Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e >

Chapter 7: Pharmacogenetics

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INTRODUCTION

It is a given that patients vary in their responses to drug therapy. Some patients derive striking and sustained benefits from drug administration; others may display no benefit, and still others display mild, severe, or even fatal adverse drug reactions (ADRs). Common sources of such variability include noncompliance, medication errors, drug interactions (see Chapter 4 and Appendix I), and genetic factors. *Pharmacogenetics* is the study of the genetic basis for variation in drug response and often implies large effects of a small number of DNA variants. *Pharmacogenomics*, on the other hand, studies larger numbers of variants, in an individual or across a population, to explain the genetic component of variable drug responses. Discovering which variants or combinations of variants have functional consequences for drug effects, validating those discoveries, and ultimately applying them to patient care and to drug discovery are the tasks of modern pharmacogenetics and pharmacogenomics.

ABBREVIATIONS

Abbreviations

ABCB1: multidrug resistance transporter (P-glycoprotein)

ACE: angiotensin-converting enzyme

ADR: adverse drug reaction

AUC: area under the curve

CBS: cystathionine β-synthase

CF: cystic fibrosis

CNV: copy number variation

cSNP: coding SNP

CYP: cytochrome P450

EGFR: epidermal growth factor receptor

EMR: electronic medical record

FDA: U.S. Food and Drug Administration

FH: familial hypercholesterolemia

GI: gastrointestinal

G6PD: glucose-6-phosphate dehydrogenase

GST: glutathione-*S*-transferase

GSTM1: glutathione-*S*-transferase M1

GWAS: genome-wide association study

HIV: human immunodeficiency virus

HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A

5HT: 5-hydroxytryptamine, serotonin

indels: insertions or deletions

INR: international normalized ratio

iPSC: induced pluripotent stem cell

LDL: low-density lipoprotein

MAF: minor allele frequency

MDR1: multidrug resistance protein 1

mRNA: messenger RNA

MTHFR: methylenetetrahydrofolate reductase

nsSNP: nonsynonymous SNP

PharmGKB: Pharmacogenomics Knowledgebase

PheWAS: phenome-wide association study

PM: poor metabolizer

RCT: randomized clinical trial

SNP: single-nucleotide polymorphism

SNV: single-nucleotide variant

sSNP: synonymous or sense SNP

TPMT: thiopurine methyltransferase

TYMS: thymidylate synthase

UDP: uridine diphosphate

UGT: UDP-glucuronosyltransferase

UTR: untranslated region

VKORC1: vitamin K epoxide reductase

IMPORTANCE OF PHARMACOGENETICS TO VARIABILITY IN DRUG RESPONSE

An individual's response to a drug depends on the complex interplay among environmental factors (e.g., diet, age, infections, other drugs, exercise level, occupation, exposure to toxins, and tobacco and alcohol use) and genetic factors. Genetic variation may result in altered protein sequence and function or in altered protein levels through regulatory variation. Key genes involved in driving variable drug actions include those encoding drug-metabolizing enzymes, drug transport molecules, the molecular targets with which drugs interact, and a host of other genes that modulate the molecular context within which drugs act, notably genes dysregulated in the disease for which the drug is administered. In some situations, variation in nongermline genomes (e.g., in cancers or in infectious agents) can be critical determinants of variable drug responses.

Drug metabolism is highly heritable, as assessed using drug exposures in monozygotic versus fraternal twins, drug exposures in cell lines from related subjects, or analysis of very large data sets using technologies such as genome-wide genotyping,

discussed further in this chapter. Twin studies suggested that up to 75% of the variability in elimination half-lives for metabolized drugs can be heritable. Some drug metabolism traits behave in a conventional "monogenic" fashion with three clearly definable (and separable) groups of drug response phenotypes: heterozygotes as well as major and minor allele homozygotes. The study of these types of responses has helped define key genetic variants that contribute to the striking variability in responses described in this chapter. However, large effect size single variants are the exception, and for many (most) drug responses, the genetic component of variable responses—although substantial—likely reflects interacting influences of many genetic variants. A major challenge to the field is to accrue large numbers of subjects with well-phenotyped drug responses to enable discovery, and subsequent replication and validation, of multigene effects or of interactions of gene (s) with environmental factors.

PRINCIPLES OF PHARMACOGENETICS

Phenotype-Driven Terminology

A trait (e.g., the CYP2D6 "poor metabolizer" [PM], as opposed to "extensive metabolizer" [EM]) may be apparent only with nonfunctional alleles on both the maternal and the paternal chromosomes. If the gene is on a nonsex chromosome, the trait is autosomal. The nonfunctional alleles may be the same; the trait is then termed *autosomal recessive*, or different, in which case the subject is a *compound heterozygote*. A trait is deemed *codominant* if heterozygotes exhibit a phenotype that is intermediate to that of homozygotes for the common allele and homozygotes for the variant allele. Many polymorphic traits (e.g., CYP2C19 metabolism of drugs such as clopidogrel and omeprazole) are now recognized to exhibit some degree of codominance; as a result, heterozygotes exhibit metabolizing activity that is intermediate between that of EM and PM subjects.

In some instances, such as clopidogrel, codeine, and irinotecan (described further in this chapter), variants in a single gene produce clearly defined and clinically important differences in drug response.

However, these high effect size examples are the exception for two reasons. First, even within a single gene, a vast array of polymorphisms (promoter, coding, noncoding, completely inactivating, or modestly modifying) is possible. Each polymorphism may produce a different effect on gene function and therefore differentially affect a measured trait. Second, even if the designations of recessive, codominant, and dominant are informative for a given gene, their utility in describing the genetic variability that underlies variability in drug response phenotype is diminished because variability is often multigenic.

Types of Genetic Variants

The major types of sequence variation are single-nucleotide polymorphisms (SNPs, sometimes termed single-nucleotide variants, SNVs), and *insertions or deletions*, which can range in size from a single nucleotide to an entire chromosome; smaller ones are generally termed *indels*, and larger ones are designated CNVs. SNPs are much more common than indels or CNVs (Figure 7–1). The term *polymorphism* was formerly applied to variants occurring at a frequency greater than 1%. However, the application of genome sequencing to large numbers of subjects has made it clear that each individual has more than 10 million sites across their genome at which they differ from some reference sequence (i.e., ~ 1 variant per 1000 base pairs). While some of these are "common" (>1% frequency), the vast majority are much rarer. For rare variants clearly associated with a genetic disease, the term *mutation* may also be used, but distinguishing between a very rare variant and a mutation may be difficult. Publically available web-based databases (e.g., http://gnomad.broadinstitute.org) aggregate sequence data in tens of thousands of subjects and highlight that MAFs may vary strikingly across ancestries (discussed later), and that for the vast majority of variants is much less than 1%.

Figure 7–1

Molecular mechanisms of genetic polymorphisms. The most common genetic variants are SNP substitutions. Coding nonsynonymous SNPs result in a nucleotide substitution that changes the amino acid codon (here proline to glutamine), which could change protein structure, stability, or substrate affinities or introduce a stop codon. Coding synonymous SNPs do not change the amino acid codon but may have functional consequences (transcript stability, splicing). Noncoding SNPs may be in promoters, introns, or other regulatory regions that may affect transcription factor binding, enhancers, transcript stability, or splicing. The second major type of polymorphism is indels. SNP indels can have any of the same effects as SNP substitutions: short repeats in the promoter (which can affect transcript amount) or indels that add or subtract amino acids. CNVs involve large segments of genomic DNA that may involve gene duplications (stably transmitted inherited germline gene replication that causes increased protein expression and activity), gene deletions that result in the complete lack of protein production, or

inversions of genes that may disrupt gene function. All of these mechanisms have been implicated in common germline pharmacogenetic polymorphisms.

