Pharmacogenomics and Pediatric Pharmacotherapy

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LEARNING OBJECTIVES

- 1. Apply basic genomic and pharmacogenomic principles to therapeutic decision-making in children.
- 2. Account for the influence of genomic variation when estimating drug exposure and response.
- 3. Evaluate factors influencing the clinical utility of a pharmacogenomic test and judge when testing is warranted in an individual patient.
- 4. Apply the principles of developmental pharmacogenomics in the interpretation of genomic information.
- 5. Design a patient-specific treatment plan using genomic data.
- 6. Critically analyze data from candidate gene and genome-wide association studies.

INTRODUCTION

Variability in drug response presents a major challenge to the delivery of quality pharmaceutical care in pediatric patients. Drug therapy outcomes in children are often unpredictable and, depending on the individual child, can range from therapeutic benefit to life-threatening toxicity. Responses to drugs within the same child can also dramatically vary throughout the course of normal growth and development. To optimize therapeutic outcomes, it is important to understand the mechanisms responsible for this variability and to account for these mechanisms in the development of an optimal pharmacotherapeutic plan. Genetics, like organ function and age, is becoming increasingly recognized as an important source of variation in drug response. Alterations in genes encoding proteins involved in drug distribution or metabolism can considerably alter the dose-exposure-response relationship. For many complex diseases, genetic variation also plays an important role in determining the key pathogenic mediators, which ultimately serve as targets for pharmacotherapeutic intervention. The potential utility of genetic information in guiding dose and drug selection is therefore increasingly apparent.

The terms pharmacogenetics and pharmacogenomics are often used interchangeably to describe the study of relationships between an individual's genetic constitution (genotype) and his or her response to drugs (phenotype). However, important fundamental differences in these two terms, as illustrated in Figure 1-1, should be recognized. Pharmacogenetics focuses on variation in drug response that occurs because of large, single-gene (monogenic) effects. Inherited differences in drug-metabolizing enzyme capacity are examples of such monogenic traits, which can alter the dose-exposure-response relationship. For most pharmacologic agents, however, response is determined by the interaction of several genes and/or gene networks encoding proteins involved in drug disposition (e.g., transporters, enzymes) and action (e.g., receptors, enzymes). This polygenic nature of drug effect is encompassed within the scope of pharmacogenomics, which seeks to deter-

BASELINE KNOWLEDGE RESOURCES

The goal of PSAP is to provide only the most recent (past 3–5 years) information or topics. Chapters do not provide an overall review. Suggested resources for background information on the topic include:

- Cavallare LH, Francis Lam YW. Pharmacogenetics. In: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM, eds. Pharmacotherapy: A Pathophysiological Approach, 7th ed. Boston: McGraw-Hill, 2008:31–46.
- McLeod HL, DeVane CL, Haga SB, Johnson JA, Knoell DL, Kolesar JM, et al. In: McInerney JD, Rogers PD, Walker JR, eds. Pharmacogenomics: Applications to Patient Care, 2nd ed. Boston: McGraw-Hill, 2009:chapters 3–6.



Abbreviations in This Chapter

ACEI	Angiotensin-converting enzyme
ADHD	Attention deficit-hyperactivity
ALOX5	Arachidonate 5-lipoxygenase (gene)
DAT1	Dopamine transporter 1 (gene)
FEV_1	Forced expiratory volume in 1 minute
GWAS	Genome-wide association studies
MDR1	Multidrug resistance 1 (gene)
SNP	Single nucleotide polymorphism
TPMT	Thiopurine methyltransferase
UGT	Uridyl glucuronosyltransferase

mine how variations in multiple genes or the entire genome contribute to variable response.

The emerging science of pharmacogenomics seeks to characterize the variability in drug response that is attributable to genetic differences; this offers the potential to develop therapeutic strategies directed toward an individual rather than an entire population. With the completion of the Human Genome Project, the information and tools to investigate the role of individual genes and gene networks in drug response are readily available. Although the field of pharmacogenomics is in its infancy, it continues to progress at a rapid pace. Pharmacists must



Figure 1-1. The concept of pharmacogenomics. Pharmacogenetics focuses on large clinical effects of single gene variants in a few patients. However, the concept of pharmacogenomics examines many genomic loci including large biologic pathways and the whole genome to identify variants that together determine variability in response to drug therapy. Reprinted from Roden DM, Altman RB, Benowitz NL, Flockhart DA, Giacomini KM, Johnson JA, et al, for the Pharmacogenetics Research Network. Pharmacogenomics: challenges and opportunities. Ann Intern Med 2006;145:749–57. therefore be prepared to interpret, critically evaluate, and judiciously apply these new discoveries to patient care.

BASIC PRINCIPLES OF GENETICS

Genes and Gene Expression

The human genetic code is written using four nucleotide bases (i.e., adenine, cytosine, thymine, and guanine) and is expressed within an individual's DNA. Specific proteins are encoded by segments of DNA, which are referred to as *genes*. Each gene occupies a particular place on a chromosome called a *locus*. Most individuals carry two *alleles* or alternative sequences of the same gene, one inherited from each parent. Within an individual gene, specific regions control gene expression (*regulatory regions*) or encode proteins (*exons*). Genes may also contain noncoding regions (*introns*), as illustrated in Figure 1-2.

The arrangement or sequence of the nucleotide bases within the coding region of a specific gene determines the amino acid sequence of the encoded protein. Expression of the nucleotide sequence is achieved by a two-step process, during which the sequence is first transcribed into RNA and then translated into protein. Nucleotide triplets, known as *codons*, specify a particular amino acid and are used to translate encoded information into a functional protein.

Variation in the Human Genome

The human genome, or the complete set of human DNA, consists of 3.2 billion nucleotide base pairs that code for 20,000–25,000 genes. Sequencing, which was completed in 2003 as part of the Human Genome Project, revealed that 99.9% of the nucleotide sequence is identical in all individuals. Consequently, variation in only 0.1% of the genome contributes to interindividual differences.

Gene variants, defined as differences in a DNA sequence compared with a reference sequence, are classified as *mutations* or *polymorphisms*. Mutations are rare gene variants typically associated with genetic diseases such as cystic fibrosis or sickle cell disease. Polymorphisms, in contrast, are variants that are relatively common, occurring in greater than 1% of the general population. The frequencies of many sequence variants differ significantly among ethnicities, and a variant may be common in one ethnic population but rare in another.

The most common type of polymorphism involves a single nucleotide change in the DNA sequence; it is referred to as a single nucleotide polymorphism or SNP. There are an estimated 12 million potential SNPs in the entire human genome that occur every 100–300 base pairs along the entire DNA sequence. These SNPs may occur in any region of a gene, and their functional consequences can vary depending on their location within the gene. Knowledge of the location and type of a particular SNP can therefore be useful in determining whether



the variant may affect drug response, particularly when the functional consequences of the SNP are not yet characterized.

Most SNPs occur in introns or noncoding regions and are silent; however, those in regulatory regions may alter the amount of protein produced. The SNPs that occur in exons have the potential to alter protein function depending on whether the resulting amino acid sequence is altered (nonsynonymous SNP) or unaltered (synonymous SNP) by the nucleotide change. Examples of nonsynonymous and synonymous SNPs are illustrated in Figure 1-3.

