DMD Fast Forward. Published on February 2, 2023 as DOI: 10.1124/dmd.122.001186
This article has not been copyedited and formatted. The final version may differ from this version.

Drug Transporters at the Human Blood-Testis Barrier

Raymond K. Hau, Stephen H. Wright, and Nathan J. Cherrington

College of Pharmacy, Department of Pharmacology & Toxicology, The University of Arizona, Tucson AZ, USA (R.K.H., N.J.C.)

College of Medicine, Department of Physiology, The University of Arizona, Tucson, AZ, USA (S.H.W.)

Running Title: Human Blood-Testis Barrier Transporters

Corresponding Author:

Nathan J. Cherrington

1703 E. Mabel St., Tucson, AZ, 85719

(520)-626-0219, cherring@pharmacy.arizona.edu

Text Pages: 22

Number of Figures: 2

Number of Tables: 2

Number of References: 117

Number of Words in Abstract: 245

Number of Words in Body of Manuscript: 5380

Abbreviations: BCRP: Breast cancer resistance protein, BTB: Blood-testis barrier, CNT: Concentrative nucleoside transporter, ENT: Equilibrative nucleoside transporter, LC: Leydig cell, MATE: Multidrug and toxin extrusion protein, MRP: Multidrug resistance-associated protein, OAT: Organic anion transporter, OATP: Organic anion transporting polypeptide, OCT: Organic cation transporter, OCTN: Organic cation transporter, novel, PMC: Peritubular myoid cell, P-gp: P-glycoprotein, SC: Sertoli cell

Abstract

Transporters are involved in the movement of many physiologically important molecules across cell membranes and have a substantial impact on the pharmacological and toxicological effect of xenobiotics. Many transporters have been studied in the context of disposition to, or toxicity in, organs like the kidney and liver; however, transporters in the testes are increasingly gaining recognition for their role in drug transport across the blood-testis barrier (BTB). The BTB is an epithelial membrane barrier formed by adjacent Sertoli cells (SCs) in the seminiferous tubules that form intercellular junctional complexes to protect developing germ cells from the external environment. Consequently, many charged or large polar molecules cannot cross this barrier without assistance from a transporter. SCs express a variety of drug uptake and efflux transporters to control the flux of endogenous and exogenous molecules across the BTB. Recent studies have identified several transport pathways in SCs that allow certain drugs to circumvent the human BTB. These pathways may exist in other species, such as rodents and nonhuman primates; however, there is (1) a lack of information on their expression and/or localization in these species and (2) conflicting reports on localization of some transporters that have been evaluated in rodents compared to humans. This review outlines the current knowledge on the expression and localization of pharmacologically relevant drug transporters in human testes and calls attention to the insufficient and contradictory understanding of testicular transporters in other species that are commonly used in drug disposition and toxicity studies.

Significance Statement

While the expression, localization, and function of many xenobiotic transporters have been studied in organs such as the kidney and liver, the characterization of transporters in the testes is scarce. This review summarizes the expression and localization of common pharmacologically-relevant transporters in human testes that have significant implications for the development of drugs that can cross the blood-testis barrier. Potential expression differences between humans and rodents highlighted here suggest rodents may be inappropriate for some testicular disposition and toxicity studies.

Introduction

The blood-testis barrier (BTB) is comprised of Sertoli cells (SCs) lining the seminiferous tubules that form intercellular junctional complexes to protect developing germ cells from external threats, such as the immune system and reproductive toxicants (Figure 1) (Dym and Fawcett, 1970). There are three components that work synergistically to achieve barrier function in the testes. First, the tight network of SC membranes and their associated junctional complexes constitutes the physical component of the BTB, which can be perturbed by certain toxicants or physical injury (Pelletier, 2011). Next, membrane transporters along the basal membrane of SCs, peritubular myoid cells (PMCs), and Leydig cells (LCs) comprise the physiological component of the BTB (Mital et al., 2011; Mruk and Cheng, 2015; Mruk et al., 2011). Lastly, the secretion of immunomodulatory factors by SCs, PMCs, and LCs to suppress the immune system from targeting haploid germ cells represents the third component of the BTB (Fijak and Meinhardt, 2006; Li et al., 2012; Zhao et al., 2014). Together, these mechanisms provide a secure compartment in the male genital tract for spermatogenesis to transpire. However, the testes also serve as a sanctuary site for some viruses (e.g., Ebola, HIV, and Zika) and cancers (e.g., leukemia) because the BTB prevents many therapeutics from gaining access to this compartment (Byrn and Kiessling, 1998; Deen et al., 2017; Houzet et al., 2014; Kiessling et al., 1998; Ma et al., 2021; Nesbit et al., 1980; Ritzen, 1990; Robinson et al., 2018).

To cross the BTB effectively, drugs must be able to passively diffuse between cells or across membranes or be substrates of membrane receptors or carrier proteins,

such as uptake transporters. Common drug uptake transporters include the concentrative and equilibrative nucleoside transporters (CNTs and ENTs), organic cation transporters (OCTs), novel organic cation transporters (OCTNs), organic anion transporters (OATs), and organic anion transporting polypeptides (OATPs). However, SCs also express efflux transporters at their basal membranes to pump drugs back into the blood, including P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and the multidrug resistance associated proteins (MRPs) are the major efflux transporters in many tissues. While the multidrug and toxin extrusion proteins (MATEs) are traditionally referred to as efflux transporters at acidic pHs, they can function as uptake transporters at physiological pH (7.4), which may be a mechanism of action in the testes. The function of these transporters has been studied in tissues such as liver, kidneys, and brain; however, many of these processes are also likely to play important roles in the testes. SC transporters are critical for maintaining normal physiological function in the testes because they mediate the flux of nutrients across the BTB to support developing germ cells. However, their presence at this membrane barrier permits some xenobiotics into the male genital tract that can treat testicular diseases or disorders and/or cause reproductive toxicity. The physiochemical properties of these small molecule drugs vary, although many are bulky and charged at physiological pH, which limit their permeability across cell membranes, thereby requiring transporters.

Although there have been several studies assessing the localization and function of selected drug transporters at the human BTB (Bakos et al., 2022; Bart et al., 2004; Bleasby et al., 2006; Hau et al., 2022; Hau et al., 2020; Huang et al., 2016; Huber et al., 2007; Klaassen and Aleksunes, 2010; Klein et al., 2013; Klein et al., 2014; Lee et al.,

2004; Melaine et al., 2002; Morgan et al., 2012; Pizzagalli et al., 2002; Suzuki et al., 2003), there are many more exploratory and functional studies across preclinical species that need to be performed. These current studies have typically relied on immunohistological/immunocytofluorescence techniques, mRNA expression profiles (Northern blots, RNA-sequencing, transcriptomics, and RT-qPCR), protein expression profiles (Western blots and proteomics), and functional transport studies in whole rodents or isolated SCs. The different strengths and weaknesses of these experimental techniques add to the complexity of data interpretation and current understanding of testicular drug transporter expression, localization, and function across species remains unclear. Typical comprehensive mRNA and protein expression profiles are based on whole testicular tissues and can be unreliable for classifying relative expression levels in specific cell types because these studies include an abundance of transcripts and proteins from SCs, PMCs, LCs, vascular endothelial cells, and germ cells (Augustine et al., 2005; Bleasby et al., 2006; Klaassen and Aleksunes, 2010; Li et al., 2011). However, SC-specific transporter expression analyses have been performed (Augustine et al., 2005; Hau et al., 2020) and can corroborate immunohistological- and immunocytofluorescence-based techniques that assess expression and localization of transporters at the BTB. It is important to note that antibody specificity is a major limitation in immunolocalization studies, although positive and negative controls in the same or independent studies should provide evidence in support of their specificity. *In vitro* studies that confirm functional transporter localization at the basal or apicolateral membranes of SCs are ideal; however, it is important to note that SCs cultured on Transwell inserts typically form weak intercellular interactions that permit greater

paracellular flux compared to conventional epithelial cell lines (Chui et al., 2011; Gerber et al., 2020; Hau et al., 2020; Mruk and Cheng, 2011; Papadopoulos et al., 2016; Prozialeck and Lamar, 1997; Siemann et al., 2017; Srinivasan et al., 2015; Tsetsarkin et al., 2020; Wu et al., 2019). This suggests that Transwell SC cultures may not accurately reflect the *in vivo* BTB, and conclusions from these studies should be carefully interpreted. Moreover, species differences in transporter localization can produce misleading disposition or toxicity predictions when extrapolating to humans (Hau et al., 2021). Independent of other data, the qualitative methods used to detect transporter expression (e.g., immunolocalization, Western blots, RT-qPCR, and transcriptomics) produce inadequate evidence to describe transporter function. On the other hand, quantitative, functional transport studies, alone, are also inappropriate for predicting transporter localization in the testes. Therefore, both analytical methods must be used to develop a clear picture of transporter expression and function in the testes. The purpose of this review is to summarize the current understanding of the expression and localization of classical drug transporters in human testes and to highlight the knowledge gaps that may fundamentally alter the conclusions drawn from testicular drug disposition or toxicity studies that use rodents.

Nucleoside Transporters

Nucleoside transporters are responsible for the bidirectional movement of endogenous nucleosides and nucleobases through cell membranes, and are fundamental to maintaining nucleoside homeostasis in the cytoplasm and in certain organelles (e.g., the mitochondria and nucleus) (Barros et al., 1995; Errasti-Murugarren et al., 2011; Govindarajan et al., 2007; Klein et al., 2013; Senyavina et al., 2016; Young et al., 2013). Nucleoside transporters also facilitate the bidirectional transport of xenobiotics, including many antivirals and antineoplastics. These types of drugs can be especially useful for treating testicular indications, including certain cancers or viral infections, in the male genital tract. Examples of these drugs include abacavir, gemcitabine, and remdesivir (Cerveny et al., 2018; Grunewald et al., 1991; Mackey et al., 1998; Mackey et al., 1999; Miller et al., 2021b). In rat and human testes, as supported by immunological localization and functional assessment, ENT1 is localized to the basal membrane of SCs, whereas ENT2 is localized to the apicolateral membrane of SCs (Figure 2, Table 1) (Klein et al., 2013). Here, ENT1 permits the bidirectional movement of nucleosides and nucleoside analogs from the blood into SCs, or out of SCs back into the blood. On the apicolateral membrane, ENT2 allows these molecules to move from SCs into, or be recycled from the lumen of seminiferous tubules. Consequently, this polarized distribution of ENT1 and ENT2 establishes a Na^+-independent transepithelial transport pathway to deliver xenobiotics across the BTB into the male genital tract for treating viral infections, cancers, and other testicular

indications. One such example of a nucleoside analog therapeutic used to treat leukemias, which can reside in the sanctuary site afforded by the BTB (Bowman et al., 1984; Chu et al., 1982; Gajjar et al., 1996; Grundy et al., 1997; Heaney et al., 1983; Kulkarni et al., 2010; Nesbit et al., 1980; Ritzen, 1990; Shaffer et al., 1992; Tombolini et al., 1986), is clofarabine. It is also known to cause toxicities in many organs, including in the seminiferous tubules of rodents and canines (U.S. Food and Drug Administration, 2004). Clofarabine has been shown to be a substrate of ENT1 and ENT2, and its disposition across the *in vivo* rat BTB is dependent on the ENTs (Miller et al., 2021a). In addition to SC expression, ENT1 and ENT2 are expressed by rat and human LCs and developing germ cells, which would support normal cellular growth and health.

