CHAPTER

6

Pharmacogenetics in Cardiovascular Diseases

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OBJECTIVES

1. Provide examples of pharmacogenetic labeling for drugs used to manage cardiovascular disease.
2. Discuss guidelines for use of genetic information to guide therapy with cardiovascular agents.
3. Describe applications of pharmacogenetics in prescribing oral antiplatelet agents and warfarin.
4. Describe the potential applications of pharmacogenetics in the management of hypertension, heart failure, and drugs that influence cardiac conduction.

INTRODUCTION

Cardiovascular disease is the most common cause of death globally and is associated with significant productivity loss and healthcare costs [1,2]. Cardiovascular drugs, including antihypertensive medications and statins, consistently rank among the top 10 most commonly prescribed drugs in the United States [3]. Guidelines

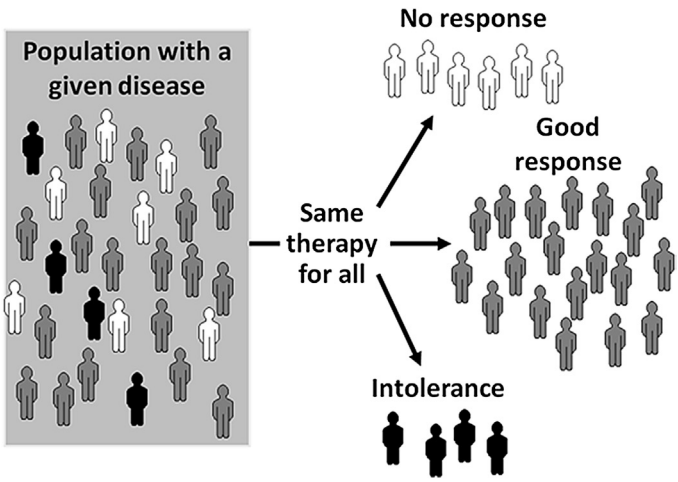

FIGURE 6.1 Current empiric approach of treating patients with cardiovascular disease. As shown, patients with a given cardiovascular disease are generally treated with similar therapy. The majority of patients will have a good response to such therapy. However, the problem with this approach is that a subset of patients will have little to no therapeutic response and another subset will develop intolerable adverse effects.

from expert consensus panels are available to guide the treatment for most cardiovascular diseases, including hypertension, heart failure, dyslipidemia, and ischemic heart disease [4–9]. These guidelines are based on data from large, randomized, placebo-controlled clinical trials demonstrating significant improvements in clinical outcomes with certain medications in clinical trial populations. As illustrated in Fig. 6.1, for some cardiovascular diseases, the same drug or drug combination is recommended for all affected persons, regardless of individual characteristics. Such is the case with renin angiotensin system inhibitors and β-blockers, which are recommended for all patients with left ventricular dysfunction in the absence of a contraindication [9]. However, although these treatments were efficacious in clinical trial populations as a whole, there is no guarantee that they will be safe or effective in an individual patient. In fact, there is significant interpatient variability in response to angiotensin-converting enzyme (ACE) inhibitors and β-blockers, with some patients deriving no benefit and other patients experiencing intolerable adverse effects with these agents. Currently, it is difficult if not impossible to predict how a patient will respond to a cardiovascular agent based on clinical factors alone.

It is now well recognized that an individual's genotype impacts his or her response to cardiovascular drugs. As of August 2017, genetic information was included in the Food and Drug Administration (FDA)-approved labeling for at least 12 drugs used to treat cardiac and vascular disorders (Table 6.1). Genotype primarily influences cardiovascular drug response by affecting drug disposition in the body (pharmacokinetics) or a patient's sensitivity to a drug (pharmacodynamics), as described in detail in Chapter 1.

This chapter reviews the pharmacogenetics of various cardiovascular agents. The strongest evidence exists for clopidogrel, warfarin, and simvastatin, and thus the most indepth discussion is devoted to these drugs. This chapter also provides an overview of pharmacogenetic application for dosing tacrolimus after cardiac transplant and the potential for pharmacogenetics to improve prescribing of antihypertensive agents, heart-failure medications, and drugs that influence cardiac conduction. Challenges and opportunities with bringing cardiovascular pharmacogenetics to the clinical arena are also highlighted.

TABLE 6.1 Cardiovascular Drugs With Genetic Labeling

Drug Class/Drug	Biomarker	Location of Label Information	Context
STATINS			
Atorvastatin	LDL receptor	Indications/Dosage and Administration/Clinical Studies	Among other indications, atorvastatin is indicated in patients with familial hypercholesterolemia that is due to mutations in the LDL receptor gene.
Pravastatin	Genotype APOE E2/E2 and Fredrickson Type III dysbetalipoproteinemia	Clinical Studies	Response to pravastatin in patients with genotype E2/E2 and Fredrickson Type III dysbetalipoproteinemia is shown.
BETA-BLOCKERS			
Carvedilol	CYP2D6	Drug Interactions/Clinical Pharmacology	Reduced carvedilol metabolism in poor metabolizers.
Metoprolol	CYP2D6	Drug Interactions/Clinical Pharmacology	Reduced metoprolol metabolism in poor metabolizers.
Propranolol	CYP2D6	Clinical Pharmacology	Reduced propranolol metabolism in poor metabolizers.
ANTIPLATELETS			
Clopidogrel	CYP2C19	Boxed Warning/Warnings and Precautions/Clinical Pharmacology	Reduced clopidogrel efficacy in poor metabolizers.
Prasugrel	CYP2C19	Use in Specific Populations/ Clinical Pharmacology/ Clinical Studies	No effect of CYP2C19 genotype on prasugrel efficacy.
Ticagrelor	CYP2C19	Clinical Pharmacology	No effect of CYP2C19 genotype on ticagrelor efficacy.
ANTICOAGULANTS			
Warfarin	CYP2C9/VKORC1	Dosage and Administration/ Drug Interactions/Clinical Pharmacology	Lower warfarin doses needed with the CYP2C9*2, CYP2C9*3, and VKORC1 -1639A alleles.
ANTIARRHYTHMICS			
Propafenone	CYP2D6	Dosage and Administration/ Warnings and Precautions/ Drug Interactions/Clinical Pharmacology	The recommended dose is the same in slow and extensive metabolizers.
Quinidine	CYP2D6	Precautions	Quinidine can convert extensive metabolizers to poor metabolizers of CYP2D6 substrates.
MISCELLANEOUS			
Hydralazine	NAT	Clinical Pharmacology	Fast acetylators have lower hydralazine exposure.

PHARMACOGENETICS OF ANTIPLATELET AGENTS

Background on Antiplatelet Agents

Antiplatelet therapy plays a major role in cardiovascular risk reduction. Antiplatelet therapy began with aspirin monotherapy and has advanced to include multiple oral antiplatelet drugs affecting different mechanisms of platelet function [10]. In addition to aspirin, currently approved oral antiplatelet drugs include ticlopidine, clopidogrel, prasugrel, and ticagrelor. Ticlopidine is rarely used because it increases the risk for neutropenia and thrombotic thrombocytopenic purpura. As such, the discussion will be limited to the other agents.

Clopidogrel has long been available; ticagrelor and prasugrel were more recently approved by the FDA. Although these agents have different pharmacokinetic and pharmacodynamic properties and indications, they all share the common mechanism of blocking the platelet $P2Y_{12}$ receptor, resulting in attenuation of adenosine diphosphate (ADP)–mediated platelet activation and aggregation. Thus, they are all classified as $P2Y_{12}$ receptor inhibitors.

Overview of Clopidogrel Metabolism and Pharmacodynamics

Clopidogrel is indicated in combination with aspirin for patients with an acute coronary syndrome (ACS) who are medically managed or undergo percutaneous coronary intervention (PCI) based on data that it reduces morbidity and mortality in these patient populations [11–13]. The combination of clopidogrel and aspirin also reduces the risk for coronary-stent thrombosis following PCI [14]. There is significant interpatient variability in clopidogrel pharmacokinetics and pharmacodynamics [15]. Clopidogrel is a prodrug requiring bioactivation by multiple cytochrome P450 (CYP450) enzymes. As shown in Fig. 6.2, clopidogrel is a p-glycoprotein substrate, and once absorbed, the majority of clopidogrel is eliminated via esterases. The remaining drug requires conversion via a two-step process to its active form. Genetic variation in pathways involved in clopidogrel absorption and bioactivation has been investigated for its effects on clopidogrel disposition and effectiveness.

Clopidogrel responsiveness can be characterized via drug effects on either platelet aggregation or clinical outcomes. Platelet aggregation tests involve ex vivo exposure of platelets to aggregating agents, including ADP. Decreased response to clopidogrel, as demonstrated by insufficient attenuation of platelet aggregation, has been linked to an increased risk of adverse cardiovascular events [15,16]. Investigators have also used clinical events, such as myocardial infarction (MI), stroke, or coronary artery stent thrombosis, as measures of clopidogrel response [16,17]. Genetic determinants of both measures of response will be discussed in this section.

CYP2C19 Genotype and Clopidogrel Responsiveness

Various isoenzymes of the CYP450 system, including cytochrome P450 3A4 (CYP3A4), cytochrome P450 3A5 (CYP3A5), and cytochrome P450 2C19 (CYP2C19), are involved in clopidogrel metabolism. However, polymorphisms within the gene for CYP2C19, which is involved in both steps of the clopidogrel bioactivation pathway and serves a major role in converting clopidogrel to its active form, have the greatest implications for clopidogrel response. In contrast, no consistent associations have been found between the cytochrome P450 3A (CYP3A) genotypes and clopidogrel pharmacokinetics or clinical response [16,18–20]. The CYP2C19 gene is located on chromosome 10q23.33. The CYP2C19*2, *3, *4, *5, *6, *7, and *8 alleles are nonfunctional (loss of function) alleles associated with absent or reduced CYP2C19 function compared to the CYP2C19*1 (normal function)

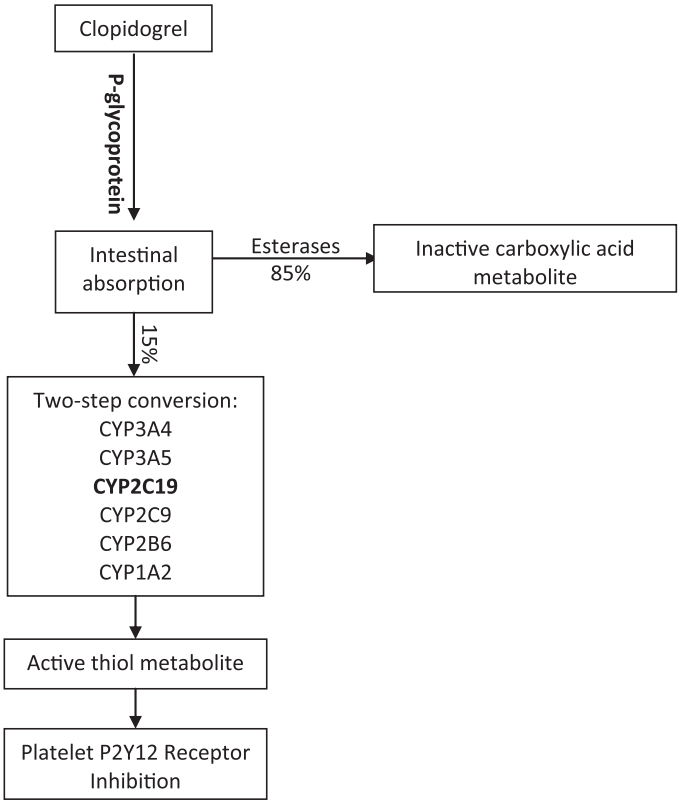

FIGURE 6.2 Proteins involved in the absorption and metabolic activation of clopidogrel. Genes for proteins shown in bold contain polymorphisms linked to clopidogrel responsiveness. *CYP*, cytochrome P450.

allele [21]. In contrast, the CYP2C19**17* allele is associated with increased CYP2C19 function. The CYP2C19**2* allele is by far the most common nonfunctional CYP2C19 variant; however, its frequency differs by ancestral origin (Fig. 6.3), with a higher frequency in Asians (approximately 30%) compared to Caucasians (13%) and African Americans (18%) [21,22]. The CYP2C19**3* allele also occurs commonly in Asian populations (~10%) but is rare in individuals of other ancestral backgrounds (<1%). Approximately 14% of Asians, 2% of Caucasians, and 4% of African Americans are CYP2C19 poor metabolizers (with two nonfunctional alleles), and 50%, 25%, and 30%, respectively, are intermediate metabolizers (with one nonfunctional allele).

In individuals with one or two nonfunctional CYP2C19 alleles, there is decreased production of the active clopidogrel metabolite and reduced clopidogrel effectiveness [23]. Studies have consistently shown that possession of a CYP2C19 nonfunctional allele increases the risk of cardiovascular events with clopidogrel (Fig. 6.4) [16,20,24–28]. In a metaanalysis of nine studies and 9685 total patients, the majority of whom underwent PCI (91%) and had an ACS (54%), carriers of at least one CYP2C19 nonfunctional allele had a higher risk of adverse cardiovascular events, with a hazard ratio of 1.57 (95% confidence interval [CI]: 1.13–2.16) compared to noncarriers (i.e., the risk for adverse cardiovascular events was approximately 1.5-fold greater in nonfunctional allele carriers) [29]. The hazard ratio for stent thrombosis was 2.81 (95% CI: 1.81–4.37) for nonfunctional allele carriers compared to noncarriers. Similarly, another metaanalysis

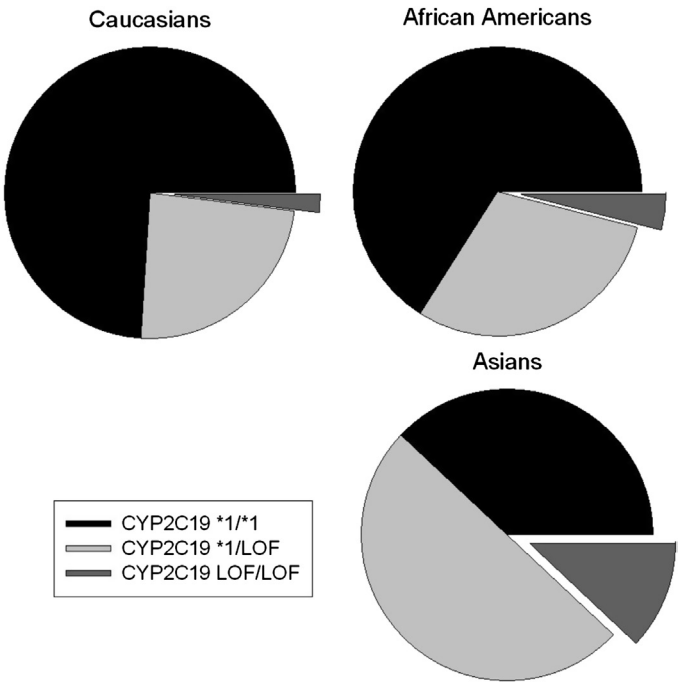

FIGURE 6.3 Cytochrome P450 2C19 (CYP2C19) allele frequencies among ethnic groups. *LOF*, loss-of-function.

