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Genetics of platelet inhibitor treatment

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Dual antiplatelet therapy with aspirin and a $P2Y_{12}$ receptor antagonist is the standard of care in patients undergoing percutaneous coronary intervention (PCI) and in patients with acute coronary syndromes (ACS) because this regimen has markedly decreased the rate of cardiovascular events. The substantial variability in pharmacodynamic response as well as the moderate antiplatelet efficacy of clopidogrel has raised major concerns, since high on-clopidogrel platelet reactivity has consistently been associated with increased risk for ischaemic events in PCI patients. Baseline demographic and clinical variables contributing to the observed variability have been identified. Besides this, research within the past decade has focused on the impact of genetic polymorphisms encoding transport systems or enzymes involved in the absorption and metabolism of these drugs. Loss-of-function polymorphisms in *CYP2C19* are the strongest individual variables affecting pharmacokinetics and antiplatelet response to clopidogrel, but explain no more than 5 to 12% of the variability in adenosine diphosphate-induced platelet aggregation on clopidogrel. No genetic variables contributing to clinical outcomes of patients treated with the newer $P2Y_{12}$ receptor antagonists, prasugrel or ticagrelor, have been identified so far. This review aims to provide an update on the current status of genotype-based personalized therapy with clopidogrel.

Introduction

Platelet activation and aggregation play an important role in the development of ischaemic events during and after acute coronary syndromes (ACS) and percutaneous coronary interventions (PCI) [1]. Acetylsalicylic acid (aspirin) was the first antiplatelet drug with proven benefit in ACS [2–4]. Studies demonstrating significant platelet activation in ACS and during PCI despite treatment with aspirin and intense anticoagulant regimens stimulated clinical studies investigating novel antithrombotic regimens using two antiplatelet drugs namely aspirin and the thienopyridine derivative ticlopidine. Ticlopidine is an irreversible inhibitor of the platelet $P2Y_{12}$ adenosine diphosphate (ADP) receptor [5, 6]. Compared with aspirin plus anticoagulation (heparin followed by vitamin K antagonists), dual antiplatelet therapy with aspirin and ticlopidine reduced not only the incidence of cardiac and vascular complications after the placement of coronary artery stents but also substantially the incidence of bleeding complications during follow-up [7]. Dual antiplatelet therapy with aspirin and a $P2Y_{12}$ receptor antagonist became therefore the standard of care for prevention of ischaemic complications in ACS patients and in patients undergoing PCI.

The clinical use of ticlopidine is hampered by serious haematological side effects. In clinical routine, ticlopidine has been widely replaced by clopidogrel which was approved in 1996/1997. Clopidogrel has demonstrated similar antiplatelet efficacy and an improved safety profile when compared with ticlopidine [8]. Two additional $P2Y_{12}$ receptor antagonists were approved within recent years by the regulatory agencies for treatment of patients with ACS, the thienopyridine derivative prasugrel (approval in

2009) and the cyclopentyltriazolopyrimidine ticagrelor (approved in 2010/2011).

A careful investigation of 700 aspirin-treated patients demonstrated that 'non-response' and/or a clinical meaningful variability in antiplatelet response is not an issue regarding aspirin [9]. Clinically meaningful residual arachidonic acid-induced platelet activation was found in approximately 2% of patients and was most likely caused by under-dosing and/or non-compliance. In contrast, a substantial variability of antiplatelet effect was observed in clopidogrel-treated patients and high on clopidogrel platelet reactivity was associated with increased risk of ischaemic events in patients after PCI with stent placement [10–13].

Low systemic availability of the active metabolite of clopidogrel has been associated with blunted antiplatelet effect and adverse ischaemic events [14, 15]. Single nucleotide polymorphisms (SNP) in genes encoding for drug transporters and drug metabolizing enzymes have been linked to antiplatelet response of clopidogrel. Therefore, current evidence regarding the genetics of antiplatelet drugs with focus on clopidogrel is the topic of this review.

