The genetic basis of platelet responsiveness to clopidogrel
A critical review of the literature

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Summary
Clopidogrel reduces ischaemic complications in a wide range of patients with coronary artery disease. However, there is much inter-individual variation in clopidogrel-induced platelet inhibition, and a substantial proportion of patients will exhibit non-responsiveness to clopidogrel. Multiple studies have demonstrated an association between the presence of genetic polymorphisms associated with suboptimal clopidogrel-active metabolite generation, decreased platelet responsiveness, and adverse clinical outcomes. However, it is not clear to what extent the genetic polymorphisms account for the observed variability in response to clopidogrel. In this review we provide a critical summary of the available evidence linking genetic factors with response to clopidogrel, and discuss the clinical implications of this association.

Keywords
Antiplatelet agents, polymorphisms, platelet pharmacology

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Platelet function testing: From bench to bedside

Introduction
Platelet activation and aggregation play a pivotal role in the pathogenesis of acute coronary syndromes (ACS) and thrombotic complications in patients undergoing percutaneous coronary interventions (PCI) (1). Clopidogrel irreversibly blocks the platelet P2Y12 receptor, thereby inhibiting sustained activation of glycoprotein (GP) IIb/IIIa receptors, leading to less stable thrombus formation at the vascular injury site (2). Major randomised controlled trials and meta-analyses have demonstrated that clopidogrel, when added to aspirin, reduces ischaemic complications in a wide range of coronary artery disease (CAD) patients (3, 4). However, clopidogrel treatment is associated with an overall variable and modest level of P2Y12 inhibition even when high loading doses are used (5–9), and a substantial proportion of patients will exhibit non-responsiveness to clopidogrel (10). Furthermore, multiple studies have demonstrated a link between clopidogrel non-responsiveness and/or high on-treatment platelet reactivity measured by multiple platelet assays and adverse clinical ischaemic events (11).

Multiple independent studies have demonstrated an association between the presence of genetic polymorphisms associated with suboptimal clopidogrel active metabolite generation, decreased platelet responsiveness, and adverse clinical outcomes. However, it is not clear to what extent genetic polymorphisms account for the observed variability in response to clopidogrel (12). Complicating this matter even further is the fact that most studies showing the attenuated benefit of clopidogrel among carriers do not include a randomised control group. In fact, the only two studies to include a randomised control group (13, 14) were less supportive of a causal link between loss-of-function alleles and adverse clinical outcomes. In this review we provide a critical summary of the available evidence linking genetic factors with response to clopidogrel, and discuss the clinical implications of this association.

Clopidogrel metabolism
Clopidogrel is a pro-drug that requires hepatic conversion into an active metabolite to exert its antplatelet effect. Approximately 85% of absorbed clopidogrel is hydrolysed by carboxylase to an inactive carboxylic acid metabolite, while the remaining clopidogrel is metabolised by hepatic cytochrome (CYP) P450 isoenzymes in a two-step process. In the first step, the thiophene ring of clopidogrel is oxidised to 2-oxo-clopidogrel, which is then hydrolysed to a highly labile active metabolite (15–17). The first step is mediated by CYP2C19, CYP1A2, and CYP2B6, while CYP2C19, CYP2C9, CYP2B6, and CYP3A are responsible for the second step (15–16). Thus, CYP2C19 and CYP2B6 participate in both metabolic steps. The active metabolite binds irreversibly to the platelet P2Y12 receptor resulting in inhibition of ADP-induced platelet activation-aggregation for the life span of the platelet (18). The following alleles are associated with loss-of-function in response to clopidogrel: CYP3A4*1B, CYP3A4*3, CYP3A4*4, CYP3A5*3,
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CYP2C19 allelic variants and clopidogrel active metabolite levels (pharmacokinetic response)

Few studies have compared levels of the clopidogrel active metabolite in carriers of the CYP reduced-function allelic variants (see Table 1). Mega et al. (19) examined plasma concentrations of the clopidogrel active metabolite in 162 healthy subjects from six studies involving thienopyridine treatment. Carriers of at least one CYP2C19 reduced-function allele (34% of the study population) had a relative reduction of 32% in plasma exposure to the active metabolite (measured as the area under the plasma concentration-time curve), compared with non-carriers (p<0.001). CYP2C19*2 was the most frequent variant allele (95%) among the reduced-function group. Likewise, Varenhorst et al. (20) genotyped 47 CAD patients treated with aspirin. Patients were genotyped for variations in six CYP genes and, based on the CYP genotype using the “star allele” nomenclature (https://www.cypalleles.ki.se), were segregated into two groups: normal function metabolisers and reduced function metabolisers. Plasma metabolite exposure levels were followed up to 29 days during maintenance dose. Active metabolite exposure levels were significantly lower (p=0.0015) in the reduced function compared with the normal function metabolisers.

