

Current Status of Pharmacogenetics in Antithrombotic Drug Therapy

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1. Introduction

"Personalized medicine" represents a conceptual change in pharmacotherapeutics, where an individual's genetic profile will determine the appropriate drug and/or dose the patient should receive. Currently, medicine is addressing this challenge through the lens of genomic technologies. In the domain of antithrombotic therapy, warfarin, clopidogrel and aspirin are still the most relevant drugs for treatment of thromboembolic cardiovascular disorders and prevention of stroke. Incorporation of pharmacogenetic approaches, particularly in the antithrombotic drug therapy, may lead to better understanding of what stands behind the individual differences in drug efficacy and adverse drug effects, with the aim of increasing benefits and reducing risks on individual level. For now, two antithrombotics, warfarin and clopidogrel are emerging as the leading examples for pharmacogenetically-guided therapeutic optimization. Several recent randomized and controlled trials have demonstrated a number of improved clinical outcomes in warfarin-treated patients undertaking pharmacogenetic testing, particularly in patients with exceptionally low or high warfarin dose requirements (outliers). In addition, there were significant achievements in identification of genetic markers of reduced clopidogrel pharmacokinetics, which can partially explain inefficiency of clopidogrel response. The American Food and Drug Association (FDA) acted quickly on these developments in approving additional labeling for warfarin and clopidogrel package inserts to include relevant genetic testing, and called for further large-scale studies on the effectiveness of pharmacogenetic approaches to therapies using these drugs. However, there is still a considerable debate on the quality, quantity, and type of evidence that are needed to encourage such changes in clinical practice. There are also pertinent questions regarding which genetic markers should be used to ensure overall population benefits from genetic testing on a population level. It is also important to establish the relationship between genetic and non-genetic factors, particularly the effects of drug-drug interactions, and also the most appropriate pharmacogenetically-based dosing algorithm designed for clinical use. This review provides an update on the most significant pharmacogenetic studies on the commonly used oral anticoagulants and antiplatelet drugs, summarizing knowledge on the known genetic polymorphisms, their therapeutic effects

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and utility of pharmacogenetic approaches in various world populations, with special emphasis on current gaps of knowledge and challenges for future research. Most recently, a new oral warfarin alternative, a direct thrombolytic dabigatran, has been approved by the FDA. This new drug is particularly relevant for warfarin dose outliers and un-stabilized patients, making warfarin pharmacogenetics especially relevant for selection of safe and efficient antithrombotic therapy for each patient.

2. Antithrombotic therapy

Cardiovascular disease (CVD) remains the leading cause of death in the modern Western societies, despite scientific and technological advancements. According to the American Heart Association (AHA) statistical update 2009, an estimated 80 million American adults (approximately 1 in 3) have one or more types of CVD (Lloyd-Jones et al., 2009). Arterial and venous thrombotic complications closely accompany CVD in contexts of myocardial infarction (MI) and stroke (Mackman, 2008). Venous thromboembolic disorders (VTE), including deep venous thrombosis and pulmonary embolism, are considered the third leading cause of CVD-related death after MI and stroke (Cushman, 2007), particularly in patients with cancer (Heit, 2005). Antithrombotic therapies with anticoagulant and antiplatelet agents have been the most important means for prevention and treatment of CVD, with validity established in a wide range of clinical conditions, including acute coronary syndrome (ACS) (Anderson et al., 2007a), ischemic stroke (Sacco et al., 2006), peripheral vascular disease (Hirsch et al., 2006), atrial fibrillation (AF) (Fuster et al., 2006), and symptomatic and asymptomatic VTE (Hirsh et al., 2008). Over the past decades, increasing resources have been devoted to the improvement of antithrombotic therapy, specifically focusing on development and validation of new antithrombotic agents. A series of anticoagulants and antiplatelet drugs with already known or totally new mechanisms of action have been developed and tested in randomized controlled clinical trials (Table 1). However, although providing support for improved clinical outcomes in a population defined by explicit clinical criteria, these trials generally did not address the issue why some patients do not respond to the antithrombotic treatment, while others have excessive pharmacologic responses and distinctive patterns of adverse effects. This broad inter-patient variability in drug response in terms of both, pharmacological efficacy and toxicological adverse effects, imposes a major concern with the use of antithrombotic drugs. In common clinical practice, physicians cope with this variability by "trial and error" approach in ruling out inappropriate types of drug or dosage for each patient. The best example over the past 50 years is warfarin (coumadin) and its derivatives, in which to avoid drug over- or under-dosing and risks of bleedings or drug insufficiency, individual dose is determined by frequent monitoring of the International Normalized Ratio (INR), especially at the initial phase of treatment. Furthermore, intermitted medical conditions and subsequent changes in concomitant medications and their interaction with warfarin produce additional difficulties in the daily management of warfarin-treated patients. In the same way, there are growing concerns over inter-individual variability in drug response to antiplatelet drugs, which have been given so far in a universal dose estimated as effective in clinical trials (Steinhubl et al., 2002; Yusuf et al., 2001). Specifically, there is an increasing awareness of the need for individualized dosing of the high-profile antiplatelet clopidogrel (Bonello et al., 2008), to which this review dedicates special discussion.

Drug class	Drug	Administration	Mechanisms of action	Metabolizing enzyme	Limitations	References
Vitamin K antagonists	Warfarin	Oral	Inhibits VKOR	Predominantly CYP2C9	Frequent INR monitoring; Sensitivity or resistance	(Mackman, 2008) (Kamali & Wynne, 2010)
Heparins	Low Molecular Weight Heparins (LMWH)/ Clezan	Intravenous	Inhibit factor Xa and thrombin	Not metabolized by CYP450 enzymes	Thrombocytopenia; Antibodies for heparin-platelet factor complex	(Mackman, 2008)
Antiplatelet agents	Aspirin	Oral	Irreversibly acetylates COX1	Not metabolized by CYP450 enzymes	Weak antiplatelet agent; Gastric ulceration Aspirin resistance	(Michelson, 2010)
	Clopidogrel/Plavix	Oral	Active metabolite irreversibly inhibits P2Y ₁₂ receptor	CYP2C9 and CYP3A4 Pro-drug activated by CYP2C19	Inter-patient variability; Clopidogrel resistance	(Michelson, 2010) (Giorgi et al. 2011)
	Prasugrel	Oral	Active metabolite irreversibly inhibits P2Y ₁₂ receptor	CYP3A4-5 and CYP2B6	Bleedings; Superior to clopidogrel in TRITON-TIMI 38 trial	(Michelson, 2010) (Giorgi et al., 2011)
	Ticagrelor	Oral	Reversibly inhibits P2Y ₁₂ receptor	CYP3A4-5	Bleedings; Superior to clopidogrel in Phase III PLATO trial	(Wallentin et al., 2009) (Giorgi et al., 2011)
	Integrin α IIb β 3 anagotists	Intravenous	Interfere with platelet activation	Not metabolized by CYP450 enzymes	Bleedings; Thrombocytopenia	(Michelson, 2010)
Direct thrombin inhibitors (DTIs)	Dabigatran/ Pradaxa	Oral	Reversibly inhibits free and clot-bound thrombin	Neither metabolized nor induced by CYP450 enzymes	Superior to warfarin in Phase III RE-LY trial	(Galanis et al., 2011); (Wallentin et al., 2010b)
	Rivaroxaban	Oral	Reversibly inhibits Factor Xa	CYP3A4	Phase III ROCKET-AF trial	(Galanis et al., 2011)
	Apixaban	Oral	Reversibly inhibits Factor Xa	CYP3A4	Phase III ARISTOTLE trial	(Galanis et al., 2011)

Abbreviations: VKORC1 vitamin K epoxide reductase; CYP2C9, CYP3A4-5 and CYP2C19 cytochrome P450 enzymes; COX1 cyclooxygenase-1; P2Y₁₂ platelet plasma membrane receptor; INR International Normalized Ratio.