SNPs

Single-nucleotide polymorphisms

Coding, nonsynonymous
e.g., *TPMT*3A*

$$\text{Pro}$$
$$\overparen{CCG}$$
$$|\ \bullet\ |$$
$$\overparen{CAG}$$
$$\text{Gln}$$

Coding, synonymous
e.g., *ABCB1 C3435T*

$$\text{Pro}$$
$$\overparen{CCG}$$
$$|\ |\ \bullet$$
$$\overparen{CCA}$$
$$\text{Pro}$$

Noncoding (promoter, intronic)
e.g., *CYP3A5*3*

GAGCATTCT
| | • | | | | | |
GATCATTCT

Indels

Insertions/Deletions

e.g., 68 bp Insertion in *CBS*, $(TA)_7$ TAA
e.g., TA repeat in *UGT1A1* $(TA)_6$ TAA

CNVs

Copy number variations

Gene Duplications

e.g., *CYP2D6*, up to 13 copies

Large Deletions

e.g., entire *GSTT1* and *GSTM1*

The SNPs in the coding region are termed *cSNPs* and are further classified as *nonsynonymous* (changing the encoded amino acid sequence) or *synonymous* (or *sense*, with no amino acid change). A nucleotide substitution in an nsSNP that changes the amino acid codon (e.g., proline [CCG] to glutamine [CAG]) can as a result change protein structure, stability, or substrate affinities. There are 64 trinucleotide codons and only 20 amino acids, so multiple codons encode the same amino acid. Often, substitutions of the third base pair, termed the *wobble position*, in a codon with 3 base pairs, such as the G-to-A substitution in proline (CCG → CCA), do not alter the encoded amino acid. Up to about 10% of SNPs display more than two possible alleles (e.g., a C can be replaced by either an A or a G), so that the same polymorphic site can be associated with amino acid substitutions in some alleles but not others. As discussed in the material that follows, assessing the functional consequences of nsSNPs can be challenging. SNPs that introduce a premature stop codon, and small indels in a coding region that disrupt the open reading frame and thereby introduce abnormal 3′ protein sequences often with early stop codons, are termed *nonsense* variants, and these are thought to be most likely to display abnormal protein function.

Synonymous polymorphisms have been reported to contribute to a phenotypic trait. One example is a polymorphism in *ABCB1*, which encodes MDR1 (also termed P-glycoprotein), an efflux pump that interacts with many clinically used drugs. In *MDR1*, a synonymous polymorphism, C3435T, is associated with various phenotypes, and some evidence indicates that the one of the resulting mRNAs is translated at a slower rate, thereby altering folding of the protein, its insertion into the membrane, and thus its interaction with drugs (Kimchi-Sarfaty et al., 2007).

The vast majority (>97%–99%) of human DNA is noncoding, and the regulatory functions of noncoding sequences are only now being defined. Polymorphisms in noncoding regions may occur in the 3′ and 5′ untranslated regions, in promoter or enhancer

regions, in intronic regions, or in large regions between genes, intergenic regions (for nomenclature guide, see Figure 7–2). Noncoding SNPs in promoter or enhancer sequences are thought to alter DNA binding by regulatory proteins to affect transcription. 3′ SNPs may alter binding of microRNAs that affect transcript stability. Noncoding SNPs may also create alternative intron-exon splicing sites, and the altered transcript may have fewer or more exons, or shorter or longer exons, than the wild-type transcript. Large consortia are defining the functions of noncoding DNA: The ENCODE project identifies functional elements (enhancers, promoters, etc.) in genome sequences; and GTEx relates genome sequence variation to tissue-specific variability in gene expression (ENCODE Project Consortium, 2012; GTEx Consortium, 2015).

Figure 7–2

Nomenclature of genomic regions.
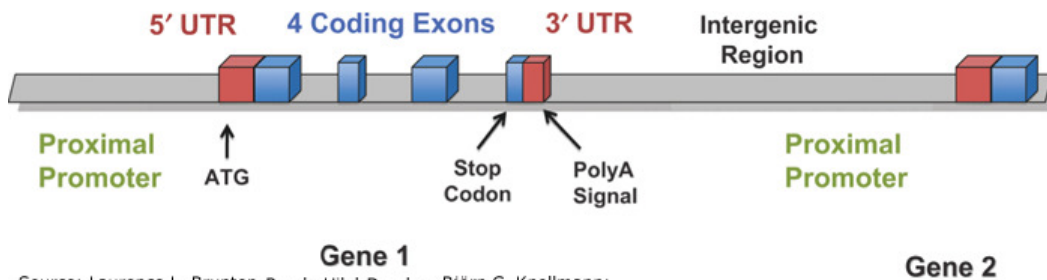
Source: Laurence L. Brunton, Randa Hilal-Dandan, Björn C. Knollmann:
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Like SNPs, indels can be short repeats in the promoter (which can affect transcript amount) or insertions/deletions that add or subtract amino acids in the coding region. The number of TA repeats in the *UGT1A1* promoter affects the quantitative expression of this important glucuronosyltransferase in liver; the most common allele has six repeats and the seven-repeat variant (*UGT1A1*28*) decreases *UGT1A1* expression. The frequency of the *28 allele is up to 30%, with up to 10% of subjects (depending on ancestry) being homozygous. Decreased *UGT1A1* transcription can modulate drug actions as described further in the chapter and also accounts for a common form of mild hyperbilirubinemia (Gilbert syndrome; see Table 6–3 and Figure 6–7).

The CNVs appear to occur in about 10% of the human genome and in one study accounted for about 18% of the detected genetic variation in expression of about 15,000 genes in lymphoblastoid cell lines (Stranger et al., 2007). The ultrarapid CYP2D6 metabolizer phenotype arises as a result of *CYP2D6* duplication(s), and individuals with more than 10 functional copies of the gene have been described. A common *GSTM1* polymorphism is caused by a large (50-kb) deletion, and the null allele has a population frequency of 30%–50%. Biochemical studies indicated that livers from homozygous null individuals have only about 50% of the glutathione-conjugating capacity of those with at least one copy of the *GSTM1* gene.

A *haplotype*—a series of alleles found at a linked locus on a chromosome—specifies the DNA sequence variation in a gene or a gene region on one chromosome. For example, consider two SNPs in *ABCB1*. One SNP is a T-to-A base-pair substitution at position 3421, and the other is a C-to-T change at position 3435. Possible haplotypes would be $T_{3421}C_{3435}$, $T_{3421}T_{3435}$, $A_{3421}C_{3435}$, and $A_{3421}T_{3435}$. For any gene, individuals will have two haplotypes, one maternal and one paternal in origin. A haplotype represents the constellation of variants that occur together for the gene on each chromosome. In some cases, this constellation of variants, rather than the individual variant or allele, may be functionally important. In others, however, a single variant may be functionally important regardless of other linked variants within the haplotype(s).

Linkage disequilibrium is the term used to describe the situation in which genotypes at the two loci are not independent of one another. With complete linkage disequilibrium, genotype at one site is a perfect predictor of genotype at the linked site. Patterns of linkage disequilibrium are population specific, and as recombination occurs, linkage disequilibrium between two alleles will decay and linkage equilibrium will result. Linkage disequilibrium has been enabling for genome-wide association studies because genotyping at a small number of SNPs ("tag SNPs") in linkage disequilibrium with many others can capture common variation across regions.

Ancestral Diversity

Polymorphisms differ in their frequencies within human populations and have been classified as either cosmopolitan or population (or race and ethnic) specific. *Cosmopolitan polymorphisms* are those polymorphisms present in all ethnic groups and are likely to be ancient, having arisen before migrations of humans from Africa, although present-day frequencies may differ among ancestral groups. The presence of *ancestry-specific polymorphisms* is consistent with geographical isolation of

human populations. These polymorphisms probably arose in isolated populations and then reached a certain frequency because they are either advantageous in some way (positive selection) or neutral to a population. Individuals descended from multiple ancestries may display haplotype structures and allele frequencies intermediate between their parents. In the U.S., African Americans have the highest number of population-specific polymorphisms (and the smallest haplotype blocks) in comparison to European Americans, Mexican Americans, and Asian Americans.