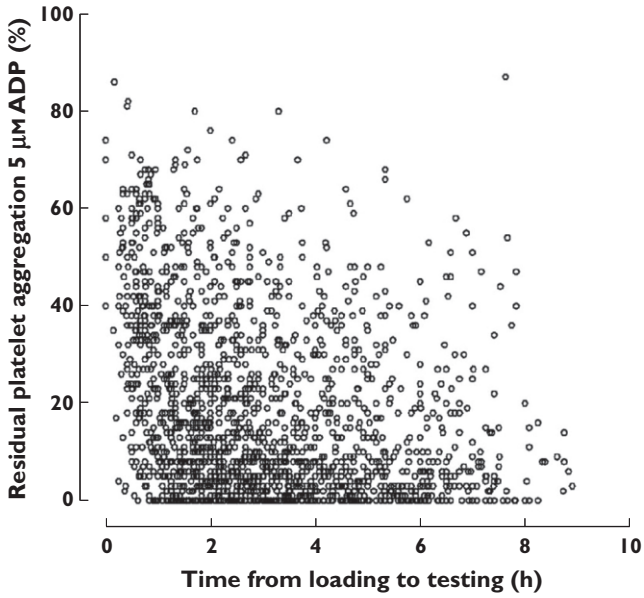

Figure 1

Residual platelet aggregation induced by 5 μM ADP in an unselected cohort of 2040 patients at elective cardiac catheterization dependent on time since administration of a loading dose of clopidogrel 600 mg [88]. ADP, adenosine diphosphate

Therapeutic limitations of clopidogrel

Despite the widespread clinical use of clopidogrel for more than 15 years, large scale clinical studies assessing the antiplatelet efficacy of the drug were performed within the last decade. Pharmacodynamic studies demonstrated only a moderate inhibition of ADP-induced platelet aggregation and a substantial variability of effect in clopidogrel-treated patients (Figure 1) [16–18]. High on-treatment platelet reactivity (HTPR) was associated with an increased incidence of adverse ischaemic/thrombotic events in patients after PCI [10, 12]. A white paper summarized the platelet function data from 28 studies comprising 11 477 patients on clopidogrel [19]. HTPR was confirmed as an important risk factor for ischaemic events post-PCI irrespective of the clinical presentation of the patient (elective PCI, PCI in ACS w/o ST-segment elevation myocardial infarction). In contrast, risk of bleeding might be increased in patients with an exaggerated response to clopidogrel [20].

Various factors have been identified that contribute to this variability. Clinical and demographic variables such as older age (>65 years), increased body mass index, diabetes mellitus, reduced left ventricular function, renal failure (serum creatinine >1.5 mg dl^{-1}) and presentation with ACS as well as drug–drug interactions were identified as predictors of HTPR [21, 22]. Clopidogrel is an inactive pro-drug requiring *in vivo* metabolism for formation of the active metabolite. Since the antiplatelet effect of clopidogrel is related to the amount of metabolite formed, recent research has focused on genetic factors with an impact on the activity of clopidogrel metabolizing enzymes and on bioavailability.

Metabolism of $P2Y_{12}$ receptor antagonists

All thienopyridines (ticlopidine, clopidogrel and prasugrel) are inactive pro-drugs requiring metabolic activation *in vivo*. The active metabolites formed bind irreversibly to the $P2Y_{12}$ receptor and therefore inhibit ADP-induced platelet activation for the life span of the platelet.

Clopidogrel is absorbed rapidly after oral ingestion. The majority of clopidogrel absorbed (~85%) is hydrolyzed by human carboxylesterase 1 (CES1) which is primarily located in the liver to an inactive acid metabolite [S26334] (Figure 2) [23, 24]. Formation of the active metabolite is catalyzed by various cytochrome P450 (CYP) enzymes with formation of 2-oxo-clopidogrel as an intermediate metabolite. While CYP1A2, CYP2B6, CYP2C19 contribute to formation of 2-oxo-clopidogrel, CYP2B6, CYP2C9, CYP2C19 and CYP3A4/5 are involved in the subsequent formation of the active metabolite [24–26]. *In vitro* experiments indicate that overall CYP2C19 is the main CYP contributing to the formation of the active metabolite of clopidogrel [26]. Furthermore, approximately 50% of 2-oxo-clopidogrel formed is metabolized

