed in FAST-MI registry, the single study to date evaluating this hypothesis in AMI patients.[12] These clinical data outline again the fact that results should be viewed with caution and considered exploratory findings that need to be replicated.

Prasugrel is a novel and potent thienopyridine that targets the same $P2Y_{12}$ ADP receptor as clopidogrel. Unlike clopidogrel, conversion of prasugrel to its active metabolite involves rapid hydrolysis by esterases followed by a single CYP-dependent step. Prasugrel is absorbed rapidly after dosing with concentrations of its active metabolite peaking ~30 min after dosing. On a molar basis, the active metabolites of clopidogrel and prasugrel are equipotent platelet inhibitors.[94] Interestingly, the pathway leading to the conversion of prasugrel and clopidogrel to their respective active metabolites differs. Prasugrel is rapidly hydrolysed by esterases to an inactive thiolactone, which is then metabolized by CYPs to the active metabolite. The responsible enzymes appear to be CYP3A4 and CYP2B6 and to a lesser extent, CYP2C9 and CYP2C19.[52]

CYP2C19 genetic polymorphisms do not affect prasugrel pharmacodynamics and pharmacokinetic parameters in healthy subjects[112] (*Table 4*). Moreover, similar rates of cardiovascular events were observed in TRITON-TIMI 38 trial among ACS

patients who were carriers and non-carriers of a *CYP2C19* loss-of-function allele, treated with prasugrel.[125]

Surprisingly, in a study with healthy subjects, lansoprazole slightly reduced the plasma level of prasugrel active metabolite without affecting the inhibition of platelet aggregation.[94] A single loading dose of prasugrel 60 mg associated with or without lansoprazole 30 mg was used in this study, with a 7-day run-in period of IPP prior to receiving the loading dose (*Table 3*). Therefore this result should be taken cautiously and needs confirmation with longer exposure and follow-up.

Other novel antiplatelets with promising and less dependent on hepatic metabolism, are still in development with currently ongoing clinical trials.[126]

Perspective of personalized medicine in antiplatelet therapies

Observational studies dating back the late 1940s onwards have unravelled the key factors that influence risk for CVD such as cigarette smoking, cholesterol levels, diabetes, and blood pressure. In addition, progress in the treatment of cardiovascular disease relates in part to greater knowledge of platelet function and the benefits of antiplatelets drugs.

However the extent of variability in response to antiplatelet drugs is proving to be a clinical problem. This is further compromised by the lack of an assay with a sufficient accuracy and predictive value in terms of platelet aggregation and clinical outcome. The promising P2Y12 assay (VerifyNow, Accumetrics Inc.) has a positive predicted value of 12% to detect ACS patients at risk of 12 month cardiovascular events (*Table 1*).[127] Thus the majority of patients with a positive test will not experience an ischaemic event. The results of the ongoing studies, such as GRAVITAS[128] will help to examine whether tailored clopidogrel therapy, using a point-of-care platelet function assay, may reduce major adverse cardiovascular events after PCI.

Interestingly, genetic variations in the pathways which govern drug metabolizing enzymes are proving to be quite relevant to clopidogrel antiplatelet therapy. Indeed genetic testing could be a new tool for identifying patients at higher risk of events.

Identification of patients at 'higher or lower risk of poor clopidogrel responsiveness' defined as carriers or non-carriers of *CYP2C19* loss-of-function alleles may help to better optimize the choice of the antiplatelet drug. As an example, for the treatment of peptic ulcer disease, clinical pharmacologists have already begun modelling the economic utility of *CYP2C19* genotyping prior to prescribing PPIs. Considering a maximum treatment duration of 3 months and an estimated genotyping cost of 10 USD per allele, investigators projected a cost saving of >5000 USD per 100 Asian patients genotyped. Due to ethnic variation in allele frequency, cost saving was lower in other populations[129] but remained significant in patients of European descent.[130] Therefore it is probable by extrapolation that in ACS patients who undergo a PCI targeting antiplatelet treatment by genotyping would probably be a cost-effective strategy. The higher benefit/risk ratio of prasugrel seems to be particularly relevant in those patients at 'higher risk of clopidogrel poor response' but is not conclusive for those patients who do not carry any CYP2C19 loss-of-function allele. In TRITON-TIMI 38, the rate of cardiovascular events was 9.8% for *CYP2C19* non-carriers in the prasugrel group[125] and 8.5% in those receiving clopidogrel during the trial follow-up.[14] Thus although there was no planned head-to-head comparison with regard to genotype data, current available results suggest that clopidogrel (300 mg LD and 75 mg thereafter) may remain the drug of choice in terms of benefit/risk and benefit/cost ratios among those homozygous *CYP2C19* wild-type patients representing the majority of treated patients. In contrast, in those patients carrying the two loss-of-function variant alleles of *CYP2C19*, prasugrel may be preferred over clopidogrel.

However, the positive predictive value of *CYP2C19* loss-of-function genetic variants is not optimal particularly among heterozygous subjects. Further studies are necessary for evaluating whether combining laboratory assay and genotyping may enhance the predictability of clopidogrel non-responsiveness among heterozygous patients.

The comparison of the effects of prasugrel and clopidogrel among heterozygous *CYP2C19* loss-of-function patients were not shown in TRITON-TIMI 38,[14,125] whereas in FAST-MI, this population receiving clopidogrel were not at higher risk of events compared with homozygous wild-type patients.[12] Therefore, among heterozygous patients, the use of prasugrel or a higher dose of clopidogrel should be discussed on an individual basis with regard to the benefit/bleeding-risk ratio. Larger prospective randomized clinical trials are needed to confirm these hypotheses.

Conclusion

Great hope has been expressed towards the development of personalized medical care strategies in terms of appropriate diagnosis, treatment, and CVD prevention. The issue of validated point-of-care testing and their ability to predict clinical outcomes remains unresolved for antiplatelet drugs. Recent research findings highlight the role of genetic variation as an important variable for optimizing the response to antiplatelet drugs such as clopidogrel. The goal of personalized medicine is to utilize in part the person's genetic makeup for selecting the best drug and dose. In addition, this approach should also include the impact of important non-genetic factors, such as the clinical status of the patient, the environmental factors including diet, and drug–drug interactions.

Funding

This work was supported in part by the Academic Medical Organization of Southwestern Ontario AHSC AFP Innovation Fund (RBK) and Post Doctorat 2007 Leem Research Fund, France (CV).

Conflict of interest: T.S. has received consulting fees from Bayer-Schering, Pfizer, sanofi-aventis, and Eli Lilly, lecture fees from Bayer-Schering, and grant support from Pfizer and Servier.

