Pharmacogenomics: The genetics of variable drug responses

Dan M. Roden, MD, Russell A. Wilke, MD, PhD, Heyo K. Kroemer, PhD, and C. Michael Stein, MD

1 Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville
2 Center of Pharmacology and Experimental Therapeutics Ernst Moritz Arndt University, Greifswald

Not all patients respond to drug therapy in a uniform and beneficial fashion. The goal of this review is to describe the contribution of genetic variation to drug response, with a focus on drugs used in cardiovascular therapy. Genetic approaches used to analyze rare and common adverse effects as well as variability in efficacy are first presented. The challenges and potential solutions to incorporating this body of knowledge into contemporary medical practice are then discussed.

History of pharmacogenetics

The notion that genetic variants might modulate variability in drug actions was first proposed by the English physiologist Garrod. He suggested that enzymatic defects lead not only to accumulation of endogenous substrates in “inborn errors of metabolism” (a term that he coined), but also to accumulation of exogenously administered substrates such as drugs, foodstuffs, and toxins, with clinical consequences. Initial examples of genetically-determined variable drug actions were the identification of pseudocholinesterase deficiency in prolonged paralysis after administration of the muscle relaxant succinylcholine, deficient N-acetylation of isoniazid, and a high incidence of hemolytic anemia among African-Americans with G6PD deficiency receiving antimalarial drugs in the South Pacific during WWII. The latter observation highlights the principle in modern genomics and pharmacogenomics that ancestry may play a key role in modulating clinically-important phenotypes.

The term “pharmacogenetics” was coined in 1959 and the first textbook was published in 1962, well before methods for studying DNA sequence variation were available. The term “pharmacogenomics” has been more recently used to transmit the idea that variable drug response may reflect sets of variants within an individual or across a population. DNA variants can modulate protein function, and hence drug response, through multiple mechanisms. Much of the initial focus in the field was on non-synonymous DNA variants, i.e. those that alter protein function by changing the encoded amino acids. Non-coding variants that modulate gene expression represent another common candidate mechanism for variable drug responses. Contemporary genomics has uncovered multiple other mechanisms...
regulating gene function and expression, such as epigenetic changes and small mRNAs, and a role for these in determining drug response seems likely.\(^7\)

**Identifying genetic contributors to variable drug actions**

**Heritability and drug responses**

Studies in families can define the extent to which common human disease phenotypes like myocardial infarction or sudden cardiac death include a heritable component. However, it is usually not possible to accumulate well-defined drug response phenotypes across multiple related patients with the same disease; as a result, the heritable component of variability in drug action may not be well-defined. An *in vitro* approach that has been useful to estimate heritability of cytotoxicity due to anticancer agents is exposure of lymphoblastoid cell lines from related subjects to the drug.\(^8,9\) Using this method, the heritability of cytotoxicity has been estimated at 0.25–0.65; one study went on to use linkage analysis to identify a potential locus mediating this toxicity.\(^9\)

One approach when heritability is not well-understood is to quantify drug responses in multiple healthy members of a family. For example, very early studies in twins demonstrated far more variability in the urinary excretion of isoniazid within dizygotic than monozygotic twins,\(^10\) thus establishing that this trait – now known to reflect genetically-determined variable N-acetylation – is heritable. Similarly, digoxin clearance was much better correlated within monozygotic than dizygotic twins; the heritability of the area under the curve was >79%.\(^11\) ADP-stimulated platelet aggregation was studied before and after clopidogrel in the Amish, a founder population with extensive genealogical records: the investigators reported that heritability was 0.33 at baseline and 0.73 during drug treatment, indicating a strong genetic component in the drug response.\(^12\)

**Experimental approaches in pharmacogenetics**

Defining mechanisms underlying variable drug concentrations and effects provides a starting point for identifying candidate genes for further pharmacogenetic study. As a result, many important examples in pharmacogenetics relate to variable drug uptake, metabolism, or elimination. Other contributors to variable drug responses identified by this physiologically-based candidate approach include variation in drug target molecules or in disease pathways. In some cases, variants in multiple genes have been implicated, as discussed below ("combinatorial pharmacogenetics"). More recently, technologies to search for previously unanticipated relationships between phenotypes and hundreds of thousands of common polymorphic sites across the genome (an unbiased approach)\(^13\) have been applied to the problem of variable drug actions; these genome-wide association studies (GWAS) have been conducted both in human cohorts as well as in cellular or model organ systems. Table 1 lists the experimental approaches that have been used in the field, along with their potential advantages and disadvantages.