Another transporter that may deliver nucleosides into the adluminal compartment is CNT2, which is located at the apicolateral membrane of SCs in human testicular tissue (Hau et al., 2022). However, it is also possible that CNT2 may be involved in nucleoside salvaging from the adluminal compartment (Gray et al., 2004). The latter hypothesis may be more plausible since CNT-mediated “efflux” is inconsistent with its concentrative role as a Na^+-nucleoside cotransporter, although it may be viewed as concentrating nucleosides into seminiferous tubule lumen. These free nucleosides can then be taken up by nucleoside transporters expressed by developing germ cells. Although there is evidence of functional CNT expression in primary rat and mouse TM4 SCs (Kato et al., 2005; Kato et al., 2006; Kato et al., 2009), positive CNT1 expression in human testicular tissue was not observed in the study conducted by Hau and colleagues (Hau et al., 2022), nor was functional CNT expression observed in rat and human SCs (Hau et al., 2020; Klein et al., 2013). In the three studies by Kato et al.

(Kato et al., 2005; Kato et al., 2006; Kato et al., 2009), CNT function was defined by their relative affinities towards purine or pyrimidine nucleosides/bases and their inhibition of uridine transport. Kato and colleagues also found that CNT3 mRNA expression was highest in primary rat SCs followed by CNT2 then CNT1; however, there were negligible levels of CNT2 or 3 (and CNT1 was not tested) in mouse TM4 SCs (Kato et al., 2005). Most of the uridine uptake in primary rat and mouse TM4 SCs appeared to be mediated by the ENTs, although Na^+-dependent uptake accounted for ~20% of total uptake in primary rat SCs. It is possible that protein expression of CNT1 is negligible in these cells, which would support the lack of CNT1 in human tissues (Hau et al., 2022). Functional characterization of each transporter in these cells were not performed, although knockdown or knockout studies could be used in the future. Nevertheless, further studies are required to clarify these observations and hypotheses concerning CNT expression and function in SCs.

Organic Ion Transporters

Organic ion transporters encompass several solute carrier subfamilies and are designated as OCTs, OCTNs, and OATs (in the SLC22A family), and the OATPs (in the SLCO families); however, their roles in the transport of anionic, cationic, zwitterionic, or neutral compounds can vary greatly. Many of these transporters have been thoroughly investigated due to their high expression and roles in the disposition of clinically relevant xenobiotics in tissues like liver, kidney, and intestine. Xenobiotic substrates of these transporters include antibiotics, antivirals, antineoplastics, statins, as well as a wide variety of plant toxins, whereas endogenous substrates include bile acids,

neurotransmitters, prostaglandins, uremic toxins, and steroids. Comprehensive mRNA expression profiles for these organic ion transporters in the testes of different species have been determined (Augustine et al., 2005; Bleasby et al., 2006; Hau et al., 2020; Klaassen and Aleksunes, 2010), but functional and cellular localization studies are incomplete and remain controversial.

The OCTs are a family of three (OCT1, 2, and 3) bidirectional transporters that mediate the facilitated diffusion of small cationic compounds such as choline, cisplatin, dopamine, metformin, and serotonin down their electrochemical gradients (Roth et al., 2012). The OCTs share several substrates with OCTN1/2 and MATE1/2, but each of these has distinct physiological roles that separate them from one another. Although OCTs are highly expressed in the liver and kidneys, OCT mRNA transcripts have also been reported in whole testicular tissue and in the SCs of different species (Augustine et al., 2005; Bleasby et al., 2006; Hau et al., 2020; Klaassen and Aleksunes, 2010; Maeda et al., 2007). A recent immunolocalization study observed OCT1, 2, and 3 expression in the PMCs and LCs, with weak expression at the basal membrane of SCs in human testes (Figure 2, Table 1) (Hau et al., 2022). Round spermatids within the seminiferous tubule also appeared to express OCT3, but its role there is unclear. An earlier study with rats observed mRNA expression of OCT1, 2, and 3 whole testes; however, only OCT1 and 3 transcripts were detected in isolated rat SCs (Maeda et al., 2007). Based on functional studies with rat SCs cultured on Transwell inserts, Maeda and colleagues hypothesized that OCT1 and OCT3 were expressed on the basal membrane and apicolateral membranes, respectively. These data contrast with the immunological observations made in human tissue (Hau et al., 2022), and, it is

important to note, that SCs cultured on Transwell inserts cannot be assumed to reflect *in vivo* physiology. Because SCs do not generate a tight *in vitro* epithelial barrier as evidenced by comparatively low transepithelial electrical resistance values to those observed in normal epithelial cell lines (e.g., MDCK and Caco-2), it is debatable if SCs polarize at all in these in these 3D culture models (Chui et al., 2011; Gerber et al., 2020; Hau et al., 2020; Mruk and Cheng, 2011; Papadopoulos et al., 2016; Prozialeck and Lamar, 1997; Siemann et al., 2017; Srinivasan et al., 2015; Tsetsarkin et al., 2020; Wu et al., 2019). Furthermore, transporter expression and localization in cultured rodent SCs may not correlate with histological observations made in intact testes, or even be on the same membrane of SCs in other species (Hau et al., 2021). Therefore, drugs that interact with the OCTs in the testes of nonhuman species must be evaluated with additional discretion until evidence suggests otherwise. Nevertheless, it is possible that some chemotherapeutics such as cisplatin and its derivatives can circumvent the BTB using OCTs to exert their toxic effects in the testes of various species (A. A. Aly and G. Eid, 2020; Filipski et al., 2009; Soni et al., 2016; U.S. Food and Drug Administration, 2019).

Although OCTN1 and OCTN2 have often been referred to as cation transporters (Kobayashi et al., 2005; Tamai et al., 1997; Wang et al., 1999), the preferred substrates for OCTN1 and OCTN2 are the zwitterions, L-ergothioneine and L-carnitine, respectively (Grundemann et al., 2005; Tamai et al., 1998; Tang et al., 1999), which are co-transported with Na^+. Both OCTN1 and OCTN2 are expressed in rodent and human testes, specifically at the basal membrane of human SCs (and in the PMCs for OCTN2) (Figure 2, Table 1) (Augustine et al., 2005; Bleasby et al., 2006; Hau et al., 2022; Hau et

al., 2020; Klaassen and Aleksunes, 2010; Kobayashi et al., 2005; Maeda et al., 2007). Functional transepithelial transport experiments with rat SCs are in good agreement with OCTN2 basal membrane localization (Hau et al., 2022; Kobayashi et al., 2005; Maeda et al., 2007). Since L-carnitine is important for SC metabolism and overall male fertility (Agarwal and Said, 2004; Mongioi et al., 2016; Palmero et al., 2000), it is reasonable that OCTN2 is localized to the basal membrane of SCs as shown in human tissues and rat SCs. Furthermore, high accumulation of carnitine in the testes compared to plasma has been observed in rats, suggesting the involvement of a carrier-mediated mechanism like OCTN2 (Bremer, 1983). On the other hand, human tissue localization of OCTN1 is inconsistent with functional transport studies with rat SCs (Hau et al., 2022; Maeda et al., 2007). Based on transepithelial transport experiments, Kobayashi et al. hypothesized that OCTN1 was responsible for L-carnitine uptake at the apicolateral membrane of rat SCs (Kobayashi et al., 2005). However, there are concerns with interpreting functional transport data collected from these Transwell SC culture experiments because L-ergothioneine is a far better substrate of OCTN1 (OCTN1-mediated transport of L-carnitine is negligible) (Grundemann et al., 2005) and, as described previously, *in vitro* SC physiology is not completely representative of the *in vivo* BTB. Thus, OCTN1 localization at the basal membrane of SCs is, arguably, more likely because L-ergothioneine is an antioxidant exclusively absorbed from dietary sources.

There are at least eight known members of the OAT family in humans (Roth et al., 2012), but only three (OAT1, 2, and 3) are frequently studied for their clinical relevance because of their expression in liver and kidney. OATs primarily transport low

molecular weight, anionic compounds against their electrochemical gradients, driven by exchange for endogenous dicarboxylates like α-ketoglutarate. While bidirectional OAT-mediated transport of selected substrates (e.g., methotrexate and indoxyl sulfate) has been observed *in vitro* (Enomoto et al., 2003; Masuda et al., 1999), the OATs typically mediate the unidirectional uptake of endogenous compounds or xenobiotics into the cytoplasm. Common substrates of the OATs include antibiotics, antihistamines, antivirals, and nonsteroidal anti-inflammatory drugs, but other classes of compounds can interact with these transporters. OAT1, 2, and 3 are highly expressed in the liver and kidneys where they are implicated in many DDIs, but they may play critical roles in other tissues, including the testes. In humans, OAT1 and OAT2 are primarily expressed at the basal membrane of SCs, but OAT1 was also observed in LCs and the luminal membrane of vascular endothelial cells of the testes (Figure 2, Table 1) (Hau et al., 2022). This observation is consistent with a previous immunolocalization study that detected high protein expression in the vascular endothelial cells and weaker expression throughout the testes (Huang et al., 2016). Unfortunately, the cellular localization of OAT3 in the testes remains unknown. Several studies were unable to detect OAT3 mRNA transcripts in the testes (Augustine et al., 2005; Bleasby et al., 2006; Klaassen and Aleksunes, 2010); however, one study did find measurable levels in immortalized human SCs by RT-qPCR and Affymetrix GeneChip microarray analysis (Hau et al., 2020). High SC-specific mRNA expression of mouse OAT6 was observed in C57BL6/J mice (Schnabolk et al., 2010), but neither its localization, nor the extent of expression of the human ortholog in SCs, is known. While it is possible that this OAT

plays a major role in organic anion transport in the testes, there is a lack of evidence to support this hypothesis.

