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REVIEW ARTICLE

Clinical Pharmacokinetics and Pharmacodynamics of Clopidogrel

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Abstract Acute coronary syndromes (ACS) remain life-threatening disorders, which are associated with high morbidity and mortality. Dual antiplatelet therapy with aspirin and clopidogrel has been shown to reduce cardiovascular events in patients with ACS. However, there is substantial inter-individual variability in the 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, which affect the pharmacokinetics of clopidogrel. While the US Food and Drug Administration has issued a boxed warning for CYP2C19 poor metabolizers because of 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 the pharmacokinetics and pharmacodynamics of clopidogrel and how they mechanistically contribute to inter-individual differences in the response to clopidogrel treatment.

Key Points

Multiple genetic and non-genetic factors contribute to the high inter-individual variability in the dose–concentration–response relationship following oral administration of the standard clopidogrel 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 and pharmacodynamics is needed.

An understanding of the variability in pharmacokinetics and pharmacodynamics requires a mechanistic-based, quantitative analysis approach that integrates available information on the clinically relevant factors that impact the pharmacokinetics and pharmacodynamics of clopidogrel.

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.

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1 Introduction

Cardiovascular disease (CVD) is currently the leading cause of death worldwide [1]. Many CVD patients develop an 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 hospitalized in the USA 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 USA 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]. Clopidogrel is a second-generation thienopyridine, which has largely replaced ticlopidine (a first-generation thienopyridine with similar efficacy) because of 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 the $P2Y_{12}$ receptor antagonists clopidogrel, prasugrel or ticagrelor has become the clinical gold standard for patients with ACS and/or undergoing percutaneous coronary intervention (PCI), because of the significant improvement in the 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 development of atherothrombotic complications [3]. This relative non-responsiveness to clopidogrel therapy has been termed '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 prodrug 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. because they are poor metabolizers or as a result of drug–drug interactions (DDIs)] have remarkably higher on-treatment platelet reactivity, which puts them at an increased risk of ischaemic events following the standard dosing regimen, prompting the US Food and Drug Administration (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 the response to clopidogrel treatment, whereas age and the 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 inter-individual variability in the 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 assays that have been used to determine the response to clopidogrel treatment are also subject to substantial between-assay variability.

The objective of this review is to comprehensively evaluate the different sources of variability in pharmacokinetics and pharmacodynamics and how they mechanistically relate to inter-individual differences in the 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 inter-individual differences in the pharmacokinetics and pharmacodynamics of clopidogrel and how they relate to the clinical outcome.

2 Pharmacokinetics and Pharmacodynamics of Clopidogrel

Following oral administration of clopidogrel, about 50 % of the dose is absorbed from the intestine, according to urinary metabolite 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 clopidogrel is delivered to the liver, a number of 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 hydrolysed 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 hydrolyses 2-oxo-clopidogrel and clop-AM [23, 24].

In vitro enzyme kinetics studies have 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 the active metabolite, clop-AM [12]. It is estimated that CYP2C19 contributes to about 50 % of the overall formation of clop-AM from clopidogrel and thus plays a substantial role in bioactivation of clopidogrel, whereas the other isozymes contribute to a lesser extent. There are 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 suggest that clopidogrel and its major inactive metabolite, SR26334, exhibit linear pharmacokinetics across a wide range of doses (50–900 mg) [25–27], Wallentin et al. [28] and Collet et al. [29] reported ~4 and ~2-fold increases in the clop-AM area under the plasma concentration–time curve (AUC) when the clopidogrel dose was increased from 75 to 600 mg and from 300 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 increases in the clop-AM AUC, respectively, in CYP2C19 extensive, intermediate and poor metabolizers [30]. These findings support the presence of non-linearity in the bioactivation processes of clopidogrel.

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 A (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 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 (see aspirin), thrombin, collagen and serotonin, also contribute to platelet activation. Therefore, any factors influencing the $P2Y_{12}$-dependent and /or $P2Y_{12}$-independent signal transduction pathways that impact 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 High On-Treatment Platelet Reactivity

Several ex vivo platelet function assays have been developed to assess patients' responsiveness to clopidogrel treatment and, ultimately, to determine which patients are at increased ischaemic risk [22]. Light transmittance aggregometry (LTA) and a variety of other methods measure overall platelet function [3, 22], whereas the VASP–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 use of different agonists at, in part, different doses, lack of reproducibility and comparability between assays, and application of different cut-off values for defining HPR, making it difficult to directly compare the different tests with respect to the determined impact on platelet reactivity and corresponding efficacy and safety outcomes [22].

