Abstract: Patients vary in their responses to drug therapy, and some of that variability is genetically determined. This review outlines general approaches used to identify genetic variation that influences drug response. Examples from specific therapeutic areas are presented, such as cholesterol management, arrhythmias, heart failure, hypertension, warfarin anticoagulation, and antiplatelet agents. A brief view of potential pathways to implementation is presented. (Circ Res. 2011;109:807-820.)

Key Words: drug therapy ■ genetics ■ pharmacogenetics ■ pharmacogenomics

There are many sources of variability in response to drug therapy, such as noncompliance and unrecognized drug interactions. This review focuses on genetic mechanisms contributing to variability in response to cardiovascular drug therapy. An introductory section discusses principles of drug action and approaches to identifying genetic contributors to drug action, followed by sections examining the relationship between genetic variation and outcomes of treatment with commonly used cardiovascular drugs. The conclusion includes a discussion of the current status of implementing this new knowledge in prescribing drugs.
Broad Principles

Principles of Drug Action

Drugs interact with specific receptors in the circulation, on the cell surface, or inside cells to exert their beneficial and detrimental effects. Variability in response to drug therapy can reflect either variability in the amount of drug delivered to receptor sites (pharmacokinetic factors) or variability in response to equivalent drug concentrations (pharmacodynamic factors). Describing the molecular basis of these processes is the critical first step to evaluating the extent to which their genetic variation leads to variable drug responses.

Pharmacokinetics

Pharmacokinetics encompasses the processes of absorption, distribution, metabolism, and elimination. The most widely studied of these processes is metabolism, most often accomplished by members of the cytochrome P450 (CYP) superfamily. CYP-mediated biotransformation generally results in metabolites that are more polar than the parent drug and, thus, more readily excreted. Metabolites may display no pharmacological activity, similar pharmacological activity to the parent drug, or different pharmacological activity from the parent drug. In some cases, the parent drug is inactive (a “prodrug”) and requires bioactivation by metabolism to exert its pharmacological effects; clopidogrel is one example. A second common pathway of drug metabolism is conjugation with specific side groups, such as methyl, acetyl, or glucuronide moieties. This is accomplished by specific transferases (eg, methyltransferases, acetyltransferases, and others).

The processes of absorption, distribution, and elimination may be passive or may depend on expression or function of specific drug transport molecules. Thus, for example, renal or biliary excretion of a drug or drug metabolite often involves active uptake of drug by specific transport molecules into the renal or biliary epithelium, followed by drug excretion into the urine or bile. Similarly, absorption from the gastrointestinal tract may involve active uptake into enterocytes, followed by excretion into the portal circulation or back into the gut by specific drug uptake and efflux molecules.

Variation in these processes by genetic factors, drug interactions, or generalized disease of organ function (eg, liver or kidney disease) may alter pharmacokinetics and, hence, drug response. The functional consequences of such variation are drug-specific. For drugs whose metabolism or elimination is dependent on the function of a single CYP or drug transport molecule, variation in that pathway may lead

Figure 1. Variability in response to cardiovascular therapies. A, Variability in low-density lipoprotein (LDL) cholesterol lowering in response to simvastatin 40 mg/d for 6 weeks. The drug is highly effective, judged by mean response, but there is substantial variability, with some subjects displaying no response and others LDL cholesterol lowering of up to 80%. Adapted by permission from Simon et al.149 B, Variability in decrease in diastolic blood pressure in response to hydrochlorothiazide (HCTZ) in 147 black subjects. As in (A), the drug is effective, judged by mean response, but there is substantial variability. C, Variability in ADP-induced platelet aggregation at baseline (top), and after clopidogrel (bottom). As in the other examples, there is an obvious drug effect but substantial variability across the population. The figures on each graph indicate population mean (±SD) response. Adapted by permission from Shuldiner et al.9
to especially large variability in drug concentrations. This is a situation that has been termed “high-risk pharmacokinetics” and applies to many commonly used drugs, including clopidogrel, warfarin, and digoxin. Variable efficacy by this mechanism is a particular risk for prodrugs. In the case of drugs inactivated by metabolism, the problem is especially acute when the margin between concentrations required for efficacy and those producing side effects is narrow, thus increasing the risk for side effects (Figure 2). However, altered function of a single CYP or transporter is unlikely to have major consequences for a drug that is eliminated by multiple pathways.

