Clinical utility of pharmacogenetic biomarkers in cardiovascular therapeutics: a challenge for clinical implementation

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Abstract

In the past decade, significant strides have been made in the area of cardiovascular pharmacogenomic research, with the discovery of associations between certain genotypes and drug-response phenotypes. While the motivations for personalized and predictive medicine are promising for patient care and support a model of health system efficiency, the implementation of pharmacogenomics for cardiovascular therapeutics on a population scale faces substantial challenges. The greatest obstacle to clinical implementation of cardiovascular pharmacogenetics may be the lack of both reproducibility and agreement about the validity and utility of the findings. In this review, we present the scientific evidence in the literature for diagnostic variants for the US FDA-labeled cardiovascular therapies, namely \textit{CYP2C19} and clopidogrel, \textit{CYP2C9/VKORC1} and warfarin, and \textit{CYP2D6/ADRB1} and \(\beta\)-blockers. We also discuss the effect of \textit{HMGCR/LDLR} in decreasing the effectiveness of low-density lipoprotein cholesterol with statin therapy, the \textit{SLCO1B1} genotype and simvastatin myotoxicity, and \textit{ADRB1/ADD1} for antihypertensive response.

Keywords

biomarker; cardiovascular; clinical utility; clopidogrel; drug label; genetics; personalized medicine; pharmacogenetics; predictive medicine; warfarin

In the past decade, significant strides have been made in the area of cardiovascular pharmacogenomic research, with the discovery of association between certain genotypes and drug-response phenotypes. While the motivations for personalized and predictive medicine are promising for patient care and support a model of health system efficiency, the implementation of pharmacogenomics for cardiovascular therapeutics on a population scale faces substantial challenges. The greatest obstacle to clinical implementation of cardiovascular pharmacogenetics may be the lack of both reproducibility and agreement about the validity and utility of the findings. In this review, we present the scientific evidence in the literature for diagnostic variants for the US FDA-labeled cardiovascular therapies, namely \textit{CYP2C19} and clopidogrel, \textit{CYP2C9/VKORC1} and warfarin, and \textit{CYP2D6/ADRB1} and \(\beta\)-blockers. We also discuss the effect of \textit{HMGCR/LDLR} in decreasing the effectiveness of low-density lipoprotein cholesterol with statin therapy, the \textit{SLCO1B1} genotype and simvastatin myotoxicity, and \textit{ADRB1/ADD1} for antihypertensive response.
drug-response phenotypes. These findings led the US FDA to add pharmacogenomic biomarkers to drug labels of many commonly prescribed drugs. In the cardiovascular and hematology therapeutic areas, these drugs include carvedilol, clopidogrel, isosorbide/hydralazine, metoprolol, prasugrel, pravastatin, propafenone, propranolol, ticagrelor and warfarin [101]. With the aim of aiding the healthcare provider in arriving at a well-informed clinical decision, these labels include boxed warnings, dosage and administration information, precautionary statements, drug interactions, clinical pharmacology information or any combination thereof. While the motivations for personalized and predictive medicine – reduction of serious adverse effects, moderation of variability in treatment efficacy, and augmentation of therapy effectiveness based on individual genetic variation – are promising for patient care and support a model of health system efficiency, the implementation of pharmacogenomics for cardiovascular therapeutics on a population scale faces substantial challenges.

One obvious hurdle is the required threshold of evidence for a particular variant prior to clinical translation. Not only does the evidence for clinical utility have to be robust, an increased value above and beyond current testing practices needs to be clearly demonstrated. The pharmacokinetic or pharmacodynamic effect of a given genotype needs to result in a predictable difference in drug efficacy, response or adverse effects. Given that a particular variant reaches a significant level of evidence in a large clinical trial, the next obstacle would be for the provider to act upon this knowledge with objective practice guidelines and easy access to the genotype of the individual patient. Both the provider and patient would need to have a positive perception and acceptance of genetic testing. The genotyping would need to be of high standards and be performed in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory. For implementation at point-of-care, the genomic information would already have to be part of the electronic medical record system, which comes with its own ethical, privacy and security issues [1]. Finally, the compensation for such testing would also need to be addressed.