Table 1. Antithrombotic drugs including generic, FDA approved and currently tested anticoagulants and antiplatelet agents

While the phenomenon of individual drug response variability has been well-recognized, its causes are not well-defined and are likely to be multifactorial, in which patient's age, sex, weight, nutrition, infections, concomitant medications and genetics play an important role (Sadée & Dai, 2005). Pharmacogenetic point of view, essentially referred to as "personalized medicine", suggests that in parallel to the development of new drugs improvement of known drugs efficacy and safety should be pursued and can be achieved by taking into account an individual's genetic make up (Aspinall & Hamermesh, 2007). Pharmacogenetics suggests that knowing more about genes implicated in drug

mechanisms, drug pharmacokinetics (drug metabolic enzymes and transporters) and pharmacodynamics (target enzymes or receptors), and genetic variations with meaningful biological and population impacts, could potentially lead to more intelligent clinical decisions on therapeutic doses and risks of adverse events for individual patients. Pharmacogenetic approaches, using only a limited number of genetic variations, are currently emerging across broad classes of antithrombotic drugs. For warfarin in particular, it has been recently demonstrated that incorporation of a pharmacogenetic rationale into the clinical decision making may hold promise for better optimization of drug benefit-to-risk outcomes (Klein et al., 2009). In the near future, pharmacogenetics together with advanced diagnostic technologies such as molecular imaging may enable to shift from the study of single genes to more comprehensive paradigms focusing on functions and interactions of multiple genes and gene products, among themselves and with an environment. The information gained from such analyses, in combination with clinical data will improve individual risk assessments, eventually guiding clinical management and decision-making to improved use of antithrombotic drugs for prevention and treatment of CVD.

This review provides an update on the most significant pharmacogenetic studies on the commonly used oral anticoagulants and antiplatelet drugs, principally including warfarin, clopidogrel and aspirin. It summarizes knowledge on the known genetic polymorphisms, their therapeutic effects and utility in pharmacogenetic approaches in various world populations, with special emphasis on current gaps of knowledge and challenges for future research. There is an ongoing debate over the utility of pharmacogenetic diagnostics in the routine clinical practice (Woodcock, 2010). This review tackles on these unresolved issues in the context of antithrombotic drug, specifically whether implementation of pharmacogenetic testing indeed improves therapy benefits, damage reduction and clinical outcomes. In a broader sense, this review relates to the place of genetics among traditional approaches to personalized clinical care, which rely on knowledge of patient's behavior, diet, social circumstances and environment, and if in the future physicians could use genetics to "personalize" treatment.

3. Pharmacogenomics

The conceptual basis of pharmacogenetics was laid more than 50 years ago (Motulsky, 1965). Since then, the science behind pharmacogenetics has contributed a great deal to basic understanding of molecular mechanisms responsible for variation in drug response and to translation of that understanding to the drug development process (Weinshilboum & Wang, 2006). Clinically relevant pharmacogenetic examples, mainly involving drug metabolism, have been recognized. With the completion of the Human Genome Project and advancement of genotyping technologies, both genomic science and its application to drug response have undergone major advances (Feero et al., 2010). The field of pharmacogenetics has evolved into "pharmacogenomics", involving a shift from candidate gene approach to whole genome studies that now can be performed with more precision in a lot more samples. Former analyses of genetic variations using lower density chromosomal markers, such as tandem nucleotide repeats (VNTRs and STRs), are now mostly focus on more ubiquitous and informative variations - single-nucleotide polymorphisms (SNPs). More efficient and accurate platforms are now adapted for ever smaller DNA samples to detect

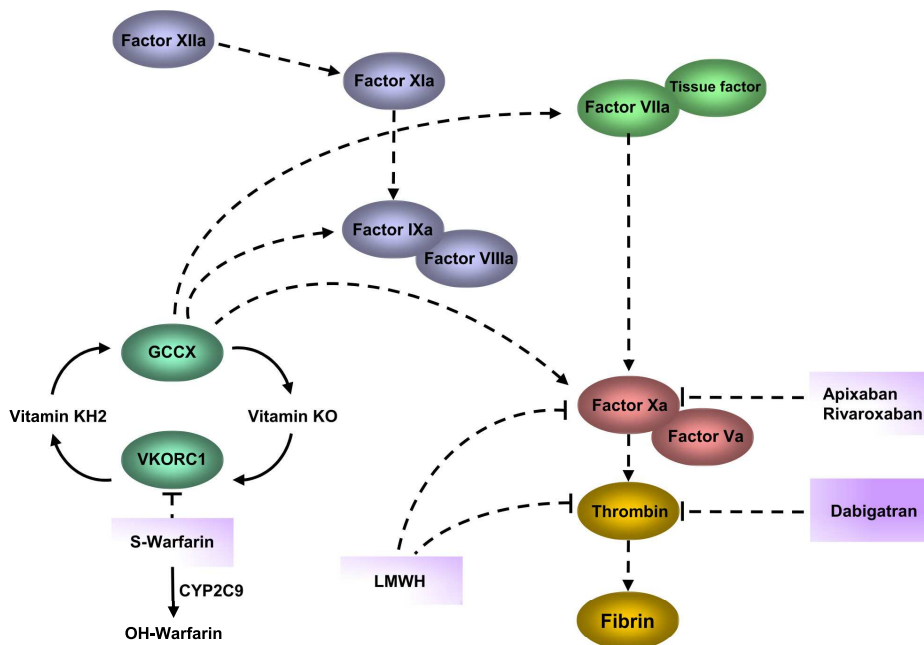
SNPs, gene copy variations (CNV) and insertion/deletion (INDEL) mutations, and also for analyses of gene expression (RNA/protein microarrays) and DNA chemical modifications (epigenomics). The HapMap project, launched in 2002, now includes a remarkable number of common human genetic variations. Most notably, new methods have dramatically increased the rates and lowered costs of DNA sequencing (Collins, 2010; Venter, 2010), facilitating the discovery of new genetic variations. More advanced bioinformatic and statistical tools have enabled genome-wide association studies (GWAS) that transformed the search for genetic factors in complex traits (Manolio, 2010), although applicability of GWAS to drug response has been hampered by the complexity and multifactorial nature of this phenotype.

It is not uncommon that drugs have a narrow therapeutic index. Warfarin and clopidogrel, the most widely prescribed antithrombotic drugs, have narrow therapeutic indexes that are influenced by genetic variations, a hallmark of drugs for which pharmacogenetic/genomic approaches can potentially provide substantial clinical benefits (Wang et al., 2011). Pharmacogenetic studies of these drugs illustrate the rapid evolution of our understanding regarding the relationships between genetic variations and drug efficacy and safety. For both these drugs, the classical candidate gene approach provided identification of important genetic markers of inter-individual variability in drug response. Additional data supporting pharmacogenetic testing for both these drugs are rapidly accumulating, among them a recent GWAS confirming the principal genetic determinants of warfarin response (Takeuchi et al., 2009) and most recent studies supporting the significance of the only known genetic factor in clopidogrel response (Mega et al., 2010b; Pare et al., 2010). Despite only partial resolution of clopidogrel pharmacogenetics, the American Food and Drug Association (FDA) acted quickly on these data by re-labeling warfarin and adding a warning on the clopidogrel label to include relevant genetic testing prior to drug use. In addition, the FDA approved several diagnostic kits for genetic testing of warfarin dosing markers, specifically those associated with warfarin sensitivity and related risk of bleedings. It is not surprising that dosing markers of warfarin and clopidogrel include variants of cytochrome P450 (CYP) enzymes that are responsible for drug metabolism or pro-drug activation. Distinct CYP polymorphisms related to reduced enzyme activity have been demonstrated as significant determinants of warfarin and clopidogrel responses and toxicity effects (Higashi et al., 2002; Mega et al., 2009b). As CYPs are responsible for metabolism of many other types of drugs (Sadee & Dai, 2005), we can presume that inclusion of genetic data on CYP polymorphisms in drug package labels is only starting to emerge. While these developments represent relative success of pharmacogenetics in the antithrombotic drug therapy, they also raised some pressing questions regarding clinical utility of pharmacogenetic testing, especially in the general population of patients (Woodcock, 2010). One problem is that the pharmacogenetic puzzle for clopidogrel is far from being complete (Fuster & Sweeny, 2010), and even more so for prasugrel, the third generation antiplatelet drug acting by the same mechanism, in addition evaluation of relative effects of genetic and non-genetic factors is still limited (Zhang et al., 2008). From an evolutionary point of view, pharmacogenetically meaningful inherited variations have most probably evolved and persisted in the human population due to ancient natural stressors such as nutrition and parasites, understanding of which may provide yet unknown and unexpected insights into the etiopathology and mechanisms of human diseases and evolutionary adaptations.

