Cardiovascular Pharmacogenomics

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Case 1: Warfarin therapy (5 mg per day) is started in a patient with deep venous thrombosis. One week later, the patient presents with an intracerebral hemorrhage, and the INR is 11.

Case 2: Sotalol therapy is begun in a 78-year-old man with paroxysmal atrial fibrillation and heart failure. Two days later, after an episode of syncope, the patient is found to be in sinus rhythm with a QT interval of 0.7 seconds. Short runs of torsades de pointes are documented.

Case 3: Lisinopril therapy is started in a 46-year-old African-American man with a blood pressure of 170/120. The blood pressure is unchanged 1 week later.

Background: Definitions

As these cases illustrate, variability in response to drug therapy is a rule by which clinicians live. The notion that genetic factors might contribute to this variability was proposed at the beginning of the twentieth century, and the earliest examples of genetically determined aberrant drug effects were highly unusual responses in individual patients, stemming from dysfunction of a single gene product, and thus defining the field of pharmacogenetics.[1,2] Although the concepts that have evolved from these initial discoveries have considerable appeal, obstacles must be overcome before they are incorporated into clinical practice.

Contemporary basic and clinical pharmacology have evolved an increasingly sophisticated molecular view of the mechanisms underlying drug action. At the same time, literally millions of variants are being identified in genes that, by their normal function, determine these mechanisms. Some variants, termed *mutations*, are associated with familiar (albeit rare) diseases, such as hypertrophic cardiomyopathy, homozygous familial hypercholesterolemia, or sickle cell anemia. Other variants, called *polymorphisms*, are much more widespread and may or may not be associated with a specific patient presentation (*phenotype*). A prior definition of polymorphism was that it represented a genetic variation present in >1% of a population. However, polymorphism frequency can vary strikingly among ethnic groups, and the distinction between rare polymorphisms and mutations is becoming blurred. The most common type of polymorphism is a single-nucleotide polymorphism, or *SNP*. The term *pharmacogenomics* is used to describe how variations in molecular function conferred by polymorphisms (and mutations) in one or more genes modulating drug actions cause variability in drug response. These variations may lie either in the coding regions (and may therefore change primary amino acid sequence to alter protein function) or in the noncoding regions (and may therefore alter protein expression).

Variability in drug action may be *pharmacokinetic* or *pharmacodynamic*. Pharmacokinetic variability refers to variability in delivery of drug to, or removal from, key molecular sites of action that mediate efficacy and/or toxicity. The molecules involved in these processes include both drug-metabolizing enzymes (such as members of the cytochrome P450, or CYP, superfamily) and drug transport molecules that mediate drug uptake into, and efflux from, intracellular sites. Pharmacodynamic variability refers to variable drug effects despite equivalent drug delivery to molecular sites of action. This may reflect variability in the function of the molecule that a drug targets to achieve its effects or in the broad pathophysiological context in which any drug interacts with its molecular target.

The ultimate goal is to use genetic information to deliver personalized medicine, "the right drug in the right dose to the right patient at the right time." Our 3 cases illustrate how pharmacogenetics is evolving and present

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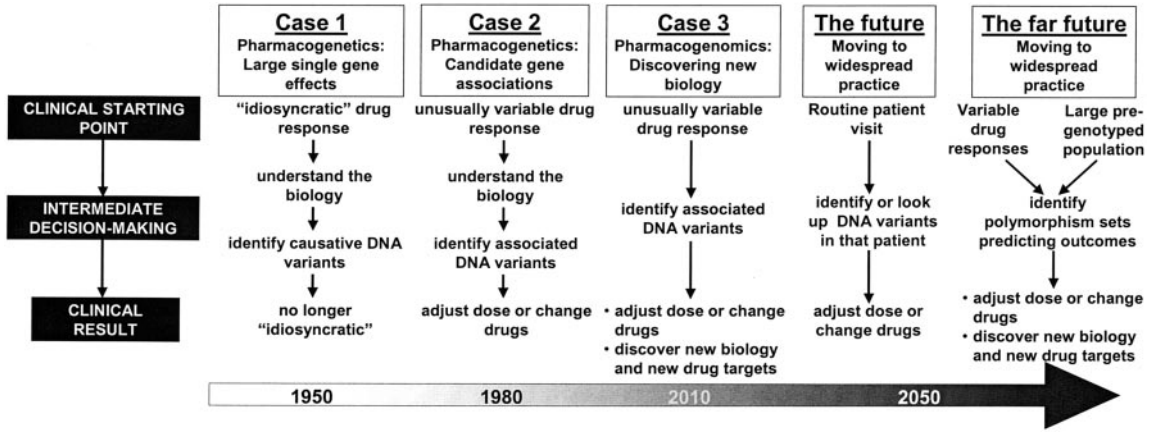

Evolution of pharmacogenomic science. The 3 cases presented at the beginning of the article illustrate 3 approaches to identifying the role of genetic factors in variable drug effects. Future possibilities are presented on the right. Each sequence begins with a clinical starting point and proceeds to a clinical result that ultimately affects practice in very few patients (left) or all patients (right).

the opportunities and obstacles to implementation of this vision (Figure).

