CHARLES UNIVERSITY IN PRAGUE

Faculty of Pharmacy in Hradec Králové

Department of Pharmacology and Toxicology

Pharmacological and physiological characteristics of organic cation transporters (OCTs) and multidrug and toxin extrusion proteins (MATEs)

Diploma Thesis

Supervisor of Diploma Thesis: Prof. PharmDr. František Štaud, Ph.D.

Hradec Králové 2013 Pavla Gregorová

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Pavla Gregorová

Acknowledgment

I would like to thank Prof. PharmDr. František Štaud, Ph.D., and Mgr. Davoud Ahmadimoghaddam for their help, advice and comments on the diploma thesis.

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Abstract:

Membrane transporters are important and life-enabling proteins of the body, which facilitate the absorption, distribution, and elimination of nutrients, metabolic waste products, drugs, and xenobiotics. Multidrug and toxin extrusion proteins (MATEs) and organic cation transporters (OCTs) belong to polyspecific transporters of the solute carrier (SLC) family and form a cooperating system of excretion in the kidney and liver. The transporters are expressed in many tissues throughout the body primarily in the kidneys, liver, heart, brain, small intestine, and placenta and transmit countless molecules from the natural neurotransmitters and hormones to exogenous compounds, such as metformine, cimetidine, 1-methyl-4-phenylpyridinium (MPP), tetraethylammonium (TEA) or acyclovir. Furthermore, there are described the most recent studies in knockout mice models and genetic polymorphisms that help identify transporters activity and pharmacokinetics with altered function. They are beneficial for discovering new safer drugs or co-medication which have reduced or any adverse effects and for improvement of the drug therapy.

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Název diplomové práce: Farmakologické a fyziologické vlastnosti transportérů organických kationtů (OCTs) a multidrug and toxin extrusion proteinů (MATEs)

Abstrakt:

Membránové transportéry jsou důležité a život umožňující proteiny těla, které usnadňují absorpci, distribuci a eliminaci živin, odpadních produktů metabolismu, léků a xenobiotik. Multidrug and toxin extrusion proteiny (MATEs) a transportéry organických kationtů (OCTs) patří mezi polyspecifické přenašeče solute carrier (SLC) rodiny a tvoří vzájemně spolupracující systém vylučování v ledvinách a játrech. Transportéry jsou exprimovány v mnoha tkáních celého těla, především v ledvinách, játrech, srdci, mozku, tenkém střevě a placentě, a přenášejí nespočet molekul, od přirozených neurotransmiterů a hormonů, po exogenní sloučeniny jako jsou metformin, cimetidin, 1-methyl-4-fenylpyridin (MPP), tetraethylammonium (TEA) nebo acyclovir. Kromě toho jsou popsány nejnovější studie na knockoutovaných myších modelech a genetické polymorfismy, které pomáhají rozpoznat aktivitu a farmakokinetiku transportérů s pozměněnou funkčností. Tyto jsou přínosné pro objevování nových bezpečnějších léků či komedikací, které mají redukované nebo žádné nežádoucí účinky, a pro zkvalitňování farmakoterapie.

Content

1 Abbreviations

AP-1	activating protein 1
AP-2	activating protein 2
cDNA	complementary DNA
cSNPs	coding single nucleotide polymorphisms
CL_{ren}	renal clearance
EMT	extraneuronal monoamine transporter
hMATE1	human multidrug and toxin extrusion 1
hMATE2	human multidrug and toxin extrusion 2
hMATE2-B	human brain specific multidrug and toxin extrusion 2
hMATE2-K	human kidney specific multidrug and toxin extrusion 2
hOCT1	human organic cation transporter 1
hOCT2	human organic cation transporter 2
hOCT3	human organic cation transporter 3
HEK	human embryonic kidney
mMate1	mouse multidrug and toxin extrusion 1
mOct1	mouse organic cation transporter 1
mOct2	mouse organic cation transporter 2
mOct3	mouse organic cation transporter 3
mRNA	messenger RNA
MATE	multidrug and toxin extrusion
MATEs	multidrug and toxin extrusion proteins

MATE1	multidrug and toxin extrusion protein 1
MATE2	multidrug and toxin extrusion protein 2
MPP	1-methyl-4-phenylpyridinium
NH_4Cl	ammonium chloride
OCs	organic cations
OCT	organic cation transporter
OCTs	organic cation transporters
OCT1	organic cation transporter 1
OCT2	organic cation transporter 2
OCT3	organic cation transporter 3
rMate1	rat multidrug and toxin extrusion 1
rMate2	rat multidrug and toxin extrusion 2
rOct1	rat organic cation transporter 1
rOct2	rat organic cation transporter 2
rOct3	rat organic cation transporter 3
RT-PCR	real time polymerase chain reaction
SLC	solute carrier
SNP	single nucleotide polymorphism
SNPs	single nucleotide polymorphisms
TEA	tetraethylammonium
TMD	transmembrane domain
TMDs	transmembrane domains

2 Introduction

Drug transporters represent a group of membrane proteins which are responsible for a number of biological processes. Their major function is the mediation of substrate transmission across the membranes. It involves a cell export, import and exchange of a variety of molecules, such as amino acids, fatty acids, nucleotides, and saccharides. Moreover, transporters have a specific physiological role in the uptake, absorption, distribution, and elimination of nutrients, drugs or foreign substances. Significant responsibility is also attributed the detoxification of environmental pollutants and metabolic waste product (You and Morris, 2007).

The goal of this diploma thesis is to focus on two types of transporters which operate in the opposite direction of substrate transmission, but mutually collaborate in excretion step of metabolic waste products, drugs and xenobiotics. My aim was to summarize the recent findings about their structure, expression, transport activity, polymorphisms and clinical implications. On one hand, there are organic cation transporters (OCTs) as the influx transporters transferring substrates into cells. On the other hand, there are multidrug and toxin extrusion proteins (MATEs) that pump the substrates out of the cells (You and Morris, 2007). Specifically in the liver the organic cations (OCs) are taken up by organic cation transporter 1 (OCT1) at the sinusoidal membrane of hepatocytes and subsequently are excreted by multidrug and toxin extrusion protein 1 (MATE1) through the bile canaliculi to the bile. In the renal epithelial cells of proximal and distal convoluted tubules, OCs are taken up by organic cation transporter 2 (OCT2) at the basolateral membrane and extruded by MATE1 and multidrug and toxin extrusion protein 2 (MATE2) at the brush-border membrane to the urine (Koepsell, et al., 2007, Moriyama, et al., 2008, Ohta, et al., 2006, Otsuka, et al., 2005) (Figure 1).

The transport of OCs mediated via OCTs is dependent on the transmembrane potential difference. By contrast, OCs export across multidrug and toxin extrusion (MATE) transporters is facilitated by transmembrane H^+ gradient. In the kidney and the liver, mammalian MATE-type transporters are electroneutral H^+/organic cation antiporters which interchange intracellular OCs with extracellular proton. For example, bacterial MATE-type transporters are H^+ or Na^+ coupled OCs exchanger (Masuda, et

al., 2006, Ohta, et al., 2006, Omote, et al., 2006, Terada and Inui, 2008, Terada, et al., 2006).

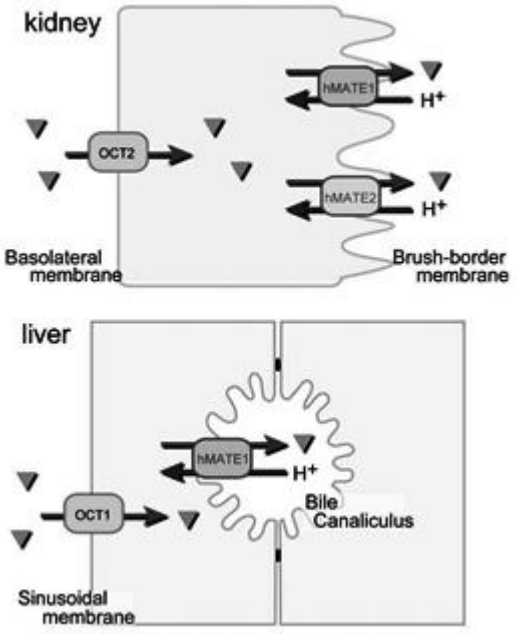

Figure 1. Simplified illustration of OCs uptake by OCTs in the interplay of OCs excretion by MATEs taking place in the kidney and the liver. Taken from: (Moriyama, et al., 2008)

3 Classification and structure

Generally speaking, multidrug transporters are branched into several groups, the best known of which are the five main families: the major facilitator superfamily, the small multidrug resistance family, the resistance nodulation cell division family, MATE family and the ATP-binding cassette family (Moriyama, et al., 2008) (Figure 2). ATP-binding cassette family is the only family which utilizes hydrolysis of ATP for substrate transport, while all the other families use an electrochemical gradient of H^+ or Na^+ as the driving force, they are also term the secondary transporters (Kuroda and Tsuchiya, 2009, Moriyama, et al., 2008, Omote, et al., 2006, Otsuka, et al., 2005).

Both MATEs and OCTs belong to the solute carrier (SLC) family (Nies, et al., 2011). In 2007, the MATE family was classified as SLC47 family by the Human Genome Organisation Gene Nomenclature Committee (Terada and Inui, 2008). The OCTs rank among the SLC representation in the major facilitator superfamily

and are marked here as the SLC22 family. The solute carriers are the second most extensive family of the membrane proteins that regulate a wide number of substances across the cell membrane. They include passive transporters, ion transporters and exchangers, but not the primary active transporters, ion channels and aquaporins. Transport via solute carriers is effected on the ion or proton gradient, not on the hydrolysis of ATP (Hoglund, et al., 2011). Members of SLC family are termed polyspecific transporters, which means that they translocate a wide range of drugs and toxins of different size and chemical constitution (Koepsell, 2004).

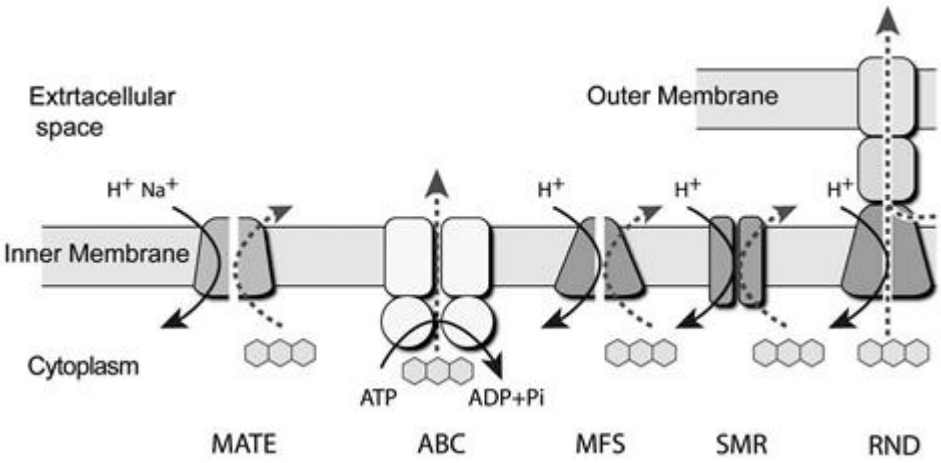

Figure 2. Summary of five main families of multidrug transporters. These are the MATE family, the ATP-binding cassette family (ABC), the major facilitator superfamily (MFS), the small multidrug resistance family (SMR), and the resistance nodulation cell division family (RND). Taken from: (Moriyama, et al., 2008)

3.1 Multidrug and toxin extrusion proteins

In 2005, Otsuka et al. revealed two genes encoding human orthologues of the bacterial MATE (SLC47A) family. The genes are found in tandem on 17 chromosome and their products are called human multidrug and toxin extrusion 1 (hMATE1/SLC47A1) protein and human multidrug and toxin extrusion 2 (hMATE2/SLC47A2) protein (Kajiwara, et al., 2009, Kuroda and Tsuchiya, 2009, Moriyama, et al., 2008, Otsuka, et al., 2005). Further, after complementary DNA (cDNA) cloning of hMATE2 human kidney-specific multidrug and toxin extrusion 2

(hMATE2-K) and human brain-specific multidrug and toxin extrusion 2 (hMATE2-B) were isolated. The above-mentioned transcripts contain 17 exons but compared to the hMATE2, hMATE2-K has a deleted part of exon 7, whereas hMATE2-B has a prolonged part of exon 7. For the sake of comparison hMATE2-K exhibits 94% amino acid identity with hMATE2, 52% with hMATE1 and 82% with hMATE2-B (Masuda, et al., 2006).

