Cardiovascular Pharmacogenetics

Invited Editorial

Cardiovascular pharmacogenetics: much ado about nothing? - M. Eichelbaum

Lead Article

Cardiovascular pharmacogenetics: opportunities and challenges - D. M. Roden

Expert Answers to Three Key Questions

Tackling pharmacogenetics: how can we select the right methods? - M. F. Sinner, S. Kääb

Pharmacogenetics of response to cardiovascular drug therapy: what is the current state of knowledge? - C. E. de Keyser, M. Eijgelsheim, A. G. Uitterlinden, B. H. Stricker

Can EMRs (electronic medical records) facilitate gene-based drug prescribing for cardiovascular disease? - J. C. Denny, R. A. Wilke

Fascinoma Cardiologica

Trails of Discovery: The evolution of tissue protection by fibrinolytic therapy - J. D. Fitzgerald

Summaries of Ten Seminal Papers - D. M. Roden

The role of genetically determined polymorphic drug metabolism in the beta-blockade produced by propafenone - J. T. Lee and others

Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo - S. Hoffmeyer and others

Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose - M. J. Rieder and others

Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects - J. S. Hulot and others

A polymorphism within a conserved β1-adrenergic receptor motif alters cardiac function and β-blocker response in human heart failure - S. B. Liggett and others

SLCO1B1 variants and statin-induced myopathy—a genome-wide study - SEARCH Collaborative Group; E. Link, and others

Estimation of the warfarin dose with clinical and pharmacogenetic data - International Warfarin Pharmacogenetics Consortium; T. E. Klein, and others

Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy - A. R. Shuldiner and others

Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis - J. L. Mega and others

A randomized and clinical effectiveness trial comparing two pharmacogenetic algorithms and standard care for individualizing warfarin dosing (CoumaGen-II) - J. L. Anderson and others

Bibliography of One Hundred Key Papers
CARDIOVASCULAR PHARMACOGENETICS: MUCH ADO ABOUT NOTHING?

Pharmacogenetics is the study of inherited differences in drug response.¹ It uses the genetic information of genes encoding proteins involved in the metabolism, transport, and action of drugs (so called “pharmacogenes”) to allow a more accurate prediction of a patient’s drug response and selection of the appropriate drug dosage to achieve the optimal therapeutic response, avoid therapeutic failure, and minimize side effects and toxicity.

Right from the beginning when the first genetic polymorphisms of drug metabolizing enzymes were described, their clinical significance has been questioned and it has been doubted whether this goal will ever be accomplished.

Characteristic of this attitude is an unsigned editorial published in the Lancet in 1984: “Polymorphic drug oxidation: much ado about nothing.” This editorial questioned the clinical relevance of the CYP2D6 and 2C19 polymorphisms that had been discovered only a few years ago²-⁶ and concluded “that there is no evidence that wide variability in plasma concentration after a fixed dose is likely to harm a patient—particularly if a reliable clinical means exists of detecting underdosage or overdosage. In the case of debrisoquine, for example, blood-pressure response can be measured, and with metoprolol heart rate can be used as an index of β-receptor activity.”² It is of interest to note that many of the drugs initially discovered to be substrates for CYP2D6 were cardiovascular drugs.⁷,⁸

Three decades later with the human genome sequenced in 2001 and the progress made in genomic sciences since, it is quite timely to ask where we stand in 2012. The articles in this issue of Dialogues in Cardiovascular Medicine provide a good account of how the field of cardiovascular pharmacogenomics has advanced. For many “pharmacogenes,”

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Keywords: adverse drug reaction; biobanks; drug-metabolizing enzyme; electronic medical record; genome-wide association study; genomics; pharmacogenetic; polymorphism

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not only have the molecular genetics and the functional consequences of the variants been elucidated, but an ever-increasing number of drugs have been identified where polymorphisms of these pharmacogenes impact their disposition and action. Although it is nice to know these data, the critical issue that has not been addressed in many studies is their clinical utility, eg, have the findings the potential to improve the drug treatment of the patients with regard to efficacy and safety? This poses the question why so many studies failed to demonstrate clinical utility.

By far the best studied examples are polymorphisms of drug metabolizing enzymes, the reasons being that drug or metabolite concentrations are an easily determined phenotype. The concept of high-risk pharmacokinetics as discussed by Dan M. Roden outlines the scenarios where genetic polymorphisms of drug metabolizing enzymes matter.

If the major pathway of metabolism is catalyzed by a polymorphic enzyme exposing poor metabolizer (PM) patients to standard doses of such a drug leads to high systemic drug exposure that can result in exaggerated drug response or toxicity as is the case with warfarin, the same poor PM phenotype will show diminished or absent response if therapeutic effects are mediated by an active metabolite—so called prodrug—formed by a polymorphic enzyme. Clinically relevant examples are clopidogrel and tamoxifen. This high-risk pharmacokinetic scenario—a major elimination pathway being impaired by diseases or drug-drug interaction—is the reason why regulatory agencies require pharmacokinetic studies for patients with liver and renal disease, in the elderly, children, and drug-drug interaction studies to see whether these conditions alter drug elimination, leading to higher drug concentrations. In this case it is recommended in the labeling to reduce the dose accordingly or to avoid the drug combination. This requirement is based on the notion that with the higher drug concentrations seen in these conditions, the probability of concentration-related side effects or toxicity is increased. However, no controlled clinical trials are required to explore: (i) whether the higher drug concentrations are associated with an exaggerated drug response or toxicity; and (ii) lowering drug concentration by dose adjustment will reduce or avoid side effects.

In the case of polymorphisms where it is not unusual to see a 100-fold difference in drug concentrations among different genotypes—which by far exceed the changes we see in disease conditions or drug-drug interactions—the concept that at higher drug concentrations exaggerated drug response and toxicity are more likely to occur is being questioned and it is demanded to prove the utility of genotype-based dose selection in clinical trials. To me it is difficult to come up with an explanation based on evidence why the same drug concentration should elicit different effects depending on the mechanism leading to elevated drug concentrations. Hence it is arguable whether we always need controlled clinical trials to prove the clinical utility of genotype-based dose selection, or if carefully conducted pharmacokinetic studies in well-defined genotypes suffice for dose selection analogous to the scenario outlined for disease conditions and drug-drug interactions.
Moreover, there is circumstantial evidence that genetic polymorphisms of drug metabolizing enzymes play a role in the pathogenesis of predictable type A adverse drug reactions (ADR), and basing drug therapy on a patient’s individual genetic makeup may result not only in an improved response, but also in a clinically important reduction in ADRs. For example, of 27 drugs frequently cited in 18 ADR studies, 59% are metabolized by at least one enzyme with a variant allele associated with decreased drug metabolism. Conversely, only 7% to 20% of randomly selected drugs are metabolized by enzymes known to exhibit functional genetic polymorphisms. Genetic variability in drug-metabolizing enzymes and transporters may therefore be an important contributor to the incidence of type A ADRs. A study from the Karolinska Institute where requests for CYP2D6 genotyping were analyzed has shown that non response in over 85% of ultrarapid metabolizers (UMs) and adverse reactions in 55% of PMs were the reasons for genotyping.

Data from both retrospective and prospective observational studies with metoprolol indicate that exaggerated drug response such as bradycardia occurs more often in PMs and a significant increased frequency of 38% PMs was observed among 24 patients with metoprolol-associated side effects necessitating discontinuation of therapy or dosage reduction. It is interesting to note that in these observational studies clinical means such as heart rate were not used to adjust the dose because irrespective of the CYP2D6 genotype PM patients were treated with nearly identical or same doses as patients with the intermediate metabolizer IM, extensive metabolizer (EM), or UM genotype. In those studies where metoprolol plasma levels were measured a gene-dose effect was seen with an up to 50-fold difference between UM and PM patients. If metoprolol levels in the EM group are taken as the levels sufficient for β-receptor blockade, the metoprolol dose would need adjustment from 12.5 mg in PM to 400 to 500 mg in UM patients. It can only be speculated why there is this little variability in metoprolol dose. But most likely physicians are afraid of underdosage and overdosage by using such low and high doses.

Thus, contrary to what has been claimed, variability in drug concentrations matters and translates into differences in response and side effects. However, if we are to use genotype-based pharmacokinetic data for dose selection, we have to realize the limitations of the data presently available. Most of the pharmacokinetic studies have been carried out mainly in healthy male volunteers, and pharmacokinetic data on rare genotypes are usually restricted to only a few subjects. Furthermore, the contribution of nongenetic factors is usually not assessed, but needs to be studied in order to develop meaningful dosing algorithms.

In the context of polymorphisms of drug metabolism and transporters we need to address how much variation in drug concentrations can be explained by variants and how predictive is the genotype for the phenotype drug concentration. Do differences in sys-
temic drug exposure translate into differences in response and toxicity? Can genotype-based dose selection increase response, avoid nonresponse, or minimize toxicity?

Most studies have been limited to associations between genotypes for drug metabolizing enzymes and quite often not well-defined drug response. Moreover, the effect sizes quite often are too small to be clinically meaningful. What is lacking are studies demonstrating how drug concentrations are related to response/side effects, what impact genetic and nongenetic factors have on systemic drug exposure, and how this is related to clinical outcome. Missing data on drug concentrations, the poor quality of clinical data, and drug response phenotype, especially in retrospective studies, has resulted in the poor predictive value of many pharmacogenetic association studies.

Furthermore, many of the studies discussed by Catherine E. de Keyser et al and Dan M. Roden suffer from serious methodological shortcomings: They have too small sample sizes, population stratification is not addressed, inclusion and exclusion criteria are not well defined, clinical data are incomplete, and drug response phenotypes are poorly defined. Quite often no replication studies have been carried out or the results could not be confirmed. Another important aspect that has been neglected and that impacts outcomes in relation to the polymorphism studied are shortcomings in genotyping with respect to the quality of the method, allele coverage, and correct genotype-phenotype assignment. For instance, in the case of the highly polymorphic CYP2D6 gene with more than 100 germline polymorphisms restricting genotyping to the most common variant, allele *4 will result in wrong genotype assignment in European populations with 4.7% PMs, 34.4% heterozygous EMs, and 60.9% homozygous EMs, as opposed to the correct genotype frequencies of 8.3%, 54.4%, and 37.3%, respectively, obtained using Amplichip, which translated into different clinical outcomes in relation to CYP2D6 genotypes. Even if genetic data can be generated retrospectively by obtaining DNA from prospective randomized clinical trials, so called “prospective-retrospective studies,” they need to meet the requirements outlined above. Neglect of these requirements has produced conflicting data.

Moreover, many pharmacogenetic association studies have focused on one gene, ignoring the fact that drug response phenotypes, like most disease phenotypes, are polygenic traits with nongenetic factors contributing to the manifestation of phenotypes.

In view of the substantial morbidity and mortality and the considerable costs for the health care system associated with severe ADRs, preemptive diagnostic tests to identify patients at risk for both type A and B ADRs would be a major step forward toward the safer use of drugs. As outlined by Dan M. Roden, drug-induced arrhythmias, severe skin lesions, hepatotoxicity, and myopathies continue to be a significant problem for many drugs during the development and postmarketing phase. The examples pro-
vided in this issue and published elsewhere demonstrate the rapid progress that has been made over the past decade in our understanding of the genetics and molecular mechanisms of severe ADRs.

At the present time, however, we have to realize that with the exception of abacavir, carbamazepine, and allopurinol, the positive and negative predictive value of the genetic markers identified so far is not this good as to advocate their routine use. The question we have to ask is whether it is justified and cost effective to test all patients in the case of very rare adverse events occurring in 1:10 000 or 1:100 000 of patients.

Do we always need controlled clinical trials or are there alternatives? Ideally, the implementation of pharmacogenomics into practice should be based on data from prospective clinical trials. To be realistic, however, for the many drugs on the market, the resources for carrying out these studies are simply not available. Hence, there is a need to develop alternative strategies. The article by Joshua C. Denny and Russell A. Wilke provides such an alternative. The innovative aspect of this approach is that it uses clinical data from comprehensive electronic medical records and links these data with biobanks (DNA). It has the potential to generate reliable data in the clinical setting without resorting to controlled clinical trials.

I consider this approach as an extremely valuable resource to establish genotype-phenotype relationships for a number of important drugs used to treat common diseases. Moreover, in view of the sample size of more than 130 000 patients that have been recruited at Vanderbilt so far, it will allow getting reliable data on the impact of rare genotypes, for instance the *3 allele of CYP2C9 on drug levels of warfarin response and bleeding complications. Furthermore, it constitutes a valuable resource for hypothesis-generating studies and for replication cohorts as recently shown for warfarin and clopidogrel. It will also be a valuable resource to study the impact of rare genotype combinations such as PMs of both CYP2D6 and CYP2C9 which occur only in 1 out of 700 to 800 subjects and require a population size of 35 000 to 40 000 to recruit 50 subjects. Furthermore, if we are going to resolve the causes of very rare idiosyncratic drug reactions such as Stevens-Johnson syndrome, toxic epidermolysis, hepatotoxicity, drug-induced arrhythmias, or rhabdomyolysis, this can only be accomplished by sharing data from various national and international medical centers that have the same operating system. By having access to millions of patients, enough index cases with well-defined phenotypes can be recruited for genome-wide association studies (GWAS).

As outlined in the article by Moritz F. Sinner and Stefan Kääb, the advances made in genomics will also advance our knowledge in pharmacogenetics. The present limitations of GWAS, which allow only analysis of the common variant, will be overcome by ExomeChip genotyping and next-generation sequencing, which can identify rare variants.
The data generated so far demonstrate that the majority of variants are rare. Even for extensively studied genes such as *CYP2D6*, with more than 100 known variants, and *CYP2C9*, with 45 known variants, the exome sequencing project has identified 58 and 22 novel nonsense or missense variants, respectively. For both P450s, there is substantial variation with respect to protein expression, catalytic function, and drug levels in a given genotype. Whether these new variants can explain aberrant drug responses in subjects with “normal genotypes” as assessed by current genotyping methods, remains to be seen.

The rapid progress being made in genomics and the other “omics” sciences, and in linking electronic medical records (EMRs) with biobanks, holds promise that molecular diagnostic tests at reasonable costs will be available in the not too distant future that can be used to improve efficacy and safety of medicines by targeting drugs to the right patient, select the correct dose, and identify patients at risk for serious ADRs.

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Cardiovascular pharmacogenetics: opportunities and challenges

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Drug therapy has revolutionized the modern management of heart and vascular diseases such as atherosclerosis, hypertension, thrombosis, and heart failure, resulting in a dramatic decrease in cardiovascular morbidity. However, even for highly effective therapies, “variability” is a sine qua non, so that although the “average” patient will benefit from therapy, the clinician can never be certain whether or not some patients will fail to respond or display adverse effects. The application of the tools of modern molecular science and genomics to the problem of variable drug response has identified genetic polymorphisms that contribute to variable drug responses. The question of establishing which of these relationships are sufficiently compelling to apply to clinical care is being addressed, and new technologies are being mobilized to implement a future view of genome-enabled health care. This report summarizes the history and current state of the field, and lays the groundwork for three accompanying more detailed Respondent Articles addressing methods in pharmacogenomics, validated genotype-drug response relations in cardiovascular pharmacogenomics, and the use of advanced information technology to deliver genomic variant data to the bedside.

Drug costs make up an enormous portion of overall medical costs across the globe. In the United States, the annual expenditure for health care in 2010 was estimated at over $2.5 trillion, with prescription drugs making up $260 million. In the Western World, cardiovascular disease and stroke remain the commonest cause of overall mortality, and rates are rising rapidly in other parts of the world. Treatments used for common cardiac conditions like hypertension, hypercholesterolemia, or thromboembolic disease often involve lifelong treatment with interventions proven in large randomized trials to have a beneficial effect on important outcomes like recurrent myocardial infarction, stroke, or death. Indeed, mortality from cardiovascular disease is dropping in Western Europe and the United States, and while many factors, such as dietary alterations or reduced rates of smoking, can be credited, drug interventions likely play a role as well. This has been one of the great success stories of the latter half of the 20th century.

EXPERT ANSWERS TO THREE KEY QUESTIONS (see page 269)

This issue of Dialogues in Cardiovascular Medicine is devoted to key emerging issues in the discipline, discussed below and in the accompanying articles. Moritz F. Sinner and Stefan Kääb address the advantages and disadvantages of a range of methodological approaches in the field. Catherine E. de Keyser and colleagues present examples from a range of disciplines within cardiovascular medicine of genetic variants with well-validated effects on cardiovascular drug therapy outcomes. Finally, in the third article, Joshua C. Denny and Russell A. Wilke present a vision for how electronic medical records (EMRs) can be used as a tool for delivering an individual’s genetic information to their health care.
Nevertheless, variability in response to therapy in individual subjects is the sine qua non of contemporary drug therapy targeting cardiovascular and many other diseases (Figure 1). Thus, modern medicine faces a paradox that “average” patients will benefit from many widely used therapies, but that an individual clinician is never certain whether a specific patient is in the “average” majority or whether that patient is more likely to show a clinically important altered response to treatment. Understanding fundamental mechanisms underlying variable responses to drugs is an important exercise since it not only holds the promise of deploying current, highly effective, therapies in a more effective fashion, but also may point to avenues for new drug development.

THE PROBLEM OF VARIABLE RESPONSES TO DRUG THERAPY

Drugs exert their pharmacologic effect by interacting with molecular receptor(s), and both pharmacokinetic and pharmacodynamic variables can impact this process (Figure 2). A desired clinical response may be absent because insufficient drug is delivered to the receptor, the receptor may not bind to or respond to drug exposure in the predicted fashion, or competing physiologic or disease-related processes may modulate response. This formulation assumes that a specific molecular receptor is expressed and is known to play a role in the disease process: for many complex cardiovascular syndromes, such as congestive heart failure or coronary artery disease, specific single molecular targets are known, but the extent to which they play a role, in an individual subject, in modulating the disease process itself is often not well understood. Drugs may also fail to produce effects because the initial diagnosis is incorrect or there is a dispensing error and the wrong drug or the wrong drug dose is administered. Failure of compliance, which may reflect complex issues such as insight into disease, or literacy or numeracy, is another common contributor.
Efficacy

Efficacy is conventionally presented as a quantitative assessment of the extent to which exposure to a drug produces a measurable response. Such metrics can be “hard” end points, such as incidence of a stroke, myocardial infarction, or death. Depending on the population studied, these may be relatively uncommon events, so another approach to gauge drug effect is to use “intermediate” end points (sometimes termed endophenotypes) that are thought to link to such serious end points: decreases in low-density lipoprotein (LDL) cholesterol or in blood pressure are examples. The logic is that a drug effect to modify in an apparently desirable fashion such endophenotypes will translate to improved drug efficacy with respect to “hard” end points. However, lessons of the last two decades have shown that drug-induced changes in such endophenotypes are not necessarily indicative of a beneficial clinical outcome. In CAST (Cardiac Arrhythmia Suppression Trial), suppression of ventricular ectopic activity by encainide or flecainide (an endophenotype thought to be desirable at the time) was associated with an unexpected increase in mortality. More recently, therapy with torcetrapib, a cholesterol ester transport protein (CETP) inhibitor designed to elevate high-density lipoprotein (HDL) cholesterol and reduce atherothrombotic-related events, also unexpectedly increased mortality. In both cases, the “disconnect” between the effect of treatment on the endophenotype and the “hard” end point likely reflected incomplete knowledge of the relationship between sodium channel block and arrhythmogenicity in the case of CAST, and torcetrapib-specific pharmacologic effects that may have contributed to an “off-target” (i.e., unrelated to CETP inhibition) effect.

Adverse events

Adverse drug reactions (ADRs) events are common during drug therapy. By one estimate, in the late 1990s, over 2 million hospitalized patients in the US suffered serious ADRs, defined as requiring hospitalization or causing disability or death, every year. In that study, there were an estimated 106 000 deaths due to unexpected drug response, making this the 4th to 6th commonest cause of death among hospitalized patients. Importantly, this estimate represents a lower limit on the more general problem of ADRs, since it was generated after eliminating cases of errors in drug administration (wrong drug or dose), noncompliance, drug abuse issues, and overdose. In addition, therapeutic failures were not considered: indeed, it may be no over exaggeration to state that the commonest serious adverse drug effect is failure of a drug to achieve its desired pharmacologic effects. A general question that variability in efficacy and especially in adverse events raises is the extent to which individual patients are susceptible due to variable genetic backgrounds. Until very recently, DNA samples have not been obtained and archived in many large clinical trials, so this question remains largely unanswered and an opportunity for future work.
Types of ADRs

One type of ADR is a simple extension of a drug’s known pharmacologic effects; hypotension with antihypertensives and excessive bleeding with anticoagulants are examples. A second type of ADR that can be very clinically important, but near-impossible to detect, is a drug-induced increase in the frequency of a serious, but common, health condition. One example is the increase in sudden cardiac death due to administration of sodium channel-blocking antiarrhythmics in CAST. Another example is an increase in myocardial infarction identified with the COX2 (cyclooxygenase 2)-inhibitor rofecoxib. In both instances, it was in a randomized clinical trial that these increases in common clinical events (sudden death, myocardial infarction) were detected and in both cases, the identification of these adverse events led not only to drug withdrawal, but also to new mechanistic insights into mechanisms of drug action. Importantly, data suggesting a potential for these adverse events were available before the results of the randomized trials, and this included mechanistic clinical pharmacology studies, clinical reports and small series, and data from large databases—including EMR-derived sets—linking drug exposure to adverse outcomes. The extent to which other agents, perhaps some in common use, are also affecting common diseases is unknown. Interestingly, pharmacopidemiologic data do suggest that some drugs, such as atypical antipsychotic agents or certain antibiotics (erythromycin, azithromycin), increase sudden cardiac death rates when deployed across a population.

