Goodman & Gilman's

The Pharmacological Basis of THERAPEUTICS

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chapter 7

Pharmacogenetics

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Kathleen M. Giacomini

Pharmacogenetics is the study of the genetic basis for variation in drug response. In this broadest sense, pharmacogenetics encompasses pharmacogenomics, which employs tools for surveying the entire genome to assess multigenic determinants of drug response. Prior to the technical advances in genomics of the last decade, pharmacogenetics proceeded using a forward genetic, phenotype-to-genotype approach. Drug response outliers were compared to individuals with "normal" drug response to identify the pharmacologic basis of altered response. An inherited component to response was demonstrated using family studies or imputed through intra- *vs*. intersubject reproducibility studies. With the explosion of technology in genomics, a reverse genetic, genotype-to-phenotype approach is feasible whereby genomic polymorphisms can serve as the starting point to assess whether genomic variability translates into phenotypic variability.

Individuals differ from each other approximately every 300-1000 nucleotides, with an estimated total of 10 million single nucleotide polymorphisms (SNPs; single base pair substitutions found at frequencies ≥1% in a population) and thousands of copy number variations in the genome (International HapMap et al., 2007; Redon et al., 2006; Stranger et al., 2007). Identifying which of these variants or combinations of variants have functional consequence for drug effects is the task of modern pharmacogenetics.

Historical Context. In the pre-genomics era, the frequency of genetic variation was hypothesized to be relatively uncommon, and the demonstration of inherited drug-response traits applied to a relatively small number of drugs and pathways (Eichelbaum and Gross, 1990; Evans and Relling, 2004; Johnson and Lima, 2003). Historically, uncommon severe drug-induced phenotypes served as the triggers to investigate and document pharmacogenetic phenotypes. Prolonged neuromuscular blockade following normal doses of succinylcholine, neurotoxicity following isoniazid therapy (Hughes et al., 1954), and methemoglobinemia in glucose-6-phosphate dehydrogenase (G6PD) deficiency (Alving et al., 1956) were discovered to have a genetic basis in the first half of the 20th century. In the 1970s and 1980s, debrisoquine hydroxylation and exaggerated hypotensive effects from that drug were related to an autosomal recessive inherited deficiency in the cytochrome P450 isoenzyme 2D6 (CYP2D6) (Evans and Relling, 2004). Since the elucidation of the molecular basis of the phenotypic polymorphism in CYP2D6 (Gonzalez et al., 1988), the molecular bases of many other monogenic pharmacogenetic traits have been identified (Meyer and Zanger, 1997).

Importance of Pharmacogenetics to Variability in Drug Response

Drug response is considered to be a gene-by-environment phenotype. That is, an individual's response to a drug depends on the complex interplay between environmental factors and genetic factors (Figure 7–1). Variation in drug response therefore may be explained by variation in environmental and genetic factors, alone or in combination. What proportion of drug-response variability is likely to be genetically determined? Classical family studies provide some information (Weinshilboum and Wang, 2004).

Because estimating the fraction of phenotypic variability that is attributable to genetic factors in pharmacogenetics usually requires administration of a drug to twins or trios of family members, data are somewhat limited. Twin studies have shown that drug metabolism is highly heritable, with genetic factors accounting for most of the variation in metabolic rates for many drugs (Vesell, 2000). Results from a twin study in which the $t_{1/2}$ of antipyrine was measured are typical (Figure 7–2). Antipyrine, a pyrazolone analgesic, is eliminated exclusively by metabolism and is a substrate for multiple CYPs. There is considerably greater concordance in the $t_{1/2}$ of antipyrine between the monozygotic (identical) twin pairs in comparison to the dizygotic (fraternal) twin pairs. Comparison of intra-twin *vs*. inter-pair variability suggests that ~75-85% of the variability in pharmacokinetic half-lives for drugs that are eliminated by metabolism is heritable (Penno et al., 1981). It has also been proposed that heritability can be estimated by comparing intra-subject *vs*. inter-subject variability in drug response or disposition in unrelated individuals (Kalow et al., 1998), with the assumption that high intra-subject reproducibility translates into high heritability; the validity of this method across pharmacologic phenotypes remains to be established. In any case, such studies provide only an estimate of

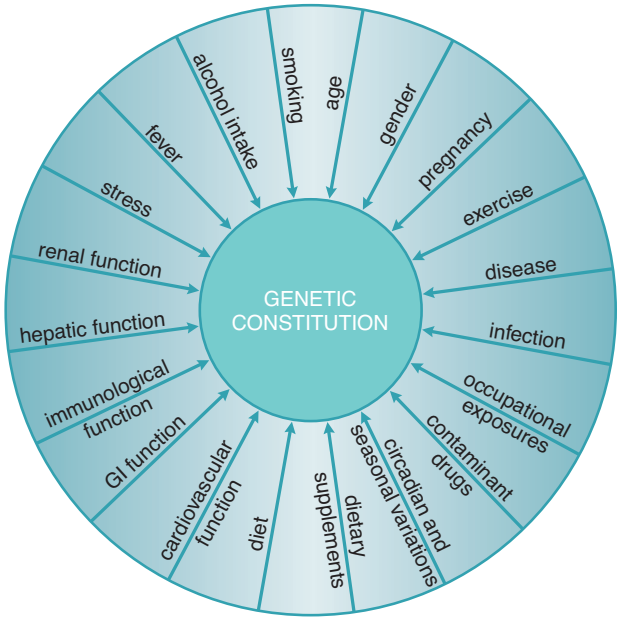


Figure 7–1. *Exogenous and endogenous factors contribute to variation in drug response.* (Reproduced with permission from Vesell, 1991. Copyright © Elsevier.)

the overall contribution of inheritance to the phenotype; because multiple gene products contribute to antipyrine disposition, most of which have unelucidated mechanisms of genetic variability, the predictability of antipyrine disposition based on known genetic variability is poor.

Extended kindreds may be used to estimate heritability. This approach to estimating the degree of heritability of a pharmacogenetic phenotype uses *ex vivo* experiments with cell lines derived from related individuals from extended multigenerational families. Inter- *vs*. intrafamily variability and relationships among members of a kindred are used to estimate heritability. Using this approach with lymphoblastoid cells, cytotoxicity from chemotherapeutic agents was shown to be heritable, with ~20-70% of the variability in sensitivity to 5-fluorouracil, cisplatin, docetaxel and other anticancer agents estimated as inherited (Hartford and Dolan, 2007; Watters et al., 2004).

For the "monogenic" phenotypic traits of G6PD deficiency, CYP2D6, or thiopurine methyltransferase (TPMT) metabolism, it is often possible to predict phenotype based on genotype. Several genetic polymorphisms of drug metabolizing enzymes result in monogenic traits. Based on a retrospective study, 49% of adverse drug reactions were associated with drugs that are substrates for polymorphic drug metabolizing enzymes, a proportion larger than estimated for all drugs (22%) or for top-selling drugs (7%) (Phillips et al., 2001). Prospective genotype determinations may result in the ability to prevent adverse drug reactions.

Defining multigenic contributors to drug response will be much more challenging. For some multigenic phenotypes, such as response to antihypertensives, the large numbers of candidate genes will necessitate a large patient sample size to produce the statistical power required to solve the "multigene" problem.

GENOMIC BASIS OF PHARMACOGENETICS

Phenotype-Driven Terminology

Because initial discoveries in pharmacogenetics were driven by variable phenotypes and defined by family and twin studies, the classic genetic terms for monogenic traits apply to some pharmacogenetic polymorphisms. A trait (e.g., CYP2D6 "poor metabolism") is deemed autosomal recessive if the responsible gene is located on an autosome (i.e., it is not sex-linked) and a distinct

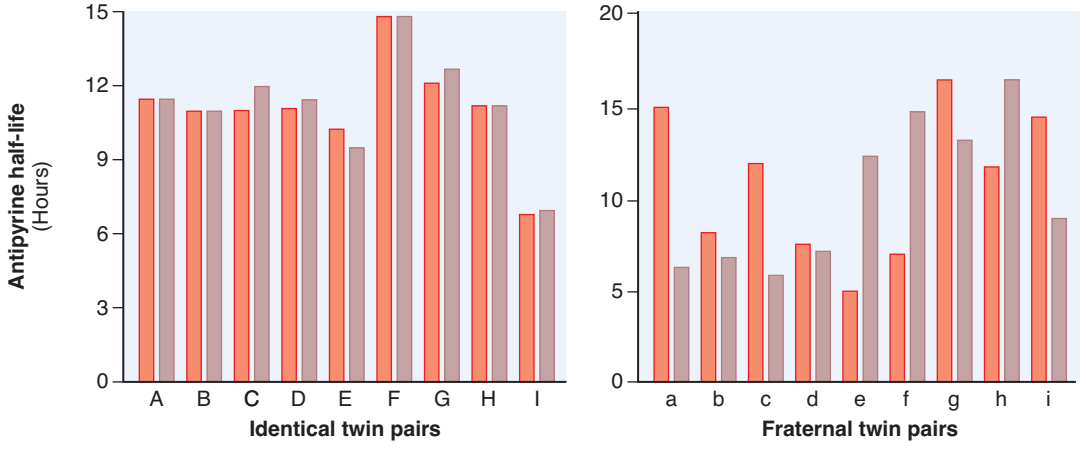


Figure 7–2. *Pharmacogenetic contribution to pharmacokinetic parameters.* $t_{1/2}$ of antipyrine is more concordant in identical in comparison to fraternal twin pairs. Bars show the $t_{1/2}$ of antipyrine in identical (monozygotic) and fraternal (dizygotic) twin pairs. (Redrawn from data in Vesell and Page, 1968.)

phenotype is evident only with nonfunctional alleles on both the maternal and paternal chromosomes. An autosomal recessive trait does not appear in heterozygotes. 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. For example, TPMT catabolism of thiopurines exhibits three relatively distinct phenotypes, and thus was deemed codominant even in the pre-molecular era. With the advances in molecular characterization of polymorphisms and a genotype-to-phenotype approach, many polymorphic traits (e.g., CYP2C19 metabolism of drugs such as mephenytoin and omeprazole) are now recognized to exhibit some degree of codominance. Some pharmacogenetic traits, such as the long QT syndrome, segregate as dominant traits; the long QT syndrome is associated with heterozygous loss-of-function mutations of ion channels. A prolonged QT interval is seen on the electrocardiogram, either basally or in the presence of certain drugs, and individuals with prolonged QT intervals are predisposed to cardiac arrhythmias (see Chapter 29).

In an era of detailed molecular characterization, two major factors complicate the historical designation of recessive, co-dominant, and dominant traits. *First*, even within a single gene, a vast array of polymorphisms (promoter, coding, noncoding, completely inactivating, or modestly modifying) are possible. Each polymorphism may produce a different effect on gene function and therefore differentially affect a measured trait. For example, the effect of a polymorphism with only a modest effect on the function of an enzyme will only be observed in individuals who are homozygous for the polymorphism. Heterozygotes will not exhibit any measureable changes in enzyme activity. In contrast, the effect of a polymorphism that exhibits complete loss of function of the enzyme will be large and may be observed phenotypically in heterozygotes. *Secondly*, most traits (pharmacogenetic and otherwise) are multigenic, not monogenic. Thus, even if the designations of recessive, co-dominant, 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 most phenotypic variability is likely to be multigenic.

