CHAPTER 1

INTRODUCTION TO PHARMACOGENOMICS OF DRUG TRANSPORTERS

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1.1   INTRODUCTION

Understanding the molecular mechanisms and clinical relevance of interindividual variability in drug response remains an important challenge. Pharmacogenomics, the study of genetic variation in the genes that influence drug effect, can provide insight into interindividual variability and a more accurate prediction of drug response than may be obtained by relying solely on a patient's clinical information. The goal of drug transporter pharmacogenomics is to understand the impact of genetic variation on the function of transporters that interact with medications. For many drugs in clinical use, transporters are important determinants of absorption, tissue accumulation, and elimination from the body, and thereby transporters significantly influence drug efficacy and toxicity. Adverse drug reactions can result from toxicity associated with high drug concentrations and lack of efficacy can result from subtherapeutic drug exposure. By understanding the genetic basis for drug transporter activity, it will be possible to enhance a predictive approach to individualization of drug therapy.

The purpose of this book is to highlight the advances in transporter pharmacogenomics that have been made since polymorphisms in drug transporter genes were first described in the late 1990s (Mickley et al., 1998; Hoffmeyer et al., 2000; Kim et al., 2001). As we enter the genomic era of medicine, pharmacogenomics will inform prescribing practices to maximize drug efficacy while minimizing risk for toxicity. Given the importance of transporters to the absorption, distribution, and elimination of many drugs, there is no doubt that transporter pharmacogenomics will make significant contributions to this aim.

1.2   OVERVIEW OF DRUG TRANSPORTERS

Membrane transporters have diverse and important roles in maintaining cellular homeostasis by the uptake and efflux of endogenous compounds to regulate solute and fluid balance, facilitate hormone signaling, and extrude potential toxins. Drug transport proteins are a functional subset of membrane transporters that also interact with drugs and their metabolites. Compounds that are most likely to rely on carrier mechanisms are polar and bulky, and less likely to pass through cell membranes by simple diffusion. Transporter substrates include numerous drugs, their hydroxylated metabolites, and the glutathione-, sulfate-, or glucuronide-conjugated products of Phase II metabolism. Transporters that are expressed in the epithelia of intestine, liver, and kidney are of particular importance for vectorial or directional movement of drugs, resulting in efficient and rapid drug absorption, distribution, metabolism, and elimination. Moreover, expression of drug transporters on the basolateral versus apical domain of polarized epithelial cells in organs such as the intestine and liver may also be critical for a drug to enter the tissue and interact with its target (Ho and Kim, 2005; Giacomini et al., 2010).

Membrane transporters are comprised of multiple transmembrane domains (TMDs) that form a pore in the membrane through which the substrates pass. These domains are joined by alternating intracellular and extracellular loops which, together with TMDs, facilitate substrate recognition, binding, and translocation. The functional mechanism and conformational changes required for transport are not completely understood, and remain an active area of investigation (Kerr et al., 2010). Of particular interest to transporter pharmacogenomics is the ability to predict the functional effect of novel mutations that are discovered in individual genomes.

Drug transporters belong to two major classes, the solute carrier (SLC) superfamily and ATP-binding cassette (ABC) superfamily. In the human genome, there are 350 transporters in the SLC superfamily and 48 ABC transporters; these transporters are divided into subfamilies based on sequence homology (Giacomini et al., 2010). ABC transporters are distinguished by the presence of an intracellular nucleotide-binding domain that catalyzes the hydrolysis of ATP to generate the energy required to transport substrates against their concentration gradient (Schinkel and Jonker, 2003). In contrast, SLC transporters utilize facilitated diffusion, ion coupling, or ion exchange to translocate their substrates. In some cases, transport relies on an ion gradient that is actively maintained by ABC transporters (Hediger et al., 2004).

Transporter function may be influenced by multiple factors, and interindividual variability in transporter function is now recognized as a major source of variability in drug disposition and response. We know that drug transporters can be inhibited by numerous compounds, typically by competition for recognition and binding, resulting in unexpected pharmacokinetics of substrate drugs, and drug–drug interactions. Genetic variants may also affect transporter function, and, in recent years, the discovery of genetic variation in drug transporters has opened up an

area of research in transporter pharmacogenomics (Giacomini et al., 2010; Sissung et al., 2010a).