Several SNPs on the same chromosome may be inherited together in groups or blocks known as *haplotypes*. When SNPs are inherited together more often than by chance alone, they are said to be in linkage disequilibrium. Haplotype blocks often contain many individual SNPs that are in high linkage disequilibrium. Determining a genotype for any SNP within a given haplotype block will therefore provide genotype information about linked SNPs within the same block.

The ability to use individual SNPs, known as tag SNPs, to establish a genotype within a haplotype block greatly enhances the ability to rapidly scan the entire genome to identify genes that may be important in determining disease susceptibility or drug response. The International HapMap Project, completed in 2007, created a haplotype map (HapMap) that can be used to facilitate these types of genome-wide association studies (GWAS).

Genomic Variation and Drug Response

Pharmacokinetics

The processes of drug absorption, distribution, metabolism, and elimination play an important role in determining drug response. These pharmacokinetic processes are also mediated by proteins that are subject to genetic variation. Alterations in the expression and/or activity of drug transporters and metabolizing enzymes can significantly influence the dose-exposure-response relationship. Variations in the genes encoding these proteins may therefore contribute to interindividual differences in drug disposition and action.

Drug Transporters

Membrane transporters are present in epithelial cells lining many absorptive surfaces (e.g., intestine, liver, kidney) and play an important role in mediating the passage of drugs across membrane barriers. In the intestine, uptake and efflux transporters in the enterocyte mediate drug absorption from the intestinal lumen, thereby influencing the bioavailability of orally administered agents. Coordinated expression and activity of transporters in the liver and kidney also regulate drug absorption from the



bloodstream into hepatocytes and proximal tubular cells, respectively. Efflux transporters localized on the canalicular membrane of the hepatocyte are similarly involved in the transport of drugs into bile for subsequent elimination. Transporters localized at plasma-tissue interfaces are important determinants of drug distribution into tissues and cells and can dramatically influence responses to drugs acting on targets located outside the systemic circulation.

Several SNPs have been identified in uptake and efflux transporter genes, and data continue to accumulate regarding the impact of these variants on drug disposition and response. At present, relationships between genetic variation and drug response have been most extensively studied for the efflux transporter P-glycoprotein.

A common SNP in the multidrug resistance 1 (MDR1) gene, which encodes P-glycoprotein, has been associated with digoxin plasma concentrations in healthy volunteers. Single nucleotide polymorphisms in the *MDR1* gene influence response to treatment with the protease inhibitor nelfinavir and susceptibility to postural hypotension during treatment with the antidepressant nortriptyline. A significant association between cyclosporine oral bioavailability (measured by the prehepatic extraction ratio) and selected *MDR1* gene polymorphisms has been similarly noted in pediatric kidney transplant recipients. However, this association was observed only in children 8 years or older, suggesting that developmental differences in P-glycoprotein expression and/or activity influence the observed *MDR1* gene phenotype.

Regarding other drug transporters, data suggest that SNPs in the organic anion uptake transporter OATP1B1 affect the efficacy and toxicity of statins. These data imply that drug transporter genotypes are useful in estimating serum drug concentrations, selecting initial drug doses, and predicting clinical responses for, at present, a small subset of select drugs.

Drug-Metabolizing Enzymes

Polymorphisms in drug-metabolizing enzymes are among the first-recognized and best-characterized examples of relationships between genetic variation and drug response. Sequence variations have been identified for enzymes involved in both phase I and phase II metabolic pathways, and many of these variants alter the expression and/or functional activity of individual enzyme isoforms.

Single nucleotide polymorphisms that cause a decrease in enzyme activity result in increased plasma concentrations of the parent drug and decreased metabolite concentrations. If the parent drug is the pharmacologically active component, the SNP may lead to an exaggerated therapeutic response and/or increased risk of toxicity. Conversely, if the compound is a prodrug, then SNPs conferring reduced enzyme activity may lead to decreased clinical effect. The impact of gene variants on drug exposure and response is likely to be most

pronounced for drugs with a narrow therapeutic index and for agents that are eliminated by a single metabolic pathway. Examples of such drugs used in children include 6-mercaptopurine, warfarin, morphine, and atomoxetine.

Although a comprehensive review of all clinically relevant drug-metabolizing enzyme SNPs is beyond the scope of this chapter, selected examples can be used to illustrate the range of potential effects that drug-metabolizing enzyme genetic variation can have on drug disposition and response. Cytochrome P450 (CYP) 2D6 is a phase I drug-metabolizing enzyme responsible for the metabolism of 25% to 30% of marketed drugs. Up to 10% of white and African American adults and children have low or absent CYP2D6 activity because of allelic variants in this enzyme isoform. These individuals, known as poor metabolizers, have higher plasma drug concentrations than individuals with normal CYP2D6 activity and are at increased risk of toxicity when given standard doses of CYP2D6 substrates such as tricyclic antidepressants, antiarrhythmics, and β -adrenergic antagonists. Adults and children who are CYP2D6 poor metabolizers also do not metabolize the prodrug codeine to its active metabolite, morphine, and hence can be relatively resistant to its analgesic effects.

The adverse effect profile of atomoxetine, a nonstimulant drug approved for the treatment of attention deficit-hyperactivity disorder (ADHD) in children, correlates with the *CYP2D6* genotype in poor metabolizers, with an increased incidence of adverse effects such as insomnia, sedation, depression, and tremor. This increase in adverse effects is most likely caused by the significant rises in peak plasma concentration (5-fold) and area under the curve (10-fold) that are observed in individuals who are CYP2D6 poor metabolizers.

Pharmacodynamics

Most drugs elicit their therapeutic responses by interacting with specific protein targets. Although the amount of drug available to interact with the target at the effector site is one determinant of therapeutic response, individuals with comparable plasma and/or tissue drug concentrations can have quite different outcomes. Variability in the expression and/or activity of protein drug targets may therefore be another important contributor to drug response. Drug targets are generally classified into three main categories: direct protein targets, signal transduction or downstream proteins, and disease pathogenesis proteins.

Direct Drug Targets

Variations in genes encoding target receptors or enzymes can result in alterations in protein expression, structure, and/or function. These alterations can, in turn, affect the interaction between the drug and its target and influence therapeutic response. Examples of such gene-response associations that are clinically relevant in children and adolescents include polymorphisms in β_2 -adrenergic receptors and sensitivity to β_2 -agonists, serotonin transporter gene variants and selective serotonin reuptake inhibitor efficacy, and vitamin K epoxide reductase gene variants and warfarin anticoagulation.

Variability in direct targets can also confer an increased susceptibility to adverse effects, as in the case of potassium channel variations and drug-induced dys-rhythmias and dopamine D3 receptor polymorphisms and drug-induced tardive dyskinesia. In contrast, certain polymorphisms in the dopamine transporter 1 (DAT1) gene reduce the incidence of stimulant-related adverse effects and improve treatment tolerability in children and adolescents with ADHD.