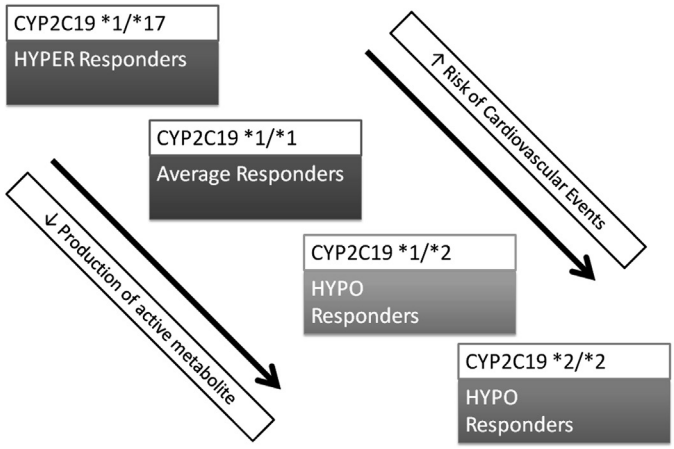

FIGURE 6.4 Effect of cytochrome P450 2C19 (CYP2C19) genotype on clopidogrel pharmacokinetics and efficacy.

of nearly 12,000 patients reported that carriers of the CYP2C19*2 allele had increased risk for major adverse cardiovascular events (odds ratio [OR]: 1.29; 95% CI: 1.12–1.49) and stent thrombosis (OR: 3.45; 95% CI: 2.14 to 5.57) compared to noncarriers (i.e., the odds of adverse events were 1.29 times greater, and the odds for stent thrombosis were more than three times greater for carriers vs. noncarriers) [30]. In contrast, two metaanalyses including more heterogeneous

patient populations found no association between CYP2C19 genotype and adverse events with clopidogrel [31,32]. However, these latter analyses have been criticized for including studies of lower-risk patients, such as those with atrial fibrillation or with an ACS managed medically (vs. with PCI). A more recent metaanalysis examined outcomes separately in patients who underwent PCI and those who did not [33]. Among clopidogrel-treated patients who underwent PCI, there was a significantly higher risk for adverse cardiovascular events in those with a CYP2C19 nonfunctional allele compared to those without a nonfunctional allele. However, no association by genotype was observed in non-PCI patients. The majority of data demonstrates that CYP2C19 nonfunctional genotype significantly impacts formation of the active clopidogrel metabolite, ex vivo inhibition of platelet aggregation with clopidogrel, and clopidogrel's effectiveness in preventing adverse cardiovascular events, particularly among patients undergoing coronary artery stent placement.

The effect of the CYP2C19*17 increased function allele on clopidogrel responsiveness has also been examined; however, the results from these studies have been inconsistent. This allele has been associated with increased production of the clopidogrel active metabolite and greater inhibition of platelet aggregation with clopidogrel [34,35]. There is some evidence that CYP2C19*17 carriers may be at greater bleeding risk [35]. However, the CYP2C19*17 and *2 alleles are in linkage disequilibrium (LD) such that the CYP2C19*17 single-nucleotide polymorphism (SNP) is not known to occur on the same allele as *2, thus complicating interpretation of effects observed in CYP2C19*17 allele carriers. Given that an independent effect of the CYP2C19*17 allele has not been clearly established, this allele is not accompanied by a specific therapy change recommendation in the currently available CYP2C19 genotype-directed practice guidelines [22,36].

ABCB1 Genotype and Clopidogrel Responsiveness

P-glycoprotein is encoded by the ATP-binding cassette, subfamily B, member 1 (ABCB1) gene. The most commonly studied ABCB1 variant is the synonymous c.3435C>T polymorphism, located in a region that encodes for a cytoplasmic loop in the transporter [37]. A lower-peak plasma concentration (Cmax) and total area under the plasma concentration–time curve (AUC) of clopidogrel and its active metabolite were noted after single 300 and 600-mg doses in subjects who were homozygous for the variant ABCB1 3435T allele [38]. Of note, increasing the clopidogrel dose to 900 mg overcame the effect of genotype on drug concentrations. Several studies have also assessed the association between ABCB1 genotype and clinical response to clopidogrel with varying results [17,19,20,24,39]. The inconsistent results from these studies render it difficult to apply ABCB1 testing to patients starting clopidogrel.

Paraoxonase-1 (PON1) Genotype and Clopidogrel Responsiveness

Paraoxonase-1 (PON1) is an esterase that has been shown to facilitate the activation of clopidogrel in vitro [41]. A nonsynonymous polymorphism in the coding region of PON1, p.Q192R, has been evaluated for its role in clopidogrel responsiveness. The 192Q allele was associated with increased clopidogrel activation in vitro in one study [41]. The same study showed that possession of a 192Q allele was associated with decreased risk of stent thrombosis. However, in contrast to most previous data, the investigators found no association between CYP2C19 genotype and stent thrombosis risk. Several studies have since demonstrated no association between *PON1* genotype and clopidogrel responsiveness [28,42–44]. Because of the lack of replication with the PON1 genotype, PON1 genotyping is not currently recommended.

Genome-Wide Association Study (GWAS) of Clopidogrel Responsiveness

Investigators for the Pharmacogenomics of Antiplatelet Intervention-1 (PAPI-1) study conducted a genome-wide association study (GWAS) of ex vivo platelet aggregation with clopidogrel in a cohort of generally healthy subjects from the Old Order Amish population ($n = 429$) [19]. Each subject was given a 300-mg clopidogrel loading dose, followed by a dose of 75 mg/day for 6 days, and platelet aggregation was measured before and after clopidogrel administration. Between 500,000 and 1 million variants were assessed for each subject to identify genetic associations with clopidogrel responsiveness based on ex vivo platelet aggregation. A cluster of 13 highly correlated variants on chromosome 10 in the genetic region encoding CYP2C18, CYP2C19, CYP2C9, and CYP2C8 were associated with clopidogrel response. These variants were in strong LD with the CYP2C19*2 allele and explained 12% of the interindividual variation in platelet aggregation. Of note, no association was seen with CYP2C19*17 or with polymorphisms in the genes encoding CYP3A, ABCB1, or PON1. In a replication cohort of 227 patients undergoing nonemergent PCI and treated with clopidogrel, the investigators found that, similar to most previous data, CYP2C19*2 was associated with residual platelet aggregation and an increased risk for cardiovascular events or death at 1 year, with a hazard ratio of 2.4 (95% CI 1.18 to 4.99), indicating a nearly 2.5-fold greater risk for events or death with the CYP2C19*2 allele [19].

Alternative Treatment Approaches in Patients With a CYP2C19 Nonfunctional Allele

Several studies have addressed whether clopidogrel dose escalation overcomes the effects of CYP2C19 nonfunctional alleles. In a multicenter, double-blind clinical trial, patients with cardiovascular disease and the CYP2C19*1/*2 or *2/*2 genotype were randomized to receive clopidogrel at varying doses (75, 150, 225, and 300 mg), each for a 14-day period. Platelet function testing was conducted with each dose, and results were compared with those from noncarriers of the CYP2C19*2 allele receiving clopidogrel 75 mg [45]. For carriers of a single CYP2C19*2 allele, a clopidogrel dose of 225 mg/day resulted in levels of platelet inhibition similar to that attained with a 75 mg/day dose in noncarriers. However, in CYP2C19*2 homozygotes, not even the 300-mg/day dose resulted in platelet inhibition comparable to the 75-mg/day dose in noncarriers. Similarly, among patients with an acute MI receiving a clopidogrel loading dose of 300 mg, significantly lower inhibition of platelet aggregation was observed in both heterozygous and homozygous carriers of the CYP2C19*2 allele compared to noncarriers [46]. A 900-mg loading dose was sufficient to inhibit platelet aggregation in heterozygotes, but not in homozygotes. A study in healthy volunteers found that poor metabolizers (CYP2C19*2/*2 or *2/*3 genotypes) receiving a clopidogrel loading dose of 600 mg, followed by a maintenance dose of 150 mg/day for 5 days, had similar inhibition of platelet aggregation compared to normal metabolizers (CYP2C19*1/*1 genotype) receiving a 300-mg loading dose and 75 mg/day dosing [47]. Intermediate metabolizers (CYP2C19*1/*2 or *1/*3 genotype) had a similar response as normal metabolizers with all clopidogrel doses tested. In contrast, a study of patients undergoing PCI after an ACS found that doubling the maintenance dose of clopidogrel in CYP2C19*2 carriers was not effective in overcoming reduced inhibition of platelet aggregation [48]. Another study of ACS patients undergoing PCI found that providing up to three additional 600-mg clopidogrel loading doses to CYP2C19*2 carriers, according to the degree of platelet reactivity, was successful in overcoming reduced response with standard 600-mg dosing in some patients [49]. However,

12% of these patients never reached the desired level of inhibition of platelet aggregation. This latter study demonstrates that although titrating clopidogrel dosing based on platelet aggregation testing in carriers of a CYP2C19 nonfunctional allele may be a viable approach to optimizing the clopidogrel loading dose for some patients, it is not an effective approach for all patients. The inconsistent results among studies are most likely due to differences in study populations (i.e., healthy subjects vs. patients with an acute cardiac event undergoing PCI).

A more effective approach to antiplatelet therapy based on CYP2C19 genotype is to treat nonfunctional allele carriers with an alternative antiplatelet agent, namely prasugrel or ticagrelor. Like clopidogrel, prasugrel is a thienopyridine that binds covalently and irreversibly to the $P2Y_{12}$ receptor and is a prodrug requiring bioactivation [50]. In contrast, ticagrelor is administered in its active form and more reversibly binds to the $P2Y_{12}$ receptor to change its conformation.

Prasugrel is currently FDA-approved for use in patients with ACS undergoing PCI. Like clopidogrel, prasugrel is a p-glycoprotein substrate that is converted to the active metabolite via multiple enzymes, and CYP3A, CYP2B6, CYP2C9, and CYP2C19 in particular. However, unlike clopidogrel, esterases convert prasugrel to an intermediate metabolite (rather than an inactivated metabolite), and the CYP450 bioactivation occurs in a single step (rather than two steps). Likely because of prasugrel's unique bioactivation pathway, common genetic variants in CYP450 enzymes do not affect the pharmacokinetics or clinical efficacy of prasugrel [51]. There is also no association between ABCB1 genotype and prasugrel pharmacokinetics, possibly because prasugrel is more rapidly metabolized compared to clopidogrel [17]. Ticagrelor is indicated for ACS, regardless of whether patients undergo PCI. The CYP3A4 enzyme is the primary enzyme responsible for ticagrelor metabolism. Similar to prasugrel, there is no evidence that common genetic variation affects ticagrelor pharmacokinetics or efficacy [40]. The data demonstrate that prasugrel and ticagrelor provide greater inhibition of platelet aggregation and greater protection against cardiovascular events compared to clopidogrel in CYP2C19 nonfunctional allele carriers [17,23,40,52,53]. In a prospective evaluation of genotype-guided antiplatelet prescribing, patients with a CYP2C19 nonfunctional allele treated with prasugrel were shown to have lower platelet reactivity compared to nonfunctional allele carriers treated with clopidogrel [54]. However, it is important to note that prasugrel use is contraindicated in patients with a history of stroke or transient ischemic attack, and its use is not recommended in patients 75 years of age or older because of increased bleeding risk.

Clopidogrel Labeling Revisions

Based on substantial data supporting an association between CYP2C19 nonfunctional alleles and reduced clopidogrel responsiveness, the FDA approved the addition of genetic information to the clopidogrel labeling in March 2010 [55]. These label changes include a boxed warning about diminished antiplatelet response to clopidogrel in CYP2C19 poor metabolizers (with two nonfunctional alleles). The labeling further states that genetic testing is available and advises consideration of alternative therapy in poor metabolizers. Although these labeling updates highlight the importance of CYP2C19 genotype in clopidogrel responsiveness, they provide no guidance on when or whom to genotype and little guidance on how to manage poor metabolizers. Further, they do not address CYP2C19 intermediate metabolizers.

Guidelines for the Clinical Use of CYP2C19 Genotyping With Clopidogrel

Several statements by expert consensus panels address CYP2C19 genotyping to determine clopidogrel responsiveness [22,55]. In 2010,

TABLE 6.2 Phenotype Classification and Therapeutic Recommendations From the CPIC Based on CYP2C19 Genotype For Patients Requiring Dual Antiplatelet Therapy. After Acute Coronary Syndrome and Percutaneous Coronary Intervention [22]

CYP2C19 genotype	Phenotype Classification	Therapeutic Recommendation	Classification of Recommendation
*1/*1	Normal metabolizer	Clopidogrel should be effective at the label-recommended dosage and administration	Strong
*1/*17 or *17/*17	Ultra-rapidmetabolizer	Clopidogrel should be effective at the label-recommended dosage and administration	Strong
*1/*2	Intermediate metabolizer	Prasugrel or ticagrelor if no contraindication	Moderate
*2/*2	Poor metabolizer	Prasugrel or ticagrelor if no contraindication	Strong

the American College of Cardiology and the American Heart Association Foundation issued a joint response to the addition of genetic information to the clopidogrel labeling, which is summarized in their guidelines for management of PCI patients [7,55]. They indicated that the available data were insufficient to recommend routine use of genetic testing for patients undergoing PCI, specifically citing the lack of outcomes data with genetic testing from large randomized control trials. However, they further stated that CYP2C19 genotyping may be considered in patients who are at moderate to high risk for poor cardiovascular outcomes, such as those undergoing elective high-risk PCI for extensive and/or very complex disease and others at the clinician's discretion. In these patients, alternative therapy (e.g., prasugrel or ticagrelor) is recommended. These recommendations are designated as Class IIb based on Level C evidence, meaning that the benefit of genotyping may be slightly greater than or equivalent to not genotyping (note to the reader: definitions of evidence levels are provided in the guidelines).

