SCHOOL OF PHARMACY
UNIVERSITY of WASHINGTON

OATP2B1: In vitro, Proteomic, and Clinical PK Relevance in GI and Liver

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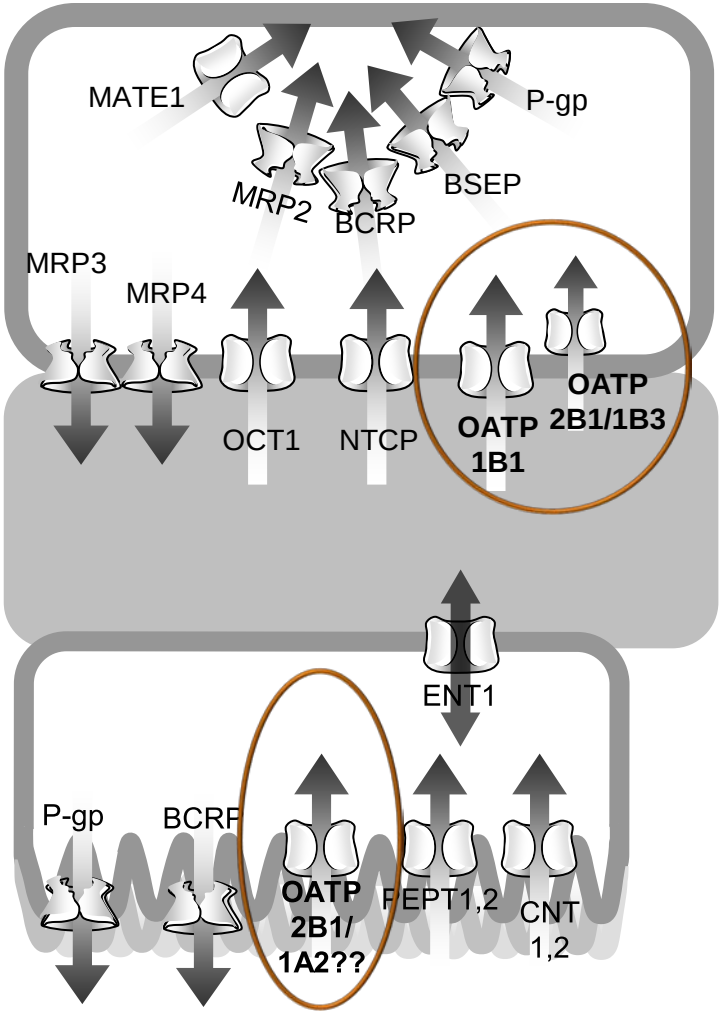


Liver

Intestinal and Hepatobiliary Transporters

Intestine

Unadkat JD, *Enzyme-and Transporter-Based Drug-Drug Interactions:* 2010.

Transporters included in FDA guidance on Drug Interactions

Solute Carrier (SLC) superfamily	ATP-binding cassette (ABC) superfamily
OATP1B1*	ABCB1* (P-gp)
OATP1B3*	ABCG2* (BCRP)
OCT2*	
OAT1/3*	
MATE1*, MATE-2K*	

OATP2B1 is missing in action!

https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table4-1

Exclusion of OATP2B1 Should Be Reconsidered

Based on data on:

1. Expression of the transporter in the liver and intestine
2. In vitro and in vivo substrate selectivity – broader than previously thought
3. In vitro and in vivo DDI

Exclusion of OATP2B1 from studies:

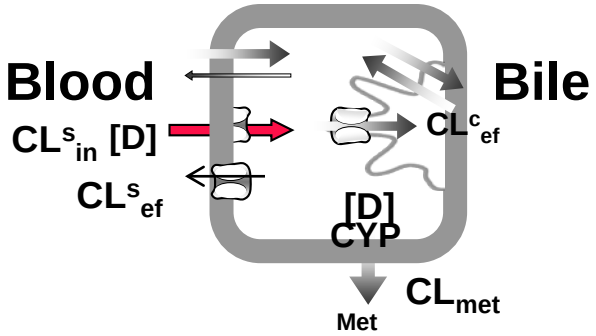
1. May produce inaccurate IVIVE of CL when transporters are rate-determining step
2. May result in inaccurate prediction of the magnitude of DDI

Transporters can Determine PK of Drugs

$$CL_h = \frac{Q_h \, fuCL^s_{in} \left(CL_{met} + CL^s_{ef} \right)}{Q_h \left(CL_{met} + CL^s_{ef} + CL^s_{ef} \right) + fuCL^s_{in} \left(CL_{met} + CL^s_{ef} \right)} + CL_{other}$$

When CLother = 0 and $CL^{s}_{ef} << CL_{met} + CL^{s}_{ef}$

$$CL_h = \frac{Q_h \, fuCL^s_{in}}{Q_h + fuCL^s_{in}}$$

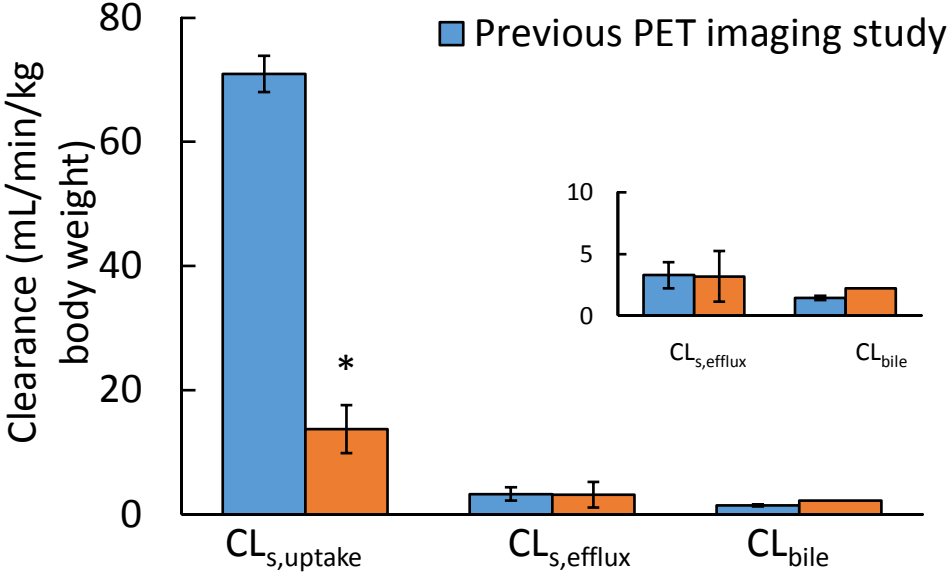


Endres et al., Mol Pharm. 2009 6:1756-65 Patilea-Vrana, Clin Pharmacol Ther In Press 2016

