**PHARMACOGENETICS**

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*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. Until the technical advances in genomics of the last few years, 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.

**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) (see Chapter 39) 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).

Individuals differ from each other approximately every 300 to 1000 nucleotides, with an estimated total of 3.2 million single nucleotide polymorphisms (SNPs; single base pair substitutions found at frequencies ≥1% in a population) in the genome (Sachidanandam et al., 2001; The International SNP Map Working Group, 2001). Identifying which of these variants or combinations of variants have functional consequence for drug effects is the task of modern pharmacogenetics.

**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 4–1). 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 half-life of *antipyrine* was measured are typical (Figure 4–2). Antipyrine, a pyrazolone analgesic, is eliminated exclusively by metabolism and is a substrate for multiple CYPs. There is considerably greater concordance in the half-life 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 approximately 75% to 85% of the variability in pharmacokinetic half-lives for drugs that are eliminated by metabolism is heritable (Penno et al., 1981). It has also
be 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 the overall contribution of inheritance to the phenotype; because multiple gene products contribute to antipyrine disposition, most of which have uneluciated mechanisms of genetic variability, the predictability of antipyrine disposition based on known genetic variability is poor.

Another approach to estimating the degree of heritability of a pharmacogenetic phenotype uses *ex vivo* experiments with cell lines derived from related individuals. 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 about 20% to 70% of the variability in sensitivity to 5-fluorouracil and docetaxel estimated as inherited, depending upon dose (Watters et al., 2004).

For the “monogenic” phenotypic traits of G6PD deficiency, CYP2D6 or thiopurine methyltransferase (TPMT) metabolism, it is 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 (Meyer, 2000).

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.

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**Figure 4–1.** Exogenous and endogenous factors contribute to variation in drug response. (Reproduced with permission from Vesell, 1991.)

**Figure 4–2.** Pharmacogenetic contribution to pharmacokinetic parameters. Half-life of antipyrine is more concordant in identical in comparison to fraternal twin pairs. Bars show the half-life of antipyrine in identical (monozygotic) and fraternal (dizygotic) twin pairs. (Redrawn from data in Vesell and Page, 1968.)
Chapter 4  /  Pharmacogenetics

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 monogenetic 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 phenotype is evident only with nonfunctional alleles on both the maternal and paternal chromosomes. For many of the earliest identified pharmacogenetic polymorphisms, phenotype did not differ enough between heterozygotes and homozygous “wild-type” individuals to distinguish that heterozygotes exhibited an intermediate (or codominant) phenotype (e.g., for CYP2D6-mediated debrisoquine metabolism). Other traits, such as TPMT, exhibit three relatively distinct phenotypes, and thus were deemed codominant even in the premolecular era. With the advances in molecular characterization of polymorphisms and a genotype-to-phenotype approach, additional 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 the individual is predisposed to cardiac arrhythmias (see Chapter 34).

In an era of detailed molecular characterization, two major factors complicate the historical designation of recessive, codominant, and dominant traits. First, even within a single gene, a vast array of polymorphisms (promoter, coding, noncoding, completely inactivating, or modestly modifying) are possible, making the assignment of “variant” vs. “wild-type” to an allele a designation that depends upon a complete survey of the gene’s polymorphisms and is not necessarily easily assigned. Secondly, most traits (pharmacogenetic and otherwise) are multigenic, not monogenic. Thus, 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 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 4–3). In comparison to base pair 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 of 1% or greater in a population are termed single nucleotide polymorphisms (SNPs) and are present in the human genome at approximately 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. cSNPs are further classified as nonsynonymous (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 4–3, do not alter the encoded amino acid. Base pair substitutions that lead to a stop codon are termed nonsense mutations. In addition, about 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.

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 4–4). 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 to 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 Tew, 2003a). The number of TA repeats in the UGT1A1 promoter affects the quantitative expression of this crucial glucuronosyl transferase in liver; although 4 to 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). Although in many of these cases the local sequence context of these insertions/deletions strongly suggests mechanisms underlying the genomic alterations (e.g., homologous recombination sites bracket the GSTM1 deletion), high allele frequencies are maintained due to Mendelian inheritance.