Measurement of ADP-induced platelet aggregation in platelet-rich plasma by the LTA assay has long been the gold standard for assessing platelet function in relation to the clinical outcome. In most studies, values of 5, 10 or 20 μmol/L of ADP have been employed and respective cut-off values have been proposed to define HPR [22]. The specificity of the 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 the LTA assay is also limited by its labour-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 whole blood by using ADP and prostaglandin E_1 (PGE_1) in order to increase the specificity to the $P2Y_{12}$ pathway. However, the experimental results of the VerifyNow $P2Y_{12}$ assay may be influenced by non-platelet blood components (e.g. haemoglobin) [3, 32]. The same holds true for other point-of-care whole-blood platelet tests, such as the Impact-R, PFA-100, Plateletworks test and Multiplate analyser [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 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 the VASP-PRI assay in prediction of cardiovascular adverse events was lower than those of the ADP-stimulated platelet function assays, suggesting that specific determination of the VASP pathway may overlook the contribution of an 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

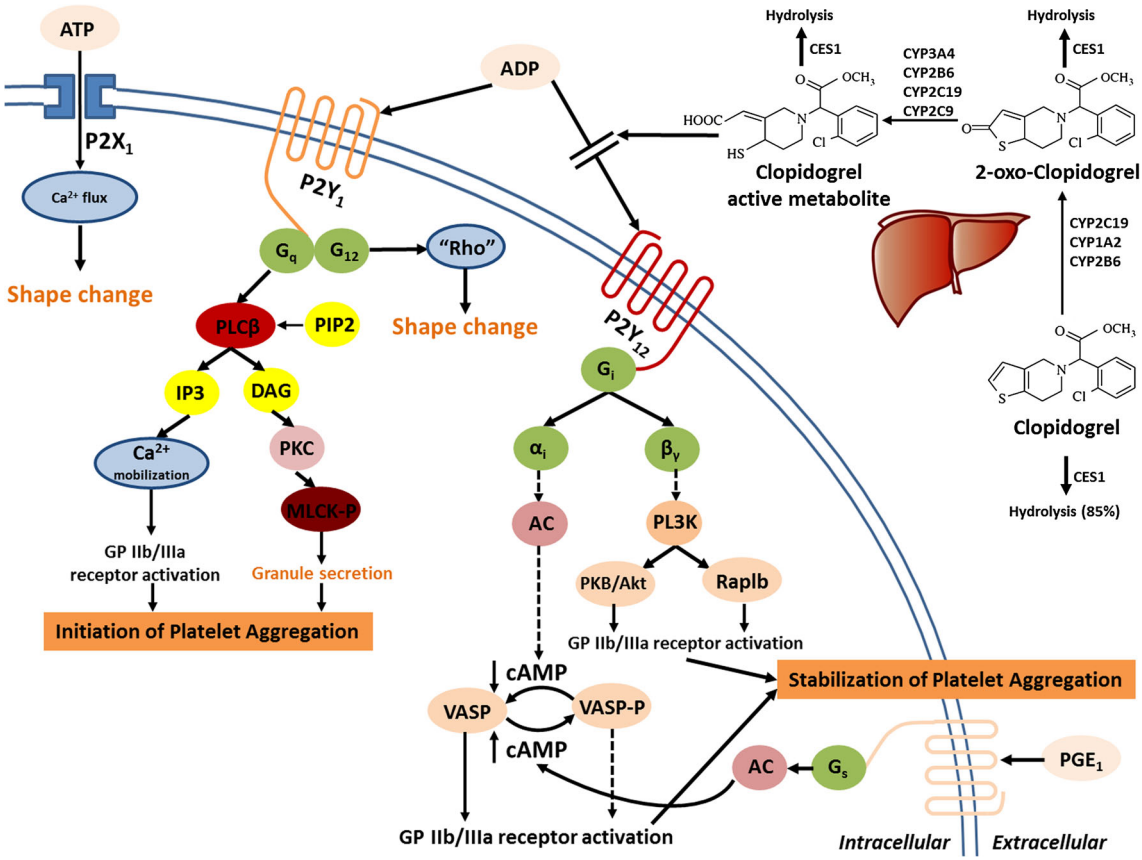

Fig. 1 Clopidogrel is an orally administered prodrug. In the liver, approximately 15 % of absorbed clopidogrel is metabolized by the cytochrome P450 (CYP) system to generate its active metabolite via a two-step bioactivation process, whereas the remaining 85 % is hydrolysed by carboxylesterase 1 (CES1) to an inactive carboxylic acid derivative. CES1 also catalyses 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 platelets and leads to irreversible inhibition of platelet aggregation. ADP binds to the G_q-coupled $P2Y_1$ receptor, activating phospholipase C (PLC), which 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 the $P2X_1$ receptor by adenosine triphosphate (ATP) [which causes extracellular calcium influx], both lead to a change in the 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 the activation of the glycoprotein (GP) IIb/IIIa receptor. The β_γ subunit activates phosphatidylinositol 3-kinase (PI3K)—which, in turn, cause activation of serine/threonine protein kinase B (PKB/Akt) and Rap1b guanosine triphosphate (GTP) binding proteins—and causes activation of the GP IIb/IIIa receptor. In addition, prostaglandin E_1 (PGE_1) elevates cAMP and VASP-P levels via activation of AC. The *solid arrows* represent activation and the *dashed arrows* represent inhibition. (This figure is modified from reference: Angiolillo et al. [3])

