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Clinical Pharmacokinetics and Pharmacodynamics of Clopidogrel

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Abstract

Acute coronary syndromes (ACS) remain life-threatening disorders that are associated with high morbidity and mortality. Dual-antiplatelet therapy with aspirin and clopidogrel has shown to reduce cardiovascular events in patients with ACS. However, there is substantial inter-individual variability in response to clopidogrel treatment in addition to prolonged recovery of platelet reactivity as a result of irreversible binding to $P2Y_{12}$ receptors. This high inter-individual variability in treatment response has primarily been associated with genetic polymorphisms in the genes encoding for cytochrome (CYP) 2C19 that affect clopidogrel's pharmacokinetics. While FDA has issued a boxed warning for CYP2C19 poor metabolizers due to a potentially reduced efficacy in these patients, results from multivariate analyses suggest that additional factors, including age, sex, obesity, concurrent diseases and drug-drug interactions, may all contribute to the overall between-subject variability in treatment response. However, the extent to which each of these factors contributes to the overall variability and how they are interrelated is currently unclear. The objective of this review article is to provide a comprehensive update on the different factors that influence clopidogrel's pharmacokinetics and pharmacodynamics and how they mechanistically contribute to inter-individual differences in response to clopidogrel treatment.

1. Introduction

Cardiovascular disease (CVD) is currently the leading cause of death worldwide [1]. Many CVD patients develop acute coronary syndrome (ACS), a life threatening condition encompassing myocardial infarction (MI) with or without ST-segment elevation (STEMI/NSTEMI), or unstable angina [1]. Approximately 1.2 million ACS patients are being hospitalized in the United States every year for cardiovascular events [2]. Elevated platelet aggregation and subsequent thrombus formation play a critical role in the pathophysiology of these patients. As a consequence, safe and effective antiplatelet therapy is essential for reducing the high morbidity and mortality of this disease [3]. Clopidogrel (Plavix®), which was the second largest selling branded drug in the US in 2010 with $8.8 billion in sales, is an irreversible $P2Y_{12}$ receptor antagonist indicated for reduction of arteriosclerotic events in patients with recent stroke or MI, and established peripheral arterial disease [4, 5].

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Clopidogrel is a second generation thienopyridine that has largely replaced ticlopidine, a first generation thienopyridine with similar efficacy, due to improved tolerability, reduced incidence of haematological side effects, more rapid onset of action and a convenient (once-daily) dosing regimen [6]. In recent years, dual antiplatelet therapy with aspirin and $P2Y_{12}$ receptor antagonists clopidogrel, prasugrel or ticagrelor has become the clinical gold standard for patients with ACS and/or undergoing percutaneous coronary interventions (PCI) due to the significant improvement of long-term clinical outcome [1, 3, 7–9]. Although clopidogrel is safe and effective in many patients, there is substantial variability in treatment response between individuals [10]. Some of these patients continue to have cardiovascular events despite clopidogrel treatment [11]. This lack of efficacy has, in part, been attributed to the reduced response to clopidogrel in patients, resulting in high on-treatment platelet reactivity (HPR) and the development of atherothrombotic complications [3]. This relative non-responsiveness to clopidogrel therapy has been coined “clopidogrel resistance” and is thought to affect 5–44% of patients receiving standard-dose clopidogrel treatment [11]. On the other hand, some patients also experience drug-induced bleeding due to excessive platelet inhibition [7].

Clopidogrel is an inactive pro-drug that requires enzymatic conversion into its active metabolite by a series of cytochrome P450 (CYP) enzymes [12]. Clinical evidence suggests that patients with deficient CYP2C19 activity (e.g. poor metabolizers or as a result of drug-drug interactions) have remarkably higher on-treatment platelet reactivity, which puts them at an increased risk of ischemic events following the standard dosing regimen, prompting the U.S. FDA to issue a boxed warning [13–16]. However, the results from a multivariate analysis of the Pharmacogenomics of Antiplatelet Intervention (PAPI) study revealed that CYP2C19 polymorphisms are responsible for about 12% of the between-subject variability in response to clopidogrel treatment, whereas age or body mass index (BMI) accounted for 3.8% and 2.3% of the variability, respectively [14]. Similar findings have been reported from other studies, which all indicate that, in addition to CYP2C19 polymorphism, multiple demographic and disease risk factors contribute to the interindividual variability in response to clopidogrel treatment [15–19]. However, the underlying mechanisms related to each of these intrinsic and extrinsic factors are not yet fully understood. It should be noted at this point though that the also the assays that have been used to determine the response to clopidogrel treatment are subject to substantial between-assay variability.

The objective of this review is to comprehensively evaluate the different sources of variability in PK and PD and how they mechanistically relate to interindividual differences in response to clopidogrel treatment. We attempt to do so in a systematic fashion by providing an overview of the known genetic and non-genetic factors that contribute to interindividual differences in clopidogrel’s pharmacokinetics (PK) and pharmacodynamics (PD) and to how they relate to clinical outcome.

2. Clopidogrel pharmacokinetics and pharmacodynamics

Following oral administration, about 50% of the dose is absorbed from the intestine based on urinary metabolites data [20]. Results from *in vitro* studies show that the uptake of clopidogrel into Caco-2 cells is limited by P-glycoprotein (P-gp, ABCB1), which suggests

that P-gp may affect the intestinal absorption and oral bioavailability of clopidogrel [21]. Once delivered to the liver, a number of cytochrome P450 (CYP) enzymes, including CYP2C19, CYP1A2, CYP2B6, CYP2C9 and CYP3A4, mediate the bioactivation of clopidogrel via a two-step process. First, 2-oxo-clopidogrel, an intermediate and pharmacologically inactive metabolite, is formed, which is then further converted into the pharmacologically active metabolite R-130964 (clop-AM) [12]. At the same time, a large portion of the absorbed clopidogrel (at least 85 – 90%) undergoes first-pass metabolism in the liver, where it is hydrolyzed by carboxylesterase 1 (CES1) to the inactive carboxylic acid metabolite SR26334 [22, 23]. As a consequence, only about 2% of the administered clopidogrel dose is converted to clop-AM and reaches the systemic circulation [20]. It should be noted at this point that CES1 also hydrolyzes 2-oxo-clopidogrel and clop-AM [23, 24].