Replication of genotype-phenotype relations can be a major issue in modern genomics, both when the effects of single candidate variants are examined\(^32–34\) as well as with genome-wide approaches.\(^35\) Pharmacogenetic studies may be especially difficult to replicate because large numbers of subjects with well-curated drug response phenotypes are often not available. Other challenges, notably for the use of GWAS, include choice of appropriate control groups matched for factors such as underlying and ancestry, contributions by DNA variants not captured by current platforms (e.g. rare variants or copy number variation), and analysis of gene-gene and gene-environment interactions in determining phenotype.
Variable drug actions and single gene variants

Large effect variants in drug-metabolizing enzymes

In the 1950s, McKusick and Price-Evans described variable N-acetylation,\(^3\) an important contributor to variable isoniazid hepatotoxicity and the lupus syndrome during treatment with procainamide and hydralazine. In the 1970s, two groups, studying different drugs (debrisoquine, an antihypertensive,\(^36\) and sparteine, being assessed as an antiarrhythmic\(^37,38\)), reported a set of 5–10% of subjects with adverse effects due to apparent absence of a key enzyme mediating drug bioactivation. The enzymes were initially termed debrisoquine 4-hydroxylase and sparteine N-oxidase, but subsequently it became clear that this was the same defect,\(^39\) now recognized to represent homozygosity for loss of function of a specific member of the cytochrome P450 (CYP) superfamily of drug metabolizing enzymes, CYP2D6.\(^40\) Dozens of variants have now been reported to reduce or eliminate CYP2D6 function (http://www.cypalleles.ki.se/cyp2d6.htm).

Coding region variants in other members of this superfamily, such as CYP2C9 and CYP2C19, generate populations of “poor metabolizers” for substrates of each of these enzymes. Interestingly, CYP3A4, the enzyme most commonly implicated in the metabolism of clinically-used drugs,\(^41\) does not include common coding region polymorphisms that alter function; nevertheless, CYP3A4 activity varies widely across individuals, and at least some of this variability likely arises from genetic variation in the regulation of CYP3A4 gene expression.\(^42\) Another contributor to variability in CYP3A activity is a common intronic single nucleotide polymorphism (SNP) in a closely-related gene, CYP3A5;\(^20,43\) the variant CYP3A5*3 allele alters mRNA by creating a new splice site.

The incidence of functionally-important CYP alleles can vary strikingly by ancestry. For example, poor metabolizers with absent CYP2D6 function are found in 5–10% of European and African populations, but are less common in Asian subjects. By contrast, CYP2C19 poor metabolizers are commoner in Asian subjects compared to the other two major ancestry groups, and the frequency of the CYP3A5*3 variant is much higher in Caucasians (0.85) compared to African Americans (0.55), which correlates with higher hepatic CYP3A5 expression in African American subjects.\(^43\)

High-risk pharmacokinetics

When drugs are eliminated by multiple pathways, absence of one of these (because of genetic variation or because of the presence of interacting inhibiting drugs) is unlikely to produce major variation in drug concentrations at the target site and thus in drug effect. However, the potential for highly variable drug concentrations increases dramatically when a drug is metabolized by a single pathway, a situation we have termed “high risk pharmacokinetics”.\(^44\) There are two scenarios in which this may occur (Figure 1). The first is the situation in which a prodrug must be metabolized, or bioactivated, to generate pharmacologic effects. In situations in which this bioactivation is accomplished by an enzyme with known loss-of-function variants, poor metabolizers will, predictably, display decreased drug action; clopidogrel and losartan are examples of cardiovascular drugs with this attribute (see Table 2), and codeine\(^59,60\) and tamoxifen\(^61,62\) are other prominent examples. Co-administration of commonly-used drugs that inhibit the bioactivating enzyme can result in a “phenocopy” of the poor metabolizer trait: that is, individuals who are genetically extensive metabolizers may display the same pharmacologic outcome as poor metabolizers if administered an interacting drug.

