Chapter 5

Pharmacogenomics and Drug Transport/Efflux

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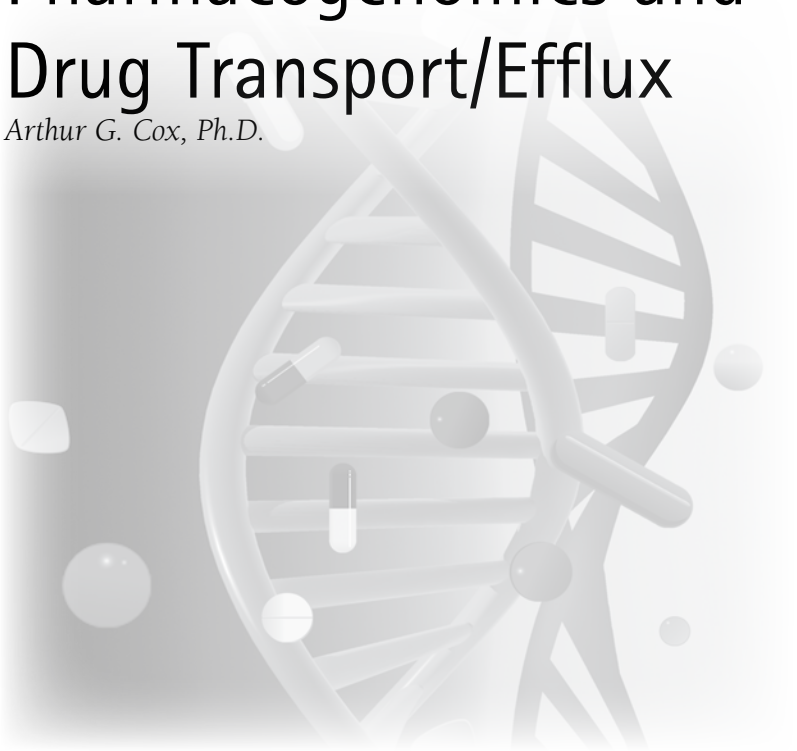


Learning Objectives

After completing this chapter, the student should be able to

- Classify drug transport proteins by their location in tissues and cells and by their physiological function.
- Discuss the general clinical relevance of the transporters and their inhibitors.
- Describe classes of drugs whose pharmacokinetics are affected by individual transporters.
- Relate genetic variants of transport proteins to variations in drug action.

Key Definitions

Transporter—A protein embedded in a cell membrane responsible for either removing substances from a cell or bringing them into the cell or membrane-bound vesicle within the cell.

Single nucleotide polymorphism (SNP)—A point mutation occurring in >1% of the population.

Syncytiotrophoblast—Multinucleated cells in the placenta that contain transport proteins serving a protective function for the fetus.

Enterocyte—Cells lining the intestine that contain transport proteins with protective roles.

Synonymous mutation—The substitution of a nucleotide within a gene that does not result in a change in an amino acid in the expressed protein.

Nonsynonymous mutation—Nucleotide substitution in a gene that results in a change in the amino acid sequence of a protein.

Missense mutation—A polymorphism that results in a different amino acid being expressed in the protein.

Nonsense mutation—A polymorphism that results in a premature stop codon.

Linkage disequilibrium—A nonrandom association of alleles, causing a certain combination to occur more or less frequently than otherwise expected.

Introduction

Genetic variability of drug metabolizing enzymes has long been recognized as a factor in both differing therapeutic response and adverse effects in individuals and patient populations. The cytochrome family of enzymes is particularly important in this regard. Another area where genotype can strongly affect drug response is that of transport proteins. This chapter will discuss the importance of transport proteins in drug absorption and response and review recent information on the effect of genetic variability on these transporters.

Transporters are those proteins that carry either endogenous compounds or xenobiotics across biological membranes. They can be classified into either efflux or uptake proteins, depending on the direction of transport. The extent of expression of genes coding for transport proteins can have a profound effect on the bioavailability and pharmacokinetics of various drugs. Additionally, genetic variation such as single-nucleotide polymorphisms (SNPs) of the transport proteins can cause differences in the uptake or efflux of drugs. In terms of cancer chemotherapy, tumor cells expressing these proteins can have either enhanced sensitivity or resistance to various anticancer drugs.[1] Transporters that serve as efflux pumps on a cell membrane can remove drugs from the cell before they can act. Transport proteins that are responsible for the vital influx of ions and nutrients such as glucose can promote growth of tumor cells if overexpressed, or lead to increased susceptibility for a drug if the transporter carries that drug into the cell. Additionally, genetic variants of transport proteins can cause or contribute to a number of diseases, such as cystic fibrosis, retinal degeneration, hypercholesterolemia, bile transport defects, and anemia.[2]

There are two superfamilies of transport proteins that have important effects on the absorption, distribution, and excretion of drugs. These are the ATP-binding cassette (ABC) and the solute-carrier (SLC) superfamilies. With the advent of high-throughput screening methods in recent years, screening of large volumes of samples for SNPs has become viable. Public

databases of the genetic variants that have been discovered are available and include those maintained by the Human Genome Gene Nomenclature Committee (HGNC), National Center for Biotechnology Information (NCBI) SNP database (dbSNP), the National Human Genome Research Institute haploid map (HapMap), the Japanese SNP database (JSNP), and the pharmacogenetics and pharmacogenomics knowledge base at Stanford University (PharmGKB).

Case Study—Irinotecan

Irinotecan is widely used in cancer chemotherapy but has been associated with unpredictable severe toxic reactions such as myelosuppression and delayed-type diarrhea. Polymorphism of the drug-metabolizing enzyme family UGT1A is a known contributor to varied response and toxicity of irinotecan in different individuals. Polymorphism of multiple drug transport proteins, such as ABCB1, ABCC1, ABCC2, ABCG2, and SLC01B1 have been suggested to have additive or synergistic effects with UGT1A1.[3-6]

Questions:

1. What are the patient parameters normally considered when determining the correct dose for irinotecan?
2. Discuss how genetic polymorphisms can affect patient response to irinotecan.
3. How can knowledge of pharmacogenomics improve the therapeutic use and safety profile of irinotecan?

Individual Transporters of Pharmacogenomic Interest

ABC Transporters

ATP-binding cassette (ABC) transporters are present in cellular and intracellular membranes and can be responsible for either importing or removing (efflux) of substances from cells and tissues. They often transport substances against a concentration gradient by using the hydrolysis of ATP to drive the transport. There are at least 49 ABC transporter genes, which are divided into seven different families (A-G) based on sequence similarity. Three of these seven gene families are particularly important for drug transport and multiple drug resistance in tumor cells[7]: (1) the *ABCB1* gene, encoding MDR1 (also known as P-glycoprotein); (2) *ABCG2* (breast cancer resistance protein); and (3) the *ABCC* family (*ABCC1* through *ABCC6*) or multidrug resistance proteins (MRP).

ABC transporters are characterized as such by the homology of their ATP binding regions. All families but one (ABCG2) contain two ATP binding regions and two transmembrane domains. The transmembrane domains contain multiple alpha helices, which span the lipid bilayer. The number of alpha helices in a transmembrane domain differs depending on the family. The ATP binding regions are located on the cytoplasmic side of the membrane (Figure 5-1). As well as being important mediators of resistance in human chemotherapy, ABC transporters are also found in bacteria and can contribute to the development of resistance to multiple antibiotics. The localization of the proteins depends on the cell type, such as hepatocyte, enterocyte, and renal proximal tubule (Figure 5-2). The majority of ABC transporters move compounds from the cytoplasm to the outside of a cell, although some move compounds into an intercellular compartment such as the endoplasmic reticulum, mitochondria, or peroxisome.