**Pharmacodynamic Factors**

The most straightforward example of genetically determined variability in drug action despite equivalent drug concentration at the receptor site is functional variation in the gene encoding the drug receptor itself. Thus, coding or regulatory variation in VKORC1, encoding the warfarin target, is a key contributor to variability in warfarin response and variants in the β-adrenergic receptor genes appear to contribute to variability in response to β-adrenergic receptor blockers (β-blockers).

More generally, any physiological variable that impacts the interaction of drug with its receptor or the downstream effects that this interaction produces can modulate drug action. Examples include changes in serum potassium influencing drug–ion channel interactions or variable catecholamines affecting a drug–adrenergic receptor interaction. This concept extends far beyond single genetic variants and encompasses the idea that drug–receptor interactions occur in a complex biological milieu whose function may be modified by multiple interacting modules, each of which may display genetic variation (Figure 3). Another factor clearly contributing to pharmacodynamic variability is the disease process that the drug is targeting.

**Approaches to Identifying Genetic Contributors to Variable Drug Actions**

**Candidate Genes**

One intuitively appealing experimental design is to identify a series of genes modulating an important biological process and to then address variability in the biological process by examining the effects of common variants in such “candidate” genes. In general, it has been difficult to replicate even

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**Figure 2. Therapeutic index.** For any drug, there is a relationship between dose and efficacy (black lines) and a second relationship between dose and toxicity (gray lines). These curves are derived from populations and efficacy may be incomplete, as indicated in these examples. The arrows on each plot identify the dose at which 50% of the response is seen, and the therapeutic index is indicated by the open arrow at the bottom of each plot. Some drugs considered here, such as warfarin and clopidogrel, have narrow therapeutic indices, whereas others (β-blockers, statins) have wider ones. DNA variants may modulate the relationship between efficacy and toxicity in populations and in individuals.

**Figure 3. Multiple genes affecting warfarin dose.** A, Steady-state warfarin dosage varies widely. Adapted by permission from Kurnik et al. B, As discussed in the text, variants in CYP2C9 and VKORC1 contribute to variable warfarin dosage. Other genes that also may play a role are illustrated here. Pharmacokinetic response genes include other cytochrome P (CYPs), although their role is likely minimal. Within the pharmacodynamic pathway, the microsomal epoxide hydroxylase (EPHX1) and γ-glutamyl carboxylase (GGCX) have been associated with warfarin response. Other genes include calumenin, which regulates the carboxylation of coagulation factors and proteins; apolipoprotein E, which facilitates cellular uptake of chylomicrons, the main vehicle of vitamin K transport to the liver; coagulation pathway genes encoding for factors II, VII, IX, and X; and genes that accelerate the inactivation of factor Va and Villa (endothelial protein C receptor [PROCR] and protein S [PROS1]).
the most biologically appealing results in candidate gene studies. However, studies of candidate variants in pharmacokinetic pathways provide an important exception to that rule: as discussed below, variants in pharmacokinetic pathways involved in variable responses to warfarin, clopidogrel, and simvastatin were first implicated by candidate gene studies based on the drugs’ pharmacokinetics, and these have since been validated in genome-wide association (GWA) studies. These studies provide further evidence for the large effects that single gene variants may exert for some drugs.

**Unbiased Approaches**

The GWA paradigm has been applied in >800 studies to identify common genetic variants modulating disease susceptibility, physiological traits, and, in a small number of studies, variable drug responses. This approach has revealed new biological mechanisms but also has been subject to a number of criticisms. Interestingly, GWA for drug response phenotypes seems less subject to these drawbacks. For example, although the signals identified in a GWA study may be statistically robust, the odds ratios that they confer are often small and validated only with very large numbers of subjects. One reasonable explanation is that single variant alleles with major detrimental effects are unlikely to propagate in a large population. However, there may be no survival disadvantage for common polymorphisms modulating drug action; hence, statistically valid associations have emerged in GWA studies of drug response traits that involve relatively small numbers of patients. A second criticism has been that loci identified by GWA studies explain only small proportions of variability in the traits being studied. However, GWA has explained relatively large proportions of variability compared to studies of traits such as disease susceptibility or physiological measurements. Thus, for example, adding pharmacogenomic variants to clinical information improves the predictability of warfarin dose from approximately 20% to 45% to 60%, and CYP2C19 explains 12% of variability in clopidogrel inhibition of ADP-induced platelet aggregation. A third criticism of the GWA approach has been that a “hit” often identifies a genomic locus but generally leaves open the question of which specific variant, or combinations of variants, within the locus actually exerts the biological effects to modulate the trait. This has not proven to be the case in pharmacogenetic-based GWA studies conducted to date, although as studies include increasing numbers of patients, such “unexplained” loci may emerge.