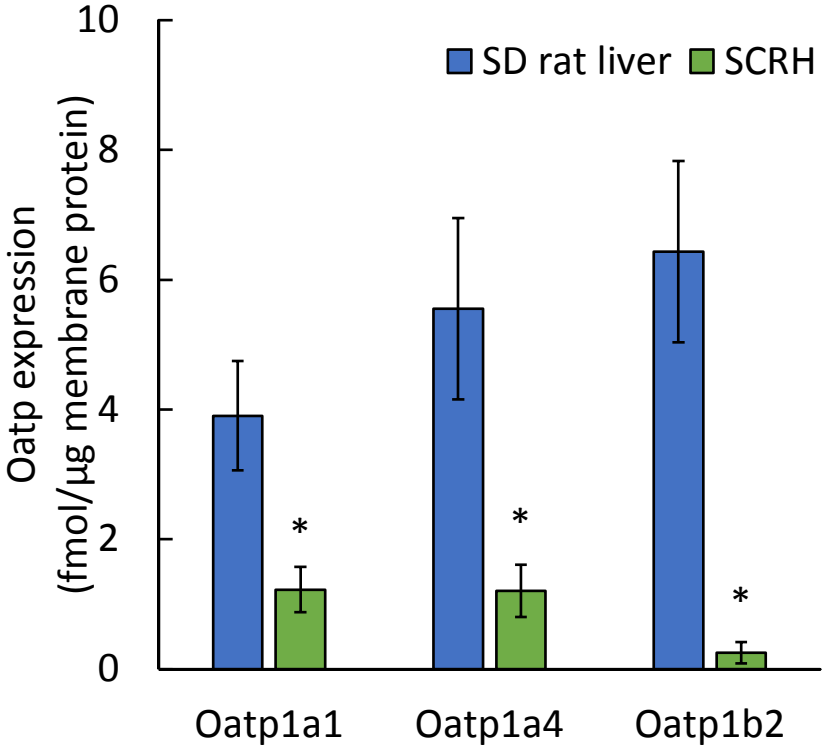
CL^{s}_{in} determines CLh

Patilea-Vrana, Clin Pharmacol Ther 2016 100:413-18

SCRH underestimate $CL_{s,uptake}$ and have lower expression of Oatps than SD rat livers

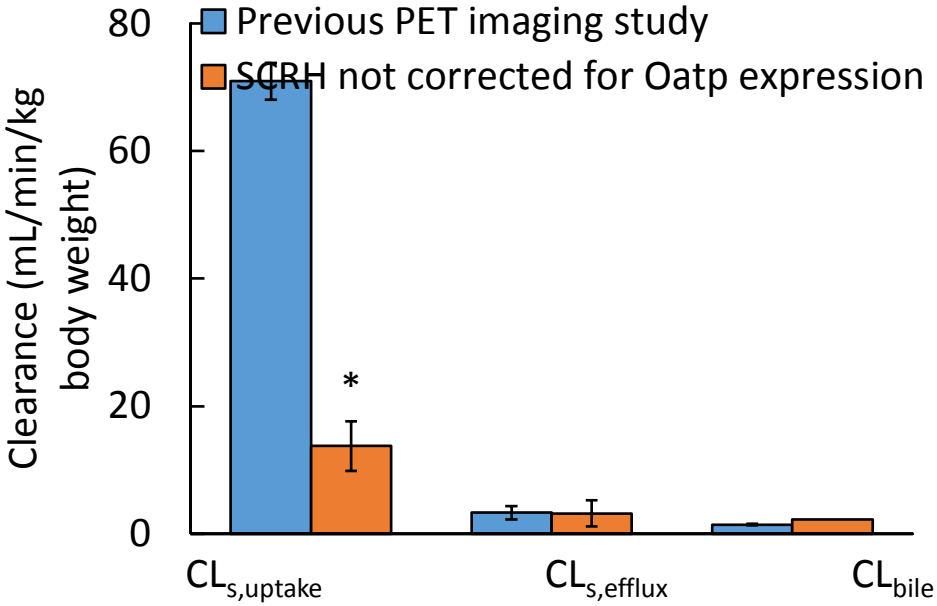


Ishsida et al., unpublished data



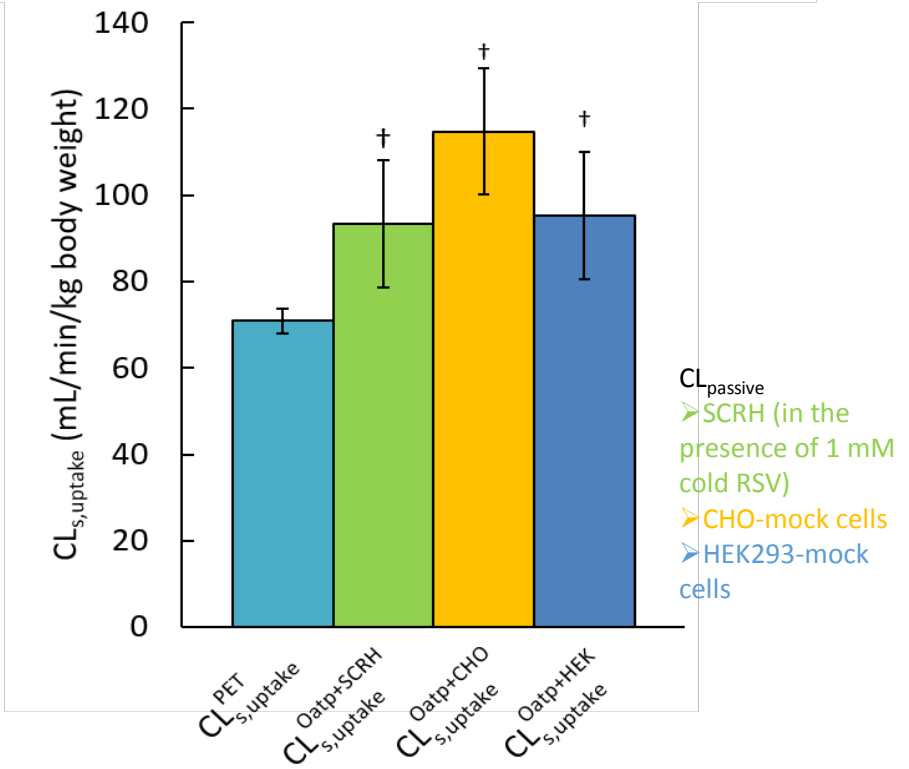
affinity for rosuvastatin Oatp1a1>1a4>1b2

Successful IVIVE Prediction of In Vivo Sinusoidal Uptake Clearance of Rosuvastatin When Corrected for Oatp Expression