link between ex vivo assay readouts and HPR. For example, results from the recent ELEVATE-TIMI 56 study indicate that the responses of 16–20 % of the patients receiving a 75 mg clopidogrel maintenance dose differed when measured at different times. In fact, 33–50 % of the patients were originally classified as non-responders but then had to be re-classified as responders following a second measurement using the same assay, or vice versa, and about 40 % of the patients showed larger than 40 score changes in $P2Y_{12}$ reaction units (PRU) following serial measurements using the VerifyNow $P2Y_{12}$ assay [38].

4 Covariates that Affect Clopidogrel Dose, Pharmacokinetics and Pharmacodynamics

4.1 Demographic Factors

4.1.1 Age

Several studies have reported a significant association between older age and a higher prevalence of HPR following clopidogrel treatment [14, 19, 39–41]. On the other hand, clinical outcome studies have 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 have reported that BMI or body weight is associated with HPR in both patients and healthy subjects [14, 41, 47]. A recent clinical pharmacokinetic study reported that, compared with 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 values, which ultimately led to higher on-treatment platelet reactivity in these obese patients (the VerifyNow $P2Y_{12}$ reaction reading in obese patients was 207 PRU, whereas that in patients of 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 than in lower–body weight ones (1.33 versus 0.89 mg/kg). In addition, down-regulation of CYP enzymes in obese subjects (e.g. CYP2C9, CYP2C19 and CYP3A4) may also play a role, as it leads to reduced bioactivation [49]. Some recent studies have also shown that the 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 on 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 BMIs (normal and underweight) had higher risks of bleeding and adverse clinical outcomes, including death and MI, than obese patients. This is because in these studies, there was a trend for patients with a higher BMI to be younger males, who usually show a tendency to seek medical care earlier and receive more aggressive initial management than older subjects [16, 42, 45, 52, 53]. On the other hand, a LEADERS trial that was conducted in discharged patients treated with clopidogrel reported that obese individuals (BMI >30 kg/m^2) had significantly more major adverse cardiac events than non-obese ones [54]. It was noteworthy that the obese patients involved in this study had a significantly higher rate of diabetes mellitus (DM), 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 to clopidogrel resistance.

4.1.3 Sex

The impact of sex on the efficacy and safety of clopidogrel has been investigated in different clinical settings. It has been reported that systemic clop-AM exposure was similar in men and women [19]. Many clinical studies have revealed that the pharmacodynamics of clopidogrel do not differ between males and females [14, 39, 57], whereas some others have reported a significantly decreased risk of HPR in males compared with females [40, 58]. Results from the FAST-MI clinical trial and another clinical study conducted in patients undergoing PCI both reported that females had a lower risk of cardiovascular events than males (including death, MI or stroke) [16, 42]. Female sex was associated with an increase in bleeding in the REPLACE-2 and ISAR-REACT 3 clinical trials [45, 59], indicating that sex might play a role in the clinical outcome of clopidogrel therapy, 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 with male patients. On the other hand, results from several other clinical studies suggest that, compared with other factors, the impact of sex on the clinical outcome of clopidogrel therapy is minimal [5, 43, 52, 60, 61].

4.2 Genetic Polymorphisms

4.2.1 Genetic Polymorphisms that Affect the Pharmacokinetics of Clopidogrel

4.2.1.1 ABCB1 The *ABCB1* [ATP-binding cassette, sub-family B (MDR/TAP), member 1] C3435T mutation has been associated with changes in the intestinal efflux of drugs and thus their oral bioavailability [62]. However, its impact on the pharmacokinetics/pharmacodynamics and clinical outcome of clopidogrel therapy remains controversial. A clinical pharmacokinetic study conducted in patients undergoing PCI reported that following administration of a single clopidogrel loading dose (300 or 600 mg), the peak plasma concentration (C_{max}) and AUC values of clopidogrel and clop-AM were significantly lower in 3435T/T homozygotes than in 3435C/T heterozygotes and C/C (wild type) homozygotes, suggesting a change in oral bioavailability due to enhanced clopidogrel efflux with 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 the C3435T polymorphism and HPR following either loading or maintenance doses of clopidogrel [19, 39, 60, 63]. The association between the C3435T mutation and cardiovascular risk is also inconsistent [16,

60, 64, 65]. These conflicting findings on the impact of the *ABCB1* C3435T mutation on cardiovascular outcomes was evaluated in two meta-analyses showing that this mutation is unlikely to play a major role in between-subject variability in the response to clopidogrel treatment [66, 67].

