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Implementing genotype-guided antithrombotic therapy

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Abstract

Genotyping has the potential to improve the efficacy and safety of major antithrombotic drugs. For warfarin, the stable maintenance dose varies from 1–10 mg/day. The *VKORC1* –1639G>A allele and the *CYP2C9**2 and *3 alleles (cumulative frequency: 90% in Asians, 65% in Europeans and 20% in Africans), explain 45% of response variability in European and 30% in African populations. The large clinical trials COAG and EU-PACT will define the extent to which pharmacogenetic dosing affects the safety and efficacy of warfarin and coumarin derivatives. The platelet inhibitor clopidogrel requires activation by the *CYP2C19* enzyme. *CYP2C19**2 and *3 alleles (cumulative frequency: 20–50%) produce null enzyme activity, and their presence attenuates platelet inhibition and increases cardiovascular events. The US FDA-mandated drug labeling recognizes the relevance of genotyping in the selection and dosing of both warfarin and clopidogrel.

Keywords

clopidogrel; *CYP2C19*; *CYP2C9*; pharmacogenetics; platelet; *VKORC1*; warfarin

One of the promises of the human genome project is individualization of patient care based on highly heterogeneous innate metabolic factors determined by DNA typing of gene polymorphisms. The practice of DNA-guided medicine requires the translation of such gene polymorphisms into clinical decision support for personalized healthcare. Pharmacogenetics serves as the foundation for the most clinically advanced application of DNA-guided medicine.

Knowledge of the patient's genetic status for common variants in the *VKORC1* and *CYP2C9* genes can already guide antithrombotic intervention with warfarin. Common variants in the *CYP2C19* gene influence antiplatelet therapy with clopidogrel, exemplifying a phase of investigation that is rapidly approaching clinical utility.

Genomic research interest is high in the area of prediction of drug responsiveness based on genetic variation. It is a useful goal to predict variability in drug responsiveness. Such

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prediction can reduce medication errors in general. Medication errors that might be ameliorated include under- or over-dosing a drug, as with warfarin, or administration of a drug that requires activation by an enzyme when gene testing results would indicate that such activation will not occur due to null enzyme activity, as in the case of a patient who receives clopidogrel and who is homozygous for *CYP2C19* minor allele *2. In the case of the anticoagulant warfarin, genetic prediction has the potential to reduce trial and error on the part of clinicians, risks of therapy, and the resources needed to treat.

Prediction of warfarin responsiveness

One of the leading examples of the translation of genetic understanding into the prediction of a drug response is the case of warfarin. Warfarin is prescribed for prophylaxis and therapy of patients with a wide variety of venous and arterial thromboembolic disorders, including those with atrial fibrillation and other cardiac arrhythmias, surgical cardiac valve replacement, deep vein thrombosis, thrombophlebitis with or without pulmonary embolism, and those at risk of stroke [1]. Over 20 million warfarin prescriptions are written per year in the USA, making it the 11th most prescribed drug in the USA [2]. Approximately two million new prescriptions are written annually, many for anticoagulant-naïve patients [3]. This figure will rise as the population of elders with atrial fibrillation continues to increase. The effective warfarin dose is that which achieves the target international normalized ratio (INR), usually 2–3. The range of effective warfarin dose varies widely from 5 to 80 mg/week.

Warfarin's narrow therapeutic window requires careful titration toward a patient's maintenance dose. The risk of bleeding associated with warfarin therapy is greatest during the commencement of therapy when the optimum dose for the patient is being evaluated [4]. Trial and error dosing at the commencement of anticoagulation therapy can lead to under- or over-dosing and the associated adverse drug reactions because of the considerable between-patient differences in warfarin dose requirement. Difference in successful outcomes during warfarin therapy is a multifactorial issue, including variables other than treatment itself. Many clinical, patient and environmental factors influence the response to warfarin and need to be considered when prescribing the drug. These include diet, alcohol intake, body mass, concomitant drug treatment, coexisting diseases and patient compliance with drug regimen and diet restrictions. Genes are also important. The determination of the initial dose is usually empiric and has not routinely taken into consideration genetic factors that affect warfarin sensitivity. However, we can now adjust a patient's warfarin dose according to a combinatorial genotype of two genes in combination with conventional nongenetic factors.

Pharmacogenetic studies from the past decade have revealed that common polymorphisms in two genes, *CYP2C9* and *VKORC1*, greatly affect warfarin dose through different mechanisms (Figure 1). Genome wide scans have confirmed these findings [5,6]. The most frequent polymorphisms of *CYP2C9* include *2 and *3, which are associated with decreases in 2C9 enzyme activity to approximately 12–70% and 5% of the normal level, respectively (Table 1) [7–39]. The US FDA revised the Coumadin® (warfarin) label in August 2007 to indicate that *CYP2C9* and *VKORC1* are covariates of interindividual variability of warfarin doses.

The result of having these most common and clinically relevant *CYP2C9* polymorphisms (i.e., *CYP2C9**2, *3, and in African–Americans *8) is excess warfarin accumulation, elevated INR (>4.0) and possible hemorrhagic complications [4]. Frequencies of *CYP2C9* variants have a significant impact on the body's ability to metabolize warfarin in Swedish, Japanese, Italian, North American, British Caucasian, Hong Kong–Chinese and African–American populations [8,10,40–48]. The *CYP2C9* status by itself accounts for approximately 15–20% of the variance in warfarin dose [42,47,49]. Frequency distributions of the most common *CYP2C9**2, *3 and *8 loss-of-function polymorphisms vary by ethnogeographic origin. It has been reported that

the gene is less polymorphic in populations of Asian or African descent than in those of European descent [19,50,51].

