

New and Emerging Research on Solute Carrier and ATP Binding Cassette Transporters in Drug Discovery and Development: Outlook From the International Transporter Consortium

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Enabled by a plethora of new technologies, research in membrane transporters has exploded in the past decade. The goal of this state-of-the-art article is to describe recent advances in research on membrane transporters that are particularly relevant to drug discovery and development. This review covers advances in basic, translational, and clinical research that has led to an increased understanding of membrane transporters at all levels. At the basic level, we describe the available crystal structures of membrane transporters in both the solute carrier (SLC) and ATP binding cassette superfamilies, which has been enabled by the development of cryogenic electron microscopy methods. Next, we describe new research on lysosomal and mitochondrial transporters as well as recently orphaned transporters in the SLC superfamily. The translational section includes a summary of proteomic research, which has led to a quantitative understanding of transporter levels in various cell types and tissues and new methods to modulate transporter function, such as allosteric modulators and targeted protein degraders of transporters. The section ends with a review of the effect of the gut microbiome on modulation of transporter function followed by a presentation of 3D cell cultures, which may enable *in vivo* predictions of transporter function. In the clinical section, we describe new genomic and pharmacogenomic research, highlighting important polymorphisms in transporters that are clinically relevant to many drugs. Finally, we describe new clinical tools, which are becoming increasingly available to enable precision medicine, with the application of tissue-derived small extracellular vesicles and real-world biomarkers.

For over a decade the International Transporter Consortium (ITC) has co-authored important review and position papers that are relevant to transporters in drug development, focusing largely on drug–drug interactions (DDIs), but also including other topics relevant to the role of transporters in drug absorption, distribution, metabolism, and excretion (ADME) as well as response and toxicity.^{1,2} In this paper, we extend the traditional purview of the ITC and for the first time focus on breakthrough research in membrane transporters with the potential to impact current and future drug development. As with all ITC papers, we concentrate on two major superfamilies of transporters: the solute carrier (SLC) superfamily and ATP-binding cassette (ABC) superfamily and, particularly, focus on transporters with known implications to pharmacokinetics and pharmacodynamics. This paper reviews the state-of-the-art in emerging technologies that are enabling transporter research, the paper is organized into three major

sections ranging from basic transporter research through translational and then clinical transporter research.

A range of breakthrough topics on transporters are co-authored by the ITC and published in this issue of *Clinical Pharmacology and Therapeutics*, therefore a few topics (emerging transporters of clinical importance,³ transcription and post-transcriptional regulation,⁴ special populations, transporter-related drug toxicities, and regulatory science perspectives) were deliberately excluded from this state-of-the-art paper. **Figure 1** depicts the topics that are discussed under each of the three major sections. Computational tools in support of research described in each of the sections are mentioned. We hope that the overview of these breakthrough topics along with detailed tables, figures, and supplementary information included in this paper will provide a rich resource for transporter biologists and pharmacologists interested in the future of transporter research in drug development.

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BASIC TRANSPORTER RESEARCH

Enabling technologies for transporter research

In recent years, the toolbox for basic as well as translational and clinical transporter research has dramatically expanded. In this section, we highlight the major new methodologies that have enabled transporter research ranging from methods to study transport function in cell or organ based systems, through the development of new small molecule modulators of transporter function, to new imaging modalities. Major advances have been made in the study of transporter function *in vitro* using novel technologies. For example, genome editing using clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated protein 9 (Cas9), CRISPR-Cas9, and similar techniques have been used to develop cell models with reduced impact from confounding transport pathways, facilitating the delineation of the mechanisms involved in drug disposition.⁵ These methods have been supplemented with a widespread adoption of increasingly advanced culture systems in order to better mimic physiologic contexts and improve long-term viability to allow experiments on scales of weeks or months—important, for example, in toxicologic assessments. Technological innovations in this area of transporter research include various 3D culture setups, such as spheroid and organoid cultures, and microfluidic devices that allow controlled circulation of drugs, nutrients and metabolites, and interconnected microtissues that mimic inter-organ communication

in vivo. Some of these technological innovations are described later in this paper. Collectively, this expanded toolbox of *in vitro* models is being increasingly used in drug ADME and toxicity studies and holds the promise to enable further knowledge and advancement in understanding the roles of transporters in drug ADME, response, and toxicity.

In addition to the availability of new *in vitro* models to study transporters, new modalities for modulation of transport function—importantly, targeted protein degraders (“PROTACs”), which will be described later, and therapeutic oligonucleotides—have been developed and have led to a new understanding of additional roles of transporters beyond simple transport functions.^{6,7} Whereas initial applications of these emerging modalities to transporters have been as tools to clarify transporter function, therapeutic applications with membrane transporters as drug targets will likely follow in the near future.

Finally, important advances in transporter research have come from an increased use of imaging technologies to clarify the dynamics of transporter-mediated drug distribution at subcellular scales *in vitro* (e.g., using fluorescence-based and Raman imaging of cultured cells) and at suborgan scales *in vivo* (e.g., using intravital fluorescence imaging, or positron or single-photon emission tomography techniques; **Table 1**).^{8–14} In addition, imaging at molecular scales have dramatically increased coverage of transporter structure space—notably driven by the advances in cryogenic

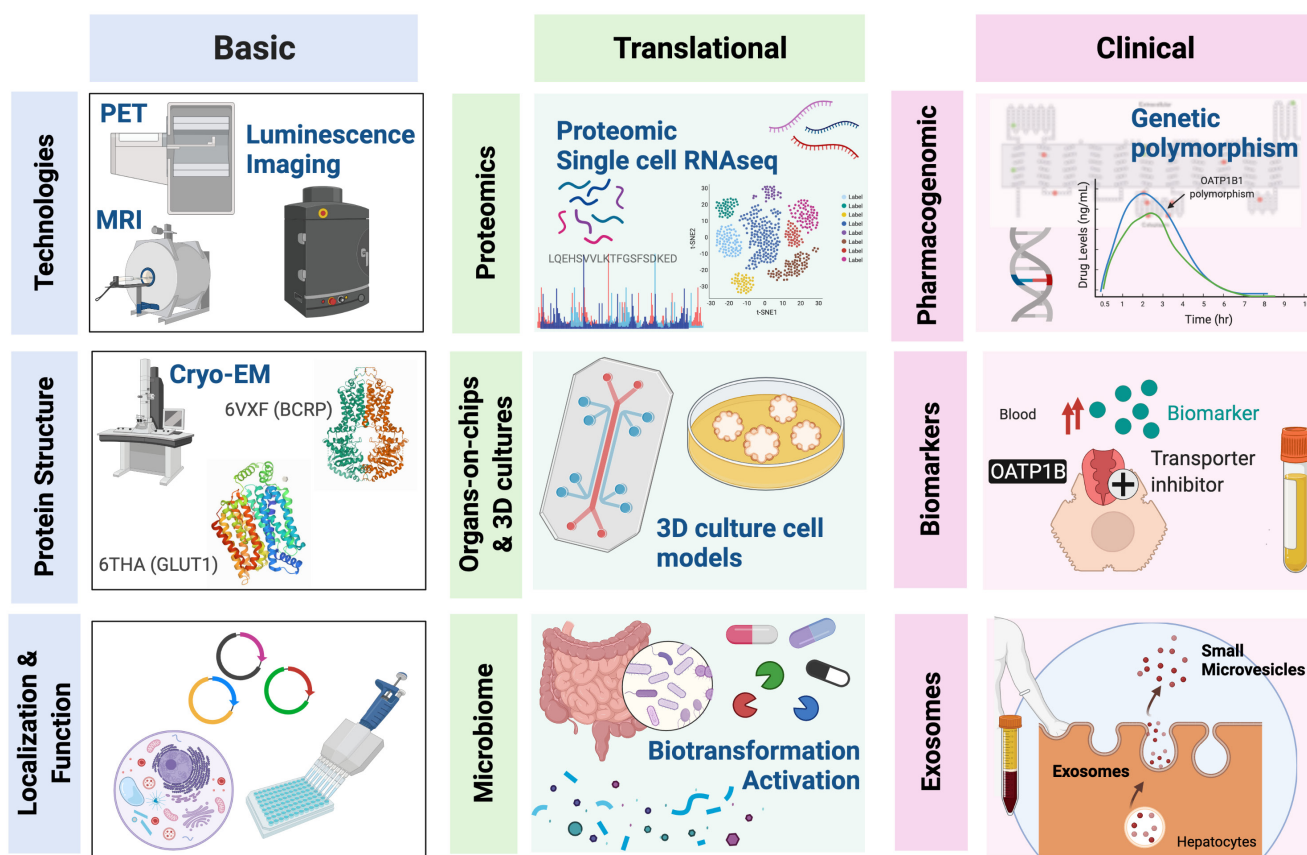


Figure 1 Various new and emerging methodologies and technologies that are used to conduct basic, translational and clinical research on SLC and ABC transporters and are described in this state of the art paper. ABC, ATP-binding cassette superfamily; cryo-EM, cryogenic electron microscopy; MRI, magnetic resonance imaging; PET, positron emission tomography; SLC, solute carrier.

Table 1 Recent examples of *in vivo* imaging modalities enabling transporter research^a

| Technique | Species | Imaging scale | Examples of transporter applications | Reference |
|-----------------------|-------------------------|-----------------|--|-----------|
| Intravital microscopy | Mouse (human xenograft) | Single-cell | Single-cell pharmacology of MDR1 inhibitors in mouse xenografts | 8 |
| | Mouse | Single-cell | BSEP function in hepatocytes of live mice | 9 |
| | Mouse | Single-cell | Wnt-dependent hepatobiliary function in mouse model of cholestatic liver disease | 122 |
| PET | Human | Sub-organ | 11 C-Csar bile acid PET in patients with cholestasis | 10 |
| | Human, mouse | Sub-organ | ABCB1/ABCG2 substrate [¹¹ C]tariquidar in healthy volunteers and wild-type, <i>Abcb1a/b</i> ^(-/-) , <i>Abcg2</i> ^(-/-) , and <i>Abcb1a/b</i> ^(-/-) <i>Abcg2</i> ^(-/-) mice | 11 |
| SPECT | Human | Organ/sub-organ | ^{99m} Tc-mebrofenin imaging in patients with NASH, compared with SLCO and MRP2 polymorphisms | 12 |
| Bioluminescence | Mouse | Organ/sub-organ | ABCG2-mediated BBB efflux using D-luciferin in mice | 123 |
| MRI | Mouse | Sub-organ | Oatp—mediated hepatobiliary transport and dysfunction in diabetes using DCE-MRI of gadoxetate disodium in mice | 13 |
| Photoacoustic imaging | Mouse (human xenograft) | Sub-organ | OATP1B3-mediated uptake of indocyanine green for photoacoustic imaging contrast in mice | 14 |

BBB, blood brain barrier; BSEP, bile salt export pump; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; MRI, magnetic resonance imaging; NASH, nonalcoholic steatohepatitis; PET, positron emission tomography; SPECT, single photon emission computed tomography.