In vitro enzyme kinetics studies revealed that CYP1A2 (35.8%), CYP2B6 (19.4%) and CYP2C19 (44.9%) contribute to the formation of 2-oxo-clopidogrel, whereas CYP2B6 (32.9%), CYP2C9 (6.79%), CYP2C19 (20.6%) and CYP3A4 (39.8%) contribute to the formation of active metabolite clop-AM, respectively [12]. It is estimated that CYP2C19 contributes to about 50% to the overall formation of clop-AM from clopidogrel and thus plays a substantial role in clopidogrel's bioactivation, whereas the other isozymes contribute to a lesser extent. There is conflicting data available in the literature on whether or not these biotransformation pathways can be saturated, i.e. whether or not clopidogrel and its active metabolite exhibit linear pharmacokinetics. While data from a variety of studies suggests that clopidogrel and its major inactive metabolite SR26334 exhibited linear PK across a wide range of doses (50 – 900 mg) [25–27], data by Wallentin *et al.* [28] and Collet *et al.* [29] reported a ~4-fold and ~2-fold increase in clop-AM AUC when increasing the clopidogrel dose from 75 mg to 600 mg and from 300 mg to 900 mg, respectively, Horenstein *et al.* reported that an increase in the clopidogrel dose from 75 to 150 or 300 mg led to ~1.5- and ~2.2-fold increase of clop-AM AUC in CYP2C19 extensive, intermediate and poor metabolizers, respectively [30]. These findings support the presence of nonlinearity in clopidogrel's bioactivation processes.

Upon activation, clopidogrel exhibits its pharmacodynamic effect by specifically and irreversibly binding to $P2Y_{12}$, a subtype of the adenosine diphosphate (ADP) receptor, on the surface of platelets [3, 31]. $P2Y_{12}$ is a G_i-protein coupled receptor. Activation of the $P2Y_{12}$ receptor triggers a complex cascade of intracellular events, resulting in reduced protein kinase (PKA) phosphorylation of vasodilator-stimulated phosphoprotein (VASP) and subsequent activation of the glycoprotein (GP) IIb/IIIa receptor, granule release, amplification of platelet aggregation, and stabilization of the platelet aggregate (Fig. 1). Irreversible binding of clop-AM to the $P2Y_{12}$ receptor consequently results in an inactivation of the GP IIb/IIIa receptor and destabilization of the thrombus for the lifespan of the platelets [3, 10, 31]. It should be noted though that other physiological agonists, such as thromboxane A_2 (cf. aspirin), thrombin, collagen, serotonin (5-HT), also contribute to platelet activation. Therefore, any factors influencing the $P2Y_{12}$-dependent and/or $P2Y_{12}$-independent signal transduction pathways that impact the platelet activation should be considered when evaluating the responsiveness of patients to clopidogrel treatment and clopidogrel resistance.

3. Assays used to determine clopidogrel resistance and HPR

Several *ex vivo* platelet function assays have been developed to assess patients' responsiveness to clopidogrel treatment and, ultimately, to determine which patient is at an increased ischemic risk [22]. Light transmittance aggregometry (LTA) and a variety of other methods measure overall platelet function [3, 22], whereas the vasodilator stimulated phosphoprotein—platelet reactivity index (VASP-PRI) assay specifically determines clopidogrel induced $P2Y_{12}$ inhibition [32–34]. Each of these assays has its advantages and limitations and none of them has been fully standardized or readily accepted for determining clopidogrel non-responsiveness [3, 11]. This is due to the fact that different agonists at in part different doses were used, lack of reproducibility and comparability between assays, and that different cut-off values for defining HPR were applied making a direct comparison of the different tests with respect to the determined impact on platelet reactivity and corresponding efficacy and safety outcomes difficult [22].

Measurement of ADP-induced platelet aggregation in platelet-rich plasma by LTA assay has long been the gold-standard for assessing platelet function in relation to clinical outcome. In most studies, 5, 10 or 20 μmol/L of ADP was employed and respective cut-off values have been proposed to define HPR [22]. The specificity of LTA assay is confounded by the fact that other ADP receptor subtypes (e.g. $P2Y_1$) can also contribute to platelet aggregation [3]. The utility of LTA assay is also limited by its labor intensive setup, operator dependent results and inconsistency between laboratories [22, 35]. The VerifyNow $P2Y_{12}$ assay, on the other hand, is a fast and standardized point-of-care method that determines platelet-induced aggregation in the whole blood by ADP and prostaglandin E_1 (PGE_1) in order to increase the specificity to $P2Y_{12}$ pathway. However, the experimental results of VerifyNow $P2Y_{12}$ assay may be influenced by non-platelet blood components (e.g. hemoglobin) [3, 32]. The same holds true for other point-of-care whole blood platelet tests, such as the Impact-R, PFA-100, the Plateletworks tests and Multiplate analyzer [3, 22, 36]. All of these point-of-care assays are relatively new and in need of more extensive qualification. Alternatively, the VASP-PRI assay measures the phosphorylation state of VASP, a specific intracellular marker of the residual $P2Y_{12}$ receptor reactivity, using flow cytometry [32–34]. However, this assay is time consuming and requires experienced staff [22, 36]. It has also been reported that the sensitivity and specificity of VASP-PRI assay in prediction of cardiovascular adverse events was lower than the ADP-stimulated platelet function assays, suggesting that specific determination of VASP pathway may overlook the contribution of alternative mechanism to platelet activation [15, 37].

In addition, a given assay may yield different results following multiple measurements in the same subject, which further complicates the establishment of a robust link between *ex vivo* assay readouts and HPR. For example, results from the recent ELEVATE-TIMI 56 study indicate that the response of 16 to 20% of the patients receiving a 75 mg clopidogrel maintenance dose differed when measured at different times. In fact, 33 to 50% of the patients who were originally classified as non-responder at first before they had to be re-classified as responder following a second measurement using the same assay, vice versa; and about 40% of the patients showed a larger than 40 scores change in PRU following serial measurement using the VerifyNow $P2Y_{12}$ assay [38].

4. Covariates that affect clopidogrel dose/PK/PD

4.1 Demographic factors

4.1.1 Age—Several studies reported a significant association between older age and higher prevalence of high on-treatment platelet reactivity following clopidogrel treatment [14, 19, 39–41]. On the other hand, clinical outcome studies revealed that old age is associated with a substantial increase in both cardiovascular events [5, 16, 42–44] and bleeding [45, 46] following clopidogrel treatment. These findings suggest that dose adjustment may become more necessary in the elderly than in younger patients to optimize platelet inhibition while avoiding bleeding events.

4.1.2 Body weight—Obesity has been shown to significantly affect clopidogrel response. Several studies report that BMI or body weight is associated with HPR in both patients and healthy subjects [14, 41, 47]. A recent clinical PK study reported that, compared to patients with lower body weight (56.4 ± 3.7 kg), patients with higher body weight (84.7 ± 14.9 kg) had about 30% lower clop-AM plasma AUC, which ultimately led to a higher on-treatment platelet reactivity in these obese patients (VerifyNow P2Y12 reaction reading in obese patients was 207 PRU, whereas that in patients with normal weight was 152 PRU) [48]. This variability can at least partially be attributed to the lower body weight normalized dose in higher body weight patients compared to lower body weight ones (1.33 vs. 0.89 mg/kg). In addition, down regulation of CYP enzymes in obese subjects (e.g. CYP2C9, CYP2C19 and CYP3A4) may also play role as it leads to reduced bioactivation [49]. Some recent studies also showed that expression level of CES1, which governs clopidogrel elimination from the body, was significantly elevated in obese subjects and could be reversed by diet-induced weight loss [50, 51], indicating that the impact of obesity in clopidogrel response may be associated with multiple mechanisms. However, the link between obesity and treatment outcome seems inconclusive and an “obesity paradox” has been reported from several clinical investigations, where patients with lower BMI (normal and underweight) had an increased risk of bleeding as well as elevated clinical outcome including death and MI than the obese ones, due to the fact that, in these studies, higher BMI was associated with a trend towards male patients with younger ages, who usually show the tendency of seeking for medical care earlier and more aggressive initial management compared to older subjects [16, 42, 45, 52, 53]. On the other hand, a LEADERS trial that was conducted in clopidogrel discharged patients reported that, the obese individuals (BMI > 30 kg/m^2) had significantly more major adverse cardiac events than those non-obese ones[54]. It was noteworthy that the obese patients involved in this study had significantly higher rate of diabetes mellitus, which itself also has been shown to significantly impact clopidogrel resistance [55, 56]. Thus, a more thorough investigation may be necessary for distinguishing the true contribution of obesity in clopidogrel resistance.