OATPs are bidirectional transporters, found in many tissues, that generally display a broad substrate selectivity that can include bile acids, hormones, various xenobiotics, and many conjugated metabolites. Some of these OATPs may play a major role in the transport of hormones across the basal and/or apicolateral membranes of SCs to regulate spermatogenesis. The substrate selectivity of some OATPs, such as OATP1C1, OATP2A1, and OATP4A1, is narrower, consistent with their specialized roles in prostaglandin or thyroid hormone transport into certain cells. In human testes, OATP1C1 and OATP4A1 are highly expressed in LCs (Hau et al., 2022; Pizzagalli et al., 2002), as determined by immunolocalization, where they can act as thyroid hormone transporters to regulate hormone signaling and homeostasis in the testes (Figure 2, Table 1) (Fujiwara et al., 2001). In the testes, the broad selectivity transporters, OATP1A2, OATP1B1, OATP1B3, OATP2A1, OATP2B1, and OATP3A1-v2, are primarily expressed at the basal membrane of human SCs, although all but OATP2A1 were also detected throughout the adluminal compartment (Hau et al., 2022). The synthesis of prostaglandins within SCs and LCs lend credence to the presence of OATP2A1 at the basal membrane of SCs to permit flux of these molecules across the BTB (Cooper and Carpenter, 1987; Samy et al., 2000; Schell et al., 2007). Notably, diffuse expression of OATP3A1-v2 in human SCs is consistent with a previous report, also in human tissues (Huber et al., 2007). It is reasonable to assume that some of these transporters are, as in the endothelial cells of the blood-brain-barrier (Ashraf et al., 2014; Gao et al., 1999; Ose et al., 2010; Sano et al., 2018), expressed in both poles

of SCs, i.e., in the basal and apicolateral membranes (as well as in developing germ cell membranes), but further studies are warranted to confirm these findings. Interestingly, the first and third OATP3A1 isoform, OATP3A1-v1 and OATP3A1-v3, are expressed in developing germ cells with weak expression in SCs of human testes (Bakos et al., 2022). The differences in the physiological role of these transcript variants are unclear; however, their affinities for some substrates vary slightly (Bakos et al., 2022; Bakos et al., 2020; Huber et al., 2007; Tamai et al., 2000). Among the remaining OATPs, it is unlikely that OATP4C1 and OATP5A1 are expressed in the testes based on whole tissue mRNA analysis (Bleasby et al., 2006). However, these data may have been influenced by the overabundance of mRNA transcripts produced by other testicular cells. One study detected mRNA transcripts for OATP5A1 in human SCs (Hau et al., 2020), but protein expression, localization, and function remain unknown. Despite high mRNA expression of the testes-specific OATP6A1 (Lee et al., 2004), the cellular localization and physiological role for this transporter remains undetermined. The rodent orthologs, OATP6B1,OATP6C1, and 6D1, share only 36-46% protein sequence identity with the human transporter (Table 2) and are highly expressed by SCs, LCs, and spermatogonia but their exact location is ambiguous (Table 1) (Suzuki et al., 2003). Along with rodent OATP1A5 and OCTN2, it is also known that these two orthologs are also partially responsible for the uptake of the male contraceptive, adjudin, in rat SCs (Su et al., 2011). However, it is unknown if OATP6A1 can transport adjudin and other endogenous or exogenous compounds. The testicular localization of the genetically similar (and potentially functional) rodent orthologs of these OATPs have been largely unexplored (Tables 1 and 2). However, it appears likely that most of these organic ion

transporters are involved in the disposition of physiologically important molecules across the BTB, although further work is necessary to confirm and define these aspects. These transporters can also be used to deliver small molecule therapeutics to treat testicular indications since many are localized to the basal membrane of SCs (Figure 2, Table 1).

Multidrug and Toxin Extrusion Proteins

The MATEs are organic cation/H^+ antiporters that mediate the uptake or efflux of substrates based on H^+ concentration gradients between the cytoplasm and extracellular space. MATE1 and MATE2 are expressed at the apical membranes of epithelial cells such as hepatocytes and renal proximal tubule epithelial cells, where they typically function as complementary efflux transporters for cationic molecules taken up by OCTs or OCTNs; however, they are also found in the testes. In human testes, MATE1 is expressed intracellularly and along the apicolateral membranes of SCs (Figure 2, Table 1) (Hau et al., 2022). These findings are in contrast to a previous study that observed only nominal levels of mouse MATE1 mRNA and protein in whole testes or in SCs (Lickteig et al., 2008). Rat MATE1 mRNA was found to be highly expressed in whole testes, but was undetectable in primary rat SCs (Maeda et al., 2007). Another study detected mouse MATE1 exclusively in LCs (Hiasa et al., 2006), which is in partial agreement with immunohistofluorescent staining in human tissues (Hau et al., 2022) that also observed MATE1 expression in LCs. On the other hand, mRNA transcripts of a MATE2 isoform have been detected in mouse (Hiasa et al., 2007; Lickteig et al., 2008) and human (Hau et al., 2020) SCs. Protein expression of the rodent MATE2 isoform

was limited to LCs (Hiasa et al., 2007); however, protein expression was not detected in human tissues (Hau et al., 2022). These observational discrepancies for MATE1 and MATE2 may be due to species differences in drug transporter expression and localization in the testes (Hau et al., 2021) or a lack of antibody specificity.

It is reasonable to assume that the MATEs act as efflux transporters at the apicolateral membrane for OCT or OCTN substrates brought in from the basal membrane of SCs. However, the seminiferous tubule fluid is slightly acidic (7.31) compared to plasma (7.50) (Levine and Marsh, 1971) and the cytoplasmic pH of SCs has been reported to be approximately 7.05 under *in vitro* experimental conditions with an extracellular buffer pH of 7.4 (Bernardino et al., 2016; Oliveira et al., 2009a; Oliveira et al., 2009b). This *in vitro* study is not representative of *in vivo* conditions and should be treated with some skepticism, although it is within the range of typical values for most cells (pH 7.0-7.4) (Madshus, 1988). This cytoplasmic to seminiferous tubule fluid H^+ gradient could drive MATE-mediated uptake from the seminiferous tubule lumen as a recycling mechanism. However, as the difference in cytoplasmic and luminal fluid H^+ concentration is relatively modest, it is possible that the electroneutral MATEs may still act as apicolateral efflux transporters that can support efflux of cationic substrates, free from the influence of the negative membrane potential of SCs.

Multidrug Resistance-associated Proteins

MRPs are unidirectional, ATP-dependent efflux transporters found at blood-tissue barriers, cancer cells, and in excretory organs where they play a significant role in the transport of a wide range of primarily anionic molecules. High expression of these

efflux transporters has been shown in the testes, and SC basal membrane localization is consistent with their protective role at the BTB (Augustine et al., 2005; Bleasby et al., 2006; Hau et al., 2022; Hau et al., 2020; Klaassen and Aleksunes, 2010; Klein et al., 2014; Koraichi et al., 2013; Su et al., 2009). MRP1 is localized to the basal membrane of SCs and LCs in humans, nonhuman primates, and rats (Figure 2, Table 1) (Klein et al., 2014). In humans, MRP3, MRP6, and MRP7 are localized to the basal membrane of SCs (Figure 2, Table 1) (Bart et al., 2004; Hau et al., 2022; Huang et al., 2016; Koraichi et al., 2013; Melaine et al., 2002; Su et al., 2009). Furthermore, MRP3, MRP6, and MRP7 are found in the PMCs in human testes, where they may serve as a first line of defense for testicular toxicants (Hau et al., 2022). A previous study found that MRP2 mRNA is extremely low in rodent testes and SCs (Augustine et al., 2005), but other studies suggest that MRP2 is, at least, weakly expressed in human PMCs and LCs of the testicular interstitium (Bart et al., 2004; Huang et al., 2016). MRP5 was exclusively localized to the LCs in humans and rats (Klein et al., 2014). Unusually, MRP8 is exclusively localized to the round spermatids in human and nonhuman primates only (Tables 1 and 2), where they may be involved in the efflux of pro-growth hormones post-meiosis (Klein et al., 2014). Although mRNA and protein expression of MRP9 has been detected in spermatozoa of mice and in human SCs (Bera et al., 2002; Chambers et al., 2022; Hau et al., 2020; Ono et al., 2007; Tammur et al., 2001), its cellular localization in human tissues remains unknown.

Interestingly, several studies have evaluated MRP4 localization in the testes; however, the findings remain contentious. Human MRP4 shares 80-96% DNA sequence identity with the rodent and nonhuman primate orthologs, whereas their

protein sequences are 87-97% identical to humans (Table 2). One study observed expression of MRP4 at the apicolateral membrane of SCs in rodent testes, in clear contrast to basal membrane expression in human and nonhuman primate testes (Klein et al., 2014). Klein and colleagues also noted that the MRP4 antibody did not detect protein expression in MRP4 knockout mice, indicating it was specific for MRP4 (Klein et al., 2014). However, several studies reported SC basal membrane localization in both human (Huang et al., 2016; Morgan et al., 2012) and rodent testes (Koraichi et al., 2013). Koraichi and colleagues noted that upon neonatal treatment of rats with the mycotoxin, zearalenone, MRP4 redistributes from the basal membrane of SCs, PMCs, and LCs exclusively to the apicolateral membrane of SCs, spermatocytes, and spermatids. This observation presents an interesting conundrum in which some external pressures can alter transporter expression and localization. To further complicate the issue, MRP4 was found to localize exclusively to LCs of mouse testes and at both the LCs and basal membrane of SCs in human testes (Morgan et al., 2012; Morgan et al., 2015). These potential species differences, combined with the observation that transporter distribution can be influenced by physiological/toxicological status, can contribute to false prediction of penetration of MRP4 substrates across the BTB in rodent species, when MRP4 may restrict penetration in humans. It is possible that antibody specificity can account for some of these differences, and additional studies are necessary to confirm MRP4 localization in the testes of these species. But the major implication for this observation is that, for at least some compounds, rodent models should not be used to extrapolate testicular disposition or toxicity in humans and, instead, make use of nonhuman primates.