On the way towards personalize medicine, pharmacogenetics ultimately aims to replace "one drug fits all" or "trial and error" methods in choosing an optimal drug at the most advantageous dose for each patient. Even if pharmacogenetics is still unable to achieve accurate predictions of therapeutic dose at an individual level, it can assist in identifying patients who are likely to benefit from a drug from those who are prone to adverse reactions that could lead to toxicity and death ("outliers"). Perhaps the most promising advances in implementation of pharmacogenetics have been made so far in the field of oncology, using a patient's genetic profile to predict the need and the choice of chemotherapy (Huang et al., 2003; Kroese et al., 2007). Adverse drug reactions are a major problem with current antithrombotic drugs and are the major cause of hospitalizations in the US today (Lloyd-Jones et al., 2009). Reducing the number of failed drug attempts and number hospitalizations due to adverse events, are all reasons why the implementation of pharmacogenetics could be beneficial and cost effective, and overall could potentially lead to decreased costs of health care (Ginsburg et al., 2005).

4. Warfarin pharmacogenetics

The most complete pharmacogenomic picture is presently available on the anticoagulant warfarin. Warfarin (coumadin), originally patented as rat poison, was introduced into the clinical practice in the 40s as an anticoagulant inhibiting the vitamin K cycle and thereby the action of vitamin K-dependent factors of the coagulation cascade, specifically factors II, VII, IX, and X (Figure 1). Warfarin and other coumarin derivatives are indicated in a wide range of clinical conditions, including prevention and treatment of venous thrombosis (VTE) and arterial thromboembolism in patients with AF and mechanical heart valves. Maintenance on warfarin most often persists for years or lifetime. Warfarin is still the most commonly prescribed oral anticoagulant in the North America and much of Europe (phenprocoumon and acenocoumarol) (Daly & King, 2003). Every year, two million patients start warfarin therapy in the US alone (Melnikova, 2009). One problem with warfarin is a narrow therapeutic index resulting in serious risks of adverse reactions at both ends of the dosing scale: low-responders to warfarin are at increased risk for embolic events and high-responders can develop intracranial hemorrhages or gastrointestinal bleeds. The other problem is an extensive variability in warfarin dose response, reflected in more than 20-fold inter-individual differences in warfarin dosing. All patients receiving warfarin are closely monitored using INR, a universal laboratory test for anticoagulation efficiency (prothrombin time). Frequent INR monitoring is especially crucial in naive patients at the beginning of warfarin administration. For most clinical indications, the therapeutic range for target INR is between 2.0 and 3.5, while INRs below or above this range are indicative of under-anticoagulation (risk of thrombosis) or over-anticoagulation (risk of bleeding), respectively. Although therapeutic control, i.e. achieving and maintaining target INR within the therapeutic range, is considered an important predictor of adverse events, it is still insufficient even in clinical trials settings, in which the average time spent in the therapeutic INR is only 50-70% (Ansell et al., 2008). Thus, it is not surprising that warfarin still ranks among the five top most "hazardous" drugs that are most often responsible for emergency room visits (Budnitz et al., 2007; Wysowski et al., 2007), making it a leading candidate for genetic testing before starting any patient on warfarin therapy.



Abbreviations: VKORC1 vitamin K epoxide reductase; GCCX γ -glutamyl carboxylase; CYP2C9 cytochrome P450 enzyme; OH-Warfarin inactive hydroxylated warfarin metabolites; LMWH low molecular weight heparins; arrows indicate activation and blocked lines inhibition.

Fig. 1. Targets of anticoagulants and direct thrombin inhibitors (DTIs)

Warfarin pharmacokinetics is predominantly determined by the hepatic CYP2C9 enzyme responsible for its metabolism. Warfarin is a racemic mixture of S and R enantiomers, S-warfarin is the main CYP2C9 substrate, has a shorter half-life and is 3-5 times more potent anticoagulant. Other drugs interfering with CYP2C9 activity and warfarin clearance (Holbrook et al., 2005), as well as age and weight (Dobrzanski et al., 1983; Wynne et al., 1995), can have significant effects on the efficacy of warfarin therapy, and also nutritional factors affecting the vitamin K cycle (Greenblatt & von Moltke, 2005). A number of genetic variants of CYP2C9 have been identified in various world populations (<http://www.cypalleles.ki.se/>), the two most important due to occurrence and functional implications are Arg144Cys (*2) and Ile359Leu (*3). CYP2C9*2 and *3 have been related to approximately 30% and 80% respective reductions in enzymatic activity *in vitro* (Rettie et al., 1994; Takahashi et al., 1998) and to reduced S-warfarin clearance *in vivo*, comparing wild type allele homozygotes *1/*1 to mutation carriers *1/*2 or *1/*3 (40-45% reduced clearance) or homozygotes *2/*2 and *3/*3 (70-85%) (Kaminsky & Zhang, 1997). Following key study (Aithal et al., 1999) suggested that a patient's CYP2C9 genetic composition is indicative of his warfarin dose requirement, in showing that hypofunctional *2 and *3 alleles were more common among patients with significantly lower steady-state doses, and that *2, *3 carriers and homozygotes had greater INR instability and more bleeding complications at warfarin induction. Gene-dose relationship between hypofunctional *2 and *3 alleles and reduced warfarin requirements was subsequently reproduced and refined in numerous

studies. A retrospective study representing an unselected patients population (Higashi et al., 2002) investigated whether patients with CYP2C9*2,*3 genotypes also demonstrate increased time to therapeutic INR, time taken to achieve stable dosing, incidence of supra-therapeutic INR and risk of serious or life-threatening bleeding events. The investigators found that carriers of *2,*3 required more time to achieve stable dosing (hazard ratio HR=0.65 [95% confidence interval CI: 0.45-0.94] with a median difference of 95 days (p=0.004), had higher risk of supra-therapeutic INR (HR=1.4 [1.03-1.90]) and moreover were more prone to bleedings at warfarin initiation (HR=3.94 [1.29-12.06]) and over the entire study (HR=2.39 [1.18-4.86]). While deciphering the relationship between patient's CYP2C9 genotype and warfarin therapeutic dose, this study suggested an optimistic perspective that the use of genotype-guided dosing can reduce the time to reach stable therapeutic dose, risk of above-range INR's and incidence of bleeding events. A recent meta-analysis (Lindh et al., 2009) summarizing data for almost 8,000 patients from 39 studies estimated that compared to patients with the wild type *1/*1 genotype, the steady-state warfarin maintenance dose was reduced by 20% [17-22%] and 34% [29-38%] for *1/*2 and *1/*3 carriers, and by 36% [30-42%], 57% [49-64%] and 78% [72-84%] for *2/*2, *2/*3 and *3/*3 homozygotes and compound heterozygotes, respectively. It is worth mentioning that CYP2C9*2,*3 have been similarly associated with reduced S-acenocoumarol clearance, lower steady state acenocoumarol dose requirements and higher risk for supra-therapeutic INRs, but not of bleeding complications (Stehle et al., 2008; Teichert et al., 2009). Thus, significant contribution of common hypofunctional CYP2C9 variants to warfarin sensitivity has been well-established, although accurate estimates of their contribution varies between studies and is dependent on inclusion of other factors.