The greatest obstacle to clinical implementation of cardiovascular pharmacogenetics may be the lack of both reproducibility and agreement about the validity and utility of the findings. In this review, we present the scientific evidence in the literature for diagnostic variants for the FDA-labeled cardiovascular therapies, namely CYP2C19 and clopidogrel, CYP2C9/VKORC1 and warfarin, and CYP2D6/ADRB1 and β-blockers (Table 1). We also discuss the effect of HMGCR/LDLR in decreasing the effectiveness of low-density lipoprotein (LDL)-cholesterol with statin therapy, the SLCO1B1 genotype and simvastatin myotoxicity, and ADRB1/ADD1 for antihypertensive response. Even with the challenges facing the clinical implementation of pharmacogenetics in cardiovascular therapeutics, personalized and predictive medicine in this arena holds great promise, and in a few centers, such as Vanderbilt University Medical Center (TN, USA) and others in the NIH Pharmacogenomics Research Network (PGRN), it has already begun. The prospective findings from these institutions that are pursuing clinical implementation will provide further insight into the challenges and future direction for pharmacogenetics. There are also ongoing prospective randomized clinical trials that will likely result in future revisions to guidelines.

**Cardiovascular therapeutics & genomic variants**

**CYP2C19 & clopidogrel**

Clopidogrel is indicated for patients at high risk for acute coronary syndrome without ST-segment elevation, ST elevation myocardial infarction and prevention of vascular ischemic events in patients with symptomatic atherosclerosis. Aspirin/clopidogrel combination therapy is the standard of care for prevention of thrombosis after placement of intracoronary stent, and clopidogrel is also an alternative antiplatelet drug to aspirin. In fact, in 2007,
Clopidogrel was the second most widely prescribed drug in the USA and worldwide sales reached US$6.6 billion in 2009.

Clopidogrel is a thienopyridine prodrug, requiring \textit{CYP2C19} [2] and other enzymes in the CYP450 superfamily to transform it to an active thiol derivative [3,4]. It is well recognized that the interindividual variation to response of clopidogrel is heritable [5–11]. Several studies have reproducibly shown that \textit{CYP2C19*2} allele (c.681G>A; rs4244285) carriers are associated with a reduced ability to metabolize clopidogrel to form the active metabolite (loss of function), a lower antiplatelet effect, and a higher risk for cardiovascular events [12–27]. Meta-analyses have shown that \textit{CYP2C19*2} carriers treated with clopidogrel were associated with higher rates of major adverse cardiovascular events and higher risk of stent thrombosis compared with noncarriers [13,26]. There has also been an association with the gain-of-function \textit{CYP2C19*17} variant (c.-806C>T; rs12248560) with increased drug sensitivity and adverse bleeding side effects [11,28,29]. In terms of population genetics, there appears to be a strong correlation with ancestry to the heritability of the alleles [11,29]. The loss-of-function \textit{CYP2C19*2} allele frequencies range from around 15% in European and African ancestries versus 29% in east Asians [11]. Conversely, the gain-of-function \textit{CYP2C19*17} alleles range from 16 to 21% in subjects of European and African ancestries compared with 3% in east Asian subjects [29]. There is also some evidence of loss-of-function *3 variant, contributing to poorer response in east Asians [11]. These results suggest that \textit{CYP2C19} testing may be even more imperative in that region of the world; however, there needs to be more education and awareness of pharmacogenomics before implementation can be carried out on a widescale in these populations.