CYP2C19 allelic variants and platelet function (pharmacodynamic response)

Numerous studies in healthy subjects have assessed the pharmacodynamic response to clopidogrel of CYP2C19 reduced-function alleles (21–24) (see Table 1). In addition to measuring the active metabolite level of clopidogrel, Mega et al. (19) and Varenhorst et al. (20) also assessed the pharmacodynamic response to clopidogrel of subjects with CYP2C19 reduced function alleles. Mega et al. (19) assessed platelet function in 162 healthy subjects using light transmission aggregometry (LTA). A total of five polymorphic genes were analysed, namely CYP2C19, CYP2C9, CYP2B6, CYP3A5, and CYP1A2. Carriers of at least one CYP2C19 reduced function allele (had a diminished pharmacodynamic response, with an absolute reduction in maximal platelet aggregation in response to clopidogrel that was 9% less than that seen in non-carriers. Varenhorst (20) evaluated platelet response to clopidogrel and prasugrel in aspirin-treated patients with CAD. Clopidogrel-treated patients with a CYP2C19 reduced metaboliser phenotype exhibited a reduced pharmacodynamic response as measured by vasodilator-stimulated phosphoprotein (VASP) and the point-of-care VerifyNow™ P2Y12 (P<0.05 for both). Giusti et al. (25), in 1,419 patients with ACS undergoing PCI on dual-anti-platelet treatment, found that carriers of the CYP2C19*2 allele had significantly higher platelet aggregation after ADP and arachidonic acid stimuli. This polymorphism was an independent predictor of antiplatelet treatment variability. In this study, all the patients were treated with a 600 mg loading dose. Therefore the authors suggest that the CYP2C19*2 polymorphism effect is not overcome by high clopidogrel loading dose. Supporting this finding, a registry of 126 PCI patients on high maintenance dose clopidogrel (150 mg/day) for at least one month found that carriers of CYP2C19*2 and *3 had significantly higher 5 and 20 μM ADP-induced platelet aggregation than did non-carriers (40.7 ± 16.8% VS 30.3 ± 12.6%, P<0.001; 54.2 ± 16.2% VS 40.5 ± 15.8%, P<0.001) (26). On the other hand, a small prospective study examined the effect of increased loading and maintenance doses of clopidogrel. In wild-type CYP2C19 carriers no difference was noted in platelet inhibition with 600 mg and 1,200 mg loading doses and 75 mg and 150 mg maintenance doses. However, in carriers of loss-of-function alleles (CYP2C19*2 and *4) increases in either loading or maintenance doses were associated with significantly greater platelet inhibition (27). The association of the CYP2C19*2 polymorphism with reduced post-treatment platelet reactivity was further confirmed in a study of 603 non-ST elevation ACS patients. In this study the CYP3A4*1B and CYP3A5*3 polymorphisms showed no such association (28). In the RECLOSE trial (29), 772 patients with a wide spectrum of CAD, including ST-elevation myocardial infarction (MI), non-ST-elevation ACS, and complex angiographic subtypes, who underwent drug-eluting stent implantation were genotyped and platelet function was assessed by LTA. Carriers of CYP2C19*2 polymorphism had significantly higher ADP-induced platelet aggregation than non-carriers (51% vs. 45%, p<0.001). Trenk et al. (30) found that 31% of 279 consecutive patients undergoing PCI were carriers of the CYP2C19*2 polymorphism, and that these patients exhibited significantly (P<0.001) higher on-clopidogrel residual platelet aggregation (5 μM ADP-induced late platelet aggregation >14%) compared with normal metabolisers, both after loading (62.4% vs. 43.4%) and at pre-discharge (41.3% vs. 22.5%). Shuldiner et al. (31) conducted a genome-wide association study in 429 healthy Amish individuals. The investigators first measured platelet aggregation using LTA in a healthy cohort at baseline and at 1 hour after the last dose of clopidogrel on day 7. They then assessed over 400,000 single nucleotide polymorphisms (SNPs) for association to platelet activity. The most significant SNP (1.5 X 10–13) clustered around chromosome 10q24, which is in high linkage disequilibrium with the CYP2C19*2 allele. ADP-induced platelet aggregation was reduced to 41%, 47%, and 65% of baseline in response to clopidogrel in participants with 0, 1, and 2 CYP2C19*2 alleles, respectively. Importantly, these SNPs did not associate with platelet aggregation at baseline, thereby confirming CYP2C19 as a major genetic mediator of clopidogrel response. The CYP2C19*2 genotype was present in its heterozygous and homozygous state in 31% and 2% of the study population, respectively.
Table 1: Studies evaluating genetic influences on clopidogrel pharmacokinetic and pharmacodynamic outcomes.