Types of Genetic Variants

A *polymorphism* is a variation in the DNA sequence that is present at an allele frequency of 1% or greater in a population. Two major types of sequence variation have been associated with variation in human phenotype: *single nucleotide polymorphisms* (SNPs) and *insertions/deletions* (indels) (Figure 7–3). In comparison to base pair

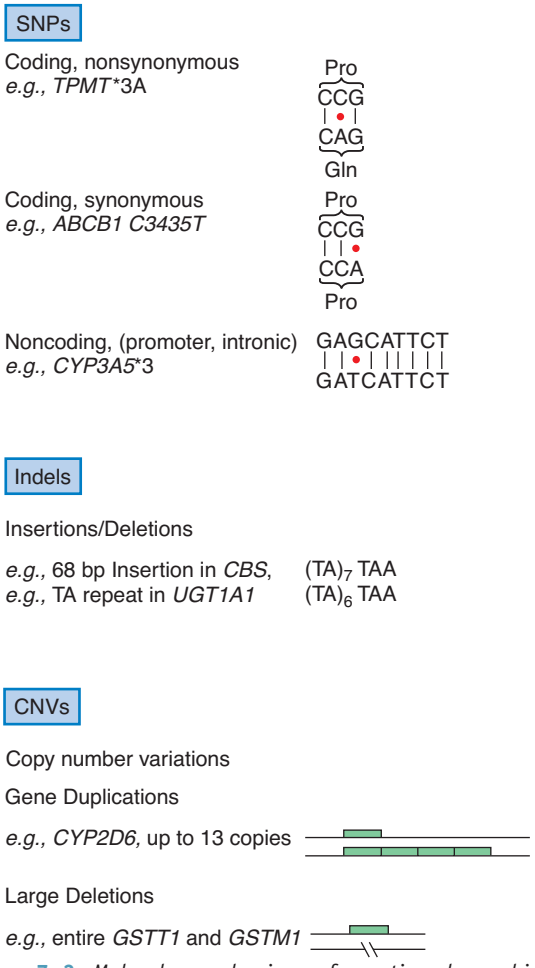


Figure 7–3. *Molecular mechanisms of genetic polymorphisms.* The most common genetic variants are single nucleotide polymorphism substitutions (SNPs). Coding non-synonymous SNPs result in a nucleotide substitution that changes the amino acid codon (here proline to glutamine), which could change protein structure, stability, 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 (insertion/deletions). SNP indels can have any of the same effects as SNP substitutions: short repeats in the promoter (which can affect transcript amount), or insertions/deletions that add or subtract amino acids. Copy number variations (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. TPMT, thiopurine methyltransferase; ABCB1, the multidrug resistance transporter (P-glycoprotein); CYP, cytochrome P450; CBS, cystathionine β-synthase; UGT, UDP-glucuronyl transferase; GST, glutathione-S-transferase.

substitutions, indels are much less frequent in the genome and are of particularly low frequency in coding regions of genes (Cargill et al., 1999; Stephens et al., 2001). Single base pair substitutions that are present at frequencies ≥1% in a population are termed single nucleotide polymorphisms (SNPs) and are present in the human genome at ~1 SNP every few hundred to a thousand base pairs, depending on the gene region (Stephens et al., 2001).

SNPs in the coding region are termed cSNPs, and are further classified as non-synonymous (or *missense*) if the base pair change results in an amino acid substitution, or synonymous (or *sense*) if the base pair substitution within a codon does not alter the encoded amino acid. Typically, substitutions of the third base pair, termed the *wobble position*, in a three base pair codon, such as the G to A substitution in proline shown in Figure 7–3, do not alter the encoded amino acid. Base pair substitutions that lead to a stop codon are termed *nonsense* mutations. In addition, ~10% of SNPs can have more than two possible alleles (e.g., a C can be replaced by either an A or G), so that the same polymorphic site can be associated with amino acid substitutions in some alleles but not others.

Synonymous polymorphisms have sometimes been found to contribute directly to a phenotypic trait. One of the most notable examples is a polymorphism in ABCB1, which encodes P-glycoprotein, an efflux pump that interacts with many clinically used drugs. The synonymous polymorphism, C3435T, is associated with various phenotypes and has been the subject of numerous studies (Hoffmeyer et al., 2000; Kim et al., 2006; Sills et al., 2005; Turgut et al., 2007). This synonymous polymorphism results in a change from a preferred codon for isoleucine to a less preferred codon. Presumably, the less preferred codon is translated at a slower rate, which apparently changes the folding of the protein, its insertion into the membrane, and its interaction with drugs (Kimchi-Sarfaty et al., 2007).

Polymorphisms in noncoding regions of genes 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 (Figure 7–4). Polymorphisms in introns found near exon-intron boundaries are often treated as a separate category from other intronic polymorphisms since these may affect splicing, and thereby affect function. Noncoding SNPs in promoters or enhancers may alter *cis*- or *trans*-acting elements that regulate gene transcription or transcript stability. Noncoding SNPs in introns or exons may create alternative exon splicing sites, and the altered transcript may have fewer or more exons, or shorter or larger exons, than the wild-type transcript. Introduction or deletion of exonic sequence can cause a frame shift in the translated protein and thereby change protein structure or function, or result in an early stop codon, which makes an unstable or nonfunctional protein. Because 95% of the genome is intergenic, most polymorphisms are unlikely to directly affect the encoded transcript or protein. However, intergenic polymorphisms may have biological consequences by affecting DNA tertiary structure, interaction with chromatin and topoisomerases, or DNA replication. Thus, intergenic polymorphisms cannot be assumed to be without pharmacogenetic importance.

A remarkable degree of diversity in the types of insertions/deletions that are tolerated as germline polymorphisms is evident. A common glutathione-S-transferase M1 (*GSTM1*) polymorphism is caused by a 50-kilobase (kb) germline deletion, and the null allele has a population frequency of 0.3-0.5, depending on race/ethnicity. Biochemical studies indicate that livers from homozygous null individuals have only ~50% of the glutathione conjugating capacity of those with at least one copy of the *GSTM1* gene (Townsend and Kew, 2003). The number of TA repeats in the *UGT1A1* promoter affects the quantitative expression of this crucial glucuronosyl transferase in liver; although 4-9 TA repeats exist in germline-inherited alleles, 6 or 7 repeats constitute the most common alleles (Monaghan et al., 1996). Cystathionine β-synthase has a common 68 base pair insertion/deletion polymorphism that has been linked to folate levels (Kraus et al., 1998). Some deletion and duplication polymorphisms can be seen as a special case of copy number variations (CNVs) (Beckmann et al., 2007; Redon et al., 2006; Stranger et al., 2007). A CNV is a segment of DNA in which a variable number of that segment has been found in one or more populations. CNVs, which range in size from 1 kb to many megabases, are caused by genomic rearrangements including duplications, deletions, and inversions. CNVs appear to occur in ~10% of the human genome and in one study accounted for ~18% of the detected genetic variation in expression of around 15,000 genes in lymphoblastoid cell lines (Stranger et al., 2007). Because of their size, CNVs are likely to affect phenotype. There are notable examples of CNVs in pharmacogenetics; gene duplications of CYP2D6 are associated with an ultra-rapid metabolizer phenotype.

A *haplotype*, which is defined as a series of alleles found at a linked locus on a chromosome, specifies the DNA sequence variation

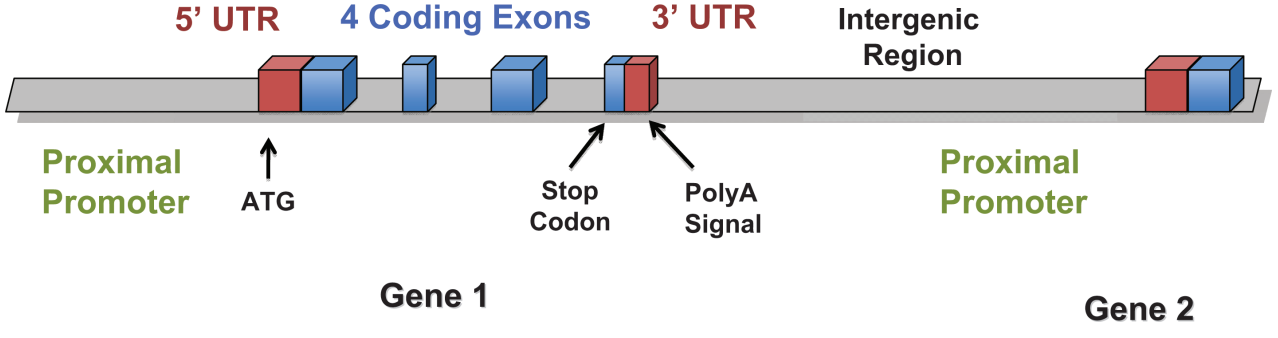


Figure 7–4. *Nomenclature of genomic regions.*

in a gene or a gene region on one chromosome. For example, consider two SNPs in *ABCB1*, which encodes for the multidrug resistance protein, P-glycoprotein. 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, which may or may not be identical. Haplotypes are important because they are the functional unit of the gene. That is, 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 mutation may be functionally important regardless of other linked variants within the haplotype(s).

Two terms are useful in describing the relationship of genotypes at two loci: linkage equilibrium and linkage disequilibrium. Linkage equilibrium occurs when the genotype present at one locus is independent of the genotype at the second locus. Linkage disequilibrium occurs when the genotypes at the two loci are not independent of one another. In complete linkage disequilibrium, genotypes at two loci always occur together. As recombination occurs then linkage disequilibrium between two alleles will decay and linkage equilibrium will result. Over many generations, with many recombination events, linkage disequilibrium will be eliminated. Patterns of linkage disequilibrium are population specific. For any gene region, linkage disequilibrium among individuals between SNPs in that region may be viewed using a software tool such as Haploview (Barrett et al., 2005) (Figure 7–5).

