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Impact of Transporter Polymorphisms on Drug Development: Is It Clinically Significant?

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Abstract

Drug transporters are becoming increasingly recognized as relevant to the drug development process. This may be a reflection of increasing target complexity and the need for high-affinity interaction with drug targets that minimize off-target side effects. Moreover, as new molecular entities (NMEs) become larger in size and amphipathic in nature, interaction with drug transporters, both uptake as well as efflux, becomes increasingly likely. In some cases transporters may limit the absorption or organ-specific entry of NMEs, whereas in other cases transporters may enhance their absorption or tissue accumulation. Indeed, in some cases, transporters may prove to be a therapeutic target. Accordingly, a better understanding of potentially clinically relevant drug transporter polymorphisms earlier in the drug development process is highly desirable. In this review we examine key transporters that are important to the absorption, distribution, and excretion of a large number of drugs in clinical use. Importantly, we provide our assessment of the potential impact of known polymorphisms in such transporters and discuss whether there is sufficient evidence to incorporate these polymorphisms in the drug development process.

Keywords

drug transporters, pharmacogenomics, drug development

Transporters are proteins localized to the plasma membrane and membranes of numerous subcellular organelles that function to facilitate the uptake or clearance of toxins, xenobiotics, and endogenous molecules important for cellular homeostasis. Transporters are classified into 2 superfamilies based on sequence homology: the ATP-binding cassette (ABC) family and the solute carrier (SLC) family. ABC transporters function as efflux transporters that directly harness energy produced by ATP hydrolysis. SLC transporters mediate import or, in some cases, export of substrates via an ion gradient established by ATP-dependent pumps or an electrochemical gradient.[1] To date, 48 ABC transporters and 350 SLC transporters have been annotated in the human genome, and approximately 30 of these are involved in the disposition of drugs.[2,3] Moreover, membrane transporters located on the intestinal epithelium, liver, and kidney modulate the overall pharmacokinetic (PK) profiles of new molecular entities (NMEs) and are therefore important considerations in drug development. In addition, membrane transporters expressed in organs that possess a barrier function, such as the blood-brain barrier, have proved to be highly relevant to drug development and therapeutic targeting.[4] The locations of key transporters are summarized in Figure 1.

The discovery and development of safe and effective NMEs require the consideration of multiple factors that impact drug disposition. Indeed, until recently, genetic variation in drug-metabolizing enzymes (DMEs) has been the major focus of pharmacogenetics. However, in the past decade, single nucleotide polymorphisms (SNPs) in membrane transporters have been demonstrated to be important, and sometimes rate-limiting, determinants of drug disposition and response.[5] Recognizing the need to evaluate the impact of membrane transporters during the drug development process, the International Transporter Consortium (ITC) developed guidelines for assessing the importance of emerging key transporters in drug development.[4] Subsequently, in 2013, the Food and Drug Administration (FDA) published a clinical pharmacogenomics guidance titled, "Clinical Pharmacogenomics: Premarketing Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling."

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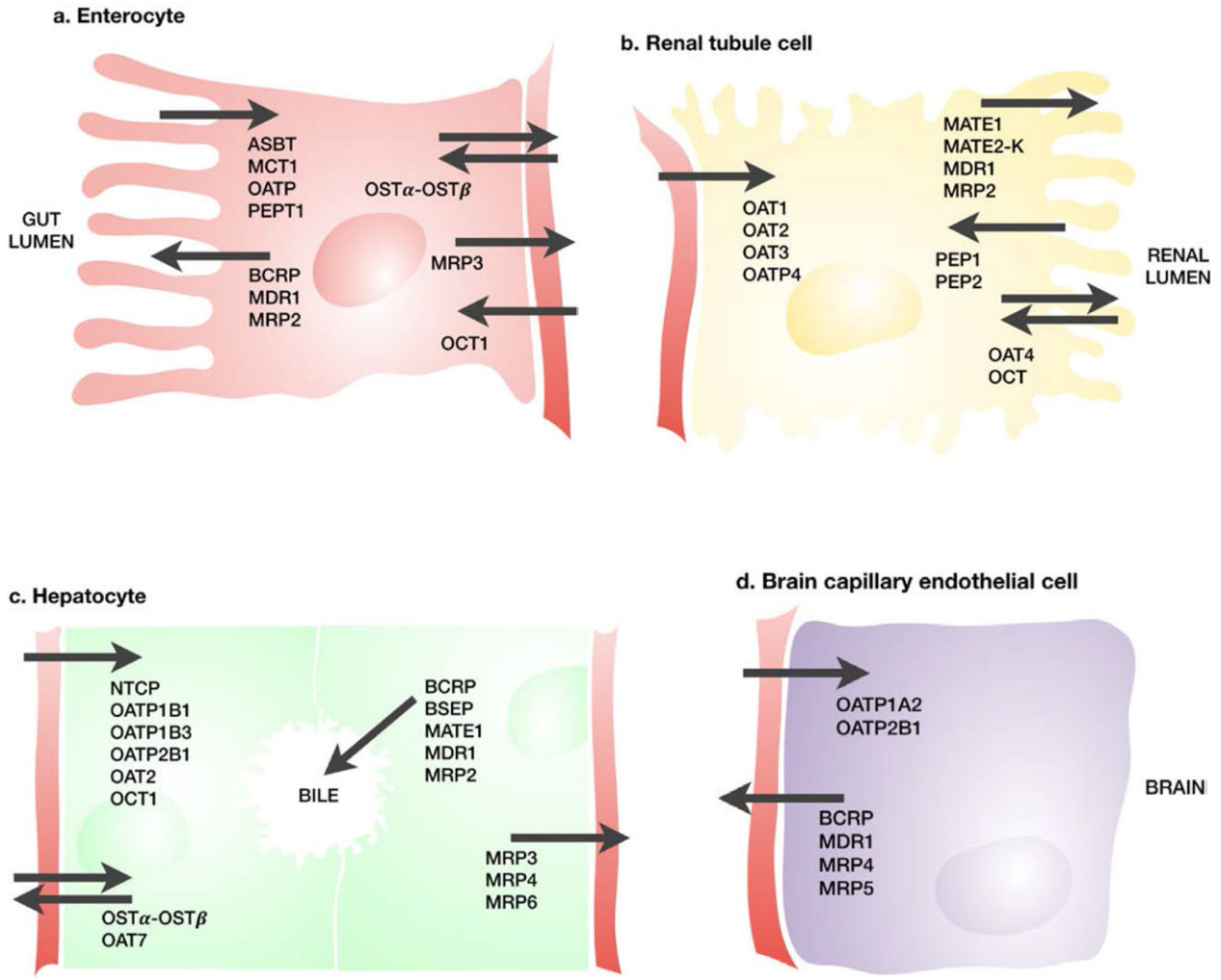

Figure 1. Illustration of important efflux and uptake transporters in the (a) intestinal epithelia, (b) proximal tubule of the kidney, (c) liver, and (d) blood-brain barrier that may be involved in the absorption, distribution, metabolism, and excretion of a drug. Abbreviations: ASBT, apical sodium-dependent bile acid transporter; MCT, monocarboxylate transporter; OATP, organic anion-transporting polypeptide; PEPT, peptide transporter; BCRP, breast cancer resistance protein; MDR, multidrug resistance; OCT, organic cation transporter; OST, organic solute transporter; OAT, organic anion transporter; MATE, multiantimicrobial extrusion protein; URAT, urate transporter; OCTN, organic zwitterion/cation transporters; NTCP, Na^+-taurocholate cotransporting polypeptide; BSEP, bile slat export pump. (Adapted from International Transporter Consortium.[4])

This work sought to provide more specific guidance to the pharmaceutical industry with regard to genetic variants in both DMEs and membrane transporters and emphasized the incorporation of pharmacogenomics into the drug development process.