<table>
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<tr>
<th>Study reference</th>
<th>Study population</th>
<th>Genetic traits evaluated</th>
<th>Methodology</th>
<th>Results</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Mega JL, et al (19)</td>
<td>162 healthy subjects</td>
<td>54 loss of function alleles classified into three phenotypic groups (ultra-rapid metabolisers, intermediate metabolisers, and poor metabolisers). Gain of function allele: CYP2C19*17</td>
<td>Optical aggregometry after stimulation with ADP (20 μM)</td>
<td>Subjects with at least one CYP2C19 loss of function allele had a relative reduction of 32.4% in plasma exposure to the active metabolite of clopidogrel and 9% less absolute reduction in maximal platelet aggregation in response to clopidogrel</td>
<td>P&lt;0.001 for all comparisons</td>
</tr>
<tr>
<td>Varenhorst C, et al (20)</td>
<td>98 patients with coronary artery disease randomly treated with clopidogrel or prasugrel</td>
<td>Loss of function alleles: CYP2C19; 2B6; 2C9; 3A5; 3A4; 1A2 categorised phenotypically as extensive (normal) metabolisers and reduced metabolisers. Gain of function allele: CYP2C19*17</td>
<td>Plasma concentration of the clopidogrel active metabolite at multiple time points over 29 days. Platelet function was assessed with VASP assay, and using the VerifyNow™ point-of-care assay.</td>
<td>For clopidogrel treated patients, reduced metabolisers had significantly lower active metabolite exposure, and significantly less reduction in platelet reactivity as assessed by VASP and VerifyNow™ as compared with normal metabolisers.</td>
<td>P&lt;0.0015 for metabolite exposure; p&lt;0.05 for platelet function assays</td>
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<tr>
<td>Giusti B, et al (25)</td>
<td>1419 ACS patients on dual antiplatelet therapy</td>
<td>CYP2C19*2, CYP3A4, T744C P2Y12 gene polymorphism</td>
<td>Optical aggregometry after stimulation with ADP (2 μM and 10 μM) and AA (0.5 mg/ml)</td>
<td>Only CYP2C19*2 was associated with higher platelet reactivity after all three stimuli.</td>
<td>AA: p=0.043 ADP: p&lt;0.001 and p=0.001 for 2 μM and 10 μM, respectively</td>
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<tr>
<td>Jeong YH, et al (26)</td>
<td>126 PCI-treated patients receiving high clopidogrel dose (150 mg/day for &gt;1 month)</td>
<td>CYP2C19, CYP3A5, ABCB1.</td>
<td>Optical aggregometry after stimulation with ADP (5 μM and 20 μM) and VerifyNow™ point-of-care assay.</td>
<td>Carriage of CYP2C19*2 and *3 was associated with higher platelet reactivity compared with non-carriers. CYP3A5 and ABCB1 did not influence platelet reactivity.</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Gladding P, et al (27)</td>
<td>60 patients undergoing PCI treated with 600 mg or 1,200 mg loading dose and 75 mg or 150 mg maintenance dose.</td>
<td>CYP2C19*1, *2, *4, *17, CYP2C9, CYP3A4, ABCB1, P2Y12, CES.</td>
<td>VerifyNow™ point-of-care assay.</td>
<td>CYP2C19*2 and *4 carriers had reduced platelet inhibition after 600 mg loading dose compared with wild type alleles but responded to higher loading (1,200 mg) and maintenance (75 mg) doses. No significant differences in platelet aggregation were noted for the other polymorphisms evaluated.</td>
<td>p=0.029 for 600 mg dose; P=0.002 for high loading dose and p=0.042 for high maintenance dose.</td>
</tr>
<tr>
<td>Trenk D, et al (30)</td>
<td>797 consecutive patients undergoing elective PCI</td>
<td>Loss of function allele: CYP2C19*2</td>
<td>Optical aggregometry after stimulation with ADP (5 and 20 μM); and flow cytometry for expression of activation dependent proteins after stimulation with ADP (20 μM).</td>
<td>Carriers of CYP2C19*2 allele were significantly more likely to exhibit higher on-clopidogrel residual platelet aggregation (&gt;14%) after clopidogrel loading and pre-hospital discharge.</td>
<td>P&lt;0.001</td>
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<tr>
<td>Shuldiner AR, et al (31)</td>
<td>Genome-wide study in 429 healthy Amish people and 227 patients undergoing non-emergent PCI</td>
<td>Loss of function alleles: CYP2C19*2, *3, *5, *17</td>
<td>Optical aggregometry after stimulation with ADP (20 μM)</td>
<td>Carriage of CYP2C19*2 was associated with reduced platelet response to clopidogrel.</td>
<td>P&lt;0.001</td>
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<tr>
<td>Sibbing D, et al (37)</td>
<td>1524 patients undergoing elective PCI (using DES)</td>
<td>Gain of function allele: CYP2C19*17</td>
<td>Multiple electrode platelet aggregometry after stimulation with ADP (6.4 μM)</td>
<td>Carriage of CYP2C19*17 was associated with increased platelet response to clopidogrel</td>
<td>P=0.009</td>
</tr>
<tr>
<td>Mega JL, et al (41)</td>
<td>321 healthy volunteers.</td>
<td>ABCB1 3435C→T; 2677G→T/A; 1236C→T. Loss-of-function alleles of CYP2C19.</td>
<td>Optical aggregometry after stimulation with ADP (20 μM) and plasma concentrations of clopidogrel and clopidogrel active metabolite were measured by liquid chromatography with mass spectroscopy</td>
<td>ABCB1 3435 TT homozygotes had a diminished pharmacodynamic effect (7.3% lower than C/T/C carriers) seen only after loading but not during maintenance therapy. No association was found with active metabolite concentrations.</td>
<td>P=0.022 for pharmacodynamic effect.</td>
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ADP, adenosine di-phosphate; ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; DES, drug-eluting stent.
CYP2C19 allelic variants and clinical outcomes