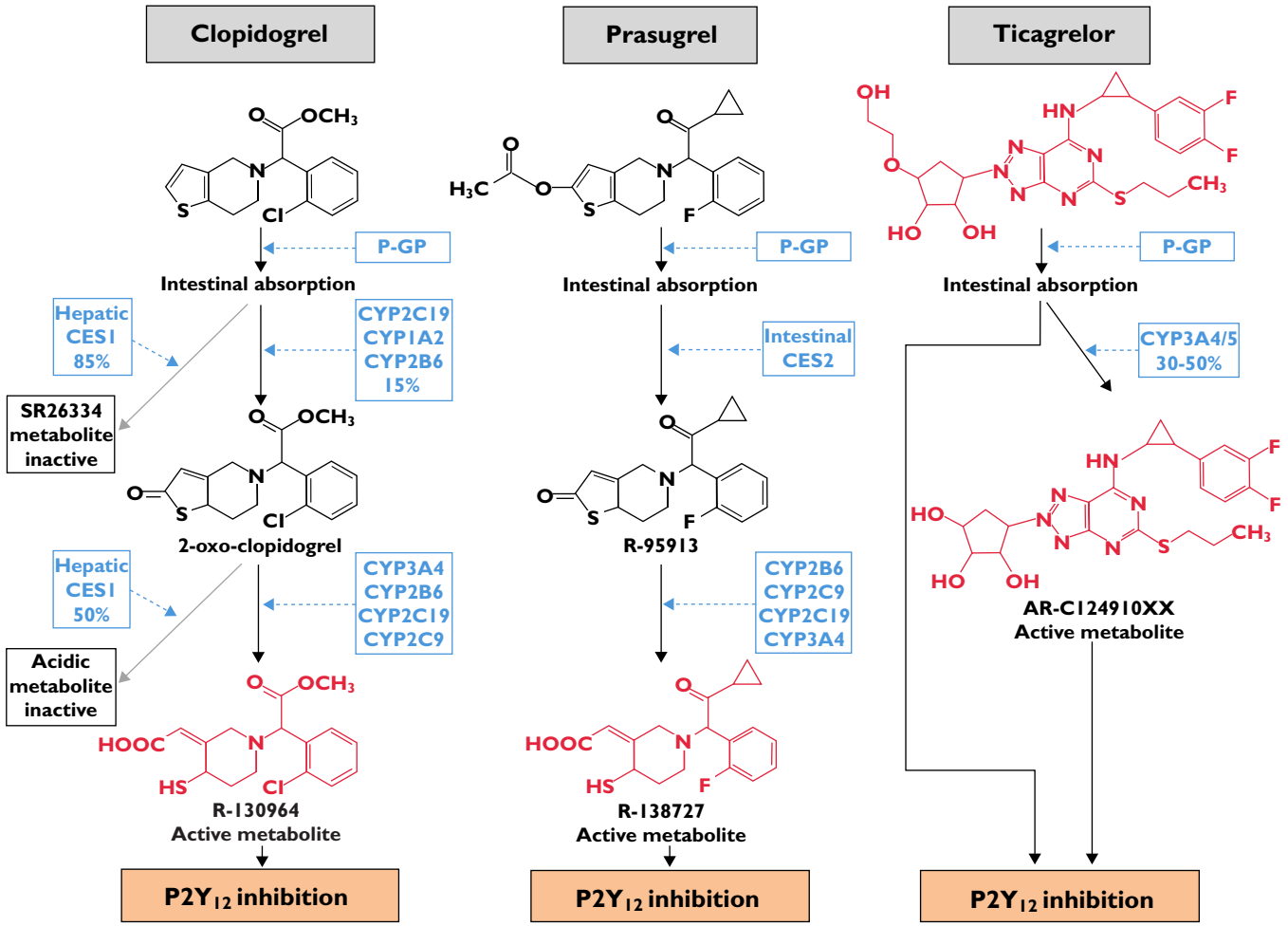

Figure 2

Metabolism of clopidogrel, prasugrel and ticagrelor [23, 24, 26, 27, 30]. Compounds with $P2Y_{12}$-receptor inhibiting properties are in red. CES, human carboxylesterase; CYP, cytochrome P450; P-GP, P-glycoprotein

by CES1 to an inactive compound thus limiting the amount of active metabolite available for an antiplatelet effect.

Prasugrel is the most recently approved member of the thienopyridine-class of platelet inhibitors. It is rapidly absorbed and similar to clopidogrel is a substrate of intestinal P-glycoprotein. After absorption, prasugrel is extensively metabolized (Figure 2), and parent drug is not detected in human or animal plasma. The intermediate thiolactone metabolite of prasugrel is formed via hydrolysis predominantly by intestinal human carboxylesterase 2 (CES2) while CYP2B6, CYP2C9, CYP2C19 and CYP3A4 catalyze the second reaction in the formation process of the active metabolite. In contrast to clopidogrel, CYP3A4 and CYP2B6 are the main contributors to the formation of the active metabolite of prasugrel with smaller contributions by CYP2C9 and CYP2C19 [27]. It is estimated that at least 50 to 70% of the dose administered are converted into the active metabolite. *In vitro* experiments with washed human platelets yield similar EC_{50} values of the active metabolites of prasugrel and clopidogrel for inhibition of ADP-induced aggregation and provide evidence that the more efficient generation of the active metabolite is responsible for the superior antiplatelet efficacy of prasugrel *in vivo* [28].

