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INVITED REVIEW

Use of genetic data to guide therapy in arterial disease

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Summary. There is considerable interindividual variation in the response to antiplatelet and anticoagulant therapies. It has been proposed that this variability in drug response may be attributable to genetic variants. Thus, pharmacogenetics may help to accurately predict response to cardiovascular disease (CVD) therapies in order to maximize drug efficacy, minimize drug toxicity, and to tailor personalized care for these patients. Although the clinical utility of pharmacogenetics is promising, its adoption in clinical practice has been slow. This resistance may stem from sometimes conflicting findings among pharmacogenetic studies. Thus, this review focuses on the genetic determinants of commonly used platelet antagonists and anticoagulants including aspirin, clopidogrel, dabigatran, and warfarin. We also explore the clinical translation of pharmacogenetics in the management of patients with CVD.

Keywords: anticoagulants; cardiovascular diseases; genetics; pharmacogenetics; platelet aggregation inhibitors.

Introduction

Cardiovascular disease (CVD) is one of the most common causes of mortality and morbidity worldwide. Despite the availability of numerous antiplatelet and anticoagulant agents, it is widely recognized that there is significant intra-individual variation in the response to these therapies. Pharmacogenetics refers to the study of how genetic variants influence drug response [1]. The primary goal of pharmacogenetics is to minimize harmful drug effects in addition to maximizing clinical benefits, in order to improve patient care. Therefore, a better understanding of the genetic polymorphisms that contribute to the variability in antiplatelet and anticoagulant drug metabolism and response would ultimately lead to identification of at-risk patients and personalized tailoring of treatments for these patients. The purpose of this review was to summarize the use of genetic data to better understand the intra-individual variation in response to commonly used platelet antagonists and anticoagulants including aspirin, clopidogrel, dabigatran, and warfarin, and to explore the clinical translation of this knowledge in management of patients with CVD.

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Aspirin

Aspirin (acetylsalicylic acid) is the most common antiplatelet agent used in the primary and secondary prevention of CVD. Aspirin decreases platelet activity by irreversibly acetylating cyclooxygenase (COX)-1 and inhibiting the production of platelet-derived thromboxane A2 [2]. Although the efficacy of aspirin has been thoroughly documented, a proportion of patients treated with aspirin experience treatment failure and have an increased risk of recurrent CVD events [2]. The variability in the response to aspirin treatment is multifactorial; however, it has been shown that the heritability of platelet aggregation response ranges from 0.266 to 0.762 among healthy Caucasians and African Americans after aspirin treatment [3]. Other reviews have explored the effect of common genetic variants associated with aspirin resistance in genes encoding glycoproteins (GPIIb/GPIIIa, GPIa/GPIIa, GPVI, and GPIbα), cyclooxygenases (COX-1 and COX-2) and adenosine diphosphate receptors (P2Y1 and P2Y12) [2,4].

Goodman *et al.* [5] conducted a meta-analysis assessing genetic effects on aspirin resistance among 2834 participants from 31 candidate gene studies. The authors included studies that measured aspirin resistance using validated laboratory methods and pooled data from at least three studies for 10 polymorphisms in six genes. The authors reported that among individuals with CVD and healthy subjects, carriers of the genetic polymorphism

PIA1 (in the GPIIIa receptor gene) did not have a significantly different level of aspirin resistance as compared to carriers of *PIA2*. However, they did observe that *PIA1* carrier status was significantly associated with aspirin resistance as compared to *PIA2* carriers among healthy subjects (OR: 2.36, 95% CI: 1.24–4.49; $N = 240$). In contrast, the authors did not observed a significant effect on aspirin resistance of *GPIa* (C807T), *COX-1* (A842G/C50T), *P2Y12* (H1/H2), and *P2Y1* (A1622G) alleles among healthy volunteers and those with CVD ($P > 0.05$ for all). Although this was a comprehensive overview of aspirin resistance among healthy and CVD patients, this study did not assess the effect of these genetic variants on the risk of CVD outcomes. Furthermore, even when pooling results from multiple studies, the meta-analysis remained underpowered to identify genetic effects. Therefore, Floyd *et al*. [6] conducted a meta-analysis to assess the effect of the PIA1/A2 (*GPIIIa*) carrier status on the risk of myocardial infarction (MI) in 57 studies among 17 911 cases and 24 584 controls. The authors found that carriers of the *PlA2* allele had a modest increased risk of MI as compared to non-carriers (OR: 1.077, 95% CI 1.024–1.132; $P = 0.004$). However, the authors reported a high degree of publication bias ($P = 0.04$). These authors also conducted a meta-analysis to assess the effect of the PIA1/A2 (*GPIIIa*) on the risk of stroke in 25 studies among 11 873 individuals. The authors reported that *PIA2* allele carrier status was associated with a higher incidence of stroke as compared to non-carriers (OR: 1.12, 95% CI: 1.03–1.22; $P = 0.011$).