References

1. Vogel F. Future problems of medical genetics. *Bull Schweiz Akad Med Wiss* 1978; **34**:411–423.
2. Meyer UA. Pharmacogenetics—five decades of therapeutic lessons from genetic diversity. *Nat Rev Genet* 2004;**5**:669–676.

3. Siest G, Marteau JB, Maumus S, Berrahmoune H, Jeannesson E, Samara A, Batt AM, Visvikis-Siest S. Pharmacogenomics and cardiovascular drugs: need for integrated biological system with phenotypes and proteomic markers. *Eur J Pharmacol* 2005;**527**:1–22.
4. Angiolillo DJ, Guzman LA. Clinical overview of promising nonthienopyridine antiplatelet agents. *Am Heart J* 2008;**156**:S23–S28.
5. Bhatt DL, Topol EJ. Scientific and therapeutic advances in antiplatelet therapy. *Nat Rev Drug Discov* 2003;**2**:15–28.
6. Barrett NE, Holbrook L, Jones S, Kaiser WJ, Moraes LA, Rana R, Sage T, Stanley RG, Tucker KL, Wright B, Gibbins JM. Future innovations in anti-platelet therapies. *Br J Pharmacol* 2008;**154**:918–939.
7. Bhatt DL, Topol EJ. Antiplatelet and anticoagulant therapy in the secondary prevention of ischemic heart disease. *Med Clin North Am* 2000;**84**:163–179, ix.
8. Jackson SP, Schoenwaelder SM. Antiplatelet therapy: in search of the 'magic bullet'. *Nat Rev Drug Discov* 2003;**2**:775–789.
9. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J Am Coll Cardiol* 2005;**45**:246–251.
10. Muller I, Besta F, Schulz C, Massberg S, Schonig A, Gawaz M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost* 2003;**89**:783–787.
11. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M, Banuelos C, Hernandez-Antolin R, Escaned J, Moreno R, Alfonso F, Macaya C. High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability. *Eur Heart J* 2004;**25**:1903–1910.
12. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N, Becquemont L. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009;**360**:363–375.
13. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, Payot L, Brugier D, Cayla G, Beygui F, Bensimon G, Funck-Brentano C, Montalescot G. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* 2009;**373**:309–317.
14. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009;**360**:354–362.
15. Geisler T, Schaeffeler E, Dippon J, Winter S, Buse V, Bischofs C, Zuern C, Moerike K, Gawaz M, Schwab M. CYP2C19 and nongenetic factors predict poor responsiveness to clopidogrel loading dose after coronary stent implantation. *Pharmacogenomics* 2008;**9**:1251–1259.
16. Maree AO, Jneid H, Fitzgerald DJ. Aspirin resistance and atherothrombotic disease. *J Am Coll Cardiol* 2006;**48**:846–847; author reply 847.
17. Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy–I. Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ* 1994;**308**:81–106.
18. Gum PA, Kottke-Marchant K, Poggio ED, Gurm H, Welsh PA, Brooks L, Sapp SK, Topol EJ. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. *Am J Cardiol* 2001;**88**:230–235.
19. Zimmermann N, Wenk A, Kim U, Kienzle P, Weber AA, Gams E, Schror K, Hohlfeld T. Functional and biochemical evaluation of platelet aspirin resistance after coronary artery bypass surgery. *Circulation* 2003;**108**:542–547.
20. Michelson AD, Furman MI, Goldschmidt-Clermont P, Mascelli MA, Hendrix C, Coleman L, Hamlington J, Barnard MR, Kickler T, Christie DJ, Kundu S, Bray PF. Platelet GP IIIa Pl(A) polymorphisms display different sensitivities to agonists. *Circulation* 2000;**101**:1013–1018.
21. Andrioli G, Minuz P, Solero P, Pincelli S, Ortolani R, Lussignoli S, Bellavite P. Defective platelet response to arachidonic acid and thromboxane A(2) in subjects with Pl(A2) polymorphism of beta(3) subunit (glycoprotein IIIa). *Br J Haematol* 2000;**110**:911–918.
22. Szczeklik A, Undas A, Sanak M, Frolow M, Wegrzyn W. Relationship between bleeding time, aspirin and the PlA1/A2 polymorphism of platelet glycoprotein IIIa. *Br J Haematol* 2000;**110**:965–967.
23. Undas A, Brummel K, Musial J, Mann KG, Szczeklik A. Pl(A2) polymorphism of beta(3) integrins is associated with enhanced thrombin generation and impaired antithrombotic action of aspirin at the site of microvascular injury. *Circulation* 2001;**104**:2666–2672.
24. Ridker PM, Hennekens CH, Schmitz C, Stampfer MJ, Lindpaintner K. PIA1/A2 polymorphism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke, and venous thrombosis. *Lancet* 1997;**349**:385–388.
25. Guthikonda S, Lev EI, Patel R, DeLao T, Bergeron AL, Dong JF, Kleiman NS. Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. *J Thromb Haemost* 2007;**5**:490–496.
26. Weber AA, Przytulski B, Schumacher M, Zimmermann N, Gams E, Hohlfeld T, Schror K. Flow cytometry analysis of platelet cyclooxygenase-2 expression: induction of platelet cyclooxygenase-2 in patients undergoing coronary artery bypass grafting. *Br J Haematol* 2002;**117**:424–426.
27. Weber AA, Zimmermann KC, Meyer-Kirchrath J, Schror K. Cyclooxygenase-2 in human platelets as a possible factor in aspirin resistance. *Lancet* 1999;**353**:900.
28. Phillips DR, Conley PB, Sinha U, Andre P. Therapeutic approaches in arterial thrombosis. *J Thromb Haemost* 2005;**3**:1577–1589.
29. Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med* 2007;**357**:2482–2494.
30. Furie B, Furie BC. Mechanisms of thrombus formation. *N Engl J Med* 2008;**359**:938–949.
31. Hagihara K, Nishiya Y, Kurihara A, Kazui M, Farid NA, Ikeda T. Comparison of human cytochrome p450 inhibition by the thienopyridines prasugrel, clopidogrel, and ticlopidine. *Drug Metab Pharmacokinet* 2008;**23**:412–420.
32. Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med* 2005;**352**:2211–2221.
33. Hass WK, Easton JD, Adams HP Jr, Pryse-Phillips W, Molony BA, Anderson S, Kamm B. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. Ticlopidine Aspirin Stroke Study Group. *N Engl J Med* 1989;**321**:501–507.
34. Gent M, Blakely JA, Easton JD, Ellis DJ, Hachinski VC, Harbison JW, Panak E, Roberts RS, Sicurella J, Turpie AG. The Canadian American Ticlopidine Study (CATS) in thromboembolic stroke. *Lancet* 1989;**1**:1215–1220.
35. Steinhubl SR, Berger PB, Mann JT III, Fry ET, DeLago A, Wilmer C, Topol EJ. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA* 2002;**288**:2411–2420.
36. Savi P, Herbert JM, Pflieger AM, Dol F, Delebassee D, Combalbert J, Defreyn G, Maffrand JP. Importance of hepatic metabolism in the antiaggregating activity of the thienopyridine clopidogrel. *Biochem Pharmacol* 1992;**44**:527–532.
37. 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**:53–59.
38. Savi P, Combalbert J, Gaich C, Rouchon MC, Maffrand JP, Berger Y, Herbert JM. The antiaggregating activity of clopidogrel is due to a metabolic activation by the hepatic cytochrome P450–1A. *Thromb Haemost* 1994;**72**:313–317.
39. Pereillo JM, Maftouh M, Andrieu A, Uzabiaga MF, Fedeli O, Savi P, Pascal M, Herbert JM, Maffrand JP, Picard C. Structure and stereochemistry of the active metabolite of clopidogrel. *Drug Metab Dispos* 2002;**30**:1288–1295.
40. Denninger MH, Necciari J, Serre-Lacroix E, Sissmann J. Clopidogrel antiplatelet activity is independent of age and presence of atherosclerosis. *Semin Thromb Hemost* 1999;**25**(Suppl. 2):41–45.
41. Taubert D, von Beckerath N, Grimberg G, Lazar A, Jung N, Goeser T, Kastrati A, Schomig A, Schomig E. Impact of P-glycoprotein on clopidogrel absorption. *Clin Pharmacol Ther* 2006;**80**:486–501.
42. Taubert D, Kastrati A, Harlfinger S, Gorchakova O, Lazar A, von Beckerath N, Schomig A, Schomig E. Pharmacokinetics of clopidogrel after administration of a high loading dose. *Thromb Haemost* 2004;**92**:311–316.
43. CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet* 1996;**348**:1329–1339.
44. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. 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.
45. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, Malmberg K, Rupprecht H, Zhao F, Chrolavicius S, Copland I, Fox KA. 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**:527–533.
46. Sabatine MS, Cannon CP, Gibson CM, Lopez-Sendon JL, Montalescot G, Theroux P, Lewis BS, Murphy SA, McCabe CH, Braunwald E. Effect of clopidogrel pretreatment before percutaneous coronary intervention in patients with ST-elevation myocardial infarction treated with fibrinolytics: the PCI-CLARITY study. *JAMA* 2005;**294**:1224–1232.
47. Sabatine MS, Cannon CP, Gibson CM, Lopez-Sendon JL, Montalescot G, Theroux P, Claeys MJ, Cools F, Hill KA, Skene AM, McCabe CH, Braunwald E. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med* 2005;**352**:1179–1189.
48. Chen ZM, Jiang LX, Chen YP, Xie JX, Pan HC, Peto R, Collins R, Liu LS. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet* 2005;**366**:1607–1621.
49. Bennett CL, Connors JM, Carwile JM, Moake JL, Bell WR, Tarantolo SR, McCarthy LJ, Sarode R, Hatfield AJ, Feldman MD, Davidson CJ, Tsai HM. Thrombotic thrombocytopenic purpura associated with clopidogrel. *N Engl J Med* 2000;**342**:1773–1777.
50. Elmi F, Peacock T, Schiavone J. Isolated profound thrombocytopenia associated with clopidogrel. *J Invasive Cardiol* 2000;**12**:532–535.

