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Pharmacogenetics of Membrane Transporters: A Review of Current Approaches

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Abstract

This chapter provides a review of the pharmacogenetics of membrane transporters, including ABC transporters and OATPs. Membrane transporters are heavily involved in drug disposition, by actively transporting substrate drugs between organs and tissues. As such, polymorphisms in the genes encoding these proteins may have a significant effect on the absorption, distribution, metabolism, excretion, and activity of compounds. Although few drug transporter polymorphisms have transitioned from the bench to the bedside, this chapter discusses clinical development of transporter pharmacogenetic markers. Finally, development of SLCO1B1 genotyping to avoid statin induced adverse drug reactions is discussed as a model case for transporter pharmacogenetics clinical development.

Keywords

ABCB1; ABCG2; ABCC1; ABCC2; OATP1B1; OATP1B3; Transport; Polymorphisms

1 Background

The fate of a drug in vivo is dictated by a variety of physiochemical properties including: size, lipophilicity, and charge. These properties determine how a drug is absorbed, distributed throughout the body, metabolized, and eventually eliminated. While movement of a drug molecule can occur through simple diffusion, there are many transporter proteins expressed on cell membranes to assist with efflux or influx via active transport. Transporters generally move substrates in an intracellular to extracellular direction, efflux transporters; however, some transporters actively move substrates in an extracellular to intracellular direction. Both efflux and influx transporters significantly affect drug disposition. For example, influx of a drug from the blood to the liver, where it is subsequently metabolized and excreted, may increase the rate of elimination. Transport proteins and the genes that encode them are essential to drug uptake, bioavailability, targeting, efficacy, toxicity, and clearance. The genes encoding these transporters are polymorphic, phenotypically resulting in transporters with different expression patterns and transport efficiency. Consequently,

common variants in genes coding for transport proteins contribute to variability in drug pharmacokinetics and ultimately the patient's response to treatment.

Many drugs undergo transport mediated by the ATP-binding cassette (ABC) family of transporters. There are a total of 49 known ABC genes including, but not limited to: *ABCB1* (P-glycoprotein, MDR-1), *ABCC1* (MRP-1), and *ABCG2* (BCRP, MXR, ABCP). ABC transporters utilize ATP to move substrates across membranes [1–5]. These transporters generally counteract uptake through the intestinal wall, efflux substrates out of tissues into the systemic circulation, and eventually promote the clearance of drugs through the kidneys and liver. Proteins in the ABC family are primarily known to be efflux transporters, moving substrates across the cell membrane and out of the cell.

ABCB1 and *ABCG2* are the best characterized polymorphic transporters to date [6, 7]. Many current FDA approved drugs are substrates of these transporters, although both transporters efflux a plethora of other compounds including naturally occurring toxins. *ABCB1* and *ABCG2* are expressed in enterocytes, the canalicular plasma membrane of hepatocytes, and the proximal renal tubule [8–12]. As such, these transporters often mediate bioavailability and exposure to their substrate drugs mentioned in Table 1 [13, 14]. Additionally, they have been shown to be expressed in hematologic tissues including hematopoietic stem cells and endothelial cells composing blood–tissue barriers of the brain, heart, nerves, testes, and placenta, where they efflux substrates out of these tissues into the systemic circulation [9, 15–17]. An exception includes the expression of *ABCB1* in the choroid plexus where it transports molecules from the circulation into the cerebrospinal fluid [18–20]. It is believed that the evolutionary role of these transporters is to limit the penetration of toxic molecules into critical organs, thereby serving a protective role in blood–tissue barriers.

Two other efflux transporters, *ABCC1* (MRP-1) and *ABCC2* (MRP-2) are also involved in drug disposition. *ABCC1* is expressed ubiquitously and is localized to the basolateral, rather than apical, membranes of epithelial cells. Due to its basolateral localization, *ABCC1* pumps drugs into the body rather than into the bile, urine, or intestine. For this reason, it is thought to serve mainly as a protective barrier in epithelial cells of tissues rather than a classic drug efflux pump [21, 22]. *ABCC2* is similar in function to *ABCB1*. *ABCC2* is expressed on the apical domain of epithelial cells. It is involved in luminal excretion in organs such as the liver, the intestine, and the kidney, but also plays a role in blood–tissue barriers. Additionally, *ABCC2* actively exports anionic drug conjugates and many unconjugated substances, making it an important part of drug detoxification [23, 24]. Both *ABCC1* and *ABCC2* primarily secrete drugs that have undergone phase II metabolism into glutathione, glucuronide, or sulfate conjugates, but both efflux wide range of drugs [25].

There are also several classes of “influx” or “uptake” transporters that mediate the cellular uptake and the reabsorption drugs by moving substrates against a concentration gradient. Uptake transporters include organic anion transporting proteins (OATPs), organic cation transporters (OCTs), concentrative nucleoside transporters (CNT), dipeptide transporters (PEPT), and monocarboxylate transporters (MCT) [24, 26]. In the interest of time, we will limit our discussion to two members of the OATP1B family of proteins, as these are well-

characterized influx transporters. OATP1B1 and OATP1B3 are expressed in liver tissues and are responsible for hepatocellular uptake of drugs from blood across the basolateral membrane [27–29]. For instance, all statins are transported from the circulation into the liver by OATP1B1, which affects the systemic exposure to many statins and thereby statin-induced myopathy [30]. Most are also transported by OATP1B3. It has previously been thought that these transporters were primarily involved in uptake of substrates into the liver where metabolism occurs [28]. However, more recent evidence suggests that OATP1B3 is overexpressed in several tumor types such as prostate, colon, lung, pancreas, breast, and liver [31–36]. Thus, since OATP1B3 influences drug treatment with docetaxel, paclitaxel, and irinotecan (along with active metabolite SN-38), it is possible that those tumors will be more sensitive to OATP1B3 substrate drugs [31, 37, 38]. Therefore, the OATP1B family is important in regulating the pharmacokinetics, toxicity, and potentially the response to several substrate drugs.