Ishsida et al., unpublished data

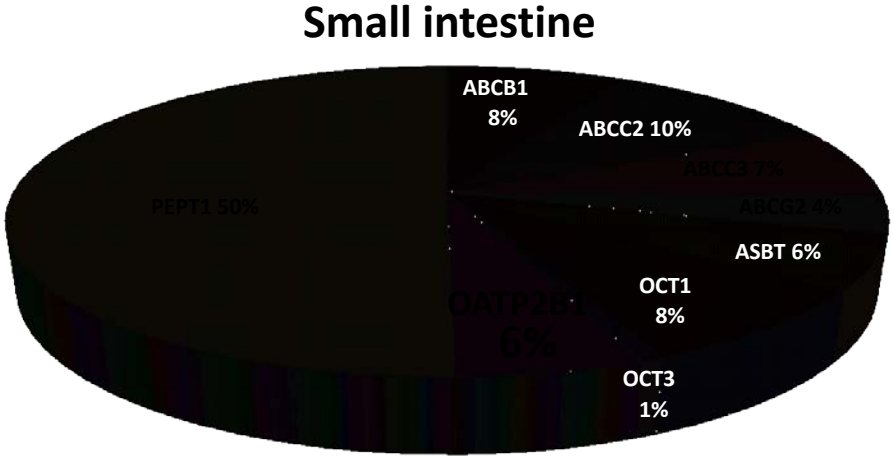
In vitro-to-*in vivo* extrapolation

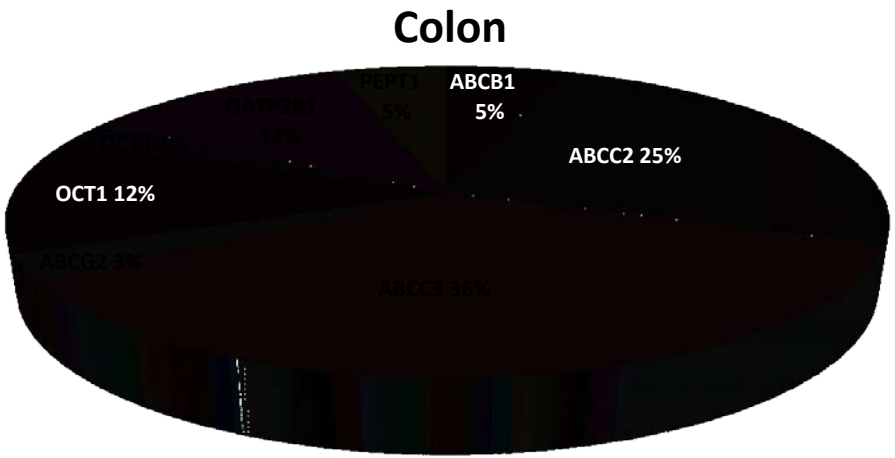


OATP2B1 is Well-Expressed in the Human Intestine

N=6, 5 males, 1 female;

Relative contribution

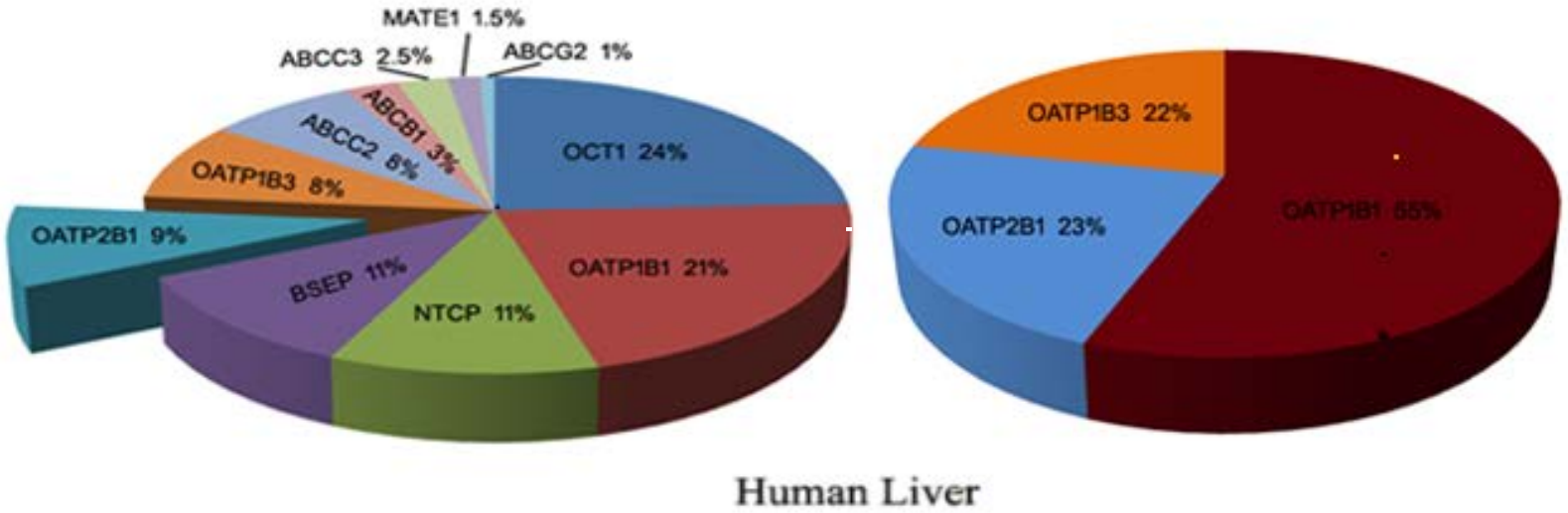




OATP1A2 could not be detected in the small intestine

Drozdzik, ..Unadkat et al., Mol Pharm. 2014 Oct 6;11(10):3547-55

Hepatic Expression of OATP2B1 is Comparable to that of OATP1B3



Examples of OATP2B1 Substrates

Substrate	K_m (μM)-OATP2B1	pH	K_m (μM)-OATP1B1	K_m (μM)-OATP1B3
Fluvastatin	0.7	7.3	2.4	7.0
Pitavastatin	1.2	7.4	3.3	3.0
Pravastatin	2.3	5.0	33.7	-
Rosuvastatin	2.4	7.4	2.6	9.8
Atorvastatin	2.84	7.3	0.77	0.73
Glyburide	6.3	7.4	-	NS
Estrone-3-sulfate	8.09	7.4	12.5	-
	13.1	5.0		
Taurocholic acid	71.8	5.9	33.8	42.2
Flavopiridol	175	7.4	66	66.8
Mesalazine	189	7.3	55.1	77.4
Dehydroepiandrosterone-3-sulfate	>200	7.4	21.5	>30
Bosentan	202	7.4	44	141

Selective Substrates of OATP2B1

Substrate	K_m (μM) for OATP2B1	pH	K_m (μM) for OATP1B1	K_m (μM) for OATP1B3
Sulfasalazine	1.7	7.4	Not a substrate	Not a substrate
Aliskiren	72	7.4	Not a substrate	Not a substrate
Unaprostone	91	7.5	Not a substrate	Not a substrate
Celiprolol	-*	7.4	Not a substrate	Not a substrate

* K_m value was not calculated, but the drug was shown to be a substrate.