Another potentially powerful approach is to use GWA to examine variability in common “intermediate” phenotypes (endophenotypes) such as electrocardiographic intervals or low-density lipoprotein (LDL) cholesterol. These studies can readily acquire tens of thousands of subjects, and thus identify multiple genomic loci with extremely low probability values that contribute to the trait being studied. Such loci can be taken forward as candidates, alone or in combination, which modulate drug response or other “hard” cardiovascular end points such as myocardial infarction or sudden arrhythmic death. The development of rapid relatively inexpensive large-scale sequencing capacity (“next-generation sequencing”) is a logical next step in this area of science.

**Combined Approaches: Moving to Systems Biology**

An intermediate experimental approach between single candidate gene variant and whole genome approaches is to consider the possibility that variability in a trait such as drug response is attributable to combinations of variants in functionally linked sets of genes. This approach may be especially appealing to dissect the contributions of variable physiological perturbations caused by disease to variable drug responses, as in the use of β-blockers in heart failure. One experimental approach is to examine associations between large numbers (hundreds or thousands) of variants in such candidate pathways and specific traits; this idea has been applied to examine variability in response to HMG-CoA reductase inhibitors (statins), among others.

Another highly promising approach is to combine information on genetic variation with other high-dimensional data, such as gene expression profiling in cell lines or specific tissues, to identify networks of genes modulating variable drug response phenotypes. Studies of drug response in mice or zebrafish with defined genetic backgrounds also have been proposed as approaches to discovery and validation in pharmacogenomics.

**Disease-Specific Cardiovascular Therapies**

**Atherosclerosis/Statins**

HMG-CoA reductase (HMGCR) inhibitors (statins) are highly efficacious in the primary and secondary prevention of cardiovascular disease. HMGCR catalyzes the rate-limiting step of cholesterol biosynthesis. By attenuating the endogenous production of cholesterol, statins also upregulate expression of the LDL receptor (LDLR) in a variety of tissues. This is the fundamental basis for the clinical effect of statins in lowering plasma LDL cholesterol.

**Cholesterol-Lowering Efficacy: In Vitro Studies**

Lymphoblastoid cell lines from individuals with specific variants have been used to characterize statin response, in vitro. For example, in vitro statin exposure of lymphoblastoid cell lines derived from black subjects expressing the L5 LDLR haplotype showed smaller induction of cell surface LDLR protein compared to noncarriers, a finding consistent with the smaller reductions in plasma LDL cholesterol with statin treatment seen clinically. Similarly, in vitro statin exposure identified a mechanism whereby the HMGCR H7 haplotype modulates response to statins; one of the three single nucleotide polymorphisms (SNPs) within this haplotype, rs3846662, was associated with differential regulation of statin-induced expression of an HMGCR splice variant lacking exon 13, HMGCR13(−). Additional studies found that cellular enrichment of HMGCR13(−) attenuated sensitivity to statin inhibition, demonstrating the likelihood that HMGCR alternative splicing is not only a marker but also a determinant of interindividual variation in LDL cholesterol reduction with statin treatment. Compared to the 1% to 2% of variation explained by most gene variants related to drug response, statin-induced HMGCR13(−) expression explained...
Cholesterol-Lowering Efficacy: Human Studies

The LDL-lowering effect of statins has been unequivocally associated with variation in two key pharmacodynamic candidate genes (HMGCR and LDLR). The H7 haplotype within HMGCR, defined by the presence of three intronic SNPs, rs17244841, rs3846662, and rs17238540, has been associated with an 11% to 19% smaller reduction in LDL cholesterol with statin treatment in multiple independent populations as well as ethnically diverse population-based cohorts.\(^{15,23-25}\) The H7 haplotype has been shown to interact with other genetic variants, including a second HMGCR haplotype, H2, defined primarily by rs3846662, as well as the LDLR L5 haplotype, defined by six SNPs within the LDLR 3’ untranslated region. LDL cholesterol-lowering with statin treatment in blacks who carry multiple copies of these haplotypes is further attenuated compared to those who carry any haplotype alone.\(^{21,24,25}\)

Additionally, genetic variants in CYP3A4, which metabolizes simvastatin, atorvastatin, and lovastatin, have been associated with variability in statin efficacy. Both a nonsynonymous polymorphism (M445T, rs4986910) as well the associated with variability in statin efficacy. Both a nonsynonymous polymorphism (M445T, rs4986910) as well the CYP3A5 0.8) with an intronic variant in CYP3A5.29

