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Pharmacogenetics of Membrane Transporters: An Update on Current Approaches

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Abstract

This review provides an overview of the pharmacogenetics of membrane transporters including selected ABC transporters (ABCB1, ABCC1, ABCC2, and ABCG2) and OATPs (OATP1B1 and OATP1B3). Membrane transporters are heavily involved in drug clearance and alters drug disposition by actively transporting substrate drugs between organs and tissues. As such, polymorphisms in the genes encoding these proteins may have significant effects on the absorption, distribution, metabolism and excretion of compounds, and may alter pharmacodynamics of many agents. This review discusses the techniques used to identify

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substrates and inhibitors of these proteins and subsequently to assess the effect of genetic mutation on transport, both in vitro and in vivo. A comprehensive list of substrates for the major drug transporters is included. Finally, studies linking transporter genotype with clinical outcomes are discussed.

Keywords

ABCBl; ABCCl; ABCC2; ABCG2; OATP1B3; OATPlB1; Pharmacogenetics; Membrane transporters

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 influx or efflux via active transport. As such, these transporters can 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. These 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 levels and transport efficiency. Consequently, polymorphisms in transporters often contribute to variability in drug pharmacokinetics and response to treatment.

Many drugs undergo transport mediated by the ATPbinding cassette (ABC) family of transporters. There are a total of 49 known ABC genes including *ABCB1* (P-glycoprotein, MDR-1), *ABCC1* (MRPl), and *ABCG2*(BCRP, MXR, ABCP), all of which utilize ATP to move substrates across membranes [1–4]. These transporters generally counteract uptake through the intestinal wall, efflux substrates out of tissues into the systemic circulation, and eventually mediate the clearance of drugs from the body. Proteins in the ABC family are primarily known to be efflux transporters, moving substrates across the cell membrane and out of the cell. The most characterized polymorphic transporters to date are ABCB1 and ABCG2 [5]. Many current FDA approved drugs are substrates of these transporters, although both transporters also efflux a plethora of other compounds including naturally occurring toxins. ABCB1 and ABCG2 are expressed in enterocytes, the colon, the intestinal epithelium, the canalicular plasma membrane of hepatocytes, and the proximal renal tubule [6–10]. As such, ABCB1 and ABCG2 often mediate bioavailability and exposure to their substrate drugs mentioned in Table 1 [3, 11]. In addition, 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 [7, 12–17]. An exception includes the expression of ABCB 1in the choroid plexus where it transports molecules from the circulation into the cerebrospinal fluid [16, 18]. 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 [19]. ABCC2 is similar in function to ABCB1. It is expressed on the apical domain of epithelial cells and is involved in luminal excretion in organs such as the liver, the intestine, and the kidney, but it also plays a role in blood-tissue barriers [20]. Both ABCC1 and ABCC2 primarily secrete drugs that have undergone phase II metabolism into glutathione, glucuronide, or sulfate conjugates, but both efflux a wide range of drugs [21].

There are also several classes of “influx” or “uptake “ transporters that mediate the cellular uptake and reabsorption of drugs by moving substrates against a concentration gradient. The main uptake transporters are the organic anion transporting proteins (OATPs), organic cation transporters (OCTs), concentrative nucleoside transporters (CNT), dipeptide transporters (PEPT), and mono carboxylate transporters (MCT) [22]. For the sake of brevity, we will discuss only 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 [23]. It was previously thought that these transporters were primarily involved in uptake of substrates into the liver where metabolism occurs [23]. However, more recent evidence suggests that OATP1B3 is overexpressed in several tumor types such as prostate, colon, and liver [24–26]. 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. Therefore, the OATP1B family is important in regulating the pharmacokinetics 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 inter-individual 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 review will provide an overview of the methods by which investigators have discovered and characterized such associations in the ABCB1, ABCG2, ABCC1, ABCC2, OATP1B1, and OATP1B13 transporters. This methodology could be readily applied to the study of many additional transporters.