A third type of ADR is one that can be clearly related to drug administration, but which occurs only rarely and the mechanism may or may not be related to the drug’s known pharmacologic effects: immunologic reactions such as hepatotoxicity or serious drug rash, or drug-induced arrhythmias are examples. Such serious ADRs are by definition rare since common serious ADRs would preclude a drug from reaching the market. The very rarity of these events, and their seeming unpredictability, raise questions about underlying genetic susceptibility, as discussed further below.

PHARMACOGENETICS: A BRIEF HISTORY

The idea that variability in response to drugs might be a heritable trait was first advanced by the English physiologist Garrod, in his studies of “inborn errors of metabolism.” The first examples of genetically determined variability in drug response came during and after World War II, and included the development of hemolytic anemia in African-American soldiers with glucose-6-phosphate dehydrogenase (G6PD) deficiency exposed to antimalarial drugs in the South Pacific; prolonged apnea after succinylcholine due to pseudocholinesterase deficiency; variable N-acetylation of isoniazid associated with hepatotoxicity; and malignant hyperthermia, now recognized to be due to mutations in the skeletal muscle ryanodine release channel encoded by RyR1, after exposure to general anesthetics. Even these first few examples highlight several principles that continue to resonate in contemporary pharmacogenomics: the importance of ancestry, an initial focus on rare ADRs, and variability in drug responses due to both pharmacokinetic (pseudocholinesterase, N-acetylation) and pharmacodynamic (Ryr1, G6pd) variants, discussed below. Early studies noted that such drug responses could be heritable and thus defined the field of “pharmacogenetics” focusing on single inherited traits with large effects. The more modern term “pharmacogenomics” extends this idea to the study of the way in which multiple genetic variants within an individual or across a population determine variability in drug response.

The early history of pharmacogenetics highlights the idea that discoveries that drove the development of the field occurred at the bedside: astute clinicians identified individuals or small groups of patients with highly aberrant drug responses and sought to pursue underlying mechanisms. In the 1970s, two groups of clinical pharmacologists, one studying variability in response to an antihypertensive agent (debrisoquine) and the other variability in response to a tocolytic (sparteine), described patients with aberrant responses. Both groups established that these variable drug responses were attributable to striking variability in plasma drug concentrations, and that outlier individuals with unusually high plasma drug concentrations were those who lacked specific enzymatic activity required to metabolize the drugs: debrisoquine 4-hydroxylase and sparteine N-oxidase. Subsequent studies have established that these enzymatic activities are in fact identical, both manifestations of what we now term CYP2D6; the patients displaying aberrant responses were CYP2D6 poor metabolizers. CYP2D6 poor metabolizers are those subjects who are homozygotes or compound heterozygotes for loss of functional alleles, i.e., those with variants on both copies of the gene. Similarly, variability in plasma concentrations of isoniazid, mephenytoin, and warfarin were initial clues to the existence of poor metabolizer traits for
There are two important “high-risk pharmacokinetics scenarios” under which variation in drug disposition pathways, determined by DNA polymorphisms or by coadministration of other drugs, may exert very large effects on drug responses (Figure 3). The first such “high-risk pharmacokinetics scenario” is the administration of drugs that require bioactivation through specific metabolic pathways to generate active metabolites that then mediate pharmacologic effects. Poor metabolizers can then display reduced or absent drug actions. Clopidogrel, tamoxifen, and codeine are examples. Interacting drugs that are inhibitors of specific drug-metabolism pathways are widely used in cardiovascular and other therapies, and thus can mimic (“phenocopy”) the poor metabolizer trait. Important drugs in this regard include ketoconazole, erythromycin, amiodarone, verapamil, diltiazem, and quinidine, which are potent inhibitors of CYP3A4, paroxetine and fluoxetine (CYP2D6), many proton pump inhibitors (CYP2C19), and phenytoin and amiodarone (CYP2C9). Further, a number of drugs (rifampin, phenobarbital, phenytoin) are potent inducers of CYP3A4 and of P-glycoprotein, and thereby lower, sometimes drastically, circulating plasma concentrations of substrate drugs. A list of inhibitor drugs (and the strength of inhibition), inducers, and substrates for a range of P450s is maintained at http://medicine.iupui.edu/clinpharm/ddis/table.aspx.

High-risk pharmacokinetics scenarios: variable bioactivation of drugs

The second “high-risk pharmacokinetics scenario” is administration of a drug that is bioinactivated by a single pathway that is subject to variability. Such variability may reflect genetic variation, drug interactions, or disease of excretory organ function, and in these situations there is accumulation of parent drug with the potential for toxicity related to unusually high drug concentrations. The use of ordinary doses of the QT-prolonging agents sotalol or dofetilide, both renally excreted, in patients with renal dysfunction, can result in increased parent plasma drug concentrations, prolonged QT intervals, and increased risk for torsades de pointes. Digoxin is eliminated by renal and biliary excretion, mediated largely by the drug efflux pump P-glycoprotein, encoded by MDR1 (or ABCB1). Coadministration of P-glycoprotein inhibitors, such as amiodarone, quinidine, verapamil, itraconazole, or erythromycin, routinely results in elevated digoxin con-

**Figure 3.** The concept of high-risk pharmacokinetics.

Two scenarios, both characterized by a drug that undergoes metabolism or elimination by a single molecular pathway, increase the risk that genomic variation, drug interactions, or abnormal excretory organ function may generate highly atypical drug concentrations and responses. One (left) is the situation of a prodrug, typified by clopidogrel, codeine, or tamoxifen. The other (right) is a drug that is biotransformed to inactive metabolites by a single enzyme system or eliminated unchanged; if the drug also has a narrow margin between the doses required for efficacy and those producing toxicity, the absence of the pathway may result in toxicity.


N-acetylation, CYP2C19, and CYP2C9, respectively. For these genes, single loss of function variants may confer clinically significant variability in response to substrate drugs, while homozygotes or compound heterozygotes are at even further increased risk. In these instances, evidence gathered from studies in families, studies in twins, and studies across populations were used to describe variability in plasma drug concentrations and drug response. This work, in turn, laid the foundation for exploitation of tools of modern molecular science to identify the genetic and molecular bases of these traits.

**MECHANISMS LEADING TO VARIABLE DRUG RESPONSES**

Figure 2 highlights the sources of variability in drug response. These can be broadly broken down into two categories: pharmacokinetic and pharmacodynamic. Pharmacokinetics is the science of describing drug concentrations in plasma or other sites as a function of time after drug administration. Processes that modulate drug concentrations include absorption, distribution, metabolism, and elimination. These processes are determined largely by the specific enzymes that determine drug metabolism (generally oxidation and conjugation reactions) and drug transport molecules that modulate drug absorption, distribution, and elimination, generally through renal or biliary excretion.
centrations and potential toxicity, and this effect is phenocopied in \textit{ABCB1} knockout mice.\textsuperscript{43} DNA variants in \textit{ABCB1} may also modulate digoxin plasma concentra-
tion and effect,\textsuperscript{44} as further discussed by de Keyser et al in this issue. The \textit{β}-blocker metoprolol\textsuperscript{45} and the sodium channel blocking antiarrhythmic propafen-
one\textsuperscript{46,47} are bio-inactivated by the hepatic P450 cyto-
chrome CYP2D6; poor metabolizers, who make up 5% to 10% of white and African-American populations, display increased concentrations of the parent drug and increased susceptibility to adverse effects such as bronchospasm or bradycardia. In each of these cases, the common thread is elimination by a single pathway and the potential for serious drug toxicity with increased drug concentrations. The potential for such toxicity is much smaller with drugs that have little potential for serious toxicity at elevated concentra-
tions, and with drugs that have multiple elimination pathways. Nevertheless, occasional patients may have lesions in more than one elimination pathway and thus display unusually elevated drug concentrations, and attendant toxicity.\textsuperscript{48} Notably, a number of currently used classes of cardiovascular therapies, such as antithrombotics and antiarrhythmics, have relatively narrow margins between the doses or plasma concentra-
tions producing efficacy and those associated with toxicity, and so this phenomenon of “high-risk” pharmacokinetics has been a particular problem in these therapeutic areas. As a result, much of our contemporary understanding of pharmacogenetic principles has risen in the course of studying such drugs.

\textbf{ASSOCIATING VARIABLE DRUG OUTCOMES WITH DNA POLYMORPHISMS}

A key discovery goal in modern genomic science is to describe the relationship between an observed trait and DNA variation. The trait can be continuous (height, LDL cholesterol, plasma drug concentration, ECG interval, etc) or discrete (red hair color, breast cancer, myocardial infarction during statin therapy, vision loss in diabetes, in-stent thrombosis during clopidogrel treatment, etc). As these examples highlight, the traits may be physiologic measures, susceptibility to disease, or outcome of a disease or disease treatment. Pharmacogenomic traits of interest include direct or indirect measures of pharmacokinetics or pharmacody-
namics (such as steady-state dose requirements during warfarin therapy or plasma drug concentrations), or beneficial or adverse outcomes during drug treatment. Two broad approaches have been used in genomics, and in pharmacogenomics, to establish relations between DNA variation and human traits: the candidate gene approach and the “unbiased” approach, usually a genome-wide association study (GWAS), discussed in detail by Sinner and Kääb. An absolute requirement for either experimental paradigm is the availability of DNA samples from patients with well-defined drug response phenotypes.

As genome science has evolved from studies of small numbers of individuals with unusual traits to larger populations, an important metric has been heritability, or the extent to which genomic variation can explain variability in a trait under study. Studies of family structure, including twin studies, have been used to estimate heritability for physiologic or disease susceptibility traits, but these have been harder to apply in pharmacogenomics, since it is unusual for multiple members of a kindred to be exposed to a drug, with a well-defined drug outcome phenotype, under identical conditions (ie, similar disease, similar age, etc). Nevertheless, heritability for drug response traits has been estimated using twin studies\textsuperscript{33,49-54} and more recently in genome-wide association studies.\textsuperscript{55}

\textbf{Candidate gene studies}

The candidate approach uses knowledge of underlying physiologic, pathophysiologic, and pharmacologic processes to generate a set of genes, and common variants (often those known to be functional) within those genes, and to then relate allele frequencies for these variants to the traits under study. The approach seems highly logical. However, experience over the last decade of genome science has repeatedly shown that, despite the apparent logical appeal of the approach, replication of seemingly high-impact associations has been very much the exception rather than the rule.\textsuperscript{56-58} Many reasons have been advanced for this observa-
tion. For example, a study that fails to identify an associa-
tion between a logical candidate variant and a human trait is unlikely to be published, whereas one that identifies a seemingly strong association may make a big and high impact “splash.” Experience has shown that under this condition, subsequent analyses of larger datasets result in weaker, and ultimately often nonsignificant associations. A second possible expla-
nation for the general failure of the candidate gene approach in genomics is that many traits studied turn out to have very complex physiologies so the expecta-
tion that single common variants will exert a large ef-
cfect on such traits seems, at least in retrospect, naïve. Further, a common DNA variant that exerts a large ef-
cfect on a disease that affects individuals at or before reproductive age would not be expected to persist in
a population. These considerations apply to candidate gene studies in general, but there are real and potential exceptions: conditions that only arise with environmental exposures, including drugs and other features of modern society (diet, lack of exercise, smoking, etc), or conditions arising after reproductive age, such as age-related macular degeneration or many cases of atrial fibrillation (AF).

In the case of drug exposure, the “high-risk” pharmacokinetic scenarios outlined above often contribute variants in single pathways that can result in very large variability in plasma concentrations of active drug, so common polymorphisms in candidate pharmacokinetic genes, such as \textit{CYP2C19}, \textit{CYP2C9}, or \textit{CYP2D6} can exert strong and highly reproducible influences on drug response traits. Similarly, variants that profoundly alter function of drug targets, and in particular those that may modulate amount or function of drug targets, can exert strong effects on drug outcomes; one good example is the way in which coding and noncoding variation in \textit{VKORC1} modulates warfarin steady-state dose requirements, discussed below. Another example is the very strong association between the human leukocyte antigen HLA-B variant *5701 and serious skin toxicity with the antiretroviral abacavir.\textsuperscript{59} As with any initially “positive” candidate gene study, any pharmacogenetic candidate gene study requires replication before it can be accepted as valid. The abacavir marker is one of the few pharmacogenomic markers whose value has been demonstrated in a randomized clinical trial.\textsuperscript{60}

**GWAS for pharmacogenomic traits**

The GWAS paradigm, discussed in detail by Sinner and Käåb, searches for associations between defined traits and common variation across the genome. A major strength of the approach is the ability to identify new genes or pathways modulating important human traits that would be inaccessible by candidate approaches because the pathophysiology of the trait is insufficiently understood. A disadvantage of the approach is that common variants interrogated by the GWAS approach rarely confer odds ratios in excess of 1.5-2, and relatively large datasets are often required to identify and replicate such signals. Another disadvantage is that single nucleotide polymorphisms (SNPs) implicated by GWAS as modulating a trait are not necessarily (and usually are not) the functional variants, but rather should be viewed as a signpost in the genome, identifying a locus within which rare or common functional variants are located. GWAS data have also been used to analyze the extent to which variability in the traits under question include a heritable component.\textsuperscript{61} As with the example of candidate gene associations, the application of GWAS to pharmacogenomic traits has provided some interesting exceptions to these generalizations. For example, application of GWAS to analysis of severe adverse drug reactions has frequently resulted in very strong (low \textit{P} value, high odds ratio) signals even when only several dozen cases were studied. As further highlighted by both de Keyser et al and Denny and Wilke, analysis of 85 cases of myopathy during high-dose simvastatin compared with 90 matched controls identified a very strong signal near \textit{SLCO1B1}, encoding a hepatic drug uptake transporter. Indeed, the encoded protein, OATP1B1, had already been implicated as a modulator of statin pharmacokinetics,\textsuperscript{62,63} and the SNP identified by GWAS was in strong linkage disequilibrium with a known functional nonsynonymous (ie, one that changes the encoded amino acid) SNP resulting in V174A in the transporter. Individuals homozygous for the risk allele made up 2.7% of the study population of over 6000 patients, but were at >20-fold increased risk for developing simvastatin myopathy: among homozygous subjects, the incidence of myopathy was 18.6% over 5 years, compared with 2.8% for the heterozygotes (who made up 24.9% of patients) and 0.6% for the remaining 73% of the population who were homozygous for the reference (“wild-type”) allele. Similarly, GWAS approaches using only several dozen cases have identified variation in the HLA-B locus in subjects developing severe skin reactions during carbamazepine\textsuperscript{64} therapy or hepatitis during treatment with the antibiotic flucloxacillin.\textsuperscript{65}

Another application of GWAS in pharmacogenomics has been to “rule out” a role for common variants modulating important pharmacogenetic traits. GWAS analysis of steady-state warfarin dose has identified variants in \textit{CYP2C9} and \textit{VKORC1},\textsuperscript{66} and conditioning the analysis on these two loci further identified variation in \textit{CYP4F2}.\textsuperscript{67} However, there was no signal in residual variation, suggesting that there are no other single loci with very large effects on warfarin steady-state dose. Similarly, analysis of over 200 cases of drug-induced torsades de pointes compared with drug-exposed controls (not developing arrhythmia) or population controls revealed no strong signal, suggesting that single common SNPs with large effect sizes do not modulate risk for this severe adverse drug event.\textsuperscript{68}

By identifying variants contributing to disease susceptibility, GWAS can also lead to candidate studies examining how those variants affect drug responses. The QT responses discussed by de Keyser et al are one
example. Another is the consistent signal associating SNPs at 4q25 with risk of AF. The closest gene is PITX2, encoding a transcription factor important for left-right differentiation early in development. Studies in knock-out mice support this as a causative gene: there is altered expression of the “pulmonary myocardium” (the segment that invaginates from the left atrium and from which AF is frequently driven), inducible AF and upregulation of pro-AF genes, such as KCNQ1 and NPPA. Further, recent studies have reported an association with 4q25 variants and a beneficial response to either ablation or antiarrhythmic drug therapy. 69,70

### Other high-dimensional approaches in pharmacogenomics

GWAS likely represents a first iteration of new tools using “high-dimensional” data to unravel the basis of variability in a range of human traits. A key challenge that statistical tests of association at 500 000-1 000 000 SNPs imposes is sorting out true from false positives, and repeated replication has the mainstay of dealing with this issue. Various methods to simplify the traditional GWAS analysis, often focusing on specific genes, specific pathways, or specific “gene modules,” 71,72 have been proposed to reduce the number of statistical tests, and thereby enable gene-gene and gene-environment interaction studies. Biological validation not only adds to the believability of a GWAS signal, but starts to unravel the underlying mechanisms.

Another interesting approach to gene discovery has been to undertake medium- or high-throughput screening directly in model organ systems. In one such study, 73 the response of wild-type zebrafish embryos to the QT-prolonging drug dofetilide was first established, and then mutagenized zebrafish lines screened for aberrant responses. The study found that fish with mutations at the GINS3 locus displayed aberrant response to drug challenge, interestingly, this locus has also been implicated as a modulator of QT in GWAS studies.

Tools to re-sequence large target regions of the genome, or the entire genome, are becoming increasingly inexpensive. In some cases, whole exome or whole genome sequencing of one or a few individuals with unusual traits from small families (previously inaccessible to traditional linkage analysis) has succeeded in identifying specific rare DNA variants likely causing unusual diseases. 74-76 Resequencing within regions identified by GWAS “hits” is another application of this new technology. Resequencing in nongermline genomes (the microbiome and other microbes; tumors) has yielded startling new biological insights and has identified new drug targets. Proposals to apply such technology to counsel individual highly curious patients or families, 77 or to supplant neonatal screening are commonplace, but the ethical challenges involved in implementing such approaches are considerable.

Modern technologies have yielded other types of “high-dimensional” data, such as circulating metabolites (“metabolomics”), or protein profiling (“proteomics”). Each of these approaches has the potential to be applied to the problem of variable drug responses and to identify new targets for drug action. Another intriguing approach is the development of methods to integrate diverse types of high dimensional data 78 to identify patterns that may mark individuals at especially high or low risk for disease or unusual drug responses. Such high-dimensional datasets can be genomic, proteomic, metabolomic, transcriptomic, etc, and may also include other types of data not generated at the “wet bench,” such as imaging data or dense phenotypic data such as represented in an EMR, the “phenome.”

### BRIEF OVERVIEW OF WARFARIN AND CLOPIDOGREL

These examples are presented here because they illustrate the pathway from discovery to candidate gene work to GWAS to potential implementation. While in both cases there is controversy over the clinical utility of the known genomic variants, an argument can also be made in both cases that patients with variant genetics would benefit from alternate treatment approaches.

#### Warfarin

Warfarin was introduced in the 1950s, and variability in dose requirements, and heritability of this trait, were recognized early. 49,79 The key enzyme responsible for bioactivation of the active enantiomer, S-warfarin, was found to be CYP2C9 35 and two nonsynonymous variants (termed *2, a reduction of function allele, and *3, a near loss of function allele) were then described, 80,81 and found to be more common among patients with low steady-state dose requirements. 82 Indeed, the data were sufficiently compelling for the US Food and Drug Administration (FDA) to propose a study in the early 2000s of the effect of guiding warfarin dose by genotyping variants in CYP2C9. However, the landscape changed in 2004 with identification of a second gene, VKORC1, at which variants contribute even more than those in CYP2C9 to variable warfarin dose requirements. VKORC1 was originally identified...
in individuals with striking resistance to warfarin who were found to have rare coding region variants in the gene, which is now recognized to encode the warfarin target in the vitamin K pathway (Figure 4A).

There are common variant haplotypes in the VKORC1 promoter, and these are associated with variability in hepatic VKORC1 mRNA, and with variable warfarin dose requirements: patients with variants associated with less mRNA require less warfarin to achieve therapeutic anticoagulation, and achieve therapeutic anticoagulation earlier than those with variants generating larger amounts of VKORC1. As described above, GWAS have identified a role for common variants in CYP2C9, VKORC1, and CYP4F2 (which is thought to play a role in vitamin K cycling), clinical covariates plus variation in these genes account for over 50% of warfarin dose variability. Further, specific variants that modulate dose requirements appear to be somewhat different in white and African subjects. Warfarin dose requirements vary by ancestry (greatest in African subjects, lowest in Asians). The International Warfarin Pharmacogenetics Consortium (IWPC) studied over 5000 subjects of variable ancestry and demonstrated that the difference in dose requirement was largely attributable to variation in VKORC1 (Figure 4B). The IWPC also compared the success of three algorithms to select warfarin dose: a fixed-dose approach, an approach that included clinical variables (age, ancestry, interacting drugs, etc) and a pharmacogenomic approach that included clinical factors plus VKORC1 and CYP2C9 variants. For “average” patients, about 70% of the population, the error in predicting warfarin dose was similar across the three approaches; however, for “outlier” patients (as in Figures 1 and 4C), the pharmacogenomic approach was superior to the other two.

Figure 4. The warfarin pathway.
A. Major genes involved in warfarin pharmacokinetics (CYP2C9, other cytochrome P450s; top) and warfarin pharmacodynamics (VKORC1 and others [CYP4F2, GGCX, EPHX1], bottom). B. Data shown here from the International Warfarin Pharmacogenetics Consortium (IWPC) highlight how a common VKORC1 promoter haplotype varies by ancestry (top): the G allele is associated with greater hepatic mRNA VKORC1 abundance. For CYP2C9 (bottom), the *1 variant is the reference allele, *2 is a reduction of function nonsynonymous SNP and *3 is a near loss of function nonsynonymous SNP. These variants are commoner in whites, and other variants have been reported in other ancestries. C. This figure shows a slightly skewed normal distribution of warfarin dose requirements in a population, as reported by the IWPC. There is substantial variability and this is largely explained by the VKORC1 and CYP2C9 variants shown in (B). However, there are also rarer variants that contribute importantly to the extremes: patients with severe loss of CYP2C9 function variants with very low dose requirements and those with nonsynonymous VKORC1 variants and very high-dose requirements as discussed in the text.