Ticagrelor is a member of the new cyclopentyltriazolopyrimidine class of agents. In contrast to the thienopyridines, ticagrelor is a direct-acting drug which interacts with the $P2Y_{12}$ receptor via reversible, non-competitive binding to a ligand-site distinct from the binding site of ADP [29]. Ticagrelor is also a substrate of P-glycoprotein. Approximately 30 to 50% of ticagrelor are eliminated via CYP3A4/5 metabolism (Figure 2) and the metabolite formed exerts antiplatelet effects, too [30]. Due to its reversible binding characteristics, restoration of platelet function after cessation of ticagrelor is

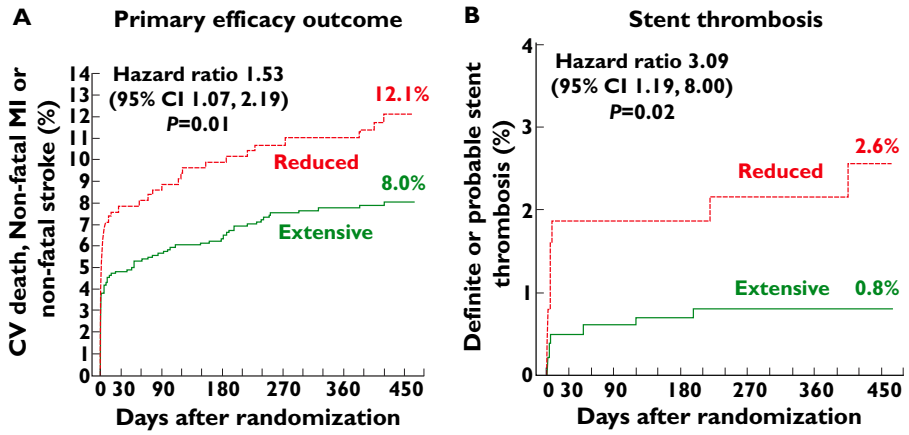

Figure 3

Risk for efficacy outcomes in non-carriers (CYP2C19*1/*1, *1/*17 and *17/*17) *vs*. carriers of CYP2C19 reduced function alleles (carriage of either *2, *3, *4,*5 or *8 allele) treated in TRITON-TIMI 38 with clopidogrel [38]. A) Risk for the primary efficacy outcome of death from cardiovascular causes, myocardial infarction or stroke (n = 1459; n = 395 carriers of at least one CYP2C19 reduced-function allele and n = 1064 non-carriers). B) Risk of stent thrombosis (n = 1389; n = 375 carriers of at least one CYP2C19 reduced-function allele and n = 1014 non-carriers)

dependent on the rate of elimination of the parent compound and respective active metabolite.

Genetic polymorphisms and clopidogrel

CYP2C19 loss of function alleles

The pro-drug characteristics of clopidogrel, the dependence of the antiplatelet activity of clopidogrel on hepatic metabolism and the contribution of cytochrome P450 enzymes to this metabolic activation has already been determined in early pre-clinical studies [31, 32]. The two separate oxidative steps required for formation of the active metabolite are dependent on various CYP enzymes (CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP3A4/5) and the genes encoding these enzymes are polymorphic. Evidence for the dominant contribution of CYP2C19 was derived in a pharmacodynamic study [33] and was confirmed in preclinical studies later [24–26]. A genome-wide association study (GWAS) identified only single nucleotide polymorphisms on chromosome 10q24 within the *CYP2C18–CYP2C19–CYP2C9–CYP2C8* cluster as being associated with clopidogrel response [34]. At least 25 SNPs of the gene encoding the CYP2C19 isoenzyme have been described (http://www.cypallelees.ki.se/cyp2c19.htm). The fully functional and most prevalent version of the gene is CYP2C19*1. Variant alleles for which reduced or loss of function (LoF) have been shown comprise CYP2C19*2 to *8. The *2 allele is the most frequent variant allele (15%) in the Caucasian and the African population while *3 to *8 have only very minor clinical impact due to their low frequency (<1%). However, frequency of the *2 and the *3 alleles is much higher in the Asian population (*2: 27%, *3: 9%). The LoF SNP in the CYP2C19*2 gene variant is a G681A mutation (rs4244285) in exon 5 which encodes for a cryptic splice variant resulting in no enzyme activity *in vivo*, while a G636A mutation in exon 4 results in a premature stop codon in CYP2C19*3 [35–37].