Warfarin exerts an anticoagulant effect through its inhibition of the vitamin K epoxide reductase multiprotein complex (VKOR), part of which is subunit 1, encoded by *VKORC1* [52,53]. VKOR is responsible for converting vitamin K epoxide to vitamin K hydroquinone, which is an essential cofactor required for the carboxylation of clotting factors II, VII, IX and X [54]. A common polymorphism of the *VKORC1* promoter sequence (−1639 G>A) results in decreased vitamin K epoxide reductase enzyme activity, as shown by *in vitro* promoter luciferase activity assays [55].

The −1639 G>A polymorphism, at position 3673 of the *VKORC1* gen (GenBank accession number AY587020 [56,57]) is in nearly perfect linkage disequilibrium with the polymorphisms 1173 C>T (also known as *VKORC1* position 6484 [56]) and 1542 G>C [40] (*VKORC1* position 6853 [57]). These three and two other single nucleotide polymorphisms (SNPs) mark *VKORC1* haplotype A/B. Table 2 lists commonly studied *VKORC1* polymorphisms to clarify otherwise convoluted nomenclature and findings [57–60]. Patients carrying haplotype A require a lower warfarin maintenance dosage. In addition, six rare coding sequence mutations result in syndromes of warfarin resistance [52,56]. In patients requiring anticoagulant therapy, the A/B haplotype independently determines 20–25% of warfarin dose variance in European patients [47,56] but considerably less in African–American populations [61,62]. Together, the *CYP2C9**2 and *3 genotypes, and the *VKORC1* A/B haplotype explain up to 45% of warfarin response variability in European [41,42,49,56] and 30% in African populations [61]. The incorporation of the *CYP2C9* *8 allele into a pharmacogenetic dosing algorithm increases accuracy in African–Americans [61]. The population frequencies of the −1639 A allele indicating haplotype A are remarkably different across populations and reported as 75–92% in East Asians [19,55,63,64], 35–47% in Europeans [56,60,65,66], 9–12% in African–Americans [19,29,48,61] and 29–48% in mixed populations [19,39]. The pronounced disparity in haplotype A frequency explains, in part, why mean effective warfarin dose is often lower in East Asian [67,68] and higher in African compared with Caucasian populations [48].

The population wide impact of combinatorial genotyping for *VKORC1* and *CYP2C9* on effective warfarin dose can be seen in Figure 2 [12]. The predicted decrease in effective dose, based on genetic and nongenetic factors, can be determined from published algorithms [41, 42,59] or online [201]. Figure 2 illustrates, in a sample of outpatients that happens to be from a preventive cardiology clinic in northeastern USA, the frequency of individual combinatorial genotypes for *CYP2C9* and *VKORC1* found in that population (horizontal axis) and the predicted decrease in effective warfarin dose associated with each combinatorial genotype (vertical axis) [12]. The bleeding risk associated with warfarin therapy increases with the number of deficient alleles [11,69–71]. Given that ethnogeographic origin affects the frequencies of combinatorial genotypes, this type of illustration makes it possible to assess the population-wide impact of the combinatorial genotypes on warfarin dose for any given population.

Emerging predictors

Other genes may augment the predictive capacity of *CYP2C9* and *VKORC1* genes. A recent study identified a non-synonymous variant (rs2108622) in exon 2 of a cytochrome P450 family 4, subfamily F, polypeptide 2 gene, *CYP4F2*, introducing a Val to Met amino acid change at position 433 (V433M), that has a small (1–2%) effect on warfarin dose [72]. *CYP4F2* is a vitamin K1 (VK1) oxidase involved in the hepatic metabolism of VK1. The rs2108622 polymorphism is linked to altered VK1 metabolism and elevated hepatic levels of VK1, hence necessitating a higher warfarin dose to elicit the same anticoagulant response. In a population largely of European ancestry, this variant predicted an additional 4–12% of the variability in

warfarin dose, or 0.5 mg/day per minor allele. The effect of rs2108622 has been confirmed in Swedish [5], Italian [73] and North American [6] patients in whom the polymorphism contributed 1–10% of the variance in dose. The effect is not present in African-Americans [61], in whom the minor allele frequency appears considerably lower [61].

A synonymous SNP of *EPHX1* (rs2292566) is a novel predictor for maintenance warfarin dose in frail elderly French inpatients [62]. *EPHX1* encodes microsomal epoxide hydrolase 1, which is a putative subunit of vitamin K epoxide reductase [74]. The rs2292566 variant accounts for 1.7% of the variability in stable therapeutic dose and patients with one or two variant alleles (GA or AA) required lower doses (up to 2.6 ± 1.3 mg; 19% reduction; $p = 0.043$). The *EPHX1* SNPs rs4653436 and rs360097 are weakly associated with warfarin dose in genome-wide scans [6,47].