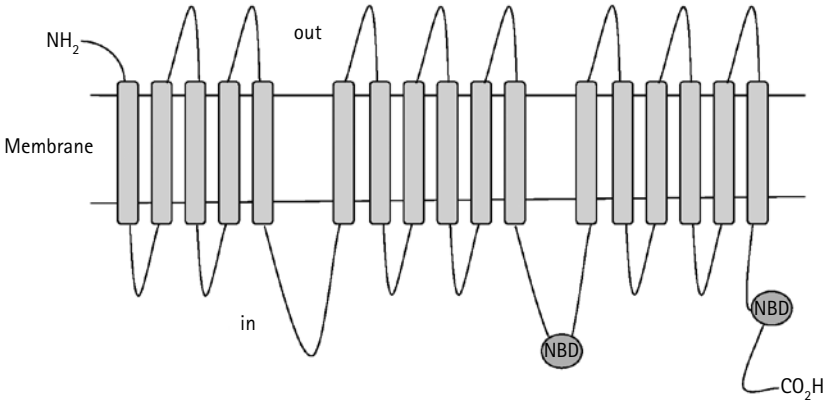
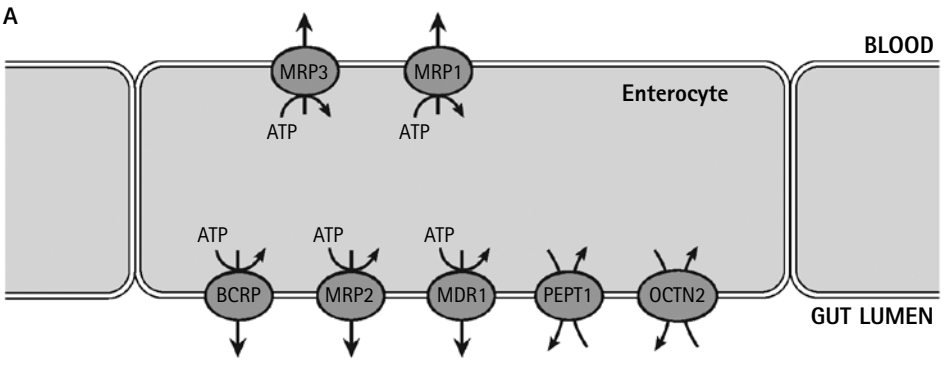


Figure 5.1 • General structure of ATP binding cassette transporters (ABC), showing transmembrane and nucleotide binding domains (NBD). Individual members of the superfamily contain differing numbers of transmembrane helices within the transmembrane domains. The example shown here illustrates MRP1 (ABCC1). ABCG2 transporters differ from the rest of the members of the superfamily in that they have only one ATP binding domain. The alpha helices making up the transmembrane segments, and the nucleotide binding regions are critical to the function of ABCs (see Figure 5-3). Figure adapted from reference 12.



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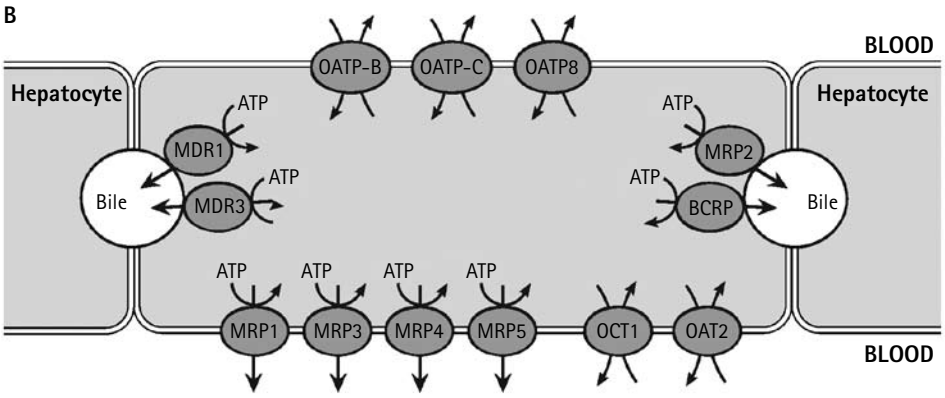

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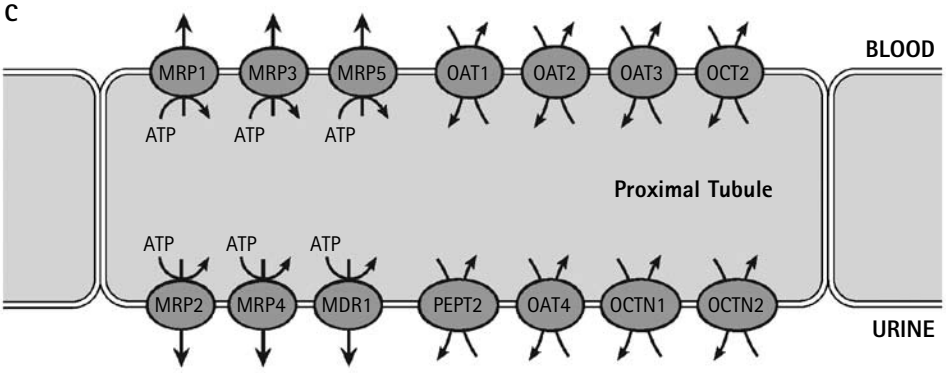

Concepts in Pharmacogenomics
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Figure 5.2 • Localization of transporters in differing cell types. A) small intestine enterocyte, B) hepatocyte with canaliculi, and C) renal proximal tubule. In addition to those transporters discussed in the text, other transport proteins with protective and possible pharmacogenomic relevance are shown. OCTN1 and OCTN2: novel organic cation transporters-1 and 2 (*SLC22A4*, *SLC22A5*), OATP-B: organic anion transporting polypeptide-B (*SLCO2B1*), OATP-C (*SLCO1B1*), OATP8 (*SLCO1B3*), OCT1 (*SLC22A1*), OAT2 (*SLC22A7*). Figure adapted from reference 15.

The exact mechanism by which ABC transporters function has not been fully elucidated. It has been proposed that there is an ATP-dependant conformational change in the protein, which causes the substrate to be pumped across the membrane. This hypothesis has been supported by recent x-ray crystallographic studies, which have shown that both import and export proteins oscillate between two conformations: one in which the substrate binding site is open to the cytoplasm, and one in which the binding site faces the opposite side of the membrane.[8] ATP binding and hydrolysis are proposed to play separate roles in the cycle. ATP binding favors the outward facing orientation, while ATP hydrolysis returns the transporter back to the inward facing conformation (Figure 5-3).[8,9] In this way, ATP can be used to drive the transport of a substance against its concentration gradient.