Genetic Variation and Genotyping Methods

More than 50 polymorphisms, three insertions/deletions, and several promoter alterations that modify gene transcription have been described in the *ABCB1* gene [27]. There are three single-nucleotide polymorphisms (SNPs) that are common in most ethnic groups and demonstrate strong linkage disequilibrium: the synonymous transition at nucleotide 1236C>T (Gly411Gly) in exon 12, the nonsynonymous tri-allelic transition 2677G>T/A (Ala893Ser/Thr) in exon 21, and the synonymous transition 3435C>T (Ile 1145Ile) in exon 26. Of these SNPs, only the 2677G>T/A (Ala893Ser/Thr) transition causes an amino acid change within a structurally important transmembrane domain of the translated protein, and the effects of this transition are controversial and drug-specific [15, 22, 28–30]. The 3435C>T SNP is associated with decreased mRNA stability and lower expression levels [31]. Recently, KimchiSarfaty et al. showed that synonymous polymorphisms in *ABCB1* may be responsible for altered protein conformations due to the phenomenon of ribosomal stalling [32]. As 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. The mechanism of this phenomenon, which may apply to other transporters in addition to ABCB1, is well described in the 2008 review by Tsai et al. [33]. When compared to single polymorphisms, haplotype combinations of these SNPs can result in greater protein function differences because each SNP has at least an additive effect on ABCB1 conformational changes. The 3435C>T polymorphism, which results in a synonymous protein change, has been shown to confer differences in ABCBl transport characteristics when combined with the 2677G>T/A and 1236C>T alleles as compared to the 2677G>T/A and 1236C>T alleles alone [32]. While this evidence is compelling, 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 be only artificially associated with inter-individual ABCB1 transport alterations.

While there are many polymorphisms in *ABCG2*, the *common ABCG2* 421C>A allele in exon 5 is by far the most well characterized. 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 wildtype [34]. The variant alleles (i.e., 421A and 141K) have also been associated with lower ATPase activity as compared with the wild-type ABCG2 [35]. 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 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%) [36]. Another SNP exists at nucleotide 34, resulting in a V12M amino acid change. This mutation results in poor localization of the ABCG2 protein [35], but does not change protein expression levels [37]. Surprisingly, this mutation does not appear to modify substrate transport [38]. Furthermore, mutations at R482 which result in non-synonymous protein changes have been identified in numerous cancer cell lines (presumably a mechanism of multidrug resistance) but have never been found in humans. This mutation affects both transport and substrate specificity [39–42].

There are several polymorphisms in *ABCC1*, many of which are non-synonymous. Those studied include C43S, T73I, S92F, Tl 17M, R230Q, V353M, R433S, R633Q, G671V, R723Q, A989T, C1047S, R1058Q, A1337T, and S1512L. The majority of these SNPs do not alter the functionality of the expressed protein and are unlikely to significantly influence the expression [43]. However, it has been noted that C43S, R433S, and A989T result in decreased ABCB1 function [43]. Others have evaluated non-synonymous polymorphisms to assess their impact on mRNA expression, but have found no significant results [44]. The *ABCC2* gene also contains several polymorphisms. In particular, patients with Dubin Johnson Syndrome (DJS) commonly have the 2302 C>T A768W polymorphism [20]. For the most part, however, *ABCC2* polymorphisms have not been significantly associated with any differences in functionality or expression with respect to drug transport [20].

There are many polymorphisms in *SLCO1B1* (which encodes OATP1B1) that have been associated with a decreased transport phenotype toward several drugs (see Table 1) and endogenous substrates [23]. In vitro assays have consistently validated altered transport efficiency in at least 13 synonymous and non-synonymous polymorphisms. At least three of these SNPs, the −11187G>A, the 388A>G (*SLCO1B1*1b*), and the 521T>C (*SLCO1B1*5*), have been shown to influence clinical outcome, although the allele frequencies differ between races. For example, while the *SLCO1B1*5* polymorphism is present in approximately 14% of the Caucasian population [45], only 1% of Japanese subjects carry this allele [46]. 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 [45, 47, 48]. The *SLCO1B3* gene has four polymorphisms (334T>G, 699G>A, 1564G>T, 1748G>A) that have been associated with altered transport and differences. It was recently determined that a common haplotype consisting of the 334T>G (Sl 12A) and 699G>A (M2331) 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 [24]. 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* [49].

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, Fluorescence Resonance Energy Transfer (FRET), etc. Table 2 includes polymerase chain reaction (PCR) and direct sequencing PCR primers that are currently used in the field to amplify polymorphic regions of DNA.

Genetic sequence variation may provide useful information to assist in making clinical decisions about drug treatment. Like all potential prognostic markers, the effect of polymorphisms on clinical endpoints must be validated through numerous preclinical and clinical processes that will be mentioned in the following subsections. Studies on the pharmacogenetics of transporters should (1) establish in silico and experimental evidence that a transporter polymorphism is associated with inter-individual variability in drug treatment, (2) establish drug interaction with a transporter, (3) establish that a polymorphism results in differential drug transport in vitro, (4) verify that transporter function is potentially

important in vivo using animal models, (5) validate that the polymorphism is associated with clinical inter-individual variability of drug treatment in specific drug-treated populations with specific measurement methods of specific endpoints, and (6) validate the precision, reproducibility, and accuracy for clinical endpoint measurement [50]. Finally, established causative variants require CUA-certified, FDA-approved genotyping methods and applications to therapy in order to be useful to the population at large. Only then these polymorphisms useful in predicting clinical outcome in the general public, and the utility of these methods are only relevant to informed physicians that can apply the results toward changes in therapy.