PHARMACOGENETIC STUDY DESIGN CONSIDERATIONS

There are many important considerations for the conduct of an experiment designed to identify sources of genetic variation contributing to variable drug responses. These include material to be studied (e.g., cells, organs, human subjects); the subjects' genetic backgrounds; the presence of confounders such as diet or variable experimental conditions; the selection of variants to be studied (ranging from a single high-likelihood candidate SNP to "agnostic" approaches that interrogate the whole genome); the methods used for genotyping and quality control; statistical analysis considerations, including effect size estimates and consideration of ancestry; and replication of findings.
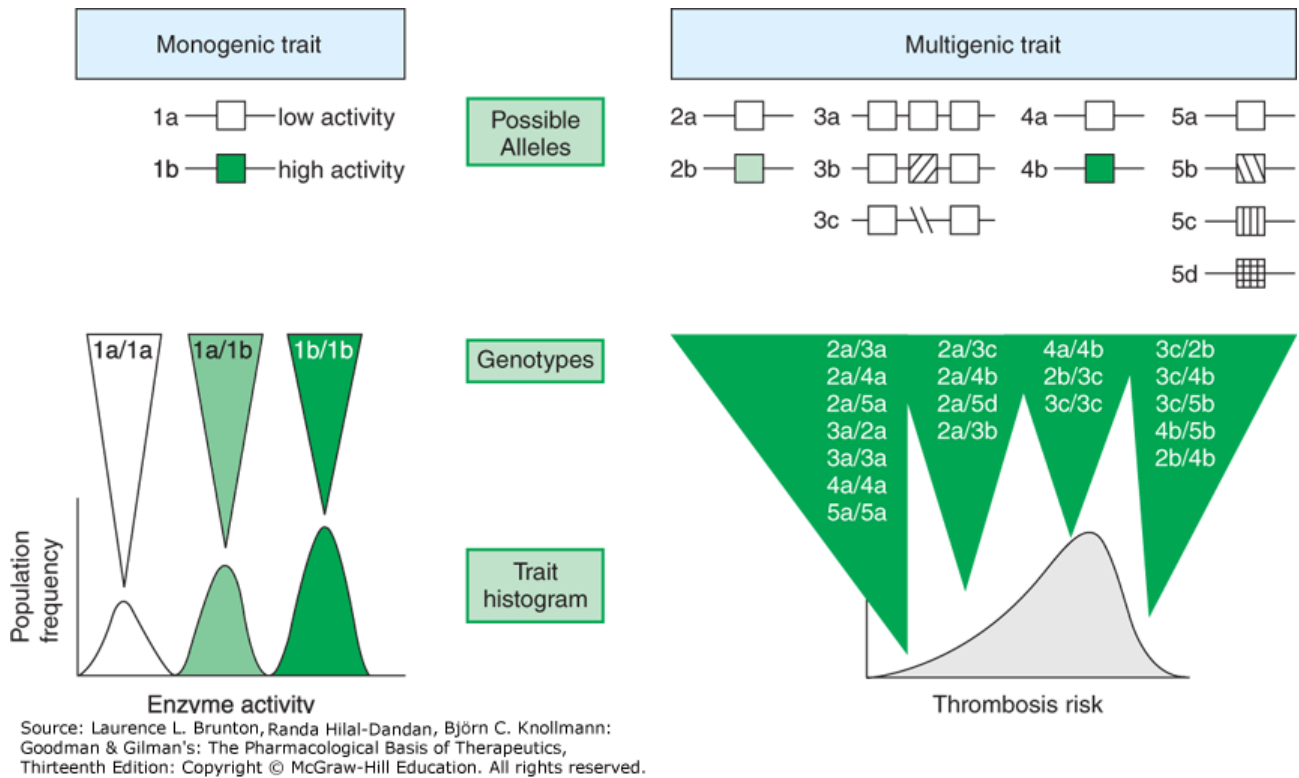
Pharmacogenetic Traits

A *pharmacogenetic trait* is any measurable or discernible trait associated with a drug. Some traits reflect the beneficial or adverse effect of a drug in a patient; lowering of blood pressure or reduction in tumor size are examples. These have the disadvantage that they reflect many genetic and nongenetic influences, but the advantage that they indicate a drug's clinical effects. Other traits represent drug response "endophenotypes," measures that may more directly reflect the action of a drug in a biologic system and thus be more amenable to genetic study but may be removed from the whole patient or a whole population. Examples of the latter include enzyme activity, drug or metabolite levels in plasma or urine, or drug-induced changes in gene expression patterns.

A variant drug metabolizer phenotype can be inferred from genotype data or in some cases directly measured by administering a "probe drug" (one thought to be metabolized by a single pathway) and measuring drug and metabolite concentrations. For example, one method to determine *CYP2D6* metabolizer status is to measure the urinary ratio of parent drug to metabolite after a single oral dose of the *CYP2D6* substrate dextromethorphan. Similarly, mephenytoin can be used as a probe drug for *CYP2C19* metabolizer phenotype. An important caveat is that other drugs can interfere with this assessment: If dextromethorphan is given with a potent inhibitor of CYP2D6, such as quinidine or fluoxetine, the phenotype may be consistent with or a "phenocopy of" the poor metabolizer genotype, even though the subject carries wild-type *CYP2D6* alleles. In this case, the assignment of a *CYP2D6* poor metabolizer phenotype would not be accurate. Another pharmacogenetic endophenotype, the erythromycin breath test (for CYP3A activity), can sometimes be unstable within a subject, indicating that the phenotype is highly influenced by nongenetic or multigenic factors. *Most pharmacogenetic traits are multigenic rather than monogenic* (Figure 7–3), and considerable effort is being made to identify the important polymorphisms that influence variability in drug response.

Figure 7–3

Monogenic versus multigenic pharmacogenetic traits. Possible alleles for a monogenic trait (*upper left*), in which a single gene has a low-activity (*1a*) and a high-activity (*1b*) allele. The population frequency distribution of a monogenic trait (*bottom left*), here depicted as enzyme activity, may exhibit a trimodal frequency distribution among low activity (homozygosity for *1a*), intermediate activity (heterozygote for *1a* and *1b*), and high activity (homozygosity for *1b*). This is contrasted with multigenic traits (e.g., an activity influenced by up to four different genes, genes 2 through 5), each of which has two, three, or four alleles (*a* through *d*). The population histogram for activity is unimodal skewed, with no distinct differences among the genotypic groups. Multiple combinations of alleles coding for low activity and high activity at several of the genes can translate into low-, medium-, and high-activity phenotypes.

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Genotyping

Most genotyping methods use DNA extracted from somatic, diploid cells, usually white blood cells or buccal cells. This "germline" DNA is extremely stable if appropriately extracted and stored, and the DNA sequence is generally (but likely not totally) invariant throughout an individual's lifetime. Any genotyping result should be subject to standard and rigorous quality control, which may include inspection of source genotyping experimental data, exclusion of SNPs with a high genotyping failure rate, exclusion of subjects in which many SNP analyses failed, assessment of Hardy-Weinberg equilibrium, and ensuring the absence of important substructure (e.g., many related individuals) in a general population study. *Hardy-Weinberg* equilibrium is maintained when mating within a population is random and there is no natural selection effect on the variant. Such assumptions are described mathematically when the proportions of the population that are observed to be homozygous for the variant genotype (q^2), homozygous for the wild-type genotype (p^2), and heterozygous ($2*p*q$) are not significantly different from that predicted from the overall allele frequencies (p = frequency of wild-type allele; q = frequency of variant allele) in the population. A deviation from Hardy-Weinberg equilibrium (i.e., from the rule that $p^2 + 2pq + q^2 = 1$) suggests a specific survival disadvantage for a particular genotype or a genotyping or other experimental error.

Candidate Gene Versus Genome-Wide Approaches

A candidate gene study uses what is known about a drug (e.g., its metabolism, transport, or mechanism of action) to test the hypothesis that variants in the underlying genes account for variable drug response phenotypes. Variants may be chosen because they are common, known (or thought) to be functional, or tag haplotype blocks. After assays are developed for a set of such variants, statistical methods are used to relate genotype to phenotype. There are several databases that contain information on polymorphisms in human genes (Table 7–1); these databases allow the investigator to search by gene for reported polymorphisms. Some of the databases, such as PharmGKB, include phenotypic as well as genotypic data.

Table 7–1

Databases Containing Information on Human Genetic Variation

DATABASE NAME	URL (AGENCY)	DESCRIPTION OF CONTENTS
Pharmacogenomics Knowledgebase (PharmGKB)	www.pharmgkb.org (National Institutes of Health –sponsored research network and knowledge database)	Genotype and phenotype data related to drug response
dbSNP	www.ncbi.nlm.nih.gov/projects/SNP (National Center for Biotechnology Information [NCBI])	SNPs and frequencies
GWAS Central	www.gwascentral.org	Genotype/phenotype associations
Genome Aggregation Database	www.gnomad.broadinstitute.org	Variants identified by sequencing >120,000 exomes and >15,000 whole genomes
Online Mendelian Inheritance in Man (OMIM)	www.ncbi.nlm.nih.gov/omim	Human genes and genetic disorders
University of California Santa Cruz (UCSC) Genome Browser	http://genome.ucsc.edu	Sequence of the human genome; variant alleles
GTEx	www.gtexportal.org/home/	Genetics of gene expression
Broad Institute Software	www.broadinstitute.org/data-software-and-tools	Software tools for the analysis of genetic studies

Large-Scale "Agnostic" Approaches

While the candidate gene approach has the intuitive appeal that known drug response pathways are studied, it has the drawback of looking only in regions of known biologic activity. Indeed, candidate genetic studies for susceptibility to common diseases have a remarkably high rate of failure to replicate, and this has been attributed to naïveté about the polygenic nature of most traits, small sizes with underpowering, and a "winner's curse" in which only positive results are published (Ioannidis et al., 2001). It has been argued that, unlike common disease studies, precedent has shown that drug responses may indeed reflect large effect sizes of a small number of genes, but these limitations should nevertheless be borne in mind in the conduct of these studies.