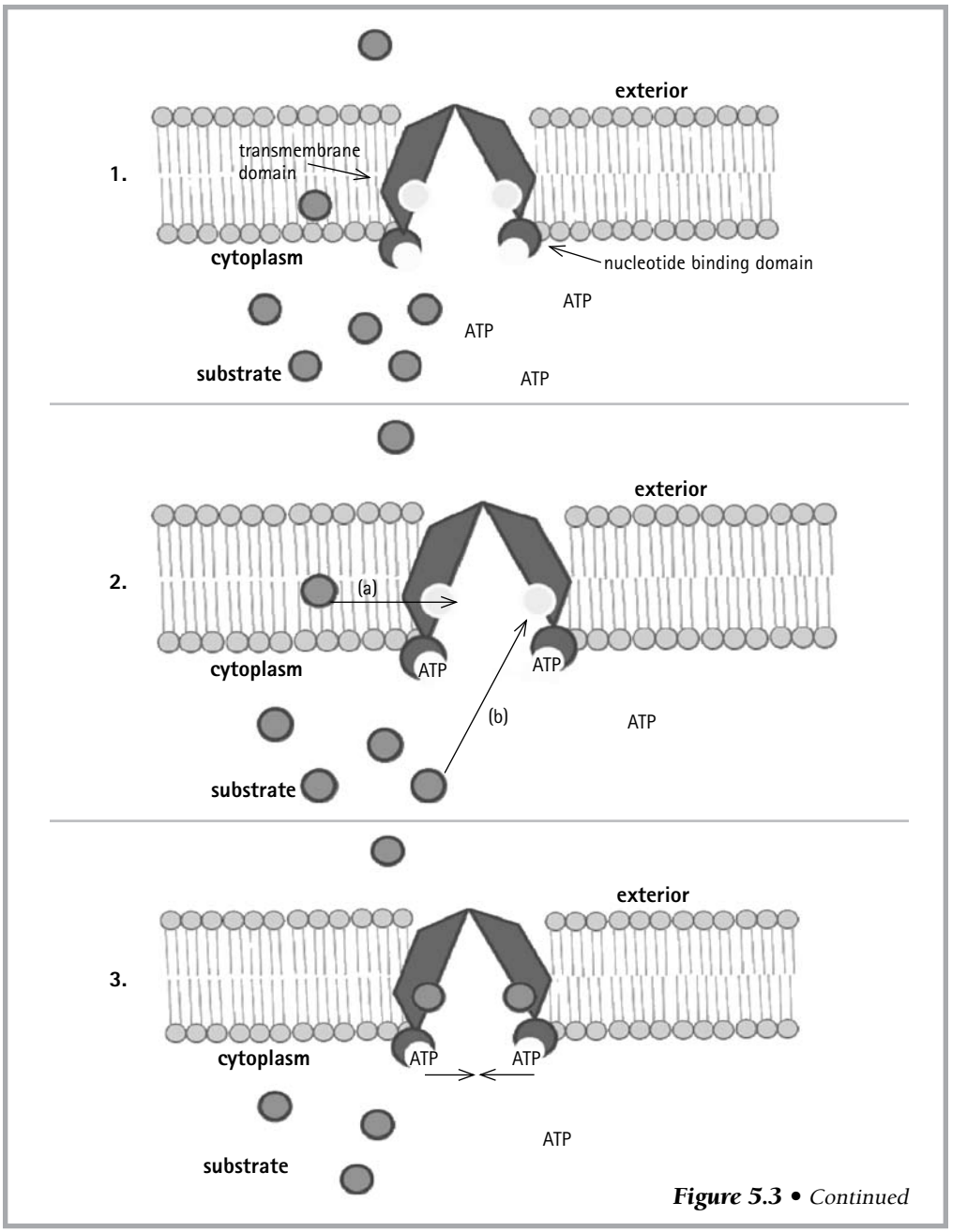

Figure 5.3 • *Continued*

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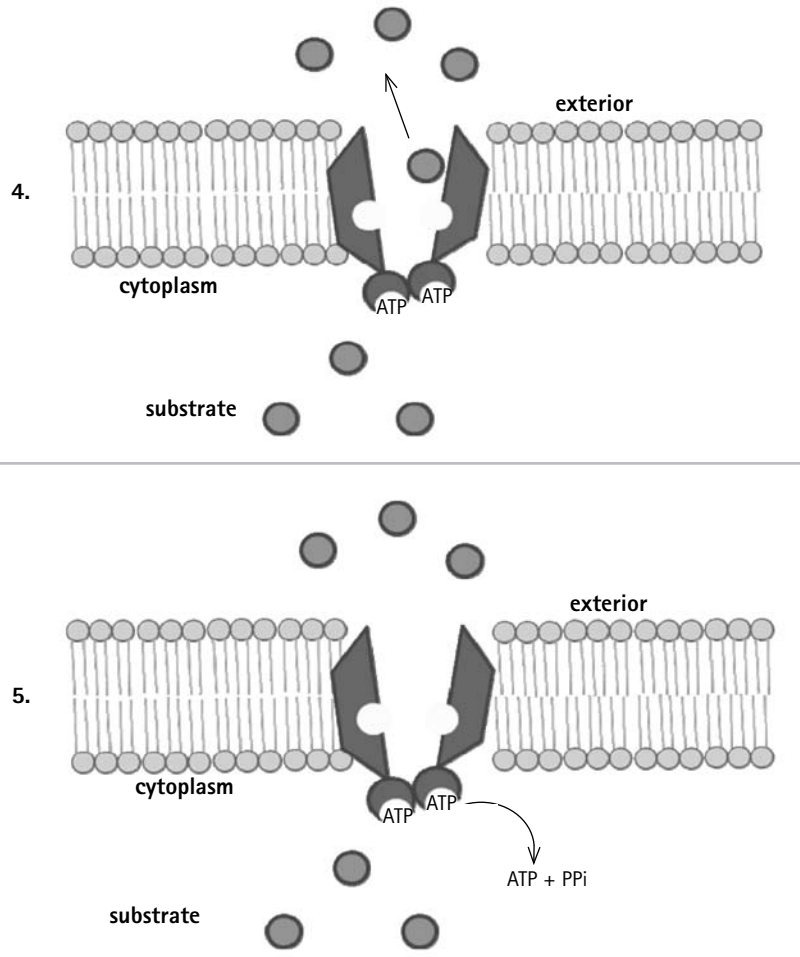

Figure 5.3 • Schematic illustration of the function of ATP efflux transporters. Step 1: The two transmembrane domains that make up the functional protein are attached to nucleotide-binding domains that are widely separated. Step 2: ATP and the substrate bind to their domains. Highly lipophilic substrates may diffuse through the plasma membrane (a). Otherwise, they can diffuse from the cytoplasm to the binding pocket (b). Step 3: The nucleotide binding regions containing ATP undergo a conformational shift, bringing them close together. Step 4: The conformational shift of the NBDs has caused a change in the conformation of the substrate binding pocket, which opens a pocket to the outside of a cell and allows efflux of the substrate. Step 5: ATP is hydrolyzed to ADP and pyrophosphate (PPi). The protein can then return to its resting state, with the substrate binding site directed inward. Figure adapted from reference 9.