Examples of established SNPs and genotyping methods can be found in those variants in *TPMT, UGT1A9, CYP2D6, CYP2C9*, and *VKORC1* that are relevant for an a priori assessment of the starting dose and/or clinical outcome of 6-mercaptopurine, irinotecan, tamoxifen, and warfarin *(CYP2C9* and *VKORC1*) treatment, respectively. Genotyping for SNPs in these genes 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, and no CUA-certified genotyping platform has been developed to genotype transporter variations. Although FDA approval and CUA 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 [51].

Substrate Identification

ABCB1 and ABCG2 substrates (see Table 1) are typically hydrophobic molecules such as lipids, peptides, steroids, and xenobiotics, and include anticancer, HIV, atypical antipsychotic, 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 [52], but ABCC2 effluxes a wider range of drugs such as cyclosporin A, cisplatin, vinblastine, and camptothecan derivatives [19, 53]. OATP1B1 and OATP1B3 also interact with a wide range of substrates (not only organic anions as the nomenclature implies) including bilirubin, bile acids [54], peptides, eicosanoids, hormones, flavonoids [55], and prescribed drugs including fexofenadine [56]. However, each transporter has distinct substrate specificity, so some compounds are transported by one transporter but not by another in the same family.

Substrates are usually identified using transfected MDCK, LLC-PK1, Caco-2, or endothelial cell lines expressing the transporter of interest. Often following selection for cells expressing the transporter of interest, 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, and drug transport across the monolayer is evaluated by sampling from the other chamber. The experiment is then repeated by applying drug to the opposite chamber. Due to the directionality of the transporters, these experimental systems allow investigators to assess the basolateral to

apical and apical to basolateral transport of drug. If the drug is a substrate for the transporter, the A:B and B:A ratios will differ significantly. Several other methods also exist to evaluate the transport capabilities toward individual drugs such as ATPase assays, and the transport of fluorescent or radiolabeled compounds into and out of cells that are native expressing, drug-selected expressing, or transfected with a transporter of interest [57, 58].

Assessing Functional Significance of Polymorphisms In Vitro

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, JAI-51, calceine, doxorubicin, and daunorubicin have been evaluated using such methods and are still used in drug–drug interaction studies [59]. 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 [60]. As the pharmacokinetics of many other drugs could potentially be altered based on polymorphic ABCB1 expression and function, 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 Vmax 893T>893S>893A, while Km 893S>893T/A [61]. 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 ABCB 1 variants have been studied and have validated the previously mentioned finding with ABCB1 [62]. The effect of different polymorphisms on substrate transport by ABCG2 has been assessed using stably transfected HEK293 cells [63]. Following incubation of the cells with the drug, concentrations can be measured via flow cytometry [41], liquid scintillation counting (if radiolabeled drug is available) [64], or LC-MS [65]. In vitro analyses of OATP1B1 functional polymorphisms were evaluated similarly [45, 46, 66–69]. Interestingly, the above assays have also been employed to address the functional consequences of polymorphisms in the ABCC family of transporters, but no notable alterations in transport capacity were found for many commonly studied SNPs in *ABCC1* [43]. It seems that while ABCC transporters contain several potentially important polymorphisms and are 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 [20].

Assessing the Cause of Phenotypic Differences

Polymorphic differences that result in altered transporter kinetics and possible subsequent changes in drug disposition can effect 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 [60, 70, 71]; 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. Possible alterations in polymorphic mRNA secondary structure could also result in inefficient translation leading to alterations in protein folding [32]. This mechanism has been suggested as one possible explanation for the effects seen with the synonymous ABCB1 3435C>T transition that nevertheless is 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 may be associated with a protein product with attenuated efflux capacity through lowered efflux efficiency.

The first hypothesis has been evaluated using mRNA expression measurements in human tissues, and researchers found that ABCB1 is generally expressed at higher levels with the 3435C allele [60, 70–72]. These observations were replicated with co-transfection of equal amounts of plasmid, and it was concluded that the 3435T allele lowers mRNA stability and is therefore responsible for decreased efflux capacity [73]. 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 [37], but this finding was not confirmed by human intestinal samples [74]. 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 [35].