Most but not all studies which assessed clinical outcomes in clopidogrel-treated patients found a correlation between CYP2C19 loss of function alleles and adverse clinical outcomes. There are methodological differences between the various studies both in terms of the study population and in the evaluated outcomes. Shuldiner at al. (31) recruited 227 patients undergoing non-emergent PCI who were pre-treated with clopidogrel. While carriers of the CYP2C19*2 genotype had no differences in baseline characteristics, they did have higher cardiovascular event rates at one year compared with non-carriers (21% vs. 10%, p=0.02), in addition to demonstrating greater residual platelet aggregation after clopidogrel therapy. This increase was limited to the 95 patients still on clopidogrel therapy at the time of the event (hazard ratio [HR] 3.4, p=0.004), compared with no increase in the event rate of those not taking clopidogrel. An additional study included 2,485 low-to-intermediate risk patients undergoing PCI after pre-treatment with clopidogrel and recruited to one of the ISAR (Intracoronary Stenting and Antithrombotic Regimen) group randomised trials (10). About one-third of the patients had ACS as the indication for PCI. The primary endpoint was definite stent thrombosis (by the Academic Research Consortium [ARC] criteria) (32) at 30 days. Baseline and procedural characteristics were well balanced between the groups: heterozygotes for CYP2C19*2 allele constituted 25%, while CYP2C19*2 homozygotes constituted 2% of the study cohort. The cumulative incidence of stent thrombosis was significantly higher in CYP2C19*2 allele carriers vs. wild-type homozygotes (CYP2C19*1) (1.5% vs. 0.4%, HR 3.84, p=0.007). The risk was highest in homozygotes, demonstrating a gene-dose effect. A multivariable Cox proportional hazard model demonstrated that carriage of the CYP2C19*2 allele was an independent predictor of 30-day stent thrombosis. In this study no significant association of CYP2C19*2 carrier status with the incidence of death or MI was observed. Another study (29) evaluated the role of the CYP2C19*2 polymorphism in the occurrence of stent thrombosis up to six months after the implantation of a drug-eluting stent in 804 consecutive patients enrolled in a one-centre prospective trial. Baseline, angiographic, and procedural characteristics were well balanced between carriers and non-carriers of the CYP2C19*2 allele. Prevalence of carriers of the loss-of-function allele was significantly higher in patients with stent thrombosis compared to those without (54% vs. 31%, p=0.025). Similar results were observed concerning the composite endpoint of cardiac mortality and stent thrombosis. In multivariate analysis adjusted for the presence of previously shown clinical and procedural risk factors for stent thrombosis, the CYP2C19*2 polymorphism remained an independent risk factor for stent thrombosis and the composite endpoint of cardiac mortality and stent thrombosis. Mega et al. (19) tested the association between carriage of a reduced-function variant in CYP2C19 and clinical outcomes in 1,477 patients with ACS assigned to clopidogrel in the TRITON-TIMI 38 trial. Carriage of at least one reduced-function allele (CYP2C19*2 accounted for 95%), found in 395 subjects (27%), was associated with significantly higher risk for the primary efficacy outcome of cardiovascular death, death, MI, or stroke compared with non-carriers (12% vs. 8%, p=0.01). The HR for each component of the combined endpoint was: cardiovascular death – 4.75; non-fatal MI – 1.38; non-fatal stroke – 3.93. The risk of stent thrombosis in carriers vs. non-carriers was almost tripled (2.6% vs. 0.8%, p=0.02). Two studies specifically enrolled acute MI patients: Simon et al. (12) recruited 2,208 patients presenting with an acute MI on clopidogrel therapy in a nationwide French registry. Patients carrying any two CYP2C19 loss-of-function alleles (*2, *3, *4, or *5), had a higher event rate than patients carrying none (22% vs. 13%, adjusted HR 1.98, confidence interval [CI] 1.1–3.58). This effect was particularly pronounced in the 1,535 patients undergoing PCI, among whom the relative risk of cardiovascular events was 3.6 times higher than non-carrier patients. Notably, in this study, as opposed to those mentioned previously, patients carrying only one CYP2C19 variant allele showed no increased risk compared with those who had no CYP2C19 variant alleles. Collet at al. (33) recruited 259 young MI patients who where exposed to clopidogrel treatment for at least one month. The primary endpoint was a composite of death, MI, and urgent coronary revascularisation. The secondary endpoint was angiographically proven stent thrombosis. Baseline characteristics were well balanced between the groups. The primary endpoint occurred more frequently in carriers than in non-carriers (HR 3.7, P=0.0005), as did stent thrombosis (HR 6.0, P=0.0009). After multivariable analysis, the CYP2C19*2 genetic variant was the only independent predictor of cardiovascular events (HR 4.0, P=0.0006). The PLATO trial was a large scale study comparing ticagrelor with clopidogrel in ACS patients. The genetic sub-study of the PLATO study included 10,285 patients. In this study, within the clopidogrel-treated patients, CYP2C19 loss-of-function alleles (*2, *3, *4, *5, *6, *7, *8) were associated with higher cardiac event rates at 30-days follow-up (5.7% vs. 3.8%, p=0.028) but not at one year (34). A negative study, published recently by Pare et al. (13), genotyped patients from two large, randomised trials showing that clopidogrel, compared with placebo, reduced the rate of cardiovascular events among 5,059 genotyped patients with ACS recruited to the CURE study, and 1,156 genotyped patients with atrial fibrillation recruited to the ACTIVe A trial. In this study, clopidogrel treatment was associated with consistent benefits, irrespective of CYP2C19 genotype both in patients with ACS and atrial fibrillation. This remained true even when analyses were restricted to homozygous patients with poor metaboliser phenotype. Also unique to this study was the observation that clopidogrel showed enhanced efficacy for the reduction of ischaemic events in patients with ACS who were carriers of the gain-in-function allele (CYP2C19*17). The lack of effect in this study is in contrast to findings in the previously quoted studies. One possible explanation posed by the authors is the difference in the rates of PCI with stenting. Only 18% of the CURE population who were genotyped underwent PCI, and only 14.5% underwent stent deployment. This is in contrast to most previously quoted studies where a majority of the patients underwent coronary stenting. Results of previous trials have consistently shown that the greatest benefit of clopidogrel, including its use in high doses, is its
Table 2: Studies evaluating genetic influences on outcome of clopidogrel-treated patients.