Despite the robust association between the \textit{CYP2C19*2} genotype with lower clopidogrel response, large-scale studies with coronary artery disease subjects with lower risks for thromboembolic events failed to show the same association [30]. Therefore, clinical utility may be limited to only patients with high risk for recurrent events [31]. Nevertheless, the FDA approved a boxed warning of clopidogrel in March 2010 (Table 2), advising that alternative antiplatelet therapy be used in patients that are homozygous for the \textit{CYP2C19*2} allele [102]. Despite this warning, the FDA has not mandated genetic testing for \textit{CYP2C19} status before initiation of clopidogrel, leading to some confusion among healthcare providers regarding clinical implementation and personalized therapy. Furthermore, the American College of Cardiology Foundation and the American Heart Association have recently suggested that in the absence of prospective randomized trials, the evidence is insufficient to recommend genetic testing [32,33]. Recently, the ELEVATE-TIMI 56 (NCT01235351 [103]), a multicenter, randomized, double-blinded trial, showed that among patients with stable cardiovascular disease, tripling the maintenance dose of clopidogrel to 225 mg daily in \textit{CYP2C19*2} heterozygotes achieved levels of platelet reactivity similar to the standard 75 mg dose in noncarriers while in \textit{CYP2C19*2} homozygotes, even a 300 mg daily dose was unlikely to result in optimal degree of platelet inhibition [34]. It will be years before the data from other ongoing prospective randomized clinical trials of genotype-directed antiplatelet therapy will become available. In the meantime, the NIH-funded Clinical Pharmacogenetics Implementation Consortium (CPIC) of PGRN has published guidelines to recommend use of genetic information to guide clopidogrel therapy, also noting that the evidence is compelling for a relationship between \textit{CYP2C19} genotype and clopidogrel response in acute coronary syndrome patients who have undergone percutaneous coronary intervention [35].

\textbf{CYP2C9, VKORC1 & warfarin}

Warfarin is the most widely prescribed oral anticoagulant drug in North America and probably the world. It is indicated for the prophylaxis or treatment of venous thrombosis and pulmonary embolism. It is also indicated for the prophylaxis or treatment of...
thromboembolic complications associated with atrial fibrillation, cardiac valve replacement and myocardial infarction. In patients with atrial fibrillation, anticoagulation with warfarin is over twice as effective in secondary prevention of stroke as any other alternatives, including other antithrombotic drugs [36] or surgical intervention [37]. Despite its effectiveness, warfarin interacts with many commonly used medications (simvastatin, metronidazole, macrolides and other broad-spectrum antibiotics), alcohol and some foods (particularly fresh plant-based foods containing vitamin K) resulting in either reduced effectiveness or increased toxicity.

Patients on this drug are monitored by a trial and error approach for safety and narrow therapeutic index based on the International Normalized Ratio. The wide interpatient variability in dosing requirement suggests a highly genetic contribution to the pharmacokinetics and pharmacodynamics of the drug [38]. The association of higher risk of bleeding as well as the under- or over-anticoagulation with certain genotypes prompted the FDA to place a warning label on the drug (Table 2) in August 2007 to include information on CYP2C9 and VKORC1 genotypes as predictors of dose response, and in January 2010, the FDA further revised the warning to include specific dosage recommendations for these genotypes [104,105]. However, as with clopidogrel, the agency stopped short of mandating pharmacogenetic testing.

Warfarin is a synthetic derivative of dicoumarol, which is a derivative from coumarin. The related 4-hydroxycoumarin-containing molecules decrease blood coagulation by inhibiting VKORC1, an enzyme that recycles the epoxide and quinine form of oxidized vitamin K to its reduced nonoxidized form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII. The onset of effect requires approximately a day before clotting factors being normally made by the liver have time to naturally disappear in metabolism, and the duration of action of a single dose of racemic warfarin is 2–5 days. Multiple linked variants in VKORC1 (−1639G>A; rs99232318 and 1173C>T; rs9934438) have been associated with variability in gene expression, increased sensitivity to warfarin, and reduction in dose requirement [39–43]. However, only the VKORC1 −1639G>A is a functional variant [44]. Additional identification of variants in CYP2C9*2 (p.Arg144Cys; rs1799853) and *3 (p.Ile359Leu; rs1057910) alleles are associated with 40–70% reduction in (S)-warfarin clearance and approximately 20–40% lower warfarin dose requirement, respectively [45–48]. As in the case of clopidogrel, there are population and racial differences in minor allele frequencies of important variants as well as sensitivity to warfarin dosage requirement [40,49,50]. The CYP2C9*2 and *3 alleles and VKORC1 −1639G>A genotype explain approximately 50–60% of the variability in dosage in Caucasians but only approximately 20–25% of variability in African–Americans [40,48,51–54]. The VKORC1 genotype has also been shown to be the strongest predictor of warfarin dosage in Japanese subjects [43,55]. Whether VKORC1 and CYP2C9 variants are the primary contributors to the drug response in African–Americans remains to be seen in studies currently in progress.