When OATP1B1 variants were expressed in HeLa cells, it was noted that *SLCO1B1*2*, *3, *5, *6, *9, *12, and *13 alleles were associated with reduced transport of OATP1B1 substrates [45]. Others noted that when the *SLCO1B1*15* variant was expressed in HEK293 cells and *Xenopus laevis* oocytes, these cells also had reduced transport capability [66, 68]. 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 [45]. It was also shown that some polymorphisms encode for impaired protein maturation that results in intracellular retention of the OATP1B1 protein [67]. A single study was published with regard to the 334T>G (S112A), the 699G>A (M233I), and the 1564G>T (G522C) in *SLCO1B3* (encoding OATP1B3) [75]. No differences were observed between wild-type and variant alleles at 334T>G or 699G>A with regard to protein localization, but the 1564G>T SNP conferred a phenotype where the protein was retained intracellularly in MDCKII cells [75].

Despite the encouraging results of these 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. However, expression levels were not associated with polymorphic variants, and it is often the case that reports evaluating the same tissues conflict [5]. Furthermore, some tissues such as cardiac endothelium actually express ABCB1 at greater levels in patients carrying variant alleles, which differs from data generated in other tissues [72]. Others have used non-human in vitro

expression systems in an attempt to validate the effect of *ABCB1* polymorphisms. However, transfected variant alleles do not seem to influence ABCBl transport in some of these experimental systems—perhaps due to differences in mRNA processing membranes in different cell lines and between species [76].

Assessing Functional Significance of Polymorphisms In Vivo

Mice carry two homologs of *ABCB1 (Abcb1a, Abcb1b)* and viable single *(Abcb1a−/−)* and double (Abcbla/b−/−) knockout mice are commercially available (Taconic Laboratories). 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 homolog of ABCG2) knockout mouse has also recently become commercially available from Taconic Laboratories, 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 [77]. These knockouts have been used as animal models of compromised blood–brain barrier function [9, 78], intestinal drug absorption [79], fetal drug exposure [80], and drug-induced damage to testicular tubules, choroid plexus epithelium [18], oropharyngeal mucosa [16], and peripheral nervous tissues [17].

Mice lacking the expression of a transporter generally have decreased ability to eliminate substrate drugs, except in cases where compensatory pathways are upregulated that circumvent transporter-mediated clearance [81, 82]. 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 hepatobilliary 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, though decreased elimination may be observed. 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. Furthermore, it is essential that agents which are transported by multiple transporters be tested in models representative of this physiology. For example, brain: plasma ratios of imatinib, a substrate for both Abcb1 and Abcg2, were only marginally higher than control in Abcg2(−/−) mice, yet 4-fold higher in Abcb1a/b(−/−) mice and 28-fold higher in double knockouts, suggesting that there is some synergy between the transporters [83].

Mice that do not express a specific transporter are generally more likely to experience benefit from treatment with a substrate drug because greater exposure to the drug is typically equated with improved efficacy. 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 [84]. Abcb1a$^{-/-}$ mice also showed 10 times higher brain–serum ratios of both risperidone and its active metabolite, 9-hydroxyrisperidone, than control mice [85], and many central nervous drugs showed 1.1- to 2.6-fold greater brain-to-plasma ratios in double knockout mice compared to wild-type mice [86].

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 [17].

Transporter Genetics in Clinical Pharmacology

Initially, investigators determined that the *ABCB1* 3435C>T transition was associated with lowered ABCB1 expression and higher digoxin levels in vivo [70]. 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 [87, 88]. 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 [13, 20, 89]. Often a polymorphism is found to be potentially important to drug treatment based on in vitro and in vivo evidence, but in clinical studies the polymorphism is not significantly associated with clinical parameters. Table 3 provides examples of transporter polymorphisms that have had an effect on certain substrates in vitro and/or in vivo that have or have not explained inter-individual variability toward these drugs in clinical pharmacology. While it is exceedingly difficult to assign a true mechanism behind the clinical findings, the in vitro and in vivo data provide powerful tools to ascertain potential mechanisms and provide the basis on which to form specific hypotheses that can be tested in the clinic. Nonetheless, one cannot say with certainty whether or not the proposed mechanism is actually occurring, and this is a major limitation of pharmacogenetics studies in general. A polymorphism that has no effect on a substrate drug in the clinic does not preclude a potential gene–drug interaction with another substrate drug.

These relationships are often dependent on route of administration, drug dosage, and schedule and can also be largely dependent on drug metabolism. For example, polymorphisms in the cytochrome P450s (CYPs) are often found to be associated with pharmacokinetics of transporter substrate drugs whereas *ABCB1* polymorphisms are not.

This is believed to occur because metabolism by the CYPs may be the rate-limiting step in drug clearance while variation in ABCB1 expression levels plays a lesser role in inter-individual variability. For example, if an impaired transporter limits transport out of the liver, then it is also possible that more metabolism occurs. However, the mechanistic relationship between transporter polymorphisms and drug plasma levels remains largely unclear, and the reasons that several drugs are more or less associated with *ABCB1* polymorphisms across multiple studies in various racial populations also remains unclear.