The second “high risk pharmacokinetic” scenario is seen when a substrate drug undergoes bioinactivation via a single metabolic pathway. In the absence of this pathway, much higher concentrations of active parent drug will accumulate. For compounds with a wide
therapeutic margin, such accumulation may be without clinical implications; conversely for other drugs such accumulation predictably results in serious toxicity. An example is the active S-enantiomer of warfarin which undergoes CYP2C9-mediated metabolism to inactive forms (Figure 2). As discussed further below, patients with common reduction-of-function alleles have higher S-warfarin concentrations and thus lower dose requirements to achieve steady state anticoagulation. However, there are rare patients with near-complete loss of CYP2C9 function (homozygotes for the *3 variant, arising from 1075A>C encoding I359L), and these patients may be very difficult to manage clinically because of low, and often unstable, warfarin dose requirements.

Large effect variants in other genes

Single variants in genes not involved in drug metabolism can also confer high risk for variable drug responses. These may involve variants in genes encoding the target molecules or pathways with which drugs interact, or those encoding genes unrelated to the therapeutic effect. In the latter group, one well-studied example is variants in the HLA system. Individuals with a single HLA B*5701 variant are at high risk for potentially fatal skin reactions during treatment with the antiretroviral drug abacavir, and similarly B*1502 (an allele seen primarily among Asians) has been linked to severe skin reactions during treatment with carbamazepine. As discussed further below, the V174A variant in SLCO1B1, that encodes a transport molecule responsible for uptake of simvastatin in liver, has been associated with a markedly increased risk for myopathy.

An example of a large effect of a variant in a drug target molecule is the reported association of the R389G variant in the beta1-adrenergic receptor gene with outcomes during treatment of heart failure with the adrenergic receptor blocker bucindolol. This variant is known to strongly modulate β1-mediated pharmacologic responses in vivo and in vitro, and outcomes in individuals with the G variant were very close to those treated with placebo. This finding suggests that pre-prescription genotyping could be used to target therapy to those predicted to derive benefit. However, the association is not replicated and other studies have implicated variants in other genes as potential contributors to outcomes of drug therapy in heart failure: examples are ACE, CYP2D6, GRK5, and alpha2C receptors. The relationship between warfarin dose and variants in VKORC1, encoding the warfarin target, are discussed below and other examples are listed in Table 2.

Combinatorial pharmacogenetics

Another approach to analyzing variability in complex traits like heart failure and its response to drugs is to study not single genetic variants, but combinations across multiple genes.

Two recent examples, warfarin and clopidogrel, illustrate how the interrogation of very large clinical datasets for candidate polymorphisms in multiple candidate genes in combination can help establish the role of these variants in determining a drug’s action. As discussed further below, the understanding that relatively large effects of single genetic variants modulate the effects of these drugs has prompted the US Food and Drug Administration to include genetic information in the labels for these and other agents, triggering a debate about how this new knowledge can be incorporated into practice.

An extension of this idea is interrogation of hundreds of SNPs in multiple genes in a candidate “pathway” to identify loci modulating a drug response. Drug-induced prolongation of the QT interval has also been analyzed in this fashion. Almost all drugs that prolong QT do so by blocking a specific cardiac potassium current, IKr. However, studies examining variation in the QT interval itself or its response to drug challenge have implicated multiple other ion channel and other genes. This supports a view in which control
of the QT interval relies on diverse mechanisms (almost all unrelated to \(I_{Kr}\)), and variation in these mechanisms then leads to variability in the extent to which \(I_{Kr}\) blockers prolong QT. This idea, termed “repolarization reserve,”\(^{77,78}\) is a specific example of the more general concept that variability in physiologic and drug response phenotypes reflects the interplay of multiple biologic pathways.