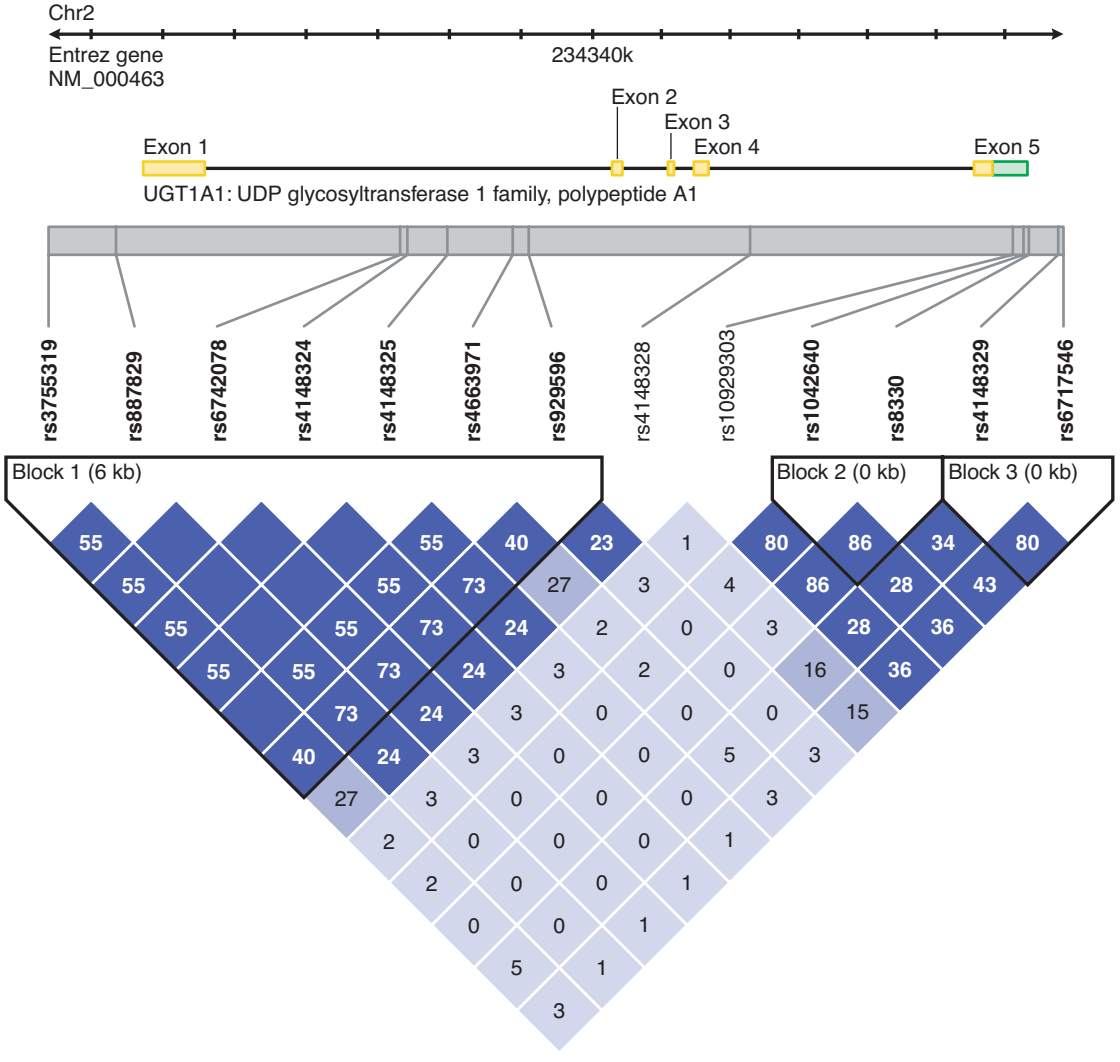


Figure 7–5. *Haplotype blocks in UGT1A1 generated by Haploview version 4.1.* Linkage disequilibrium between SNPs in *UGT1A1* in Europeans is shown. SNPs present at allele frequencies of 20% or greater are included and identified by rs numbers. The r^2 values indicating linkage disequilibrium values between any two SNPs are shown in the blocks below as whole numbers (e.g., 86 = r^2 of 0.86 between SNPs at rs4148238 and rs8330. Those that are dark blue without numbers have an $r^2 = 1.0$. The relationships among the SNP genotypes in this population for this gene indicate that there are three primary linkage disequilibrium blocks (Block 1, Block 2, and Block 3), which in this case, were generated by the Haploview program. (Source: Broad Institute, http://www.broad.mit.edu/haploview/haploview.)

Ethnic Diversity

Polymorphisms differ in their frequencies within human populations (Burchard et al., 2003; Rosenberg et al., 2002, 2003). Frequencies of polymorphisms in ethnically or racially diverse human populations have been examined in whole genome scanning studies (Cargill et al., 1999; Stephens et al., 2001). In these studies, polymorphisms have been classified as either cosmopolitan or population (or race and ethnic) specific. Cosmopolitan polymorphisms are those polymorphisms present in all ethnic groups, although frequencies may differ among ethnic groups. Cosmopolitan polymorphisms are usually found at higher allele frequencies in comparison to population-specific polymorphisms. Likely to have arisen before migrations of humans from Africa, cosmopolitan polymorphisms are generally older than population-specific polymorphisms.

The presence of ethnic and race-specific polymorphisms is consistent with geographical isolation of human populations (Xie et al., 2001). These polymorphisms probably arose in isolated populations and then reached a certain frequency because they are advantageous (positive selection) or more likely, neutral, conferring no advantage or disadvantage to a population. Large-scale sequence studies in ethnically diverse populations in the U.S. demonstrate that African Americans have the highest number of population-specific polymorphisms in comparison to European Americans, Mexican Americans, and Asian Americans (Leabman et al., 2003; Stephens et al., 2001). Africans are believed to be the oldest population and therefore have both recently derived, population-specific polymorphisms, and a large number of older polymorphisms that occurred before migrations out of Africa.

Consider the coding region variants of two membrane transporters identified in 247 ethnically diverse DNA samples (Figure 7–6).

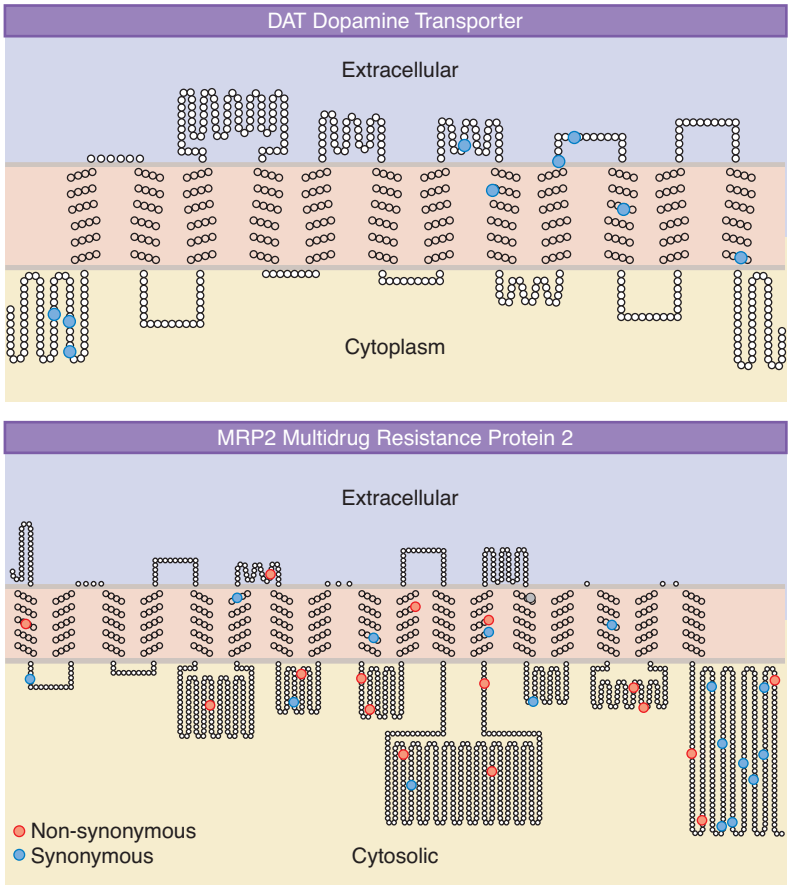


Figure 7–6. *Coding region polymorphisms in two membrane transporters.* Shown are the dopamine transporter, DAT (encoded by *SLC6A3*) and multidrug resistance associated protein, MRP2 (encoded by *ABCC2*). Coding region variants were identified in 247 ethnically diverse DNA samples (100 African Americans, 100 European Americans, 30 Asians, 10 Mexicans, and 7 Pacific islanders). Shown in blue circles are synonymous variants, and in red circles, non-synonymous variants.

Shown are non-synonymous and synonymous SNPs; population-specific non-synonymous cSNPs are indicated in the figure. The multidrug resistance associated protein, MRP2, has a large number of non-synonymous cSNPs. There are fewer synonymous variants than non-synonymous variants, but the allele frequencies of the synonymous variants are greater than those of the non-synonymous variants (Leabman et al., 2003). By comparison, DAT, the dopamine transporter, has a number of synonymous variants but no non-synonymous variants, suggesting that selective pressures have acted against substitutions that led to changes in amino acids.

In a survey of coding region haplotypes in 313 different genes in 80 ethnically diverse DNA samples, most genes were found to have between 2 and 53 haplotypes, with the average number of haplotypes in a gene being 14 (Stephens et al., 2001). Like SNPs, haplotypes may be cosmopolitan or population specific and ~ 20% of the over 4000 identified haplotypes were cosmopolitan (Stephens et al., 2001). Considering the frequencies of the haplotypes, cosmopolitan haplotypes actually accounted for over 80% of all haplotypes, whereas population-specific haplotypes accounted for only 8%. Similarly, recent studies suggest that population-specific CNVs and cosmopolitan CNVs also exist (Redon et al., 2006). As with SNPs and haplotypes, African populations have the greatest numbers of CNVs.

Polymorphism Selection

Genetic variation that results in penetrant and constitutively evident biological variation sometimes causes a "disease" phenotype. Cystic fibrosis, sickle-cell anemia, and Crigler-Najjar syndrome are examples of inherited diseases caused by single gene defects (Pani et al., 2000). In the case of Crigler-Najjar syndrome, the same gene (*UGT1A1*) that is targeted by rare inactivating mutations (and associated with a serious disease) is also targeted by modest polymorphisms (and associated with modest hyperbilirubinemia and altered drug clearance) (Monaghan et al., 1996). Due to the disease, some evolutionary selection against these single-gene polymorphisms is present. Polymorphisms in other genes have highly penetrant effects in the drug-challenged but not in the constitutive state, which are the causes of monogenic pharmacogenetic traits. There is unlikely to be any selective pressure for or against these polymorphisms (Evans and Relling, 2004; Meyer, 2000; Weinshilboum, 2003). The vast majority of genetic polymorphisms have a modest impact on the affected genes, are part of a large array of multigenic factors that impact on drug effect, or affect genes whose products play a minor role in drug action relative to a large nongenetic effect. For example, phenobarbital induction of metabolism may be such an overwhelming "environmental" effect that polymorphisms in the affected transcription factors and drug-metabolizing genes have modest effects by comparison.

PHARMACOGENETIC STUDY DESIGN CONSIDERATIONS

Pharmacogenetic Measures

What are pharmacogenetic traits and how are they measured? A *pharmacogenetic trait* is any measurable or discernible trait associated with a drug. Thus, enzyme activity, drug or metabolite levels in plasma or urine, blood pressure or lipid lowering produced by a drug, and drug-induced gene expression patterns are examples of pharmacogenetic traits. Directly measuring a trait (e.g., enzyme activity) has the advantage that the net effect of the contributions of all genes that influence the trait is reflected in the phenotypic measure. However, it has the disadvantage that it is also reflective of nongenetic influences (e.g., diet, drug interactions, diurnal or hormonal fluctuation) and thus, may be "unstable."