There is significant variation in the genes encoding all of the aforementioned transporters. Several of these genetic variants result in alterations in mRNA expression levels (e.g., promoter variants), translational efficiency (e.g., alterations in mRNA folding), and protein function (e.g., coding polymorphisms). Such genetic variability in transporters often explains a component of the interindividual variability in drug disposition, ultimately resulting in differences in clinical endpoints including toxicity and response. The field of transporter pharmacogenetics is concerned with elucidating the mechanisms by which genetic variation in transporters determines individual differences in drug transport, with a goal of eventually personalizing treatment with substrate drugs based on genotype. This chapter provides an overview of the methods by which investigators have discovered and characterized such associations in the ABCB1, ABCG2, ABCC1, ABCC2, OATP1B1, and OATP1B3 transporters. This methodology could be readily applied to the study of many additional transporters.

2 Genetic Variation and Genotyping Methods

More than 66 coding Single Nucleotide Polymorphisms (SNPs), three insertions/deletions, and several promoter alterations that modify gene transcription have been described in the ABCB1 gene. Twenty-four of the SNPs are synonymous and forty-two are non-synonymous [39, 40]. Three of the SNPs are common in most ethnic groups and demonstrate strong linkage disequilibrium: the synonymous transition at nucleotide 1236C>T (Gly411Gly) in exon 12, the non-synonymous tri-allelic transition 2677G>T/A (Ala893Ser/Thr) in exon 21, and the synonymous transition 3435C>T (Ile1145Ile) in exon 26. Studies have found evidence to suggest that these three SNPs may be implicated in altered transcription of mRNA [23], folding of ABCB1 protein [41], and the pharmacokinetics of drugs [42]. Other studies, however, have failed to confirm these findings [43]. Of the three SNPs mentioned above, only the 2677G>T/A (Ala893Ser/Thr) polymorphism causes an amino acid change. This change is located in a structurally important transmembrane domain of the translated protein. The effects of this transition are controversial and drug specific [26, 44–47]. The 3435C>T SNP is associated with decreased mRNA stability and expression levels [48]. Synonymous polymorphisms in ABCB1 may be responsible for altered protein conformations due to ribosomal stalling [41]. Because the genetic code is degenerate and

relative frequencies of codons vary, there is occasion for frequent-to-rare synonymous codon substitutions to appear. The substitution of a rarer codon can lead to pauses in ribosomal translation, during which the protein can adopt different secondary structures that may result in functional changes. This mechanism may apply broadly to several transporters [49].

Additive effects cause haplotype combinations to potentially result in greater protein functional differences when compared to single polymorphisms alone. The combination of the 3435C>T, 1236C>T, and the 2677G>T/A polymorphisms, also known as ABCB1*13, has been found to result in a change of ABCB1 transporter characteristics when compared to the polymorphisms alone [41]. Overall, nearly 64 haplotypes have been identified. Linkage between SNPs should be studied for confounding factors. For example, the 1236C>T polymorphism is in ~90 % D' linkage with the 2677G>T/A polymorphism in several populations, and by virtue of that linkage may only be artificially associated with interindividual ABCB1 transport alterations.

While there are many polymorphisms in ABCG2, the most characterized and common is the ABCG2 421C>A allele in exon 5. This SNP results in an amino acid change of Gln to Lys at codon 141 and has been shown in Flp-In-293 cells to have half the protein expression of the wild-type [50]. The variant alleles (i.e., 421A and 141K) have also been associated with lower ATPase activity compared with the wild-type ABCG2 [51]. Thus the ABCG2 421C>A SNP, much like the ABCB1 2677G>T/A allele, may alter both expression and activity of the encoded protein. The variant alleles have also been associated with reduced transport of tyrosine kinase inhibitors because of lower protein concentrations [52]. The frequency of this mutation varies significantly by race; it occurs at 35 % frequency in Chinese populations, whereas the mutation is very rare in African Americans (1 %) [53]. Another potentially important SNP exists at nucleotide 34 and results in an amino acid of V12M. This mutation is most notably associated with poor ABCG2 protein localization [51, 54]. Conclusions vary on whether or not the change results in a difference in expression levels [52, 55]. Surprisingly, this mutation does not appear to modify substrate transport [56]. Furthermore, mutations at R482 which result in non-synonymous protein changes have been identified in numerous cancer cell lines (presumably a mechanism of multi drug resistance) but have never been found in humans. This mutation affects both transport and substrate specificity [57–60].

There are several polymorphisms in *ABCC1*, many of which are non-synonymous. Those studied include C43S, T73I, S92F, T117M, R230Q, V353M, R433S, R633Q, G671V, R723Q, A989T, C1047S, R1058Q, A1337T, and S1512L. A majority of these SNPs do not alter the functionality of the expressed protein and are unlikely to significantly influence the expression [61, 62]. However, it has been noted that C43S, R433S, and A989T result in decreased ABCB1 function [61]. The G1299T mutation of exon 10 resulted in decreased transport of multiple organic anions but increased the resistance of doxorubicin [62]. It has been noted that C43S, R433S, and A989T all result in decreased ABCC1 function [61]. In vitro analysis has shown that the G128C mutation causes changes in membrane localization [62]. Others have evaluated non-synonymous polymorphisms to assess their impact on mRNA expression, but have found no significant results [63].

The *ABCC2* gene also contains several polymorphisms. In particular, patients with Dubin Johnson Syndrome (DJS) commonly have the 2302C>T A768W polymorphism [23]. Four other SNPs have recently been studied more extensively. They are –24C> T, 1249G>A, 3972C>T, and 4544G>A. In vitro studies with the –24C> T polymorphism showed a 20 % reduction in transcription in HepG2 cell lines. In kidney tissues carrying the –24C> T polymorphism, lower *ABCC2* mRNA levels were detected [64].