An alternate approach to the candidate gene approach is a GWAS, in which genotypes at more than 500,000 SNP sites (generally tagging haplotype blocks across the genome) are compared across a continuous trait or between cases and controls (e.g., those with or without a therapeutic response or an ADR). A GWAS requires large numbers of subjects, must consider the appropriate statistical approaches to minimize type I (false-positive) errors, and, if successful, identifies loci of interest that require further investigation to identify causative variants and the underlying biology. While associations identified by GWASs generally have modest effect sizes (odds ratios < 2), even with very low P values, pharmacogenetic GWASs provide some exceptions; for example, a GWAS in 51 cases of flucloxacillin-induced hepatotoxicity and 282 controls identified risk SNPs in the HLA-B locus with an odds ratio greater than 80 (Daly et al., 2009). Not all pharmacogenetic GWASs have successfully identified signals with this strength, but the approach has some promise and is increasingly used (Karnes et al., 2015; Mosley et al., 2015; Motsinger-Reif et al., 2013; Van Driest et al., 2015).

The GWAS analyses have also provided strong support for candidate gene studies that implicate variants in *CYP2C9* and *VKORC1* in warfarin dose requirement (Cooper et al., 2008; Takeuchi et al., 2009; see Figure 32–6 and Table 32–2) and variants in CYP2C19 in clopidogrel clinical response (Shuldiner et al., 2009). Newer genotyping platforms can capture both rare coding region variants and tags for common haplotype blocks, and the availability of increasing amounts of sequence data allows

reasonable inferences (by a statistical method called imputation) of up to 10 million genotypes from a GWAS genotyping experiment.

While single experimental approaches can suggest a relationship between variable drug responses and a variant in a specific locus or gene, the use of multiple complementary approaches provides the strongest evidence supporting such relationships. One method is to establish that putative variants do in fact display altered function in an in vitro system, as discussed in the material that follows. Another approach is to integrate genotype data (by GWAS) with other large-scale measures of gene function, such as the abundance of mRNAs (transcriptomics) or proteins (proteomics). This has the advantage that the abundance of signal may itself directly reflect some of the relevant genetic variation. One such study identified six loci at which exposure to simvastatin in cell lines changed gene expression, and variants in one of these genes, glycine amidinotransferase, was associated with simvastatin myotoxicity in a clinical trial (Mangravite et al., 2013). However, both mRNA and protein expression are highly influenced by choice of tissue type, which may not be available; for example, it may not be feasible to obtain biopsies of brain tissue for studies of CNS toxicity. The GTEx project described previously couples whole-genome sequence to mRNA transcript levels across multiple tissues and should enable further such studies.

Large-scale coupling of genotypes to phenotypes in EMR systems with associated DNA biobanks represents another potential resource for pharmacogenomic studies. One interesting approach using such biobanks is to turn the GWAS paradigm "on its head" and to ask with what human phenotype is a particular genetic variant associated. This PheWAS can be used to replicate a GWAS result or to identify entirely new associations (Denny et al., 2013) and has been used to "repurpose" (suggest new indications for) marketed drugs (Rastegar-Mojarad et al., 2015).

Functional Studies of Polymorphisms

Once a gene or a locus modulating a drug response phenotype is identified, a major challenge is to establish which coding or regulatory variants contribute. Comparative genomics and functional studies of individual polymorphisms in vitro and in animal models are commonly used approaches. Precedents from Mendelian diseases suggest that the variants with the greatest potential effect sizes are rare nonsense variants or missense variants that drastically alter evolutionarily conserved residues. For example, studies of variants in membrane transporters and ion channels suggested that those conferring with the greatest change in function are at low allele frequencies and change an evolutionarily conserved amino acid residue. These data indicate that SNPs that alter evolutionarily conserved residues are most deleterious. For example, substitution of a charged amino acid (Arg) for a nonpolar, uncharged amino acid (Cys) is more likely to affect function than substitution of residues that are more chemically similar (e.g., Arg to Lys). The data also suggest that rare nsSNPs are more likely to alter function than common ones.

The link between Mendelian disease and variant drug responses is highlighted by the fact that one of the first pharmacogenetic examples to be discovered was G6PD deficiency, an X-linked monogenic trait that results in severe hemolytic anemia in individuals after ingestion of fava beans or various drugs, including many antimalarial agents. G6PD is normally present in red blood cells and regulates levels of the antioxidant glutathione. Antimalarials such as primaquine increase red blood cell fragility in individuals with G6PD deficiency, leading to profound hemolytic anemia; the trait is more common in African Americans. The severity of the deficiency syndrome varies among individuals and is related to the amino acid variant in G6PD. The severe form of G6PD deficiency is associated with changes at residues that are highly conserved across evolutionary history. *The information in* Table 7–2 *on genetic polymorphisms influencing drug response at the end of the chapter can be used as a guide for prioritizing polymorphisms in candidate gene association studies.*

Table 7–2

Examples of Genetic Polymorphisms Influencing Drug Response

GENE PRODUCT (GENE)	DRUGS[a]	RESPONSES AFFECTED
Drug metabolism and transport		
CYP2C9	Tolbutamide, warfarin,[a] phenytoin, nonsteroidal anti-inflammatory	Anticoagulant effect of warfarin
CYP2C19	Mephenytoin, omeprazole, voriconazole,[a] hexobarbital, mephobarbital, propranolol, proguanil, phenytoin, clopidogrel	Peptic ulcer response to omeprazole; cardiovascular events after clopidogrel
CYP2D6	β blockers, antidepressants, antipsychotics, codeine, debrisoquine, atomoxetine,[a] dextromethorphan, encainide, flecainide, fluoxetine, guanoxan, N-propylajmaline, perhexiline, phenacetin, phenformin, propafenone, sparteine, tamoxifen	Tardive dyskinesia from antipsychotics, narcotic side effects, codeine efficacy, imipramine dose requirement, β-blocker effect; breast cancer recurrence after tamoxifen
CYP3A4/3A5/3A7	Macrolides, cyclosporine, tacrolimus, Ca^{2+} channel blockers, midazolam, terfenadine, lidocaine, dapsone, quinidine, triazolam, etoposide, teniposide, lovastatin, alfentanil, tamoxifen, steroids	Efficacy of immunosuppressive effects of tacrolimus
Dihydropyrimidine dehydrogenase	Fluorouracil, capecitabine[a]	5-Fluorouracil toxicity
N-acetyltransferase (NAT2)	Isoniazid, hydralazine, sulfonamides, amonafide, procainamide, dapsone, caffeine	Hypersensitivity to sulfonamides, amonafide toxicity, hydralazine-induced lupus, isoniazid neurotoxicity
Glutathione transferases (GSTM1, GSTT1, GSTP1)	Several anticancer agents	Decreased response in breast cancer, more toxicity and worse response in acute myelogenous leukemia
Thiopurine methyltransferase (TPMT)	Mercaptopurine,[a] thioguanine,[a] azathioprine[a]	Thiopurine toxicity and efficacy, risk of second cancers
UDP-glucuronosyl-transferase (UGT1A1)	Irinotecan,[a] bilirubin	Irinotecan toxicity
P-glycoprotein (ABCB1)	Natural product anticancer drugs, HIV protease inhibitors, digoxin	Decreased CD4 response in HIV-infected patients, decreased digoxin AUC, drug resistance in epilepsy
UGT2B7	Morphine	Morphine plasma levels
Organic anion transporter (SLC01B1)	Statins, methotrexate, ACE inhibitors	Statin plasma levels, myopathy; methotrexate plasma levels, mucositis
Catechol-O-methyltransferase	Levodopa	Enhanced drug effect