Most of the MATE transporters, especially hMATE1, have predicted the membrane topology of twelve α-helical transmembrane domains (TMDs) with C- (terminate with COOH group) and N-terminus (terminate with NH_2 group) located in the cells (Hiasa, et al., 2006, Otsuka, et al., 2005, Terada and Inui, 2008). But there is no identical sequence conserved in all types of MATEs, although all MATEs share a 40% sequence similarity (Omote, et al., 2006). On the other hand, Terada and Inui argue that the use of other transmembrane domain (TMD) predicting programs revealed thirteen TMDs for hMATE1. This would mean that the carboxylic acid residue was found on the other side, thus on the extracellular side (Terada and Inui, 2008) (Figure 3). The secondary structure of hMATE1, the prototype of MATE transporters from all kingdoms, was analysed by multiple sequence alignment. It shows highly conserved regions in the vicinity of TMD1 and 7, the extracellular loops between TMD1 and 2, and also between TMD7 and 8, and the intracellular loops between TMD2 and 3, between TMD3 and 4, between TMD8 and 9, and between TMD10 and 11 (Figure 4). The arrangement of the conserved regions of the molecule suggests that MATEs originate from a common ancestral gene (Moriyama, et al., 2008, Omote, et al., 2006). Human genes for MATE1 and MATE2 are localized on 17p11.2 chromosome (Koepsell, et al., 2007).

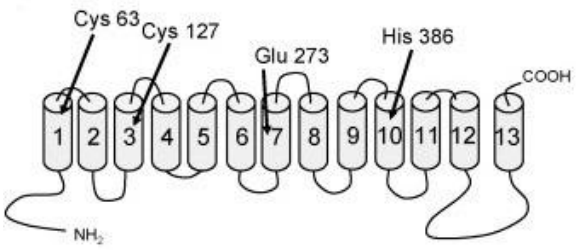

Figure 3. Secondary structure of hMATE1 with thirteen TMDs. At the beginning is situated N-terminus representing the amino group while C-terminus representing the carboxyl group is situated at the end outside in the extracellular space. Taken from: (Terada and Inui, 2008)

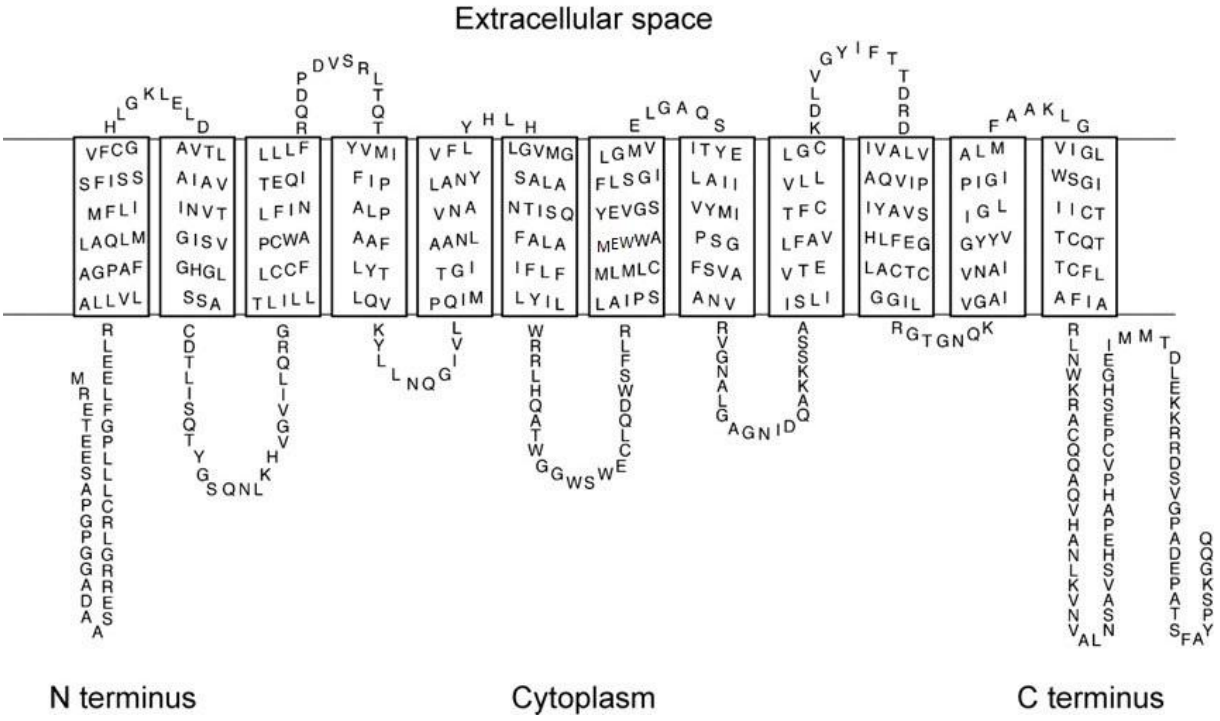

Figure 4. Secondary structure of hMATE1 with twelve TMDs. N-terminus representing the NH_2 group is located at the beginning in the cytoplasm. C-terminus representing COOH group is located at the end also in the cytoplasm. Modified from: (Hiasa, et al., 2006)

3.2 Organic cation transporters

According to the substrate and transport mechanism, SLC22A family comprises three OCTs: OCT1 which is also termed SLC22A1, OCT2/SLC22A2 and organic cation transporter 3 (OCT3 /SLC22A3). Other members of this family are organic anion transporters, carnitine and cation transporters and a few of transporters which are unidentified and their function is not determined (Koepsell and Endou, 2004) (Table 1).

Table 1. A short recapitulation of all polyspecific transporters covered in this work.

Gene	Protein
SLC22A1	OCT1
SLC22A2	OCT2
SLC22A3	OCT3
SLC47A1	MATE1
SLC47A2	MATE2

OCTs have predicted membrane topology consisting of twelve α-helical TMDs, a large glycosylated extracellular loop between TMDs 1 and 2, a large intracellular loop with phosporylation sites between TMDs 6 and 7, an intracellular N-terminus and C-terminus (Koepsell, et al., 2007) (Figure 5). Human gene for OCT1 and OCT2 is localized in a cluster on chromosome 6q26 (Koehler, et al., 1997), gene for human organic cation transporter 3 (hOCT3) on 6q27 (Grundemann, et al., 1998). Human organic cation transporter 1 (hOCT1) gene consists of 7 exons and 6 introns (Hayer, et al., 1999), human organic cation transporter 2 (hOCT2) and hOCT3 consist of 11 exons and 10 introns (Grundemann and Schomig, 2000).

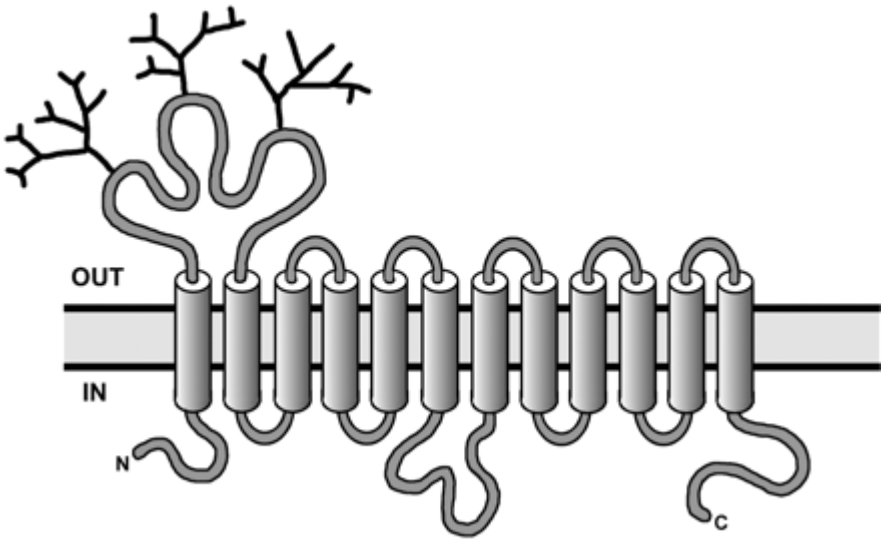

Figure 5. Secondary structure of hOCT1. Both N-terminus and C-terminus are located inside the cell. Taken from: (Jonker and Schinkel, 2004)

4 Tissue distribution and transporter localization

4.1 Multidrug and toxin extrusion protein 1

In humans, MATE1 is a frequently occurring member of MATE family throughout the body. The strongest expression was founded in the kidney, exactly at the apical membrane of the proximal and distal convoluted tubules. Other organs with abundant expression of hMATE1 are the adrenal gland, testis, skeletal muscle, fetal and adult liver, specifically at the canalicular membrane of hepatocytes. Weak messenger RNA (mRNA) levels were expressed in the heart, lung and uterus (Masuda, et al., 2006, Omote, et al., 2006, Terada and Inui, 2008).

Northern blot analysis elucidates that rat orthologue of multidrug and toxin extrusion 1 (rMate1) is significantly expressed only in the kidney. The presence of rMate1 was detected in the renal proximal convoluted tubule and the proximal straight tubule. Nevertheless, real-time polymerase chain reaction (RT-PCR) proved that rMate1 mRNA was primarily expressed in the kidney, subsequently in the placenta, pancreas, spleen, bladder and lung (Ohta, et al., 2006, Terada and Inui, 2008, Terada, et al., 2006). Recently, the highest rMate1 expression was observed in the placenta,

by RT-PCR thirteen times and by Western blot 1,4-fold higher compared to the maternal kidney (Ahmadimoghaddam, et al., 2012).

Mouse multidrug and toxin extrusion 1 (mMate1) is largely expressed by Northern blot in the kidney, liver and heart, and less prominently in the skeletal muscle. Other findings of rMate1 by reverse transcriptase PCR were shown in the adrenal gland, brain, small intestine, stomach, testes, thyroid gland and urinary bladder, as well as in the kidney and liver. More accurate transporter location were demonstrated by immunohistochemical staining mainly at the apical regions of cortical collecting ducts, proximal convoluted tubules and thin limb of Henle's loop in the kidney, and in the luminal membrane of bile canaliculi and at the apical region of bile duct in the liver. In addition, mMate1 transporters are localized in brain glial-like cells and capillaries, pancreatic alpha cells of the Langerhans islets, intermediate cells urinary bladder epithelium, Leydig cells of testes and throughout the cortex of adrenal glands (Hiasa, et al., 2006, Otsuka, et al., 2005, Terada and Inui, 2008).

4.2 Multidrug and toxin extrusion protein 2

Abundant expression of hMATE2, strictly speaking hMATE2-K, was found only in the kidney where it was localized in the brush border membrane of the proximal tubule. On the other hand, hMATE2-B is expressed in many organs but not in the kidney (Koepsell, et al., 2007, Masuda, et al., 2006, Otsuka, et al., 2005).

From animal kingdom, rodent MATE2 is specifically expressed in the testis with localization to the Leydig cells. By RT-PCR, the expression of rat multidrug and toxin extrusion 2 (rMate2) was also confirmed in the kidney and was six times higher in the placenta compared to the kidney (Ahmadimoghaddam, et al., 2012). To clarification, rodent MATE2, it includes mouse and rat Mate2, has been recently classified as a third member of MATE family: multidrug and toxin extrusion 3 (Staud, et al., 2012, Terada and Inui, 2008).

4.3 Organic cation transporter 1

Regardless of the species, OCT1 is primarily expressed in liver (Koepsell, et al., 2003). The highest rat organic cation transporter 1 (rOct1) mRNA expression is in the kidney, in the basolateral membrane of proximal tubules (Karbach, et al., 2000). Moderate in the skin, spleen, and liver localized to the sinusoidal membrane of hepatocytes (Meyer-Wentrup, et al., 1998). Low tissue distribution of rOct1 was detected in the brain, gastrointestinal tract, lung, thymus, muscle, bladder, and blood vessels (Slitt, et al., 2002).

In humans, hOCT1 is mainly located in the liver (Gorboulev, et al., 1997, Nies, et al., 2009), specifically in the basolateral membrane of hepatocytes (Nies, et al., 2008). Furthermore, considerable expression of hOCT1 mRNA occurs within the adrenal gland and the luminal membrane of ciliated epithelial cells of lung (Lips, et al., 2005). Lower tissue distribution was found then in the kidney, spleen, pancreas and lymph nodes (Nies, et al., 2009).

4.4 Organic cation transporter 2

Unlike OCT1 and OCT3, the OCT2 shows a limited expression pattern. The strongest expression of hOCT2 was found in the kidney, at the luminal membrane of the distal tubule (Gorboulev, et al., 1997) and at the basolateral membrane of proximal tubule epithelial cells (Nies, et al., 2008). Further hOCT2 localization was revealed in the brain, especially in the pyramidal cells of the cortex and hippocampus (Busch, et al., 1998), or in the luminal membrane of pulmonary epithelial cells like hOCT1 (Lips, et al., 2005).