Despite these advances, robust estimates of bleeding risks for specific CYP2C9 genotypes are still ambiguous, due to the rarity of severe bleeding events and the need of large cohort studies. In order to circumvent this limitation, most studies use grouping of CYP2C9 genotypes. For instance, a 365 patients study (Sanderson et al., 2005) reported relative bleeding risk RR=2.26 [1.36-3.75] for carriers of *2 or *3 variants, while a 446 patients study (Limdi et al., 2008) reported hazard ratio HR=3.0 [1.1-8.0] for major bleeding that was highest during induction (5.3-fold) but remained increased (2.2-fold) after stabilization. A large prospective Swedish Warfarin Genetics (WARG) cohort of 1496 patients (Wadelius et al., 2009) reported that 1 of 8 (12.5%) patients homozygous for *3 experienced a serious bleeding event, compared to 4 of 1482 (0.27%) with other CYP2C9 genotypes (p=0.066). Judging from these and other observations, it was clear that CYP2C9 polymorphisms can not explain the entire inter-individual variability in warfarin dose response. In addition, CYP2C9*2,*3 allele frequencies in various world populations were not entirely matching to previous epidemiological findings of ethnic differences in warfarin dose requirements, suggesting that individuals of Asian origin are relatively low dose requirers and individuals of African origin are high dose requirers compared to Caucasians (Absher et al., 2002; Dang et al., 2005). Conversely, CYP2C9*2 and *3 were found prevalent in Caucasians (12% and 8% respectively), but were hardly present in African or Asian populations (Moyer et al., 2009; Stehle et al., 2008).

Recent studies suggested implication of yet another cytochrome P450 in warfarin dose, CYP4F2, showing that coding and exceptionally common Val433Met polymorphism (up to 30% allele frequency in white populations) is associated with 4-12% increase in warfarin dose requirements (Borgiani et al., 2009; Caldwell et al., 2008). Contribution of this factor

was further supported in recent GWAS showing that when CYP2C9 and VKORC1 effects were removed through multiple regression adjustments, an additional signal for CYP4F2 was observed (Takeuchi et al., 2009). Although the effect of this CYP4F2 polymorphism is probably small (about 1.1% variability explained), the suggested molecular explanation of this effect is interesting. CYP4F2 was shown to catalyze vitamin K oxidation, while the presence of 433Met variation reduced its catalytic ability, potentially leading to accumulation of the VKORC1 substrate - vitamin K epoxide and larger warfarin doses required for inhibition of this pathway (McDonald et al., 2009). CYP4F2 Val433Met was also associated with acenocoumarol dose requirements with similar modest effect (1.2-1.3%) (Perez-Andreu et al., 2009; Teichert et al., 2009).

A major step forward has been taken with discovery of an enzyme responsible for warfarin pharmacodynamics and direct warfarin target, the vitamin K epoxide reductase (VKOR). When VKOR is blocked by warfarin, vitamin K epoxide cannot be reduced to replenish the active form of vitamin K, which is necessary for activation of coagulation factors by γ -carboxylation (Figure 1). Therefore, lack of active vitamin K eventually results in less activated coagulation factors and decreased coagulation activity. After more than 50 years of search for the warfarin target, the gene encoding the VKOR catalytic subunit (VKORC1) was identified in parallel by two independent groups (Li et al., 2004; Rost et al., 2004), the latter also provided first evidence of rare VKORC1 mutations in patients with exceptionally high warfarin dose requirements (i.e. warfarin resistance). Shortly after, several studies reported that VKORC1 polymorphisms affect warfarin dose response (Bodin et al., 2005; D'Andrea et al., 2005; Sconce et al., 2005) and studies considering both CYP2C9 and VKORC1 polymorphisms suggested that they provide relatively good explanation of low dose requirements conditional on enzymes insufficiencies and a total of about 50% variability explained (Bodin et al., 2005; Sconce et al., 2005). A landmark study (Rieder et al., 2005) revealed a series of VKORC1 polymorphisms (10 SNPs including previously reported) that construct high-linkage disequilibrium haplotype structure with distinct frequencies among human populations. Specifically, haplotypes H1 and H2 containing promoter -1639G>A (also 3673G>A), and intragenic 1173C>T (also 6486C>T), 6853G>C and 7566C>T variations were associated with reduced VKORC1 transcription and lower warfarin doses, consistent with the notion that lower target enzyme production leads to lower requirement of its specific inhibitor. Since then, numerous studies have supported the notion that H1 and H2 haplotype variants (also VKORC1*2 (Geisen et al., 2005) are associated with warfarin sensitivity. Studies examining VKORC1 haplotype frequencies in various world populations confirmed that VKORC1 alleles/haplotypes are important genetic factors in determining individual as well as populational warfarin dose response variability, particularly the occurrence of warfarin sensitivity (Mushiroda et al., 2006; Takahashi et al., 2006; Veenstra et al., 2005). A paramount analysis of VKORC1 alleles/haplotypes in 8,750 patients from 11 countries partaking in the International Warfarin Pharmacogenetics Consortium (IWPC) , the largest cohort representing three racial groups (Asians, whites and blacks) (Limdi et al., 2010), showed that the -1639G>A marker is sufficient to explain the variance across all three racial groups. In fact, the -1639G>A marker has been incorporated into all warfarin genetic testing kits approved by the FDA. However this study acknowledged that the contribution of VKORC1 to dose requirements is higher in whites than in non-whites. The most compelling evidence for VKORC1 contribution to warfarin sensitivity were provided by the IWPC study of over 5,000 patients (Klein et al., 2009), showing that patients with -1639 GG,

GA, and AA genotypes had mean warfarin weekly doses of 42.6mg [41.5-43.7], 30.7mg [29.9-31.5] and 20.3mg [19.8-20.8], respectively, corresponding to approximately 25% dose reduction per A allele. Moreover, the -1639A allele was associated with other clinical outcomes indicative of increased warfarin sensitivity, specifically with higher INR values and shorter time spent within the therapeutic range, but not with bleeding complications (Limdi et al, 2008; Schwarz et al, 2008; Wadelius et al, 2009), as previously mentioned, evaluation of bleeding risks may require larger studies. VKORC1 alleles/haplotypes were shown to have similar effects on increased sensitivity to acenocoumarol (Stehle et al., 2008).

Thus, added genetic data on CYP2C9 and VKORC1 provided sufficiently good resolution of warfarin sensitivity but not of warfarin resistance, showing by default that patients of African origin (African-Americans), in which warfarin resistance is common, are essentially lacking markers of warfarin sensitivity. In addition, use of correlation analyses and more complex models accounting for other genetic and non genetic factors in African-American patients, did not reach the values achieved in Caucasian and Asian patients (Momary et al., 2007; Schelleman et al., 2007). Rare VKORC1 mutations identified in singular families with multiple coagulation factor deficiency (Rost et al., 2004) and rare patients with severe warfarin resistance (Harrington et al., 2005) also could not explain the relatively common occurrence of warfarin resistance in patients of African origin. Other gene variants, such as polymorphisms in the microsomal epoxide hydrolase (EPHX1) and calumenin (CALU), were shown to have only marginal contribution to higher warfarin doses (Loebstein et al., 2005; Wadelius et al., 2007). This gap of knowledge was resolved by two studies reporting a common warfarin resistance marker, the VKORC1 Asp36Tyr mutation, with significant contribution to high warfarin doses (>70 mg/week) and dominant effect over warfarin sensitivity markers in the same individual (Loebstein et al., 2007; Scott et al., 2008). Initially described in the Jewish Ashkenazi (4% allele frequency), Asp36Tyr was further found surprisingly prevalent in individuals from Ethiopia (15%) (Aklillu et al., 2008). Most recent study specifically focusing on high-dose coumarins requirers reported that Asp36Tyr is the most common VKORC1 mutation also among European warfarin resistant patients and appears to affect phenprocoumon therapeutic in the same way (Watzka et al., 2011).

