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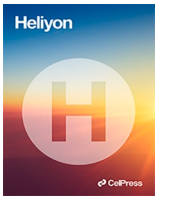

Review article

Advances and Perspectives in methods for identifying high platelet reactivity

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ABSTRACT

Antiplatelet therapy is the foundational treatment for the prevention and treatment of coronary and cerebrovascular ischemic events in patients with coronary heart disease, ischemic stroke, and transient ischemic attack (TIA). However, with more and more studies reporting an increased risk of thrombosis in some patients due to poor response to therapeutic agents, the selection of appropriate P2Y12 inhibitors has become a major challenge that needs to be addressed urgently. Currently, commonly used oral P2Y12 inhibitors include clopidogrel, ticagrelor, and prasugrel. Assessing patients' risk factors before the development of treatment regimens by effectively predicting the risk of high platelet reactivity with specific P2Y12 inhibitors in advance to avert the occurrence of major adverse cardiovascular and cerebrovascular events (MACCE) is the key point to the problem. Up to now, methods available for predicting platelet reactivity include genetic testing, platelet function testing, and risk scores. This review provides a summarization of the existent available identification methods and analyzes the advantages and drawbacks of different methods in specific clinical settings, intending to guide the rational clinical application of P2Y12 receptor inhibitors.

1. Introduction

Ischemic cardiovascular and cerebrovascular diseases are common and frequent diseases that endanger human health. Atherosclerotic thrombosis is an important causative factor, and platelet adhesion and aggregation are important pathways of thrombosis, so antiplatelet therapy plays an important role in reducing the risk of major adverse cardiovascular and cerebrovascular events (MACCE) [1]. Nowadays, dual antiplatelet therapy (DAPT) consisting of aspirin combined with one P2Y12 inhibitor or P2Y12 inhibitor monotherapy is not only the cornerstone for the prevention of coronary and cerebrovascular ischemic events after percutaneous coronary

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intervention (PCI) in patients with coronary artery disease but also the secondary prevention strategies to reduce the recurrence, disability or death of patients with ischemic stroke and transient ischemic attack (TIA). However, owing to the inter-individual heterogeneity, some patients develop HPR during treatment due to poor responsiveness to the antiplatelet agents [2], which results in inadequate inhibition of platelet function and a significantly increased risk of adverse clinical outcome events [3,4]. Some studies have reported that the incidence of high platelet reactivity (HPR) during clopidogrel, ticagrelor, and prasugrel therapy is expected to reach 30 %, 3–15 %, and 0–3%, respectively, which seriously affects patients' clinical outcomes and prognosis [5,6]. Therefore, identifying patients prone to HPR by using effective methods in the development of treatment regimens and maximizing the therapeutic effect with effective interventions is a major challenge in promoting individualized treatment with P2Y12 inhibitors.

In the early stage, platelet reactivity is mainly assessed by platelet function testing (PFT) assays, then determines the patient's platelet function status based on the detection value, and different assays have specific operating procedures and monitoring ranges. Afterward, with the development of pharmacogenomics, some investigators found that certain specific alleles showed a high correlation to HPR as well as thrombotic events. Thereafter, based on previous findings, researchers constructed different clinical prediction models consisting of clinical risk factors, genetic factors, or a combination of clinical and genetic factors to achieve the goal of improving the predictive power in high-risk patients. This review summarizes the evidence that different methods in antiplatelet therapy can be used to predict the risk of developing HPR, provides an outline of currently available methods, as well as an analysis of the advantages and drawbacks of each method in the context of the clinical setting, and provides a reference for further research directions.

2. Hereditary factors and genetic testing

With the advancement of pharmacogenomics knowledge and gene sequencing technologies, genetic information has played an increasingly significant role in the performance of individualized therapy [7]. Pharmacogenomics focuses on the problem of individualized differences in drug efficacy caused by genetic polymorphisms. Individualized therapy is based on the results of pharmacogenomics and gene sequencing, combined with the clinical factors affecting the body's response to drugs, and optimizes the selection of drugs and dosage adjustments by predicting the safety and efficacy of the drugs, intending to achieve the best therapeutic effect while minimizing the risk of adverse reactions. Simultaneously, owing to the availability of disparate assays and rapid bedside assays, feasibility, as well as the results, are inherent in the patients, the genetic testing technology has become widely used in clinical practice [8]. Gene polymorphism mainly affects the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs *in vivo* by changing the number and activity of drug-metabolizing enzymes, transporters, and/or targets. Consequently, the differences between the individual genetic information have a momentous impact on the clinical effects of the drugs and the risk of adverse drug reactions.

2.1. Clopidogrel

Clopidogrel, as a prodrug, after being transported to the intestinal by P-glycoprotein (P-gp), about 85 % is hydrolyzed by carboxylesterase-1 (CES1) to inactive carboxylic acid derivatives, and the rest is activated by cytochrome P450 (CYP450) enzymes [9]. The activation process consists of 2 steps [10]. In the first step, clopidogrel is metabolized to 2-oxo-clopidogrel by CYP1A2 (36 %), CYP2B6 (19 %), and CYP2C19 (45 %) [11,12]. In the second step, under the action of the rate-limiting enzyme paraoxonase 1 (PON1), the bulk of 2-oxo-clopidogrel is transformed by CYP2B6 (33 %), CYP2C9 (7 %), CYP2C19 (20 %), and CYP3A4 (40 %) to generate active 5-thiol-clopidogrel [11,12]. The irreversibly binding of active metabolites to Adenosine Diphosphate (ADP) receptor P2Y12 on platelet membrane inhibits the binding of fibrinogen to GPIIb/IIIa receptor and blocks the activation of GPIIb/IIIa complex mediated by ADP, thus playing an antiplatelet aggregation role.

As an antiplatelet drug, clopidogrel is mainly used to prevent thrombosis in patients with cardiovascular and cerebrovascular diseases, and changes in polymorphisms and expression levels of genes of drug metabolism, transport, and target sites of action all affect the blood concentration and sensitivity of its active metabolites *in vivo*. If the blood concentration of the active metabolite reaches the therapeutic window, it can effectively inhibit platelet aggregation and prevent the occurrence of adverse ischemic events; conversely, the risk of adverse clinical outcome events increases. It should be noted that excessive antiplatelet activity can disrupt the body's coagulation-hemorrhage balance and increase the occurrence of bleeding events. In 2010, the U.S. Food and Drug Administration (FDA) added a boxed warning to the label for clopidogrel suggesting that patients carrying the CYP2C19 Loss-of-Function (LOF) gene have attenuated antiplatelet effects. The warning made people realize the importance of accurately identifying the differences in genetic information between individuals to optimize clopidogrel efficacy, and further studies were carried out on its pharmacogenetic characteristics.