Taken together, these data have laid the groundwork for proposals to use genotypes to guide dose selection in individuals receiving warfarin. Several small studies have suggested this approach does predict steady-state dose and may reduce bleeding, and the National Institutes of Health’s COAG trial (Clarification of Optimal Anticoagulation through Genetics) is assessing the use of point-of-care genotyping to implement this approach. The studies to date have focused on common variants in these genes, and variation in other genes in the warfarin pathway (Figure 4C) may need to be considered. Further, rare nonsynonymous coding region variants in VKORC1 have now been described in patients with unusually high warfarin dose requirements. One, resulting in D36Y, is present in 4% of Ashkenazi Jews and has an enormous impact (up to doubling warfarin dose requirement). Similarly, rare patients with the CYP2C9*3/*3 genotype have severely reduced dose requirements and have been said to be very difficult if not impossible to anticoagulate safely. Thus, the approach discussed by Wilke and Denny in this monograph, to preemptively embed genotypic information in an EMR system that also includes advanced decision support capabilities, may have increasing utility as the complexities of understanding the impact of multiple genotypic variants become better understood.

**Clopidogrel**

The platelet ADP P2Y12 receptor inhibitor clopidogrel was approved for US marketing in 1997, but the first publication to describe the fact that its bioactivation by CYP2C19 to platelet inhibiting metabolites was in 2006. Homozygotes for a common loss of function variant, termed CYP2C19*2, are present in 2% to 3% of white and Africans, 5% to 15% of Asians, and 10% to 20% of Inuit and Australian aborigines. Three large candidate gene studies identified higher event rates among patients carrying one or two copies of the *2 variant (and in one case those with an ABCB1 variant) treated with clopidogrel for acute coronary syndromes (ACS), compared with those with reference alleles. In a GWAS studying the endophenotype of clopidogrel inhibition of ADP-mediated platelet aggregation in 429 healthy Amish subjects, Shuldiner et al identified a strong signal at the CYP2C19 locus; the *2 variant accounted for 12% of total variability in clopidogrel effect and no other common contributing variants were identified. They also further validated the findings by showing an effect of genotype on outcome during ACS. These candidate gene and GWAS findings led to a rewriting of the clopidogrel FDA label to suggest alternate therapies in individuals with CYP2C19 loss of function alleles, but the nature of the alternate strategies was not presented and these recommendations have been very controversial.

Arguments supporting the use of CYP2C19 testing include the clinical pharmacology data showing reduced CYP2C19 activity in the presence of one or two *2 alleles. A meta-analysis also supports the influence of CYP2C19*2 on outcome in patients treated invasively for coronary artery disease, the odds ratios for a commonly-used composite end point (in-stent thrombosis, myocardial infarction, stroke, death), as well as for in-stent thrombosis alone, were increased (1.55 to 3.97) along those with one or two *2 variants compared with those with the *1/*1 genotype. However, another meta-analysis, which included larger numbers of patients drawn from trials demonstrating clopidogrel efficacy over placebo in a range of clinical settings, found a much smaller effect size (odds ratio 1.18) and suggested that the evidence supporting an important effect of the *2 allele was weak. The reason may be different effect sizes across multiple studies analyzed or differing criteria for accepting studies into one or the other meta-analysis. The data speak to an effect of the *2 allele, but—as in many other areas of modern genome science—the effect is not black and white, but rather probabilistic. Taken as a whole, the data support the view that the CYP2C19*2 genotype is a modulator of the probability of successful outcome with clopidogrel therapy, but do not address whether patients with variants genotypes would do better with alternate strategies. Open questions in this area include the role of other (rarer) variants in mediating both failure of efficacy (eg, *3/*8) and bleeding risk (eg, the *17 allele discussed further by Sinner and Kääb), and the role of other genomic variants in contributing to variability in clopidogrel clinical actions.

A COAG-like trial to prospectively address the contribution of genotype to overall management of patients receiving clopidogrel might involve randomization of patients destined to receive the drug to a genotype-guided and a nongenotype-guided arm. Those in the nongenotype-guided arm would receive standard of care and those in the genotype-guided arm would receive standard of care (with the wild type genotype) and alternate therapy in the presence of variant genotypes. Such alternate therapy might include prasugrel, ticagrelor, or (perhaps only for heterozygotes) an increased dose of clopidogrel. A difficulty with this trial design is that many patients would need to be enrolled in order to identify an effect in a small subset. The
demonstration of an effect would depend on the choice of alternate therapies and it is conceivable that some of the alternate therapies would be more or less effective in some subsets of patients receiving clopidogrel. For both warfarin and clopidogrel, there are also functional tests, the international normalized ratio (INR) for warfarin and various forms of platelet function tests for clopidogrel, and a therapeutic strategy wherein initial dose selection is guided by genotype and subsequent dose adjustment by functional testing seems plausible.114

PHARMACOCENOMICS AND DRUG DEVELOPMENT

The identification of functionally important and common polymorphisms in drug metabolism pathways, notably CYP2D6, has led to the increasingly accepted notion in drug development that candidate molecules that are substrates for these pathways (and especially those in which alternate pathways are not present, the “high-risk pharmacokinetics scenario”) are not suitable for further development. Rather, these structures should be modified to eliminate dependence on polymorphic pathways to avoid a predicted problem of variable drug response and susceptibility to adverse reactions during therapy with inhibiting or inducing drugs.

Another way in which genetic studies may inform the drug development process comes from disease genetics. An excellent example is the identification of relatively rare (minor allele frequency < 5%) variants in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene that are associated with decreased LDL cholesterol and striking resistance to myocardial infarction, particularly among African-Americans.115 This initial finding has subsequently been validated116 and clearly points to PCSK9 as a viable drug target to lower LDL117 and thus potentially reduce myocardial infarction risk. Interestingly, variants that increase HDL cholesterol have also been described, however, these do not seem to be associated with a decrease in myocardial infarction risk,118 and thus may be less desirable as targets for further drug development. The evolving PCSK9 dataset highlights the way in which identification of rare, functional variants, through GWAS or candidate approaches (in the case of PCSK9, a candidate approach) can lead to strong associations that then lay the groundwork for developing new approaches to treatment of common diseases.

DELIVERING PHARMACOCENOMICS TO THE BEDSIDE

This review and the accompanying articles highlight the way in which the discipline of pharmacogenomics is evolving from an interesting intellectual curiosity to an important consideration in drug development and in potential clinical adoption.

In some health care sectors, the data are now in routine use; testing for HLA-B*5701 is routine and recommended in guidelines prior to starting abacavir. As described above and in the accompanying articles, there is accumulating evidence for the impact of DNA on clinically important responses to cardiovascular agents such as warfarin, clopidogrel, simvastatin, and QT-prolonging agents. One scenario is to test for such variants when a drug is prescribed (Figure 5). The difficulty with this “reactive” approach is that it adds considerable

![Figure 5. Reactive versus preemptive use of pharmacogenomic data in workflow.](image)
cost and complexity to decision making, but, that for any given trait, most patients are “average” and will respond in a gratifying fashion to “average” therapies established by population studies.

Modern genomic science is proving that each of us is not average for at least some traits. This fundamental insight, combined with increasing computational power and technologic advances that can deliver hundreds (and eventually millions) of highly reliable genotypes for the same cost as a single genotype test today (Figure 6), supports consideration of an alternate “preemptive” strategy (Figure 5), described further by Wilke and Denny. As appealing as such a vision may be, there are considerable barriers to widespread adoption. As discussed by Wilke and Denny, these include developing and validating the evidence, performing and delivering the genotype data in a timely fashion, and providing advice on how to act on the data. Other challenges include how to assess outcomes in such an enterprise and what to do with variants of unknown significance (often termed “VOUS”) particularly those in genes where other variants have been associated with clinically significant outcomes such as increased risk for cancer, sudden arrhythmic death, or Alzheimer’s disease. Importantly, the accumulation of a large cohort of patients with genomic data coupled to EMRs may not only provide a mechanism for delivering genomic information to practitioners, but could also serve as a tool for discovery and validation of new associations. There are also important and difficult ethical issues in generating biologic test data in the absence of evidence of how to act on such information; however, data on potential pharmacogenomic variants suffer less from this issue.

The problem of high accuracy genotyping is a barrier to the implementation of a vision of using large genomic sequences in practice. While the first draft of the human genome was reported over 10 years ago, there has not been, to date, a single full-length complete human genome sequence generated. Some regions of the genome are difficult to sequence with currently available technologies, and specific base pair and calling of short and long insertions and deletions may be problematic. While the technology to produce highly reliable genomic sequences is now coming online, it is vital that genomic variant data used in clinical decision making be as close to 100% reliable as possible. Advising health care providers or patients about changes in drug therapy must be predicated on accurate genotyping. This is even more the case should the genomic data suggest major health care interventions, such as mastectomy or oophorectomy in BRCA1 carriers or implantable cardioverter defibrillator use in patients with high-risk ion channel gene mutations.

The application of whole exome or whole genome sequencing is highlighting the fact that the vast majority of genomic variation is rare. This finding, in turn, creates a potential for misclassification of anticipated drug response from genomic data. For example, the Exome Sequencing Project analyzed samples from ≈12 000 white and African-American subjects (http://evs.gs.washington.edu/EVS/). There were 67 nonsense or missense (non-synonymous) variants in CYP2C19 and 22/67 were novel. Similarly, there were 172 nonsense or missense CYP2D6 variants, and 98/172 had never been reported previously. It is not a given all such variants are necessarily function-altering, so a challenge arising from new sequencing technologies is to establish the func-
tional significance of each new variant. Clearly, some patients harboring such rare variants—not interro-
gated by current genotyping platforms—may display aberrant drug responses.

SUMMARY

Cardiovascular pharmacotherapy has undergone a true revolution in the past decades, with increasing and widespread use of highly effective new therapies for common and serious disorders such as atherosclerosis, hypertension, thrombosis, and heart failure, and widespread use of these therapies has contributed to decreased cardiovascular morbidity. However, even for highly effective therapies, some patients will fail to respond or display adverse effects. The tools of modern molecular science and genomics are being applied to this problem of variable drug response, and validated and replicated associations are now being described. The question of establishing which of these relationships are sufficiently compelling to apply to clinical care is being addressed, and new technologies are being mobilized to implement a future view of genome-enabled health care. It is an interesting paradox that very large datasets are required to identify and validate believable genotype-drug response relationships; that is, to enable personalized medicine requires studies in very large patient sets. Community (or country-wide) cohorts and electronic medical records are the likely sources of such very large sets, and developing methods to fully exploit such resources will be an important step to bringing genomic and pharmacogenomic variant data to the bedside.

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Cardiovascular Pharmacogenetics

Expert Answers to Three Key Questions

1

Tackling pharmacogenetics: how can we select the right methods?

M. F. Sinner, S. Kääb

2

Pharmacogenetics of response to cardiovascular drug therapy: what is the current state of knowledge?

C. E. de Keyser, M. Eijgelsheim, A. G. Uitterlinden, B. H. Stricker

3

Can EMRs (electronic medical records) facilitate gene-based drug prescribing for cardiovascular disease?

J. C. Denny, R. A. Wilke
Tackling pharmacogenetics: how can we select the right methods?

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Pharmacogenetics investigates the role of genetic factors determining and modulating the response of patients to drugs. A multitude of techniques were developed to examine genetic factors affecting diseases, including drug response. Two questions are important: First, should the approach conceptually follow a candidate gene design, or is the study design hypothesis-free? Second, how many genetic variants need to be studied? Whereas so far technology focused on genotyping single variants, most recently large-scale sequencing has become feasible, raising pharmacogenetics to the next level. With all techniques, there is a need for independent replication and functional validation. This article introduces the most important methods used in pharmacogenetics.

Keywords: candidate gene; common variant; genotyping; GWAS; mutation; pharmacogenetics; rare variant; sequencing; SNP

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Genetic variation of genes and regulatory elements of the genome have the potential to modify the pharmacodynamics and pharmacokinetics of many drugs used in clinical practice. Pharmacogenetics aims at describing and deciphering this variation in drug response. The field continuously gains importance, as drug safety is becoming a major concern. Pharmacogenetics is highly relevant for the development of novel drugs, but is also defining and limiting the use of existing agents.

The recent years, and the publication of the human genome in 2001 in particular, have markedly pushed the area of genetics forward. Advances in genotyping methods made it possible to reliably detect genetic variation and identify single nucleotide polymorphisms (SNPs) and mutations that contribute to altered drug response. With these assets in hand, it is possible to inform and promote the field of individualized medicine, guided by genotypic biomarkers. Such biomarkers could be used in many ways, finally reaching the level of single-patient personalized (private) diagnosis and treatment. Intermediate steps, however, are warranted and include: (i) genotype-based stratification of larger groups of patients; (ii) genome-based information on disease related traits; (iii) the assessment of the individual disease risk of patient groups; and (iv) the application of therapeutic interventions to selected groups. This article is intended to familiarize the reader with the most important methodology for conducting genetic association studies, using examples from the field of pharmacogenetics. A selection of methods is also concisely presented in Table I (page 272).

CANDIDATE GENE STUDIES VERSUS GENOME-WIDE ASSOCIATION STUDIES

When deciding on suitable methodology, an important conceptual decision on study design is required. In a candidate gene study, investigators base their research on the prespecified hypothesis that variants in a single gene or a group of genes are associated with drug response or a related outcome. Thus, only relatively few SNPs will be genotyped, which reside in or in close proximity to the candidate gene(s). In contrast, genome-wide association studies (GWAS) are conducted based on the general assumption that SNPs in previously unspecified genomic regions are associated with drug response. GWAS are therefore not bound to a preformulated hypothesis. However, to cover genetic variation across the entire genome, many more SNPs need to be ana-
lyzed. Accordingly, different genotyping methods have to be applied. Candidate gene studies require methods where researchers are able to individually select variants for genotyping. The final number of variants will depend on the number of genes to be studied, but will range from 1 to a few thousand. Conversely, GWAS aim to cover all SNPs in the genome. Depending on the SNP definition, there are approximately 15 million such variants listed in databases like dbSNP\(^2\) or the HapMap.\(^3\) Due to the relatedness (linkage disequilibrium [LD]) between SNPs, not all of them need to be genotyped; most current genotyping arrays contain 500 000 to 1 million SNPs. Using the LD information retrievable from the HapMap,

<table>
<thead>
<tr>
<th>Genotyping method</th>
<th>Ideal for</th>
<th>Customizable</th>
<th>Major advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restriction fragment length polymorphism</td>
<td>Genotype single SNPs</td>
<td>Yes, depends on the presence of suitable restriction sites</td>
<td>Easy to use</td>
<td>No high-throughput possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No special equipment required</td>
<td>No multiplexing</td>
</tr>
<tr>
<td>TaqMan</td>
<td>Genotype single SNPs</td>
<td>Yes, predesigned or customizable assays available</td>
<td>Easy to use</td>
<td>High call rate</td>
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<td></td>
<td></td>
<td></td>
<td>Only real-time PCR cycler required</td>
<td>Multiplexing very limited</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>No high throughput</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Comparably high cost per genotype</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Genotype medium number of SNPs (4-40)</td>
<td>Yes, almost every single genomic base is designable</td>
<td>High design flexibility</td>
<td>Multiplexing possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High throughput</td>
<td>Expensive equipment</td>
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<td></td>
<td></td>
<td>Usable in core labs only</td>
</tr>
<tr>
<td>GoldenGate</td>
<td>Genotype medium to high number of SNPs (96-1536)</td>
<td>Yes, almost every single genomic base is designable</td>
<td>High design flexibility</td>
<td>High call rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Slow design, order, and genotyping process</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No postdesign modification of the assay possible</td>
</tr>
<tr>
<td>Genechip</td>
<td>GWAS</td>
<td>No; some more recent products offer customizability for up to 50,000 SNPs</td>
<td>Standardized genotyping procedure</td>
<td>Genotype imputation possible to &gt;2.5 million HapMap SNPs</td>
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<tr>
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<td>Low cost per genotype</td>
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<td>High absolute cost</td>
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<td></td>
<td>Statistical analysis limited in small datasets</td>
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<td></td>
<td>Usable in core labs only</td>
</tr>
<tr>
<td>Infinium</td>
<td>GWAS</td>
<td>No; some more recent products offer customizability for up to 50,000 SNPs</td>
<td>Standardized genotyping procedure</td>
<td>Genotype imputation possible to &gt;2.5 million HapMap SNPs</td>
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<td>Usable in core labs only</td>
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<tr>
<td>ExomeChip</td>
<td>Exome-wide association study</td>
<td>No</td>
<td>Comprehensive analysis of large parts of the gene encoding region</td>
<td>Particularly low statistical power in smaller datasets</td>
</tr>
<tr>
<td></td>
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<td>Possibility to analyze rare and low frequency variants</td>
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<td></td>
<td>Usable in core labs only</td>
</tr>
<tr>
<td>Sanger Sequencing</td>
<td>Individual re-sequencing</td>
<td>Yes</td>
<td>Highly flexible</td>
<td>Available in most laboratory settings</td>
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<tr>
<td></td>
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<td></td>
<td>High cost</td>
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<td></td>
<td></td>
<td>Limited throughput</td>
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<td>Time-consuming analysis</td>
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<tr>
<td>Next-generation sequencing</td>
<td>Large-scale exome, whole-genome, or custom re-sequencing</td>
<td>Yes, predesigned assays also available</td>
<td>Generation of very large amounts of data</td>
<td>Very high cost</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High-speed and throughput</td>
<td>Analytical models under development</td>
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<td>Usable in core labs only</td>
</tr>
</tbody>
</table>

Table 1. Overview of selected genotyping and sequencing methods relevant to pharmacogenetic research.
researchers can impute their directly genotyped variants to a by far larger number of common variants (>2.5 million). In consequence, imputed datasets of current GWAS are suited to cover close to the entire common variation of the human genome.

CANDIDATE GENE METHODS IN PHARMACOGENETICS

As opposed to GWAS, candidate gene studies are hypothesis-driven. Since such a hypothesis is usually founded on prior knowledge, an advantage of the candidate gene results are their easier interpretability. Another general advantage of candidate gene studies is the lower absolute cost for genotyping owing to the lower number of variants under investigation. Also, the analytic complexity of candidate gene studies is lower. The statistical methods per se are largely the same as in GWAS. However, the computational power required to calculate a limited number of candidate gene associations rather than millions of associations as in GWAS is straightforward.

The disadvantages of candidate gene studies are conceptual. Since the study hypothesis is founded on prior results that imply a gene to be involved, the study results can only support or discourage a suspected association. However, it is hardly possible to identify novel, previously unsuspected relations. Also, false-positive findings are common. The human genome contains approximately 20,000 to 25,000 genes and an estimated 15 million common variants. About 2.5 million of these variants are considered common. SNPs with allele frequencies >10%, the remainder occur less frequently. The likelihood of correctly selecting the significantly associated, or even functionally relevant SNP, is extremely low, unless compelling prior evidence is available.

Several genotyping methods are available for candidate gene studies. The method of choice depends on the local technological infrastructure. Other than that, the most important factor is the number of variants that need to be genotyped. In the following, three of the most commonly used methods are presented.

Single SNP assay

The administration of platelet aggregation inhibitors like clopidogrel is an important therapy to prevent the thrombosis of stents implanted during percutaneous coronary intervention. Severe side effects of antithrombotic therapy are bleeding events. The metabolism of clopidogrel depends on the cytochrome P450 system, including its isoenzyme CYP2C19. Sibbing et al could show in a prospective clinical trial of patients undergoing coronary interventions that a common variant in CYP2C19 (CYP2C19*17) is significantly associated with an increased incidence of bleeding complications after up to 30 days of follow up.

The CYP2C19*17 variant is a known SNP (rs12248560) that was genotyped in this study by an Applied Biosystems TaqMan assay. The assay is typically performed in a single tube per SNP and subject. The manufacturer offers predesigned assays for >1 million SNPs, but custom design is also possible. Technically, genotyping involves the so-called 5' nuclease assay. Allele-specific oligonucleotide probes bind to the product of a real time polymerase chain reaction (PCR). The probes are labeled with fluorescent dyes that emit light of different frequencies upon excitation, depending on the alleles present in the reaction. Dedicated software enables the accurate detection of the genotype.

The TaqMan assay is ideal if one—or at the most few—variants need to be genotyped. Advantages include a reproducibly high call rate of >99%. Also, the chemistry is simple, and the most complex equipment needed is a real-time PCRycler available in many laboratories. Thus, the technology is widely applicable. Disadvantages are limited options to process several variants at a time, and the comparatively high costs per genotype ranging around US$ 0.50 to 0.80 per genotype and sample.

Multiple SNPs assay (small scale, iPLEX)

Many cardiovascular conditions, like atrial fibrillation, deep venous thrombosis, pulmonary embolism or stroke, require long-term anticoagulation, and warfarin is still the most widely used drug for this purpose. However, the drug has a narrow therapeutic range and the dose
required to reach this range varies widely between patients.\textsuperscript{8} Besides environmental and nutritional factors, genetics plays an important role explaining this variability. Two genes, the cytochrome P450 enzyme \textit{CYP2CP}, and the vitamin K epoxide reductase complex, subunit 1 (\textit{VCORCl}) have been shown to be relevant. In a study involving a discovery cohort (\textit{n}=4043) and subsequent validation in 1009 independent patients, the investigators of the International Warfarin Pharmacogenetics Consortium investigated 9 SNPs in \textit{CYP2CP} (\textit{n}=2) and \textit{VCORCl} (\textit{n}=7) to predict the required warfarin dose.\textsuperscript{9} They found that knowledge about the genes' variants significantly improved the prediction, particularly in patients requiring low (\leq 21 mg/week) or high (\geq 49 mg/week) doses.