Guidelines by the Clinical Pharmacogenetics Implementation Consortium (CPIC) do not address whether to order CYP2C19 testing for patients undergoing PCI, leaving this to the discretion of the physician [22]. Rather, they provide recommendations for therapy based on available genotype results. The guidelines focus on patients with an ACS who undergo PCI. For these patients, either prasugrel or ticagrel is recommended in the presence of a CYP2C19 nonfunctional allele, in the absence of contraindications. For patients without a nonfunctional allele, clopidogrel is expected to be effective (Table 6.2).

Randomized Controlled Trials Examining Outcomes With CYP2C19-Guided Antiplatelet Therapy

Two large randomized controlled trials are addressing the efficacy of genotype-guided clopidogrel use. The Tailored Antiplatelet Therapy Following PCI (TAILOR-PCI) trial is examining the effect of genotype-guided antiplatelet therapy on adverse cardiovascular events (ClinicalTrials.gov Identifier: NCT01742117). Patients undergoing PCI are randomized to either genotype-guided antiplatelet therapy or to treatment with clopidogrel. In the genotype-guided arm, patients with a nonfunctional allele are prescribed ticagrelor, and those without a nonfunctional allele are prescribed clopidogrel. The primary outcome is composite of nonfatal myocardial infarction, nonfatal stroke, cardiovascular mortality, severe recurrent ischemia, or stent thrombosis during the 12 months following PCI. The trial is targeting 5270 patients and began in 2012, with anticipated completion in 2020.

In the Cost-effectiveness of Genotype Guided Treatment with Antiplatelet Drugs in STEMI Patients: Optimization of Treatment (POPular Genetics) trial (ClinicalTrials.gov NCT01742117), patients with ST-segment elevation myocardial infarction who undergo PCI are randomized to a genotype-guided group or control group. Patients in the genotype-guided group with a nonfunctional CYP2C19 allele are treated with prasugrel or ticagrelor, whereas clopidogrel is prescribed to patients without a nonfunctional allele. Patients in the control arm are prescribed either prasugrel or ticagrelor. The primary endpoint is death, recurrent myocardial infarction, stent thrombosis, stroke, or major bleeding at 1 year. The trial is targeting an enrollment of 2700 patients and expected to be completed in 2019.

Two smaller trials in Chinese patients also examined a genotype-guided approach to antiplatelet prescribing. In one trial, 600 patients who underwent PCI were randomized to clopidogrel without genotyping or to genotyping, with high-dose clopidogrel prescribed for IMs and high-dose clopidogrel plus cilostazol prescribed for PMs [56]. The genotype-guided group had a significantly lower risk for the composite endpoint of death, MI, or stroke at 6 months. The second trial included 628 patients and had similar treatment arms except that ticagrelor was prescribed for PMs [57]. Similar to the first trial, there was a significantly lower risk for the composite endpoint of death, MI, stroke, or target vessel revascularization in the genotype versus conventional treatment arm.

Clinical Implementation of CYP2C19-Guided Antiplatelet Therapy

Based on the strong and consistent evidence of reduced clopidogrel effectiveness in patients with a CYP2C19 nonfunctional allele, a number of institutions have starting offering CYP2C19 genotyping to assist with antiplatelet prescribing decisions for patients undergoing PCI [58–62]. Some institutions are taking a preemptive approach whereby patients undergoing cardiac catheterization or at high risk for cardiovascular events are genotyped so that genotype is readily available in the event that the patient requires PCI. Other institutions are genotyping in a more reactive manner at the time of PCI. Regardless of the approach, most institutions are following CPIC guidelines and recommending alternative antiplatelet therapy with prasugrel or ticagrelor for patients with the PM or IM phenotype. Many sites have built clinical decision support rules into their electronic health record so that in the event that clopidogrel is prescribed for a patients with a PM or IM phenotype on record, then the physician is alerted to the genotype result and risk for poor response to clopidogrel. Examples of clinical decision-support tools are available through the National Institute of Health sponsored Implementing Genomics in Practice (IGNITE) website (https://ignite-genomics.org/).

Outcome data are beginning to emerge from pragmatic and observational studies of clinical implementation of CYP2C19 genotyping. Unlike randomized controlled trials, which often have strict eligibility criteria and occur in controlled settings that limit the generalizability of results, pragmatic studies provide data in the context of routine clinical practice, thus reflecting the effectiveness of an intervention in a real-world setting and maximizing generalizability [63]. There is less control for sources of bias in pragmatic studies, and propensity score matching and other statistical techniques are often required to account for differences between treatment groups. As part of the National Institutes of Health (NIH)-funded IGNITE Network, investigators from seven institutions in the United States pooled data on cardiovascular events for over 1800 patients who underwent either emergent or elective PCI and were genotyped as part of clinical care [64]. The median time from PCI to genotype results being available across sites was 1 day, demonstrating the feasibility of providing genotype-guided antiplatelet therapy.

Alternative antiplatelet therapy was recommended for patients with one or two CYP2C19 nonfunctional alleles (e.g., IM or PMs), but the ultimate choice of antiplatelet therapy was left to the decision of the prescriber. Approximately 30% of patients had a nonfunctional allele, and 60.5% of these were prescribed alternative therapy. In contrast, 85% of patients without a nonfunctional allele were prescribed clopidogrel. After propensity scoring to account for differences between groups, the risk for major adverse cardiovascular events (defined as the composite outcome of death, myocardial infarction, or ischemic stroke) over the 12-month follow-up period after PCI was significantly higher in carriers of a nonfunctional allele prescribed clopidogrel versus alternative therapy (adjusted hazard ratio 2.26, 95% CI 1.18–4.32). In contrast, there was no difference in outcomes between carriers of a nonfunctional allele prescribed alternative therapy and those without a nonfunctional allele.

Similar results were observed in a Dutch study that included over 3200 patients who underwent elective PCI. In contrast to the U.S. study, recommendations for alternative antiplatelet therapy were confined to PMs [59]. Over the follow-up period of up to 18 months, 31% of PMs treated with clopidogrel versus 5% of PMs treated with alternative therapy had an adverse cardiovascular event ($P = .003$).

An additional study was conducted in Spain and compared outcomes between approximately 300 patients who received genotype-guided antiplatelet therapy and approximately 400 historical controls who underwent PCI prior to genotype implementation [65]. Both the CYP2C19 and ABCB1 genotypes were determined in the genotype group, with alternative therapy prescribed to those with a CYP2C19 nonfunctional allele or the ABCB1 TT genotype. Most of the patients in the control group were treated with clopidogrel. The investigators reported a significantly lower risk for cardiovascular death, MI, or stroke in the genotype group compared to historical controls.

Opportunities and Challenges With Clopidogrel Pharmacogenetics

The data supporting CYP2C19 genotype associations with clopidogrel response have accumulated to the extent that institutions have started offering genotyping as part of clinical practice. Data from small randomized controlled trials and observational and pragmatic studies demonstrate improved outcomes with a genotype-guided approach to antiplatelet therapy after PCI. However, current guidelines for the management of patients with PCI do not recommend CYP2C19 genotyping to guide antiplatelet therapy because of the lack of data from large randomized controlled trials. Data from recent pragmatic studies or the ongoing TAILOR-PCI and POPular-Genetics trials may lead to changes in future PCI guidelines, prompting adoption that is more widespread of genotype-guided antiplatelet therapy after coronary intervention.

In addition to the evidence barrier, another factor hindering genotype adoption is concern that genetic testing may disrupt workflow in the busy cardiac catheterization laboratory. The availability of rapid genetic testing helps to overcome this concern [60]. Preemptive testing, so that results are available at the time of PCI, is another approach to limiting workflow disruption [61].

WARFARIN PHARMACOGENETICS

Challenges With Warfarin

Warfarin is an oral anticoagulant indicated for the prevention and treatment of venous thrombosis and thromboembolic complications associated with atrial fibrillation or heart-valve replacement. Even with the availability of newer oral anticoagulants, warfarin remains commonly prescribed, especially for individuals unable to tolerate or afford newer agents or with indications not covered by newer agents. Although in use for over 60 years, warfarin remains a difficult drug to manage primarily because of its narrow

therapeutic index and the wide interpatient variability in the dose required to obtain optimal anticoagulation. For most indications, warfarin is dosed to achieve an international normalized ratio (INR, a measure of its anticoagulant activity) of 2–3. Failure to achieve optimal anticoagulation significantly increases the risk for adverse sequelae. Specifically, subtherapeutic anticoagulation increases the risk for thromboembolism, and supratherapeutic anticoagulation (particularly when the INR exceeds 4) increases the risk for bleeding [66,67]. Because of the difficulty in achieving therapeutic anticoagulation with warfarin, warfarin consistently ranks among the leading causes of serious drug-related adverse events, prompting a boxed warning in its FDA-approved labeling. Achieving therapeutic anticoagulation in an efficient manner is, therefore, a priority for clinicians managing warfarin therapy.

The warfarin dose required to achieve an INR within the therapeutic INR range varies by as much as 20-fold among patients [68]. There are also significant differences in warfarin-dose requirements by ancestral origin, with African Americans generally requiring higher doses and Asians requiring lower doses compared to those of European descent [69]. Thus, a major challenge with initiating warfarin therapy is predicting the dose that will produce therapeutic anticoagulation for a particular patient. Traditionally, warfarin is initiated at a similar dose for all patients, typically 5 mg/day, with the dose adjusted according to INR results. The problem with this trial-and-error dosing approach is that it often leads to overanticoagulation during the initial months of therapy when the risk for bleeding is greatest [70]. Alternatively, for patients requiring doses higher than 5 mg/day, it can delay attainment of therapeutic anticoagulation. Clinical factors, including age, body size, diet, medications that interfere with warfarin metabolism, and renal and hepatic function, influence warfarin-dose requirements [71–73]. However, clinical factors alone account for only 15%–20% of the overall variability in warfarin dose, and considering these factors alone is often insufficient to predict the dose of warfarin a patient will require [74,75].

Genes Affecting Warfarin Pharmacokinetics and Pharmacodynamics

It is widely recognized that genotype significantly influences the pharmacokinetics and pharmacodynamics of warfarin and contributes to the interpatient variability in warfarin-dose requirements [76,77]. The major genes influencing warfarin pharmacokinetics and pharmacodynamics are cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit 1 (VKORC1), respectively. As shown in Fig. 6.5, CYP2C9 metabolizes the *S*-enantiomer of warfarin to the inactive 7-hydroxy warfarin protein. The *S*-enantiomer possesses approximately three to five times the anticoagulant effects of *R*-warfarin [70]. The VKORC1 gene encodes for the target site of warfarin. Specifically, warfarin inhibits vitamin K epoxide reductase complex 1

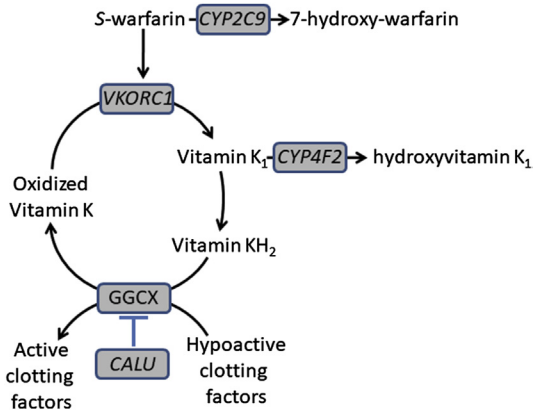

FIGURE 6.5 Genes involved in warfarin pharmacokinetics and pharmacodynamics. The CYP2C9 gene influences the drug's pharmacokinetics; other genes affect warfarin's pharmacodynamics. CYP2C9, cytochrome P450 2C9; VKORC1, vitamin K epoxide reductase complex subunit 1; GGCX, gamma-glutamyl carboxylase; CALU, calumenin; CYP4F2, cytochrome P450 4F2; vit, vitamin.

(VKORC1), thus preventing formation of vitamin K hydroquinone, a necessary cofactor for the gamma-carboxylation and activation of clotting factors II, VII, IX, and X.

Data from numerous candidate gene and GWASs consistently show that the CYP2C9 and VKORC1 genotypes affect warfarin-dose requirements [74–76,78,79–82]. There are additional data that the rs12777823 G > A polymorphism on chromosome 10 near the CYP2C18 gene influences warfarin-dose requirements in African Americans [83]. Other genes, including CYP4F2 and calumenin, produce lesser effects on warfarin pharmacodynamics and provide minor contributions to the variability in warfarin-dose requirements [84–87]. These genes are described in detail in the following sections. The major goal of warfarin pharmacogenetics is to improve the accuracy of warfarin dosing and, consequently, to reduce the risk for adverse sequelae with warfarin therapy.

CYP2C9

The CYP2C9 gene is located on chromosome 10q24.1, and approximately 60 CYP2C9 alleles have been described, as detailed in Chapter 1. The CYP2C9*2 and *3 alleles are the most extensively studied and result from variants in the coding regions of the gene, as shown in Table 6.3. The CYP2C9*2 amino acid substitution occurs on the outer surface of the enzyme, and the *3 substitution occurs internally [88,89]. Neither substitution appears to affect substrate binding. Rather, evidence suggests that the

TABLE 6.3 Nucleotide Base Pair Or Amino Acid Substitution, Location, and Minor Allele Frequencies of Variants Associated With Warfarin Dose Response in Various Populations [69,78,85,100,101,230,231]

			Minor Allele Frequency			
Polymorphism	**Base Pair or Amino Acid Substitution**	**Location**	**Caucasian**	**African American**	**Asian**	**Egyptian**
CYP2C9						
*2 (rs1799853)	p.R144C	Exon 3	0.13–0.14	0.01–0.02	<0.01	0.12
*3 (rs1057910)	p.I359L	Exon 7	0.06–0.11	0.01	0.02–0.04	0.09
*5 (rs28371686)	p.D360E	Exon 7	<0.01	0.01	<0.01	0.01
*6 (rs9332131)	10601delA	Exon 5	<0.01	0.01	<0.01	NR
*8 (rs7900194)	p.R150H	Exon 3	<0.01	0.05–0.07	0.01	<0.01
*11 (rs28371685)	p.R335W	Exon 7	<0.01	0.01–0.04	<0.01	NR
VKORC1						
rs9923231	c.1639G>A	Promoter	0.39	0.11	0.91	0.46
rs9934438	c.1173C>T	Intronic	0.40	0.10	0.90	NR
CYP4F2						
rs2108622	p.V433M	Exon 11	0.24	0.07	0.23	0.42
CALU						
rs339097	c.A>G	Intronic	<0.01	0.14	0.02	0.02

APOE, apolipoprotein E; *CALU*, calumenin; *CYP*, cytochrome P450; *VKORC1*, vitamin K epoxide reductase complex subunit 1.