ABCB1 Transporters: P-glycoprotein

The *ABCB1* gene codes for a glycosylated membrane protein originally detected in cells that had developed resistance to cancer chemotherapy agents. The protein is commonly referred to as P-glycoprotein (P-gp), PGY1, or multidrug resistance protein-1 (MDR1). It is designated as a multidrug resistance protein due to the fact that its expression in a cell may confer resistance to multiple classes of drugs with differing chemical structures and mechanisms of action. Various cancers tend to display low initial levels of P-gp with levels of expression increasing after chemotherapy and relapse. Of the wide variety of transport proteins that have been discovered and studied, P-gp is the best characterized in terms of distribution and function. Some drugs (e.g., cyclosporine) act as both substrates and inhibitors of P-gp. Other drugs act only as substrates or as inhibitors. The substrates for P-gp are often hydrophobic drugs with a polyaromatic skeleton and a neutral or positive charge.[10] P-gp functions as a dimer of 1280 residue polypeptides, forming a pore across the cell membrane. In addition to cytotoxic chemotherapeutic agents, many other drugs are transported across membranes by P-gp. These include protease inhibitors, immunosuppressants, calcium channel blockers, beta blockers, statins, steroids, antihistamines, anticonvulsants, and antidepressants. The importance of P-gp for pharmacotherapy has led to great interest in its pharmacogenomics.[11-13]

Clinical Pearl

P-glycoprotein translocates multiple structurally unrelated drugs out of cells, including anticancer drugs, immunosuppressants, HIV protease inhibitors, cardiac drugs, and β-adrenoreceptor antagonists. Expression of P-gp in a cell may result in resistance to the effects of a wide variety of drugs, and genetic variation of the protein may result in differing susceptibility to pharmacotherapy. Ethnic background can also increase or decrease the likelihood of interaction between P-gp and a drug.

Besides being expressed in cancer cells, P-glycoprotein is expressed in multiple normal tissues with excretory or protective function including intestine, kidney, liver, blood-brain barrier, spinal cord, testes and placenta. P-gp has an important role in forming a protective barrier against absorption of xenobiotics in these tissues. The broad substrate specificity of P-gp is shared with cytochrome P450 3A4 (CYP3A4), which is well known to metabolize a diverse set of drugs. This broad specificity, coupled with the tissue localization and function of both proteins, has led to the hypothesis that they work in concert, protecting the body from absorption of harmful compounds by acting synergistically in the small intestine.

Significant interindividual variability of the amount of P-gp expressed (two- to eightfold) has been demonstrated in healthy volunteers during intestinal biopsy, suggesting the possibility of variable bioavailability of its substrates. Numerous SNPs of the human *MDR1* gene have been discovered and studied during systematic screening. The frequencies of these SNPs in a population can vary according to racial/ethnic background.[14] At least 29 SNPs have been found, 19 of them located in exonic regions and 11 of them coding for nonsynonymous mutations.[15] Interest in the clinical and functional relevance of polymorphisms of *MDR1* has led to a number of recent reviews.[15-17] Two SNPs of particular interest are a mutation in exon 26 at position 3435 (3435C>T) and a mutation in exon 21 (2677G>T/A). 3435C>T has been exten-

sively studied since it is associated with differences in expression or function of P-gp. Change in nucleotide sequence from C to T at position 3435 does not result in a change of amino acids but is a silent mutation located in the wobble position of the codon. Although there is no change in the expressed protein, both the level of its expression and function can be variable. For instance, a twofold reduction of intestinal P-gp was observed in patients who were homozygous for 3435T.[18] In a number of studies, reduction in P-gp level has been correlated with differences in pharmacokinetic parameters for substrates such as digoxin. There are several possible explanations for the reduction in P-gp expression with homozygous 3435T genotype. One is that there may be a reduction in translation of the protein in this polymorphism.[19]

Other studies have probed the relationship of SNPs with various anticancer agents that are substrates for P-gp transport. For example, one recent study investigated the correlation of *MDR1* polymorphisms with clinical response to docetaxel-cisplatin in nonsmall cell lung cancer (NSCLC) in Han Chinese patients. This study found the 2677 GG genotype was associated with significantly better response to chemotherapy compared with the combined 2677 GT and TT genotypes.[20] The haplotype of 2677G-3435C was also found to be a significant predictor of treatment response in this same study. A demonstrated linkage disequilibrium between the synonymous SNP C3435T and the nonsynonymous SNP 2677G>T/A may explain observed functional differences in P-gp that have previously been attributed to the 3435C>T.[21]

The variation in frequency of SNPs for MDR1 has been studied in different racial/ethnic populations. It has been found that the allelic frequency can differ among these groups. The incidence of *C/T* and *C/C* genotypes at position 3435 has been found to be much higher in African than Caucasian or Asian populations. For instance, in one study 83% of Ghanaians and 61% of African Americans were homozygous for the C allele, while only 26% of Caucasians and 34% of Japanese shared this trait.[22] Individuals that are homozygous for the T allele have substantially lower intestinal P-gp than those that are homozygous for the C allele.[18] Lower intestinal P-gp may increase the bioavailability of P-gp substrates. This seems to be supported by studies that show that the maximum plasma concentrations of the P-gp substrate cyclosporine is substantially lower in African Americans than Caucasians.[23] It has been hypothesized that the higher frequency of the *C/C* genotype in African populations compared to Japanese or Caucasians could result from a selective advantage of this genotype against gastrointestinal-tract infections endemic to tropical regions.[22] On the other hand, the high frequency of the C3435 allele in African populations may explain a high prevalence of more aggressive tumors in breast cancer and the high incidence of resistance to cancer chemotherapy seen in African populations.[14,24,25]

The effect of MDR1 polymorphism on digoxin absorption has been probed in a number of studies.[26,27] Since digoxin is not subject to metabolic transformation, it has been used as a model substrate for the study of phenotype-genotype relationships of *MDR1* polymorphs. A sizable Dutch study (195 elderly patients) involving chronic dosing of digoxin rather than single dose kinetics examined the effect of *MDR1* genotype on digoxin levels.[26] The 3435C>T, 1236C>T, and 2677G>T/A SNPs were identified in peripheral blood DNA. All three variants were associated with serum digoxin concentration of 0.18-0.21 mcg/L per additional T allele. The association was even stronger for the 1236-2677-3435 TTT haplotype and absent from other haplotypes examined. The results of this study agree with another study in healthy Japanese subjects.[28] In this study, a single oral dose of digoxin was administered and the serum concentration of digoxin was monitored. Individuals harboring a T allele at 3435 had significantly lower AUC_4 than those homozygous for C at this position.

It should be noted that not all studies support the association of the 3435C>T SNP with reduced P-gp function or clinical outcome of patients treated with known P-gp substrates. For instance, in a study conducted in Korea of 200 patients with acute myeloid leukemia (AML) undergoing a standard induction chemotherapeutic regimen, no correlation was found between 3435C>T polymorphism and P-glycoprotein function in leukemic blasts or in clinical outcomes.[29] This inconsistency in correlating clinical outcomes with 3435C>T polymorphism in AML and other diseases suggests that other genetic or nongenetic factors also play an important role.

In addition to race and ethnicity, the patient gender can also significantly affect the expression of P-gp. For instance, hepatic P-gp levels are 2- to 2.4-fold lower in females than males.[30] In the case of antineoplastics such as vinca alkaloids, etoposide, doxorubicin, and docetaxel, this means increased risk for myelosuppression and gastrointestinal toxicity in females, as well as prolonged drug exposure.[31] Thus females may have an increased response to a drug, as well as increased toxicity.

Clinical Pearl

Patient gender can influence the rate of clearance and efficacy for drugs that are transported by P-glycoprotein.