However, the field of drug transporters, particularly in relation to clinically relevant transporter polymorphisms, continues to evolve rapidly and merits ongoing assessment to aid in future drug development. Although the definition of clinically significant transporter polymorphisms remains debatable, based on observed drug-drug interactions associated with transporters, a 3-fold or higher increase in AUC of transporter substrate drug would fit such a definition. However, for many drugs in clinical use, clinical relevance may occur even at much lower AUC increase of substrate drugs. Accordingly, in this review, we summarize the key drug transporter polymorphisms associated with variations in drug pharmacokinetics and response. Specifically, we focus on SNPs within members of the organic cation transporters (OCTs), organic anion-transporting polypeptides (OATPs) family of SLC transporters, in addition to multidrug-resistant proteins (MRPs), breast cancer resistance protein (BCRP), and P-glycoprotein (P-gp, *MDR1*, *ABCB1*).

SLC Transporter Polymorphisms of Potential Clinical Significance

Organic Anion-Transporting Polypeptides

In humans, 12 organic anion-transporting polypeptides (OATP proteins; encoded by the SLCO gene

family) have been identified with a wide substrate specificity for amphipathic molecules, including endogenous compounds such as bile acids and hormones, and xenobiotic substrates including statins, antidiabetics, and chemotherapeutic agents.[6,7] Of these, OATP1B1 (*SLCO1B1*), OATP1B3 (*SLCO1B3*), and OATP2B1 (*SLCO2B1*) are largely expressed at the sinusoidal endothelium of the liver and mediate hepatocellular uptake of substrates from portal circulation; OATP2B1 is also expressed on intestinal endothelia, where it facilitates the absorption of its substrates.[8] Interestingly, OATP1A2 (*SLCO1A2*) is expressed in the distal nephron, apical membrane of cholangiocytes, ciliary body epithelium of the eye, and capillary endothelia of the brain and is now thought to facilitate brain entry of certain medications.[9] Select OATP variants and their effects are summarized in Table 1.

OATP1B1. Of the 40+ nonsynonomous variants that have been identified in human *SLCO1B1*, c.521T>C (rs4149056) in exon 5, first identified by Tirona et al, is the most extensively characterized.[6,10] *SLCO1B1* c.521T>C has an allele frequency of approximately 8% to 20% in European individuals with lower frequencies in East Asian and African-American individuals.[11] In vitro work suggests that the c.521T>C variant significantly impairs transport activity of several OATP1B1 substrates through reduced transporter expression at the plasma membrane.[10] Clinically, the significance of *SLCO1B1* c.521T>C is best exemplified by its effects on statins. For example, the areas under the plasma drug concentration-time curves (AUCs) for the pharmacologically active form of simvastatin (simvastatin acid) were 120% and 221% higher in c.521C/C healthy volunteers than in those with the c.521C/T and c.521T/T genotypes, respectively.[12] Furthermore, the c.521T>C variant was strongly associated with the risk for simvastatin-induced myopathy in a genome-wide association study (GWAS) that compared 85 patients with myopathy on high-dose simvastatin with 90 matched controls.[13] These studies, in conjunction with others, spurred the Clinical Pharmacogenetics Implementation Consortium (CPIC) to include recommendations for genotype-informed dosing of statins in the 2014 guideline update.[14]

Another commonly studied SNP in *SLCO1B1* is c.388A>G (rs2306283), which has been shown to cause increased liver OATP1B1 expression in a white population.[15] Allele frequencies for *SLCO1B1* c.388A>G appear to range from 26% to 77% in European and sub-Saharan individuals, respectively.[11] Unlike c.521T>C, c.388A>G is associated with lower plasma concentrations of various statins,[16,17] and homozygous variant carriers have shown increased low-density lipoprotein (LDL) reduction in response to atorvastatin treatment when compared with heterozygous and homozygous wild-type individuals.[18] Interestingly, Teft et al found that although c.521C was significantly associated with increased exposure to the active metabolite of irinotecan, the progression-free survival (PFS) was significantly longer in c.388G/G advanced cancer patients treated with irinotecan-based regimes.[19] Moreover, c.521T>C and c.388A>G can be in linkage disequilibrium and result in 4 functionally distinct haplotypes with varying effects on drug disposition.[8]

OATP1B3. c.334T>G (rs4149117) and c.699G>A (rs7311358) in SCLO1B3 are frequently observed across diverse ethnic populations and have been shown to alter the pharmacokinetics of certain medications.[20] For example, both variants have been associated with increased dose-adjusted AUC of the immunosuppressant mycophenolate mofetil (MMF) in Japanese renal transplant patients.[21] In contrast, Picard et al investigated MMF plasma exposure in kidney transplant patients who were coadministered tacrolimus or sirolimus and found that carriers of at least 1 c.334T allele had a 1.4-fold higher AUC than carriers of the GG genotype.[22] More recently, 334T>G has also been associated with increased imatinib clearance and increased accumulation within the leukocytes in chronic myeloid leukemia patients.[23,24] Interestingly, inactivating mutations of *SLCO1B1* and *SLCO1B3* result in Rotor syndrome, a benign but very rare autosomal recessive liver disease associated with conjugated hyperbilirubinemia, impaired hepatic uptake of diagnostic dyes, and delayed clearance of certain anionic drugs.[25] Clinical impact of SLCO1B3 genetic variation, particularly of relevance to drug development, remains to be clarified.