^aFurther examples of imaging modalities applied in transporter research were listed in ref.124

electron microscopy (cryo-EM) imaging described below. The integration of time-resolved imaging data into pharmacokinetic models is yielding new insights into drug distribution mechanisms and the impact of transporter-mediated DDIs, and through the continuous technological advances in super-resolution imaging, we can expect new light shed on unresolved questions in transporter research, including the impact of transporters on subcellular drug distribution and efficacy. The use of many of the technologies described here will be described in later sections.

New structures and protein dynamics

The availability of structures for membrane transporters has lagged behind many other classes of proteins in large part because of technical difficulties in isolating, purifying, and crystallizing membrane proteins to high resolution. However, with improvements in protein purification and crystallization methods and the increasing availability of cryo-EM methods, many new structures of transporters are available. Below, we highlight new structures in the two major superfamilies, SLC and ABC.

Structural advancements for SLC transporters. Despite comprising one of the largest gene families with over 450 members categorized

into 65 subfamilies by homology and function, SLC transporters are understudied¹⁵ and three dimensional structures are under-represented in the Protein Data Bank.¹⁶ Protein structure determination has many uses in drug discovery and development: high-resolution structures can provide templates for computational chemistry and docking of potential ligands to drug targets, inform structure-activity relationships in medicinal chemistry, reveal allosteric sites for target modulation, or provide insight into molecular mechanisms of substrate translocation and inhibitory interactions (see review in ref. 17). Until recently, crystallography was the most utilized method for structure determination; however, multiple challenges act as a barrier to the crystallization of membrane proteins, including SLC transporters—for one, few transporters have high-fidelity antibodies.¹⁶ As recently as 2018, only nine mammalian SLC structures had been solved, including five human structures (SLC2A2 (GLUT1), SLC2A3 (GLUT3), SLC4A1 (Band 3), SLC6A4 (SERT), and RhCG (SLC42A3)), two from the cow (SLC14A1 (UT-B) and SLC25A4 (ANT1)), one from both the cow and rat (SLC2A5 (GLUT5)), and one from the mouse (LeuT).¹⁸ Since the last review of published human SLC transporter structures in 2018,¹⁸ structures for 25 new human SLC transporters have been published to our knowledge (Table S1).

The most recently published structures are highlighted in [Table 2](#). Transport mechanisms, which include rocker switch, alternative access, and elevator models, have been reviewed recently.¹⁶ Interestingly, most structures are not monomers, instead solved as homodimers, homotrimers, heterodimers, or heterotetramers, possibly reflecting a crystallization bias for SLC transporters. Cryo-EM has been emerging as the most common technique for solving SLC structures. Structures have been determined with and without ligands and with the inward-facing (i.e., pore open toward the cytosol) conformation being the most prevalent. It has been noted that some apo structures prefer the inward-facing conformation, as in the case of EAAT3 (SLC1A1).¹⁹ A variety of protein folds are observed, including two of the most common structural categories: the LeuT fold named for the bacterial homolog of the sodium-dependent leucine transporter with two inverted structural repeats of 5 transmembrane domains each for a total of 10 transmembrane domains, and the major facilitator superfamily (MFS) fold whereby 12 transmembrane domains can be classified into either N- or C-terminal domains with pseudo-symmetry between the two terminal domains.

Although structures for most human SLC transporters have yet to be solved, non-human structures exist for eukaryotic homologues of some clinically important transporters. Of note, a number of eukaryotic plant multidrug and toxin exclusion (MATE) structures were solved in recent years with ~32% protein sequence identity to the human MATE transporter (MATE1, SLC47A1),²⁰ which as an efflux transporter has important roles in drug disposition and excretion. Additionally, the chicken structure of the proton-coupled folate transporter (SLC46A1) was solved with 54% protein sequence identity to the human proton-coupled folate transporter, relevant in the transport of anti-folate drugs like methotrexate and pemetrexed.²¹

Despite the uptick of human SLC structures solved in recent years, experimental techniques used to solve protein structures remain time and cost intensive. Artificial intelligence has the potential to bridge the gap between proteins with solved structures and those that are yet to be solved. Recent advances have enabled highly accurate 3D structural prediction of proteins, such as with DeepMind's AlphaFold algorithm,²² which uses deep neural networks to predict the structure of a protein based on the primary amino acid sequence and has been applied to predict the structure of SLC transporters. For example, Killer *et al.* determined the structure of human PepT2 by cryo-EM ([Table 2](#)) and compared it to the structure predicted by AlphaFold2. Interestingly, the AlphaFold2 structure for PepT2 in the inward-facing open apo state was similar to the cryo-EM determined PepT2 structure in the inward-facing partially occluded substrate bound state. The authors used the AlphaFold2 structure's apo state to complement their understanding of the molecular shifts necessary for cytoplasmic release of the substrate and in the refinement of a large extracellular domain of PepT2 for which cryo-EM had poor resolution. With several transporters bearing clinical relevance in drug disposition and over 100 SLC transporters implicated in disease, artificial intelligence -predicted structures have the potential to enable structure-based drug discovery with molecular docking and other techniques and revolutionize novel therapies for rare diseases. Low

resolution cryo-EM structures have recently been combined with modeling to guide drug discovery.²³

Structural advancements for ABC transporters. For ABC transporters, cryo-EM has provided a powerful tool for solving multiple structures at the atomic level (reviewed in refs. [24, 25](#)). In this section, we summarize the structures of the two most studied transporters, P-gp (ABCB1) and BCRP (ABCG2), which play critical roles in drug efflux. Their structures have been captured in the apo state and in nucleotide-, substrate-, and inhibitor-bound states, revealing important features of the catalytic transport cycle and determinants of substrate and inhibitor recognition. Vincristine- and paclitaxel-bound structures of P-gp revealed that substrate binding induces an occluded conformation accompanied by closure of the nucleotide binding domains.^{26,27} The binding cavity is globular in shape and allows substrate binding in multiple orientations. P-gp structures which are elacridar-, tariquidar-, and zosuquidar-bound revealed multiple binding sites for these potent inhibitors, with two inhibitor molecules bound in each structure.²⁶ Elacridar and tariquidar both adopt a globular conformation that binds within the substrate binding cavity and a second L-shaped conformation that binds at the intersection between the central cavity and a cytoplasmic gate in an "access tunnel" which allows access of solutes in the cytoplasm to the central cavity. This suggests that binding of a second inhibitor molecule outside the central cavity engages additional regions of the transporter in a noncompetitive fashion, accounting for their observed potent inhibition. An ATP-bound structure of P-gp captured an outward facing conformation, consistent with ATP binding, and not hydrolysis, being the determining step for substrate release on the extracellular side.²⁸ Structures of BCRP have revealed some key differences between ABC transporters with pseudo-symmetric halves encoded by a single gene and those that must homodimerize to generate a functional protein. The substrate binding cavity is more slit-like and lies on the two-fold symmetry axis of the BCRP dimer, with a leucine plug at the top of the binding cavity formed by L554 of opposing monomers.^{29,30} Substrates lie between opposing phenyl rings of F439, consistent with its preference for flat polycyclic structures. Upon ATP binding, the leucine plug is opened to allow substrate release.^{29,30} Two molecules of more potent inhibitors, like MZ29, and a single molecule of a larger inhibitor, like MB136, can fill the complete binding cavity, locking the transporter in an inward-facing conformation that prevents substrate access.³¹ BCRP structures obtained under turnover conditions with estrone-3-sulfate or topotecan identified 2 distinct states, with a more closed conformation favored for the smaller endogenous substrate and a more open conformation favored for the larger drug, consistent with the higher rate of transport for the conjugated estrone.³⁰ These structures also revealed movement of the polymorphic R482 residue upon binding of substrate and ATP that indirectly influence substrate interactions with F439.³⁰ Collectively, the results of these structural studies will facilitate the design of more potent and selective inhibitors and provide a framework for interpretation of polymorphic variants. Other references to crystal structures of human ABC transporters are available in [Table S2](#).

Recently orphaned transporters

Transporters designated "orphans" lack information about their substrates or ligands. In recent years, increasing numbers of papers

Table 2 Recently solved^a human SLC transporter structures

| Gene | Protein | PDB entry | Method (Res Å) | Complex | Ligand | Conformation | Transport mechanism | Fold | Reference |
|---------|---|-----------|---------------------------|------------------------------------|--|----------------------------------|---------------------|------|-----------|
| SLC1A1 | Excitatory amino acid transporter 3 (EAAT3) | 6X2L | Cryo-EM (2.85) | Homotrimer | Na+ | Inward | Elevator | — | 19 |
| | | 6X3F | Cryo-EM (3.03) | | None | Inward | | — | |
| | | 6X3E | Cryo-EM (3.42) | | None | Asymmetric | | — | |
| | | 6X2Z | Cryo-EM (3.03) | | Aspartate | Outward | | — | |
| SLC1A2 | Excitatory amino acid transporter 2 (EAAT2) | 7VFR8 | Cryo-EM (3.60) | Homotrimer | None | Inward-open | Elevator | — | 125 |
| | | 7VR7 | Cryo-EM (3.49) | Homotrimer | WAY213613 | Inward-facing | | — | |
| SLC6A9 | Sodium and glycine dependent transporter 1 (GlyT1) | 6ZBV | X-ray diffraction (3.4) | Heterodimer with sybody Sb_GlyT1#7 | Inhibitor Cpmid1 + sybody inhibitor + sybody + Na ⁺ + Cl ⁻ | Inward | Alternating access | LeuT | 126 |
| | | 6ZPL | X-ray diffraction (3.945) | | | | | | |
| SLC7A5 | Large neutral amino acids transporter small subunit 1 (LAT1) | 7DSQ | Cryo-EM (3.4) | Heterodimer with 4F2hc (SLC3A2) | 3,5-diiodo-L-tyrosine | Outward-facing occluded | — | — | 127 |
| | | 7DSN | Cryo-EM (3.1) | | JX-119 | | | — | |
| | | 7DSL | Cryo-EM (2.9) | | JX-078 | | | — | |
| | | 7DSK | Cryo-EM (2.9) | | JX-075 | | | — | |
| SLC12A2 | Na ⁺ -K ⁺ -2Cl ⁻ cotransporter 1 (NKCC1) | 7D10 | Cryo-EM (3.52) | Homodimer | None | Inward-open inactive | — | LeuT | 128 |
| SLC12A5 | Electroneutral K ⁺ -Cl ⁻ cotransporter 2 (KCC2) | 6M23 | Cryo-EM (3.20) | Homodimer | None | Inward-facing | — | LeuT | 129 |
| SLC12A6 | Electroneutral K ⁺ -Cl ⁻ cotransporter 3 (KCC3) | 6M1Y | Cryo-EM (3.30) | Homodimer | None | Inward-facing | — | LeuT | 129 |
| | | 6M22 | Cryo-EM (2.70) | | DIOA | | — | — | |
| SLC13A5 | Sodium citrate cotransporter (NaCT) | 7JSJ | Cryo-EM (3.12) | Homodimer | PF2 inhibitor | Inward-facing | elevator | — | 130 |
| | | 7JSK | Cryo-EM (3.04) | | Citrate | | | | |
| SLC15A1 | Peptide transporter 1 (Pept1) | 7PN1 | Cryo-EM (3.90) | Monomer | None | Outward-facing open | rocker-switch | MFS | 131 |
| | | 7PMX | Cryo-EM (3.50) | Monomer | Ala-Phe dipeptide | Outward-facing open | | | |
| | | 7PMW | Cryo-EM (4.10) | Monomer | Ala-Phe dipeptide | Outward-facing occluded | | | |
| SLC15A2 | Peptide transporter 2 (Pept2) | 7PMY | Cryo-EM (3.80) | Monomer | Ala-Phe dipeptide | Inward-facing partially occluded | Rocker-switch | MFS | 131 |