GENE PRODUCT (GENE)	DRUGS[a]	RESPONSES AFFECTED
Organic cation transporter (*SLC22A1*, *OCT1*)	Metformin	Pharmacologic effect and pharmacokinetics
Organic cation transporter (*SLC22A2*, *OCT2*)	Metformin	Renal clearance
Novel organic cation transporter (*SLC22A4*, *OCTN1*)	Gabapentin	Renal clearance
CYP2B6	Cyclophosphamide	Ovarian failure
Targets and receptors		
Angiotensin-converting enzyme (ACE)	ACE inhibitors (e.g., enalapril)	Renoprotective effects, hypotension, left ventricular mass reduction, cough
Thymidylate synthase	5-Fluorouracil	Colorectal cancer response
Chemokine receptor 5 (CCR5)	Antiretrovirals, interferon	Antiviral response
β_2 adrenergic receptor (*ADBR2*)	β_2-Antagonists (e.g., albuterol, terbutaline)	Bronchodilation, susceptibility to agonist-induced desensitization, cardiovascular effects (e.g., increased heart rate, cardiac index, peripheral vasodilation)
β_1 adrenergic receptor (*ADBR1*)	β_1-Antagonists	Blood pressure and heart rate after β_1 antagonists
5-Lipoxygenase (ALOX5)	Leukotriene receptor antagonists	Asthma response
Dopamine receptors (D_2, D_3, D_4)	Antipsychotics (e.g., haloperidol, clozapine, thioridazine, nemonapride)	Antipsychotic response (D_2, D_3 D_4), antipsychotic-induced tardive dyskinesia (D_3) and acute akathisia (D_3), hyperprolactinemia in females (D_2)
Estrogen receptor α	Estrogen hormone replacement therapy	High-density lipoprotein cholesterol
Serotonin transporter (5HTT)	Antidepressants (e.g., clomipramine, fluoxetine, paroxetine, fluvoxamine)	Clozapine effects, 5HT neurotransmission, antidepressant response
Serotonin receptor ($5HT_{2A}$)	Antipsychotics	Clozapine antipsychotic response, tardive dyskinesia, paroxetine antidepression response, drug discrimination
HMG-CoA reductase	Pravastatin	Reduction in serum cholesterol
Vitamin K oxidoreductase (VKORC1)	Warfarin[a]	Anticoagulant effect, bleeding risk
	Glucocorticoids	Bronchodilation, osteopenia

GENE PRODUCT (GENE)	DRUGS[a]	RESPONSES AFFECTED
Corticotropin-releasing hormone receptor (CRHR1)		
Ryanodine receptor (RYR1)	General anesthetics	Malignant hyperthermia
Modifiers		
Adducin	Diuretics	Myocardial infarction or strokes, blood pressure
Apolipoprotein E	Statins (e.g., simvastatin), tacrine	Lipid lowering; clinical improvement in Alzheimer disease
Human leukocyte antigen	Abacavir, carbamazepine, phenytoin	Hypersensitivity reactions
G6PD deficiency	Rasburicase,[a] dapsone[a]	Methemoglobinemia
Cholesteryl ester transfer protein	Statins (e.g., pravastatin)	Slowing atherosclerosis progression
Ion channels (*HERG, KvLQT1, Mink, MiRP1*)	Erythromycin, cisapride, clarithromycin, quinidine	Increased risk of drug-induced torsades de pointes, increased QT interval (Roden, 2003, 2004)
Methylguanine-methyltransferase	DNA methylating agents	Response of glioma to chemotherapy
Parkin	Levodopa	Parkinson disease response
MTHFR	Methotrexate	GI toxicity (Ulrich et al., 2001)
Prothrombin, factor V	Oral contraceptives	Venous thrombosis risk
Stromelysin-1	Statins (e.g., pravastatin)	Reduction in cardiovascular events and in repeat angioplasty
Inosine triphosphatase	Azathioprine, mercaptopurine	Myelosuppression
Vitamin D receptor	Estrogen	Bone mineral density

[a]
Information on genetics-based dosing, adverse events, or testing added to FDA-approved drug label (Grossman, 2007).

With increasing application of exome or whole-genome sequencing in populations, millions of DNA variants are being identified, and methods to establish their function are evolving. One approach uses computational algorithms to identify potentially deleterious amino acid substitutions. Earlier methods (e.g., BLOSUM62, SIFT, and PolyPhen) use sequence comparisons across multiple species to identify and score substitutions, especially at highly conserved residues. More recent approaches use structural predictions (Kircher et al., 2014) or integrate multiple predictors (e.g., CADD). While these programs are becoming increasingly sophisticated, they have not yet reached the point that they can substitute for experimental verification.

The functional activity of amino acid variants for many proteins can be studied in isolation, in cellular assays, or in animal models. A traditional step in a cellular study of a nonsynonymous variant is to isolate the variant gene or to construct the variant by site-directed mutagenesis, express it in cells, and compare its functional activity (enzymatic activity, transport kinetics, ion channel gating, etc.) to that of the reference or most common form of the protein (Figure 7–4). Figure 7–5 shows an

example of how the combination of population studies, in vitro functional assays, and in silico simulations can be integrated to identify a variant that modulates the risk of drug-induced arrhythmias.

Figure 7–4

Simulated concentration-dependence curves for the common genetic form of an enzyme and two nonsynonymous variants. Compared to the common form of the enzyme, variant A exhibits an increased K_m, likely reflecting an altered substrate-binding site of the protein by the substituted amino acid. Variant B exhibits the same K_m as the common form but a reduced maximum rate of metabolism of the substrate (V_{max}). Because these measurements were made on cell extracts, the reduced V_{max} may be due to a reduced expression level of the enzyme. If similar data were obtained with purified protein, then the reduced activity of variant B could be ascribed to a structural alteration in the enzyme that affects its maximal catalytic rate but not its affinity for the substrate under these assay conditions.
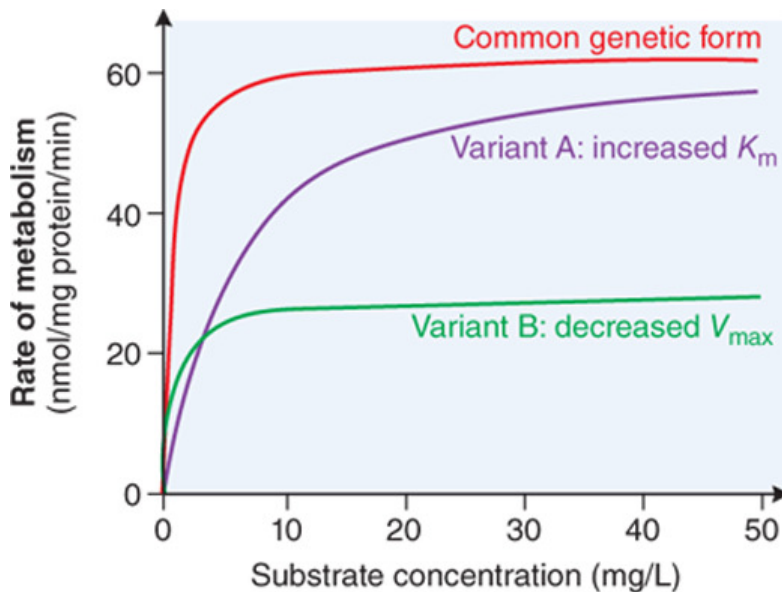
Figure 7–5

Functional evaluation of an ion channel variant. A population study implicated an nsSNP resulting in D85N in *KCNE1* as a modulator of the risk for arrhythmias when blockers of the KCNH2 K^+ channel are administered to patients (Kääb et al., 2012).