For CYP2D6, if a patient is given an oral dose of dextromethorphan, and the urinary ratio of parent drug to metabolite is assessed, the phenotype is reflective of the genotype for CYP2D6 (Meyer and Zanger, 1997). However, if dextromethorphan is given with quinidine, a potent inhibitor of CYP2D6, the phenotype may be consistent with a poor metabolizer genotype, even though the subject carries wild-type CYP2D6 alleles. In this case, quinidine administration results in a drug-induced phaloinsufficiency, and the assignment of a CYP2D6 poor metabolizer phenotype would not be accurate for that subject in the absence of quinidine. If a phenotypic measure, such as the erythromycin breath test (for CYP3A), is not stable within a subject, this is an indication that the phenotype is highly influenced by nongenetic factors, and may indicate a multigenic or weakly penetrant effect of a monogenic trait.

Because most pharmacogenetic traits are multigenic rather than monogenic (Figure 7–7), considerable effort is being made to identify the important genes and their polymorphisms that influence variability in drug response.

Genetic Testing. Most genotyping methods use constitutional or germline DNA, i.e., DNA extracted from any somatic, diploid cells, usually white blood cells or buccal cells (due to their ready accessibility). DNA is extremely stable if appropriately extracted and stored, and unlike many laboratory tests, genotyping need to be performed only once, because DNA sequence is generally invariant throughout an individual's lifetime. Progress continues in moving genotyping tests from research laboratories into patient care. Because genotyping tests are directed at specific known polymorphic sites using a variety of strategies, and not all known functional polymorphisms are likely to be known for any particular gene, it is critical that the methodology for interrogating the polymorphic sites be understood, so that the probability of a negative genotyping test being falsely negative can be estimated.

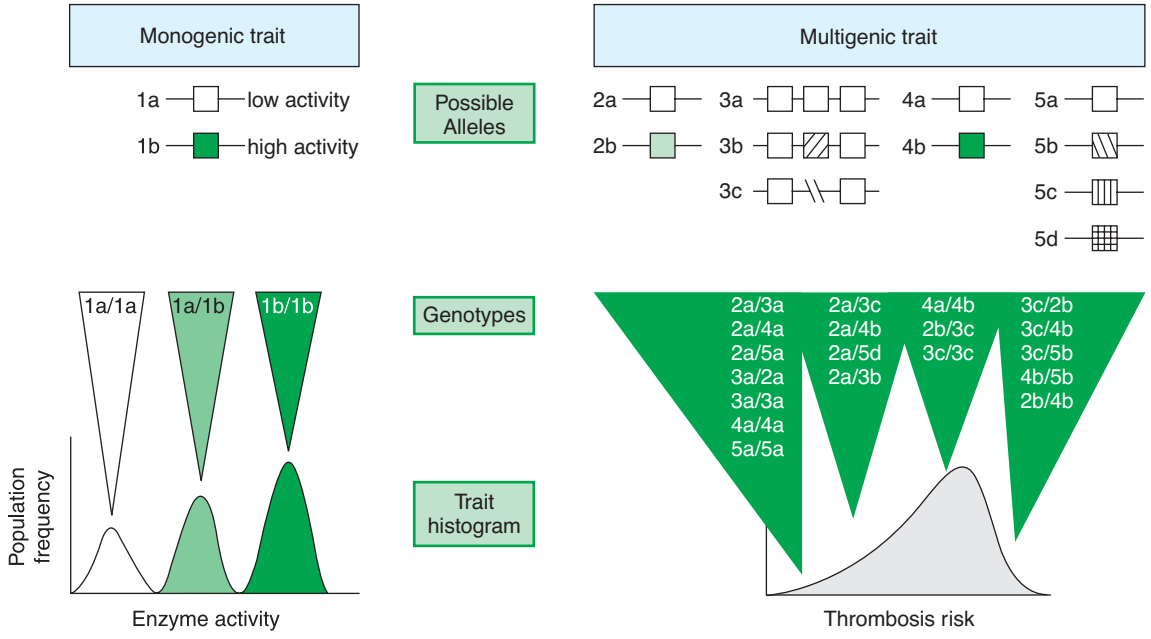


Figure 7–7. *Monogenic versus multigenic pharmacogenetic traits.* Possible alleles for a monogenic trait (*upper left*), in which a single gene has a low-activity (1a) and a high-activity (1b) allele. The population frequency distribution of a monogenic trait (*bottom left*), here depicted as enzyme activity, may exhibit a trimodal frequency distribution with relatively distinct separation 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 2, 3, or 4 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.

One method to assess the reliability of any specific genotype determination in a group of individuals is to assess whether the relative number of homozygotes to heterozygotes is consistent with the overall allele frequency at each polymorphic site. *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. Proportions of the observed three genotypes must add up to one; significant differences from those predicted may indicate a genotyping error.

Candidate Gene Versus Genome-Wide Approaches

Because pathways involved in drug response are often known or at least partially known, pharmacogenetic studies are highly amenable to candidate gene association studies. After genes in drug response pathways are identified, the next step in the design of a candidate gene association pharmacogenetic study is to identify the genetic polymorphisms that are likely to contribute to the therapeutic and/or adverse responses to the drug. There are several databases that contain information on polymorphisms and mutations in human genes (Table 7–1); these databases allow the investigator to search by gene for reported polymorphisms. Some of the databases, such as the Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB), include phenotypic as well as genotypic data.

In candidate gene association studies, specific genes are prioritized as playing a role in response or adverse response to a drug, it is important to select polymorphisms in those genes for association studies. For this purpose, there are two categories of polymorphisms. The first are polymorphisms that do not, in and of themselves, cause altered function or expression level of the encoded protein (e.g., an enzyme that metabolizes the drug or the drug receptor). Rather, these polymorphisms are linked to the variant allele(s) that produces the altered function. These polymorphisms serve as biomarkers for drug-response phenotype. One way to select SNPs in each gene is to use a tag SNP approach. That is, all SNPs in a gene including SNPs in and around the gene (e.g., 25 kb upstream and downstream of the

Table 7–1

Databases Containing Information on Human Genetic Variation

DATABASE NAME	URL (AGENCY)	DESCRIPTION OF CONTENTS
Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB)	www.pharmgkb.org (NIH Sponsored Research Network and Knowledge Database)	Genotype and phenotype data related to drug response
EntrezSNP (Single Nucleotide Polymorphism) (dbSNP)	www.ncbi.nlm.nih.gov/SNP (National Center for Biotechnology Information [NCBI])	SNPs and frequencies
Human Genome Variation Database (HGVbase)	www.hgvbaseg2p.org	Genotype/phenotype associations
HuGE Navigator	www.hugenavigator.net	Literature annotations for genotype/phenotype associations
Online Mendelian Inheritance in Man	www.ncbi.nlm.nih.gov/sites/entrez/?db=OMIM (NCBI)	Human genes and genetic disorders
International HapMap Project	www.hapmap.org	Genotypes, frequency and linkage data for variants in ethnic and racial populations
UCSC Genome Browser	http://genome.ucsc.edu	Sequence of the human genome; variant alleles
Genomics Institute of Novartis Research Foundation	http://symatlas.gnf.org/SymAtlas/	Gene expression data for human genes in multiple tissues and cell lines
The Broad Institute Software	http://www.broad.mit.edu/science/software/software	Software tools for the analysis of genetic studies

gene) are identified from SNP databases (e.g., HapMap Database: http://www.hapmap.org/). SNPs with allele frequencies equal to or greater than a target allele frequency are selected. From this set of SNPs, tag SNPs are selected to serve as representatives of multiple SNPs that tend to be in linkage disequilibrium. These tag SNPs are then genotyped in the candidate gene studies.

The second type of polymorphism is the causative polymorphism, which directly precipitates the phenotype. For example, a causative SNP may change an amino acid residue at a site that is highly conserved throughout evolution. This substitution may result in a protein that is nonfunctional or has reduced function. If biological information indicates that a particular polymorphism alters function, e.g., in cellular assays of non-synonymous variants, this polymorphism is an excellent candidate to use in an association study. When causative SNPs are unknown, tag SNPs can be typed to represent important, relatively common blocks of variation within a gene. Once a tag SNP is found to associate with a drug response phenotype, the causative variant or variants, which may be in linkage with the tag SNP, should be identified. Because the causative variant may be an unknown variant, sequencing the gene may be necessary to identify potential causative variants. These additional causative variants may be uncovered by further deep resequencing of the gene.

Genome-Wide and Alternative Large-Scale Approaches. A potential drawback of the candidate gene approach is that the wrong genes may be studied. Genome-wide approaches, using gene expression arrays, genome-wide scans, or proteomics, can complement and feed into the candidate gene approach by providing a relatively unbiased survey of the genome to identify previously unrecognized candidate genes. For example, RNA, DNA, or protein from patients who have unacceptable toxicity from a drug can be compared with identical material from identically treated patients who did not have such toxicity. Differences in gene expression, DNA polymorphisms, or relative amounts of proteins can be ascertained using computational tools, to identify genes, genomic regions, or proteins that can be further assessed for germline polymorphisms differentiating the phenotype. Gene expression and proteomic approaches have the advantage that the abundance of signal may itself directly reflect some of the relevant genetic variation; however, both types of expression are highly influenced by choice of tissue type, which may not be available from the relevant tissue; e.g., it may not be feasible to obtain biopsies of brain tissue for studies on CNS toxicity. DNA has the advantage that it is readily available and independent of tissue type, but the vast majority of genomic variation is not in genes, and the large number of polymorphisms presents the danger of *type I error* (finding differences in genome-wide surveys that are false positives). Current research challenges include prioritizing among the many possible differentiating variations in genome-wide surveys of RNA, DNA, and protein to focus on those that hold the most promise for future pharmacogenomic utility.

Functional Studies of Polymorphisms

For most polymorphisms, functional information is not available. Therefore, to select polymorphisms that are

likely to be causative, it is important to predict whether a polymorphism may result in a change in expression level of a protein or a change in protein function, stability, or subcellular localization. One way to gain an understanding of the functional effects of various types of genomic variations is to survey the mutations that have been associated with human Mendelian disease. The greatest numbers of DNA variations associated with Mendelian diseases or traits are missense and nonsense mutations, followed by deletions. Further studies suggest that among amino acid replacements associated with human disease, there is a high representation at residues that are most evolutionarily conserved (Miller and Kumar, 2001; Ng and Henikoff, 2003).

These data have been supplemented by a large survey of genetic variation in membrane transporters important in drug response (Leabman et al., 2003). That survey shows that non-synonymous SNPs that alter evolutionarily conserved amino acids are present at lower allele frequencies on average than those that alter residues that are not conserved across species. A functional genomics study of almost 90 variants in membrane transporters demonstrated that the variants that altered function were likely to change an evolutionarily conserved amino acid residue and to be at low allele frequencies (Urban et al., 2006; SEARCH Group et al., 2008). These data indicate that SNPs that alter evolutionarily conserved residues are most deleterious. The nature of chemical change of an amino acid substitution determines the functional effect of an amino acid variant. More radical changes in amino acids are more likely to be associated with disease than more conservative changes. For example, substitution of a charged amino acid (Arg) for a non-polar, 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 SNPs, at least in the coding region, are likely to alter function. New sequencing methods to identify SNPs in pharmacogenetic studies will likely uncover many new rare SNPs which cause variation in drug response.