P-glycoprotein and Breast Cancer Resistance Protein

P-gp and BCRP are expressed in a wide range of tissues as the major efflux pumps for many clinically important drugs and endogenous molecules. These transporters are involved in drug-drug interactions and modulate the pharmacokinetics of many drugs, which is why regulatory agencies consider them clinically relevant (European Medicines Agency, 2013; Pharmaceuticals and Medical Devices Agency, 2019; U.S. Food and Drug Administration, 2020). Consistent with their cytoprotective role in other tissues, P-gp and BCRP are expressed by several cell types throughout rodent and human testes (Bart et al., 2004; Enokizono et al., 2007; Huang et al., 2016; Melaine et al., 2002; Qian et al., 2013; Su et al., 2009). P-gp has been observed at both the basal and apicolateral membranes of SCs, as well as in PMCs, LCs, developing germ cells, and luminal membrane of vascular endothelial cells in humans and rodents (Figure 2, Table 1) (Bart et al., 2004; Huang et al., 2016; Melaine et al., 2002; Su et al., 2009). However, despite 81-97% DNA and protein sequence identity of other species relative to humans (Table 2), there are major BCRP expression differences between species that require further analysis. In humans, BCRP has been detected at the basal and apicolateral membranes of SCs, PMCs, developing germ cells, and luminal membrane of vascular endothelial cells (Figure 2, Table 1) (Huang et al., 2016; Melaine et al., 2002). In rats, BCRP was found in the same locations as in humans except, importantly, at the basal membrane of SCs (Qian et al., 2013). Unlike rats or humans, BCRP was exclusively localized to the vascular endothelial cells in mouse testes (Enokizono et al., 2007). These findings present a similar issue to that noted above for

MRP4 regarding the use of rodent models for efficacy or toxicity studies in the testes, because misleading results could be costly in the future. Therefore, additional studies are warranted to address the differences in BCRP localization in rodents and humans.

Conclusions

The expression and localization of the ENTs, CNTs, OCTs, OCTNs, OATs, OATPs, MATEs, MRPs, P-gp, and BCRP at the human BTB are summarized in this review. There is growing evidence that expression of drug transporters is not limited to SCs, and other cell types may contribute to overall drug disposition across the BTB. However, it is important to note that transporters expressed by SCs are still generally required for drug delivery across the BTB. There are also major discrepancies in transporter expression and localization between rodents and humans that would, for at least some classes of compounds, preclude the use of rodent models for testicular disposition and toxicity studies. The transporter expression and localization differences between species must be fully understood to accurately extrapolate the pharmacokinetic and pharmacodynamic difficulties presented by the BTB. For example, there are conflicting reports for the expression profiles of two major efflux transporters,

BCRP and MRP4, which participate in the transport of many clinically relevant drugs. If these previous observations are accurate, testicular disposition and/or reproductive toxicity data for BCRP or MRP4 substrates derived from rodent studies will be misleading when extrapolating to humans. Therefore, studies using primates may be mandatory to accurately predict *in vivo* disposition of these drugs. Unfortunately, the map of drug transporter expression in human testes (Figure 2, Table 1), limited as it is, is largely unavailable for rodents and nonhuman primates. Additional histological studies with tissues from preclinical species and humans are necessary to develop a complete picture of transporters in the testes. Functional transport studies are also warranted to corroborate the histological observations made in different species, although the lack of representative *in vitro* models challenges the observations from these confirmatory studies. Altogether, these findings will provide valuable insight into the testicular transport processes in different species and guide experimental design to correctly predict testicular disposition and/or toxicity in humans.

DMD Fast Forward. Published on February 2, 2023 as DOI: 10.1124/dmd.122.001186
This article has not been copyedited and formatted. The final version may differ from this version.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Hau, Wright, and Cherrington

References

A. A. Aly H and G. Eid B (2020) Cisplatin induced testicular damage through mitochondria mediated apoptosis, inflammation and oxidative stress in rats: impact of resveratrol. *Endocr J* **67:**969-980.

Agarwal A and Said TM (2004) Carnitines and male infertility. *Reprod Biomed Online* **8:**376-384.

Ashraf T, Kao A, and Bendayan R (2014) Functional expression of drug transporters in glial cells: potential role on drug delivery to the CNS. *Adv Pharmacol* **71:**45-111.

Augustine LM, Markelewicz RJ, Jr., Boekelheide K, and Cherrington NJ (2005) Xenobiotic and endobiotic transporter mRNA expression in the blood-testis barrier. *Drug Metab Dispos* **33:**182-189.

Bakos É, Német O, Kucsma N, Tőkési N, Stieger B, Rushing E, Tőkés AM, Kele P, Tusnády GE, and Özvegy-Laczka C (2022) Cloning and characterization of a

novel functional organic anion transporting polypeptide 3A1 isoform highly expressed in the human brain and testis. *Front Pharmacol* **13:**958023.

Bakos E, Tusnady GE, Nemet O, Patik I, Magyar C, Nemeth K, Kele P, and Ozvegy-Laczka C (2020) Synergistic transport of a fluorescent coumarin probe marks coumarins as pharmacological modulators of Organic anion-transporting polypeptide, OATP3A1. *Biochem Pharmacol* **182:**114250.

Barros LF, Yudilevich DL, Jarvis SM, Beaumont N, Young JD, and Baldwin SA (1995) Immunolocalisation of nucleoside transporters in human placental trophoblast and endothelial cells: evidence for multiple transporter isoforms. *Pflugers Arch* **429:**394-399.

Bart J, Hollema H, Groen HJ, de Vries EG, Hendrikse NH, Sleijfer DT, Wegman TD, Vaalburg W, and van der Graaf WT (2004) The distribution of drug-efflux pumps, P-gp, BCRP, MRP1 and MRP2, in the normal blood-testis barrier and in primary testicular tumours. *Eur J Cancer* **40:**2064-2070.

Bera TK, Iavarone C, Kumar V, Lee S, Lee B, and Pastan I (2002) MRP9, an unusual truncated member of the ABC transporter superfamily, is highly expressed in breast cancer. *Proc Natl Acad Sci U S A* **99:**6997-7002.

Bernardino RL, Costa AR, Martins AD, Silva J, Barros A, Sousa M, Sa R, Alves MG, and Oliveira PF (2016) Estradiol modulates Na(+) -dependent HCO3 (-) transporters altering intracellular pH and ion transport in human Sertoli cells: A role on male fertility? *Biol Cell* **108:**179-188.

Bleasby K, Castle JC, Roberts CJ, Cheng C, Bailey WJ, Sina JF, Kulkarni AV, Hafey MJ, Evers R, Johnson JM, Ulrich RG, and Slatter JG (2006) Expression profiles

of 50 xenobiotic transporter genes in humans and pre-clinical species: a resource for investigations into drug disposition. *Xenobiotica* **36:**963-988.

Bowman WP, Aur RJ, Hustu HO, and Rivera G (1984) Isolated testicular relapse in acute lymphocytic leukemia of childhood: categories and influence on survival. *J Clin Oncol* **2:**924-929.

Bremer J (1983) Carnitine--metabolism and functions. *Physiol Rev* **63:**1420-1480.

Byrn RA and Kiessling AA (1998) Analysis of human immunodeficiency virus in semen: indications of a genetically distinct virus reservoir. *J Reprod Immunol* **41:**161-176.

Cerveny L, Ptackova Z, Ceckova M, Karahoda R, Karbanova S, Jiraskova L, Greenwood SL, Glazier JD, and Staud F (2018) Equilibrative Nucleoside Transporter 1 (ENT1, SLC29A1) Facilitates Transfer of the Antiretroviral Drug Abacavir across the Placenta. *Drug Metab Dispos* **46:**1817-1826.

Chambers IG, Kumar P, Lichtenberg J, Wang P, Yu J, Phillips JD, Kane MA, Bodine D, and Hamza I (2022) MRP5 and MRP9 play a concerted role in male reproduction and mitochondrial function. *Proc Natl Acad Sci U S A* **119**.

Chu JY, O'Connor DM, Cradock TV, and Gale GB (1982) Isolated testicular relapse five years after cessation of chemotherapy for acute lymphoblastic leukemia. *Clin Pediatr (Phila)* **21:**376-377.

Chui K, Trivedi A, Cheng CY, Cherbavaz DB, Dazin PF, Huynh AL, Mitchell JB, Rabinovich GA, Noble-Haeusslein LJ, and John CM (2011) Characterization and functionality of proliferative human Sertoli cells. *Cell Transplant* **20:**619-635.

Cooper DR and Carpenter MP (1987) Sertoli-cell prostaglandin synthesis. Effects of (follitropin) differentiation and dietary vitamin E. *Biochem J* **241:**847-855.

Deen GF, Broutet N, Xu W, Knust B, Sesay FR, McDonald SLR, Ervin E, Marrinan JE, Gaillard P, Habib N, Liu H, Liu W, Thorson AE, Yamba F, Massaquoi TA, James F, Ariyarajah A, Ross C, Bernstein K, Coursier A, Klena J, Carino M, Wurie AH, Zhang Y, Dumbuya MS, Abad N, Idriss B, Wi T, Bennett SD, Davies T, Ebrahim FK, Meites E, Naidoo D, Smith SJ, Ongpin P, Malik T, Banerjee A, Erickson BR, Liu Y, Liu Y, Xu K, Brault A, Durski KN, Winter J, Sealy T, Nichol ST, Lamunu M, Bangura J, Landoulsi S, Jambai A, Morgan O, Wu G, Liang M, Su Q, Lan Y, Hao Y, Formenty P, Stroher U, and Sahr F (2017) Ebola RNA Persistence in Semen of Ebola Virus Disease Survivors - Final Report. *N Engl J Med* **377:**1428-1437.

Dym M and Fawcett DW (1970) The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol Reprod* **3:**308-326.

Enokizono J, Kusuhara H, and Sugiyama Y (2007) Effect of breast cancer resistance protein (Bcrp/Abcg2) on the disposition of phytoestrogens. *Mol Pharmacol* **72:**967-975.

Enomoto A, Takeda M, Taki K, Takayama F, Noshiro R, Niwa T, and Endou H (2003) Interactions of human organic anion as well as cation transporters with indoxyl sulfate. *Eur J Pharmacol* **466:**13-20.

Errasti-Murugarren E, Diaz P, Godoy V, Riquelme G, and Pastor-Anglada M (2011) Expression and distribution of nucleoside transporter proteins in the human syncytiotrophoblast. *Mol Pharmacol* **80:**809-817.

Guideline on the investigation of drug interactions. European Medicines Agency. <https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions-revision-1_en.pdf> (2013). Accessed November 30, 2022.

Fijak M and Meinhardt A (2006) The testis in immune privilege. *Immunol Rev* **213:**66-81.

Filipski KK, Mathijssen RH, Mikkelsen TS, Schinkel AH, and Sparreboom A (2009) Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. *Clin Pharmacol Ther* **86:**396-402.