### The warfarin example (Figure 2)

In 2004, the disease gene for a very rare pharmacogenetic syndrome, warfarin resistance (in which patients displayed little change in INR upon challenge with extremely high doses of warfarin) was identified.\(^{79}\) The gene, \(VKORC1\), encodes the component of the vitamin K receptor complex that is the warfarin target, thus explaining the rare genetic trait. Identification of \(VKORC1\) as the warfarin target led rapidly to identification of common variant promoter haplotypes which correlated well with variable liver expression of the protein,\(^{24}\) and are associated with decreased steady state dose requirement and shorter time to therapeutic anticoagulation.\(^{24,25}\) One study in 539 Caucasian patients receiving steady state warfarin therapy reported that 25% of the variability in warfarin dose could be accounted for by common \(VKORC1\) promoter SNPs, and 9% by variants in \(CYP2C9\); these are very large genetic effects.\(^{24}\) Warfarin dose requirements vary strikingly by ancestry (highest in African-Americans, lowest in Asians), and much of the difference can be attributed to the frequency of common \(VKORC1\) promoter variants (Figure 2B).\(^{80}\) In addition, rare \(VKORC1\) coding region variants have been described in some subjects with unusually high warfarin dosages; for example, in one study, 4.3% of Ashkenazi subjects were found to have a variant resulting in D36Y, associated with high dose requirements (>10mg/day).\(^{81}\)

The International Warfarin Pharmacogenetics Consortium studied the relationship between genotypes and steady state warfarin dose in >5,000 patients of diverse ancestries.\(^{63}\) There was clear ancestry-dependent variation in dose requirement (highest in subjects of African descent, lowest in subjects of Asian descent), and the differences could be attributed to variation in \(VKORC1\) and \(CYP2C9\) (Figure 2B). As discussed below, trials are now underway to compare outcomes in patients using genetically- versus clinically-guided therapy.

### The clopidogrel example

Several studies in early 2009 reported that reduction-of-function variants in \(CYP2C19\) (the enzyme responsible for the bioactivation of clopidogrel) increase the risk of cardiovascular events after stent placement.\(^{16–18}\) One of these\(^{18}\) also examined the potential contribution of multiple other candidate genes to variable clopidogrel effects, including those encoding other CYPs, the P2Y12 receptor (the drug target), other molecules known to interact with the receptor, and the drug efflux transporter P-glycoprotein. The latter is encoded by \(ABCB1\), and P-glycoprotein is known to modulate absorption and elimination of many other drugs. The result of that study was that, in addition to the \(CYP2C19\) effect, individuals homozygous for a variant \(ABCB1\) coding region allele were more likely to display failure of efficacy during clopidogrel therapy. Although the role of \(CYP2C19\) is now described in the clopidogrel label, the way in which clinicians should respond to this information remains uncertain.\(^{82,83}\)

### Unbiased approaches to identifying genes modulating drug actions

The Human Genome Project and subsequent increasingly detailed maps of human genetic variation are providing tools to interrogate the relationship between genetic variation across the human genome and important human physiology and disease traits in a relatively...
unbiased fashion. A conventional GWAS design generates a set of SNPs associated with the trait under study, and then seeks to replicate these associations in other (often larger) clinical datasets. The contribution of variants identified by GWAS to susceptibility to common diseases is usually modest: it is likely that high risk alleles do not accumulate in populations because of the evolutionary disadvantage such accumulation confers.

Applying the GWAS paradigm to pharmacogenomics faces the obstacle that very large sets of patients with well-defined drug response phenotypes are unusual (Table 1). On the other hand, since there may be no selection pressure on genes encoding proteins mediating drug action, functionally-important variants with large effects may have accumulated in populations. Another distinctive feature of GWAS studies of drug response is the nature of the signals identified. GWAS approaches to disease have often identified new susceptibility loci. By contrast, many (but not all)\(^\text{84–86}\) GWAS studies in pharmacogenomics have yielded signals in previously-studied pathways, presumably reflecting large effects readily derived from candidate gene approaches.