Similar to rodents, the highest rat organic cation transporter 2 (rOct2) mRNA levels were found in the kidney on basolateral membrane of proximal tubules (Karbach, et al., 2000). Comparing sex, in the female kidney there is only a quarter of that present in a male one. A significantly lower expression of rOct2 is in the liver, thymus and duodenum (Slitt, et al., 2002).

4.5 Organic cation transporter 3

OCT3 shows the widest tissue distribution of OCTs. For the first time, the OCT3 transporter was cloned from the rat placenta. There is abundant representation of rat organic cation transporter 3 (rOct3) right here in the placenta (Kekuda, et al., 1998). As confirmed by Leazer and Klaassen, rOct3 protein is expressed more than twice the amount in the placenta than in the kidney and five times more than in the liver (Leazer and Klaassen, 2003). Ahmadimoghaddam et al. state even 345-fold higher expression in the placenta compared to the maternal kidney analysed by RT-PCR (Ahmadimoghaddam, et al., 2012). The highest rOct3 expression was found in blood vessels, skin and thymus. A notable expression was ascertained in the lungs, spleen, heart, muscle, gastrointestinal tract, brain, and kidney, unlike that of rOct1 and rOct2 (Slitt, et al., 2002). It was not detected in the liver (Kekuda, et al., 1998).

In humans, the highest amount of OCT3 occurs in the adrenal gland. Other tissues with medium expression include the uterus, prostate and liver, where hOCT3 protein is localized on the basolateral hepatocyte membrane. A minor expression was found in the lung, placenta, penis, vagina, and stomach (Nies, et al., 2009). RT-PCR analysis has revealed that OCT3 is the only organic cation transporter (OCT) isoform which is expressed in the human placenta (Sata, et al., 2005). On the other hand, Bottalico et al. claim that a small amount of hOCT1 and hOCT2 mRNA has been detected in the intima layer of some of the placental blood vessels, primarily in veins, along with low expression levels of both transporters, mentioned above, in the central placenta (Bottalico, et al., 2004). By Western blot analysis hOCT3 mRNA was expressed only in the placental basal membrane vesicles, but not in the microvillous membrane vesicles. Consequently, hOCT3 localizes to the basolateral membrane of the trophoblast cells (Sata, et al., 2005).

4.6 Overview of transporters

In the study by Koepsell et al., there are summarized all the polyspecific transporters and their presence in human and rodent tissues demonstrated by Northern blot, RT-PCR, Western blot or immunohistochemistry (Koepsell, et al., 2007). This can

help to outline the situation throughout the body. I have attempted to streamline the information, adapt it to the text, and focus on MATE and OCT isoforms (Table 2).

Table 2. Tissue distribution of polyspecific transporters.

	OCT1		OCT2		OCT3		MATE1		MATE2-K	MATE3
	hu	rod	hu	rod	hu	rod	hu	rod	hu	rod
Stomach	+	+	–	–	+	+	–	+	–	–
Small intestine	+	+	+	+	+	+	–	+	–	–
Large intestine	+	+	Ø	–	Ø	+	–	–	–	–
Rectum	–	–	–	–	–	–	–	+	–	–
Liver	+	+	Ø	+	+ [c]	+ [*]	+	+	–	–
Pancreas	+	–	–	–	–	–	–	+	–	–
Spleen	+	+	+	–	Ø	+	–	+	–	–
Trachea	+	+	+	+	+	+	–	–	–	–
Lung	+	+	+	+	+	+	+	+	–	–
Kidney	+ [c]	+	+	+	+ [c]	+	+	+	+	+
Urinary bladder	+	+	Ø	Ø	+	+	–	+	–	–
Prostate	–	+	–	–	+	+	–	+	–	–
Testis	–	+	–	Ø	–	+	+	+	–	+
Sertoli cells	–	+	–	Ø	–	+	–	–	–	–
Skin	+ [b]	+ [b]	+ [b]	+ [b]	+ [b]	+ [b]	–	–	–	–
Skeletal muscle	+	+	Ø	Ø	+	+	+	+	–	–
Heart	+	+	–	Ø	+	+	+	+	–	–
Blood vessels	–	+	–	Ø	+	+	–	+	–	–
Brain	+	+	+	+	+	+	–	+	–	–
Choroid plexus	–	+	–	+	–	+	–	–	–	–
Adrenal gland	+	–	–	+	+	–	+	+	–	–
Mammary gland	+	+	Ø	Ø	+	+	–	–	–	–
Uterus	–	Ø	–	Ø	+	+	+	–	–	–
Placenta	+	Ø	+	Ø	+	+	Ø	+	–	+
Ovary	–	Ø	–	Ø	–	+	–	–	–	–
Thymus	–	+	–	+	Ø	+	–	–	–	–

	OCT1		OCT2		OCT3		MATE1		MATE2-K	MATE3
	hu	**rod**	**hu**	**rod**	**hu**	**rod**	**hu**	**rod**	**hu**	**rod**
Epithelial cells	+	+	+	+	+	+	–	+	–	–
Neurons	–	–	+	+	+	+	–	–	–	–
Glial cells	–	–	–	–	+	–	–	+	–	–
Muscle cells	–	–	ø	ø	+	+	–	+	–	–
Granulocytes	–	+	–	–	–	+	–	–	–	–
Tumor cells	+	–	+	–	+	–	–	–	–	–

+ bold face indicates very strong expression, ø no expression detected , - expression has not been investigated. Positive expression shown by Nies, et al., 2009 +, Lips, et al., 2005 +, Meyer-Wentrup et al., 1998 +, Slitt, et al., 2002 +, Masuda, et al., 2006 +. * no expression by Kekuda, et al.,1998. [b] Lips, K.S., Zapf, F., Volk, C., Müller, J., Brandsch, M., and Koepsell, H. unpublished data. [c] Lips., K.S., Kummer, W., Ciarimboli, G., Schlatter, E., and Koepsell, H., unpublished data. Modified from: (Koepsell, et al., 2007)

5 Substrate specificity

MATE transporters are responsible for excretion of toxic endogenous and exogenous compounds comprising various OCs. On the other hand, they are able to transmit not only the cationic substrates but also the anionic and zwitterionic ones. Tetraethylammonium (TEA) is considered the prototype substrate of OCs exporter (Tanihara, et al., 2007).

There exist many substrates that are transported across OCTs. To begin with, there are OCs, from which they get their name, then also positively-charged weak bases or non-charged compounds. They are biogenic amines, foreign substances, and drugs. Some of them are translocated only by one type of the OCT isoform, but we can find some structures common for all kinds. An example of this is molecule of 1-methyl-4-phenylpyridinium (MPP). It is a model compound which is transported by all OCTs from various species. Some molecules are transmitted, others inhibit the transporter. Thus, the inhibitor blocks the carrier, but it alone is not transferred. In addition, it depends not only on the type of transporters isoform but also on interspecies differences (Koepsell, et al., 2007, Koepsell, et al., 2003).

5.1 Multidrug and toxin extrusion proteins

The substrates transported by hMATE1 and hMATE2-K are the cationic compound TEA, neurotoxin MPP, H_2-receptor antagonist cimetidine, antidiabetics metformine, endogenous metabolites creatinine and guanidine, antiarrhythmic drug procainamide, vitamin B_1 (thiamine), and chemotherapeutic agent topotecan. Other substrates from anionic representatives are estrone sulfate, antiviral guanosine analogue acyclovir and 2-deoxyguanosine analogue ganciclovir. Both transporters have similar affinity to their substrates. Considerably higher affinity to hMATE1 than hMATE2-K is assigned to the zwitterionic β-lactam antibiotics cephalexin and cephradine. These antibiotics are never transported via hMATE2-K. Conversely, the anticancer agent oxaliplatin has bigger affinity to hMATE2-K than hMATE1 (Tanihara, et al., 2007) (Table 3). In addition, the substrateof hMATE2-K is the metabolite of vitamin nicotinamide: *N*-methylnicotinamide (Masuda, et al., 2006).

In rMate1, there was detected different substrate specificity from the human counterparts. The significant uptake by human embryonic kidney (HEK) 293 cells transfected with rMate1 cDNA possessed *N*-methylnicotinamide, TEA, cimetidine, metformin and cephalexin. No transport proved by MPP, creatinine, guanidine, procainamide, the diagnostic agent para-aminohippuric acid and cephalosporin cefazolin (Terada, et al., 2006).

Table 3. The substrates of hMATE1 and hMATE2-K.

	Substrate
Transported by hMATE1 and hMATE2-K	**TEA, MPP, cimetidine, metformin, creatinine, guanidine, procainamide, thiamine, topotecan, estrone sulfate, acyclovir, ganciclovir**
hMATE1 > hMATE2-K	**Cephalexin, cephradine**
hMATE2-K > hMATE1	**Oxaliplatin**

Modified from: (Tanihara, et al., 2007)

All previously described substrates belong to rat or hMATE1 and hMATE2-K. This is due to no uptake of TEA in HEK293 cells stably expressing hMATE2-B. Both hMATE2-K and hMATE2-B cDNA were co-transfected into HEK293 cells and examined for TEA uptake. Interestingly, hMATE2-B has no impact on hMATE2-K or its own activity as OCs exporter (Masuda, et al., 2006).

The uptake of substrate can be supported by addition of ammonium chloride (NH_4Cl). It would be appropriate to distinguish two different ways of adding NH_4Cl to the incubation medium. First, NH_4Cl is added at some time and subsequently removed. This is called pre-treatment. The transporters are preincubated with preincubation medium, then the intracellular pH decreases and the uptake becomes greater because the intracellular acidification facilitates the exchange of intracellular proton with extracellular substrate. The second method is called acute treatment, rMate1 expressing cells aren't pre-treated but they are exposed to NH_4Cl for the whole incubation period. It causes alkalization of the intracellular pH and a reduction of substrate uptake. Moreover, the best extracellular pH for uptake is at 8,4 and the lowest between 6,0-6,5 pH values (Ohta, et al., 2006, Terada, et al., 2006). As well as in humans, the maximal transport activity occurs with extracellular pH 8,0-8,5 and the lowest is pH 6,0 (Otsuka, et al., 2005).

One of the most potent inhibitors of human MATEs is a neutral steroid hormone, corticosterone. In general, steroid hormones such as testosterone, corticosterone and progesterone are inhibitors of mMate1. Although, other authors state that these hormones are recognized as the substrates of mouse and human MATE-type transporters (Hiasa, et al., 2006, Ohta, et al., 2009, Omote, et al., 2006). Likewise, cimetidine is a good substrate with high affinity to both rMate1 and human MATE transporters and can be used as their probe substrate. But it is also referred to be an inhibitor (Hiasa, et al., 2006, Ohta, et al., 2009, Otsuka, et al., 2005). Furthermore, the strong inhibitors of human MATEs are the quinolone antibiotics, levofloxacin and ciprofloxacin. Other antibiotics, cephalexin and cephadrine, are substrates of hMATE1 as pointed out above. In addition, in TEA uptake by hMATE1 operate as the inhibitors and cephadrine alone inhibit TEA uptake by hMATE2-K (Tanihara, et al., 2007). Inhibitory effect on cimetidine uptake by human MATEs have fluorescent 4′,6-diamino-2-phenylindole, ethidium bromide and propidium iodide. Surprisingly,

thiamine has also potent inhibitory properties, even though it was reported as substrate of human MATEs (Ohta, et al., 2009).

In rodents, effective inhibitors of rMate1 are the potassium-sparing diuretic amiloride, centrally acting α_2-adrenergic receptor agonist clonidine, antidepressant imipramine, antiarrhythmic quinidine, antimalarial quinine, thiamine and calcium channel blockers verapamil and diltiazem (Ohta, et al., 2006).

5.2 Organic cation transporters

In humans, the most ordinary substrates are the endogenous monoamines choline, acetylcholine, dopamine, histamine, serotonin, and endogenous compounds creatinine, guanidine and thiamine. Another large group of substrates comprises drugs such as acyclovir and ganciclovir, antidiabetic drugs metformine and phenformin, glutamate *N*-methyl-*D*-aspartate receptor antagonist memantine, and cimetidine (Koepsell, et al., 2003). Moreover, oxybutynin and trospium, used for the treatment of an overactive bladder (Wenge, et al., 2011), and as well nucleoside reverse transcriptase inhibitor lamivudine (Minuesa, et al., 2009).

The steroid hormones corticosterone, deoxycorticosterone and β-estradiol are the common inhibitors of human OCTs (Koepsell, et al., 2003). Other potent human OCTs inhibitor is quinidine (Bourdet, et al., 2005).