Predicators of Myotoxicity

The spectrum of statin-related myotoxicity includes stopping the drug for muscle aches to serum creatine kinase elevations to the rare occurrence of rhabdomyolysis. Initial studies identified variants in CYP3A4 and SLCO1B1 as potential predictors of myotoxicity.\(^{35}\) A GWA study examined 85 individuals with myotoxicity during high-dose (80 mg/d) simvastatin and implicated a SNP in SLCO1B1: resequencing and subsequent replication indicated that a nonsynonymous variant, resulting in Val174Ala, conferred an odds ratio for myopathy of 16.9 in homozygotes (95% confidence interval, 4.7–61.1) and 4.5 (95% confidence interval, 2.6–7.7) in heterozygotes.\(^{8}\) Homozygotes comprised 2.1% of the study group and heterozygotes comprised 24.9%; this single variant accounted for approximately 60% of risk. This association has since been replicated in two independent study populations.\(^{36,37}\)

Predictors of Clinical Outcome

A missense SNP, Trp719Arg, in KIF6 has been associated with increased risk of coronary artery disease, coronary heart disease, and myocardial infarction,\(^{38,39}\) although the mechanisms for the association are unknown and the association is disputed.\(^{40}\) In multiple trials,\(^{38,39}\) statin treatment has been shown to significantly reduce coronary events in carriers of Trp719Arg, and SNPs in high linkage disequilibrium with it, with relative risk reduction of approximately 30%, whereas no benefit of statin treatment is reported in noncarriers.

Clinical Utility

SLCO1B1 genotype exerts a large effect on risk for simvastatin myotoxicity and may be useful in some settings. Current studies identifying genomics of LDL response are a key step to addressing the broader question of whether genomic markers identify subjects with coronary events developing during statin therapy.

Antiarrhythmics and Drug-Induced Arrhythmias

Drug-Induced Long QT Syndrome

Striking QT interval prolongation and the polymorphic ventricular tachycardia, torsades de pointes, develops in 1% to 5% of patients exposed to QT-prolonging antiarrhythmic drugs;\(^{41}\) this also occurs with a range of “noncardiovascular” therapies, including some anti-psychotics, antibiotics, and methadone.\(^{41}\) Many clinical features of drug-induced torsades de pointes resemble those seen in the congenital long QT syndromes, diseases caused by mutations in genes (13 have now been described) encoding ion channels or function-modifying subunits and characterized by incomplete penetrance.\(^{42}\)

Small studies have estimated that 10% to 40% of subjects with drug-induced torsades de pointes have subclinical congenital long QT syndrome gene mutations.\(^{43-45}\) A study using targeted next-generation sequencing to screen all 13 congenital long QT syndrome disease genes and other arrhythmia susceptibility genes identified rare variants predicted to be deleterious to protein function in 20 of 31 patients (64.5%).\(^{46}\) Thus, there seems little doubt that congenital long QT syndrome mutations contribute to risk for drug-induced torsades de pointes, but the extent to which they explain the risk is uncertain. The β-adrenergic receptor polymorphisms discussed were not associated with drug-induced torsades de pointes.\(^{47}\)

GWA Approaches in Electrophysiology

GWA has implicated multiple loci modulating variability in the normal QT interval duration.\(^{48-50}\) These are in two broad groups. The first includes “obvious” candidates that encode cardiac ion channels and in which mutations cause the congenital long QT or other arrhythmia susceptibility syndromes. These include SCN5A, encoding the cardiac sodium channel as well as genes encoding a series of cardiac potassium channels (KCNH2, KCNQ1, KCNJ2, KCNE1). The second group includes genes not previously implicated as
modulating the QT interval. One of these is NOS1AP, encoding an accessory protein for neuronal nitric oxide synthase, and another is GINS3, now implicated as a potential modulator of the extent to which challenge with a QT-prolonging drug (dofetilide) modulates action potential duration in zebrafish.51 The convergence of the zebrafish experiment on the GINS3 locus provides biological validation for the GWA result, although the underlying physiology remains to be explored. One study suggested that a NOS1AP variant was associated with total and cardiovascular mortality during treatment with dihydropyridine calcium channel blockers.52 Variants in NOS1AP also have been reported to modulate the risk of arrhythmias, at equivalent QT interval durations, in patients with the congenital long QT syndrome53,54 and to modulate risk for sudden death in the general population.55 To date, the mechanisms whereby NOS1AP variation affects QT remain uncertain; one study suggested that the NOS1AP is a modulator of L-type calcium current.56 Variability in PR and QRS durations have also been analyzed by GWA. As with QT, the results point to previously implicated genes, as well as to loci previously not implicated in cardiac electrophysiology. One locus, at chromosome 4q25, has consistently been associated with risk of atrial fibrillation.57 Preliminary data have linked 4q25 variants to outcome for ablation58 and of antiarrhythmic drug therapy for atrial fibrillation.59 Candidate gene studies have also suggested a role for β-adrenergic receptor polymorphisms in rate control during atrial fibrillation.60