The clinical impact was initially observed in pharmacodynamic studies which showed that carriage of CYP2C19 LoF alleles is associated with a decreased antiplatelet activity of clopidogrel [11, 33]. Subsequently, pharmacokinetic/pharmacodynamic studies linked the attenuated efficacy of clopidogrel to a decreased systemic availability of the active metabolite [38]. Clinical studies showed that patients undergoing PCI carrying CYP2C19 LoF alleles are at increased risk for adverse cardiovascular events (Figure 3) [38–41]. A meta-analysis comprising 9685 cardiac patients with the vast majority (91%) enrolled after PCI confirmed this association and provided some evidence for a gene–dose effect [42]. The risk of major adverse cardiovascular events and, in particular, the risk of stent thrombosis was increased in carriers of one or two reduced-function CYP2C19 alleles (Table 1).

The PREDICT (Residual Platelet Aggregation after Deployment of Intracoronary Stent) score provides an estimate for prediction of antiplatelet response after administration of a loading dose of clopidogrel 600 mg. The score comprises the weighted contribution of non-genetic variables and carriage of CYP2C19*2 [43]. The predictive value of CYP2C19 LoF alleles as well as baseline and demographic variables for an insufficient antiplatelet response were analyzed to determine the contribution of these factors to variability in antiplatelet response. Carriage of a

Table 1

Incidence of cardiovascular death, myocardial infarction or ischaemic stroke and stent thrombosis by CYP2C19 genotype comprising 9685 patients

	Hazard ratio	95% confidence interval	*P* value
Cardiovascular death, myocardial infarction or ischaemic stroke			
Carriers of one or two CYP2C19 alleles with reduced function *vs.* non-carriers	1.57	1.13, 2.16	0.006
Carriers of one CYP2C19 allele with reduced function *vs.* non-carriers	1.55	1.11, 2.17	0.01
Carriers of two CYP2C19 alleles with reduced function *vs.* non-carriers	1.76	1.24, 2.50	0.002
Stent thrombosis			
Carriers of one or two CYP2C19 alleles with reduced function *vs.* non-carriers	2.81	1.81, 4.37	<0.00001
Carriers of one CYP2C19 allele with reduced function *vs.* non-carriers	2.67	1.69, 4.22	<0.0001
Carriers of two CYP2C19 alleles with reduced function *vs.* non-carriers	3.97	1.75, 9.02	0.001

Determined CYP2C19 alleles with reduced function: CYP2C19*2, CYP2C19*3, CYP2C19*4, CYP2C19*5 and CYP2C19*8. Data adopted from Mega *et al.*, 2010 [42].

CYP2C19*2 allele was the strongest individual factor contributing to variability in the *ex vivo* determined platelet-inhibitory effect of clopidogrel, but this polymorphism accounts only for 5.2 to 12% of the variation of ADP-induced aggregation [34, 43, 44]. Overall, CYP2C19*2 carrier status together with all demographic and clinical predictors for high on-clopidogrel could only explain no more than 12–22% of platelet reactivity on treatment with clopidogrel [34, 44].

CYP2C19 gain of function alleles

Polymorphisms within the CYP2C19 system comprise not only LoF alleles but also a gain-of-function (GoF) mutation (CYP2C19*17). This allelic variant, a C806T mutation in exon 5, is responsible for an increased catalytic activity most likely due to a higher transcription rate of the gene [45]. Carriage of CYP2C19*17 allele(s) should result in an increased antiplatelet effect of clopidogrel due to an enhanced formation of the active metabolite which in turn may potentially expose the patients to an increased risk of bleeding. However, data on the impact of CYP2C19 GoF SNPs are conflicting. While three independent studies showed an association of CYP2C19*17 allele carriage with lower on-clopidogrel platelet reactivity [46–48], CYP2C19*17 was not associated with ADP-stimulated platelet aggregation in healthy Amish subjects treated with clopidogrel [34]. However, an independent association of CYP2C19*17 allele carriage with risk of bleeding was observed in a large registry cohort as well as in the genetic substudy of the PLATO trial in patients randomized to clopidogrel [49, 50]. Two recently published meta-analysis confirmed that carriers of the CYP2C19*17 allele had a lower risk of cardiovascular events but an increased risk of bleeding [51, 52].

ABCB1

Clopidogrel is a substrate of the intestinal ATP-dependent efflux transporter P-glycoprotein (MDR-1) and an increased activity of this transport system decreases bioavailability of clopidogrel and other substrates [53, 54]. P-glycoprotein is encoded by the *ABCB1* gene which is expressed highly polymorphic with more than 50 variants residing in the coding region [55]. Various studies investigated the impact of the C3435T SNP on pharmacokinetics and/or antiplatelet effect of clopidogrel as well as the impact on clinical outcome in patients on clopidogrel.