(Continued)

Table 2 (Continued)

| Gene | Protein | PDB entry | Method (Res Å) | Complex | Ligand | Conformation | Transport mechanism | Fold | Reference |
|---------|--------------------------------------|--------------|----------------------------------|--|--------------------|------------------------------|---------------------|------|----------------|
| SLC16A1 | Monocarboxylate transporter 1 (MCT1) | 6LYY 6LZO | Cryo-EM (3.20) Cryo-EM (3.30) | Heterodimer with Basigin-2 Heterodimer with Basigin-2 | AZD3965 Lactate | Outward-open Outward-open | Alternating access | MFS | ¹³² |
| | | 7CKO | Cryo-EM (2.95) | Heterodimer with Basigin-2 | 7ACC2 | Inward-open | | | |
| | | 7CKR | Cryo-EM (3.00) | Heterodimer with Basigin-2 | BAY-8002 | Outward-open | | | |
| | | 7DA5 | Cryo-EM (3.30) | Heterodimer with Basigin-2 | None | Inward-open | | | |

4F2hc, 4F2 cell-surface antigen heavy chain (SLC3A2); Cryo-EM, cryogenic electron microscopy; EM, electron microscopy; GltpH, sodium-coupled aspartate transporter; LeuT, Leucine transporter; MFS, major facilitator superfamily; MFSD, major facilitator superfamily domain containing protein (atypical SLC); PDB, Protein Data Bank; Res Å, resolution in angstroms; SLC, solute carrier; Sybody, synthetic single-domain antibody.

^aPublished in 2021. Human SLC structures published before 2021 are listed in the (Table S1). To our knowledge, these include SLC1A3 (EAAT1), SLC1A5 (ASCT2), SLC4A4 (NBCe1), SLC16A9 (BOAT1), SLC7A9 (b0,+LAT1), SLC12A7 (KCC4), SLC16A7 (MCT2), SLC26A9, SLC28A3 (CNT3), SLC29A1 (ENT1), SLC30A8 (ZnT8), SLC40A1 (FPN), and MFSD10 (TETRAIN). Entries marked “—” indicate the information was not reported in the referenced article.

have described the deorphaning of transporters in the SLC superfamily. The relevance of SLC transporters in drug discovery has been increasingly recognized, given their emerging roles as drug targets.³² The knowledge of their physiological function and substrate specificity will help to understand their therapeutic potential and potential roles in drug disposition and toxicity. Among the 446 SLC transporters in the human genome, ~ 122 have unknown substrates, as annotated in this SLC annotation resource, <https://opendata.cemmm.at/gsflab/slcontology/>.¹⁵ In contrast, information on substrates for the majority of the 48 members of the ABC superfamily in the human genome is available except for members in the ABC subfamily E and F, which do not have transmembrane domains (see review in ref. 33). These proteins seem to regulate protein synthesis and expression (Table S2) and seem to be devoid of transport activity. Of the 48 ABC transporters with known functions, many have multiple ligands with 11 playing critical roles in multidrug resistance (see review in ref. 34).

The successful identification of ligands for orphan transporters has relied on a plethora of methodologies and technologies. Of these, human genetic association studies have provided a powerful method to begin to identify substrates of orphan transporters, particularly when mutations in orphan transporters have been associated with changes in the systemic or tissue levels of metabolites or inorganic ions (see review in ref. 32). Deorphaning several orphan transporters began with associations of the genetic polymorphisms or mutations in the transporters with various metabolites, which were subsequently tested as substrates of the transporters (Table S3). Several examples highlighted below illustrate the approaches to deorphan SLCs and identify their potential pharmacological relevance (see more references related to these transporters in Table S3).

- Using metabolomic genome-wide association studies (GWAS), substrates for two previous orphan transporters, SLC16A9 and SLC22A24, were identified. Deorphaning both transporters began by testing metabolites associated with genetic polymorphisms in the transporters in metabolomic GWAS. Polymorphisms in SLC16A9 were associated with uric acid and carnitine levels and the transporter was shown to transport both ligands in cellular assays. A stop codon in SLC22A24 was associated with low systemic levels of steroid conjugates in metabolomic GWAS and the transporter was shown to potently transport steroid glucuronides.³⁵
- Using GWAS for human disease and in particular associations of genetic polymorphisms in SLC16A11 with type 2 diabetes in Mexican subjects,³⁶ researchers identified pyruvate as a substrate of SLC16A11 explaining the mechanisms for the association with type 2 diabetes.³⁷ Recently, follow-up studies have shown that the orphan transporter SLC16A13, which is located next to SLC16A11 on chromosome 17p13.1, is a lactate transporter and also plays a role in type 2 diabetes.³⁸
- Using knockout mice, riboflavin was identified as the major substrate of SLC22A14, which was an orphan at the time.³⁹ Detailed studies showed that riboflavin plays an important role in the testis and reduced uptake of riboflavin in male SLC22A14 knockout mice resulted in infertility.

- Using *in silico* analysis, the gene *SLCO1B7* previously considered as pseudogene, was predicted to have 11 transmembrane domains. The gene was cloned and overexpressed in HeLa cells.⁴⁰ *In vitro* studies showed that *SLCO1B7* shared similar substrates as *SLCO1B1* and *SLCO1B3* (e.g., DHEAS and estradiol glucuronide).⁴⁰ *SLCO1B7* is expressed on the plasma membrane and in the smooth endoplasmic reticulum of hepatocytes and potentially plays a role in accumulation of drugs, such as ezetimibe in the endoplasmic reticulum.⁴⁰

In addition to genetic methods, other approaches have been applied in deorphaning transporters. For example, the RESOLUTE Consortium⁴¹ deorphaned *SLC25A51* using a genetic interactions approach to infer gene function in a “guilt-by-association” principle.^{42,43} The group used the human cell line HAP1, where the isogenic cell lines lacking 1 of 141 highly expressed SLC genes were combined with a CRISPR/Cas9 library targeting 390 SLC genes. This large resource resulted in > 55,000 SLC-SLC combinations and was used to develop a hypothesis for the substrates and function of the previous orphan transporter, *SLC25A51*. Based on the genetic interaction network, the group followed up with various experiments, such as targeted metabolomics and various measurements in mitochondria, to show that *SLC25A51* plays an important role in determining nicotinamide adenine dinucleotide (NAD⁺) levels in mitochondria (Table S3).

In general, orphan transporters within a family transport similar substrates as other family members. However, just testing similar substrates of family members to deorphan a transporter is often not fruitful, likely because if it were, the transporter would have been deorphaned already. For example, although *SLC10A1* and *SLC10A2* are known bile acid transporters, their paralogs, *SLC10A5* and *SLC10A7*, did not transport bile acids in an attempt to deorphan these transporters.⁴⁴ The challenges to deorphan these transporters may require more complex methodologies or multiple experimental conditions to identify their substrates. It is worthwhile pointing out that assignment of transporters to protein (sub)families is done based on overall sequence similarity, whereas substrate selectivity is determined by the small subset of amino acids in the translocation pathway. Emerging measured and predicted structure for orphan transporters will thus likely prove important for deorphanization. In addition, if the transporters are not expressed on the plasma membrane, such as *SLC25A1*, *SLC22A14*, and *MFS12*, different methods are needed to study transporter function (Table S3).

Lysosomal and mitochondrial transporters

The majority of the membrane transporter proteins in the ABC and SLC superfamilies are expressed on the plasma membrane. However, there are several transporters that are specifically expressed on lysosomal or mitochondrial membranes. These include members of the ABCA (e.g., *ABCA2* and *ABCA3* in the lysosomes) and ABCB (e.g., *ABCB7* and *ABCB8* in mitochondria), family and members of the SLC15 (e.g., *SLC15A3* and *SLC15A4* in lysosome), and SLC29 (e.g., *SLC29A3* in lysosomes) families. In addition, the majority of the 53 members of the SLC25 family

are mitochondrial transporters (<https://opendata.cemmm.at/gsflab/slcontology/#explore>; Table S2).⁴⁵ This review will highlight recent progress on studying lysosomal and mitochondrial transporters. For further information, we recommend these review articles focused on diseases caused by mutations in lysosomal transporters,⁴⁶ diseases caused by mutations in SLC25 family members⁴⁷ and general reviews on the SLC25 family,⁴⁸ and on lysosomal transporters.⁴⁵

Lysosomal transporters. Lysosomes are important membrane-bound enclosed organelles which contain an internal acidic environment (pH 5) and a variety of acid hydrolases to degrade materials that accumulate within their intra-organelle space.⁴⁹ Mutations in genes expressed in lysosomes result in various lysosome disorders.⁴⁶ The majority of proteins expressed in lysosomes are lysosomal hydrolases⁴⁹; however, ~ 33 proteins from the ABC or SLC superfamilies are also expressed in lysosomes (Table S2, <https://opendata.cemmm.at/gsflab/slcontology/>). Methods used to identify and characterize lysosomal transporters include (i) confocal microscopy using lysosomal membrane markers (e.g., LAMP1)⁵⁰; (ii) site-directed mutagenesis to mutate lysosomal sorting motifs (e.g., two dileucine motifs found in the lysosomal sorting consensus sequence, (D/E)XXXL(L/I) (Asp/Glu-X-X-X-Leu-Leu/Ile, where X indicates any amino acid), allowing lysosomal transporters to be expressed and then characterized on the plasma membrane). These methods have been used to functionally characterize *SLC15A3*, *SLC15A4*, *SLC17A5*, and *SLC29A3*^{51–53}; (iii) purification of lysosomal transporters and expression in proteoliposomes has been used to characterize lysosomal transporters (e.g., Niemann-Pick C1 (NPC1) and *SLC65*)⁵⁴; (iv) isolation of lysosomes (melanosomes) from melanoma cells with and without knockdown of the transporter gene has been used to functionally characterize the lysosomal transporter, *MFS12*.⁵⁵ This latter study revealed that loss of *MFS12* reduced melanosomal cystine and cysteinyl dopas levels and resulted in differences in skin pigmentation, as previously identified from GWAS (Table S3); and (v) untargeted metabolomics has been used to profile the plasma and urine from *SLC29A3* wild type and knockout mice. *SLC29A3* is a lysosomal transporter that transports nucleoside analogs⁵² and has recently been shown to transport bile acids, providing a potential mechanism for the association of *SLC29A3* mutations with human genetic disorders.⁵⁶