5.2.1 Organic cation transporter 1

Other substrates of hOCT1 are H_2-blockers, ranitidine and famotidine (Bourdet, et al., 2005), and anticonvulsant drug lamotrigine (Dickens, et al., 2012).

Inhibition potency was determined with a range of n-tetraalkylammonium compounds (tetramethylammonium, TEA, tetrapropylammonium and tetrabutylammonium) on the mouse, rat, rabbit and human OCT1 transporter. The relationship was found between the length of alkyl chain and degree of inhibition. Alkyl chain extension increases the rate of inhibition. This fact can be generalized to all investigated species (Dresser, et al., 2000). Further, inhibitors from the group of nonsteroidal anti-inflammatory drugs that suppress hOCT1-mediated organic cation (in this experiment TEA) uptake, are diclofenac, ibuprofen, indomethacin, ketoprofen,

mefenamic acid and sulindac (Khamdang, et al., 2002). Inhibitors with pronounced affinity to hOCT1 than hOCT2 and 3 are for example the antidepressant desipramine, anticholinergic atropine, *N*-methyl-*D*-aspartate receptor antagonist phencyclidine, or H_1-receptor antagonist diphenylhydramine (Koepsell, et al., 2007).

5.2.2 Organic cation transporter 2

The substrates translocated by hOCT2 are the antiviral and antiparkinsonic drug amantadine, cytostatic cisplatin and antihypertensive debrisoquine (Koepsell, et al., 2007). Significant substrate of hOCT2 is an antiparkinsonic pramipexole (Diao, et al., 2010). Ranitidine is also hOCT2 substrate, but compared to hOCT1 is slightly weaker (Bourdet, et al., 2005).

Activation of certain signalling pathways may affect the transmission through the hOCT2 transporter. The agents of inhibitors are the protein kinase: phosphatidylinositol-3-kinase, cAMP-dependent protein kinase A and phospholipase C, not the protein kinase C. Vice versa, hOCT2 is activated by Ca^{2+}/calmodulin-dependent protein kinase II, myosin light chain kinase and Ca^{2+}/calmodulin complex (Cetinkaya, et al., 2003).

A potent inhibition of MPP uptake was detected by beta-blockers carvediol and propanolol, while that of metoprolol and bisoprolol was negligible. Compared to the OCT2-mediated metformin uptake, all these beta-blockers are significant inhibitors (Bachmakov, et al., 2009). The other ones are imipramine, clonidine and verapamil (Zolk, et al., 2009). Inhibitors from nonsteroidal anti-inflammatory drugs are indomethacin, naproxen, piroxicam and sulindac (Khamdang, et al., 2002).

5.2.3 Organic cation transporter 3

The OCT3 transporter is also marked as the extraneuronal monoamine transporter (EMT) independent of transmembrane Na^+ and Cl^- gradients, which means that the native substrates are the monoamines dopamine, histamine, norepinephrine and serotonin (Ganapathy, et al., 2000, Wu, et al., 1998). Pramipexole is taken up by hOCT3, but not in a high-affinity manner, in comparison with hOCT2 (Diao, et al., 2010). Ganapathy et al. state that other possible substrates could be amiloride, amphetamines, clonidine, cimetidine, antidepressant desipramine, and imipramine

(Ganapathy, et al., 2000). Unlike Ganapathy et al., other authors report just the opposite; see the next paragraph below to ascertain if the appropriate representatives are substrates or inhibitors.

Famotidine is one of the strongest OCT3 inhibitors described (Bourdet, et al., 2005). Significant inhibition is caused by corticosterone, decynium 22 and disprocynium 24 (Gasser, et al., 2006, Hayer-Zillgen, et al., 2002, Koepsell, et al., 2007, Wu, et al., 1998). The hypothalamic cells expressing OCT3 transporters were used to demonstrate the further inhibitors, such as the antidepressant fluoxetine, tomoxetine and desipramine (Gasser, et al., 2006, Wu, et al., 1998). The other compounds with an inhibitory effect are amphetamine, methamphetamine, dopamine, serotonin and cimetidine (Kekuda, et al., 1998, Wu, et al., 1998). Some of the weaker compounds are for instance atropine, clonidine, cocaine, diphenylhydramine (Koepsell, et al., 2007, Wu, et al., 1998).

For an overview of substrates see Figure 6. For inhibitors see Figure 7.

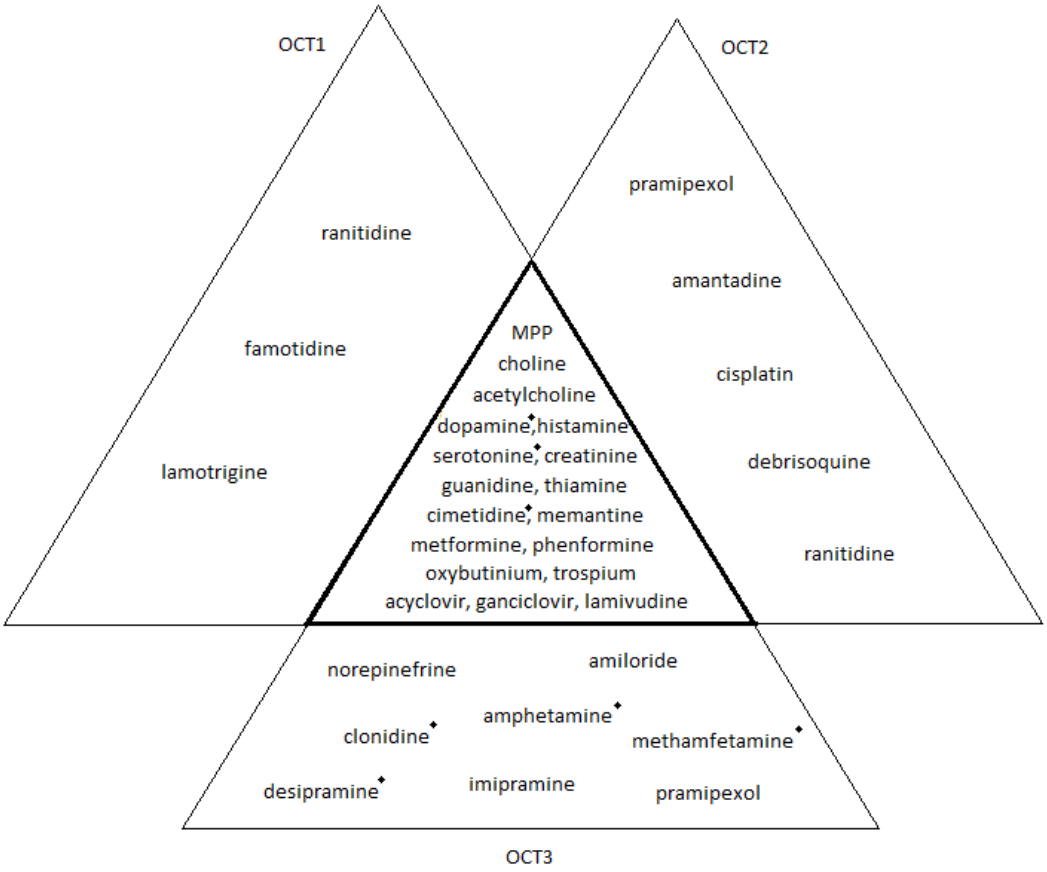

Figure 6. Substrates of OCTs. Compounds marked with an asterisk occur in the literature with different interpretation as to whether they are substrates or inhibitors. See the text for more details.

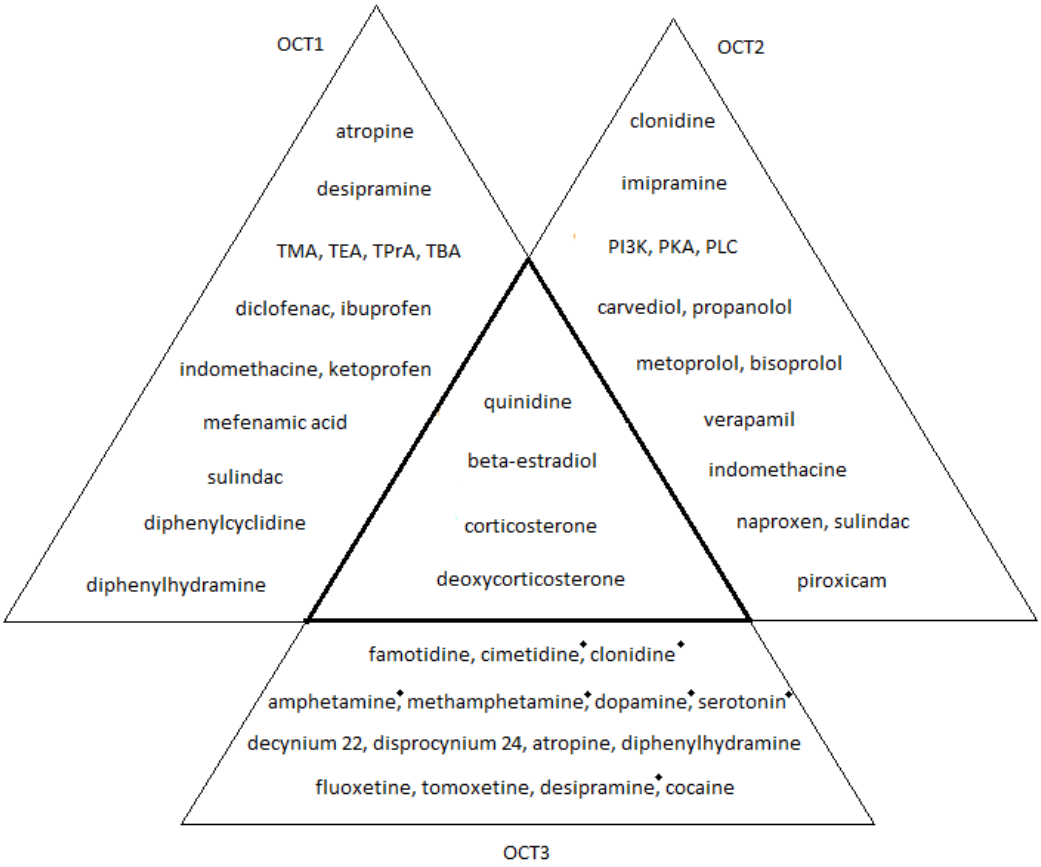

Figure 7. Inhibitors of OCTs. Compounds marked with an asterisk occur in the literature with different interpretation as to whether they are substrates or inhibitors. See the text for more details. TMA tetramethylammonium, TPrA tetrapropylammonium, TBA tetrabutylammonium, PI3K phosphatidylinositol-3-kinase, PKA protein kinase A, PLC phospholipase C.

6 Physiological and pathophysiological significance

The kidneys and liver belong to important elimination organs that get the body rid of endogenous metabolites, drugs and xenobiotics. They are essential for native physiological functioning. Excretion is, among others, ensured by members of SLC22A and SLC47A families, abundantly expressed in the kidney and liver. To remind, hOCT1 and hOCT2 are located in the hepatic and renal basolateral membranes and mediate the uptake of OCs from blood into the cells. For maintaining the balance, hMATE1 and hMATE2-K are expressed on the luminal sides of bile canaliculi and renal proximal tubules and mediate the extrusion of cellular OCs into the bile and urine. Lest we forget, hOCT3 is ubiquitous and its expression levels are smaller than those of OCT1 in the liver and OCT2 in the kidney (Tsuda, et al., 2009, Yonezawa and Inui, 2011).

Human OCT3 is also called EMT, since it enables extraneuronal uptake-2. There are two uptake systems regulating extracellular concentrations of released monoamines, namely adrenaline, noradrenaline, dopamine and serotonin. Uptake-1 is the neuronal system that takes place at the presynaptic nerve endings and mediates the reuptake of released neurotransmitters from the synaptic cleft. In contrast, uptake-2 takes place outside the nervous system, especially in mice in the heart and recently also in the placenta (Zwart, et al., 2001).

Targeted disruptions of the transporters are the commonest usage in research of physiological and pharmacological importance. These experiments are performed on knockout mice. In contrast to the wild type mice, the knockout mouse models have a specific deficiency caused by a modification of the genetic information. Single knockout or double knockout mice were generated. Single knockout mice lack in Oct1 [Oct1(-/-)], Oct2 [Oct2(-/-)], Oct3 [Oct3(-/-)] or Mate1 [Mate1(-/-)] and double knockout mice deficient in Oct1 and Oct2 together [Oct1/2(-/-)]. They are viable, healthy and fertile without evident physiological variances. These animal models belong to the *in vivo* approaches and can help us to predict or explore the impact resulting from absence of some transporter in humans. Knockout mice models bring new perspectives in the characterisation of transporters and maybe in the future, the results of the studies could be extrapolated to humans. It might help to elucidate diversity of drug metabolism or toxicity (Jonker and Schinkel, 2004, Jonker, et al., 2003, Staud, et al., 2012).