4.1.3 Sex—The impact of sex on clopidogrel’s efficacy and safety has been investigated in different clinical settings. It has been reported that the systemic clop-AM exposure was similar in men and women [19]. Many clinical studies revealed that clopidogrel PD was not different between males and females [14, 39, 57], whereas some others reported a significant decreased risk of HPR in males compared to females [40, 58]. Results from the FAST-MI

clinical trial and another clinical study conducted in patients undergoing PCI both reported that females had lower risk of cardiovascular events than males (including death, MI or stroke) [16, 42]. Female sex was associated with an increase in bleeding from REPLACE-2 and ISAR-REACT 3 clinical trials [45, 59], indicating that sex might play a role in clopidogrel clinical outcome, which may be associated with the “one size fits all” dosing of clopidogrel in all patients and the relatively lower body weight of female patients compared to male patients. On the other hand, results from several other clinical studies suggest that, compared to other factors, the impact of sex on clopidogrel clinical outcome is minimal [5, 43, 52, 60, 61].

4.2 Genetic polymorphisms

4.2.1 Genetic polymorphisms that affect clopidogrel PK

4.2.1.1 ABCB1: The *ABCB1* C3435T mutation has been associated with changes in the intestinal efflux of drugs and thus their oral bioavailability [62]. However, its impact on clopidogrel’s PK/PD and clinical outcome remains controversial. A clinical PK study conducted in patients undergoing PCI reported that following administration of a single clopidogrel loading dose (300 mg or 600 mg), peak plasma concentrations (C_{max}) and area under the curve (AUC) of clopidogrel and clop-AM were significantly lower in 3435T/T homozygotes than that in 3435C/T heterozygotes and C/C (wild type) homozygotes, suggesting a change in oral bioavailability due to enhanced clopidogrel efflux for the C3435T mutation [21]. However, these results could not be reproduced by subsequent studies following clopidogrel 75 or 150 mg maintenance doses [16, 19]. Similarly, several studies in both healthy adults and patients undergoing PCI failed to show a clear correlation between C3435T polymorphism and HPR following either loading or maintenance doses of clopidogrel [19, 39, 60, 63]. The association between C3435T mutation and cardiovascular risk is also inconsistent [16, 60, 64, 65]. These conflicting findings on the impact of ABCB1 C3435T mutation for cardiovascular outcome was evaluated in two meta-analyses showing that this mutation is unlikely to play a major in between subject variability in response to clopidogrel treatment [66, 67].

4.2.1.2 CES1: Hepatic CES1 is a serine hydrolase with broad substrate spectrum, which is involved in biotransformation of both endobiotic and xenobiotic substrates. In addition to its role in cholesterol metabolism and trafficking, CES1 also processes metabolism and bioactivation of numerous drugs, such as clopidogrel, methylphenidate, and oseltamivir [23]. A recent *in vitro* study reported that hydrolysis of clopidogrel and 2-oxo-clopidogrel was completely impaired in CES1 G143E allelic isozyme; suppression of CES1 activity greatly enhanced generation of 2-oxo-clopidogrel and clop-AM from clopidogrel in human liver S9 fraction [23]. Consistently, further analysis of PAPI study data revealed that, following clopidogrel treatment, CES1 143E-allele carriers have significantly higher clop-AM levels resulting in a more pronounced PD response than patients that are homozygous for CES1 143G (wild-type) [68]. In patients with acute coronary disease under clopidogrel treatment, the on-treatment platelet reactivity in the individuals carrying the CES1 143E-allele was also significantly lower than that in 143G-homozygotes [68]. On the other hand, the CES1A -816A/C allele, which has been reported to cause significantly enhanced transcriptional activity of CES gene [69], has been found to be associated with either significantly increased

or reduced on treatment platelet reactivity in patients with coronary heart disease [70, 71]. These findings suggests that more research needs to be done to conclusively characterize the impact of genetic polymorphism in CES1 on clopidogrel response.

4.2.1.3 CYP enzymes: CYP2C19 is one of the most important polymorphic CYP enzymes across different populations. To date, over 20 genetic variants have been identified for the CYP2C19 gene. CYP2C19*2 (G681A) and CYP2C19*3 (G636A) mutations are the two most functionally important variants, which in combination account for more than 90% of CYP2C19 loss-of-function (LOF) alleles, whereas other CYP2C19 LOF alleles occur far less frequently [72, 73]. On the other hand, the gain-of-function mutation CYP2C19*17 (C806T) has been associated with elevated enzyme expression and thus increased catalytic capacity [74]. The PAPI study and several other clinical PK/PD investigations conducted in healthy volunteers all revealed that the subjects carrying CYP2C19 reduced-function alleles (e.g. CYP2C19*2 or CYP2C19*3) had significantly lower systemic exposure to clop-AM and anti-platelet aggregation effect compared to wild-type individuals [4, 30, 75–77]. Similarly, studies conducted in patients with cardiovascular diseases treated with clopidogrel all reported that CYP2C19 reduced-function alleles were associated with significantly higher on-treatment platelet reactivity [17, 18, 39, 58, 78–82] and worse clinical outcomes including cardiovascular death, MI, stroke and ST [14, 16, 18, 65, 78, 81, 82], as confirmed by several meta-analyses [83–85]. In comparison, the impact of CYP2C19*17 mutation on clopidogrel PK may be minimal, as shown in PAPI study, which reported that clop-AM levels were similar in subjects carrying CYP2C19*17 alleles and the corresponding peers carrying CYP2C19*1 allele [77]. Conflicting results have been also reported in regard to the association between CYP2C19*17 allele and enhancement of platelet inhibition, reduction of major cardiovascular risk or increase in bleeding events from different studies [15, 65, 74, 77, 83, 86, 87]. A recent pharmacogenetic study identified a linkage between *CYP2C19*17* allele and *CYP2C19*4*, a loss-of-function mutation, which suggest that the high metabolic capacity of CYP2C19*17 carriers is altered if these subjects show also the *CYP2C19*4B* haplotype [88]. As a result, further studies are needed to fully delineate the overall impact of new CYP2C19 genotype/haplotype on clopidogrel's treatment response.