Inhibitors of OATP2B1 (also substrates)

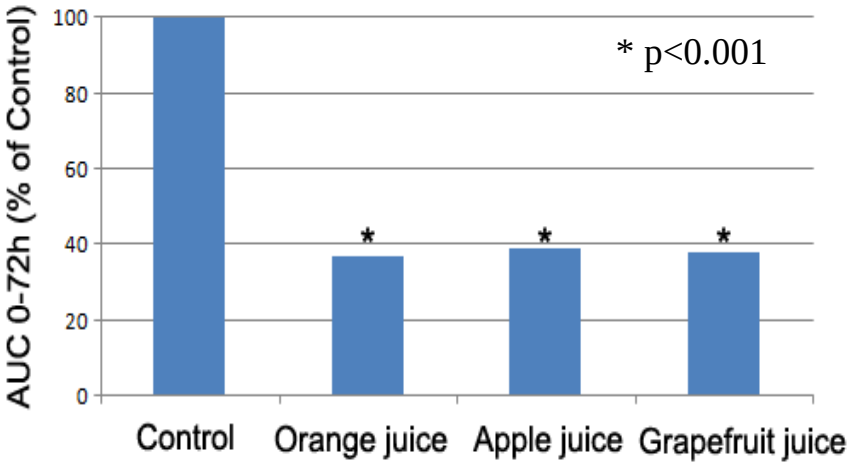
Inhibitor	Substrate	IC_{50} or K_i (μM)
Asunaprevir	E3S	0.27
Atorvastatin	E3S	0.7
BSP	E3S	-
DHEAS	E3S	-
Eltrombopag	E3S	8.5
Glyburide	BSP	2
Montelukast	E3S	-
Penicillin G	E3S	-
Pravastatin	E3S	-
Sulfasalazine	E3S	3.0
Taurocholic acid	E3S	-

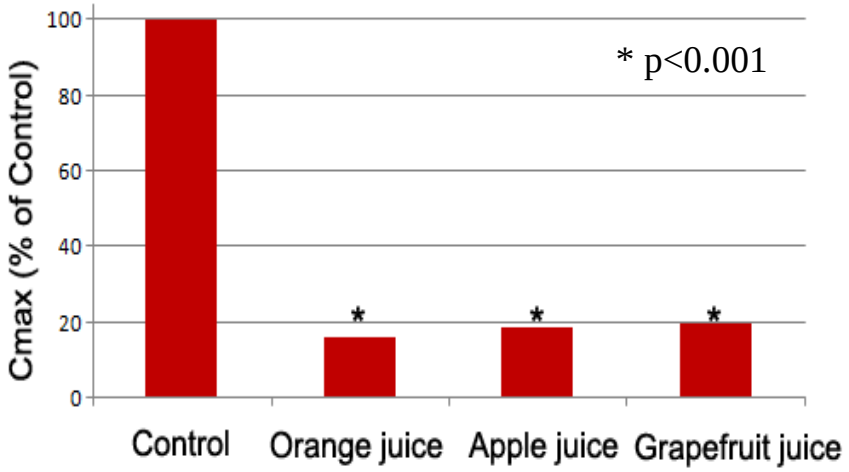
Inhibitors of OATP2B1 (not substrates)

Clinically used drugs				Natural products		
Inhibitor	**Substrate**	**IC_{50} or K_i (μM)**		**Inhibitor**	**Substrate**	**IC_{50} or K_i (μM)**
Neratinib	E3S	2.68		Quercetin	BSP	8.7
Nilotinib	E3S	2.67			E3S	9.47
Pelitinib	E3S	2.01			Atorvastatin	14.1
Rifampin	Flavopiridol	1.36		Rutin	E3S	60.7
	E3S	65		Scutellarin	E3S	2.0
Ritonavir	E3S	6.1		Silybin A	E1S	4.5
Ronacaleret	Rosuvastatin	12		Silybin B	E1S	0.8
Talinolol	E3S	-		Silychristin	E1S	3.6
Testosterone	E3S	-		Silymarin	E1S	0.3

Role of Intestinal OATP2B1 In In Vivo Drug-Drug Interactions

Victim: Aliskiren, 150mg, p.o, Perpetrator: Fruit juice



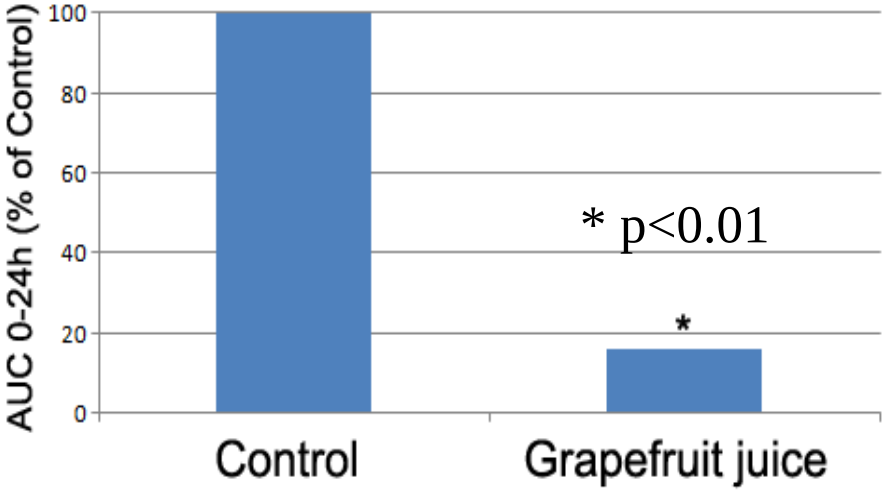


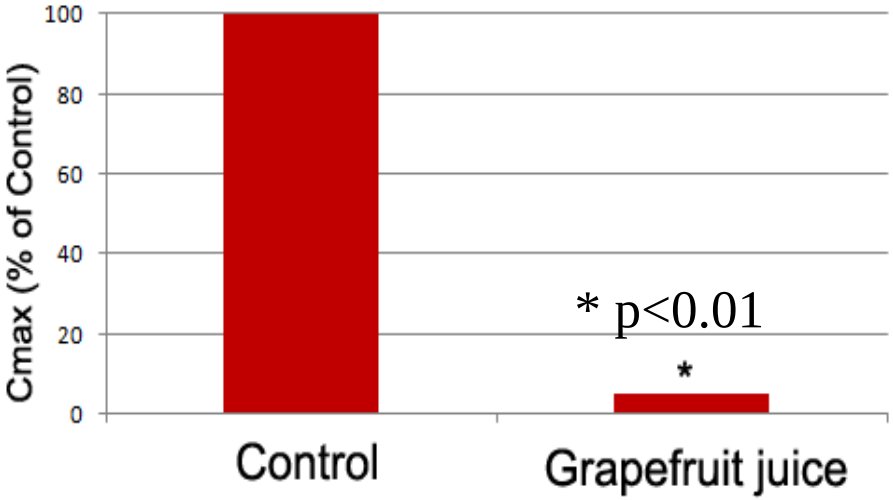
The participants ingested 200 ml of orange/apple/ grapefruit juice three times a day for 5 days. On day 3, they ingested a single 150-mg dose of aliskiren with 200 ml of orange/apple/grapefruit juice.