The gene *SLCO1B1*, which codes for transporter OATP1B1, contains many polymorphisms that have been associated with a decreased transport phenotype towards several drugs (see Table 1) and endogenous substrates [28, 65]. It was also noted that nearly five variants have been shown to effect expression of OAT1B1 on the membrane surface (*SLCO1B1* *2, *3, *5, *6, *9) [65]. A few of the SNPs have been well studied. These are the –11187G>A, the 388A>G (*SLCO1B1* *1b), and the 521 T>C (*SLCO1B1* *5). These three variants have been shown to influence clinical outcomes. Studies found that *SLCO1B1* *5 affected the maximum transport velocity, not the substrate affinity of transport kinetics [65]. The affects of *SLCO1B1* *1b however remain controversial. Studies have been published showing increased activity, decreased activity, or no activity change at all. These results were very experiment and substrate specific [65]. The polymorphism –11187G>A found in a promoter region has not been shown to reduce or increase expression levels [65]. Allele frequencies differ between populations. The *SLCO1B1* *5 polymorphism is present in approximately 14 % of the Caucasian population [66] but only 1 % in Japanese populations [67]. For this reason, studies evaluating associations between *SLCO1B1* *5 and clinical outcome in Caucasians have been more statistically powered and have resulted in clearer clinical outcomes [66, 68, 69]. *SLCO1B1* *b1 and *SLCO1B1* *5 may be in linkage disequilibrium. This leads to four functionally distinct haplotypes. The 388G/521C variant is classified as *SLCO1B1* *15 and showed nearly a decrease in activity of 70 % when transporting estradiol-17β-d-glucuronide in vitro compared to the wild-type allele [65].

Genetic variations for *SLCO1B1* are limited and have been much less characterized [65]. Three variations found in the Caucasian population are 334T>G, 699G>A, and 1563G>T and have been studied in HEK293 and MDCKII cells. 334T>G and 699G>A variants did not result in altered expression of six substrates. The frequency of the variants in the Caucasian population are 334T>G 74 %, 699G>A 71 %, and 1563G>T 1.9 % [65]. Variant 699G>A was associated with decreased uptake of cholecystokinin-8 and rosuvastatin in HeLa cells [70]. Recently, variants 332T>G and 699G>A have shown to decrease uptake of testosterone and mycophenolic acid [65]. Two other variants, 1679T>C and 1559A>C lead to decreased cell surface expression and for this reason lower transport functions when compared to the wild-type. In vivo, 334T>G and 699G>A have not been associated with differences in clearance or exposure of paclitaxel or docetaxel [65]. A common haplotype consisting of the 334T>G (S112A) and 699G>A (M233I) SNPs was related to altered OATP1B3 transport characteristics in COS-7 cells, while no differences in the transport of cells transfected were observed with either variant alone [71]. However, this observation may be substrate- or assay-specific given that paclitaxel transport was not altered based on any of the SNPs (334T>G, 699G>A, 1564G>T) or haplotype combinations thereof in *Xenopus oocytes* [72].

Many of the recent publications regarding transporter genotyping have utilized restriction fragment length polymorphism (RFLP) analysis or direct sequencing, although several other methods of genotyping are available such as resequencing, allele-specific PCR, TaqMan PCR, and Fluorescence Resonance Energy Transfer (FRET). Next generation sequencing has brought many new methods including; DNA nanoball sequencing, pyrosequencing, Illumina sequencing, Single Molecule Real-Time (SMRT) sequencing, ion torrent semiconductor sequencing, SOLiD sequencing, and HeliScope single molecule sequencing.

Genotyping for SNPs in genes that may have an effect on drug transport is recommended by the FDA and can be achieved by CLIA-certified genotyping services, many of which use the AmpliChip P450 or the CodeLink P450 genotyping platforms. However, to our knowledge, no genetic variation in a drug transporter has yet been evaluated by the FDA. Recently, some hospitals have begun to offer SLCO1B1 genotyping assays for statin use, these assays must be CLIA-certified if they will be used to inform a patients clinical decisions. Although FDA approval and CLIA certification remain to be worked out, the drug metabolizing enzyme transporter (DMET) platform may provide a basis to evaluate hundreds of polymorphisms in drug transporters and factors that regulate transporter expression (i.e., PXR) in future clinical trials. A brief overview of these genotyping platforms is reviewed in [73].

3 Substrate Identification

ABCB1 and ABCG2 substrates (*see* Table 1) are typically hydrophobic molecules including lipids, peptides, steroids, and xenobiotics—such as anticancer, HIV, atypical antipsychotics, and immunosuppressant drugs. There is often broad overlap between ABCB1 and ABCG2 substrates. The ABCC proteins are multispecific anion transporters. ABCC1 is known to be involved in anthracycline transport [74], but ABCC2 effluxes a wider range of drugs such as cyclosporine, cisplatin, vinblastine, and camptothecin derivatives [21, 75]. OATP1B1 and OATP1B3 interact with a wide range of substrates (not only organic anions as the nomenclature implies) including bilirubin, bile acids [76], peptides, eicosanoids, hormones, and prescribed drugs, including fexofenadine [77]. However, each transporter has distinct substrate specificity, so some compounds are transported by one transporter but not another in the same family.

For investigational drugs, the FDA recommends that all investigational drugs should be evaluated whether they are substrates for drug transporters [78]. In short, all investigational drugs should be tested whether they are ABCB1 and/or ABCG2 substrates in vitro. If results are positive, these drugs should undergo further testing in humans. This does not apply to highly permeable and highly soluble drugs since intestinal absorption is not a rate-limiting step. In addition, drugs that undergo extensive (e.g., 25 % of total clearance) hepatic or biliary secretion should be investigated whether they are substrates of OATP1B1/OATP1B3. Several test systems are used to identify ABC and OATP substrates.

3.1 ABCB1 and ABCG2 Substrates

Substrates for ABC drug transporters can be identified using several assays, which can be classified into membrane-based assay systems (including ATPase assay, vesicular transport assay and photoaffinity labeling) and cell-based assay systems (including monolayer assay,

cytotoxicity assay, and sandwich-cultured hepatocytes) [79]. The FDA recommends to perform a bidirectional transporter assay using cell lines overexpressing the transporter of interest (e.g., transfected polarized cells: MDCK, Caco-2, LLC-PK1, endothelial cell lines; or unpolarized cells: HEK293, CHO) [78]. These cell types are grown in a monolayer on a membrane separating two chambers of culture medium (i.e., the Transwell Cell Culture Assay, Corning Costar Corp., Cambridge, MA). Drug is administered into one chamber in the presence or absence of a specific inhibitor of the transporter of interest, and drug transport across the monolayer is evaluated by sampling from the other chamber. The experiment is then repeated applying drug to the opposite chamber. Due to the directionality of the transporters, these experimental systems allow investigators to assess the basolateral to apical (B-A), and apical to basolateral (A-B), transport of drug. A drug is considered be a substrate for ABCB1 or ABCG2, if the efflux ratio B-A to A-B is ≥ 2 . In addition, ABCB1- or ABCG2-mediated inhibition is further confirmed when specific inhibitors (e.g., itraconazole and verapamil for ABCB1; and fumitremorgin C for ABCG2) reduce the efflux ratio by more than 50% [78, 80]. In that case, clinical drug–drug interaction studies may be warranted.