No clear association was found between genetic polymorphism of CYP2B6, CYP2C9, CYP1A2 and CYP3A4, which all contribute to clopidogrel bioactivation to some extent *in vitro* [12], and clopidogrel PK, PD as well as clinical outcome. Results from *in vitro* studies indicate that both clopidogrel and 2-oxo-clopidogrel irreversibly inhibit CYP2B6 [89, 90]. An *in vivo* study also revealed that repeated dose (4 days) of clopidogrel significantly suppressed the CYP2B6-catalyzed bupropion hydrolyzation in healthy adults [91], suggesting that long term exposure to clopidogrel may suppress the function of CYP2B6, which consequentially attenuates the impact of CYP2B6 polymorphism. Consistently, reports from two clinical studies showed that the CYP2B6 reduced-function alleles (*1B, *1C, *5, *6, *9 or *13) had a significant impact on clopidogrel bioactivation and PD following short-term clopidogrel treatments, but didn't impact the long-term clopidogrel PD or clinical outcome [18, 39]. Conflicting findings have been reported when assessing the impact of CYP2C9 polymorphism on clopidogrel PK, PD and clinical outcome [18, 61, 75, 80]. No significant association with CYP1A2 polymorphism has been reported [17, 18, 75,

80]. Inconsistent results have also been reported for the impact of CYP3A4/5 polymorphism on clopidogrel PD or clinical outcome [18, 61, 75, 80, 81, 92, 93]. Nevertheless, a clinical PK study conducted in healthy volunteers revealed that CYP3A4 inhibitor itraconazole showed stronger inhibitory effect on clopidogrel PD in healthy volunteers carrying CYP3A5 non-expressor genotype than those carrying CYP3A5 expressor genotype [93], another CROSS-VERIFY clinical study also reported that calcium channel blocker amlodipine, which is a CYP3A4 inhibitor, only exhibited adverse effect on clopidogrel response and clinical outcome in CYP3A5 non-expressors as CYP3A5 may act as a "backup system" once CYP3A4 is inhibited [94], suggesting a potential interplay between CYP3A4 and CYP3A5 functional variations.

4.2.1.4 Paraoxonase-1 (PON1): Paraoxonase-1 (PON1) is an aromatic esterase that is thought to have antioxidant and cardioprotective properties [95]. It has been reported that its gain-of-function mutation Q192R and elevated PON1 activity were both associated with a significantly lower incidence of major adverse cardiovascular events [95]. Bouman *et al.* first reported that PON1 Q192R mutation was identified as a new determinant in converting clopidogrel to clop-AM and risk of ST in patients undergoing PCI [24]. However, inconsistent results were observed in several subsequent studies when assessing the association between PON1 Q192R mutation and clop-AM formation, antiplatelet activity or clinical outcome [65, 76, 79, 82, 96–98], suggesting that the role of PON1 on clopidogrel resistance may need further investigation.

4.2.2 Genetic Polymorphisms that affect clopidogrel PD—The interplay between ADP and $P2Y_{12}$ receptor that is located on the surface of platelet plays an essential role in platelet activation [3, 22]. To date, several $P2Y_{12}$ gene mutations have been identified and investigated with respect to their impact on clopidogrel resistance. However, their association with clopidogrel resistance is inconclusive. It has been reported that the frequency of the $P2Y_{12}$ H2 haplotype (consisting of intronic [i]-C139T, [i]-T744C, [i]-ins801A and G52T SNPs) was significantly higher in coronary artery disease (CAD) patients [99]. Although some clinical PK/PD studies revealed that the H2 haplotype was associated with HPR in both healthy subjects and patients undergoing PCI [100, 101], such relationship couldn't be demonstrated in several other studies [81, 102, 103]. H2 haplotype also failed to show impact on worse clinical outcome in clopidogrel treated patients undergoing PCI [104, 105]. Similarly, inconsistent results have also been reported when assessing the association between the $P2Y_{12}$ C34T mutation and clopidogrel PD or clinical outcome [16, 39, 106, 107]. A study conducted in Chinese ACS-PCI patients receiving clopidogrel treatment reported that the impact of *P2Y12* C34T mutation on clinical outcomes became significant only in patients also carrying CYP2C19*2 (G681A) allele [107]. Rudez *et al.* reported that the s6787801 mutation (c.−217+2739T>C) of $P2Y_{12}$ receptor was associated with significantly lower on-treatment platelet reactivity in 1031 clopidogrel treated CAD patients under PCI [108]. However, their one-year clinical follow-up study failed to show the impact of such mutation in cardiovascular events [109], whereas two other studies suggested that this mutation might be associated with a significantly increased HPR or rate of target vessel revascularization in patients undergoing PCI [57,

105]. Therefore, further studies are necessary for the establishment of the relation of $P2Y_{12}$ receptor genetic polymorphisms and clopidogrel non-responsiveness.

The ITGB3 gene encodes for the integrin β3 of the GP IIb/IIIa platelet receptor, which is the major membrane receptor for platelet aggregation. Inconsistent results have been reported when evaluating the association between ITGB3 and clopidogrel resistance. One study reported that ITGB3 PLA2 mutation carriers showed higher on-treatment platelet reactivity following clopidogrel loading dose (300 mg) [110]. Another study revealed that ITGB3 mutation was associated with decreased risk of early ST [104]. However, results from other studies suggested that there was no association between ITGB3 PLA2 mutation and clopidogrel response [16, 111]. In addition to $P2Y_{12}$ receptors, ADP also stimulates platelet $P2Y_1$ receptors, which causes platelet conformational change and initiate weak and transient platelet aggregation [3]. Yet, no association was observed between P2Y1 A1622G genotype and altered clopidogrel response in patients [61, 111, 112].

4.2.3 Race—Race is probably the most important demographic covariate that explains differences in response to clopidogrel treatment as it not only accounts for genetic differences between subjects but also for other associated factors, such as diet, life style, comorbidity and medical practice [113]. It is well-known that allele frequencies of CYP2C19 variants are subject to significant interracial differences. CYP2C19*2, the most frequent LOF allele, for example, is present in 13% of Caucasians, 20% of African-Americans and 28% of Asians. Other LOF mutations, such as CYP2C19*3, are also more prevalent in Asians compared to other racial groups (~5% vs. <1%) [72, 73]. CYP2C19*17, a gain-of-function mutation, on the other hand, is expressed to a lesser extent in Asians (~6%) compared to African-Americans (~18%) or Caucasians (~16%) [72]. In addition to CYP2C19, LOF mutations in other CYP enzymes involved in clopidogrel's biotransformation, such as CYP2C9*2 and *3, have also been reported to vary by race [114]. Substantial differences have also been reported in the allelic frequency of ABCB1 C3435T mutations in European Americans (62%) and in African Americans (13%) [115]. As a result, the prevalence of clopidogrel resistance is expected to be higher in Asians compared to Caucasians. In fact, several clinical studies conducted in Chinese, Japanese and Korean patients revealed that the frequency of clopidogrel resistance in Asian population ranged from 20 to 65%, which was remarkably higher than the results obtained from clinical trials that majorly included Caucasian patients [116]. However, direct comparison of these study result may not be feasible, since these studies have relatively small sample sizes, applied different HPR cut-off values, or utilized different clinical settings. In addition, difference in body weight, diet, life style and comorbidities in different racial groups should be also taken into consideration when investigating the impact of race factor in clopidogrel resistance.