Tapaninen et al. Br J Clin Pharmacol. 2011,71:718. Tapaninen et al Clin Pharmacol Ther. 2010, 88:339.

Role of OATP2B1 In In Vivo Drug-Drug Interactions

Victim: Celiprolol, 100mg, p.o. Perpetrator: Grape fruit juice





participants took 200 mL of grapefruit juice 3 times a day after meals for 2 days. On day 3, the participants received 100 mg celiprolol hydrochloride with 200 mL of grapefruit juice. They also took 200 mL of grapefruit juice at 0.5 and 1.5 hours after dosing.

Iieri et al. J Clin Pharmacol. 2012, 52(7):1078-89.

Other OATP2B1 and Drug-Drug (or food) Interactions

Victim (dose)	Perpetrator (dose)	AUC	C_{max}
Aliskiren(150mg, p.o.)	Apple juice	63% ↓	84% ↓
Aliskiren (150mg, p.o.)	Grapefruit juice	61% ↓	81% ↓
Aliskiren (150mg, p.o.)	Orange juice	62% ↓	80% ↓
Aliskiren (300mg×7d, p.o.)	Atorvastatin (10mg×4d, p.o.)	1.5-fold ↑	1.5-fold ↑
Celiprolol (100mg, p.o.)	Grapefruit juice	84% ↓	95% ↓
Rosuvastatin(10mg, p.o.)	Ronacaleret (400mg, p.o.)	47% ↓	34% ↓
	Ronacaleret (400mg×10d, p.o.)	49% ↓	33% ↓
Asunaprevir (200mg, p.o.)	Rifampicin (600mg, p.o.)	15-fold ↑	21-fold ↑
Atorvastatin (10mg×4d, p.o.)	Aliskiren (300mg×7d, p.o.)	9% ↓	23% ↓

Conclusions and Recommendations

- The assumed narrower substrate specificity of OATP2B1 compared to other OATPs maybe only be because of limited research
- OATP2B1 appears to be crucial for the intestinal absorption of some drugs – but the role of other transporters cannot be discounted
- Based on the expression and function of OATP2B1, it likely contributes to drug absorption and hepatic clearance of drugs to a greater extent than currently predicted
- NME should be screened to determine if it is a substrate or inhibitor of OATP2B1

Major Contributors

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Bhagwat Prasad

Anand Deo

Other Collaborators

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Dept. of Radiology: Jeanne Link, David Mankoff, Todd Richards, Janet Eary, Satoshi Minoshima, Ken Maravilla, Mark Muzi, Steve Shoner and the PET suite team

Dept. of Medicine: Ann Collier and her team

Dept. of Anesthesiology: Karen Domino

Dept. of Pharmaceutics: Bhagwat Prasad, Edward Kelly, Carol Collins, Joanne Wang

Kidney Research Institute: Jonathan Himmelfarb

Univ. of Greifswald: Stefan Oswald and team

Children's Mercy Hospitals: Steven Leeder and team

Newcastle Univ.: Colin Brown

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UWRAPT
ATP
ADP + Pi

Unadkat Team

Jiake He, Bhagwat Prasad, Vineet Kumar, Gabriela Patilea

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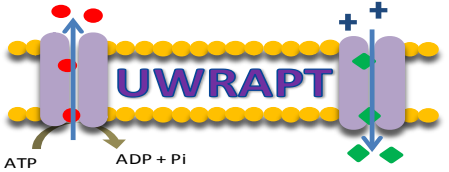
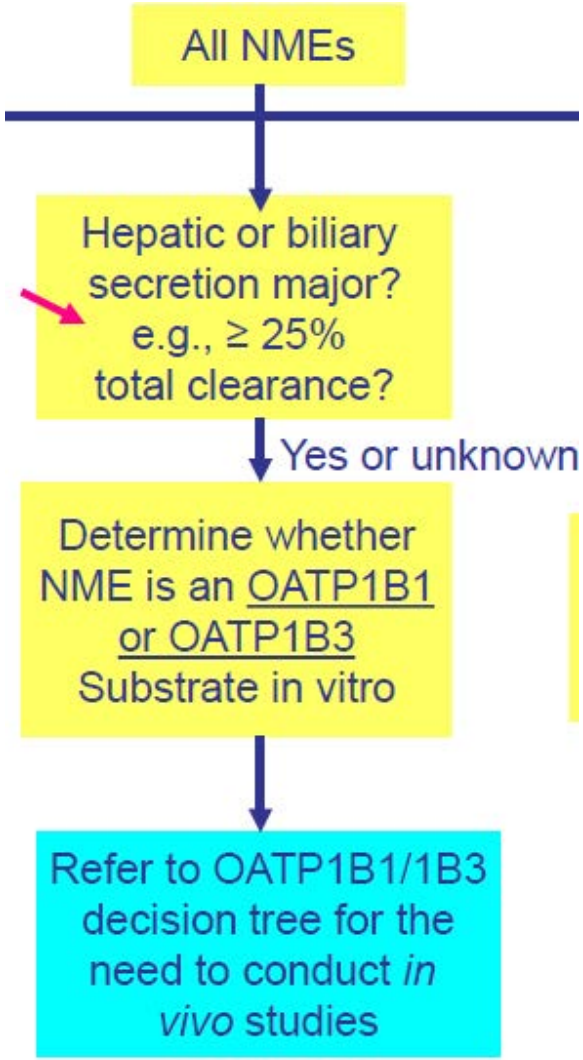
OATP2B1 and drug-drug(food) interactions