Future work should address ethnic differences in the genetic variants that influence warfarin dose as well as subtle intra-ethnic differences and admixture that may exist in other populations such as Hispanics. Unidentified genes may influence warfarin dose in other ethnicities, and some rare dose-altering variants in genes such as *CYP4F2* and *EPHX1* may exist in only a subset of populations. The sequencing of greater numbers of human genomes [75] will reveal many SNPs with frequencies of approximately 1%. Knowledge of these SNPs will help to further refine warfarin dosing algorithms.

Clinical utility

Pharmacogenetic dosing & outcomes—To date, there are no studies adequately powered to tell whether pharmacogenetic testing (PGt; here, including the *VKORC1* –1639G>A allele and the *CYP2C9**1, *2 and *3 alleles) provides an advantage over the nongenetic approach to dosing with respect to clinical outcomes. A meta-analysis of three randomized trials totaling 423 patients showed no statistically significant difference in bleeding rates or time within the therapeutic INR range [76]. The highest quality study found a trend toward more rapid achievement of a stable dose (14.1 vs 19.6 days; $p = 0.07$) in the pharmacogenetic arm [69]. Results from ongoing clinical trials will help to clarify the role of genetic testing in warfarin management. For a listing of ongoing trials, see Kangelaris *et al.* [76].

Pharmacogenetic dosing algorithms & prediction of maintenance warfarin dose

—A recent multicenter study on four continents reported moderate success using a pharmacogenetic algorithm [77]. The International Warfarin Pharmacogenetics Consortium (IWPC) tested PGt and clinical algorithms on 4043 patients who had achieved the stable therapeutic dose of warfarin, defined as the steady-state dose that led to stable anticoagulation levels. The IWPC tested the PGt and clinical algorithms in 1009 patients also at the stable therapeutic dose. The criteria for success was prediction to within 20% of the dose ultimately needed to achieve target INR. The PGt algorithm surpassed the nongenetic approach in subsets of patients at the extreme ranges of warfarin dose. For patients who required a low dose (≤ 21 mg of warfarin/week), the PGt approach correctly predicted the stable therapeutic dose in 49.4% of patients compared with 33.3% for the clinical approach ($p < 0.001$). For patients requiring a high dose (≥ 49 mg/week), the PGt predicted the dose in 24.8% of patients compared with 7.2% for the clinical approach ($p < 0.001$). These two groups of patients represent 46% of all patients. For the remaining 54% of patients receiving between 21 and 49 mg of warfarin per week, the PGt algorithm provided no advantage. The number of patients needed to genotype was 13.2 in order to obtain an improvement with the pharmacogenetic algorithm in one patient.

The penultimate question is, for which patient is pharmacogenetic testing useful? The answer is becoming clearer as the products of genetic discovery are becoming increasingly relevant to the practice of clinical medicine. Genotype-guided warfarin prescribing is positioned to set

a precedent for how integration of genetic technologies in clinical practice will proceed. In the case of warfarin, it seems intuitive that adjusting warfarin dose to match patients' genetic makeup will result in fewer complications; however, additional clinical trial data are needed prior to endorsing a new standard of care for warfarin dosing.

Cost-effectiveness

The cost of pharmacogenetic testing must be offset by the savings associated with the reduction in major bleeding. Patients with atrial fibrillation represent the largest group (30%) of all patients receiving warfarin therapy [78]. Anticoagulation with warfarin decreases the risk of embolic stroke by over 50%; however, warfarin poses a risk for major hemorrhagic events such as intracranial hemorrhage and gastro intestinal bleeding [78,79]. Major and minor embolic strokes associated with atrial fibrillation are estimated to cost US\$1,758,548 and \$19,352, respectively, per event (in 2003 US dollars), whereas severe lower gastrointestinal hemorrhages are estimated to cost \$193,804 per event [80]. Eckman *et al.* have stated that “Warfarin-related genotyping is unlikely to be cost effective for typical patients with nonvalvular atrial fibrillation, but may be cost effective in patients at high risk for hemorrhage who are starting warfarin therapy” [81], where hemorrhage risk is assessed using the HEMORR₂HAGES tool [82]. For pharmacogenetic dosing to be cost effective in a 69-year-old patient with nonvalvular atrial fibrillation, it would have to prevent more than 32% of major bleeding events, be available within 24 h and cost less than \$200 [81].

Approaching the clinic: prediction of platelet responsiveness

Heritable factors play a major role in the lack of response to pharmacological therapy for treatment of platelet-dependent thrombosis [83]. However, genetic variants in platelet receptors have not consistently been shown to influence platelet function, alter drug response or be associated with cardiovascular disease [84].

The thienopyridine clopidogrel is a widely used antithrombotic agent that inhibits the platelet P2Y₁₂ ADP receptor [85]. It is a prodrug that requires activation by the CYP2C19 isoenzyme to form the active compound R-130964 (Figure 3) [86,87]. The gene encoding the CYP2C19 isoenzyme is polymorphic, and carriers of the *2 and other specific alleles of *CYP2C19* have a diminished response to the antiplatelet effects of clopidogrel (Table 3) [88–120].

In the case of *CYP2C19**17, metabolism is increased [95] but with little apparent impact on the antiplatelet efficacy of clopidogrel [92]. Reduced responsiveness and/or efficacy in carriers of the minor *2 allele of the *CYP2C19* gene has been demonstrated in patients after coronary stent placement [121,122], in patients with acute coronary syndrome [86], in patients with myocardial infarction (MI) [123,124] and after MI without ST elevation [125].