One notable success using the GWAS approach in cardiovascular pharmacogenomics was a study that examined simvastatin-associated myopathy.\(^\text{28}\) Among 6033 patients receiving 20 mg/day, there were only 8 possible cases, so the study focused on 98/6031 patients who developed myotoxicity during high dose (80 mg/day) therapy. A GWAS comparing 85 cases of definite or incipient myopathy to 90 controls identified a single SNP (rs4363657) in \(SLCO1B1\) at genomewide significance. This SNP is in near perfect linkage disequilibrium with a previously-studied non-synonymous variant (V174A) in OATP1B1, the drug uptake transporter that \(SLCO1B1\) encodes; studies prior to the GWAS showed that V174A impairs elimination of simvastatin acid, and thus implicated it as a potential modulator of drug efficacy and toxicity.\(^\text{15}\) Patients with the rare (2.1%) homozygous phenotype had an 18% 5-year incidence of myopathy, compared to 3% among heterozygotes, and 0.6% among those lacking the risk allele. The findings were replicated in a separate study of subjects a lower dose, 40 mg/day, with a smaller effect size (relative risk 2.6 per C allele).\(^\text{28}\) To date, one group has reported a similar finding across multiple statin drugs in a smaller trial.\(^\text{87}\)

A recent GWAS examining predictors of therapeutic response to statin therapy in three randomized treatment trials has implicated multiple known lipid control loci, as well as identifying a novel association near calmin, a gene not previously known to influence lipid homeostasis.\(^\text{88}\) This analysis of thousands of patients exposed to atorvastatin, pravastatin or simvastatin used Bayesian statistical approaches to suggest that the relationship between genetic variability at the calmin locus and statin-related change in fasting lipid levels may represent a class effect.

**GWAS results to aid study design**

In 2003, the FDA proposed a prospective evaluation of the utility of \(CYP2C9\) genotyping as a guide to warfarin therapy. This effort was suspended with the recognition that \(VKORC1\) contributes importantly to variability in warfarin action. While interest in a prospective trial remained high, the new finding raised the question of how many other as-yet-unidentified genes might also contribute to warfarin actions. GWAS studies examining steady state warfarin dose demonstrated that \(VKORC1\) variants were the most important contributors to this phenotype, and while a number of other genes were implicated, none except \(CYP2C9\) (and in one study \(CYP4F2\)) survived replication.\(^\text{29,30}\) Thus, although variants in other genes may still contribute to variability in warfarin actions (\(CYP4F2,\text{89} CYP2C18,\text{90} EPHX1,\text{91} GGCX\text{92}\) these contributions seem to be small or affect only a small number of individuals and prospective trials are now ongoing in the US and Europe to examine the utility of genotyping to guide dosage of warfarin and other vitamin K antagonists. Similarly, a GWAS examining clopidogrel effects on ADP-mediated platelet aggregation ex vivo
demonstrated that variability at a single locus, encompassing CYP2C19, was the major contributor to the phenotype. Interestingly, ~70% of the variability in clopidogrel dose was found to be heritable, and this locus contributed ~15% to that variability. This is a very large effect of a single common polymorphism, that also highlights the “missing heritability” that may reflect gene-gene interactions or multiple rare genetic variants.

Model organisms and mechanisms

In addition to the cell-based approaches mentioned above (immortalized lymphoblastoid cell lines), unbiased studies in model organisms have also been used to identify contributions of loci across the genome to drug response phenotypes. Milan et al. demonstrated that QT prolonging drugs reproducibly produce bradycardia and atrioventricular block in zebrafish embryos. Challenging wild-type of mutagenized fish with dofetilide, a prototypical QT prolonging agent, identified multiple loci modulating this drug response phenotype. Interestingly, one of these, GINS3, also was implicated as a modulator of the normal QT duration in GWAS analyses of tens of thousands of subjects. An example of how unbiased discovery approaches can lead to new understanding new mechanisms is the finding that a SNP in the HMG-CoA reductase gene, initially identified as a modulator of response to inhibitor drugs, generates an alternatively spliced mRNA that encodes a protein with reduced drug sensitivity.

How will pharmacogenetic information be incorporated into the fabric of healthcare?