2.1.1. ATP-binding cassette subfamily B member 1 (ABCB1)

Located on human chromosome 7 q21.1, the ABCB1 gene encodes the transporter P-gp involved in the efflux process of various drugs [13]. The P-gp, also known as ABCB1 transporter, is expressed on intestinal epithelial cells, and increased expression or function can alter the bioavailability of substrate drugs [14]. The ABCB1 gene has about 50 single nucleotide loci, among which C3435T, C1236T, and G2677T are associated with P-gp, and the C3435T locus is the most studied [15,16]. Interindividual variation in P-gp expression due to ABCB1 gene polymorphisms may alter clopidogrel absorption *in vivo*, affecting the amount of parent drug entering the liver [17].

For C3435T, one study discovered that patients carrying the TT + CT genotype had reduced clopidogrel absorption and increased risk of adverse cardiovascular events compared to patients with the CC genotype, suggesting that the 3435T-allele may be related to

clopidogrel hyporesponsiveness [18]. Another study conducted in patients with acute coronary syndrome (ACS) after PCI yielded similar results, with the TT genotype associated with clopidogrel hyperresponsiveness and the risk of recurrent ischaemic events [19]. A recent study performed in the ACS, as well as coronary artery disease (CAD) patients, found an increased risk of major adverse cardiovascular events (MACE) in patients carrying the TT genotype, whether compared to genotype CC or CC + CT [20]. However, the results of Karaźniewicz et al. [14] and Sridharan et al. [21] showed that the ABCB1 C3435T gene polymorphism has no effect on the degree of inhibition of platelet aggregation by clopidogrel. Similarly, some studies also did not find a correlation between ABCB1 gene polymorphisms and clopidogrel efficacy [22,23], indicating that the ABCB1 genotype does not affect platelet reactivity in clopidogrel-treated patients [24,25], and is no correlation with the occurrence of clopidogrel resistance (CR)/nonresponse [26].

The effect of ABCB1 C3435T gene polymorphism on the antiplatelet effect of clopidogrel remains highly controversial and is not yet supported as a predictor of HPR after clopidogrel treatment. However, for patients with poor response to clopidogrel, the presence or absence of the TT genotype can be one of the references to adjusting the treatment regimen.

2.1.2. Carboxylesterase 1 (CES1)

CES1 plays an important role in the metabolic conversion of clopidogrel [27]. Mutations in the CES1 gene, which encodes the carboxylesterase family, a hepatic enzyme that plays a major role in drug clearance, can cause changes in enzyme activity. The most studied single nucleotide loci are rs71647871 and rs 2244613.

Earlier studies have shown that the rs71647871 (c.428G > A, p.Gly143Glu) single nucleotide variation (SNV) attenuates the metabolic capability of CES1 [28]. A study conducted in patients with CAD showed that polymorphisms in the CES1 c.428G > A gene were associated with the antiplatelet effect of clopidogrel, the carriers of the CES1 143E-allele had elevated levels of active metabolites and enhanced inhibitory effect on ADP-induced platelet aggregation [29]. Similar findings were subsequently reached in other studies that the carriage of the E allele significantly reduces the inactive metabolic process of clopidogrel [30,31], resulting in increased clopidogrel active metabolite (Clop-AM) plasma concentrations [32], inhibition of platelet reactivity [33], and decreased risk of CR [30,33]. There are relatively few studies on the PD effects of CES1 G143E polymorphism on clopidogrel. A study exploring factors influencing clopidogrel responsiveness found no difference in the genotype distribution of G143E between patients who developed MACE and those who did not, suggesting that the genetic variabilities in CES1 G143E may not influence the development of MACE [34]. In contrast to CES1 G143E, the carriers of the CES1 rs2244613 C-allele with enhanced enzymatic activity, elevated levels of platelet reactivity, and increased risk of CR [35]. However, other researches has shown that the CES1 rs2244613 single nucleotide polymorphism (SNP) are not correlated to the risk of MACE events and does not affect the clopidogrel inhibition efficacy on platelet aggregation [36,37].

On balance, polymorphisms in the CES1 gene lead to alterations in CES1 enzyme activity, which negatively correlates with the production of active metabolites of clopidogrel, thereby affecting antiplatelet effects [27]. Owing to the mutation in the CES1 G143E gene leading to the weakened enzyme catalytic activity, the 143E-allele is expected to be a predictor of platelet hyporesponsiveness to clopidogrel [27]. The CES1 rs2244613 is associated with strengthening enzyme activity, but given the paucity of relevant studies and inconsistent results at present, further studies are needed to investigate its specific influence and correlation with HPR.

2.1.3. Paraoxonase-1 (PON1)

PON1 is an esterase synthesized in the liver and present in the serum, mainly encoded by the PON1 gene, whose genetic polymorphism determines the rate of clopidogrel active product formation. There are two-locus included Q192R and L55 M in the PON1 gene [38], and the current studies on clopidogrel mainly focused on Q192R, which has two genotypes with opposite enzymatic activities, QQ and RR [39].

A study exploring the effect of PON1 Q192R gene polymorphism on clinical antiplatelet indicators and outcome events found that patients carrying the QQ genotype had reduced PON1 enzyme activity, diminished antiplatelet effects, and a higher risk of HPR and instent thrombosis [40]. Subsequently, a study carried out on ACS patients and non-ACS patients discovered that the PON1 Q-allele was highly associated with adverse cardiovascular outcomes but not with the antiplatelet function of clopidogrel [41]. However, unlike the previous studies, the recent studies revealed that PON1 192R variants but not PON1 192Q attenuated the effect of clopidogrel on platelet aggregation [42], and could serve as an independent risk predictor for HPR [43]. A study exploring the effect of polymorphisms in genes encoding metabolic enzymes on clopidogrel efficacy and MACE noted a higher risk of CR in patients with the RR genotype, but no correlation with clinical outcomes was found [44]. The same results were validated in patients with UA [45]. In addition, the RR genotype has been found to serve as a risk predictor for PCI followed by revascularisation in patients with ACS [46]. In contrast, it has also been shown that PON1 Q192R does not affect the PK and antiplatelet effect of clopidogrel [47,48], and is independent of low responsiveness to clopidogrel [49]. There was no difference in the activity of platelets as well as the incidence of MACE including stent thrombosis in patients carrying different genotypes of PON1 Q192R [50].

From what has been described above, the current controversy regarding the effect of the PON1 Q192R gene polymorphism on clopidogrel response is characterized by two main points. Firstly, the culprit gene (Q or R) is still unclear. Secondly, the results of studies exploring the effect of the R gene on platelet reactivity after clopidogrel treatment have been inconsistent. More studies are needed to confirm the concrete effects of different genotypes of PON1 Q192R on the ADP-induced platelet aggregation effect in clopidogrel-treated patients.

2.1.4. Cytochrome P450 2C19 (CYP2C19)

CYP2C19 occupies an important part in the transformation of clopidogrel [51], contributing to about 12 % of the variability of clopidogrel efficacy [52]. The CYP2C19 gene is located at q24.1 ~ q24.3 of human chromosome 10 [53], and 42 alleles have now been

discovered [54]. These alleles were separated into four categories composed of normal function (*1), decreased function (*9), no function (*2∼*8), and increased function (*17) [55]. Based on various types and numbers of alleles carried, each individual is classified as having different metabotropic. Individuals carrying two normal-function alleles are categorized as normal metabolizers (NM), carrying one no-function allele with one normal allele or one increased allele are pigeonholed as intermediate metabolizers (IM), carrying two no-function alleles are defined as poor metabolizers (PM) [55].