Cyclooxygenase (COX) enzymes are involved in the formation of prostaglandins, prostacyclin, and thromboxane [2]. It has been proposed that variability in aspirin response may be attributed to the *COX-1* (*PTGS1*) genetic variants as the COX-1 enzyme is a specific target of aspirin. Halushka *et al*. [7] demonstrated that two *COX-1* polymorphisms, rs10306114 and rs3842787, which are in complete linkage disequilibrium, were associated with reduced arachidonic acid-induced platelet aggregation in 38 healthy participants ($P = 0.01$). These results were also replicated among 144 patients with stable coronary artery disease (CAD) receiving aspirin [8]. Clappers *et al*. [9] assessed whether the rs3842787 polymorphism (*COX-1*) was associated with the risk of MI, stroke, or cardiovascular death in 496 patients with CVD receiving aspirin. The authors reported that the *COX-1* polymorphism was not associated with an increased risk of CVD outcomes (hazard ratio [HR]: 1.07, 95% CI: 0.62–1.85; $P = 0.80$). It would appear that genetic variation in *COX-1* is associated with changes in platelet function and aspirin response; however, there is a lack of well-powered studies assessing the effect of *COX-1* polymorphisms on clinical outcomes.

In contrast, the COX-2 enzyme is believed to have cardioprotective effects because it facilitates the production of prostacyclin [10]. However, the role of COX-2 in atherothrombosis remains controversial. For example, randomized controlled trials (RCTs) have shown that selective COX-2 inhibitors are associated with an increased risk of cardiovascular events [11]. Furthermore, some animal studies suggest that genetic inhibition of the COX-2 enzyme decreases the risk of atherosclerosis [12], whereas others demonstrate an increased risk of thrombosis [13]. A genetic polymorphism (rs20417) (*COX-2*) has been associated with lower COX-2 activity in the atherosclerotic plaque and a decreased risk of MI and stroke [14]. However, early *COX-2* genetic studies had small sample sizes, and only some [15–18] but not all [19–23] have replicated these findings. Furthermore, an interaction between aspirin use and carriage of the *COX-2* polymorphism has been reported. Lee *et al*. [24] reported an interaction between aspirin use and the rs20417 SNP (*COX-2*) for the risk of CVD events in 2212 participants from the ARIC study (P for interaction: 0.072), a finding later replicated by Lemaitre *et al*. [16]. Based on these findings, Ross *et al*. assessed whether *COX-2* carrier status was associated with risk of major cardiovascular outcomes among and reported that rs20417 carrier status was associated with decreased CVD outcomes (OR: 0.78, 95% CI: 0.70–0.87; $P = 1.2 \times 10^{-5}$) in 49 232 participants. The authors also observed that aspirin use (P for interaction: 0.004) and previous CAD (P for interaction: 0.015) appeared to modify the association between rs20417 carrier status and the risk of CVD outcomes. Additionally, carriers had significantly lower urinary levels of thromboxane and prostacyclin metabolites as compared to non-carriers ($P = 0.01$ and $P = 0.01$, respectively).