CYP2C9*2 and *3 alleles disrupt formation of intermediate compounds in the CYP2C9 catalytic cycle leading to significant reductions in enzyme activity [90]. As a result, the clearance of *S*-warfarin is reduced approximately 40% with the CYP2C9*1/*2 genotype, up to 75% with the *1/*3 genotype, and up to 90% with the *3/*3 genotype [77,91–93]. Accordingly, individuals with the CYP2C9*1/*2 or *1/*3 genotypes require dose reductions of 30%–47%, respectively, compared to those with the CYP2C9*1/*1 (wild-type) genotype [77]. Individuals with the CYP2C9*3/*3 genotype need up to 80% lower warfarin doses than CYP2C9*1 homozygotes [91,92].

The CYP2C9*2 and *3 alleles are the most common variant CYP2C9 alleles in Caucasians but are much less prevalent among Asians and African Americans, as shown in Table 6.3. The CYP2C9*8 allele is one of the most common variant CYP2C9 alleles in African Americans but is virtually absent in other populations [94]. The CYP2C9 *5, *6, and *11 alleles also occur almost exclusively in African Americans but at much lower frequencies than the *8 allele. The CYP2C9*5, *8, and *11 alleles result from nonsynonymous variants in gene coding regions, whereas CYP2C9*6 results from a nucleotide deletion (Table 6.3). Decreased enzyme activity and clearance of CYP2C9 substrates have been reported with the CYP2C9*5, *6, *8 and *11 alleles [95–98]. However, allele effects appear to be substrate specific. For example, CYP2C9*8 decreases enzyme activity toward warfarin and phenytoin, increases enzyme activity toward tolbutamide, and has no effect on losartan metabolism [95,96,98,99]. The CYP2C9*8 allele decreases clearance of *S*-warfarin by 25%–30% [98]. This decrease coincides with about a 20% reduction in warfarin-dose requirements with the CYP2C9*8 allele [78]. Similarly, lower warfarin-dose requirements have been reported in individuals with a CYP2C9*5, *6, or *11 allele [78,80,100].

In addition to affecting warfarin-dose requirements, the CYP2C9 genotype is associated with the risk of overanticoagulation and bleeding during warfarin therapy [91,101,102]. Specifically, warfarin-treated patients with a CYP2C9 variant allele have about a twofold greater risk for bleeding compared to CYP2C9*1 homozygotes [101,103]. Although the risk for bleeding with a CYP2C9 variant allele is highest during the initial months of warfarin therapy, there is evidence that it persists during chronic therapy [101]. Thus, patients with a CYP2C9 variant allele should be monitored closely for signs and symptoms of bleeding throughout warfarin therapy.

VKORC1

The VKORC1 gene is located on chromosome 16p11.2 and was initially discovered in the context of warfarin resistance, in which exceptionally high doses of warfarin (e.g., >20 mg/day) are needed to achieve therapeutic anticoagulation [104]. Warfarin resistance is due to nonsynonymous (or missense) mutations in the VKORC1 coding region. Variants contributing to warfarin resistance are commonly referred to as "mutations" because they are rare in most populations. An exception is in the Ashkenazi Jewish population, in which individuals have a relatively high prevalence (8%) of the VKORC1 p.D36Y variant, leading to a higher prevalence of warfarin resistance in this population [105].

In 2005, investigators identified common VKORC1 variants, c.1639G>A (rs9923231) and c.1173C>T (rs9934438), that contribute to warfarin-dose variability across the general population [76,106]. These variants occur in VKORC1 regulatory regions and are in near complete LD [69]. In vitro studies in liver tissue showed that the -1639G>A and 1173C>T variants were associated with twofold allelic mRNA expression imbalance (e.g., twofold lower gene expression) [107]. Numerous studies have consistently demonstrated that the -1639A and 1173T alleles are associated with significantly lower warfarin-dose requirements in these populations [68,69,74,75,78,80,108–110]. On average, the -1639

AA, AG, and GG genotypes predict warfarin maintenance doses of 3, 5, and 6 mg/day, respectively. The -1639G>A and 1173C>T SNP are equally predictive of dose requirements [69]. Thus, only one of these Variants needs to be considered for warfarin-dosing decisions. This greatly simplifies genotype-guided warfarin dosing compared to dosing based on VKORC1 haplotype because only one SNP needs to be genotyped.

As shown in Table 6.3, the frequency of the VKORC1 -1639A allele differs significantly by ancestry, with a greater frequency in Asians and lower frequency in African Americans compared to Caucasians. Approximately 50% of Caucasians have the -1639AG genotype, associated with intermediate VKORC1 sensitivity and usual (i.e., 5 mg/day) warfarin-dose requirements. The -1639 AA genotype is the most common genotype in Asians and is associated with high VKORC1 sensitivity and low warfarin-dose requirements. The most common genotype in African Americans is -1639 GG, which is associated with lower VKORC1 sensitivity and high-dose requirements. The difference in VKORC1 genotype distribution among ancestral groups contributes to the higher mean warfarin maintenance dose in African Americans and lower mean dose in Asians, compared to Caucasians, independent of the effects associated with CYP2C9 genotype [69].

CYP4F2

The CYP4F2 enzyme is responsible for metabolizing vitamin K_1 to hydroxyvitamin K_1, as shown in Fig. 6.5 [111]. This process results in less vitamin K_1 being available for reduction to vitamin KH_2, which is necessary for clotting-factor activation. Thus, increased CYP4F2 activity leads to reduced clotting-factor activation. The CYP4F2 p.V433M SNP in exon 11 leads to lower CYP4F2 protein concentration and consequently to greater vitamin K availability [111].

In an initial study of three independent Caucasian cohorts, the CYP4F2 433M/M genotype was associated with approximately 1 mg/day higher warfarin-dose requirements compared to the V/V genotype, with heterozygotes requiring intermediate doses [84]. Subsequent studies in Caucasians and Asians confirmed the association between V433M genotype and warfarin-dose requirements [81,82,112–114]. The CYP4F2 V433M genotype explains approximately 1%–3% of the overall variability in warfarin dose in these populations [82,112]. Interestingly, the association between CYP4F2 genotype and warfarin-dose requirements was not observed in African Americans, Indonesians, Egyptians, or children [78,115–117]. The lack of association in African Americans is likely due to the low frequency of the 433M allele in individuals of African ancestry. However, the 433M allele is common in Indonesians and Egyptians, and the explanation for the negative association in these groups is unclear. Body size provides a greater contribution to warfarin-dose variability in children versus adults, potentially explaining the negative findings with the CYP4F2 genotype in a pediatric population.

CALU

Calumenin inhibits gamma-carboxylation of vitamin K–dependent proteins, suggesting that CALU may influence warfarin-dose requirements [118]. The CALU variant, rs339097 A>G, was associated with warfarin maintenance dose in a diverse patient cohort [87]. Specifically, the minor rs339097G allele was significantly overrepresented among patients requiring high (mean dose of 13 mg/day) versus low (mean dose of 2.6 mg/day) warfarin doses. The association between the rs339097 variant and warfarin-dose requirements was validated in a separate diverse cohort and in a cohort of African Americans [87]. In a pooled analysis of 241 African Americans, the G allele was associated with an 11% higher warfarin dose than predicted based on clinical factors, CYP2C9, and

VKORC1. The correlation of the rs339097G allele with higher warfarin doses was confirmed in a separate study of Egyptian patients, in whom the variant allele was associated with 14 mg/week higher dose requirements [116]. The rs339097G allele is common among African Americans but rare in other populations, as shown in Table 6.3.

Genome-Wide Association Studies

Several GWASs with warfarin have been completed in varying populations and confirm that the CYP2C9 and VKORC1 genes are the primary contributors to warfarin-dose requirements [79,81,82]. In an initial GWAS, investigators surveyed over 538,000 Variants in a discovery cohort of 181 Caucasians and 2 independent replication cohorts consisting of 374 Caucasians taking warfarin [79]. An SNP in complete LD with the VKORC1 -1639G>A variant had the most significant effect on warfarin dose in the index population and explained approximately 25% of the overall variance in dose requirements. The CYP2C9*2 and CYP2C9*3 alleles provided modest contributions to warfarin dose and explained an additional 9% of the variability. These associations were validated in the replication cohort. The combination of VKORC1, CYP2C9, and clinical factors (age, sex, weight, amiodarone use, and losartan use) explained 47% of total variance in warfarin maintenance dose [79].

In a second GWAS, over 325,000 variants were tested for their association with warfarin dose in 1053 Swedish patients [82]. Similar to the first GWAS, the VKORC1 locus had the strongest association with warfarin dose, followed by variants clustered around CYP2C9. After adjustment for VKORC1, CYP2C9, age, and gender, the only other SNP reaching genome-wide significance with warfarin dose was CYP4F2 V433M, which explained an additional 1%–2% of the variability. Results were confirmed in a replication cohort of 588 Swedish patients.

A third GWAS was conducted in Japanese patients [81]. Similar to the studies in Caucasians, VKORC1 was found to provide the greatest contribution to warfarin maintenance dose, with CYP2C9 and CYP4F2 providing lesser contribution.

A fourth GWAS was conducted in African Americans and identified a novel association between the rs12777823G>A polymorphism in the CYP2C cluster on chromosome 10 and warfarin-dose requirements [83]. In addition, to its association with lower warfarin-dose requirements, the rs12777823A allele was correlated with lower *S*-warfarin clearance. Approximately 40% of African Americans carry the rs12777823A allele. Although it is also common in Europeans and Asians, the rs12777823G>A polymorphism has not been associated with warfarin-dose requirements in these populations, suggesting that it does not directly influence warfarin response, but rather may be in linkage disequilibrium with a functional variant or variants influencing warfarin response in African Americans.

Warfarin Pharmacogenetics Dosing Algorithms

There are a number of published algorithms to assist clinicians with warfarin dosing when genotype is known [74,75,109,119–124]. Most contain the VKORC1 -1639G>A or 1173C>T SNP, CYP2C9 *2 and *3 alleles, and clinical factors, including age, body size, and amiodarone use. The two algorithms derived from the largest populations and most commonly cited are those by the International Warfarin Pharmacogenetics Consortium (IWPC) [75] and by Gage and colleagues [74]. The latter is commonly referred to as the warfarindosing.org algorithm. It was derived from a population of 1015 warfarin-treated patients, 83% of whom were Caucasian, and validated in 292 patients with similar characteristics. The IWPC was formed by members of the Pharmacogenomics Knowledge Base (PharmGKB) in collaboration with investigators from the international community with the initial purpose of creating a dosing equation that would

have global clinical utility (see Chapter 3 for further information about the PharmGKB) [75]. Researchers from 21 groups representing 11 countries and four continents pooled genotype and phenotype data for over 5000 chronic warfarin-treated patients (55% Caucasian, 30% Asian) [69,75]. Data from 4043 patients were used to derive the IWPC algorithm, with validation in the remaining 1009 patients.

The Gage et al. and IWPC algorithms include clinical factors and the CYP2C9*2 and *3 and VKORC1 -1639G>A genotypes and provide similar dose estimations. Both are freely available through the www.warfarindosing.org website. The algorithm available through the www.warfarindosing.org. Website allows for refinement of dose estimation based on INR response to previous warfarin doses and thus may be preferred over the use of other algorithms when genotype results are not immediately available [125].

The Gage et al. and IWPC algorithms explain between 30% and 60% of the variability in warfarin dose requirements in Caucasians but less of the variability in African Americans and Asians [69,123]. They are superior to other dosing methods, especially for patients requiring low (≤3 mg/day) or high (≥7 mg/day) warfarin doses [75,126]. However, warfarin pharmacogenetic algorithms have several limitations. First, they estimate doses within 20% of the actual dose only about 50% of the time [127–129]. Pharmacogenetic algorithms do not include all of the factors known to affect warfarin-dose variability, such as vitamin K intake and many of the drugs known to interact with warfarin. In addition, most algorithms, including the www.warfarindosing.org and IWPC algorithms, do not contain genetic variables that are common or specifically affect dose in African Americans (e.g., CYP2C9*8, rs12777823), likely contributing to lesser accuracy in this population [129,130]. Also, many algorithms do not include genetic variants associated with warfarin resistance and are thus less accurate at predicting higher than usual doses [131]. Finally, pharmacogenetic algorithms may overestimate doses in elderly patients (>65 years) who often require warfarin doses of less than 2 mg/day [132]. As such, pharmacogenetic algorithms are useful to reduce uncertainty about initial warfarin doses. However, they should not replace routine INR monitoring and clinical judgment.

Warfarin Labeling Revisions

In August 2007, the FDA approved the addition of pharmacogenetic data to the warfarin labeling. The pharmacogenetic content of the label was further revised in January 2010, with the addition of a dosing table based on the CYP2C9*2 and *3 alleles and VKORC1 genotypes (Fig. 6.6). The table may be used to estimate initial warfarin dose when genotype is known, with subsequent dose adjustment based on INR results. An advantage of the table over dosing algorithms is its ease of use. However, it does not include clinical factors that influence dose variability and has been shown to be less accurate at predicting warfarin-dose requirements compared to pharmacogenetics algorithms [126].