Another widely applied cell-based system is the cytotoxicity assay. In this system, the cytotoxic effect of the investigational compound is determined after incubation of ABC transporter expressing cells. This assay can be performed with the test compound alone (*direct*) or in the presence of a cytotoxic ABC transporter substrate (*indirect*). The test compound is considered a ABC transporter substrate, when the inhibitory drug concentration causing 50 % cell death (IC_{50} value) is increased in ABC-transporter-expressing cells compared to wild-type parental cells. When using the indirect method, the IC_{50} value of the ABC transporter substrate is decreased when the test compound is a competing substrate.

The third cell-based approach concerns sandwich-cultured rat or human hepatocytes (SCH) [80], which closely mimic the hepatic environment in terms of expression of transporters and metabolizing enzymes. In the SCH model, hepatocytes are cultured in a sandwich configuration between two layers of gelled matrix to form intact bile canaliculi [81]. The advantage of this model is that both hepatic uptake and biliary excretion can be studied.

Among the membrane-based assay systems, the ATPase assay can be used to identify ABC substrates, since ABC transporters require ATP to transport substrates across the cell membrane. Using isolated membranes containing the ABC transporter of interest or reconstituted ABC protein preparations, ABC substrates would be revealed by an observed increase in ATPase activity (colorimetric detection of inorganic phosphate). In an alternative, inhibition-type (indirect) setup, the test compound is added to a well-established ABC substrate, which creates high ATPase activity. If the test compound is also an ABC substrate, the increased ATPase activity will decrease.

By use of the vesicular transport assay, the direct transport of ABC substrates into inside-out plasma membrane vesicles can be detected. These vesicles can be derived from several different cell lines, such as drug-selected cells, transfected cells, and baculovirus-infected

insect cells [80]. Similar to the cytotoxicity assay, this assay can be executed using a direct or indirect setup.

3.2 OATP181 and OATP183 Models

Identification of OATP1B/3 substrates is usually performed in stable OATP1B1- or OATP1B3-overexpressing systems, such as Chinese Hamster Ovary (CHO) and Human Embryonic Kidney 293 (HEK293) cells, *X. Laevis* oocytes, and recombinant virus [80, 82]. The criterion for test compounds to be considered as OATP substrates is a ratio of uptake in OATP-expressing cells versus control (or empty vector cells) statistically greater than 1 [80]. Furthermore, uptake should be inhibited by a known inhibitor of the transporter.

In addition, similar to the identification of ABC substrates the uptake of OATP substrates can also be studied in primary isolated hepatocytes and the SCH model [82].

The FDA utilizes the following criteria to determine whether an investigational drug is a substrate of OATP1B1 or OATP1B3: “uptake in OATP1B1- or OATP1B3-transfected cells greater than 2-fold of that in empty vector transfected cells and is inhibitable (e.g., >50% reduction to unity) by a known inhibitor (e.g., rifampin) at a concentration at least 10 times of its K_i . Michaelis–Menten studies may be conducted in the transfected cells to determine the kinetic parameters of the investigational drug. A positive control should be included. In an acceptable cell system, the positive control should show a 2 fold increase in uptake compared to vector-transfected cells. An uptake ratio (transporter transfected vs. empty vector transfected cells) other than 2 may be used if a ratio of 2 is deemed non-discriminative as supported by prior experience with the cell system used.”[78].

4 Assessing Functional Significance of Polymorphisms In Vitro

4.1 Cell-Based Assays

Polymorphic efflux of ABCB1 substrates was initially evaluated using flow cytometry, although such assays are limited in that only fluorescent compounds can be assayed and differences in polymorphic transporter expression and function are not made clear. To date, the influx of Rhodamine 123, calcein, doxorubicin, and daunorubicin have been evaluated using such methods, and are still used in drug–drug interaction studies (covered later). The same technique has been used with mitoxantrone to assess transport by, and inhibition of, ABCG2. Such assays were initially used in the field of transporter pharmacogenetics to show that Rhodamine 123 transport is lower in 3435TT human CD56⁺ cells [83]. As the pharmacokinetics of many other drugs could potentially also be differentially altered based on polymorphic ABCB1 expression and function, with ensuing clinical implications, many have evaluated ABCB1 efflux using other in vitro assays. Some have used transfected cell lines to evaluate the functional significance of non-synonymous polymorphisms in ABCB1 and have demonstrated that differences in activity exist between proteins carrying a single amino acid difference brought on by these SNPs. For example, using this technique, it was found that the 2677G> T/A (893S>T/A) polymorphism results in activity differences toward vincristine such that V_{max} 893T>893S>893A, while K_m 893S>893T/A [84]. Other investigators have employed ATPase assays to evaluate the ATP-dependent active transport

of substrates. In this assay, vesicles obtained from Sf9 cells transfected with ABCB1 variants have been studied and have validated the previously mentioned finding with ABCB1 [85]. The effect of different polymorphisms on substrate transport by ABCG2 has been assessed using stably transfected HEK293 cells [86]. Following incubation of the cells with the drug, concentrations can be measured via flow cytometry [59], liquid scintillation counting if radiolabelled drug is available [87], or LC-MS [88]. In vitro analyses of OATP1B1 functional polymorphisms were evaluated similarly [66, 67, 89–92]. Interestingly, the above assays have been employed to address the functional consequences of polymorphisms in the ABCC family of transporters, but to no notable alterations in transport capacity have been found [61]. It seems that while ABCC transporters contain several potentially important polymorphisms and are very important in drug transport overall, functional variability is actually quite low. This is perhaps the reason for the multiple negative studies that have assessed ABCC polymorphisms as they relate to drug bioavailability [23].