The no-function allele is also known as the LOF gene. Due to the high mutation in the population, the research on CYP2C19*2 and *3 is more mature and in-depth [56], and genetic variants of other CYP2C19 leading to no function are relatively rare [57]. The inactivation of the enzyme associated with the CYP2C19*2 allele is due to the G > A mutation of the base at position 681 of exon 5 of the allele, forming an abnormal cutting site, causing the loss of base pairs at the beginning of the exon and accompanying loss of 215–227 amino acids during translation, result in premature termination of protein synthesis, then a nonfunctional protein with loss of enzyme catalytic activity is produced [58]. Because the G/A mutation at the 636th base of exon 4 creates a premature stop codon and then results in the termination of protein synthesis, the carrier of CYP2C19*3 allele leads to the loss of CYP2C19 enzymatic activity [59]. Thus, at standard therapeutic doses, NM patients have normal enzyme activity and generate levels of active metabolites that achieve effective blood concentrations.IM and PM patients have relative or absolute deficiencies in enzyme activity due to the carriage of the LOF gene, rendering the antiplatelet efficacy of clopidogrel attenuated to varying degrees.

Studies have found that the presence of the CYP2C19 LOF gene can reduce the activity of the enzyme with a subsequent decrease of clopidogrel active metabolites [60], resulting in the lack of safety and effectiveness of treatment [61]. One study in coronary heart disease (CHD) patients treated with clopidogrel indicated that the CYP2C19 LOF gene had the potential to predict the occurrence of adverse cardiovascular events [62]. Similarly, Liu et al. found the CYP2C19*2 and *3 alleles could be regarded as the independent risk factors of stroke recurrence in ischemic stroke patients [63]. Additionally, the results of multiple studies demonstrated the patients carrying the CYP2C19 LOF gene showed a higher incidence of ischemic events and increased risk of stent thrombosis [64,65]. The conclusions of the two meta-analyses were consistent with the above findings [66,67], thus confirming the predictive value of the CYP2C19 LOF gene for cardiovascular events.

The CYP2C19 LOF gene also associated with platelet reactivity, affects the antiplatelet function of clopidogrel. In patients with cardiovascular disease or ischemic stroke, the CYP2C19*2 polymorphism was correlated with lower clopidogrel antiplatelet reactivity [68] and could be used to predict clopidogrel responsiveness [69]. One study with patients who underwent percutaneous neurointervention attained similar findings [16]. Besides, studies also revealed that polymorphism in the CYP2C19*3 allele increased platelet reactivity in clopidogrel-treated patients [70]. Although Hou et al. discovered that CYP2C19*3 was associated with a higher risk of HPR while CYP2C19*2 was not [25], numerous studies implied the CYP2C19*2 and *3 mutant alleles increased the risk of CR [71–73], could serve as an independent predictor for high platelet reactivity in patients with clopidogrel [42,70].

In conclusion, the CYP2C19 LOF gene composed of CYP2C19*2 and *3 mutant alleles was not only related to the platelet reactivity to clopidogrel but also had an adverse effect on the clinical outcomes [44], has the potential to predict HPR in clopidogrel-treated patients. For the patients treated with clopidogrel, current guidelines, and expert consensus do not recommend routine genetic testing [74,75], but the genetic testing should be thinking highly of physicians because the results will be conducive to identifying high-risk patients, predict the curative effect, and make optimum individualized management, preventing patients from developing cardiovascular outcomes due to poor response to clopidogrel.

2.1.5. P2Y12

The P2Y12 gene is located on human chromosome 3 q24-25 including two exons and one intron [76]. At present, it has been found that there are 5 mutated loci in this gene, namely I–C139T, I-T744C, I-INS801A, C34T, and G52T [76]. Most of the existing studies on the P2Y12 gene placed emphasis on the T744C, C34T, and G52T locus. One study by Wang et al. pointed out that the presence of P2Y12 744T-allele contributes to a higher risk of high on-treatment platelet reactivity (HTPR) and MACE in patients after PCI [77]. Apart from that, another study found the C allele had a lower risk of CR, which means that T is the risk factor for CR [69]. Nevertheless, a meta-analysis failed to discover the effect T744C polymorphisms of P2Y12 bring on CR [78]. The studies of Nie et al. [79] and Cuisset et al. [80] obtained analogous conclusions. Simultaneously, two observational studies developed in patients who underwent percutaneous neurointervention showed no association between the T744C allele and HTPR [81]. For the C34T and G52T, the study implemented in Chinese patients undergoing PCI suggested the genetic variations of the C34T and G52T were linked to lower clopidogrel responsiveness and adverse clinical events [82]. Concomitantly, a meta-analysis indicated the T-allele of the C34T and G52T had the potential to be viewed as the risk factors for higher platelet reactivity in clopidogrel-treated patients [78]. Of note, in another meta-analysis, the investigators found in the Chinese population the variabilities in P2Y12C34T and G52T genes showed an effect on cardiovascular events while in the Caucasian population showed no effect [83]. Maybe the difference in the mutation frequency of the P2Y12 gene between races is partly responsible for the difference in results.

In summary, the results of current studies on the effect of P2Y12 T744C on clopidogrel efficacy are controversial. Although there are some positive results from studies of C34T and G52T, they also cannot be used as direct evidence to guide clinical treatment, considering the small base of relevant original studies. The correlation studies of the P2Y12 gene provide research ideas for predicting platelet reactivity after clopidogrel treatment, but the current evidence is not enough to support its clinical application.

2.2. Ticagrelor

Ticagrelor is an active drug, mainly metabolized by CYP3A4 and a small proportion by CYP3A5, its main metabolite AR-C124910XX (ARC) has been shown to with equivalent potency *in vitro* [84].

Studies revealed the CYP3A4*22 allele displayed a strong antiplatelet effect due to the attenuation of CYP3A4 enzyme activity [85], which may increase the risk of adverse bleeding events but does not correlate with clinical ischemic endpoints [86,87]. By contrast, the CYP3A4*1G enhanced the activity of the CYP3A4 enzyme, which led to the accelerated metabolism of ticagrelor, but had less impact on the inhibition of platelet aggregation because its metabolite had similar antiplatelet potency as the parent drug [88]. In addition, it was found that CYP3A4*1G added removal for its metabolite ARC [89], but had no impact on the PK and PD of the ticagrelor [90]. For the CYP3A5*3, most studies found no effect on either PD or PK of ticagrelor [85–87].

The effect of ticagrelor on platelet activation was not affected by ABCB1 gene polymorphisms [91]. Zhu et al. [92] discovered the allele CYP2C19*3 was associated with the decline of Tmax of the ticagrelor and CYP2C19*2 was relevant to the reduction of the Cmax of the ARC. Other studies obtained similar results, indicating that the CYP2C19 polymorphisms affect the PK of ticagrelor but not its antiplatelet effects [93,94]. As the drug target, current findings do not support a correlation between polymorphisms in the P2Y12 gene and responsiveness to ticagrelor [91,95,96].