The role of membrane transporters in facilitating lysosomal sequestration of drugs is worth mentioning. The acidic environment in lysosomes acts as a sink for weakly basic drugs. Studies have shown that transporters in the lysosome play a role in drug resistance. Examples of drugs that are known to sequester into lysosomes include hydroxychloroquine⁵⁷ and imatinib.⁵⁸ ABC transporters play an active role in lysosome-mediated drug resistance especially for a number of anti-cancer drugs (e.g., daunorubicin and doxorubicin, for a review see ref. 59). For example, the lysosomal transporter *ABCA3* contributes to imatinib intrinsic resistance by facilitating sequestration of imatinib in lysosomes of leukemia cells.⁵⁸ Mutations or polymorphisms in lysosomal transporters that are implicated in human diseases present an opportunity for drug targeting. For example, polymorphisms in *SLC15A4* are associated with autoimmune diseases. Using *Slc15a4* knockout

mice, researchers showed a reduction in inflammatory biomarkers, such as interferons and interleukins, and in the development of systemic lupus erythematosus.⁶⁰

Mitochondrial transporters. Similarly, mitochondria are also essential organelles in cells playing critical role in energy production, apoptosis, cellular metabolism, and more. There are ~ 65 members in the ABC or SLC superfamilies which are mitochondrial transporters. These include the majority of the 53 members of the SLC25 family as well as members of the SLC8B, SLC55, and SLC56 family. In addition, the ABC transporters, ABCB7, ABCB8, and ABCB10 are found in mitochondria.⁴⁸ Members in the SLC25 family play critical roles as carriers on the inner mitochondrial membrane to transport substrates, such as ADP/ATP, amino acids, ornithine, carnitine, and thiamine pyrophosphate. The Kunji laboratory and colleagues have developed protocols for expression and purification of human mitochondrial membrane proteins in *S. cerevisiae* and *Lactococcus lactis*.⁶¹ In brief, following expression in these organisms, methods to characterize mitochondrial reference and mutant transporters include isolation of membranes containing the proteins from *Lactococcus* strains, preparation of liposomes, and mixing membranes from lactococcal strains with liposomes to create membrane vesicle fusions, which can then be used in transporter assays. These studies have been widely used for studying mitochondrial transporters (e.g., SLC25A1, SLC25A4, and SLC25A21).^{62–64} Commonly used methods to determine the localization of mitochondrial transporters are to co-transfect a GFP chimera transporter cDNA with the cDNA of the mitochondrial marker, COX8⁶⁵ and determine the transporter expression levels after isolating mitochondrial fractions.⁶⁶

Mutations in mitochondrial transporters are known to cause many diseases (for a review see ref. 47), furthermore, drug-induced mitochondrial toxicity has been well-recognized to affect the liver, kidneys, and heart (see review ref. 67). Transporters in the ABC and SLC superfamily play critical roles in modulating intracellular concentrations of drugs and toxins and as such play roles in mitochondrial toxicity. Commonly used drugs for cholesterol lowering, diabetes, and antibiotics are known to interfere with mitochondrial function and cause drug toxicity. Mitochondrial toxicity is one of the causes of drug-induced liver injury and drug-induced cardiotoxicity. The ABC transporter, BSEP (ABCB11), a plasma membrane transporter, plays a critical role in the efflux of bile acids from hepatocytes. Accumulation of bile acids in hepatocytes can occur by inhibiting BSEP. For example, troglitazone is a drug that inhibits BSEP and causes bile acid accumulation and drug-induced liver injury.⁶⁸ Similarly, drugs can induce cardiotoxicity through mitochondrial toxicity in cardiac cells. Through controlling intracellular levels of doxorubicin, several transporters have been implicated in its cardiotoxicity, for example, SLC22A3 (OCT3)⁶⁹ and ABCC1.⁷⁰ The mitochondrial accumulation of fialuridine, a nucleoside drug which caused severe hepatotoxicity was shown to be mediated by SLC29A1 (ENT1), a transporter that is expressed in both mitochondrial and plasma membranes.⁶⁵

There is growing interest among researchers to determine whether drug-induced mitochondrial toxicity is due to inhibition

of mitochondrial transporters.⁷¹ For example, prescription drug inhibitors of ADP/ATP carriers, SLC25A4 and SLC25A5, may cause mitochondrial toxicity by triggering mitochondrial apoptosis.^{72,73} The development of inhibitors of the mitochondrial pyruvate carrier (MPC1; SLC54) to treat hair loss also represents a new area of research.⁷⁴ Candidate MPC inhibitors were able to increase hair growth in shaved mice and also to inhibit oxygen consumption in the presence of pyruvate.⁷⁴

TRANSLATIONAL TRANSPORTER RESEARCH

Proteomic information on expression levels of membrane transporters

For many years, expression levels of transporters in various tissues were assessed based solely on their mRNA levels, first qualitatively through Northern blotting, then more quantitatively through reverse transcription polymerase chain reaction methods followed by RNAseq. However, recently, technological advances have led to quantitative measurements of actual protein levels (proteomics) and not simply transcript levels in various tissues.⁷⁵ Proteomic methods are increasingly transitioning from targeted proteomics, in which selected proteins of interest are quantified to global proteomics, in which the levels of all quantifiable proteins in a sample are assessed.⁷⁵ Proteomic methods have been applied to assess the expression levels of transporters in various tissues and within tissues, to specific cell types, greatly enhancing our understanding of the physiologic and pharmacologic mechanisms of drug transport. In the **Supplementary Material S1**, we provide an overview of the emerging knowledge of transporter protein expression levels in key ADME-related organs, such as the intestines, liver, and kidneys followed by a short description of transporter proteomics in the blood brain barrier (BBB) and other tissues.

Novel methods for modulation of transporter activity

With the emerging efforts in targeting transporters for therapeutic drug development, there is growing interest in developing novel methods and reagents for modulation of their activity. In this brief section, these methods and strategies are described.

New ligands and chemical probes of transporters. Our knowledge of the SLC superfamily has been limited by the lack of available tools and, in particular, chemical probes that modulate transporter function.⁷⁶ In fact, of the 20 families in the SLC superfamily that have been targeted by new compounds in the last few years, only four represent previously untargeted transporter families.⁷⁶ The vast majority represent an established cadre of transporter families that have been studied for many years (e.g., SLC5 family), which includes the sodium dependent glucose transporters, SGLT1 and SGLT2. However, interesting approaches have led to the discovery of novel inhibitors for some of these well-studied transporters. These approaches include unbiased cell-based high throughput screening for pathway inhibitors, 3D microarrays, and homology modeling coupled with virtual screening. These methods have led to the discovery of inhibitors of nutrient transporters in cancer cells, which represents a vibrant area of drug discovery. For example,

chromopyrone and indomorphane derivatives as inhibitors of the glucose transporter (SLC2A1), an attractive target in tumors, have been discovered using target agnostic high throughput screening coupled with structure-activity relationships. Other areas of active targeting of SLC transporters include targeting neurotransmitter transporters in the SLC1 and SLC6 families for treatment of multiple diseases that may be associated with mutations in the transporters.⁷⁶

Allosteric modulators and pharmacochaperones. Enhancers of transporter function are greatly needed especially for the over 100 rare genetic disorders that are caused by reduced or nonfunctional mutations in SLC transporters.³² Recent studies have focused on the development of positive allosteric modulators for the glutamate transporter, SLC1A2 (EAAT2) with the goal to enhance neuroprotection by increasing the reuptake of the excitatory amino acid, glutamate. A high-resolution pharmacophore model of EAAT2 was created and four small molecule enhancers were discovered and tested. Their putative mechanism was thought to be enhancement of EAAT2-mediated glutamate translocation rate without affecting its binding, consistent with an allosteric mechanism.⁷⁷ These exciting results bode well for the development of positive allosteric modulators for other transporters. Pharmacochaperones have received a great amount of attention in the literature for a variety of membrane proteins. Although pharmacochaperones have not been greatly applied to SLC mutant transporters, derivatives of the natural product, ibogaine, have been developed and found to enhance the activity of misfolded mutants of the dopamine transporter, DAT (SLC6A3).⁷⁸ These promising results bode well for the future of the development of pharmacochaperones for SLC transporters (see review in ref. 79).

Targeted degradation of SLC transporters. Knocking down the function of SLC transporters represents a critical tool for understanding their biological roles. However, there are significant limitations of many technologies designed to knockdown gene function. Most importantly, loss of one gene leads to compensatory adaption by other genes; therefore, the effect of gene loss cannot be accurately evaluated particularly in the context of dynamic and rapid processes, such as cellular metabolism. Recent techniques for targeted degradation of specific proteins have been evaluated and one such technique, heterobifunctional small-molecule degraders or PROTACs (proteolysis-targeting chimeras) has been recently applied to control the abundance of a number of transporters including SLC38A2 and various members of the SLC9 family.⁶ In brief, the method applied-dTAG PROTACs-involves tagging the SLC transporter with a mutated FKBP (FK506-Binding Protein) domain. The dTAGed protein is subject to degradation by bispecific degrader ligands that simultaneously bind to dTAG and to an E3 ligase. The induced molecular proximity leads to polyubiquitination of the transporter and subsequent degradation by the proteasome.⁶ These proof of concept studies will undoubtedly lead to a new understanding of SLC transporter biology.

The role of the gut microbiome in human drug transporter regulation

Increasingly, evidence has been obtained suggesting that the human gut microbiome affects pharmacokinetics through microbial biotransformation, resulting in drug activation,⁸⁰ inactivation,⁸¹ and toxicity.⁸² These microbial transformed products may modulate transporter function through induction of expression, or inhibition or enhancement of activity. Alternatively, microbes may metabolize parent compounds that modulate transporter function to metabolites with no effect on membrane transporters. However, to date, interactions between the gut microbiome and drug transporters are poorly understood. This section aims to summarize the current understanding of the interaction of the gut microbiome with human membrane transporters, with emphasis on clinically relevant drug transporters from the SLC and ABC superfamilies (Figure 2). This information is critical for understanding individual variability in drug response and achieving optimal drug therapy.

Microbiome-mediated metabolism of xenobiotics and membrane transporter function. Numerous studies have shown that xenobiotics, such as diet, drugs, drug metabolites, and excipients undergo microbial biotransformation. The inactivation of the cardiac drug digoxin⁸¹ and the Parkinson's disease medication, levodopa,⁸³ and the reactivation of the cancer drug irinotecan,⁸² demonstrated that the gut microbiome affects pharmacokinetics and pharmacodynamics in humans (Table S4). Notably, a recent study showed that about two thirds of the assayed 271 orally administered drugs are chemically modified *in vitro* by at least one strain of human gut bacteria.⁸⁴ Although it is well established that drug metabolites formed by human enzymes can be substrates and inhibitors of drug transporters, few studies have examined the relationship between drug metabolites formed by gut microbiota and drug transporters. Further, excipients, which are formulated with oral drug products, may also undergo metabolism by the gut microbiome. Recently, it was shown that azo dyes, commonly included in drug products and metabolized by gut microbiota, inhibited the intestinal uptake transporter, OATP2B1, resulting in decreased absorption of the antihistamine drug, fexofenadine in mice.⁸⁵ Although the azo dyes themselves inhibited OATP2B1, their metabolites derived from the microbiota did not, suggesting a complex interplay between gut metabolism of azo dyes and transporter inhibition. These studies suggest that drugs and excipients may be metabolized in the gut by microbes and that both the parent compounds or the metabolites may interact with intestinal transporters as substrates or through inhibition or induction. Further studies are warranted to determine whether gut microbiota-derived compounds (parent and metabolites) affect drug transporter activity in a clinical setting.