1.3   OVERVIEW OF PHARMACOGENOMICS

The study of inherited differences in drug response dates back to observations made in the 1950s; in the late 1980s, molecular advances provided a mechanistic explanation for these findings (Evans and Relling, 1999; Weinshilboum and Wang, 2006). Many early achievements in pharmacogenetics were in cytochrome P450 (CYP) drug-metabolizing enzyme research and the effect of genetic variation in these enzymes on metabolite concentrations. Pharmacogenomics studies have benefited from having well-defined phenotypes: a pharmacokinetic measure such as the plasma or urine concentration of a drug or its metabolite, or a measure of drug response, such as a change in blood pressure or heart rate. For monogenic traits, this approach has led to new insights into our understanding of the factors underlying drug disposition and response, and provided a solid foundation to study traits that are influenced by multiple genes and other clinical factors. Today, pharmacogenomics encompasses a broad spectrum of genes involved in metabolism as well as transport, and in drug targets and related pathways (Sim and Ingelman-Sundberg, 2011).

Genetic variants include single-nucleotide polymorphisms (SNPs), which are typically present in less than 1% of the population, while more rare variants are considered to be genetic mutations. SNPs in the coding regions of proteins may be classified as synonymous or nonsynonymous, depending on whether the amino acid sequence is altered in the variant allele. SNPs may also come in the form of small insertions or deletions, which result in frameshift of amino acid sequence or premature truncation of the protein, and likely a nonfunctional product (Urban et al., 2006). Duplication or deletion of larger regions of genomic sequence (>50 bp) are classified as copy number or structural variants (Alkan et al., 2011). A classic example of copy-number variation comes from the field of pharmacogenomics: CYP2D6 is commonly duplicated or deleted, resulting in profound differences in the rate of metabolism of its substrate drugs in individuals with these alleles (Zanger et al., 2004). There is a growing appreciation for the importance of structural differences as a source of variation in the human genome, and further study of this variation, as it relates to transporter genes, is expected (Alkan et al., 2011).

Pharmacogenomic information may be used to predict treatment outcomes and choose the best drug and its optimal dose. Pharmacogenomics may also be used to predict a patient's risk for an adverse drug reaction, including drug–drug interactions that may be more severe due to the genetics of the proteins involved. At the time of writing, the US Food and Drug Administration (FDA) listed nearly 80 drugs for which pharmacogenomic biomarkers in over 30 genes were included in some part of the label recommendations. To date, the FDA has focused on drug-metabolizing enzymes and target proteins; however, transporter genes are expected to be added in

the future, following the work of the International Transporter Consortium, sponsored by the FDA's Critical Path Initiative (Giacomini et al., 2010).

1.4   PHARMACOGENOMICS OF DRUG TRANSPORTERS

Transporter polymorphisms may increase or reduce an individual's overall exposure to a substrate, depending on the tissue expression and localization of the transporter. For example, reduced function of an uptake transporter on the luminal membrane of the intestine would result in reduced systemic exposure of its substrate, whereas reduced function of an uptake transporter on the basolateral membrane of the liver or kidney may result in increased systemic exposure if the drug in question relies on these organs for its elimination. On the other hand, reduced function of an ABC efflux transporter present on the luminal membrane of the intestine will result in increased plasma concentration of the substrate drug, as less drug is returned to the intestinal lumen by the transporter. In some cases, the precise *in vivo* contribution of a transporter may be difficult to define, particularly if the transporter is present in multiple tissues, or has overlapping function with transporters of similar expression patterns. The extent of phenotypic variation observed will depend on how much the substrate relies on the single transporter in question, and the extent of genetic variation present in the other transporters, metabolizing enzymes and targets that interact with the drug.