4.3 Drug-Drug Interactions

Patients undergoing clopidogrel treatment are often required to take concomitant medication. Therefore, the PK and PD level drug-drug interactions (DDI) that affect plasma levels of clop-AM and platelet activation & aggregation may consequently all contribute to differences in clopidogrel response and clinical outcome.

4.3.1 Proton pump inhibitors—Since gastrointestinal (GI) bleeding is a common side effect of clopidogrel, in particular when combined with aspirin [117], proton pump inhibitors (PPIs) are often co-prescribed with clopidogrel and aspirin, which significantly decreased the drug-induced gastrointestinal bleeding [117, 118]. *In vitro* studies revealed that some of the PPIs, such as omeprazole, esomeprazole and lansoprazole, but not pantoprazole are mechanism-dependent inhibitors of CYP2C19, which suppress clopidogrel's bioactivation [12, 119]. Clinical PK/PD studies conducted in healthy subjects and patients all confirmed that concurrent omeprazole led to significant decrease in systemic exposure to clop-AM as well as in suppression of antiplatelet activity [120–123]. On the other hand, esomeprazole, but not other PPIs including dexlansoprazole and pantoprazole, significantly interfered with clopidogrel PK and PD [120–122]. Conflicting results have been reported when assessing impact of lansoprazole on clopidogrel PD [121, 123–125]. Interestingly, the inhibitory effect of omeprazole and lansoprazole on clopidogrel PD was diminished in *CYP2C19*2* carriers, suggesting the impact of PPIs on clopidogrel response may be dependent on CYP2C19 genotype [123, 125]. In 2009 and 2011, the FDA issued warnings to avoid concomitant use of omeprazole or esomeprazole with clopidogrel "because of the effect on clopidogrel's active metabolite levels and anti-clotting activity" [126, 127]. However, the impact of concomitant PPI on clopidogrel clinical outcome still remains controversial and the degree of interaction between PPIs and clopidogrel seems to depend on the PPI [118, 128, 129]. Although several recent meta-analyses showed that there is no clinically significant interaction between clopidogrel and PPI, which suggests that this combination is a safe treatment choice for patients at high risk of GI bleeding, these analyses face the following limitations: inclusion of lower risk population (e.g. only 42% were taking clopidogrel for ACS [128]), usage of fixed dose formulations or early termination of the study. [130–132]. As a result, the findings of these meta-analyses should be interpreted with caution and preference may be given to PPIs that minimally inhibit CYP2C19 for patients taking clopidogrel who are considered to be at increased risk of upper gastrointestinal bleeding.

4.3.2 Statins—Clopidogrel is often co-prescribed with 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors or statins. Concerns that these statins could potentially affect clopidogrel bioactivation and response have been voiced in recent years due to the fact that some lipophilic statins (e.g. atorvastatin, lovastatin and simvastatin) are majorly metabolized by CYP3A4. Other statin drugs including atorvastatin, simvastatin, rosuvastatin, lovastatin and pravastatin can induce CYP2B6 and CYP2C9 in addition to CYP3A4 via activation of the pregnane X receptor (PXR) [133, 134]. Two clinical studies reported that continuous treatment of 80 mg atorvastatin significantly enhanced clopidogrel bioactivation and efficacy in both healthy volunteers and PCI patients with or without diabetes mellitus [135, 136]. Several other clinical studies also suggested that atorvastatin didn't show negative effect or even showed positive effect on the antiplatelet activity of clopidogrel in patients [137–145]. Similarly, no other statins including lovastatin and simvastatin, fluvastatin, rosuvastatin or pravastatin have shown a significant effect on clopidogrel's antiplatelet activity [137–144]. These findings are in agreement with most clinical reports showing that concomitant atorvastatin and other statin drugs have no negative impact on clinical outcome in patients taking clopidogrel [141, 142, 145–148].

4.3.3 Calcium channel blockers—Some of calcium channel blockers (CCBs) including amlodipine, nicardipine and verapamil are CYP3A4 substrates and inhibitors [149]. It has been reported that concurrent use of CCBs was associated with significant decrease of the antiplatelet potency of clopidogrel and increase of cardiovascular risk in CAD patients [63, 150]. However, several subsequent studies showed that CCBs didn't affect clopidogrel's antiplatelet effect or clinical outcome in ACS patients [151–153]. Interestingly, the prospective POPular study reported that, in CAD patients undergoing PCI, concurrent CCBs were only significantly associated with both HPR and increased cardiovascular events (death, non-fatal MI, ST and ischemic stroke) in patients, who were CYP2C19*2-carriers but not in those, who were CYP2C19*2-noncarriers [154]. Similarly, a CROSS-VERIFY clinical study also revealed that amlodipine only had a significant impact on clopidogrel response and clinical outcome in CYP3A5 non-expressors [94], both indicating that further prospective research is still needed to conclusively determine the clinical significance of clopidogrel-CCBs interactions.

4.3.4 CYP inhibitor and inducers that may interact with clopidogrel—Since the pharmacological effect of clopidogrel is closely linked to its bioactivation via CYP enzymes, other concomitant medications that suppress the activity of relevant CYP enzymes (e.g. CYP2C19, CYP3A4, CYP2C9, CYP2B6 and CYP1A2) may interrupt the antiplatelet activity of clopidogrel and, thus, negatively impact clinical outcome. For example, platelet inhibition was significantly reduced when clopidogrel was co-administered with sulfonylureas (CYP2C9 substrates) [155], phenprocoumon (CYP3A4 and CYP2C9 substrate) [156] or other CYP3A4 inhibitors, such as ketoconazole, erythromycin and troleandomycin [157, 158]. It has been shown that the concurrent intake of grapefruit juice causes a more than 80% decrease in clopidogrel bioactivation due to a suppression of CYP2C19 in addition to its well-established effect on CYP3A4 [159]. Interestingly enough, the CYP3A4 inhibitor itraconazole showed a stronger inhibitory effect on clopidogrel PD in healthy volunteers carrying the CYP3A5 non-expressor genotype than in those carrying the CYP3A5 expressor genotype [93].