51. Cadroy Y, Bossavy JP, Thalamas C, Sagnard L, Sakariassen K, Boneu B. Early potent antithrombotic effect with combined aspirin and a loading dose of clopidogrel on experimental arterial thrombogenesis in humans. *Circulation* 2000;**101**: 2823–2828.
52. Rehmel JL, Eckstein JA, Farid NA, Heim JB, Kasper SC, Kurihara A, Wrighton SA, Ring BJ. Interactions of two major metabolites of prasugrel, a thienopyridine antiplatelet agent, with the cytochromes P450. *Drug Metab Dispos* 2006;**34**: 600–607.
53. Wallentin L, Varenhorst C, James S, Erlinge D, Braun OO, Jakubowski JA, Sugidachi A, Winters KJ, Siegbahn A. Prasugrel achieves greater and faster P2Y12receptor-mediated platelet inhibition than clopidogrel due to more efficient generation of its active metabolite in aspirin-treated patients with coronary artery disease. *Eur Heart J* 2008;**29**:21–30.
54. Payne CD, Li YG, Small DS, Ernest CS II, Farid NA, Jakubowski JA, Brandt JT, Salazar DE, Winters KJ. Increased active metabolite formation explains the greater platelet inhibition with prasugrel compared to high-dose clopidogrel. *J Cardiovasc Pharmacol* 2007;**50**:555–562.
55. Niitsu Y, Jakubowski JA, Sugidachi A, Asai F. Pharmacology of CS-747 (prasugrel, LY640315), a novel, potent antiplatelet agent with in vivo P2Y12 receptor antagonist activity. *Semin Thromb Hemost* 2005;**31**:184–194.
56. Herbert JM, Dol F, Bernat A, Falotico R, Lale A, Savi P. The antiaggregating and antithrombotic activity of clopidogrel is potentiated by aspirin in several experimental models in the rabbit. *Thromb Haemost* 1998;**80**:512–518.
57. Makkar RR, Eigler NL, Kaul S, Frimerman A, Nakamura M, Shah PK, Forrester JS, Herbert JM, Litvack F. Effects of clopidogrel, aspirin and combined therapy in a porcine ex vivo model of high-shear induced stent thrombosis. *Eur Heart J* 1998; **19**:1538–1546.
58. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003;**107**:2908–2913.
59. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Barrera-Ramirez C, Sabate M, Hernandez R, Moreno R, Escaned J, Alfonso F, Banuelos C, Costa MA, Bass TA, Macaya C. Identification of low responders to a 300-mg clopidogrel loading dose in patients undergoing coronary stenting. *Thromb Res* 2005; **115**:101–108.
60. Snoep JD, Hovens MM, Eikenboom JC, van der Bom JG, Jukema JW, Huisman MV. Clopidogrel nonresponsiveness in patients undergoing percutaneous coronary intervention with stenting: a systematic review and meta-analysis. *Am Heart J* 2007;**154**:221–231.
61. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, Costa MA. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol* 2007;**49**: 1505–1516.
62. Angiolillo DJ. Antiplatelet therapy in diabetes: efficacy and limitations of current treatment strategies and future directions. *Diabetes Care* 2009;**32**:531–540.
63. Angiolillo DJ. Variability in responsiveness to oral antiplatelet therapy. *Am J Cardiol* 2009;**103**:27A–34A.
64. Li XQ, Andersson TB, Ahlstrom M, Weidolf L. Comparison of inhibitory effects of the proton-pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos* 2004;**32**:821–827.
65. 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.
66. Gladding P, Webster M, Zeng I, Farrel H, Stewart J, Ruygrok P, Ormiston J, El-Jack S, Armstrong G, Kay P, Scott D, Gunes A, Dahl ML. The antiplatelet effect of higher loading and maintenance dose regimens of clopidogrel: the PRINC (Plavix Response in Coronary Intervention) trial. *J Am Coll Cardiol Intv* 2008;**1**:612–619.
67. Aleil B, Jacquemin L, De Poli F, Zaehringer M, Collet J, Montalescot G, Cazenave J, Dickele M, Monassier J, Gachet C. Clopidogrel 150 mg/day to overcome low responsiveness in patients undergoing elective percutaneous coronary intervention: results from the VASP-02 (Vasodilator-Stimulated Phosphoprotein-02) randomized study. *J Am Coll Cardiol Intv* 2008;**1**:631–638.
68. Montalescot G, Sideris G, Meuleman C, Bal-dit-Sollier C, Lellouche N, Steg PG, Slama M, Milleron O, Collet JP, Henry P, Beygui F, Drouet L. A randomized comparison of high clopidogrel loading doses in patients with non-ST-segment elevation acute coronary syndromes: the ALBION (Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation and Ongoing Necrosis) trial. *J Am Coll Cardiol* 2006;**48**:931–938.
69. von Beckerath N, Taubert D, Pogatsa-Murray G, Schomig E, Kastrati A, Schomig A. Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) Trial. *Circulation* 2005;**112**:2946–2950.