The CYP4F2 gene is located on chromosome 19, encoding the synthesis and function of the CYP4F2 enzyme. The CYP4F2 enzyme plays a critical role in the course of catalyzing arachidonic acid (AA) to 20-hydroxyeicosatetraenoic acid (20-HETE), which antagonizes platelet surface membrane thromboxane receptor and thus affects platelet aggregation process [97]. The CYP4F2 enzyme is also responsible for the metabolism of vitamin K1 to hydroxyvitamin K1, a process that leads to a decrease in the production of vitamin KH2 via vitamin K1 reduction, which has an impact on the process of coagulation factor activation. Previous studies suggested genetic variations of CYP4F2 had an impact on platelet aggregation and were correlated with stent thrombosis [97,98]. With the aim to explore the influence of factors on the antiplatelet effect of ticagrelor, Vacis et al. discovered that the CYP4F2 rs3093135 TT genotype was associated with low platelet aggregation values as well as bleeding events [99,100]. However, the results of a study exploring the association of genetic polymorphisms with bleeding events during tegretol treatment showed no association between CYP4F2 rs3093135 and the occurrence of bleeding events [101]. Apart from that, one study conducted on healthy Chinese volunteers showed that the single nucleotide variability of the CYP4F2 rs2074900 was related to the PK of ticagrelor, AA genotype carriers showed higher Cmax and AUC [102].

It has been demonstrated that ticagrelor can affect platelet aggregation by mediating adenosine levels by the following possible mechanisms. Inhibition of sodium-dependent equilibrative nucleoside transporter 1 (ENT1) expressed by erythrocytes [103] and platelets [104] by ticagrelor reduces the transport of adenosine into the cell, increasing the level of adenosine bound to the A2a receptor on the platelet surface [105]. Activation of the A2a receptor by ticagrelor causes an increase in the level of cAMP, which attenuates the binding of glycoproteins to fibrinogen, thus exerting an antiplatelet effect [106]. Additionally, some studies have also explored the effect of A2a receptor polymorphism and platelet aggregation. Nardin et al. observed the carriers with A2a receptor rs5751876 CC genotype showed a higher risk of HPR in patients treated with ticagrelor, indicating that C-allele may be a risk factor for low reactivity to the treatment of ticagrelor [107]. Then, given the paucity of relevant studies, it is not yet supported to conclude correlation.

The SLCO1B1 gene, located on chromosome 12 of the human body, encodes the hepatic superficial transmembrane transporter organic anion transporter polypeptide (OATP1B1), mainly mediating the transport and elimination of endogenous and exogenous substances. A previous study reported that the SLCO1B1*5 (rs4149056, c.521T > C) results in reduced OATP1B1 transporter protein activity, affected the level of ticagrelor and ARC, but not related to the occurrence of clinical outcomes [108]. However, in a recent study, the investigators found no statistically significant effect of SLCO1B1*5 on the PK parameters of ticagrelor and ARC [92]. Li et al. acquired the same result that SLCO1B1*5 did not affect both PD and PK of ticagrelor [90]. In addition, Zhu et al. found that the Cmax of the parent drug and ARC was elevated by approximately 39.0 % in carriers of the SLCO1B1 388A > G (rs2306283) GG genotype compared to the AA genotype, but a statistically significant difference due to the smaller proportion of AA genotype carriers cannot be excluded [92].

UGT2B7 gene mainly encodes the protein concerning drug biotransformation. Christoph et al. found the UGT2B7 rs61361928 was related to the high concentration of ARC, but not the ticagrelor, speculating that the UGT2B7 enzyme may be involved in metabolic processes downstream of ARC [108]. However, another study failed to discover the effect of UGT2B7 rs7439366 and UGT2B7 rs12233719 on the PD and PK of the ticagrelor as well as ARC [92]. Consequently, a correlation between the UGT2B7 gene and ticagrelor including its metabolite cannot yet be demonstrated.

ITGA2B and IT-GB3 genes mainly participated in the encoding of the receptor complex integrin αIIbβ3 on the platelet surface. An *ex-vivo* study exploring the effect of the ITGA2B (rs5911 T > G) genetic variant on platelet reactivity after ticagrelor treatment found that the inhibitory effect of the GG genotype on platelet aggregation was significantly attenuated [109]. The studies explored the genotypic variations of the IT-GB3 on antiplatelet effect have not found a correlation [95,109]. Platelet endothelial aggregation receptor-1 (PEAR1) is an integral membrane protein engaging in platelet aggregation [110,111]. It was found that PEAR1 rs12041331 had an impact on antiplatelet function and PEAR1 rs2768759 can be used to predict the clinical outcome in ACS and CAD patients [111,112]. Besides, a recent study indicated genetic polymorphisms in PEAR1 rs77235035 were associated with ticagrelor PK [102].

Based on the current research evidence, it is not enough to support the application of genetic testing in a clinical setting to evaluate the correlation between gene polymorphisms and PK as well as PD of ticagrelor [113]. It is worth noting that the genetic differences of CYP3A4, SLCO1B1, UGT2B7, ITGA2B, PEAR1, CYP4F2, A2a receptor, and P2Y12 among individuals cannot be ignored. Although there is no definitive conclusion, further studies are still needed to clarify the dependence between genetic variability and antiplatelet effect as well as clinical outcome, so as to provide a valuable reference for clinical decision-making.

2.3. Prasugrel

As a P2Y12 receptor prodrug, prasugrel is metabolized through cytochrome P-450 including CYP3A4, CYP2B6, CYP2C9 as well as CYP2C19, and then combined with the targeted receptor to inhibit the clumping of platelets [114,115]. For the moment, the coherence studies on the effects of prasugrel metabolizing enzymes and transporters gene polymorphisms on its PK and PD have not yet reached a unified conclusion.

A number of previous studies have shown that genetic variabilities of the CYP2C19 enzyme are associated with platelet reactivity in prasugrel-treated patients [116,117], the LOF gene carriers with higher residual platelet activity than non-carriers [117]. However, some researchers found that the genetic differences of CYP2C19 did not affect the PK and antiplatelet effect of prasugrel [118,119], nor did it change the evaluation parameter P2Y12% inhibition after treatment [120]. At the same time, some studies have found that ABCB1 gene polymorphism has no effect on the PK process and therapeutic effect of prasugrel [121,122], which may be mainly contributing to the complete absorption of prasugrel in the intestine.

For the cytochrome P450 CYP3A4 enzyme, a study by Holmberg et al. demonstrated that CYP3A4*22 does not imply the absence of an effect of biotransformation on prasugrel [85]. No studies have shown an association between the genetic variations of CYP3A4 and the antiplatelet effect of prasugrel, however, a study implemented by Máchal et al. pointed out that the metabolic activity of CYP3A4 is associated with platelet reactivity after prasugrel treatment [115].