Another anticancer drug that has been extensively studied with respect to pharmacogenomics is irinotecan.[3-6] Irinotecan is a prodrug, transformed to the active metabolite 7-ethyl-10-hydroxycamptothecan (SN-38) by carboxylesterase enzymes. SN-38 is thought to be responsible for most of the activity of irinotecan. SN-38 is transformed in phase II metabolism to the glucuronide conjugate by UDP-glucuronosyltransferase (UGT) enzymes. The resultant conjugate is more hydrophilic than the parent and is subsequently eliminated in the bile or urine by transport proteins. These proteins include ABCB1, ABCC1, ABCC2, ABCG2, and SLC01B1 (OATP1B1). Standard dosing regimens of irinotecan rely on calculation of patient body surface area, which correlates with blood volume. However, it has been found that there is tremendous interindividual variability of response to irinotecan, with some patients developing severe life-threatening diarrhea and neutropenia. Correct dosing is critical since reduced plasma levels will not provide effective treatment, while elevated levels produce toxicity. Modifications of dosing regimens are recommended based on the observed individual toxicity. Polymorphisms of UGT1A1 that reduce glucuronidation and thus increase plasma levels have been definitively identified. Because of this, in 2005 the package labeling was revised to recommend reduced dosing in patients known to be homozygous for the *UGT1A1*28* allele. This includes approximately 10% of the North American population. In 2005 the FDA also approved a genetic test to aid the detection and identification of *UGT1A1*28* (Invader UGT1A1 by Third Wave Technologies Inc.). Polymorphisms of transport proteins with reduced activity would naturally be expected to further modify pharmacokinetics and possibly increase toxicity. This supposition has been supported for *ABCB1* (1236C>T), *ABCC2* (3972T>C), *ABCG2* (delCTCA -19572-19576 and 421C>A), and *SLC01B1*1b* in various ethnic groups.[32-38] This data indicates that testing for transporter polymorphisms may further improve quality of treatment for irinotecan.

ABCC Transporter Family

The protein product of ABCC genes are commonly known as MRPs or multidrug resistance proteins. In contrast to the neutral and cationic hydrophobic compounds that P-gp transports, MRPs often transport anionic compounds. Ten members of the MRP family are known and at least seven may be involved in conferring resistance to cancer chemotherapeutics (MRP1 to MRP7).[12] MRP1 has the most likely significance in clinical anticancer drug resistance. MRPs are located in various tissues with protective and excretory function such as the brain, liver, kidney, and intestines. They transport a structurally diverse set of endogenous substances, xenobiotics, and metabolites. Genetic polymorphisms of ABCC1-5 have been subject to intensive study recently.[39]

ABCC1 Transporters

The ABCC1 (MRP1) transport protein has broad substrate specificity and is expressed in many tissues of the body. It was originally discovered in small-cell lung cancer cells that showed multidrug resistance without overexpressing ABCB1 (MDR1). Similarly to MDR1, it is able to confer resistance to anthracyclines and vinca alkaloids. MRP1 transports primarily neutral and anionic hydrophobic compounds and their glutathione, sulfate, and glucuronide conjugates. A few cationic substances can also be transported. Many unconjugated substances are cotransported with reduced glutathione (G-SH). The oxidized form of glutathione (G-SS-G) is also transported by MRP1. In most polarized cells, localization of the protein is on basolateral membranes for efflux of substrates into the blood. It occurs in many epithelial tissues, such as the testes, skeletal muscle, heart, kidney, and lung, and may have a protective role for the central nervous system. Physiologically relevant endogenous compounds that are transported by MRP1 include leukotriene C4, which is important for inflammatory reactions.

There are a number of nonsynonymous genetic variants of the transporter that have been studied for functional significance by in vitro methods. For instance, Arg433Ser decreased the transport of leukotriene C4 and estrone sulfate but not estradiol 17-βglucuronide.[40] This same SNP conferred a 2.1-fold resistance to doxorubicin compared to cells expressing the wild type MRP1. Another SNP, Cys43Ser, has been associated with a decrease in vincristine resistance. In this case, the polymorphism led to loss of localization to the correct cell membrane.[41] Polymorphisms in the promoter region of *ABCC1* have also been found, raising the possibility of differences in promoter activity and gene expression.[42]

ABCC2 Transporters

The ABCC2 transporter is also known as multidrug resistance protein-2 (MRP2) or canalicular multispecific organic anion transporter (cMOAT). It is the most studied member of the ABCC family. This protein is expressed in the liver, kidneys, and intestines. It plays an important role in chemoprotection by transporting the products of phase II metabolism out of cells. Thus glucuronide, glutathione, and sulfate conjugates of drugs are predominant substrates of MRP2. These conjugates are transported from hepatic cells into the canaliculi and then to the bile for excretion. Unconjugated drugs are also transported, as are the conjugates of bilirubin. Unlike other members of the ABCC family, ABCC2 is expressed in apical membranes of absorptive and excretory cells, such as hepatocytes, enterocytes, renal proximal tubules, and syncytiotrophoblasts of the placenta.

Mutations in the *ABCC2* gene are associated with the rare autosomal recessive disorder Dubin-Johnson syndrome (DJS). These mutations may cause DJS through a variety of mechanisms. The most obvious is the formation of nonfunctional forms of the protein, which results in the inability for hepatocytes to secrete conjugated bilirubin into the bile. Many of the mutations associated with DJS occur on the ATP binding region, which is critical for protein function. Other mutations result in impaired transcription and localization of the protein or reduced substrate binding. The results of the dysfunction are conjugated hyperbilirubinemia and consequent deposition of pigment into hepatocytes. Occurrence of DJS is most common in males, but in women pregnancy or oral contraceptive use may result in jaundice. The prevalence of DJS varies among racial/ethnic populations, and it is most commonly seen in Iranian Jewish patients. Besides modification of hepatic function, DJS patients have been thought to have reduced expression and function of intestinal MRP2, although there is little evidence of this.[43] Wide ranging studies concerning the effect of DJS polymorphisms on drug pharmacokinetics are not yet available, but some small scale studies have been completed.

In a case study of a patient with DJS being treated for large B-cell lymphoma with methotrexate, a threefold reduction in methotrexate elimination rate was observed, resulting in severe overdosing and reversible nephrotoxicity. Genetic analysis of the *ABCC2* gene revealed a heterozygous SNP Arg412Gly, which occurs in a region of the protein associated with substrate binding. Functional analysis revealed that this mutation conferred loss of transport activity.[44] This case is illustrative of a situation where effective pharmacogenomic screening might be successfully applied to improve patient care.

Other studies have attempted to correlate the expression of MRP2 with both intrinsic and acquired resistance to other cancer chemotherapeutics, for instance cisplatin in the treatment of pancreatic cancer.[45] In resected pancreatic cancer tissues only MRP2 mRNA, and not MRP1 or MRP3, was expressed, and it was overexpressed compared to normal pancreatic tissue. In this same study when pancreatic cancer cells were cultured in the presence of cisplatin, they began to overexpress MRP2 but not MRP1 or MRP3 proteins.