Among the first pharmacogenetic examples to be discovered was glucose-6-phosphate dehydrogenase (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 (Alving et al., 1956). G6PD is normally present in red blood cells and helps to regulate levels of glutathione (GSH), an antioxidant. Antimalarials such as primaquine increase red blood cell fragility in individuals with G6PD deficiency, leading to profound hemolytic anemia. Interestingly, 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. Chemical change is also more radical on average in mutations associated with severe G6PD deficiency in comparison to mutations associated with milder forms of the syndrome. Collectively, studies of Mendelian traits and polymorphisms suggest that non-synonymous SNPs that alter residues that are highly conserved among species and those that result in more radical changes in the nature of the amino acid are likely to be the best candidates for causing functional changes. The information in Table 7–2 (categories of polymorphisms and the likelihood of each polymorphism to alter function) can be used as a guide for prioritizing polymorphisms in candidate gene association studies.

With the increasing number of SNPs that have been identified in large-scale SNP discovery projects, it is clear that computational methods are needed to predict the functional consequences of SNPs. To this end, predictive algorithms have been developed to identify potentially deleterious amino acid substitutions. These methods can be classified into two groups. The first group relies on sequence comparisons alone to identify and score substitutions according to their degree of conservation across multiple species; different scoring matrices have been used (e.g., BLOSUM62, SIFT and PolyPhen) (Henikoff and Henikoff, 1992; Ng and Henikoff, 2003; Ramensky, 2002). The second group of methods relies on mapping of SNPs onto protein structures, in addition to sequence comparisons (Mirkovic et al., 2004). For example, rules have been developed that classify SNPs in terms of their impact on folding and stability of the native protein structure as well as shapes of its binding sites. Such rules depend on the structural context in which SNPs occur (e.g., buried in the core of the fold or exposed to the solvent, in the binding site or not), and are inferred by machine learning methods from many functionally annotated SNPs in test proteins.

Functional activity of amino acid variants for many proteins can be studied in cellular assays. An initial step in characterizing the function of a non-synonymous variant would be to isolate the variant gene or construct the variant by site-directed mutagenesis, express it in cells, and compare its functional activity to that of the reference or most common form of the protein. Large-scale functional analyses have been performed on genetic variants in membrane transporters and phase II enzymes. Figure 7–8 shows the function of all non-synonymous variants and coding region insertions and deletions of two membrane transporters, the organic cation transporter, OCT1 (encoded by *SLC22A1*) and the nucleoside transporter, CNT3 (encoded by *SLC28A3*). Most of the naturally occurring variants have functional activity similar to that of the reference transporters. However, several variants exhibit reduced function; in the case of OCT1, a gain-of-function variant is also present. Results such as these indicate heterogeneity exists in the functionality of natural amino acid variants in normal healthy human populations.

For many proteins, including enzymes, transporters, and receptors, the mechanisms by which amino acid substitutions alter function have been characterized in kinetic studies. Figure 7–9 shows simulated curves depicting the rate of metabolism of a substrate by two amino acid variants of an enzyme and the most common genetic form of the enzyme. The kinetics of metabolism of substrate by one variant enzyme, Variant A, are characterized by an

Table 7–2

Predicted Functional Effect and Relative Risk That a Variant Will Alter Function of SNP Types in the Human Genome

TYPE OF VARIANT	LOCATION	FREQUENCY IN GENOME	PREDICTED RELATIVE RISK OF PHENOTYPE	FUNCTIONAL EFFECT
Nonsense	Coding region	Very low	Very high	Stop codon
Nonsynonymous Evolutionarily conserved	Coding region	Low	High	Amino acid substitution of a residue conserved across evolution
Nonsynonymous Evolutionarily unconserved	Coding region	Low	Low to moderate	Amino acid substitution of a residue not conserved across evolution
Nonsynonymous Radical chemical change	Coding region	Low	Moderate to high	Amino acid substitution of a residue that is chemically dissimilar to the original residue
Nonsynonymous Low to moderate chemical change	Coding region	Low	Low to high	Amino acid substitution of a residue that is chemically similar to the original residue
Insertion/deletion	Coding/ noncoding region	Low	Low to high	Coding region: can cause frameshift
Synonymous	Coding region	Medium	Low	Can affect mRNA stability or splicing
Regulatory region	Promoter, 5′ UTR, 3′ UTR	Medium	Low to High	Can affect the level of mRNA transcript by changing rate of transcription or stability of transcript
Intron/exon boundary	Within 8 bp of intron	Low	High	May affect splicing
Intronic	Deep within intron	Medium	Unknown	May affect mRNA transcript levels through enhancer mechanism
Intergenic	Noncoding region between genes	High	Unknown	May affect mRNA transcript levels through enhancer mechanisms

Data adapted from Tabor et al., 2002.

increased K_m. Such an effect can occur if the amino acid substitution alters the binding site of the enzyme leading to a decrease in its affinity for the substrate. An amino acid variant may also alter the maximum rate of metabolism (V_{max}) of substrate by the enzyme, as exemplified by Variant B. The mechanisms for a reduced V_{max} are generally related to a reduced expression level of the enzyme, which may occur because of decreased stability of the protein or changes in protein trafficking or recycling (Shu et al., 2003; Tirona et al., 2001; Xu et al., 2002).

In contrast to the studies with SNPs in coding regions, we know much less about noncoding region SNPs. The principles of evolutionary conservation that have been shown to be important in predicting the function of non-synonymous variants in the coding region need to be refined and tested as predictors of function of SNPs in noncoding regions. New methods in comparative genomics are being refined to identify conserved elements in noncoding regions of genes that may be functionally important (Bejerano et al., 2004; Boffelli et al., 2004; Brudno et al., 2003). SNPs identified in genome-wide association studies as being associated with clinical phenotypes including drug response phenotypes have largely been in noncoding regions, either intergenic or intronic regions, of the genome (Figure 7–10). It is a challenge in human genetics and pharmacogenetics to understand the functional effects of noncoding region variants. Such variants may be in potential enhancer regions of the genome and may enhance (or repress) gene transcription.

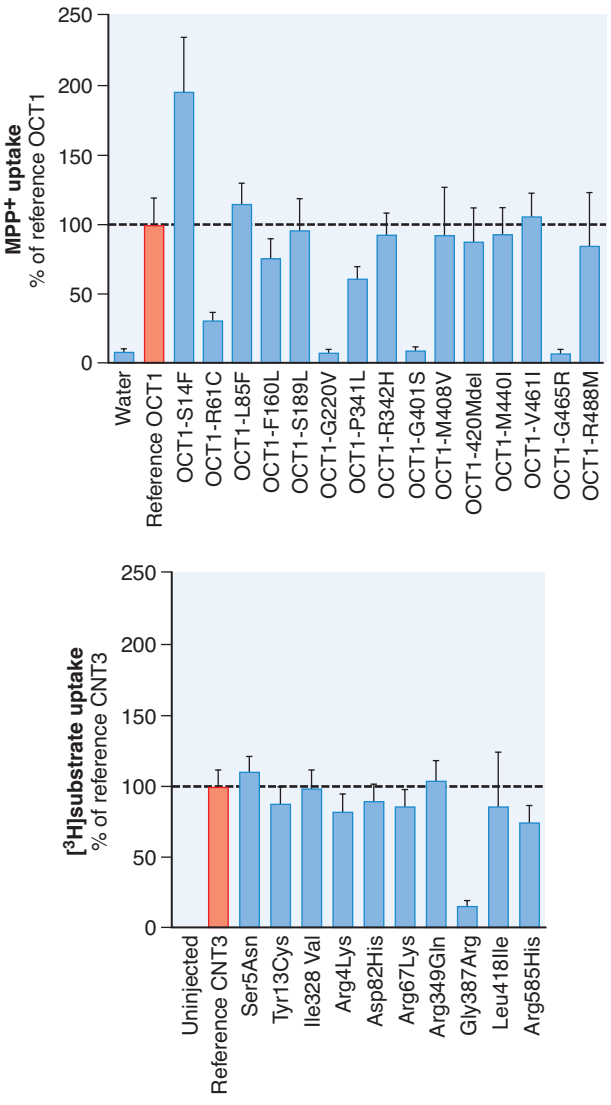


Figure 7–8. *Functional activity of natural variants of two membrane transporters.* Data for the organic cation transporter (OCT1, *top panel*) and the nucleoside transporter (CNT3, *bottom panel*). Variants, identified in ethnically diverse populations, were constructed by site-directed mutagenesis and expressed in *Xenopus laevis* oocytes. Blue bars represent uptake of the model compounds by variant transporters. Red bars represent uptake of the model compounds by reference transporters. MPP^+, 1-methyl-4-phenylpyridium. (Reproduced with permission from Shu et al., 2003. Copyright © National Academy of Sciences, USA.)

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. It was well known that only ~10% of whites but a higher percentage of blacks expressed CYP3A5. The SNP accounting for variation in CYP3A5 protein lies in intron 3, 1618 nucleotides 3′ from exon 3 and 377 nucleotides 5′ of exon 4. This SNP creates an alternative

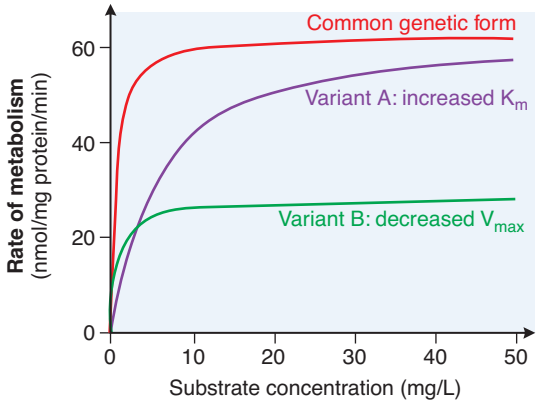


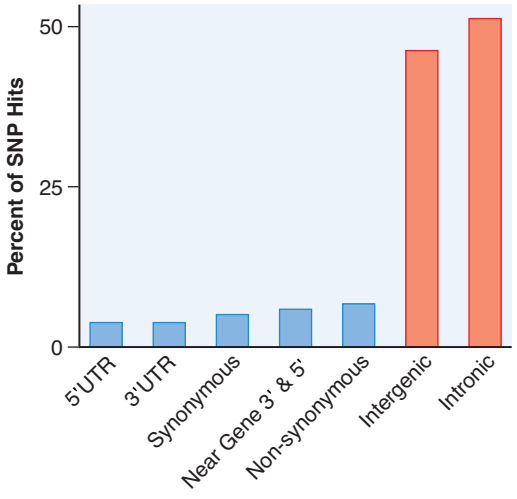
Figure 7–9. *Simulated concentration-dependence curves showing the rate of metabolism of a hypothetical substrate by the common genetic form of an enzyme and two non-synonymous variants.* Variant A exhibits an increased K_m and likely reflects a change in the substrate binding site of the protein by the substituted amino acid. Variant B exhibits a change in the maximum rate of metabolism (V_{max}) of the substrate. This may be due to reduced expression level of the enzyme.

splice site, resulting in a transcript with a larger exon 3 but also the introduction of an early stop codon in this 13 exon transcript (Figure 7–11). The resultant protein, in the majority of whites who are homozygous for the *3 nonfunctional allele, is thus truncated so early that the protein is completely non-detectable. Thus, even SNPs quite distant from intron/exon borders can profoundly affect splicing and thus affect protein function (Kuehl et al., 2001).

Pharmacogenetic Phenotypes

Candidate genes for therapeutic and adverse response can be divided into three categories: *pharmacokinetic*, *receptor/target*, and *disease modifying*.