Clinical Utility

Identifying patients at risk for long QT-related arrhythmias during drug therapy may become possible as platforms to identify both common and rare variants are increasingly deployed. Early studies suggest that available atrial fibrillation therapies may be less effective in some genetically defined subsets, and these patients thus may be candidates for alternate approaches.

Hypertension

Pharmacogenomics of the Antihypertensive Response61

One major focus of candidate gene studies has been two common and nonsynonymous SNPs, resulting in Ser49Gly and Arg389Gly, in the gene encoding the β1 adrenergic receptor (ADRB1). These variants demonstrate altered biological function in vitro,62 including enhanced agonist-induced adenyl cyclase activation by Gly49 compared to Ser49 and by Arg389 compared to Gly389. The effects in humans, in whom multiple haplotypes are the rule, have been more difficult to dissect. Many, but not all, studies have shown that the Arg389Arg genotype, or Ser49/Arg389 haplotype, is associated with the greatest blood pressure-lowering.4,63,64 These findings are consistent with β-blocker response associations in heart failure outcomes discussed.

A common functional polymorphism resulting in Gly460Trp in the α-adducin gene ADD165 has been associated with response to thiazides in some but not all studies. This association led to the development of a novel antihypertensive drug class targeting adducin.65 NEDD4L is also a candidate gene with a documented functional SNP, a role in sodium reabsorption, and several studies have found an association between this SNP and blood pressure response with thiazides.66,67

There has been one GWA study conducted to date examining antihypertensive response, and this revealed an association between thiazide response in blacks and a locus at chromosome 12q15.68 This association has since been replicated in an independent cohort.69 Whereas no genes in the region are obvious thiazide response genes, an interesting candidate is FRS2, which is involved in fibroblast growth factor signaling, which plays a role in vascular smooth muscle cell regulation.

Myocardial Infarction, Stroke, and Death During Antihypertensive Therapy

The ADD1 Gly460Trp polymorphism has been associated with increased risk of myocardial infarction or stroke during thiazide diuretic treatment.70 However, analyses from both ALLHAT and INVEST were unable to replicate this finding.71,72 In the INVEST cohort, the ADRB1 Ser49/Arg389 haplotype was associated with increased risk of death, and the risk of this allele was offset by treatment with the β-blocker atenolol compared to the calcium channel blocker, verapamil.4 A cohort study similarly found significant interactions between SNPs in ADRB1 and risk for myocardial infarction or stroke.

Other studies have suggested potential associations between SNPs in CACNA1C, CACNB2, and KCNMB1 (involved in calcium signaling) and myocardial infarction or stroke with β-blockers versus calcium channel blockers.73-75 CACNB2 was also one of ten genes that replicated in large GWA studies of hypertension.76 Other reported genetic associations include variable stroke risk by genotype for an MMP3 promoter polymorphism in patients treated with lisinopril,77 an ACE gene polymorphism, and differential outcomes by genotype during treatment with an angiotensin-converting enzyme inhibitor78 and different treatment-related outcomes with thiazides and β-blockers, but not diltiazem, by NEDD4L genotype.66

Clinical Utility

Genetic determinants of blood pressure and long-term outcomes in hypertensive patients are being identified. Their role in choosing among therapies is undergoing active investigation.

Heart Failure

The largest body of literature centers on the genetic associations between ADRB1 SNPs (discussed previously) and either β-blocker–mediated improvements in left ventricular ejection fraction or clinical outcomes.4 Some studies have suggested that the Arg389Arg genotype is associated with the greatest improvement in left ventricular ejection fraction, but this has not been consistently observed.4 In BEST, a large trial of bucindolol in heart failure, the survival benefit was confined to Arg389Arg patients, whereas there was no difference for Gly389 carriers using therapy versus those using placebo.79 The sponsor for bucindolol has announced plans for a superiority trial in 3200 ADRB1 Arg389Arg patients who will be randomized to long-acting metoprolol or bucindolol.80 A population cohort suggested that Arg389Arg patients using high-dose β-blocker had significantly better outcomes than...
those with the same genotype on no or low-dose β-blocker, also consistent with the findings in BEST.81 However, other studies have not documented an association between Arg389Gly genotype and improved outcome with β-blockers.82,83