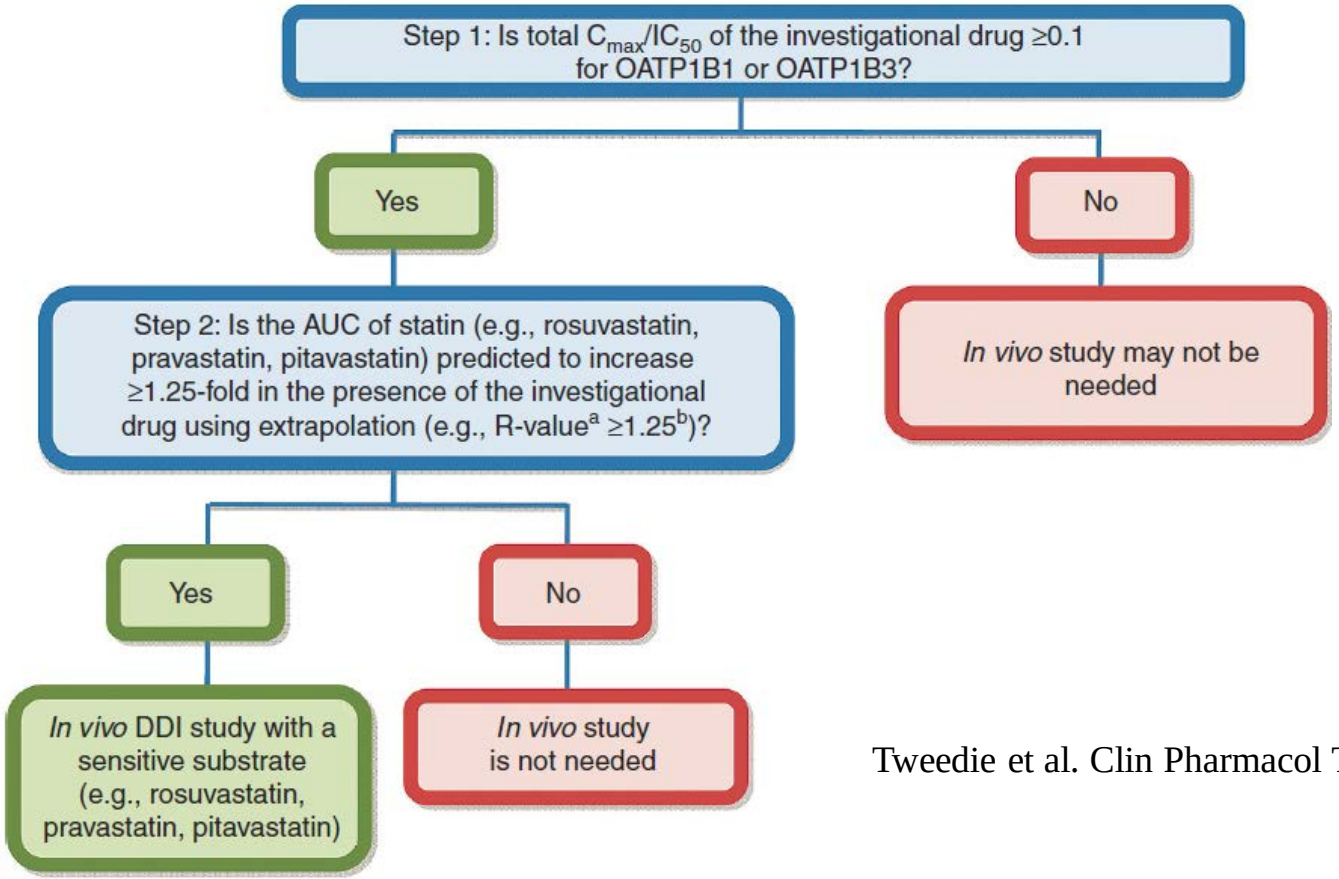
Victim (dose)	Perpetrator (dose)	Change in AUC	Change in C_{max}
Glyburide (1.25mg, p.o.)	Rifampicin (600mg, i.v.)	1.8-fold ↑	2.2-fold ↑
Montelukast (10mg, p.o.)	Gemfibrozil (1200mg×3d, p.o.)	4.5-fold ↑	1.5-fold ↑
Montelukast (10mg, p.o.)	Orange juice	21% ↓	16% ↓
Rosuvastatin(10mg, p.o.)	Asunaprevir (400mg×10d, p.o.)	1.4-fold ↑	1.9-fold ↑



OATP2B1 Not Included in FDA guidance on Drug Interactions

From Lei Zhang, FDA presentation to ASCPT 2015

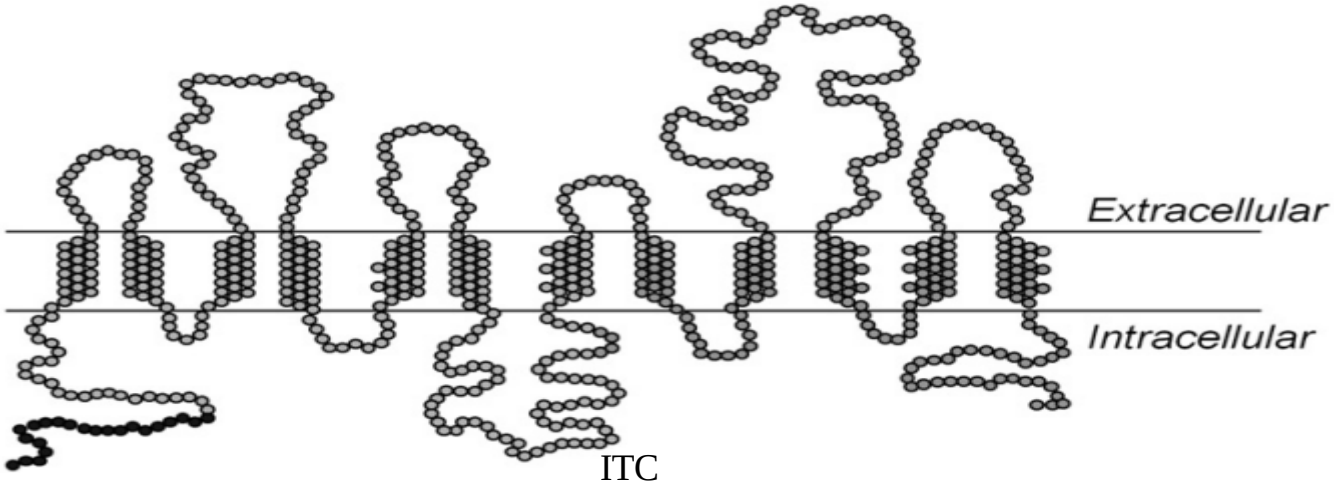
OATP2B1 Not Included in FDA guidance on Drug Interactions



Tweedie et al. Clin Pharmacol Ther. 2013 Jul;94(1):113-25

Organic Anion Transporting Polypeptide 2B1 (OATP2B1)

- A member of the the organic anion transporting polypeptide (OATP) family, solute carrier (SLC) superfamily.
- originally isolated from human brain in 2000.
- a 709-amino acid glycoprotein, 12 putative transmembrane-spanning domains.

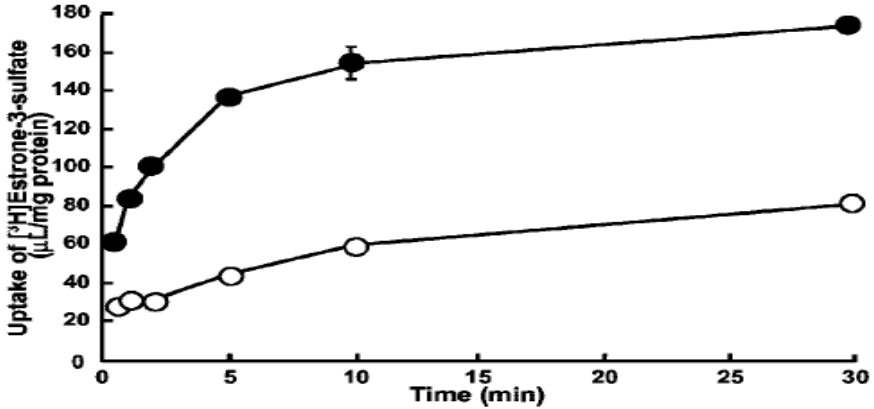


Mol Pharmacol. 2013,83(6):1218-28.