<table>
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<tr>
<th>Study/ reference</th>
<th>Study population</th>
<th>Genetic traits evaluated</th>
<th>Endpoint evaluated</th>
<th>Results</th>
<th>HR/OR P-value</th>
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<tbody>
<tr>
<td>Sibbing D, et al (10)</td>
<td>2,485 patients undergoing PCI</td>
<td>Loss of function allele: CYP2C19*2</td>
<td>Definite ST (ARC criteria) within 30 days</td>
<td>Incidence of ST was significantly higher in carriers of CYP2C19*2 allele.</td>
<td>HR 3.81, P=0.007</td>
</tr>
<tr>
<td>Simon T, et al (12)</td>
<td>2,208 patients with acute MI treated with clopidogrel</td>
<td>CYP3AS and CYP2C19 *2-*5 affecting metabolic activation; P2RY12 and ITGB3 affecting biologic activity; and ABCB1 affecting clopidogrel absorption</td>
<td>MACE: death, nonfatal stroke, or MI within one year of follow-up</td>
<td>Patients with two variant alleles of ABCB1 had a higher rate of MACE compared with wild type; Patients carrying any two CYP2C19 loss of function alleles (*2, *3, *4, or *5) had a higher event rate than patients with none (HR 1.98) and among 1,535 patients undergoing PCI, the HR was 3.58</td>
<td>P=0.007 for ABCB1; P=0.003 for CYP2C19</td>
</tr>
<tr>
<td>Pare G, et al (13)</td>
<td>5,059 patients with ACS from the CURE study and 1,156 patients with AF from the ACTIVE A study</td>
<td>CYP2C19 loss of function alleles *2 and *3, and gain-of-function allele *17.</td>
<td>For CURE patients-MACE including CV death, nonfatal MI, or stroke. For AF patients- composite of stroke, systemic embolism, MI, or vascular death.</td>
<td>Reduction of primary endpoint was similar among patients with loss-of-function alleles. For ACS patients, carriage of CYP2C19<em>17 was associated with more benefit from clopidogrel treatment as compared to placebo than for non-carriers. For AF patients, there was no difference in efficacy measures. For both ACS and AF patients, CYP2C19</em>17 carriage was not associated with increased bleeding.</td>
<td>P=0.12 for primary outcome in ACS patients, p=0.64 for bleeding in ACS patients. P=0.32 for primary outcome in AF patients, p=0.08 for bleeding in AF patients.</td>
</tr>
<tr>
<td>Mega JL, et al (19)</td>
<td>1,477 ACS subjects treated with clopidogrel and planned PCI in the TRITON-TIMI 38 trial</td>
<td>54 loss of function alleles classified into 3 phenotypic groups (ultra-rapid metabolisers, intermediate metabolisers, and poor metabolisers). Gain of function allele: CYP2C19*17</td>
<td>Primary endpoint: Composite of cardiovascular death, MI, or stroke. Secondary endpoint: definite or probable ST (ARC criteria). Safety endpoint: TIMI minor or major non- CABG related bleeding</td>
<td>Among clopidogrel treated patients, carriers of at least 1 CYP2C19 reduced function allele (95% were *2) had a relative increase of 53% in the primary endpoint, and a HR of 3.09 for ST. No significant associations were noted for the other genotypes. No differences in bleeding were noted with any of the CYP genotypes.</td>
<td>For carriers of CYP2C19*2 – primary outcome: HR-1.42, p=0.04; ST – HR 3.33, p=0.004.</td>
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<tr>