Microbiome-derived endogenous compounds and membrane transporter function. The gut microbiome has co-evolved with humans for thousands of years and, as such, plays a critical role in human physiology and diseases by regulating the development and function of the immune, metabolic, and nervous systems. One of the prominent examples is bile salt

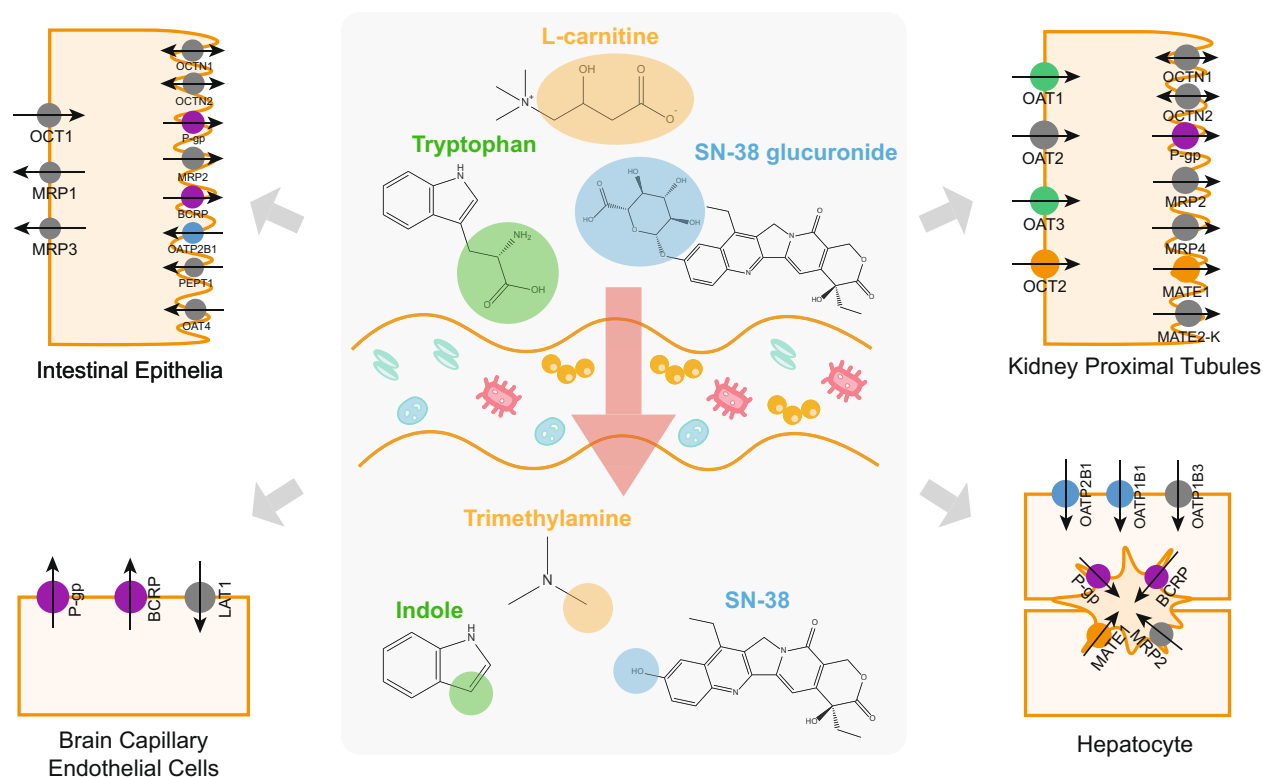


Figure 2 Interactions of representative gut microbial metabolites with membrane transporters. Membrane transporters expressed in epithelial cells of the intestines, liver, kidneys, and in the endothelium of the blood–brain barrier are shown in the shaded circles. The arrow represents the net direction of substrate movement for each transporter. The green color circles represent transporters with known interactions with indoxyl sulfate, the precursor of which, indole, is exclusively produced by gut microbes. The orange color represents transporters known to interact with trimethylamine N-oxide, the precursor of which, trimethylamine, is exclusively produced by gut microbes. The blue color represents transporters known to interact with SN-38, which is formed in the intestine by microbial glucuronidases. The purple color represents the transporters that interact with at least two of the microbial metabolites (or host derivatives). The grey color represents additional membrane transporters that are well-known for playing important roles in drug/nutrient disposition, but are not currently known to interact with the aforementioned gut microbial metabolites.

biotransformation by human intestinal bacteria. Secondary bile acids, produced solely by intestinal bacteria, can accumulate to high levels and may contribute to the pathogenesis of colon cancer, gallstones, and other gastrointestinal diseases.⁸⁶ Studies show that glycine- or taurine-conjugated secondary bile acids, such as glycolithocholic acid and taurothiocholic acid, are substrates of OATP1B1 and OATP1B3 with K_m values $<1 \mu\text{M}$ ⁸⁷ (Table S4). Interestingly, higher levels of several secondary bile acids in humans have been correlated with increased plasma concentrations of the cholesterol lowering drug, simvastatin.⁸⁸ These data suggest that secondary bile acids may decrease OATP1B1-mediated simvastatin acid uptake in hepatocytes resulting in higher plasma exposure. Further mechanistic studies are needed to extend our understanding of the impact of microbial biotransformation of endogenous compounds on drug transporter activities.

Gut microbiome and expression of membrane transporters. Several studies have examined the impact of the gut microbiome on the expression of membrane transporters, using germ-free mice or animals treated with antimicrobial agents.^{89,90} These studies have shown that gene or protein expression levels of

Bcrp1 and Mdr1a decreased⁸⁹ and increased,⁹⁰ respectively, in these microbiome-lacking animal models. More studies are needed to confirm these findings in humans. Although several approaches, such as biopsies from patients, organoids, the gut on a chip model, and human cell cultures, have been applied to examine the influence of the gut microbiome on human gene expression,⁹¹ many more studies are needed to fully understand the impact of the gut microbiome on expression levels of membrane transporters in humans.

In summary, emerging evidence suggests that metabolism of drugs and excipients by the gut microbiome affects membrane transporter function in humans and that the gut microbiome itself plays a critical role in regulation of expression levels of human intestinal transporters. To translate these observations into clinical applications, further studies are needed to understand the complex interactions between gut microbiome composition, microbial biotransformation of chemicals, and their impact on transporter expression and function in humans. See Table S4 for more examples and references.

3D cell culture and microphysiological systems

The rapid advances in cell biology, tissue engineering, and microfabrication technologies have enabled the development of

state-of-the-art 3D cell culture models, such as spheroids, organoids, scaffolds, and microphysiological system (MPS; also known as organs-on-chips). Compared with traditional 2D cell models, these novel 3D cell systems, with varying levels of complexity, can better recapitulate morphological, microenvironmental, and functional features of human tissues, and therefore have demonstrated great promise as next generation *in vitro* tools to investigate physiology and disease mechanisms, drug disposition, toxicity, and response.^{92,93} To date, a variety of 3D cell models and MPS, as stand-alone or interconnected organ systems, have been developed to represent major human ADME-related organs, such as the liver, gut, kidneys, and BBB. Their key features, limitations, and potential applications in drug discovery and development have been extensively reviewed.^{92,94}

With increasing recognition of the clinical implications of transporters in drug disposition, toxicity, and efficacy, recapitulating or retaining the expression and functional activity of drug transporters in 3D-cell culture models are critically important.^{95,96} Compared with spheroids and organoids, which are in microstructures, MPS provides a compartmentalized platform and is more suitable to study vectorial transport of substrates across apical and basolateral compartments of polarized cells mediated by multiple transporters in a dynamic, physiologically relevant microenvironments. Furthermore, MPS can be designed to incorporate multiple cell types to create more organ-like models and allow interconnectivity between different organ platforms to study transporter activity across different organ systems.

Currently, the characterization and validation of these 3D models in ADME settings largely focus on the end points of morphologic features, gene expression profile, and metabolic activity, whereas the evaluation of transporters is still emerging, in particular at the functional level.^{95,96} In **Table 3**, we summarized recent examples of liver, gut, kidney, and brain 3D/MPS platforms with the focus on the expression and functional characterization of drug transporters, as well as their applications and limitations for transporter research. For example, in a recently developed human duodenum intestine-Chip⁹⁷ established from the organoid-derived cells of three independent donors, mRNA expression of major intestinal efflux (P-gp, BCRP, MRP2, and MRP3) and uptake transporters (PepT1, OATP2B1, OCT1, and SLC40A1) on day 8 of chip culture are comparable to those in the freshly isolated human duodenum tissue. The luminal localization P-gp and BCRP, and functional activity P-gp were also demonstrated. These data suggest that this model could improve *in vitro* to *in vivo* extrapolation for better predictions of human intestinal absorption mediated by these transporters. In another example, primary human kidney proximal tubule cells cultured in a dual channel proximal tubule-on-a-chip model retained epithelial polarization and functional activity of basolateral localized uptake transporter OCT2.⁹⁸ This was demonstrated by the observation that cisplatin, a substrate of OCT2, induced renal toxicity when perfused from the basolateral, but not from the apical compartment, suggesting the dual-channel construction of renal MPS are physiologically and mechanistically relevant models to study renal transporter related toxicity. Last, it has been widely recognized that transporters expressed at the BBB play critical roles in modulating brain penetration of drugs. Given

the complex nature of the brain microvasculature, MPS models could offer significant advantages over existing *in vitro* BBB models to study transporter function in a more *in vivo* relevant environment by incorporating shear stress and multiple cell types. Recently, various cell types of the neurovascular unit differentiated from induced pluripotent stem cells were used to create isogenic BBB models. These models can be made with induced pluripotent stem cells derived from healthy and diseased patients and promise the attractive aspect of studying BBB transporter and barrier functions in relevant physiological contexts.⁹⁹ However, the characterization of the expression and activity of transporters in these models is still limited, and further evaluation is warranted.

As an emerging technology, 3D cell culture and MPS systems still have limitations and challenges for transporter studies, especially for quantitative *in vitro* to *in vivo* extrapolation. Whereas many transporter gene expression profiling data are promising, transporter characterization at protein and functional levels are still limited. Unlike conventional *in vitro* transporter models, determining transporter kinetic parameters—such as initial uptake/efflux rates—may not be feasible in current MPS models due to the small sample volume and low flow rates of the incubation media. On the other hand, it may be more applicable to study transport activity at the steady-state condition in MPS models. Nonspecific binding of lipophilic drugs to cells and microfluidic devices further complicates the accurate measurement drug concentrations and the determination of kinetic parameters. Optimization of assay conditions and development of *in silico* mechanistic modeling for MPS settings are needed in order to apply these systems for quantitative prediction of transporter-mediated effects. Furthermore, there are remarkable differences in 3D cell culture and MPS systems in terms of the design of the platforms, types of materials, cell sources, and extracellular matrices, which makes it challenging to standardize the assays and obtain reproducible data across different systems. Finally, the generation of parameters for use in predictive physiologically-based pharmacokinetic models will require reproducible 3D cultures, conditions, and results. Until that time, it is likely that well-defined 2D cultures will continue to be used.