Clopidogrel

Clopidogrel is a prodrug that inhibits the ADP-induced platelet activation and aggregation pathways [25]. Dual therapy with clopidogrel and aspirin is the current standard of care in the prevention and management of acute coronary syndromes (ACS), especially in patients with percutaneous coronary intervention (PCI) [26]. The majority of clopidogrel pharmacogenetic studies have focused on genetic variants associated with the hepatic cytochrome P450 (CYP) 2C19 enzyme [27]. Carriers of the loss-of-function *CYP2C19* alleles (*CYP2C19*2* and *CYP2C19*3*) have been associated with poor responsiveness to clopidogrel. For example, Geisler *et al*. reported that carriers of the *CYP2C19*2* had increased residual platelet aggregation as compared to non-carriers and incorporating *CYP2C19*2* carrier status into a risk prediction score with non-genetic factors (i.e. age > 65, type 2 diabetes mellitus, decreased left ventricular function, renal failure, and ACS) improved the predication of clopidogrel responsiveness [28]. Furthermore, studies have also shown that carriers of *CYP2C19*2* and *CYP2C19*3* have been associated with an increased risk of CVD [29],

while carriers of the gain-of-function alleles (*CYP2C19*17*) have been associated with an increased risk of bleeds [30,31]. However, the effects of the loss-of-function and gain-of-function alleles have not been consistent across studies.

Mega *et al*. [29] conducted a meta-analysis to explore the association between loss-of-function carrier status and the risk of major adverse CVD outcomes (MACE) in 9685 patients with ACS who underwent PCI in nine studies. This study reported a significant association between carriers of at least one or two loss-of-function allele treated with clopidogrel with an increased risk of MACE (HR: 1.55, 95% CI: 1.11–2.17; $P = 0.01$ and HR: 1.76, 95% CI: 1.24–2.50; $P = 0.002$, respectively). However, in this analysis, the authors included observational studies and studies without control groups, which makes it difficult to determine whether the risk of CVD is due to *CYP2C19* carrier status or mechanisms that are independent of clopidogrel [32,33]. In contrast, Paré *et al*. [30] reported a non-significant association between loss-of-function allele carrier status and risk of CVD events and bleeds when comparing effects of clopidogrel vs. placebo in carriers and non-carriers from the CURE and ACTIVE studies. To further address these issues, Holmes *et al*. [34] conducted a meta-analysis to assess the effect of loss-of-function carrier status on CVD outcomes in four placebo-controlled RCTs among 11 477 participants. The authors observed that loss-of-function carrier status did not appear to modify the association between treatment with clopidogrel and risk of CVD events or bleeds (P for interaction > 0.05 for all).

In light of these results, it has been suggested that *CYP2C19* genetic testing may help to guide clopidogrel therapy. These previous studies have indicated that genotyping may be more useful in high-risk populations where the same relative risk difference between genotype groups translates into a larger absolute risk as compared to low-risk populations. Thus, a few studies have examined the effect of incorporating pharmacogenetic testing to guide clopidogrel therapy. For instance, Roberts *et al*. [35] conducted a prospective, randomized, proof-of-concept trial to assess the effect of point-of-care testing for personalized antiplatelet treatment (RAPID GENE trial) in 187 patients and reported that point-of-care testing was able to reduce high on-treatment platelet reactivity among *CYP2C19*2* allele carriers. However, the authors used a surrogate outcome to assess the efficacy of point-of-care testing as opposed to measuring a clinical outcome, which hinders drawing any definitive conclusions on the clinical utility of this test. The Individual Application of Clopidogrel after PCI (IAC-PCI) trial also assessed the effect of personalized antiplatelet therapy compared to conventional antiplatelet treatment in 600 Chinese patients receiving PCI and stent implantation for CAD [36]. The authors reported that the genotype-guided arm had significantly reduced incidence of MACE or cerebrovascular events as compared to the control group (9.03% vs. 2.66%; $P = 0.001$) and no difference in the incidence of bleeding or stroke between these groups ($P > 0.05$ for all). These studies have aimed to demonstrate that point-of-care testing may be feasible in clinical settings; yet, there is still insufficient evidence for the clinical utility of *CYP2C19* testing to guide clopidogrel therapy.