One simple approach to the problem of drugs with genetically-determined variable outcomes is to replace them with other drugs that lack these genetic features. In fact, because of delineation of the role of common variants in CYP2D6, candidate drugs whose elimination occurs largely via this enzyme are generally not developed. For available drugs that display genetically-determined variable metabolism, dose adjustment or use of alternate drugs seems a logical strategy to reduce that variability. For example, the clinical effects of the P2Y12 inhibitor prasugrel appear to be independent of CYP2C19, and so this drug may be especially desirable in patients who would ordinarily receive clopidogrel but have variant enzyme. Conversely, it may be cheaper and just as effective to use the older (off-patent) drug in patients lacking the clinically important genetic variants.

Another approach to adopting pharmacogenomic data to practice is to design clinical trials that do not simply examine the effect of genotypes on outcomes post hoc, but use genotypes as entry or stratification criteria. This idea has only occasionally been attempted to date in cardiovascular medicine, although it is rapidly becoming an important part of cancer therapeutics, where mutations in the tumor genome are increasingly identified as drivers of disease and of response to therapy. The evidence that genomic variants can clearly influence the outcome of drug therapy has led the Food and Drug Administration to include this information in drug labels. However, it is fair to say that application of this information to the bedside care of patients has been slow to be adopted. Multiple reasons can be proposed for this apparent paradox:

- It is burdensome for healthcare providers to incorporate genetic testing into prescribing. The results of genetic testing take at least hours (if not days or longer) to generate and once the result is obtained, the patient must be recontacted to inform them that the drug and dose selected are or are not appropriate. Further, consensus recommendations on dose adjustment may not be available, even when large effects of genetic variants on drug response have been identified.
• The level of evidence required to adopt a specific genetic test as a guide to practice is not established. The gold standard for evaluating therapeutic approaches in populations is the randomized clinical trial (RCT); however, given the number of variants implicated as modulators of drug action, it is not possible to mount an RCT to evaluate each one. Moreover, variants that are relatively uncommon across populations may still exert large and clinically important effects in individual subjects, and there is ample precedent for incorporating these into practice in the absence of RCTs: the clinical practice of decreasing dose of renally-excreted drugs in patients with elevated creatinine is one simple example.

• The numbers of variants and their potential effects during treatment with specific drugs is growing extraordinarily rapidly. It is not possible for individual practitioners to keep track of these without assistance from information technology systems. Medical school and post-graduate education is only now beginning to incorporate new genomics into curricula.

In the face of these obstacles, a positive and potentially enabling development is that the costs of genotyping at target loci or across large sections of the genome are plummeting: the cost for the first full human genome sequence, completed a decade ago, was ~$3,000,000,000, and is expected to fall to $1000 within a year (a cost that begins to compare favorably to that for individual genotyping tests). A number of companies are now offering direct to consumer wide-scale genotyping, and this includes some of the common CYP and other variants described above.

These developments set the stage for a potential paradigm shift in the use genomic, and in particular pharmacogenomic, information in clinical medicine. Currently (Figure 3; left), genetic information is used on an ad hoc and as needed basis: the prescriber must remember or be reminded to order a specific genetic test, and once the result is delivered, recontact the patient. Figure 3 (right) illustrates the new paradigm: genomic data is deposited preemptively into individual subjects’ electronic medical records (EMRs), to be accessed at the point of care, as needed. The incorporation of genetic information in this fashion into EMRs could also enable a future vision in which drug outcomes can be not only queried retrospectively but also followed prospectively, to generate new genotype-drug response relationships. Francis Collins enunciated this vision after being appointed NIH director, when he said: “The limiting factor right now is that often times, if you are ready to write a prescription, you don’t want to wait a week to find out the genotype before you decide whether you’ve got the right dose and the right drug. But if everybody’s DNA sequence is already in their medical record and it is simply a click of the mouse to find out all the information you need, then there is going to be a much lower barrier to beginning to incorporate that information into drug prescribing. If you have the evidence, it will be hard, I think, to say that this is not a good thing. And once you’ve got the sequence, it’s not going to be terribly expensive. And it should improve outcomes and reduce adverse events.”