70. Bonello L, Camoin-Jau L, Armero S, Com O, Arques S, Burignat-Bonello C, Giacomoni MP, Bonello R, Collet F, Rossi P, Barragan P, Dignat-George F, Paganelli F. Tailored clopidogrel loading dose according to platelet reactivity monitoring to prevent acute and subacute stent thrombosis. *Am J Cardiol* 2009;**103**:5–10.
71. Mehta SR, Bassand JP, Chrolavicius S, Diaz R, Fox KA, Granger CB, Jolly S, Rupprecht HJ, Widimsky P, Yusuf S. Design and rationale of CURRENT-OASIS 7: a randomized, 2x2 factorial trial evaluating optimal dosing strategies for clopidogrel and aspirin in patients with ST and non-ST-elevation acute coronary syndromes managed with an early invasive strategy. *Am Heart J* 2008;**156**: 1080–1088, e1081.
72. Yong G, Rankin J, Ferguson L, Thom J, French J, Brieger D, Chew DP, Dick R, Eccleston D, Hockings B, Walters D, Whelan A, Eikelboom JW. Randomized trial comparing 600- with 300-mg loading dose of clopidogrel in patients with non-ST elevation acute coronary syndrome undergoing percutaneous coronary intervention: results of the Platelet Responsiveness to Aspirin and Clopidogrel and Troponin Increment after Coronary intervention in Acute coronary Lesions (PRACTICAL) Trial. *Am Heart J* 2009;**157**:60, e61–69.
73. Kurihara A, Hagihara K, Kazui M. In vitro metabolism of antiplatelet agent clopidogrel: cytochrome P450 isoforms responsible for two oxidation steps involved in the active metabolite formation. *Drug Metab Rev* 2005;**37**(Suppl. 2):99.
74. Farid NA, Payne CD, Small DS, Winters KJ, Ernest CS II, Brandt JT, Darstein C, Jakubowski JA, Salazar DE. Cytochrome P450 3A inhibition by ketoconazole affects prasugrel and clopidogrel pharmacokinetics and pharmacodynamics differently. *Clin Pharmacol Ther* 2007;**81**:735–741.
75. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001;**27**:383–391.
76. Lee SJ, Usmani KA, Chanas B, Ghanayem B, Xi T, Hodgson E, Mohrenweiser HW, Goldstein JA. Genetic findings and functional studies of human CYP3A5 single nucleotide polymorphisms in different ethnic groups. *Pharmacogenetics* 2003;**13**:461–472.
77. Neubauer H, Gunesdogan B, Hanefeld C, Spiecker M, Mugge A. Lipophilic statins interfere with the inhibitory effects of clopidogrel on platelet function–a flow cytometry study. *Eur Heart J* 2003;**24**:1744–1749.
78. Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, Tait AR, Carville DG, Guyer KE, Bates ER. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. *Circulation* 2003;**107**: 32–37.
79. Piorkowski M, Weikert U, Schwimmbeck PL, Martus P, Schultheiss HP, Rauch U. ADP induced platelet degranulation in healthy individuals is reduced by clopidogrel after pretreatment with atorvastatin. *Thromb Haemost* 2004;**92**:614–620.
80. Muller I, Besta F, Schulz C, Li Z, Massberg S, Gawaz M. Effects of statins on platelet inhibition by a high loading dose of clopidogrel. *Circulation* 2003;**108**: 2195–2197.
81. Mitsios JV, Papathanasiou AI, Rodis FI, Elisaf M, Goudevenos JA, Tselepis AD. Atorvastatin does not affect the antiplatelet potency of clopidogrel when it is administered concomitantly for 5 weeks in patients with acute coronary syndromes. *Circulation* 2004;**109**:1335–1338.
82. Gorchakova O, von Beckerath N, Gawaz M, Mocz A, Joost A, Schomig A, Kastrati A. Antiplatelet effects of a 600 mg loading dose of clopidogrel are not attenuated in patients receiving atorvastatin or simvastatin for at least 4 weeks prior to coronary artery stenting. *Eur Heart J* 2004;**25**:1898–1902.
83. Serebruany VL, Midei MG, Malinin AI, Oshrine BR, Lowry DR, Sane DC, Tanguay JF, Steinhubl SR, Berger PB, O'Connor CM, Hennekens CH. Absence of interaction between atorvastatin or other statins and clopidogrel: results from the interaction study. *Arch Intern Med* 2004;**164**:2051–2057.
84. Trenk D, Hochholzer W, Frundi D, Stratz C, Valina CM, Bestehorn HP, Buttner HJ, Neumann FJ. Impact of cytochrome P450 3A4-metabolized statins on the antiplatelet effect of a 600-mg loading dose clopidogrel and on clinical outcome in patients undergoing elective coronary stent placement. *Thromb Haemost* 2008;**99**:174–181.
85. Saw J, Brennan DM, Steinhubl SR, Bhatt DL, Mak KH, Fox K, Topol EJ. Lack of evidence of a clopidogrel-statin interaction in the CHARISMA trial. *J Am Coll Cardiol* 2007;**50**:291–295.
86. Saw J, Steinhubl SR, Berger PB, Kereiakes DJ, Serebruany VL, Brennan D, Topol EJ. Lack of adverse clopidogrel-atorvastatin clinical interaction from secondary analysis of a randomized, placebo-controlled clopidogrel trial. *Circulation* 2003;**108**:921–924.
87. Serebruany VL, Steinhubl SR, Hennekens CH. Are antiplatelet effects of clopidogrel inhibited by atorvastatin? A research question formulated but not yet adequately tested. *Circulation* 2003;**107**:1568–1569.