Early Studies of Genotype-Guided Warfarin Dosing

A comparative effectiveness study showed that patients who were offered free CYP2C9 and

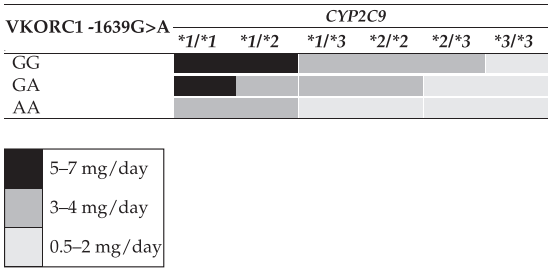

FIGURE 6.6 Warfarin dose by CYP2C9 and VKORC1 genotypes, as reproduced from the FDA-approved warfarin label.

VKORC1 genotyping, with results provided to their physician with an interpretive report, had fewer hospitalizations for any cause and fewer hospitalizations for bleeding or thromboembolism during the initial 6 months of warfarin therapy compared to historical controls [133]. In contrast, two small, randomized trials showed no benefit with a genotype-guided approach over traditional dosing [131,134]. In particular, both trials showed that the percent of time spent within the therapeutic range, which is often used as a marker of bleeding or thrombotic risk, was similar between patients dosed based on genotype plus clinical factors or clinical factors alone. However, these trials were small in size, including only 206 to 230 patients. In addition, an exploratory analysis of one trial, called the CoumaGen-I trial, showed a benefit with pharmacogenetic dosing for two groups of patients: those with more than one variant allele and those with the wild-type genotype (VKORC1 -1639 CC and CYP2C9 **1/*1*) [131]. In contrast, single-variant allele carriers appeared to have no benefit from genotype-guided dosing, likely because patients with a single variant usually require a warfarin dose of about 5 mg/day, which is the dose commonly started in patients new to warfarin. In contrast, those with multiple variant alleles usually require lower doses (e.g., 3–4 mg/day), and those with the wild-type genotype usually require higher doses (6–7 mg/day). Thus, starting a dose of 5 mg/day in individuals with multiple or no variant allele would probably result in over- and undercoagulation, respectively.

The subsequent CoumaGen-II involved (1) a blinded, randomized comparison of two pharmacogenetic dosing algorithms; and (2) a clinical effectiveness comparison of genotype-guided warfarin dosing ($n = 504$) versus standard dosing ($n = 1911$) [135]. For the comparison of dosing algorithms, a modified version of the IWPC algorithm (taking into account smoking status and different INR targets) was compared to a three-step algorithm in which the CYP2C9 genotype was not taken into account until day 3, and a dose-revision algorithm was used starting on day 4, taking into account warfarin dosing history and INR. The three-step algorithm was found to be noninferior, but not superior, to the modified IWPC algorithm in terms of the percent of out-of-range INR values at one and 3 months. Thus, the two pharmacogenetic dosing approaches were combined for comparison with standard dosing. Genotype-guided therapy (using either algorithm) was superior to standard warfarin dosing in reducing the percent of out-of-range INRs and the percent of INRs greater than or equal to 4 or less than or equal to 1.5. An additional analysis suggested that there were fewer serious adverse events at 3 months in the genotype-guided arm.

Large Randomized Clinical Trials of Genotype-Guided Warfarin Therapy

Two multicenter, randomized trials assessing the clinical efficacy of genotype-guided warfarin dosing were published in 2013 with differing results. The details of these trials are shown in Table 6.4. The European Pharmacogenetics of Anticoagulation Therapy (EU-PACT) trial was conducted in a homogenous European population and randomized participants to genotype-guided warfarin dosing, with use of a pharmacogenetic algorithm, or to a traditional fixed-dose approach (e.g., 5 mg/day) [136]. The trial showed significantly greater time in therapeutic range, the primary endpoint, with use of a pharmacogenetics algorithm. The Clarification of Optimal Anticoagulation through Genetics (COAG) trial was conducted in a diverse U.S. population and randomized participants to dosing with a pharmacogenetics algorithm, including both genotype and clinical factors or to dosing with a clinical algorithm, containing clinical factors only [137]. In contrast to the EU-PACT trial, the COAG trial showed no difference in the primary endpoint of time in therapeutic range between dosing strategies. Among African

TABLE 6.4 Clinical Trials that Assessed the Clinical Utility of Genotype-Guided Warfarin Dosing

Trial Name or Acronym	Intervention	Outcomes	Study Population	Findings
Clarification of Optimal Anticoagulation Through Genetics (COAG) [137]	Genotype guided dosing with a pharmacogenetics dosing algorithm (including the CYP2C9*2 and *3, VKORC1 -1639G>A) variants versus clinically guided dosing with a dosing algorithm including clinical factors only	Time spent within the therapeutic INR range in the first 4 weeks	n = 1015 patients	Mean percent of time in range was 45.2% in the genotype-guided group and 45.4% in the clinically-guided group ($P = .91$) Among blacks, the mean time in range was 35.2% in the genotype-guided group versus 43.5% in the clinically guided group ($P = .01$).
European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) [136]	Genotype-guided warfarin dosing, with use of a pharmacogenetic algorithm (including the CYP2C9*2 and *3, VKORC1 -1639G>A> variants) versus standard dosing consisting of 10 mg on day 1 (5 mg for patients older than 75 years), 5 mg on days 2 and 3, then dosing according to usual practice	Percent of time in an INR range of 2.0–3.0 during the initial 12 weeks	n = 455 patients (61% male, 98.5% white, mean age 67 years)	Primary: Mean percent of time in therapeutic range was 67.4% in the genotype-guided group and 60.3% in the control group ($P < .001$)
Genetics Informatics Trial (GIFT) of Warfarin to Prevent DVT [139]	Genotype guided dosing with a pharmacogenetic dosing algorithm (including the CYP2C9*2 and *3, VKORC1 -1639G>A>, and CYP4F2 Val 433Met variants) versus clinically guided dosing with a dosing algorithm including clinical factors only	Primary: Composite of death, venous thromboembolism, major bleeding, and INR ≥ 4 during the initial 4–6 weeks	N = 1597, age ≥65 years, 64% women, 91% Caucasian, undergoing elective knee or hip replacement surgery	Event rate was 14.7% in the clinical arm and 10.8% in the genotype-guided arm, representing a 27% reduction in the primary endpoint with genotype-guided dosing

DVT, deep vein thrombosis; *GI*, gastrointestinal; INR, international normalized ratio; *PE*, pulmonary embolism.

Americans, who composed 27% of the population, pharmacogenetic dosing resulted in less time in therapeutic range and more time with an INR over 3. The occurrence of INRs ≥4 and the composite secondary outcome of any INR ≥4, major bleeding, or thromboembolism was similar between groups in the population overall as well as in the African American subset. Although there was no difference between groups in the individual secondary endpoint of major bleeding, there were numerically more bleeds in the clinically dosing arm, and the difference between groups was statistically significant at 6 months (4% vs. 1%, $P = .021$).

There were several differences between the two trials that might have contributed to the disparate results, including differences in the comparator arm, differences in patient populations, and lack of a loading dose in the COAG trial. Dosing in the genotype-guided arm of both trials was based on the CYP2C9*2, *3, and VKORC1 -1639G>A genotypes. These are the primary genotypes influencing warfarin dose in persons of European ancestry, and thus appropriate for the EU-PACT trial. Additional variants, namely the CYP2C9*5, *6, *8, *11 and CYP2C rs12777823 variants, contribute to warfarin-dose requirements in African Americans. Data published since the COAG trial show that failure to account for these variants leads to significant overprediction of warfarin doses in African Americans, likely contributing to the greater likelihood of supratherapeutic anticoagulation with genotype-guided dosing among African American participants of the COAG trial [130]. Additional data suggest that loading doses may be especially important to efficiently attain therapeutic anticoagulation for patients with one or no genetic variants associated with decreased warfarin-dosing requirements [138]. Most patients of European or African ancestry would fall into this category. Thus, failure to use loading doses in the COAG trial may have contributed to the inability to detect differences in time in therapeutic range in the initial weeks of therapy.

The primary endpoint for both the EU-PACT and COAG trials was time in therapeutic range, which is a surrogate marker for risk of venous thromboembolism or bleeding (Table 6.4). In contrast, the more recent Genetics InFormatics Trial (GIFT), which enrolled more patients than the EU-PACT and COAG trials combined, was powered to examine venous thromboembolism and major bleeding with genotype-guided dosing [139]. GIFT included older patients requiring prophylaxis for venous thromboembolism after hip or knee arthroplasty. Similar to the COAG trial, participants in GIFT were randomized to dosing with use of a pharmacogenetics versus clinical algorithm. Patients in the pharmacogenetic dosing arm spent significantly more time in the therapeutic INR range through the first 4 weeks of therapy. The investigators reported a 27% reduction in the composite endpoint of venous thromboembolism, major bleeding, INR ≥4, and death with pharmacogenetic versus clinical dosing. This was driven mainly by a reduction in supratherapeutic INR values with pharmacogenetic dosing. Genotyping in GIFT was similar to that for the COAG and EU-PACT trials with the addition of the CYP4F2 Val433Met genotype. The majority of GIFT participants (91%) were of European ancestry, and thus the exclusion of CYP2C variants common in African Americans unlikely had a significant impact on the results. However, refinement of warfarin-dosing algorithms through the inclusion of additional variants influencing dose requirements across populations would be expected to further improve dosing accuracy and hence clinical outcomes with genotype-guided dosing.

Pharmacogenetic Guidelines

The CPIC guidelines for dosing warfarin based on genotype were originally published in 2011, and were updated in 2017 to reflect the more recent data with genotypes important for African Americans [121]. Similar to CPIC guidelines for clopidogrel, the warfarin CPIC guidelines do not address when to order genotyping, leaving that to the discretion of the clinician. Based on the strong and consistent evidence that genotype influences warfarin-dose requirements, the guidelines recommend dosing warfarin based on genotype when appropriate genotype information is available. Separate recommendations are provided for patients of African and non-African ancestry, as outlined in Fig. 6.7. For those of non-African ancestry, the recommendation is to dose warfarin based on the VKORC1 -1639G>A (or 1173C>T), CYP2C9*2, and CYP2C9*3 genotypes using one

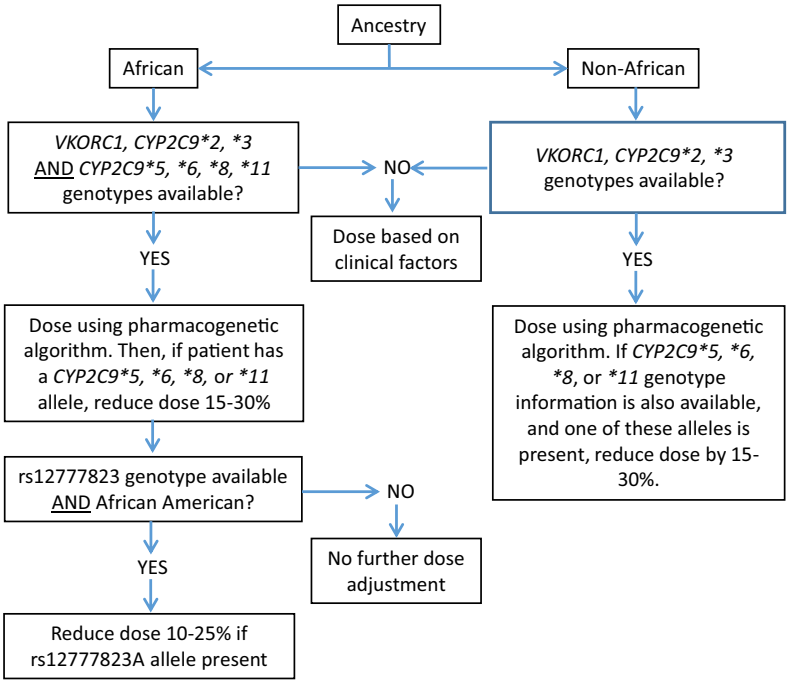

FIGURE 6.7 Clinical Pharmacogenetics Implementation Consortium Guidelines for genotype-guided warfarin dosing.

of the published pharmacogenetic dosing algorithms. Recognizing the importance of the CYP2C9*5, *6, *8, and *11 variants in persons of African ancestry, the recommendation for this population is to only use genotype information to dose warfarin when these additional variants are also tested. Otherwise, the recommendation is to dose warfarin based on clinically factors alone. For African Americans, an additional dose reduction is recommended when genotyping includes the rs12777823G>A variant, and the rs12777823A allele is present.

Opportunities and Challenges for Warfarin Pharmacogenetics

Genotype-guided warfarin dosing has the potential to improve time to reach therapeutic anticoagulation and reduce the risk for adverse events during the warfarin initiation period. Thus, despite the inconsistent clinical trial data, some institutions have started to offer genotyping to guide warfarin initiation based on the large body of evidence supporting genetic associations with warfarin-dose requirements and bleeding risk [58,140]. However, several challenges remain that limit broader initiation at present. One of the biggest challenges is the lack of reimbursement for genetic testing by most insurers. In particular, the Centers for Medicare and Medicaid Services has announced that coverage for genetic testing to guide warfarin therapy would be denied unless testing is provided in the context of a controlled clinical study. Whether recent outcomes from GIFT will alter this position remains to be determined. Cost-effectiveness data are important for policy decisions, and studies have demonstrated the cost-effectiveness of genotype-guided warfarin dosing in the setting of atrial fibrillation [141]. In cases in which genotyping is done during hospitalization, which is often for patients newly

starting warfarin, coverage for the cost of testing may fall to the hospital. In these cases, it may be important to demonstrate the benefits of genotyping to hospital administrators, in terms of effects on time to therapeutic INR or clinical outcomes (e.g., bleeding or thrombotic events). This is especially important during the initial 30 days following discharge when hospitals may not be reimbursed for readmissions under the Center for Medicare and Medicaid Services Hospital Readmissions Reduction Program.

Another challenge is obtaining timely genotype results, ideally before the first dose of warfarin. This would require either rapid genotyping or preemptive genotyping ahead of the need for warfarin. With a preemptive approach, genotype results may be placed in the medical record so that they are available in the event that warfarin is needed. Another approach that has been used is to base the initial warfarin dose on clinical factors alone, and then obtain genotype results prior to the second dose [140].

TRIALS AND TRIBULATIONS OF PHARMACOGENETICS OF AGENTS USED TO TREAT DYSLIPIDEMIA

Overview of Statin Pharmacokinetics and Pharmacodynamics

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also known as statins, are commonly prescribed to reduce low-density lipoprotein (LDL) cholesterol. Multiple randomized, placebo-controlled, clinical trials have demonstrated that statins reduce the relative risk of major coronary events [142]. However, there is substantial variability in LDL cholesterol lowering and clinical outcomes with statin therapy [143,144]. In addition, although these medications are well tolerated, a small percentage of patients can experience the serious adverse event of rhabdomyolysis. Candidate genes associated with the pharmacokinetics and pharmacodynamics of statins have been studied for their contribution to this variability.