The effect of *CYP2C19* loss-of-function carrier status on clopidogrel response only accounts for 12% of the variability in platelet aggregation [37], prompting the search for other genetic variants that predict the response to clopidogrel. Another commonly studied genetic variant is the gain-of-function allele of *CYP2C19* (*CYP2C19*17*). Gain-of-function allele carriers have been associated with increased platelet response to clopidogrel and decreased risk of ischemic events [30,31]. Zabalza *et al*. [38] conducted a meta-analysis on the association between *CYP2C19* with risk of adverse CVD outcomes and bleeding among 6584 patients with CAD and reported that gain-of-function allele carrier status was significantly associated with a lower risk of CVD events (HR: 0.75, 95% CI: 0.66–0.87; $P < 0.001$) and a higher risk of bleeding (HR: 1.26, 95% CI: 1.05–1.50; $P = 0.011$). Other studies have also indicated that genes such as *ABCB1*, *P2Y12*, and *PON1* contribute to the variability of clopidogrel response. The *ABCB1* polymorphism rs1045642 has been associated with impaired P-glycoprotein function, reduced absorption of clopidogrel, and increased risk of CVD outcomes [39,40] while the effects of the *P2Y12* and *PON1* polymorphisms are inconclusive [41,42]. For a more comprehensive overview of the genetic effect of the *CYP*, *ABCB1,* and *P2Y12,* please refer to these reviews [43,44]. There have also been conflicting reports with regard to the effect of *PON1* in clopidogrel response. Bouman *et al*. [45] proposed that the PON1 enzyme was primarily involved in clopidogrel biotransformation and showed that the *PON1* Q192R polymorphism was associated with a decrease in the conversion of clopidogrel to its active metabolite. However, contrary to the primary report, Gong *et al*. [46] demonstrated that PON1 mediates the formation of the thiol metabolite, Endo, which does not mediate the formation of clopidogrel active metabolite or antiplatelet action. Furthermore, numerous studies have also illustrated that the *PON1* Q192R polymorphism does not appear to modify the metabolism of clopidogrel nor was it associated with risk of thrombosis or adverse cardiovascular events [30,47–51]. These conflicting results demonstrate the importance of incorporating all genetic variants to provide a comprehensive estimate of the genetic determinants of clopidogrel metabolism, as well as the inclusion of randomized comparison groups from pharmacogenetic studies to reduce the potential effects of confounding.

To date, the use of *CYP2C19* allele carrier status for guiding clopidogrel therapy has not transitioned into clinical practice. This is most likely due to insufficient

evidence regarding the impact loss-of-function allele carrier status on the clinical benefit of clopidogrel treatment. In addition, newer antiplatelet agents that target the P2Y12 receptor, such as prasugrel and ticagrelor, appear to be more effective than standard-dose clopidogrel, irrespective of genotype [52,53]. On the other hand, clopidogrel is now off-patent and there may be potential cost benefits in optimizing clopidogrel treatment among those who respond to it and providing alternative non-patent therapies, such as prasugrel and ticagrelor, for non-responders. In other words, the use of *CYP2C19* testing in clinical settings may be beneficial if the choice of therapy (i.e. standard clopidogrel treatment or an alternative antiplatelet therapy) differs depending on genotype, either because of improved outcomes (Fig. 1) or for pharmacoeconomic reasons. However, there is a need for additional large, well-designed prospective trials to assess the efficacy and safety of *CYP2C19* testing using clinical outcomes, such as the Tailored Antiplatelet Therapy Following PCI (TAILOR-PCI) trial (NCT01742117) and the Genotyping Infarct Patients to Adjust and Normalise Thienopyridine Treatment (GIANT) trial (NCT01134380).