Co-administration of the proton-pump inhibitor omeprazole, which is metabolized by *CYP2C19*, decreases the platelet inhibitory effect of clopidogrel [126], but others, such as pantoprazole and esomeprazole, do not [127]. Going further, the concomitant use of a proton-pump inhibitor that inhibits cytochrome P450 2C19 (omeprazole, lansoprazole or rabeprazole) was associated with an increased risk of recurrent MI in patients receiving clopidogrel therapy in an observational study [128]. However, no such association was found in the preliminary results of the only randomized trial comparing outcomes in clopidogrel with omeprazole or placebo [129].

Recent studies have advanced the case for geno typing to aid clopidogrel therapy [86,122–124]. As a result of these, the Plavix® (clopidogrel bisulfate) label was revised by the FDA in May 2009 to include a pharmacogenetics section. In this section there is a thorough discussion

on the variance of potency related to *CYP2C19* gene polymorphisms and a connection to pharmacogenetic testing to identify genotypes of *CYP2C19*.

Simon *et al.* examined 2208 patients with acute MI after receiving clopidogrel 300 mg/day mean loading dose and a maintenance dose of 75 mg/day upon hospital discharge over a 1-year period in the French registry of Acute ST elevation or non-ST-elevation Myocardial Infarction (FAST-MI) study [124]. Of these patients, 10.2% (n = 225) died and 4.3% (n = 94) had a MI or nonfatal stroke during follow-up. Patients who are double carriers of any possible combination of the *2, *3, *4 and *5 loss-of-function alleles had a higher event rate (21.5 vs 13.3%, adjusted hazard ratio [HR]: 1.98; 95% CI: 1.10–3.58).

Mega *et al.* studied 1477 patients with acute coronary syndromes receiving clopidogrel 300 mg loading dose in the hospital and 75 mg/day upon discharge [86] in the Trial to assess Improvement in Therapeutic Outcomes by optimizing platelet Inhibition with prasugrel Thrombolysis In Myocardial Infarction 38 (TRITON-TIMI 38) study [130]. Carriers of at least one *CYP2C19* loss-of-function allele had a 32.4% decrease in levels of the active clopidogrel metabolite and there was 9% less reduction in platelet aggregation, compared with non-carriers. In those with the *2 allele, the risk of stent thrombosis was three-times that of non-carriers. Event curves diverged within the first month of treatment with clopidogrel, consistent with the effect expected from antiplatelet therapy.

The presence of at least one copy of the *CYP2C19**2 allele in 259 young survivors of acute MI (<45 years of age) treated with clopidogrel was associated with a greatly increased risk of recurrent arterial thrombotic events [123]. Baseline characteristics were balanced between carriers (heterozygous *1/*2, n = 64; homozygous *2/*2, n = 9) and non-carriers (n = 186) of the *CYP2C19**2 variant. Mean time on clopidogrel therapy was 1.07 years. The primary end point was a composite of cardiovascular death, nonfatal MI, and urgent coronary revascularization occurring during exposure to clopidogrel with follow-up every 6 months. The primary end point occurred more frequently in carriers than in non-carriers (15 vs 11 events; HR: 3.69; 95% CI: 1.69–8.05; p = 0.0005), as did stent thrombosis (eight vs four events; HR: 6.02; 95% CI: 1.81–20.04; p = 0.0009). This detrimental effect of the *CYP2C19**2 variant on the rates of first cardiovascular event, observed in the initial 6 months following clopidogrel initiation, also persisted through the period 6–12 months after clopidogrel initiation (HR: 3.00; 95% CI: 1.27–7.10; p = 0.009). After multivariable analysis, the *CYP2C19**2 genetic variant was the only independent predictor of cardiovascular events (HR: 4.04; 95% CI: 1.81–9.02; p = 0.0006). This remarkable effect in younger patients raises the question of whether *CYP2C19* activity has a more important role in the response to clopidogrel in younger individuals compared with the majority of older patients with acute coronary syndrome [131].

Platelet responsiveness

Recently developed point-of-care, *ex vivo* platelet function tests allow a functional assessment of the effect of thienopyridines soon after dosing begins [132,133]. The *CYP2C19**2 allele has been linked to reduced platelet responsiveness to clopidogrel in a healthy cohort of patients of European ancestry and in a series of patients undergoing coronary intervention [122]. In the patients who were carriers of the *CYP2C19**2 allele, there was also a greater than twofold increased rate of subsequent ischemic events during 1 year of follow-up [122]. In patients undergoing percutaneous coronary intervention (PCI), other studies have also linked high residual platelet reactivity during clopidogrel therapy to poorer outcomes at 30-day [134], 6-month [135] and 12-month time points [136]. Platelets respond to many factors such as smoking [137] and other medications [138]. A significant proportion of carriers of the loss-of-function *CYP2C19* allele do not have high post-clopidogrel residual platelet reactivity, and high residual reactivity is present in patients with wild-type alleles [139]. Functional tests therefore furnish a dynamic result that supplements the genotyping result that is innate to the patient.