KCNE1 encodes a function-modifying subunit for a different cardiac K^+ channel (encoded by *KCNQ1*), and the ion currents generated at a range of voltages by heterologous coexpression of *KCNQ1* plus the wild-type or mutant *KCNE1* are shown in **A** and **B**, respectively. While there are subtle differences in activation kinetics and overall current amplitude, it is not clear whether these are functionally important. **C.** Results of numerical action potential simulations incorporating either the experimentally determined wild-type or variant K^+ current. At baseline (black and green tracings), there is no difference in computed action potential duration. However, when drug block of the KCNH2 K^+ channel is superimposed and the stimulation rate is slowed (orange tracings), an arrhythmogenic afterpotential (*arrow*) is seen with the mutant but not the wild-type *KCNE1*. Taken together, these functional data therefore provide support for the population study. (Data from Drs. Al George and Yoram Rudy.)
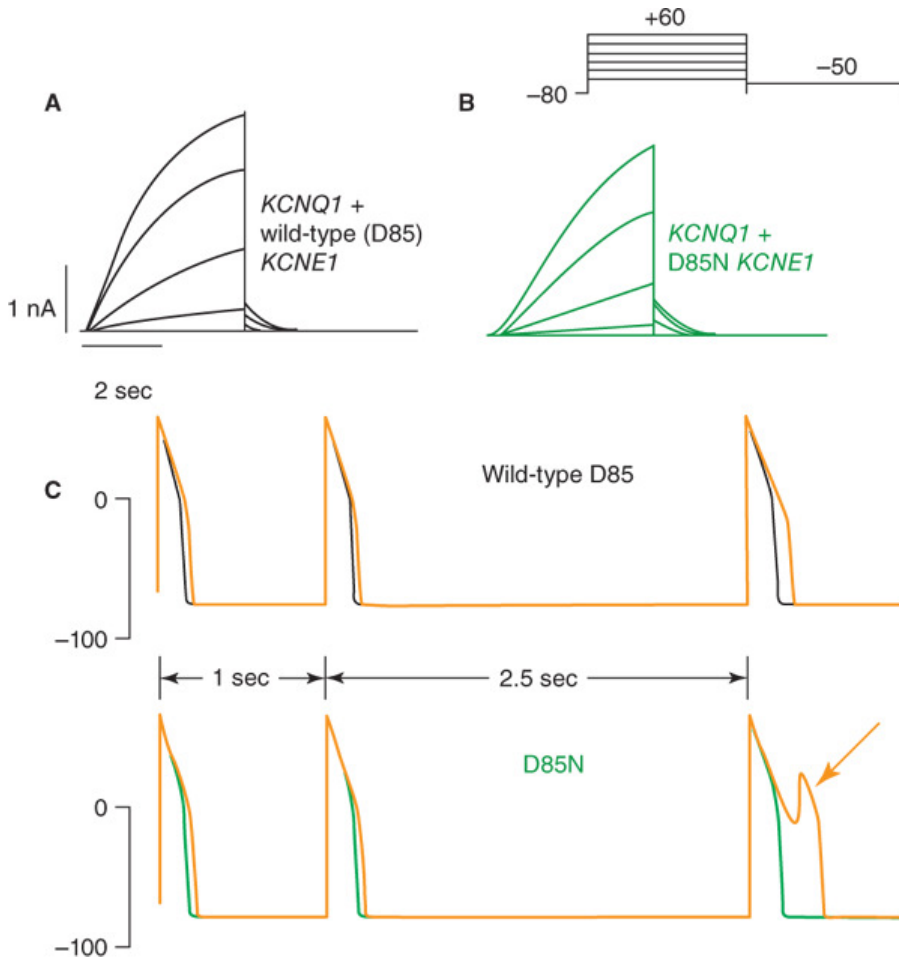
The SNPs identified in GWASs as associated with clinical phenotypes, including drug response phenotypes, have largely been in noncoding regions. An example of profound functional effect of a noncoding SNP is provided by *CYP3A5*; a common noncoding intronic SNP in *CYP3A5* accounts for its polymorphic expression in humans. The SNP accounting for variation in CYP3A5 protein creates an alternative splice site, resulting in not only a transcript with a larger exon 3 but also the introduction of an early stop codon (Figure 7–6). The nonfunctional allele is more common in subjects of European ancestry compared to those of African ancestry; as a result, CYP3A5 activity is lower in individuals expressing the noncoding intronic SNP (i.e., for a given dose of a drug that is a substrate of CYP3A5, concentrations of the drug will be higher in Europeans). Increased rates of transplant rejection in subjects of African descent may reflect decreased plasma concentrations of the antirejection drug tacrolimus, a substrate for CYP3A5 (the higher activity form lacking the noncoding intronic SNP) (Birdwell et al., 2012).

Figure 7–6

An intronic SNP can affect splicing and account for polymorphic expression of CYP3A5. A common polymorphism (A > G) in intron 3 of CYP3A5 defines the genotypes associated with the wild-type CYP3A5*1 allele or the variant nonfunctional CYP3A5*3 allele. This intronic SNP creates an alternative splice site that results in the production of an alternative CYP3A5 transcript carrying an additional intron 3B (**B**), with an early stop codon and truncated CYP3A5 protein. The wild-type gene (more common in African than Caucasian or Asian populations) results in production of active CYP3A5 protein (**A**); the *3 variant results in a truncated and inactive protein. Thus, metabolism of CYP3A5 substrates is diminished in vitro (**C**), and blood concentrations of such substrates (medications) are higher in vivo (**D**) for those with the *3 than the *1 allele. (Data from Haufroid et al., 2004; Kuehl et al., 2001; Lin et al., 2002.)
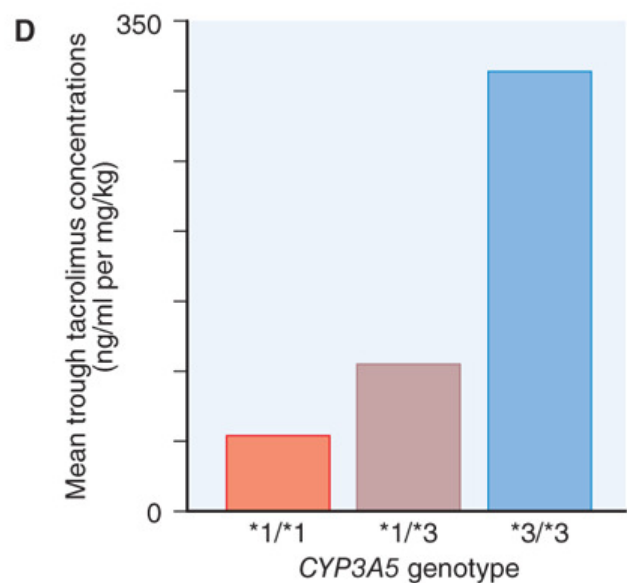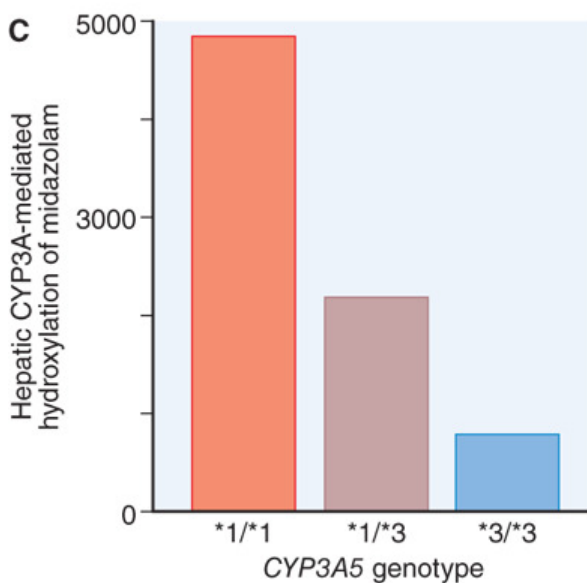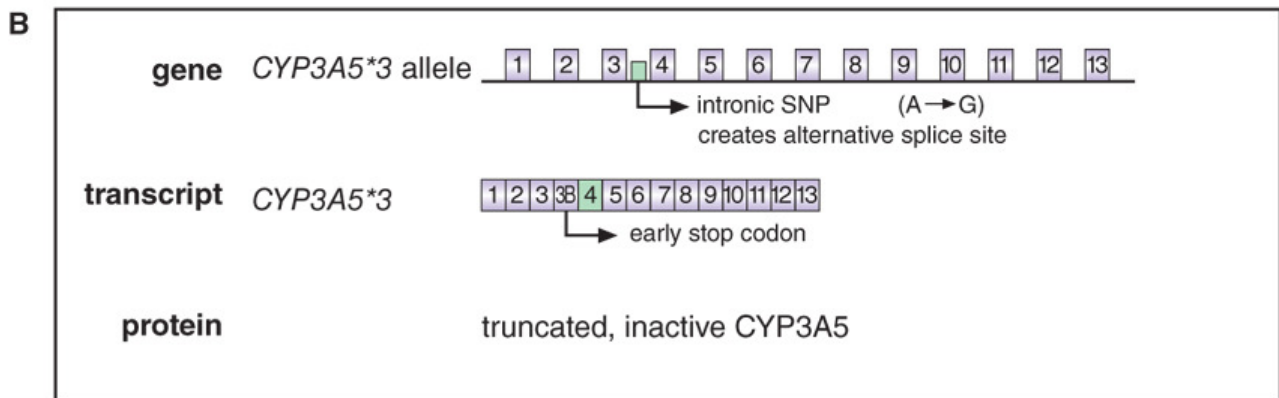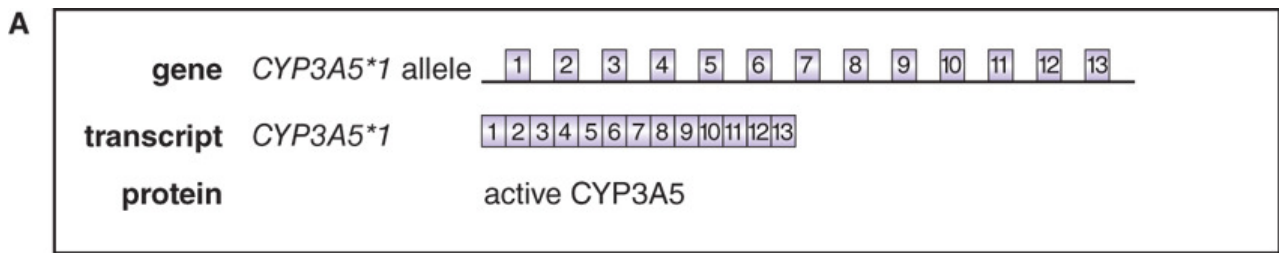
Two new technologies appear poised to revolutionize functional studies. The first is the ability to generate iPSCs from any individual and then use the cells to generate specific cell types (hepatocytes, cardiomyocytes, neurons, etc.), thereby enabling studies of that individual's cellular physiology. The second is rapid and efficient genome editing using CRISPR/cas9 in iPSCs or any other cell system (see Chapter 3). Multiple exciting applications of genome-editing technology, from rapid generation of genetically modified animals to curing genetic disease in humans, are being explored. Genome editing holds the promise that the function of individual coding or noncoding variants, alone or in combination, can be rapidly assessed in cellular systems.