A haplotype, which is defined as 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, 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<sub>3421</sub>C<sub>3435</sub>, T<sub>3421</sub>T<sub>3435</sub>, A<sub>3421</sub>C<sub>3435</sub>, and A<sub>3421</sub>T<sub>3435</sub>. 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).

**Ethnic Diversity**

Polymorphisms differ in their frequencies within human populations (Burchard et al., 2003; Rosenberg et al., 2002; Rosenberg et al., 2003). Among coding region SNPs, synonymous SNPs are present, on average, at higher frequencies than nonsynonymous SNPs. Thus, for most genes, the nucleotide diversity, which reflects the number of SNPs and the frequency of the SNPs, is greater for synonymous than for nonsynonymous SNPs. This fact reflects selective pressures (termed negative or purifying selection), which act to preserve the functional activity of proteins, and therefore the amino acid sequence. 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 United States 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 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 4–5). Shown are nonsynonymous and synonymous SNPs; population-specific nonsynonymous cSNPs are indicated in the figure. The multidrug resistance associated protein, MRP2, has a large number of nonsynonymous cSNPs. There are fewer nonsynonymous variants than nonsynonymous variants, but the allele frequencies of the synonymous variants are greater than those of the nonsynonymous variants (Leabman et al., 2003). By comparison, DAT, the dopamine transporter, has a number of synonymous variants but no nonsynonymous 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 about 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%.

**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) (Monganhan 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; Weinsilboum, 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 in 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 haplo-insufficiency, 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

*Figure 4–5. 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 light gray are synonymous variants, and in black, nonsynonymous variants. (Reproduced with permission from Shu et al., 2003.)
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 4–6), considerable effort is being made to identify the important genes and their polymorphisms that influence variability in drug response.

Most genotyping methods use germline DNA, that is, 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 be performed only once, because DNA sequence is generally invariant throughout an individual’s lifetime. Although tremendous progress has been made in molecular biological techniques to determine genotypes, relatively few are used routinely in patient care. Genotyping tests are directed at each specific known polymorphic site using a variety of strategies that generally depend at some level on the specific and avid annealing of at least one oligonucleotide to a region of DNA flanking or overlapping the polymorphic site (Koch, 2004). Because genomic variability is so common (with polymorphic sites every few hundred nucleotides), “cryptic” or unrecognized polymorphisms may interfere with oligonucleotide annealing, thereby resulting in false positive or false negative genotype assignments. Full integration of genotyping into therapeutics will require high standards for genotyping technology, perhaps with more than one method required for each polymorphic site.

One method to assess the reliability of genotype determinations 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 ($2pq$) are not significantly different from that predicted from the overall allele frequencies ($p = fre-
equency of wild-type allele; \( q \) = frequency of variant allele) in the population. If proportions of the observed three genotypes, which must add up to one, differ significantly from those predicted, it may indicate that a genotyping error may be present.

Because polymorphisms are so common, haplotype (the allelic structure that indicates whether polymorphisms within a gene are on the same or different alleles) may also be important. Thus far, experimental methods to unambiguously confirm whether polymorphisms are allelic has proven to be feasible but technically challenging (McDonald et al., 2002). Most investigators use statistical probability to assign putative or inferred haplotypes; e.g., because the two most common SNPs in TPMT (at 460 and 719) often are allelic, a genotyping result showing heterozygosity at both SNPs will have a >95% chance of reflecting one allele wild-type and one allele variant at both SNP positions (resulting in a “heterozygote” genotype for TPMT). However, the remote prospect that each of the two alleles carries a single SNP variant, thereby conferring a homozygous variant/deficient phenotype, is a theoretical possibility.

**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 4–1), which allow the investigator to search by gene for polymorphisms that have been reported. Some of the databases, such as the Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB), include phenotypic as well as genotypic data.