To date, the best studied transporter polymorphisms have been those in the coding regions of transporter genes. Some variants cause reduced trafficking of the transporter to the cell membrane, resulting from incorrect folding or an inability to interact with molecular chaperones, and other variants may affect substrate recognition or binding. Certain amino acid changes, particularly in substrate binding regions, have been shown to alter transport in a substrate-specific fashion, making it difficult to fully predict the effect of a polymorphism on transport of a particular compound without testing that compound directly. Although numerous polymorphisms in transporter genes have been identified, not all polymorphisms appear to affect transporter function. One method to test the function of a SNP is to express its protein product and measure its transport function *in vitro*. Of the 88 protein-altering variants studied in 11 SLC transporters, 14% had decreased or total loss of functional activity in *in vitro* assays (Urban et al., 2006). This is likely an underestimation, due to the possibility of substrate-specific differences in effect.

Analysis of large numbers of SNPs in the coding regions of transporters demonstrated that genetic diversity is significantly higher in loop domains compared to TMDs, suggesting that there is selective pressure against amino acid changes in these regions (Leabman et al., 2003). Polymorphisms may also occur in intronic regions, affecting splicing, or in promoter and enhancer regions, affecting RNA expression. Analysis of proximal promoter region variation showed that SLC transporter promoters are more likely to contain variants than ABC transporter promoters, and highly active promoters are more likely to contain variants than less active ones (Hesselson et al., 2009). Genetic diversity in transporter genes also appears to be related to ethnicity. In a study of 680 SNPs identified from samples representing five

ethnic populations, only 83 SNPs were present in all the five populations (Leabman et al., 2003). Thus, differences in transporter polymorphism frequency may account for some variability in drug response observed across ethnicities.

The pharmacogenomics of SLC transporters of particular importance to drug transport are described in the following chapters: organic anion transporting polypeptides (OATPs/SLCO; Chapters 6 and 7), organic anion transporters (OATs/SLC22A; Chapter 6), organic cation transporters (OCTs/SLC22A; Chapter 8), organic cation and carnitine transporters (OCTN/SLC22A; Chapter 8), multidrug and toxin extrusion transporters (MATEs/SLC47; Chapter 9), peptide transporters (PEPTs/SLC15A; Chapter 10), and nucleoside transporters (NTs/SLC28 and SLC29; Chapter 11). The pharmacogenomics of ABC transporters important to drug transport are covered in subsequent chapters: P-glycoprotein (ABCB1; Chapter 12), bile salt export pump (BSEP/ABCB11; Chapter 13), breast cancer resistance protein (BCRP/ABCG2; Chapter 14), multidrug resistance-associated proteins MRP2 (ABCC2; Chapter 15), MRP3 (ABCC3; Chapter 15), MRP4 (ABCC4; Chapter 16), and MRP8 (ABCC11; Chapter 17). Significant advances in transporter biology and pharmacogenomics of transporters have been made through the contributions of individual labs as well as large multi-investigator projects such as the Pharmacogenomics of Membrane Transporters project, funded by the National Institutes of Health as part of the Pharmacogenomics Research Network, and described in Chapter 4 (Kroetz et al., 2010).

1.5   TECHNIQUES TO STUDY DRUG TRANSPORTER FUNCTION

The application of advances in molecular biology techniques to the study of transporters over the last 20 years has made a significant contribution to our understanding of transporter biology. *In vitro*, transporter activity is often characterized in primary cells and in expression systems, including transiently and stably transfected cultured human cell lines, inside-out membrane vesicles, and insect cells. One challenge to studying transporters *in vivo* is the overlapping substrate specificity and tissue distribution of many transporters, which can lead to difficulties in the precise identification of the transporter(s) responsible for a particular effect. Knockout mouse models of transporters have proven to be useful to delineate the contribution of certain transporters to drug disposition (DeGorter and Kim, 2011). Knockout mice exist for many of the SLC and ABC transporters, and double and triple ABC transporter knockout models have been used to characterize the contribution of multiple transporters with overlapping substrate specificities (Keppler, 2011). It is important to bear in mind that there are species-related differences in transporter expression and substrate specificity that may make it difficult to interpret and extrapolate the results obtained in mice to the human situation. The relative contribution of a given transporter *in vivo* has also been examined by drug-specific pharmacokinetic and pharmacodynamic studies in individuals with and without polymorphisms in the transporter gene of interest.