6.1 Targeted genetic disruptions of the multidrug and toxin extrusion proteins

Tsuda and his colleagues compared the plasma and renal concentrations of metformin in Mate1 knockout and wild type mice. In Mate1(-/-) mice, resultant absence of Mate1 transporter did not lead to any expression in the kidney and liver, at the same time no or negligible differences in mRNA expression of OCTs in the kidney and liver and no genotype-related anomalies in eighteen tissues were observed. Only, high values of plasma creatinine and blood urea nitrogen were confirmed in Mate1(-/-) mice. They detected significantly higher plasma, renal and hepatic concentration of metformin in sixtieth minute after the intravenous

administration of metformin in Mate1(-/-) mice compared to Mate1(+/+) mice. Whereas, the urinary excretion, total body clearance, renal clearance (CL_{ren}) and renal secretory clearance of metformin were strongly decreased under the same conditions. Because of these results, there could occur a mild nephropathy in Mate1(-/-) mice. Thus we can conclude that MATE1 has an important role in the renal secretion of metformin (Tsuda, et al., 2009). It is one of the first studies of physiological and pharmacological roles in MATE1 and next ones should be required (Staud, et al., 2012).

6.2 Targeted genetic disruptions of the organic cation transporters

The functional studies of OCTs were carried out on single or double knockout mice (Jonker, et al., 2003, Zwart, et al., 2001). In Oct1(-/-) mice after intravenous administration of TEA strongly decreased its accumulation in the liver and its direct small intestinal excretion compared to the wild type mice. It indicates that mouse organic cation transporter 1 (mOct1) plays an important role for TEA excretion in the liver and also into the gut lumen (Jonker and Schinkel, 2004, Jonker, et al., 2001). That mOct1 is major sinusoidal uptake system for TEA in the liver, was verified by other researches (Jonker and Schinkel, 2004). The absence of mOct1 induces a significant decrease of metformin concentration in the liver, but its plasma concentration were not affected (Tsuda, et al., 2009). Metformin is good substrate of mOct1 but occasionally has toxic side effects, the lactic acidosis which caused accumulation of lactate in the plasma. Thus in Oct1(-/-) mice hepatic uptake of metformin may be reduced and is followed by decrease in glucose-lowering effects of metfomin and increase in metformin-induced blood lactate levels (Jonker and Schinkel, 2004, Tsuda, et al., 2009).

Another crucial place of TEA elimination is the kidney. In Oct1 knockout mice the concentration of TEA in urine dramatically increased to about 80% of the dose, compared to the wild type mice. How is it possible when the very opposite was expected? Jonker and his team explain this as the secondary effect of absence of mOct1 in the liver, leading to an elevated TEA uptake in the kidney and functional predominance of mouse organic cation transporter 2 (mOct2) (Jonker, et al., 2001). This is another highly expressed transporter in the kidney and has common substrates

with mOct1 (Jonker and Schinkel, 2004, Jonker, et al., 2001). Therefore, other studies with Oct2(-/-) and Oct1/2(-/-) mice were undertaken. The lack of each of mOct1 or mOct2 separately has no pronounced effect on the renal excretion of TEA, but the absence of both mOct1 and mOct2 causes substantial reduction of renal elimination of TEA and significant elevation of TEA plasma concentration. These results show that the above-mentioned transporters can decrease the systemic and tissue toxicity of the harmful drugs transported through them. Furthermore, mOct1 and mOct2 together are essential for the renal tubular secretion of the substrates. To compare this with humans, only hOCT2 is expressed in the kidney and therefore it can be estimated that the deficiency in hOCT2 will have similar influence on renal elimination as deficiency in both mOct1 and mOct2 in mice (Jonker, et al., 2003).

Oct3(-/-) mice have also been created; homozygous mutant mice do not display any substantial disproportions of noradrenaline and dopamine. The heart was determined as one of the main uptake-2 sites, and after intravenous application of MPP in Oct3(-/-) mice the accumulation into heart was strongly decreased compared to the wild type ones. Only the adult heart has an essential function from the variety sites of expression. A novel uptake-2 site was identified in the placenta. The accumulation of MPP, administered to pregnant females with mouse organic cation transporter 3 (mOct3) heterozygous cross was decreased into Oct3(-/-) foetuses compared to the wild type ones. In addition, mOct3 is colocalized with the monoamine degrading enzyme of monoamine oxidase A in the placenta and maybe together form a functional monoamine metabolizing pathway from the embryo (Jonker and Schinkel, 2004, Zwart, et al., 2001). Likewise, Ahmadimoghaddam et al. suggest that rOct3 together with rMate1 represent an effective eliminatory route of OCs across the placenta and play significant role in fetal protection and detoxification (Ahmadimoghaddam, et al., 2012). Other sites with mOct3 expression indicate no differences in the uptake of MPP. It could be due to interference with the next transporters that the further studies with double knocknout mice models should be performed (Jonker and Schinkel, 2004, Zwart, et al., 2001).

7 Genetic polymorphism

In general, a characteristic conditioned by at least two variants of gene could be considered genetically polymorphic. The polymorphic allele occurs commonly in a particular population, precisely with a frequency higher than 1%, by contrast the mutant allele rarely occurs. Genetic variability results from differences in DNA sequence. Only small part of genetic variability is reflected in the phenotype. Since the coding exons form a fraction of genomic DNA, the rest belongs to the non-coding sequences, including introns. Even the degeneration of the genetic code, which does not lead to the amino acid exchange in nucleotide of coding segment, fails to manifest itself in the phenotype. There is a large number of genetic polymorphisms, which are detectable using the methods of molecular genetics.

One of the most important polymorphisms in human genetics is the single nucleotide polymorphism (SNP). It is caused by point mutations involving the substitution, deletion or insertion of a specific place in DNA, which leads to confusion between one nucleotide and another and a change in the sequence of bases. Single nucleotide polymorphisms (SNPs) are usually distinguished as synonymous and non-synonymous. The synonymous polymorphism results from the substitution of one nucleotide with another while the coding strand does not alter the meaning and the final codon encodes the same amino acid. Conversely, the non-synonymous polymorphism, the swapping of nucleotide, leads to a change in the amino acid. A frequently used technique of detection of SNPs is known as PCR-restriction fragment length polymorphism. The need to map out polymorphisms led to setting up the International HapMap Consortium in 2002 (http://snp.cshl.org). Thus far it has credited 3.4 milion SNPs, or about 25 to 35 percent of total 9 to 10 milion SNPs in the human genome. The haplotype map, or HapMap, provides a database of genetic variations for the general public and scientists to help them find the gene or genetic variations that affect health, disease and individual responses to treatment and environmental factors (http://snp.cshl.org).

7.1 Single nucleotide polymorphisms in multidrug and toxin extrusion proteins

Kajiwara and his group recognized eight hMATE1 coding SNPs (cSNPs) and five hMATE2-K cSNPs in the coding regions of 89 Japanese citizens, including five and two non-synonymous respectively (Table 4). All of the mutated proteins, except for hMATE1 V10L, demonstrated considerable reduction in the transport activity, particularly hMATE1 G64D and hMATE2-K G211V variants, whose transport activities were totally abolished. It was elucidated on the basis of the cell surface biotinylation why the membrane expression levels of hMATE1 G64D, D328A and hMATE2-K G211V, K64N variants were substantially decreased, compared to the wild type ones. It is safe to conclude that a reduced cell surface expression is responsible for the loss of transport activity (Kajiwara, et al., 2009). The aforementioned team also identified regulatory SNPs, marked G-32A at a Sp 1-binding site of hMATE1 promoter region. Sp 1 is important for the regulation and stimulation of the basal promoter activity of hMATE1 and the inhibition of the Sp 1-biding site by the antitumor antibiotic mithramycin A. These are the reasons of a significant decline in promoter activity. Moreover, the G-32A substitution downregulates hMATE1 promoter activity by a disruption of Sp 1 binding (Kajiwara, et al., 2007).

Another group identified four synonymous and six non-synonymous cSNPs of hMATE1 (Figure 8). This group furthered the uptake studies with four hMATE1 substrates (metformin, oxaliplatin, paraquat and TEA) and detected two genetic variants, namely G64D and V480M, which have completely lost their function. This attributes to an amino acid change at decisive position or to expression of G64D and V489M variants, which is mostly localized in the intracellular space, compared to hMATE1 reference localized exclusively on the plasma membrane. It is worth noting that oxaliplatin induced an increased toxicity by hMATE1 reference and four transporter variants, while G64D and V480M ones did not. Even if these genetic variants are rare and indeed negligible at the population level, they could lead to a modification in the pharmacologic action of oxaliplatin. Further, L125F variant has a decreased transport capability for metformin, paraquat and TEA, while the ability to transport oxaliplatin was conserved. The hMATE1 variant V338I had a reduced

transport activity of metformin and TEA and at the same time maintained the transport activity of oxaliplatin and paraquat, similarly to C479S variant. Chen et al. assumed that reduced transport function is also apparently due to the fact that the variants are located on both the plasma membrane and intracellularly, unlike hMATE1 reference, which is primarily located on the plasma membrane. As confirmed by confocal microscopy studies, the modified function of genetic polymorphisms was due to altered localization to the plasma membrane. Hence the findings suggest that the non-synonymous polymorphisms of hMATE1 might influence the drug disposition and subsequently the clinical drug response (Chen, et al., 2009).

Table 4. Overview of cSNPs of hMATE1 and hMATE2.

Location	*SNP*	*dbSNP (NCBI)*	*Effects*
MATE1			
Exon1	28G>T	ss104806851	V10L
Exon1	33C>T	ss104806852	R11R
Exon1	126T>C	ss104806853	A42A
Exon2	191G>A	ss104806854	G64D
Exon8	708C>T	ss104806855	L236L
Exon11	929C>T	ss104806856	A310V
Exon11	983A>C	ss104806857	D328A
Exon16	1421A>G	ss104806858	N474S
MATE2-K			
Exon2	192G>T	ss104806859	K64N
Exon2	207G>A	ss104806860	S69S
Exon4	345C>A	ss104806861	G115G
Exon8	632_633GC>TT	ss104806862	G211V
Exon10	885C>T	ss104806863	Y295Y

Non-synonymous polymorphisms are placed in the frame. Modified from: (Kajiwara, et al., 2009)

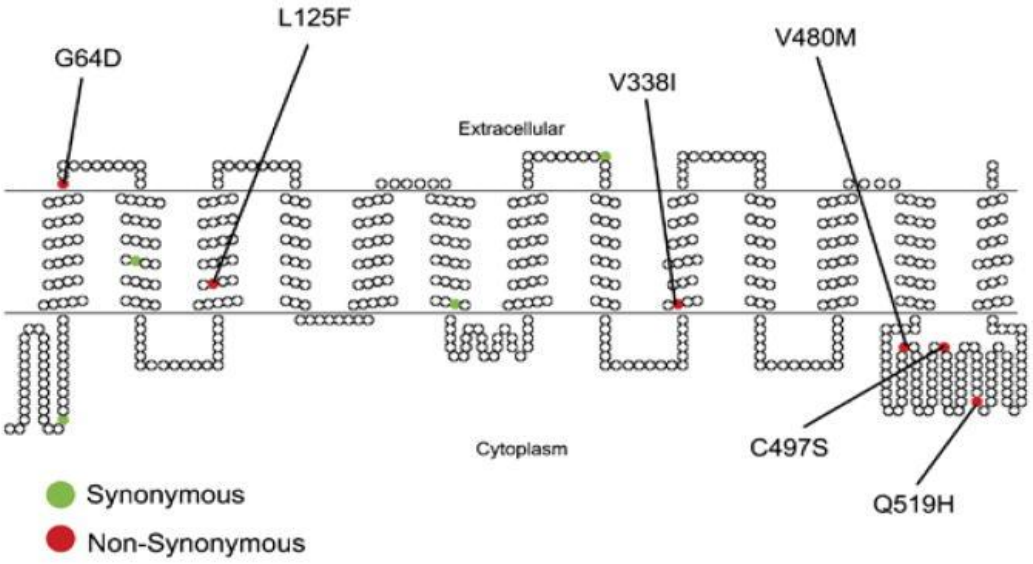

Figure 8. Four synonymous and six non-synonymous cSNPs of hMATE1 simulated on the putative secondary structure model. Taken from: (Chen, et al., 2009)

In hMATE1 there was identified the rs2289669 G>A SNP, related to the glucose-lowering effect of metformin by means of reducing levels of glycated hemoglobin after initiation of metformin therapy. Metformin is a drug of first choice in patients with the second type diabetes and a good substrate of hMATE1 transporter. The impairment of hMATE1 causes a decrease in metformin efflux which in the kidney leads to a rise in the metformin plasma levels, hence reducing the glucose levels and in the liver leads to an increase of metformin levels in the hepatocytes and to an inhibition of the gluconeogenesis, also resulting in the reduction of glucose levels. These data show that polymorphism in hMATE1 can play a significant role in the pharmacokinetics and pharmacodynamics of metformin. However, further studies are necessary because this was the preliminary essay (Becker, et al., 2009).