Collectively, the findings linking *CYP2C19* variants to unfavorable outcomes with clopidogrel are promising, but genotyping raises management considerations: can the loss of effect associated with a single deficient or null allele be overcome by increasing the dose of clopidogrel? Would patients who are homozygous for loss-of-function CYP variant alleles have improved platelet function and clinical outcomes (e.g., thrombosis and hemorrhage) under therapy with an alternative drug that is not dependent on CYP2C19 transformation? Would dose adjustment based on genotyping results enhance efficacy? Additional studies are needed to evaluate the applications of *CYP2C19* genotyping in clopidogrel therapy.

Prasugrel therapeutics

On July 9 2009, the thienopyridine prasugrel (Effient®), also a P2Y₁₂-receptor inhibitor, was approved by the FDA for the reduction of thrombotic cardiovascular events (including stent thrombosis) in patients with acute coronary syndrome (ACS). Compared with clopidogrel, prasugrel has demonstrated greater inhibition of platelet function after high-load dosing in healthy [140] and diseased patients [141], greater reduction in stent thrombosis [130] and greater prevention of ischemic events in STEMI patients undergoing PCI [142].

The pharmacogenetic aspects of prasugrel and clopidogrel differ. Both are prodrugs and require oxidation by similar CYP enzymes. Esterases shunt the majority of clopidogrel to a dead-end inactive pathway, with the remaining prodrug requiring two separate CYP-dependent oxidative steps, one of which is through cytochrome p450 2C19 [14]. By contrast, esterases are part of the activation pathway with prasugrel, and prasugrel is oxidized to its active metabolite in a single CYP-dependent step that may include 3A4/5, 2D6, 2C9 or 2C19, without an apparent dead-end inactive pathway [143]. There is no effect of the common functional CYP genetic variants on active drug metabolite levels or clinical cardiovascular event rates in persons treated with prasugrel. These pharmacogenetic findings are in contrast to observations with clopidogrel, which may explain, in part, the different pharmacological and clinical responses to the two medications [143].

Although prasugrel has been demonstrated to be more efficacious than clopidogrel, it causes more bleeding complications than clopidogrel. Bleeding complications associated with prasugrel are increased in the first 30 days after coronary artery bypass graft surgery [142]. The TRITON-TIMI 38 trial randomized 13,608 moderate- to high-risk ACS patients scheduled for PCI to receive prasugrel (60-mg loading dose and then 10-mg daily maintenance dose) or clopidogrel (300-mg loading dose and then 75-mg maintenance dose) for 6 to 15 months [144]. Bleeding complications were increased in moderate- to high-risk patients with ACS, including a threefold increase in fatal bleeding episodes [144]. However, bleeding complications were not increased in diabetic patients [145], perhaps due in part to a loss of platelet responsiveness to insulin [146], which increases their adhesiveness and aggregability.

Pharmacoeconomics of genotype-guided medicine

The example of warfarin

In August 2007, the FDA revised the warfarin label to include a pharmacogenomic subsection in which the metabolic impact of the *CYP2C9* and *VKORC1* gene polymorphisms is outlined [147]. The recommendations suggest beginning warfarin therapy with the standard starting dose (usually 5 mg/daily), obtaining *CYP2C9* and *VKOR1* genotype information and adjusting the dose according to the specifications discussed in the drug labeling. Then, it is suggested to combine the pharmacogenetics results and critical nongenetic covariates such as age and body size with a target dose estimated by published algorithms [59,41,42] or online [202]. Community pharmacists involved with a collaborative practice agreement with physicians may be in a position to eventually utilize this information in order to individualize warfarin therapy.

The warfarin pharmacogenetic test examines a person's inherited capacity to metabolize/ breakdown this medication along with the sensitivity of the site where warfarin acts. The combination of these factors, along with age, height and sometimes weight, determines the initial target dose. This may help minimize the chance of administering an excessive dose and the risk for side effects such as bleeding. The *CYP2C9* and *VKORC1* polymorphisms are additionally referenced in the Precautions and Dosage and Administration sections of the label, noting that the presence of polymorphisms in those two genes may lead to the need for more frequent INR monitoring and reduced warfarin doses [147].

Given the narrow therapeutic range of the INR among patients receiving warfarin therapy, dose estimate empiricism is risk prone. The consequences of over- or under-anticoagulation can be serious, ranging from life-threatening bleeding to cerebrovascular emboli, and require intensive treatment. Major and fatal bleeding events occur at rates of 7.2 and 1.3 per 100 patient-years, respectively, according to a meta-analysis of 33 studies [148]. The cost-effectiveness of warfarin genotyping hinges on improved INR control compared with standard care, and costs associated with bleeding complications must not exceed those associated with nongenotype-guided dosing. One analysis has estimated that if DNA typing were performed before warfarin is prescribed, 85,000 serious bleeding events and 17,000 strokes could be avoided annually in the USA alone, saving over \$1 billion in healthcare spending [3]. This analysis optimistically assumed a high rate of bleeding (27.6%) among patients with variant alleles, that DNA typing would result in 100% efficacy in preventing major bleeding during maintenance dosing, that 10% of stroke discharges are preventable with warfarin therapy and that more aggressive anticoagulation therapy would be pursued in stroke discharge patients than is actually the case [3].