Taubert and co-workers investigated the antiplatelet effects and pharmacokinetics of clopidogrel after 300, 600 and 900 mg bolus doses of clopidogrel and their association with ABCB1 C3435T genotype [53]. C_{max} and AUC values of clopidogrel and its active metabolite in the 300 mg and 600 mg groups (but not in the 900 mg group) were lower in subjects homozygous for the ABCB1 C3435T variant alleles compared with subjects with the 3435C/T and 3435C/C genotypes. However, subsequently performed clinical studies yielded conflicting results. Carriers of one or two ABCB1 C3435T variant alleles in the FAST-MI registry had an increased risk of the combined endpoint of cardiovascular death, myocardial infarction, or stroke [40] while an increase in risk in the genetic substudy of the TRITON–TIMI 38 study was evident only in TT homozygotes treated with clopidogrel [56]. ABCB1 C3435T genotype was not associated in two studies with ADP-stimulated platelet aggregation and patients with the C/C genotype on treatment with clopidogrel in the PLATO genetic substudy had the highest risk for the combined ischaemic endpoint [34, 50, 57]. A recently performed meta-analysis also failed to show an association between ABCB1 C3435T genotype and risk of overall recurrent ischaemic events, stent thrombosis or bleeding in clopidogrel treated patients [58].

Only one study analyzed the association between G2677T SNP and platelet reactivity in a small cohort of patients after elective PCI. This study could not show any difference in platelet reactivity between the genotypes [59].

Carboxylesterase 1 G143E

The vast majority of absorbed clopidogrel is shunted by carboxylesterase 1 (CES1) to inactive metabolites. Thus, inhibition of CES1 *in vitro* increased formation of the active metabolite of clopidogrel [60]. A missense single nucleotide polymorphism (SNP) in exon 4 of CES1 (rs71647871) results in a catalytic site glycine (G)-to-glutamic acid (E) amino acid change at position 143 (G143E). The G143E mutation severely decreased CES1 catalytic function [61]. A recently published study showed that systemic availability of the active metabolite of clopidogrel was increased and ADP-induced platelet aggregation was amplified in carriers of the CES1 143E-allele [62]. Due to lack of adequately powered studies it is currently unknown whether CES1 G143E mutations impact on clinical outcome of clopidogrel treated patients.

PON1

Paraoxonase-1 (PON1) is an esterase synthesized in the liver and associated with HDL-cholesterol in blood [63]. The catalytic activity of PON1 is determined by a common SNP in PON1 c.575A>G resulting in an amino acid substitution Gln>Arg at position 192 (Q192R). A case-cohort study in survivors of a stent thrombosis on clopidogrel and matched controls linked PON1 Q192R polymorphism with the antiplatelet activity of clopidogrel and the risk of stent thrombosis [64]. The link between PON1 Q192R polymorphism and the risk of cardiovascular events on treatment with clopidogrel was supported by a replication study using a prospective cohort of 1982 individuals with ACS. Furthermore, investigation of the pharmacokinetics of clopidogrel, *ex vivo* determination of ADP-induced platelet aggregation and a series of *in vitro* experiments using microsomal expression system of metabolizing enzymes corroborated the hypothesis. The authors suggested that PON1 and not CYP2C19 seems to be the key enzyme involved in metabolism of 2-oxo-clopidogrel to the active metabolite. They estimated that more than 70% of variability in platelet aggregation could be attributed to PON1 Q192R polymorphism which thus seems to be the primary predictor of clopidogrel response [64]. A series of other studies and a meta-analysis did not find an association between PON1 Q192R polymorphism either with the antiplatelet effect of clopidogrel regardless of the test used or with risk of cardiovascular events in patients treated with clopidogrel [59, 65–70]. The reason for the discrepancy between the original work and the subsequently performed studies is unclear up to now. It was hypothesized that differences in the study populations might be an issue because the distribution of Q192R genotypes in the study of Bouman *et al*. [64] differed from the subsequently performed studies [59, 65–70]. The specificity of the analytical methods used by Bouman *et al*. [64] in their *in vitro* studies was questioned by Dansette and co-workers [71]. These authors investigated the impact of CYP enzymes and PON1 on the complex stereochemistry of the metabolism of 2-oxo-clopiodgrel to the active thiol metabolite. They showed that formation of the most active *cis*-diastereoisomers of the thiol metabolite of clopidogrel is catalyzed by CYP enzymes. In contrast, contribution of esterases such as PON1, results in formation of a *trans*-diastereoisomer metabolite which exhibits only minor antiplatelet properties [71].