Despite the plethora of information from multiple genome-wide association studies, the strong support from the International Warfarin Pharmacogenetics Consortium (IWPC) and CPIC, in addition to the FDA warning labels and the availability of several FDA-approved platforms for warfarin genotyping, the clinical implementation of genetic testing for warfarin dosing has not only been disappointing in practice, but also the current consensus guidelines by the American College of Chest Physicians actually warn against the routine use of genetic data to guide dosing [38]. Similarly, the American College of Medical Genetics endorses testing only in cases of unusual warfarin response [56]. These conclusions have generally cited the suboptimal predictive value of the pharmacogenetic test over large populations [57]. There is a lack of evidence from large-scale prospective randomized trials,
but such studies are currently under way – COAG (NCT00839657 [106]), GIFT (NCT01006733 [107]) and EU-PACT (NCT01119300 [108]) trials. Randomized clinical trials thus far have found the benefit of a genotype-guided dosing algorithm as a predictor of final stable dose compared with the clinical-only algorithm; however, the studies have not demonstrated a clear advantage of pharmacogenetic dosing on anticoagulation control [40,51,58,59]. By contrast, two prospective studies show a relationship between reduced hospitalization and better prediction of maintenance dose with the healthcare provider having knowledge of warfarin genetic information [45,60].

The clinical evidence shows that there is clinical and analytical validity of warfarin pharmacogenetics for Caucasians and possibly for other racial groups. However, whether the clinical utility of genetic testing for warfarin has been unequivocally established is still debatable. Even if the evidence is favorable for testing from prospective randomized trials and the guidelines are modified to reflect that, the challenge still remains in obtaining quality (CLIA-approved) clinical laboratory genotyping with quick turnaround time, compared with the turnaround time for international normalized ratio, if not eventually necessitating pre-emptive genotyping information in the medical electronic system for point-of-care access.

**CYP2D6, ADRB1 & β-blockers**

Clinicians have long known about the relationship of ancestry and drug response, especially in the case of heart failure, exemplified by the improvement in survival in African–Americans treated with hydralazine and nitrate, in addition to standard therapy. Three commonly prescribed β-blockers for heart failure (metoprolol, carvedilol and propranolol) have FDA labeling regarding the CYP2D6 polymorphisms [109–111]. However, the evidence for pharmaco-kinetic effect of this genotype does not appear to translate into differences in efficacy, response or adverse effects, where dose titration is based on clinical surrogates such as blood pressure and heart rate.

In heart failure therapeutic pharmaco-genomics, the most robust evidence concerns the association of ADRB1 variants (two common nonsynonymous polymorphisms Ser49Gly; rs1801252 and Arg389Gly; rs1801253) and left ventricular ejection fraction or clinical outcomes mediated by β-blockade [61–63]. The BEST (NCT00000560) suggested that Arg389Arg patients had significant benefits from bucindolol, whereas Gly389 carriers had no significant benefit [61]. This has led to probably the first case of pharmacogenomically guided drug development for the treatment of cardiovascular disease. The pharmaceutical company plans to launch a superiority trial in 3200 ADRB1 Arg389Arg patients randomized to metoprolol or bucindolol. Other studies, however, have not documented an association between Arg389Gly genotype and improved outcome with β-blockade [64,65]. There are also studies suggesting association of β-blockers for heart failure with ADRB2, ADRA2C and GRK5 [66–68]. Thus, the literature suggests that while some subjects with heart failure may derive little benefit from β-blockade, in other patients, genetic variability in the adrenergic signaling pathway may have an important influence on the benefits of the drug. Nevertheless, it is difficult to clinically implement testing based on the consensus guideline use of β-blockers in patients with heart failure with the suggestion to withhold therapy without providing an alternative therapeutic option.