Since most enzymes (e.g. CYP3A4, CYP2C19, CYP2B6 and CYP1A2) involved in clopidogrel bioactivation are regulated by xenobiotic receptors including aryl hydrocarbon receptor (AhR), PXR and constitutive androstane receptor (CAR) [160], any xenobiotic that can activate these xenobiotic receptors has the potential to enhance the antiplatelet effect of clopidogrel via upregulation of enzymatic activity. At the same time, the risk of experiencing bleeding events can also expected to be higher for these drug-drug interactions. For example, rifampicin, a potent PXR and CAR ligand, has been shown to significantly promote the antiplatelet activity of clopidogrel [158]. Concordantly, St John's wort, a PXR ligand, remarkably induced CYP3A4 activity and magnified the antiplatelet activity of clopidogrel in both healthy volunteers and post-coronary stent patients [161]. Smoking is known to cause significant induction of CYP1A2 activity via AhR pathway [160]. Several studies showed that smokers exhibited enhanced platelet inhibition [144, 162, 163]. However, the association between smoking and clopidogrel clinical outcome is inconclusive, as summarized by a recent review paper [163].

4.3.5 Anticoagulants—Blood clots are the result of elevated platelet aggregation and activation of coagulation system [164]. Blockage of both systems by anticoagulants (e.g. warfarin) and antiplatelet agents (e.g. aspirin and clopidogrel) as, for example during triple antithrombotic therapy (anticoagulant drugs + clopidogrel + aspirin) recommended for atrial fibrillation patients presenting with ACS and/or PCI [165], causes an increase in antithrombotic efficacy in addition to an increased bleeding risk. This expectation is confirmed by the results from two meta-analyses that showed that this drug combination resulted in a more efficacious protection from major cardiovascular risk but also remarkably elevated bleeding events compared to antiplatelet or anticoagulant treatment alone [166, 167]. The results from WOEST clinical trial also reported that, compared with the triple antithrombotic therapy, dual antithrombotic therapy (clopidogrel + anticoagulant) significantly decreases the risk of bleeding complications while leaving the rate of thrombotic events unchanged [168]. These findings indicate that aspirin may have to be excluded from combination therapy in order to achieve the desired benefit/risk profile.

4.3.6 Selective serotonin reuptake inhibitors—It has been reported that about 20% of CVD patients suffer from depression, which is frequently treated with selective serotonin reuptake inhibitors (SSRIs, e.g. fluoxetine, citalopram and sertraline) [169]. SSRIs inhibit the serotonin transporter in the central nervous system and thereby suppress the uptake of synaptic serotonin into the presynaptic neuron. In the blood, SSRIs block the entry of serotonin into platelets, which leads to a depletion of intra-platelet serotonin stores, thereby reducing the efficiency of ADP-induced platelet aggregation [170]. Results from the SADHART trial ACS in patients with depression showed that in addition to antiplatelet regimens including aspirin and clopidogrel, concomitant sertraline was associated with a further reduction in platelet/endothelial activation, suggesting that SSRIs might bring additional advantage in CAD patients with comorbid depression [171]. On the other hand, a recent DDI study conducted with healthy adults revealed that concurrent fluoxetine significantly reduced clop-AM plasma levels and clopidogrel response [172] due to the inhibition of CYP2C9, CYP2C19 and CYP3A4 [12, 173]. Inconsistent results were reported from several clinical outcome studies. The ENRICHD clinical trial and two following clinical outcome studies reported that concurrent SSRIs was associated with a significant reduced risk of death or recurrent MI as well as an increased risk in bleeding in clopidogrel treated patients [174–176]. However, conflicting results were reported from several other studies [169, 177, 178], suggesting the necessity of further evaluation of the potential impact of SSRIs on clopidogrel antiplatelet treatment.

4.4 Comorbidities

4.4.1 Diabetes mellitus—A significant portion of ACS patients (~30%) also suffer from diabetes mellitus (DM), which puts them at an increased atherothrombotic risk and higher mortality rates compared to their non-diabetic peers [44, 55, 56, 82, 179]. The EXCELSIOR study showed that DM was most relevant independent indicator of HPR next to the patient's *CYP2C19*2* carrier status and that individuals with DM had a significantly higher prevalence of HPR than non-diabetic subjects at all BMI and age groups (e.g. age ≥ 70 & BMI ≤ 25 or age ≤ 60 & BMI ≥ 30) (Figure 2) [41]. Although the exact mechanism is still unclear, several factors, such as endothelial dysfunction, increased coagulation, impaired

fibrinolysis and platelet hyper-reactivity, contribute to prothrombotic conditions in DM patients and are summarized elsewhere [180]. A study conducted by Angiolillo *et al.* reported that a mutation (rs956115) of insulin receptor substrate-1 was associated with significantly higher prevalence of HPR and major adverse cardiac events in patients with type 2 DM and stable CAD following treatment of clopidogrel and aspirin [181]. On the other hand, Erlinge *et al.* reported that the poor clopidogrel response in patients with DM was attributed to the lower systemic exposure to clop-AM rather than changes in platelet response [182]. These findings were confirmed in a PK study conducted in healthy subjects and type 2 diabetes patients with CYP2C19 substrate R483, which showed that DM may cause a significant suppression of CYP2C19 catalytic capacity [183] and potentially increase clopidogrel resistance in DM patients. Consistently, a recent study conducted in ACS patients also reported that CYP2C19 loss-of-function mutation significantly impacted clopidogrel clinical outcome in non-DM individuals compared to DM patients [184].

4.4.2 Chronic kidney disease—Renal insufficiency (creatinine clearance < 70 mL/min) has been reported for 35 to 40% of ACS patients [185]. The impact of chronic kidney disease (CKD) on clopidogrel response has been studied by multiple investigators but the results remain inconclusive. This is partly due to the fact that different assays used provide different results. For example, CKD was determined to have a significant impact on HPR when using the VerifyNow P2Y12 assay, whereas no difference between patients with or without CKD was found when using the LTA assay or the VASP-PRI assay [186–191]. The differences in test results were attributed to varying hemoglobin levels in CKD patients, which may cause interference in this assay when whole blood is used [186–188]. On the other hand, CKD was found to be associated with an increased risk in clinical outcome (e.g. death, adverse cardiovascular events and ST) and bleeding clopidogrel treated patients [43, 44, 59, 192–195]. A recent meta-analysis also reported that the benefits of antiplatelet therapy in CKD patients are uncertain and potentially outweighed by bleeding hazards [196], suggesting that caution should be needed when CKD patients seek for antiplatelet therapy.

4.5 Patient compliance

Timely initiation of therapy and rigorous adherence to the prescribed treatment are imperative for successful management of ACS. Failure to comply with these requirements, e.g. in terms of delayed onset of therapy, failure in timely refill of prescriptions or premature discontinuation of clopidogrel or dual antiplatelet therapy, were identified as “hidden factors” that contribute to clopidogrel resistance, an elevated risk of adverse cardiac events and even death [5, 44, 197]. Ho *et al.* reported that delay in filling clopidogrel prescriptions resulted in significantly increased death/MI rates in patients following stent implantation [198]. Interventions, such as follow up by phone, checking refill histories or monitoring clinical registries, have resulted in significantly improved compliance to clopidogrel therapy [199, 200].