Fujiwara K, Adachi H, Nishio T, Unno M, Tokui T, Okabe M, Onogawa T, Suzuki T, Asano N, Tanemoto M, Seki M, Shiiba K, Suzuki M, Kondo Y, Nunoki K, Shimosegawa T, Iinuma K, Ito S, Matsuno S, and Abe T (2001) Identification of thyroid hormone transporters in humans: different molecules are involved in a tissue-specific manner. *Endocrinology* **142:**2005-2012.

Gajjar A, Ribeiro RC, Mahmoud HH, Sandlund JT, Liu Q, Furman WL, Santana VM, Crist WM, Rivera GK, and Pui CH (1996) Overt testicular disease at diagnosis is associated with high risk features and a poor prognosis in patients with childhood acute lymphoblastic leukemia. *Cancer* **78:**2437-2442.

Gao B, Stieger B, Noe B, Fritschy JM, and Meier PJ (1999) Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain. *J Histochem Cytochem* **47:**1255-1264.

Gerber J, Rode K, Hambruch N, Langeheine M, Schnepel N, and Brehm R (2020) Establishment and functional characterization of a murine primary Sertoli cell line deficient of connexin43. *Cell Tissue Res* **381:**309-326.

Govindarajan R, Bakken AH, Hudkins KL, Lai Y, Casado FJ, Pastor-Anglada M, Tse CM, Hayashi J, and Unadkat JD (2007) In situ hybridization and immunolocalization of concentrative and equilibrative nucleoside transporters in the human intestine, liver, kidneys, and placenta. *Am J Physiol Regul Integr Comp Physiol* **293:**R1809-1822.

Gray JH, Owen RP, and Giacomini KM (2004) The concentrative nucleoside transporter family, SLC28. *Pflugers Arch* **447:**728-734.

Grundemann D, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A, and Schomig E (2005) Discovery of the ergothioneine transporter. *Proc Natl Acad Sci U S A* **102:**5256-5261.

Grundy RG, Leiper AD, Stanhope R, and Chessells JM (1997) Survival and endocrine outcome after testicular relapse in acute lymphoblastic leukaemia. *Arch Dis Child* **76:**190-196.

Grunewald R, Abbruzzese JL, Tarassoff P, and Plunkett W (1991) Saturation of 2',2'-difluorodeoxycytidine 5'-triphosphate accumulation by mononuclear cells during a phase I trial of gemcitabine. *Cancer Chemother Pharmacol* **27:**258-262.

Hau RK, Klein RR, Wright SH, and Cherrington NJ (2022) Localization of Xenobiotic Transporters Expressed at the Human Blood-Testis Barrier. *Drug Metab Dispos* **50:**770-780.

Hau RK, Miller SR, and Cherrington NJ (2021) Implications of species differences in function and localization of transporters at the blood-testis barrier. *Toxicol Sci* **181:**1-2.

Hau RK, Miller SR, Wright SH, and Cherrington NJ (2020) Generation of a hTERT-Immortalized Human Sertoli Cell Model to Study Transporter Dynamics at the Blood-Testis Barrier. *Pharmaceutics* **12:**1005.

Heaney JA, Klauber GT, and Conley GR (1983) Acute leukemia: diagnosis and management of testicular involvement. *Urology* **21:**573-577.

Hiasa M, Matsumoto T, Komatsu T, and Moriyama Y (2006) Wide variety of locations for rodent MATE1, a transporter protein that mediates the final excretion step for toxic organic cations. *Am J Physiol Cell Physiol* **291:**C678-686.

Hiasa M, Matsumoto T, Komatsu T, Omote H, and Moriyama Y (2007) Functional characterization of testis-specific rodent multidrug and toxic compound extrusion 2, a class III MATE-type polyspecific H+/organic cation exporter. *Am J Physiol Cell Physiol* **293:**C1437-1444.

Houzet L, Matusali G, and Dejucq-Rainsford N (2014) Origins of HIV-infected leukocytes and virions in semen. *J Infect Dis* **210 Suppl 3:**S622-630.

Huang Y, Hoque MT, Jenabian MA, Vyboh K, Whyte SK, Sheehan NL, Brassard P, Belanger M, Chomont N, Fletcher CV, Routy JP, and Bendayan R (2016) Antiretroviral drug transporters and metabolic enzymes in human testicular tissue: potential contribution to HIV-1 sanctuary site. *J Antimicrob Chemother* **71:**1954-1965.

Huber RD, Gao B, Sidler Pfandler MA, Zhang-Fu W, Leuthold S, Hagenbuch B, Folkers G, Meier PJ, and Stieger B (2007) Characterization of two splice variants of human organic anion transporting polypeptide 3A1 isolated from human brain. *Am J Physiol Cell Physiol* **292:**C795-806.

Kato R, Maeda T, Akaike T, and Tamai I (2005) Nucleoside transport at the blood-testis barrier studied with primary-cultured sertoli cells. *J Pharmacol Exp Ther* **312:**601-608.

Kato R, Maeda T, Akaike T, and Tamai I (2006) Characterization of novel Na+-dependent nucleobase transport systems at the blood-testis barrier. *Am J Physiol Endocrinol Metab* **290:**E968-975.

Kato R, Maeda T, Akaike T, and Tamai I (2009) Characterization of nucleobase transport by mouse Sertoli cell line TM4. *Biol Pharm Bull* **32:**450-455.

Kiessling AA, Fitzgerald LM, Zhang D, Chhay H, Brettler D, Eyre RC, Steinberg J, McGowan K, and Byrn RA (1998) Human immunodeficiency virus in semen arises from a genetically distinct virus reservoir. *AIDS Res Hum Retroviruses* **14 Suppl 1:**S33-41.

Klaassen CD and Aleksunes LM (2010) Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev* **62:**1-96.

Klein DM, Evans KK, Hardwick RN, Dantzler WH, Wright SH, and Cherrington NJ (2013) Basolateral uptake of nucleosides by Sertoli cells is mediated primarily by equilibrative nucleoside transporter 1. *J Pharmacol Exp Ther* **346:**121-129.

Klein DM, Wright SH, and Cherrington NJ (2014) Localization of multidrug resistance-associated proteins along the blood-testis barrier in rat, macaque, and human testis. *Drug Metab Dispos* **42:**89-93.

Kobayashi D, Goto A, Maeda T, Nezu J, Tsuji A, and Tamai I (2005) OCTN2-mediated transport of carnitine in isolated Sertoli cells. *Reproduction* **129:**729-736.

Koraichi F, Inoubli L, Lakhdari N, Meunier L, Vega A, Mauduit C, Benahmed M, Prouillac C, and Lecoeur S (2013) Neonatal exposure to zearalenone induces long term modulation of ABC transporter expression in testis. *Toxicology* **310:**29-38.

Kulkarni KP, Marwaha RK, Trehan A, and Bansal D (2010) Testicular relapse in childhood acute lymphoblastic leukemia: the challenges and lessons. *Indian J Cancer* **47:**134-138.

Lee SY, Williamson B, Caballero OL, Chen YT, Scanlan MJ, Ritter G, Jongeneel CV, Simpson AJ, and Old LJ (2004) Identification of the gonad-specific anion transporter SLCO6A1 as a cancer/testis (CT) antigen expressed in human lung cancer. *Cancer Immun* **4:**13.

Levine N and Marsh DJ (1971) Micropuncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. *J Physiol* **213:**557-570.

Li J, Liu F, Liu X, Liu J, Zhu P, Wan F, Jin S, Wang W, Li N, Liu J, and Wang H (2011) Mapping of the human testicular proteome and its relationship with that of the epididymis and spermatozoa. *Mol Cell Proteomics* **10:**M110 004630.

Li N, Wang T, and Han D (2012) Structural, cellular and molecular aspects of immune privilege in the testis. *Front Immunol* **3:**152.

Lickteig AJ, Cheng X, Augustine LM, Klaassen CD, and Cherrington NJ (2008) Tissue distribution, ontogeny and induction of the transporters Multidrug and toxin extrusion (MATE) 1 and MATE2 mRNA expression levels in mice. *Life Sci* **83:**59-64.

Ma X, Guan C, Chen R, Wang Y, Feng S, Wang R, Qu G, Zhao S, Wang F, Wang X, Zhang D, Liu L, Liao A, and Yuan S (2021) Pathological and molecular examinations of postmortem testis biopsies reveal SARS-CoV-2 infection in the testis and spermatogenesis damage in COVID-19 patients. *Cell Mol Immunol* **18:**487-489.

Mackey JR, Mani RS, Selner M, Mowles D, Young JD, Belt JA, Crawford CR, and Cass CE (1998) Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. *Cancer Res* **58:**4349-4357.

Mackey JR, Yao SY, Smith KM, Karpinski E, Baldwin SA, Cass CE, and Young JD (1999) Gemcitabine transport in xenopus oocytes expressing recombinant plasma membrane mammalian nucleoside transporters. *J Natl Cancer Inst* **91:**1876-1881.

Madshus IH (1988) Regulation of intracellular pH in eukaryotic cells. *Biochem J* **250:**1-8.

Maeda T, Goto A, Kobayashi D, and Tamai I (2007) Transport of organic cations across the blood-testis barrier. *Mol Pharm* **4:**600-607.

Masuda S, Takeuchi A, Saito H, Hashimoto Y, and Inui K (1999) Functional analysis of rat renal organic anion transporter OAT-K1: bidirectional methotrexate transport in apical membrane. *FEBS Lett* **459:**128-132.

Melaine N, Lienard MO, Dorval I, Le Goascogne C, Lejeune H, and Jegou B (2002) Multidrug resistance genes and p-glycoprotein in the testis of the rat, mouse, Guinea pig, and human. *Biol Reprod* **67:**1699-1707.

Miller SR, Jilek JL, McGrath ME, Hau RK, Jennings EQ, Galligan JJ, Wright SH, and Cherrington NJ (2021a) Testicular disposition of clofarabine in rats is dependent on equilibrative nucleoside transporters. *Pharmacol Res Perspect* **9:**e00831.

Miller SR, McGrath ME, Zorn KM, Ekins S, Wright SH, and Cherrington NJ (2021b) Remdesivir and EIDD-1931 Interact with Human Equilibrative Nucleoside Transporters 1 and 2: Implications for Reaching SARS-CoV-2 Viral Sanctuary Sites. *Mol Pharmacol* **100:**548-557.

Mital P, Hinton BT, and Dufour JM (2011) The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biol Reprod* **84:**851-858.

Mongioi L, Calogero AE, Vicari E, Condorelli RA, Russo GI, Privitera S, Morgia G, and La Vignera S (2016) The role of carnitine in male infertility. *Andrology* **4:**800-807.