Pharmacokinetics. 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 (Table 7–3; Nebert et al., 1996). Multiple enzymes and transporters may be involved in the pharmacokinetics of a single drug. Several polymorphisms in drug metabolizing enzymes were discovered as monogenic phenotypic trait variations, and thus may be referenced using their phenotypic designations (e.g., slow *vs*. fast acetylation, extensive *vs*. poor metabolizers of debrisoquine or sparteine) rather than their genotypic designations that reference the polymorphic gene (NAT2 and CYP2D6, respectively) (Grant et al., 1990). CYP2D6 is now known to catabolize the two initial probe drugs (sparteine and debrisoquine), each of which was associated with exaggerated responses in 5-10% of treated



	Percent of SNP Hits
5' UTR	3
3' UTR	3
Synonymous	4
Near Gene	5
Non-synonymous	6
Intergenic	45
Intronic	50

Figure 7–10. *Types of genetic variants that have been significantly associated with complex human traits and disease in 208 genome-wide association studies.* Approximately 500 SNPs were associated with human disease and complex traits. Intergenic and intronic SNPs comprise the largest fraction of associated variants. See www.genome.gov/gwastudies/.

individuals. The exaggerated responses are an inherited trait (Eichelbaum et al., 1975; Mahgoub et al., 1977). At present, a very large number of medications (estimated at 15-25% of all medicines in use) have been shown to be substrates for CYP2D6 (Table 7–3 and Figure 6–3A). The molecular and phenotypic characterization of multiple racial and ethnic groups has shown that seven variant alleles account for well over 90% of the "poor metabolizer" low-activity alleles for this gene in most racial groups; that the frequency of variant alleles varies with geographic origin; and that a small percentage of individuals carry stable duplications of CYP2D6, with "ultra-rapid" metabolizers having up to 13 copies of the active gene (Ingelman-Sundberg and Evans, 2001). Phenotypic consequences of the deficient CYP2D6 phenotype (Table 7–3) include increased risk of toxicity of antidepressants or antipsychotics (catabolized by the enzyme), lack of analgesic effects of codeine (anabolized by the enzyme), and lack of activation of tamoxifen, leading to a greater risk of relapse or recurrence in breast cancer (Borges et al., 2006; Goetz et al., 2008; Ingle, 2008). Conversely, the ultra-rapid phenotype is associated with extremely rapid clearance and thus inefficacy of antidepressants (Kirchheiner et al., 2001).

A promoter region variant in the enzyme UGT1A1, UGT1A1*28, which has an additional TA in comparison to the more common form of the gene, has been associated with a reduced transcription rate of *UGT1A1* and lower glucuronidation activity of the enzyme. This reduced activity has been associated with higher levels of the active metabolite of the cancer chemotherapeutic agent *irinotecan* (see Chapters 6). The metabolite, SN38, which is eliminated by glucuronidation, is associated with the risk of toxicity (Iyer et al., 2002; Rosner and Panetta, 2008), which will be more severe in individuals with genetically lower *UGT1A1* activity (see Figures 6–5, 6–7, and 6–8).

CYP2C19, historically termed mephenytoin hydroxylase, displays penetrant pharmacogenetic variability, with just a few SNPs accounting for the majority of the deficient, poor metabolizer phenotype (Mallal et al., 2002). The deficient phenotype is much more common in Chinese and Japanese populations. Several proton pump inhibitors, including omeprazole and lansoprazole, are inactivated by CYP2C19. Thus, the deficient 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 (Figure 7–12).

Both pharmacokinetic and pharmacodynamic polymorphisms affect warfarin dosing. The anticoagulant warfarin is catabolized by CYP2C9, and its action is partly dependent upon the baseline level of reduced vitamin K (catalyzed by vitamin K epoxide reductase; Figures 7–13 and 30–7). Inactivating polymorphisms in *CYP2C9* are common (Goldstein, 2001), with 2-10% of most populations being homozygous for low-activity variants, and are associated with lower warfarin clearance, a higher risk of bleeding complications, and lower dose requirements (see Table 30–2 and Aithal et al., 1999). Combined with

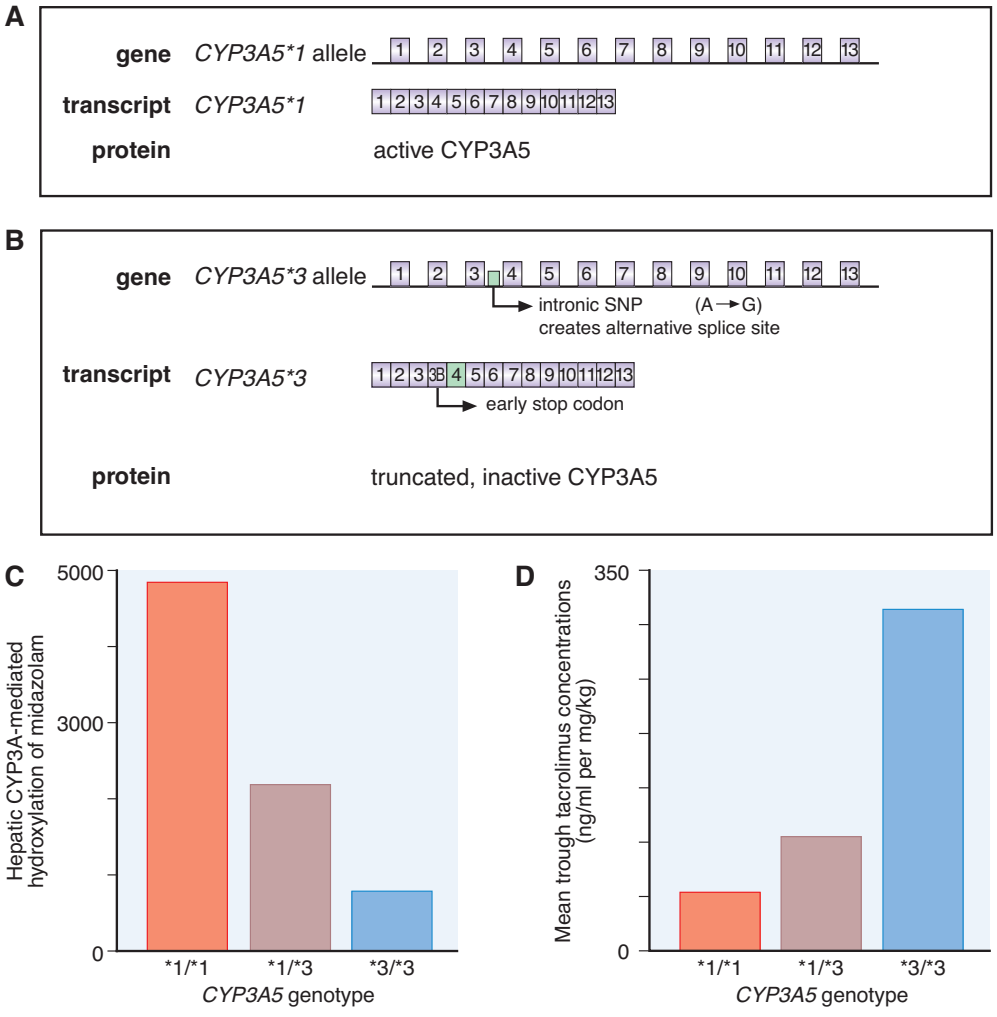


Figure 7–11. *An intronic SNP can affect splicing and account for polymorphic expression of CYP3A5.* A common polymorphism (A>G) in intron 3 of CYP3A5 defines the genotypes associated with the wild-type CYP3A5∗1 allele, or the variant nonfunctional CYP3A5∗3 allele. This intronic SNP creates an alternative splice site that results in the production of an alternative CYP3A5 transcript carrying an additional intron 3B (panel B), with an accompanying early stop codon and truncated CYP3A5 protein. Whereas the wild-type gene (more common in African than Caucasian or Asian populations) results in production of active CYP3A5 protein (panel A), the ∗3 variant results in a truncated and inactive CYP3A5 protein. Thus, metabolism of CYP3A5 substrates is diminished *in vitro* (panel C, shown for midazolam) and blood concentrations of such medications are higher *in vivo* (panel D, shown for tacrolimus) for these with the ∗3 than the ∗1 allele. (Based on data from Haufroid et al., 2004; Kuehl et al., 2001; Lin et al., 2002.)

genotyping for a common polymorphism in *VKORC1*, inherited variation in these two genes account for 20-60% of the variability in warfarin doses needed to achieve the desired INR, and use of these tests in the clinic can result in fewer bleeding complications and shorter time of trial-and-error to achieve the desired steady state level of anticoagulation. (Caraco et al., 2008; Lesko, 2008; Schwarz et al., 2008).

Thiopurine methyltransferase (TPMT) methylates thiopurines such as mercaptopurine (an anti-leukemic drug that is also the product of azathioprine metabolism; Figure 47–5). One in 300 individuals is homozygous deficient, 10% are heterozygotes, and ~90% are homozygous for the wild-type alleles for *TPMT* (Weinshilboum and Sladek, 1980). Three SNPs account for over 90% of the inactivating alleles (Yates et al., 1997). Because methylation of mercaptopurine competes with activation of the drug to thioguanine nucleotides, the concentration of the active (but also toxic) thioguanine metabolites is inversely related to

Table 7–3

Examples of Genetic Polymorphisms Influencing Drug Response

GENE PRODUCT *(GENE)*	DRUGS*	RESPONSES AFFECTED
Drug Metabolism and Transport		
CYP2C9	Tolbutamide, warfarin,* phenytoin, nonsteroidal anti-inflammatory	Anticoagulant effect of warfarin
CYP2C19	Mephenytoin, omeprazole, voriconazole*, hexobarbital, mephobarbital, propranolol, proguanil, phenytoin, clopidogrel	Peptic ulcer response to omeprazole; cardiovascular events after clopidogrel
CYP2D6	β blockers, antidepressants, anti-psychotics, codeine, debrisoquine, atomoxetine*, 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*	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*, thioguanine*, azathioprine*	Thiopurine toxicity and efficacy, risk of second cancers
UDP-glucuronosyl-transferase *(UGT1A1)*	Irinotecan*, 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
COMT	Levodopa	Enhanced drug effect
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

(*Continued*)

Table 7–3

Examples of Genetic Polymorphisms Influencing Drug Response (*Continued*)

GENE PRODUCT *(GENE)*	DRUGS*	RESPONSES AFFECTED
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 (5-HTT)	Antidepressants (e.g., clomipramine, fluoxetine, paroxetine, fluvoxamine)	Clozapine effects, 5-HT neurotransmission, antidepressant response
Serotonin receptor ($5\text{-}HT_{2A}$)	Antipsychotics	Clozapine antipsychotic response, tardive dyskinesia, paroxetine antidepression response, drug discrimination
HMG-CoA reductase	Pravastatin	Reduction in serum cholesterol
Vitamin K oxidoreductase (VKORC1)	Warfarin*	Anticoagulant effect, bleeding risk
Corticotropin releasing hormone receptor (CRHR1)	Glucocorticoids	Bronchodilation, osteopenia
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's disease
Human leukocyte antigen	Abacavir, carbamazepine, phenytoin	Hypersensitivity reactions