Pharmacogenetic Phenotypes

Candidate genes for therapeutic and adverse response can be divided into three categories:

those modifying drug disposition (*pharmacokinetic*)

those altering the function of the molecules with which drugs interact to produce their beneficial or adverse effects (*receptor/target*)

those altering the broad *biologic milieu* in which the drugs interact with target molecules, including the changes associated with the diseases for which the drug is being prescribed

This section summarizes important examples of each type but cannot be all inclusive. Web-based resources such as PharmGKB (Table 7–1) can be consulted for specific genes, variants, drugs, and diseases.

Pharmacokinetic Alterations

Germline variability in genes that encode determinants of the pharmacokinetics of a drug, in particular metabolizing enzymes and transporters, affect drug concentrations and are therefore major determinants of therapeutic and adverse drug response (at the end of the chapter, see Table 7–2 on genetic polymorphisms influencing drug response). A particularly high-risk situation is a drug with a narrow therapeutic margin eliminated by a single pathway: Loss of function in that pathway can lead to drastic increases in drug concentrations (and decreases in metabolite concentrations) with attendant loss of efficacy and an increased likelihood of ADRs (Roden and Stein, 2009). The loss of function can be genetic or can arise as a result of drug interactions or dysfunction of excretory organs (e.g., renal failure will elevate plasma concentrations of renally excreted drugs unless dosages are reduced).

CYP2C9-mediated metabolism of the more active *S*-enantiomer of warfarin is an example. Individuals with the loss of function *3 allele require lower steady-state warfarin dosages and are at increased risk of bleeding (Aithal et al., 1999; Kawai et al., 2014; see also Table 32–2). When multiple enzymes and transporters are involved in the pharmacokinetics of a drug, single variants are unlikely to produce large clinical effects.

Another high-risk situation is a drug that requires bioactivation to achieve pharmacological effect. Individuals with increased or decreased bioactivation, because of genetic variants or drug interactions, are at risk for variant drug responses. Clopidogrel, bioactivated by CYP2C19, and tamoxifen, bioactivated by CYP2D6, are examples (see Table 7–2 and Figure 6–3A). PM subjects homozygous for a common loss function variant in *CYP2C19* display decreased antiplatelet effects and increased stent thrombosis during clopidogrel treatment (Mega et al., 2010; Shuldiner et al., 2009). In heterozygotes (~20%) receiving clopidogrel, adequate antiplatelet effects can be achieved by increasing the dose, whereas in homozygotes (2%–3%) an alternate antiplatelet drug should be used because even large dose increases do not affect platelet function. Other loss-of-function variants (notably *3) are common in Chinese and Japanese populations. Several proton pump inhibitors, including omeprazole and lansoprazole, are inactivated by CYP2C19. Thus, PM patients have higher exposure to active parent drug, a greater pharmacodynamic effect (higher gastric pH), and a higher probability of ulcer cure than heterozygotes or homozygous wild-type individuals.

A variation on this theme is the use of codeine (a prodrug bioactivated to morphine by CYP2D6). In PMs, analgesia is absent. Perhaps more important, excess morphine is generated in ultrarapid metabolizers, and death due to respiratory arrest has been reported (Ciszkowski et al., 2009). A large number of medications (estimated at 15%–25% of all medicines in use) are substrates for CYP2D6.

The *UGT1A1 *28* variant, encoding the 7-TA reduced function *UGT1A1* promoter mentioned previously, has been associated with higher levels of the active metabolite SN-38 of the cancer chemotherapeutic agent *irinotecan* (see Chapter 66), and this increased concentration has been associated with an increased risk of serious toxicities (see Figures 6–6, 6–8, and 6–9).

Drug Receptor/Target Alterations

Warfarin exerts its anticoagulant effect by interfering with the synthesis of vitamin K–dependent clotting factors, and the target molecule with which warfarin interacts to exert this effect is encoded by *VKORC1*, an enzyme in the vitamin K cycle (Figure 7–7). Rare coding region variants in the gene lead to partial or complete warfarin resistance; interestingly, these variants are common (5% allele frequency) in Ashkenazi patients and may account for high dosage requirements in carrier subjects. The *VKORC1* promoter includes common variants that strongly modulate its expression; in subjects with reduced expression, lower steady-state warfarin doses are required. These variants are more common in Asian subjects than in Caucasians or Africans. Inherited variation in *CYP2C9* and *VKORC1* account for more than 50% of the variability in warfarin doses needed to achieve the desired coagulation level. *VKORC1* is one example of how both rare and common variants in genes encoding drug targets can exert important effects on drug actions.

Figure 7–7

Pharmacogenetics of warfarin dosing. Warfarin is metabolized by *CYP2C9* to inactive metabolites and exerts its anticoagulant effect partly via inhibition of *VKORC1*, an enzyme necessary for reduction of inactive to active vitamin K. Common polymorphisms in both genes, *CYP2C9* and *VKORC1*, have an effect on warfarin pharmacokinetics and pharmacodynamics, respectively, to affect the population mean therapeutic doses of warfarin necessary to maintain the desired degree of anticoagulation (often measured by the INR blood test) and minimize the risk of too little anticoagulation (thrombosis) or too much anticoagulation (bleeding). See also Figure 32–6 and Table 32–2. (Data from Caraco et al., 2008; Schwarz et al., 2008; Wen et al., 2008.)
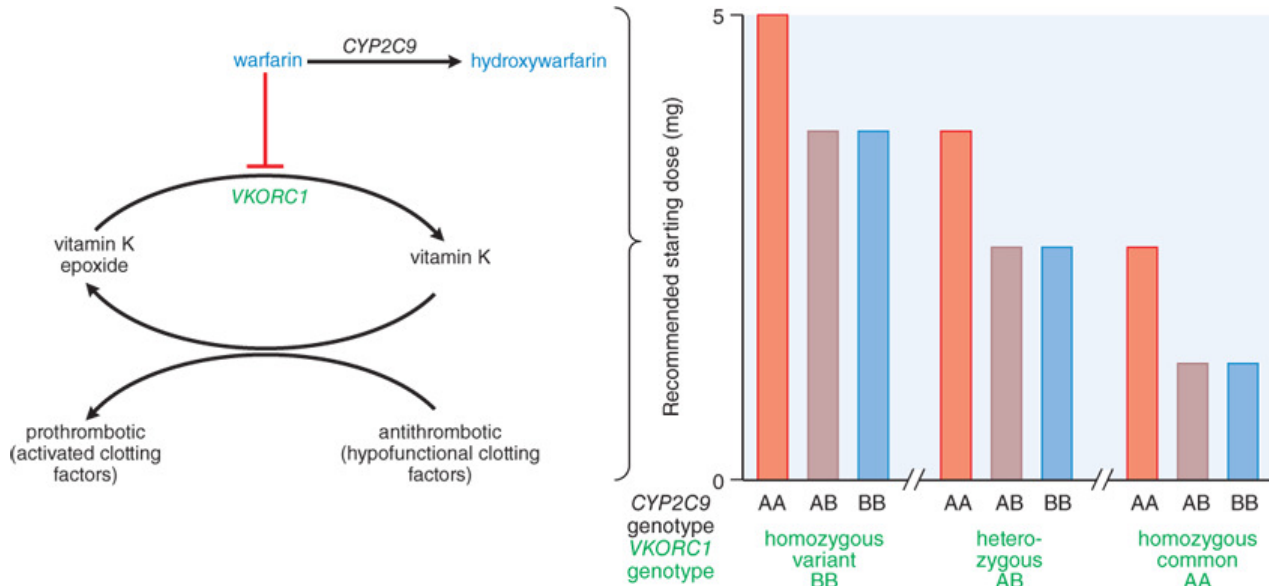
In some instances, highly penetrant variants with profound functional consequences may cause disease phenotypes that confer negative selective pressure; more subtle variations in the same genes can be maintained in the population without causing disease but nonetheless causing variation in drug response. For example, rare loss-of-function mutations in MTHFR cause severe mental retardation, cardiovascular disease, and a shortened life span. Conversely, the 677C→T SNP causes an amino acid substitution that is maintained in the population at a high frequency (40% allele frequency in most white populations) and is associated with modestly lower MTHFR activity (~30% less than the 677C allele) and modest but significantly elevated plasma homocysteine concentrations (~25% higher). This polymorphism does not alter drug pharmacokinetics but does appear to modulate pharmacodynamics by predisposing to GI toxicity to the antifolate drug methotrexate in stem cell transplant recipients.