Pharmacogenetics of prasugrel and ticagrelor

The impact of the ABCB1 C3435T polymorphism and SNPs in genes encoding for cytochrome P450 enzymes was analyzed in various studies within the clinical study programme of the new $P2Y_{12}$ receptor antagonists, prasugrel and ticagrelor. Neither the pharmacodynamics nor the pharmacokinetics of prasugrel or ticagrelor were altered in patients with this genetic background [72–74]. A recent study showed that the antiplatelet effect of prasugrel might also be modulated by CYP2C19*2 and *17 if platelet reactivity index vasodilator-stimulated phosphoprotein (PRI VASP) is used for assessment [75]. The latter observation requires confirmation since the same authors reported in a preceding paper that this association holds true only for PRI VASP assay while no significant influence of CYP2C19 genotype on platelet reactivity assessed by ADP-induced platelet aggregation was observed [76].

At present, there are no data supporting any impact of genetic polymorphisms in pharmacokinetics of prasugrel or ticagrelor on clinical outcomes of patients. Large subgroups of patients enrolled in the large scale phase III clinical outcomes studies TRITON-TIMI 38 (prasugrel) and PLATO (ticagrelor) consented for additional genetic analysis. Prasugrel as well as ticagrelor provided overall a significant beneficial effect by reducing the incidence of the combined endpoint (cardiovascular death, myocardial infarction, stroke) compared with clopidogrel [77, 78]. The observed net clinical benefit of prasugrel/ticagrelor over clopidogrel was not affected by ABCB1 C3435T polymorphism or carriage of CYP2C19 LoF allele(s) [50, 73]

Current status of therapeutic strategies based on genotype

Triggered by the compelling data on the association between CYP2C19 LoF polymorphism and increased risk for ischaemic events in clopidogrel-treated patients after PCI the FDA announced a 'Boxed warning' on March 12, 2010, informing physicians and patients about the reduced effectiveness of clopidogrel in patients with an impaired ability to convert the drug into its active form (FDA 03-12-2010). This warning was based upon the CYP2C19 LoF genetic background of attenuated antiplatelet efficacy of clopidogrel and triggered several actions:

- US [American College of Cardiology Foundation/American Heart Association (ACCF/AHA)] and European Society of Cardiology (ESC) implemented recommendations for genotyping in their respective guidelines [79, 80]. However, one has to admit that classes of recommendation and levels of evidence attributed to genotyping as part of antiplatelet therapy are weak (ACCF/AHA: class IIb, level of evidence C; ESC: class IIb, level of evidence B).
- Bedside tests for the rapid assessment of CYP2C19*2 genotype were developed.
- Clinical studies were initiated to investigate if HTPR in carriers of CYP2C19 LoF allele(s) can be overcome by high dose regimens of clopidogrel or treatment with alternate $P2Y_{12}$ receptor antagonists.

Clinical implementation of CYP2C19 genotyping

A prerequisite for implementation of genotype based antiplatelet therapy especially in patients presenting with ACS is the option for a simple bedside assay which can be performed in the coronary angiography laboratory with a rapid turnover of the test and, of course, reliability of test results.

At present, at least two point-of-care assays are available for CYP2C19*2 genotyping: the Verigene CYP2C19 XP system (Nanosphere, Northbrook, IL, USA) and the Spartan RX CYP2C19 system (Spartan Bioscience Inc, Ottawa, Ontario, Canada). The CE-certified SPARTAN RX assay uses a buccal swap and results are available within 60 min. CYP2C19*2 carrier status is provided as homozygous for the wild-type allele (*1/*1), heterozygous (*1/*2) or homozygous for the *2 allele (*2/*2). Assessment of the technical performance of the SPARTAN RX assay provided a sensitivity of 100% (95% CI 92.3, 100) and a specificity of 99.3% (95% CI 96.3, 100) using direct DNA sequencing as reference. A proof of concept study (RAPID GENE) was published recently [81]. Patients undergoing PCI for ACS or stable angina were randomly assigned to rapid CYP2C19*2 allele screening at randomization or conventional treatment with subsequent CYP2C19*2 genotyping. Patients randomized to rapid screening and carrying CYP2C19*2 alleles were treated with prasugrel 10 mg daily, while non-carriers and patients in the conventional treatment arm received clopidogrel 75 mg daily. The proportion of patients with HTPR (PRU >234 by VerifyNow P212™ test) was analyzed after 1 week of treatment. None of the CYP2C19*2 carriers in the rapid genotyping group treated with prasugrel had HTPR, while 30% of CYP2C19*2 patients in the conventional group treated with clopidogrel had HTPR. However, 9.9% and 9.4% of non-carriers of CYP2C19*2 alleles in both groups had HTPR after 1 week of clopidogrel treatment.

The Verigene CYP2C19 XP assay is performed in a blood sample from the patient and provides data on the presence of either CYP2C19*2 or *3 allele(s). Turnaround time is about 3 h.