Signal Transduction Proteins

Responses to many drugs are mediated by intracellular signaling pathways, and variability in the proteins involved in these pathways can influence treatment outcomes. G protein–coupled receptors, such as β -adrenoreceptors, are examples of direct drug targets that elicit their responses through activation or inhibition of intracellular signal transduction pathways. Single nucleotide polymorphisms in the regulatory subunits of the G protein influence blood pressure response to β -blockers and antidepressant response to serotonin reuptake inhibitors and tricyclic antidepressants.

Variations in proteins downstream in the signaling cascade can also influence drug response, as shown by the bradykinin B2 receptor and angiotensin-converting enzyme inhibitor (ACEI)-induced cough. Although not its primary mechanism of effect, ACEIs prevent the breakdown of bradykinin, the substance responsible for ACEI-induced cough. The actions of bradykinin are mediated through its interaction with its own G protein-coupled receptor (β_2 -receptor), and SNPs in this receptor have been associated with susceptibility to cough during ACEI therapy.

Disease Pathogenesis Proteins

Proteins involved in disease pathogenesis and susceptibility are important determinants of therapeutic response even if they are not directly involved with the pharmacologic actions of a drug. In some instances, the risk of drug-induced adverse events can be increased by genetic polymorphisms that predispose an individual to that same adverse event. Examples include increased risk of oral contraceptive-induced thrombosis in individuals with factor V Leiden mutations, enhanced susceptibility to drug-induced torsades de pointes in patients with mutations in cardiac sodium and potassium channel genes associated with congenital long QT syndrome, and susceptibility to abacavir hypersensitivity in patients with certain major histocompatibility complex haplotypes.

For many complex diseases, genetic variation also plays an important role in determining the key pathogenic mediators that serve as targets for pharmacotherapeutic intervention. Given important interindividual variability in disease presentation, severity, and response to drug treatment, it is plausible that many common diseases such as asthma are not homogeneous conditions but rather represent an overlapping spectrum of mechanistically different conditions at the molecular level. Within a single condition, there may therefore be many distinct phenotypes that are genetically determined and respond differently to a given therapeutic intervention.

The specific disease phenotype may be useful in guiding drug selection. For example, neonates with a certain polymorphism in *KCNJ11*, a component of a sulfonylurea-sensitive potassium channel, have a form of diabetes that is especially sensitive to sulfonylureas. Similarly, mutations in hepatic nuclear factor 1 α are important determinants of responsiveness to sulfonylurea therapy in children with maturity onset diabetes of the young. Associations between SNPs in the guanine nucleotide binding protein beta 3 gene and the efficacy of the antiobesity drug sibutramine have also been reported, suggesting that genetics is an important component of childhood obesity pharmacotherapy.

IDENTIFYING GENE-RESPONSE Associations

Candidate Gene Studies

The candidate gene approach evaluates whether gene polymorphisms occur more often in individuals with a specific drug response phenotype. Genes are selected for study on the basis of their known or suspected involvement in disease pathogenesis and/or drug response. Prior knowledge about the function of the candidate gene is therefore necessary. The most biologically plausible gene candidates are those with SNPs that are known to affect the expression and/or functional activity of enzymes, receptors, or transporters involved in drug disposition and/or action.

Candidate gene studies are relatively simple and inexpensive to perform, and the resultant data are straightforward. Primary limitations of this approach include the requirement of prior knowledge of gene function and the failure to consider other genes/ gene networks that may be important contributors to response.

An alternative to the candidate gene study is the tag SNP approach. In this approach, certain SNPs are selected as tag SNPs because they are in linkage disequilibrium with other SNPs in the chromosomal region of interest, therefore tagging or providing information about the other SNPs. Tag SNPs allow the identification of a genotype within a given haplotype block, thereby decreasing the number of genotypes that must be determined and enhancing the ability to scan the entire genome rapidly.

The case-control association study is the most common design in which the candidate gene approach is used to identify gene-response relationships. Typically, patients enrolled in the active treatment arm of a clinical trial are stratified into groups on the basis of their response (i.e., present or absent). These groups, in turn, constitute the cases and controls that are genotyped for a particular candidate SNP. Although simple to perform, case-control association studies are subject to substantial biases or difficulties in interpretation. Pharmacists should be aware of these issues and be able to critically evaluate and interpret data from these types of investigations. A list of questions that can be used to evaluate case-control association studies is presented in Table 1-1. It is admittedly difficult for investigators to address all of these considerations in a given study, and in some cases, these biases cannot be completely avoided. Failure to consider these biases when interpreting study data, however, can negatively affect study conclusions. This impact can be illustrated by comparing the outcomes of two case-control candidate gene studies designed to investigate associations between polymorphisms in the gene encoding *N*-acetyltransferase 2, a phase II enzyme involved in histamine degradation, and atopic dermatitis.

Both studies compared the frequencies of two candidate SNPs (C481T and G590A) in white individuals with and without atopic dermatitis. However, one study (in adults) concluded that there was no association between either of these SNPs and atopic dermatitis, whereas the other study (of children) indicated that the risk of disease

Issue	Key Questions	Possible Solutions
Selection of cases and controls	How were cases and controls selected?	Population-based selection
	Do cases meet appropriate criteria for dis- ease status?	Family-based association design
	Are controls free of disease and potential confounders?	
	Do cases and controls have similar demo- graphic and environmental factors?	
Sample size	Is there an adequate number of cases and controls?	Minimum number of cases and controls included
Population stratification	Are cases and controls matched?	Matching on ethnicity
		Family-based association designs
		Negative results with multiple unlinked markers
Selection of candidate gene polymorphism	Biologically reasonable?	Demonstration of biologically functional effect
	Positional candidate? All variants identified?	Within linked region in human or syntenic from animal model
		Complete sequencing of the gene
Observation bias	How was the phenotyping and genotyp- ing done?	Blind assessment of genotype and phenotype
Linkage disequilibrium	Are there other genes?	Haplotypes
	Are there other polymorphisms in this gene?	Family-based association
Allele or genotype-based analysis	How are the heterozygotes treated in analysis?	Use appropriate genetic model
Multivariate analysis	Are relevant covariates identified?	Use appropriate genetic model
Gene by environment interaction	Is sample large enough to detect a gene environment?	Stratification by environmental exposure
		Multivariate analysis interaction
Multiple comparisons	How many alleles were tested?	Bonferroni correction
	How many phenotypes were tested?	Estimation of empirical P values
	How many genetic loci were tested?	

J 2001;1:157–8.

was associated with SNPs in the *N*-acetyltransferase 2 gene. Given that the study populations were of different ages, it is possible that differences in outcomes reflect age dependence in the gene-disease association. However, important differences in study design/data analysis may also have contributed to the disparate outcomes.

The criteria used to select case subjects were not clearly defined in the study with the negative (no association) outcome, whereas the positive outcome study carefully selected cases using specific diagnostic tests (serum and skin immunoglobulin G [IgG] concentrations). It is therefore possible that some cases in the negative study did not have atopic dermatitis. Given that the sample in the negative outcome study was also substantially smaller (20 subjects per group) than in the positive outcome study (80 cases and 101 controls), the study may have been underpowered to detect a significant gene-disease association. Failure to consider the combined influence of both SNPs in the N-acetyltransferase 2 gene may have also led to the conclusion of no association because the risk of disease was altered only in individuals with homozygous genotypes for both SNPs in the positive outcome study.