88. Bhatt DL, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD, Johnson DA, Mahaffey KW, Quigley EM. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. *Circulation* 2008;**118**:1894–1909.
89. Robinson M, Horn J. Clinical pharmacology of proton pump inhibitors: what the practising physician needs to know. *Drugs* 2003;**63**:2739–2754.
90. Furuta T, Ohashi K, Kamata T, Takashima M, Kosuge K, Kawasaki T, Hanai H, Kubota T, Ishizaki T, Kaneko E. Effect of genetic differences in omeprazole metabolism on cure rates for Helicobacter pylori infection and peptic ulcer. *Ann Intern Med* 1998;**129**:1027–1030.
91. Gilard M, Arnaud B, Cornily JC, Le Gal G, Lacut K, Le Calvez G, Mansourati J, Mottier D, Abgrall JF, Boschat J. 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**:256–260.
92. Gilard M, Arnaud B, Le Gal G, Abgrall JF, Boschat J. Influence of omeprazol on the antiplatelet action of clopidogrel associated to aspirin. *J Thromb Haemost* 2006;**4**:2508–2509.
93. Barragan P, Bouvier JL, Roquebert PO, Macaluso G, Commeau P, Comet B, Lafont A, Camoin L, Walter U, Eigenthaler M. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. *Catheter Cardiovasc Interv* 2003;**59**:295–302.
94. Small DS, Farid NA, Payne CD, Weerakkody GJ, Li YG, Brandt JT, Salazar DE, Winters KJ. Effects of the proton pump inhibitor lansoprazole on the pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel. *J Clin Pharmacol* 2008;**48**:475–484.
95. Siller-Matula JM, Spiel AO, Lang IM, Kreiner G, Christ G, Jilma B. Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel. *Am Heart J* 2009;**157**:148, e141–145.
96. Juurlink DN, Gomes T, Ko DT, Szmitko PE, Austin PC, Tu JV, Henry DA, Kopp A, Mamdani MM. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. *CMAJ* 2009;**180**:713–718.
97. Ho PM, Maddox TM, Wang L, Fihn SD, Jesse RL, Peterson ED, Rumsfeld JS. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. *JAMA* 2009;**301**:937–944.
98. Pezalla E, Day D, Pulliadath I. Initial assessment of clinical impact of a drug interaction between clopidogrel and proton pump inhibitors. *J Am Coll Cardiol* 2008;**52**:1038–1039, author reply 1039.
99. Sibbing D, Morath T, Stegherr J, Braun S, Vogt W, Hadamitzky M, Schomig A, Kastrati A, von Beckerath N. Impact of proton pump inhibitors on the antiplatelet effects of clopidogrel. *Thromb Haemost* 2009;**101**:714–719.
100. Stanek E. Society of Cardiovascular Angiography and Interventions. SCAI Statement on 'A National Study of the Effect of Individual Proton Pump Unhibitors on Cardiovascular outcomes in Patients Treated with Clopidogrel Following Coronary Stenting: The Clopidogrel Medco Outcomes Study'. http://wwwscaiorg/drlt1aspx?PAGE_ID_5870 (May 2009).
101. Lai KC, Lam SK, Chu KM, Wong BC, Hui WM, Hu WH, Lau GK, Wong WM, Yuen MF, Chan AO, Lai CL, Wong J. Lansoprazole for the prevention of recurrences of ulcer complications from long-term low-dose aspirin use. *N Engl J Med* 2002;**346**:2033–2038.
102. Schwartz KA, Schwartz DE, Ghosheh K, Reeves MJ, Barber K, DeFranco A. Compliance as a critical consideration in patients who appear to be resistant to aspirin after healing of myocardial infarction. *Am J Cardiol* 2005;**95**:973–975.
103. Hankey GJ, Eikelboom JW. Aspirin resistance. *Lancet* 2006;**367**:606–617.
104. Maree AO, Fitzgerald DJ. Variable platelet response to aspirin and clopidogrel in atherothrombotic disease. *Circulation* 2007;**115**:2196–2207.
105. Halushka MK, Walker LP, Halushka PV. Genetic variation in cyclooxygenase 1: effects on response to aspirin. *Clin Pharmacol Ther* 2003;**73**:122–130.
106. Maree AO, Curtin RJ, Chubb A, Dolan C, Cox D, O'Brien J, Crean P, Shields DC, Fitzgerald DJ. Cyclooxygenase-1 haplotype modulates platelet response to aspirin. *J Thromb Haemost* 2005;**3**:2340–2345.
107. Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, Aiach M, Lechat P, Gaussem P. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood* 2006;**108**:2244–2247.
108. Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* 2002;**54**:1271–1294.
109. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Cavallari U, Trabetti E, Sabate M, Hernandez R, Moreno R, Escaned J, Alfonso F, Banuelos C, Costa MA, Bass TA, Pignatti PF, Macaya C. 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**:1895–1900.
110. Suh JW, Koo BK, Zhang SY, Park KW, Cho JY, Jang IJ, Lee DS, Sohn DW, Lee MM, Kim HS. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. *CMAJ* 2006;**174**:1715–1722.
111. Smith SM, Judge HM, Peters G, Armstrong M, Fontana P, Gaussem P, Daly ME, Storey RF. Common sequence variations in the P2Y12 and CYP3A5 genes do not explain the variability in the inhibitory effects of clopidogrel therapy. *Platelets* 2006;**17**:250–258.
112. Brandt JT, Close SL, Iturria SJ, Payne CD, Farid NA, Ernest CS II, Lachno DR, Salazar D, Winters KJ. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. *J Thromb Haemost* 2007;**5**:2429–2436.
113. Kim KA, Park PW, Park JY. Effect of CYP3A5*3 genotype on the pharmacokinetics and antiplatelet effect of clopidogrel in healthy subjects. *Eur J Clin Pharmacol* 2008;**64**:589–597.
114. Wedlund PJ. The CYP2C19 enzyme polymorphism. *Pharmacology* 2000;**61**:174–183.
115. Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M. 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**:103–113.
116. Sibbing D, Stegherr J, Latz W, Koch W, Mehilli J, Dorrler K, Morath T, Schomig A, Kastrati A, von Beckerath N. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur Heart J* 2009;**30**:916–922.
117. Giusti B, Gori AM, Marcucci R, Saracini C, Sestini I, Paniccia R, Buonamici P, Antoniucci D, Abbate R, Gensini GF. Relation of cytochrome P450 2C19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis. *Am J Cardiol* 2009;**103**:806–811.
118. Trenk D, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, Stratz C, Schmiebusch P, Bestehorn HP, Buttner HJ, Neumann FJ. 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**:1925–1934.
119. Jin J, Daniel JL, Kunapuli SP. Molecular basis for ADP-induced platelet activation. II. The P2Y1 receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. *J Biol Chem* 1998;**273**:2030–2034.
120. Daniel JL, Dangelmaier C, Jin J, Ashby B, Smith JB, Kunapuli SP. Molecular basis for ADP-induced platelet activation. I. Evidence for three distinct ADP receptors on human platelets. *J Biol Chem* 1998;**273**:2024–2029.
121. Woulfe D, Jiang H, Mortensen R, Yang J, Brass LF. Activation of Rap1B by G(i) family members in platelets. *J Biol Chem* 2002;**277**:23382–23390.
122. Smyth SS, Woulfe DS, Weitz JI, Gachet C, Conley PB, Goodman SG, Roe MT, Kuliopulos A, Moliterno DJ, French PA, Steinhubl SR, Becker RC. G-protein-coupled receptors as signaling targets for antiplatelet therapy. *Arterioscler Thromb Vasc Biol* 2009;**29**:449–457.
123. Hetherington SL, Singh RK, Lodwick D, Thompson JR, Goodall AH, Samani NJ. Dimorphism in the P2Y1 ADP receptor gene is associated with increased platelet activation response to ADP. *Arterioscler Thromb Vasc Biol* 2005;**25**:252–257.
124. Fontana P, Gaussem P, Aiach M, Fiessinger JN, Emmerich J, Reny JL. P2Y12 H2 haplotype is associated with peripheral arterial disease: a case-control study. *Circulation* 2003;**108**:2971–2973.
125. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias WL, Braunwald E, Sabatine MS. Cytochrome P450 Genetic Polymorphisms and the Response to Prasugrel. Relationship to Pharmacokinetic, Pharmacodynamic, and Clinical Outcomes. *Circulation* 2009;**119**:2553–2560.
126. Angiolillo DJ. Emerging insights on antiplatelet therapy. Introduction. *Am J Cardiol* 2009;**103**:1A–3A.
127. Marcucci R, Gori AM, Paniccia R, Giusti B, Valente S, Giglioli C, Buonamici P, Antoniucci D, Abbate R, Gensini GF. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: a 12-month follow-up. *Circulation* 2009;**119**:237–242.
128. Price MJ, Berger PB, Angiolillo DJ, Teirstein PS, Tanguay JF, Kandzari DE, Cannon CP, Topol EJ. Evaluation of individualized clopidogrel therapy after drug-eluting stent implantation in patients with high residual platelet reactivity: design and rationale of the GRAVITAS trial. *Am Heart J* 2009;**157**:818–824. 824 e811.
129. Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet* 2002;**41**:913–958.
130. Schwab M, Schaeffeler E, Klotz U, Treiber G. CYP2C19 polymorphism is a major predictor of treatment failure in white patients by use of lansoprazole-based