Six of the 9 SNPs were genotyped using Sequenom's iPlex MassARRAY matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.\textsuperscript{10} This technology is suitable for multiplexing, up to 40 SNPs can be assayed into a single experiment. In a first step, the genomic regions containing the selected SNPs are amplified by PCR. In a second step, for each SNP allele-specific oligonucleotides of known molecular weight are hybridized to the PCR product. The product is finally inserted into a mass spectrometry tube, where it is activated by laser pulses, and the desorbed particles are accelerated along the tube. Depending on their known weight, the time of flight is corresponding to the underlying genotype, which can be called by a software.

The iPlex technology is a method of choice for projects involving medium numbers of SNPs. The selection of variants is highly flexible. Almost every single base of the genome could be determined in theory, as the allele-specific oligonucleotides can be generically synthesized. Other advantages include the high-throughput capacity of \textgreater 150 000 genotypes/2 days, the simple nature of the laboratory methods, and the cheaper costs per genotyped SNP. Cost estimates range around US$ 0.20 to 0.11 per sample and SNP, depending on the plex-grade of the assay. A disadvantage is the high cost of the required hardware equipment. Other than TaqMan, the iPlex system is thus the privilege of dedicated genotyping centers.

**Multiple SNPs assay (large scale, array)**

The drug-induced long QT syndrome (diLQTS) is a drug-triggered prolongation of cardiac repolarization. Its danger is the increased arrhythmogenicity, which can culminate in a distinct ventricular arrhythmia known as torsades de pointes. Since torsades de pointes can degenerate into lethal ventricular fibrillation and sudden cardiac death, it is a major concern for drug development.\textsuperscript{11} The congenital long QT syndrome is caused by mutations in a number of genes; mutations in these genes are not uncommon in patients with diLQTS, but cannot explain all cases or the phenotypic variability.\textsuperscript{12} In a large genetic association study, Kääb et al sought to determine whether common variants in genes related to the congenital long QT syndrome, cardiac repolarization, or sudden death can explain diLQTS in patients who experienced torsades de pointes.\textsuperscript{13} The authors genotyped 1424 SNPs selected from 18 candidate genes. They found a low frequency variant in the gene \textit{KCNE1} (D85N, rs1805128), which encodes the beta-subunit of the cardiac slow delayed rectifier potassium channel. The SNP was consistently associated with torsades de pointes, increasing the risk about 9-fold (Figure 1).\textsuperscript{13} The 1424 variants investigated in the study were genotyped using the Illumina Golden Gate method.\textsuperscript{14} This method involves a custom-made array capable of processing 96-1536 variants. Methodologically crucial is a combination of 3 oligonucleotides per SNP. A first one specifically binds to the target sequence, the other 2 are allele-specific for their SNP and bind to the target sequence depending on which allele is present in the sample. The target-specific and the respective allele-specific oligonucleotides leave a small gap between them, which is then closed by DNA extension and ligation. The result is a longer DNA segment that is subsequently amplified by PCR using generic primers and fluorescent labeling. Finally, the PCR product is hybridized to sequence-specific beads that are manufactured into the array.\textsuperscript{14} Allele-specific fluorescence detection then allows determining the underlying genotype using the Illumina Beadstudio software.

Custom-made array solutions like the Golden Gate system are filling the gap between multiplexed assays like the iPlex approach, and genome-wide genotyping. They are thus ideal for large-scale candidate-gene studies at a reasonable cost. The cost per genotyped SNP per sample can be as low as US$ 0.08 for a high plex grade of 1536 SNP, but is higher for lower plex grades.

Other advantages include the user-friendly design-process with company support, and high-quality genotypes with call rates >99%. Disadvantages are the fact that only about 80% of common SNPs are suitable for Golden Gate genotyping. Also, the laboratory processing requires complex hardware and the time from array design to assay delivery is long.
GENOME-WIDE SNP ANALYSIS

The recent years have seen a tremendous publication success of GWAS. As of September 2011, 1617 GWAS have been published on 249 different traits.\(^5\)\(^6\) The success has several explanations. The genotyping methodology has made great progress in the last decade, enabling researchers to conveniently assess genetic variation across the entire human genome and to detect novel genetic associations.\(^7\) Only rarely were previously expected associations identified. The majority of detected genetic loci were located in intronic or intergenic regions, and in chromosomal proximity to potentially involved genes that were not suspected based on prior knowledge.\(^8\) A major disadvantage of GWAS is the high cost. Although the cost per genotype is low, the genotyping arrays themselves are not.

One has to consider that high numbers of subjects are required to identify the usually low effect sizes of common variants with sufficient statistical power.\(^9\) Also, the analysis of GWAS comes with a strong penalty to correct for the multiple comparisons. With \(>2.5\) million imputed SNPs (calculated genotypes based on LD [see introduction]) to be analyzed in a current GWAS, each SNP corresponds to a separate experiment. Thus, associations have to be highly significant to be credible. A \(P\)-value threshold of \(5\times10^{-8}\), corresponding to an adjustment for 1 million independent tests, is commonly accepted.\(^9\)

Over the past years, various GWAS have been performed on pharmacogenetics traits (Figure 2, page 276). One of the most successful studies has been published in 2008 by the SEARCH Collaborative Group (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine).\(^10\) The authors conducted a GWAS stage in a comparably very small sample of 85 patients with statin-induced myopathy and 90 controls. They identified a common SNP in the gene \(SLOC1B1\), encoding the solute carrier organic anion transporter. This protein is expressed in the liver, where it is involved in the uptake of statins into the organ. The initially identified SNP (rs4363657) itself is not in the coding sequence, but is in high LD \((r^2=0.97)\) with an amino acid exchanging (missense) SNP (rs4149056, valine \(\rightarrow\) alanine). The association was subsequently replicated in approximately 20 000 individuals on statin therapy. The missense SNP increased the odds of acquiring a statin-induced myopathy 4.5-fold, when 1 variant allele was present, and 16.9-fold in homozygous individuals.\(^10\) Effect sizes in this order of magnitude are huge compared to common diseases.
to the median odds ratio of 1.33 conferred by common variants. Statins are among the most widely prescribed drugs in patients with cardiovascular conditions. In combination with important clinical information, e.g., muscle pain or elevated creatinine kinase laboratory values, and changes in complaints following modifications of the statin therapy, the additional insights gained form the genetic results might have clinical relevance.

The cited work applied a GWAS using the Illumina HumanHap300 Bead Chip. While other genome-wide genotyping platforms exist, the companies Illumina and Affymetrix are the best established in the field. Although their genotyping methods are different, both can be considered generally comparable. Current products process approximately 2 million SNPs to reach genome-wide coverage. Thereby, Affymetrix selects markers distributed randomly across the genome, and in their current 6.0 array reach a median spacing between SNPs of 680 base pairs. Illumina orient their SNP selection on the HapMap project, and intends to use LD measures to systematically select SNPs. Regarding the genotyping technique, the Affymetrix method first digests the DNA with restriction enzymes. Following a PCR step, the amplification products are hybridized to the array after undergoing allele la-

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**Figure 2. Genomic susceptibility loci identified by the GWAS method in pharmacogenetics related traits.**

The figure displays the 22 human autosomes and the X and the Y chromosomes. Black lines indicate where in the genome any GWAS signal has been detected. Dots mark susceptibility loci that have been reported for a variety of pharmacogenetic traits. Their genomic location is only an approximation. Displayed pharmacogenetic traits are not considered to be complete and include: Adverse response to carbamazepine (n=2); Adverse response to lamotrigine and phenytoin (n=12); Antipsychotic drug-induced weight gain (n=1); Butyrylcholinesterase levels (n=3); Drug-induced liver injury (amoxicillin-clavulanate) (n=2); Fibrinogen (n=7); Hepatitis B vaccine response (n=2); Heparin levels (n=3); IFN-related cytopenia (n=1); Immune response to smallpox (n=106); Insulin resistance/response (n=14); QT interval (n=25 distinct loci); Neutropine-induced rash (n=3); Response to angiotensin II receptor blocker therapy (n=22); Response to antidepressants (n=28); Response to antineoplastic agents (n=9); Response to antipsychotic therapy (n=59); Response to hepatitis C treatment (n=12); Response to IFN-β therapy (n=2); Response to irinotecan in non–small-cell lung cancer (n=2); Response to metformin (n=1); Response to platinum-based agents (n=4); Response to statin therapy (n=39); Response to tamoxifen in breast cancer (n=1); Response to TNF-alpha inhibitors in rheumatoid arthritis (n=8); Response to tocilizumab in rheumatoid arthritis (n=20); Response to vitamin E supplementation (n=8); Sexual dysfunction (SSRI/SNRI-related) (n=5); vWF and FVIII levels (n=11).

**Abbreviations:** FVIII, antihemophilic factor; GWAS, genome-wide associated study; IFN, interferon; SSRI, selective serotonin reuptake inhibitor; TNF, tumor necrosis factor; vWF, von Willebrand factor.

tion of the human genome is likely to be sufficiently covered by the frequency variants are potentially missed.  

Whereas most common variants are covered by the GWAS approach, rarer and lower among the approximately 15 million SNPs in the human genome. SNPs are the ones most common across platforms. The 2.5 million SNPs are the ones most common among the approximately 15 million SNPs in the human genome. Whereas this most common variation of the human genome is likely to be sufficiently covered by the GWAS approach, rarer and lower frequency variants are potentially missed.

**ANALYZING RARE GENETIC VARIANTS (IN CANDIDATE GENES AND IN GENOME-WIDE APPROACHES)**

A current concept in genetic studies is the understanding that common variants occur at a higher frequency in the population, but thereby confer a small effect explaining the genetic variability of a disease or trait. Conversely, rare variants only occur in a small share of the population, but their effect size is larger.

To explain genetic variability of a disease, a combination of common and rare variants is required: one fraction of the variability is due to common variants shared by many individuals; the other fraction stems from several rare variants. However, subgroups of patients can carry different such rare variants.

Regarding the methodology for the analysis and both types of variants—common and rare—the TaqMan, iPlex, and customizable array solutions are suitable options. The problem is to recognize the presence or potential importance of the variant. This is the domain of sequencing technologies and future methods discussed in the following.

**FUTURE METHODS IN PHARMACOGENETICS**

All methods described so far focused on detecting and genotyping common genetic variants. Two novel methods promise to shed light particularly on rare variants: ExomeChip genotyping and next-generation sequencing.

**ExomeChip genotyping**

The ExomeChip is a genotyping array marketed by Illumina. The competitor Affymetrix is planning to launch a similar product shortly.

The genotyping methodology of the current ExomeChip is comparable to other Illumina GWAS arrays as described above. The main difference is the composition of the array. Instead of SNPs, rare variants in exons of genes throughout the genome, and to a lesser extent, in regulatory elements like microRNA binding sites have been included.

The variants on the chip have primarily been identified through the United States funded National Heart Lung and Blood Institute’s Exome Sequencing Project Grant Opportunity (ESP-GO). This project detected the rare variants by large-scale sequencing of exomes (ie, all exons present in the human genome) in ~12,000 individuals, mostly recruited from population-based studies.

**Next-generation sequencing**

Next-generation sequencing promises to become another method promoting genetic and pharmacogenetics research. Unlike classic Sanger sequencing, which allows the analysis of PCR products of a length of around 1000 bp, the novel approach exponentiates that.

Currently available next-generation sequencing products can provide up to 500,000 bp in a single experiment. Predesigned products are available covering the entire human exome, or even the whole human genome. Also, custom assays are available that can be defined by the investigators themselves to study the genetic regions of their particular interest. Next-generation sequencing methods are still new and undergoing rapid development. Relevant studies exploring pharmacogenetics topics have not yet been published, but are expected in the near future.
Despite the anticipated success of next-generation sequencing and ExomeChip analyses for the assessment of rare variants, the statistical power required to reliably identify associations is huge. Even to identify moderate effect sizes (e.g., an odds ratio of 2), very large samples will be necessary (n>10 000). Since functionally relevant variants are more likely to reside in the coding sequence of the genome, approaches like ExomeChip genotyping or next-generation sequencing of the human exome (exome sequencing) seem to be most promising. Yet, particularly with the ExomeChip, problems remain, which include the uneven coverage of exons, or technical difficulties to cover insertions/deletions, inversions, and duplications. One way to optimize the statistical power is the investigation of families, which can dramatically reduce the number of variants detected by sequencing that need to be considered potentially causal. For example, sequencing the entire human exome in 1 individual reveals around 10 000 nonsynonymous coding variants, 500 of which have never been described in existing databases and are thus considered novel. Sequencing 2 suitable family members can reduce the number of nonsynonymous variants shared by both to about 4000, and the number of novel ones to 3. Three variants can then potentially be assessed in more detail for their concrete functional effect.

As described earlier, the effect sizes conferred by common genetic variants are usually low. Also, millions of variants exist in the human genome. Both facts increase the likelihood that initially identified variants that appear being significantly associated with a disease, condition or outcome, are merely false positive. To reduce the likelihood of reporting false positives, a critical principle of state-of-the-art association studies is to aim for replication of the positive findings in an independent sample. Conversely, to avoid false-negative non-replication, the replication sample needs to be of sufficient size, and the investigated phenotype should be highly comparable. Certain situations might prevent investigators from attempting replication analysis. In such instances, the reported
associations need to be interpreted considering the lower level of evidence. Examples for situations where replication might be omitted are: (i) The associated variant has been examined before, and compelling evidence from replicated studies or functional characterizations is available, (ii) the investigated phenotype is extremely rare or hard to ascertain, and recruiting a suitable replication sample is not feasible, (iii) the detected effect, derived from a large and well-powered sample, is large and the association is highly significant (ideally exceeding the threshold of genome-wide significance).

The type of genetic studies described in this article report associations between genotypes and phenotypes. Yet, they do not prove causation, which can only be achieved through functional characterization of the associated variants in suitable model systems. For some of the successful and methodologically sound candidate-gene studies, the candidates have been selected in part because such functional data were already available. However, the results of GWAS rarely imply susceptibility loci that reside in genes, which have previously been studied for the phenotype of interest, or even at all. Functional characterization is therefore of high importance to substantiate GWAS findings and elucidate the pathophysiologic pathways behind the association signals. Various steps will be helpful to pave the way to successful characterizations. The generation of large-scale, standardized, and affordable phenotyping and biobanking is pivotal. Bioinformatics approaches are required to establish a knowledge base of all associations between genotypes, phenotypes, and across phenotypes. Finally, the gained knowledge has then to be transferred back to the patient by performing prospective clinical trials, which can ultimately lead to the approval of genotype-guided procedures established in routine clinical practice (Figure 3).

CONCLUSIONS

The methodology available today already enables the successful conduction of genetic association studies in pharmacogenetics, using both candidate gene and GWAS approaches. Depending on the needs of the specific project, a variety of genotyping platforms can be selected. Whereas so far studies mainly focused on common SNPs, the near future will enable researchers to also study rare variants, either by sequencing or by special genotyping solutions.

All genetic association studies bring along the risk of false-positive results. Thus, the independent replication of findings is crucial. Identifying the pathophysiologic mechanisms underlying associated variants will require functional studies in model systems.

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Pharmacogenetics of response to cardiovascular drug therapy: what is the current state of knowledge?

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Cardiovascular disease (CVD) is a major cause of morbidity and mortality in developed countries. Besides well-known environmental factors such as smoking and overconsumption of saturated fat, genetic factors contribute to the risk of developing CVD. In addition, genetic factors may modify both the pharmacokinetics and pharmacodynamics of cardiovascular drugs (pharmacogenetics). The most important genetic polymorphisms that influence response to cardiovascular treatment are highlighted, with regard to effectiveness and risk of adverse reactions. Insight into individual genetic risk factors for disease and treatment response could lead to “personalized medicine” in the future.

Keywords: β-blocking agent; ACE-inhibitor; antiarrhythmic; anticoagulant; cardiovascular disease; diuretic; HMG-CoA reductase inhibitor; pharmacogenetics; platelet-inhibiting drug; therapy

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Pharmacogenetics of response to cardiovascular drug therapy - de Keyser and others

tabolized by the same cytochrome P450 (CYP450) isoenzyme or if another drug is an inducer or inhibitor of the enzyme by which the cardiovascular drug is metabolized. Interindividual differences in pharmacodynamic response may occur via a difference in the molecular structure of a drug receptor or a smaller number of receptors. Although it is assumed that aging is associated with a decrease in numbers of receptors, determinants for differences in pharmacodynamics are less well documented.

Probably the most important factor that can explain interindividual variability in pharmacokinetics and pharmacodynamic response to drug therapy is genetic variation.2-3 Pharmacogenetics focuses on genetic variants and polymorphisms that influence response to drug therapy. A single nucleotide polymorphism (SNP) is a DNA sequence variation, in which one single nucleotide differs between individuals. If this variation occurs in 1% or greater of the population, it is called a genetic polymorphism or variant.4 A SNP or a combination of SNPs (haplotype) can help predict susceptibility to environmental factors and the risk of developing a particular disease, but also the pharmacokinetic and pharmacodynamic response of individuals to certain drugs. Other reasons for genetic variation may be the presence (“insertion”) or absence (“deletion”) of a series of DNA bases or the presence of “copy number variations” (CNV). In case of genetic variation that modifies pharmacokinetics, the SNP is located in a gene involved in the absorption, distribution, metabolism, or excretion of a drug. For example, genetic variation in one of the important CYP450 isoenzymes, such as CYP2C9 or CYP2D6, or genetic variation in a liver transporter that is involved in the active uptake or excretion of a particular drug, modifies the pharmacokinetics of a drug. As a consequence of this variation, the plasma concentration of the drug changes, which alters efficacy or toxicity risk, because less or more drug, respectively, is available at the receptor site. Regulatory variation in the gene encoding the drug uptake receptor at its target organ can influence the active uptake of the drug to its main organ where it is acting. In this case, the plasma concentration of the drug has not changed, but the concentration in the primary organ of acting drug is diminished or increased, depending on whether the SNP increases or decreases the number of uptake transporters, respectively.5-7 A pharmacodynamic example of a potential genetic variation is the encoding for the structure of cardiovascular and respiratory β-adrenoceptors, which is supposed to lead to differences in response to drugs.

Over the past decades, many studies have focused on identifying genetic determinants that influence response to therapy, using different techniques for analysis. Candidate gene studies investigating SNPs in “biologically plausible” genes, eg, SNPs in genes involved in the biological pathway of a disease, and genome-wide association studies (GWAS) without an a priori hypothesis of the underlying genetic variation involved in treatment response, are two often used methods.8 Furthermore, new analyzing techniques, “next-generation sequencing” like exome sequencing and whole-genome sequencing, are currently upcoming and promising techniques to unravel rare coding variants involved in the genetics of complex traits.9 In future perspective, markers that are predictive of drug efficacy or the occurrence of adverse drug reactions could be useful in tailoring treatment for individual subjects or certain patient subgroups, so-called “personalized medicine.”

In the following paragraphs, we discuss the most important pharmacogenetic associations that were discovered in the past years for the different cardiovascular drugs, regarding treatment response. This includes both the efficacy of drugs, and the risk of developing adverse reactions. Although there are many drugs with cardiovascular adverse effects, the discussion will be restricted to drugs with a cardiovascular indication, notably antiarrhythmics, diuretics, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin blockers, β-blockers, lipid-lowering drugs, and anticoagulants. Importantly, we restricted ourselves to those associations that were confirmed in other studies.

### SELECTED ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>CHARGE</td>
<td>Cohorts for Heart and Aging Research in Genomic Epidemiology [Consortium]</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>GWAS</td>
<td>genome-wide association study</td>
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<tr>
<td>HCTZ</td>
<td>hydrochlorothiazide</td>
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<tr>
<td>RAAS</td>
<td>renin-angiotensin-aldosterone system</td>
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<tr>
<td>SEARCH</td>
<td>Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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<tr>
<td>TdP</td>
<td>torsades de pointes</td>
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because many incidental findings in the pharmacogenetic literature have not been confirmed by others.

**ANTIARRHYTHMICS: PROLONGED QT INTERVAL DURATION, CALCIUM ANTAGONISTS, AND DIGOXIN**

Several drugs are associated with prolongation of the electrocardiographic QT interval duration, but only some of them have cardiovascular indications. The QT interval is a measure of myocardial repolarization time, and prolongation of the QT interval duration is associated with a higher risk of drug-induced arrhythmias and sudden cardiac death (SCD). In particular, in individuals with the congenital long QT syndrome (a term encompassing more than 10 different mutations, for instance, in the genes KCNQ1, KCNH2, and SCN5A), it is associated with an increased risk of torsades de pointes (TdP), a specific type of ventricular arrhythmia. Although the risk of TdP might also be increased in cases of drug-induced QT prolongation, this seems to be less well documented. Moreover, there are rare and more common genetic loci for QT prolongation. In 2009, a meta-analysis of three GWAS in 13,685 individuals of European ancestry discovered 14 independent variants at 10 loci, together explaining 5.4% to 6.5% of the variation in QT interval. The results of this meta-analysis are represented in Figure 1, the Manhattan plot for the QT interval association analysis, a meta-analysis of three GWAS in 13,685 individuals from three independent cohorts.

 qt interval association results for 2,543,686 imputed SNPs in 13,685 individuals from 3 cohorts. Results are shown on the \(-\log_{10}(P)\) scale and are truncated at \(-\log_{10}(P) = 10\) for display purposes. The solid bar corresponds to the genome-wide significance threshold of \(5 \times 10^{-8}\).

**Abbreviations:** CHS, Cardiovascular Heart Study; FHS, Framingham Heart Study; GWAS, genome-wide association study; RS, Rotterdam Study; SNP, single nucleotide polymorphism.