Case 1: Pharmacogenetics—Large Single-Gene Effects

Warfarin is administered as a racemate, and the active enantiomer, S-warfarin, is eliminated almost exclusively by CYP2C9-mediated oxidation. CYP2C9 variants that decrease the catalytic activity of the enzyme are well recognized, and very rare individuals, homozygous for such variants, display striking elevations of S-warfarin plasma concentration, and thus markedly exaggerated warfarin effects, with standard dosages. More generally, the case illustrates the concept of "high-risk pharmacokinetics" as follows: When a drug with a narrow margin between effective and toxic concentrations is eliminated by a single pathway, genetic variation in that pathway may lead to large (sometimes orders of magnitude) changes in drug clearance, concentrations, and effects. Such high-risk pharmacokinetics also underlie large changes in drug effects with drug interactions, such as quinidine + digoxin[3] or terfenadine + ketoconazole.[4]

Case 2: Pharmacogenetics—Candidate Gene Associations

Torsades de pointes can occur as a consequence of administration of QT-prolonging drugs or in the congenital long-QT syndrome (LQTS), which is caused by mutations in 1 of at least 7 different genes. Risk factors include bradycardia, hypokalemia, and heart failure. Thus, variations in a small set of "candidate" genes—those modulating these risk factors as well as those causing congenital LQTS—might alter susceptibility to drug-induced LQTS. Indeed, there are now well-documented examples, such as in Case 2, of individuals who have LQTS mutations and yet have no manifest QT prolongation or family history of sudden death until drug challenge or other repolarization stressor is superimposed.[5–7] Unlike manifest congenital LQTS, these cases may present in later life, presumably as the incidence of drug prescribing increases. Although cases with such mutations represent proof of principle for the idea of screening a limited candidate gene set, they are not very informative when applied to predict variability in drug actions in large populations. A more exciting prospect is that common polymorphisms in these genes might also modulate risk. One such variant is Y1102S in the cardiac sodium channel gene. The S allele was detected only in African Americans and was overrepresented in a series of patients with a range of arrhythmias, including some related to QT-prolonging drugs, compared with controls.[8]

One way of conceptualizing this variability in response to sotalol is that the ability to maintain a short QT interval in the face of challenge with a QT-prolonging drug is a function of the complex biology that determines normal repolarization. Thus, patients with risk factors such as bradycardia, hypokalemia, heart failure, or DNA variants of the type just described may have reduced protective mechanisms but nevertheless display no manifest phenotype (ie, have normal QT intervals) until drug challenge; the term *reduced repolarization reserve* has been proposed to describe this situation.[9]

This logic can be extended to analyze variability in the way that other complex biological systems respond variably to drugs or to other exogenous stressors, such as myocardial ischemia, infection, or elevated blood pressure. Thus, understanding the complexities of such biology is a key first step in identifying candidate genes in which variants may modulate baseline or drug-response phenotypes. A common approach to evaluating the role of such

Examples of DNA Polymorphisms Implicated in Variable Outcomes of Drug Therapy in Cardiovascular Medicine

Drug	Gene	Reported Association
Pharmacokinetic mechanisms		
Digoxin	MDR1	Variable drug levels due to variable bioavailability and clearance
Warfarin	CYP2C9	Greater anticoagulation with hypofunctional alleles
Losartan, irbesartan	CYP2C9	Greater blood pressure drop with hypofunctional alleles
Metoprolol, timolol, propafenone	CYP2D6	Poor metabolizers display greater β-blockade
Procainamide	NAT2	Poor acetylators at greater risk for drug-induced lupus
Pharmacodynamic mechanisms		
QT-prolonging drugs	KCNH2, KCNE2, KCNQ1, KCNE1, SCN5A	Increased torsades de pointes risk
β-Blockers	β_1-and β_2-Adrenergic receptor	Altered extent of heart rate slowing or blood pressure lowering
ACE inhibitors	ACE	Decreased response in subjects with the "DD" genotype
β-Blockers	ACE	Increased response in subjects with the "DD" genotype
Fluvastatin	ABCA1 transporter	Fluvastatin resistance
Pravastatin	Cholesteryl ester transfer protein	Variable regression of atherosclerosis
Estrogen	Estrogen receptor	Variable HDL elevation during estrogen therapy
Lipid-lowering therapy	Hepatic lipase	Variable lipid lowering
Antiplatelet drugs	Platelet glycoprotein IIIa	Variable antiplatelet effects ex vivo
Antihypertensive drugs	AT1 receptor	No relation to antihypertensive effects
Amiloride	Epithelial sodium channel	Antihypertensive effect in African-American subjects
Antihypertensive drugs	$G_s\alpha$	Variable blood pressure lowering
Diuretics	α-Adducin	Variable stroke incidence Variable blood pressure response (especially when analyzed as a function of ACE polymorphism)

candidate genes is an association study design in which statistically significant differences in polymorphism frequency between control and affected populations are sought. This requires identification of polymorphisms in candidate genes as well as access to relatively large sets of well-phenotyped patients (affected as well as controls), appropriately consented DNA samples, and sophisticated genetic epidemiology techniques. Notably, this algorithm may not require any understanding of how the polymorphisms studied alter gene function; indeed, significant polymorphisms may simply be markers for the biologically relevant variants. A general rule is that even the strongest association studies should be regarded with skepticism until reproduced.[10] Examples of how this approach has been applied in contemporary cardiovascular pharmacogenomics are presented in the Table.