Genome-Wide Association Studies

Genome-wide association studies, like the candidate gene approach, seek to determine whether polymorphisms occur more often in individuals with a specific drug response phenotype. Instead of a few candidates, however, GWAS analyses characterize many SNPs (100,000–1,000,000) spanning the entire genome. An advantage is that this approach does not require knowledge of gene function or mechanisms of drug action. Therefore, it can be used to investigate gene-response associations for drugs that are still under clinical development or for which mechanisms of action are not well understood. Genome-wide association studies can also be used as hypothesis-generating tools to identify a list of candidate SNPs that can be further investigated in additional studies.

Limitations of GWAS include technical complexity, high cost, and the need for large samples to identify significant gene-response associations. A recent GWAS of methylphenidate response in 187 children with ADHD illustrates the need for large samples to detect modest genetic effects that influence drug response. The study, which evaluated 319,722 SNPs, failed to identify any significant gene-response associations; however, it was only powered to detect variants that account for a large portion (33%) of the phenotypic variance in treatment response. To detect a more modest effect, the investigators estimated that a sample of 1300 children would be required.

Another important limitation of GWAS is the high risk of spurious or false-positive associations. Given the large number of comparisons being performed (between 350,000 and 1,000,000), it is likely that several significant findings (17,500–50,000) will occur by random chance when using p values less than 0.05. Therefore, most GWAS require p values of less than 10^{-6} to 10^{-7} to avoid false-positive results.

False-positive associations may also occur because of systematic biases such as the confounding effect of ethnic ancestry (known as population stratification). The validity of results from any GWAS is therefore best reflected by the consistency and strength of the association across one or more large-scale replication studies of independent populations. A recent GWAS of children with acute lymphoblastic leukemia used this replication approach to confirm its finding of a significant association between SNPs in the organic anion transporter polypeptide gene and gastrointestinal toxicity of methotrexate. A total of 500,568 SNPs were evaluated first in a "discovery" cohort of 434 children with acute lymphoblastic leukemia treated with methotrexate; these were then validated in an independent cohort of 206 children who were similar to the discovery cohort in disease, treatment, and demographic characteristics.

Examples of Gene-Response Associations Relevant to Pediatrics

Thiopurine Methyltransferase and 6-Mercaptopurine

An estimated 3250 children in the United States receive a diagnosis of leukemia each year, and of these, about 75% have acute lymphoblastic leukemia. Thiopurines such as 6-mercaptopurine are part of the standard chemotherapeutic treatment regimen used in this patient population. The thiopurines are converted to inactive metabolites by the phase II drug-metabolizing enzyme thiopurine methyltransferase (TPMT). The activity of TPMT is inherited as an autosomal recessive trait and varies substantially among individuals. About 0.3% of whites have low TPMT activity, 6% to 11% have intermediate activity, and 89% to 94% have high activity.

Of the more than 20 identified *TPMT* allelic variants, three (*2, *3A, and *3C) are common and collectively account for more than 95% of the inherited variation in enzyme activity. The *TPMT**2 and *3C alleles result in a single amino acid change, whereas the *3A allele results in a change of two amino acids. The resultant proteins are nonfunctional because they produce an enzyme with an increased susceptibility to cellular degradation. Several studies have shown that children with a homozygous variant *TPMT* genotype (i.e., two alleles conferring low TPMT activity) are at high risk of developing hematopoietic toxicity after treatment with standard 6-mercaptopurine dosages that are used in children with high TPMT activity may not achieve an optimal therapeutic effect.

The U.S. Food and Drug Administration (FDA)approved labeling for the thiopurines recommends that clinicians consider a dosage reduction in patients heterozygous for a nonfunctional allele and alternative treatment in patients with a homozygous variant genotype. Specific recommendations for adjusting 6-mercaptopurine dosages based on TPMT genotype are currently lacking in the product labeling. However, clinical trial data suggest that 6-mercaptopurine dosage reductions of around 90% are required in children with homozygous variant and 50% in heterozygous variant genotypes to achieve comparable overall survival without dose-limiting hematopoietic toxicity. Given the comparable survival and response rates, the addition of a second chemotherapeutic agent is not needed when 6-mercaptopurine dosages are reduced on the basis of TPMT genotype. Administering the full dosage is appropriate only in individuals with two functional TPMT alleles.

Cysteinyl Leukotrienes and Leukotriene Modifiers

Asthma is one of the most common chronic diseases of childhood, affecting an estimated 6 million children in the United States. Chronic airway inflammation is a hallmark of asthma disease pathogenesis that, if uncontrolled, can significantly alter lung function. Cysteinyl leukotrienes are potent inflammatory mediators that contract airway smooth muscle, increase vascular permeability and mucus secretion, and attract and activate inflammatory cells in the airways of patients with asthma. Synthesis of the cysteinyl leukotrienes from arachidonic acid is mediated, in part, by the enzymes 5-lipoxygenase and leukotriene C₄ synthase.

Targeted disruption of the leukotriene pathway, through inhibition of synthesis or antagonism at the receptor, is one strategy for controlling leukotriene-mediated inflammation. Leukotriene receptor antagonists exert their effects by binding to cysteinyl leukotriene receptor 1 and antagonizing the inflammatory actions in the airway. Currently, two leukotriene receptor antagonists (montelukast and zafirlukast) are available for clinical use. Zileuton, the only marketed leukotriene synthesis inhibitor, works by inhibiting the 5-lipoxygenase pathway.

Several polymorphisms in genes that encode proteins in the leukotriene pathway have been identified and are thought to influence therapeutic response. A SNP in the leukotriene C_4 synthase gene promoter creates an additional binding site for transcription factors; this, in turn, leads to increased leukotriene production and inflammation. Individuals with a genotype conferring increased leukotriene C_4 synthase activity are expected to have a superior response to pharmacologic modification of the leukotriene pathway, and this response occurs for the leukotriene C_4 receptor antagonist pranlukast (not available in the United States).

Allelic variation in the promoter region of the arachidonate 5-lipoxygenase (ALOX5) gene is similarly associated with response to leukotriene synthesis inhibitors. Single nucleotide polymorphisms in the *ALOX5* gene inhibit the 5-lipoxygenase pathway and reduce leukotriene production. Individuals with these SNPs may not be as responsive to 5-lipoxygenase inhibition because the activity of this enzyme is already low. Indeed, reduced responses to an investigational 5-lipoxygenase inhibitor in individuals with polymorphic forms of the *ALOX5* gene have been described.

Maternal *CYP2D6* Genotype and Opioid Exposure in Breastfeeding Infants

Codeine, alone or in combination with acetaminophen, is often used during the immediate postpartum period for pain associated with episiotomy and cesarean section. Given that codeine is classified by the American Academy of Pediatrics as safe to use during breastfeeding, many women continue to nurse during analgesic treatment. However, the safety of codeine was recently questioned after a report of fatal opioid toxicity in a 13-day-old breastfed infant whose mother was taking a codeine-containing analgesic.

Several published case reports concern central nervous system depressive effects (e.g., somnolence, apnea) in infants exposed to codeine through breast milk. A prospective follow-up of adverse reactions in breastfed infants exposed to maternal drugs also found that about 20% of nursing mothers taking codeine-containing analgesics report drowsiness in their infants. Although the impact of maternal drug therapy on the infant is determined by several factors, recent data suggest that genetics plays an important role.