Because it is currently not practical to analyze all polymorphisms in a candidate gene association study, it is important to select polymorphisms that are likely to be associated with the drug-response phenotype. For this purpose, there are two categories of polymorphisms. The first are polymorphisms that do not, in and of themselves, cause altered function of the expressed protein (e.g., an enzyme that metabolizes the drug or the drug receptor). Rather, these polymorphisms are linked to the variant allele that produces the altered function. These polymorphisms serve as biomarkers for drug-response phenotype. However, their major shortcoming is that unless they are in 100% linkage with the causative polymorphism, they are not the best markers for the drug-response phenotype.

The second type of polymorphism is the *causative polymorphism*, which directly precipitates the pheno-

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**Table 4–1**

_Databases Containing Information on Human Genetic Variation_

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<td><a href="http://www.pharmgkb.org">www.pharmgkb.org</a> (NIH Sponsored Research Network and Knowledge Database)</td>
<td>Genotype and phenotype data related to drug response</td>
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<tr>
<td>Human Genome Variation Database (HGVbase)</td>
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<td>Genotype/phenotype associations</td>
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<tr>
<td>Human Gene Mutation Database (HGMD)</td>
<td>www hgmd.org/</td>
<td>Mutations/SNPs in human genes</td>
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type. 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. Whenever possible, it is desirable to select polymorphisms for pharmacogenetic studies that are likely to be causative (Tabor et al., 2002). If biological information indicates that a particular polymorphism alters function, for example, in cellular assays of nonsynonymous variants, this polymorphism is an excellent candidate to use in an association study.

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 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. Patterns of gene expression, clusters of polymorphisms or heterozygosity, 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; for example, 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 SNPs raises the danger of type I error (finding differences that are false-positives). Nonetheless, technology is rapidly evolving for genome-wide surveys of RNA, DNA, and protein, and such approaches hold promise for future pharmacogenomic discoveries.

**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 protein function, stability, or subcellular localization. One way that we can 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 number of DNA variations associated with diseases or traits are missense and nonsense mutations, followed by deletions (Figure 4–7).

Further studies have suggested that of 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 nonsynonymous 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. This suggests 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 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).

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 4–2 (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 and SIFT).
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 nonsynonymous 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. In the past few years, large-scale functional analyses have been performed on genetic variants in membrane transporters and phase II enzymes. Figure 4–8 shows the function of all nonsynonymous 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). As shown, most of the naturally occurring variants have similar functional activity as 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 4–9 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, is characterized by an 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, predicting the function of SNPs in noncoding regions of genes represents a major new challenge in.
human genetics and pharmacogenetics. The principles of evolutionary conservation that have been shown to be important in predicting the function of nonsynonymous 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).

**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 enzymes and transporters, affect drug concentrations, and are therefore major determinants of therapeutic and adverse drug response (Table 4–3; 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 gene that is the target of polymorphisms in each case (NAT2 and CYP2D6 polymorphisms, 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% to 10% of treated 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% to 25% of all medicines in use) have been shown to be substrates for CYP2D6 (Table 4–3). 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 include increased risk of toxicity of antidepressants or antipsychotics (catabolized by the enzyme), and lack of analgesic effects of codeine (anabolized by the enzyme); 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 3 and 51). The metabolite, SN38, which is eliminated by glucuronidation, is associated with the risk of toxicity (Iyer et al., 2002), which will be more severe in individuals with genetically lower UGT1A1 activity.