OATP2B1. Compared to the extensive knowledge on the clinical significance of polymorphisms in *SLCO1B1,* data surrounding the functional consequences of *SLCO2B1* polymorphisms are limited. One of the first nonsynonymous variants described in *SLCO2B1* was c.1457C>T, which has an allele frequency of approximately 3% and 31% in white and Asian individuals, respectively.[8] Although c.1457C>T does not appear to affect OATP2B1 protein expression,[15] it has been linked to decreased AUC of both celiprolol (β-blocker),[26] and fexofenadine (antihistamine) in healthy volunteers.[27] In contrast, Akamine et al found lower levels of S-fexofenadine in healthy Japanese variant carriers following a 60-mg dose of fexofenadine.[28] Similarly, *SLCO2B1* c.935G>A (rs12422149) was associated with decreased plasma concentrations of montelukast (leukotriene receptor antagonist) and decreased symptom improvement in asthma patients in 1 study,[29] and another study found no association between the variant and montelukast pharmacokinetics.[30] These conflicting findings

Table 1. Organic Anion-Transporting Polypeptides

Variant	Effect on mRNA/Protein	Drug Class	In Vitro/ In Vivo	Allele Effect/Association	Drug/Effect Association[a]
Select Variants and Variant Effects Within *SLCO1A2* (OATP1A2)					
113T, 38T>C rs10841795		Antimetabolites	In vitro	**Methotrexate**: ↑ uptake (*Xenopus laevis* oocytes)[104]	Low/Preliminary
		Tyrosine kinase inhibitors	In vivo	**Imatinib**: No effect on average steady-state concentration in white cancer patients (n = 94)[32]	Low/Preliminary
Q172D, 516A>C rs11568563	Variant appears to cause a reduction in cell surface transporter expression[9]	δ-Opioid receptor agonists	In vitro	**Deltorphin II and [D-penicillamine-2,5]-enkephalin (DPDE)**: ↓ uptake (HeLa cells)[31]	Low/Preliminary
		Tyrosine kinase inhibitors	In vitro	**Imatinib**: ↓ uptake (HeLa cells)[32]	Low/Preliminary
			In vivo	**Imatinib**: No effect on drug levels in white cancer patients (n = 94)[32]	
–1105G>A rs4148977		Tyrosine kinase inhibitors	In vivo	**Imatinib**: ↓ clearance in CML patients (n = 34)[33]	Low/Preliminary
–1032G>A rs4148978		Tyrosine kinase inhibitors	In vivo	**Imatinib**: ↓ clearance in CML patients (n = 34)[33]	Low/Preliminary
–361G>A rs3764043		Tyrosine kinase inhibitors	In vivo	**Imatinib**: ↑ clearance in CML patients (n = 34)[33]	Low/Preliminary
Select Variants and Variant Effects Within *SLCO1B1* (OATP1B1)					
V174A, 521T>C rs4149056	Variant causes reduced membrane transporter expression[10]	Statins	In vivo	**Simvastatin Acid**: ↑ AUC and C_{max} (n=32);[105] variant strongly associated with ↑ simvastatin-induced myopathy risk (n = 175)[13]	High
			In vivo	**Pravastatin**: ↑ drug plasma concentration in healthy white volunteers (n = 41)[106]	Moderate
			In vitro	**Pravastatin**: ↓ V_{max} (HEK 293 cells)[107]	
			In vivo	**Atorvastatin and Rosuvastatin**: ↑ AUC in healthy volunteers (n = 32)[108]	Moderate
		Meglitinides	In vivo	**Repaglinide**: ↑ AUC in both homozygous and heterozygous variant carriers when compared to wild-type individuals (n = 56)[109]	Low/Preliminary
		Antimetabolites	In vivo	**Methotrexate**: ↓ clearance in children diagnosed with acute lymphoblastic leukaemia (n = 434)[110]	Low/Preliminary
		Alkaloids	In vivo	**Irinotecan**: ↑ plasma exposure of active metabolite in advanced cancer patients (n = 127)[19]	Low/Preliminary
N130D, 388A>G rs2306283	Variant causes increased liver protein expression in white population[15]	Statins	In vivo	**Pravastatin**: ↓ AUC in healthy Japanese volunteers (n = 23)[16]	Low/Preliminary
			In vivo	**Atorvastatin**: ↑ LDL reduction for GG variant carriers when compared with GA and AA carriers (n=136)[18]	Low/Preliminary

(*Continued*)

Table 1. Continued

Variant	Effect on mRNA/Protein	Drug Class	In Vitro/ In Vivo	Allele Effect/Association	Drug/Effect Association[a]
			In vivo	**Lovastatin Acid**: ↓ AUC in healthy white volunteers (n = 27)[17]	Low/Preliminary
		Alkaloids	In vivo	**Irinotecan**: ↑ PFS in advanced cancer patients (n = 103)[19]	Low/Preliminary
		Cholesterol absorption inhibitors	In vivo	**Ezetimibe**: gene dose ↓ in AUC in healthy volunteers (n = 17)[111]	Low/Preliminary
		Meglitinides	In vivo	**Repaglinide**: ↓ AUC and C_{max}, and ↑ mean blood glucose concentration in healthy volunteers (n = 8)[112]	Low/Preliminary
P155T, 463C>A rs11045819		Antimycobacterials	In vivo	**Rifampin**: ↓ AUC in adults with pulmonary tuberculosis from Africa, North America, and Spain (n = 72)[113]	Low/Preliminary
–11187G>A , rs4149015		Statins	In vivo	**Pravastatin**: heterozygous carriers have a 98% ↑ drug plasma concentration than homozygous wild-type individuals in healthy white volunteers (n = 41)[106]	Moderate
		Alkaloids	In vivo	**Irinotecan**: ↑ AUC of active metabolite in Korean patients with advanced non-small-cell lung cancer (n = 81)[114]	Low/Preliminary
Select Variants and Variant Effects Within *SLCO1B3* (OATP1B3)					
S112A, 334T>G rs4149117	Does not alter membrane protein localization[115]	Immune suppressants	In vivo	**Mycophenolate mofetil**: ↑ dose-adjusted AUC and ↓ oral clearance in Japanese renal transplant recipients (n = 87)[21]; in contrast, a study in kidney transplant patients found ↓ plasma concentrations (n = 70)[22]	Low/Preliminary
		Tyrosine kinase inhibitors	In vivo	**Imatinib**: ↑ clearance in Japanese CML patients (n = 34)[24]	Low/Preliminary
			In vivo	**Imatinib**: ↑ intracellular accumulation in leukocytes of CML patients (n = 15)[23]	
M233I, 699G>A rs7311358	Does not alter membrane protein localization[115]	Immune suppressants	In vivo	**Mycophenolate mofetil**: ↑ dose-adjusted AUC and ↓ oral clearance in Japanese renal transplant recipients (n = 87)[21]	Low/Preliminary
IVS12-5676A>G rs11045585	Does not affect protein expression[15]	Mitotic inhibitors	In vivo	**Docetaxel**: ↑ AUC, ↓ oral clearance, and ↑ risk of docetaxel-induced adverse events in Japanese cancer patients (n = 84)[116]	Low/Preliminary
Select Variants and Variant Effects Within *SLCO2B1* (OATP2B1)					
R312Q, 935G>A rs12422149	Variant does not appear to affect protein expression[15]	Leukotriene receptor antagonists	In vivo	**Montelukast**: ↓ plasma concentrations and ↓ symptom improvement in asthma patients (n = 489)[29]; in contrast, a study done in healthy volunteers found no association between the variant and montelukast pharmacokinetics (n = 16)[30]	Low/Preliminary

(*Continued*)

Table 1. Continued

Variant	Effect on mRNA/Protein	Drug Class	In Vitro/ In Vivo	Allele Effect/Association	Drug/Effect Association[a]
			In vitro	**Montelukast**: ↓ transport (Caco-2 cells)[29]	
S486F, 1457C>T rs2306168	Variant does not appear to affect protein expression[15]	β-Blockers	In vivo	**Celiprolol**: ↓ AUC in healthy participants (n = 30)[26]	Low/Preliminary
		Antihistamine	In vivo	**Fexofenadine**: ↓ AUC in healthy individuals (n = 14) [27]; in contrast, the variant was associated with ↑ AUC of S-fexofenadine in healthy Japanese volunteers (n = 24)[28]	Low/Preliminary