4.2 Assessing the Cause of Phenotypic Differences

Polymorphic differences that result in altered transporter kinetics, and possibly subsequent changes in drug disposition, can affect this change via multiple mechanisms, including modulated tissue expression. For example, the *ABCB1* 2677TT genotype was associated with decreased mRNA expression in several human tissues as compared to the wild-type allele [83, 93, 94], and thus the functional consequences of the 2677G>T/A polymorphism may be explained by expression alterations alone and not necessarily by altered substrate binding or transport efficiency of the protein. Some postulate that polymorphisms encoding rarer codons for the same amino acid (a synonymous or silent mutation) result in decreased translation efficiency of the mRNA, resulting in lower protein levels, and that it is possible that alterations in polymorphic mRNA secondary structure could also result in inefficient translation. This mechanism has been suggested as one possible explanation for the effects seen with a synonymous mutation in ABCB1 because the 3435C>T transition does not result in an amino acid change, but is still associated with differential drug efflux capability. An alternate, though not mutually exclusive explanation has also been proposed; the 3435C>T SNP is in linkage with the non-synonymous 2677G>T (893T>S) transition and therefore, it may be associated with a protein product with attenuated efflux capacity through lowered efflux efficiency.

The former hypothesis has been evaluated using mRNA expression measurements in human tissues and it was found that ABCB1 is generally expressed at higher levels with the 3435C [83, 93–95]. These observations were replicated with cotransfection of equal amounts of plasmid and it was concluded that the 3435T allele lowers mRNA stability and is therefore responsible for decrease efflux capacity [96]. In the case of ABCG2, the effect of the 421C>A polymorphism has been debated. Originally, the resulting amino acid change was believed to reduce protein expression, due to instability [55], but this finding was not confirmed by human intestinal samples that did not reveal a difference [97]. Subsequently, it has been shown that the transport efficiency of the protein is decreased. This was demonstrated by measuring ATPase activity in wild-type and mutant cells, normalizing for expression [51].

When OATP1B1 variants were expressed in HeLa cells, it was noted that *OATP1B1**2, *3, *5, *6, *9, *12, and *13 alleles were associated with reduced transport toward OATP1B1 substrates [66]. Others noted that when the OATP1B1 *15 variant was expressed in HEK293 cells, and *Xenopus laevis* oocytes, these cells also had reduced transport capability [89, 91]. The reasons for the reduced transport capacity of these alleles was made clear after it was demonstrated that the plasma membrane localization of many of these polymorphic transporters was impaired due to a cell surface trafficking defect [66]. It was also shown that some polymorphisms encode for impaired protein maturation that results in the encoded OATP1B1 protein to be retained intracellularly [90]. Studies evaluating OATP1B3 polymorphisms are currently undergoing similar validation, but no significant results have yet been reported.

Despite the encouraging results of the above investigations, not all studies using the above experimental systems have consistently validated these observations in other tissues and cell types. For example, associations between genotype and expression seem to be tissue-specific, as lymphocytes and the small intestine both express ABCB1, but expression levels were not associated with polymorphic variants, and it is often the case that reports evaluating the same tissues conflict [7]. Furthermore, some tissues such as cardiac endothelium actually express ABCB1 at greater levels in patients carrying variant alleles which is the direct opposite of data generated in other tissues [95]. Others have used nonhuman in vitro expression systems in an attempt to validate the effect of ABCB1 polymorphisms although transfected variant alleles do not seem to influence ABCB1 transport in some of these experimental systems—perhaps due to differences in mRNA processing membranes in different cell lines and between species [98].

5 Assessing Functional Significance of Polymorphisms In Vivo

Mice carry two homologues of *ABCB1* (*Abcb1a*, *Abcb1b*), and viable single (*Abcb1a*), and double knockout mice are commercially available (Taconic Laboratories). Additionally, triple knockout (TKO) mice have recently become available in which homologous genes encoding ABCB1, and ABCC family members (covered later) have been removed from the mouse genome. An *Abcg2* (the mouse homologue of ABCG2) knockout mouse is also commercially available from, in addition to a triple knockout, null for *Abcb1a*, *Abcb1b* and *Abcg2*. Many have utilized such mice to evaluate the influence of ABC transporters on the pharmacokinetics and toxicity of drugs. Based on data obtained from these mice, ABCB1 has been shown to play a major role in detoxification and serves as a protective barrier against the toxic effects of xenobiotics [99]. Mice lacking *Oatp1a* and mice lacking *Oatp1a/1b* are also available. However, while these uptake transporters are expressed in human liver and few other tissues and tumors, mice express these transporters more ubiquitously thereby limiting the usefulness of this model in drug-drug interaction studies and other “translational” endpoints [100, 101]. Several humanized models are also readily available. Genetically engineered mice have been used as animal models of compromised blood–brain barrier function [11, 102], intestinal drug absorption [103], fetal drug exposure [104], and drug-induced damage to testicular tubules, choroid plexus epithelium [19], oropharyngeal mucosa [18], and peripheral nervous tissues [105].

Mice lacking the expression of a transporter generally have less ability to eliminate substrate drugs, except in cases where compensatory pathways are upregulated that circumvent transporter-mediated clearance [106, 107]. Alterations in plasma pharmacokinetics result from the lack of transporter expression in gut, liver, and renal tissues where several transporters are involved in the elimination of substrate drugs through hepatobiliary pathways, and glomerular filtration. Such mice generally also demonstrate increased uptake of oral substrate drugs as efflux transporters are involved in the excretion of toxic substances back into the gut lumen in normal mice. As such, bioavailability and exposure are usually increased in knockout mice, while clearance is decreased. This can have both positive and negative effects and can allow translational researchers to make clinical decisions based on the outcome of these drug-treated mouse models. However, this is not necessarily always the case. Compounds that are highly bioavailable in wild-type mice are unlikely to show great increases in absorption when the transporter protein is impaired. Also, as mentioned previously, many drugs have alternate routes of elimination, which may become more important when the primary transport mechanism is not functioning. As such, it is critical that in vivo testing is carried out for each compound, rather than assuming that because a drug is a substrate, it will be greatly affected by these polymorphisms.

