Genetic variability in response to clopidogrel therapy and its clinical implications

Liana Yukhanyan1; Matthias K. Freynhofer1; Jolanta Siller-Matula2; Karsten Schrör3; Kurt Huber1
13rd Department of Medicine, Cardiology and Emergency Medicine, Wilhelminen Hospital, Vienna, Austria; 2Department of Cardiology, Medical University of Vienna, Vienna, Austria; 3Institute for Pharmacology und Clinical Pharmacology, Heinrich-Heine-Universität, Düsseldorf, Germany

Summary
This article concentrates on individual genetic differences responsible for variations of action of clopidogrel, which have been found to be partially responsible for increased cardiovascular events in patients with coronary artery disease under dual antiplatelet therapy. According to these results, genotyping for the relevant gene polymorphisms, especially for the CYP2C19 loss-of-function alleles, has been discussed to be an effective method of individualising and optimising clopidogrel treatment. However, due to the facts that 1) there are no prospective studies demonstrating a clinical benefit of personalising antiplatelet therapy based on genotyping; 2) CYP2C19 polymorphisms account for only approximately 12% of variability in clopidogrel platelet response; 3) the positive predictive value of CYP2C19 loss-of-function polymorphisms for cardiovascular events in patients with acute coronary syndrome undergoing percutaneous coronary intervention is only approximately 12% – 20%; 4) it is likely that other clinical factors and risk constellations might be of greater clinical importance; and 5) it is unknown whether a specific genetic polymorphism is capable of influencing outcome for the individual patient; genetic profiling cannot be recommended for routine use at present but will remain of considerable scientific interest.

Keywords
Antiplatelet agents, coronary syndrome, gene mutations, thrombosis

Introduction
Dual antiplatelet therapy (DAPT) with aspirin and clopidogrel is the current standard of treatment both in patients with acute coronary syndromes (ACS), namely unstable angina (UA), non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI) and also in stable patients referred for percutaneous coronary intervention (PCI) and stent implantation (1–3). DAPT has demonstrated important clinical benefit by significantly reducing the risk of hard clinical endpoints i.e. myocardial infarction (MI) by 25% and death by 20%, respectively (4). Furthermore, DAPT has effectively reduced the risk of thrombotic complications such as early or late stent thrombosis among patients undergoing PCI and coronary stenting (5).

Unfortunately, despite the consequent use of DAPT, the rate of adverse cardiovascular events (ACE), namely cardiovascular death, MI, stroke, early or late stent thrombosis remains high (>10%) (4, 6–8). Moreover, it is obvious that these cardiovascular events may be associated with a poor response to clopidogrel raising the concern of clopidogrel ‘resistance’ (4, 9). More than 40 million patients around the world receive clopidogrel but, based on different platelet function assays, about 20–30% of clopidogrel-treated patients have been defined as either poor- or non-responders, which has been associated with an increased risk of recurrent cardiovascular events, especially in patients undergoing PCI (10–13).

Reasons for a less than expected action of clopidogrel are multifactorial and besides under-dosing, non compliance, drug-drug-interactions, and several co-morbidities (e.g. diabetes mellitus) include several gene polymorphisms that interact with intestinal absorption, metabolic activation in the liver and pharmacodynamics (5, 14).

This article concentrates on genetic disorders responsible for such variations of action of clopidogrel.

Methods
Literature from 2006 to February 2011 was reviewed. Various internet sources such as PubMed, Medline or Medscape were used. Important scientific journals such as The New England Journal of Medicine, The Lancet, European Heart Journal as well as most American Heart Association journals were screened. Prospective clinical controlled trials, peer-reviewed full articles, letters and
meta-analyses have been selected if they appeared to have a similar content to the objective of the study. The following keywords were used: clopidogrel, CYP2C19, CYP2B6, CYP1A2, CYP2C9, CYP3A4, ABCB1 genes as well as polymorphism in the ADP-receptor P2Y12 gene, respectively.