The analgesic effects of codeine depend on its conversion to morphine by CYP2D6. Morphine, in turn, is converted to morphine-3-glucuronide and morphine-6-glucuronide by the phase II enzyme uridyl glucuronosyltransferase (UGT) 2B7. Morphine-6-glucuronide is an active metabolite and is produced almost exclusively by UGT2B7. Several variants in the genes encoding CYP2D6 and UGT2B7 have been identified and have been associated with analgesic response and/or susceptibility to adverse central nervous system effects.

Individuals homozygous for the *UGT2B7**2 allele, a variant arising from a SNP in the coding region of the *UGT2B7* gene, show higher morphine-6-glucuronide/ morphine ratios than those homozygous for the wild-type allele. Individuals with several copies of a functional *CYP2D6* gene (known as gene duplication) have an ultr-arapid drug-metabolizing enzyme phenotype and are more susceptible to toxicity given enhanced CYP2D6-mediated conversion of codeine to morphine.

Maternal genotype is an important determinant of opioid exposure in nursing infants. Together with infant genotype and drug-metabolizing enzyme phenotype, maternal genotype can significantly influence an infant's risk of developing central nervous system toxicity. In the one reported infant death, the mother was a CYP2D6 ultrarapid metabolizer, and both the mother and infant were homozygous for the *UGT2B7*2* allele. Although the infant had two functional *CYP2D6* alleles, the level of CYP2D6 activity was probably more consistent with that of a poor metabolizer given the inherent immaturity of drug-metabolizing enzyme activity during the first few weeks of life. The interaction of genetic and developmental variability in drug-metabolizing enzyme activity led to a level of morphine exposure in the infant that was about 5 times higher than normally expected in nursing infants.

After the publication of this report, the FDA issued a public health advisory warning that the use of codeine by nursing mothers who are CYP2D6 ultrarapid metabolizers may increase the risk of serious adverse events in some breastfed infants. A subsequent case-control study confirmed the relationship between codeine use, maternal genotype, and risk of adverse central nervous system effects in nursing infants and identified codeine dose, treatment duration, and infant age as other factors that might also contribute to the risk of adverse effects.

INCORPORATING PHARMACOGENOMICS INTO PEDIATRIC PHARMACOTHERAPY

Factors Influencing the Clinical Utility of Pharmacogenomics Testing *Predictive Value*

Many factors can influence the application of pharmacogenomics discoveries to patient care. For a pharmacogenomics test to be useful in clinical practice, it must be able to accurately measure the genotype of interest (analytic validity) and predict drug response (clinical validity). For drug responses that can be measured as a dichotomous variable (i.e., present or absent), diagnostic test criteria such as sensitivity, specificity, and predictive value (positive and negative) are useful for assessing the potential clinical utility of a pharmacogenomics test. These criteria, however, are usually unreported in the literature. In addition, responses to many drugs cannot be classified as all-or-none phenomena. The relative contribution of the genotype to the variability in response (the percentage of explained variance) may therefore be a more useful parameter to assess a test's predictive value. Given the complex nature of most drug responses, a combination of SNPs in multiple genes may account for a greater proportion of response variability (and be more predictive) than individual SNPs.

Impact on Patient Outcomes and Treatment Costs

The ability of a pharmacogenomics test to improve patient outcomes and decrease costs is also an important determinant of its clinical utility. To date, however, there is limited scientific evidence documenting that pharmacogenomics testing has a positive impact on

clinical outcomes or health care costs. Most existing data that support the clinical application of pharmacogenomics testing pertain to predicting adverse drug reactions that have potentially life-threatening consequences, severe effects on quality of life, and/or high financial costs (e.g., abacavir hypersensitivity and HLA-5701*B variants). Pharmacogenomic testing is also most likely to be of benefit for drugs that are difficult to monitor (narrow therapeutic window and/or inadequate monitoring methods), exhibit large interindividual variability in response, have a consistent pharmacokinetic/ pharmacodynamic relationship, and are administered as long-term therapy. There should also be a strong association between a relatively common gene variant and outcome that can be measured using a rapid and inexpensive assay. Whether pharmacogenomics testing will be useful for responses that are mild in both health-related and financial consequences is undetermined. Third-party payers, however, will ultimately require this information to justify reimbursement for routine pharmacogenomics testing.

Test Regulation, Availability, and Reimbursement

Factors specific to the tests themselves can also significantly influence the clinical applicability of pharmacogenomics. Some pharmacogenomics tests have been approved by the FDA as in vitro diagnostic devices or test kits that are manufactured, produced, and packaged with all the materials to perform the pharmacogenomics test. However, most clinically available pharmacogenomics tests have been developed by individual clinical laboratories and are not regulated by the FDA. The quality of these "home brew" tests is regulated under the Clinical and Laboratory Improvement Act of 1988.

Although the typical turnaround time for genotype determination results is 2–6 hours, the time to receive test results can differ substantially, depending on whether the test is conducted at the institution or must be sent to another laboratory. For some drugs, it may not be feasible to delay initiation of treatment until genotype results are received. Reimbursement is also an issue. In the United States, the cost of pharmacogenomics testing is \$250–\$500, if the test is required in the FDA-approved product labeling, the cost is usually reimbursed by an insurance plan. However, most third-party payers consider pharmacogenomics tests experimental and do not reimburse associated genotyping costs.

Challenges in the Clinical Application of Pharmacogenomics

Identifying Relevant Genes/Gene Networks

A fundamental challenge to the clinical application of pharmacogenomics is identifying genes and/or gene networks important in determining drug response. Information from the Human Genome Project and the International HapMap Project provides an initial framework regarding human genomic variation. Because responses to most drugs are polygenic, an understanding of relationships between variations in individual genes or gene networks and biologic effects is necessary for pharmacogenomics to be widely applicable in clinical practice.

Defining Clinical Phenotype

Defining and identifying a response phenotype is also a difficulty inherent with clinical pharmacogenomics. For a given drug, several outcomes (e.g., reduced hospitalizations, disease-free interval, increase in forced expiratory volume in 1 minute $[FEV_1]$ by 10%) could be considered a positive response. Currently, no standards exist for methods of measuring or defining clinical phenotypes. Pharmacists therefore have a unique opportunity to help establish the criteria to use in defining drug response phenotypes.

Specifically, pharmacists can use their knowledge of drug response mechanisms and pharmacodynamics to help physicians identify the physiologic parameters that most accurately reflect drug response and the magnitude of response expected in the average patient. This information can then be used to develop specific criteria for defining responders and nonresponders in pharmacogenomics studies.

Another challenge in the clinical application of pharmacogenomics lies in unequivocally defining genotype-phenotype relationships. Many diverse factors can interact to produce the same phenotypic result, a phenomenon referred to as *phenocopy*. Individuals with the same phenotype may therefore not always have the same genotype. Similarly, the same gene can produce different outcomes in different patients (*genocopy*) because of the influence of modifier genes, development, and the environment.