**ADRB1, ADD1 & antihypertensives**

As with heart failure and β-blockade, there are consistent data in several clinical studies showing association in differential blood pressure lowering levels with polymorphisms of the ADRB1 gene, namely Ser49Gly and Arg389Gly [63,69–71]. In atenolol-treated patients with hypertension, the Ser49/Arg389 haplotype was associated with improved outcome,
especially in lower death rate, in comparison with verapamil [71]. It may be that these variants demonstrate enhanced agonist-induced adenylyl cyclase activation by Gly49 compared with Ser49 and by Arg389 compared with Gly389 as shown in vitro. There has been equivocal data regarding the association of Gly460Trp variant in the a-adducin gene ADD1 with response to thiazide [71–76]. This polymorphism in ADD1 has also been associated with increased risk of myocardial infarction or stroke during thiazide diuretic treatment [77]. But analyses in both the ALLHAT (NCT00000542 [112]) and the INVEST (NCT00133692 [113]) studies were unable to replicate this finding [78,79]. Nevertheless, this association led to the development of a novel antihypertensive drug class targeting adducin, namely ouabain, which has shown impressive Phase II data regarding blood pressure lowering efficacy [80,81].

**HMGCR, LDLR, SLCO1B1 & statins**

Another class of widely prescribed drugs for the prevention of cardiovascular events in coronary artery disease is the HMGCR inhibitors. The mechanism by which these inhibitors lower plasma LDL-cholesterol is via attenuation of endogenous production of cholesterol by increasing expression of the LDLR [82]. The H7 haplotype involves three intronic polymorphisms – rs17244841, rs3846662, rs17238540 – in the HMGCR and has been associated with 11–19% decreased reduction in LDL-cholesterol in both ethnically diverse and multiple independent populations [72,83–88]. Other haplotypes – HMGCR H2 and LDLR L5 [86–88], have also been associated with decreased attenuation of LDL-cholesterol. Additional studies have shown association with variability in statin efficacy with variants in HMGCR and LDLR [89]. The FDA recently recommended that the higher dose of 80 mg/day of simvastatin be restricted to patients taking the medication for 12 months or longer [114]. Most patients who are at risk for developing myopathy or fatal rhabdomyolysis would most likely show clinical symptoms of adverse side effects within a few weeks of treatment. The FDA recommendation was based on the finding from the SEARCH (NCT00124072 [115]) consortium, where patients homozygous or heterozygous for a variant (rs4363657) in the SLCO1B1 gene were found to have a 16.9- and 4.5-fold increase, respectively, in developing myopathy or rhabdomyolysis at the 80 mg/day higher dose [91]. SLCO1B1 encodes the uptake transporter organic anion-transporting polypeptide 1B1 [92]. The identified polymorphism tags a known nonsynonymous variant SLCO1B1*5 (p.Val174Ala; rs4149056), which has been replicated in a separate cohort in the original study [91] and subsequently in two other studies [93,94]. Another study also showed that Val174Ala predicted the discontinuation of another popular statin drug, atorvastatin, due to adverse side effects [93]. Cerivastatin-associated rhabdomyolysis has also been associated with this variant in SLCO1B1 [95]. These studies demonstrate how dosage and duration of therapy interact with genetics to produce a drug response phenotype. However, it also appears that a biomarker that can modulate risk may not necessarily be of high clinical utility because of low predictive value (<20% for developing myopathy in patients homozygous for the Val174Ala allele). The SLCO1B1 genotype may predict a large effect on risk of simvastatin-associated myotoxicity in some settings and not in others. Current studies are exploring whether genomic markers have predictive value in identifying subjects with developing coronary events while on statin therapy.