The relativity between CYP2B6 and CYP2C9 gene polymorphisms with the antiplatelet effect of prasugrel remains controversial. In one study, Franken et al. indicated that CYP2B6*6 and CYP2C9*2 exhibit a correlation with high on-prasugrel platelet reactivity [123]. Subsequently, Fiore et al. reported the first case of a patient with prasugrel resistance owing to the carry of the heterozygous mutant gene of CYP2B6 (G516T) and CYP2C9*3 [124]. Besides, a recent case report by Yamagata et al. pointed out that CYP2B6*2 (C64T) may weaken the metabolic conversion of prasugrel [125]. Nevertheless, some studies have also pointed out that CYP2B6 and CYP2C9 gene polymorphisms do not affect the inhibitory effect of prasugrel on platelet aggregation [51,60].

Pharmacogenomics in prasugrel label information updated by FDA 2021 states that genetic variants in CYP2B6, CYP2C9, CYP2C19, or CYP3A5 have no relevant effect on the PK of prasugrel's active metabolite or its inhibition of clumping of thrombocytes [126]. In addition to this, the clinical outcomes in prasugrel-treated patients were not influenced by gene polymorphisms of the CYP450enzyme [20,51]. It suggests that it is infeasible to carry out genetic testing to appraise the platelet reactivity of prasugrel-treated patients in clinical practice. Further clinical studies are still needed to prove the application value of genetic testing technology in patients on prasugrel.

From what has been discussed above, genetic polymorphisms can affect the PK course and PD approaches of drugs *in vivo*, leading to differences in drug responsiveness among individuals. Thus, with pharmacogenetic testing, it is possible to predict in advance the possible risks of patients during treatment and adjust the treatment regimen to minimize the occurrence of adverse outcome events. For clopidogrel, a great deal of research has proved that CYP2C19*2 and *3 can assist physicians in predicting the risk of HPR in patients during antiplatelet therapy. Genetic testing to guide treatment with ticagrelor and prasugrel is not supported at this time. In addition, as an important link to precision medication administration, the potential risks of genetic testing during treatment cannot be ignored. The foremost thing is the accuracy and reliability of the results. If the genetic phenotype test results are wrong due to machine or reagent problems, or if the patient's genotype is not consistent with the phenotype, it will cause long-term unpredictable and adverse health effects to the patients [55]. Furthermore, incomplete information on genetic testing, the existence of uncertain factors affecting treatment decisions, and the lack of standardized guidance on the sequencing process and interpretation of results can all pose certain risks. Therefore, it is momentous to strictly standardize the operation process to ensure high-quality deoxyribonucleic acid (DNA) sequencing results.

3. Platelet function testing

3.1. Light transmittance aggregometry (LTA)

The principle of the LTA is to induce platelet aggregation and sedimentation by adding different pro-aggregation substances to platelet-enriched plasma and to detect the changes in light transmission due to reduced plasma turbidity by optical turbidimetry. Platelet reactivity is reflected by reporting the maximum platelet aggregation rate (MAR,%). The advantages are that it is a whole blood assay, inexpensive, results correlate well with clinical adverse events, is widely used, and is considered the gold standard for measuring platelet aggregation function [127,128]. The disadvantages are the complexity of the procedure, the time-consuming and labor-intensive sample pre-treatment, the poor reproducibility of the results, the unsuitability of certain special samples (e.g. hemolysis, lipidemia, jaundice, etc.), and the fact that the process of centrifugation also activates platelets [127,128,129,130].

3.2. Thrombelastogram (TEG)

TEG [131,132] reflects platelet aggregation, thrombosis, and fibrinolysis by simulating the changes of the whole coagulation process *in vitro*, calculating the inhibition rate of the corresponding pathway based on the maximum thrombus amplitude of different aggregation inducers, qualitatively assessing the different stages of the coagulation process, and quantitatively assessing the reactivity of platelets by reporting the maximum amplitude (MA). The advantages are that the test sample is whole blood, easy to standardize, can be done immediately at the bedside, reproducibility is better, and helps to assess the risk of bleeding and ischemia [133,134]. The disadvantages are that it is expensive, there is no uniform standard, specificity is low, and the consistency of the test results with those

of the LTA method is still controversial.

3.3. Vasodilator stimulated phosphoprotein (VASP)

The detection principle of VASP is to determine the degree of inhibition of P2Y12 receptors on the platelet surface by adding prostaglandin E1 to induce aggregation and subsequently measuring the phosphorylation status of intra-platelet protein using flow cytometry [135]. The residual platelet response index (PRI), calculated from the proportion of P2Y12 receptors inhibited, reflects the anti-platelet effect of P2Y12 inhibitors. The advantages of the VASP assay are whole blood detection, rapid operation, relatively longer sample storage time [136], good repeatabilities, and good agreement with the LTA assay results. The disadvantages are the high cost of the assay, the complexity of the operation, and the vulnerability of the results to human interference.

3.4. VerifyNow-P2Y12 assay

Verify Now-P2Y12 Assay [137] is the same as LTA, both use the turbidimetric method as the optical principle to measure the change of light transmission caused by platelet aggregation. After the addition of the activator, cross-linking of platelet surface IIb/IIIa receptor complexes with fibrinogen-coated beads allows platelets to aggregate, enhancing light transmission. The degree of platelet aggregation is reflected by the Platelet Reaction Units (PRU) [132]. The advantages are that it is quick and easy to perform, fully automated, can be used at the bedside, requires a small sample size, does not require sample processing, and the procedure is easily standardized [138]. The disadvantages are that it is limited by the hematocrit and platelet count, there is no standardized threshold value, and it is expensive [139].

3.5. Plateletworks

The plateletworks assay is based on the difference in platelet counts before and after aggregation and uses a continuous dynamic PFT method to compare the platelet counts measured in control tubes of ethylenediamine tetraacetic acid (EDTA)-anticoagulated whole blood with the counterpart obtained in sodium citrate anticoagulated whole blood after aggregation with an inducer. The level of platelet function is expressed by the maximum platelet aggregation rate (MAR). The method does not require the processing of blood samples and can be used for immediate detection. However, the test needs to be performed within 10 min after blood collection results to ensure timely detection difficulty, and additional platelet counts are also required which all limit the clinical application [140].

3.6. Platelet function Analyzer

The PFA assay system uses hemodynamic principles *in vitro* to simulate platelet adhesion and aggregation during a vascular injury *in vivo*. Under high shear, whole blood samples flow through a membrane pore coated with collagen and pro-aggregating substances (ADP, epinephrine), which activates platelets, causing them to adhere and aggregate at the membrane pore to form a thrombus, ultimately blocking blood flow. The system measures the time required for the pore to be completely occluded from the start of the assay and reports the closure time (CT) to reflect the platelet aggregation function [141]. The advantages are that the sample is whole blood, the sample size required is small, and the test time is short. The disadvantages are that the results are easily influenced by hematocrit, platelet count, fibrinogen, etc., and are poorly standardized.