5. Implementation of warfarin pharmacogenetics

Prior to the genetic era, warfarin dose prediction at the initiation of therapy used a clinical algorithm, including variables such as age, weight or height, race, concomitant medication and dietary vitamin K consumption, all together accounting for 20-30% of warfarin dose variability (Gage et al., 2008). Taken together, CYP2C9 and VKORC1 genotypes could explain additional 20-30% of warfarin dose variability (Wu, 2007) and by other estimates even 30-40% (Manolopoulos et al., 2010), again showing an overriding effect of genetic factors. These observations raised the possibility that genetic testing of patients prior to therapy initiation might provide information that could enhance the clinical algorithm. Several prospective studies examined potential clinical utility of the pharmacogenetic algorithm including genetic and clinical data. The first prospective randomized study comparing between the pharmacogenetically-guided and standard dosing algorithms for 206 patients initiating warfarin therapy (Anderson et al., 2007b) failed to show significant differences between groups for the primary endpoint, i.e. the number of out-of-range INR standardized by the number of INRs obtained ($30.7\% \pm 22.9$ in pharmacogenetic-guided

versus 33.1%±22.9 in standard dosing group). However, the investigators succeeded to show that the pharmacogenetic algorithm slightly, but significantly, decreased the number of dose adjustments from 3.6 to 3.0 per patient (mean decrease of 0.62 adjustments [0.04–1.19], $p=0.035$). While it was clear that a patient's genetics influences warfarin dosing, it was still unclear how this data could be utilized in the clinic. Several groups suggested other pharmacogenetic algorithms, using genetic and clinical factors (Gage et al., 2008; Limdi et al., 2008; Takahashi et al., 2006). The IWPC pharmacogenetic algorithm was constructed on the basis of analysis of 4000 patients of various ethnicities, accounting for patients' genetic (VKORC1 and CYP2C9) and clinical data (age, weight and early INR values) (Klein et al., 2009). The predictive value of this pharmacogenetic algorithm was then validated in a cohort of 1000 patients, calculating the percentage of patients whose predicted dose was within 20% of the actual stable therapeutic dose. The investigators found that the pharmacogenetically-guided dosing was more accurate compared to the traditional approach. The greatest predictive value of the pharmacogenetic algorithm was seen in patients receiving weekly doses of 21mg or less, and 49mg or more to achieve the target INR, 49.4% in pharmacogenetically-guided *versus* 33.3% in traditional among patients requiring ≤ 21 mg and 24.8% *versus* 7.2% among patients requiring ≥ 49 mg ($p<0.001$ for both comparisons). Thus, the conclusion was that the addition of genotype information enhanced outcomes, especially for patients who required unusually high or low warfarin doses (outliers). CYP4F2 was not included in this algorithm but has been included in several algorithms developed later (Sagreiya et al., 2010; Zambon et al., 2011). Probably the most direct evidence for benefits of the pharmacogenetically-guided approach were provided in the latest study comparing nearly 900 patients for whom genetic information on CYP2C9 and VKORC1 was made available to prescribing physicians with matched 2,690 patients control group who started warfarin therapy without genetic information (Epstein et al., 2010). Six months after warfarin initiation, the genotyped cohort had 31% fewer hospitalizations overall (HR=0.69 [0.58-0.82], $p<0.001$) and 28% fewer hospitalizations for bleeding or thromboembolism (HR=0.72 [0.53-0.97], $p=0.029$)

In February 2010, the FDA revised warfarin label providing genotype-specific ranges of doses and recommending, but not requiring, that genotypes be taken into consideration when the drug is prescribed. The wide availability of CYP2C9 and VKORC1 genotyping and the release of both Web-based and personal decision-support tools have facilitated clinical use of this information. Nevertheless, clinical adoption of genotype-guided administration of warfarin has been slow (Ansell et al., 2008). Several prospective clinical trials are currently ongoing to fill the need for prospective assessment of the value of genetic information in warfarin therapy (Ginsburg & Voora, 2010). Alternative anticoagulant therapies are also being developed that might replace warfarin, perhaps in patients with genotypes associated with extreme warfarin dose response (Kanagasabapathy et al., 2010).

6. Antiplatelet therapies

Platelets play a central role in cardiovascular arterial thrombosis caused by endothelial damage due to a ruptured atherosclerotic plaque, they adhere to the damaged sub-endothelial matrix and aggregate with each other to form a prothrombotic surface that promotes clot formation and subsequently vascular occlusion. Treatment of cardiovascular arterial disease has been using drugs targeting key pathways of platelet activation,

including thromboxane A₂ synthesis and ADP-mediated and integrin $\alpha_{IIb}\beta_3$ signaling pathways. The most common antiplatelet agents include aspirin, clopidogrel and integrin $\alpha_{IIb}\beta_3$ antagonists (Figure 2). Numerous clinical trials have accumulated substantial evidence for efficacy of aspirin and clopidogrel, or both, in the primary and secondary prevention of MI, stroke and cardiovascular death (Wang et al., 2006). However, these trials have also demonstrated subsets of patients in which failure of antiplatelet therapy increased risks of vascular event and death. It has been estimated that 10-15% of the population is resistant to aspirin and close to 30% to clopidogrel, while resistance to both aspirin and clopidogrel occurs in 9% (Dupont et al., 2009). A lot of focus has been drawn to defining antiplatelet drug resistance and understanding how it develops. The term 'resistance' has been coined for lack of ability to attain the expected pharmacologic effect in the laboratory *in vitro* tests of platelet function (Barragan et al., 2003; Mehta et al., 1978; Muller et al., 2003). However, lack of agreement on a standardized definition for antiplatelet resistance contributed to the disparity in its incidence among different studies. Multiple assays for platelet function have been developed, among them the test considered the gold standard for aspirin response - light transmittance aggregometry (LTA), the point-of-care platelet function analyzer PFA-100 device and the newly introduced 'VeryfyNow' assays for aspirin and clopidogrel. One problem is the extent to which these laboratory methods correlate with one another, recent study using six different platelet function test has demonstrated that their results are weakly comparable regarding aspirin response (Lordkipanidze et al., 2007). The other problem is that the phenomenon of resistance is not well understood and, apart from genetic factors, is highly dependent on drug-drug interactions, diet and clinical conditions associated with high platelet turnover, such as inflammation, chronic infection and other disorders (Musallam et al., 2011). Therefore, a more subtle term, i.e. "non-responsiveness", have been suggested (Hennekens et al., 2004) until the reasons for antiplatelet treatment failure are better recognized.

7. Aspirin

For over 50 years, aspirin has been the foundation of antiplatelet therapy. Aspirin (acetylsalicylic acid) irreversibly acetylates the platelet cyclooxygenase-1 (COX1) at serine 529, which reduces the production of thromboxane A₂, a potent platelet activator (Figure 2). Oral aspirin is rapidly absorbed from the stomach and small intestine, reaching peak plasma levels in 1-4 hours, its plasma half-life is only 15-20 minutes, but the platelet inhibitory effect lasts for platelets lifespan because of the irreversible inactivation of COX1 (Patrono et al., 2008). In high-risk patients, aspirin reduces vascular death by approximately 15% and non-fatal vascular events by 30% (Patrono et al., 2008). Aspirin may also be of benefit in the primary prevention of cardiovascular events, but the effect is more modest (Patrono et al., 2008). Consensus guidelines on the role of laboratory testing for aspirin response remain lacking, as evaluation of platelet function for aspirin is highly test specific. The very low cost of the drug is a major advantage.