**Figure 1.** Manhattan plot for the QT interval association analysis, a meta-analysis of three GWAS in 13,685 individuals from three independent cohorts.


Not only studies on QT interval duration, but also GWAS in different populations showed genetic variation that was associated with other electrocardiographic measures such as PR-interval and ORS interval. Also, genetic variants associated with the risk of atrial fibrillation were discovered in GWAS. Testing these polymorphisms on interaction with drugs used in atrial fibrillation, such as calcium channel blockers, would be an interesting topic for future research.
Digoxin is most frequently used in chronic heart failure with atrial fibrillation. It is a known substrate for the ATP-binding cassette B1 (ABCB1) transporter (P-gp, P-glycoprotein), encoded by the ABCB1 gene, formerly known as multidrug resistance 1 (MDR1) gene. A study showed that three common variants in the ABCB1 gene—1235C>T, 2673G>T, and 3435C>T—and the associated TTT haplotype were associated with increased digoxin serum concentrations. Other studies also showed that TTT haplotype and the 3435TT genotype were associated with higher digoxin serum concentrations. Thereby, the effect of haplotype analysis seemed superior to single SNP analysis in the prediction of digoxin pharmacokinetics.

**DIURETICS**

Thiazide diuretics are the most commonly used diuretics in the treatment of hypertension. However, large differences in blood pressure–lowering response between individuals exist. In 2008, a GWAS discovered a region of chromosome 12q15 that was significantly associated with blood pressure–lowering response in black individuals using hydrochlorothiazide (HCTZ). After fine mapping of the three genes in the region (FRS2, YEATS4, LYZ), the variation in YEATS4 appeared to be most strongly associated with blood pressure response. This gene is involved in the regulation of the initiation of transcription, and a priori this gene was not expected to be involved in thiazide response.

The study was replicated in an individual population, and variation in the YEATS4 rs7297610 polymorphism contributed most to the variation in response to HCTZ. In expression analyses, HCTZ-treated African-Americans showed different YEATS4 expression patterns post-treatment between the rs7297610 genotypes, which could explain the HCTZ response variability. Other candidate gene studies on the blood pressure–lowering response to thiazides revealed several polymorphisms at different loci. Two polymorphisms in the sodium channel γ-subunit promoter gene (SCNN1G, rs5729, and rs5723), and a polymorphism in the endothelial nitric oxide synthase gene (eNOS, rs1799983) were significantly associated with blood pressure response to HCTZ. A combination of genetic variation in the alpha adducin (ADD1) gene (Gly460Trp) and NEDD4L gene (rs4149601, G>A), both genes regulating renal sodium absorption, was associated with a modified antihypertensive response to thiazides. This effect has also been demonstrated for the two polymorphisms separately.

Regarding pharmacogenetics of response to loop diuretics, there are several candidate genes possibly relevant for interindividual variability in drug pharmacokinetics and pharmacodynamics, such as genetic variation in the organic anion transporters OAT1 (SLC22A6 gene) and OAT3 (SLC22A8 gene), and the primary target of loop diuretics, the Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2, SLC12A1 gene). Some associations were described, however, there is little evidence and more research is needed.

**DRUGS INVOLVED IN THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS): ACE INHIBITORS AND ANGIIOTENSIN RECEPTOR BLOCKERS**

The most frequently investigated polymorphism in the treatment response to ACE inhibitors is the ACE gene insertion/deletion (I/D) polymorphism (rs1799752). This polymorphism is strongly associated with serum ACE levels, and accounts for almost 50% of the phenotypic variance of serum ACE levels. This suggests that the polymorphism might be a good candidate for modifying the response to ACE-inhibitor therapy. However, studies on this topic show conflicting results on blood pressure–lowering effect, cardiovascular events, and mortality risk, and currently no final conclusions can be drawn on an association with therapeutic response.

A recent review on the effect of genetic variants in the RAAS system on the blood pressure–lowering response to RAAS-blocking drugs (ACE inhibitors, angiotensin receptor blockers) also failed to show an association between the ACE I/D polymorphism and antihypertensive effects from RAAS blockade. Another frequently investigated variant in the RAAS system is the Met235Thr polymorphism in the angiotensinogen (AGT) gene, which is associated with elevated serum levels of angiotensinogen. But after review, again no association with antihypertensive effects of RAAS blockade could be demonstrated. Furthermore, in the Rotterdam Study, both the ACE I/D polymorphism and the AGT Met235Thr polymorphism did not significantly modify the risk of atherosclerosis.

Several other genetic variation involved in the RAAS system (AT1 A1166C and haplotype, AT2 variants, AGT rs7079, REN and ACE2 variants) might be involved in the response to ACE inhibitors and angiotensin receptor blockers, but as confirmative studies are lacking or conflicting, further evaluation within larger populations is needed to confirm associations before definite conclusions can be drawn.
**β-BLOCKING AGENTS**

β-Adrenoreceptor antagonists or β-blockers have an important role in the treatment of cardiovascular diseases. Major indications are heart failure, hypertension, angina pectoris, and myocardial infarction (MI). β-Blockers can selectively act on the β1-adrenergic receptor (ADRB1) or on the β2-adrenergic receptor (ADRB2), or on both receptors.

Regarding the efficacy of β-blocker treatment, ADRB1 and ADRB2 are potentially interesting candidate genes for investigation. Within the ADRB1 gene, the linked polymorphisms rs1801252 (Ser49Gly) and rs1801253 (Arg389Gly) are clinically relevant, and for the ADRB2 gene three clinically relevant polymorphisms were described: rs1042713 (Arg16Gly), rs1042714 (Gln27Glu), and rs1800888 (Thr164Ile). Many studies have investigated these polymorphisms and the results are extensive and diverse. For instance, ADRB1 polymorphisms have been associated with blood pressure reduction,38-39 with overall the largest blood pressure–lowering response for users with the homozygous Arg389 genotype or Ser49/Arg389 haplotype. Furthermore, they have been associated with mortality in heart failure patients on the β-blocker carvedilol,40 and the homozygous Arg389 genotype is associated with significantly better improvement of left ventricular ejection fraction (LVEF) during β-blocker therapy within heart failure patients.41-45

However, other studies did not find an association, and further investigation in larger populations or studying the combination of different alleles is needed. Regarding the ADRB2 polymorphisms, these are described in relationship with survival, and the Glu27 allele of the rs1042714 polymorphism seems associated with improved LVEF in response to β-blocker therapy in heart failure patients, compared with the Gln27 allele.46 Patients heterozygous for the Ile164 rs1800888 genotype may have impaired heart failure survival during β-blocker treatment.47 No association with blood pressure response is found.48-49 Also, for these ADRB2 polymorphisms, results of studies are inconsistent and more research should be performed.

Many β-blockers are metabolized by the polymorphic isoenzyme cytochrome P450 2D6 (CYP2D6), encoded by the CYP2D6 gene. To date, more than 70 genetic variants within this gene have been described. Several of these variants lead to diminished or absent function of the enzyme. Patients with two inactive alleles are associated with the so-called “poor metabolizer” phenotype, which includes 5% to 10% of the white population. Poor metabolism results in higher plasma concentrations of β-blockers, with a higher risk of toxicity, but also a potentially increased efficacy of the drug. Studies showed that poor metabolizers have a lower heart rate than extensive metabolizers in response to β-blockers that are metabolized by CYP2D6 (eg, metoprolol), but not in nonmetabolized β-blockers such as atenolol.50 Also, the blood pressure reduction was larger in individuals with the poor metabolizing phenotypes, reflecting a better efficacy of the drug.50-52 An example of a study in which poor metabolizers showed a stronger lowering of the heart rate than extensive metabolizers is given in Figure 2 (page 286). A stronger heart rate–lowering response increases the risk of adverse reactions: a study showed an almost fourfold increased risk of bradycardia with metoprolol in poor metabolizers compared with extensive metabolizers.50 Another study demonstrated that CYP2D6 poor metabolizers had a fivefold increased risk for the development of adverse reactions during metoprolol treatment in comparison with patients who were not poor metabolizers.53 The consequences of genetic variation in the CYP2D6 gene and their clinical effect were mainly demonstrated for metoprolol, which could be explained from the fact that this β-blocker is most extensively metabolized by CYP2D6.54 As the CYP2D6 gene may demonstrate copy number variations with ultra-extensive metabolism, high doses of metoprolol might be required for a clinical effect in some individuals. Since there is a large difference between β-blockers in their receptor specificity, and in their affinity for metabolizing enzymes and transporters, it is difficult to draw definitive conclusions about the pharmacogenetics of β-blocker response. Currently, genetic testing for the CYP2D6 poor metabolizing genotype is performed only occasionally. In many patients, the dosage will be titrated downward if needed on clinical grounds. According to some, dose adjustments should be considered in certain patients, depending on the particular drug or underlying disease.55

**LIPID-LOWERING THERAPY WITH STATINS: CHOLESTEROL-LOWERING EFFECT AND MUSCLE TOXICITY**

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, have a beneficial effect on the primary and secondary prevention of cardiovascular morbidity and mortality, primarily by lowering the concentration of circulating low-density lipoprotein (LDL).56 Statins exert their effect by inhibition of HMG-CoA reductase (HMGCR), the rate-limiting enzyme
in the cholesterol biosynthesis pathway. Therefore, the HMGCR gene is a good candidate for studies on genetic variation influencing the cholesterol-lowering effect of statin therapy. Studies have shown that polymorphisms in the HMGCR gene are associated with a lower reduction in levels of total and LDL cholesterol, within different populations and settings.57-59

On the other hand, within the CYP3A4 gene, polymorphisms showing a diminished lipid-lowering response to statin therapy were also described.60-65 Regarding the apolipoprotein E (APOE) ε2/ε3/ε4 variants (a combination of genetic polymorphisms rs429358 and rs7412) and response to statin therapy, in several studies the ε2 variant seemed to be associated with better cholesterol-lowering response to statin therapy.63-64

In general, statins are well-tolerated and safe drugs, although adverse reactions do occur. A relatively common adverse reaction is myopathy, which in its severe form may evolve into rhabdomyolysis with muscle necrosis and release of myoglobin. This serious condition can lead to renal failure and death. The SEARCH Collaborative Group (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine) published a GWAS on the development of myopathy in simvastatin users and identified a strong significant association for the rs4363657 variant in the solute carrier organic anion transporter family 1B1 (SLCO1B1) gene, as shown in Figure 3.72 This polymorphism was in almost complete linkage disequilibrium with the rs4149056 c.521T>C polymorphism, which had already been described in the literature in relation to statin metabolism. Patients homozygous for the minor allele had a 17 times higher risk of myopathy than...
patients homozygous for the major allele. It is established that the homozygous minor allele genotype of this polymorphism is associated with a higher risk of simvastatin-induced adverse reactions, since other studies also find an association. Thus, the question remains whether there is a class effect, since currently no association could be found between the SLCO1B1 rs4149056 polymorphism and adverse reactions during use of other statins such as atorvastatin.

**ANTICOAGULANT THERAPY WITH COUMARIN DERIVATIVES AND PLATELET-INHIBITING THERAPY WITH CLOPIDOGREL**

Although in a strict sense one might question whether anticoagulants and platelet inhibitors can be considered as cardiovascular drugs, they are often used in combination with such drugs. In this topic, we mention the most important polymorphisms in the response to oral anticoagulants and platelet inhibitors.

Coumarin derivatives (vitamin K antagonists) are widely used in the prevention and treatment of venous thromboembolism. In models for dose prediction of the coumarin derivative warfarin, polymorphisms in genetic variation in the CYP2C9 gene. These results are also represented in Figure 4 (page 288). The variant alleles of the VKORC1 polymorphisms—1639A>G (rs9923231) and 1173T>C (rs9934438)—result in patients with this genetic trait being less sensitive to warfarin therapy. Vitamin K epoxide reductase complex subunit 1, encoded by VKORC1, is responsible for converting inactive vitamin K back to its active form, and coumarins are in competition with vitamin K for receptors that activate vitamin K–dependent clotting factors. Therefore, genetic variation in the VKORC1 gene that decreases the function of the VKORC1 enzyme requires higher doses of warfarin to elicit the same effect as in patients with the major genotype.

Cytochrome P450 2C9 (CYP2C9) is the most important enzyme for the elimination of warfarin. The CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) alleles are
Figure 4. Genetic variation in the VKORC1 gene has a stronger effect on variability in response to warfarin therapy, than genetic variation in the CYP2C9 gene.

Association between specific genetic variants and study outcomes. The graphs show the association between the time to the first international normalized ratio (INR) within the therapeutic range and the time to the first INR of more than 4 for patients carrying genetic variants for vitamin K epoxide reductase (VKORC1) (Panels A and B) and for cytochrome P-450 2C9 (CYP2C9) (Panels C and D).


Pharmacogenetics of response to cardiovascular drug therapy - de Keyser and others
to reach their optimal effect.\textsuperscript{81-87} Whether these lower plasma levels also lead to increased risk of cardiovascular events due to inefficiency of the drug is questionable: large studies or reviews find an increased risk of cardiovascular events in carriers of a reduced-function allele,\textsuperscript{84-85} but other studies could not demonstrate an association with cardiovascular events.\textsuperscript{86-87}

**OVERVIEW AND FUTURE CHALLENGES**

In this paper, we have summarized current knowledge concerning the most important genetic associations in the response to cardiovascular drug therapy. There is also much literature about pharmacogenetic determinants of cardiovascular effects by drugs with other indications, such as by tricyclic antidepressants, which may prolong the QT interval duration, but these associations were not covered in this paper.\textsuperscript{88} A couple of conclusions can be made.

First, it is clear that despite abundant literature we know relatively little about pharmacogenetic determinants of drug response. Much pharmacogenetic literature is contradictory and gives a scattered picture of the topic. Probably, many apparently contradictory results come from lack of power in candidate gene studies, lack of standardization, and lack of collaboration between studies within a large consortium. Also, the likelihood that for complex phenotypes the contribution of pharmacogenetic determinants may be difficult to disentangle from other risk factors may have contributed to the relative lack of consistency between studies. Consequently, most clinically relevant knowledge pertains to pharmacogenetics of drug metabolism by the cytochrome P450 system, where a relative wealth of clinical pharmacological literature facilitates the performance of candidate gene studies with blood levels as an outcome. Second, despite the fact that during the past decades much effort has been put into pharmacogenetic research and many studies have been published, the pharmacogenetics of cardiovascular drug therapy has not had substantial clinical consequences. Apart from the abovementioned argument of contradictory literature, this is explained by the fact that most drugs can be titrated on clinical symptoms (eg, digoxin) or biomarkers such as the INR (eg, warfarin). Although health authorities such as the US Food and Drug Administration give dose recommendations based on genetic polymorphisms for some drugs (eg, warfarin), there is no indication that this has led to substantial implementation in clinical practice. Although this might seem to be a negative appraisal, it does not mean that pharmacogenetic research was a waste of time, effort, and resources. Pharmacogenetic research provided us with important scientific insights into drug metabolism and actions. For instance, pharmaceutical companies will be reluctant to develop a drug that is metabolized by CYP2D6 because of the high prevalence of “poor metabolizers.” Moreover, pharmacogenetic research is only at an early stage because progressive cost reduction of DNA analyses, and increasing international cooperation in study consortia such as CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology)\textsuperscript{89} are leading to discovery of important polymorphisms in candidate genes studies as well as in GWAS. Moreover, new genetic approaches, the “next-generation sequencing,” will undoubtedly have an important role in revealing new and also rare loci of genetic variation. Especially, pharmaceutical companies should play a more active role than they currently do by focusing on variations of known pharmacological entities. New genetic associations, and their synergism with patient characteristics, should be investigated more proactively to determine specific groups of patients for whom genetic testing is relevant. Hopefully, future findings will make it possible that, based on a pharmacogenetics profile, drug therapy can be individualized. This would improve efficacy of therapy, reduce the risk of adverse drug reactions, and minimize costs. However, before we reach that point, if ever, there is still a long way to go.

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Can EMRs (electronic medical records) facilitate gene-based drug prescribing for cardiovascular disease?

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The application of electronic medical records (EMRs) is expanding at an unprecedented rate in the US and abroad. In 2009, only a small minority of US physicians utilized a basic EMR. In 2010, US federal legislators set an aggressive 5-year timeline for their widespread implementation. At present, health care providers are being offered financial incentives if they fulfill prespecified criteria for the “meaningful use” of these EMRs. The meaningful use rules for 2011 and 2012 were released by the US Department of Health and Human Services on July 13, 2010. Stage I included a two-track approach, containing several criteria that were mandatory (e.g., maintenance of active medication lists) and several that were optional (e.g., decision support software capable of flagging drug-drug interactions [DDIs]). The Centers for Medicare and Medicaid Services are now in the process of formulating the rules for Stage II.

It is anticipated that one of the key Stage II meaningful use criteria will be computerized provider order entry (CPOE) with integrated decision support. This requirement will change the field of translational genomics, particularly in the context of treatment outcome. The availability of highly structured medication data will increase the use of EMRs for large-scale discovery in the area of pharmacoepidemiology. When DNA is linked to these resources, clinical data from EMRs can also be used to further the field of pharmacogenomics. Practice-based DNA biobanks allow investigators to assess the generalizability of emerging genotype-phenotype associations—retrospectively—within their community of interest prior to moving these associations into routine clinical care. EMRs can then be leveraged—prospectively—to provide decision support. When deemed advantageous, software already in place from Stage I of the meaningful use initiative (e.g., for DDIs) can...
Be modified in Stage II to support the rollout of gene-based drug prescribing (i.e., drug-gene interactions [DGIs]). As such, there is currently great interest in prioritizing these DDIs and DGIs, based on the strength of the evidence.

**ASSESSING THE EVIDENCE**

In 2007, the US Food and Drug Administration (FDA) began incorporating genomic information into drug labels, including several “black box warnings.” Five years later, in macrogenomics is moving forward from discovery using biological materials archived within randomized treatment trials, to a rigorous assessment of the generalizability using DNA linked to clinical data from existing EMRs.

Many of these EMRs are in place at institutions capable of moving the findings forward prospectively. International efforts are therefore being coordinated to standardize the process of implementation. One such effort has been the Clinical Pharmacogenomics Implementation

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**Biobanks linked to EMRs**

Advances in genomic medicine are increasingly being made through studies in observational cohorts. EMRs can enable traditional genomic “discovery” research as well as clinical outcomes research through the collection and tracking of treatment response. Many models have emerged for the construction of biobanks linked to EMRs, including models based on specific geographic regions or practice communities. Novel informatics strategies have also been used to completely de-identify EMRs. De-identification software can be employed to link scrubbed EMR data to archived biological material in a secure cost-effective manner, rigorous enough to comply with stringent policy protections. The eMERGE network (electronic Medical Records and Genomics) represents a group of nine large academic medical institutions within the US that have collected DNA linked to clinical data extracted from dense longitudinal medical records. EMR-based genetic studies have been shown to both replicate existing studies found in research cohorts and to advance science through new discoveries, including the ability to perform broadly and evaluate variants against many phenotypes. In many cases, these data are available along with self-reported race and family structure across multiple generations to allow for robust study of admixed populations. Similar efforts are also under way at other US institutions, large health care systems, and internationally.

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2012, the FDA includes genomic information in nearly 100 medication labels. As our understanding of pharmacogenomics increases, this field has the potential to transform the clinical practice of medicine. However, a clear path toward implementation has not yet been defined. A variety of bidirectional efforts are being organized to facilitate discovery in practice-based data and move the most compelling findings forward into routine clinical care (summarized in Box above). Thus pharmaConsortium (CPIC). This consortium reviews the evidence and publishes guidelines for the most well-characterized drug-gene outcome relationships on a regular basis. All CPIC guidelines are simultaneously published and updated on-line.

To date, six such guidelines have been published, and three of these pertain to cardiovascular medications: simvastatin, clopidogrel, and warfarin. Nine more are under construction. The evidence summarized in each CPIC guideline manuscript is graded using the three-tiered system: HIGH = consistent results from well-designed studies; MODERATE = evidence sufficient to determine the effect, but the strength of evidence is limited by number, quality, or consistency of the studies; LOW = evidence insufficient to assess the effects on health outcomes. For all six CPIC guidelines published to date, and for many similar guidelines under construction, the strength of the evidence is HIGH. When the strength of evidence is HIGH, even relatively safe drugs (wide therapeutic indices and rare adverse event rates) can be linked to genetic variants that are highly clinically actionable.

Consider the example of 3-hydroxy-3-methylglutaryl coenzyme A [HMG CoA] reductase inhibitors (statins). These drugs are among the most commonly prescribed drugs in the industrialized world. Although mild adverse drug reactions (ADRs, e.g., muscle pain) occur in up to 10% of patients taking statins, severe ADRs (muscle damage) occur very frequently. Clinical factors that influence the risk of statin-induced muscle damage include increased age, race, sex, body mass index, physical activity, clinical comorbidities, and concomitant medications. In general, the severity of statin-related muscle problems is strongly influenced by other drugs altering statin disposition (absorption, distribution, metabolism, and elimination, ADME). Pharmacokinetic handling of statins differs on a drug-by-drug basis (Figure 1). While many statins undergo a great deal of phase I oxidation (atorvastatin, fluvastatin, lovastatin, simvastatin), the impact of phase I oxidation on others (pitavastatin, pravastatin, rosuvastatin) is limited. Correspondingly, drugs known to inhibit cytochrome P450 (CYP) enzyme activity (e.g.,
CYP3A inhibitors, such as azole antifungals, increase the severity of muscle ADRs for many of these agents. As a result of phase I drug metabolism, many statins form hydroxyl intermediates (e.g., atorvastatin is converted to 2-OH atorvastatin and 4-OH atorvastatin). These hydroxyl-statin derivatives then undergo further modification, through phase II drug metabolism including conjugation (Figure 1). Thus, competitive UGT1A1 substrates (e.g., gemfibrozil) also alter the kinetic handling of most statins.