**Future perspective**

With the ‘$1000’ whole-genome sequencing within reach in the next few years, it will not be the cost of genotyping or sequencing that will deter the progress of personalized and predictive medicine. The greatest obstacle to clinical implementation of cardiovascular pharmaco-genetics may be the lack of both reproducibility and agreement about the validity and utility of the findings. Once the scientific and medical communities are in agreement as to the clinical validity and utility of these pharmacogenetic tests, objective practice
guidelines may be developed. Instantaneous access to the genotype information for point-of-care treatment of the individual patient may also be a great obstacle as the genomic information needs to be in the electronic medical record system pre-emptively, which raises its own ethical, legal and social issues. In the end, clinical implementation of pharmacogenetics in the cardiovascular field or any other therapeutic arena on a population-wide scale across cultures, nations and ethnicities may very well be limited by the social acceptance and public perception of genetic testing.

References

Papers of special note have been highlighted as:

▪ of interest
▪ of considerable interest


34· Mega JL, Hochholzer W, Frelinger AL 3rd, et al. Dosing clopidogrel based on CYP2C19 genotype and the effect on platelet reactivity in patients with stable cardiovascular disease. JAMA. 2011; 306(20):2221–2228. A recent clinical trial showing that tripling the maintenance dose of clopidogrel to 225 mg daily in CYP2C19*2 heterozygotes achieved levels of platelet reactivity similar to the standard 75 mg dose in noncarriers while in CYP2C19*2 homozygotes, even a 300 mg daily dose was unlikely to result in optimal degree of platelet inhibition. [PubMed: 22088980]


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77. Psaty BM, Smith NL, Heckbert SR, et al. Diuretic therapy, the alpha-adducin gene variant, and the risk of myocardial infarction or stroke in persons with treated hypertension. JAMA. 2002; 287(13):1680–1689. Showed that in carriers of the adducin variant, diuretic therapy was associated with a lower risk of combined myocardial infarction or stroke than other antihypertensive therapies. Suggests that this large subgroup of the hypertensive population may be especially likely to benefit from low-dose diuretic therapy. [PubMed: 11926892]


Websites

101. Table of pharmacogenomic biomarkers in drug labels. www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm

102. Label and approval history, clopidogrel bisulfate. www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm236280.htm


104. Label and approval history, warfarin sodium, CYP2C9. www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm238046.htm

105. Label and approval history, warfarin sodium, VKORC1. www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm238050.htm


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110. Label and approval history, carvedilol. www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm236255.htm
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   www.fda.gov/Drugs/DrugSafety/ucm256581.htm
Executive summary

Background

- Significant advances in cardiovascular pharmacogenomic research with discovery of association between genotype and drug response phenotype.
- Challenges include the required threshold of evidence for a particular variant prior to clinical translation, increased value above and beyond current testing practices, genotype predictability in drug efficacy, response, adverse effects, objective practice guidelines, point-of-care access to patient genomic information, compensation and reimbursement, and ethical, legal, privacy and security issues.

Cardiovascular & genomic variants

- Clopidogrel and CYP2C19:
  - Association of loss-of-function *2 allele carriers with a reduced ability to metabolize clopidogrel to form the active metabolite, a lower antiplatelet effect, and a higher risk for cardiovascular events.
  - Association of the gain-of-function *17 variant with increased drug sensitivity and adverse bleeding side effects.
  - Strong correlation of ancestry to the heritability of the alleles.
  - Some evidence of loss-of-function *3 variant contributing to poorer response in east Asians.
  - Despite the robust association between the CYP2C19*2 genotype and lower clopidogrel response, large-scale studies with coronary artery disease subjects with lower risks for thromboembolic events failed to show the same association. Therefore, clinical utility may be limited to only patients with high risk for recurrent events.
  - Despite the boxed warning, the US FDA has not mandated genetic testing for CYP2C19 status before initiation of clopidogrel, leading to some confusion among healthcare providers regarding clinical implementation.
  - The American College of Cardiology Foundation and the American Heart Association have recently suggested that in the absence of prospective randomized trials, the evidence is insufficient to recommend genetic testing.
  - Recently, the ELEVATE-TIMI 56 (NCT01235351), a multicenter, randomized, double-blinded trial, showed that among patients with stable cardiovascular disease, tripling the maintenance dose of clopidogrel to 225 mg daily in CYP2C19*2 heterozygotes achieved levels of platelet reactivity similar to the standard 75 mg dose in noncarriers while in CYP2C19*2 homozygotes, even a 300 mg daily dose was unlikely to result in optimal degree of platelet inhibition.