These differences may be explained by whether studies examine outcomes in patients treated with β-blocker compared to a control (low-dose or placebo) or whether they examine outcome by genotype in a cohort of patients all treated with β-blocker. One interpretation of the data are that Gly389 carriers may be at lower risk for adverse outcomes, and so garner less benefit from β-blockers than Arg389Arg patients; in this case, β-blocker treatment benefits Arg389Arg patients, making their outcomes similar to that of Gly389 carriers (treated or untreated). This is consistent with the treatment-related outcomes in hypertension for this gene in which β-blocker treatment reduced the risk of the Arg389Arg genotype.

The adrenergic signaling pathway includes a number of other genes that have been associated with severity of the heart failure phenotype or with benefits of response to treatment. A 12-nucleotide (4-amino acid) insertion deletion polymorphism in ADRA2C has been shown to lead to a loss of feedback inhibition, resulting in increased norepinephrine, and in combination with the ADRB1 Arg389Arg genotype has been associated with increased risk of heart failure in blacks.84 A study of metoprolol reported that the combination of ADRB1 Arg389Arg/ADRA2C Del carrier status led to the greatest improvement in left ventricular ejection fraction.85 By contrast, ADRA2C Del carrier status was associated with reduced efficacy (death and hospitalization outcomes) in BEST.86 The difference in effect of ADRA2C genotype of efficacy in these two studies may reflect differences in the pharmacological properties of bucindolol versus metoprolol.

The Leu41Gln variant in GRK5 is common in blacks and has been found to blunt catecholamine-induced responses in vitro and in experimental animals.57 In three reported cohort studies,82,87 carriers of the Leu41 variant had improved outcomes compared to those with Gln41Gln. In addition, Leu41 carriers did not appear to derive benefit from β-blocker therapy, whereas Gln41Gln seemed to derive substantial survival benefit.

Clinical Utility
Some subjects with heart failure may derive little benefit from β-blocker therapy. Trials are underway to further address this issue.

Warfarin

Genetics of Warfarin Response
CYP2C9 is largely responsible for the metabolic clearance of (S)-warfarin, the more active of the two warfarin enantiomers. Two nonsynonymous reduction-of-function variants in CYP2C9, designated *2 and *3, are clearly associated with lower warfarin dose requirements and increased bleeding risk in white populations;3,88–90 they are much less prevalent in blacks and thus do not have as large of a population effect on dose.91

VKORC1 is the warfarin-sensitive and rate-limiting enzyme of the vitamin K cycle that recycles the epoxide and quinone form of vitamin K to the reduced nonoxidized form. Multiple linked variants in the VKORC1 promoter have been associated with variability in gene expression and dose requirement;3 rs9934438 in intron 1 (also designated 1173C/T) is as informative as haplotypes for predicting warfarin dose in whites and blacks. Additional VKORC1 variants and CYP2C9 variants (eg, CYP2C9*5, *6, *8, and *11 alleles) may improve prediction, particularly in blacks.92 Rare coding region variants that generate relative or absolute warfarin resistance have also been reported.93

GWA identified variants in CYP2C9, VKORC1, and CYP4F2, now recognized as a vitamin K1 oxidase, as determinants of increased warfarin dose requirements.10,94 Carriers of the CYP4F2 V433M allele (rs2108622) were found to have reduced capacity to metabolize vitamin K1, suggesting that the variant may be associated with elevated hepatic levels of vitamin K, thus explaining the higher dose requirements in those with this variant.95 The effects of CYP4F2 on warfarin dosing have not been consistent across studies.96 Other genes implicated in variable warfarin dosages are highlighted in Figure 3.

Dosing Algorithms
Numerous dosing algorithms have been developed that incorporate both clinical and genetic information to predict warfarin dose requirements. Two serve as useful examples: the International Warfarin Pharmacogenetics Consortium (IWPC) algorithm13 and an algorithm developed by Gage et al.97 Both groups developed models using clinical parameters alone and then developed models with both clinical and genetic information, and both demonstrated clinically meaningful improvements in dose prediction with the addition of genetic data. These models have been externally validated.98,99

Research on dosing algorithms has demonstrated three important findings. First, the majority of models include both clinical parameters and a single VKORC1 SNP plus CYP2C9*2 and CYP2C9*3 variants. Second, most recent models have similar overall predictive ability, explaining approximately 40% to 65% of warfarin dose variability (in mostly white populations), compared with only approximately 20% variability for algorithms that use clinical information alone.98–100 Whether the residual variability is genetic or environmental (eg, because of diet) is undergoing study. Third, they perform substantially better in nonblack populations than they do in black populations.13,92,99,100 All of the findings are consistent with the known associations between genetic variants and warfarin dosing across different populations.