4.2.1.2 CES1 Hepatic CES1 is a serine hydrolase with a 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 the enzymatic activity of the CES1 variant G143E in catalysing the hydrolysis of clopidogrel and 2-oxo-clopidogrel was completely impaired; suppression of CES1 activity greatly enhanced generation of 2-oxo-clopidogrel and clop-AM from clopidogrel in human liver S9 fractions [23]. Consistently, further analysis of the PAPI study data revealed that, following clopidogrel treatment, *CES1* 143E allele carriers have significantly higher clop-AM levels, resulting in a more pronounced pharmacodynamic response than that seen in patients who are homozygous for *CES1* 143G (wild-type) [68]. In patients with acute coronary disease receiving clopidogrel treatment, the on-treatment platelet reactivity in individuals carrying the *CES1* 143E allele was also significantly lower than that in 143G homozygotes [68]. On the other hand, the *CES1* −816A/C allele, which has been reported to cause significantly enhanced transcriptional activity of the *CES1* 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 polymorphisms in *CES1* on the response to clopidogrel.

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 of the *CYP2C19* gene have been identified. *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 pharmacokinetic/pharmacodynamic investigations conducted in healthy volunteers all revealed that subjects carrying CYP2C19 reduced-function alleles (e.g. *CYP2C19**2 or *CYP2C19**3) had significantly lower systemic exposure to clop-AM and the antiplatelet aggregation effect than wild-type individuals [4, 30, 75–77]. Similarly, studies conducted in patients with CVD 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 stent thrombosis (ST) [14, 16, 18, 65, 78, 81, 82], as confirmed by several meta-analyses [83–85]. In comparison, the impact of the *CYP2C19**17 mutation on the pharmacokinetics of clopidogrel may be minimal, as shown in the PAPI study, which reported that clop-AM levels were similar in subjects carrying the *CYP2C19**17 allele and corresponding peers carrying the *CYP2C19**1 allele [77]. Conflicting results have also been reported in regard to the association between the *CYP2C19**17 allele and enhancement of platelet inhibition, reduction of major cardiovascular risk or increases in bleeding events in different studies [15, 65, 74, 77, 83, 86, 87]. A recent pharmacogenetic study identified a linkage between the *CYP2C19**17 allele and *CYP2C19**4, an LOF mutation, which suggest that the high metabolic capacity of *CYP2C19**17 carriers is altered if these subjects also show the *CYP2C19**4B haplotype [88]. As a result, further studies are needed to fully delineate the overall impact of new *CYP2C19* genotypes/haplotypes on clopidogrel treatment response.

No clear association was found between genetic polymorphisms of *CYP2B6*, *CYP2C9*, *CYP1A2* and *CYP3A4*, which all contribute to bioactivation of clopidogrel to some extent in vitro [12], and the pharmacokinetics, pharmacodynamics and clinical outcome of clopidogrel therapy. 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 dosing of clopidogrel (for 4 days) significantly suppressed CYP2B6-catalysed bupropion hydrolysation in healthy adults [91], suggesting that long-term exposure to clopidogrel may suppress the function of CYP2B6, which consequently attenuates the impact of *CYP2B6* polymorphisms. Consistently, reports from two clinical studies showed that CYP2B6 reduced-function alleles (*1B, *1C, *5, *6, *9 or *13) had a significant impact on clopidogrel bioactivation and pharmacodynamics following short-term clopidogrel treatment but did not impact the long-term pharmacodynamics or clinical outcome of clopidogrel therapy [18, 39]. Conflicting findings have been reported in assessments of the impact of *CYP2C9* polymorphisms on the pharmacokinetics, pharmacodynamics and clinical outcome of clopidogrel therapy [18, 61, 75, 80]. No significant association with *CYP1A2* polymorphisms has been reported [17, 18, 75, 80]. Inconsistent results have also been reported for the impact of *CYP3A4* and *CYP3A5*

polymorphisms on the pharmacodynamics or clinical outcome of clopidogrel therapy [18, 61, 75, 80, 81, 92, 93]. Nevertheless, a clinical pharmacokinetic study conducted in healthy volunteers revealed that the CYP3A4 inhibitor itraconazole showed a stronger inhibitory effect on the pharmacodynamics of clopidogrel in healthy volunteers carrying the CYP3A5 non-expressor genotype than in those carrying the CYP3A5 expressor genotype [93]. Another CROSS-VERIFY clinical study also reported that the calcium channel blocker (CCB) amlodipine, which is a CYP3A4 inhibitor, exhibited adverse effects on clopidogrel response and clinical outcome only 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 PON1 Paraoxonase-1 (PON1) is an aromatic esterase, which 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 the *PON1* Q192R mutation was identified as a new determinant in converting clopidogrel to clop-AM and the risk of ST in patients undergoing PCI [24]. However, inconsistent results were observed in several subsequent studies that assessed the association between the *PON1* Q192R mutation and clop-AM formation, antiplatelet activity or clinical outcome [65, 76, 79, 82, 96–98], suggesting that the role of PON1 in clopidogrel resistance may need further investigation.