The clinical significance of platelet function testing is to be able to identify the functional status of platelets in patients after taking therapeutic drugs so that appropriate preventive and curative measures can be given to minimize the risk of adverse events in patients [142]. However, given different assays in platelet function determination have no uniform criteria for operational standards and judgment cut-off values, further improvements are still needed in the field of platelet function testing. Firstly, to continue to explore and establish an assay with high specificity, rapid and simple operation, easy standardization of the process, and good consistency with clinical outcome events. Secondly, to improve the deficiencies of existing assay methods in the application, such as standardizing the process of collecting and processing samples to reduce the interference of external factors on platelet stability, standardizing the operation process of the instrument and equipment to improve the credibility and accuracy of the results, and developing a reasonable time interval for the assay, etc. Thirdly, for different antiplatelet drugs, their specific thresholds under different assay methods should

Table 1
Monitoring indicators of common oral P2Y12 receptor inhibitors.

P2Y12 receptor inhibitors	Detection method	HPR monitoring range	
clopidogrel	LTA	ADP(5umol/L)	MAR≥50 % [144]
ticagrelor		ADP(20μmol/L)	MAR≥60 % [144]
prasugrel	TEG	MA_{ADP}>47 mm [134]	
	VerifyNow P2Y12	PRU>208 [144]/230 [145]	
	VASP	PRI>50 % [146]	

Abbreviations: HPR: high platelet reactivity; LTA: light transmittance aggregometry; TEG: thrombelastogram; VASP: vasodilator-stimulated phosphoprotein; ADP: adenosine diphosphate; MAR: maximum platelet aggregation rate; MA: maximum amplitude; PRU: platelet reaction units; PRI: platelet response index.

be obtained through experimental exploration of large samples to improve the specificity of treatment [143]. The monitoring range of HPR of different assays is shown in Table 1.

4. Risk score

4.1. Clopidogrel

4.1.1. ABCD-GENE score

ABCD-GENE score is a simple risk score derivative from the dataset of the GRAVITAS study and conducted external validation in 2 substantive DAPT-treated cohorts [147]. The risk score, via a cut-off score≥10 related to the optimal sensitivity and specificity, integrating genetic and clinical factors to identify patients with HPR on clopidogrel and who are at increased risk of ischemic events, which is comprised of Age, Body Mass Index, Chronic Kidney Disease, Diabetes Mellitus and Genotyping 5 independent variables [147] (Fig. 1).

In a posthoc analysis of the TALOR-PCI that patients with PCI on clopidogrel, the ABCD-GENE score was found to be helpful in raising the precision treatment of P2Y12 inhibitors and identifying the patients at high risk [148]. In view of the ABCD-GENE score was built and validated in mostly Caucasian populations, two clinical studies with the aim to authenticate the predictive power of it in Asian populations revealed the result that the risk score significantly predicted high-risk patients on clopidogrel who are CAD or ACS patients underwent PCI [149,150]. Notably, the addition of 5-day HPR (blood samples were drawn for platelet function testing after 5 days of maintenance clopidogrel administration) could slightly improve the diagnostic accuracy of the score [150]. For patients with minor stroke or TIA, the clinical effectiveness of clopidogrel would be diminished with the ABCD-GENE score ≥10 [151]. Beyond that, given only the CYP2C19*2 LOF allele was accounted for in the assessment of the score, a validation trial consisted of a diverse real-world population of PCI patients on clopidogrel who completed genetic testing incorporated CYP2C19*2 and *3 alleles arrived at the conclusion that patients with at least one LOF allele or an ABCD-GENE score ≥10 are at incremental risk of weakening clopidogrel therapeutic effects [152].

It reminds us that maybe a combination of the ABCD-GENE score and CYP2C19 LOF gene CYP2C19*3 could effectively improve safety and efficacy in clopidogrel-treated patients when physicians practice the risk core in real and complex clinical practice. From what has been pictured above, for patients with cardiovascular and cerebrovascular diseases requiring long-term antiplatelet therapy, clopidogrel should be substituted by potent P2Y12 inhibitors such as prasugrel and ticagrelor when the ABCD-GENE score ≥10 [147]. Owing to a majority of the existing studies on the ABCD-GENE score being retrospective studies, more prospective studies are still needed to evaluate the predictive ability of the risk score in patients with HPR on clopidogrel.

4.1.2. GeneFA score

The GeneFA score [153] is a novel score stemming from a study population of 445 patients who were diagnosed with ACS undergoing coronary stenting and validated in an independent cohort enrolled 196 patients with ACS. The risk score is the combination of age, fibrinogen, and CYP2C19 genotype (*2/*3) for the sake of connecting genetic and clinical features with a total score was 4 points (Fig. 2). The GeneFA score had a moderate predictive ability to discern patients with a high incidence of HPR (C-statistic: 0.855) and prone to recurrent ischemic events (C-statistic:0.726) based on a cut-off value ≥ 3.

In comparison with the ABCD-GENE score (C-statistic:0.843), the GeneFA score had a slightly high prognostic value for the HPR on clopidogrel-treated patients. The following reasons may account for the phenomenon. Firstly, the CYP2C19 genotype of the GeneFA score incorporated the CYP2C19*2 and *3 alleles while the genotype of the ABCD-GENE score only included CY2C19*2 allele, omitting

ABCD-GENE Score		Points
Clinical Factors	Age >75 years	+4
	Body mass index >30 kg/m^2	+4
	CKD (GFR <60 ml/min)	+3
	Diabetes mellitus	+3
Genetic Factors	Carrying 1 CYP2C19*2 allele	+6
	Carrying 2 CYP2C19*2 alleles	+24
Points <10: clopidogrel Points ≥10: other P2Y12 receptor inhibitors		

Fig. 1. Parameters and recommendations for the ABCD-GENE score. Abbreviations: CKD, chronic kidney disease; glomerular filtration rate. For clinical predictors, age >75 years and body mass index >30 kg/m^2 were scored as 4, and CKD and diabetes were scored as 3. For genetic predictors, carriage of 1 CYP2C19*2 allele was scored as 6, and carriage of 2 CYP2C19/2 alleles was scored as 24. clopidogrel is appropriate when the ABCD-GENE score is < 10. When the score is ≥ 10, the risk of HPR with clopidogrel is a high and alternative treatment with other oral P2Y12 inhibitors should be considered.

GeneFA Score		Points
Clinical Factors	Age > 60 years	+1
	Fibrinogen value > 310mg/dl	+1
Genetic Factors	Carrying 0 CYP2C19 LOF gene	+0
	Carrying 1 CYP2C19 LOF gene	+1
	Carrying 2 CYP2C19 LOF genes	+2
Points <3: clopidogrel Points ≥3: other P2Y12 receptor inhibitors		

Fig. 2. Parameters and recommendations of the GeneFA Score. Parameters and recommendations for the GeneFA score. For clinical variables: age >60 years and fibrinogen value > 310 mg/dl were scored as 1. For genetic variables: carriage of 0 CYP2C19 LOF gene was scored as 0, carriage of 1 CYP2C19 LOF gene was scored as 1, and carriage of 2 CYP2C19-LOF genes was scored as 2. Therapeutic decisions are based on a threshold value of ≥3. Clopidogrel is favourable with a score of <3. When the score is ≥ 3, it should be replaced with other P2Y12 receptor inhibitors.

the patients harboring *3 alleles in high risk of HPR. Secondly, the age limit of the GeneFA score is 60 years old, however, the ABCD-GENE score sets the boundary of age to 75 years old, this may cause the former to include the low-risk patients with the age >60 years old and the latter excludes the high-risk patients under the age of 75 years old. Thirdly, the derivation cohort, as well as the validation cohort of the GeneFA score, were all from the population consisting of ACS patients, while the derivation and validation population of the ABCD-GENE score was comprised of ACS and CAD patients.