(*Continued*)

Table 7–3

Examples of Genetic Polymorphisms Influencing Drug Response (*Continued*)

GENE PRODUCT (*GENE*)	DRUGS*	RESPONSES AFFECTED
G6PD deficiency	Rasburicase*, dapsone*	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; Roden, 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 (ITPA)	Azathioprine, mercaptopurine	Myelosuppression
Vitamin D receptor	Estrogen	Bone mineral density

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

TPMT activity and directly related to the probability of pharmacologic effects. Dose reductions (from the "average" population dose) may be required to avoid myelosuppression in 100% of homozygous deficient patients, 35% of heterozygotes, and only 7-8% of those with homozygous wild-type activity (Relling et al., 1999). The rare homozygous deficient patients can tolerate 10% or less of the mercaptopurine doses tolerated by the homozygous wild-type patients, with heterozygotes often requiring an intermediate dose. Conversely, homozygous wild-type patients show less anti-leukemic response to a short course of mercaptopurine than

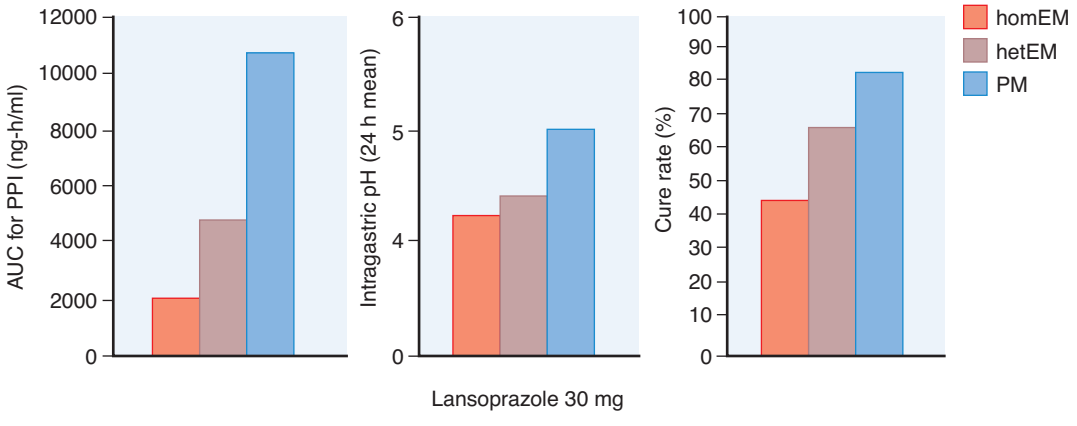


Figure 7–12. *Effect of CYP2C19 genotype on proton pump inhibitor (PPI) pharmacokinetics (AUC), gastric pH, and ulcer cure rates.* Depicted are the average variables for *CYP2C19* homozygous extensive metabolizers (homEM), heterozygotes (hetEM), and poor metabolizers (PM). (Reproduced with permission from Furuta T et al. Pharmcogenomics of proton pump inhibitors. *Pharmacogenomics*, **2004**, 5: 181–202. Copyright © 2004 Future Medicine Ltd. All rights reserved.)

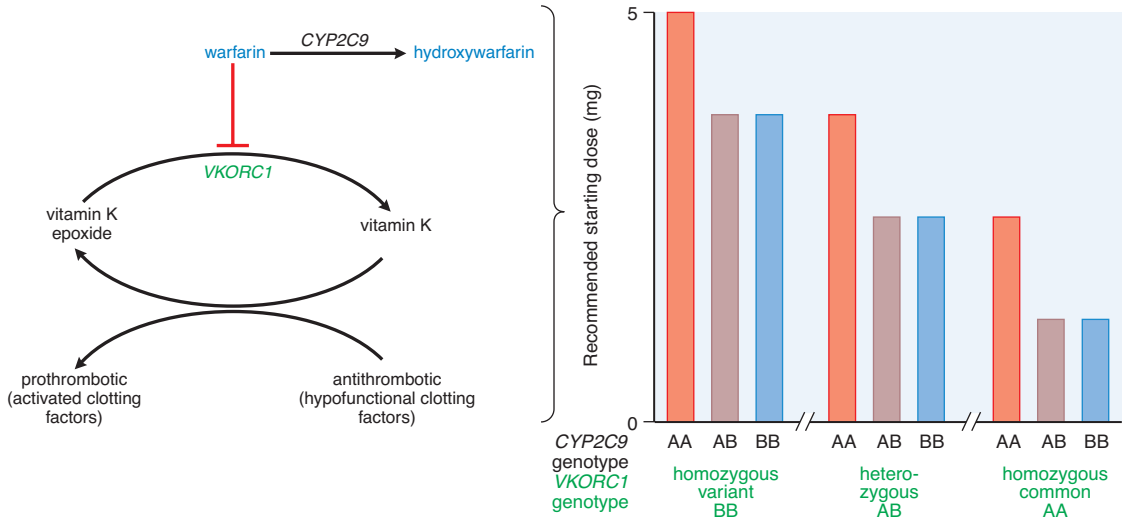


Figure 7–13. *Pharmacogenetics of warfarin dosing.* Warfarin is metabolized by *CYP2C9* to inactive metabolites, and exerts its anticoagulant effect partly via inhibition of *VKORC1* (vitamin K epoxide hydrolase), an enzyme necessary for reduction of inactive to active vitamin K. Common polymorphisms in both genes, *CYP2C9* and *VKORC1*, impact 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 international normalized ratio [INR] blood test) and minimize the risk of too little anticoagulation (thrombosis) or too much anticoagulation (bleeding). (Based on data from Caraco et al., 2008; Schwarz et al., 2008; Wen et al., 2008.)

those with at least one inactive *TPMT* allele (Stanulla et al., 2005). Mercaptopurine has a narrow therapeutic range, and dosing by trial and error can place patients at higher risk of toxicity; thus, prospective adjustment of *thiopurine* doses based on *TPMT* genotype has been suggested (Lesko and Woodcock, 2004). Life-threatening toxicity has also been reported when thiopurines have been given to patients with nonmalignant conditions (such as Crohn's disease, arthritis, or for prevention of solid organ transplant rejection) (Evans and Johnson, 2001; Evans and Relling, 2004; Weinshilboum, 2003).

Pharmacogenetics and Drug Receptors/Targets. Gene products that are direct targets for drugs have an important role in pharmacogenetics (Johnson and Lima, 2003). Whereas highly penetrant variants with profound functional consequences in some genes 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, complete inactivation by means of rare point mutations in methylenetetrahydrofolate reductase (MTHFR) causes severe mental retardation, cardiovascular disease, and a shortened lifespan (Goyette et al., 1994). MTHFR reduces 5,10-CH_2- to 5-CH_3-tetrahydrofolate, and thereby interacts with folate-dependent one-carbon synthesis reactions, including homocysteine/methionine metabolism and pyrimidine/purine synthesis (see Chapter 61). This pathway is the target of several antifolate drugs. For details, see the methotrexate pathway at www.pharmGKB.org.

Whereas rare inactivating variants in *MTHFR* may result in early death, the 677C→T SNP causes an amino acid substitution that is maintained in the population at a high frequency (variant allele, q, frequency in most white populations = 0.4). This variant is associated with modestly lower MTHFR activity (~30% less than the 677C allele) and modest but significantly elevated plasma homocysteine concentrations (about 25% higher) (Klerk et al., 2002). 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. Following prophylactic treatment with methotrexate for graft-versus-host disease, mucositis was three times more common among patients homozygous for the 677T allele than those homozygous for the 677C allele (Ulrich et al., 2001).

Factors Modifying Methotrexate Action. The methotrexate pathway involves metabolism, transport, drug modifier, and drug target polymorphisms. Methotrexate is a substrate for transporters and anabolizing enzymes that affect its intracellular pharmacokinetics and that are subject to common polymorphisms (see methotrexate pathway at www.pharmGKB.org). Several of the direct targets (dihydrofolate reductase, purine transformylases, and thymidylate synthase [TYMS]) are also subject to common polymorphisms.

A polymorphic indel in *TYMS* (two vs. three repeats of a 28-base pair repeat in the enhancer) affects the amount of enzyme expression in both normal and tumor cells. The polymorphism is quite common, with alleles equally split between the lower-expression two-repeat and the higher-expression three-repeat alleles. The *TYMS* polymorphism can affect both toxicity and efficacy of anticancer agents (e.g., fluorouracil and methotrexate) that target TYMS (Krajinovic et al., 2002). 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 Polymorphisms. Many drug target polymorphisms have been shown to predict responsiveness to drugs (Table 7–3). Serotonin receptor polymorphisms predict not only the responsiveness to antidepressants, but also the overall risk of depression (Murphy et al., 2003). β Adrenergic receptor polymorphisms have been linked to asthma responsiveness (degree of change in 1-second forced expiratory volume after use of a β agonist) (Tan et al., 1997), renal function following angiotensin-converting enzyme (ACE) inhibitors (Essen et al., 1996), and heart rate following β blockers (Taylor and Kennedy, 2001). Polymorphisms in 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase have been linked to the degree of lipid lowering following statins, which are HMG-CoA reductase inhibitors (see Chapter 31), and to the degree of positive effects on high-density lipoproteins among women on estrogen replacement therapy (Herrington et al., 2002). Ion channel polymorphisms have been linked to a risk of cardiac arrhythmias in the presence and absence of drug triggers (Roden, 2004).

Polymorphism-Modifying Diseases and Drug Responses. Some genes may be involved in an underlying disease being treated, but do not directly interact with the drug. Modifier polymorphisms are important for the *de novo* risk of some events and for the risk of drug-induced events. The *MTHFR* polymorphism, e.g., is linked to homocysteinemia, which in turn affects thrombosis risk (den Heijer, 2003). The risk of a drug-induced thrombosis is dependent not only on the use of prothrombotic drugs, but on environmental and genetic predisposition to thrombosis, which may be affected by germline polymorphisms in *MTHFR*, factor V, and prothrombin (Chanock, 2003). 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., *HERG*, KvLQT1, Mink, and *MiRP1*) may affect the overall risk of cardiac dysrhythmias, which may be accentuated in the presence of a drug that can prolong the QT interval in some circumstances (e.g., macrolide antibiotics, antihistamines) (Roden, 2003). These modifier polymorphisms may impact on the risk of "disease" phenotypes even in the absence of drug challenges; in the presence of drug, the "disease" phenotype may be elicited.

Cancer As a Special Case. Cancer pharmacogenetics have an unusual aspect in that tumors exhibit somatically acquired mutations in addition to the underlying germline variation of the host. Thus, the efficacy of some anticancer drugs depends on the genetics of both the host and the tumor. For example, non-small-cell lung cancer is treated with an inhibitor of epidermal growth factor receptor (EGFR), gefitinib. Patients whose tumors have activating mutations in the tyrosine kinase domain of *EGFR* appear to respond better to gefitinib than those without the mutations (Lynch et al., 2004). Thus, the receptor is altered, and at the same time, individuals with the activating mutations may be considered to have a distinct category of non-small-cell lung cancer. Breast cancer patients with expression of the Her2 antigen (as an acquired genetic changes) are more likely to benefit from the antibody trastuzumab than are those who are negative for Her2 expression, and this results in a common tailoring of anticancer therapy in patients with breast cancer based on tumor genetics. As an example of a gene that affects both tumor and host, the presence of two instead of three copies of a *TYMS* enhancer repeat polymorphism increases the risk of host toxicity but also increases the chance of tumor susceptibility to thymidylate synthase inhibitors (Evans, and McLeod, 2003; Relling and Dervieux, 2001; Villafranca et al., 2001).