Mice that do not express a specific transporter are generally more likely to experience benefit from treatment with a substrate drug because bioavailability and exposure to the drug are usually increased along with the beneficial aspects of treatment. Lack of transporter function may also allow penetration into tissues that were previously impermeable to the agent. For example, *Abcb1* knockout mice with brain metastases can be successfully treated with drugs that otherwise would not penetrate the blood–brain barrier such as paclitaxel [108]. *ABCB1a*^{−/−} mice also showed ten times more brain–serum ratios of both risperidone and its active metabolite, 9-hydroxyrisperidone than control mice [109], and most central nervous drugs showed 1.1- to 2.6-fold greater brain-to-plasma ratios in double knockout mice compared to wild-type mice [110].

Although the efficacy of drug treatment may increase, this is counterbalanced by increases in toxicity through routes other than increased plasma concentrations as blood-tissue barriers are disrupted allowing increased penetration of drugs into organs—especially the brain where *ABCB1* is an important mediator of drug exposure. In drugs with a narrow therapeutic window (e.g., many anticancer agents), the toxicity can outweigh the beneficial aspects of drug treatment. Following the above example, *ABCB1* knockout mice treated with paclitaxel are more susceptible to treatment-related peripheral neuropathy due to increases in drug concentrations in nerve cells [105].

6 Transporter Genetics in Clinical Pharmacokinetics

Numerous clinical trials have investigated the effects of *ABCB1* polymorphisms on the pharmacokinetics of *ABCB1* substrates [111]. Initially, investigators determined that the *ABCB1* 3435C>T SNP was associated with lowered *ABCB1* expression and higher digoxin levels in human volunteers [93]. The association was stronger when the *ABCB1* 2677G>T/A and 3435C>T polymorphisms were evaluated together as a haplotype—those patients variant at both alleles having both the lowest *ABCB1* expression and the highest digoxin

AUC [112, 113]. Since then, many investigators have found similar associations between these polymorphisms and plasma concentrations of several other drugs, although these observations have not been consistently confirmed [23, 114, 115]. Overall, the relationship between ABCB1 polymorphisms (e.g., common coding SNPs 1236T>C, 2677T>G/A, and 3435T>C) and the pharmacokinetics of ABCB1 substrates is yet unclear, since clinical studies often report discordant results [80, 116]. It should be noted that polymorphic ABCB1 expression not only influences plasma pharmacokinetics, but also the degree to which drugs are able to penetrate into tissues that express ABCB1 (e.g., tumors, brain, HIV-infected cells, etc.) [117, 118]. As previously mentioned, drug penetration into tissues can be both efficacious (i.e., by increasing therapeutic efficacy) and deleterious (i.e., by increasing toxicity).

For ABCG2, the most common and best-studied polymorphism concerns the 421C>A SNP, which is associated with reduced protein expression levels and impaired ABCG2 activity [119]. Thus far, associations between the 421C>A mutation and plasma pharmacokinetics have been evaluated for several drugs. As reviewed by Schnepf and Zolk [119], individuals with the ABCG2 421AA genotype displayed significantly higher systemic levels of statins (rosuvastatin, atorvastatin, fluvastatin), compared with the ABCG2 421CC genotype. Concordant with these results, impaired transporter activity by the 421C>A SNP also led to increased bioavailability of the anticancer drugs topotecan, diflomotecan, gefitinib, and sunitinib [120–123]. In contrast, for certain other drugs (e.g., pitavastatin, irinotecan, sulfasalazine) no significant association was found between the ABCG2 421AA variant and their pharmacokinetics [53, 124, 125].

The clinical consequences of OATP1B1 polymorphisms on drug exposure have been investigated in several studies [126, 127]. In particular, the relatively common 521 T>C SNP is associated with decreased transporter activity and consequently higher systemic exposure to OATP1B1 substrates, including statins, repaglinide, lopinavir, and erythromycin [127]. For example, individuals expressing the 521 CC genotype displayed twofold and threefold higher plasma levels of simvastatin acid than those with the TC and TT genotype, respectively [128]. In line with this finding, the 521 T>C SNP is associated with simvastatin-induced myopathy [129]. In addition, the effects of genetic ABCC1 variants on drug transport are largely unknown, while ABCC2 polymorphisms seem less likely to alter transporter expression or function [130].

Table 2 shows the effects of common transporter polymorphisms on certain substrates in vitro and in the clinical setting. This table demonstrates that for certain drugs, data obtained in vitro are not always extrapolatable to humans. For example, the ABCG2 421C>A variant significantly increased imatinib accumulation in cells, while this polymorphism did not significantly affect imatinib pharmacokinetics in patients carrying this variant [131]. In addition, conflicting clinical results with the same drug substrate are shown (e.g., OATP1B3 effects on docetaxel pharmacokinetics) [38, 132].

7 Transporter Genetics in Clinical Endpoint Analysis

The ultimate research goal of transporter pharmacogenetics is to further our understanding of the ways in which transporter genetics influences clinical endpoints so that current drug treatment can be made safer and more efficacious, and investigational therapies can be better developed. The literature consists of a multitude of studies that have evaluated drug efficacy and toxicity and have made associations between these parameters and polymorphisms in drug transporters [7, 23, 133]. The FDA recommends several endpoints to evaluate specific diseases and those endpoints should be evaluated when making associations between a genetic variation and the treatment of diseases with drugs (see: www.fda.gov/cder/guidance; last accessed November 25, 2013). In pharmacogenetic studies, these endpoints should be evaluated in a standard fashion in similar populations in order to establish the predictive value of a polymorphism. Unfortunately, the literature has not typically been consistent mainly due to the availability of samples for analysis, and perhaps this is the reason that transporter polymorphisms have not been consistently validated. Thus far, all studies linking pharmacogenomics of membrane transporters with clinical outcome have been retrospective, taking place in eclectic populations with relatively low statistical power. It is essential that well-powered and prospective studies are undertaken, prior to any treatment modification, to assess the true effects of these polymorphisms and determine whether the effect is drug-specific or disease related.

8 Case Study: SLCO1B1 Genotyping for Statin-Induced Adverse Drug Reactions

The FDA has listed numerous markers of variability in: drug exposure, clinical response, adverse event risk, dosing, drug action, targeting, and disposition (<http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm>; last accessed 10 January 2013). Notably, while this list consists of over 100 gene/drug pairings, not a single drug transporter is listed. This is in stark contrast to the number of CYP enzymes and other regulators of absorption, distribution, metabolism, and elimination that are frequently listed by the FDA as important regulators of drug disposition and outcome. It is also important to consider that over 35,000 articles have been published on ATP-binding cassette (ABC) drug transporters alone while a similar search of “cytochrome P450” only reveals approximately 75,000 articles. Clearly, the disparity between the clinical utility of information about a patient’s genetic status at a transporter versus a CYP is not for a lack of research; rather, current methodologies are not breaking the barrier between discovery, development, and ultimate translation of transporter pharmacogenetics.