5. Summary and future perspectives

In recent years, variability in clopidogrel response has become an increasingly important clinical issue with potentially severe consequences [3]. Therefore, it becomes imperative to understand the key factors that contribute to the high between-subject variability in response to clopidogrel treatment, particularly to clopidogrel resistance. In this paper we systematically reviewed known pharmacokinetic, pharmacodynamic as well as genetic and non-genetic factors that contribute to interindividual differences in response to clopidogrel therapy and evaluated how they relate to clinical outcome (cf. Table 1 & 2).

To date, numerous clinical studies have been conducted to investigate the potential cause of clopidogrel resistance. Most of these studies were either conducted to answer one specific question (e.g. age, DDI) or to investigate the impact of multiple impact factors to a qualitative manner, as manifested by statistical significance. Our review of the literature clearly indicates that despite a multiplicity of research efforts, no clear cut answer is available yet that allows to sufficiently answer all the open questions and, ultimately, to reliably identify optimal treatment/dosing regimens for individual patients prior to the start of therapy. This in part due to the fact that suboptimal response to clopidogrel treatment, both in terms of efficacy and safety, is a multifactorial problem that is difficult to address in one-off clinical trials that evaluate only one or only a few factors at a time. The harmonized use of quantitative analysis strategies, such as population and physiologically based modeling and simulation approaches in corporation with systems biology/pharmacology modeling, may provide a quantitative characterization for the multiple genetic, demographic and disease risk factors that affect clopidogrel response, and the interaction between them with a dynamic manner. These quantitative approaches in combination with clinical trials may help to overcome this limitation as they allow to interpret and to compare information from head-to-head clinical trials and to evaluate the impact of different genetic and non-genetic factors as well as their interplay on clinical outcome. Once identified and qualified, these models have the potential to serve as bedside-ready decision support tools for physicians and other health care professionals for optimizing patients' clopidogrel dosing regimens based on the individuals' genetics, demographics, medication and disease history. The use of quantitative approaches may further allow to performing cost-effectiveness analyses for single as well as combination antiplatelet therapy and, ultimately, guide clinical and health-policy decision-making.

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Key messages

- Multiple genetic and non-genetic factors contribute to the high inter-individual variability in clopidogrel's dose-concentration-response relationship following oral administration of the standard dosing regimen (300 mg loading dose, 75 mg maintenance dose)
- In order to understand the relative contribution of each of these factors to the overall variability in treatment response, sufficient understanding of the underlying pharmacokinetics (PK) and pharmacodynamics (PD) is needed
- An understanding of the variability in PK and PD requires a mechanistic-based, quantitative analysis approach that integrates available information on the clinically relevant factors that impact clopidogrel's PK and PD
- Once established and qualified, this qualitative and quantitative link can then be used to translate genetic and clinical information into actionable dosing recommendations and thus help to personalize clopidogrel therapy on a patient-by-patient basis.

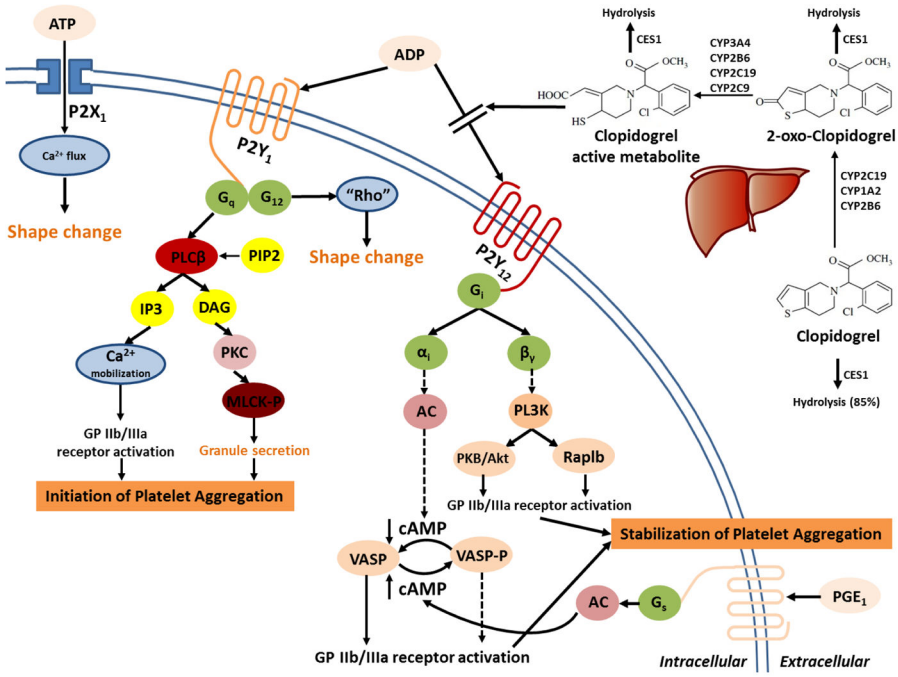

Figure 1.

Clopidogrel is an orally administered pro-drug. In the liver, approximately 15% of absorbed clopidogrel is metabolized by the cytochrome P450 (CYP) system to generate its active metabolite via a 2-step bioactivation process, whereas the rest 85% is hydrolyzed by carboxylesterase 1 (CES1) to an inactive carboxylic acid derivative. CES1 also catalyzes the hydrolysis of the intermediate metabolite 2-oxo-clopidogrel and the active metabolite. The active metabolite binds to the adenosine diphosphate (ADP) $P2Y_{12}$ receptor on the surface of platelet and leads to an irreversible inhibition of platelet aggregation. ADP binds to the G_q-coupled $P2Y_1$ receptor, which activates phospholipase C (PLC) that forms inositol triphosphate (IP3) and diacylglycerol (DAG) from phosphatidylinositol bisphosphate (PIP2). IP3 causes mobilization of intracellular calcium, whereas DAG activates protein kinase C (PKC) and leads to phosphorylation of myosin light chain kinase (MLCK-P). These two processes both lead to initiation of platelet aggregation. On the other hand, activation of $P2Y_1$ receptor coupled G_{12}, another G-protein, which activates the “Rho” protein, as well as activation of $P2X_1$ receptor by adenosine triphosphate (ATP), which causes extracellular calcium influx, both lead to change of platelet shape. Activation of the Gi-coupled $P2Y_{12}$ receptor by ADP leads to release of the α_i and β_γ subunits, which ultimately lead to stabilization of platelet aggregation. The α_i subunit inhibits adenylyl cyclase (AC), which decreases intracellular levels of cyclic adenosine monophosphate (cAMP), reduces cAMP-mediated phosphorylation of vasodilator-stimulated phosphoprotein (VASP) (VASP-P), and modulates activation of glycoprotein (GP) IIb/IIIa receptor. The β_γ subunit activates the phosphatidylinositol 3-kinase (PI3K), which in turn, cause activation of a serine-threonine protein kinase B (PKB/Akt) and of Rap1b GTP binding proteins and causes activation of GP IIb/IIIa receptor. In addition, Prostaglandin E_1 (PGE_1) elevates cAMP and VASP-P levels via activation of AC. Solid arrows represent activation, dashed arrows represent inhibition (This figure is modified from Angiolillo, DJ et al. Variability in individual responsiveness to

clopidogrel: clinical implications, management, and future perspectives. Am Coll Cardiol. 2007 Apr 10;49(14):1505–16 [3]).