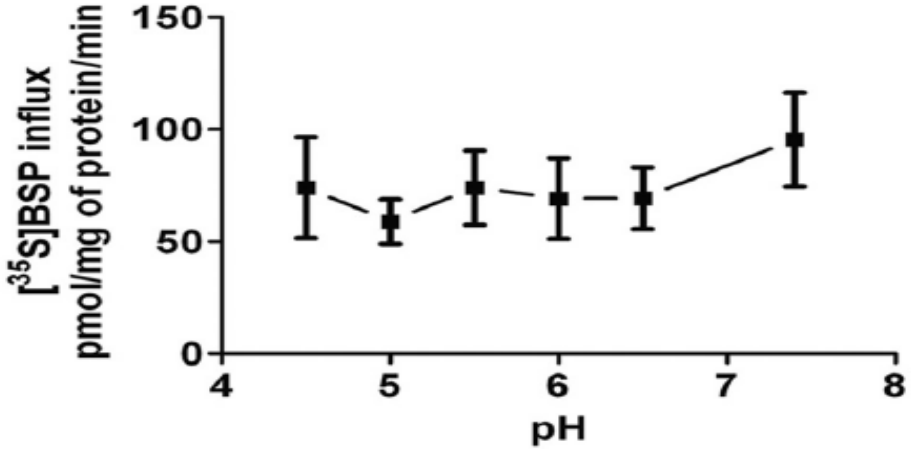
OATP2B1 uptake activity

Two characteristics:

- pH-dependent (substrate-



OATP2B1-induced estrone-3-sulfate uptake is pH-dependent



OATP2B1-induced BSP uptake is pH-independent.

pH	Km (μM)	Vmax (pmol/mg protein/min)
5.0	13.1± 3.2	2136±410
7.4	8.09± 1.67	300±46

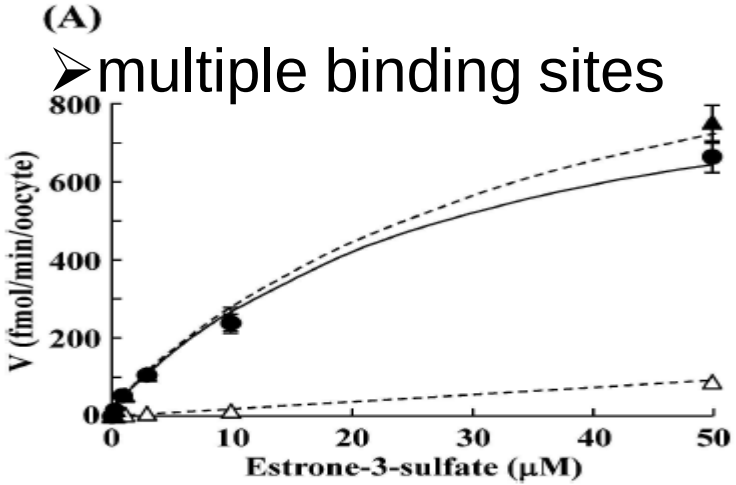
ITC

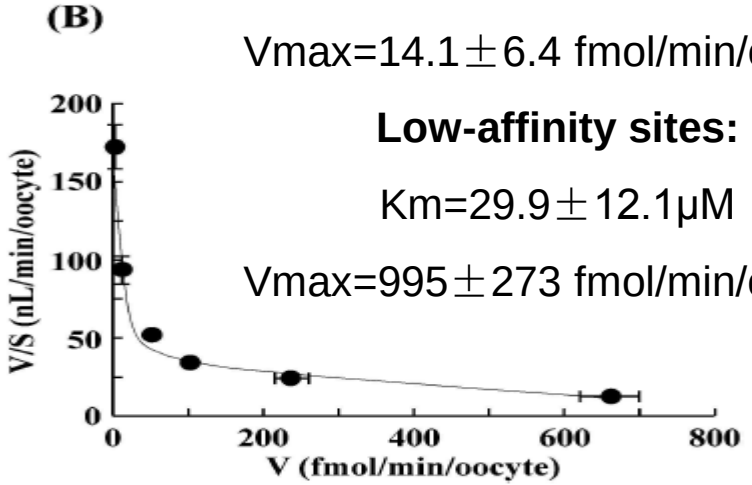
J Pharmacol Exp Ther. 2004,308(2):438-45.
Mol Pharmacol. 2012, 81(2):134-42

OATP2B1 uptake activity

Two characteristics:

- pH-dependent
- multiple binding sites





High-affinity sites:

Km=0.10±0.05 μM

Vmax=14.1±6.4 fmol/min/oocyte

Low-affinity sites:

Km=29.9±12.1μM

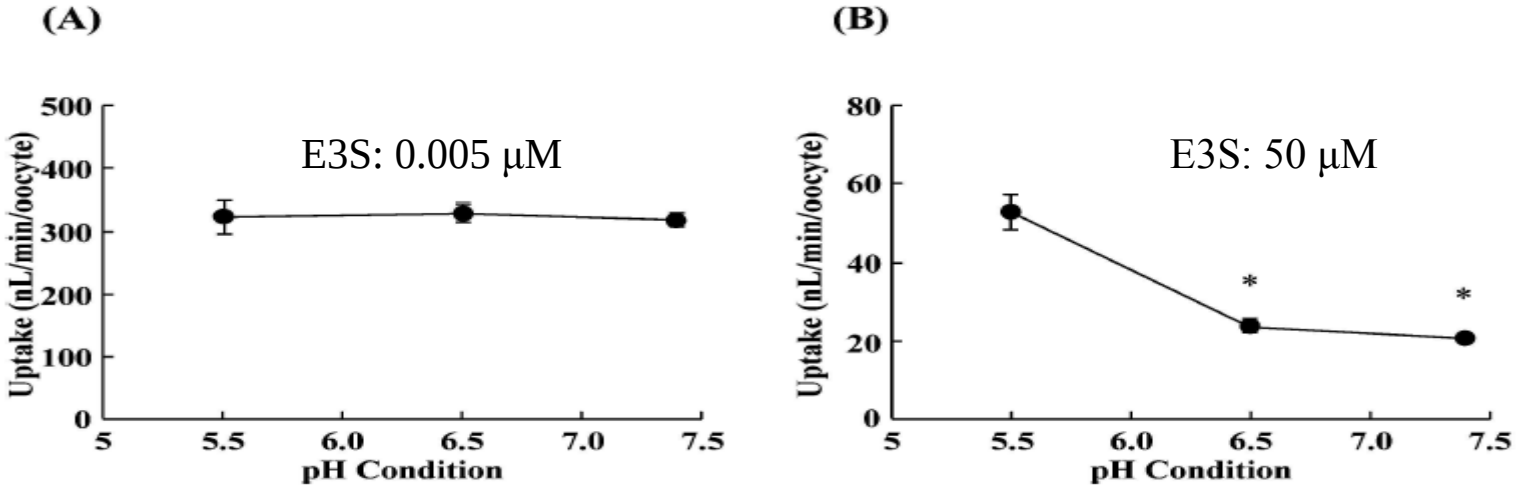
Vmax=995±273 fmol/min/oocyte

(A) Michaelis-Menten and (B) Eadie-Hofstee plots of estrone-3-sulfate uptake by Xenopus oocytes expressing OATP2B1 (pH6.5). Open triangles, water-injected; Filled triangles, OATP2B1 cRNAinjected; Filled circles, OATP2B1-mediated uptake.