Morgan JA, Cheepala SB, Wang Y, Neale G, Adachi M, Nachagari D, Leggas M, Zhao W, Boyd K, Venkataramanan R, and Schuetz JD (2012) Deregulated hepatic metabolism exacerbates impaired testosterone production in Mrp4-deficient mice. *J Biol Chem* **287:**14456-14466.

Morgan JA, Lynch J, Panetta JC, Wang Y, Frase S, Bao J, Zheng J, Opferman JT, Janke L, Green DM, Chemaitilly W, and Schuetz JD (2015) Apoptosome

activation, an important molecular instigator in 6-mercaptopurine induced Leydig cell death. *Sci Rep* **5:**16488.

Mruk DD and Cheng CY (2011) An in vitro system to study Sertoli cell blood-testis barrier dynamics. *Methods Mol Biol* **763:**237-252.

Mruk DD and Cheng CY (2015) The mammalian blood-testis barrier: its biology and regulation. *Endocr Rev* **36:**564-591.

Mruk DD, Su L, and Cheng CY (2011) Emerging role for drug transporters at the blood-testis barrier. *Trends Pharmacol Sci* **32:**99-106.

Nesbit ME, Jr., Robison LL, Ortega JA, Sather HN, Donaldson M, and Hammond D (1980) Testicular relapse in childhood acute lymphoblastic leukemia: association with pretreatment patient characteristics and treatment. A report for Childrens Cancer Study Group. *Cancer* **45:**2009-2016.

Oliveira PF, Sousa M, Barros A, Moura T, and Rebelo da Costa A (2009a) Intracellular pH regulation in human Sertoli cells: role of membrane transporters. *Reproduction* **137:**353-359.

Oliveira PF, Sousa M, Barros A, Moura T, and Rebelo da Costa A (2009b) Membrane transporters and cytoplasmatic pH regulation on bovine Sertoli cells. *J Membr Biol* **227:**49-55.

Ono N, Van der Heijden I, Scheffer GL, Van de Wetering K, Van Deemter E, De Haas M, Boerke A, Gadella BM, De Rooij DG, Neefjes JJ, Groothuis TA, Oomen L, Brocks L, Ishikawa T, and Borst P (2007) Multidrug resistance-associated protein 9 (ABCC12) is present in mouse and boar sperm. *Biochem J* **406:**31-40.

Ose A, Kusuhara H, Endo C, Tohyama K, Miyajima M, Kitamura S, and Sugiyama Y (2010) Functional characterization of mouse organic anion transporting peptide 1a4 in the uptake and efflux of drugs across the blood-brain barrier. *Drug Metab Dispos* **38:**168-176.

Palmero S, Bottazzi C, Costa M, Leone M, and Fugassa E (2000) Metabolic effects of L-carnitine on prepubertal rat Sertoli cells. *Horm Metab Res* **32:**87-90.

Papadopoulos D, Dietze R, Shihan M, Kirch U, and Scheiner-Bobis G (2016) Dehydroepiandrosterone Sulfate Stimulates Expression of Blood-Testis-Barrier Proteins Claudin-3 and -5 and Tight Junction Formation via a Gnalpha11-Coupled Receptor in Sertoli Cells. *PLoS One* **11:**e0150143.

Pelletier RM (2011) The blood-testis barrier: the junctional permeability, the proteins and the lipids. *Prog Histochem Cytochem* **46:**49-127.

Guideline on drug interaction for drug development and appropriate provision of information. Pharmaceuticals and Medical Devices Agency. <https://www.pmda.go.jp/files/000228122.pdf> (2019). Accessed November 30, 2022.

Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, and Meier PJ (2002) Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. *Mol Endocrinol* **16:**2283-2296.

Prozialeck WC and Lamar PC (1997) Cadmium (Cd2+) disrupts E-cadherin-dependent cell-cell junctions in MDCK cells. *In Vitro Cell Dev Biol Anim* **33:**516-526.

Qian X, Mruk DD, Wong EW, and Cheng CY (2013) Breast cancer resistance protein regulates apical ectoplasmic specialization dynamics stage specifically in the rat testis. *Am J Physiol Endocrinol Metab* **304:**E757-769.

Ritzen EM (1990) Testicular relapse of acute lymphoblastic leukemia (ALL). *J Reprod Immunol* **18:**117-121.

Robinson CL, Chong ACN, Ashbrook AW, Jeng G, Jin J, Chen H, Tang EI, Martin LA, Kim RS, Kenyon RM, Do E, Luna JM, Saeed M, Zeltser L, Ralph H, Dudley VL, Goldstein M, Rice CM, Cheng CY, Seandel M, and Chen S (2018) Male germ cells support long-term propagation of Zika virus. *Nat Commun* **9:**2090.

Roth M, Obaidat A, and Hagenbuch B (2012) OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. *Br J Pharmacol* **165:**1260-1287.

Samy ET, Li JC, Grima J, Lee WM, Silvestrini B, and Cheng CY (2000) Sertoli cell prostaglandin D2 synthetase is a multifunctional molecule: its expression and regulation. *Endocrinology* **141:**710-721.

Sano Y, Mizuno T, Mochizuki T, Uchida Y, Umetsu M, Terasaki T, and Kusuhara H (2018) Evaluation of Organic Anion Transporter 1A2-knock-in Mice as a Model of Human Blood-brain Barrier. *Drug Metab Dispos* **46:**1767-1775.

Schell C, Frungieri MB, Albrecht M, Gonzalez-Calvar SI, Kohn FM, Calandra RS, and Mayerhofer A (2007) A prostaglandin D2 system in the human testis. *Fertil Steril* **88:**233-236.

Schnabolk GW, Gupta B, Mulgaonkar A, Kulkarni M, and Sweet DH (2010) Organic anion transporter 6 (Slc22a20) specificity and Sertoli cell-specific expression

provide new insight on potential endogenous roles. *J Pharmacol Exp Ther* **334:**927-935.

Senyavina NV, Gerasimenko TN, Fomicheva KA, Tonevitskaya SA, and Kaprin AD (2016) Localization and Expression of Nucleoside Transporters ENT1 and ENT2 in Polar Cells of Intestinal Epithelium. *Bull Exp Biol Med* **160:**771-774.

Shaffer DW, Burris HA, and O'Rourke TJ (1992) Testicular relapse in adult acute myelogenous leukemia. *Cancer* **70:**1541-1544.

Siemann DN, Strange DP, Maharaj PN, Shi PY, and Verma S (2017) Zika Virus Infects Human Sertoli Cells and Modulates the Integrity of the In Vitro Blood-Testis Barrier Model. *J Virol* **91**.

Soni KK, Kim HK, Choi BR, Karna KK, You JH, Cha JS, Shin YS, Lee SW, Kim CY, and Park JK (2016) Dose-dependent effects of cisplatin on the severity of testicular injury in Sprague Dawley rats: reactive oxygen species and endoplasmic reticulum stress. *Drug Des Devel Ther* **10:**3959-3968.

Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, and Hickman JJ (2015) TEER measurement techniques for in vitro barrier model systems. *J Lab Autom* **20:**107-126.

Su L, Cheng CY, and Mruk DD (2009) Drug transporter, P-glycoprotein (MDR1), is an integrated component of the mammalian blood-testis barrier. *Int J Biochem Cell Biol* **41:**2578-2587.

Su L, Mruk DD, Lee WM, and Cheng CY (2011) Drug transporters and blood--testis barrier function. *J Endocrinol* **209:**337-351.

Suzuki T, Onogawa T, Asano N, Mizutamari H, Mikkaichi T, Tanemoto M, Abe M, Satoh F, Unno M, Nunoki K, Suzuki M, Hishinuma T, Goto J, Shimosegawa T, Matsuno S, Ito S, and Abe T (2003) Identification and characterization of novel rat and human gonad-specific organic anion transporters. *Mol Endocrinol* **17:**1203-1215.

Tamai I, Nezu J, Uchino H, Sai Y, Oku A, Shimane M, and Tsuji A (2000) Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem Biophys Res Commun* **273:**251-260.

Tamai I, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y, and Tsuji A (1998) Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. *J Biol Chem* **273:**20378-20382.

Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M, and Tsuji A (1997) Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. *FEBS Lett* **419:**107-111.

Tammur J, Prades C, Arnould I, Rzhetsky A, Hutchinson A, Adachi M, Schuetz JD, Swoboda KJ, Ptacek LJ, Rosier M, Dean M, and Allikmets R (2001) Two new genes from the human ATP-binding cassette transporter superfamily, ABCC11 and ABCC12, tandemly duplicated on chromosome 16q12. *Gene* **273:**89-96.

Tang NL, Ganapathy V, Wu X, Hui J, Seth P, Yuen PM, Wanders RJ, Fok TF, and Hjelm NM (1999) Mutations of OCTN2, an organic cation/carnitine transporter, lead to deficient cellular carnitine uptake in primary carnitine deficiency. *Hum Mol Genet* **8:**655-660.

Tombolini V, Banelli E, Capua A, Giona F, and Vitturini A (1986) Radiation treatment of testicular relapse in acute lymphoblastic leukemia. *Acta Radiol Oncol* **25:**29-32.

Tsetsarkin KA, Acklin JA, Liu G, Kenney H, Teterina NL, Pletnev AG, and Lim JK (2020) Zika virus tropism during early infection of the testicular interstitium and its role in viral pathogenesis in the testes. *PLoS Pathog* **16:**e1008601.

Clolar (Clofarabine) FDA Package Insert. U.S. Food and Drug Administration. <https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/021673s023lbl.pdf > (2004). Accessed November 30, 2022.

Cisplatin FDA Package Insert. U.S. Food and Drug Administration. <https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/018057s089lbl.pdf > (2019). Accessed November 30, 2022.

In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry. U.S. Food and Drug Administration. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/in-vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions> (2020). Accessed November 30, 2022.

Wang Y, Ye J, Ganapathy V, and Longo N (1999) Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. *Proc Natl Acad Sci U S A* **96:**2356-2360.

Wu H, Jiang X, Gao Y, Liu W, Wang F, Gong M, Chen R, Yu X, Zhang W, Gao B, Song C, and Han D (2019) Mumps virus infection disrupts blood-testis barrier through the induction of TNF-alpha in Sertoli cells. *FASEB J* **33:**12528-12540.

Young JD, Yao SY, Baldwin JM, Cass CE, and Baldwin SA (2013) The human concentrative and equilibrative nucleoside transporter families, SLC28 and SLC29. *Mol Aspects Med* **34:**529-547.

Zhao S, Zhu W, Xue S, and Han D (2014) Testicular defense systems: immune privilege and innate immunity. *Cell Mol Immunol* **11:**428-437.