Dabigatran

Dabigatran etexilate is an oral anticoagulant prodrug used in the prevention of stroke among patients with arterial fibrillation (AF), as well as an alternative therapy for patients with acute venous thromboembolism. Unlike vitamin K antagonists, such as warfarin, dabigatran etexilate is given in fixed doses without coagulation monitoring. In 2009, the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial showed that dabigatran etexilate (110 mg twice daily and 150 mg twice daily) was as effective as standard-dose warfarin and 150 mg doses were superior to standard-dose warfarin for stroke prevention in AF patients [54]. However, there is approximately 30% interindividual variability in blood concentrations of the active metabolite (dabigatran) [55]. Polymorphisms of the carboxylesterase 1 (*CES1*) gene have been shown to affect the metabolism of dabigatran etexilate. Paré *et al.* [56] conducted a genomewide association analysis to assess the effect of genetic variants associated with dabigatran etexilate in 2944 RE-LY trial participants. The authors reported that the *CES1* SNP rs2244613 was associated with trough concentrations, while the *ABCB1* SNP rs4148738 and *CES1* SNP rs8192935 were associated with peak concentrations of dabigatran etexilate ($P < 9 \times 10^{-8}$ for all). The authors also reported that each minor allele of rs2244613 (*CES1*) was associated with reduced risk of any bleeds (OR: 0.67, 95% CI: 0.55–0.82; $P = 7 \times 10^{-5}$). In contrast, neither rs4148738 nor rs8192935 was associated with bleeding or ischemic events ($P > 0.05$ for all). The authors also reported that *CES1* carrier status appeared to modify the differential response to warfarin vs. dabigatran treatment (P for interaction: 0.002). Among carriers, those treated with dabigatran had a lower risk of bleeding as compared to those treated with warfarin (HR: 0.59, 95% CI: 0.46–0.76; $P = 5.2 \times 10^{-5}$), while there was no difference in risk of bleeding in non-carriers (HR: 0.96, 95% CI: 0.81–1.14; $P = 0.65$). Again, these results emphasize the potential importance of pharmacogenetics to guide the treatment of narrow therapeutic index drugs, such as anticoagulants. However, these results also point to the decreasing probability of finding genetic determinants of clinical importance with newer, highly efficacious and very safe drugs. Indeed, despite the large sample size, only 66 dabigatran etexilate-treated individuals suffered from any ischemic event in the genetic analysis subgroup of the RE-LY trial, and thus, power to detect a genetic effect was limited. In other words, no genetic variant of clinical importance can be expected to be found if a drug is both perfectly effective and safe.

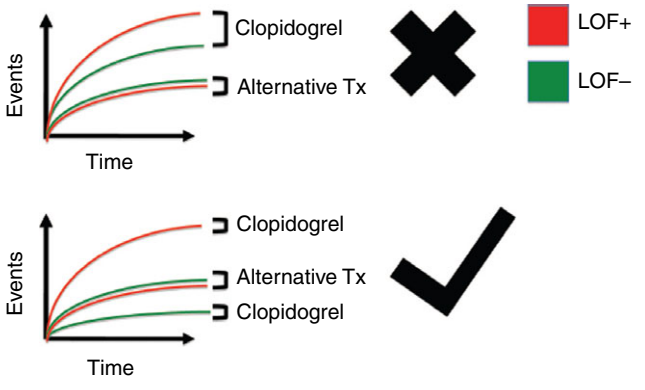

Fig. 1. Two hypothetical scenarios illustrating situations in which pharmacogenetic testing is or is not of clinical utility. LOF represents loss of function. This is a reprinted figure from *Circ Cardiovasc Interv* 2011; 4(5): 505–13.

Warfarin

Warfarin, a vitamin K antagonist, is used in the secondary prevention of venous thromboembolism and the primary and secondary prevention of systemic embolism in patients with AF. Warfarin treatment has been associated with a 70% reduction in stroke as compared to placebo [57]. However, despite its effectiveness, warfarin treatment is typically complicated by both intra- and interindividual variability and requires regular monitoring. Initial warfarin dosage is typically determined by a therapeutic algorithm or by a fixed dose, and is then further adjusted based on the patient's anticoagulation response measured by laboratory assays, namely the international normalized ratio (INR). Studies have shown that clinical variables only account for 17–21% of variation in warfarin response, while genetic polymorphisms influence 30–35% of the variability [58,59]. Thus, better management of inadequate or excessive anticoagulation may lead to a decrease in the risk of CVD events or bleeding complications.