In the last decade, the field of genomics has developed rapidly, with the sequencing of the human genome (International Human Genome Sequencing Consortium,

2001; Venter et al., 2001) and subsequent efforts to determine haplotype structure by the HapMap project (The International HapMap Consortium, 2007), and sequence variation by the 1000 Genomes project (1000 Genomes Project Consortium, 2010). Genome-wide association studies (GWAS) incorporating clinical and genetic data have been widely used to identify genetic variants that predict risk for disease and also to assess drug response or toxicity. For pharmacogenomics studies, GWAS offer to identify candidate genes unrelated to our current knowledge of drug mechanism (Motsinger-Reif et al., 2010).

Methods for detecting transporter polymorphisms and predicting the functional consequences of unique polymorphisms in real time will be required to use pharmacogenomics in the clinical setting. To address this need, genotyping platforms for a focused set of important pharmacogenetic genes are being developed for clinical use (Sissung et al., 2010b). QSAR and molecular dynamics simulations are in silico approaches that are active areas of research aimed at addressing this challenge of SNP prediction (Ishikawa et al., 2010); see Chapters 5 and 18 for emerging technologies with applications to transporter pharmacogenomics.

1.6   TRANSPORTER PHARMACOGENOMICS IN DRUG DISCOVERY AND DEVELOPMENT

An understanding of transporter pharmacogenomics is important for the design and development of new drugs that are safe and effective. Transporters interacting with drug candidates may be identified during the preclinical stage of drug development, taking into consideration the limitations inherent to extrapolating *in vitro* and animal data to predict human response. For this reason, pharmacogenomic studies in later phases of drug development and postmarketing surveillance are crucial to identify potential transporter-mediated drug interactions, and individuals with transporter polymorphisms who may require dose adjustment or an alternative compound (Stingl Kirchheiner and Brockmoller, 2011). The International Transporters Consortium is a group of academic, industry, and regulatory leaders formed to create guidelines for the systematic inclusion of transporter studies in the drug development and approval process (Giacomini et al., 2010). Transporter pharmacogenomics and the role of diagnostic tests to support the clinical use of pharmacogenomics is discussed in Chapter 2, and a regulatory perspective on the contribution of drug transporters to the drug development process is provided in Chapter 3.

1.7   CLINICAL IMPLICATIONS OF TRANSPORTER PHARMACOGENOMICS

As our understanding of transporter pharmacogenomics matures, and pharmacogenomics technologies are more widely adopted in the clinic, transporter genomics could be used to select an appropriate dose, or the best medication from a particular class of compounds, and identify those individuals who may be at increased risk for an adverse drug reaction. Transporters that affect drug response are numerous

and diverse in their effect; key examples from the SLC and ABC superfamilies are summarized in Tables 1.1 and 1.2, respectively.

P-glycoprotein is an example of an efflux transporter that can significantly limit the accumulation of its substrates in certain tissues. The expression of P-glycoprotein at the blood–brain barrier prevents the CNS accumulation of drugs such as protease inhibitors, and its overexpression in cancer cells is associated with a multidrug-resistant phenotype (Cascorbi, 2011). Genetic variants in the cation transporter OCT1 (SLC22A1) have been associated with reduced efficacy of metformin, an antidiabetic drug that targets the liver as its site of action (Shu et al., 2007). The OATP1B1 (SLCO1B1) polymorphism c.521T>C has been associated with increased risk for statin-induced muscle toxicity (Link et al., 2008) and genotyping patients for this variant has been proposed to identify those at greater risk for side effects (Niemi, 2010).

Transporter pharmacogenomics have not yet been widely used in a clinical setting. Moving forward, studies are needed to show that the risk–benefit ratio of a drug is improved by pharmacogenomic testing, and some efforts are being made to determine the key components to be included in pharmacoeconomic evaluations of pharmacogenomic tests (Beaulieu et al., 2010). As sequencing becomes more cost-efficient, the possibility of sequencing relevant genes or even genomes in a clinical setting poses a new challenge of interpreting pharmacogenomic information on an individual level (Ashley et al., 2010).