4.2.2 Genetic Polymorphisms that Affect the Pharmacodynamics of Clopidogrel

The interplay between ADP and the $P2Y_{12}$ receptor located on the surface of platelet plays an essential role in platelet activation [3, 22]. To date, several *P2RY12* (purinergic receptor P2Y, G-protein coupled, 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 *P2RY12* H2 haplotype (consisting of intronic [i]-C139T, [i]-T744C, [i]-ins801A and G52T single nucleotide polymorphisms) was significantly higher in coronary artery disease (CAD) patients [99]. Although some clinical pharmacokinetic/pharmacodynamic studies have revealed that the H2 haplotype was associated with HPR in both healthy subjects and patients undergoing PCI [100, 101], such a relationship could not be demonstrated in several other studies [81, 102, 103]. The H2 haplotype also failed to show an impact on the clinical outcome of clopidogrel-treated patients undergoing PCI [104, 105]. Similarly, inconsistent results have also been reported from assessments of the association between the *P2RY12* C34T mutation and the pharmacodynamics or clinical outcome of clopidogrel therapy [16, 39, 106, 107]. A study conducted in Chinese ACS–PCI patients receiving clopidogrel therapy reported that the impact of the *P2RY12* C34T mutation on the clinical outcome became significant only in patients who also carried the *CYP2C19**2 (G681A) allele [107]. Rudez et al. [108] reported that the s6787801 mutation (c. −217 + 2739T>C) of the $P2Y_{12}$ receptor was associated with significantly lower on-treatment platelet reactivity in 1,031 clopidogrel-treated CAD patients treated with PCI. However, their 1-year clinical follow-up study failed to show an impact of such a mutation on cardiovascular events [109], whereas two other studies have suggested that this mutation might be associated with a significantly increased HPR or target-vessel revascularization rate in patients undergoing PCI [57, 105]. Therefore, further studies are necessary for establishment of the relation between *P2RY12* genetic polymorphisms and non-responsiveness to clopidogrel.

The *ITGB3* [integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)] gene encodes for integrin β3 of the GP IIb/IIIa platelet receptor, which is the major membrane receptor for platelet aggregation. Inconsistent results have been reported from evaluations of the association between *ITGB3* and clopidogrel resistance. One study reported that *ITGB3* PLA2 mutation carriers showed higher on-treatment platelet reactivity following a clopidogrel loading dose (300 mg) [110]. Another study revealed that *ITGB3* mutation was associated with a decreased risk of early ST [104]. However, results from other studies have suggested that there is no association between the *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 initiates weak and transient platelet aggregation [3]. Yet no association has been observed between the *P2RY1* (purinergic receptor P2Y, G-protein coupled, 1) 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 accounts not only for genetic differences between subjects but also for other associated factors, such as diet, lifestyle, co-morbidity and medical practice [113]. It is well known that allele frequencies of CYP2C19 variants are subject to significant inter-racial differences. For example, *CYP2C19**2, the most frequent LOF allele, 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 than in other racial groups (~5 versus <1 %) [72, 73]. On the other hand, *CYP2C19**17, a gain-of-function mutation, is expressed to a lesser extent in Asians (~6 %) than in African Americans (~18 %) or Caucasians (~16 %) [72]. In addition to *CYP2C19*, LOF mutations in other CYP enzymes involved in biotransformation of clopidogrel, 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 African Americans (13 %) [115]. As a result, the prevalence of clopidogrel resistance is expected to be higher in Asians than in Caucasians. In fact, several clinical studies conducted in Chinese, Japanese and Korean patients revealed that the frequencies of clopidogrel resistance in Asian populations ranged from 20 to 65 %, which was remarkably higher than the frequencies reported from clinical trials that majorly included Caucasian patients [116]. However, direct comparison of these study result may not be feasible, since these studies had relatively small sample sizes, applied different HPR cut-off values or utilized different clinical settings. In addition, difference in body weight, diet, lifestyle and co-morbidities in different racial groups should be taken into consideration in investigations of the impact of the race factor on clopidogrel resistance.

4.3 Drug–Drug Interactions

Patients undergoing clopidogrel treatment are often required to take concomitant medication. Therefore, the pharmacokinetics- and pharmacodynamics-level DDIs that affect plasma levels of clop-AM and platelet activation and aggregation may consequently all contribute to differences in clopidogrel response and clinical outcome.