CLINICAL TRANSPORTER RESEARCH

Pharmacogenomics and functional genomics of transporters

Pharmacogenomics of transporters. Several recent reviews have been published on the effects of polymorphisms in transporters on drug disposition and response.² Notably, eight GWAS reporting significant associations ($P < 5 \times 10^{-8}$) between transporter polymorphisms and drug response or disposition were cited in the 2018 review on transporter polymorphisms published by the ITC.² Since then, a few additional GWAS have been published that further support the evidence that reduced function polymorphisms in SLCO1B1 and ABCG2 are associated with statin toxicity and disposition and allopurinol response, respectively (**Table S3**). Recently, GWAS meta-analysis identified an SLCO1B1 locus associated with greater reduction in HbA1c upon sulfonylurea treatment in patients with type 2 diabetes.¹⁰⁰ Unlike other pharmacogenomic GWAS where SLCO1B1 p.V174A polymorphism is the

Table 3 Examples of 3D culture cell models and MPS systems for transporter evaluation

| Organs (references) | 3D models | Cell type | Cell culture platform | Transporter expression (mRNA and protein) | Functional end point | Key features | Limitations/Comments |
|---------------------------|----------------------|--|---|--|--|--|---|
| Gut ^{97,133-136} | Organoid systems | Intestinal/colonal stem cell or iPSC-differentiated tissues | <ul style="list-style-type: none"> Altitis GI crypt stem cell-differentiated organoids dissociated and cultured on transwells Stemcell technologies GI organoids differentiated from crypt stem cells or iPSCs | <ul style="list-style-type: none"> mRNA expressions of BCRP, MCT1, P-gp, BCRP, PEPT1, MRP3, OCT1, OSTα, and OSTβ were detected in intestinal organoids | <ul style="list-style-type: none"> Barrier function was confirmed with FITC-dextran and TEER (Altitis) P-gp mediated directional transport of digoxin was observed in monolayers (Altitis) Transporters-mediated glucose and fructose uptake was observed PEPT1-mediated Gly-SAR uptake was observed | <ul style="list-style-type: none"> Crypt stem cells are capable of differentiating into different cell types, including enterocytes, goblet cells, and enteroendocrine cells Differentiated organoids form polarized monolayer when plated on transwells Tight junction proteins, such as E-cadherin, ZO-1, and occludin, and microvilli were observed Crypt stem cells and iPSCs can be expanded and genetically modified | <ul style="list-style-type: none"> Intact organoids might not be suitable for certain transporter assays due to the format Expanding and differentiating stem cells are technically challenging Donor to donor variability was observed and pooled organoids are currently not available Source of stem cells and differentiation, and assay protocols could alter barrier and transporter function |
| | Microfluidic systems | Primary cells, immortalized cells, intestinal/colonal stem cell or iPSC-differentiated tissues | <ul style="list-style-type: none"> EmulateOrganoids differentiated from intestinal/colonal stem cells cultured in perfused channels separated by semi-permeable membrane Mimetas A microtiter-plate-based microfluidic chip platform enabling 40 chips per plate; with one chip presenting two perfusion channels and one ECM channel | <ul style="list-style-type: none"> Increased mRNA expressions of P-gp, BCRP, OATPs, and MCTs were observed under shear stress condition mRNA expressions of MRP2, MRP3, PEPT1, OCT1, OATP2B1 were observed | <ul style="list-style-type: none"> Barrier function, as measured by fluorescent probes and TEER, was maintained up to 12 days P-gp-mediated Rhodamine 123 efflux was observed | <ul style="list-style-type: none"> Primary and immortalized GI cells showed increased expression of polarization and differentiation markers under shear stress Improved barrier function was observed under shear stress Crypt stem cells and iPSCs are capable of differentiating into different cell types, including enterocytes, goblet cells, and enteroendocrine cells | <ul style="list-style-type: none"> High nonspecific binding to device material, such as PDMS Preparing and seeding chips can be technically challenging Lower throughput in some systems compared with traditional tools Donor to donor variability was observed and pooled organoids are currently not available Source of stem cells and differentiation, and assay protocols could alter barrier and transporter function |

(Continued)

Table 3 (Continued)

| Organs (references) | 3D models | Cell type | Cell culture platform | Transporter expression (mRNA and protein) | Functional end point | Key features | Limitations/Comments |
|--------------------------|----------------------|--|---|---|---|---|--|
| Liver ¹³⁷⁻¹⁴³ | Spheroid systems | Primary hepatocytes, hepatic NPCs | <ul style="list-style-type: none"> InSphero pooled donor liver microtissues in 96-well or 384-well formats RegeneMed Primary hepatocytes and NPCs cultured on porous 3D scaffolds in 24-well format | <ul style="list-style-type: none"> mRNA expressions of OATP1B1, OATP1B3, OCT1, P-gp, BCRP, MRP2 were detected and most were at levels comparable to native liver over 5 weeks Proteomics detected OATPs, OCT1, OAT2, MRP2, MRP3, P-gp, and MDR3. The levels of expression were time and culture condition dependent MRP2 and BSEP colocalization with canalicular membrane were detected by immunostaining | <ul style="list-style-type: none"> Estrone-3-sulfate uptake by OATPs and OATs was inhibited by cyclosporine A, verapamil and MK571 MRP2 function was demonstrated with CMFDA accumulation in bile canaliculi | <ul style="list-style-type: none"> Recapitulate tissue architecture and cell-cell interactions Formation of bile canaliculi-like structures and polarized expression of efflux transporters Cells are viable for at least 2 weeks Utility for hepatotoxicity assessment was demonstrated with species-specific toxicity of fenofibrate and troglitazone in human and rat organoids Long-term incubation for clearance determination | <ul style="list-style-type: none"> Most ADME/toxicity relevant genes were comparable to liver, however, down regulation was observed for OATP1B3 Determination of transporter-mediated efflux and biliary excretion were difficult due to the organization of organoids |
| | Microfluidic systems | Immortalized hepatocyte cell lines, Primary hepatocytes only, or primary hepatocytes with NPC, iPSC-differentiated hepatocytes | <ul style="list-style-type: none"> CnBio Primary hepatocytes and NPCs cultured on porous scaffold perfused with recirculating flow Emulate Primary hepatocytes and NPCs cultured in perfused channels separated by semi-permeable membrane Mimetas Primary or immortalized hepatocyte line (HepaRG) cultured in glass substrate embedded in 384-well plates forming a 2-lane or 3-lane channels separated by phaseguides | <ul style="list-style-type: none"> mRNA expression of OCT1, NTCP, OATPs, BCRP, BSEP, MRP2, and P-gp were detected. Expression levels of most transporters recovered and remained stable over time but OATP1B3 and BSEP were down-regulated MRP2 and BSEP were detected by immunostaining | <ul style="list-style-type: none"> Bile canaliculi formation was confirmed by the accumulation of fluorescent substrates for MRP2 and BSEP (5-CFDA, 5-CF) Inhibition of BSEP-mediated efflux of fluorescence substrate CLF by bosentan was observed | <ul style="list-style-type: none"> Incorporation of flow and shear stress to systems Recapitulate tissue architecture and cell-cell interactions Formation of bile canaliculi-like structures and polarized expression of efflux transporters Hepatocyte functions, measured in albumin secretion and CYP3A4 activity, were more stable than organoids and traditional 2D systems Albumin secretion was comparable in liver chips (20-70 µg per day per million cells) and in vivo (50 µg per day per million cells) Cells are viable for at least 2 weeks Pre-clinical species liver-on-chips are available on some platforms | <ul style="list-style-type: none"> High nonspecific binding to device material, such as PDMS (polydimethylsiloxane) Preparing and seeding chips can be technically challenging Lower throughput in some systems compared with traditional tools Hepatocytes exhibited different sensitivity to toxins depending on the culture platform Some CYPs and transporters expressions were downregulated over time (e.g., OATP1B3, BSEP) |

(Continued)

Table 3 (Continued)

| Organs (references) | 3D models | Cell type | Cell culture platform | Transporter expression (mRNA and protein) | Functional end point | Key features | Limitations/Comments |
|------------------------------|----------------------|--|---|---|---|--|---|
| Kidney ^{98,144-146} | Microfluidic systems | <ul style="list-style-type: none"> Human renal proximal tubule epithelial cells (HRPTEC) Conditionally immortalized PTEC overexpressing OAT1 (ciPTEC-OAT1) | <ul style="list-style-type: none"> Nortis dual channel microphysiological chips OrganoPlate, a microtiter-plate-based microfluidic chip platform enabling 40 chips per plate; with one chip presenting two perfusion channels and one ECM channel | <ul style="list-style-type: none"> HRPTECs cultured for 7 days in chips compared with 2D HRPTECs cultured for the same time; ↑ mRNA expression of MATE1, MATE2K, BCRP, and megalin endocytosis receptor by 19.9-, 23.2-, 4.3- and 106-fold and comparable OCT2 but reduced P-gp mRNA expression OAT1 mRNA expression variable in different studies; OAT3 was not detected in either 2D or chip-cultured HRPTECs | <ul style="list-style-type: none"> Membrane integrity; apparent permeability; Efflux transport of P-gp and MRP2/4 tested using fluorescence-based transport assay OCT2 -dependent transport activity of cisplatin and reduced kidney toxicity with cimetidine treatment; confirmed OCT2 activity with 4-di-1-ASP trans-cellular transport Trans-epithelial transport of OAT1/3 substrate PAH and indoxyl sulfate (Nortis MPS) | <ul style="list-style-type: none"> Structural recapitulation of the proximal tubule microenvironment; recaptulation of proximal tubule physiological functions Polarize with transporters selectively localized to the basolateral and apical membrane Sustained functional activity over longer incubation (+28 days) Increased tight-junction formation (zona occludens-1 ZO-1 expression), and increased number of cilia and microvilli at the apical membrane Potentially be implemented for routine assessment of kidney toxicity and DDI studies for transporter substrates | <ul style="list-style-type: none"> Some inter- and intra-MPS variability of transport functional activity Possible low assay sensitivity due to limited amounts of cells and small sample volume Limited experience for quantitative IVIVE |

(Continued)