Potential contribution of genetic factors to aspirin resistance has been investigated in numerous studies, but has not been entirely resolved. Early studies suggested that polymorphisms in the COX1 gene could be responsible for partial resistance to low dose aspirin (Eikelboom et al., 2002; M.K. Halushka & P.V.Halushka, 2002). Further study of 144 CVD patients on aspirin using LTA for platelet activity studies (Maree et al., 2005)

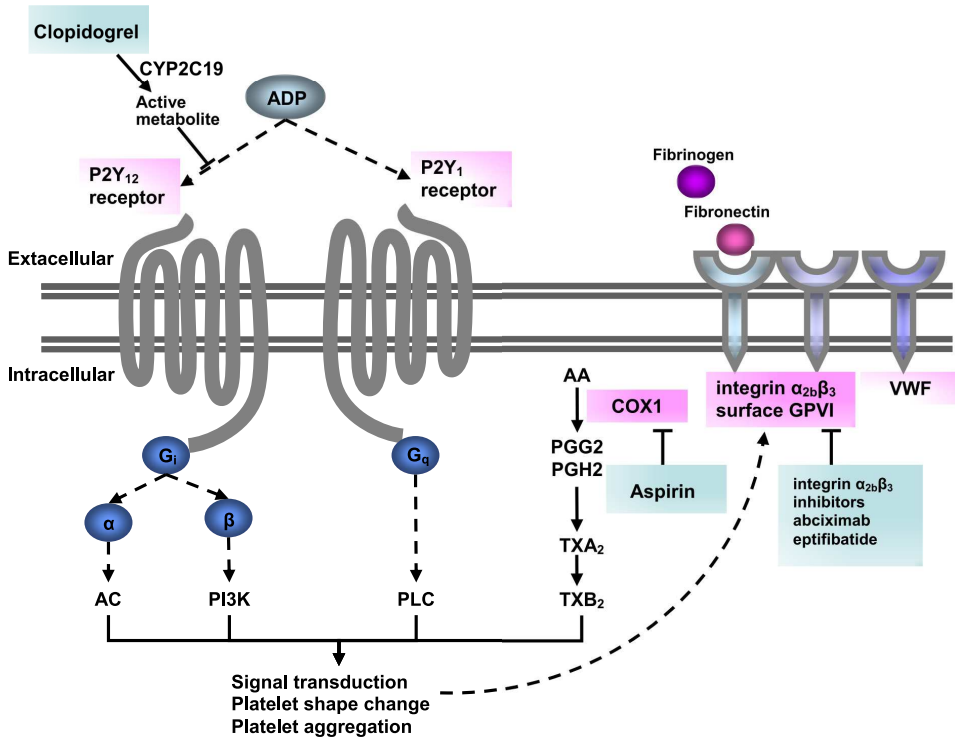
confirmed that polymorphisms in COX1 significantly affect arachidonic acid (AA)-induced platelet aggregation and serum thromboxane A₂ levels ($p=0.004$). However, more recent systematic review has not supported the association between COX1 polymorphisms and aspirin resistance (Goodman et al., 2008). Candidate polymorphisms in platelet glycoprotein receptors (GPIa/IIa, GP Iba and GPIIIa) have been also considered as potential contributors to variability in aspirin response. An original study of 100 patients on low dose aspirin using PFA-100 method for measuring platelet-induced hemostasis *in vitro* (Macchi et al., 2003) reported that patients with poor platelet response to aspirin therapy had significantly more often GPIIIa A1/A1 genotype (86.2%) than good responders (59.4%; $p = 0.01$). No relation was found between aspirin resistance and other GP genotypes. Association between homozygosity for the GPIIIa A1 allele and resistance to aspirin inhibition was further supported by several studies (Dropinski et al., 2007; Feher et al., 2009; Papp et al., 2005), but refuted by others (Lev et al., 2007). Another interesting study re-assessing the effects of various polymorphisms in COX1 or platelet glycoprotein receptors on variable response to aspirin, used both PFA-100 and LTA platelet activity assays (Lepantalo et al., 2006). This study emphasized the effect that the two methods may have on association findings, in addition, the authors suggested that the poor response to aspirin was also associated with female gender ($p=0.019$). Several studies using female platelets have shown increased platelet reactivity at baseline and a less effective inhibition of platelet aggregation by aspirin (Zuern et al., 2009). The mechanisms underlying these differences are still to be elucidated, but influences of female sex hormones may play an important role. As a consequence, inhibition of platelet aggregation in women treated with aspirin may be insufficient, and female patients might benefit from higher maintenance dosages or the use of alternative antiplatelet medications.

Thus, the potential causes, incidence and clinical impact of aspirin resistance are still obscure. Measured variability in response to aspirin is most probably multifactorial, with genetics playing what appears to be a small, undefined role. Others suggest that the actual incidence of true clinical aspirin resistance is very low, and that aspirin failure has little to do with *ex vivo*-determined responsiveness (Cuisset et al., 2009). Alternate pathways for platelets activation that are not inhibited by aspirin, such as erythrocyte induced platelet activation (Santos et al., 1991), may be responsible for aspirin resistance. Based on these notions and the mixed results shown in the above studies, there is currently no defined role for pharmacogenetic testing to dose aspirin.

8. Clopidogrel high-risk pharmacokinetics

Thienopyridines, such as clopidogrel and prasugrel, irreversibly bind to the purinoceptor P2Y₁₂ receptor resulting in inhibition of platelets activation in response to adenosine diphosphate (ADP) and inhibition of platelet aggregation (Figure 2). Clopidogrel is given orally in a daily universal dose, it has substantial benefits in patients after PCI and stent implantation (Anderson et al., 2007a). Dual antiplatelet therapy (aspirin plus clopidogrel) is the standard of care for patients with acute coronary syndrome managed medically after coronary stenting or by PCI (Anderson et al., 2007a). However, major adverse cardiovascular events including stent thrombosis can occur despite antiplatelet therapy, recent meta-analysis showed that persistent platelet reactivity on clopidogrel treatment confers a five-fold increased risk of major adverse cardiovascular events (Sofi et al., 2010). All

thienopyridines are pro-drugs requiring activation by the hepatic cytochrome P450 enzyme system. Clopidogrel is metabolized to its active metabolite through a two-step process mediated by various CYPs, among which CYP2C9 and CYP2C19 play a major role (Brandt et al., 2007), the activation of prasugrel, in contrast, is mediated by esterases and by CYP3As with lesser contribution of CYP2C9 and CYP2C19 (Jakubowski et al., 2007). Inter-patient response variability to clopidogrel became evident from platelet function assays *in vitro* (Barragan et al., 2003; Muller et al., 2003) and from associations of poor clopidogrel response *in vitro* (clopidogrel resistance or none-responsiveness) to poor clinical response evidenced by major adverse events (Snoep et al., 2007).



Abbreviations: CYP2C19 cytochrome P450 enzyme; ADP adenosine diphosphate; P2Y₁₂ and P2Y₁ purinoreceptor receptors; coupled G_i and G_q proteins; G_i protein α and β subunits; AC adenylyl cyclase; PI3K phosphatidylinositol 3-kinase; PLC phospholipase C; AA arachidonic acid; PGG₂ and PGH₂ endoperoxides; TXA₂ TXB₂ thromboxanes; COX1 cyclooxygenase 1; surface GPVI glycoprotein VI and integrin α₂β₃; VWF von Willebrand factor; arrows indicate activation and blocked lines inhibition.

Fig. 2. Targets of several antiplatelet agents, clopidogrel, aspirin and integrin α₂β₃ inhibitors

Multiple factors have been implicated in high-on clopidogrel platelet reactivity, including drug compliance, drug-drug interactions, age, diabetes, body-mass index, left ventricle ejection function and inflammation (Giusti et al., 2010). Several studies have demonstrated that common and functional polymorphisms in CYPs responsible for clopidogrel pharmacokinetics can affect clopidogrel responsiveness. In a key study (Brandt et al., 2007),

the investigators hypothesized that polymorphisms inducing loss-of-function of CYP2C19, CYP2C9, and CYP3A5 could contribute to decreased formation of the active clopidogrel metabolite and thereby affect inhibition of platelet activation. They examined the effect of loading doses of clopidogrel and prasugrel on platelet function *in vitro*, showing significant association between the CYP2C19*2 allele encoding a truncated protein product with little enzymatic activity and poor response to clopidogrel, but not to prasugrel. CYP2C9*2 and *3 showed similar tendencies. Carriers of CYP2C19*2 were more frequently poor responders compared to patients without the allele (72% versus 41% respectively, $p=0.030$). A similar trend was observed among CYP2C9*2,*3 homozygotes compared to patients with the wild type genotype (75% versus 41.4%, $p=0.024$). Overall, the presence of either CYP2C19*2 or CYP2C9 (*2/*2 or *3) was strongly associated with poor clopidogrel response ($p<0.001$). No association was found between CYP3A5 polymorphisms and clopidogrel response. In addition, the presence of these or any other CYP polymorphisms had no effect on response to prasugrel. The effect of CYP3A5 polymorphisms on clopidogrel response is still elusive, a follow up study of 348 patients treated with clopidogrel after stent placement (Suh et al., 2006) suggested that the CYP3A5*3 'non-expressor' allele contributed to significantly increased risk of atherothrombotic events, however these findings could not be reproduced by others (Simon et al., 2009). Since CYP3A5 and A4 have an overlapping substrate specificity (Lamba et al., 2002), variability in CYP3A4 activity was also associated with clopidogrel response (Lau et al., 2004). As a result, other drugs that are metabolized by CYP3A4, e.g. certain statins commonly used in patients with atherosclerosis, could interfere with clopidogrel activation.