**Genetic disorders as cause of high on-treatment platelet reactivity**

Genetic disorders, particularly genetic polymorphisms in genes coding for isoenzymes of the CYP family involved in metabolism, transport proteins involved in drug transport across biological membranes and/or target proteins for the drug ADP-receptor P2Y12, may have a significant influence on clopidogrel responsiveness (21). Recent studies have especially concentrated on polymorphisms of CYP2C19 isoenzyme and have demonstrated a relationship between carriage of CYP2C19 loss-of-function alleles and a higher rate of ACE (22).

The first study, which has shown a relationship between CYP2C19 loss-of-function polymorphisms and diminished response to clopidogrel, was conducted among young healthy male volunteers (23).

Carriers of at least one CYP2C19 reduced-function allele (approximately 25–30% of the Caucasian population) exhibited about 30% reduction of the plasma concentration of the active metabolite levels of clopidogrel as compared to non-carriers (24–26). This was associated with 25% relative reduction in platelet aggregation response to clopidogrel and high on-treatment platelet reactivity (25, 26, 27). Moreover, carriers of one of the CYP2C19 loss-of-function alleles or any two of them (*2, *3, *4, or *5) were associated with a significantly higher rate of ACE than carriers without them (21.5% vs. 13.3%) (14, 24, 26, 28).

Thereby, CYP2C19*2 seems as the most frequent mutant allele (95%) (26, 29) and the major determinant among the carriers with high on-treatment platelet reactivity (30). The first genome-wide association study (The Amish Pharmacogenomics of Anti-platelet Intervention [PAPi]-Study) has proved that CYP2C19*2 has counted for 12% of the variation in response to clopidogrel and has determined that approximately 30% of the general population with CYP2C19*2 mutant allele have had poorer platelet response and relatively increased risk of ACE (20.9% vs. 10%) (30).

Occurrence of stent thrombosis under DAPT is more often seen in carriers of both of CYP2C19*2 and CYP2C9*3 loss-of-function alleles (31–33). According to the study of Harnsze et al. even the single-nucleotide polymorphism of CYP2C9*3 has led to two-fold increased risk of stent thrombosis. Consequently, CYP2C9*3 is an independent risk factor for high on-treatment platelet reactivity (31).

Carriers of a reduced function CYP2B6 allele have also demonstrated lower plasma exposure of the active metabolite of clopidogrel (a relative reduction of 15.7%) and less reduction of platelet aggregation in response to clopidogrel (26). Alleles in CYP2C18, CYP2C19*3 and CYP2C8 are associated with diminished response to clopidogrel, with a high degree of statistical significance (30, 34). Nevertheless, no significant correlation between these genetic variants and occurrence of stent thrombosis can be shown (31). The other CYP enzymes and alleles either have shown no polymorphisms (CYP3A4) or have not been associated with pharmacokinetic and pharmacodynamic responses to clopidogrel (CYP3A5, CYP1A2, CYP2C19*4, *5, *6) (26, 27).

**Clopidogrel**

Clopidogrel is an ADP receptor inhibitor (15) and a prodrug undergoing two steps of hepatic biotransformation by cytochrome P-450 (CYP) enzymes to its active metabolite, which inhibits platelet activation through an irreversible blockade of the platelet (ADP) P2Y12 receptor (16). After intestinal absorption, the majority (85%) of the prodrug is metabolised and inactivated by esterases, and only the remaining 15% of clopidogrel is transformed into the intermediate 2-oxo-clopidogrel metabolite (17). This intermediate metabolite is further hydrolysed into the highly unstable active thiol derivative R-130964, which inhibits platelet aggregation through an irreversible blockade of the ADP P2Y12 receptors on the platelet surface (14).

As already is mentioned, two sequential oxidative steps in the liver are required to transform clopidogrel into its active metabolite (11). The first metabolic step, which leads to 2-oxo-clopidogrel, is catalyzed by three isoenzymes (CYP1A2, CYP2B6 and CYP2C19), whereas the second step, which forms the active metabolite involves four isoenzymes (CYP2B6, CYP2C9, CYP2C19 and CYP3A4) (18). The CYP2C19 isoenzyme is involved in both sequential metabolic steps, contributing to an estimated 45% of 2-oxo-clopidogrel metabolite and 21% of its conversion to the active metabolite (18). Accordingly, CYP2C19 genotype represents a major factor contributing to clopidogrel response variability and may, therefore, affect clinical outcome particularly in patients who undergo PCI (19).