Genotyping according to bleeding risk stratification

The heterogeneity of patients with respect to diagnoses, ethnogeographic origin and bleeding risk makes it reasonable to consider warfarin genotyping by bleeding risk stratification. The economic viability of warfarin testing can be assessed through an analysis of the cost of warfarin testing per quality-adjusted life-year (QALY). A QALY cost of US\$50,000 is considered the upper limit that society is 'willing to pay' for improved medical care [149]. Recently, two independent analyses concluded that the cost of warfarin testing per QALY currently exceeds \$50,000 [81,150]. One analysis assumed a patient of European ancestry with nonvalvular atrial fibrillation and low bleeding risk, used the current cost of \$400 for *CYP2C9* and *VKORC1* genotyping, 3 days as the turnaround time for test results and presumed 32% efficacy in the prevention of subdural hemorrhage to generate a QALY cost of \$170,000 [81]. The scenario can change according to diagnoses and characteristics of the patient, but here the achievement of a QALY cost of less than \$50,000 would require:

- Halving the genotyping cost from \$400 to \$200;
- That test results be returned within 24 h instead of 3 days;
- That testing be limited to nonvalvular atrial fibrillation patients with a risk for hemorrhage of 4% per 100 patient-years (the risk corresponding to a HEMORR₂HAGES score of 1 to 2 [151]).

This magnitude of risk is well within the annual estimate of 7.2% per 100 patient-years for major bleeding for all patients [148]; in a similar analysis, You *et al.* assumed a genotyping cost of \$177, but conservatively assumed that genotyping would lead to a relatively small 7.3% decrease in out-of-range INRs in all patients [150]. That assumption led to an estimated cost per QALY of \$347,000 [79]. In their model, three factors effectively lower the QALY cost [150]:

- Assumption of a greater reduction in out-of-range INRs due to genotyping;

- Restriction of testing to patients with poor baseline INR control (high percentage of out-of-range INRs);
- Lowering the genotyping cost.

At a genotyping cost of \$47, the cost per QALY is \$50,000.

The US National Heart, Lung and Blood Institute is currently sponsoring the Clarification of Optimal Anticoagulation Through Genetics (COAG) trial, a prospective genotype-guided warfarin dosing protocol [203]. In this trial, initial dosing of warfarin and readjustment after three to four doses will be determined by algorithms that use clinical and genetic information. The primary outcome is the percentage of time in the first 4 weeks that participants spend within the therapeutic INR range. The European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial will assess, in a single-blinded and randomized controlled trial with a follow-up period of 3 months, the safety and clinical utility of genotype-guided dosing in daily practice for the three main coumarin derivatives used in Europe. The primary outcome measure is the percentage time in the therapeutic range for INR. Secondary outcomes include major hemorrhage, the cost-effectiveness of pretreatment genotyping and the clinical utility of the rapid genotyping instrument [152]. The results of these efforts will inform of the healthcare benefits of genotype-guided warfarin therapy.

Public policy & ethical considerations on genotype-guided medicine

Regulatory oversight

Hudson and Javitt explain that the success of genotyping depends on the development of a coherent framework for oversight which provides both adequate assurance of the safety and effectiveness of genetic tests and an equitable and stable regulatory playing field [153]. The Secretary's Advisory Committee on Genetics, Health and Society (SACGHS), which advises the Secretary of Health and Human Services (HHS), has recently reviewed pharmacogenetic testing [154]. The Committee has considered three aspects of pharmacogenetic test evaluation including Analytic Validity, Clinical Validity and Clinical Utility. Analytic Validity addresses accurate and reliable measurement of the genotype; Clinical Validity the ability to detect or predict the associated disorder; and Clinical Utility the risks and benefit of test use. In general, Analytic Validity is quite high for chip- and bead-based genomic methodologies with sensitivity and specificity in excess of 99%. On July 26 2007, the FDA issued a draft guidance for '*In vitro* Diagnostic Multivariate Index Assays (IVDMIA)' indicating an intent to require IVDMIA to meet pre- and post-market device requirements under FDA regulations [155].

Progress in the development of genotype-guided warfarin dosing has been further accelerated by FDA approval of two commercial kits from two different companies. On 17 September 2007, Nanosphere obtained approval for a nanotechnology-based assay, bringing a revolutionary technology to clinical laboratory practice [156]. On 4 May 2008, ParagonDx obtained FDA approval for a rapid assay to measure allelic variability, potentially allowing dose adjustment even during the induction phase of warfarin treatment [202].

Ethical considerations

Personalized medicine relies on the physician–doctor relationship for implementation. The intimacy and trust inherent to this relationship is the ideal setting in which to divulge and discuss genetic data. No subject matter will rely more heavily on this relationship than genetic privacy and genotype-guided treatments.

However, personalized medicine will require safeguarding the privacy of genetic information beyond the doctor–patient relationship. For treatment purposes, the ethical 'risks' of various

types of genetic information should be practically differentiated. Pure diagnosis of intractable genetic disease poses the greatest ethical risk, and profiling of drug metabolism the least. Consent for drug metabolism genotyping requires that information on its intended use needs to be provided to the patient by the physician.

The collection of genetic data will challenge the current definitions of ancestry based on 'race'. It will instill an appreciation and respect for one's genetic legacy with the ultimate intent of empowering, not stigmatizing, the individual. In countries with widely heterogeneous populations, such as the USA and Britain, and with admixed populations, such as Latin America, diversity of ethnogeographic origin poses particular limitation to the existing definitions of 'race', which will trigger reassessment of population groups defined with genetic markers.