CYP2C19 codes for a cytochrome P450, historically termed mephenytoin hydroxylase, that 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
<table>
<thead>
<tr>
<th>GENE PRODUCT (GENE)</th>
<th>DRUGS</th>
<th>RESPONSES AFFECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug metabolizers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Tolbutamide, warfarin, phenytoin, nonsteroidal antiinflammatories</td>
<td>Anticoagulant effect of warfarin (Aithal et al., 1999; Roden, 2003; Weinshilboum, 2003)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Mephenytoin, omeprazole, hexobarbital, mephobarbital, propranolol, proguanil, phenytoin</td>
<td>Peptic ulcer response to omeprazole (Kirchheiner et al., 2001)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>β blockers, antidepressants, anti-psychotics, codeine, debrisoquine, dextromethorphan, encaïnide, flecainide, fluoxetine, guanoxan, N-propylajmaline, perhexiline, phenacetin, phenformin, propafenone, sparteine</td>
<td>Tardive dyskinesia from antipsychotics, narcotic side effects, codeine efficacy, imipramine dose requirement, β blocker effect (Kirchheiner et al., 2001; Weinshilboum, 2003)</td>
</tr>
<tr>
<td>CYP3A4/3A5/3A7</td>
<td>Macrolides, cyclosporine, tacrolimus, Ca²⁺ channel blockers, midazolam, terfenadine, lidocaine, dapsone, quinidine, triazolam, etoposide, teniposide, lovastatin, alfenatil, tamoxifen, steroids</td>
<td>Efficacy of immunosuppressive effects of tacrolimus (Evans and Relling, 2004)</td>
</tr>
<tr>
<td><strong>Dihydropyrimidine dehydrogenase</strong></td>
<td>Fluorouracil</td>
<td>5-Fluorouracil neurotoxicity (Chibana et al., 2002)</td>
</tr>
<tr>
<td><strong>N-acetyltransferase (NAT2)</strong></td>
<td>Isoniazid, hydralazine, sulfonamides, aminoflavine, procainamide, dapsone, caffeine</td>
<td>Hypersensitivity to sulfonamides, aminoflavine toxicity, hydralazine-induced lupus, isoniazid neurotoxicity (Roden, 2003; Grant et al., 1990)</td>
</tr>
<tr>
<td><strong>Glutathione transferases (GSTM1, GSTT1, GSTP1)</strong></td>
<td>Several anticancer agents</td>
<td>Decreased response in breast cancer, more toxicity and worse response in acute myelogenous leukemia (Townsend and Tew, 2003b)</td>
</tr>
<tr>
<td><strong>Thiopurine methyltransferase (TPMT)</strong></td>
<td>Mercaptopurine, thioguanine, azathioprine</td>
<td>Thiopurine toxicity and efficacy, risk of second cancers (Relling and Dervieux, 2001; Weinshilboum, 2003)</td>
</tr>
<tr>
<td><strong>UDP-glucuronosyltransferase (UGT1A1)</strong></td>
<td>Irinotecan, bilirubin</td>
<td>Irinotecan toxicity (Iyer et al., 1998; Relling and Dervieux, 2001)</td>
</tr>
<tr>
<td><strong>P-glycoprotein (ABCB1)</strong></td>
<td>Natural product anticancer drugs, HIV protease inhibitors, digoxin</td>
<td>Decreased CD4 response in HIV-infected patients, decreased digoxin AUC, drug resistance in epilepsy (Fellay et al., 2002; Quirk et al., 2004; Roden, 2003; Siddiqui et al., 2003)</td>
</tr>
<tr>
<td><strong>UGT2B7</strong></td>
<td>Morphine</td>
<td>Morphine plasma levels (Sawyer et al., 2003)</td>
</tr>
<tr>
<td><strong>COMT</strong></td>
<td>Levodopa</td>
<td>Enhanced drug effect (Weinshilboum, 2003)</td>
</tr>
<tr>
<td><strong>CYP2B6</strong></td>
<td>Cyclophosphamide</td>
<td>Ovarian failure (Takada et al., 2004)</td>
</tr>
<tr>
<td><strong>Targets and receptors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin-converting enzyme (ACE)</td>
<td>ACE inhibitors (e.g., enalapril)</td>
<td>Renoprotective effects, hypotension, left ventricular mass reduction, cough (van Essen et al., 1996; Roden, 2003)</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>GENE PRODUCT (GENE)</th>