Recent evidence suggests 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, HIVinfected cells, etc.) [90, 91]. As previously mentioned, drug penetration into tissues can be both efficacious (i.e., by increasing therapeutic effect) and deleterious (i.e., by increasing toxicity). ABCB1 is also deterministic of the intracellular concentration of drugs as it efftuxes drugs from the cytoplasm into the extracellular matrix in several cell types. Based on the above findings, the hypothesis can be formed that ABCB1 expression levels are associated with pharmacokinetic parameters. Since the efficacy and toxicity of drugs is ultimately determined by plasma pharmacokinetic parameters and by the degree to which drugs are able to penetrate into tissues, studies investigating *ABCB1* polymorphisms as they relate to drug administration are likely to become increasingly important in the clinical setting. The effect of *ABCG2* polymorphisms on clinical pharmacology has only recently begun to be evaluated. Thus far, associations between the 421C>A mutation and plasma pharmacokinetics have been evaluated for several drugs including topotecan, irinotecan, and imatinib. This polymorphism was shown to increase bioavailability of topotecan [17] and more than double exposure of rosuvastatin, a statin commonly used in the treatment high cholesterol [92]. Findings for other drugs were less deterministic. Increasingly, investigators have sought to correlate genotype with toxicity. It has been shown that variant haplotypes for ABCB1 (3435C/T, 1236C/T, 2677G/T) or ABCG2 (−15622C/T, 1143C/T) increased the likelihood of sunitinib toxicity, including hand-foot syndrome [93].

The clinical consequences of OATPs are still being investigated, although several studies have confirmed that *SLCO* polymorphisms are associated with inter-individual variability in drug treatment [23]. This is especially true for pravastatin, which reduces cholesterol biosynthesis. It has been suggested that reduced hepatic uptake of pravastatin resulting from decreased transport through OATP1B1 is responsible for the increased plasma AUC of pravastatin [94], the unfavorable plasma pharmacokinetics of cholesterol synthesis biomarkers [95], and the resulting decreased efficacy of the drug. Interestingly, an *SLCO1B1* allele that was associated with lower pravastatin AUC (and presumably greater liver uptake) was also associated with increased efficacy [96], although in vitro assays do not confirm an altered transport efficiency [68]. An indirect gene–drug interaction between OATP1B3 and androgendeprivation therapeutics (ADT; e.g., leuprolide and goserelin) was recently proposed where OATP1B3 may scavenge low levels of testosterone during ADT, thereby opposing the effects of ADT on androgen receptor signaling [97]. Thus, endogenous processes affected by transporters may also alter drug treatment in some cases.

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 that 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 [5, 20, 23]. The FDA recommends several endpoints to evaluate specific diseases which should be used when making associations between a genetic variation and the treatment of diseases with drugs (see www.fda.gov/cder/guidance; last accessed September 16, 2009). 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. The genotypes should also be evaluated using a consistent set of SNPs in the relevant transporters. 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. However, the advent of genotyping technologies such as DMET may offer standardization of major pharmacogenetics studies in the future. Thus far, all studies linking pharmacogenomics of membrane transporters with clinical outcome have been retrospective and have taken place in eclectic populations with relatively low statistical power. It is essential that prospective studies are conducted, prior to any treatment modification, in order to assess the true effects of these polymorphisms and to determine whether the effect is drug-specific or disease related.

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Table 1

Substrates of ABCB1, ABCG2, ABCC1, ABCC2, OATP1B1, OATP1B3

	Substrates	Inhibitors
ABCB1		
Antibiotics	Actinomycin D	
	Erythromycin	
	Gramicidin D	
	Rifampin	
	Salinomycin	
	Sparfloxacin	
	Valinomycin	
Anti-cancer drugs	Bisantrene	Sunitinib
	Daunorubicin	Tamoxifen
	Diflomotecan	
	Docetaxel	
	Doxorubicin	
	Epirubicin	
	Etoposide	
	Gefitinib	
	Imatinib	
	Irinotecan	
	Mitoxantrone	
	Paclitaxel	
	Romidepsin	
	Teniposid	
	Tipifarnib	
	Vinblastine	
	Vincristine	
Antifungals	Itraconazole	Ketoconazole
	Ketoconazol	
Antihistamines	Certirizine	
	Fexofenadine	
	Loratadine	
	Terfenadine	
Antihypertensive drugs	Losartin	Nicardipine
	Talinolol	Quinidine
		Verapami
CNS drugs	Chlorpromazine	
	Clozapine	
	Fluphenazine	
	Olanzapine	
	Quetiapine	