ABCC3 Transporters

The *ABCC3* gene, which codes for MRP3, has not been studied as extensively as either MDR1 or MDR2. In contrast to the MDR1, MRP3 does not transport glutathione and is a poor transporter for glutathione conjugates.[46] Glucuronide conjugates are transported, such as estradiol-17-βglucuronide. MRP3 is localized in the liver, kidneys, and intestines. Location in polarized cells is in basolateral membranes, similar to MRP1. A number of different polymorphs have been investigated for their effect on MRP3 expression levels. One of the SNPs frequently found in the promoter region, 211C>T, has possible relevance for pharmacotherapy and disease progression.[47] Individuals homozygous or heterozygous for this SNP showed significantly lower MRP3 mRNA levels than individuals with a wild-type allele. This SNP has been studied for its association with adult acute myelogenous leukemia (AML) as a predictor for disease predisposition or prognosis.[48] It was found that 211C>T had a negative effect on prognosis. Conflicting results have been obtained for the correlation of 211C>T with treatment outcome in childhood AML.[49]

ABCC4 and ABCC5 Transporters

These proteins, also known as MRP4 and MRP5, respectively, are much less studied than MRP1, MRP2, and MRP3. Tissue localization is shown in Table 5-1. Substrates for both transporters are anticancer/antiviral nucleoside and nucleotide analogs as well as various organic anions. A number of SNPs have been identified for these transporters. Some of these have been suggested to have relevance for pharmacotherapy. For instance, the SNP in MRP4 (rs3765534) was found to dramatically reduce MRP4 function through impairment of membrane localization.[50] This SNP is relatively common in Japanese patients (>18%) and may play a role in the high sensitivity that some patients have for thiopurines.

Table 5-1
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Transporter (common name)	Gene Name (systematic protein name)	Tissue Localization and Position in Polarized Cells	Representative Substrates	Example Polymorphisms and Phenotype Effect
MDR1, P-gp	ABCB1	Apical: kidney, liver, brain, intestine, placenta	Anthracyclines, cyclosporine, taxanes, vinca alkaloids, doxorubicin[82]	3435C>T (↓ intestinal expression, ↓ substrate bioavailability)[18] 2677G>T/A (↑ response to docetaxel/cisplatin)[20]
MRP1	ABCC1	Lung, ubiquitous on basolateral membrane epithelial: e.g., choroid plexus (blood-cerebrospinal fluid barrier), testes	Anthracyclines, vinca alkaloids, methotrexate, glutathione conjugates, leukotriene C4, bilirubin, glutathione, saquinavir, ritonavir, difloxacin	Arg433Ser (↑ doxorubicin resistance)[40] Cys433Ser (↓ vincristine resistance)[41]
MRP2, cMOAT	ABCC2	Apical: liver, proximal tubule, small intestine, placenta	Bilirubin conjugates, glucuronide, sulfate & glutathione conjugates of various drugs, unconjugated anionic drugs (e.g., methotrexate): broad substrate specificity	↑ cisplatin resistance[45] 2302C>T, 2439T>C (Dubin-Johnson syndrome)[83] c.3972C>T (↑ hepatocellular carcinoma)[84]

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MRP3	ABCC3	Basolateral: liver, kidneys, intestines	Glucuronidated substrates (acetaminophen,[85] morphine,[86] estradiol, bilirubin)	-211C>T (↓ expression),[47] (worsen prognosis: lung cancer)[87] Arg1381Ser, Ser346Phe, & Ser607Asn (↓ transport activity)[88]
MRP4 and MRP5	ABCC4 and ABCC5	Prostate[89] (asolateral), kidney,[90] lung,[91] brain,[92] pancreas,[93] lymphocytes,[94] platelets,[95] heart (MRP5)[96]	Azidothymidine, mercaptopurine, thioguanine, cladribine, abacavir[39]	MRP4: rs3765534 (↑ thiopurine sensitivity), Gly187Trp, Gly487Glu (↓ azidothymidine transport),[97] A3463G (↓ tenofovir efflux)[98]
MRP6	ABCC6	Basolateral: liver, kidney	Glutathione conjugates, leukotriene C_4	Many: e.g., c.3421C>T (pseudoxanthoma elasticum)[99]
BCRP, MXR, ABCP	ABCG2	Placenta syncytiotrophoblasts, hepatocyte canalicular, apical intestinal epithelia, vascular endothelia	Doxorubicin, daunorubicin, mitoxantrone, topotecan, prazosin, uric acid[58]	↑ anthracyclines, mitoxantrone, SN-38 resistance 421C>A (worsen prognosis: lung cancer & cisplatin),[87] (↑ gefitinib-induced diarrhea)[57] Gln141Lys (↑ chemotherapy-induced diarrhea)[100]
Serotonin transporter	SLC6A4	Neurons, heart valve, intestine (apical)[101]	Serotonin	"l" allele (↑ psychopathology)[102] "s" allele (↓ antidepressant efficacy, citalopram-induced diarrhea)[65]
Reduced folate carrier (RFC-1)	SLC19A1	Apical: kidney, leukemic cells, wide distribution	Methotrexate, leucovorin, pemetrexed	80AA (↑ methotrexate polyglutamation)[103]
OATP1B1	SLCO1B1	Basolateral: liver, brain	Pravastatin, atorvastatin, lovastatin, cerivastatin, bilirubin, digoxin, estradiol, thyroid hormones, mycophenolate	521T>C (↓ pravastatin AUC)[104]

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OATP1B3	SLCO1B3	Basolateral: liver	Methotrexate, glucuronidated estradiol, mycophenolate	334T>G (GG ↑ mycophenolate AUC)[105]
PEPT1 and PEPT2	SLC15A1, SLC15A2	PEPT1: small intestine, duodenum (apical) PEPT2: broad distribution[106]	Cephalexin, other β-lactam antibiotics, ACE inhibitors (?), valacyclovir, peptides	Arg57His (transport function loss)[107]
RFC-1	SLC19A1	Broad distribution	Methotrexate	80A>G (AA ↑ plasma folate)[77](↑ remission of rheumatoid arthritis with methotrexate)[78]
CNT1, CNT2, CNT3	SLC28A1, SLC28A2, SLC28A3	Intestinal/renal epithelia, liver, macrophages, leukemic cells[1]	Didanosine, idoxuridine, zidovudine, cladribine, fludarabine, gemcitabine, capecitabine[1]	Unclear relevance for polymorphisms
ENT1, ENT2, ENT3, ENT4	SLC29A1, SLC29A2, SLC29A3, SLC28A4	Intestine, liver, kidney, placenta[108]	Pyrimidine and/or purine nucleosides, adenosine, gemcitabine, cladribine, fludarabine	Unclear relevance for polymorphisms

ABCC6 Transporters

ABCC6 encodes MRP6 protein, also known as MRP-like protein 1 (MLP-1), anthracycline resistance associated protein (ARA), and multispecific organic anion transporter-E (MOAT-E). It is expressed primarily in the liver and kidneys. Mutations in the ABCC6 gene are associated with pseudoxanthoma elasticum, a disease that causes mineralization of elastic fibers in some tissues.