Footnotes

This work was supported by funding from the National Institutes of General Medical Sciences [Grants R01GM123643 and R01GM129777], National Institute of Environmental Health Sciences [Grants T32ES007091 and P30ES006694], and National Cancer Institute [Grant P30CA023074]. The authors declare no conflicts of interest.

Reprint Requests

Nathan J. Cherrington, Department of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85719. Email: cherring@pharmacy.arizona.edu

Figure Legends

Figure 1: Illustration of a seminiferous tubule cross section and a drug transporter pathway to bypass the BTB. LCs and vascular endothelial cells (blood capillary) occupy the interstitial tissue around the seminiferous tubules. PMCs line the outer edge to provide structural stability and contractility for moving spermatids through the seminiferous tubule. Within the seminiferous tubule are SCs that form intercellular junctional complexes that comprise the physical component of the BTB. Spermatogonia lie along the basal membranes of SCs exposed to the basal compartment, where they undergo mitotic division into primary spermatocytes and migrate past the junctional complexes. Primary spermatocytes undergo meiotic division to form secondary spermatocytes that will divide again into spermatids, which all occur in the adluminal compartment. Drug transporters expressed at the basal and apicolateral membranes of SCs can form a transport pathway for drugs in the blood to circumvent the BTB. Figure created with BioRender.com.

Figure 2: Localization of drug transporters in human testes. Summary of the localization of pharmacologically relevant drug transporters and their directionality in SCs, PMCs, LCs, developing germ cells, and vascular endothelial cells (blood capillary) in human testes. Question marks (?) indicate unknown directionality (CNT2 and MATE1) due to lack of functional transport analysis. Asterisks (*) indicate transporters (OATPs) that are speculated to localize to both the basal and apicolateral membranes

of SCs based on positive immunostaining in human tissues but lack functional analysis to confirm this observation. Figure created with BioRender.com.

Tables

Table 1: Testicular transporter expression/localization similarities and differences between species.

	Expression/Localization (supporting analytical methods)			
Transporter	**Human**	**Nonhuman Primate**	**Rat**	**Mouse**
CNT1	• Testis - unknown location **(Western blot and mRNA analysis)**		• Sertoli cells - unknown location **(mRNA analysis)**	
CNT2	• Sertoli cell apicolateral membrane **(immunolocalization and mRNA analysis)**		• Sertoli cells - unknown location **(mRNA analysis)**	• Sertoli cells - unknown location **(mRNA analysis)**
CNT3			• Sertoli cells - unknown location **(mRNA analysis)**	• Undetected **(mRNA analysis)**
ENT1	• Sertoli cell basal membrane • Leydig cells • Developing germ cells **(immunolocalization, mRNA and functional**		• Sertoli cell basal membrane • Leydig cells • Developing germ cells **(immunlocalization, mRNA and functional**	• Sertoli cell basal membrane **(functional Transwell transport analysis)**

	transport analysis)		**Transwell transport analysis)**	
ENT2	• Sertoli cell apicolateral membrane • Leydig cells • Developing germ cells **(immunolocalization, Western blot, and mRNA analysis)**		• Sertoli cell apicolateral membrane • Leydig cells • Developing germ cells **(immunolocalization and mRNA analysis)**	• Sertoli cells - unknown location **(mRNA analysis)**
OCT1	• Sertoli cell basal membrane • Peritubular myoid cells • Leydig cells **(immunolocalization and mRNA analysis)**		• Sertoli cell basal membrane **(mRNA and functional Transwell transport analysis)**	
OCT2	• Sertoli cell basal membrane • Peritubular myoid cells • Leydig cells **(immunolocalization and mRNA analysis)**		• Undetected[1] **(mRNA analysis)**	
OCT3	• Sertoli cell basal		• Sertoli cell apicolateral	

	membrane • Peritubular myoid cells • Leydig cells • Round spermatids **(immunolocalization and mRNA analysis)**		membrane[1] **(mRNA and functional Transwell transport analysis)**	
OCTN1	• Sertoli cell basal membrane **(immunolocalization and mRNA analysis)**		• Sertoli cell apicolateral membrane[1] **(mRNA and functional Transwell transport analysis)**	
OCTN2	• Sertoli cell basal membrane **(immunolocalization and mRNA analysis)**		• Sertoli cell basal membrane **(Western blot, mRNA and functional Transwell transport analysis)**	
OAT1	• Sertoli cell basal membrane • Leydig cells • Vascular endothelial cell luminal membrane **(immunolocalization,**		• Undetected[1] **(mRNA analysis)**	

		Western blot, and mRNA analysis)			
OAT2		• Sertoli cell basal membrane **(immunolocalization and mRNA analysis)**		• Sertoli cells - unknown location **(mRNA analysis)**	
OAT3		• Sertoli cells - unknown location **(mRNA analysis)**		• Undetected[1] **(mRNA analysis)**	
mOAT6					• Sertoli cells - unknown location **(mRNA analysis)**
OATP1A2	r/mOATP1A1	• Sertoli cell basal membrane • Sertoli cell apicolateral membrane **(immunolocalization and mRNA analysis)**		• Rodent OATP1A1 and OATP1A6 Undetected[1] **(mRNA analysis)** • Rodent OATP1A4 and OATP1A5 Sertoli cells - unknown location **(mRNA analysis)**	
	rOATP1A3				
	r/mOATP1A4				
	r/mOATP1A5				
	r/mOATP1A6				
OATP1B1	r/mOATP1B2	• Sertoli cell basal membrane • Sertoli cell apicolateral		• Rodent OATP1B2 Undetected[1] **(mRNA analysis)**	

		membrane **(immunolocalization)**			
OATP1B3		• Sertoli cell basal membrane • Sertoli cell apicolateral membrane **(immunolocalization and mRNA analysis)**		• Rodent OATP1B2 Undetected[1] **(mRNA analysis)**	
OATP1C1		• Leydig cells **(immunolocalization)**			
OATP2A1		• Sertoli cell basal membrane **(immunolocalization and mRNA analysis)**		• Sertoli cells - unknown location **(mRNA analysis)**	
OATP2B1		• Sertoli cell basal membrane • Sertoli cell apicolateral membrane **(immunolocalization, Western blot, and mRNA analysis)**			
OATP3A1-v1	rOATP3A1	• Developing germ cells			

		(immunolocalization)			
OATP3A1-v2		• Sertoli cell basal membrane • Sertoli cell apicolateral membrane **(immunolocalization and mRNA analysis)**			
OATP3A1-v3		• Developing germ cells **(immunolocalization)**			
OATP4A1		• Leydig cells **(immunolocalization)**			
OATP4C1					
OATP5A1		• Sertoli cells - unknown location **(mRNA analysis)**			
OATP6A1	r/mOATP6B1	• Sertoli cells - unknown location **(mRNA analysis)**		• Rodent OATP6B1 and OATP6C1 Sertoli cells - unknown location **(mRNA and functional Transwell transport analysis)**	
	r/mOATP6C1				

	r/mOATP6D1			• Rodent OATP6B1 and OATP6C1 Peritubular myoid cells • Rodent OATP6B1 and OATP6C1 Leydig cells **(mRNA analysis)**	
MATE1		• Sertoli cell apicolateral membrane • Sertoli cell intracellular membranes • Leydig cells **(immunolocalization and mRNA analysis)**		• Testis - unknown location **(mRNA analysis)**	• Leydig cells **(immunolocalization)**
MATE2		• Sertoli cells - unknown location **(mRNA analysis)**		• Sertoli cells - unknown location **(mRNA analysis)** • Leydig cells[1] **(immunolocalization)**	• Leydig cells[1] **(immunolocalization)**
MRP1		• Sertoli cell basal membrane • Leydig cells **(immunolocalization,**	• Sertoli cell basal membrane • Leydig cells **(immunolocalization)**	• Sertoli cell basal membrane • Leydig cells **(immunolocalization)**	

	Western blot, and mRNA analysis)			
MRP2	• Peritubular myoid cells • Leydig cells **(immunolocalization and mRNA analysis)**			
MRP3	• Sertoli cell basal membrane • Peritubular myoid cells **(immunolocalization and mRNA analysis)**			
MRP4	• Sertoli cell basal membrane • Leydig cells **(immunolocalization, Western blot, and mRNA analysis)**	• Sertoli cell basal membrane **(immunolocalization)**	• Sertoli cell basal membrane • Sertoli cell apicolateral membrane[1] • Peritubular myoid cells • Leydig cells • Round spermatids • Spermatocytes **(immunolocalization)**	• Sertoli cell apicolateral membrane[1] • Leydig cells **(immunolocalization)**
MRP5	• Leydig cells **(immunolocalization)**		• Sertoli cells - unknown location[1]	

			(mRNA analysis) • Leydig cells **(immunolocalization)**	
MRP6	• Sertoli cell basal membrane • Peritubular myoid cells **(immunolocalization and mRNA analysis)**			
MRP7	• Sertoli cell basal membrane • Peritubular myoid cells **(immunolocalization and mRNA analysis)**		• Sertoli cells - unknown location **(mRNA analysis)**	
MRP8	• Round spermatids **(immunolocalization)**	• Round spermatids **(immunolocalization)**	• No ortholog[1]	• No ortholog[1]
MRP9	• Sertoli cells - unknown location **(mRNA analysis)**		• Sertoli cells - unknown location **(mRNA analysis)**	• Spermatozoa[1] **(immunolocalization, Western blot, and mRNA analysis)**

P-gp/MDR1	r/mMDR1A	• Sertoli cell basal membrane • Sertoli cell apicolateral membrane • Peritubular myoid cells • Leydig cells • Developing germ cells • Vascular endothelial cell luminal membrane **(immunolocalization, Western blot, and mRNA analysis)**		• Sertoli cell basal membrane • Sertoli cell apicolateral membrane • Peritubular myoid cells • Leydig cells • Developing germ cells • Vascular endothelial cell luminal membrane **(immunolocalization, Western blot, mRNA and functional transport analysis)**	• Sertoli cell basal membrane • Sertoli cell apicolateral membrane • Peritubular myoid cells • Leydig cells • Developing germ cells • Vascular endothelial cell luminal membrane **(immunolocalization, Western blot, and mRNA analysis)**
	r/mMDR1B				
BCRP		• Sertoli cell basal membrane[1] • Sertoli cell apicolateral membrane • Peritubular myoid cells • Leydig cells • Developing germ cells • Vascular endothelial cell luminal membrane		• Sertoli cell apicolateral membrane • Peritubular myoid cells • Leydig cells • Developing germ cells • Vascular endothelial cell luminal membrane **(immunolocalization, Western blot, and mRNA**	• Vascular endothelial cell luminal membrane **(immunolocalization)**

	(immunolocalization, Western blot, and mRNA analysis)		**analysis)**	

[1]Indicates an observed species difference in testicular transporter localization based on currently available information. Emboldened text indicates the various experimental methods used to as evidence towards predicting transporter expression/localization.