	Substrates	Inhibitors
	Risperidone	
Flavonoids		Biochanin A
		Genistein
		Oroxylin A
Heart medications	Digoxin	Gallopamil
	Diltiazem	
	Ouabain	
	Quinidine	
	Verapamil	
HIV-1 protease inhibitors	Abacavir	
	Amprenavir	
	Aquinavir	
	Darunavir	
	Indinavir	
	Lopinavir	
	Nelfinavir	
	Ritonavir	
	Saquinavir	
Immunosuppressants	Cyclosporin A	Cyclosporin A
	Dexamethasone	Valspodar
	D-penicillamine enkephalin	
	FK 506	
	Hydrocortisone	
	Prednisolone	
	Rapamycin	
	Tacrolimus	
	Triamcinolone	
Sedatives		Midazolam
Statins	Atorvastatin	
	Cerivastatin	
	Lovastatin	
Miscellaneous	Asimadoline	Dexverapamil
	Cimetidine	Emopamil
	Colchicine	JAI-51
	Domperidine	Quinacrine
	Eletriptan	Tariquidar
	Flesinoxan	
	Glabridin	
	Ivermectin	
	Loperamide	
	Ondansetron	
	Quinacrine	

	Substrates	Inhibitors
	Ranitidine	
	Topiramate	
ABCG2		
Antibiotics	Ciprofloxacin	Novobiocin
	Erythromycin	Rapamycin
	Nitrofurantoin	
	Norfloxacin	
	Ofloxacin	
Anti-cancer drugs	9-Aminocamptothecin	Biricodar
	Bisantrene	Diethyls tilbestrol
	Cladribine	Elacridar
	Daunorubicin	Fumitremorgin
	Diflomotecan	Gefitinib
	Doxorubicin	Ginsenoside
	Epirubicin	Ortataxel
	Erlotinib	Sunitinib
	Etoposide	Tamoxifen
	Flavopiridol	Tryprostatin
	Gefitinib	Vandetanib
	Gimatecan	
	Homocamptothecin	
	Imatinib	
	Methotrexate	
	Mitoxantrone	
	SN-38 (irinotecan metabolite)	
	Teniposide	
	Tomudex	
	Topotecan	
Antihypertensive drugs	Olmesartan	Dihydropyridine
		Dipyridamole
		Reserpine
Anti-inflammatory drugs		Chrysin
		Curcumin
Antiplatelets	Dipyridamole	
Calcium channel blockers	Azidopine	Nicardapine
	Dipyridamole	Nimodipine
	Nitrendipine	Nitrendipine
Flavonoids	Seravastatin	Acacetin
		Apigenin
		Genistein
		Naringenin
		Quercetin

	Substrates	Inhibitors
		Silymarin
		Techochrysin
HIV-1 protease inhibitors	Abacavir	Abacavir
	Lamivudine	Amprenavir
	Nelfinavir	Atazanavir
	Zidovudine (AZT)	Delavirdine
		Efavirenz
		Lopinavir
		Nelfinavir
		Ritonavir
		Saquinavir
Immunosuppressants	Cyclosporin A	Cyclosporin A
	Lefunomide	Sirolimus
	Sirolimus	Tacrolimus
	Sulfasalazine	
	Tacrolimus	
Specific inhibitors		GF120918
		Ko143
		Tariquidar (XR9576)
Statins	Pitavastatin	Rosuvastatin
	Posuvastatin	
	Seravastatin	
Miscellaneous	Glyburide	Pantoprazole
	Protoporphyrin	
ABCC1		
Antibiotics	Berberine	
	Ciprofloxacin	
	Difloxacin	
	Grepafloxacin	
	Pirarubicin	
Anti-cancer drugs	Apicidin	Vandetanib
	Camptothecin	
	Chlorambucil	
	Chlorambucil	
	Cyclophosphamide	
	Daunorubicin	
	Depsipeptide (FK228)	
	Doxorubicin	
	Edatrexate	
	Epirubicin	
	Etoposide	
	Flutamide	

	Substrates	Inhibitors
	Hydroxyflutamide	
	Idarubicin	
	Irinotecan	
	Melphalan	
	Methotrexate	
	Paclitaxel	
	SN-38 (irinotecan metabolite)	
	Vinblastine	
	Vincristine	
	ZD1694	
Antihypertensive drugs		Verapamil
Anitiinflammatory drugs		Indomethacin
		Quercetin
		Sulindac
Flavonoids		Biochanin A
		Genistein
HIV-1 protease inhibitors	Indinavir	Kaempferol
	Ritonavir	
	Saquinavir	
Immunosuppressants		Cyclosporin A
Miscellaneous		Probenecid
		Sulfinpyrazone
ABCC2		
Antibiotics	Ampicillin	Azithromycin
	Azithromycin	
	Cefodizime	
	Ceftriaxone	
	Grepafloxacine	
Anti-cancer drugs	Camptothecin	BTK
	Cisplatin	Lonafarnib
	Doxorubicin	
	Etoposide	
	Irinotecan	
	Methotrexate	
	Mitoxantrone	
	Vinblastine	
	Vincristine	
Antihypertensive drugs	Olmesartan	
Antiflammatory drugs		Curcumin
Blood-glucode lowering drugs		Glibenclamide
HIV-1 protease inhibitors	Adefovir	
	Cidofovir	