<td>Giusti B, et al (29)</td>
<td>772 consecutive patients undergoing PCI (using DES)</td>
<td>Loss of function allele: CYP2C19*2</td>
<td>Prevalence of CYP2C19*2 among patients exhibiting ST or cardiac mortality within six months</td>
<td>Carriage of CYP2C19*2 was independently associated with ST and ST and cardiac death at 6 months.</td>
<td>OR for ST 3.43, p=0.047; OR for ST and cardiac death 2.7, p=0.049.</td>
</tr>
<tr>
<td>Trenk D, et al (30)</td>
<td>797 consecutive patients undergoing elective PCI</td>
<td>Loss of function allele: CYP2C19*2</td>
<td>Cumulative one-year death and myocardial infarction in patients with high (&gt;14%) on-clopidogrel residual platelet aggregation</td>
<td>Patients with high on-clopidogrel residual platelet aggregation (&gt;14%) had significantly greater risk of death and MI at 1 year follow-up.</td>
<td>HR-3, P=0.004</td>
</tr>
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<td>Shuldiner AR, et al (31)</td>
<td>227 patients undergoing non-emergent PCI</td>
<td>CYP2C19*2</td>
<td>Cardiovascular ischaemic event or death at one year</td>
<td>Carriers of CYP2C19*2 had higher cardiovascular event rates compared with non-carriers. This effect was limited to patients still taking clopidogrel.</td>
<td>HR-3.4, P=0.004</td>
</tr>
<tr>
<td>Collet JP, et al (33)</td>
<td>Young (&lt;45 years) post-MI patients on clopidogrel for at least one month</td>
<td>Loss of function allele: CYP2C19*2</td>
<td>Primary endpoint: MACE (death, MI, urgent revascularisation). Secondary endpoint: Angiography proven ST</td>
<td>Primary and secondary endpoints occurred more frequently in CYP2C19*2 carriers as compared with non-carriers</td>
<td>Primary endpoint: HR-3.69, P&lt;0.001; Secondary endpoint – HR 6.02, P&lt;0.001</td>
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effect on reducing the rate of stent thrombosis (35). Cases in point are studies by Collet et al. (33) and the genetic sub-study of the TRITON-TIMI 38 study (19), where the greatest effect of loss-of-function alleles on the outcome in clopidogrel-treated patients was on stent thrombosis. Importantly, all published studies, other than that of Pare et al. (13), did not include a randomised control group. A recently published meta-analysis (36) evaluating 10 clinical studies (11,959 patients) investigating the influence of CYP2C19 genetic variants, found that 28% were carriers of a CYP2C19*2 allele. These subjects displayed a 30% increase in the risk for major adverse cardiac events (MACE) compared with non-carriers. In this meta-analysis, this single gene variant was also associated with excess mortality and stent thrombosis. This increase was apparent in both heterozygotes and homozygotes and was independent of the baseline cardiovascular risk. One study specifically assessed the gain-of-function allele CYP2C19*17 in 1524 clopidogrel-treated patients undergoing PCI and found a significantly increased risk of bleeding in carriers of this allele with the highest risk observed in carriers of CYP2C19*17 higher rates of bleeding. No association of ABCB1 polymorphism and outcomes was noted. This increase was not associated with TLR or MACE.