<th>DRUGS</th>
<th>RESPONSES AFFECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymidylate synthase</td>
<td>Methotrexate</td>
<td>Leukemia response, colorectal cancer response (Krajnovic et al., 2002; Relling and Dervieux, 2001)</td>
</tr>
<tr>
<td>β2 Adrenergic receptor (ADBR2)</td>
<td>β2 Antagonists (e.g., albuterol, terbutaline)</td>
<td>Bronchodilation, susceptibility to agonist-induced desensitization, cardiovascular effects (e.g., increased heart rate, cardiac index, peripheral vasodilation) (Roden, 2003; Tan et al., 1997)</td>
</tr>
<tr>
<td>β1 Adrenergic receptor (ADBR1)</td>
<td>β1 Antagonists</td>
<td>Response to β1 antagonists (Johnson and Lima, 2003)</td>
</tr>
<tr>
<td>5-Lipoxgenase (ALOX5)</td>
<td>Leukotriene receptor antagonists</td>
<td>Asthma response (Drazen et al., 1999)</td>
</tr>
<tr>
<td>Dopamine receptors (D2, D3, D4)</td>
<td>Antipsychotics (e.g., haloperidol, clozapine, thioridazine, nemonapride)</td>
<td>Antipsychotic response (D2, D3, D4), antipsychotic-induced tardive dyskinesia (D3) and acute akathisia (D3), hyperprolactinemia in females (D3) (Arranz et al., 2000; Evans and McLeod, 2003)</td>
</tr>
<tr>
<td>Estrogen receptor α</td>
<td>Estrogen hormone replacement therapy</td>
<td>High-density lipoprotein cholesterol (Herrington et al., 2002)</td>
</tr>
<tr>
<td>Serotonin transporter (5-HTT)</td>
<td>Antidepressants (e.g., clomipramine, fluoxetine, paroxetine, fluvoxamine)</td>
<td>Clozapine effects, 5-HT neurotransmission, antidepressant response (Arranz et al., 2000)</td>
</tr>
<tr>
<td>Serotonin receptor (5-HT2A)</td>
<td>Antipsychotics</td>
<td>Clozapine antipsychotic response, tardive dyskinesia, paroxetine antidepressant response, drug discrimination (Arranz et al., 2000) (Murphy et al., 2003)</td>
</tr>
<tr>
<td>HMG-CoA reductase</td>
<td>Pravastatin</td>
<td>Reduction in serum cholesterol</td>
</tr>
<tr>
<td>Adducin</td>
<td>Diuretics (e.g., simvastatin), tacrine</td>
<td>Myocardial infarction or strokes (Roden, 2003)</td>
</tr>
<tr>
<td>Apolipoprotein E</td>
<td>Statins (e.g., pravastatin)</td>
<td>Lipid-lowering; clinical improvement in Alzheimer’s (Evans and McLeod, 2003)</td>
</tr>
<tr>
<td>Human leukocyte antigen Cholesterol ester transfer protein</td>
<td>Abacavir</td>
<td>Hypersensitivity reactions (Mallal et al., 2002)</td>
</tr>
<tr>
<td>Ion channels (HERG, KvLQT1, Mink, MIRP1)</td>
<td>Erythromycin, cisapride, clarithromycin, quinidine</td>
<td>Slowing atherosclerosis progression (Evans and McLeod, 2003)</td>
</tr>
<tr>
<td>Methylguanine-deoxyribonucleic acid methyltransferase</td>
<td>Carmustine</td>
<td>Increased risk of drug-induced torsades de pointes, increased QT interval (Roden, 2003; Roden, 2004)</td>
</tr>
<tr>
<td>Parkin</td>
<td>Levodopa</td>
<td>Response of glioma to carmustine (Evans and McLeod, 2003)</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Methotrexate</td>
<td>Parkinson’s disease response (Evans and McLeod, 2003)</td>
</tr>
<tr>
<td>Prothrombin, factor V</td>
<td>Oral contraceptives</td>
<td>Gastrointestinal toxicity (Ulrich et al., 2001)</td>
</tr>
<tr>
<td>Tromboprol, factor V</td>
<td>Statins (e.g., pravastatin)</td>
<td>Venous thrombosis risk (Evans and McLeod, 2003)</td>
</tr>
<tr>
<td>Vitamin D receptor</td>
<td>Estrogen</td>
<td>Reduction in cardiovascular events and in repeat angioplasty (Evans et al., 2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone mineral density (Hustmyer et al., 1994)</td>
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heterozygotes or homozygous wild-type individuals (Figure 4–10).