quadruple therapy for eradication of Helicobacter pylori. *Clin Pharmacol Ther* 2004;**76**:201–209.
131. Gurbel PA, Bliden KP, Samara W, Yoho JA, Hayes K, Fissha MZ, Tantry US. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST Study. *J Am Coll Cardiol* 2005;**46**:1827–1832.
132. Ajzenberg N, Aubry P, Huisse MG, Cachier A, El Amara W, Feldman LJ, Himbert D, Baruch D, Guillin MC, Steg PG. Enhanced shear-induced platelet aggregation in patients who experience subacute stent thrombosis: a case-control study. *J Am Coll Cardiol* 2005;**45**:1753–1756.
133. Buonamici P, Marcucci R, Migliorini A, Gensini GF, Santini A, Paniccia R, Moschi G, Gori AM, Abbate R, Antoniucci D. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. *J Am Coll Cardiol* 2007;**49**:2312–2317.
134. Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I, Novikov I, Pres H, Savion N, Varon D, Hod H. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* 2004;**109**:3171–3175.
135. Gurbel PA, Bliden KP, Guyer K, Cho PW, Zaman KA, Kreutz RP, Bassi AK, Tantry US. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. *J Am Coll Cardiol* 2005;**46**:1820–1826.
136. Cuisset T, Frere C, Quilici J, Morange PE, Nait-Saidi L, Carvajal J, Lehmann A, Lambert M, Bonnet JL, Alessi MC. Benefit of a 600-mg loading dose of clopidogrel on platelet reactivity and clinical outcomes in patients with non-ST-segment elevation acute coronary syndrome undergoing coronary stenting. *J Am Coll Cardiol* 2006;**48**:1339–1345.
137. Lev EI, Patel RT, Maresh KJ, Guthikonda S, Granada J, DeLao T, Bray PF, Kleiman NS. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: the role of dual drug resistance. *J Am Coll Cardiol* 2006;**47**:27–33.
138. Cuisset T, Frere C, Quilici J, Barbou F, Morange PE, Hovasse T, Bonnet JL, Alessi MC. High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome. *J Thromb Haemost* 2006;**4**:542–549.
139. Hochholzer W, Trenk D, Bestehorn HP, Fischer B, Valina CM, Ferenc M, Gick M, Caputo A, Buttner HJ, Neumann FJ. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol* 2006;**48**:1742–1750.
140. Geisler T, Langer H, Wydymus M, Gohring K, Zurn C, Bigalke B, Stellos K, May AE, Gawaz M. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. *Eur Heart J* 2006;**27**:2420–2425.
141. Bliden KP, DiChiara J, Tantry US, Bassi AK, Chaganti SK, Gurbel PA. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? *J Am Coll Cardiol* 2007;**49**:657–666.
142. Cuisset T, Frere C, Quilici J, Morange PE, Nait-Saidi L, Mielot C, Bali L, Lambert M, Alessi MC, Bonnet JL. High post-treatment platelet reactivity is associated with a high incidence of myonecrosis after stenting for non-ST elevation acute coronary syndromes. *Thromb Haemost* 2007;**97**:282–287.
143. Bonello L, Paganelli F, Arpin-Bornet M, Auquier P, Sampol J, Dignat-George F, Barragan P, Camoin-Jau L. Vasodilator-stimulated phosphoprotein phosphorylation analysis prior to percutaneous coronary intervention for exclusion of post-procedural major adverse cardiovascular events. *J Thromb Haemost* 2007;**5**:1630–1636.
144. Angiolillo DJ, Bernardo E, Sabate M, Jimenez-Quevedo P, Costa MA, Palazuelos J, Hernandez-Antolin R, Moreno R, Escaned J, Alfonso F, Banuelos C, Guzman LA, Bass TA, Macaya C, Fernandez-Ortiz A. Impact of platelet reactivity on cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Coll Cardiol* 2007;**50**:1541–1547.
145. Frere C, Cuisset T, Quilici J, Camoin L, Carvajal J, Morange PE, Lambert M, Juhan-Vague I, Bonnet JL, Alessi MC. ADP-induced platelet aggregation and platelet reactivity index VASP are good predictive markers for clinical outcomes in non-ST elevation acute coronary syndrome. *Thromb Haemost* 2007;**98**:838–843.
146. Price MJ, Endemann S, Gollapudi RR, Valencia R, Stinis CT, Levisay JP, Ernst A, Sawhney NS, Schatz RA, Teirstein PS. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. *Eur Heart J* 2008;**29**:992–1000.
147. Bonello L, Camoin-Jau L, Arques S, Boyer C, Panagides D, Wittenberg O, Simeoni MC, Barragan P, Dignat-George F, Paganelli F. 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**:1404–1411.
148. Patti G, Nusca A, Mangiacapra F, Gatto L, D'Ambrosio A, Di Sciascio G. Point-of-care measurement of clopidogrel responsiveness predicts clinical outcome in patients undergoing percutaneous coronary intervention results of the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) study. *J Am Coll Cardiol* 2008;**52**:1128–1133.
149. von Beckerath N, Kastrati A, Wieczorek A, Pogatsa-Murray G, Sibbing D, Graf I, Schomig A. A double-blind randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. *Eur Heart J* 2007;**28**:1814–1819.
150. Sibbing D, Braun S, Morath T, Mehilli J, Vogt W, Schomig A, Kastrati A, von Beckerath N. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. *J Am Coll Cardiol* 2009;**53**:849–856.
151. Chen BL, Chen Y, Tu JH, Li YL, Zhang W, Li Q, Fan L, Tan ZR, Hu DL, Wang D, Wang LS, Ouyang DS, Zhou HH. Clopidogrel Inhibits CYP2C19-Dependent Hydroxylation of Omeprazole Related to CYP2C19 Genetic Polymorphisms. *J Clin Pharmacol* 2009;**49**:574–581.
152. Fontana P, Dupont A, Gandrille S, Bachelot-Loza C, Reny JL, Aiach M, Gaussem P. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. *Circulation* 2003;**108**:989–995.
153. Lau WC, Gurbel PA, Watkins PB, Neer CJ, Hopp AS, Carville DG, Guyer KE, Tait AR, Bates ER. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of clopidogrel resistance. *Circulation* 2004;**109**:166–171.
154. Fontana P, Hulot JS, De Moerloose P, Gaussem P. Influence of CYP2C19 and CYP3A4 gene polymorphisms on clopidogrel responsiveness in healthy subjects. *J Thromb Haemost* 2007;**5**:2153–2155.
155. Giusti B, Gori AM, Marcucci R, Saracini C, Sestini I, Paniccia R, Valente S, Antoniucci D, Abbate R, Gensini GF. 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**:1057–1064.
156. Aynacioglu AS, Sachse C, Bozkurt A, Kortunay S, Nacak M, Schroder T, Kayaalp SO, Roots I, Brockmoller J. Low frequency of defective alleles of cytochrome P450 enzymes 2C19 and 2D6 in the Turkish population. *Clin Pharmacol Ther* 1999;**66**:185–192.
157. Scordo MG, Caputi AP, D'Arrigo C, Fava G, Spina E. Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. *Pharmacol Res* 2004;**50**:195–200.
158. Ozawa S, Soyama A, Saeki M, Fukushima-Uesaka H, Itoda M, Koyano S, Sai K, Ohno Y, Saito Y, Sawada J. Ethnic differences in genetic polymorphisms of CYP2D6, CYP2C19, CYP3As and MDR1/ABCB1. *Drug Metab Pharmacokinet* 2004;**19**:83–95.
159. Goldstein JA, Ishizaki T, Chiba K, de Morais SM, Bell D, Krahn PM, Evans DA. Frequencies of the defective CYP2C19 alleles responsible for the mephenytoin poor metabolizer phenotype in various Oriental, Caucasian, Saudi Arabian and American black populations. *Pharmacogenetics* 1997;**7**:59–64.
160. Luo HR, Poland RE, Lin KM, Wan YJ. Genetic polymorphism of cytochrome P450 2C19 in Mexican Americans: a cross-ethnic comparative study. *Clin Pharmacol Ther* 2006;**80**:33–40.
161. Bravo-Villalta HV, Yamamoto K, Nakamura K, Baya A, Okada Y, Horiuchi R. Genetic polymorphism of CYP2C9 and CYP2C19 in a Bolivian population: an investigative and comparative study. *Eur J Clin Pharmacol* 2005;**61**:179–184.
162. Persson I, Aklillu E, Rodrigues F, Bertilsson L, Ingelman-Sundberg M. S-mephenytoin hydroxylation phenotype and CYP2C19 genotype among Ethiopians. *Pharmacogenetics* 1996;**6**:521–526.
163. Sameer AE, Amany GM, Abdela AA, Fadel SA. CYP2C19 genotypes in a population of healthy volunteers and in children with hematological malignancies in Gaza Strip. *Can J Clin Pharmacol* 2009;**16**:e156–e162.
164. Nowak MP, Sellers EM, Tyndale RF. Canadian Native Indians exhibit unique CYP2A6 and CYP2C19 mutant allele frequencies. *Clin Pharmacol Ther* 1998;**64**:378–383.
165. Tassaneeyakul W, Mahatthanatrakul W, Niwatananun K, Na-Bangchang K, Tawalee A, Krikreangsak N, Cykleng U. Tassaneeyakul W. CYP2C19 genetic polymorphism in Thai, Burmese and Karen populations. *Drug Metab Pharmacokinet* 2006;**21**:286–290.
166. Yamada S, Onda M, Kato S, Matsuda N, Matsuhisa T, Yamada N, Miki M, Matsukura N. Genetic differences in CYP2C19 single nucleotide polymorphisms among four Asian populations. *J Gastroenterol* 2001;**36**:669–672.
167. Chen L, Qin S, Xie J, Tang J, Yang L, Shen W, Zhao X, Du J, He G, Feng G, He L, Xing Q. Genetic polymorphism analysis of CYP2C19 in Chinese Han