The barriers to executing this idea are considerable. Whole genome sequencing is likely to be very inexpensive, but the ethical issues inherent in use of large scale sequencing in clinical practice are daunting, especially at the dawn of the era of individualized genomes, when multiple rare variants of unknown significance will be routinely discovered in each of us. One solution might be to acquire whole genome sequence data but to mask all but the “actionable” portions of the genome, such as high-impact pharmacogenetic variants. The sequence data will need to meet standards for use in clinical settings, and extensive annotation and curation will be required to ensure that traits like “poor metabolizer” are correctly inferred. As whole genomes become more widely available, methods to ensure that costs do not spiral in pursuit of incidental findings must be developed. It is clear that this vision of preemptive genotyping cannot be executed without electronic systems to manage
the data. Levels of evidence that justify acting on a particular genotype or set of genotypes and smart informatics systems to deliver prescribing advice in a simple but comprehensive format will need to be developed. New algorithms to mine outcomes in EMRs and to link these to embedded genomic markers would be highly desirable. The costs of preemptive genotyping and the extent to which costs associated with ineffective or dangerous drug therapy can be avoided will need to be assessed. Overcoming all these barriers will enable a new paradigm of practice that moves away from one size fits all medicine towards a more personalized approach to therapy.

Acknowledgments

Grant Support

Supported in part by grants from the US National Institutes of Health (U01 HL065962, U01 HL069757, R01 DK080007) and the Deutsche Forschungsgemeinschaft (SFB TR19)

References


Figure 1.
High-risk pharmacokinetics. Drugs that are eliminated by a single pathway can generate aberrant responses if that pathway is absent on a genetic basis, or because of co-administration of inhibiting drugs. This figure illustrates the two scenarios underlying such “high-risk” pharmacokinetic situations. One (left) is the administration of a drug that is itself not active but requires drug metabolism to generate an active metabolite; the absence of the pathway can lead to failure to generate the desired drug effect. This is thought to underlie variability in response to clopidogrel, tamoxifen, losartan, and codeine, as described in the text. The second scenario (right) is the administration of a drug eliminated by single pathway. Absence of this pathway will result in accumulation of the parent drug and thus drug-related toxicity. Adapted, by permission.44
Figure 2.
A framework for analyzing contributions of multiple genes to a clinical phenotype. The example of warfarin maintenance dose requirement is shown here. A. A simple pathway analysis of the key molecular determinants of warfarin response. The drug is administered as a racemate, and most anticoagulant action is mediated by the more potent S-enantiomer. S-warfarin is bioinactivated primarily by CYP2C9. The pharmacologic target for the drug is encoded by VKORC1, important for maintaining active Vitamin K. The role for other drug metabolizing pathways and for other enzymes that influence Vitamin K metabolism (EPHX1, GGCX) are shown in gray. B. Distribution of CYP2C9 and VKORC1 variants as a function of ancestry. For CYP2C9 (top panel), the *1 allele has the highest activity, *2 is a reduction of function variant, and *3 is a near loss of function variant. For the VKORC1 promoter variant shown, the G allele results in greater liver expression than does the A allele. These distributions of genotypes largely explain ancestry-dependent variability in warfarin dosing. C. Contribution of common and rare variants to warfarin dose requirements in a population. The normally-distributed dose requirements predominantly reflect the common variants shown in panel (B). However, individuals with rare VKORC1 coding region variants and individuals with the rare CYP2C9*3/*3 genotype may display unusually high or low dose requirements.
Figure 3.
Contrasting approaches to incorporating genomic information into prescribing. The pathway on the left illustrates current practice, genetic testing on an as needed basis. The pathway on the right illustrates how preemptive deposit of genotypic data into a genome-enabled electronic medical record would result rapid and efficient genotype-guided therapy.
## Table 1