Significantly, clarifying the specific applicable population of the two risk scores will help to improve the accuracy of the results and the value of the clinical application. In addition, due to the restricted sample size and the limitations of the study group, emphasis should be placed on the further validation and application of the risk score in large-scale and multicenter clinical studies, especially prospective studies.

4.1.3. POPular risk score

The POPular Risk Score [154] is the first scoring prediction model that integrates platelet function, genetic and clinical factors (Fig. 3). It is primarily used to identify the risk of vulnerability to HPR in non-urgent PCI patients treated with clopidogrel to guide the

POPular Risk Score		Points
Clinical Factors	(Adjoined) Length of stent(s) >30mm	+0.5
	Left ventricular ejection fraction <30%	+0.5
	Diabetes mellitus	+0.5
Genetic Factors	EM (carrying 0 CYP2C19 LOF gene)	+0
	IM (carrying 1 CYP2C19 LOF gene)	+1
	PM (carrying 2 CYP2C19 LOF genes)	+2
VerifyNow P2Y12-assay	PRU ≥ 236	+2
Points <2: clopidogrel Points ≥2: prasugrel		

Fig. 3. Parameters and recommendations for the POPular risk score. Abbreviations: EM, extensive metabolizer, also known as normal metabolizer (NM, individuals carrying 0 CYP2C19 LOF gene); IM, intermediate metabolizer (individuals carrying 1 CYP2C19 LOF gene); PM, poor metabolizer (individuals carrying 2 CYP2C19 LOF genes); and PRU, platelet reaction unit. For clinical factors, each parameter was assigned a score of 1. For genetic factors, the specific score was determined by the patient's metabolic phenotype. The scores for EM, IM, and PM were 0, 1, and 2, respectively. For the VerifyNow P2Y12 assay, PRU ≥236 was defined as HPR, which corresponded to a score of 2. When the total score is < 2, clopidogrel is recommended. When the total score is ≥ 2, prasugrel is suitable.

appropriate use of P2Y12 receptor inhibitors. Platelet function was measured by the VerifyNow P2Y12 assay, and Platelet Reaction Units (PRUs) ≥236 were determined as HPR. CYP2C19 was the main genetic factor, and the patient's specific metabolic phenotype was determined and assigned a corresponding score based on the number of LOF genes. Clinical risk factors consisted of diabetes mellitus, stent length >30 mm, and left ventricular ejection fraction <30 %. When the total score was less than 2 points, the responsiveness of patients to clopidogrel was predicted to be positive, and clopidogrel was recommended. If the score was ≥2 points, prasugrel was recommended. Under the scoring rule, clopidogrel is not indicated if the patient is CYP2C19 p.m. or if platelets function is rated as HPR.

After constructing the score, investigators validated its predictive power in a single-center prospective study comparing the score-guided DAPT medication adjustment cohort with the cohort treated routinely with clopidogrel. The results showed a significantly lower incidence of thrombotic events and no increase in bleeding events in the score-guided group compared with the conventional treatment group, suggesting that the risk score helps to predict patients at high risk of poor response during clopidogrel therapy [154]. Hernandez-Suarez et al. [155] added 2 new items to the original score if the patient carried 1 PON1 p.Q192R allele or hematocrit (Hct) > 50 %, 0.5 points were subtracted from the original score, with no change in the overall judging threshold. This study was a multicenter, prospective, nonrandomized clinical trial in which investigators compared the occurrence of endpoint events during the follow-up period in the score-guided group with the standard treatment group to predict the value of the score for clinical application. However, the study is still ongoing and not yet conclusive. A study implemented in Asian patients undergoing drug-eluting stents (DES) implantation found that developing a DAPT regimen based on the POPular Risk Score improved clinical outcomes and reduced the frequency of adverse cardiovascular outcome events [156]. It is worth mentioning that this trial applied LTA to assess platelet reactivity, suggesting that switching to other assays could be an alternative way for regions where the VerifyNow P2Y12 test cannot be applied.

Although the score can be used to instruct the individualized selection of P2Y12 receptor inhibitors, several limitations remain. First, the platelet function assays used in different regions are not entirely consistent, and the operational criteria and judgment thresholds for different assays are still controversial. Further validation is needed to verify whether different assays are directly interchangeable with each other. Second, the current validation studies on this score are mostly single-center observational studies, and multi-center, randomized controlled prospective studies are needed to confirm its predictive value in clinical practice.

4.1.4. STIB score

The STIB score [157] is a clinical evaluation score mainly used to identify clopidogrel nonresponders in non-ACS patients. The risk score was derived from the STIB trail population composed of 844 stable angina patients undergoing PCI. By performing the univariate, multivariate, and conditional logistic regression analysis, researchers screened the three parameters consisting of diabetes, hemoglobin <13.9 g/dl, and BMI >28 kg/m^2 (Fig. 4). These three factors not only showed significant association with high platelet reactivity but also manifested similar predictive power to identify high reactivity to clopidogrel. Moreover, the study indicated that compared to the patients with 0 or 1 factor (38.5 % and 44.1 %, respectively), the patients with 2 or 3 factors demonstrated a high probability for CR (77.8 %). Thus, the STIB score was comprised of biological and clinical factors related to low responsiveness to clopidogrel with a score of 1 for each parameter. For patients with at least 2 factors, clopidogrel is unrecommended, the patients may benefit from ticagrelor or prasugrel.

Notably, the researchers found that the incidence of HPR in patients with stable angina pectoris treated with clopidogrel after PCI reached 50.2 % [157]. This phenomenon reminds us although stable angina pectoris patients occupy a small proportion of patients with coronary heart disease, it's necessary to identify high-risk patients prone to have adverse outcomes during treatment and optimize the therapy plans. The proposal of the STIB score provides a reference for the improvement of the curative effect of this kind of patient (non-ACS patients). Furthermore, the risk score was only composed of clinical and biological characteristics, which can assist physicians in quickly screening out high-risk patients at the beginning of treatment. However, there are still some defects in the risk score. First, the score can only be considered as a hypothesis due to the lack of external validation, and further validation of the predictive value and feasibility of clinical application is needed. Second, the relatively small sample size of the derivation cohort may affect the population representation of the study cohort. Third, the researchers evaded genetic factors in the analysis, while improving clinical feasibility, but reduced the predictive power of the score [158].