Table 2: Canonical transporter DNA and protein sequence percent identity between species relative to humans by BLAST analysis.

Transporter	**Nonhuman Primate**		**Rat**		**Mouse**	
	DNA	**Protein**	**DNA**	**Protein**	**DNA**	**Protein**

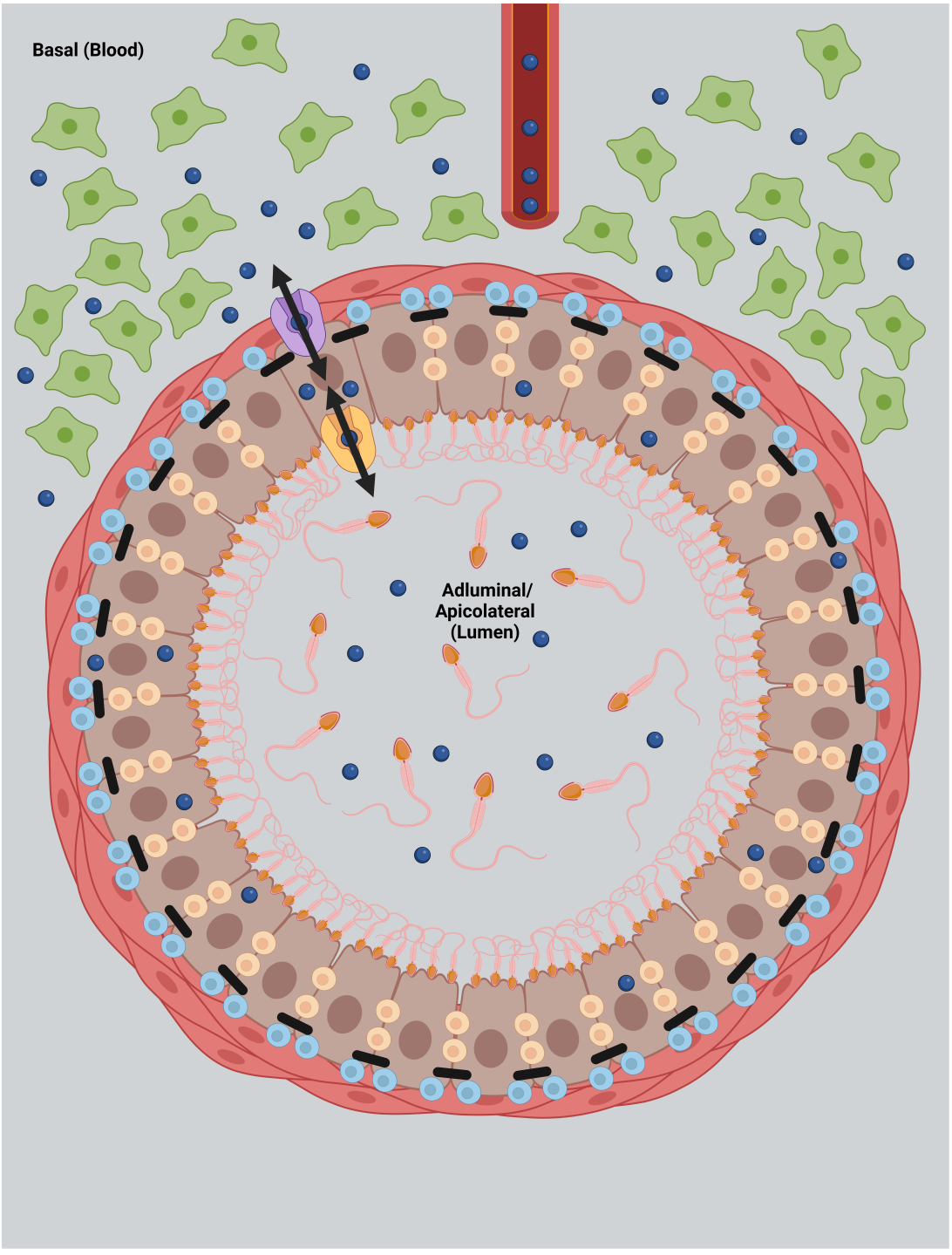
CNT1		95.8%	94.4%	83.5%	82.8%	83.4%	84.1%
CNT2		93.6%	94.5%	83.2%	80.9%	83.1%	80.2%
CNT3		94.9%	95.8%	80.0%	78.2%	78.5%	78.2%
ENT1		96.6%	99.3%	78.7%	78.1%	80.0%	79.4%
ENT2		95.7%	96.9%	86.6%	88.0%	86.5%	88.5%
OCT1		94.6%	94.2%	80.3%	77.9%	79.7%	77.7%
OCT2		95.0%	93.9%	77.6%	82.0%	82.8%	83.5%
OCT3		95.2%	97.7%	81.0%	86.3%	84.6%	86.5%
OCTN1		96.7%	98.4%	85.1%	85.4%	86.0%	84.8%
OCTN2		96.2%	94.3%	86.3%	81.9%	86.3%	81.9%
OAT1		95.3%	94.7%	83.6%	86.0%	81.4%	83.3%
OAT2		95.3%	96.5%	83.0%	79.0%	81.7%	79.0%
OAT3		96.7%	96.3%	79.6%	79.7%	78.9%	78.2%
mOAT6							
OATP1A2	r/mOATP1A1	94.9%	96.1%	76.2%	67.0%	77.2%	68.8%
	rOATP1A3			76.4%	68.4%		
	r/mOATP1A4			76.2%	72.6%	78.2%	72.8%
	r/mOATP1A5			78.1%	72.4%	78.3%	72.5%
	r/mOATP1A6			75.0%	66.4%	75.9%	65.7%
OATP1B1	r/mOATP1B2	94.5%	92.0%	75.4%	64.4%	76.0%	66.2%
OATP1B3		95.5%	92.9%				
OATP1C1		97.7%	98.8%	84.3%	86.7%	83.9%	85.7%

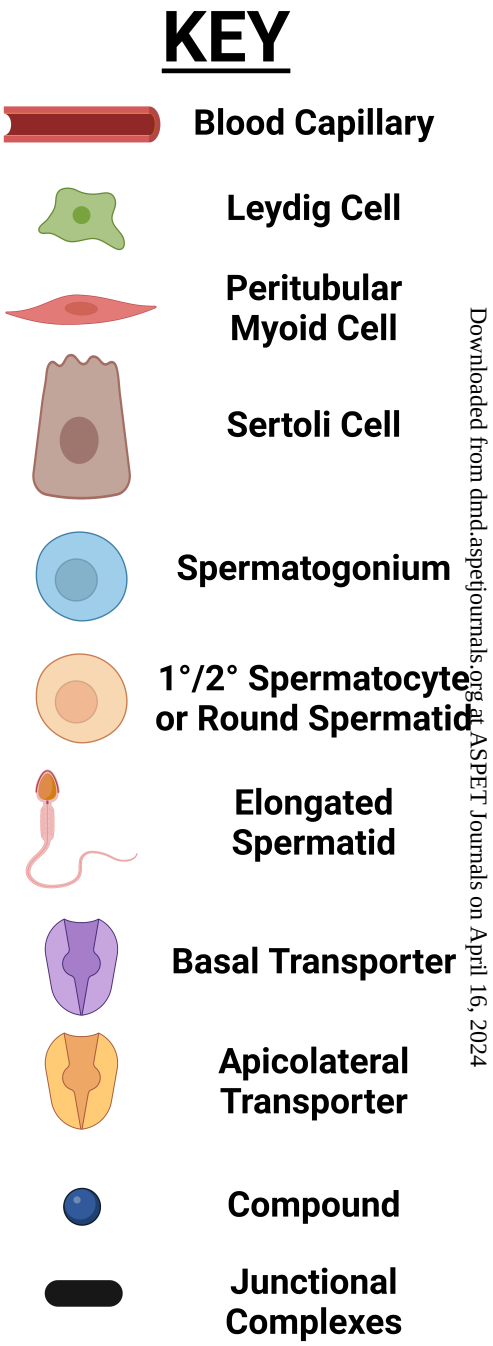
OATP2A1		97.0%	97.5%	84.0%	82.6%	84.2%	83.1%
OATP2B1		95.7%	96.9%	80.5%	76.1%	79.3%	74.4%
OATP3A1-v1	rOATP3A1	96.4%	99.3%	90.7%	95.2%	83.9%	97.9%
OATP3A1-v2		97.7%	97.6%			85.9%	97.8%
OATP3A1-v3		97.6%					
OATP4A1		93.4%	94.2%	78.4%	73.1%	79.0%	74.7%
OATP4C1		93.9%	94.3%	80.4%	80.7%	81.3%	80.8%
OATP5A1		94.8%	98.0%	81.4%	87.6%	81.2%	88.6%
OATP6A1	r/mOATP6B1	91.4%	81.6%	68.0%	46.1%	70.4%	
	r/mOATP6C1			66.7%	41.1%	67.1%	38.8%
	r/mOATP6D1			68.0%	38.9%	64.2%	36.2%
MATE1		94.6%	95.3%	80.3%	78.4%	80.7%	78.1%
MATE2		95.2%	89.5%	73.6%	45.4%	74.2%	45.7%
MRP1		95.2%	97.7%	83.3%	87.4%	83.2%	88.1%
MRP2		97.0%	95.5%	80.8%	77.5%	80.9%	77.6%
MRP3		95.8%	95.5%	81.5%	79.3%	82.1%	80.8%
MRP4		95.9%	97.4%	80.0%	86.9%	79.5%	87.1%
MRP5		98.1%	99.7%	85.6%	94.8%	85.6%	95.0%
MRP6		95.6%	96.1%	80.6%	78.8%	80.5%	79.0%
MRP7		94.8%	96.4%	83.9%	85.6%	83.5%	84.5%
MRP8		95.1%	92.2%				
MRP9		96.0%	96.2%	81.4%	83.5%	82.1%	84.5%

P-gp/MDR1	r/mMDR1A	97.1%	96.2%	83.8%	87.0%	84.1%	87.3%
	r/mMDR1B			82.4%	80.3%	82.4%	80.6%
BCRP		97.1%	96.6%	82.5%	81.0%	81.9%	81.6%

DMD Fast Forward. Published on February 2, 2023 as DOI: 10.1124/dmd.122.001186
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 1





DMD Fast Forward. Published on February 2, 2023 as DOI: 10.1124/dmd.122.001186
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 2