Clinical Utility
It is currently unknown if pharmacogenetic-based dosing of warfarin will improve anticoagulation control and clinical outcomes. The largest published randomized clinical trial to date included only 206 patients and did not demonstrate an advantage of pharmacogenetic dosing on anticoagulation control, despite the fact that the dosing algorithm clearly predicted the final stable dose better than a clinical-only algorithm.101 Another small study that used CYP2C9 only to determine dosing did show some benefit on anticoagulation control and minor bleeding, but it had a high dropout rate.102 An observational study reported a relationship between pro-
viding warfarin genetic information to clinicians and reduced hospitalizations. Large ongoing and future randomized studies are addressing this issue.

Antiplatelet Agents

Pharmacogenomics of Antiplatelet Medications

Dual antiplatelet therapy with aspirin and clopidogrel is the standard of care for prevention of thromboembolic events in patients at high risk for myocardial infarction. However, there is marked interindividual variation in response to aspirin and clopidogrel. Ex vivo quantitative measures of changes in platelet aggregation in response to aspirin and clopidogrel indicate that response is normally distributed in the population. Mounting evidence supports a genetic component to both baseline platelet function and antiplatelet drug response. In healthy Amish families, the heritability (h²) of baseline ADP-stimulated platelet aggregation and changes in response to clopidogrel and clopidogrel plus aspirin was 0.33 (P=0.005), 0.73 (P<0.001), and 0.83 (P<0.001), respectively. The heritability of response to aspirin monotherapy appears to be more modest (h²=0.19; P<0.01) for changes in collagen-induced platelet aggregation.

Genetics of Aspirin Response

Most reports of aspirin response to date have studied one or a few candidate variants, and these have not yielded reproducible results. A meta-analysis of 50 polymorphisms in 11 genes reported in 31 studies and a combined sample size of 2834 subjects suggested that the common PLA1/2 polymorphism (Leu59Pro; rs5918) in glycoprotein IIIa (GPIIIa) does confer aspirin resistance (odds ratio in healthy subjects=2.36; P=0.009); however, when combining both healthy subjects and those with cardiovascular disease, the odds ratio was 1.14 (P=0.40). The meta-analysis found no effect on aspirin sensitivity with variants in COX1, GP1a, GP1b, GPIIa, GPIIIa, FXIII, P2Y1, and P2Y12.

Genetics of Clopidogrel Response

Clopidogrel is a prodrug, requiring biotransformation in the liver to an active thiol derivative primarily by CYP2C19, CYP3A4/5, CYP1A2, and CYP2B6. The active metabolite irreversibly binds to platelet ADP P2Y12 receptors. Early candidate gene studies implicated variants in the P2Y12 receptor and CYP3A4 as potential determinants of clopidogrel resistance; however, these studies were not consistently replicated. More recently, the loss-of-function CYP2C19*2 allele (rs4244285) has been reproducibly shown to be associated with a decreased conversion of clopidogrel into its active metabolite, antiplatelet effect, and increased risk for cardiovascular events in patients using clopidogrel. A GWA study identified CYP2C19*2 as the single major genetic determinant of biochemical response to clopidogrel, accounting for approximately 12% of the variation in ADP-stimulated platelet aggregation during drug treatment. In a large meta-analysis CYP2C19*2 carriers treated with clopidogrel have an increased risk for major adverse cardiovascular events compared to noncarriers (hazard ratio, 1.55; 95% confidence interval, 1.11–2.17 for heterozygotes; hazard ratio, 1.76; 95% confidence interval, 1.24–2.50 for homozygotes) and increased risks of stent thrombosis (hazard ratio, 2.67; 95% confidence interval, 1.69–4.22 for heterozygotes; hazard ratio, 3.97; 95% confidence interval, 1.75–9.02 for homozygotes). In whites, blacks, and Mexicans, CYP2C19*2 is present is 18% to 33% (2%–3% homozygotes), and the allele frequency is higher in Asians. The studies showing a relationship between CYP2C19 genotype and clopidogrel response have been conducted in relatively high-risk patients and thus may not apply to all indications for clopidogrel, such as atrial fibrillation, stroke, peripheral artery disease, or chronic stable angina.