populations from different geographic areas of mainland China. *Pharmacogenomics* 2008;**9**:691–702.
168. Zand N, Tajik N, Moghaddam AS, Milanian I. Genetic polymorphisms of cytochrome P450 enzymes 2C9 and 2C19 in a healthy Iranian population. *Clin Exp Pharmacol Physiol* 2007;**34**:102–105.
169. Lamba JK, Dhiman RK, Kohli KK. Genetic polymorphism of the hepatic cytochrome P450 2C19 in north Indian subjects. *Clin Pharmacol Ther* 1998;**63**:422–427.
170. Adithan C, Gerard N, Vasu S, Rosemary J, Shashindran CH, Krishnamoorthy R. Allele and genotype frequency of CYP2C19 in a Tamilian population. *Br J Clin Pharmacol* 2003;**56**:331–333.
171. Takakubo F, Kuwano A, Kondo I. Evidence that poor metabolizers of (S)-mephenytoin could be identified by haplotypes of CYP2C19 in Japanese. *Pharmacogenetics* 1996;**6**:265–267.
172. Lee SS, Lee SJ, Gwak J, Jung HJ, Thi-Le H, Song IS, Kim EY, Shin JG. Comparisons of CYP2C19 genetic polymorphisms between Korean and Vietnamese populations. *Ther Drug Monit* 2007;**29**:455–459.
173. Herrlin K, Massele AY, Jande M, Alm C, Tybring G, Abdi YA, Wennerholm A, Johansson I, Dahl ML, Bertilsson L, Gustafsson LL. Bantu Tanzanians have a decreased capacity to metabolize omeprazole and mephenytoin in relation to their CYP2C19 genotype. *Clin Pharmacol Ther* 1998;**64**:391–401.