Like warfarin, methotrexate's clinical effects are dependent on a number of polymorphisms affecting metabolism, transport, drug modifiers, and drug targets. Several of the direct targets (dihydrofolate reductase, purine transformylases, and TYMS) are also subject to common polymorphisms. A polymorphic indel in *TYMS* (two vs. three repeats of a 28–base pair sequence in the enhancer) affects the amount of enzyme expression in both normal and tumor cells. The *TYMS* polymorphism can affect both toxicity and efficacy of anticancer agents (e.g., fluorouracil and methotrexate) that target TYMS. Thus, the genetic contribution to variability in the pharmacokinetics and pharmacodynamics of methotrexate cannot be understood without assessing genotypes at a number of different loci.

Other examples of drug target variants affecting drug response are presented in Table 7–2 at the end of the chapter. Serotonin receptor polymorphisms have been implicated as predictors of responsiveness to antidepressants and of the overall risk of depression. β adrenergic receptor polymorphisms have been linked to asthma responsiveness, changes in renal function following ACE inhibitors, sinus heart rate following β blockers, and the incidence of atrial fibrillation during β blocker therapy. The degree of lowering of LDL by statins has been linked to polymorphisms in HMG-CoA reductase, the statin target (see Chapter 31). Ion channel polymorphisms have been linked by both candidate gene and exome sequencing approaches to a risk of cardiac arrhythmias in the presence and absence of drug triggers (Kääb et al., 2012; Weeke et al., 2014).

Modifiers of the Biologic Milieu

The *MTHFR* polymorphism is linked to homocysteinemia, which in turn affects thrombosis risk. The risk of drug-induced thrombosis is dependent not only on the use of prothrombotic drugs but also on environmental and genetic predisposition to thrombosis, which may be affected by germline polymorphisms in *MTHFR*, factor V, and prothrombin. These polymorphisms do not directly act on the pharmacokinetics or pharmacodynamics of prothrombotic drugs such as glucocorticoids, estrogens, and asparaginase but may modify the risk of the phenotypic event (thrombosis) in the presence of the drug. Likewise, polymorphisms in ion channels (e.g., *KCNQ1, KCNE1, KCNE2*) that are not themselves the targets of drugs that prolong QT intervals may affect the duration of the baseline QT interval and the overall risk of cardiac arrhythmias; this may in turn increase risk of long QT arrhythmias seen with antiarrhythmics and a number of other "noncardiovascular" drugs (e.g., macrolide antibiotics, antihistamines).

Cancer as a Special Case

Cancer appears to be a disease of genomic instability. In addition to the underlying variation in the germline of the host, tumor cells exhibit somatically acquired mutations, some of which generate mutant protein kinases that are drivers for the development of cancer. Thus, tumor sequencing is becoming standard of care for choosing among anticancer drugs in certain settings (see Chapters 65–68).

For example, patients with lung cancer with activating mutations in *EGFR*, encoding the epidermal growth factor receptor, display increased responses to the EGFR inhibitor gefitinib (Maemondo et al., 2010). Thus, the EGFR is altered, and patients with the activating mutation have, in treatment terms, a distinct pharmacogenetic category of lung cancer. The Her2 antibody trastuzumab can produce cardiomyopathy in all exposed patients. Patients with breast cancer whose tumors express the Her2 antigen may benefit from trastuzumab, whereas those whose tumors do not express Her2 do not benefit but are nevertheless susceptible to cardiomyopathy. Similarly, only patients with melanoma whose tumors express the mutant BRAF V600E respond to vemurafinib; interestingly, vemurafinib may also be effective in other tumors (thyroid cancer, hairy cell leukemia) that express BRAF V600E. Some genetic alterations affect both tumor and host: The presence of two instead of three copies of a *TYMS* enhancer repeat polymorphism not only increases the risk of host toxicity but also increases the chance of tumor susceptibility to TYMS inhibitors (Evans and McLeod, 2003).

Genomics as a Pathway to Identification of New Drug Targets

The identification of genetic pathways in normal physiology and in disease can provide important clues to new drug targets. Seminal studies of patients with the rare disease FH identified HMG-CoA reductase as the key rate-limiting enzyme in LDL cholesterol biosynthesis; now, inhibitors of that enzyme (the statins) are among the most effective and widely used medications in cardiovascular therapy (see Chapter 33). PCSK9 contributes to the degradation of LDL receptors, which are responsible for removing LDL cholesterol from the circulation; an increase in PCSK9 activity results in reduction of LDL receptor function and an increase in LDL cholesterol. One rare cause of FH is gain-of-function mutations in *PCSK9.* Conversely, work in the Dallas Heart Study showed that individuals carrying nonsense mutations in *PCSK9* had lower LDL cholesterol values and decreased risk for coronary artery disease compared to noncarriers (Cohen et al., 2006). This result, in turn, identified PCSK9 as a potential drug target. In 2015, two antibodies that target PCSK9, alirocumab and evolocumab, were approved by the FDA for clinical use in FH and other lipid disorders. These PCSK9 inhibitors prevent degradation of LDL receptors and enhance their recycling to the hepatocyte membrane, thereby facilitating removal of LDL cholesterol and lowering blood LDL cholesterol levels (see Figure 33–4).

In a similar fashion, new drug targets have been identified by work showing that rare loss-of-function variants in *APOC3* lower triglycerides and reduce the risk of coronary artery disease (Stitziel et al., 2014), and loss-of-function variants in *SLC30A8* reduce risk for type 2 diabetes (Flannick et al., 2014). Patients homozygous for *SCN9A* loss-of-function variants are pain insensitive (Cox et al., 2006); inhibitors of SCN9A might be useful analgesics. Hundreds of mutations in the chloride transporter encoded by CFTR cause CF, but through diverse mechanisms. Ivacaftor partially corrects abnormal gating of certain rare variants of CFTR (G551D and others), while lumacaftor improves cell surface expression of the most common variant, ΔF508. Ivacaftor (Ramsey et al., 2011) and the ivacaftor/lumacaftor combination (Wainwright et al., 2015) improve symptoms and outcomes in patients with CF; both agents have now been approved in genotyped patients.

PHARMACOGENETICS IN CLINICAL PRACTICE

The increasing understanding of genetic contributors to variable drug actions raises questions of how these data might be used by healthcare providers to choose among drugs, doses, and dosing regimens. One approach is point-of-care testing, in which genotyping is ordered at the time of drug prescription; platforms that reliably deliver relevant genotypes rapidly (often in less than an hour) now make such approaches feasible. However, one difficulty in this approach is that each drug requires a separate assay. An alternate approach envisions genotyping at multiple loci relevant for responses to large numbers of drugs, embedding this information in each patient's EMR, and using clinical decision support to advise on drug selection and dosing when a relevant drug is prescribed to a patient with a variant genotype. This approach is being tested in a number of "early adopter" sites (Pulley et al., 2012; Rasmussen-Torvik et al., 2014).

There are several barriers that must be addressed if such an approach is to become widely adopted. First, the evidence linking a variant to a variable drug response must be solid, the variable outcome must be clinically important, and some form of genetically guided advice should be provided (choose another drug, choose another dose, etc.). Drug gene pairs such as *CYP2C19*2*/clopidogrel or *CYP2C9*3*/warfarin may fall into this category; the Clinical Pharmacogenomics Implementation Consortium provides guidelines on such advice by genotype across multiple drugs (Relling and Klein, 2011). Second, the strength of the evidence supporting a genotype-specific prescribing strategy varies. The strongest level of evidence comes from RCTs, in which a clinically important, genotype-guided treatment strategy is compared to a standard of care. Using this approach, genotyping for HLA-B5701 has been shown to eliminate the risk for severe skin reactions (such as the Stevens-Johnson syndrome) during treatment with the antiretroviral agent abacavir (Mallal et al., 2008). A number of trials have studied the utility of genotyping for *CYP2C9* and *VKORC1* variants during warfarin therapy. The main outcome metric has been duration of drug exposure in therapeutic range during the first 30–90 days of therapy; the results have been inconsistent, with none showing a huge effect (Kimmel et al., 2013; Pirmohamed et al., 2013). These studies have few bleeding events, but EMR-based case-control studies looking at this problem have implicated variants in CYP2C9 or CYP4F2 as risk alleles (Kawai et al., 2014; Roth et al., 2014). Nonrandomized study designs are weaker than RCTs, but performing RCTs to target small subsets of patients carrying uncommon variants may not be feasible.

Acknowledgment

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