There is some evidence that the loss-of-function *3 variant, present in Asians, is also associated with poorer response. Other loss-of-function alleles are rare. By contrast, the gain-of-function *17 variant may be associated with increased response (and thus adverse bleeding outcomes). However, it is unclear whether the *17 variant has effects that are independent of the *1 allele attributable to linkage disequilibrium.

Variants in ABCBI, which is involved in clopidogrel absorption, have been implicated in clopidogrel response, but these findings have not been replicated in all studies. A recent study reported that the common decreased function Gln192Arg (rs662) allele in paraoxonase 1 may be associated with decreased active metabolite levels, decreased inhibition of ADP-stimulated platelet aggregation, and increased risk for stent thrombosis. That study did not find an effect of CYP2C19 and the results await replication by others.

Clinical Utility

Guided by the replicated findings of CYP2C19*2 as a determinant of clopidogrel response in patients undergoing percutaneous coronary intervention for atherosclerotic heart disease, the Food and Drug Administration approved new labeling of clopidogrel in March 2010. This includes a boxed warning alerting physicians to the genetic findings and suggests alternative antiplatelet therapy in CYP2C19*2 homozygotes. These may include prasugrel and the investigational agent ticagrelor, which are not appreciably affected by CYP2C19 genotype. The idea of increasing clopidogrel dose has also been evaluated, but not specifically in CYP2C19*2 homozygotes.

Summary

The focus of this brief review has been to emphasize that even for widely deployed and clinically familiar therapies, there is substantial variability in response across patients: one size cannot fit all. Substantial work, particularly over the past decade, has elucidated genetic contributors to this variability. These include variants in pharmacokinetic and pharmacodynamic pathways, with an increasing view that drugs act in a complex biological milieu. The technology to allow accumulation of high-dimensional datasets, such as genomic tissue-specific expression, and drug response phenotypes in large groups of patients are enabling for this approach. Next-generation sequencing will similarly expand our catalog of variants associated with variable drug responses. There are substantial barriers to applying pharmacogenomic information to practice (Table), and these include...
Table. Issues in Implementing Pharmacogenomics in Practice

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<td>Implementation</td>
<td>Establishing interactions among genes and between genes and the environment</td>
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<td>Translation</td>
<td>Establishing which genetic variants should be used in which settings</td>
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<td>At the point of care or preemptively</td>
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<td>Analyzing outcomes</td>
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challenges in discovery, translation, and implementation. One significant obstacle is determining the level of evidence that is required before a variant is moved into clinical practice. It is an interesting paradox that the ability to personalize therapy—to prescribe with high confidence that specific variant alleles influence the outcome in an individual patient—must rely on analysis of large datasets to enable discovery and replication of response in genetically defined subsets. Another set of challenges is logistic: determining genotype at the time the drug is prescribed means the practitioner must get the result, know how to act on it, and contact the patient, if needed, to change drug or dose. An alternate approach is a preemptive one, in which genotypic information is deposited into an electronic medical record before drug prescription. The preemptive scenario has the potential appeal of automated delivery of point-of-care decision support to practitioners and the ability to acquire and manage large amounts of genomic information that ultimately may be accessible to an electronic medical record system. Thus, advanced information technology must be part of a vision for developing, analyzing, and ultimately applying new genomic data to health care.33,143 Identifying outcomes and analyzing costs are other challenges. Translating fundamental discovery in genome science to individual patients and populations is a challenge for the field, and genotyping for selection of appropriate drug therapy is one of the first ways this is being accomplished. In oncology, tumor genotyping is rapidly becoming standard of care to identify specific mutations that then dictate selection of therapy.144 Prescreening germline genotyping is becoming standard of care to reduce the risk of serious adverse reactions to carbamazepine145 and abacavir.146 In cardiovascular therapy, a number of centers are now deploying programs to use CYP2C19 genotypes to guide clopidogrel therapy. National Institutes of Health is making major investments in discovery of new pharmacogenomic pathways and in how that information can be used to improve health care. These include the warfarin trials and efforts such as the Pharmacogenomics Research Network147 and the Electronic Medical Records and Genomics Network.148 The newly developed NHGRI strategic plan includes an emphasis on clinical implementation of new genomic knowledge.149 Bio-medical discovery over the past several decades has included studies to understand sources of individual variability in response to drug therapy. We are clearly entering an era in which this knowledge will be increasingly leveraged to improve health care.

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