Approaches to identifying and validating genetic influences on drug response

<table>
<thead>
<tr>
<th>Approach</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological candidates</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Candidate gene based on variable pharmacokinetics | Variability in these processes logically determine variable drug effects | Identification and replication of associations between variant genotypes and drug responses may require large populations, depending on the size of the genetic effect and the frequency of the variant allele | Warfarin/CYP2C9<sup>14</sup>  
Simvastatin/SLCO1B1<sup>15</sup>  
Clopidogrel/CYP2C19<sup>16-18</sup>  
Metoprolol/CYP2D6<sup>19</sup>  
Atorvastatin/CYP3A5<sup>20</sup> |
| Candidate gene based on variable pharmacodynamics | Candidate genes often identified                                             |                                                                                                     | Bucindolol/ADRB1<sup>21</sup>  
Beta-blockers in heart failure /ACE<sup>22</sup>  
Antiarrhythmics in atrial fibrillation/ACE<sup>23</sup>  
Warfarin/VKORC1<sup>24,25</sup> |
| Candidate pathway analysis                    | Possibly less biased than single gene approaches                             | Requires interrogation of large numbers of SNPs; replication may be difficult                        | HMG-CoA reductase haplotype as a predictor of statin response<sup>26</sup>                          |
| Unbiased approaches                           |                                                                           |                                                                                                     |                                                                                                    |
| Candidate gene selected from GWAS or other unbiased approach | GWAS result must be available  
Replication may be difficult                                                      | Unbiased                                                                                            | NOS1AP as a predictor of mortality during calcium channel blocker therapy<sup>27</sup>             |
| Genome-wide association study                  | Unbiased                                                                  | Sets of cases and controls generally need to be large; replication may be difficult                 | Simvastatin/SLCO1B1<sup>28</sup>  
Warfarin/VKORC1, CYP2C9, CYP4F2<sup>29,30</sup>  
Clopidogrel/CYP2C9/19 locus<sup>12</sup> |
| Drug response in model organisms with manipulated genetic background | Assay may be difficult to establish  
Translation from model organism to human may be imperfect                         |                                                                                                     | QT prolongation/GINS3 locus<sup>31</sup>                                                            |
### Table 2
Genetic variants influencing cardiovascular drug therapy: examples

<table>
<thead>
<tr>
<th>Gene</th>
<th>Drug</th>
<th>Clinical effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug metabolism</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Losartan</td>
<td>Decreased bioactivation and effects (PMs)танк</td>
</tr>
<tr>
<td></td>
<td>Warfarin</td>
<td>Decreased dose requirements; possible increased bleeding risk (PMs)танк</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Clopidogrel</td>
<td>Decreased bioactivation and effect in PMsтанк</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Metoprolol, carvedilol, timolol, propafenone</td>
<td>Increased beta-blockade in PMsтанк</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>Atorvastatin, simvastatin, lovastatin</td>
<td>Increased lipid lowering efficacyтанк, increased severity of myotoxicityтанк</td>
</tr>
<tr>
<td>NAT2</td>
<td>Hydralazine, procainamide</td>
<td>Increased risk of toxicity in PMsтанк</td>
</tr>
<tr>
<td><strong>Drug transport</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLCO1B1</td>
<td>Simvastatin</td>
<td>Variant non-synonymous single nucleotide polymorphism alters efficacy and increases myopathy riskтанк</td>
</tr>
<tr>
<td>ABCG2</td>
<td>Many statins</td>
<td>Altered pharmacokineticsтанк</td>
</tr>
<tr>
<td><strong>Drug targets</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMG-CoA reductase</td>
<td>Pravastatin</td>
<td>Haplotype-dependent LDL loweringтанк</td>
</tr>
<tr>
<td>VKORC1</td>
<td>Warfarin</td>
<td>Decreased dose requirements with variant promoter haplotypeтанк</td>
</tr>
<tr>
<td>ADRB1, ADRB2</td>
<td>Many beta-blockers</td>
<td>Altered vascular and heart rate effectsтанк</td>
</tr>
<tr>
<td>ACE</td>
<td>ACE inhibitors</td>
<td>No effect on drug responseтанк</td>
</tr>
<tr>
<td><strong>Other genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>Beta-blockers in heart failure Antiarrhythmics in atrial fibrillation</td>
<td>Decreased response in subjects with DD genotypeтанк</td>
</tr>
<tr>
<td>G-protein β3 subunit, kininogen, other loci</td>
<td>Thiazide diuretics</td>
<td>Greater reduction in diastolic and systolic blood pressureтанк</td>
</tr>
</tbody>
</table>

As discussed in the text, there is variability in the size of the genetic effects and in the extent to which these findings have been replicated.

Further data at the Pharmacogenetics Research Network/Knowledge base: http://www.pharmgkb.org