Table 3 (Continued)

| Organs (references) | 3D models | Cell type | Cell culture platform | Transporter expression (mRNA and protein) | Functional end point | Key features | Limitations/Comments |
|--------------------------|----------------------|---|--|---|--|--|---|
| Brain ¹⁴⁷⁻¹⁵⁰ | Microfluidic systems | Primary, immortalized, or iPSC-differentiated brain microvascular endothelial cells, with or without neurons, astrocytes, pericytes | <ul style="list-style-type: none"> Mimetic iPSC-differentiated brain microvascular endothelial cells and primary pericytes and astrocytes cultured in separate channels in glass substrate embedded in 384-well plates divided by extracellular matrix Emulate iPSC-differentiated brain microvascular endothelial cells and primary pericytes and astrocytes cultured in perfused channels separated by semi-permeable membrane SynVivo Immortalized brain microvascular endothelial cells and primary astrocytes cultured in perfused compartments separated by 3 μm slits | <ul style="list-style-type: none"> mRNA expression of P-gp, BCRP, and MRPs, OCTs, MCTs, • OATPs were detected P-gp and BCRP were detected by immunostaining | <ul style="list-style-type: none"> TEER values were maintained above 1,000 Ω × cm² for 5 days The model was capable of differentiating BBB permeable and impermeable peptides based on anti-inflammation PD readout Glutamate transporter function was observed Inhibitable P-gp efflux of rhodamine 123 by vinblastine | <ul style="list-style-type: none"> Improved barrier function over static models in TEER values and endothelial markers such as OCLN, PECAM1, caveolins and Von Willebrand factor Cell types heterogeneity and cell-cell interactions resulted in improved physiological characteristics Interindividual variability in dextran permeability was observed in BBB chips differentiated from cells of diseased patients Incorporation of shear stress and mechanical cues which affected the expression of SLC and ABC transporters | <ul style="list-style-type: none"> Transcript levels of some transporters in iPSC differentiated brain endothelial cells were lower than in freshly purified human brain endothelial cells Nonspecific binding to material (such as PDMS) were observed in some systems Forming leak-tight monolayer tubules is technically challenging Principal component analysis comparing transcriptomes indicated that iPSC-differentiated BMEC most closely correlates with HUVECs and LSECs Source of iPSC cells and differentiation, and assay protocols could alter barrier and transporter function |

ABC, ATP-binding cassette superfamily; ADME, absorption, distribution, metabolism, and excretion; BBB, blood brain barrier; BCRP, breast cancer resistant protein; BSEP, bile salt export pump; CMFDA, 5-chloromethylfluorescein diacetate; DDI, drug-drug interaction; ECM, extracellular matrix; FITC, fluorescein isothiocyanate; GI, gastrointestinal; HUVECs, human umbilical vein endothelial cells; iPSC, induced pluripotent stem cells; IVIVE, *in vitro to in vivo* extrapolation; LSEC, liver sinusoidal endothelial cells; MRP, multidrug resistance-associated protein; MPS, microphysiological system; NPC, non-parenchymal cells; NTCP, sodium/bile acid cotransporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; PAH, para-aminohippurate; PD, pharmacodynamic; PDMS, polydimethylsiloxane; P-gp, P-glycoprotein; SLC, solute carrier.

significant single-nucleotide polymorphism (SNP) in the locus, this study identified an intronic SNP, rs10770791, with weak linkage disequilibrium with p.V174A. Association of ABCG2 polymorphisms with caffeine consumption is a new finding (Table S5). Caffeine, a purine with a similar structure to uric acid, has previously been reported to inhibit BCRP.¹⁰¹ Interestingly, the ABCG2 missense variant, p.Q141K, strongly associated with reduced caffeine consumption, presumably due to higher levels of caffeine, which resulted in a feedback mechanism that reduced caffeine intake. The higher caffeine levels were a result of increased absorption in individuals with p.Q141K. In the previous ITC review, the effect of multiple missense variants of SLC22A1 encoding OCT1 on the pharmacokinetics and response to several prescription drugs were cited.² Since then, SLC22A1 missense variants have been found to be significantly associated with other phenotypes, including proguanil's active metabolite, cycloguanil¹⁰² (Table S5). Recent significant associations of polymorphisms in ABCG2, SLCO1B1, and SLC22A1 with drug response or disposition from GWAS or genotype to phenotype studies are summarized in Table S5. Other ABC and SLC transporters that have been associated with pharmacogenomics traits in GWAS include ABCB1 (dabigatran plasma levels and chemotherapy-induced alopecia), SLCO1A2 (rocuronium dose requirement), SLC16A5 (cisplatin-induced ototoxicity in patients with testicular cancer), and SLC38A7 (anastrozole levels) are shown in Table S5.

Increasingly, pharmacogenomic GWAS have involved larger sample sizes, multiple ethnic groups and functional genomics. For example, the discovery of the association of an SLC2A2 intronic variant with metformin response was made possible by a sample size of > 10,000, multiple ethnic groups, and a large meta-analysis.¹⁰³ Phenotypic data extracted from the electronic health records from various clinical centers were combined in the meta-analysis.

The well-characterized reduced function ABCG2-Q141K (rs2231142) and SLCO1B1-V174A (rs4149056) are present at very low allele frequencies (~ 1–5%) in African populations. Interestingly, recent studies have demonstrated that other SNPs in SLCO1B1 are associated with various phenotypes in the African population. For example:

- SLCO1B1 rs114419265 (in strong linkage to SLCO1B1 p.Gly-488A) is strongly associated with the SLCO1B1 biomarker, glycochenodeoxycholate glucuronide in African populations.¹⁰⁴ This variant is specific to populations of African ancestry.
- SLCO1B1 loss-of-function variant (c.481+1G>T; rs77271279) is strongly associated with the SLCO1B1 biomarker, hexadecanedioic acid in African Americans.¹⁰⁵ This variant is specific to African Americans and the reduced function variant is associated with higher levels of the biomarker (beta = 0.38, $P = 2.2 \times 10^{-9}$).

There are increasing numbers of studies supporting the association of the SLCO1B1 reduced function variant, rs4149056 with statin levels and statin-induced myopathy. Hopefully, in the future, African ancestry-specific variants in SLCO1B1 will be assessed

for their effects on statin levels and toxicity in this understudied population.

Functional genomics of transporters. Recent advances in technologies have resulted in new information on the function of genetic variants in transporters. For example, next generation sequencing together with high-throughput functional readouts have been used to determine the function of hundreds to thousands of genetic variants in a variety of genes. These methods developed by Fowler and colleagues have been applied to various drug metabolizing enzymes and transporters, such as OATP1B1.¹⁰⁶ Over 130 missense variants in OATP1B1 were functionally characterized in a single experiment to elucidate the expression of the GFP-tagged OATP1B1 to identify mutations that caused significantly reduced expression of GFP.

The availability of a CRISPR-Cas9 library targeting 390 human SLC genes utilized by Superti-Furga and colleagues allowed genetic screening to elucidate SLC transporter function.^{42,107} This resource has also been used successfully to determine cell cytotoxicity of various anti-cancer drugs upon targeting each of the SLC genes. The results showed that artemisinin toxicity was dependent on the expression SLC11A2 and SLC16A1 and the sensitivity to cisplatin was dependent on the expression of SLC35A2 and SLC38A5.¹⁰⁷ Motivated by the increasing potential to discover SLCs as drug targets, the RESOLUTE consortium has made significant progress in developing accessible SLC tools, reagents, and protocols. These tools and reagents include codon-optimized cDNAs for SLC transporters, antibodies, and transcriptomic and proteomic information.⁴¹

Electronic health record biomarkers and transporter-mediated drug–drug interactions

In 2018, the ITC published a perspective on endogenous substrates of transporters as biomarkers to monitor DDIs during early phases of drug development.¹⁰⁸ Since then, many studies have been published demonstrating the use of biomarkers for transporters in the liver and kidneys to predict or validate transporter-mediated DDIs.¹⁰⁹ However, because regulatory authorities did not require studies of transporter-mediated DDIs besides P-gp until 2012, many current drugs on the market have never been studied as potential perpetrators of transporter-mediated DDIs. Such studies are urgently needed. Recently, 25 drugs in clinical repurposing trials for the treatment of coronavirus disease 2019 (COVID-19) were evaluated as potential perpetrators of transporter-mediated DDIs.¹¹⁰ Interactions of the drugs with 11 drug transporters, including the 9 transporters recommended for study by the US Food and Drug Administration (FDA) were characterized in standardized *in vitro* assays. The *in vitro* half-maximal inhibitory concentrations were then compared with the relevant clinical concentrations of the drugs and criteria recommended by the FDA were applied to predict clinical DDIs. The results were striking, demonstrating that many of these drugs interact with multiple transporters at clinically relevant concentrations suggesting potential to perpetrate clinical DDIs. In fact, 20 of the 25 drugs met the FDA criteria to trigger consideration of a clinical DDI trial. Notably, 40 potential clinical DDIs were predicted for the 14 compounds that were approved before 2010.

The above studies were then validated with the use of biomarkers commonly reported in electronic health records. These real-world biomarkers typically represent solutes that are substrates of transporters and are routinely measured during clinical care. For example, uric acid is a known substrate of BCRP (ABCG2) and is routinely measured in people with suspected or actual inflammatory arthritis and gout. In the COVID-19 drug study, sildenafil, which is used to treat pulmonary hypertension associated with later stages of COVID-19, was shown to inhibit BCRP at concentrations, which are expected to be achieved in the intestines following therapeutic doses. Indeed, uric acid levels in patients on sildenafil were significantly increased compared with age, sex, and diagnosis matched patients not on sildenafil.¹¹⁰ Control studies validating uric acid as a real-world biomarker were performed in studies that showed that cyclosporin and eltrombopag, the FDA recommended clinical inhibitors of BCRP,¹¹¹ were associated with elevated uric acid levels. Collectively, these data suggest that uric acid could be a potential biomarker to predict DDIs mediated by BCRP. This new finding from real-world data is exciting as endogenous biomarkers to predict BCRP-mediated DDIs have not yet been identified. Further studies are needed to characterize the sensitivity and selectivity of uric acid as the biomarker for BCRP DDI evaluation. Other biomarkers commonly measured in electronic health records include bilirubin for OATP1B1 and creatinine for OCT2/MATE1 (Table 4). Biomarkers shown in Table 4 have been validated in clinical genetic studies as being associated with reduced function polymorphisms of the transporters.^{110,112–114}

Beyond drug transporters, genetic variants in other transporters not generally involved in drug absorption or disposition have been associated with plasma levels of various endogenous solutes, including a number of amino acids, oligopeptides, neurotransmitters, and heavy metals.^{35–38} Many of these solutes are not routinely measured in a clinical care setting and, therefore, cannot be used in observational studies. However, these solutes may serve as

indicators of transporter activity or perturbations in transporter activity, for example, in the presence of prescription drugs to assess the effects of drugs on important micro and macro nutrient transporters.