[a]**High**: annotation for a variant-drug combination where most evidence demonstrates an association. The association must be replicated in more than 1 cohort with significant *P*-values. **Moderate**: annotation for a variant-drug combination with moderate evidence of an association. The association must be replicated in more than 1 cohort, but there may be some studies that do not show statistical significance. **Low/Preliminary**: annotation for a variant-drug combination based on a single significant study or annotation for a variant-drug combination evaluated in multiple cohorts but lacking clear evidence of an association; this classification also encompasses associations that have been studied only in vitro.

underscore the need for further investigations into *SLCO2B1* polymorphisms and their effects on drug disposition. Current knowledge does not support a major role of *SLCO2B1* SNPs in drug disposition; thus, a major focus on *SLCO2B1* genetic variation during development is unlikely to more fully predict clinical drug response or variation in the PK profile of a OATP2B1 substrate NME.

OATP1A2. To date, OATP1A2 has been the only human OATP transporter detected in the brain capillary endothelium and thus has the potential to play a role in the central nervous system penetration of certain medications.[31] Our group had demonstrated decreased transport of δ-opioid receptor agonists (deltorphin II and [D-penicillamine-2,5]-enkephalin, DPDE) in HeLa cells expressing the *SLCO1A2* variant c.516A>C (rs11568563).[31] *SLCO1A2* c.516A>C has an allele frequency of approximately 2% and 5% to 7% in African and white individuals, respectively, and appears to cause a reduction in cell surface transporter expression.[8,9] The effects of *SLCO1A2* c.516A>C have also been investigated in relation to imatinib (a tyrosine kinase inhibitor) pharmacokinetics. However, a significant change in imatinib plasma concentrations in 94 white cancer patients was not observed.[32] Interestingly, another study found that common promoter polymorphisms in *SLCO1A2* were associated with decreased (c.–361G>A, rs3764043) and increased (c.–1105G>A, rs4148977; c.–1032G>A, rs4148978) clearance of imatinib in Japanese myeloid leukemia patients.[33] Taken together, although OATP1A2 is likely to play an important role in the CNS entry of certain drugs, there is no compelling evidence to suggest functional SNPs in this transporter will have a major impact on substrate drug response, particularly in terms of PK.

In general, among the *SLCO* variants, the *SLCO1B1* variants c.521T>C and –11187G>A appear to have the greatest evidence in support of their impact on statin PK and toxicity profiles.

Organic Cation Transporters

The organic cation transporters (OCTs) belong to the SLC22 family.[34] In humans, the members of this subgroup include OCT1 (*SLC22A1*), OCT2 (*SLC22A2*), and OCT3 (*SLC22A3*). These proteins share a very similar structure and translocate a number of endogenous and exogenous substrates bidirectionally across cellular membranes, primarily through facilitated diffusion.[35,36] OCT1 is predominantly expressed on the hepatocyte sinusoidal membrane, whereas OCT2 is positioned mainly within the proximal tubule of the kidney, and OCT3 is more broadly distributed.[35,37] The OCTs have been implicated in regulating the disposition of numerous drugs including metformin, lamivudine, and numerous chemotherapeutics.[35]

A number of SNPs in the genes encoding OCT1 and OCT2 have been identified and are associated with changes in the mRNA or protein expression of these transporters.[38–42] Furthermore, many of these variants appear to be associated with reduced protein and/or mRNA expression resulting in changes in the in vitro and in vivo PK of biguanides, tyrosine kinase inhibitors, and dopamine precursors (Table 2).

OCT1. In vitro work using HEK293 cells transfected with either *SLC22A1* p.M420del (rs35191146)[43] or *SLC22A1* c.181C>T (rs12208357)[44] has demonstrated decreased uptake of metformin, a blood-glucose-lowering medication that exerts its effect by increasing peripheral insulin sensitivity.[45] Moreover, healthy volunteer data have demonstrated that

Table 2. Organic Cation Transporters

Variant	Effect on mRNA/Protein	Drug Class	In Vitro/ In Vivo	Allele Effect/Association	Drug/Effect Association[a]
Select Variants and Variant Effects Within *SLC22A1* (OCT1)					
M420del, 1258delG rs35191146	↓ protein expression despite no difference in the amount of mRNA transcript[39]	Tyrosine kinase inhibitors	In vivo	**Imatinib**: ↑ probability of treatment failure in chronic myeloid leukemia patients (n = 336)[39]	Low/Preliminary
			In vitro	**Imatinib**: ↓ uptake, but this effect was countered if the M408V (rs628031) SNP was also present (KCL22-hOCT1 cells)[39]	
		Biguanides	In vitro	**Metformin**: ↓ uptake and ↑ sensitivity to metformin transport inhibition by medications such as verapamil and amitriptyline (HEK293 cells)[43]	Low/Preliminary
M408V, 1222A>G rs628031	↓ hepatic protein expression; no association with mRNA expression[38,49]	Tyrosine kinase inhibitors	In vivo	**Imatinib**: ↓ treatment response, 5-year survival, and event-free survival in chronic myeloid leukemia patients (n = 167)[48]	Low/Preliminary
		Biguanides	In vivo	**Metformin**: positive predictor of hematological, cytogenic, and major molecular responses in patients with type 2 diabetes (n =33)[49]; variant was not associated with glucose or lipid control in patients with type 2 diabetes (n = 135)[50]	Low/Preliminary
			In vitro	**Metformin**: no change in uptake (HEK293 cells)[43]	
R61C, 181C>T rs12208357	↓ protein expression and membrane localization[40,42]	Biguanides	In vivo	**Metformin**: ↑ AUC and C_{max} in healthy white volunteers[44]; another study found no association between the variant and metformin pharmacokinetics in patients with type 2 diabetes (n = 120), healthy white individuals (n = 16), or healthy Malaysian individuals (n = 169)[46]	Low/Preliminary
			In vitro	**Metformin**: ↓ uptake (HEK293 calls)[42]	
1386C>A rs622342		Biguanides	In vivo	**Metformin**: ↓ efficacy in white (n = 102)[51] and South Indian (n = 122)[117] patients with type 2 diabetes	Moderate
		Dopamine Precursor	In vivo	**Levodopa**: ↑ doses and ↑ mortality in Parkinson patients (n = 99)[118]	Low/Preliminary
F160L, 480C>G rs683369	No association with altered mRNA expression[38]	Tyrosine kinase inhibitors	In vivo	**Imatinib**: ↓ in apparent drug clearance in chronic myeloid leukemia patients carrying at least 1 polymorphic allele (n = 60)[119]	Low/Preliminary

(*Continued*)