- Warfarin and CYP2C9 and VKORC1:
  - Multiple linked variants in VKORC1 have been associated with variability in gene expression, increased sensitivity to warfarin and
reduction in dose requirement. However, only VKORC1 –1639G>A is a functional variant.

- Additional identification of variants in CYP2C9*2 and *3 alleles are associated with 40–70% reduction in (S)-warfarin clearance and approximately 20–40% lower warfarin dose requirement.

- Despite the plethora of information from multiple genome-wide association studies, the strong support from the International Warfarin Pharmacogenetics Consortium (IWPC) and Clinical Pharmacogenetics Implementation Consortium (CPIC), in addition to the FDA warning labels and the availability of several FDA-approved platforms for warfarin genotyping, the clinical implementation of genetic testing for warfarin dosing has been disappointing in practice.

- The current consensus guidelines by the American College of Chest Physicians actually warn against the routine use of genetic data to guide dosing. Similarly, the American College of Medical Genetics endorses testing only in cases of unusual warfarin response.

- There is a lack of evidence from large-scale prospective randomized trials, but current such studies are underway.

  • β-blockers and CYP2D6 and ADRB1:
    - In heart failure therapeutic pharmacogenomics, the most robust evidence concerns the association of ADRB1 variants (two common nonsynonymous polymorphisms Ser49Gly; rs1801252 and Arg389Gly; rs1801253) and left ventricular ejection fraction or clinical outcomes mediated by β-blockade.

  • Antihypertensives and ADRB1 and ADD1:
    - There are consistent data in several clinical studies showing association of differential blood pressure lowering levels with polymorphisms of the ADRB1 gene, namely Ser49Gly and Arg389Gly.

  • Statins and HMGCR, LDLR and SLCO1B1:
    - The H7 haplotype in the HMGCR has been associated with 11–19% decreased reduction in low-density lipoprotein cholesterol in both ethnically diverse and multiple independent populations; HMGCR H2 and LDLR L5, have also been associated with decreased attenuation of low-density lipoprotein cholesterol.
    - The FDA recommendation that the higher dose of 80 mg/day of simvastatin be restricted to patients taking the medication for 12 months or longer was based on the finding where patients homozygous or heterozygous for a variant (rs4363657) in the SLCO1B1 gene were found to have a 16.9- and 4.5-fold increase, respectively, in developing myopathy or rhabdomyolysis at the 80 mg/day higher dose. The identified polymorphism tags a known nonsynonymous variant SLCO1B1*5.
    - Other studies have shown that variants in SLCO1B1 predict adverse side effects to atorvastatin and cerivastatin-associated rhabdomyolysis.
A biomarker that can modulate risk may not necessarily be of high clinical utility because of low predictive value (<20% for developing myopathy in patients homozygous for the Val174Ala allele).