Nonetheless, there is a single drug transporter where genetic testing has led to clinically actionable information. The Pharmacogenetics Research Network (PGRN) has also listed annotations for polymorphisms that have either been endorsed by a medical society, have been implemented in a major hospital site, or where the preponderance of the evidence suggests that there is an association between polymorphic variation and clinical drug use (<http://www.pharmgkb.org/search/clinicalAnnotationList.action?levelOfEvidence=top>; last accessed 10 January 2013). While there are 45 polymorphism/drug pairings listed on this

Web site, the PGRN lists a single transporter polymorphism *SLCO1B1* (rs4149056) which encodes an amino acid transition in the OATP1B1 protein. This, rather common, allelic variant alters the disposition of various statins and is a strong marker of the risk to develop statin-induced myopathy. It is widely expected that the FDA will embrace this genetic approach to adverse drug reaction (ADR) avoidance in certain clinical situations. Therefore, OATP1B1-related statin pharmacogenetics may serve as a rubric for successful translation of transporter pharmacogenetics.

In 2001, Tirona et al. identified and functionally characterized polymorphisms in *SLCO1B1*. Although several SNPs altered OATP1B1 transport activity, the 521T>C SNP (V174A) caused a significant decrease in transporter activity toward multiple substrates (Tirona et al. [66]). Haplotype analysis was also conducted in several world populations by various investigators [27]. Statins were later discovered to be OATP1B1 substrates using several transwell and overexpression systems [27]. In vivo studies were conducted concurrently with retrospective clinical pharmacogenetics studies in humans. The *Slco1b2* knockout mouse model showed that there was a fourfold decrease in the liver–plasma ratio of pitivastatin although humanized mice were never studied [134]. Several candidate SNP studies also largely demonstrated that plasma exposure of statins was significantly increased in individuals carrying 521CC, and modestly in those carrying 521CT [27] suggesting, by and large, that the *5 and *15 haplotypes were both associated with increased plasma AUC of several statins. Several other studies suggested that the 521C allele was also related to attenuated cholesterol response [151]. A very large retrospective GWAS study was published in 2008 [136] that suggested the 521C allele was associated with both an impaired cholesterol response and an increased incidence of statin-induced myopathy. This led to ultimate clinical guidelines for implementation published by the Clinical Pharmacogenetics Implementation Consortium, consisting of individuals from academia, the pharmaceutical industry, and clinicians from numerous institutions [137].

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Table 1

Substrates and inhibitors of ABCB1, ABCG2, ABCC2, OATP1B1, and OATP1B3

	Substrates	Inhibitors ^a
<i>ABCB1</i> (<i>P-gp</i>) Antibiotics	Ciprofloxacin (Systemic, UTD) Erythromycin (UTD) Rifampicin (UTD)	Azithromycin [116, 138] Clarithromycin [138, 139] Erythromycin [138, 139] Telithromycin [1]
Antifungals	Posaconazole [138, 139]	Itraconazole [138, 139] Ketoconazole [138, 139]
Antihistamines	Fexofenadine [138, 139] Cetirizine (UTD) Desloratadine (UTD) Loratadine (UTD)	
Antihypertensive drugs	Aliskiren [138, 139] Ambrisentan [138, 139] Talinolol [138, 139] Amiodarone (UTD) Carvedilol (UTD) Diltiazem (UTD) Nadolol (UTD) Nicardipine (UTD) Verapamil (UTD)	Captopril [138, 139] Carvedilol [138, 139] Conivaptan [138, 139] Diltiazem [138, 139] Felodipine [138, 139] Verapamil [138, 139] Reserpine [138] Nicardipine (UTD) Propranolol (UTD)
Heart medications	Digoxin [138, 139] Ranolazine [139] Tolvaptan [138, 139] Quinidine [138] Digitoxin (UTD) Quinine (UTD)	Amiodarone [138, 139] Dronedarone [138, 139] Quinidine [138, 139] Ranolazine [138, 139] Quinine (UTD)
Antiviral drugs	Maraviroc [138, 139] Ritonavir (UTD) Indinavir [138] Fosamprenavir (UTD) Nelfinavir (UTD) Saquinavir (UTD) Telaprevir (UTD)	Lopinavir [138, 139] Indinavir [138] Ritonavir [138, 139] Nelfinavir [138] Saquinavir [138] Telaprevir [139] Tipranavir [138] Cobicistat (UTD) Darunavir (UTD)
Immunosuppressants	Sirolimus [138] Ciclosporin (UTD) Hydrocortisone (UTD) Dexamethasone (UTD) Tacrolimus (UTD)	Ciclosporin [138, 139] Tacrolimus [138]
Platelet aggregation inhibitors	Dabigatran etexilate [138, 139] Rivaroxaban (UTD)	Ticagrelor [139]
Flavonoids		Dipyridamole (UTD) Quercetin [138, 139]
Anticancer drugs	Everolimus [138, 139] Imatinib [138, 139] Lapatinib [138, 139] Nilotinib [138, 139] Topotecan [138, 139] Paclitaxel [138] Vincristine [138] Vinblastine [138] Crizotinib [143] Erlotinib [144]	Valsopodar (PSC833) [138] Lapatinib [139] Everolimus [140] Bosutinib [79] Nilotinib [79] Dasatinib [79] Crizotinib [141] Erlotinib [142] Gefinitib Abiraterone acetate (UTD + product label)