**Clopidogrel “resistance”**

At present, a clear and precise definition of poor- or non-response to clopidogrel does not exist and the term “resistance” seems to be a source of controversy and confusion (7, 9). According to different observations, the term “resistance” can be divided into clinical “resistance” meaning that a cardiovascular event occurs in a patient despite the use of DAPT (4) and laboratory “resistance”, meaning that cardiovascular event is not or only poorly visible despite the use of DAPT anticipated platelet aggregation response measured by platelet function tests (20). It should be noted that these two forms of resistance are not necessarily in parallel (4).
In contrast to the above mentioned genetic variants, CYP2C19*17 has been encoded for an increased enzyme activity due to an enhanced transcription rate (35). Thus, CYP2C19*17 carriers are extensive metabolizers resulting in increased antiplatelet response to clopidogrel (27, 35). There is evidence in the literature that this superior antiplatelet effect may be associated with an increased risk of bleeding (36).

However, no association has been found with polymorphisms of the P2Y12 receptor or with glycoprotein IIb/IIIa receptors (14).

According to these results, genotyping for the relevant gene polymorphisms may be an effective method of individualizing and optimizing clopidogrel treatment (37, 38).

**Ethnicity**

One of the causes for variable response to clopidogrel is ethnicity. CYP2C19 genetic polymorphism has a wide interethnic variability, ranging from 20–30% among Caucasians to 30–45% among African-Americans and approximately 50–65% in East Asians (5, 26). However, CYP2C19*2 seems to be the most frequent defective allele (75–85% in Caucasians and East Asians) for inter-ethnic differences and is responsible for diminished pharmacokinetic and pharmacodynamic responses to clopidogrel by approximately 25–30% (26).

**Under-dosing and polymorphisms**

Despite the fact that observational studies have indicated association between clopidogrel low- or non-responsiveness and ACE, most of these studies are limited by varying clopidogrel dosing (39).

Recent randomised clinical studies have indicated that there is a clopidogrel dose-effect relationship (40, 41).

Carriers of the CYP2C19*2 and *4 alleles have shown reduced platelet inhibition after a 600-mg clopidogrel loading dose (LD) but have responded to higher LD and maintenance dose (MD) regimens (21). Occurrence of ACE was also significantly lower in carriers who have received up to three additional 600-mg LDs of clopidogrel versus a control group, in which clopidogrel dose was not adapted (42). Moreover, using up to three additional 600-mg LD of clopidogrel may enable 88% of CYP2C19*2 carriers to overcome high on-treatment platelet reactivity and to reach higher platelet inhibition (41).

The results were similar in respect to higher MD (150 mg/day) of clopidogrel versus a usual MD (75 mg/day) (8). The results of the CURRENT-OASIS 7 study with 25,086 patients have shown no significant dose-effect relationship regarding cardiovascular death, MI or stroke (4.2% vs. 4.4%; \(p=0.61\)), but there was a significant reduction of the occurrence of stent thrombosis (1.6% vs. 2.3%; \(p=0.001\)) (43).

Thus, increase in dosing of clopidogrel in carriers with high on-treatment platelet reactivity can increase its antiplatelet effect and reduce the risk of ACE (37, 42), but often it does not lead to a complete restoration of clopidogrel function in low-responders.

**Poor absorption**

Variants of genes (e.g. ABCB1) modulating clopidogrel absorption can also have an impact on clopidogrel response (6, 44).

A study conducted by the French Registry of Acute ST-Elevation or Non-ST-Elevation Myocardial infarction (FAST-MI) with 2,208 subjects has shown that carriers with two mutant alleles of ABCB1 have higher rates of ACE (15.5% vs. 10.7%). Moreover, the presence of any two CYP2C19 loss-of-function alleles (*2,*3,*4, or *5) and either one or two ABCB1 mutant alleles has been associated with the highest risk for cardiovascular death, MI, or stroke (14).

In the PLATO-trial with 13,408 patients occurrence of cardiovascular events was consistently lower in carriers with CYP2C19 genotype versus in those who have shown additional ABCB1 genotype (11.2% vs. 11.9%) (45).