Table 2. Continued

Variant	Effect on mRNA/Protein	Drug Class	In Vitro/ In Vivo	Allele Effect/Association	Drug/Effect Association[a]
Select Variants and Variant Effects Within *SLC22A2* (OCT2)					
A270S, 808G>T rs316019	Inconclusive data regarding changes in protein expression; variant may alter substrate recognition and translocation[47,120,121]	Alkylating agents	In vivo	**Cisplatin**: no association between variant and pharmacokinetic parameters in white cancer patients (n = 106)[122]; variant was found to be protective against cisplatin-induced ototoxicity in 64 pediatric patients and 66 adult patients[123]; variant was found to be protective against nephrotoxicity in cancer patients (n = 53)[124]	Low/Preliminary
		Biguanides	In vivo	**Metformin**: ↑ AUC/C_{max} and ↓ renal clearance in healthy Korean individuals (n = 26)[54]; variant associated with ↓ renal clearance in healthy white and African-American individuals (n = 23)[120]	Low/Preliminary
			In vitro	**Metformin**: ↓ uptake (oocytes)[53]	

[a]**High**: annotation for a variant-drug combination where most evidence demonstrates an association. The association must be replicated in more than 1 cohort with significant *P*-values. **Moderate**: annotation for a variant-drug combination with moderate evidence of an association. The association must be replicated in more than 1 cohort, but there may be some studies that do not show statistical significance. **Low/Preliminary**: annotation for a variant-drug combination based on a single significant study or annotation for a variant-drug combination evaluated in multiple cohorts but lacking clear evidence of an association; this classification also encompasses associations that have been studied only in vitro.

individuals carrying a reduced-function *SLC22A1* c.181C>T allele had an increased AUC and higher maximum plasma concentration of metformin compared to those homozygous wild-type individuals;[44] however, patient data did not replicate these findings.[46] The prevalence of these 2 polymorphisms is variable depending on the ethnic group being evaluated. *SLC22A1* p.M420del is more commonly seen in white (18.5%) and Mexican (21.4%) Americans than in African Americans (2.9%), whereas c.181C>T is less prevalent with frequencies of 7.2% and 5.6% for white and Mexican Americans, respectively.[47] Furthermore, the presence of variant *SLC22A1* p.M420del in KCL22-hOCT1 cells was also associated with reduced imatinib uptake, although the effect was obliterated in the presence of *SLC22A1* c.1222A>G (rs628031).[39] This effect translated to findings in imatinib-treated chronic myeloid leukemia (CML) patients, where an increased risk of treatment failure was seen in those carrying the variant allele.[48]

SLC22A1 c.1222A>G (rs628031) is found in 74% of African American, 60% of white American, and 74% to 80% of Asian individuals.[47] Although in vitro studies did not correlate with reduced uptake of metformin in transfected HEK293 cells,[43] a small study in 33 patients with type 2 diabetes on metformin revealed that the variant allele was a positive predictor of metformin efficacy.[49] This finding was not supported by a larger, more recent patient-based study, which showed that the *SLC22A1* variant c.122A>G did not correlate with blood glucose levels or lipid control in 135 diabetic subjects.[50] Additionally, a fourth *SLC22A1* variant, c.1386C>A (rs622342), has been associated with reduced metformin efficacy in white[51] and south Indian diabetic patients.[52]

OCT2. In comparison to *SLC22A1*, few variants within *SLC22A2* have been assessed for drug associations. However, *SLC22A2* c.808G>T (rs316019), which has an overall prevalence of approximately 7% to 16%,[47] has been associated with decreased metformin transport in vitro.[53] Moreover, Song et al determined that the *SLC22A2* c.808G>T variant was associated with increased metformin AUC and decreased renal clearance in healthy Korean individuals.[54] However, this association has yet to be validated in a patient population.

Overall, there is sufficient evidence to suggest that a number of commonly occurring SNPs in *SLC22A1* should be considered early in the drug development process, when an NME is deemed to be a substrate of this transporter. However, evidence does not suggest appreciable drug-variant associations for OCT2.

Overall, our understanding of the impact of genetic variation in the OCTs is still in its infancy, with only preliminary or single-study evidence available in most cases. The exception appears to be the *SLC22A1* variant 1386C>A, which appears to have a moderate association with metformin efficacy.

Efflux Transporter Polymorphisms of Potential Clinical Significance

P-Glycoprotein

P-Glycoprotein (P-gp) is highly studied in drug development because of its widely appreciated capability for mediating the efflux transport of a broad array of structurally divergent endogenous and xenobiotic compounds.[55] P-gp substrates include various antiepileptics, statins, selective estrogen receptor modulators (SERMs), protease inhibitors, and a number of chemotherapeutic agents and immunosuppressants.[56]

The genetic variability of *MDR1* has been extensively studied in vivo and in vitro, and a number of *MDR1* SNPs have been identified (Table 3).[57] Most notably, nonsynonymous SNPs in exons 12 (c.1236C>T), 21 (g.2677G>T), and 26 (c.3435C>T) have been researched extensively with regard to their impact on P-gp substrate pharmacokinetics.[58] Although not shown in vitro, of 15 identified MDR1 SNPs, Groups have linked homozygous variant carriers of c.3435T to reduced P-gp tissue expression and higher drug plasma concentrations.[58,59] Moreover, several groups have shown that c.3435C>T, g.2677G>T, and c.1236C>T are inherited in linkage disequilibrium.[60,61]

Not all substrates of P-gp are impacted by genetic variation in *MDR1*. Hoffmeyer et al showed an increase in digoxin plasma concentrations in individuals homozygous for the *MDR1* c.3435T variant, but this has not been replicated in other studies.[56,58] The effect of the *MDR1* c.3435C>T SNP on the efficacy of antiepileptics such as carbamazepine has been extensively reviewed. Although 1 systematic review of 4269 Chinese adult patients revealed an association between the CC genotype and anti-epileptic resistance, similar reviews in larger and more diverse populations have failed to show any association between *MDR1* c.3435C>T and antiepileptic drug response.[62–64]

Limited data exist regarding the effect of *MDR1* polymorphisms on statin pharmacokinetics and patient drug response. Homozygous variant carriers of *MDR1* c.3435C>T and g.2677G>T have both been linked to increased statin exposure and reduced statin effect. Both the AUC and maximum concentrations of pivastatin and rosuvastatin were increased in healthy Chinese volunteers with the *MDR1* g.2677TT and c.3435TT genotypes, respectively.[65,66] Similarly, a small study in Bosnian volunteers found a smaller statin effect in individuals homozygous for the c.3435T variant.[67]

The P2y12 inhibitor clopidogrel has been shown to be a substrate of P-gp in vitro, and its oral absorption is affected by the inhibition or upregulation of P-gp.[68] The *MDR1* c.3435C>T SNP has been shown to modulate the pharmacokinetics and efficacy in multiple patient cohorts; however, the findings were contradictory to the posited physiologic effect of the variant. In a group of 60 white acute coronary syndrome (ACS) patients, individuals with the TT genotype had a lower clopidogrel AUC and maximal concentration.[68] This was duplicated in a group of 401 Chinese ACS patients, where the c.3435T variant was associated with lower plasma concentrations of both clopidogrel and its active metabolite and resulted in reduced platelet inhibition.[69]