Finally, it is important to bear in mind that many factors contribute to variability in drug responsiveness, including renal and hepatic functions, underlying disease processes, and drug interactions. At the end of the day, a patient's actual drug-response phenotype, in terms of efficacy and toxicity, is the key clinically relevant endpoint, and pharmacogenomics should be integrated with other parameters such as drug levels, biomarkers, and measures of drug response in order to provide truly personalized medicine.

1.8   CONCLUSION

Genetic variation in transporters contributes significantly to observed interindividual variability in drug response. In future, systematic inclusion of drug transporter studies that include genetic variation, whether affecting transporter function or expression, will be essential to the development of drugs that are safe and effective. There is little doubt that drug transporter pharmacogenomics is expanding rapidly and new insights will continue to inform improved drug prescribing and thereby enhance the delivery of optimal medical care.

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TABLE 1.1   Drug Transporters of the Solute Carrier Superfamily

Transporter (gene)	Tissue(s) of predominant expression in humans	Key drug substrates	Key inhibitors	SNPs associated with drug response	References
OCT1 (SLC22A1)	Hepatocyte (basolateral)	Metformin, oxaliplatin	Quinine	Multiple associated with metformin response	Nies et al. (2011)
OCT2 (SLC22A2)	Renal proximal tubule (basolateral)	Metformin, oxaliplatin	Cimetidine	None to date	Nies et al. (2011)
MATE1 (SLC47A1)	Hepatocyte (canalicular membrane); renal proximal tubule (luminal)	Cimetidine, metformin, procainamide	Cimetidine, pyrimethamine	Possibly rs2289669	Nies et al. (2011)
OAT1 (SLC22A6)	Renal proximal tubule (basolateral)	Acyclovir	Probenecid, NSAIDs	None to date	Burckhardt and Burckhardt (2011)
OAT3 (SLC22A8)	Renal proximal tubule (basolateral)	NSAIDs, furosemide	Probenecid, NSAIDs	None to date	Burckhardt and Burckhardt, 2011
OATP1B1 (SLCO1B1)	Hepatocyte (basolateral)	Statins, repaglinide	Rifampicin, gemfibrozil cyclosporine	c.521T>C (rs4149056)	Niemi et al. (2011)
OATP1B3 (SLCO1B3)	Hepatocyte (basolateral)	Statins, taxanes	Rifampicin, cyclosporine	Possibly c.334T>G (rs4149117)	Kalliokoski and Niemi (2009)
OATP2B1 (SLCO2B1)	Hepatocyte (basolateral); enterocyte (luminal)	Statins, fexofenadine	Cyclosporine	Possibly c.935G>A (rs12422149)	Kalliokoski and Niemi (2009)

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TABLE 1.2 Drug Transporters of the ATP-binding Cassette Superfamily

Transporter (gene)	Tissue(s) of predominant expression in humans	Key drug substrates	Key inhibitors	SNPs associated with drug response	References
P-gp (ABCB1)	Hepatocyte (canalicular); enterocyte (luminal); blood–brain barrier	HIV protease inhibitors, antineoplastics	Cyclosporine, verapamil	Possibly c.3435T>C (rs1045642)	Cascorbi (2011)
BSEP (ABCB11)	Hepatocyte (canalicular)	Pravastatin	Cyclosporine, glibenclamide	Possibly p.V444A (rs2287622)	Klaassen and Aleksunes (2010); Stieger (2011)
MRP2 (ABCC2)	Hepatocyte (canalicular)	B-lactam antibiotics, methotrexate, multiple Phase II conjugates	Cyclosporine	None to date	Klaassen and Aleksunes (2010)
MRP4 (ABCC4)	Hepatocyte (basolateral); renal proximal tubule (luminal)	Nucleoside-based antivirals, methotrexate, topotecan	Dipyridamole, losartan	Possibly p.E757K (rs3765534)	Russel et al. (2008)
BCRP (ABCG2)	Hepatocyte (canalicular); enterocyte (luminal); blood–brain barrier	Statins, antineoplastics	Dipyridamole, cyclosporine	c.421C>A (rs2231142)	Schwabedissen and Kroemer (2011)

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