### Table 2: Continued

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<tr>
<td>Wallentin L, et al (34)</td>
<td>10,285 patients enrolled in the PLATO study evaluating ticagrelor vs clopidogrel in patients with ACS</td>
<td>Loss of function alleles: CYP2C19*2,*3,*4,*5,*6, *7,<em>8. Gain of function allele: CYP2C19</em>17. ABCB1 polymorphism affecting intestinal absorption of clopidogrel</td>
<td>MACE (cardiovascular death, myocardial infarction, or stroke) up to 12 months.</td>
<td>In clopidogrel treated patients, MACE rate at 30 days was higher in patients with any loss of function allele. This difference was no longer statistically significant at 1 year. Carriers of CYP2C19*17 had higher rates of bleeding. No association of ABCB1 polymorphism and outcomes was noted.</td>
<td>HR at 30 days for loss of function alleles – 1.5, P=0.028. HR for bleeding in patients with *17 allele 1.25, P=0.022.</td>
</tr>
<tr>
<td>Sibbing D, et al (37)</td>
<td>1524 patients undergoing elective PCI (using DES)</td>
<td>Gain of function allele: CYP2C19*17</td>
<td>30-day incidence of major and minor bleeding (TIMI definition) and incidence of definite or probable ST (ARC criteria) and MACE</td>
<td>Carriers of CYP2C19*17 had higher risk for TIMI bleedings at 30-days. No difference noted in rates of ST or MACE between carriers and non-carriers.</td>
<td>OR for bleeding: 1.85–3.41, P=0.006</td>
</tr>
<tr>
<td>Mega, JL, et al (41)</td>
<td>1,471 ACS patients undergoing PCI and treated with clopidogrel within the TRITON-TIMI 38 trial</td>
<td>ABCB1 3435C→T; 2677G→T/A; 1236C→T. CYP2C19 loss-of-function alleles.</td>
<td>Primary endpoint- MACE (CV death, MI, or stroke).</td>
<td>ABCB1 3435C→T was significantly associated with MACE. TT homozygotes had a 72% increased risk compared with CT or CC. ABCB1 3435C→T and/or CYP2C19 genotypes were significant predictors of the primary endpoint (HR 1.97).</td>
<td>P=0.0064 for primary endpoint. p=0.002 for TT homozygotes. P=0.0002 for CYP2C19 and/or ABCB1 3435C→T.</td>
</tr>
<tr>
<td>Harmsze AM, et al (42)</td>
<td>176 subjects who developed ST while on clopidogrel and 420 control subjects who did not</td>
<td>Genes involved in absorption (ABCB1 C1236T, G2677T/A, C3435T), metabolism (CYP2C19<em>2 and <em>3, CYP2C9</em>2,<em>3, CYP3A4</em>1B, CYP3A5</em>3), and pharmacodynamics (P2Y1 A1622G).</td>
<td>Case control study comparing frequencies of genetic polymorphisms in patients with and without ST.</td>
<td>Carriage of CYP2C19<em>2 and CYP2C9</em>3 was most strongly associated with ST. No significant associations of the other genetic variations and ST were found.</td>
<td>CYP2C19<em>2 - OR 2.5, P= 0.026; CYP2C9</em>3 - OR 3.3, P= 0.031</td>
</tr>
<tr>
<td>Tiroch KA, et al (43)</td>
<td>928 acute MI patients</td>
<td>ABCB1 T-allele, CYP2C19<em>2, CYP2C19</em>17</td>
<td>Primary endpoint of the need of clinically-driven target lesion revascularisation sation (TLR). Secondary endpoints of MACE (death, MI, TLR). Both evaluated at one year.</td>
<td>Gain-of-function CYP2C19<em>17 allele carriers had a 37% relative reduction in the primary endpoint (TLR) (14.0% vs 22.3%), and a 22% relative reduction of the secondary endpoint (22.0% vs 28.1%) compared with noncarriers. ABCB1 and CYP2C19</em>2 carriage was not associated with TLR or MACE.</td>
<td>Primary endpoint – P= 0.002. Secondary endpoint – P= 0.04.</td>
</tr>
</tbody>
</table>

PCI- percutaneous coronary intervention; ST- stent thrombosis; MI- myocardial infarction; MACE- major adverse cardiac event; AF- atrial fibrillation; TIMI- thrombolysis in myocardial infarction; DES- drug eluting stent.