Statins further interact with gemfibrozil at the level of cellular uptake. Organic anion transporters including SLC01B1 facilitate the hepatic uptake of most statins, and drugs known to inhibit SLC01B1 activity (e.g., cyclosporine) also increase the severity of statin-related muscle damage. Genetic variability in SLC01B1 is associated with altered hepatic uptake of most statins, and this effect appears to be largest for simvastatin (Figure 2). Much of this variability can be attributed to a single coding variant in the SLC01B1 gene. The clinical impact of this variant is so striking that it was identified in a genome-wide association study (GWAS) using only 85 myopathy cases (and 90 statin-exposed nonmyopathic controls), achieving genome-wide significance with a large effect size (odds ratio 4.5 for heterozygotes, and 16.9 for homozygotes). As a result, several large medical centers have begun moving this relationship into clinical practice through automated decision support linked to EMRs.

This same gene variant also alters statin efficacy. Carriers experience decreased efficacy with regard to low-density lipoprotein (LDL) lowering when taking statins, and again the genetic effect appears largest for simvastatin. We recently leveraged EMR-based data to construct full dose-response curves, and we extracted rigorous parameters representing efficacy (Emax) and potency (ED50) for subjects exposed to multiple doses of simvastatin during...
ing the course of routine care. For the entire cohort, Emax was 60.5 ± 0.5 mg/dL and ED50 was 7.4 ± 0.1 mg/day. Simvastatin potency was reduced in subjects of African ancestry (AA), when compared with subjects of European ancestry (EA) (P < 0.0001). Overall, ED50 was associated with genetic variability in SLCO1B1 in this large racially diverse cohort (an EMR biobank representing ~150,000 unique individuals); however, when these genotype-phenotype association findings were stratified by race, the SLCO1B1 association only remained significant only in EA subjects.

This observation underscores another important principle that must be addressed in gene-based drug prescribing: ancestry influences outcome. Even the most accurate drug-gene outcome models improve with the addition of information characterizing biogeographical race. We have previously reviewed analytical strategies that leverage population structure to more fully characterize genetic determinants of outcome in EMR-based cohorts. The success of this approach depends upon three key factors: (i) the availability of outcome data from groups of admixed individuals (i.e., populations recombined over multiple generations); (ii) a measurable difference in treatment outcome (for efficacy as well as and toxicity end points); and (iii) a measurable difference in allele frequency between the ancestral populations.

Although the allele frequency of SLCO1B1 gene variants differs by ancestry, our observation that the strength of the relationship between these variants and simvastatin potency increases with race stratification suggests that additional factors contribute. For other statins like rosvastatin, this difference appears to be at least partly attributable to variability in efflux transporters such as ABCG2 (Figure 1), and gene-environment interactions not yet defined. At present, the US FDA recommends that providers consider ancestry when initiating rosuvastatin. Patients of Asian ancestry should have their dose reduced for two reasons: they exhibit a 2-fold increase in rosuvastatin blood levels compared with patients of European ancestry, and they have greater lipid-lowering efficacy at lower doses of rosuvastatin compared with patients of European ancestry. As a result, the FDA has concluded that Asian-Americans are one of three important groups with an elevated risk/benefit ratio (the others were patients on calcineurin inhibitors and patients with severe kidney failure). All of these risk determinants—genetics, comorbidity, and concomitant medication—can be flagged using EMRs in real time, at the point of prescribing.

IMPLEMENTING THE FINDINGS

A decade ago the role of EMRs in pharmacogenetics was fairly narrow and limited to the identification of case patients for small case-control studies in the context of toxicity. As noted above, the role of EMRs is rapidly expanding, and EMR-linked biobanks now provide robust resources for: (i) assessing the generalizability of associations identified in treatment trials; and (ii) discovery of new associations in the context of large observational cohorts. Because many existing EMRs also contain efficient decision support platforms for flagging DDIs during the process of clinical workflow, they represent a powerful scaffolding for the construction of prospective implementation efforts that present health care providers with DDIs as well. At present a variety of approaches to the real-time application of DDIs are being explored around the world. Many of these approaches recommend that the provider consider genotyping when attempting to electronically prescribe a drug with an outcome known to be influenced by a pharmacogene (“just-in-time” genotyping). Others are developing preemptive approaches, based upon clinical risk criteria, that genotype patients on large arrays of potentially useful pharmacogenes then keep these multiplexed genotypes archived in an ancillary database behind the EMR so the data can be accessed rapidly when needed (“just-in-case” genotyping).

This latter preemptive model, assumes that—at some point in the not too distant future—dense genotype data will be available on a patient-by-patient basis, eventually through electronic access to whole-genome sequencing data linked to comprehensive EMRs. If the information is already embedded in each individual patient’s EMR, automated decision support algorithms can access that information in real time when a provider initiates a prescription for relevant medications via CPOE, as has been suggested by Dr Francis Collins in a 2009 interview. The example below illustrates a CPOE advisor activated in early 2012 for gene-based simvastatin prescribing. Since the internal representations of these results are based on the genotype results, the phenotypic descriptions (and electronic advisors) can be updated without altering the core results stored in each chart as new knowledge becomes available (e.g., if a new drug were found relevant to an existing variant in the EMR).

There are a variety of ways to determine who should have preemptive genotyping. Until issues such as cost
and third-party payer/coverage are addressed, this approach may not be universally embraced. In the interim, providers can target genomic testing based on estimates of risk (likelihood of needing a particular medication), and this more focused approach can be batched within different clinical arenas. For example, patients about to undergo cardiac catheterization can be preemptively genotyped for all known pharmacogenes, and data relevant to cardiovascular medications can be imported in the event that gene-based drug dosing is needed after the procedure. Three medication labels recently modified by the FDA (with published guidelines featured by the Pharmacogenomics Research Network) have available CPOE-implemented pharmacogenetic guidance for patients with cardiovascular disease (clopidogrel, warfarin, and simvastatin). In one embodiment of this approach, the data can be brought forward, gene-by-gene, at the point of prescribing, when any physician attempts to prescribe a drug from within a preselected group determined by the local Pharmacy and Therapeutics Committee. Such a committee must meet regularly to review the dynamic drug list impacted by each pharmacogene.24

Clearly, gene-based health care delivery has the potential to shift the allocation of medical resources in the coming decade, and EMRs will play a pivotal role in this transformation.27 However, several obstacles must be overcome. Decision support software must be sufficiently flexible to allow reinterpretation of clinical genetic data based upon discoveries not yet made. Further, these data (while dynamic) need to be accessible within the EMRs and stored in structured, computable representations that leverage common terminologies. At present, genetic results often take the form of a faxed result from a third party reference laboratory described in human-readable, but noncomputable, language. To ease the transition of this information into routine clinical practice, bolt-on modules are being constructed to facilitate the addition of genetic information (ie, in the form of DGIs) to existing DDI databases.

This approach is clinically appealing. The real challenge will be determining when and how to use the information. Application paradigms are not limited to drug selection and dose selection; they include medication reconciliation and “push” phenotyping (electronic recognition of risk or disease prior to clinical recognition). There is also a considerable need to educate and train health
care professionals in the use of such an approach. A recent survey carried out by the American Medical Association, in collaboration with Medco Health Solutions Inc, found that while the majority of provider participants agreed about the utility of genetic testing in drug therapy, only a small minority felt that they had been adequately informed about the process.\(^\text{28}\) This stands in strong contrast to data emerging from patient interviews indicating that most patients expect their health care provider to explain the risks and benefits of gene-based drug prescribing.

**TRACKING OUTCOMES**

Genetic predictors of drug response typically fall into two categories: pharmacodynamic (PD) predictors and pharmacokinetic (PK) predictors. The former (PD variants) influence a drug’s mechanism of action while the latter (PK variants) influence a drug’s disposition. Because many of the early successes in this field were realized within the latter framework, most models of implementation focus on PK genes like *SLCO1B1*. Thus array-based genotyping platforms have been developed to meet this need, and these platforms are moving into clinical practice while the scientific community grapples with the many challenges that will eventually accompany the integration of complete sequencing data. Array-based single nucleotide polymorphism (SNP) genotyping platforms may contain a large number of the pharmacogene variants for which published guidelines exist, but it is important to note that not all of these medications are used for cardiovascular indications, and not all of the critical gene variants are readily amenable to SNP genotyping (Table I). In some cases (eg, for adverse skin reactions

<table>
<thead>
<tr>
<th>Drugs:</th>
<th>Simvastatin</th>
<th>Clopidogrel</th>
<th>Warfarin</th>
<th>Codeine</th>
<th>Thiopurines</th>
<th>Abacavir</th>
<th>Allopurinol</th>
<th>Carbamazepine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene:</td>
<td><em>SLCO1B1</em></td>
<td>CYP2C19</td>
<td>CYP2C9, VKORC1</td>
<td>CYP2D6</td>
<td>TPMT</td>
<td>HLA-B*5701</td>
<td>HLA-B*5801</td>
<td>HLA-B*1502</td>
</tr>
<tr>
<td>Adverse Outcome:</td>
<td>myopathy/rhabdomyolysis</td>
<td>stent thrombosis/MACE</td>
<td>bleeding/out of range INR</td>
<td>morphine toxicity/efficacy</td>
<td>myelosuppression</td>
<td>skin hypersensitivity</td>
<td>skin hypersensitivity</td>
<td>Stevens-Johnson Syndrome</td>
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<tr>
<td>Event rate:</td>
<td>&lt;1%</td>
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<td>13%/45%</td>
<td>7%/10%</td>
<td>7%</td>
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<td>suggested</td>
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<td>recommended</td>
<td>none</td>
<td>black box</td>
</tr>
</tbody>
</table>

**Table I. Steps for clinical implementation for key cardiovascular and noncardiovascular drug-gene interactions (DGIs).**

Abbreviations: CLIA, Clinical Laboratory Improvement Amendment; DGI, drug-gene interactions; EMR, electronic medical record; FDA, Food and Drug Administration; INR, international normalized ratio; MACE, major adverse cardiac event; TBD, to be determined.
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Advances in therapeutics depend heavily on the application of basic science advances to specific clinical indications. Experience indicates that bench-to-bedside innovation follows a complex and somewhat erratic path from concept to exploitation. The recent review of how long it takes to apply scientific discoveries to commercial exploitation is about seventeen years. The story of the discovery and development of fibrinolytic agents is perhaps a classic example of the challenge of translational medicine.

The existence of a potential endogenous mammalian fibrinolytic system was postulated as long ago as 1889 by Denys and de Marbaix. They observed the spontaneous dissolution of clots during postmortem studies and also showed that exposure to chloroform accelerated the dissolution of clots. The term “fibrinolysis” was first proposed by Dastre in 1893. Thus, from the 1890s until the 1950s, there was a long-standing scientific interest in trying to understand the mechanisms responsible for the endogenous dissolution of fibrin. Christensen and McLeod showed in 1945 that the enzyme that dissolved fibrin, which was generated in serum by exposure to fibrates of the bacterial product streptokinase, was the same as that formed when serum was exposed to chloroform, the latter experiment being performed almost fifty years previously! This proteolytic enzyme was termed “plasmin” and was shown to be generated from a precursor, plasminogen, by exposure to streptokinase. The crucial observation concerning the dissolution of fibrin was made in 1933 when Tillett and Garret, working in the John Hopkins Medical School (USA) exposed fibrin clots to filtrates of broth cultures of selected strains of streptococcus bacteria. Their paper provides a classic example of bedside-to-bench research in that they obtained 28 strains of Streptococcus haemolyticus from patients suffering from streptococcus infection. Purified broth cultures derived from these patients were shown to rapidly liquify normal human fibrin clots. In contrast, of eighteen strains of Streptococcus haemolyticus of animal origin, only three caused dissolution of the clot. Furthermore, when the plasma of patients who had recovered from the streptococcal infection was clotted in the presence of active cultures, the plasma was highly resistant to fibrinolysis. They also demonstrated that the response was species-dependent in that normal rabbit fibrin clot was totally resistant to dissolution. Tillett named the material “streptococcal fibrinolysin.” The mechanism of streptococcal-mediated fibrinolysis was first described by Christensen, who discovered that the effects of streptococcal fibrinolysin were mediated by a serum enzyme activated by streptococcal fibrinolysin. Christensen and McLeod showed that “streptococcal fibrinolysin” acted on a precursor enzyme present in human plasma, which they termed “plasminogen,” which converted it to the fibrinolytic enzyme plasmin. A pivotal contribution to this complex field was made by Macfarlane and Biggs who, in a masterly review in 1948, postulated that blood fluidity was maintained by a homeostatic balance between thrombus formation and thrombus dissolution. They reviewed the extensive literature of the previous fifty years, and proposed that there is an endogenous homeostatic system that determines the activity of the protease plasmin, which breaks down preformed fibrin that has been formed from the thrombin-mediated activation of fibrinogen. A model proposed at that time is depicted in Figure 1 (page 302). It postulates that plasmin arises from a precursor, plasminogen, whose activity is constrained by an albumin-bound antiplasmin. Interestingly, it was shown many years previously that plasmin-mediated fibrinolysis could be triggered not only by chloroform, but also by exercise and epinephrine. From the point of view of translational medicine, the fibrinolytic activity of streptokinase was attributed to activation of globulin-bound plasminogen.

An important element in the Macfarlane and Biggs review of 1948 is clarification about the terminology in the fibrinolytic system. Thus, fibrinolysis was defined as “the aseptic dissolution of fibrin brought about by the direct ac-

**Keywords:** coagulation; fibrinolysis; myocardial infarction; plasmin; streptokinase; therapeutics; thrombin; thrombolysis; urokinase

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McLeod showed that “streptococcal fibrinolysin” acted on a precursor enzyme present in human plasma, which they termed “plasminogen,” which converted it to the fibrinolytic enzyme plasmin. A pivotal contribution to this complex field was made by Macfarlane and Biggs who, in a masterly review in 1948, postulated that blood fluidity was maintained by a homeostatic balance between thrombus formation and thrombus dissolution. They reviewed the extensive literature of the previous fifty years, and proposed that there is an endogenous homeostatic system that determines the activity of the protease plasmin, which breaks down preformed fibrin that has been formed from the thrombin-mediated activation of fibrinogen. A model proposed at that time is depicted in Figure 1 (page 302). It postulates that plasmin arises from a precursor, plasminogen, whose activity is constrained by an albumin-bound antiplasmin. Interestingly, it was shown many years previously that plasmin-mediated fibrinolysis could be triggered not only by chloroform, but also by exercise and epinephrine. From the point of view of translational medicine, the fibrinolytic activity of streptokinase was attributed to activation of globulin-bound plasminogen.

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tion of a mechanism existing in normal blood. The scientific challenge for the next fifteen years was to identify the components involved in this mechanism. Previously, a number of different terms had been used such as serum tryptase, fibrinolysin, and thrombolyisin, which reflects the conflicting hypotheses generated by in vitro and in vivo experiments on fibrinolysis.

The following definitions were proposed by Christensen and McLeod in 1945:

- Plasmin: a proteolytic enzyme in plasma that is activated by chloroform.
- Plasminogen: the precursor of plasmin.
- Streptokinase: isolated from streptococcal culture filtrates instead of "fibrinolysin."
- Antiplasmin: the inhibitor of proteolytic enzymes present in normal plasma.

**FIRST-GENERATION FIBRINOLYICS (1947-1960)**

First-generation fibrinolytics were of two origins: bacterial (streptokinase) and tissular (urokinase and plasmin) (Table I).

### Streptokinase

The first clinical studies with streptokinase were performed at the New York University Bellevue Hospital by a multidisciplinary group led by William Tillett, who had made the original discovery of streptokinase in 1933. A key member of the team was the bacteriologist Millstone, who was able to provide sterile preparations of the broth used to culture the hemolytic streptococci that generated streptokinase. Only about 10% of the sterile solution comprised streptokinase. The remainder of the solution contained other unidentified protein enzymes, which were subsequently identified as streptodornase, hyaluronidase, etc. The initial therapeutic target for bacterial streptokinase was the dissolution of intrapleural adhesions, which were secondary to pulmonary infection. Part of the therapeutic objective was to dissolve adhesions that bound localized areas of infected tissue so that the lesions were drained more effectively. A subsequent target was the dissolution of fibrotic tissue secondary to tuberculous meningitis within the central nervous system.

The translational medicine operating environment in the 1940s could hardly have been more different compared with the 21st-century processes. The streptokinase used had poor batch quality control. There is no evidence that the classic preclinical toxicological evaluation was performed, and no evidence that ethical committee consultation and approval was performed. Christensen, working at the New York University College of Medicine, published a series of papers between 1944 and 1948 that described techniques for improving the purification of streptokinase. His group showed for the first time that streptokinase caused fibrinolysis by activating an endogenous serum protease. They proposed that the activated enzyme be called "plasmin" and that the inactive enzyme be called "plasminogen." "

The first clinical publication in 1949 describes in detail the effects of streptokinase preparations in 23 patients suffering from either acute fibrinous pleurisy, bacterial empyema, or postoperative hemothorax. The study

<table>
<thead>
<tr>
<th>Preparation</th>
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<th>Company</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varidase</td>
<td>1957</td>
<td>Lederle Co (USA)</td>
<td>Streptokinase/streptodornase mixture</td>
</tr>
<tr>
<td>Kinalysin</td>
<td>1962</td>
<td>Merck Sharpe &amp; Dohme (USA)</td>
<td>Plasmin + streptokinase</td>
</tr>
<tr>
<td>Kabikinase</td>
<td>1963</td>
<td>AB Kabi (Stockholm)</td>
<td></td>
</tr>
<tr>
<td>Streptase</td>
<td>1964/69</td>
<td>Boehringer Werke (Germany)</td>
<td></td>
</tr>
<tr>
<td>Aurelysin</td>
<td>1976</td>
<td>UEB Arzneimittelwerke (Germany)</td>
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</tr>
</tbody>
</table>

Table I. Pharmaceutical companies developing streptokinase preparations in the 1950s-1960s.
was started in 1947 using the relatively crude preparation of streptokinase made by the bacteriologist Christensen. He collaborated with scientists in the Lederle company, who subsequently supplied Tillett and his colleagues with improved preparations of streptokinase that also contained an additional enzyme, streptodornase. The latter enzyme, which is also produced by *Streptococcus haemolyticus* (strain Lansfield group C), was believed to add to the effectiveness of streptokinase because it was known the fluid in the pleural infections of patients contained desoxynucleoproteins, which could be liquefied by the streptodornase enzyme. It is perhaps worth emphasizing that these concentrated filtrates were used as adjunctive to resolving acute or chronic topical localized infections, often requiring surgical intervention. Given the subsequent development of fibrinolytics for vascular diseases, these initial clinical targets may, in retrospect, seem a little paradoxical. It is perhaps even harder to comprehend today that this combination therapy isolated from biological sources was even used intrathecally, administered over 24 days to patients suffering from tuberculous meningitis who had also received streptomycin. The streptokinase/streptodornase preparation, subsequently provided in a more purified form by the Lederle pharmaceutical company (USA), was used in surgical indications as a new biologic approach to infections and clotted hemothorax.¹²

The majority of patients in the study showed only modest clinical improvement, though the patient with hemothorax responded with dramatic benefit. The streptokinase solution containing between 20 000 and 400 000 units was administered by local administration into the pleural space or the infected area. It frequently caused increase in body temperature accompanied by hypotension and leukocytosis. These adverse reactions were attributed to the difficulty in isolating a reliable purified preparation, which it was believed would not cause such adverse effects.

Sherry’s group was in fact more interested in determining if streptokinase might be beneficial in acute coronary thrombosis.¹³ They showed experimentally in the rabbit ear vein thrombosis model that intravenous streptokinase would dissolve such clots.¹³ So it seems that if a well-tolerated fibrinolytic preparation could be developed, there was a strong pathophysiological rationale for evaluating it in coronary thrombosis associated with acute myocardial infarction.¹⁶ There were three options at this time for the clinical scientists:

- To use highly purified streptokinase supplied by the Lederle company.
- To use urokinase supplied by the Leo laboratory (Denmark) (vide infra).
- To use highly purified streptokinase made by the Abbott Laboratories (USA) in the form of prourokinase, which was a recombinant molecule based on the sequence of urokinase.

**Urokinase**

In 1885, Sahl first reported the presence of proteases in normal human urine.¹⁷ The potential clinical utility of urokinase was only described in 1947, and in 1952 the term “urokinase” was proposed by Sobel to describe a plasminogen activator excreted in urine.¹⁸ In the ensuing ten years, the properties of urokinase were extensively evaluated.

Appropriate experimental and clinical studies could only be performed once the major hurdle of identifying a technology that provided a highly purified preparation was overcome. A number of complex steps were required in order to eliminate pyrogens, viruses, and proteins with thrombolytic activity.¹⁹ Pure urokinase is a 54-kDa single-chain glycoprotein that is nonantigenic. It is a specific fibrinolytic activator with a triple active site, so its efficacy is more predictable than that of streptokinase. Despite its predictive effects in activating plasminogen, urokinase was not shown to be effective in some relatively small clinical trials in infarction.