Much progress in the field has been maligned by fears of intrusion into one's life by the readout of the genetic material. One approach to deal with this legitimate concern is to limit the genetic readout to the markers required for a given treatment choice or diagnosis to be collected, and to discard the sample afterwards. In the context of preventive care, one may desire as comprehensive a readout as possible, where all observations are germane, and none incidental. The costs of reading again the genetic transcript become less onerous than the storage and safeguarding of the entire genetic complement.

Prospects

Cardiovascular medicine has the advantage of having a drug at once familiar and challenging, warfarin, modernized by genotype-guided dosing. In parallel, major advances in antiplatelet pharmacogenetics herald the era of genotype-guided antiplatelet selection and prescription within 5 years.

The rationale for genotype-guided management of antithrombotic therapy is that the cost of providing the genotyping would be offset by means of at least two major applications in healthcare. As a side effect diagnostic, genotype-guided therapy can reduce laboratory testing, physician visits, emergency room visits, hospitalizations and noncompliance. As a side effect, prognostic, genotype-guided therapy will enable utilization of the safest drug in the first place, therefore reducing side effects altogether. For example, with thienopyridine therapy, genotype-guided management can be employed prognostically before prescribing clopidogrel or diagnostically to manage clopidogrel resistance.

Future perspective

The FDA revised the Coumadin (warfarin) label in 2007 and the Plavix (clopidogrel) label in 2009 to report the differentiation of drug response and treatment outcome by gene variants. Warfarin has been prescribed for some 50 years without genotype guidance. While polymorphisms in the *VKORC1* and *CYP2C9* genes clearly predict 35–45% of the individual variation in the stable therapeutic warfarin dose, the broad embrace of genotype testing is hampered by the long experience of prescribing without genotype guidance, the absence of hard data to define the clinical benefits of genotyping, the cost of testing and the turnaround time for results (typically 3 days), and perhaps the absence of a universal pharmacogenetic dosing algorithm. Changes in these factors may drive an increase in genotype testing and pharmacogenetic warfarin dosing. Progress hinges on two large multicenter studies (COAG and EU-PACT), whose results will be known in 2012. They will help define the extent to which pharmacogenetic dosing affects the safety and efficacy of coumarin derivatives used in antithrombotic therapy. In the interim, the refinement of pharmacogenetic algorithms will continue as other gene variants gleaned from the study of various populations are incorporated.

With respect to drugs that inhibit platelet aggregation, clopidogrel (Plavix) is the most widely prescribed, having appeared in the USA in 1997. Clopidogrel is a P2Y₁₂ receptor antagonist whose activation depends on cytochrome p450 2C19 activity. Clopidogrel's effectiveness is decreased in the presence of *CYP2C19**2 and *3 alleles, which mark deficient metabolism. These alleles have a combined frequency of 20–30% in persons of European or African ethnogeographic origin, and 50% in Asian populations. Clopidogrel's competitor drug prasugrel (Effient) was approved in the USA in 2009. It is unaffected by variation in the *CYP2C19* gene or, to date, other genes. Clinical practice guidelines for the treatment of ACS and PCI strongly influence the extent to which clopidogrel and prasugrel are used. In the next few years, guidelines may evolve to include the recommendation for genotype testing in clopidogrel treatment. Concurrently, P2Y₁₂-receptor agonists with different pharmacokinetic and pharmacodynamic properties, such as ticagrelor and cangrelor, will emerge as competitors to both drugs. Current evidence does not indicate a strong influence of variants of the *P2Y12* or other platelet genes on the platelet response to any of these drugs. Plavix will become generic in 2011. Generic status may even further increase clopidogrel prescription driven by substantially reduced prices. As a result, the demand for genotype testing for *CYP2C19* will increase in the next few years. On 12 March 2010, the FDA added a boxed warning to the Plavix label to inform that *CYP2C19* poor metabolizers do not effectively convert clopidogrel to its active form, and that tests are available to identify genetic differences in *CYP2C19* function.

Executive summary

- Warfarin and clopidogrel are widely prescribed antithrombotic therapies. The current US FDA label for each drug reflects genotypic differentiation of response.
- In general, genotype guidance has the potential to reduce medication errors. In the case of warfarin, under- or over-dosing can be reduced. In the case of clopidogrel, the administration of a drug that requires activation by an enzyme when gene testing results would indicate that innate capacity for such activation is decreased or absent, can be avoided.
- Common variants in *VKORC1* and *CYP2C9* explain 30–45% of the interpatient variability in stable therapeutic warfarin dose in Caucasian, African, Asian and mixed populations such as Latin Americans. *CYP4F2* and *EPHX1* gene variants may be useful predictors of response to antithrombotic medication. Large trials in the US (COAG) and Europe (EU-PACT) are underway to specifically define the benefits of pharmacogenetic dosing of coumarin derivatives with respect to safety and efficacy within the first 4 weeks (COAG) and 3 months of therapy (EU-PACT). Results are expected by 2012.
- Plavix® (clopidogrel) and Effient® (prasugrel) are prodrugs whose active metabolites inhibit platelet aggregation through inhibition of the platelet P2Y₁₂ receptor. The activation of clopidogrel is dependent on the *CYP2C19* enzyme. Prasugrel activation is unaffected by these variants. Studies show that the effectiveness and clinical outcomes associated with clopidogrel treatment are reduced in carriers of the *2 or *3 minor alleles of *CYP2C19*. Testing for these variants can guide the selection of antiplatelet medication.
- In summary, genetic prediction of antithrombotic medication response has the potential to reduce trial and error on the part of clinicians, risks of therapy and the resources needed to treat.