Translating Genotypes into Clinical Recommendations

The biggest hurdle to the clinical implementation of pharmacogenomics testing may be the lack of information correlating the results of pharmacogenomics testing with dosing recommendations. Interpretation of genotype information is relatively straightforward when the goal of testing is to identify a group of patients to include or exclude from receiving a particular drug therapy. It becomes more difficult, however, when the goal of testing is to guide drug dosing given the complexity of factors (genetic and nongenetic) that can influence drug response.

Although more than 120 drug labels include pharmacogenomics information, most do not recommend a specific dosage modification based on genotype; this is because of the relative lack of information regarding safe and effective dosage adjustments for patients with different genotypes. The lack of genotype-based dosing guidelines for drugs with known genetic contributions to response variability (e.g., warfarin) considerably limits the practical value of pharmacogenomics testing.

Atomoxetine, an agent often used in children for the treatment of ADHD, is one of the few drugs that include genotype-based dosing information in its product information. This information is included for this drug because the pharmacokinetic and pharmacodynamic profiles of atomoxetine in patients with different CYP2D6 metabolizer phenotypes were characterized during clinical development in both adult and pediatric patients. Dosage recommendations or adjustments for antidepressants and antipsychotics according to *CYP2D6* and *CYP2C19* genotypes have also been proposed, although these guidelines have not yet been widely adopted for clinical use; this is at least partly because of the clinician's lack of familiarity with and understanding of genotype data and the interpretation of such data.

Developmental Considerations Ontogeny of Gene Expression and Activity

Children present a unique challenge to the clinical application of pharmacogenomics because of the dynamic changes in gene expression that accompany the process of normal growth and development. Although an individual's genomic information remains relatively stable during a lifetime, patterns of gene expression and the nature of gene interactions change considerably as an individual matures and develops (Figure 1-4).

Expression of individual genes also does not occur in isolation but rather as an integral component of larger, complex gene networks that interact during the maturational process. Different gene products may therefore be important determinants of drug response in children and adults. Certain gene products involved in disease pathogenesis and/or drug response may also be discernible or relevant only at specific points in the developmental continuum. Application of pharmacogenomics in pediatric patients therefore requires an appreciation and understanding of the dynamic nature of gene expression that accompanies normal human development. The concept of developmental pharmacogenomics, in which the developmental process is considered a network of interacting genes that is operative at different developmental stages, factors in the dynamic nature of human maturation.

Genotype-Phenotype Correlation

Because of changes in gene expression during development, genotype-phenotype correlations may not be readily apparent if the gene of interest is yet unexpressed or is undergoing change caused by maturation. The phenotypic consequences of a specific gene variant may therefore only be apparent at certain points during the developmental process. For example, a specific drugmetabolizing enzyme gene variant may produce distinct clinical phenotypes in adults that are not readily discernible in neonates or infants because of the developmental



Figure 1-4. Developmental pharmacogenomics as a determinant of interindividual variability. A, Developmental changes in gene expression and/or functional activity of a hypothetical gene for the first 25 years of life for 20 individuals. B, Gene expression and/ or functional activity at specific ages of development. In adults (III), allelic variation within the coding region of the gene gives rise to two distinct phenotypes, high activity in 92% of the population (black circles) and low activity in 8% of the population (white circles). However, there is interindividual variability in the rate at which functional activity is acquired postnatally such that the two phenotypes are not readily distinguishable shortly after birth (I). In addition, there might be discrete developmental stages during which the gene expression or activity appears greater than that observed in adults (II).

Adapted with permission from Leeder JS. Translating pharmacogenetics and pharmacogenomics into drug development for clinical pediatrics and beyond. Drug Discov Today 2004;9:567–73. delay in the acquisition of expression and activity of that particular drug-metabolizing enzyme isoform (see Figure 1-4). Pharmacogenomic information in children must therefore be interpreted in the context of the developmental and environmental factors that can also influence drug disposition and response.

Knowledge of the parents' genotypes may provide some insight regarding a child's inherent risk of adverse or unexpected treatment responses, although this information must be considered in conjunction with the child's developmental status. Similarly, studies of adults can be used to identify common gene variants and to characterize allele frequencies in different ethnic populations. Developmental biology studies of children are needed to characterize patterns of gene expression. This information can then be integrated with information about genetic variation to understand the factors influencing drug disposition and response throughout the developmental continuum.

Some pediatric diseases have no known correlates (e.g., patent ductus arteriosus, Kawasaki's disease) or are rarely encountered in adults (e.g., acute lymphoblastic leukemia, neuroblastoma); for these diseases, pharmacogenomics studies in children will be required.

Identifying Appropriate Indications for Pharmacogenomics Testing

The ultimate goal of pharmacogenomics testing is to individualize drug therapy by determining the likelihood of efficacy and/or toxicity before therapy initiation (Figure 1-5). This personalized or stratified medicine approach requires the prospective screening of a large population of patients with a given disease to inform drug and/or dosage selection. Although there are examples in which this approach has been successfully applied, most pharmacogenomics tests are currently performed retrospectively in an individual patient to identify reasons for treatment failure or an unexpected adverse event.

Both prospective and retrospective pharmacogenomics testing can provide useful information if used appropriately. The decision to perform pharmacogenomics testing therefore requires a systematic and critical evaluation of the potential clinical utility of genotype information. Given their knowledge and understanding of the factors influencing the disposition and action of drugs, pharmacists are in a unique position to make recommendations regarding appropriate indications for pharmacogenomics testing. Table 1-2 lists important factors to consider when determining if pharmacogenomics testing is warranted in the individual patient.

Special Pediatric Considerations Sample Collection

Genomic DNA can be obtained from any nucleated cell (i.e., any cell in the body except red blood cells and platelets). Given the large amount of genetic material



obtained, collection of whole blood by venipuncture is considered the standard sample collection method. In general, 1 mL of whole blood yields about 20–30 mcg of genomic DNA.

On average, genotyping procedures require 10–100 ng of genomic DNA. In adults, a 5-mL blood sample provides DNA for thousands of genotype determinations. Collection of this volume of blood in young children is often unfeasible, however, particularly in newborns. Strategies for circumventing these volume limitations include scavenging whole blood or cellular fractions from laboratory samples collected as part of standard medical care but no longer needed for clinical purposes; and using alternative sources of genomic DNA such as buccal epithelial cells. Buccal cell samples are usually collected by cheek swab or a mouthwash method. Although easy to perform, cheek swabs often yield a low amount of DNA and have a higher rate of nonhuman DNA contamination from oral microbial flora. The mouthwash method, which is most appropriate for older children who can follow instructions, involves asking the child to swish a small volume of commercially available mouthwash for 1 minute and then expectorate into a sterile container. In general, 15–30 mL of mouthwash yields a median of 25 mcg of genomic DNA, an amount sufficient for around 250 genotyping reactions.