ABCG2 Transporters

ABCG2 is alternatively known as Breast Cancer Resistance Protein (BCRP), placenta-specific ABC transporter (ABCP), and mitoxantrone resistance protein (MTX). It was originally identified in a resistant breast cancer cell line. It is very important in limiting bioavailability of certain drugs, concentrating drugs in breast milk, and protecting the fetus from drugs in maternal circulation.[7] It is highly expressed in the gastrointestinal tract, liver, and placenta, and influences the absorption and distribution of a wide variety of drugs and organic anions.[51-53] The substrate specificity for ABCG2 is broad and overlaps that of P-glycoprotein but is distinct from it. In contrast to the rest of the ABC transporter family, ABCG2 contains only one binding site for ATP and one transmembrane domain, rather than two of each. It is assumed to function as a dimer and is therefore referred to as a *half-transporter.* ABCG2 confers resistance to a broad range of hydrophobic anticancer drugs, similar to P-gp and MDR1, and is considered one of the

most important ABC transporters mediating multidrug resistance in cancer cells. Resistance can be brought about by either reduced absorption or increased biliary excretion of the drug.

Various polymorphisms of ABCG2 are known to exist, some of which are associated with increased resistance to anticancer drugs such as mitoxantrone, the anthracyclines, and camptothecin derivatives. Some SNPs that have been associated with altered transport activity are Arg428Gly and Arg428Thr, Cys421Ala, Val12Met, Gln141Lys, Gln126X.[54,55] Other drugs that act as inhibitors of ABCG2 are antiviral nucleoside analogs such as zidovudine (AZT), lopinavir, nelfinavir, etc.[56] One SNP of ABCG2 has been associated with adverse reactions in patients treated with gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase used in nonsmall-cell lung cancer.[57] Thus, 44% of patients that were heterozygous for the Cys421Ala polymorphism developed diarrhea after treatment with gefitinib, versus 12% of patients homozygous for the wild type protein.

Besides being associated with adverse drug reactions and variations in therapeutic efficacy, SNPs of ABCG2 have been found to be highly predictive of plasma uric acid levels in one large study.[58] In this study a genome-wide scan was made for SNPs associated with serum uric acid concentration and gout. The study used phenotype and genotype results from a cohort of the Framingham Heart Study as well as a Rotterdam cohort. SNPs identified as being associated with uric acid concentration and gout were identified in ABCG2, SLC17A3, and SLC2A9. The results of this study were used to calculate a risk score for an individual based on whether they have the polymorphisms associated with hyperuricemia. The risk score was generated based on the number of alleles associated with high uric acid concentration. Mean uric acid concentration rose linearly with the number of risk alleles. For individuals with no risk alleles, prevalence of gout was 1% to 2% across the cohorts examined. The prevalence increased to 8% to 12% with six risk alleles. Individual common genetic variants were found to confer only a modest risk of gout, but their combination resulted in a large association with uric acid and gout. Ultimately the risk score may be used to help identify patients with asymptomatic hyperuricemia and guide therapeutic intervention.

One of the primary tissues in which ABCG2 is expressed in humans is in the placenta. The precise physiological function of the protein in this location is not clear, but it likely plays a strong role in protecting the fetus from xenobiotics, toxins, and metabolites by expelling them across the placental barrier.[59] Wide variations in the expression level of BCRP has been found in human placenta, which may lead to considerable variation in fetal exposure to drugs and xenobiotics. Such variation may be caused by polymorphisms in BCRP.[60]

Solute Carrier Proteins

Solute carrier proteins (SLCs) are important in transport of ions and organic substances across biological membranes in the maintenance of homeostasis. Members of the SLC superfamily consist of membrane channels, facilitative transporters, and secondary active transporters. Examples of some of the endogenous solutes that are transported include steroid hormones, thyroid hormones, leukotrienes, and prostaglandins. Additionally, SLCs are important in the transport of a large number of drugs. The solute carrier protein class includes the transporters known as OATs (organic anion transporters), the OATPs (organic anion transporting polypeptides, which are structurally different from OATs), OCTs (organic cation transporters), and PepTs (peptide transport proteins). In all, more than 40 families of transporters make up the SLC superfamily. Members within an individual SLC family have >20% to 25% sequence homology. However, the homology between families is low or nonexistent. Thus inclusion of

a family into the SLC group is not dependent on evolutionary or structural relationship, but rather is a functional classification. Individual members of the SLCs are expressed in a variety of tissues such as liver, kidney, brain, and intestine and transport substances either into or out of cells.

SLCO1B1

Genetic variants of solute carrier proteins, such as SLCO1B1, have been associated with pharmacogenomic relevance. For example, a genome-wide scan of 300,000 genetic markers in a study of statin-induced myopathy found a strong correlation with the rs4363657 SNP located within the SLCO1B1 gene.[61] SLCO1B1 encodes the sodium-independent organic anion transporting peptide OATP1B1. An increased risk of myopathy was associated with simvastatin use in patients expressing this particular variation. Polymorphisms of solute transporter genes have also been associated with pharmacokinetic variance for other statin drugs. For example, altered uptake of pravastatin into the liver has been associated with polymorphisms of SLC21A6 (OATP-C) and SLC22A8 (OAT3).[62] A variant of SLCO1B1 has also been associated with functionally relevant SNPs important for the pharmacokinetics of other drugs, such as the irinotecan metabolite SN-38, estrone 3-sulfate, and estradiol 17-beta glucuronide. Methods have been developed to rapidly identify the relevant SNPs.[63]

SLC6 Family

Members of the SLC6 family are sodium-dependant transporters for neurotransmitters such as dopamine, serotonin, norepinephrine, glycine, and GABA. The *SLC6A4* gene codes for the serotonin transporter (SERT). The best evidence for pharmacogenomic relevance within the SLC6 family has been found for SERT, which is a cotransporter for serotonin and sodium ions. Its physiological function at the synapse is serotonin reuptake and thus termination of the signal. Since this protein is the site of action of the serotonin reuptake inhibitors, there has been much interest in the effect of polymorphisms of SERT on drug action and pathology.

The 5HTTLPR (serotonin transporter linked promoter region) of the *SLC6A4* gene has been extensively studied for association with neuropsychiatric disorders. This polymorphism occurs in the promoter region of the gene rather than in the protein coding region. It is associated with short *s* and long *l* repeats in this region. The short variation contains fourteen repeats of a particular sequence, while the long version contains sixteen repeats. The short version leads to reduced promoter activity and less transcription of *SLC6A4*, while the l allele has the opposite effect. A number of studies and meta-analyses have found that the ss genotype or s allele is predictive of reduced antidepressant efficacy, while the LL genotype is associated with better response to therapy. Other studies have found that presence of the s allele is associated with greater numbers of side effects during treatment of depression with selective serotonin reuptake inhibitors.[64] For instance, in one large study, adverse effects of citalopram were strongly associated with the 5HTTLPR s allele and ss genotype. Interestingly, in this study there was no differentiation between therapeutic responses for the different alleles.[65] In summary, there is mounting evidence that genetic screening may soon become useful for predicting if a given antidepressant will be effective or produce adverse effects in a patient. This would be a major advancement for individualizing the pharmacotherapy of depression.