The major genetic variants involved in the metabolism of warfarin are the vitamin K epoxide reductase complex subunit 1 (*VKORC1*) and *CYP2C9* polymorphisms. The *VKORC1* gene encodes the enzyme involved in conversion of vitamin K epoxide to vitamin K, which is the target of warfarin [60]. Studies have demonstrated that carriers of the *VKORC1* variant rs9923231 have reduced liver expression of VKORC1 and are more sensitive to warfarin, while rare *VKORC1* mutations have been associated with warfarin resistance and an increased risk of adverse ischemic events [61,62]. The CYP2C9 enzyme is involved in the metabolism of the (S)-isomer of warfarin [24]. Loss-of-function genetic variants of *CYP2C9* (*CYP2C9*2* and *CYP2C9*3*) have been associated with reductions in warfarin metabolism and over-anticoagulation and an increased risk of bleeding [63]. In addition, the *CYP4F2* gene has been associated with warfarin dosing, where rs2108622 is associated with both higher maintenance doses of warfarin and elevated vitamin K concentrations [64]. The CYP4F2 enzyme is thought to have a role in the vitamin K/warfarin pathway by inactivation of vitamin E by hydroxylating the vitamin K phytyl side chain, which alters vitamin K metabolism [65,66].

Several studies have assessed the effect of incorporating genetic information into already established clinical algorithms to increase the accuracy of dose prediction. For instance, the International Warfarin Pharmacogenetics Consortium (IWPC) developed a pharmacogenetic algorithm to predict a stable warfarin maintenance dose, while Avery *et al*. [67] proposed a pharmacogenetic algorithm to predict an initial warfarin dose and the International Warfarin Dose Refinement Collaboration developed a genetic algorithm to improve dose prediction on day 4 or 5 of warfarin therapy. Although these studies provided promising effects of genotype-guided care, the implementation of pharmacogenetic testing ultimately depends on clear evidence for improved clinical outcomes. For instance, these studies did not assess the effect on CVD outcomes, such as thromboembolic events or bleeding, and therefore, more research is needed to assess how pharmacogenetic algorithms impact hard clinical outcomes.

Based on this work, genotype-guided randomized control trials (RCTs) were conducted to assess the clinical utility of genotype-guided warfarin dosing. To date, two genotype-guided trials have assessed the effect of genotype-guided warfarin dosing [68,69], namely the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial and Clarification of Optimal Anticoagulation through Genetics (COAG) trial. The EU-PACT trial was a single-blind RCT that assessed whether genotype-guided warfarin dosing was superior to standard dosing among 455 warfarin-naive patients with either AF or venous thromboembolism [69]. In the genotype-guided dosing arm, patients received point-of-care testing for *CYP2C9*2*, *CYP2C9*3,* and rs9923231 (*VKORC1*). Warfarin dose was determined by a modified version of the IWPC [67,70] loading dosing algorithm and a dose-revision algorithm [71]. In contrast, in the control group, patients received standard warfarin dosing. The authors reported that the mean percentage of time in the therapeutic range for patients randomized to the genotype-guided treatment arm was significantly increased as compared to those randomized to the control group after 12 weeks (67.4% vs. 60.3%; $P < 0.001$). Furthermore, the genotype-guided group had a reduced risk of excessive anticoagulation (INR $\geq$ 4.0) (27% vs. 37%) and a shorter median time to reach therapeutic range as compared to the control group (21 days [IQR: 8–36] and 29 days [IQR: 14–58], respectively). The COAG trial also assessed the effect of genotype-guided dosing on anticoagulation control [68]. COAG was a multicenter, double-blind RCT that compared genotype-guided warfarin dosing with clinically based dosing among 1015 patients. In the genotype-guided dosing arm, warfarin dose was determined by a modified dose-initiation algorithm based on a model by Gage *et al*. [72] and a dose-revision algorithm [71]. In the control group, warfarin dose was based on modified versions of the dose-initiation algorithm and the dose-revision algorithm using only clinical variables. The authors reported that the mean percentage of time in the therapeutic range for patients randomized to the genotype-guided treatment arm was not significantly different than in those randomized to the control group (45.2% vs. 45.4%) after 28 days of follow-up. The authors also noted a significant interaction between ethnicity and dosing algorithm (P for interaction: 0.003), where African Americans in the genetically guided dosing group had a lower mean percentage of time in therapeutic range as compared to those in the control group (35.2% vs. 43.5%; $P = 0.01$, respectively). This was not significant among Europeans. In addition, the authors did not observe significant associations between the risks of excessive anticoagulation (INR $\geq$ 4.0), major bleeding, or thromboembolism between the dosing strategy groups ($P > 0.05$ for all).