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Feer, Matetzky: Genetics of clopidogrel response

Platelet function testing: From bench to bedside

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Paraoxanase-1 Q192R genotypes, platelet response, and clinical outcomes

Very recently, Bouman et al. (38) described a new mediator of clopidogrel’s platelet effect, a genetic variant (Q192R) within the gene encoding paraoxanase-1 (PON1). Using in vitro metabonomic profiling technique, the authors identified PON1 as the crucial enzyme for the second step of clopidogrel bioactivation and, in contrast to previous studies (10, 12, 19, 29, 31, 33), found no evidence for the involvement of CYP2C19 in any of the steps of clopidogrel metabolism. In this study, the PON1 Q192R polymorphism was tested in a population of patients with CAD who underwent stent implantation. These patients had lower concentrations of clopidogrel’s active metabolite, lower platelet inhibition, and considerably higher risk of stent thrombosis. Contesting this provocative finding, Sibbing et al. (39) assessed the impact of PON1 Q192R and CYP2C19*2 genotypes on platelet aggregation and stent thrombosis. The clinical impact of genetic variants was investigated by comparing genotype frequencies of both genetic variants in a registry of 127 cases with early stent thrombosis versus an early stent thrombosis-free control cohort. For PON1 Q192R genotypes, platelet aggregation values (assessed with multiple electrode aggregometry) were similar across all genotype groups (p=0.65), whereas for CYP2C19*2 genotypes, significantly higher aggregation values were found in carriers of the *2 genotype compared with patients homozygous for wild-type alleles (p<0.0001). Comparing stent thrombosis cases and controls, no differences were observed for PON1 Q192R genotype distributions (p=0.23), whereas the genotype distribution differed for CYP2C19*2 genotypes (p=0.019). See Table 2 for an overview of studies evaluating genetic influences on outcome of clopidogrel-treated patients.

Clinical effect of other allelic variants

Variation in the levels of active metabolite generation following clopidogrel administration may also be due to variable or limited intestinal absorption. The C3435T polymorphism is associated with increased expression of P-GP, a membrane transport protein which results in reduced levels of environmental toxins and drugs, including clopidogrel (40). Simon et al. (12) found that acute MI patients with two variant alleles of ABCB1 (TT at nucleotide 3435) had a higher rate of death, non-fatal MI, or stroke at one year than those with ABCB1 wild-type genotype (15.5% vs. 10.7%; adjusted HR 1.72, CI 1.2–2.47). Also the TRITON-TIMI 38 showed a 72% increased risk of cardiovascular death, MI, or stroke at 15 months in these patients (41). However, a number of other studies have failed to show such an association (27, 34, 42, 43).

Clinical relevance

Functional variability in P450 isoenzyme activity may be influenced by drug-drug interactions. Exposure to rifampin and St. John’s wort, and smoking, have been shown to enhance clopidogrel anti-platelet activity (44, 45). Conversely, agents that inhibit CYP attenuate the antiplatelet effect of clopidogrel, as has been observed with the co-administration of proton-pump inhibitors, lipophilic statins, and calcium-channel blockers that are metabolised by the CYP3A4 isoenzymes (46–48). In addition, various epidemiologic and clinical factors, such as diabetes (49), renal failure (50), body mass index (51) and smoking (52) can substantially modify the antiplatelet effect of clopidogrel. Overall, Shuldiner et al. (31), in a genome-wide association study, found that CYP2C19 allelic variants accounted for about 12% of the variation in clopidogrel response, while most of the variation remained unexplained. While there is overwhelming evidence regarding the presence and clinical significance of inter-individual variability in platelet response to clopidogrel (53), the question of how much of this variability can be accounted for by allelic variants and, more importantly, whether routine individual genetic testing for allelic variants could affect clinical outcomes, remains to be determined. However, the introduction of newer and more potent antiplatelet agents (e.g. prasugrel and ticagrelor) which seem to be less affected by genetic polymorphisms (34, 41, 54) may obviate the need for genetic testing.

Conflict of interest

None declared.

References