Table 6.5 shows the various enzymes involved in statin transport and metabolism. The CYP3A4 enzyme plays an important role in the metabolism of lovastatin, simvastatin, and atorvastatin; fluvastatin and rosuvastatin are metabolized primarily by CYP2C9 [145]. Pravastatin is primarily eliminated unchanged in the feces and urine, and pitavastatin is a substrate for UGT1A3 and UGT2B7. Most statins are transported by OATP1B1 into hepatocytes, in which they are competitive inhibitors of HMG-CoA reductase, the rate-limiting enzyme involved in cholesterol synthesis. All statins share this uniform mechanism of action.

Pharmacogenetics of Statin Safety

Statins are generally well tolerated but can facilitate myopathies in some individuals, with symptoms ranging from mild myalgias to life-threatening rhabdomyolysis. In clinical trials, the reported incidence of statin-associated myalgias is 3%–5%, with greater risk with the use of high-dose statin therapy [146]. Fatal rhabdomyolysis is rare, occurring in an estimated 1.5 patients per 10 million prescriptions [146].

The mechanism underlying statin-associated myopathies is unknown but likely is related to increased statin concentrations [146]. Statin concentrations are affected by extensive first-pass uptake into hepatocytes and the rate of metabolism by hepatic CYP450 enzymes. Hepatic uptake appears to be necessary for statin clearance. Genetic variants for hepatic uptake and statin metabolism have been associated with altered statin concentrations and risk for myopathy [145].

The strongest genetic association with statin-induced myopathy has been detected with genes involved in statin hepatic uptake. Statins are transported into hepatocytes by OATP1B1, which is encoded by the SLCO1B1 gene. Organic anion transporting polypeptides or solute carrier

TABLE 6.5 Drug Metabolizing Enzymes and Transporter Proteins for Various Statins

Statin	Metabolizing CYP450 Enzymes	Active Metabolite	Transporter Proteins
Atorvastatin	3A4, 3A5, 7A1	Yes	OATP1B1, ABCG2
Fluvastatin	2C9, 3A4, 2C8	No	OATP1B1, ABCG2
Lovastatin	3A4, 3A5, 2C8	Yes	OATP1B1, ABCB1
Pitavastatin	2C9		OATP1B1, ABCB1
Pravastatin	None	No	OATP1B1, ABCB1, ABCG2, ABCC2
Rosuvastatin	2C9, 2C19	Yes	OATP1B1, ABCG2
Simvastatin	3A4, 3A5, 2C8	Yes	OATP1B1, ABCB1, ABCG2

Approximately 10% of rosuvastatin is metabolized by CYP2C.

organic (SLCO) anion transporters are vital for drug uptake into tissues and organ systems. These transporters are found in the liver, intestine, and central nervous system. All statins are transported by this mechanism into hepatocytes.

A genome-wide analysis in participants of the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) study demonstrated an association between SLCO1B1 genotype and myopathy risk with statin therapy [147]. More than 300,000 variants were genotyped in 85 patients who developed confirmed myopathy (cases) and 90 patients who did not develop myopathy (controls) during treatment with simvastatin 80 mg/day. The only variant reaching genome-wide significance for association with statin-induced myopathy was rs4363657, a noncoding SNP located within the SLCO1B1 gene on chromosome 12. The rs4363657 variant was in near complete LD with the nonsynonymous rs4149056 (c.521T>C, p.V174A) variant. The odds ratio for myopathy was 4.5 (95% CI: 2.6 to 7.7) with a single rs4149056C allele and nearly 17 (95% CI: 4.7 to 61) with the CC versus TT genotype. In a replication cohort of patients who received simvastatin 40 mg/day as part of the Heart Protection Study, rs4149056 remained associated with statin-induced myopathy (OR: 2.6, 95% CI: 1.3 to 5.0).

The haplotypes containing the SLCO1B1 521C allele include SLCO1B1*5, **15* and **17* [148]. The 521C allele is associated with low OATP1B1 activity and increased plasma concentrations of relevant substrates [148]. Consistent with previous data, in a study of patients receiving atorvastatin, simvastatin, or pravastatin, SLCO1B1*5 was associated with increased adverse effects from statins, defined as statin discontinuation for any side effect, myalgia, or creatinine kinase greater than three times the upper limit of normal [149]. The association between SLCO1B1*5 and statin-induced myopathy was further validated in two additional studies [150,151]. Data from one of these studies suggest the association may be stronger for simvastatin than atorvastatin [150]. In contrast, there is little evidence that SLCO1B1 rs4149056 is associated with myopathy for pravastatin or rosuvastatin [149,152].

The CPIC published guidelines related to simvastatin dosing when SLCO1B1 genotype results are available [148]. Regardless of genotype, the simvastatin 80-mg dose should be avoided. For heterozygous (CT genotype) and homozygous variant carriers (CC genotype), CPIC recommends using a lower simvastatin dose or considering an alternative statin (e.g., pravastatin or rosuvastatin) and considering routine creatine kinase (CK) surveillance. The guidelines also states that factors other

than genotype are implicated in statin-induced myopathy and should be considered. These factors include increased statin dose, advanced age, small body-mass index, female gender, metabolic comorbidities (e.g., hypothyroidism), intense physical exercise, and Asian or African ancestry.

Variants in other transporter genes have also been found to be associated with statin-induced myopathy. A ABCC2 variant was associated with simvastatin discontinuation and dose reduction [153]. There is also a theoretical role for ABCB1 in statin myopathy. In addition, some genetic variants in the CYP450 system have been associated with statin-induced myopathy, but this relates specifically to the metabolic pathway of each statin [154].

The role of genetics in antibody-mediated myopathy with statin therapy has also been evaluated [154–156]. This form of myopathy is far less common but does persist even after statins have been discontinued. The HLA Class II DRB1*11:01 allele was associated with this unique form of myopathy; however, it is unclear if statin exposure is the sole trigger for this disease state. Until further evidence is available, genotyping to predict this unique form of statin myopathy is not recommended.

Genetic Contributors to Plasma Lipid Levels

Plasma lipid levels are highly heritable traits, with over 50% of the interindividual variation in LDL cholesterol levels attributed to genetic factors [157]. Mutations in single genes with severe functional consequences contribute to Mendelian lipid disorders (also referred to as familial hypercholesterolemia); polymorphisms in multiple genes, each with fairly weak to moderate effects, contribute to variation in lipid levels across the general population. Among the most notable discoveries from Mendelian studies were genetic mutations in the LDL receptor that cause significantly elevated LDL cholesterol and premature coronary heart disease [158]. Information about variants in the LDL receptor gene and other variants associated with Mendelian lipid disorders are included in the product labeling for some statins (Table 6.1).

As evidence of multigenic contributions to cholesterol levels across the population, a large GWAS examining approximately 2.6 million variants in over 100,000 individuals identified variants at 95 loci associated with lipid levels [159]. In addition, to genotype, lipid levels are also affected by lifestyle, diet, and other environmental factors, thus underscoring the complexity of dyslipidemia [160]. This complexity renders it difficult to identify the genetic factors that influence statin response.

Pharmacogenetics of Statin Efficacy

Given the important role of statins in reducing cardiovascular disease risk, pharmacogenetic studies of statins are plentiful. The majority of data are related to statin efficacy. There are two major outcomes in these studies: LDL cholesterol lowering or clinical event risk lowering with statin therapy. The efficacy-related studies follow either a candidate-gene approach (single or multiple genes) or GWAS. There are several plausible candidate genes that have been well studied for their role in statin response. These include genes encoding for HMG-CoA reductase (HMGCR), the target of statin therapy; apolipoprotein E (ApoE), which transports cholesterol through the bloodstream; and organic anion transporting polypeptide 1B1 (OATP1B1), which transports statins to the liver [161–167]. However, the data with these genotypes are inconsistent. In addition, a large metaanalysis of GWASs was published [168]. The authors analyzed two separate cohorts of patients from randomized controlled trials and observational studies of statin therapy via two steps of genome-wide analysis. The first and second cohorts included 18,596 and 21,975 patients, respectively. Metaanalysis of the first cohort

found three loci with 13 variants that reached genome-wide significance ($P < 5 \times 10^{-8}$) for association with low-density lipoprotein cholesterol (LDL-C) response to statin treatment. The three loci were in the genes encoding apolipoprotein E (ApoE), lipoprotein (a) (LPA), and the rapamycin-insensitive companion of mammalian target of rapamycin (mTOR) (RICTOR). The association with APoE and LPA loci persisted in the second cohort and two new loci (SORT1/CELSR2/PSRC1 and SLCO1B1) were detected. The authors also performed a genome-wide conditional analysis of these polymorphisms to detect combined effects. They found 14 variants that were independently associated with LDL-C response to statin therapy including those from LPA, APoE, SLCO1B1, and SORT1/CELSR2/PSRC1. Those 14 variants explained approximately 5% of the variability in LDL-C response to statin therapy. The majority of genes identified in this study were associated previously with statin efficacy. These results further underscore that variation in LDL-C reduction from statin therapy is genetically complex.

Despite the many studies assessing the pharmacogenetics of statin responsiveness, no concrete genotype associations with statin efficacy have been made. There are several reasons why genetic association studies with statins are difficult. First, each statin has its own specific metabolic process. Therefore, genetic variation in a particular metabolizing enzyme will not affect response to all statins. In addition, baseline lipid levels are affected by many factors beyond genetics. Thus, the effect of statin therapy on lipid levels is laid over the backdrop of an already complex physiology. Because each study assesses a different statin and a different patient population, with varying underlying pathophysiologies, it is difficult to find genotypes that consistently affect statin response. A composite of variants from several genes and clinical factors, each explaining some small portion of statin response, will likely be necessary to truly predict statin response.

Ezetimibe Pharmacogenetics and NPC1L1 Genotype

Ezetimibe lowers LDL-C by blocking the Niemann–Pick C1-like 1 (NPC1L1) intestinal cholesterol transporter. The first genetic association reported with ezetimibe was in a treatment-resistant patient who was found to have rare nonsynonymous NPC1L1 gene mutation [169]. The gene was subsequently sequenced in additional patients, and 140 variants and five insertion/deletion polymorphisms were identified.

Multiple studies have assessed the association between NPC1L1 genotype and LDL-C response to ezetimibe. The first study found a haplotype, consisting of three variants (1735C, 25342A, and 27677T), associated with the percent of LDL-C reduction from baseline [170]. Specifically, subjects possessing at least one copy of the NPC1L1 haplotype had smaller LDL cholesterol reduction from baseline with ezetimibe ($-23.6 \pm 1.6\%$ vs. $-35.9 \pm 4.0\%$, $P < .01$). The second study also used three NPC1L1 variants to create haplotype groups, albeit different variants from the previous study [171]. They found that possession of the haplotype −133A/-18A/1679G was associated with greater ezetimibe-induced LDL-C lowering. However, because each study found different NPC1L1 variants and haplotypes to be associated with ezetimibe response, it is yet unclear which polymorphism(s) is actually underlying altered LDL-C response. In addition, there were impressive differences in the allele frequencies for studied variants by ancestral origin. Thus, whether ancestral differences exist in the genotype–ezetimibe response association is unclear.

Other variants in NPC1L1 have been associated with baseline cholesterol absorption and lipid profile [172,173]. The exons of NPC1L1 were resequenced in 7364 patients with coronary heart disease and 14,728 controls of varied ancestry, and 15 distinct variants were identified [173]. Heterozygous carriers of inactivating mutations had a mean LDL-C level that was

12 mg/dL lower than noncarriers, which was a statistically significant difference. In addition, carrier status was associated with a relative reduction in coronary heart-disease risk of 53%. This makes it difficult to distinguish between the baseline and pharmacogenetic effects of these variants. Another group of investigators discovered similar associations between NPC1L1 genotype and cardiovascular events that persisted after controlling for total cholesterol, LDL-C, and other cardiovascular risk factors [174].

At this time, because of these issues, regular genotyping for NPC1L1 polymorphisms to predict ezetimibe response cannot be recommended. In addition, as discussed with statins, lipid homeostasis involves several pathways with many different genes. Therefore, a polygenetic approach will likely be necessary to assess ezetimibe response.

Opportunity in Pharmacogenetics: Potential to Improve Management of Dyslipidemia

In clinical trials, statins have been shown to reduce the risk for adverse cardiovascular events in patients with established cardiovascular disease as well as those at high risk for cardiovascular disease [8]. However, not all patients derive protection against cardiovascular events with statins, and some patients experience intolerable (e.g., myopathy) and potentially life-threatening (e.g., rhabdomyolysis) adverse effects. Pharmacogenetics offers the potential to identify patients who will either not benefit from statin therapy or who are at high risk for experiencing adverse statin-induced effects, in whom a statin may be avoided. At present, the evidence most strongly supports a genetic determinant of adverse statin-induced effects for simvastatin (e.g., SLCO1B1 genotype and statin-induced myopathy), and several institutions have implemented SLCO1B1 genotyping into clinical practice, either as a standalone test or as part of a comprehensive genotype panel, to predict risk for simvastatin-induced myopathy [175,176].

TACROLIMUS PHARMACOGENETICS

Tacrolimus is a widely prescribed immunosuppressant indicated after solid organ transplant, including heart transplant. Tacrolimus has a narrow therapeutic index, with subtherapeutic blood concentrations increasing the risk for organ rejection and supratherapeutic concentrations increasing the risk for hypertension, nephrotoxicity, and other adverse drug effects. CYP3A4 and CYP3A5 are involved in the metabolism of tacrolimus, and CYP3A5 genotype has been consistently associated with variability in tacrolimus blood concentrations [177].

The CYP3A5*3 allele is a nonfunctional allele that creates an aberrant splice site in intron 3. Approximately 85% of individuals of European ancestry are homozygous for the *3 allele and have no CYP3A5 activity. These individuals are deemed CYP3A5 nonexpressers. However, individuals with the CYP3A5 *1/*1 or *1/*3 genotype are CYP3A5 expressers, with partial to full CYP3A5 activity. African Americans and Asians are more likely than Caucasians to be CYP3A5 expressers.