All 3 exonic SNPs, *MDR1* c.3435C>T, c.1236C>T, and g.2677G>T, have been linked to changes in protease inhibitor pharmacokinetics and effect, although the evidence is not robust. In vitro, HEK293T cells carrying the g.2677T variant did not transport telaprevir, and higher telaprevir trough plasma concentrations were seen among 29 hepatitis C patients carrying a variant allele.[70] A Brazilian study of 187 HIV patients, among whom 27 were protease inhibitor nonresponders, were assessed for their *MDR1* c.3435C>T genotype. Conversely, individuals carrying the c.3435T variant were more likely to fail first-line HIV treatment regimens compared to those carrying a wild-type allele.[71]

Moreover, the immunosuppressants and chemotherapeutics cyclosporine, methotrexate, and imantinib have been shown to be affected by genetic variation in *MDR1*. Renal transplant patients homozygous for the *MDR1* c.3435T or the g.2677T variant had higher cyclosporine trough plasma concentrations compared to individuals carrying a wild-type allele.[72] In a small Korean population of post–stem-cell-transplant patients, individuals carrying 2 variant *MDR1* c.3435T alleles had lower methotrexate clearance.[73] Similarly, a small Spanish cohort showed that the *MDR1* c.34335CC genotype was associated with primary failure of imantinib treatment in CML.[74] A larger Chinese meta-analysis contradictorily found that the *MDR1* c.1236T variant was a possible risk factor for nonoptimal clinical response to imantinib treatment in Asian patients with CML.[75]

Overall, although the c.3435T variant appears to be linked to digoxin and chemotherapeutic pharmacokinetics, the evidence for other *MDR1* variant-drug associations needs more study in larger cohorts.

Table 3. P-Glycoprotein

Variant	Effect on mRNA/Protein	Drug Class	In Vitro/In Vivo	Allele Effect/Association	Drug/Effect Association[a]
Select Variants and Variant Effects Within *MDR1* (P-gp)					
3435C>T rs1045642	↓ duodenal protein expression[58]	Glycosides	In vivo	**Digoxin**: ↑ AUC in healthy white volunteers (n = 21)[58]	Moderate
		Antiepileptics	In vivo	**Multiple AEDs**: CC genotype associated with drug resistance in a Chinese population (n = 4269)[63]	Low/Preliminary
		HMG-CoA reductase inhibitors	In vivo	**Simvastain and Atorvastatin**: ↓ benefit of statin therapy in dyslipidemic patients (n = 180)[67]	Low/Preliminary
		P2y12 inhibitor	In vivo	**Clopiogrel**: ↓ AUC in patients with acute coronary syndrome (n = 401)[69]	Low/Preliminary
		Glucocorticoid	In vivo	**Prednisone**: variant associated with ↑ risk for steroid-induced osteonecrosis of the femoral head in Chinese population (n = 200)[125]	Low/Preliminary
		Protease inhibitors	In vivo	**Multiple Protease Inhibitors:** variant associated with ↑ failure of first-line regimes in Brazilian HIV-infected patients (n = 187)[71]	Moderate
		Antimetabolites	In vivo	**Methotrexate**: ↓ clearance in Korean patients after hematopoietic stem cell transplantation (n = 20)[73]	Moderate
		Tyrosine kinase inhibitors	In vivo	**Imatinib**: CC genotype associated with primary failure in chronic myeloid leukemia patients (n = 65)[74]	Low/Preliminary
S893A/T, 2677G>T/A rs2032582		Protease inhibitors	In vivo	**Telprevir**: ↑ trough concentrations in hepatitis C patients (n = 29)[70]	Low/Preliminary
		Immune suppressant	In vivo	**Cyclosporine**: ↑ trough concentrations in TT vs GG/GT renal transplant recipients (n = 101)[72]	Low/Preliminary
		Behavior modifier	In vivo	**OROS-methylphenidate**: TT genotype found to be an independent determinant of adverse drug reactions in children with attention-deficit hyperactivity disorder (n = 134)[126]	Low/Preliminary
		HMG-CoA reductase inhibitors	In vivo	**Pivastatin**: ↑ AUC and C_{max} in non-G carriers (n = 12)[66]	Low/Preliminary
1236C>T rs1128503		Protease inhibitors	In vivo	**Telprevir**: ↑ trough concentrations in hepatitis C patients (n = 29)[70]	Low/Preliminary
		Tyrosine kinase inhibitors	In vivo	**Imatinib**: T variant associated with nonoptimal clinical response in Asian chronic myeloid leukemia patients (meta-analysis)[75]	Moderate

[a]**High**: annotation for a variant-drug combination where most evidence demonstrates an association. The association must be replicated in more than 1 cohort with significant *P*-values. **Moderate**: annotation for a variant-drug combination with moderate evidence of an association. The association must be replicated in more than 1 cohort, but there may be some studies that do not show statistical significance. **Low/Preliminary**: annotation for a variant-drug combination based on a single significant study or annotation for a variant-drug combination evaluated in multiple cohorts but lacking clear evidence of an association. This classification also encompasses associations that have been studied only in vitro.

The Multidrug Resistance Proteins MRP2, MRP3, and MRP4

Common genetic variants in *ABCC2* (MRP2), *ABCC3* (MRP3), and *ABCC4* (MRP4) have been linked to changes in mRNA and/or protein expression as well to changes in the disposition of many drugs in vivo including statins and antimetabolites such as methotrexate and azathiopine/6-mp (Table 4). However, genetic variation within *ABCC2* is most widely studied. This may be due, in part, to the propensity of *ABCC2* to tolerate major loss of function with genetic variation. Indeed, complete loss of MRP2 function is the molecular basis for Dubin-Johnson syndrome.[76]

Interestingly, *ABCC2* c.1249G>A (rs2273697) is associated with reduced mRNA expression in human placenta but increased mRNA expression in human liver.[77,78] *ABCC2* c.1249G>A is found in 9% of Asian, 22% of sub-Saharan African, and 24% of white individuals.[79] Conversely, *ABCC2* variant, rs717620 is associated with reduced *ABCC2* mRNA in healthy kidney but not in liver or intestine, and variant rs4793665 is associated with reduced *ABCC3* mRNA expression in liver tissue samples.[77,80] The frequency of these variants is 20% in Asian, white, and African populations for the former and 56% to 86% across these groups for the latter.[81,82]

Genetic variations in *ABCC2* and *ABCC4* have been linked to alterations in methotrexate pharmacokinetics and efficacy. Specifically, *ABCC2* variants c.1249G>A (rs2273697) and c.3972C>T (rs3740066) were associated with an increased risk of gastrointestinal toxicity such as nausea and vomiting as well as hepatotoxicity in a cohort of 65 children suffering from acute lymphoblastic leukemia (ALL).[83] Similarly, in a cohort of 112 Chinese children with ALL, the *ABCC2* variant c.–24C>T (rs717620) was associated with increased plasma concentrations as well as an increased risk of clinical adverse drug reactions.[84] The *ABCC4* variant c.1372A>C (rs9516519) is also associated with elevated methotrexate plasma concentrations as well as drug toxicity in children suffering from ALL.[85]

Taken together, SNPs in *ABCC2* and possibly *ABCC4* should be considered as potentially relevant during the drug development process if these transporters are considered to be essential or rate limiting to the elimination of a NME in development.