Future perspective

- The greatest obstacle to clinical implementation of cardiovascular pharmacogenetics may be the lack of both reproducibility and agreement about the clinical validity and utility of the findings.
- Population-wide implementation across cultures, nations and ethnicities may be limited by the social acceptance and public perception of genetic testing as well as ethical, legal and social issues.
Table 1
Cardiovascular therapeutics, associated genes and gene variants, associated allele effect and minor allele frequency in different populations.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Associated gene(s)</th>
<th>Associated gene variant(s)</th>
<th>Associated allele effect</th>
<th>Minor allele frequency in different populations&lt;sup&gt;†&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clopidogrel</td>
<td>CYP2C19</td>
<td>CYP2C19*2 (c.681G&gt;A; rs4244285)</td>
<td>Loss-of-function, lower antiplatelet effect, higher risk of cardiovascular events</td>
<td>CEU 14%; YRI/ASW 14%; CHB/JPT 26–28%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP2C19*17 (c.-806C&gt;T; rs12248560)</td>
<td>Gain-of-function, increased drug sensitivity, increased adverse bleeding side effect</td>
<td>CEU 23%; YRI/ASW 29%; CHB/JPT 0-2%</td>
</tr>
<tr>
<td>Warfarin</td>
<td>VKORC1/CYP2C9</td>
<td>VKORC1 (~1639G&gt;A; rs9923231)</td>
<td>Increased drug sensitivity, reduced dose requirement</td>
<td>CEU 40%; YRI/ASW 3–10%; CHB/JPT 91–95%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP2C9*2 (p.Arg144Cys; rs1799853)</td>
<td>Reduced drug clearance, reduced dose requirement</td>
<td>CEU 10%; YRI/ASW 0%; CHB/JPT 0%</td>
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<td></td>
<td>CYP2C9*3 (p.Ile359Leu; rs1057910)</td>
<td>Reduced drug clearance, reduced dose requirement</td>
<td>CEU 6%; YRI/ASW 0–3%; CHB/JPT 2–4%</td>
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<tr>
<td>β-blockers</td>
<td>ADRB1</td>
<td>ADRB1 (p.Ser49Gly; rs1801252)</td>
<td>More optimal blood pressure control, increased left ventricular ejection fraction</td>
<td>CEU data not available; YRI/ASW 1%; CHB/JPT 0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADRB1 (p.Arg389Gly; rs1801253)</td>
<td>More optimal blood pressure control, increased left ventricular ejection fraction</td>
<td>CEU 31%; YRI/ASW 42%; CHB/JPT 15–25%</td>
</tr>
<tr>
<td>Statins</td>
<td>HMGCR/SLCO1B1</td>
<td>HMGCR H7 (c.451-174A&gt;T; rs17244841)</td>
<td>Decreased reduction in LDL-cholesterol</td>
<td>Data not available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HMGCR H7 (c.1564–106A&gt;G; rs3466662)</td>
<td>Decreased reduction in LDL-cholesterol</td>
<td>CEU 43%; YRI/ASW 88–95%; CHB/JPT 54%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HMGCR H7 (c.2298+117T&gt;G; rs17238540)</td>
<td>Decreased reduction in LDL-cholesterol</td>
<td>Data not available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLCO1B1*3 (p.Val174Ala; rs4149056)</td>
<td>Increased risk of developing myopathy or rhabdomyolysis</td>
<td>CEU 15%; YRI/ASW 1–5%; CHB/JPT 12–14%</td>
</tr>
</tbody>
</table>

<sup>†</sup> Data from International HapMap Project Data Rel 28 Phases II and III, August 2010 [116], on NCBI B36 assembly, dbSNP b126.

ASW: African ancestry in southwestern USA; CEU: Utah residents with northern and western European ancestry from the CEPH collection; CHB: Han Chinese in Beijing, China; JPT: Japanese in Tokyo, Japan; LDL: Low-density lipoprotein; YRI: Yoruban in Ibadan, Nigeria.
Table 2

US FDA warning label for clopidogrel and warfarin and FDA-approved testing platforms and assays for the analyte(s).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Biomarker(s)</th>
<th>Label sections</th>
<th>Date of FDA warning</th>
<th>FDA-approved pharmacogenetic testing platforms and assays</th>
<th>Effective date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clopidogrel</td>
<td>CYP2C19</td>
<td>Boxed warning, Warnings and Precautions, Dosage and Administration</td>
<td>March 2010</td>
<td>Affymetrix GeneChip System 3000DX (Roche AmpliChip CYP450 Test)</td>
<td>January 2005</td>
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<td></td>
<td></td>
<td>AutoGenomics Infiniti™ Analyzer</td>
<td>November 2010</td>
</tr>
</tbody>
</table>

† US FDA drug label.
‡ US FDA Center for Devices and Radiological Health Clinical Laboratory Improvement Amendments database.