The key question is what will be the role for genotyping in personalized antiplatelet therapy given the background of the new $P2Y_{12}$ receptor antagonists, prasugrel and ticagrelor? The more potent $P2Y_{12}$ receptor antagonists prasugrel or ticagrelor decreased the incidence of ischaemic endpoints but at the cost of a slightly increased rate of non-CABG related major bleedings [77, 78]. These benefit–risk considerations and the availability of substantially less expensive generic clopidogrel in most countries provide an attractive stimulus for optimizing the therapeutic benefit of clopidogrel not only from a pharmacoeconomic point of view.

So far, only pharmacodynamic studies investigating treatment algorithms based on genotype are available. Three non-randomized open label studies determined antiplatelet response to increased loading doses of clopidogrel [82–84]. The frequency of patients with high on clopidogrel platelet reactivity can be reduced in CYP2C19*2 carriers by increasing the loading dose of clopidogrel. Bonello and co-workers used a target approach: They administered additional day by day 600 mg loading doses of clopidogrel up to a total dose of 2400 mg until the pre-defined antiplatelet effect assessed by vasodilator-stimulated phosphoprotein (VASP) assay was obtained. This attempt was successful in 88% of patients carrying at least one CYP2C19*2 allele and in five out of six CYP2C19*2 homozygous patients [83].

ELEVATE-TIMI used a randomized, double-blind study design to investigate if increasing the maintenance doses of clopidogrel up to 300 mg daily can improve platelet reactivity in CYP2C19*2 carriers with stable cardiovascular disease [85]. A maintenance dose of 225 mg clopidogrel daily provided in CYP2C19*2 heterozygotes levels of platelet reactivity assessed with the VerifyNow P212™ assay which were similar to that determined on the standard 75 mg dose in non-carriers. It seems that CYP2C19*2 homozygous patients did not achieve degrees of platelet reactivity comparable with non-carriers despite treatment with clopidogrel doses up to 300 mg daily. However, the number of patients with this genotype was insufficient to draw any firm conclusions.

There are no studies available that investigated the time course of platelet reactivity in patients with CYP2C19 LoF genotype in the first days after switching from clopidogrel to prasugrel or ticagrelor. The RAPID GENE study showed that a superior antiplatelet effect is achieved at day 7 after switching from clopidogrel to prasugrel 10 mg daily in CYP2C19*2 patients [81]. There is no dedicated switching study from clopidogrel to ticagrelor in CYP2C19*2 patients available. A pharmacodynamic study confirmed that the antiplatelet response to ticagrelor is not influenced by CYP2C19 genotype [74].

Summary and conclusions

High on-treatment platelet reactivity is associated with increased risk for ischaemic events in patients undergoing PCI with stent placement. Data supporting the impact of ABCB1 genotype on antiplatelet effect and clinical outcome of patients are controversial while the majority of studies obviate any impact of the PON1 Q192R polymorphism. So far, no clinical outcome studies investigating the contribution of carboxylesterase 1 G143E genotype are available.

Carriage of CYP2C19 LoF allele(s) is the strongest individual variable associated with HTPR on clopidogrel but CYP2C19*2 carrier status accounted for only 5 to 12% of the variability in on-clopidogrel platelet reactivity. Easy to use validated bedside tests for rapid assessment of this genetic background are available. Prospective double-blind randomized clinical outcome studies should be the next step for assessment of the clinical utility of personalized CYP2C19 genotype-guided antiplatelet therapy and patients presenting with ACS should be the target patient population. Currently, a combination of genotyping and phenotyping (i.e determination of platelet reactivity) seems to be the most promising approach to personalize antiplatelet therapy and improve efficacy and safety. This approach should be compared with the one size fits all strategy by using the new $P2Y_{12}$ platelet antagonists prasugrel or ticagrelor in ACS patients. At present, the latter strategy is favoured above clopidogrel in the current guidelines of ACCF/AHA and ESC for ACS [79, 86, 87]. In order to progress pharmacogenetics in antiplatelet therapy, utilization of next generation sequencing technologies seems promising after clear phenotype definitions balancing risk of thrombosis *vs*. bleeding risk have been determined.

Competing Interests

Both authors of the paper have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted manuscript. DT reported receiving consulting fees or paid advisory board fees and lecture fees from Eli Lilly, Daiichi Sankyo, AstraZeneca, Bayer Vital, and lecture fees from Boehringer Ingelheim KG, Bristol Myers Squibb, and Merck Sharp & Dohme in the previous 3 years. WH reported serving on a consultant/advisory board for Sanofi-Aventis in the previous 3 years.

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