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Figure 1. The vitamin K cycle

The *VKORC1* gene product reduces vitamin K and the cytochrome p450 2C9 protein metabolizes warfarin. The (red) X indicates inhibition by warfarin.

Figure 2. Predicted mean decreases in warfarin dose (mg/day) in 127 outpatients from a preventive cardiology clinic with reference sequences and single, double, triple or quadruple polymorphisms for *CYP2C9* and *VKORC1* genes

The decreases were calculated using published formulae [41,42] that employ age, gender, weight, height, *CYP2C9* genotype and *VKORC1* genotype. The purple areas indicate ranges of predicted dose reductions. The upper and lower borders represent the reductions according to the formulae of Zhu *et al.* [41] and Sconce *et al.* [42], respectively.

Figure taken from [12].

Figure 3. Clopidogrel activation to R130964 by CYP2C19: essential step for therapeutic effect

Table 1

CYP2C9 alleles resulting in deficient metabolism of warfarin.

Allele	cDNA change	Amino acid change	Enzymatic activity	Frequency (%)				
				European	Asian	African	Native American	Mixed other
*1	Reference	Reference	Normal	70–86	88–99	82–99	91–100	85–93
*2	430C>T	Arg144Cys	Deficient	8–19	0–4	0–12	0–3	0–8
*3	1075A>C	Ile359Leu	Deficient	5–16	1–8	0–6	0–6	3–7
*4	1076T>C	Ile359Tyr	Deficient	0.9	Rare	Rare	Rare	Rare
*5	1080C>G	Asp360Glu	Deficient	Rare	Rare	0.8–1.8	Rare	Rare
*6	818delA	Frameshift	Null	Rare	Rare	0.6–1.0	Rare	1.1
*8	449G>A	Arg150His	Deficient	Rare	Rare	4.7	Rare	Rare
*11	1003C>T	Arg335Trp	Deficient	Rare	Rare	1.3	Rare	Rare

European [13–22]; Asian: [19,20,23–28] African: [20,29–34]; Native American [35]; Mixed or other: [19,36–39].
Data taken from [12].

Table 2
VKORC1 haplotypes A and B require low and high effective doses of warfarin, respectively.

	<i>VKORC1</i> polymorphic sites	<i>VKORC1</i> haplotype							
		Other equivalent designations				B (high dose)			
		A (low dose)							
		H1	H2	H7	H8	H9			
381 [58]		C	C	T	T	T			
861		C	C	C	A	A			
3673	–[639 G>A [41,42]	A	A	G	G	G			
5808		T	G	T	T	T			
6484 [‡]	1173 C>T [‡]	T	T	C	C	C			
6853 [‡] [59]	1542 G>C [40]	C	C	G	G	G			
7566		T	T	C	C	C			
9041	3730 G>A [§] [60]	G	G	A	A	G			

Liver mRNA is increased threefold in haplotype B. Presumably, these regions act as regulatory sequences that may bind transcription factor binding sites, but additional studies will be required [56].

[‡]These single nucleotide polymorphism flank exon 2, but fall outside the canonical regions required for exon splicing.

[‡]Located within intron 1.

[§]A single nucleotide polymorphism in the 3'UTR of *VKORC1*.

Table 3

CYP2C19 alleles.

Allele	cDNA change	Amino acid change	Enzymatic activity	Frequency (%)					
				European	Asian	African	Native American	Oceania	Mixed other
*1	Wild-type	Reference	Normal	Common	Common	Common	Common	Not common	Common
*2	681G>A	Splicing defect	Null	9–15	21–39	10–25	19	57	8–25
*3	636G>A	Stop codon	Null	0–2	0–13	0–2	0	25	0.1
*4	1A>G	Start codon	Null	Rare	0.4	Rare	Rare	Rare	Rare
*5	1297C>T	Arg433 Trp	Null	Rare	Rare	Rare	Rare	Rare	Rare
*6	395G>A	Arg132 Glu	Null	Rare	Rare	Rare	Rare	Rare	Rare
*7	IVS5+2T>A	Splicing defect	Null	Rare	Rare	Rare	Rare	Rare	Rare
*8	358T>C	Trp120 Arg	Null	Rare	Rare	Rare	Rare	Rare	Rare
*17	–806C>T	Promoter variant	Increased	18–27	1.4–4.0	19–24	?	?	?

*1 connotes functionality only with regard to the absence of deficient or null alleles. Allele frequencies are taken from studies that directly assayed for an allele.

The *17 allele was recently discovered [95] and its frequency is still unknown for some populations. For allele *1, definitive frequency ranges are omitted due to the use of a method by some studies to infer *1 status by exclusion of the others, without having assayed for *17.

References used to determine ranges corresponding to allele frequencies: European: [13,15,16,18,95–105]; Asian: [24,27,100,102,106–111], African: [95,97,100,112–115]; Native American: [116], Oceania: [117], Mixed other: [36,118–120].