Ethical Concerns

Several health care provider groups, including medical geneticists, nurses, and pediatricians, have issued guidelines or recommendations on how to address ethical concerns pertaining to genetic testing in children. Three basic principles are common themes across the multidisciplinary recommendations. (1) The primary justification for genetic testing in children and adolescents should produce a timely medical benefit to the child. (2) Genetic testing should be preceded by informed parental consent and assent in children. (3) Genetic testing for adult-onset conditions should be deferred until adulthood or until the child can make an informed choice about testing. When considered within this framework, the use of pharmacogenomics testing to guide treatment appears to be an optimal use of genetic testing in pediatric patients. Concern may arise, however, if pharmacogenomics test results provide unwanted predictive or susceptibility information. Many genes, particularly those involved in drug metabolism, have several effects. Determining a genotype at a particular locus that provides information about drug response may therefore simultaneously provide information about susceptibility

to future disease or phenotype.

Pharmacogenomic information has implications not only for the child but also for family members who have not consented to genetic testing, and the potential impact of test results on relatives must be considered. Collection of sensitive information from children also raises issues of privacy and discrimination. Access of third parties (e.g., insurers, employers, schools) to genetic information has the potential to result in discrimination or stigmatization. There is also concern that access to particular drugs could be limited based on the results of genetic testing. The benefits of improved drug selection and/or dosage determination must therefore be carefully balanced with the potential ethical implications.

Factor	Characteristics Supporting Pharmacogenomics Testing
Drug	Large interindividual variability in response
	Narrow therapeutic index
	Long-term or chronic use
	Response difficult to predict with available methods
	Alternative treatments available
	Disposition/action influenced by genetic factors
Consequences of genetic variation	High mortality
	Significant changes in quality of life
	Increased medical costs
Prevalence of variant allele	Common in the general population
	Occurs often in ethnic group of interest
Genotype-phenotype association	Strong association between variant allele and patient outcome
	Validated in independent patient populations
Pharmacogenomics test	High sensitivity, specificity, and predictive value
	Rapid and relatively inexpensive assay available
	Acceptable turnaround time
Developmental considerations	Developmental expression and activity of the gene product have been characterized
	Relationship between development and drug disposition/response has been described
Interpretation of results	Guidelines or dosing algorithms are available to adjust treatment based on genotype

Role of the Pharmacist

Pharmacogenomics offers new challenges and opportunities for pediatric clinical pharmacists. Pharmacists have a responsibility to ensure that pharmacogenomics tests are used appropriately and that genotype information is applied correctly in therapeutic decision-making. The unique skill set that pharmacists possess relative to drug disposition and action affords the ability to integrate genotype data with other patient-specific developmental and/or environmental factors to estimate a response phenotype and assist prescribers in drug and/or dose selection. Pharmacists may also work with other health care professionals to establish criteria for defining a response phenotype, identify candidate genes for further study, and develop dosing algorithms incorporating genotype data.

Given their easy accessibility, pharmacists play an important role in educating patients and their families about pharmacogenomics testing, particularly in the community practice setting. Direct-to-consumer advertising of genetic testing services is becoming increasingly common, and pharmacists should take an active role in ensuring that parents understand the validity, implications, and limitations of each test. Some genetic tests, such as the Identigene DNA Paternity Test, are also sold directly to consumers in community/retail pharmacies, and it is expected that the number of off-the-shelf tests will only increase. Pharmacists must therefore be prepared to educate parents on the use, risks, and uncertainties of these tests and to provide information regarding available resources for pre- and posttest genetic counseling and result interpretation.

To promote the rational application of pharmacogenomics testing in clinical practice, pediatric pharmacists must be able to integrate pharmacokinetic, pharmacodynamic, developmental physiology, and gene-response association information to determine whether pharmacogenomics testing may benefit an individual patient. A conceptual framework that can be used to determine whether pharmacogenomics testing may be valuable for a specific drug is outlined in Table 1-2. Criteria for critically evaluating results of candidate gene and GWAS are described earlier in the chapter.

Given the rapid pace of pharmacogenomics discoveries, pharmacists who do not specialize in this area may find it difficult to stay current with new advances and recommendations. However, many online resources, such as those offered by the Human Genome Project, can help pharmacists stay up to date. Recently, the Pharmacogenomics Research Network was funded by the National Institute of General Medical Sciences. This group supports the Pharmacogenetics and Pharmacogenomics Knowledge Base and links to other resources, such as the National Center for Biotechnology Information, that are good sources of information regarding pharmacogenomics. Organizations such as the American College of Clinical Pharmacology and the American Medical Association also offer free online continuing education programs related to pharmacogenomics. In addition, most national pharmacy organizations now offer pharmacogenomics-related continuing education programming at their national meetings; this is a good opportunity for pharmacists to learn more about new advances and clinical applications.

Conclusion

Pharmacogenomics offers significant potential to improve the delivery of quality pharmaceutical care to pediatric patients. Using information from the Human Genome and International HapMap Projects, allelic variants responsible for interindividual variability in drug response are being identified at a rapid pace, and relationships between these variants and patient outcomes continue to be investigated. Ultimately, pharmacogenomics may improve the quality of patient care and reduce health care costs by decreasing the number of treatment failures and unexpected adverse drug reactions. Substantial challenges must be overcome, however, before pharmacogenomics can be routinely applied in clinical practice. Nevertheless, the field of pharmacogenomics continues to progress rapidly, and pharmacists must be prepared to apply these new discoveries to patient care.

ANNOTATED BIBLIOGRAPHY

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This article describes the results of a pharmacogenomics investigation designed to investigate associations between polymorphisms in the DAT1 gene and methylphenidate-induced adverse effects in children with ADHD (n=177). Adverse event and DAT1 genotype data were combined from two previously published studies. Significant genotype-dependent differences in the adverse effect profile were noted. The data are of potential clinical importance because they suggest that the types and severity of adverse effects are determined by genetic variation in the DAT1 gene. Limitations of the study include failure to account for differences in methylphenidate dosages and formulations between study sites, subjectivity of parental adverse effect assessment, and risk of false-positive associations because of several statistical comparisons. Although this study provides an example

of how candidate gene studies can be applied in children, the results should be interpreted cautiously until they are replicated in an independent study population.

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This study illustrates the combined influences of genetics and development on drug disposition. Pediatric kidney transplant recipients (n=104) were genotyped for 17 sequence variations in six candidate genes thought to influence cyclosporine pharmacokinetics. Prehepatic extraction ratios in children 8 years or older differed significantly because of the ABCB1 genotype. A SNP in this gene (which encodes P-glycoprotein) accounted for 33% of the variation in the prehepatic extraction ratio. In contrast, no association was observed in children younger than 8 years, likely because of developmental differences in P-glycoprotein expression. Strengths of this study include consideration of important clinical covariates that might affect cyclosporine pharmacokinetics and inclusion of a detailed sample and power calculation. However, many genotypes and haplotypes were evaluated, and no corrections for multiple comparisons were made. Ethnicity was also not considered a potential confounder.

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This article describes a population pharmacokinetic/ pharmacogenetic model developed to guide the dosing of tacrolimus in pediatric patients after kidney transplantation. The model was developed using pharmacokinetic and pharmacogenomics data obtained from 50 kidney transplant recipients (ages 2–18 years) and indicated that tacrolimus dosing should be based on body weight, hematocrit concentrations, and *CYP3A5* genotype. This study is the first to examine the combined effects of age and genetic variation on the disposition of tacrolimus. The study also illustrates the multifactorial nature of drug disposition and the need to simultaneously consider the potential influences of environment, genetics, and development when developing dosing recommendations and guidelines.