Breast Cancer Resistance Protein

This efflux transporter, encoded by *ABCG2* gene, is an ATP-binding cassette efflux half transporter that likely functions as a homodimer.[4] Similar to P-glycoprotein, breast cancer resistance protein (BCRP) appears to serve as a barrier to limit the absorption, entry, or retention of diverse substrates including both endogenous (eg, dietary flavonoids, porphyrins, estrone 3-sulfate) and exogenous (eg, statins, antineoplastics) molecules into various tissue compartments.[86]

Pharmacogenetic studies have identified a number of common SNPs in *ABCG2* that are associated with changes in the PK parameters for medications (Table 5). Among the SNPs identified to date, a nonsynonymous SNP c.421C>A (rs22331142) in exon 5 of BCRP, which results in a glutamine-to-lysine change at amino acid position 141, is the most extensively studied.[87] *ABCG2* c.421C>A has an allele frequency of approximately 10% with higher frequencies in Asian individuals and lower frequencies in sub-Sahra African and African-American individuals.[88,89] In vitro work suggests that the c.421C>A variant impairs protein expression by 30% to 40% but maintains mRNA expression when compared with wild-type controls.[90] Reduced BCRP expression is possibly due to enhanced susceptibility to ubiquitin-mediated proteasomal degradation and incomplete trafficking to the plasma membrane.[91,92]

Carriers of the c.421C>A polymorphism have demonstrated increased bioavailability of marketed BCRP substrates. For example, the AUC for atorvastatin and fluvastatin were 72% greater, for simvastatin lactone 111% greater, and for rosuvastatin 144% greater in *ABCG2* c.421A/A individuals than in c.421C/C individuals.[93,94] Furthermore, the *ABCG2* c.421C>A variant has been found to correlate in a gene-dose–dependent manner with reductions in LDL cholesterol in individuals taking rosuvastatin to manage their hypercholesterolemia.[95,96] The c.421C>A polymorphism also appears to impact the disposition and efficacy of antineoplastic medications. For example, Sparreboom et al determined that heterozygous cancer patients receiving a 20-minute infusion of diflomotecan had significantly higher plasma concentrations of the medication when compared to homozygous wild-type individuals.[97] Similar effects were also seen in vitro for another topoisomerase inhibitor, topotecan, where expression of the variant was associated with 30% reduction in efflux transport of topotecan in HEK293 cells when compared to wild type.[98] Similarly, *ABCG2* c.421C>A has also been associated with increased accumulation of imatinib, a tyrosine kinase inhibitor, in HEK293 cells.[99] More recently, Koo et al investigated the 5-year PFS rate in 209 gastrointestinal stromal tumor patients being treated with imatinib and found that PFS was vastly superior in AA-carrying individuals when compared with patients carrying the CC or CA genotypes.[100] Interestingly, another recent study suggested that *ABCG2* c.421C>A polymorphism may affect substrate distribution to the brain, as the mean cerebrospinal fluid concentration of raltegravir, an anti-HIV medication, was significantly lower among AA and CA patients when compared with those individuals with the CC genotype.[101]

Table 4. Multidrug-Resistant Proteins

Variant	Effect on mRNA/Protein	Drug Class	In Vitro/ In Vivo	Allele Effect/Association	Drug/Effect Association[a]
Select variants and variant effects within *ABCC2* (MRP2)					
V417I, 1249G>A rs2273697	Variant associated with reduced mRNA expression in human preterm placenta[78] and associated with increased mRNA expression in human hepatic tissue[77]	Antimetabolites	In vivo	**Methotrexate**: variant associated with ↑ GI toxicity in children with acute lymphoblastic leukemia (n = 65)[83]	Low/Preliminary
		Antiepileptics	In vivo	**Multiple antiepileptics**: meta-analysis indicated that the variant genotypes (in Asian or white populations) were associated with a significantly ↓ risk of antiepileptic resistance[127]; no association between the variant and antiepileptic response was found in Croatian epilepsy patients (n = 97)[128]	Low/Preliminary
–24C>T rs717620	Variant associated with lower ABCC2 mRNA levels in the normal human kidney[129] but not in the intestine[130] or liver[77]	Antimetabolites	In vivo	**Methotrexate**: ↑ plasma concentrations ↑ risk of hematologic and nonhematologic adverse events in Chinese children with acute lymphoblastic leukemia (n = 112)[84]	Low/Preliminary
		Statins	In vivo	**Simvastatin and atorvastatin**: variant associated with a dose ↓ or switches to other cholesterol-lowering drugs in simvastatin, but not atorvastatin, users (n = 1014)[131]	Low/Preliminary
1132A1, 3972C>T rs3740066		Antimetabolites	In vivo	**Methotrexate**: variant associated with ↑ hepatotoxicity in children with acute lymphoblastic leukemia (n = 65)[83]	Low/Preliminary
Select Variants and Variant Effects Within *ABCC3* (MRP3)					
–211C>T rs4793665	Lower mRNA expression in liver samples[81,132]	Opiates	In vivo	**Morphine**: associated with ↓ levels of morphine-6-glucoronide and morphine-3-glucoronide in children undergoing outpatient adenotonsillectomy (n = 220)[133]	Low/Preliminary
Select Variants and Variant Effects Within *ABCC4* (MRP4)					
E857K, 2269G>A rs3765534	Impaired membrane localization[134]	Immune suppressants/ antimetabolites	In vivo	**Azathioprine/6-MP**: ↑ hematopoitic toxicity in IBD patients (n = 235)[135]	Low/Preliminary
3225+1243C>A rs9561778		Alkylating agents	In vivo	**Cyclophosphamide**: ↑ adverse drug reactions (ie, gastrointestinal toxicity and leukopenia/neutropenia) in 403 breast cancer patients[136]	Low/Preliminary

(*Continued*)

Table 4. Continued

Variant	Effect on mRNA/Protein	Drug Class	In Vitro/ In Vivo	Allele Effect/Association	Drug/Effect Association[a]
1372A>C rs9516519		Antimetabolites	In vivo	**Methotrexate**: variant associated with ↑ methotrexate levels and toxicity in children with acute lymphoblastic leukemia (n = 151)[85]	Low/Preliminary

[a]**High**: annotation for a variant-drug combination where most evidence demonstrates an association. The association must be replicated in more than 1 cohort with significant *P*-values. **Moderate**: annotation for a variant-drug combination with moderate evidence of an association. The association must be replicated in more than 1 cohort, but there may be some studies that do not show statistical significance. **Low/Preliminary**: annotation for a variant-drug combination based on a single significant study or annotation for a variant-drug combination evaluated in multiple cohorts but lacking clear evidence of an association. This classification also encompasses associations that have been studied only in vitro.