	Substrates	Inhibitors ^a
	Barasertib [145] Vismodegib (UTD)	Sunitinib (UTD)
	Afatinib (UTD)	Tamoxifen (UTD)
	Bosutinib (UTD)	Vandetanib (UTD)
	Carfilzomib (UTD)	Vemurafenib (UTD)
	Gefitinib	
	Daunorubicin (UTD)	
	Docetaxel (UTD)	
	Doxorubicin (UTD)	
	Etoposide (UTD)	
	Idarubicin (UTD)	
	Irinotecan (UTD)	
	Methotrexate (UTD)	
	Mitomycin (UTD)	
	Pazopanib (UTD)	
	Pomalidomide (UTD)	
	Romidepsin (UTD)	
	Temsirolimus (UTD)	
	Teniposide (UTD)	
	Trabectedin (UTD)	
	Vemurafenib (UTD)	
Statins and other cholesterol-lowering drugs	Atorvastatin (UTD)	Atorvastatin (LTTD)
	Lovastatin (UTD)	Lomitapide (LTTD)
	Pravastatin (UTD)	
Miscellaneous	Colchicine [138] Saxagliptin [138, 139] Sitagliptin [138, 139] Loperamide [138]	Elacridar (GF120918) [138] Tariquidar (XR9576) [146] Zosuquidar (LY335979) [138] Laniquidar (R101933) [147, 148]
	Cimetidine (LTTD)	Grapefruit juice (LTTD)
	Estradiol (UTD)	Ivacaftor (LTTD+product label)
	Ivermectin (LTTD)	Mefloquine (LTTD)
	Linagliptin (LTTD)	Progesterone (LTTD)
	Ondansetron (LTTD)	Ulipristal (UTD)
	Paliperidone (LTTD)	
	Risperidone (LTTD)	
	Ranitidine (LTTD)	
	Silodosin (LTTD)	
<i>ABCG2 (BCRP)</i>	Daunorubicin [138] Doxorubicin [138] Methotrexate [138, 139] Mitoxantrone [138, 139] Imatinib [138, 139] Irinotecan [138, 139] Lapatinib [138, 139] Topotecan [138, 139] Barasertib [145] Nilotinib [79] Dasatinib [79] Erlotinib [144]	Gefitinib [138, 139] Lapatinib [140] Everolimus [140] Nilotinib [79] Dasatinib [79] Bosutinib [79] Erlotinib [142]
Anticancer drugs		

	Substrates	Inhibitors ^a
	Gefinitib	
Immunosuppressants		Ciclosporin [138]
Statins	Rosuvastatin [139]	
Miscellaneous	Sulfasalazine [138, 139]	Eltrombopag [138] Elacridar (GF120918) [138, 139]
<i>ABCC2</i>		
Anticancer drugs	Cisplatin [138]	
Antiviral drugs	Indinavir [138]	
Immunosuppressants		Ciclosporin [138]
<i>OATP1B1</i>		
Antibiotics	Rifampicin [138, 139]	Rifampicin [139] Clarithromycin [1]
Anticancer drugs	Atrasentan [139] Methotrexate [138] SN-38 (active metabolite of irinotecan) [139]	
Antihypertensive drugs	Bosentan [139] Valsartan [139] Olmesartan [139]	
Antiviral drugs		Atazanavir [139] Lopinavir [139] Ritonavir [139] Saquinavir [139] Tipranavir [139]
Blood-glucose lowering drugs	Glibenclamide (Glyburide) [139] Repaglinide [139]	
Immunosuppressants		Ciclosporin [139]
Statins and lipid lowering drugs	Atorvastatin [139] Ezetimibe [139] Cerivastatin Fluvastatin [139] Rosuvastatin [138, 139] Simvastatin acid [139] Pitavastatin [139] Pravastatin [138, 139]	Gemfibrozil [1, 138, 139]
Miscellaneous	Thyroxine [139]	Eltrombopag [139]
<i>OATP1B3</i>		
Antibiotics	Rifampicin [138]	Rifampicin [139] Erythromycin [1]
Anticancer drugs	Methotrexate [138]	
Antihypertensive drugs	Telmisartan [139] Valsartan [139] Olmesartan [139]	
Antiviral drugs		Atazanavir [139] Lopinavir [139] Ritonavir [139] Saquinavir [139]
Heart medications	Digoxin [138]	
Immunosuppressants		Ciclosporin [139]
Statins	Atorvastatin [139] Rosuvastatin [139] Pitavastatin [139] Pravastatin [1]	

^aInhibitors listed for P-gp are those that showed >25 %increase in digoxin/fexofenadine/talinolol AUC.

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Table 2

Examples of in vitro and clinical studies that have investigated the effects of common drug transporter polymorphisms on drug transport

Drug transporter and polymorphism	Drug	In vitro effect on drug transport	Reference	Clinical effect on PK	Reference
<i>ABCB1</i>					
3435C>T	Digoxin	No sign, change in digoxin transport in LLC-PK1 cells expressing SNP	[150]	Increased digoxin C_{max} in T/T genotype Decreased digoxin AUC in T/T genotype	[93] [151]
<i>ABCG2</i>					
421C>A	Topotecan	Increased topotecan accumulation in cells transfected with 421A	[121]	Increased AUC_{0-24h} of topotecan in heterozygous C421A patients	[131]
	Imatinib	Increased imatinib accumulation in cells transfected with 421A	[131]	No significant differences in PK parameters of imatinib in heterozygous C421A patients (vs. wild-type)	[131]
<i>OATP1B1</i>					
521T>C 388A>G	Simvastatin, Pravastatin, Atorvastatin, Cerivastatin	Decreased statin uptake in HEK293 cells transfected with OATP1B1*5 (521T>C), *15 (388A>G, 521T>C) and *15+C1007G	[152]	Increased pravastatin AUC in heterozygous OATP1B1*5 and *15 healthy volunteers compared to noncarriers of these variants	[153]
	Docetaxel	Decreased docetaxel uptake in Flp-In T-Rex293 cells transfected with OATP1B1*5 or *15	[38]	No significant association between docetaxel CL and 521T>C or 388G>A variants in cancer patients	[38]
<i>OATP1B3</i>					
334T>G 699G>A IVS 12-5676A>G	Docetaxel	No in vitro studies regarding OATP1B3 genetic variants and docetaxel transport		In 334T>G, 699G>A, IVS 12-5676A>G variants no association with docetaxel CL was observed Homozygous IVS 12-5676A>G variant showed sign. higher AUC and lower CL of docetaxel compared to AA +AG genotypes	[38] [132]

PK pharmacokinetics, C_{max} maximum plasma concentration, CL systemic clearance, AUC area under the concentration vs. time curve, SNP single-nucleotide polymorphism