4.3.1 Proton Pump Inhibitors

Since gastrointestinal 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 has been shown to significantly decrease drug-induced gastrointestinal bleeding [117, 118]. In vitro studies have revealed that some PPIs, such as omeprazole, esomeprazole and lansoprazole, but not pantoprazole, are mechanism-dependent inhibitors of CYP2C19, which suppress bioactivation of clopidogrel [12, 119]. Clinical pharmacokinetic/pharmacodynamic studies conducted in healthy subjects and patients all confirmed that concurrent omeprazole led to a significant decrease in systemic exposure to clop-AM, as well as suppression of antiplatelet activity [120–123]. On the other hand, esomeprazole, but not other PPIs (including dexlansoprazole and pantoprazole), significantly interfered with the pharmacokinetics and pharmacodynamics of clopidogrel [120–122]. Conflicting results have been reported from assessments of the impact of lansoprazole on the pharmacodynamics of clopidogrel [121, 123–125]. Interestingly, the inhibitory effect of omeprazole and lansoprazole on the pharmacodynamics of clopidogrel was diminished in *CYP2C19**2 carriers, suggesting that the impact of PPIs on clopidogrel response may be dependent on the *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 use on the clinical outcome of clopidogrel therapy 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 have shown that there is no clinically significant interaction between clopidogrel and PPIs, which suggests that this combination is a safe treatment choice for patients at high risk of gastrointestinal bleeding, these analyses faced the following limitations: inclusion of a lower-risk population (e.g. only 42 % were taking clopidogrel for ACS [128]), use 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 use in 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-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins. Concerns that these statins could potentially affect clopidogrel bioactivation and response have been voiced in recent years because 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 with atorvastatin 80 mg significantly enhanced clopidogrel bioactivation and efficacy in both healthy volunteers and PCI patients with or without DM [135, 136]. Several other clinical studies also suggested that atorvastatin did not show a negative effect or even showed a positive effect on the antiplatelet activity of clopidogrel in patients [137–145]. Similarly, no other statins, including lovastatin, simvastatin, fluvastatin, rosuvastatin or pravastatin, have shown a significant effect on

the antiplatelet activity of clopidogrel [137–144]. These findings are in agreement with most clinical reports, showing that concomitant use of atorvastatin or other statin drugs have no negative impact on the clinical outcome of patients taking clopidogrel [141, 142, 145–148].

4.3.3 Calcium Channel Blockers

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

4.3.4 CYP Inhibitors 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 the clinical outcome. For example, platelet inhibition was significantly reduced when clopidogrel was co-administered with sulfonylureas (CYP2C9 substrates) [155], phenprocoumon (a CYP3A4 and CYP2C9 substrate) [156] or other CYP3A4 inhibitors, such as ketoconazole, erythromycin or troleandomycin [157, 158]. It has been shown that concurrent intake of grapefruit juice causes a more than 80 % decrease in clopidogrel bioactivation because of 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 the pharmacodynamics of clopidogrel 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 up-regulation of enzymatic activity. At the same time, the risk of experiencing bleeding events can also be expected to be higher with these DDIs. 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 the AhR pathway [160]. Several studies have shown that smokers exhibited enhanced platelet inhibition [144, 162, 163]. However, the association between smoking and the clinical outcome of clopidogrel therapy 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 the 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 (clopidogrel plus aspirin plus an anticoagulant) 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, which showed that this drug combination resulted in more efficacious protection from major cardiovascular risk but also remarkably elevated the incidence of bleeding events, compared with antiplatelet or anticoagulant treatment alone [166, 167]. The results from the WOEST clinical trial also reported that, compared with triple antithrombotic therapy, dual antithrombotic therapy (clopidogrel plus an anticoagulant) significantly decreased 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 a selective serotonin reuptake inhibitor (SSRI), such as fluoxetine, citalopram or sertraline [169]. SSRIs inhibit the serotonin