	Substrates	Inhibitors
	Indinavir	
	Lopinavir	
	Nelfinavir	
	Ritonavir	
	Saquinavir	
Immunosuppressants		Cyclosporin A
Statins	Pravastatin	
Miscellaneous	Temocaprilate	MK-571
	Valproate	Furosemide
		PAK-104P
		Phenobarbital
		Probenecid
OATP1B1		
Antibiotics	Benzylpenicilliin	Clarithromycin
	Rifampin	Erythromycin
		Hyperforin
		Rapamycin
		Rifampin
		Rifamycin SV
		Roxithromycin
		Telithromycin
Anti-cancer drugs	ACU-154	Antamanide
	Atrasentan	Ketoconazole
	Bamet-R2	Paclitaxel
	Bamet-UD2	PKI-166
	Demethylphalloin	SN-38
	Dihydromicrocystin-LR	
	Irinotecan	
	Methotrexate	
	SN-38	
Anti-diabetics		Glibenclamide
		Pioglitazone
		Rosiglitazone
Antifungals	Caspofungin	Clotrimazole
Antihistamines	Fexofenadine	
Antihypertensive drugs	Bosentan	Telmisartan
	Enalapril	
	Olmesartan	
	Temocapril	
	Valsartan	
Anti-inflammatory drugs	D-penicillamin encephalin	Troglitazone
	Troglitazone sulfate	Troglitazone sulfate

	Substrates	Inhibitors
Blood-glucose lowering drugs	Repaglinide	
Fibrates		Gemfibrozil
		Gemfibrozil-1-O-glucuronide
Flavonoids		Biochanin A
Heart medications		Digoxin
HIV-1 protease inhibitors		Indinavir
		Nelfinavir
		Ritonavir
		Saquinavir
Immunosuppressants		Cyclosporin A
		Tacrolimus
Statins	Atorvastatin	Atorvastatin
	Cerivastatin	BMS-241423 (atorvastatin analog)
	Fluvastatin	BMS-243887 (atorvastatin analog)
	Pitavastatin	Lovastatin
	Pravastatin	Lovastatin acid
	Rosuvastatin	Lovastatin lactone
	Simvastatin acid	Pravastatin
		Simvastatin
		Simvastatin lactone
Miscellaneous	BQ-123	Carbamazepine
	Bromosulphophthalein	Glycyrrhizin
		Metyrapone
		Mifepristone
		Sildenafil
OATP1B3		
Antibiotics	Rifampin	Clarithromycin
		Erythromycin
		Hyperforin
		Rifampin
		Rifamycin
		Roxithromycin
		Telithromycin
Anti-cancer drugs	Demethylphalloin	
	Dihydromicrocystin-LR	
	Docetaxel	
	Imatinib	
	Irinotecan	
	Methotrexate	
	Paclitaxel	
	SN-38	
Antihistamines	Fexofendadine	

	Substrates	Inhibitors
Antihypertensives	Bosentan	
	Enalapril	
	Olmesartan	
	Telmisartan	
	Valsartan	
Anti-inflammatory drugs	D-penicillamine	Troglitazone sulfate
	enkephalin	
Blood-glucose lowring drugs	Repaglinide	
Heart medications	Digoxin	
	Ouabain	
Immunosuppressants		Cyclosporin A
Statins	Fluvastatin	Pravastatin
	Pitavastatin	
	Pravastatin	
	Rosuvastatin	
Miscellaneous	BQ-123	Bromosulphophthalein
	Bromosulphophthalein	Glycyrrhizin

Table 2

Primers used to amplify selected polymorphisms in the ABCB1, ABCG2, OATP1B1, and OATP1B3 genes