Choi et al. described a novel and simultaneously most common genetic variant in the proximal promoter region of hMATE1, -66T>C, which showed a strong diminution in the promoter activity and minor expression levels of the transporter in the kidney (Ha Choi, et al., 2009). Interestingly, -32G>A polymorphism previously identified by people in Japan (Kajiwara, et al., 2007) was not detected in this study with samples from an ethnically diverse U.S. population. Reduced transcription activity happens due to the binding of two transcription factors, activating protein-1 (AP-1)

and activating protein-2 (AP-2) repressor, to the basal promoter region close to the -66T>C variant. The mechanism of reduction is associated with a decrease in the bond strength of a transcriptional activator, AP-1 and an increase in bond strength of the AP-2 repressor. Therefore, they proposed that AP-1 and AP-2 repressor regulate the transcriptional activity of hMATE1 and the genetic variant -66T>C may influence the drug response in the kidney (Ha Choi, et al., 2009).

7.2 Single nucleotide polymorphisms in organic cation transporters

7.2.1 Organic cation transporter 1

One of the earliest studies of genetic variants in hOCT1 were performed on 57 Caucasians, whereby a total of 25 SNPs were discovered, and three of them (Arg 61Cys, Cys 88Arg, Gly401Ser) were non-synonymous with the reduced uptake of MPP compared to the wild type (Kerb, et al., 2002). Another team functionally characterized fifteen non-synonymous polymorphisms in hOCT1 on ethnically diverse volunteers. They noticed three non-functional variants (G220V, G401S, G465R), two variants exhibiting a decreased function (R61C, P341L), and one SNP with an increased function (S14F). Side-directed mutagenesis helped to verify that the amino acid substitution was responsible for the modified activities. Subsequently they jumped on the conclusion that there likely exist a relationship between the allele frequency and the transport activity, in part supported by that the common variants (presence above 10%) rather exhibited normal function than the rare ones exhibiting altered function, and that the change of evolutionarily conserved glycine residues by G220V, G401S, and G465R variants resulted in an abolition of transport activity while none of variants altering evolutionarily unconserved residues led to a decrease of function, thus, a chemical change at evolutionarily conserved position could be considered as a strong predictor of function (Shu, et al., 2003). Itoda et al. found twenty genetic polymorphisms of hOCT1 in 116 Japanese patients. Eight of them were also detected by Kerb et al. in their study, but with demonstrably different frequencies. For this reason, they assume that some genetic variants can be ethnically specific. Itoda and his group also revealed one novel non-synonymous SNP, namely Pro117Leu, by which has

not yet been proven that the amino acid change alters the transport activity. Accordingly, other explorations of this SNP are necessary (Itoda, et al., 2004).

The previous studies of genetic polymorphisms in hOCT1 used MPP as a probe substrate. Shu et al. in their follow-up study described an influence of hOCT1 variants on the response to metformin. They measured metformin uptake by twelve non-synonymous SNPs and found seven variants (S14F, R61C, S189L, G220V, G401S, M420del, and G465R) with reduced metformin uptake compared to the reference. They also administered oral glucose to healthy individuals and observed the glucose-lowering effect of metformin after its administration. This effect is detectable only after increased glucose plasma levels by administration of oral glucose in nondiabetic subjects. The results indicated that volunteers carrying reduced function polymorphisms of hOCT1 exhibited significantly higher plasma glucose levels compared to the volunteers carrying hOCT1 reference. Therefore, they suggested that hOCT1 is crucial for therapeutic action of metformin and SNPs in hOCT1 contribute to varied response to metformin. Likewise, they subscribe to the idea that transporter variants could interact differently with different substrates because several hOCT1 variants had various transport activities of MPP and metformin (Shu, et al., 2007). A proof of this statement is also the Choi and Song's study, which examined the transport activities of MPP, TEA, metformin and lamivudine in hOCT1 variants, P283L and P341L, recognized in a Korean population. Uptake of MPP was considerably reduced in P283L polymorphism while P341L polymorphism remained unchanged compared to the wild type. The transport activity of TEA, as well as of lamivudine, was significantly decreased in both P283L and P341L variants compared to the wild type. Metformin uptake did not show altered function in P283L and P341L variants in comparison with the wild type (Choi and Song, 2012). The above mentioned genetic polymorphisms in human SLC22A1 gene are summarized in Table 5.

Table 5. A few non-synonymous SNPs in *SLC22A1* gene and their in vitro transport activity.

Amino acid change	TRANSPORT ACTIVITY* (in vitro)			
	MPP[a,b,c,e,f]	**TEA**[e]	**metformin**[d,e]	**lamivudine**[e]
S14F	increase	NA	decrease	NA
R61C	decrease	NA	decrease	NA
C88R	decrease	NA	NA	NA
P117L	NA	NA	NA	NA
S189L	similar**	NA	decrease	NA
G220V	no function	NA	decrease	NA
P283L	decrease	decrease	similar	decrease
P341L	decrease[a]/similar[e]	decrease	similar	decrease
G401S	decrease[a]/no function[b]	NA	decrease	NA
M420del	similar**	NA	decrease	NA
G465R	no function	NA	decrease	NA

Amino acid deletion in red, NA not available. * compared to the wild-type variants. ** data not shown in the text. [a] Kerb, et al., 2002; [b] Shu, at al.,2003; [c] Itoda, et al., 2004; [d] Shu, et al., 2007; [e] Choi and Song, 2012; [f] Choi and Song, 2008. Inspired by: (Choi and Song, 2008)

7.2.2 Organic cation transporter 2

In hOCT2, there were identified 28 variants from ethnically various samples. Only eight of them were non-synonymous (Figure 9), thus, they carried out next analysis and discerned a major prevalence of synonymous polymorphisms over the non-synonymous ones in SLC22A2 compared to the other genes (Fujita, et al., 2006, Leabman, et al., 2002). Four of non-synonymous variants (Met165Ile, Ala270Ser, Arg400Cys, and Lys432Gln) were common, with the allele frequencies greater than or equal to one percent, and three of them (Met165Ile, Arg400Cys, and Lys432Gln) displayed functional differences from the hOCT2 reference via decreased transport activity or changed interactions with organic cations (Fujita, et al., 2006). A fourth variant, Ala270Ser, showed unchanged transport function compared to the reference (Koepsell, 2004). The remaining rare variants, namely Pro54Ser, Phe161Leu,

Met165Val, and Ala297Gly, showed similar transport activity to the reference and it was not suggested that the rare variants were likely to exhibit any altered function than the more common ones, mentioned in hOCT1 (Fujita, et al., 2006). Kang and his colleagues identified three SNPs of hOCT2 (T199I, T201M, and A270S) in the Korean subjects and reported that all these variants had a decreased uptake of MPP and TEA compared to the wild type. Although there appeared a contradiction in transport function of A270S with a foregoing study, a slight difference in the inhibitor constant K_i, it can be stated that A207S mutant showed reduced transport activity because functional activity is assessed on the basis of intrinsic clearance, V_{max} and K_m values which confirmed this (Kang, et al., 2007). Among the Japanese population there were determined 27 SNPs in hOCT2, of which two were synonymous, Thr130Thr and Val502Val, while no one was non-synonymous (Saito, et al., 2002). Another study on 116 Japanese arrhythmic patients demonstrated fourteen novel SNPs in SLC22A2 gene. Two of them were non-synonymous (Thr199Ile and Thr201Met) but the effect of the amino acid substitution on transport function was not investigated (Fukushima-Uesaka, et al., 2004).

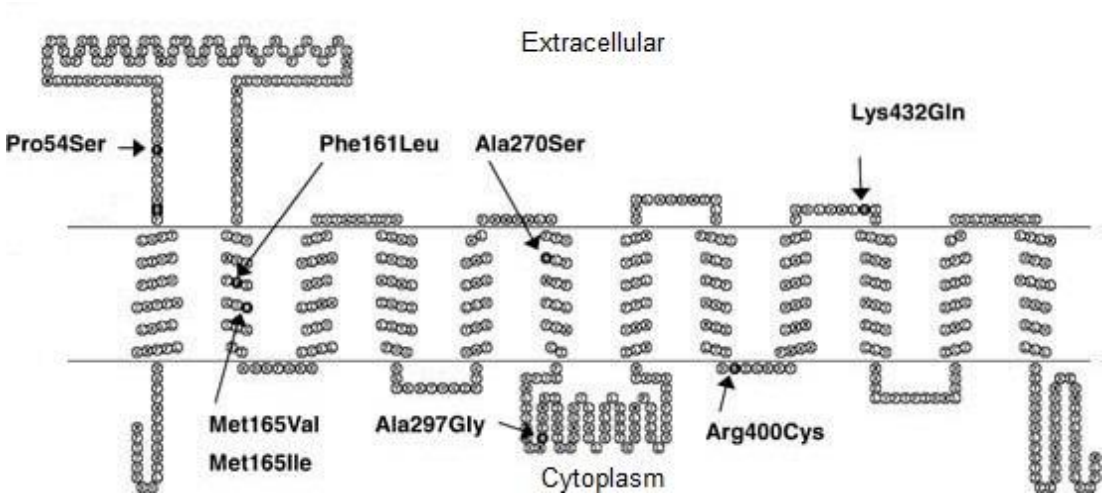

Figure 9. Eight non-synonymous SNPs in hOCT2 typified on predicted secondary structure. Modified from: (Fujita, et al., 2006)

A highly polymorphic variant A207S, present in all ethnic groups, had according to some authors reduced ability to transport metformin. In detail it reduced tubular clearance or CL_{ren} (Song, et al., 2008, Wang, et al., 2008), but other authors detected increased CL_{ren} compared to the cells expressing hOCT2 reference (Chen, et al., 2009). Both studies were performed on healthy volunteers and the discrepancy has not been elucidated. Not only did A207S mutant possess a substantially decreased transport activity of metformin, but T199I and T201M also exhibited a decreased CL_{ren} and increased plasma concentrations of metformin, compared to the reference. Consequently, these polymorphisms may contribute to the alteration in metformin pharmacokinetics (Song, et al., 2008). Recently, the previously mentioned genetic variants OCT2-T119I, -T201M, and -A270S have been tested for the uptake of their substrates: MPP, TEA, metformin, and lamivudine. Surprisingly, in contrast with hOCT1, the uptake of all the substrates was significantly reduced compared to hOCT2 wild type (Choi and Song, 2012).

7.2.3 Organic cation transporter 3

The first investigation of genetic polymorphisms in hOCT3 transporter was conducted on one hundred healthy, unconnected Caucasians. Four noncoding and three synonymous SNPs without amino acid changes were detected in the putative core promoter. No correlation between identified polymorphisms and functional importance can be found (Lazar, et al., 2003). Recently, five non-synonymous SNPs of hOCT3 have been listed in the public database of SNPs (Figure 10). Three of these variants (A116S, A439V and T400I) showed reduced uptake of histamine and MPP. Altered transport activities originate not from the modified localization of expression, because both hOCT3 variants and wild types are located to the plasma membrane equally to the cytoplasm, but from the reduced transport activity itself. Therefore the altered functional properties of hOCT3 variants could contribute to the differences in the disease processes, for example in hypertension, because hOCT3 is important for salt-intake regulation corresponding to blood pressure (Sakata, et al., 2010).