Following FDA-label recommended dosing of tacrolimus, lower blood concentrations have been reported in CYP3A5 expressers compared to nonexpressers, placing expressers at an increased risk for organ rejection [177]. Although no data are available in heart-transplant recipients, a randomized controlled trial in kidney-transplant recipients showed that a genotype-guided approach to tacrolimus dosing with higher doses started in individuals with the CYP3A5 *1/*1 or *1/*3 genotype, decreased time to achieve therapeutic drug concentrations compared to a traditional (nongenotype-guided) dosing approach [178]. CPIC guidelines addressing tacrolimus dosing based on CYP3A5 genotype were published in 2015 and recommend increasing the tacrolimus dose by 1.5–2 times the label recommended dose in CYP3A5 expressers [177]. However, the total daily dose should not exceed 0.3 mg/kg day

given the risk for serious adverse effects with supratherapeutic concentrations.

PHARMACOGENETICS OF ANTIHYPERTENSIVES

Hypertension is the most common chronic disease in the United States, affecting more than 85 million Americans [1]. Thus, agents to treat hypertension are among the most commonly prescribed drugs in the United States and other countries. There is significant interpatient variability in response to antihypertensive agents, and factors underlying this variability are not well understood [179,180]. Clinicians currently treat hypertension with a largely trial-and-error approach. It is often necessary to try several agents or combinations of agents before achieving adequate blood pressure control with acceptable tolerability for a given patient. The ability to predict antihypertensive response may allow for earlier initiation of effective antihypertensive therapy, thus reducing the time to adequate blood pressure control and potentially reducing the risk for adverse sequelae from prolonged untreated hypertension. In addition, it may also help to decrease adverse event risk with antihypertensive therapy. With this idea in mind, a number of investigators are searching for genetic determinants of antihypertensive responses. However, in contrast to pharmacogenetic data with warfarin and clopidogrel, pharmacogenetic data with antihypertensives are often inconsistent and even conflicting, which is particularly true with agents that antagonize the renin–angiotensin system. Thus, the potential for improving blood pressure control with pharmacogenetics is largely unrealized.

The International Consortium for Antihypertensive Pharmacogenomics Studies (ICAPS) was formed in 2012 to assist in replication of previously identified genetic variants and the discovery of new variants [179]. ICAPS includes 29 cohorts with more than 345,000 participants from 22 different research groups based in 10 countries on three continents. The work from this group is facilitating the identification and validation of pharmacogenetic markers in hypertension, and some of the data will be summarized in this section.

The following section discusses only the most consistently replicated genetic associations with blood-pressure-lowering effects with antihypertensive agents. In addition, emerging data on genetic determinants of clinical outcomes and adverse drug effects with antihypertensive agents will be discussed.

Genetic Determinants of β-Blocker Response

β-blockers are indicated for the treatment of a number of cardiovascular disorders, including hypertension, coronary artery disease, heart failure, and cardiac arrhythmias. Many β-blockers are metabolized to some degree by CYP2D6 to inactive metabolites, and all β-blockers exert their therapeutic effects by primarily antagonizing the β1-adrenergic receptor, encoded by the ADRB1 gene. Both the CYP2D6 and ADRB1 genes are highly polymorphic and can have significant effects on β-blocker plasma concentration and therapeutic effects, respectively [179,181].

Metoprolol is the β-blocker most extensively metabolized by the CYP2D6 enzyme. A description of the CYP2D6 genetic variants is provided in Chapter 1. The clinical relevance of alterations in β-blocker plasma concentration due to CYP2D6 polymorphism is questionable, given that β-blockers have a wide therapeutic index. Nonetheless, investigators have reported a higher risk of adverse effects with β-blocker therapy among CYP2D6 poor metabolizers compared to normal metabolizers, with normal CYP2D6 function [182]. Specifically, in a cohort of more than 700 metoprolol users, the PM phenotype was associated with a significantly lower heart rate and diastolic blood pressure and a nearly fourfold higher risk of bradycardia compared to the normal metabolizer phenotype [182].

Common variants in the ADRB1 gene, p.S49G and p.R389G, have been correlated with blood pressure lowering effects of β-blocker therapy. These variants are in strong LD, as described in detail in Chapter 1. The R389 allele has been associated with hypertension in multiple large studies [180]. In addition, the majority of studies have shown greater blood pressure reduction with β-blocker therapy with the homozygous RR389 genotype and the S49-R389 haplotype [183–188]. This change in blood pressure response is likely due to an increased coupling of the β1-adrenergic receptor to the second messenger adenylyl cyclase with the R389 allele [180]. Data also suggest that the S49 allele encodes for a receptor that undergoes less internalization resulting in greater downstream signaling.

Given that blood pressure is a surrogate marker and the ultimate goal of antihypertensive therapy is to reduce hypertension-related morbidity and mortality, genetic associations with clinical outcomes have particular relevance. The influence of ADRB1 genotype on the incidence of death, nonfatal myocardial infarction, or nonfatal stroke was examined in participants in the International VErapail SR/Trandolapril (INVEST) study [189]. Patients in this trial had both hypertension and coronary heart disease and were assigned to either atenolol- or verapamil-sustained release (SR)-based treatment. The ADRB1 S49-R389 haplotype was associated with an increased risk for death among patients randomized to verapamil but not those randomized to atenolol. These data suggest that atenolol exerts a protective effect in individuals with hypertension, coronary heart disease, and the S49-R389 genotype. Another study found that polymorphisms in the promoter region of ADRB1 were associated with increased risk of adverse cardiovascular events in patients taking β-blockers [190]. Lastly, R389 and S49-R389 haplotype have been associated with improved clinical outcomes in patients receiving β-blocker therapy for the treatment of atrial fibrillation, ventricular arrhythmias, and heart failure [191–193].

The ability to predict response to β-blockade based on genotype could have important clinical implications. Specifically, in the absence of compelling indications for β-blocker therapy, β-blockers could be reserved for hypertension management in individuals expected to have a good blood pressure response to this drug class based on ADRB1 genotype. Alternative antihypertensive agents could be used in those expected to have little to no blood pressure reduction with β-blockade. β-blockers could also be used as first-line therapy for hypertensive patients with coronary heart disease and the ADRB1 genotype predictive of poor survival. However, further confirmatory data are necessary before genotype will be used clinically for antihypertensive therapy.

Genetic Determinants of Response to Thiazide Diuretics

Thiazide diuretics exert their effect by blocking the reabsorption of sodium and chloride in the distal tubule and therefore an accompanying amount of water. NEDD4L encodes the NEDD4-2 protein, which plays a role in controlling receptor expression of the epithelial sodium channel, ENaC, and potentially other sodium transporters [180]. There is a common functional variant in the NEDD4L gene, rs4149601G>A. The rs4149601G allele increases expression of the ENaC and has been associated with salt-sensitive hypertension with lower plasma renin activity and increased cardiovascular mortality [194,195]. A genetic substudy of the Nordic Diltiazem (NORDIL) study examined the impact of the rs4149601 variant on blood pressure response to diuretic and β-blocker therapy, given the effects of these drugs on inhibiting sodium reabsorption and renin release, respectively. The investigators found that carriers of the NEDD4L rs4149601G allele treated with either a thiazide diuretic or β-blocker had greater systolic and

diastolic blood pressure reduction than similarly treated patients with the AA genotype [196]. A subsequent study confirmed the association of the G allele with blood pressure lowering with thiazide diuretics, but not with β-blockers [195].

The *NEDD4L* rs4149601G allele has also been evaluated for its association with treatment-related clinical outcomes. In the NORDIL genetic substudy, patients with the G allele treated with a β-blocker and/or diuretic had a significant reduction in the risk for MI or stroke compared to those with AA genotype [196]. Consistent with this finding, carriers of the G allele in the International Verapamil SR Trandolapril (INVEST) Study who were not taking a thiazide diuretic had a significantly higher risk of cardiovascular disease than those with AA genotype [195]. Taken together, the data suggest that the *NEDD4L* rs4149601G allele is associated with worse cardiovascular outcomes and that thiazide diuretics and β-blockers may ameliorate this risk. These data suggest that there may be a role for *NEDD4L* genotype in predicting blood pressure response to thiazide diuretics and cardioprotective effects of both diuretics and β-blockers.

Opportunity in Pharmacogenetics: A Look to the Future of Hypertension Management

There are a number of antihypertensive agents available, and it is often difficult to choose which agent to prescribe for a particular patient. Even when following guideline recommendations, there is no guarantee that the prescribed drug will effectively lower blood pressure and prevent adverse outcomes in a given patient. The ability to predict response to antihypertensive therapy based on genotype could eliminate the trial and error approach to hypertension management. Further, an improved understanding of genetic contributions to the mechanisms underlying hypertension could lead to the development of novel therapies to combat the disease.

PHARMACOGENETIC POTENTIAL IN HEART FAILURE

Current Approach to Heart-Failure Management

As shown in Fig. 6.8, standard therapy for heart failure generally consists of an ACE inhibitor or an angiotensin receptor blocker (ARB) (or combination of an ARB plus neprilysin inhibitor) and a β-blocker for morbidity and mortality reduction, with the addition of a diuretic for symptom control. Other agents, including aldosterone antagonists and the combination of isosorbide dinitrate (ISDN) and hydralazine have been shown to further improve outcomes when added to the standard heart-failure drug regimen in select patients [9,197]. Thus, patients may require three to four or more medications for their heart failure alone, in addition, to therapy needed to treat any concomitant diseases.

There are several limitations with our current approach to heart-failure treatment. First, patients often have difficulty adhering to the multidrug regimens that have become the norm in heart failure. Second, many patients cannot safely take target doses of all recommended heart-failure therapies because of low blood pressure. Thus, clinicians must decide which drug to uptitrate and which drug to continue at suboptimal doses or abandon all together. Third, although data from multiple randomized trials demonstrate reductions in morbidity and mortality with vasodilators and β-blockers in overall heart-failure study populations, not all study participants derived benefits from these agents, and some experienced serious adverse effects requiring drug discontinuation. For example, approximately 13% of enalapril-treated subjects in the Studies of Left Ventricular Dysfunction (SOLVD) discontinued the drug because of worsening heart failure or adverse drug effects [198]. Similarly, 14% of patients in the β-blocker arm of the Metoprolol Controlled Release/Extended Release (CR/XL) Randomized Intervention

Current Approach to Heart Failure Treatment			Potential of Pharmacogenetics
Place in Therapy	**Drug Class**	**Limitations**	
Recommended for all patients (in the absence of contra-indications) to reduce morbidity and mortality	ACE inhibitors	• Does not improve outcomes in all patients. • Produces serious (angioedema, hyperkalemia) or intolerable (cough) side effects in some patients.	
	B-blockers	• Does not improve outcomes in all patients. • Worsens symptoms in some patients.	
Useful in many patients to control symptoms	Diuretics	• Significant inter-patient variability in response. • Increases risk for electrolyte derangements and renal dysfunction with supra-therapeutic doses.	
	Digoxin	• Increases risk for ventricular arrhythmias.	
Appropriate in select patients to reduce morbidity and mortality	Aldosterone antagonists	• Does not improve outcomes in all patients. • Produces hyperkalemia and worsening renal dysfunction in some patients.	Streamline treatment based on genetic signatures to include the combination of medications most likely to improve outcomes without causing toxicity for a given patient.
	Hydralazine/ nitrates	• Does not improve outcomes in all patients • Produces significant hypotension in some patients	
	Angiotensin receptor blockers	• Does not improve outcomes in all patients. • Produces serious side effects (angioedema, hyperkalemia) in some patients.	
	Angiotensin neprilysin receptor inhibitors	• Does not improve outcomes in all patients. • Produces serious side effects (angioedema, hypotenion) in some patients.	

FIGURE 6.8 Current approach and potential of pharmacogenetics in the treatment of heart failure.

Trial in Congestive Heart Failure (MERIT-HF) discontinued the drug prematurely because of poor tolerability [199]. Thus, although ACE inhibitors and β-blockers improved outcomes in clinical trial populations as a whole, there is no guarantee that they will improve outcomes without causing harm in an individual patient. Currently, there is no reliable method of predicting response to heart-failure medications, and all patients are treated with a similar "cocktail" of medications. Pharmacogenetics in heart failure aims to identify the combination of drugs most likely to be of benefit without causing harm for a particular patient based on genotype.

Pharmacogenetics of ACE Inhibitors in Heart Failure

The genes discussed thus far in this chapter primarily influence drug response by altering drug pharmacokinetics or pharmacodynamics. However, there are also examples of genes associated with disease prognosis, in which the adverse consequences attributed to a gene are modified by drug therapy. One such example is the ACE gene. Most studies of the ACE gene have focused on a 287-bp insertion/deletion (I/D) polymorphism in intron 16 of the gene, which occurs commonly in persons of European and

TABLE 6.6 Minor Allele Frequencies for Genes Associated With Responses to Hypertension and Heart-Failure Therapies [218,232,233]

Gene	Variant	Caucasians	African Americans
ACE	I/D	0.42	0.56
ADRB1	S49G	0.15	0.13
	R389G	0.27	0.42
ADRA2C	Del322-325	0.04	0.40
NOS3	E298D	0.37	0.14

African descent (Table 6.6). The ACE D allele has been consistently correlated with higher ACE activity and has been shown to confer increased risk for cardiac transplant or death in heart-failure patients, likely because of the deleterious effects of the renin–angiotensin system on heart-failure progression [200–204]. Inhibition of the renin–angiotensin system appears to attenuate the detrimental effects of the ACE D allele. For example, a study of patients with systolic heart failure showed that the adverse effect of the ACE D allele on transplant-free survival was greatest among patients who were not taking β-blockers or were taking less than or equal to 50% of the recommended target ACE inhibitor dose (dose associated with mortality reduction in clinical trials) [202]. Both ACE inhibitors and β-blockers attenuate the renin–angiotensin system. Use of β-blockers and higher ACE inhibitor doses, defined as doses greater than 50% of the target dose, attenuated the detrimental effects of the ACE D allele. A subsequent study in diastolic heart failure revealed similar findings [202].