So in the period between 1955 and 1966, a number of critical studies were performed in order to compare the relative merits of streptokinase and urokinase. The potential desirable properties of urokinase were that, unlike streptokinase, it was nonantigenic and nonpyrogenic. This direct activator of plasminogen was provided in appropriate quantities by both the Abbott and Sterling Winthrop pharmaceutical companies. Experimental studies confirmed that both compounds, when sufficiently pure, were effective thrombolytics.²⁰,²¹ In the 10 years beyond 1975, analogs of urokinase were made by the Abbott Laboratories (USA) in the form of prourokinase, which was a recombinant

**Plasmin**

The third alternative potential thrombolytic agent was purified plasmin, which is the actual fibrinolytic agent generated either by streptokinase or urokinase from plasminogen.²² Logically, plasmin should be the preferred therapeutic agent, and was therefore produced by the Merck Sharp & Dohme pharmaceutical company as thrombolysin, and by the Lederle company as “activase.” However, subsequent studies demonstrated that these commercial preparations also contained streptokinase.²³ As a consequence activase was withdrawn, but thrombolysin continued to be used despite variable therapeutic responses. A significant disadvantage of the use of purified plasmin is that it also acts on fibrinogen as a substrate, resulting in its depletion as well as that of other clotting factors.²³
The rationale for the clinicalevaluation of experimental thrombolytic therapy relies on the complexity of clinical application. Initially, the dissolution of pulmonary emboli by systemic thrombolysis with streptokinase showed that significant dissolution could be achieved. Once adequate supplies of pure streptokinase or urokinase became widely available, a series of clinical trials of their effects in acute myocardial infarction were performed in the USA, Europe, and Australia.

One of the earliest studies with Vari-dase by Tillett’s group described in detail the hematomal effects of 100,000 units of streptokinase given over 4 to 5 hours in 11 patients pre-treated with amidopyrine to control the febrile response, as well as an antihistamine to prevent hypotension. The paper makes no mention of what the clinical conditions were, requiring thrombolytic therapy. Perhaps these hematological studies were based on the patients described in an earlier Tillett paper (1949), but this is not clarified in their 1954 paper. This example is quoted to indicate that progress in the clinical application of fibrinolysis was critically dependent on technical advances, both in diagnostic technology, pathophysiological studies, as well as sophisticated purification techniques.

The rationale for the clinical evaluation of the therapeutic effects of streptokinase was based firstly on convincing preclinical experimental studies that demonstrated that intravenous streptokinase (40,000 units) could cause resolution of experimental thrombi in veins or arteries within 1 to 3 hours of starting the infusion, depending upon the individual dose of streptokinase. The dose of streptokinase was individualized for each patient depending on the pretreatment streptokinase antibody titer level. The dose therefore ranged between 35,000 and 1.5 million units, which were administered over 6 to 48 hours. The streptokinase formulation was provided by the Lederle company (USA) and was much more highly purified than the previous streptokinase preparations. One of the earliest reports of clinical studies of fibrinolytic enzymes from Sherry’s group described effects of intravenous streptokinase in 23 patients (Table II). In this uncontrolled study, many patients appeared to undergo a beneficial response, characterized by a reduction in acute inflammation. In the same period (1957), Tillett’s group in Bellevue Hospital, New York, reported that extensive ECG monitoring in patients receiving streptokinase for a range of indications showed no evidence of abnormality. Perhaps it was observations such as this that encouraged Sherry’s group to examine the effects of streptokinase in patients with acute myocardial infarction.

The first report of the effects of streptokinase on patients with acute myocardial infarction was by Sherry’s group in 1959, and this triggered widespread interest in this novel approach. This paper, while emphasizing studies in myocardial infarction, also included patients with distal thrombotic arterial occlusion, pulmonary embolism, and thrombophlebitis. The authors in their discussion emphasize that their careful observations suggest that plasma thrombolytic states are harmless to the infarcted myocardium, and that the treatment of myocardial infarction will not be impeded by deleterious consequences secondary to its primary effect. Over the next 12 years a large number of clinical trials, mainly with streptokinase, but also with urokinase, were reported administering the agents either systematically or into the coronary artery. A pooled analysis of these trials by Yusuf et al in 1985 concluded that intravenous thrombolysis (mainly streptokinase) caused a 22% reduction in mortality (0.001). However, many of the individual clinical trials were insufficient in number to provide more than a positive trend.

It is perhaps somewhat surprising today that in the 1950s there was considerable controversy as to the pathophysiology of acute myocardial infarction. There had been controversy as to whether coronary thrombosis was a result of myocardial infarction, rather than its cause. For example, in the National Institute of Health, a sponsored workshop held in 1973 described the conflicting opinions and concluded “most evidence continues to affirm the basic concept that myocardial infarction can result from acute ischemia.

**Table II. Diagnoses in 23 patients “treated” with intravenous streptokinase.**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Acute thrombophlebitis</td>
</tr>
<tr>
<td>1</td>
<td>Chronic cellulitis</td>
</tr>
<tr>
<td>4</td>
<td>Cerebral vascular accident</td>
</tr>
<tr>
<td>1</td>
<td>Chronic arachnoiditis</td>
</tr>
<tr>
<td>2</td>
<td>Auricular fibrillation (rheumatic heart disease)</td>
</tr>
<tr>
<td>2</td>
<td>? Subacute bacterial endocarditis</td>
</tr>
<tr>
<td>1</td>
<td>Popliteal artery thrombosis</td>
</tr>
<tr>
<td>1</td>
<td>Chronic organizing pneumonia</td>
</tr>
</tbody>
</table>

Clinical diagnosis of patients treated with streptokinase (SK), supplied by the Lederle laboratories (1954-1958). Individual doses, calculated on the estimated patient’s plasma volume, were infused over 2 to 3 hours. Fibrinolysis was demonstrated in 75% of patients (based on reference 26).
produced by thrombotic occlusion of a coronary artery. However, subsequent comments in the conclusion remained somewhat equivocal. Much of the uncertainty in this field was resolved by the publication by de Wood et al in 1980, which showed that the prevalence of transmural myocardial infarction, studied by coronary angiography in order to establish the time sequence, provided definitive evidence that the thrombosis preceded the infarct. It would seem that the de Wood paper finally resolved the debate.

During this period, there was a concomitant large multicenter study of the effects of streptokinase in acute myocardial infarction, which showed a highly significant reduction in mortality. This GISSI trial (Gruppo Italiano per lo Studio della Streptochinas nell’Infarto Miocardico) involved 11,806 patients. The subsequent publication in 1988 of the ISIS-2 (Second International Study of Infarct Survival Collaborative Group) involving 17,187 cases of suspected acute myocardial infarction showed a similar highly significant reduction in mortality.

**THE IMPACT OF RECOMBINANT TECHNOLOGY ON FIBRINOLYTIC THERAPY**

Advances in different technologies had a major impact on vascular thrombolytic therapy between 1970 and 1990. Thus, improved intra-arterial catheters led to trials of both streptokinase and urokinase in pulmonary embolism and acute myocardial infarction. Despite these advances, efforts continued to improve the outcomes of interventional therapy. In this period, the combination of markedly purified plasminogen activators, combined with improved catheter technology, led to multicenter studies showing improvements in morbidity and mortality. In the same period, a new source of plasminogen activator was identified by Désiré Collen’s group in the University of Leuven based on tissue rather than bacterial sources of plasminogen activator. It had been shown in the 1970s that plasminogen activators (tPA) isolated from tissue were much more fibrin-specific than either streptokinase or urokinase, which raised the possibility of improved efficacy and reduced hemorrhagic liability. The technical challenge was to produce sufficient quantities of pure specific tissue plasminogen activator. Collen’s group had characterized tPA isolated from uterine tissue in 1979. They also received samples of a tPA purified by Rifkin in the United States from a melanoma cell line, which had first been cultured by Moore from a patient called Bowes. This stable human melanoma cell line was used to study the properties of tPA isolated and purified from this cultured cell line. Several groups worked with the Bowes tPA and, furthermore, a sample was provided to Collen in 1978 by Rifkin of the New York University Medical School.

The Leuven group used sophisticated purification procedures that permitted detailed characterization of the biological properties of the Bowes tPA. The initial scientific findings were presented at a congress on fibrinolysis in Malmo in 1980. The congress was also attended by Pennica, working at the Genentech company (United States), who contacted Collen’s group in order to discuss potential scientific collaboration. This collaboration resulted in the cloning of the tPA gene and its expression in *Escherichia coli*. Subsequently, the gene was expressed in Chinese hamster ovary cells (CHO), which enabled the large-scale production of human tPA. This was subsequently commercialized by Genentech Inc as Actilyse, and by the Böhringer Ingelheim company in Germany as Actilyze. While the early work with Bowes tPA was progressing in Leuven, Burt Sobel (Washington University) also collaborated with the Leuven group and showed that recombinant tPA (rtPA) caused effective dissolution of experimental coronary infarcts in a canine model. This restoration of coronary flow was not accompanied by the systemic hemorrhagic effects associated with both streptokinase and urokinase.

The first trial of rtPA in acute myocardial infarction involved material isolated from the Bowes melanoma cell line, which was used in the pilot study in 6 patients at Washington University, was organized by Sobel. Based on this pilot study, several multicenter clinical trials, sponsored both by Genentech and Böhringer Ingelheim, were performed over the next 10 years, successfully establishing the efficacy and improved safety of human rtPA in acute myocardial infarction.

**21st-CENTURY FIBRINOLYTICS**

The ability to clone tPA genes led to an impressive increase in understanding of the peptidic structural requirements for enhancing the properties of rtPA. While the major focus of research was in finding improved tPA, the Cuban Center of Genetic Engineering and Biotechnology applied recombinant technology to make streptokinase, which they termed Heberkinase. Recombinant streptokinase was extensively evaluated in multicenter studies in Cuba between November 1992 and May 1995. It is currently the only thrombolytic agent used to treat thrombotic disorders in Cuba, and has an attractive pharmacoeconomic justification in that it is about 40% cheaper than imported preparations. In the same time period, recombinant staphylokinase was generated by recombinant technology by Collen’s group in Leuven. The rationale was to develop an agent with a simple bolus regimen and acceptable cost effectiveness. A positive aspect of staphylokinase was that it did not enhance fibrinogen degradation, and the pegylated form...
(PEG-SAK) was less immunogenic. While efficacy was established in clinical trials between 2000 and 2004, this compound has not progressed much further.45

Many pharmaceutical companies are working to produce a “best-in-class” fibrinolytic agent. The profile of such an agent includes rapid complete restoration of coronary flow following a bolus injection, with low bleeding liability and high fibrin specificity, as well as a long pharmacokinetic half-life. The fibrinolytics currently marketed or in advanced clinical trials are summarized in Table III. It seems fair to conclude that, at present, the perfect fibrinolytic has yet to be developed.

**EPILOGUE**

This account of the discovery and clinical application of fibrinolytics for cardiovascular diseases illustrates some of the classic characteristics of bench-to-bedside research. These include such factors as slow exploitation of the initial novel properties of streptokinase between 1933 and 1948, including the initial application in pleural fibrotic lesions. As with most novel therapeutic advances, there needs to be a scientific champion determined to ensure progress despite critical rejection of the novel ideas by the scientific community. In this instance, the teams of Dr Tillett and Sherry in the USA, and Professor Collen’s team in Europe, played a key role. The transatlantic collaboration, including Dr Sobel’s experimental and clinical studies, was pivotal in ensuring progress.

The contribution made by the pharmaceutical industry in the early stages of fibrinolytic drug development is difficult to document. The purification of streptokinase by the Lederle company in collaboration with Tillett’s group was clearly important, and their product Varidase is still available today for topical application.46,47 Other important contributions were made by Merck Sharp and Dohme (thrombolysin) as well as the Schering Plough and Kabi companies. Possibly the most important commercial contribution was made by Genentech’s collaboration with Professor Collen, leading to the genetic expression of tPA in 1981. The extensive long-term clinical trials in acute myocardial infarction were mainly funded by the pharmaceutical industry.30,34 It will be interesting to see if the ideal fibrinolytic agent is finally identified.

It may be that advances in concomitant technology will enhance the tissue selectivity of tPA, as exemplified by a recent study in which microencapsulated tPA was shown to be selectively

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**Table III. Summary of profiles of current thrombolytic agents based on recombinant technologies.**

<table>
<thead>
<tr>
<th>Agent</th>
<th>PK profile (T½ min)</th>
<th>Fibrin selectivity (scale 0-5)</th>
<th>Potential advantages/disadvantages versus SK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alteplase</td>
<td>4-8</td>
<td>2</td>
<td>Nonimmunogenic large-scale pure preparation</td>
</tr>
<tr>
<td>Montepase</td>
<td>23</td>
<td>2</td>
<td>Much lower dose, but ↑ hemorrhagic stroke</td>
</tr>
<tr>
<td>Tenecteplase</td>
<td>11-20</td>
<td>3</td>
<td>Much lower dose but ↑ hemorrhagic stroke</td>
</tr>
<tr>
<td>Reteplase</td>
<td>14-18</td>
<td>1</td>
<td>Similar trial outcomes to SK</td>
</tr>
<tr>
<td>Lanotoprase</td>
<td>23-37</td>
<td>1</td>
<td>Higher incidence of hemorrhagic stroke Not inhibited by PAI</td>
</tr>
<tr>
<td>Palmitiplase</td>
<td>40-47</td>
<td>2</td>
<td>Anti-genicity greatly reduced Much improved TIMI-3 flow</td>
</tr>
<tr>
<td>Staphylokinase</td>
<td>6</td>
<td>4</td>
<td>Anti-genicity greatly reduced Much improved TIMI-3 flow</td>
</tr>
</tbody>
</table>

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**Figure 2. Current understanding of the endogenous fibrinolytic system (2004).**

Diagram of stimulating and inhibitory factors in mammalian plasma, which either generate fibrin (A) or plasmin (B), depending on the extent of inhibitor activity, ie, plasminogen activator inhibitor (PAI), α2-antiplasmin, or thrombin-activatable fibrinolysis inhibitor (TAFI) (based on reference 49).
targeted to arterial thrombi due to their tissue-specific distribution, which was determined by local shear stress factors. 48 Figure 2 illustrates the current concepts of the endogenous fibrinolytic system. 49

This essay has focused entirely on the application of fibrinolytic therapy in acute myocardial infarction and no attempt has been made to discuss other clinical applications, including pulmonary embolism, stroke, and peripheral arterial disease.

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Cardiovascular Pharmacogenetics

Summaries of Ten Seminal Papers

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1. The role of genetically determined polymorphic drug metabolism in the beta-blockade produced by propafenone

2. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations...
   S. Hoffmeyer and others. Proc Natl Acad Sci. 2000

3. Effect of VKORCI haplotypes on transcriptional regulation and warfarin dose

4. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects
   J. S. Hulot and others. Blood. 2006

5. A polymorphism within a conserved β1-adrenergic receptor motif alters cardiac function and β-blocker response in human heart failure
   S. B. Liggett and others. Proc Natl Acad Sci. 2006

6. SLC01B1 variants and statin-induced myopathy—a genome-wide study

7. Estimation of the warfarin dose with clinical and pharmacogenetic data

8. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy
   A. R. Shuldiner and others. JAMA. 2009

9. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI...
   J. L. Mega and others. JAMA. 2010

10. A randomized and clinical effectiveness trial comparing two pharmacogenetic algorithms and standard care for individualizing warfarin dosing...

Selection of seminal papers by Dan M. Roden, MD
Director - Oates Institute for Experimental Therapeutics - Assistant Vice-Chancellor for Personalized Medicine - Nashville TN - USA

Highlights of the years by Ian Mudway, MD
Lung Biology - Division of Life Sciences - Franklin Williams Building
150 Stamford Street - London SE1 9NN - UK
The role of genetically determined polymorphic drug metabolism in the beta-blockade produced by propafenone


The sodium channel–blocking antiarrhythmic agent propafenone is well tolerated, but side effects such as profound bradycardia or bronchospasm occur occasionally. The drug has some structural features in common with the β-blocker propranolol, raising the possibility that some of these adverse events are related to clinically observed β-blockade during treatment with the drug. A complicating issue in evaluating clinical effects of propafenone is that the drug undergoes metabolism by CYP2D6, which is known to have a polymorphic distribution in the population with 5% to 10% of white subjects being deficient in CYP2D6 activity ("poor metabolizers," PMs).

This study was conducted in 14 normal volunteers who were exposed to placebo and a range of dosages of propafenone for 5 days each. When the study was conducted, the molecular genetics of CYP2D6 were just beginning to be defined, and subjects’ phenotypic status (extensive metabolizer [EM] or PM) was determined by their ability to metabolize a prototypical CYP2D6 substrate drug, debrisoquine. β-Blockade was assessed by reduction in peak heart rate during treadmill exercise and challenge with isoproterenol. All subjects displayed β-blockade, but the extent of β-blockade was greater in PMs than in EMs at low dosages. At high dosages, there was greater β-blockade, and this was evident in both groups. Analysis of plasma drug and metabolite profiles showed that EMs rapidly metabolized the parent drug to the 5-hydroxy metabolite, while at high dosages this metabolism was saturated and there were disproportionate increases in propafenone concentrations. In vitro studies demonstrated that the parent drug propafenone had higher affinity for β receptors than either of its major metabolites, whereas other studies had shown the metabolite and the parent drug had equivalent sodium channel–blocking properties.

This study demonstrated that variability in propafenone effects was readily explicable by CYP2D6 phenotype. Extensive metabolizers receiving low dosages of the drug demonstrated little β-blockade, because the parent was rapidly biotransformed to the active sodium channel–blocking, non-β-blocking metabolite. By contrast, poor metabolizers at low dose, and both groups at higher dosages, accumulated parent drug with readily demonstrable β-blockade. The study shows the value of precise assessment of drug effects in a controlled clinical research center environment, and the value of assessing a range of drug dosages, integrating that information with plasma concentration measurements and with in vitro assessment of drug activity across a range of experimental systems.

A treaty ending the production of chemical weapons and instigating the destruction of existing stockpiles in the USA and Russia is signed by George Bush and Mikhail Gorbachev; in the first free election in Czechoslovakia since 1946, the Civic Forum wins the most seats, but fails to secure a parliamentary majority; and Metropolitan Alexy of Leningrad is elected the 15th Russian Orthodox Patriarch of Moscow and Russia.
The idea that intracellular drug concentrations could be mediated by specific drug uptake and efflux transport proteins emerged in the 1970s. One of the first transport proteins to be identified and functionally characterized was P-glycoprotein, an efflux pump associated with multidrug resistance in cancer; accordingly, the gene was termed \textit{MDR1} (and is also termed \textit{ABCB1}). Studies in the 1980s and 1990s demonstrated not only that \textit{MDR1}-expressing cancer cells could survive chemotherapy (by pumping drug out of cells), but also that \textit{MDR1} was expressed in normal tissue, notably intestinal endothelium, hepatocytes, and renal tubules. Emerging evidence then demonstrated that, at these sites, P-glycoprotein mediated bioavailability as well as biliary and renal excretion. One key probe drug was digoxin, and the well-recognized effect of a range of structurally unrelated drugs (quinidine, amiodarone, verapamil, erythromycin, itraconazole, etc) to strikingly elevate digoxin’s serum concentrations and predispose to toxicity can be attributed to inhibition of P-glycoprotein and thus increased bioavailability and decreased drug excretion.

This paper addressed an obvious and interesting question arising from an emerging understanding of P-glycoprotein as a prototypical drug efflux transporter: do genetic variants account for variability in plasma concentrations or effects of substrate drugs? Expression of P-glycoprotein was assessed by assaying protein in intestinal biopsy samples and function was assessed by plasma concentrations during orally administered digoxin. Fifteen polymorphisms were identified, but minor allele frequencies were in general too low to assess a relationship to function. However, there was a single synonymous single nucleotide polymorphism (SNP) in exon 26 that correlated with P-glycoprotein expression in intestine, the extent of P-glycoprotein induction by rifampin, and mean serum digoxin concentration.

This was one of the first studies to begin to examine the role of DNA variation in P-glycoprotein function. Hundreds of polymorphisms have now been described at the \textit{MDR1} locus and the haplotype structure of the gene has been well defined. Subsequent studies of the exon 26 synonymous SNP have supported the idea that this variant affects mRNA stability and thereby modulates protein expression. Further, these initial studies with P-glycoprotein established a role for drug efflux (and subsequently uptake) transporters in drug disposition and in pharmacogenomics. In addition, structurally related proteins, such as CFTR (cystic fibrosis transmembrane conductance regulator) and members of the sulfonylurea receptor (SUR) family, are now well recognized to play a role in a wide range of disease susceptibility and variable drug responses.

The last known free-roaming Pyrenean ibex is found dead, crushed by a falling tree; the Constitution of Finland is rewritten; and 235 members of a Ugandan doomsday cult commit mass suicide.
Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose


By the early 2000s, it had become clear that common variants in CYP2C9 reduced the function of the encoded enzyme and were associated with lower steady state warfarin dose requirement. However, although it was known that warfarin inhibited the vitamin K epoxide reductase complex, the precise molecular target for the drug was not known. In 2004, studies in rare patients with two familial syndromes, combined deficiency of vitamin K–dependent clotting factors and a familial syndrome of warfarin resistance (very high-dose requirements to achieve therapeutic anticoagulation) identified mutations in VKORC1, encoding a subunit of the complex, as the disease gene in these entities and as the molecular target for warfarin. Patients with combined deficiency vitamin K clotting factors were homozygous for point mutations in the gene, whereas patients with warfarin resistance were heterozygous for rare non-synonymous variants. The VKORC1 variants identified were obviously of interest to scientists studying sources of variability in warfarin response, but these are rare diseases and therefore these variants were not thought to contribute importantly to the variability in warfarin dose requirements observed clinically.

This paper explored the hypothesis that variation in regulatory regions of VKORC1 could contribute to variability in expression of the encoded protein and thus variability in warfarin dose requirements. The authors surveyed a group of 186 white subjects receiving warfarin and identified one with a rare nonsynonymous variant in VKORC1; this individual had very high maintenance warfarin dose requirements (the warfarin resistance syndrome). Ten single nucleotide polymorphisms (SNPs), each occurring at a frequency greater than 5%, were identified in the promoter region and two haplotype groups were then derived, a low-dose group (A) and a high-dose group (B). Individuals with the AA, AB, and BB haplotypes required 2.7±0.2, 4.9±0.2, and 6.2±0.3 mg per day to achieve stable anticoagulation, respectively (*P*<0.001). VKORC1 mRNA levels in the liver varied by haplotype, lowest in AA and highest in BB, suggesting that variability in gene transcription contributed to variability in the amount of warfarin target protein synthesized and therefore the amount of warfarin required to inhibit its activity. The BB haplotype was commoner in African-Americans and the AA haplotype in Asian-Americans, suggesting, as later confirmed by many other studies, that variability in VKORC1 promoter haplotype contributes importantly to ancestry-dependent variability in warfarin dose requirements.