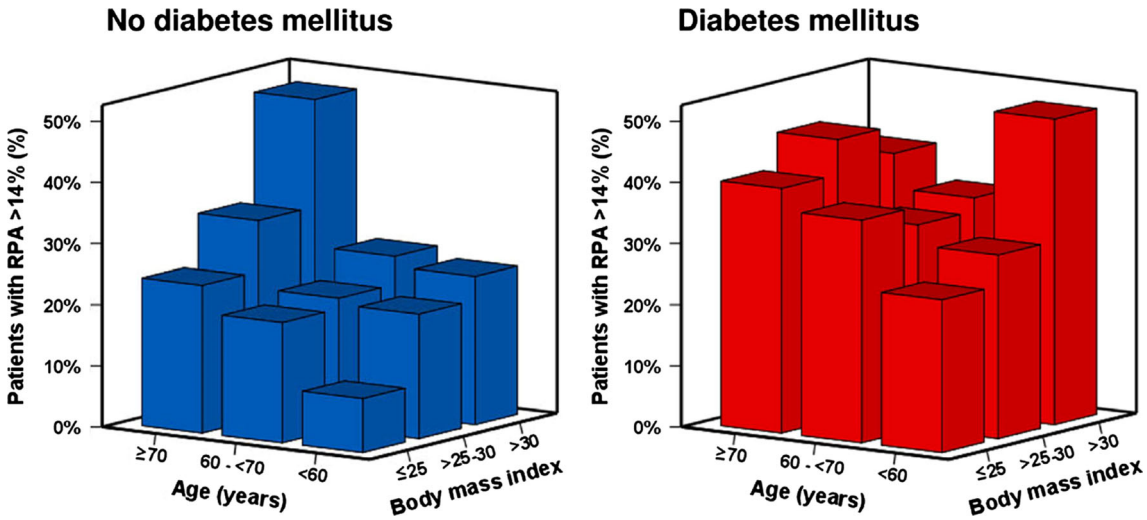

Fig. 2 Prevalence of patients with high on-treatment platelet reactivity (HPR) according to age, body mass index and diabetes mellitus status. HPR was defined as residual platelet aggregation (RPA) >14 %, which was assessed by a light transmittance aggregometry (LTA) assay with adenosine diphosphate (ADP) 5 μmol/L following a 600 mg clopidogrel loading dose in 760 patients undergoing elective coronary stent implantation [41]

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 depletion of intraplatelet serotonin stores, thereby reducing the efficiency of ADP-induced platelet aggregation [170]. Results from the SADHART trial in ACS 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 offer an additional advantage in CAD patients with co-morbid depression [171]. On the other hand, a recent DDI study conducted in healthy adults revealed that concurrent fluoxetine significantly reduced clop-AM plasma levels and clopidogrel response [172] because of inhibition of CYP2C9, CYP2C19 and CYP3A4 [12, 173]. Inconsistent results have been reported from several clinical outcome studies. The ENRICHD clinical trial and two following clinical outcome studies reported that concurrent use of SSRIs was associated with a significantly reduced risk of death or recurrent MI, as well as an increased risk of bleeding in clopidogrel-treated patients [174–176]. However, conflicting results have been reported from several other studies [169, 177, 178], suggesting the necessity for further evaluation of the potential impact of SSRIs on clopidogrel antiplatelet treatment.

4.4 Co-morbidities

4.4.1 Diabetes Mellitus

A significant proportion of ACS patients (~30 %) also suffer from DM, which gives them an increased atherothrombotic risk and higher mortality rates, compared with their non-diabetic peers [44, 55, 56, 82, 179]. The EXCELSIOR study showed that DM was the 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 in all BMI and age groups [e.g. age ≥70 years and BMI ≤ 25, or age ≤ 60 years and BMI ≥ 30] (Fig. 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. [181] reported that a mutation (rs956115) of *IRS1* (insulin receptor substrate 1) was associated with significantly higher prevalences of HPR and major adverse cardiac events in patients with type 2 DM and stable CAD following treatment with clopidogrel and aspirin. On the other hand, Erlinge et al. reported that the poor clopidogrel response in patients with DM was attributable to the lower systemic exposure to clop-AM rather than changes in the platelet response [182]. These findings were confirmed in a pharmacokinetic study conducted in healthy subjects and type 2 DM patients with the CYP2C19 substrate R483, which showed that DM may cause significant suppression of CYP2C19 catalytic capacity [183] and may potentially increase clopidogrel resistance in DM patients. Consistently, a recent study conducted in ACS patients also reported that CYP2C19 LOF mutations significantly impacted the clinical outcome of clopidogrel therapy in non-DM individuals compared with DM patients [184].

Table 1 Summary of factors that may affect the pharmacokinetics, pharmacodynamics and clinical outcome of clopidogrel therapy

Potential factors		Influence on pharmacokinetics and pharmacodynamics	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, 40, 57, 58, 87]	Minimal/inconclusive [5, 16, 42, 43, 45, 52, 59–61]
Pharmacogenetics			
ABCB1	C3435T	Minimal/inconclusive [16, 19, 21, 39, 60]	Minimal/inconclusive [16, 60, 64–67]
CES1	G143E	Lower on-treatment platelet reactivity [23, 68]	NA
	A-618C	Inconclusive [70, 71]	
CYP2C19	G681A (*2)	Lower systemic exposure to clop-AM [4, 75–77]	Increase in cardiovascular risk [14, 16, 18, 65, 78, 81–85]
	G636A (*3)	Higher on-treatment platelet reactivity [17, 18, 39, 58, 78–82]	
	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 and *CYP3A5*	*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]
P2RY12	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		NA	Decrease in cardiovascular risk but increase in bleeding events [166, 167]
Antidepressants		Conflicting/inconclusive [171, 172]	Conflicting/inconclusive [169, 174–178]
Co-morbidities			
Diabetes		Lower systemic exposure to clop-AM [182] Higher on-treatment platelet reactivity [41, 55, 82, 179, 181]	Increase in cardiovascular risk [41, 44, 56, 82, 181]
Chronic kidney disease		Higher on-treatment platelet reactivity (more significant when using VerifyNow^{TM} $P2Y_{12}$ assay) [91, 186–191]	Increase in both cardiovascular events and bleeding [43, 44, 59, 192–196]