In contrast to candidate gene studies linking the ACE I/D genotype to adverse outcomes in heart failure, a GWAS examining over 2.4 million variants in nearly 21,000 Caucasians and 3000 African Americans found no association between the ACE gene and heart-failure prognosis [205]. However, the investigators did not account for heart-failure treatment, which has been shown to modify the effect of ACE genotype on outcomes. Nonetheless, it is certainly premature to suggest that ACE inhibitors may not be necessary in individuals without an ACE D allele. However, if a patient is known to carry the ACE D allele, it may be particularly beneficial to use ACE inhibitors at recommended target doses to potentially ameliorate adverse consequences of this genotype.

ADRB1 Genotype: A Case for Targeted β-Blocker Therapy?

β-blockers are well recognized to reduce morbidity and mortality in heart failure by inhibiting the excessive sympathetic nervous system activity that propagates heart-failure progression. However, not all patients benefit from β-blocker therapy [206]. In addition, because β-blockers inhibit cardiac contractility, they must be started in very low doses with careful uptitration to help prevent worsening heart failure. Nonetheless, some patients still suffer cardiac decompensation during β-blocker initiation.

The ADRB1 gene has been extensively studied for its effects on β-blocker response. Although the data are not always consistent, the ADRB1 R389G genotype is associated with the degree of improvement in left ventricular ejection fraction with either metoprolol or carvedilol, with the greatest improvement observed with the RR389 genotype [207,208]. The RR389 genotype has also been associated with greater survival benefits with the β-blocker, bucindolol. Unlike metoprolol, carvedilol, and bisoprolol, which significantly improved survival in heart-failure clinical trials, bucindolol was shown to have a neutral effect on survival [209,210]. However, unlike clinical trials with the other β-blockers, the trial with bucindolol included a larger number of African Americans, and a subgroup analysis revealed improved survival with bucindolol in Caucasians but not African Americans [209]. A subsequent genetic analysis showed that response to bucindolol was dependent on ADBR1 genotype, with a reduced risk

for hospitalization and death with bucindolol in RR389 homozygotes, but not G389 allele carriers [193]. The G389 allele is more common among African Americans than Caucasians (Table 6.6), potentially accounting for the negative effects of bucindolol in persons of African descent. Other studies demonstrate RR389 homozygotes have a significant decrease in all-cause mortality or cardiac transplantation, new onset atrial fibrillation, and ventricular tachycardia and fibrillation burden when treated with bucindolol [191,192,211].

In contrast to data with bucindolol, the ADRB1 genotype was not associated with clinical outcomes with metoprolol or carvedilol [212–216]. However, there are important pharmacological differences among β-blockers, including a sympatholytic effect with bucindolol, which may contribute to differential genotype interactions with response to various drugs. Based on the pharmacogenetic data with bucindolol, ARCA Biopharma sought FDA approval of bucindolol in patients with the ADRB1 RR389 genotype. However, their initial request was denied.

Pharmacogenetics of Nitrates/Hydralazine

In the African-American Heart Failure Trial (A-HeFT), the addition of isosorbide dinitrate (ISDN)/hydralazine to standard therapy with an ACE inhibitor plus/minus a β-blocker significantly improved the primary composite endpoint of death, hospitalizations for heart failure, and quality of life compared to placebo [217]. Based on these data, the ISDN/hydralazine combination was FDA-approved for the treatment of heart failure in self-identified African Americans. Because the effects of adjunctive ISDN/hydralazine therapy has been examined only in African Americans, the benefits of the combination in individuals of other descents are unknown. Consistent with the FDA-approved labeling, current joint guidelines from the American College of Cardiology and American Heart Association recommend ISDN/hydralazine for African Americans with continued symptoms despite optimal treatment with ACE inhibitors, β-blockers, and diuretics [9,197].

Ancestral origin is a poor and controversial marker of drug response. Because any difference in drug response may be attributable, at least in part, to genotype, investigators have attempted to identify a genetic marker for response to ISDN/hydralazine in the African American heart-failure trial (A-HeFT) population. ISDN/hydralazine is believed to exert its beneficial effects by increasing nitric oxide availability, and as such, several variants in the endothelial nitric oxide synthase (eNOS) gene have been examined for their effects on ISDN/hydralazine response. Of these, only the p.E298D variant in exon 7 was found to influence response to ISDN/hydralazine [218]. The EE298 genotype is more common in African Americans than Caucasians and was associated with a greater improvement in the study's composite endpoint with ISDN/hydralazine, an association largely driven by the improvement in the quality-of-life score with the EE298 genotype. An additional study focused on the guanine nucleotide-binding protein β-3 subunit (GNB3) genotype, which is involved in adrenergic receptor signaling. The c.C825T polymorphism, which occurs more commonly in African Americans, was associated with greater response to ISDN/hydralazine, with the greatest benefit observed with the TT genotype [219]. These data suggest that eNOS and GNB3 genotypes, rather than ancestral background, may be useful as predictors of response to ISDN/hydralazine therapy. In the future, studies determining outcomes by self-reported race will likely be replaced by genetic studies. Until then, studies will continue to collect both race and genetic information in the hopes of effectively predicting drug response.

Opportunity in Pharmacogenetics: Potential to Streamline Heart-Failure Therapy

At this point, data are insufficient to support withholding any heart-failure therapy because

of a potential lack of benefit based on genotype. However, future prospective studies evaluating the benefits of pharmacogenetic-based prescribing compared to traditional prescribing of heart-failure medications are conceivable. Ultimately, results of pharmacogenetic research efforts could lead to genotype-guided prescribing of heart-failure therapy. Specifically, rather than initiating the same "cocktail" of drugs for all patients, regimens might be tailored according to each individual's genetic predisposition for obtaining benefit or experiencing harm from a particular drug. Drugs predicted to be of minimal to no benefit could be avoided, thus simplifying drug regimens and reducing the associated costs, while potentially improving overall patient outcomes.

GENETIC INFLUENCES OF DRUG-INDUCED ARRHYTHMIA

Cardiac arrhythmias are potentially fatal if not treated appropriately. However, the drugs used to treat arrhythmias are themselves arrhythmogenic. Thus, there has been significant study of the genetics of cardiac arrhythmia and the pharmacogenetics of antiarrhythmic therapy to improve drug effectiveness and limit proarrhythmic effects.

Antiarrhythmic Medications

Antiarrhythmic agents in general have a narrow therapeutic index. Thus, they are often susceptible to drug–drug interactions and can cause significant adverse events. Polymorphisms in the genes encoding drug-metabolizing enzymes have been examined for their role in antiarrhythmic efficacy and toxicity. The highly polymorphic CYP2D6 enzyme metabolizes propafenone and quinidine. Propafenone is a class IC antiarrhythmic that exerts its effects by blocking the fast-inward sodium current in addition to having some β-receptor blocking properties at higher concentrations. Propafenone is primarily metabolized by CYP2D6, though CYP1A2 and CYP3A4 also contribute to its metabolism. Genetic classification of CYP2D6 activity is complex and can be determined via genotyping or phenotyping. Patients are generally classified as poor, intermediate, normal, or ultrarapid metabolizers. Patients who are classified as CYP2D6 poor metabolizers have decreased propafenone clearance, which leads to an increase in propafenone serum concentrations. However, because this increase is balanced by a decrease in production of an active metabolite, the recommended dosing regimen is the same regardless of CYP2D6 phenotype.

The greater variability in plasma concentrations of propafenone and its metabolites in CYP2D6 poor metabolizers does require that propafenone be titrated carefully and the echocardiogram (ECG) be monitored for evidence of toxicity [220]. Importantly, the additional β-blockade seen in CYP2D6 poor metabolizers can potentially lead to adverse events in asthmatic patients. In addition, although the data are not entirely consistent, there is evidence that subjects with paroxysmal atrial fibrillation classified as CYP2D6 poor metabolizers are more likely to maintain normal sinus rhythm with propafenone compared to normal metabolizers [221].

In contrast, quinidine is not a CYP2D6 substrate. However, the prescribing information for quinidine contains information on CYP2D6 pharmacogenetics because quinidine is a potent CYP2D6 inhibitor even at a subtherapeutic dose (Table 1). Quinidine can convert patients who are normal metabolizers to poor metabolizers. Therefore, it is important to monitor for adverse events when quinidine is coadministered with CYP2D6 substrates. Although pharmacogenetic studies have been done for several other antiarrhythmic medications, no strong and reliable associations have been observed.

Pharmacogenetics of Drug-Induced Long QT Syndrome

The proarrhythmic effects of medications (both those used to treat arrhythmia and those

used for other indications) have been well studied given that they can be life threatening and require a significant amount of patient monitoring. Proarrhythmia is generally defined as the worsening of the arrhythmia being treated or generation of a new arrhythmia with drug therapy [222,223]. Genetic studies have focused on drug-induced increases in the QT interval on the ECG and the life-threatening arrhythmia, torsades de pointes. The knowledge gained from many years of studying and evaluating congenital long QT syndromes has aided the study of genetic factors associated with drug-induced prolonged QT intervals.

The QT interval on an ECG represents the action potential of ventricular myocytes. The ventricular action potential is made up of several currents produced by different ion channels. The action potential is prolonged when there is either increased inward current or decreased outward current. The heart has significant built-in redundancy, as several ion channels participate in the ventricular action potential. This redundancy is termed "repolarization reserve." Thus, variation in one ion channel will not necessarily lead to an increase in the QT interval. A combination of factors is generally necessary for patients to exhibit congenital or drug-induced long-QT syndromes.

Genetic variation in ion channels associated with ventricular action potential has been well studied in congenital long-QT syndromes because of the possible effect on "repolarization reserve" [224]. Mutations found in genes encoding potassium (KCNQ1, KCNH2, KCNE1, and KCNE2) and sodium (SCN5A) voltage-gated channels have been associated with risk for congenital long QT syndrome. In addition, medications can prolong the QT interval by blocking the ion channel pore, inducing conformational changes in the ion channel pore, and/or decreasing production of the proteins encoding the ion channels. The amino acid structure of KCNH2 appears to make this ion channel pore particularly susceptible to drug blockade. Polymorphisms in the gene encoding KCNH2 may affect its susceptibility to drug binding and contribute to risk of long-QT syndrome [224]. In the FDA guidance for industry for screening non-antiarrhythmic drug potential to cause delay in cardiac repolarization, they recommend considering genotyping for cardiac ion channel mutations for patients who experience marked QT-interval prolongation or torsades de pointes in early clinical trials [225].

GWASs have identified additional genes associated with congenital long-QT syndrome. Nitric Oxide Synthase 1 Adaptor Protein (NOS1AP), which encodes for an accessory protein for the nitric oxide synthase type 1 gene, was initially linked to variability of QT interval across the normal population [226]. Variants in NOS1AP have also been linked to arrhythmia risk, sudden cardiac death, congenital long-QT syndrome, and the QT-interval prolonging effects of verapamil and amiodarone, though the mechanism underlying these associations remains unclear [226,227].

Clinical factors, such as hypokalemia, recent conversion of atrial fibrillation, and advanced heart disease may potentiate risk of drug-induced long-QT syndromes. The risk of drug-induced long-QT syndrome may also be increased if the clearance of a drug is decreased via either a drug interaction or genetic variants in hepatic enzyme systems. Clinicians should be particularly vigilant in monitoring for drug interactions with medications known to prolong the QT interval. In addition, if genetic variability in hepatic enzyme systems for a patient is known, this should be considered as well [228].

Currently, using genetic information to predict drug-induced long-QT syndrome cannot be recommended. However, evidence on this topic is growing rapidly, and with validated genetic markers, genotyping may in the future be clinically useful. However, it is unlikely that polymorphisms in a single gene or a single clinical risk factor will be sufficient to predict risk because of the redundancy in the system. Predicting

drug-induced long-QT syndrome will likely require a complex combination of multiple polymorphisms and clinical and environmental information. In support of this, a combination of 61 genetic variants was recently found to predict risk of QT prolongation with multiple drugs [229].

Opportunities in Pharmacogenetics: Potential to Resurrect Old Drugs

One of the primary reasons that drugs in development do not succeed or that approved drugs are withdrawn from the market is because of proarrhythmic effects. The ability to predict risk for proarrhythmia with a drug could potentially revive some agents, particularly if few other treatment options are available. In this case, genetic testing for the "at-risk" variant(s) would likely be required prior to drug use. The drug could then be avoided in patients genetically at risk for drug-induced proarrhythmia.

CONCLUSION

After nearly two decades of research in the area of cardiovascular pharmacogenetics, the evidence has accumulated to the extent of informing clinical implementation of genotype-guided prescribing for several drugs. An increasing number of institutions are implementing clinical pharmacogenetics programs for cardiovascular drugs, with clopidogrel being the primary focus to date [175]. One approach to pharmacogenetic implementation is to genotype variants related to a specific drug at the time of drug prescribing. Another approach is to genotype a panel of variants influencing responses to numerous drugs preemptively. This way, genetic information is available at the time various drugs are prescribed. Although fewer variants need to be tested with the former approach, there are substantial personnel costs associated with the need to obtain genotype results efficiently. For example, in the case of CYP2C19 testing for clopidogrel, genotype results need to be obtained quickly to inform antiplatelet therapy early after the coronary intervention when the risk for adverse cardiovascular events is highest. Dedicated personnel are needed to efficiently process the sample with preemptive panel-based testing. Multiple samples can be batched and run at one time, because there is no urgency in obtaining genotype results, significantly reducing personnel time and associated costs. Both approaches are being used in clinical practice, and guidelines by the CPIC are available to assist with translating genotype results into actionable prescribing decisions for drugs with the greatest evidence supporting genotype-guided decisions. Research continues for other drugs to identify variants influencing risk for toxicity or likelihood of therapeutic response. With efforts such as the NIH-funded eMERGE and IGNITE Networks, the incorporation of genotype data into drug-therapy decisions is expected to be increasingly utilized to optimize treatment.

DISCUSSION POINTS

1. Contrast the effect of a poor drug-metabolizer phenotype on response to warfarin versus clopidogrel.
2. Describe feasible approaches to implement warfarin and clopidogrel pharmacogenetics into clinical practice.
3. Describe the data supporting use of genetic data to assist with clopidogrel, warfarin, simvastatin, and tacrolimus dosing.
4. Describe CPIC guideline recommendations for genotype-guided clopidogrel, warfarin, simvastatin, and tacrolimus prescribing.

QUESTIONS FOR DISCUSSION

1. What are examples of novel pharmacogenetic findings from genome-wide association studies?