Interestingly, the researchers made a point. Patients with a score ≥2 have a decreased response to clopidogrel and a decreased risk

STIB Score		Points
Clinical Factors	Hemoglobin < 13.9g/dl	+1
	Diabetes mellitus	+1
	Body mass index > 28 kg/m^2	+1
Points <2: clopidogrel Points ≥2: other P2Y12 receptor inhibitors		

Fig. 4. Parameters and recommendations for STIB score. A score of 1 was given for diabetes mellitus, hemoglobin <13.9 g/dl, and BMI >28 kg/m^2 respectively. When the score was <2, clopidogrel was recommended. When the score is ≥ 2, ticagrelor or prasugrel is more suitable.

of bleeding, if surgery is urgently needed, the preoperative management of antiplatelet drugs in such patients will also be affected [157]. The management of perioperative antiplatelet agents in patients prone to P2Y12 receptor inhibitor resistance needs to be further explored.

4.1.5. Weighted polygenic risk score (wPGxRS)

The wPGxRS [159] was developed in clopidogrel-treated CAD/ACS patients undergoing PCI or not, consisting of multiple genetic loci including PON1 p.Q192R (rs662), ABCB1/MDR1 rs2032582, CYP2C19*2 (rs4244285), and PEAR1 rs12041331. Compared with the wild-type gene, the PRU values of the carriers of the first three gene variants mentioned above are relatively high, and the PEAR1 rs12041331 variant is associated with low PRU values. The wPGxRS was obtained by calculating the product of the effect values (β coefficient) of the genes involved in the linear regression model and the number of corresponding mutant alleles and then adjusting it according to the corresponding proportion. It was found that the higher the number of risk gene types or mutations carried, the higher the risk of HPR and the significantly higher the incidence of adverse cardiovascular outcomes. Although the polygenic prediction model is complex and cumbersome to apply and less clinically accessible, it can distinguish good responders from poor responders to clopidogrel and provides a reference for the application of polygenic guidance for clopidogrel therapy.

4.2. Prasugrel

4.2.1. HHD-GENE score

HHD-GENE score [160] is a novel scoring system containing hemodialysis, hypertension, diabetes, and the number of CYP2C19 LOF alleles (*2/*3) to discriminate CAD patients at high risk for HPR who are taking prasugrel (Fig. 5). Based on the receiver operating characteristic curve analysis illustrated that the area under the curve (AUC) was 0.74 as the best cutoff value was 5 ($p < 0.001$), and the risk score showed moderate diagnostic capability.

However, because of the limitations of the original study design, investigators were unable to verify the correlation of the risk factors of the score with clinical outcomes. Apart from the above, the study population of the risk score is Asian, which restricted the application of the score in the Caucasian population. At the same time, the study only considered the CYP2C19 LOF alleles, ignoring the effects of the other LOF alleles. It is important to note that the score incorporates the genetic risk factor CYP2C19, which is one of the major influences on clopidogrel metabolism. Therefore, clopidogrel is not indicated when the score results suggest that prasugrel is not indicated or when the score items include the CYP2C19 LOF gene. Given all of that, the HHD-GENE score, in view of the shortage of external validation, should only be deemed as a hypothesis that needs further studies formed by large-scale sample size and multicenter design.

To recap, as a quantitative tool for risk and benefit assessment, risk scores can facilitate individualized treatment. In clinical practice, risk scores can quantify the genetic and/or clinical risk factors present in patients, providing physicians with more intuitive information to help them quickly determine and select the appropriate antiplatelet agent during treatment planning. During follow-up, some of the patient's indicators may change as the disease progresses and therapeutic interventions are made, the physician could use the risk score to dynamically assess and adjust the treatment plan based on the changes in the results. It is important to note that good predictive ability and operability determine the value of a risk score in the clinical setting. On the one hand, if the score has low predictive power for outcome events, it is not only not clinically meaningful, but can mislead physicians to make poor decisions, especially for high-risk patients who cannot be accurately identified due to the low sensitivity of the score. On the other hand, if the scores are cumbersome, time-consuming, or expensive to apply, they can significantly affect the efficiency of treatment and reduce accessibility.

The risk scores mentioned above, by quantifying risk factors, not only help to identify high-risk patients who are prone to platelet hyper reactivity during P2Y12 inhibitor therapy but also provide a reference for further exploration of related subjects. However, the

HHD-GENE Score		Points
Clinical Factors	Hemodialysis	+2
	Hypertension	+2
	Diabetes mellitus	+2
Genetic Factors	Carrying 1 CYP2C19 LOF gene	+1
	Carrying 2 CYP2C19 LOF genes	+2
Points <5: prasugrel Points ≥5: ticagrelor		

Fig. 5. Parameters and recommendations for the HHD-GENE Score. Clinical predictors consisted of hemodialysis, hypertension, and diabetes, each of which was scored as 1. Genetic predictors were scored depending on the number of CYP2C19 LOF genes carried, with scores of 0, 1, and 2 for carrying 0, 1, and 2 LOF genes, respectively. The decision threshold for scoring is 5 points. If the scoring score is less than 5, prasugrel is applicable. If the score is greater than or equal to 5, other potent P2Y12 receptor inhibitors should be selected.

current risk scores still have the following limitations: the accuracy and completeness of the data selected for constructing the scores cannot be scientifically judged; the selection of the scoring parameters is limited to known risk factors; the results derived from analyzing and constructing models based on retrospectively collected data may have a lag; the limitations of the populations from which the models are constructed affect the external applicability; and the model bias or error exists in all computer modeling. Therefore, in the future, risk scoring can be explored and innovated based on data science and artificial intelligence, combined with a number of aspects such as high-throughput technologies, biomarkers, medical imaging and imaging studies, health behaviors and lifestyles. Of course, the practical validation of large-sample, multi-center clinical studies are also needed.

5. Conclusion

Considering that platelet reactivity is vulnerable to multiple factors [161], a comprehensive strategy combining 2 or more effective identification methods is conducive to screening high-risk patients who are prone to HPR after P2Y12 inhibitors therapy and to assist in the decision-making process for the individualized selection of antiplatelet agents in treatment regimens to improve the precision of antiplatelet therapy [8]. Future research should focus on the following points. Firstly, standardizing the process of data extraction and processing, so that the quality of the data can be scientifically assessed. Second, use in silico analysis to assist in optimizing the performance of risk scores, such as analyzing the predictive ability of risk scores for different patient groups, determining the optimal cut-off value of risk scores, and assessing the interactions between the included variables, with a view to minimizing model bias/error. Finally, future research on risk scoring should be explored and innovated based on existing identification methods and risk factors, with a data science orientation, integrating multidimensional data from high-throughput technologies, biomarkers, medical images, and imaging, to improve the accuracy, precision, and clinical applicability of its assessment.

Data availability statement

Data included in article/referenced in article.

Declaration of Interest's statement.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Hua Gao: Writing – original draft. **Nan Yang:** Writing – original draft. **Libo Yang:** Writing – review & editing. **Hui Wang:** Writing – original draft. **Guoshan Zhang:** Writing – review & editing. **Xueping Ma:** Conceptualization. **Ning Deng:** Conceptualization.

Declaration of competing interest

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