The concept of high-risk pharmacokinetics in response to clopidogrel and specifically the role of CYP2C19*2 became increasingly recognized owing to several recent studies. Sub-group analysis of the EXCELSIOR study examined whether the loss-of-function CYP2C19*2 allele is associated with increased platelet reactivity despite clopidogrel treatment in patients undergoing elective PCI with stent placement (Trenk et al., 2008). CYP2C19*2 was significantly associated with residual platelet aggregation (RPA>14%) before hospital discharge. Patients with RPA>14% had significantly increased risk of death or MI (HR=3.0 [1.4-6.8], $p=0.004$) 1-year post-procedure. The authors concluded that patients carrying at least one CYP2C19*2 allele are more prone to high-on clopidogrel platelet reactivity, although this study was not adequately powered to determine the effect of variant alleles on clinical outcomes. A consecutive study (Shuldiner et al., 2009) clarified the association between CYP2C19*2 and clinical outcomes, by doing GWAS and CYP2C19*2 genotyping in conjunction with platelet function assays in 429 healthy Amish volunteers on clopidogrel from the Amish Pharmacogenomics of Antiplatelet Intervention (PAPI) study, and then re-examining PAPI findings in relation to cardiovascular outcomes in an independent cohort of 227 clopidogrel-treated patients after PCI. The investigators established that CYP2C19*2 was associated with reduced clopidogrel response in PAPI study accounting for 12% of the variability in ADP-induced platelet aggregation ($p=4.3\times 10^{-11}$). The relation between CYP2C19*2 genotype and platelet aggregation was replicated in the patients cohort ($p=0.02$). Moreover, patients with the CYP2C19*2 variant were more likely (20.9% versus 10.0%) to have cardiovascular ischemic events or death during the 1-year follow-up period (HR=2.42 [1.18-4.99], $p=0.02$) (Shuldiner et al., 2009). In patients no longer taking clopidogrel after 1-year, no increase was observed between carriers and non-carriers of CYP2C19*2 (Mega et al.,

2009a). Apart from the CYP2C19*2 contribution (12%), age, body mass, and lipid levels accounted for additional 10% of clopidogrel response. Essential confirmation of CYP2C19*2 as an important determinant of clopidogrel response was provided by a sub-analysis of the randomized TRITON-TIMI 38 trial of clopidogrel and prasugrel outcomes using similar two-phase approach (Mega et al., 2009a). This study tested associations between CYP2C19 reduced-function alleles (five alleles were tested) and measurements of active drug metabolite in plasma and platelet aggregation in response to clopidogrel in healthy individuals (n=162), and then re-evaluated these associations in a separate cohort of patients with acute coronary syndrome (n=1477) considering cardiovascular outcomes. In healthy individuals, carriers of at least one CYP2C19 reduced-function allele (approximately 30% of the population) had 32.4% reduction in plasma exposure to the active drug metabolite and reduction in maximal platelet aggregation in response to clopidogrel, as compared to non-carriers (both $p < 0.001$). Among TRITON-TIMI 38 patients, carriers of the CYP2C19 reduced-function allele had 53% increase in the risk of death from cardiovascular causes, MI or stroke compared to non-carriers (12.1% versus 8.0%, HR=1.53 for carriers [1.07-2.19], $p=0.01$) and increase in the risk of stent thrombosis (2.6% versus 0.8%, HR=3.09 [1.19-8.00], $p=0.02$). These findings imply that in patients receiving clopidogrel reduced-function CYP2C19 alleles can lead to reduced exposure to the active metabolite, less platelet inhibition and reduced protection from ischemic events, including stent thrombosis. Interestingly, no associations were found with any of these CYP variants among patients randomized to prasugrel therapy in the TRITON trial (Mega et al., 2009b). One drawback raised in a parallel study of the French FAST-MI registry including 2,208 patients (Simon et al., 2009), suggested that only carriers of two loss-of-function alleles CYP2C19*2, *3, *4, or *5 (i.e. homozygotes or compound heterozygotes) have increased risk of death, nonfatal MI or stroke during 1-year period (21.5% versus 13.3%, HR=1.98 [1.10-3.58]). This risk was increased even further in PCI (HR=3.58 [1.71-7.51]). This question has been resolved in the meta-analysis including 9,685 patients with acute coronary syndrome or PCI (Mega et al., 2010b), showing that carriers of even one reduced-function CYP2C19 allele may have significantly increased risks of major adverse cardiovascular events, particularly stent thrombosis.

Platelet response to clopidogrel is not fully explained by the CYP2C19 loss-of-function alleles. Other pharmacokinetic sources of inter-patient variability have been suggested, specifically the effect of the 3435C>T polymorphism in the p-glycoprotein ABCB1 on clopidogrel absorption and metabolism (Hoffmeyer et al., 2000; Owen et al., 2005; Taubert et al., 2006). However, this issue remains controversial in the recent sub-analysis of TRITON-TIMI 38 and PLATO trials considering ABCB1 genotypes and clinical outcomes of patients on clopidogrel (Mega et al., 2010a; Wallentin et al., 2010a). Most recently, a novel determinant of clopidogrel efficacy was proposed (Bouman et al., 2011), namely the esterase PON1, a key enzyme in the rate limiting step of clopidogrel bioactivation and that a coding Q192R PON1 polymorphism can affect plasma concentrations of active metabolite, clopidogrel inhibition and risk of stent thrombosis. Large randomized replication studies are needed to confirm these interesting and new observations. In addition, few contrasting data are available on pharmacodynamic factors, particularly polymorphisms in genes encoding platelet glycoproteins involved in thienopyridines intestinal absorption and platelet receptors that serve as thienopyridines targets. Effects of polymorphisms in

glycoprotein GPIIIa, and platelet receptors P2Y₁₂ and P2Y₁ related to aspirin and clopidogrel response were evaluated in a preliminary study (Lev et al., 2007), finding no definitive associations between various polymorphisms and clopidogrel response assessed by platelet aggregation studies. Another study (Shuldiner et al., 2009) relating to clinical outcomes also did not find an association between the P2Y₁₂ polymorphism and the risk of death, non-fatal MI, or stroke in patients treated with clopidogrel.

9. Implementation of clopidogrel pharmacogenetics

Early in 2010, the FDA added a boxed warning to prescribing information for clopidogrel, stating that persons with the low-rate metabolizing *CYP2C19* variant might require dose adjustment or the use of another drug. After this FDA action, the American Heart Association and the American College of Cardiology issued a joint endorsement of *CYP2C19* genotyping for patients at moderate or high risk for cardiovascular events who are treated with clopidogrel (Holmes et al., 2010), this genetic test is now widely available in the US. Despite that, there are studies that challenge the clinical impact of polymorphisms on the effectiveness of clopidogrel. Most recently, the CHARISMA genomic sub-study reported at the TCT 2009 meeting <http://www.theheart.org/article/1008623.do> that while patients homozygous for the loss-of-function *CYP2C19* allele appeared to have an increased risk of ischemia when compared with patients with the wild-type allele, they also had fewer bleeding events. Conflicting results of many different studies exemplify many unanswered questions regarding clinical significance of pharmacogenetics in antiplatelet drugs. Being able to predict the specific response of an individual patient based on his or her genetic code has yet to be defined, especially for clopidogrel. Despite the lack of clear guidance regarding how clinicians could utilize the pharmacogenetic information on clopidogrel, some clinical laboratories, especially in the US, now offer genetic screening for markers associated with response to antithrombotics including clopidogrel.