Based on the conflicting findings between the EU-PACT and COAG trials, it is difficult to determine whether pharmacogenetic guidance of anticoagulant therapies may be effective in a clinical setting. However, these conflicting results may be due to differences in the study populations or trial designs. For instance, in the EU-PACT trial, the study population was mainly composed of Caucasian individuals from Europe, while the COAG trial consisted of both Caucasian and African American study participants from North America. Thus, the reported differences between these two trials may have resulted from genetic factors based on ancestry, as well as other factors that are known to influence warfarin variability, such as lifestyle factors or socioeconomic status In addition, the dosing algorithms used in the genotype-guided arms of the EU-PACT trial and the

COAG trial were different, as well as the treatment provided for the controls. The dose algorithms used in these trials were mainly developed in European patient populations and therefore may not be directly applicable to the COAG trial, which was composed of both Caucasians and African Americans from North America. Furthermore, among controls, the study protocols used in these trials required intensive INR measurements and frequent dose adjustments, which may not be reflective of clinical care practices in Europe or the United States. Thus, the frequent monitoring may have obscured differences between the intervention and control groups, resulting in an underestimation of the variant effect sizes. There were also differences in the length of follow-up between the two studies (12 weeks vs. 28 days), which suggest that the dosing algorithms may have a more clinically meaningful impact over time or it may reflect differences in patient care between the two trials. Finally, both studies used a surrogate outcome to assess the effect of genetically guided dosing algorithms. Although the COAG trial did not report a significant association between major bleeding and thromboembolic events, the study was not powered to detect these secondary outcomes. Based on the discrepancies between these two trials, future trials that assess the effectiveness of pharmacogenetic algorithms should be population specific as the generalization of results appears to be difficult.

Future directions

The ultimate goal of pharmacogenetics is to improve the efficacy and safety profile of drugs, including antiplatelet and anticoagulant therapies. However, the adoption of pharmacogenetics for treatment and prevention of arterial thrombosis has been slow. Although regulatory bodies and other pharmacogenetic groups, such as the FDA and Clinical Pharmacogenetics Implementation Consortium, have proposed clinical recommendations for pharmacogenetic testing, there is a lack of evidence from randomized trials and its impact on clinical outcomes. Thus, future pharmacogenetic studies require much larger sample sizes in order to have enough power to detect drug–gene interactions. There is also a need for well-designed, prospective clinical trials that assess the effects of genotype-guided antiplatelet and anticoagulant therapies on clinical outcomes. However, a more cost-effective approach may be to obtain biological samples from completed or ongoing trials that have evaluated the efficacy and safety of antithrombotic therapies, as well as collecting genetic information from patients when reporting rare or severe side effects, as is the case in the analysis of electronic records [73]. Evidence from these types of trials will help to guide future recommendations on the clinical utility of genotype-guided clinical care.

The recent advancements in exome sequencing have also provided a unique opportunity to identify rare mutations and novel genetic variants in the field of CVD pharmacogenetics, which was not possible using genotyping-based technologies [74]. Although these genetic variants may be rare at the population level, they may have a greater importance among individual carriers. Indeed, Daneshjou *et al.* [75] performed exome sequencing and reported that the rs7856096 SNP (*FPGS*) was associated with lower warfarin dose in 103 African Americans. Furthermore, the authors also showed that by incorporating rs7856096 carrier status into the IWPC, pharmacogenetic algorithm resulted in a 5.8 mg week^{-1} ($P = 3.93 \times 10^{-5}$) decrease in warfarin dose. This indicates that better detection of population-specific genetic variants has the ability to improve prediction and response to pharmacogenetic tests.

The goal of pharmacogenetics is to improve the efficacy and safety of antiplatelet and anticoagulant by taking into account the patient's unique genetic profile. Although the utility of pharmacogenetics in clinical practice is promising, there is still a need for a better understanding how genetic determinants contribute to antiplatelet and anticoagulant treatment. Indeed, only a small fraction of predicted heritability of drug response is currently explained by known variants. As our knowledge of genes and variants involved in drug metabolism and response improves, so will our ability to predict drug response. Furthermore, rare mutations with marked effect might provide a clearer path to clinical translation than common genetic variants of modest effect currently known and studied.

Disclosure of Conflict of Interests

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