Case 3: Pharmacogenomics—Discovering New Biology

Astute clinicians treating hypertension would recognize that an angiotensin-converting enzyme (ACE) inhibitor might not be an appropriate drug of first choice to treat hypertension in an African-American patient. This is an example of how ethnic background is used in contemporary medicine to select among therapeutic choices, and failure of an African-American patient to respond to an ACE inhibitor is no surprise. Nevertheless, this is not a hard and fast rule; a more accurate statement would be that African-American patients are less likely to respond to ACE inhibitors than are Caucasian patients.[11] The question of whether ethnicity can or should be used in contemporary genomic studies has been the subject of high profile debates.[12,13] It seems likely that a deeper understanding of genetic factors that determine the pathogenesis of hypertension, and thus (as in Case 2) its response to drug therapy, could resolve this problem. Rather than stratifying on ethnicity, one could envision a therapeutic approach based on specific sets of polymorphisms that may have different frequencies across ethnicities.

The candidate gene approach could be used to identify such polymorphism sets. An alternative approach would be to ask whether there are loci anywhere in the genome that can be linked to specific drug-response phenotypes. The power of modern genetics lies in identifying disease loci, and ultimately mutations in specific genes, in monogenic diseases. The approach has been applied successfully to identify genetic loci and occasionally specific genes modulating complex cardiovascular diseases like hypertension and stroke. The candidate gene logic identifies these loci as potential modulators of drug response, as well. To extend the

whole genome approach to specifically identify new genes modulating drug responses will be especially challenging. Very large numbers of patients with well-characterized drug responses will be required; studies in genetically manipulable animal models of human disease may be a promising alternative approach. The very large numbers of polymorphisms in the human genome are another challenge because high-throughput cheap genotyping will be required and because the sheer numbers of polymorphisms may generate many false positives.

Moving to Widespread Practice

Case 1 illustrates that single DNA variants may occasionally underlie truly spectacular adverse drug effects. As technology evolves and studies are conducted to validate cost-effectiveness, incorporation of genetic information into routine prescribing may become standard of care, both to avoid serious toxicity with narrow–therapeutic range drugs such as warfarin and to maximize the likelihood of a beneficial response. Indeed, current labeling for azathioprine and 6-mercaptopurine suggests preprescription genotyping for a rare polymorphism in the thiopurine methyltransferase (TPMT) gene, because TPMT deficiency can lead to fatal agranulocytosis with the use of these drugs in rheumatoid arthritis or leukemia. An alternative to avoid these serious adverse drug effects is to understand the underlying biology and to then develop drugs that lack the potential for such toxicity. Indeed, the pharmaceutical industry has been sufficiently sensitized to the issue of high-risk pharmacokinetics that new drugs are screened for this characteristic and generally not developed if it is likely.

This concept is readily extended from single mutations to individual polymorphisms or sets of polymorphisms that could then be used to identify patients at risk for unusual drug responses, such as marked QT prolongation, or variant therapeutic responses, as in ACE inhibitors in hypertensives. The challenges to implementation of these approaches are considerable. They include ethical and social considerations with regard to DNA research,[14] implementation of new clinically reliable and cost-effective clinical genetic testing, education to the provider and patient communities, and statistical considerations with regard to trial size. Research in this area is by definition interdisciplinary, and an increasing amount of information is available on the web; the National Institutes of Health–sponsored Pharmacogenetics Research Network (http://www.nigms.nih.gov/pharmacogenetics/) and its Knowledge Base (http://www.pharmgkb.org/index.jsp) are examples. When we reach the era in which each patient's genome is sequenced once, software to identify the variants and their clinical significance will remain as major barriers. It is important to note that sequence information need be generated only once in a lifetime, and with sufficiently large databases, clinical outcomes could actually feed back to genomic information to identify new polymorphism sets for clinical trials and practice (Figure).

The field of pharmacogenetics has its roots in the clinical community, which identifies very unusual responses to drugs and translates these into research identifying underlying mechanisms. The development of genomic science raises the prospect that this approach can be much more widely applied to therapeutics. Pharmacogenomics is an extraordinarily young field, impressive successes have already been recorded, and the pace at which new technologies are being developed supports some optimism. One aspect does seem irreplaceable: The clinician-scientist is key to establishing a sophisticated understanding of the nuances of distinct disease phenotypes and responses to drug therapy.

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