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This study provides a good example of how clinical and pharmacogenomics information may be integrated to improve the prediction of complex drug responses in children. In this investigation, the ability of a single clinical parameter (i.e., baseline FEV_1) to predict bronchodilator response was evaluated alone and in combination with a pharmacogenomics test consisting of eight SNPs in several genes. The combination of clinical and genetic parameters was significantly more accurate in predicting response than the clinical parameter alone. An important strength of this study is that it used diagnostic test criteria to measure predictive ability. The study also considered the polygenic nature of drug response by evaluating the contribution of multiple SNPs. Although the performance of this combined test may be below the normal discriminatory threshold, these data provide initial evidence that adding genetic information to clinical indicators improves predictive ability.

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This article describes an innovative national surveillance network designed to identify novel predictive genomic biomarkers of severe adverse drug reactions in children. The goal of the project, known as the Genotypic Approaches to Therapy in Children (GATC) network, is to identify children experiencing adverse drug reactions and matched controls, collect DNA, and apply genomics-based technologies to identify genetic biomarkers. The authors briefly review the epidemiology of adverse drug reactions in children and present examples of pharmacogenomics tests currently used in clinical practice. The goals, structure, and creation of the network are also reviewed, and challenges faced by the network are discussed. Results of preliminary genomic analyses and success stories from the GATC project are reviewed. This article provides a good framework for pharmacists and physicians interested in developing similar programs on a local, regional, or even national level.

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Although written within the context of antiepileptic treatment, this article provides a foundation regarding gene expression profiling approaches in children. After reviewing basic information about antiepileptic therapy, treatment resistance, and adverse event phenotypes, the authors discuss sources of biologic variability and technologies used to assess this variability. However, the real value of this article is its description of a study that evaluated changes in gene expression patterns in children with excessive weight gain during valproic acid therapy. Although no predictive genomic pattern was identified, the description of the approach and methods used is useful. Because changes in gene expression occur with normal growth and development, gene expression profiling will likely be required to fully understand generesponse relationships. This article is a good example of how these types of investigations can be successfully conducted in pediatric patients.

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This article is a comprehensive review of current genotyping technologies and is a useful resource for clinicians involved in pharmacogenomics. The article discusses platforms used for whole genome studies and presents basic methodologies, followed by the advantages and limitations of each platform. Of particular interest for pharmacogenomics studies is the discussion of GeneChip assay platforms, many of which are available for CYP genotyping. The article also discusses various custom SNP assays (e.g., the TaqMan assay) often used in pharmacogenomics studies. An overview is presented of methods for haplotype determination, and comparisons are made between the various platforms available for whole genome studies. The article is not written within the context of pharmacogenomics, but the methods and techniques described are directly applicable to these types of studies. Written in an easy-to-understand format, the article includes definitions of key terms and has an extensive, partly annotated bibliography.

 Flockhart DA, O'Kane D, Williams MS, Watson MS, Flockhart DA, Gage B; ACMG Working Group on Pharmacogenetic Testing of CYP2C9, VKORC1 Alleles for Warfarin Use. Pharmacogenetic testing of CYP2C9 and VKORC1 alleles for warfarin. Genet Med 2008;10:139–50.

This consensus document from the American College of Medical Genetics is included as an example of a policy statement that was recently developed regarding the clinical application of pharmacogenomics testing. The statement is based on information obtained during an expert review that evaluated the following areas of evidence: (1) analytic validity, (2) clinical validity, (3) clinical utility, and (4) ethical, legal, and social implications. The consensus document presents the results in each of the four review areas and highlights current limitations and/or gaps in information. After presentation of the evidence, the article provides recommendations regarding appropriate indications for warfarin pharmacogenomics testing. Although this guideline pertains to warfarin, the approach presented provides a general systematic framework for critically evaluating the utility of any pharmacogenomics test.

 Avard D, Silverstein T, Sillon G, Joly Y. Researchers' perceptions of the ethical implications of pharmacogenomics research in children. Public Health Genomics 2009;12:191–201.

This article presents the results of a qualitative study designed to assess researchers' perceptions of a preselected group of ethical issues raised by the conduct of pharmacogenetic research in children. Researchers were asked questions guided by the following themes: (1) benefits and risks of inclusion, (2) consent/assent process, and (3) return of research results. The study itself is rather simply designed and descriptive. Although the focus is on research, the study addresses issues that are also important for clinicians to consider when deciding whether pharmacogenomics testing is warranted in an individual child. The article includes a discussion of drug development in children and the ethics of pediatric clinical pharmacology research in general. Also of particular relevance to practitioners is the discussion of communicating pharmacogenomics test results.

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This review provides a comprehensive overview of the conduct and interpretation of GWAS and is a good resource for clinicians to use when interpreting and critically evaluating GWAS data. Although not directed specifically at pharmacogenomics studies, the information provided can be applied to any type of GWAS. The article begins with a brief overview of GWAS, as well as common study designs and their advantages and disadvantages. The limitations of GWAS are presented, and the clinical applicability is reviewed. A table defining terms often used in GWAS is especially valuable for individuals new to this type of data. A list of 10 questions to ask about GWAS investigations is provided and can be used to guide the evaluation of data obtained from GWAS.

11. U.S. Food and Drug Administration. Guidance for industry. Pharmacogenomic data submissions. Available at www.fda.gov/downloads/RegulatoryInformation/ Guidances/ucm126957.pdf. Accessed August 31, 2010.

This guidance document provides recommendations to pharmaceutical sponsors regarding the submission of pharmacogenomics data to the FDA and explains how the FDA will use this information in regulatory decisionmaking. The document represents the FDA's position on pharmacogenomics and provides an interesting viewpoint on the status of personalized medicine from the regulatory perspective. This document is a good resource for understanding the scientific rigor with which pharmacogenomics studies should be conducted. The document also provides a good resource for understanding the information that will ultimately be required to integrate genomic data into drug labeling. The guidance points out that many pharmacogenomics tests are not yet well enough established scientifically to be appropriate for regulatory decision-making, and it outlines the types of information required for specific types of drug submissions (investigational new drug applications, new drug applications, biologic license applications).

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This review provides a comprehensive overview of membrane transporter pharmacogenetics. The techniques used to identify substrates and inhibitors of these proteins and to assess the effect of genetic variation on their functional activity are discussed. Studies linking transporter genotype with clinical outcomes are also reviewed. A useful feature of this review is a table listing individual transporters and their common drug substrates organized by therapeutic class. Common SNPs in the various transporters are also discussed. This article is a good general resource, but it focuses more on methods than on clinical consequences of transporter polymorphisms.

13. Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 2009;41:89–295.

This article, which provides an extensive overview of CYP pharmacogenomics, is an up-to-date resource for current knowledge in this area. The authors discuss each enzyme isoform individually, review known polymorphisms, discuss their clinical significance, and provide relevant examples. The entire review is quite long (208 pages) and is difficult to absorb when reading from beginning to end. However, it can be a useful resource for individuals interested in learning more about a specific enzyme isoform and the potential consequences of its genetic variation. A limitation of this review is that it focuses only on the CYP system and does not discuss the pharmacogenomics of other important phase I or phase II metabolic pathways.