Tissue-derived plasma small extracellular vesicles

Small extracellular vesicles (sEVs), such as exosomes (30–150 nm diameter), are secreted by most organs into the systemic circulation, and are present in biological fluids, such as plasma, serum, urine, breast milk, saliva, bile, and feces. Exosomal cargo contains proteins, DNA species (mitochondrial and nuclear DNA), RNA species (mRNA, microRNA, and lncRNA), lipids, and metabolites derived from the originating organs.¹¹⁵ As such, exosomes carry unique signals from their originating organs. Exosomes can be isolated by various methods/techniques, such as differential ultracentrifugation, size-exclusion chromatography, ultrafiltration, polyethylene glycol-based precipitation, microfluidics-based nanofiltration and immunoaffinity capture, and immunoprecipitation¹¹⁶ (Figure 3). Since the discovery of exosomes in 1983,¹¹⁷ many studies have demonstrated their promising utility as mediators of intercellular communication, biomarkers of various diseases, and potential therapeutic targets and drug delivery carriers.¹¹⁸

Very recently, potential applications of tissue-derived plasma sEVs as a liquid biopsy to understand drug metabolizing enzyme (DME) and transporter profiles in ADME-related organs have emerged, and were summarized in a review article by Rodrigues and Rowland.¹¹⁵ The sEVs for ADME-related purposes are particularly useful as there are limitations to the feasibility of directly measuring the protein expression and functional activity of transporters and DMEs in tissues during clinical studies. Hence, profiling and quantifying functional proteins of transporters and DMEs in isolated sEVs derived from ADME-related tissues can potentially serve as a methodology to understand interindividual variability in pharmacokinetics, address complex DDIs involving DMEs and transporters, and evaluate the impact of age and diseases on the expression and ultimately the functional activity of ADME-related proteins in different tissues.

Several recent examples have illustrated the value of sEVs in transporter research and their potential application in drug development. Achour *et al.*¹¹⁹ isolated plasma exosomes from the blood samples collected from 29 patients with liver cancer. The mRNA or protein expression of multiple enzymes and transporters in plasma exosomes and matched liver samples was measured. To normalize for the variability in exosome shedding from liver to blood among individuals, a shedding factor was used based on the measurement of plasma RNA of 13 liver-specific markers. A good correlation was observed between normalized plasma exosome mRNA levels and protein expression in matched liver tissues for 4 transporters (OATP1B1, MRP2, P-gp, and BCRP) and 12 DMEs. This work suggests that plasma exosomes are promising tools to determine the variability in expression of hepatic transporters and enzymes in humans. Rodrigues *et al.*¹²⁰ isolated liver-specific sEVs in human serum samples using a novel two-step method, which included the use of size exclusion chromatography to obtain global

Table 4 Potential biomarkers commonly reported in EHRs that could be used for study of transporter-mediated drug–drug interactions

| Transporter | Potential real-world biomarkers | Validation (genetic or inhibitor studies) | References |
|-------------------|---------------------------------|---|------------|
| BCRP | Uric acid | Genetic and inhibitor | 110,112 |
| OATP1B1 | Bilirubin | Genetic and inhibitor | 110 |
| OATP1B3 | Bilirubin | Genetic and inhibitor | 110 |
| OCT1 ^a | Cholesterol, Triglycerides | Genetic | 113 |
| OCT2 | Creatinine | Genetic and inhibitor | 110 |
| MATE1 | Creatinine | Genetic and inhibitor | 110 |
| OAT1 | Uric Acid | Genetic | 114 |
| OAT3 | Uric Acid | Genetic | 114 |

EHR, electronic health record.

^aOCT1 is not currently included in the US Food and Drug Administration (FDA) draft guidance for transporter-mediated drug–drug interaction studies. However, the International Transporter Consortium (ITC) has previously recommended that OCT1 be evaluated as a mediator of clinical DDI based on mounting evidence.¹

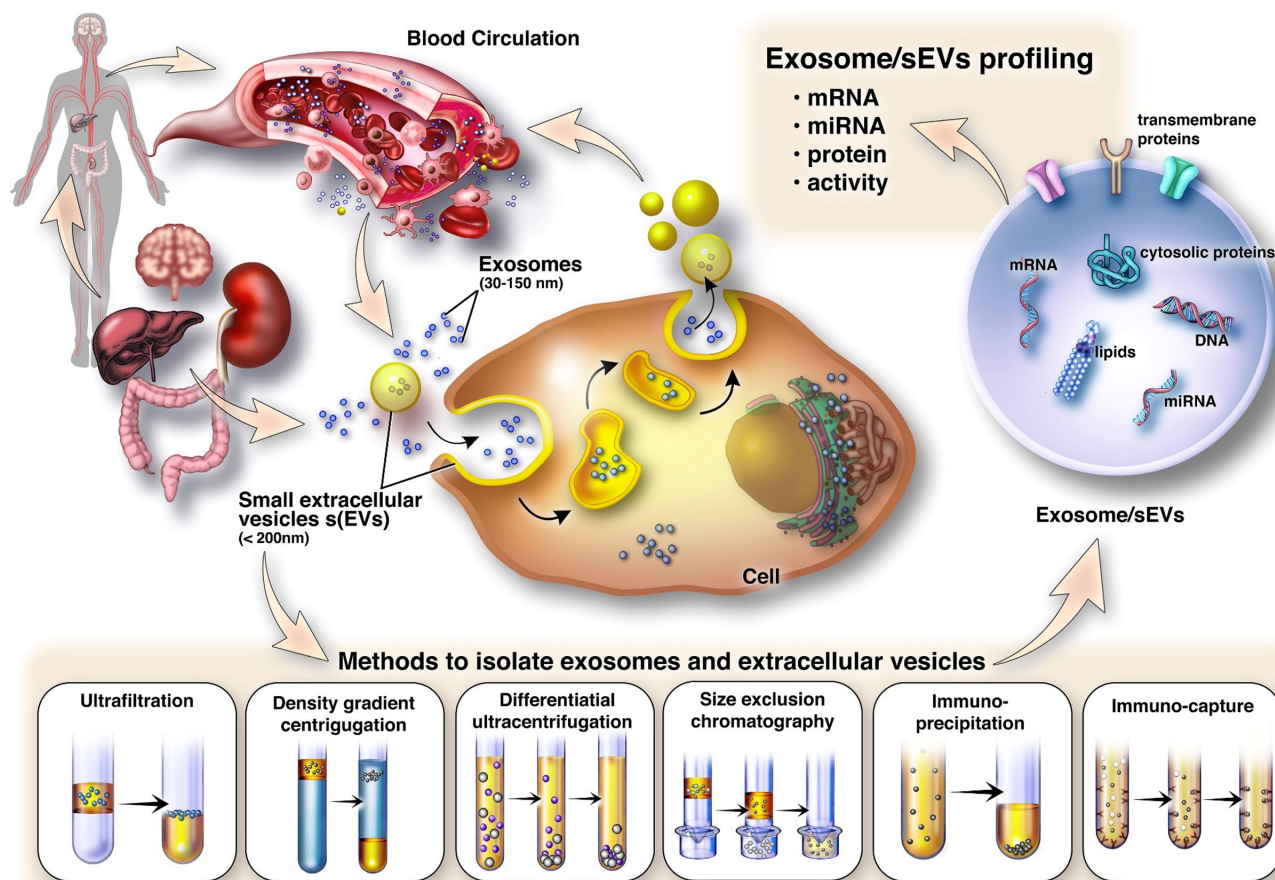


Figure 3 Concept of tissue derived plasma small extracellular vesicles and methods for their isolation. Tissue-derived small extracellular vesicles are derived from organs in the body and circulate. Methods to isolate them from the circulating plasma are shown. sEVs, small extracellular vesicles.

EVs, followed by incubating the global EVs with the beads coated with a biotin labelled anti-asiaglycoprotein receptor 1 (ASGR1) polyclonal antibody. ASGR1 is highly enriched in the liver and serves as a vector protein for the immunocapture of hepatic sEVs. The data confirmed the induction of CYP3A4 but the lack of induction of hepatic OATP1B1 and -1B3, following multiple doses of rifampin, a well-known inducer for CYP3A and P-gp. BCRP, primarily expressed in the intestines and liver, can limit intestinal absorption and biliary excretion of substrates. Expression of a microRNA called miR-328 has been shown to negatively correlate with BCRP mRNA and protein levels.¹²¹ In one study, intestinal-derived exosomal miR-328 was isolated from plasma using immunoprecipitation. The change of intestinal exosomal miR-328 correlated with plasma area under the curve (AUC) of sulfasalazine, a substrate of BCRP, in a clinical study,¹²¹ suggesting that intestine-derived exosomal miR-328 could be a potential biomarker to estimate intestinal BCRP function in humans.

As exploration of sEVs in ADME and transporter research is still at a very early stage, there are considerable technical challenges and knowledge gaps in the isolation, quantification, and integration of exosomal data with other ADME-related data for quantitative translation. Importantly, it is critical to develop

reliable and robust methods to isolate plasma exosomes specific for selected tissues, such as the liver, intestines, kidneys, and brain, in order to deconvolute the change of transporter expression/function in multiple tissues (e.g., liver vs. gut for BCRP). Isolation and characterization of exosomes are labor intensive, the availability of well-validated commercial isolation kits will ultimately promote the broader use of exosome data in drug development. Plasma exosomes have relatively low expression of transporters, which require the development of highly sensitive methods to detect their mRNA and protein expression. Furthermore, it remains challenging to study functional activity of transporters using exosomes. To begin to use exosomal data for quantitative prediction of ADME profiles of drugs, specific ADME-related proteins need to be measured in exosomes harvested from plasma samples from many individuals to understand between and within subject variability, as well as the factors associated with the variability. Importantly, correlation with protein expression in relevant tissues needs to be established.

Overall, sEVs have emerged as a novel next generation tool, which can provide rich information on ADME and transporter-related genes and proteins in various tissues. Exosome-based approaches, integrated with *in vitro*, preclinical, clinical studies,

and mechanistic modeling holds great promise to advance our understanding and the capability for quantitative prediction of the impact of transporters on pharmacokinetics, DDIs, efficacy, and toxicity of drugs in healthy and special populations. Further characterization and validation of sEVs is required to define their utility and limitation in transporter research and drug development.

CONCLUSIONS

In conclusion, dramatic breakthroughs in the past 5 years have enabled research at all levels of transporter biology. In the basic area, new structures have paved the way to understanding transporter-ligand interactions at a molecular level. It is envisioned that future research will continue to employ cryo-EM methods to understand transporter ligand interactions for many of the transporters in both the SLC and ABC superfamilies and that these structures will be used in developing novel therapeutics. The substrates of transporters previously designated as orphans are rapidly being discovered and validated using novel metabolomic and genomic methods. Many of these newly deorphaned transporters may play critical roles in drug absorption, disposition and response. Future research will focus on deorphaning additional transporters and the biological roles of recently deorphaned transporters in human physiology and pathophysiology and in pharmacokinetics and pharmacodynamics. Simultaneously, new information about previously understudied transporters in lysosomes and mitochondria is accumulating as technologies have advanced for the isolation and study of transporters in these subcellular organelles. In the next decade, new studies on transporters in subcellular organelles and their roles as determinants of cellular and subcellular levels of drugs and endogenous ligands will be performed. Translational tools are being developed and applied to probe and modulate transporter function *in vivo*, to model transporter-mediated drug disposition in three-dimensions, and to quantify protein levels of individual transporters in various tissues. Research in the 3D models will continue to evolve and it is envisioned that those models will be used in drug development to more precisely understand drug absorption and elimination. A nascent understanding of the role of the microbiome in modulating the expression levels of transporters as well as in producing metabolites that may inhibit or induce transporters is emerging and will continue to emerge. Finally, new clinical tools will continue to become available to enable precision medicine, with the application of sEVs, real-world biomarkers, and DNA testing for transporter polymorphisms.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

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