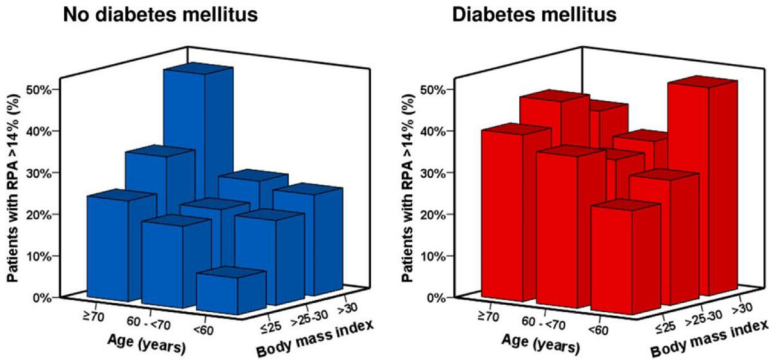

Figure 2.

Prevalence of patients with high on-treatment platelet reactivity (HPR) according to age, body mass index and diabetes mellitus status. HPR was defined as residual platelet aggregation (RPA) > 14%, which was assessed by light transmittance aggregometry (LTA) assay with ADP 5 μmol/L following a 600 mg of clopidogrel loading dose in 760 patients undergoing elective coronary stent implantation [41].

Table 1

Summary of factors that may affect clopidogrel pharmacokinetics, pharmacodynamics and clinical outcome.

Potential Factors		Influence on Clopidogrel PK/PD	Influence on Clinical Outcome
Demographics			
Older Age		Higher on-treatment platelet reactivity [14, 19, 39–41].	Increase in both cardiovascular events [5, 16, 42–44] and bleeding [45, 46].
Obesity		Lower systemic exposure to clop-AM [136] Higher on-treatment platelet reactivity [14, 41, 47].	Inconclusive (obesity paradox) [16, 42, 45, 52, 53].
Sex		Minimal/inconclusive [14, 19, 39, 57, 87] [40, 58].	Minimal/inconclusive [16, 42] [5, 43, 45, 52, 59–61].
Pharmacogenetics			
ABCB1	C3435T	Minimal/inconclusive [16, 19, 21, 39, 60]	Minimal/inconclusive [16, 60, 64–67]
CES1	G143E A-618C	Lower on-treatment platelet reactivity [23, 68] Inconclusive [70, 71]	N/A
CYP2C19	G681A (**2*) G636A (**3*)	Lower systemic exposure to clop-AM [4, 75–77] Higher on-treatment platelet reactivity [17, 18, 39, 58, 78–82]	Increase of cardiovascular risk [14, 16, 18, 65, 78, 81–85]
	C806T (**17*)	Minimal/inconclusive [15, 77, 86, 87]	Minimal/inconclusive [15, 65, 74, 83, 87]
CYP1A2	**1C-1F*, **7*, **11*, **16* and others	Minimal/inconclusive [17, 18, 75, 80]	Minimal/inconclusive [18]
CYP2B6	**1B*, **1C*, **5*, **6*, **9*, **11* and others	Minimal/inconclusive [18, 39]	Minimal/inconclusive [18]
CYP2C9	C430T (**2*) A1075C (**3*) and others	Minimal/inconclusive [18, 75, 80]	Minimal/inconclusive [18, 61]
CYP3A4/5	**2*, **3*, **17* and others (CYP3A4) **2*, **3*, **6* and others (CYP3A5)	Minimal/inconclusive [18, 75, 80, 81, 92, 93].	Minimal/inconclusive [18, 61, 93].
PON1	Q192R	Minimal/inconclusive [24, 76, 79, 82, 96, 97],	Minimal/inconclusive [65, 79, 82, 96–98],
P2Y12	H2 haplotype and others	Minimal/inconclusive [57, 100, 101, 104, 105, 108]	Minimal/inconclusive [16, 39, 105–107, 109]
Drug-Drug Interactions			
Proton pump inhibitors		Lower systemic exposure to clop-AM [120, 121, 124] Higher on-treatment platelet reactivity [120–127].	Minimal impact on cardiovascular risk [118, 130–132]
Statins		No negative effect on systemic exposure to clop-AM [135]. No negative effect on on-treatment platelet reactivity [127, 135, 137–145].	No negative effect on clinical outcome [141, 146–148]
Calcium channel blockers		Minimal/inconclusive [63, 94, 150, 151, 154]	Minimal/inconclusive [94, 150–154]
Anticoagulants		N/A	Decrease of cardiovascular risk while increase of bleeding events [166, 167]
Antidepressants		Conflicting/inconclusive [171, 172]	Conflicting/inconclusive [169, 174–178]
Comorbidities			
Diabetes		Lower systemic exposure to clop-AM [182] Higher on-treatment platelet reactivity [41, 55, 82, 179, 181].	Increase of cardiovascular risk [41, 44, 56, 82, 181]

Potential Factors	Influence on Clopidogrel PK/PD	Influence on Clinical Outcome
Chronic kidney disease	Higher on-treatment platelet reactivity (more significant when using VerifyNow™ P2Y12 assay) [91, 186–191]	Increase in both cardiovascular events and bleeding [43, 44, 59, 192–196]

Table 2

Summary of demographic, genetic, drug- and disease-mediated factors influencing antiplatelet therapy with clopidogrel.

Potential factors		Influence on clopidogrel HPR	Influence on cardiovascular risk	Influence on bleeding risk
Demographics				
Older age		↑	↑	↑
Obesity		↑↑	↑↓	-
Sex		↔	↔	↔
Pharmacogenetics				
ABCB1	C3435T	↔	↔	↔
CES1	G143E A-618C	↓ ↔	N/A ↔	N/A N/A
CYP2C19	G681A (**2*) G636A (**3*)	↑↑	↑↑	↓
	C806T (**17*)	↔	↔	↔
CYP1A2	**1C-1F*, **7*, **11*, **16* and others	↔	↔	↔
CYP2B6	**1B*, **1C*, **5*, **6*, **9*, **11* and others	↔	↔	↔
CYP2C9	C430T (**2*) A1075C (**3*) and others	↔	↔	↔
CYP3A4/5	**2*, **3*, **17* and others (CYP3A4) **2*, **3*, **6* and others (CYP3A5)	↔	↔	↔
PON1	Q192R	↔	↔	↔
P2Y12	H2 haplotype and others	↔	↔	↔
Drug-Drug Interactions				
Proton pump inhibitors		↑	↔	↓
Statins		↔	↔	↔
Calcium channel blockers		↔	↔	↔
Anticoagulants		N/A	↓	↑
Antidepressants		↔	↔	↔
Comorbidities				
Diabetes		↑↑	↑↑	↓
Chronic kidney disease		↑	↑	↑↑

N/A not available, ↑ and ↓ indicate an increase and decrease, respectively, ↔ indicates no effect.