The anticoagulant warfarin is catabolized by CYP2C9. Inactivating polymorphisms in CYP2C9 are common (Goldstein, 2001), with 2% to 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 (Aithal et al., 1999).

Thiopurine methyltransferase (TPMT) methylates thiopurines such as mercaptopurine (an antileukemic drug that is also the product of azathioprine metabolism). One in 300 individuals is homozygous deficient, 10% are heterozygotes, and about 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 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% to 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. 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 azathioprine has 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).

**Figure 4–10.** 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 et al., 2004.)

Pharmacogenetics and Drug 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 via 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-CH2- to 5-CH3-tetrahydrofolate, and thereby interacts with folate-dependent one-carbon synthesis reactions, including homocysteine/methionine metabolism and pyrimidine/purine synthesis (see Chapter 51). This pathway is the target of several antifolate drugs (Figure 4–11). Whereas rare 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 (about 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 gastrointestinal 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).

Methotrexate is a substrate for transporters and anabolizing enzymes that affect its intracellular pharmacokinetics and that are subject to common polymorphisms (Figure 4–11). 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.

Many drug target polymorphisms have been shown to predict responsiveness to drugs (Table 4–3). Serotonin receptor polymorphisms predict not only the responsiveness to antidepressants (Figure 4–12), 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 one-second forced expiratory volume after use of a β agonist) (Tan et al., 1997), renal function following angiotensin-converting enzyme (ACE) inhibitors (van Essen et al., 1996), and heart rate following β-blockers (Tay-
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 35), and to the degree of positive effects on high-density lipoproteins among women on estrogen replacement therapy (Herrington et al., 2002; Figure 4–13). 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, for example, 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 aspirin, 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.

Figure 4–12. Pharmacodynamics and pharmacogenetics. The proportion of patients requiring a dosage decrease for the antidepressant drug paroxetine was greater ($p = 0.001$) in the approximately one-third of patients who have the C/C genotype for the serotonin 2A receptor (5HT2A) compared to the two-thirds of patients who have either the T/C or T/T genotype at position 102 (Murphy et al., 2003). The major reason for dosage decreases in paroxetine was the occurrence of adverse drug effects. (Reproduced with permission from Greer et al., 2003.)

![Figure 4–12. Pharmacodynamics and pharmacogenetics.](image)

Figure 4–13. Effect of genotype on response to estrogen hormone replacement therapy. Depicted are pretreatment (base line) and posttreatment (follow-up) high-density lipoprotein (HDL) cholesterol levels in women of the C/C vs. C/T or T/T HMG-CoA reductase genotype. (Reproduced with permission from Herrington et al., 2002.)

![Figure 4–13. Effect of genotype on response to estrogen hormone replacement therapy.](image)
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 (see Chapter 51). 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. As another example, the TYMS enhancer repeat polymorphism affects not only host toxicity, but also tumor susceptibility to thymidylate synthase inhibitors (Evans and McLeod, 2003; Villafranca et al., 2001; Relling and Dervieux, 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 interindividual variability may lead to genotype-specific development of new drugs, and to genotype-specific dosing regimens.

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 antileukemic 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 $\beta_2$ agonists has been linked to $\beta_2$ 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) could theoretically identify a subset of patients who should receive alternative therapy, and thereby minimize or even abrogate 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 is rarely utilized in clinical practice (Evans and Johnson, 2001; Weinshilboum and Wang, 2004). There are three major types of evidence that should accumulate in order to implicate a polymorphism in clinical care (Figure 4–14): 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; and multiple supportive clinical phenotype/genotype 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 takes place using 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 4–3), 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, distrust of the genetic tests (which are constantly being refined), 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 4–1).

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 combina-

Figure 4–14. 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.)
tions. 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 4–15. 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 three anticancer drugs, 11 drug regimens can easily be generated. Nonetheless, the potential utility of pharmacogenetics to optimize drug therapy is great. Once adequate genotype/phenotype studies have been conducted, molecular
diagnostic tests will be developed that detect >95% of the important genetic variants for the majority of polymorphisms, 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 likely to become a fundamental component of diagnosing any illness and guiding the choice and dosage of medications.

**BIBLIOGRAPHY**