Transporter polymorphism	Forward primer	Reverse primer	Ref.
ABCB1 1236C>T			
PCR amplification	5′-GGCACAAACCAGATAATATTAAGG-3′	5′-TATCCTGTCCATCAACACTGACC-3′	#
Nested PCR	5′-GTTCACTTCAGTTACCCATCTCG-3′	5′-TCCTGTCCATCAACACTGACCTG-3′	#
Sequencing PCR	5′-GTCAGTTCCTATATCCTGTGTCTG-3′	5′-TCGCATGGGTCATCTCACCATC-3′	#
ABCB1 2677G>T/A (A893S/T)			
PCR amplification	5′-AGGCTATAGGTTCCAGGCTTGC-3′	5′-AGAACAGTGTGAAGACAATGGCC-3′	#
Nested PCR	5′-CCCATCATTCGAAT AGCAGGAG-3′	5′-GAACAGTGTGAAGACAATGGCCT-3′	#
Sequencing PCR	5′-ATCCTTCATCTATGGTTGGCAAC-3′	5′-TGAGTCCAAGAACTGGCTTTGC-3′	#
ABCB1 3435C>T			
PCR amplification	5′-ATCTCACAGTAACTTGGCAGTTTC-3′	5′-AACCCAAACAGGAAGTGTGGCC-3′	#
Sequencing PCR	5′-GCTGGTCCTGAAGTTGATCTGTG-3′	5′-AAACAGGAAGTGTGGCCAGATGC-3′	#
ABCG2 421C>T			
PCR amplification	5′-TGGCAAATCCTTGTATGAAGCAG-3′	5′-TTCACGTACAACACCACATTGCC-3′	#
Sequencing PCR	5′-GCAGGTTCATCA TTAGCTAGAAC-3′	5′-CCTACTTATGCTGATCATGAGC-3′	#
*OATP1B1**1b			
PCR amplification	5′-GCAAATAAAGGGGAATATTTCTC-3′	5′-AGAGATGTAATTAAATGTATAC-3′	[45]
RFLP enzyme	*Cla*I		
*OATP1B1**5			
PCR amplification	5′-GTTAAATTTGTAATAGAAATGC-3′	5′-GTAGACAAAGGGAAAGTGATCATA-3′	[45]
Allele specific PCR primers	WT 5′-CATACATGTGGATATATGT-3′	*N/A*	[45]
	MT 5′-CATACATGTGGATATATGC-3′D		
OATP1B3 334T>G (S112A)			
PCR amplification	5′-CCTTCACAGTTAAATTACATGGTC-3′	5′-TATTCATTTCATATAAAACTGTATACC-3′	#
Sequencing PCR	5′-GGGCATATTTGCATTCATTTGGG-3′	5′-CATGATAAATAAAGAAATACATGATG-3′	#
OATP1 B3 699G>A (M2331)			
PCR amplification	5′-TCCTTGTATTTAGGT AACGTACAG-3′	5′-TCAAGTTTGGTTA TTTTGGATCAAG-3′	#
Sequencing PCR	5′-GATCTACCCTTGAAATAATAATGTC-3′	5′GTAAAAGCAAAGTATAAATAGGAGC-3′	#
OATP1B3 1564G>T (G522C)			

Transporter polymorphism	Forward primer	Reverse primer	Ref.
PCR amplification	5′-ATATACAGAATTTCATACACTAATTTC-3′	5′-AATTCTAAGAAAATGCATTCTCAAAG-3′	#
Sequencing PCR	5′-TATTTTGCCTTCACTATTAAGCAAC-3′	5′-AATATGAATTTGAGCTCAAAATACAG-3′	#

Primers are used for direct sequencing following amplification from genomic DNA, unless otherwise indicated

#Denotes previously unpublished primer

Table 3

Selected examples of studies that have either verified, or detracted from the importance of transporter polymorphisms on drug treatment after in vitro or in vivo validation

Transporter	Drug	In vitro or in vivo verification of polymorphic effects	Ref.	Clinical verification of inter-individual variability	Ref.
ABCB1					
	Fexofenadine	Increased transport with 893Ser Increased transport with 893Thr	[98]	Decreased AUC in patients with 2677TT and 2677AA genotypes	[30, 99]
	Docetaxel	Decreased expression of ABCB1 in survival unchanged.	[70, 71]	Docetaxel AUC and overall liver with 3435TT genotype	[100–102]
		Docetaxel is transported by ABCB1	[103]		
ABCG2					
	Topotecan	Increased accumulation of drug in cells transfected with 421A (Q141K)	[104]	Increased bioavailability in heterozygous C421A patients	[104]
	Imatinib	Increased accumulation of drug in cells transfected with 421A (Q141K)	[105]	No significant difference in AUC or Cmax in heterozygous C421 patients	[105]
ABCC1					
	N/A	None to date			
ABCC2					
	N/A	None to date			
OATP1B1					
	Pravastatin	Reduced membrane expression of OATP1B1*5 variant	[45]	Increased AUC in OATP1B1*5	[94, 106]
OATP1B3	Leuprolide and goserelin	Reduced transport of testosterone with the 334G/699A haplotype	[24]	Increased progression-free survival in patients with 334G/699A haplotype	[97]