This paper translated basic discovery in the genetics of a rare syndrome to identify a key gene in which common variants contribute to interindividual variability in warfarin dose requirements. Taken together, variants in CYP2C9 and VKORC1 account for up to 50% of variability in warfarin dose requirements. Genome-wide association studies examining warfarin dose requirements have also identified variants in CYP4F2, involved in vitamin K-dependent clotting factor synthesis, as a possible other contributor, but no other loci with common variants. The role of CYP2C9 and VKORC1 testing in clinical practice is now being assessed in randomized clinical trials. Open questions include the role of variation in these and other genes across other ancestries, and the extent to which rare variation in CYP2C9, VKORC1, and other components of the vitamin K-dependent clotting factor cycle contribute to variability in warfarin dose requirements, particularly in individuals requiring unusually high dosages.

A new aria by Johann Sebastian Bach is discovered among documents in a German library; Emperor Akihito of Japan visits the memorial to the Korean War dead in Saipan; and Jamaican sprinter Asafa Powell breaks the world 100 meters record, recording a time of 9.77 in Athens, Greece.
Clopidogrel was approved for adjunctive therapy in patients with atherosclerotic vascular disease in the late 1990s. In 2005, TIMI-28 (Thrombolysis in Myocardial Infarction–28) reported that 30-day event rates (myocardial infarction, death, stroke, in-stent thrombosis) in patients treated for coronary syndrome were 11.6% at 30 days with clopidogrel (n=1752) and 14.1% with placebo (n=1739). Thus, clopidogrel rapidly became standard of care in patients receiving contemporary treatment for acute coronary syndrome, most commonly involving placement of intracoronary stents.

Clopidogrel was known to be a prodrug, requiring bioactivation to achieve inhibition of ADP-induced platelet aggregation. The steps involved in its bioinactivation were not clearly defined until 2006 when Hulot and colleagues reported this study in 28 normal volunteers. The authors examined the relationship between variable clopidogrel effect (inhibition of ADP-induced platelet aggregation) and a number of common variants in enzymes previously implicated as possible mediators of clopidogrel's bioactivation. The major finding was that subjects heterozygous for the common \textit{CYP2C19*2} loss-of-function allele displayed significantly less clopidogrel effect after a week of treatment than did subjects with the reference (*1/*1) genotype.

This study was the first to define the potential role for loss-of-function variation in \textit{CYP2C19} as an important contributor to variability in clopidogrel efficacy. This paper then led to subsequent evaluations of the role of \textit{CYP2C19*2} in mediating variable clinical actions of clopidogrel, in the acute coronary syndrome and other settings. This is one important example of the general concept that prodrug bioactivation by a single metabolic pathway is a setting in which variable drug effects may arise due to DNA polymorphisms or to drug interactions inhibiting the bioactivating pathway. Other prodrugs for which this holds true include tamoxifen and codeine.

Another interesting aspect of this study is that it was relatively simple to demonstrate variability in clopidogrel action even among \textit{CYP2C19*2} heterozygotes. Subsequent studies have demonstrated that homozygotes, who constitute 2% to 3% of a white population, have even more severe deficits in clopidogrel action, that such poor metabolizers are more common in some nonwhite ancestries (notably Asians, Aborigines, and Inuit), and that other variants besides *2 may contribute to this trait in those ancestries.

\textbf{Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects}

\textbf{J. S. Hulot, A. Bura, E. Villard, M. Azizi, V. Remones, C. Goyenvalle, M. Aiach, P. Lechat, P. Gaussem}

\textit{Blood.} 2006;108(7):2244-2247

Andrew Fire and Craig Mello are awarded the Nobel Prize in Physiology or Medicine for the discovery of RNA interference; Google purchases YouTube for US$1.65 billion; and seven times World Formula One Champion Michael Schumacher retires
A polymorphism within a conserved β₁-adrenergic receptor motif alters cardiac function and β-blocker response in human heart failure


Proc Natl Acad Sci. 2006;103(7):11288-11293

Polymorphisms in adrenergic receptor genes are obvious candidates for mediating susceptibility to a wide range of common cardiovascular diseases such as hypertension and heart failure, and responses to β-blocker and other therapies in those conditions. One common nonsynonymous single nucleotide polymorphism (R389G) in the β₁-adrenergic receptor gene is located at position 389 where an arginine residue, conserved across multiple species, is substituted by a glycine. The minor allele frequency is approximately 30% in whites and Asians and somewhat higher (approximately 40%) in African subjects. A range of in vitro studies have generally agreed that adenyl cyclase activity is greater with the R389 allele compared to the GLY389 allele, likely reflecting enhanced coupling to stimulatory G proteins (Gs).

This study used right ventricular trabeculae from failing and nonfailing hearts, genotyped at the 389 locus, to define significantly greater agonist-promoted contractility in hearts with the R389 genotype. Based on these, and previous, in vitro studies, the investigators examined the effect of R389G genotype on outcome in BEST (Beta-Blocker Evaluation of Survival Trial), a large randomized placebo-controlled trial in 2708 subjects examining the effects of the β-blocker bucindolol on outcome in patients with heart failure. Overall, there was no statistically significant effect of bucindolol on mortality in BEST. Among 1040 patients who consented to provide DNA samples, there was a discernible effect of genotype on outcome: patients homozygous for the R389 genotype demonstrated reduced mortality with bucindolol compared with placebo, whereas those either homozygous or heterozygous for a G389 allele demonstrated no effect of the drug compared with placebo. The mortality-sparing effect seen with drug in the R389 group was most evident among patients with higher baseline norepinephrine levels and those who demonstrated the greatest change in norepinephrine concentration during treatment with the drug. This study combines both in vitro and clinical trial data to support the idea that genotype is an important determinant of outcome during therapy with the β-blocker bucindolol in patients with heart failure.

While the results of the genetic analysis are consistent with in vitro findings, the substudy involved only a minority of patients, and subsetting this group further (eg, by genotype, and then by tertiles with change in norepinephrine) led to study cells with small numbers. These data highlight a fundamental challenge in moving pharmacogenomic data to the bedside: subsetting patients even within very large trials will result in smaller subgroups, and so the statistical and clinical significance of any differences observed may be difficult to establish and confirm using a traditional randomized trial paradigm.

Studies of the impact of the R389G polymorphism on susceptibility to cardiovascular disease have yielded weak associations, whereas studies examining response to β-blocker therapy have yielded more consistent results, similar to those described above. A number of studies have examined the effect of metoprolol or carvedilol by genotype, but interpretation is confounded here by the fact both of these are CYP2D6 substrates. Further, there are other common polymorphisms displaying variant function in vitro and reported to modulate the effect of R389G in the β₁-adrenergic receptor gene (at position 49), in the β₂-adrenergic receptor gene, and in the α₂-adrenergic receptor gene. Thus even in diseases where variable adrenergic tone may clearly play a role, the functional R389G polymorphism is one of many contributors to clinically important and complex phenotypes.

Russia hosts the 32nd G8 summit in Saint Petersburg; US cyclist Floyd Landis, winner of the 2006 Tour de France, fails a drug test, leading to his dismissal from the Phonak team and suspension from professional cycling; and negotiations by the World Trade Organization aimed at liberalizing of world trade collapse.
The introduction of HMG-CoA reductase inhibitors (statins) into clinical practice has revolutionized the care of patients with vascular disease. There are two reasons for the drugs' success: their efficacy both in lowering LDL cholesterol and in decreasing cardiovascular events, and the fact that they are so well tolerated. While side effects are rare, a range of muscle symptoms from pain to myopathy to very rare cases of frank rhabdomyolysis do occur. While these symptoms are uncommon, they nevertheless represent limiters of statin use. Elucidating genetic risk factors for such rare adverse drug reactions (ADRs) has traditionally involved accrual of cases from registries or loose consortia of interested investigators and comparing these with population or drug-exposed controls.

The SEARCH investigators (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine) randomized 12,064 patients to low-dose (20 mg per day) or high-dose (80 mg per day) simvastatin and examined cardiovascular outcomes. The genome-wide association study (GWAS) paradigm was used to study cases meeting predefined criteria for myopathy, defined prospectively using scheduled measurement of creatine kinase (CK) with or without muscle symptoms, and controls exposed to the same drug dose and not developing symptoms or CK elevations. The ADR was dose-dependent: there were 8 cases of myopathy among the 6033 participants assigned to 20 mg per day and 98 cases among the 6031 assigned to 80 mg per day. The GWAS compared 96 cases from the high-dose group to 96 controls matched for age, sex, renal function, and amiodarone use. Despite these very small numbers, a single nucleotide polymorphism (SNP) was associated at genome-wide significance with myopathy. The variant was located in SLCO1B1, encoding a hepatic drug uptake transporter, and was in strong linkage disequilibrium with a known nonsynonymous SNP previously implicated in variability in simvastatin pharmacokinetics. Homozygote carriers of the variant, representing 2.1% of the population, the heterozygotes (24.9%) were intermediate in risk. The incidence of myopathy for those with the at-risk genotype was 18.6% over 5 years compared with 0.63% for those homozygote for the reference allele.

The SEARCH investigators replicated the trend to risk with the SLCO1B1 variant in a separate cohort, and others have since also reported that the variant confers risk of myopathy or an inability to continue long-term simvastatin therapy. This study demonstrates the power of the GWAS paradigm to identify high-impact signals even when very low numbers of patients are studied. Moreover, they highlight the value of prospectively defining such ADRs during large clinical trials and of obtaining DNA samples, and appropriate consent for their use, on a prospective basis in a large clinical trial.

Rwanda formally accuses the late French President François Mitterrand and former Prime Minister Dominique de Villepin with complicity in the Rwandan genocide; the 2008 Summer Olympics begins with the lavish opening ceremony at the Beijing National Stadium; and Irish golfer Pádraig Harrington wins the 2008 US PGA Championship.
Estimation of the warfarin dose with clinical and pharmacogenetic data


Relatively small studies through the 1990s and later established a role for variants in \textit{CYP2C9}, responsible for warfarin bioinactivation, and \textit{VKORC1}, encoding the warfarin target. Reports from across the world strongly suggested that warfarin dose requirements varied by ancestry, smallest in Asians and greatest in African populations. Algorithms had been developed to integrate clinical datasets and genetic information to predict steady state warfarin dose requirements. However, these had not been tested across ancestries.

The International Warfarin Pharmacogenetics Consortium (IWPC) consolidated clinical factors, \textit{CYP2C9} and \textit{VKORC1} genotypes, and steady state warfarin dose requirements in 5051 patients of varying ancestry accrued from 21 sites from around the world. The group selected 4043 subjects in which to develop two algorithms, one incorporating clinical factors only (such as age, sex, ancestry, interacting drugs, etc) and the other incorporating both clinical factors as well as pharmacogenetic variants. The performance of the algorithms was then tested in the remaining 1009 subjects.

The IWPC found that \textit{VKORC1} variants predicting lower warfarin steady state dose requirements were much more common in Asians than in African subjects, and these data suggest that ancestry-dependent variability in warfarin dose requirements may be largely attributable to this genetic factor. The performance of the two algorithms was compared with the performance of an “algorithm” in which 5 mg of warfarin was administered daily. The algorithms were compared by examining the proportion of patients in which steady state warfarin dose was correctly predicted to within ±20%. For “average” patients, in whom the actual daily warfarin dose requirement was >3 or <7 mg per day, all three algorithms performed well and there was no difference among them. However, for patients whose warfarin steady state dose requirement was ≥7 mg per day, the pharmacogenomic algorithm similarly outperformed the clinical algorithm (24.8% vs 7.2%, \( P < 0.0001 \)) but neither performed especially well. Overall, almost half the population required ≤3 mg or ≥7 mg per day.

One key lesson from this study was that large numbers of subjects can be accrued from across the globe to study the role of pharmacogenomics and outcome for a commonly used drug. A second lesson was that readily identifiable genetic factors may strongly contribute to apparent ancestry-dependent variability in drug requirement or drug response. A third important lesson was that even after correction for common genomic variants, there remained substantial and unexplained variability in dose requirement especially in patients requiring high dosages. Whether this reflects poorly defined clinical covariates or rare variants in \textit{CYP2C9}, \textit{VKORC1}, or other genes is an area of very active investigation.
Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy


JAMA. 2009;302(8):849-857

Candidate gene studies examining variability in clopidogrel efficacy focused on pathways of bioactivation, and identified variants in CYP2C19 as major modulators of inhibition of ADP-induced platelet aggregation by clopidogrel in normal volunteers. However, the extent to which common variants in other genes might contribute to variability in clopidogrel action was not well captured by this candidate gene paradigm.

In this study, 429 healthy individuals from the Amish community were studied at baseline and after 7 days of therapy with clopidogrel, whose major mechanism of action is inhibition of ADP-induced platelet aggregation. A genome-wide association study (GWAS) analysis was then performed using the change in ADP-induced platelet aggregation as the primary outcome measure. Because the Amish subjects studied were all descended from founder immigrants and have extensive genealogical records, it was possible to estimate heritability of the trait, and this was quite high, 0.73; that is, 73% of the variability in change in ADP-induced platelet aggregation by clopidogrel could be explained by heritable factors. The GWAS analysis identified a cluster of single nucleotide polymorphisms (SNPs) in chromosome 10q24 that encompassed the CYP2C19 locus and a variant in strong linkage disequilibrium with CYP2C19*2 accounted for 12% of variation in platelet aggregation. Conditioning the GWAS analysis on CYP2C19*2 (ie, including it as a covariate) eliminated the association signal entirely; that is, the association was due to CYP2C19*2 alone and not to other common variants within this locus. These investigators also demonstrated that the CYP2C19*2 variant predicted decreased clopidogrel efficacy among patients undergoing coronary intervention and that the variant also predicted a higher incidence of recurrent ischemia or death in 1 year of follow (hazard ratio 2.42; 95% confidence interval level 1.18-4.99) in a small group of patients studied with coronary disease.

The importance of this study was the demonstration that clopidogrel’s pharmacologic effect is variable, but highly heritable, and that CYP2C19*2 accounted for a modest amount of this heritability. An interesting question, then, is whether rare variants in CYP2C19, or in other genes involved in generation or delivery of active clopidogrel metabolites to the platelet or factors intrinsic to the platelet itself, account for the remainder of the variability. The power of the GWAS paradigm to identify, in an unbiased fashion, modulators of an endophenotype related to clopidogrel clinical action and the conduct of the study in a genealogically defined large cohort are other strengths of the approach, which should lend itself to evaluating variability in response to other drug therapies.

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Mahmoud Ahmadinejad is officially sworn in as President of Iran for a second term; a giant carnivorous plant discovered in the highlands of the central Philippines is named after Sir David Attenborough, Nepenthes attenboroughi; and seventeen-year-old English sailor Michael Perham becomes the youngest person to complete a solo circumnavigation of the world.
Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis


JAMA. 2010;304(16):1821-1830

The platelet ADP receptor antagonist clopidogrel was marketed in 1997 and its efficacy in patients treated for acute coronary syndrome was first reported in the mid-2000s. In 2006, a study in normal volunteers (detailed elsewhere in this issue) showed that carriers of a single copy of the CYP2C19*2 loss-of-function allele demonstrated significantly less inhibition of ADP-induced platelet aggregation than did individuals with the reference (*1/*1) genotype. Three papers published simultaneously in late 2009 demonstrated higher event rates among individuals with acute coronary syndromes treated with clopidogrel and carrying the *2 allele compared with rates in *1/*1 patients. The *1/*2 genotype is found in 20% to 25% of the white population, but none of the original studies included sufficient numbers of patients with the *2/*2 variant, comprising 2% to 3% of white subjects, to infer whether there was a gene-dose related effect on outcome.

This study performed a meta-analysis of nine individual studies in which data were available on clinical outcomes and CYP2C19 genotype among patients treated with clopidogrel. There were 9685 subjects, and greater than 90% underwent percutaneous coronary intervention and over half were treated for acute coronary syndrome. Of these, 863 experienced cardiovascular death, myocardial infarction, or stroke. In addition, 5894 patients were evaluated for possible in-stent thrombosis, which was identified in 84. There was a significantly increased risk for the composite end point among carriers of 1 or 2 copies of CYP2C19*2: the hazard ratios comparing rates with those in *1/*1 patients were 1.55 (95% confidence interval 1.11-2.17) for *1/*2 and 1.76 (1.24-2.50) for *2/*2 individuals. The hazard ratios for in-stent thrombosis were higher: 2.67 (1.69-4.22) for *1/*2 and 3.97 (1.75-9.02) for *2/*2.

In spring 2010, the US Food and Drug Administration relabeled clopidogrel to include the statement that practitioners should “consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers.” The statement evoked considerable controversy, since many felt that the data were not particularly strong and that alternative treatment strategies were not offered. In addition, a subsequent large meta-analysis examined 32 studies comprising over 42,000 subjects and over 3500 cardiovascular events and found a much lower effect of CYP2C19 genotype during clopidogrel (relative risk 1.18; 95% confidence interval 1.09-1.28). This small relative risk was eliminated when only studies comprising greater than 200 individuals were considered. These authors concluded that CYP2C19 genotype was not an important modulator of clopidogrel efficacy. The larger meta-analysis has been criticized because it included large numbers of subjects accrued from studies that showed only a very small effect of clopidogrel over placebo: if the drug does not produce a major pharmacologic effect then it has been argued that it should be no surprise that its effects are not modulated by genotype. The contrasting outcomes highlight controversies and tensions within the broad field of pharmacogenomics. Mounting large trials to identify variant outcomes in small subsets of patients does not lend itself well to the randomized clinical trial paradigm which is widely viewed as the gold standard for changing therapy. One way forward for clopidogrel may be to integrate genotype with platelet function testing during therapy. This is analogous to a warfarin strategy of choosing the starting dose based on genotype and subsequently adjusting dose based on INR (International Normalized Ratio).

2010

British physiologist Robert Edwards wins the Nobel Prize in Physiology or Medicine for his pioneering work on in vitro fertilization; Northern Irish golfer Graeme McDowell defeats Hunter Mahan (USA) to win the 2010 Ryder Cup; and Australian opera singer Dame Joan Sutherland, the “Voice of the Century,” dies in Switzerland
A randomized and clinical effectiveness trial comparing two pharmacogenetic algorithms and standard care for individualizing warfarin dosing (CoumaGen-II)


Anticoagulant therapy is the classic double-edged sword of modern therapeutics: subtherapeutic dosing can translate into fatal thrombosis, while excessive doses raise the risk of hemorrhage. Despite this balancing act, the introduction of warfarin in the 1950s, and the subsequent clear demonstration that warfarin administration, with dosages adjusted by the International Normalized Ratio (INR), to patients with atrial fibrillation strikingly reduces stroke rate with an acceptable incidence of hemorrhage made warfarin anticoagulation a mainstay of modern therapeutics. A vast amount of experience over decades of warfarin therapy demonstrated striking interindividual variation in dosages required to achieve therapeutic anticoagulation. Up to 50% of this variability can be attributed to common DNA polymorphisms in \( CYP2C9 \), which encodes the enzyme responsible for bioinactivation of the active warfarin enantiomer (S-warfarin) and in the promoter region of \( VKORC1 \), which encodes the protein with which warfarin interacts to achieve inhibition of vitamin K-dependent synthesis of coagulation factors. While algorithms have been developed to use genetic information to predict steady state warfarin dosage, and these also predict the time required to achieve therapeutic anticoagulation with the drug after it is started, data actually attesting to the clinical utility of such algorithms in practice have been lacking.

A number of trials have therefore been mounted to develop such algorithms and to compare their utility in practice to standard of care, ie, initiation of a standard dose of warfarin and subsequent dose adjustment using the INR. This study was one of the first to identify a potential benefit of pharmacogenomically-guided warfarin dose adjustment in practice. Investigators compared two separate algorithms to predict warfarin dosage in 504 patients, and compared outcomes in this group to a group of 1866 patients receiving standard warfarin dosing. The primary outcome of INR out of range (≥4 or ≤1.5) was lower in the pharmacogenomic arm and there were fewer serious adverse events of three months with pharmacogenomic guidance (4.5% vs. 9.4%, pos 0.01).

The investigators concluded that these data supported further large clinical trials, now under way, to assess the role of pharmacogenomic guidance for warfarin therapy. Newer anticoagulants (dabigatran, rivaroxaban, apixaban) have now been tested at fixed doses compared to dose-adjusted warfarin and found to be equivalent to or superior to the older drug. Thus, advances in warfarin pharmacogenomics, and their utility are coming at a time when the drug itself may become less used.

One of the arguments against the use of warfarin is the need to monitor INR to insure that the drug’s effect remains in the therapeutic range. For agents such as anticoagulants, the idea that one size fits all seems misguided in an era increasing appreciation for sources of variability in drug outcome. The fact that newer anticoagulants “beat” warfarin in large RCTs clearly adds to their appeal, and suggests that they could, in fact, be even more effective were doses adjustable to maximize efficacy and minimize risk of hemorrhage-related adverse events.

Miguel de la Madrid, former President of Mexico dies aged 77; Hungarian president Pál Schmitt resigns following accusations that he plagiarized significant parts of his doctorate; and Raymond Aubrac, one of the leaders of the French Resistance during the Second World War, dies at the age of 97
Cardiovascular Pharmacogenetics

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