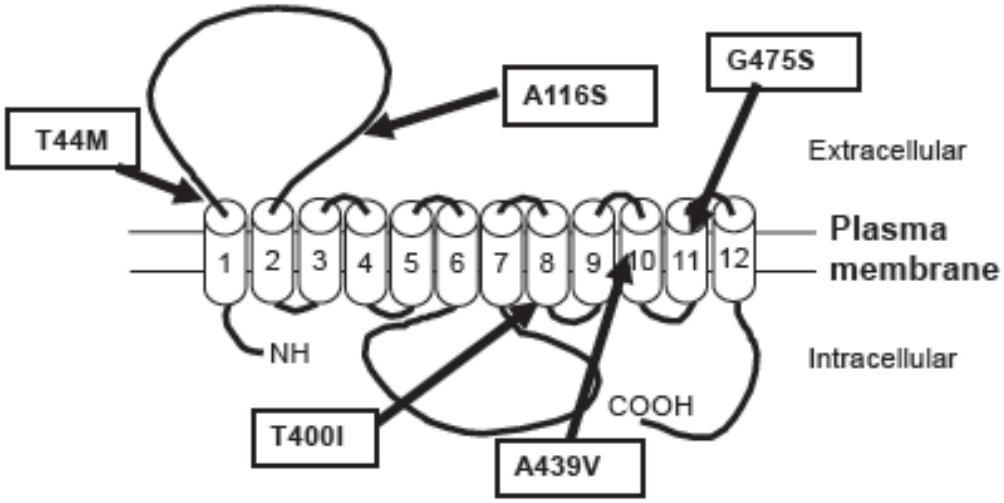

Figure 10. Five non-synonymous SNPs shown on hOCT3 transmembrane topology. Taken from: (Sakata, et al., 2010)

8 Possible consequences and perspectives

The non-synonymous variants located in the coding regions of the exons which result in an amino acid change cause mostly a significant alteration in physiology. These genetic polymorphisms should be the subject of further research because they cause variations in the elimination of substrate across the carrier and in the clearance and potential toxicity of the substrate, simply in drug response and disposition.

For the case of illustration, the loss-of-function polymorphisms and mutations in hOCT1 affect the biliary excretion of cationic drugs in the liver. In case of hMATE1, there occurs an increase of intracellular concentrations of hOCT1/hOCT3 substrates which may bring about hepatotoxicity or hepatic tumors (Koepsell, 2004, Koepsell, et al., 2007). Polymorphism in hOCT2 influences for example metformin disposition or the cisplatin-induced nephrotoxicity (for more details see next paragraph). Metformin is eliminated primarily through hOCT2 in the kidney and the genetic variants of hOCT2 showed a decreased uptake of metformin, leading to altered pharmacokinetics and therapeutic effect of metformin (Choi and Song, 2008). The SLC22A2 gene mutation reduced transport across the hOCT2; therefore cisplatin did not penetrate into the kidney and did not cause the adverse effect (Zhang and Zhou, 2012).

It is worth mentioning the relation between platinum agents and the transmission across the transporters in the kidney. Carboplatin, cisplatin, nedaplatin and oxaliplatin are frequently used powerful chemotherapeutic drugs for the treatment of solid malignant tumors (Terada and Inui, 2008, Zhang and Zhou, 2012). But only cisplatin provokes a severe adverse effect, namely nephrotoxicity. It is explained by an intracellular accumulation of cisplatin in the kidney, because cisplatin is a substrate of hOCT2 but not of hMATE2-K. Neither carboplatin nor nedaplatin are transported by any of these transporters. Although oxaliplatin is transported by hOCT2, it is also strongly transported by hMATE2-K, which enables an efflux transfer out of the cells and that's why it is not nephrotoxic (Terada and Inui, 2008). A cisplatin-induced nephrotoxicity can be eased by co-administration of cimetidine without any influence on an antitumor activity of cisplatin. It probably happens through the mechanism of competitive inhibition of cisplatin by cimetidine (Katsuda, et al., 2010). Such a drug-drug interaction could be utilized for the mitigation of the adverse effects. On the other hand, a similar competitive inhibition of metformin by cimetidine via hOCT2 in the renal tubules causes increased plasma concentration and reduced renal elimination of metformin resulting in lethal side effect lactic acidosis (Choi and Song, 2008, Wang, et al., 2003). Drug interactions on the renal tubular level are very frequent especially in hOCT2 and hOCT3 and maybe in the future they will be important for the clinical use and drug therapies (Koepsell, et al., 2007).

Further on, hOCT3 was also described as EMT influencing the regulation of the circulating and centrally released neurotransmitters. Several psychoactive drugs are substrates or inhibitors of hOCT3, thus EMT can be related to psychiatric disorders and could become the target for the treatment of central-nervous diseases (Koepsell, et al., 2007, Lazar, et al., 2003).

In the rat placenta Oct3 and Mate1 were found to have synchronized activity in the term placenta and play the role in fetal protection because there were observed predominantly fetomaternal transport of MPP, more precisely rOct3 takes up MPP from the fetal circulation into the placenta while rMate1 pump MPP out from the placenta into the maternal circulation (Ahmadimoghaddam, et al., 2012). Subsequently were found the changing placental levels of drug transporters in the course of pregnancy between species and how it influenced fetal protection and detoxification. The rat fetus is protected especially in the later stages of gestation, which was also confirmed

by the rapid decrease of MPP in the fetal tissue beyond the twelfth gestation day after its administration to the pregnant rats. Otherwise in humans, they hypothesized higher placental protection of the fetus at the earlier stages of pregnancy, suggesting a different fetal protective mechanism in different species (Ahmadimoghaddam, et al., 2013). Recently was also verified significant role of rOct3 and rMate1 in transplacental transport of metformin (Ahmadimoghaddam and Staud, 2013).

9 Discussion

In this study, I endeavoured to gather and compare the current information about transporters from the MATE and OCT families which are important members of membrane carriers transmitting a variety of molecules. I focus on their characterization, locations in tissues, substrate specificity, function, genetic variants, and perspectives. All the literary sources, from which I drew, were quite comprehensible to me and explained in detail. I seldom had problems with finding information; however certain problems were encountered in regard of tissue locations of transporters, but such information noise can be attributed to differences in applied techniques of the researches.

Thanks to advanced technologies, we can normally observe the human body at the cellular or molecular level. I deal with membrane transporters which can be comfortably investigated by means of modern techniques, such as the Western and Northern blotting, multiple sequence alignment, RT-PCR or reverse transcriptase PCR, and many others. Of course, *in vitro* methods are not the only ones that can be used. For example, the study of gene expression and function of MATE and OCT transporters in placenta are performed *ex vivo* on perfused placentas because there are ethical limitations; pregnant women are prohibited from taking clinical trials. Other approaches are *in vitro*, as evidenced by the frequently used animal knockout models. They are used for the sake of better physiological and pharmacological characterization of the transporters and have their limitations. We cannot always obtain data from knockout models extrapolated to humans and also can be these models considered as a controversial research tool. Therefore, studies involving experimental animals are carried out in conformity with legislation and code of ethics.

The genetic polymorphisms are budding new methods that screen the genetic background of transporter variants and describe possible changes in the expression and function of the transporters. Nevertheless, the results of these studies cannot be uniformly applied to human kind. These are other limitations that have to be taken into account in determining the conclusions. Each race has its specific genetic variants and that is why further explorations are essential for the identified findings to be used for benefit of man.

10 Conclusion

The polyspecific MATEs and OCTs of the SLC22 family represent the membrane proteins that participate in the transmission and excretion of OCs and foreign substances. Their expression is broadly distributed; a significant role being played in the kidney and liver where they collectively mediate the secretion of cationic drugs and toxins. Transporters also interact with structurally diverse substrates and inhibitors and these recognitions can be used for the drug-transporter and drug-drug interactions. A recent identification of targeted disruptions and polymorphisms contributes to the unveiling of potential adverse drug reactions and inter-individual variations in pharmacokinetics and pharmacodynamics of the substrates. However, further transport and functional studies are necessary to find some application into the clinical use and the obtained results can be extrapolated to humans.

11 References

Ahmadimoghaddam D, Hofman J, Zemankova L, Nachtigal P, Dolezelova E, Cerveny L, et al. Synchronized activity of organic cation transporter 3 (Oct3/Slc22a3) and multidrug and toxin extrusion 1 (Mate1/Slc47a1) transporter in transplacental passage of MPP+ in rat. Toxicol Sci 2012;128:471-81.

Ahmadimoghaddam D, Staud F. Transfer of metformin across the rat placenta is mediated by organic cation transporter 3 (OCT3/SLC22A3) and multidrug and toxin extrusion 1 (MATE1/SLC47A1) protein. Reprod Toxicol 2013;39:17-22.

Ahmadimoghaddam D, Zemankova L, Nachtigal P, Dolezelova E, Neumanova Z, Cerveny L, et al. Organic Cation Transporter 3 (OCT3/SLC22A3) and Multidrug and Toxin Extrusion 1 (MATE1/SLC47A1) Transporter in the Placenta and Fetal Tissues: Expression Profile and Fetus Protective Role at Different Stages of Gestation. Biol Reprod 2013;88:55.

Bachmakov I, Glaeser H, Endress B, Morl F, Konig J, Fromm MF. Interaction of beta-blockers with the renal uptake transporter OCT2. Diabetes Obes Metab 2009;11:1080-3.

Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose-lowering effect of metformin in patients with diabetes: a preliminary study. Diabetes 2009;58:745-9.

Bottalico B, Larsson I, Brodszki J, Hernandez-Andrade E, Casslen B, Marsal K, et al. Norepinephrine transporter (NET), serotonin transporter (SERT), vesicular monoamine transporter (VMAT2) and organic cation transporters (OCT1, 2 and EMT) in human placenta from pre-eclamptic and normotensive pregnancies. Placenta 2004;25:518-29.

Bourdet DL, Pritchard JB, Thakker DR. Differential substrate and inhibitory activities of ranitidine and famotidine toward human organic cation transporter 1 (hOCT1; SLC22A1), hOCT2 (SLC22A2), and hOCT3 (SLC22A3). J Pharmacol Exp Ther 2005;315:1288-97.

Busch AE, Karbach U, Miska D, Gorboulev V, Akhoundova A, Volk C, et al. Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. Mol Pharmacol 1998;54:342-52.

Cetinkaya I, Ciarimboli G, Yalcinkaya G, Mehrens T, Velic A, Hirsch JR, et al. Regulation of human organic cation transporter hOCT2 by PKA, PI3K, and calmodulin-dependent kinases. Am J Physiol Renal Physiol 2003;284:F293-302.

Diao L, Shu Y, Polli JE. Uptake of pramipexole by human organic cation transporters. Mol Pharm 2010;7:1342-7.

Dickens D, Owen A, Alfirevic A, Giannoudis A, Davies A, Weksler B, et al. Lamotrigine is a substrate for OCT1 in brain endothelial cells. Biochem Pharmacol 2012;83:805-14.

Dresser MJ, Gray AT, Giacomini KM. Kinetic and selectivity differences between rodent, rabbit, and human organic cation transporters (OCT1). J Pharmacol Exp Ther 2000;292:1146-52.

Fujita T, Urban TJ, Leabman MK, Fujita K, Giacomini KM. Transport of drugs in the kidney by the human organic cation transporter, OCT2 and its genetic variants. J Pharm Sci 2006;95:25-36.

Fukushima-Uesaka H, Maekawa K, Ozawa S, Komamura K, Ueno K, Shibakawa M, et al. Fourteen novel single nucleotide polymorphisms in the SLC22A2 gene encoding human organic cation transporter (OCT2). Drug Metab Pharmacokinet 2004;19:239-44.

Ganapathy V, Prasad PD, Ganapathy ME, Leibach FH. Placental transporters relevant to drug distribution across the maternal-fetal interface. J Pharmacol Exp Ther 2000;294:413-20.

Gasser PJ, Lowry CA, Orchinik M. Corticosterone-sensitive monoamine transport in the rat dorsomedial hypothalamus: potential role for organic cation transporter 3 in stress-induced modulation of monoaminergic neurotransmission. J Neurosci 2006;26:8758-66.

Gorboulev V, Ulzheimer JC, Akhoundova A, Ulzheimer-Teuber I, Karbach U, Quester S, et al. Cloning and characterization of two human polyspecific organic cation transporters. DNA Cell Biol 1997;16:871-81.

Grundemann D, Schechinger B, Rappold GA, Schomig E. Molecular identification of the corticosterone-sensitive extraneuronal catecholamine transporter. Nat Neurosci 1998;1:349-51.

Grundemann D, Schomig E. Gene structures of the human non-neuronal monoamine transporters EMT and OCT2. Hum Genet 2000;106:627-35.

Ha Choi J, Wah Yee S, Kim MJ, Nguyen L, Ho Lee J, Kang JO, et al. Identification and characterization of novel polymorphisms in the basal promoter of the human transporter, MATE1. Pharmacogenet Genomics 2009;19:770-80.

Hayer-Zillgen M, Bruss M, Bonisch H. Expression and pharmacological profile of the human organic cation transporters hOCT1, hOCT2 and hOCT3. Br J Pharmacol 2002;136:829-36.

Hayer M, Bonisch H, Bruss M. Molecular cloning, functional characterization and genomic organization of four alternatively spliced isoforms of the human organic cation transporter 1 (hOCT1/SLC22A1). Ann Hum Genet 1999;63:473-82.