The *SLC6A3* gene encodes for the dopamine transporter DAT1. Polymorphisms have been found for this transporter, and an attempt has been made to correlate genotype with neuropsychiatric disorders, such as attention deficit hyperactivity disorder (ADHD). One study suggested an association of a particular haplotype with adult ADHD.[66] The haplotype implicated was the

9-6 *SLC6A3*-haplotype, formed by the 9-repeat allele of the variable number of tandem repeat (VNTR) polymorphism in the 3' untranslated region of the gene and the 6-repeat allele of the VNTR in intron 8 of the gene. Polymorphisms of DAT1 have also been implicated in variability of response to methylphenidate in ADHD, although there has been conflicting results presented by a number of studies. In one meta-analysis, a significant relationship was seen between low rates of methylphenidate response and a homozygotic 10R VNTR polymorphism.[67]

The *SLC6A2* gene encodes for the norepinephrine transporter (NET). Because of the wide implications of this neurotransmitter in neuropsychiatric disorders and drug action, a number of studies have focused on finding polymorphisms for this gene and correlating them to therapeutic response. One study that examined predictive antidepressant response to the mixed serotonin/norepinephrine reuptake inhibitor milnacipran found that a polymorphism of NET was associated with superior response.[68] Substantially more research is needed in this area to make firm predictions regarding antidepressant response.

SLC15 Family

The PEPT1 and PEPT2 transporters (SLC15A1 and SLC15A2) are proton-coupled oligopeptide transporters. They carry small peptides of two to three residues, as well as peptide-like drugs that would otherwise not cross lipid membranes. Intestinal PEPT1 is involved in uptake of cephalexin and other β-lactams. The nucleoside prodrugs valacyclovir and valganciclovir have enhanced bioavailability due to transport by PEPT1.[69] Significant interindividual variation in intestinal absorption of valacyclovir suggests the presence of genetic factors.[70] Angiotensin-converting enzyme inhibitors are also often considered to be substrates for PEPT1 and PEPT2; however, data supporting this claim is inconsistent.[71,72] Besides being localized in the intestine, PEPT transporters are found in the kidney and liver, and in the case of PEPT2, in the central and peripheral nervous system, lung, heart, and mammary glands.

Clinical relevance for genetic variation of PEPT1 or PEPT2 remains murky, but several researchers have studied polymorphisms of these loci. In one study, in a panel of 44 ethnically diverse individuals, nine nonsynonymous and four synonymous polymorphisms were identified in PEPT1.[73] When transfected into an immortal cell line and analyzed for transport capacity, only one rare SNP (Pro586Leu) was found to be associated with reduced activity, which resulted from post-translational reduction of protein expression in the plasma membrane. The results of this study have been confirmed and extended to 247 individuals of various ethnic origins.[74] This study found that there were additional genetic variants of PEPT1, but concluded genetic factors played only a small role in determining interindividual variation in PEPT1 transport activity in the intestine. Because of the vital role that PEPT1 plays in normal homeostasis, mutations that result in loss of activity likely have a high negative evolutionary selection pressure. This does not, however, preclude future discovery of polymorphs with variation in expression or activity. In the case of PEPT2, polymorphs have been identified that lack transport function and have differing affinity and pH sensitivity. Variable mRNA expression has also been observed, likely due to *cis* acting polymorphisms.[75] Thus there is a considerable variability in the PEPT2 gene, with a possible influence on the pharmacokinetics of drugs transported by PEPT2.

SLC19A1

SLC19A1, also known as reduced folate carrier-1 (RFC-1), is involved in the transport of folate and antifolate drugs into human cells. Resistance to folate anticancer drugs may be mediated by point mutations of this transporter. Since lack of nutritional folate is strongly associated with birth defects such as cleft palate, it would be expected that variants of the folate carrier might also be associated with these defects. While one study failed to show a strong correlation between genetic variants of RFC-1 and cleft palate, this same study did show modest evidence for an interaction between infant RFC-1 genotype and risk of certain congenital heart defects.[76] The specific variant examined was the SNP 80A>G, which results in the replacement of a histidine residue with an arginine in the protein.[77] The functional result of this replacement on the transport protein is unknown, but higher plasma folate levels were observed in individuals homozygous for A80 compared to individuals with a G80/G80 genotype.

Methotrexate is an example of a drug that is transported by the reduced folate carrier-1. The 80G>A polymorphism in RFC-1 has been associated with altered treatment efficacy in patients with rheumatoid arthritis treated with methotrexate. In one study, the probability of remission was 3.3-fold higher in patients with the 80AA genotype compared to those with the 80GG genotype. The frequency of the A allele was also found to be 14% higher in patients that responded to methotrexate compared to nonresponders. Additionally, aminotransferase activity was noted more frequently in carriers of the 80AA genotype.[78] All of this information suggests that evaluation of RFC-1 polymorphism could be useful for optimization of methotrexate therapy.

Another study examined the effect of the Gly80Ala polymorphism in RFC-1 in relation to risk for thrombosis.[79] Since folate lowers homocysteine, which is thrombogenic, reduction in the transport of folate might be expected to have an effect on the prevalence of thrombosis. This study did find a significant protective effect of the A allele against thrombosis. No effect on homocysteine plasma level was observed, but an increased extracellular to intracellular ratio of folate was seen. This is consistent with the biological role of RFC-1 and may explain the protective effect of the polymorph against thrombosis.

SLC28

There are three members of the *SLC28* gene family in humans: *SLC28A1, SLC28A2*, and *SLC28A3*. All of them encode nucleoside transporters coupled to ion gradients (i.e., concentrative nucleoside transporters CNT1, CNT2, and CNT3). CNT1 translocates pyrimidines, while CNT2 translocates purines. Both of them are coupled to sodium ion transport. The CNT3 transporter has broad selectivity and can transport nucleosides coupled to either sodium ions or protons. This coupling allows the transporters to move nucleosides against their concentration gradient. Besides transporting naturally occurring nucleosides such as adenosine, these transporters are vitally important in transporting anticancer/antiviral nucleosides into cells. Since nucleoside drugs are mostly hydrophilic molecules, cellular uptake is dependent on transport proteins. Along with the SLC29 family, SLC28 members are important for salvage processing of nucleosides. SNPs of the CNTs have been identified, but pharmacogenomic relevance is currently poorly defined.

SL29

The family *SLC29* genes code for equilibrative nucleoside transporter proteins (ENT). There are four members of this family in humans: SLC29A1 to SLC29A4 (ENT1 to ENT4). ENT1 is independent of sodium ion concentration, in contrast to concentrative nucleoside transporters. This transporter plays a role in cellular uptake of anticancer nucleoside analogs.[80] The clini-

cal relevance of this finding is currently unclear, although a number of polymorphisms at this gene have been uncovered. In one study on a population of 256 Japanese cancer patients, 39 variations of the gene were found, with a highest frequency of 0.051.[81]

Summary

In summary, evidence is accumulating of the many ways in which genotype for individual transport proteins affects the response to a variety of medications. Additionally, the profound effect of racial/ethnic heritage on distribution of genotypic variation can no longer be ignored. This chapter has reviewed some of the transporters with accumulated evidence for pharmacogenomic relevance. Many other transporters have been identified that also may ultimately be found to be important for determining individual therapeutic response. Also, many more SNPs for transport proteins have been identified than have been studied in vivo. The advent of inexpensive broad genetic screening for transport protein polymorphisms will no doubt be instrumental in a new era of truly personalized therapy. For instance, DNA chips are now available that screen 100,000 SNPs in a matter of hours. In order to solidify treatment guidelines for genetically diverse populations, tremendous amounts of research continue to be needed in this area. Since few drugs are transported by just one carrier protein, other carriers may compensate for a deleterious SNP. Thus a single SNP is often not capable of altering the pharmacokinetics of a drug. For this reason, future studies that are more comprehensive in scope will offer more insight into the genetics of drug response.

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