ABCB1 ATP-binding cassette, sub-family B (MDR/TAP), member 1, *CES1* carboxylesterase 1, *clop-AM* clopidogrel active metabolite R-130964, *CYP* cytochrome P450, *NA* not available, *P2RY12* purinergic receptor P2Y, G-protein coupled, 12, *PON1* paraoxonase 1

4.4.2 Chronic Kidney Disease

Renal insufficiency (creatinine clearance <70 mL/min) has been reported in 35–40 % 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 the different assays that were used provided different

Table 2 Summary of demographic, genetic, drug-mediated and disease-mediated factors influencing antiplatelet therapy with clopidogrel

Potential factors		Influence on high on-treatment platelet reactivity	Influence on cardiovascular risk	Influence on bleeding risk
Demographics				
Older age		↑	↑	↑
Obesity		↑↑	↑↓	↔
Sex		↔	↔	↔
Pharmacogenetics				
ABCB1	C3435T	↔	↔	↔
CES1	G143E	↓	NA	NA
	A-618C	↔	↔	NA
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 and *CYP3A5*	*2, *3, *17 and others (*CYP3A4*)	↔	↔	↔
	*2, *3, *6 and others (*CYP3A5*)			
PON1	Q192R	↔	↔	↔
P2RY12	H2 haplotype and others	↔	↔	↔
Drug–drug interactions				
Proton pump inhibitors		↑	↔	↓
Statins		↔	↔	↔
Calcium channel blockers		↔	↔	↔
Anticoagulants		NA	↓	↑
Antidepressants		↔	↔	↔
Co-morbidities				
Diabetes		↑↑	↑↑	↓
Chronic kidney disease		↑	↑	↑↑

ABCB1 ATP-binding cassette, sub-family B (MDR/TAP), member 1, *CES1* carboxylesterase 1, *CYP* cytochrome P450, *NA* not available, *P2RY12* purinergic receptor P2Y, G-protein coupled, 12, *PON1* paraoxonase 1, ↑ increase, ↓ decrease, ↔ no effect

results. For example, CKD was determined to have a significant impact on HPR when the VerifyNow $P2Y_{12}$ assay was used, whereas no difference between patients with or without CKD was found when the LTA assay or the VASP-PRI assay was used [186–191]. The differences in test results were attributed to varying haemoglobin levels in CKD patients, which may cause interference in the assay when whole blood is used [186–188]. On the other hand, CKD was found to be associated with increased risks of adverse clinical outcomes (e.g. death, cardiovascular events and ST) and bleeding in 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 are potentially outweighed by bleeding hazards [196], suggesting that caution is needed when CKD patients require 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 to obtain timely refills of prescriptions or premature discontinuation of clopidogrel or dual antiplatelet therapy) was identified as a 'hidden factor' that contributes to clopidogrel resistance, an elevated risk of adverse cardiac events and even mortality [5, 44, 197]. Ho et al. [198] reported

that delays in filling clopidogrel prescriptions resulted in significantly increased death/MI rates in patients following stent implantation. Interventions such as follow-up by telephone, checking refill histories or monitoring via clinical registries have resulted in significantly improved compliance with 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 the response to clopidogrel treatment, particularly clopidogrel resistance. In this paper, we systematically review the known pharmacokinetic and pharmacodynamic factors, as well as genetic and non-genetic factors, that contribute to inter-individual differences in the response to clopidogrel treatment, and we evaluate how they relate to the clinical outcome (see Tables 1, 2).

To date, numerous clinical studies have been conducted to investigate the potential cause of clopidogrel resistance. Most of these studies were conducted either to answer one specific question (e.g. age, DDIs) or to investigate the impact of multiple impact factors in 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 us to sufficiently answer all of the open questions and, ultimately, to reliably identify optimal treatment/dosing regimens for individual patients prior to the start of therapy. This is, in part, due to the fact that suboptimal response to clopidogrel treatment, in terms of both efficacy and safety, is a multifactorial problem, which is difficult to address in one-off clinical trials that evaluate only one factor or only a few factors at a time. Harmonized use of quantitative analysis strategies, such as population and physiologically based modelling and simulation approaches in conjunction with systems biology/pharmacology modelling, may provide a quantitative characterization for the multiple genetic, demographic and disease risk factors that affect clopidogrel response, and the interaction between them, in a dynamic manner. These quantitative approaches, in combination with clinical trials, may help to overcome this limitation, as they will allow researchers to interpret and to compare information from head-to-head clinical trials and to evaluate the impacts of different genetic and non-genetic factors, as well as their interplay, on the 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 on the basis of their individual genetics, demographics, medication and disease history. The use of quantitative approaches may further allow performance of cost-effectiveness analyses for single as well as combination antiplatelet therapy and, ultimately, guide clinical and health-policy decision-making.

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