Hiasa M, Matsumoto T, Komatsu T, Moriyama Y. 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 2006;291:C678-86.

Hoglund PJ, Nordstrom KJ, Schioth HB, Fredriksson R. The solute carrier families have a remarkably long evolutionary history with the majority of the human families present before divergence of Bilaterian species. Mol Biol Evol 2011;28:1531-41.

Chen Y, Li S, Brown C, Cheatham S, Castro RA, Leabman MK, et al. Effect of genetic variation in the organic cation transporter 2 on the renal elimination of metformin. Pharmacogenet Genomics 2009;19:497-504.

Chen Y, Teranishi K, Li S, Yee SW, Hesselson S, Stryke D, et al. Genetic variants in multidrug and toxic compound extrusion-1, hMATE1, alter transport function. Pharmacogenomics J 2009;9:127-36.

Choi MK, Song IS. Genetic variants of organic cation transporter 1 (OCT1) and OCT2 significantly reduce lamivudine uptake. Biopharm Drug Dispos 2012;33:170-8.

Choi MK, Song IS. Organic cation transporters and their pharmacokinetic and pharmacodynamic consequences. Drug Metab Pharmacokinet 2008;23:243-53.

Itoda M, Saito Y, Maekawa K, Hichiya H, Komamura K, Kamakura S, et al. Seven novel single nucleotide polymorphisms in the human SLC22A1 gene encoding organic cation transporter 1 (OCT1). Drug Metab Pharmacokinet 2004;19:308-12.

Jonker JW, Schinkel AH. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). J Pharmacol Exp Ther 2004;308:2-9.

Jonker JW, Wagenaar E, Mol CA, Buitelaar M, Koepsell H, Smit JW, et al. Reduced hepatic uptake and intestinal excretion of organic cations in mice with a targeted disruption of the organic cation transporter 1 (Oct1 [Slc22a1]) gene. Mol Cell Biol 2001;21:5471-7.

Jonker JW, Wagenaar E, Van Eijl S, Schinkel AH. Deficiency in the organic cation transporters 1 and 2 (Oct1/Oct2 [Slc22a1/Slc22a2]) in mice abolishes renal secretion of organic cations. Mol Cell Biol 2003;23:7902-8.

Kajiwara M, Terada T, Asaka J, Ogasawara K, Katsura T, Ogawa O, et al. Critical roles of Sp1 in gene expression of human and rat H+/organic cation antiporter MATE1. Am J Physiol Renal Physiol 2007;293:F1564-70.

Kajiwara M, Terada T, Ogasawara K, Iwano J, Katsura T, Fukatsu A, et al. Identification of multidrug and toxin extrusion (MATE1 and MATE2-K) variants with complete loss of transport activity. J Hum Genet 2009;54:40-6.

Kang HJ, Song IS, Shin HJ, Kim WY, Lee CH, Shim JC, et al. Identification and functional characterization of genetic variants of human organic cation transporters in a Korean population. Drug Metab Dispos 2007;35:667-75.

Karbach U, Kricke J, Meyer-Wentrup F, Gorboulev V, Volk C, Loffing-Cueni D, et al. Localization of organic cation transporters OCT1 and OCT2 in rat kidney. Am J Physiol Renal Physiol 2000;279:F679-87.

Katsuda H, Yamashita M, Katsura H, Yu J, Waki Y, Nagata N, et al. Protecting cisplatin-induced nephrotoxicity with cimetidine does not affect antitumor activity. Biol Pharm Bull 2010;33:1867-71.

Kekuda R, Prasad PD, Wu X, Wang H, Fei YJ, Leibach FH, et al. Cloning and functional characterization of a potential-sensitive, polyspecific organic cation transporter (OCT3) most abundantly expressed in placenta. J Biol Chem 1998;273:15971-9.

Kerb R, Brinkmann U, Chatskaia N, Gorbunov D, Gorboulev V, Mornhinweg E, et al. Identification of genetic variations of the human organic cation transporter hOCT1 and their functional consequences. Pharmacogenetics 2002;12:591-5.

Khamdang S, Takeda M, Noshiro R, Narikawa S, Enomoto A, Anzai N, et al. Interactions of human organic anion transporters and human organic cation transporters with nonsteroidal anti-inflammatory drugs. J Pharmacol Exp Ther 2002;303:534-9.

Koehler MR, Wissinger B, Gorboulev V, Koepsell H, Schmid M. The two human organic cation transporter genes SLC22A1 and SLC22A2 are located on chromosome 6q26. Cytogenet Cell Genet 1997;79:198-200.

Koepsell H. Polyspecific organic cation transporters: their functions and interactions with drugs. Trends Pharmacol Sci 2004;25:375-81.

Koepsell H, Endou H. The SLC22 drug transporter family. Pflugers Arch 2004;447:666-76.

Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. Pharm Res 2007;24:1227-51.

Koepsell H, Schmitt BM, Gorboulev V. Organic cation transporters. Rev Physiol Biochem Pharmacol 2003;150:36-90.

Kuroda T, Tsuchiya T. Multidrug efflux transporters in the MATE family. Biochim Biophys Acta 2009;1794:763-8.

Lazar A, Grundemann D, Berkels R, Taubert D, Zimmermann T, Schomig E. Genetic variability of the extraneuronal monoamine transporter EMT (SLC22A3). J Hum Genet 2003;48:226-30.

Leabman MK, Huang CC, Kawamoto M, Johns SJ, Stryke D, Ferrin TE, et al. Polymorphisms in a human kidney xenobiotic transporter, OCT2, exhibit altered function. Pharmacogenetics 2002;12:395-405.

Leazer TM, Klaassen CD. The presence of xenobiotic transporters in rat placenta. Drug Metab Dispos 2003;31:153-67.

Lips KS, Volk C, Schmitt BM, Pfeil U, Arndt P, Miska D, et al. Polyspecific cation transporters mediate luminal release of acetylcholine from bronchial epithelium. Am J Respir Cell Mol Biol 2005;33:79-88.

Masuda S, Terada T, Yonezawa A, Tanihara Y, Kishimoto K, Katsura T, et al. Identification and functional characterization of a new human kidney-specific H+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. J Am Soc Nephrol 2006;17:2127-35.

Meyer-Wentrup F, Karbach U, Gorboulev V, Arndt P, Koepsell H. Membrane localization of the electrogenic cation transporter rOCT1 in rat liver. Biochem Biophys Res Commun 1998;248:673-8.

Minuesa G, Volk C, Molina-Arcas M, Gorboulev V, Erkizia I, Arndt P, et al. Transport of lamivudine [(-)-beta-L-2',3'-dideoxy-3'-thiacytidine] and high-affinity interaction of nucleoside reverse transcriptase inhibitors with human organic cation transporters 1, 2, and 3. J Pharmacol Exp Ther 2009;329:252-61.

Moriyama Y, Hiasa M, Matsumoto T, Omote H. Multidrug and toxic compound extrusion (MATE)-type proteins as anchor transporters for the excretion of metabolic waste products and xenobiotics. Xenobiotica 2008;38:1107-18.

Nies AT, Herrmann E, Brom M, Keppler D. Vectorial transport of the plant alkaloid berberine by double-transfected cells expressing the human organic cation transporter 1 (OCT1, SLC22A1) and the efflux pump MDR1 P-glycoprotein (ABCB1). Naunyn Schmiedebergs Arch Pharmacol 2008;376:449-61.

Nies AT, Koepsell H, Damme K, Schwab M. Organic cation transporters (OCTs, MATEs), in vitro and in vivo evidence for the importance in drug therapy. Handb Exp Pharmacol 2011:105-67.

Nies AT, Koepsell H, Winter S, Burk O, Klein K, Kerb R, et al. Expression of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) is affected by genetic factors and cholestasis in human liver. Hepatology 2009;50:1227-40.

Ohta KY, Inoue K, Hayashi Y, Yuasa H. Molecular identification and functional characterization of rat multidrug and toxin extrusion type transporter 1 as an organic cation/H+ antiporter in the kidney. Drug Metab Dispos 2006;34:1868-74.

Ohta KY, Inoue K, Yasujima T, Ishimaru M, Yuasa H. Functional characteristics of two human MATE transporters: kinetics of cimetidine transport and profiles of inhibition by various compounds. J Pharm Pharm Sci 2009;12:388-96.

Omote H, Hiasa M, Matsumoto T, Otsuka M, Moriyama Y. The MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations. Trends Pharmacol Sci 2006;27:587-93.

Otsuka M, Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y. A human transporter protein that mediates the final excretion step for toxic organic cations. Proc Natl Acad Sci U S A 2005;102:17923-8.

Saito S, Iida A, Sekine A, Ogawa C, Kawauchi S, Higuchi S, et al. Catalog of 238 variations among six human genes encoding solute carriers (hSLCs) in the Japanese population. J Hum Genet 2002;47:576-84.

Sakata T, Anzai N, Kimura T, Miura D, Fukutomi T, Takeda M, et al. Functional analysis of human organic cation transporter OCT3 (SLC22A3) polymorphisms. J Pharmacol Sci 2010;113:263-6.

Sata R, Ohtani H, Tsujimoto M, Murakami H, Koyabu N, Nakamura T, et al. Functional analysis of organic cation transporter 3 expressed in human placenta. J Pharmacol Exp Ther 2005;315:888-95.

Shu Y, Leabman MK, Feng B, Mangravite LM, Huang CC, Stryke D, et al. Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. Proc Natl Acad Sci U S A 2003;100:5902-7.

Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, Castro RA, et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. J Clin Invest 2007;117:1422-31.

Slitt AL, Cherrington NJ, Hartley DP, Leazer TM, Klaassen CD. Tissue distribution and renal developmental changes in rat organic cation transporter mRNA levels. Drug Metab Dispos 2002;30:212-9.

Song IS, Shin HJ, Shim EJ, Jung IS, Kim WY, Shon JH, et al. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. Clin Pharmacol Ther 2008;84:559-62.

Staud F, Cerveny L, Ceckova M. Pharmacotherapy in pregnancy; effect of ABC and SLC transporters on drug transport across the placenta and fetal drug exposure. J Drug Target 2012;20:736-63.

Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. Biochem Pharmacol 2007;74:359-71.

Terada T, Inui K. Physiological and pharmacokinetic roles of H+/organic cation antiporters (MATE/SLC47A). Biochem Pharmacol 2008;75:1689-96.

Terada T, Masuda S, Asaka J, Tsuda M, Katsura T, Inui K. Molecular cloning, functional characterization and tissue distribution of rat H+/organic cation antiporter MATE1. Pharm Res 2006;23:1696-701.

Tsuda M, Terada T, Mizuno T, Katsura T, Shimakura J, Inui K. Targeted disruption of the multidrug and toxin extrusion 1 (mate1) gene in mice reduces renal secretion of metformin. Mol Pharmacol 2009;75:1280-6.

Wang DS, Kusuhara H, Kato Y, Jonker JW, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in the lactic acidosis caused by metformin. Mol Pharmacol 2003;63:844-8.

Wang ZJ, Yin OQ, Tomlinson B, Chow MS. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. Pharmacogenet Genomics 2008;18:637-45.

Wenge B, Geyer J, Bonisch H. Oxybutynin and trospium are substrates of the human organic cation transporters. Naunyn Schmiedebergs Arch Pharmacol 2011;383:203-8.

Wu X, Kekuda R, Huang W, Fei YJ, Leibach FH, Chen J, et al. Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. J Biol Chem 1998;273:32776-86.

Yonezawa A, Inui K. Importance of the multidrug and toxin extrusion MATE/SLC47A family to pharmacokinetics, pharmacodynamics/toxicodynamics and pharmacogenomics. Br J Pharmacol 2011;164:1817-25.

You G, Morris ME. Overview of Drug Transporter Families. In: You G, Morris ME, editors. Drug Transporters: Molecular Characterization and Role in Drug Disposition. New Jersey: John Wiley & Sons, Inc, 2007. p. 1-10.

Zhang J, Zhou W. Ameliorative effects of SLC22A2 gene polymorphism 808 G/T and cimetidine on cisplatin-induced nephrotoxicity in Chinese cancer patients. Food Chem Toxicol 2012;50:2289-93.

Zolk O, Solbach TF, Konig J, Fromm MF. Structural determinants of inhibitor interaction with the human organic cation transporter OCT2 (SLC22A2). Naunyn Schmiedebergs Arch Pharmacol 2009;379:337-48.

Zwart R, Verhaagh S, Buitelaar M, Popp-Snijders C, Barlow DP. Impaired activity of the extraneuronal monoamine transporter system known as uptake-2 in Orct3/Slc22a3-deficient mice. Mol Cell Biol 2001;21:4188-96.