Pharmacogenetics and Drug Development

Pharmacogenetics will likely impact drug regulatory considerations in several ways (Evans and Relling, 2004; Lesko and Woodcock, 2004; Weinshilboum and Wang, 2004). Genome-wide approaches hold promise for identification of new drug targets and therefore new drugs. In addition, accounting for genetic/genomic inter-individual variability may lead to genotype-specific development of new drugs, and to genotype-specific dosing regimens. Recently, the U.S. Food and Drug Administration (FDA) altered the labels of several drugs in clinical use to indicate a pharmacogenetic issue (Table 7–3). With time and study, other drug labels will likely be changed as well.

Pharmacogenomics can identify new targets. For example, genome-wide assessments using microarray technology could identify genes whose expression differentiates inflammatory processes; a compound could be identified that changes expression of that gene; and then that compound could serve as a starting point for anti-inflammatory drug development. Proof of principle has been demonstrated for identification of anti-leukemic agents (Stegmaier et al., 2004) and antifungal drugs (Parsons et al., 2004), among others.

Pharmacogenetics may identify subsets of patients who will have a very high or a very low likelihood of responding to an agent. This will permit testing of the drug

in a selected population that is more likely to respond, minimizing the possibility of adverse events in patients who derive no benefit, and more tightly defining the parameters of response in the subset more likely to benefit. Somatic mutations in the *EGFR* gene strongly identify patients with lung cancer who are likely to respond to the tyrosine kinase inhibitor gefitinib (Lynch et al., 2004); germline variations in 5-lipoxygenase (*ALOX5*) determine which asthma patients are likely to respond to ALOX inhibitors (Drazen et al., 1999); and vasodilation in response to β_2 agonists has been linked to β_2 adrenergic receptor polymorphisms (Johnson and Lima, 2003).

A related role for pharmacogenomics in drug development is to identify which genetic subset of patients is at highest risk for a serious adverse drug effect, and to avoid testing the drug in that subset of patients (Lesko and Woodcock, 2004). For example, the identification of HLA subtypes associated with hypersensitivity to the HIV-1 reverse transcriptase inhibitor abacavir (Mallal et al., 2002, 2008) identifies a subset of patients who should receive alternative antiretroviral therapy, and this has been shown to decrease the frequency of hypersensitivity as an adverse effect of this agent. Children with acute myeloid leukemia who are homozygous for germline deletions in GSH transferase (*GSTT1*) are almost three times as likely to die of toxicity as those patients who have at least one wild-type copy of *GSTT1* following intensively timed antileukemic therapy but not after "usual" doses of antileukemic therapy (Davies et al., 2001). These latter results suggest an important principle: pharmacogenetic testing may help to identify patients who require altered dosages of medications, but will not necessarily preclude the use of the agents completely.

Pharmacogenetics in Clinical Practice

Despite considerable research activity, pharmacogenetics are not yet widely utilized in clinical practice. There are three major types of evidence that should accumulate in order to implicate a polymorphism in clinical care (Figure 7–14):

- screens of tissues from multiple humans linking the polymorphism to a trait
- complementary preclinical functional studies indicating that the polymorphism is plausibly linked with the phenotype
- multiple supportive clinical phenotype/genotype association studies

Because of the high probability of type I error in genotype/phenotype association studies, replication of clinical findings will generally be necessary. Although the impact of the polymorphism in TPMT on mercaptopurine dosing in childhood leukemia is a good example of a polymorphism for which all three types of evidence are available, proactive individualized dosing of thiopurines based on genotype has not been widely incorporated into clinical practice (Lesko et al., 2004).

Most drug dosing relies on a population "average" dose of drug. Adjusting dosages for variables such as renal or liver dysfunction is often accepted in drug dosing, even in cases in which the clinical outcome of such adjustments has not been studied. Even though there are many examples of significant effects of polymorphisms on drug disposition (e.g., Table 7–3), there is much more hesitation from clinicians to adjust doses based on genetic testing than on indirect clinical measures of renal and liver function. Whether this hesitation reflects resistance to abandon the "trial-and-error" approach that has defined most drug dosing, concern about genetic discrimination, or unfamiliarity with the principles of genetics is not clear. Nonetheless, broad public initiatives, such as the NIH-funded Pharmacogenetics and Pharmacogenomics Knowledge Base (www.pharmGKB.org), provide useful resources to permit clinicians to access information on pharmacogenetics (see Table 7–1). The passage of laws to prevent genetic discrimination (Erwin, 2008) may also assuage concerns that genetic data placed in medical records could penalize those with "unfavorable" genotypes.

The fact that functionally important polymorphisms are so common means that complexity of dosing will be likely to increase substantially in the postgenomic era. Even if every drug has only one important polymorphism to consider when dosing, the scale of complexity could be large. Many individuals take multiple drugs simultaneously for different diseases, and many therapeutic regimens for a single disease consist of multiple agents. This situation translates into a large number of possible drug-dose combinations. Much of the excitement regarding the promise of human genomics has emphasized the hope of discovering individualized "magic bullets," and ignored the reality of the added complexity of additional testing and need for interpretation of results to capitalize on individualized dosing. This is illustrated in a potential pharmacogenetic example in Figure 7–14. In this case, a traditional anticancer treatment approach is replaced with one that incorporates pharmacogenetic information with the stage of the cancer determined by a variety of standardized pathological criteria. Assuming just one important genetic polymorphism for each of the

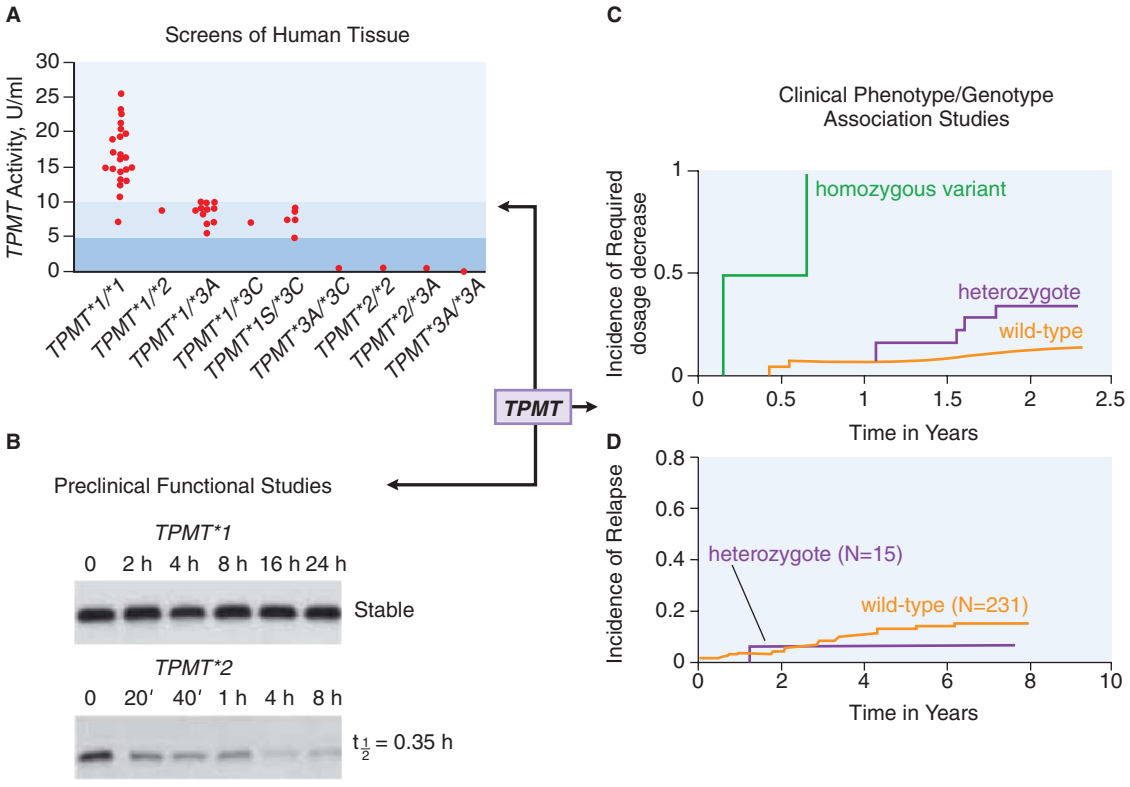


Figure 7–14. *Three primary types of evidence in pharmacogenetics.* Screens of human tissue (***A***) link phenotype (thiopurine methyltransferase activity in erythrocytes) with genotype (germline *TPMT* genotype). The two alleles are separated by a slash (/); the ∗1 and ∗1S alleles are wild-type, and the ∗2, ∗3A, and ∗3C are nonfunctional alleles. Shaded areas indicate low and intermediate levels of enzyme activity: those with the homozygous wild-type genotype have the highest activity, those heterozygous for at least one ∗1 allele have intermediate activity, and those homozygous for two inactive alleles have low or undetectable TPMT activity (Yates et al., 1997). Directed preclinical functional studies (***B***) can provide biochemical data consistent with the *in vitro* screens of human tissue, and may offer further confirmatory evidence. Here, the heterologous expression of the *TPMT∗1* wild-type and the *TPMT∗2* variant alleles indicate that the former produces a more stable protein, as assessed by Western blot (Tai et al., 1997). The third type of evidence comes from clinical phenotype/genotype association studies (***C*** and ***D***). The incidence of required dosage decrease for thiopurine in children with leukemia (***C***) differs by *TPMT* genotype: 100%, 35%, and 7% of patients with homozygous variant, heterozygous, or homozygous wild-type, respectively, require a dosage decrease (Relling et al., 1999). When dosages of thiopurine are adjusted based on *TPMT* genotype in the successor study (***D***), leukemic relapse is not compromised, as indicated by comparable relapse rates in children who were wild-type *vs*. heterozygous for *TPMT*. Taken together, these three data sets indicate that the polymorphism should be accounted for in dosing of thiopurines. (Reproduced with permission from Relling et al., 1999. Copyright © Oxford University Press.)

three different anticancer drugs, 11 individual drug regimens can easily be generated.

Nonetheless, the potential utility of pharmacogenetics to optimize drug therapy is great. After adequate genotype/phenotype studies have been conducted, molecular diagnostic tests will be developed, and genetic tests have the advantage that they need only be conducted once during an individual's lifetime. With continued incorporation of pharmacogenetics into clinical trials, the important genes and polymorphisms will be identified, and data will demonstrate whether dosage individualization can improve outcomes and decrease short- and long-term adverse effects. Significant covariates will be identified to allow refinement of dosing in the context of drug interactions and disease influences. Although the challenges are substantial, accounting for the genetic basis of variability in response to medications is already being used in specific pharmacotherapeutics decisions, and is likely to become a fundamental component of diagnosing any illness and guiding the choice and dosage of medications.

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