Data regarding ABCB1 polymorphism as an independent factor are significantly different. Specifically, various studies have shown that there is no considerable effect on platelet inhibition as an independent factor (14, 46).

Meanwhile, data of the study by Mega et al. which assessed the effect of ABCB1 polymorphisms both separately and in combination with CYP2C19 mutant alleles seems more realistic. The results showed that ABCB1 and CYP2C19 genotypes are independent predictors, and carriers of those mutant alleles are at increased risk of cardiovascular death, MI, or stroke (47).

Accordingly, in addition to CYP2C19, ABCB1 genotype allows better prediction of clopidogrel high on-treatment platelet reactivity (48).

**Prasugrel, ticagrelor and influences of genetic variants**

At present, new ADP receptor antagonists, such as prasugrel and ticagrelor have been investigated and are commercially available (prasugrel) or close to commercial use (ticagrelor). Both agents have demonstrated better clinical results compared to clopidogrel (49, 50). The main advantage compared to clopidogrel is the reduced platelet function variability, which is also explained by low importance of genetic variations (51).

Similar to clopidogrel, prasugrel is converted into its active metabolite by cytochrome P450 (CYP) enzymes (52). As a first step, the produg is hydrolysed by carboxylesterases into an intermediate metabolite, which is then catalysed to the active metabolite by five different CYP isoenzymes (52). This second oxidative step is performed with major contributions from the CYP3A4/5 and CYP2B6 isoenzymes and minor contributions from CYP2C19 and CYP2C9. Most importantly, these isoenzymes can be compensated...
by each other (53). As a consequence, genetic polymorphisms of single CYP-enzyme alleles do not affect the generation of the active metabolite of prasugrel (53).

These hypotheses have meanwhile been confirmed by a study from Varenhorst et al. which demonstrated that loss-of-function polymorphism of CYP2C19 has led on the one hand to a reduced concentration of the active metabolite of clopidogrel and a corresponding reduction of platelet inhibition, while there was no such impact on active metabolites of prasugrel and related inhibition of platelet function (53). Moreover, prasugrel-treated carriers of the CYP2C19 loss-of-function allele are not at increased risk of MI, stent thrombosis or the other cardiovascular complications (17, 19).

Unlike clopidogrel and prasugrel which bind irreversibly to the platelet surface P2Y12 receptors, ticagrelor is a reversible active agent that does not require metabolic activation (54).

Different studies have proved that treatment with ticagrelor has been associated with more rapid and greater platelet inhibition compared to clopidogrel in both clopidogrel responders and non-responders (55). These effects may explain a reduced rate of cardiovascular events in patients treated with ticagrelor compared to clopidogrel (50). Consequently, treatment with ticagrelor is more effective for ACS than treatment with clopidogrel, irrespective of CYP2C19 and ABCB1 polymorphisms (56).

Conclusion

Based on the fact that in the majority of studies CYP2C19 loss-of-function polymorphisms have shown to reduce clopidogrel metabolism and its clinical effectiveness, personalised therapy has been discussed to help improve clinical outcome after stent implantation.

However, for the clinical role of genetic profiling multiple unknown factors still remain (57). At present, no prospective studies exist to demonstrate a clinical benefit of personalised antiplatelet therapy based on genotyping. Moreover, commercially available genetic tests that can determine CYP2C19 genotype variants are not routinely reimbursed. In addition, CYP2C19 polymorphisms account for only approximately 12% of variability in clopidogrel platelet response (30), and the positive predictive value of CYP2C19 loss-of-function polymorphisms for cardiovascular events in patients with ACS undergoing PCI is approximately only 12–20% (19, 30). Among demographic and clinical factors age, body mass index, and especially co-existing diabetes mellitus impact significantly on platelet reactivity in treatment with clopidogrel (58–60).

Furthermore, there is no evidence that a specific genetic polymorphism is capable of influencing clinical outcome for the individual patient. Genetic profiling as a routine measure to identify patients at an increased risk for future cardiovascular events and to tailor the most reliable antiplatelet strategy is currently investigated by interventional trials and will serve also as basis for future research.

Conflict of interest

None declared.

References


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