10. New generation antithrombotic drugs

Novel orally active antiplatelet agents are now available. Prasugrel, the third-generation thienopyridine, has been associated with greater active metabolite generation, superior inhibition of platelet aggregation and less response variability than clopidogrel (Gurbel & Tantry, 2008). In the TRITON-TIMI 38 trial of patients with acute coronary syndrome undergoing PCI, the prevalence of cardiovascular death, non-fatal MI or stroke was lower with prasugrel than with clopidogrel, although rates of bleeding were higher in the prasugrel group (Wiviott et al., 2007). A novel selective inhibitor of P2Y₁₂-receptor, ticagrelor has been also evaluated against clopidogrel in patients with acute coronary syndrome in the PLATO trial (Wallentin et al., 2009). Ticagrelor was associated with significant reduction in cardiovascular death, MI and stroke, without any difference in the overall incidence of major bleeding, but with increase in major bleeding related to noncoronary-artery bypass graft.

In October 2010, the FDA approved the oral anticoagulant dabigatran (Pradaxa; Boehringer Ingelheim), a direct thrombin inhibitor (DTI), for stroke prevention in patients with AF (SPAF). The clinical community is excited at the prospect of having an alternative for warfarin therapy, the current gold standard therapy for stroke prevention, a challenge that

has taken more than 50 years. SPAF is not the only indication for dabigatran and other new DTIs, ongoing trials are evaluating DTIs for the treatment of acute coronary syndromes and VTE after major orthopaedic surgery (Hughes, 2010). Initially, dabigatran will probably substitute warfarin in patients who have problems with INR management, investigators of the Phase III RE-LY trial comparing between dabigatran and warfarin effects in 18,113 patients suggest that the rates of adverse events were similar for dabigatran and warfarin in patients with good INR control, whereas dabigatran was always superior to warfarin in patients with poor INR control (Wallentin et al., 2010b). Thus, successful entry of dabigatran may benefit from identification of warfarin dose outliers by genetic testing for CYP2C9 and VKORC1 markers and in the same way, the next generation DTIs, rivaroxaban and apixaban that are currently tested in the Phase III ROCKET-AF and ARISTOTLE trials, respectively (<http://clinicaltrials.gov/>).

11. Conclusions

Pharmacogenetics is one of the major components of personalized therapy. However, even though the conceptual basis for pharmacogenetics has existed for over half century and recent scientific and technological advancements in the field, and the FDA awareness of the necessity to integrate genomic data into regulatory review, the translation of pharmacogenetics into the clinic has been slow. The cardiovascular field and particularly the antithrombotic drug therapy have provided some excellent examples of clinical utility of pharmacogenetic approaches. The impact of VKORC1 and CYP2C9 variants on warfarin response, established the value of genetic variability to predict the appropriate warfarin dose for improving and easing the transition to a therapeutic INR level. In fact, the labeling for warfarin now includes recommendations for genetic testing. Nevertheless, the clinical application of this information has yet to become universal, in part due to ethical and confidentiality issues regarding genetic information, logistic issues with obtaining timely genotyping, and resolution of appropriate genetically-guided dosing algorithms for warfarin in various populations. Specifically to this last point, the validity of the existing genetically-guided dosing algorithms in ethnically heterogenous populations, such as in the US, has been seriously compromised by ethnic stratification of certain genetic warfarin dosing markers and inability to predict with equivalent degree of confidence in individual dose response. In addition, as warfarin pharmacogenetics is extensively affected by environmental interactions, differences in lifestyle, nutrition and traditional medical routines may have significant impact on how warfarin genetic testing is translated to clinical decision making in various population. Once again, large-scale prospective studies are needed to confirm the usefulness and pharmaco-economic benefits of personalized genetically-guided treatment for warfarin on a population basis.

Similar to the evolution of personalized medicine for the anticoagulant warfarin, the antiplatelet drug clopidogrel has also demonstrated strong potential for improving therapy by pharmacogenetic approach. For now, however, clopidogrel pharmacogenetics is even farther from obtaining widespread application than warfarin. There is a much smaller percentage of variability explained by the current paradigm and fewer prospective studies confirming the worthiness of genetic information for improving clinical outcomes. Likewise, any potential economic savings of this strategy have not been demonstrated. Most importantly, it is unclear how the genetic information on CYP2C19 can be utilized for

adjustment of the treatment regime, for example by increasing the dosing of clopidogrel or by substituting clopidogrel for the recently approved prasugrel or for the potentially soon to be available ticagrelor. The appropriate use of these strategies is difficult to assess in the absence of genetic information on clopidogrel alternatives, and incomplete understanding about other potential predictors, i.e. genetic, epigenetic and environmental modulators of response to antiplatelet agents. This problem is moreover accentuated by lack of reliable and validated assays for measuring platelet function with sensitivity, consistency, standardization and correlation with clinical outcomes, in order to tailor with confidence personalized antiplatelet therapy.

There has been significant discussion in the scientific press (Collins, 2010; Varmus, 2010; Venter, 2010; Woodcock, 2010) about the slow pace of the application of genomics to clinical practice. All parties point to the need for increasingly large and complex studies to test pharmacogenomic paradigms in the clinical setting; economic disincentives for pharmaceutical industry to accept the implications of individualized drug response; and the slow pace of the incorporation of pharmacogenomics into the drug evaluation process. In line with this critique, the FDA has recently released several regulatory guidelines (Guidance for Industry: Pharmacogenomic Data Submission 2005 and Companion Guidance 2007) on integration of genomic data in the evaluation process of new drug applications. Finally, there is a need for concerted effort directed at the education of healthcare professionals as well as patients to understand, accept and utilize genomic information

As time progresses, technology will continue to decrease the cost of whole-genome scans and other genetic tools, allowing more efficient and secure transfer of genetic information. Challenges that are associated with replication of study findings and evaluation of the clinical significance of genetic variants, underscore the importance of functional experiments to test their biological implications and to extend our understanding of drug mechanisms. These advances, along with development of logistical platforms for universal application of genetic information will allow realization of personalized medicine across all therapeutic areas, antithrombotic drug therapy. Finally, additional scientific, regulatory, and psychological factors must be addressed before pharmacogenomic tests will become a routine part of medicine. The FDA-mandated incorporation of pharmacogenomic information in drug labeling will remain an important step in the acceptance of pharmacogenomics in clinical practice. Perhaps equally important will be the willingness of physicians to reexamine suboptimal pharmacologic management programs.

12. References

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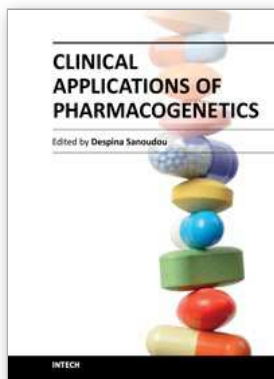
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The rapidly evolving field of Pharmacogenetics aims at identifying the genetic factors implicated in the inter-individual variation of drug response. These factors could enable patient sub-classification based on their treatment needs thus expediting drug development and promoting personalized, safer and more effective treatments. This book presents Pharmacogenetic examples from a broad spectrum of different drugs, for different diseases, which are representative of different stages of evaluation or application. It has been designed so as to serve both the unfamiliar reader through explanations of basic Pharmacogenetic concepts, the clinician with presentation of the latest developments and international guidelines, and the research scientist with examples of Pharmacogenetic applications, discussions on the limitations and an outlook on the new scientific trends in this field.

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