Of particular importance to drug development is the recent association of Jr(a–) blood group with null-allelic mutations in *ABCG2*.[102,103] In essence, those who have the Jr(a–) could be considered *ABCG2* knockout humans. The Jr(a–) phenotype is mainly detected in Japanese individuals with region-dependent frequencies between 0.3% and 1.7%.[103] In the Jr(a–) population, the production of anti-Jr(a) antibodies is known to be involved in hemolytic disease, but little is known about the effect the nonfunctional BCRP protein has on drug disposition and efficacy. The Jr(a–) population may aid in establishing the precise role of *ABCG2* in the pharmacokinetics of a NME.

Therefore, given the presence of common as well as rare functional genetic variations in BCRP in the human population, and the presence of moderate to strong variant-drug associations, SNPs in *ABCG2* should most certainly be considered as a part of the overall drug development process for a substrate NME.

Drug Discovery and Development Strategies

There have been a number of excellent publications in the past 5 years, in terms of in vitro as well as in vivo guidance for transporter-related studies during drug discovery and development.[2–10] Shown below is a very brief 6-step summary of what should be viewed as our opinion, with regard to drug transporters and clinically relevant polymorphisms.

Step 1: Figure Out Extent of Metabolism vs Transport

In vitro or liver microsome studies should quickly demonstrate the likely extent of metabolism. If the NME is shown to be metabolically stable, anionic, cationic, zwitterionic, or amphipathic, with a molecular weight between 300 and 1000 daltons, transporters are likely to be relevant in vivo.

Step 2: Transporter(s) to Screen

It is not possible to screen all the transporters. The decision with regard to which subset of transporter to screen should be based on therapeutic target and likely organ(s) involved in the elimination of the NME. For CNS-active compounds, P-gp and BCRP should be considered. For those cleared by liver or targeting liver, consider OATP1B1, P-gp, or BCRP. If the NME is cleared by the kidney, particularly if the renal clearance appears to exceed GFR, consider OAT1/3 for anionic NMEs and OCT2 and MATE2-K for cationic NMEs in addition to P-gp.

Step 3: OATP1B1, P-gp, and BCRP

These are the top 3 transporters to keep in mind if a broader screening effort for various NMEs is to be carried out earlier in the discovery process. These transporters have shown the most clinical relevance to date. Additional transporters might be considered later in discovery or during development.

Step 4: Determine Transporter Inhibition–Associated DDI Risk

There is now significant guidance with regard to in vitro transporter studies and prediction of in vivo relevance. In vitro inhibition data could be used as a surrogate for loss of function polymorphism(s) in such transporters.

Step 5: If Worried About OATP1B1, P-gp, or BCRP Polymorphisms

A number of functional SNPs in these transporters result in loss of cell surface trafficking of the variant transporter. Thus, instead of worrying about whether there may be polymorphism-dependent differences in substrate specificity, it may be easier to skip in vitro analysis of variant transporters and directly carry out in vivo assessment in relevant rodent knockout models.

Table 5. Breast Cancer Resistance Protein

Variant	Effect on mRNA/Protein	Drug Class	In Vitro/In Vivo	Allele Effect/Association	Drug/Effect Association[a]
Select Variants and Variant Effects Within *ABCG2* (BCRP)					
Q141K, 421C>A rs2231142	↓protein expression and trafficking to plasma membrane[90,91]	HMG-CoA reductase inhibitors	In vivo	**Rosuvastatin**: ↑ AUC in healthy Chinese (n = 7)[137] and Finnish (n = 32)[94] volunteers; superior LDL-C lowering in Chinese patients with hypercholesterolemia (n = 305)[96] and white patients who had recently suffered a myocardial infarction (n = 601)[95]	High
			In vivo	**Atorvastatin**: ↑ AUC in healthy Finnish (n = 32)[94] volunteers	Moderate
		Topoisomerase inhibitors	In vivo	**Diflomotecan**: ↑ AUC and C_{max} in white cancer patients receiving 20-minute infusion (n = 22)[97]	Low/Preliminary
			In vitro	**Topotecan**: ↓ efflux transport (HEK293 cells)[98]	Low/Preliminary
		Tyrosine kinase inhibitors	In vivo	**Imatinib**: 5-year PFS rate in gastrointestinal stromal tumor patients with the AA variant significantly superior to that of patients with CC/CA genotypes (n = 209)[100]	Low/Preliminary
			In vitro	**Imatinib**: ↑ drug accumulation (HEK293 cells)[99]	
		Anivirals	In vivo	**Raltegravir**: ↓ CSF concentrations in HIV-infected patients (n = 14)[101]	Low/Preliminary
V12M, 34G>A rs2231137	Possible ↓ localization to apical plasma membrane[138]	Tyrosine kinase inhibitors	In vivo	**Gefitinib, Erlotinib, Icotinib**: ↑ median overall survival of Chinese advanced non-small-cell lung cancer patients (n = 100)[139] **Imatinib**: homozygous wild-type carriers showed impaired cytogenetic response when compared to variant carriers (n = 229)[140]	Low/Preliminary
–15994C>T rs7699188	↑ multitissue protein expression[141]	Tyrosine kinase inhibitors	In vivo	**Imatinib**: ↑ oral clearance in gastrointestinal stromal tumor patients (n = 82)[141]	Low/Preliminary
18271G>A rs1564481	No association with mRNA expression[38]	CDK9 kinase inhibitor	In vivo	**Flavopiridol**: ↑ AUC and improved response to treatment (n = 51)[142]	Low/Preliminary

[a]**High**: annotation for a variant-drug combination where most evidence demonstrates an association. The association must be replicated in more than 1 cohort with significant *P*-values. **Moderate**: annotation for a variant-drug combination with moderate evidence of an association. The association must be replicated in more than 1 cohort, but there may be some studies that do not show statistical significance. **Low/Preliminary**: annotation for a variant-drug combination based on a single significant study or annotation for a variant-drug combination evaluated in multiple cohorts but lacking clear evidence of an association. This classification also encompasses associations that have been studied only in vitro.

Step 6: In Phase 1 and 2 Studies, Collect DNA and Assess Impact on PK Relative to Transporter Genotype(s)

For NMEs that are subject to transport, having genotype-vs-PK data as a part of the overall development process will provide meaningful data in terms of likely clinical impact. The key will be to include DNA collection and genotyping strategies as a part of the development process. Note that effect of

genotype and DDI may provide additional insights relating to the overall clinical impact because many drugs are substrates of multiple transporters, and a number of clinical DDI studies focus specifically on key efflux transporters such as P-gp.

Conclusion

Predicting all the factors that can account for interpatient variation in drug response is an important goal of the drug development process. It is now becoming clear that genetic variations within drug transporters are clinically relevant. Specifically, in 2014, international statin-dosing guidelines were changed to reflect the impact of OATP1B1 variants on statin PK profile and toxicity. Similar to CYP enzymes, it appears that a handful of key drug uptake and efflux transporters account for the absorption, distribution, and elimination of the majority of currently prescribed drugs as well as NMEs. Systematic inclusion of transporter studies, including the consideration of polymorphisms in relevant transporters, will result in better candidate NME selection as well as a more robust prediction of intersubject variation in the response to the NME when it is released for general use.

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