CARDIOVASCULAR FLASHLIGHT

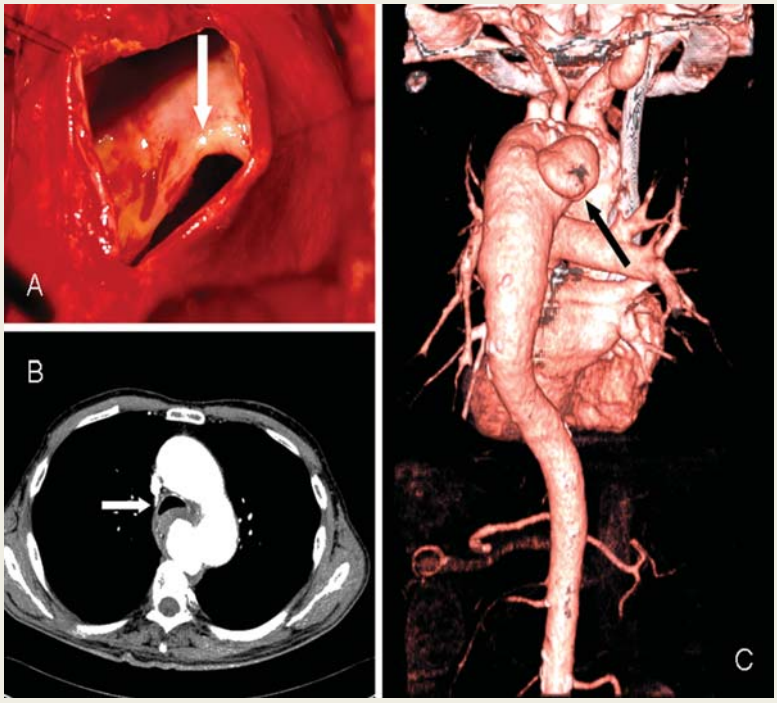
doi:10.1093/eurheartj/ehp202
Online publish-ahead-of-print 19 May 2009

Luetic mycotic pseudoaneurysm of the aortic isthmus

Sandor Szabados[1], Edit Varady[2], and Laszlo Göbölös[1]*

[1]Heart Institute, Faculty of Medicine, University of Pécs, Pécs, Hungary and [2]Department of Radiology, Faculty of Medicine, University of Pécs, Pécs, Hungary

* Corresponding author. Tel: +36 20 9889 949, Fax: +36 72 536 388, Email: isartor@hotmail.com



Since the wide range introduction of antibiotic treatment, development of the systemic luetic disease with mycotic aortitis has become extremely rare. Atraumatic, spontaneous aortic rupture is a rare but potentially lethal event. In this report, we present an unusual case of aortic isthmus pseudoaneurysm resulting from luetic infiltration of the aortic wall elements. Thoracic aorta false aneurysms are a surgical challenge; in view of the severe effect of any open thoracic surgical intervention, exact pre-operative diagnosis is crucial. We demonstrate a 62-year-old male patient who was accidentally diagnosed with a pseudoaneurysm of the atherosclerotic aortic isthmus, presented with irritative coughs, problems with swallowing and weight loss (*Panel B*). He had no major diseases in the past history, except a treated luetic event decades before. The pre-operative serum analysis also showed treponema-antibody positivity. The appearance of aortic isthmus pseudoaneurysm and the rupture area were clearly visualized with transoesophageal echocardiography, as well as by three-dimensional reconstruction of multislice computed tomography images (*Panel C*). The saccular false aneurysm originating from a mural defect at the site of an ulcerated atherosclerotic plaque in a diameter of 6 cm was resected through an anterolateral thoracotomy electively. The patient received a 9 mm heparin-bounded Gott's shunt linking the ascending aorta with the descending part distal to the lesion; the aortic rupture (*Panel A*) was closed by an antibiotic-soaked Dacron patch. The intraoperative microbiological analysis revealed a pseudoaneurysm of the aorta with luetic origin. The patient had an uneventful post-operative course and the control computed tomography scan showed no evidence of recurrent aneurysm or vascular leakage after 6 months and 2 years.

Panel A. Intraoperative lateral view of the aortic isthmus; arrow indicates the intramural wall defect.
Panel B. Computed tomography image at level of false aneurysm; arrow marks the significantly compressed trachea.
Panel C. Posterior aspect of three-dimensionally reconstructed aorta; arrow points on pseudoaneurysm.