Drug Metab Pharmacokinet. 2012,27(3):360-4.

OATP2B1 uptake activity

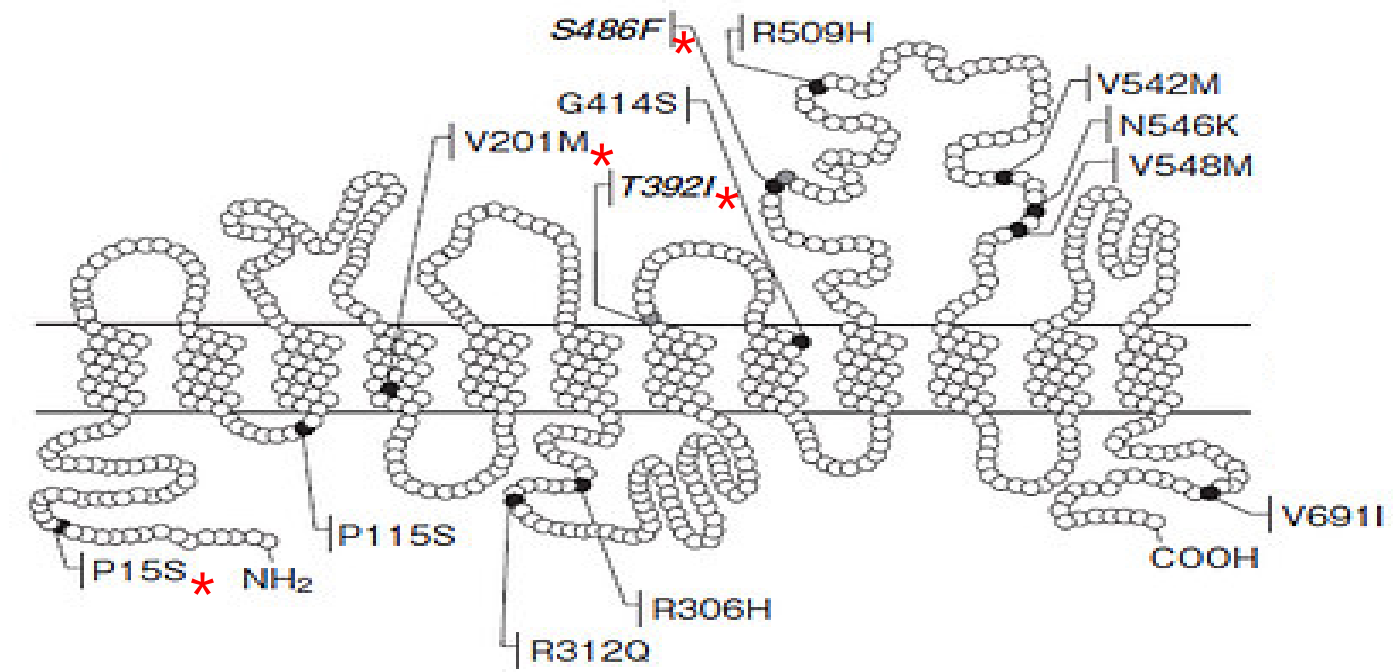
Different binding site shows different pH-sensitivity.



Uptake of estrone-3-sulfate mediated by (A) high- and (B) low-affinity sites on OATP2B1 by Xenopus oocytes expressing OATP2B1.

Drug Metab Pharmacokinet. 2012,27(3):360-4.

Pharmacogenetics of *SLCO2B1*



* Results in decreased OATP2B1 activity *in vitro*.

Drug Metab Pharmacokinet. 2012,27(1):106-21.

Pharmacogenetics of *SLCO2B1*

Parameter	*SLCO2B1* c.1457C>T genotype			
	CC ($n=5$)	CT ($n=5$)	TT ($n=4$)	CT + TT ($n=9$)
$AUC_{0-\infty}$ (ng·h/ml)	1762 ± 542	1088 ± 449	1136 ± 225	1110 ± 347[c]
Ratio vs. CC genotype		0.61	0.66	0.63
(95% CI)		(0.34–1.05)	(0.42–1.05)	(0.42–0.94)
C_{max} (ng/ml)	343 ± 127	224 ± 139	179 ± 42.0[c]	204 ± 104
Ratio vs. CC genotype		0.58	0.54	0.57
(95% CI)		(0.25–1.37)	(0.32–0.91)	(0.32–1.00)
t_{max} (h)	1.5 (1.5–2.5)	1.5 (1.0–3.0)	1.8 (1.5–2.5)	1.5 (1.0–3.0)
$t_{1/2}$ (h)	3.2 ± 0.6	3.0 ± 0.4	3.9 ± 1.1	3.4 ± 0.9
Ratio vs. CC genotype		0.94	1.19	1.05
(95% CI)		(0.73–1.20)	(0.81–1.76)	(0.81–1.36)
CL/F/weight (l/h/kg)	0.6 ± 0.2	1.0 ± 0.4	0.8 ± 0.2	0.9 ± 0.3
Ratio vs. CC genotype		1.61	1.42	1.52
(95% CI)		(0.92–2.80)	(0.84–2.41)	(1.00–2.32)

[c]P<0.05 (compared with CC group).

Inconsistent effects of c.1457C>T polymorphism of SLCO2B1 on fexofenadine pharmacokinetics after 60mg oral administration.

	n	C_{max} (ng ml^{-1})	p Values	$t_{1/2}$ (h)	p Values	AUC_{0-24} (ng h l^{-1})	p Values
S-Fexofenadine							
SLCO2B1							
*1/*1	14	111 (27–186)	0.931	2.6 (2.0–4.9)	0.12	446 (112–643)	0.031
*1/*3+*3/*3	10	113 (53–152)		3.6 (1.8–7.7)		675 (298–1123)	
R-Fexofenadine							
SLCO2B1							
*1/*1	14	148 (40–269)	0.183	3.3 (2.5–5.7)	0.134	764 (241–1113)	0.212
*1/*3+*3/*3	10	133 (61–179)		4 (2.5–6.2)		916 (496–1366)	

ITC

Pharmacogenet Genomics. 2011,21(2):84-93.

Pharmacogenetics of *SLCO2B1*

	SLCO2B1 c.1457C>T genotype			
Parameter	CC ($n=5$)	CT ($n=5$)	TT ($n=4$)	CT + TT ($n=9$)
$AUC_{0-\infty}$ (ng·h/ml)	1762 ± 542	1088 ± 449	1136 ± 225	1110 ± 347[c]
Ratio vs. CC genotype		0.61	0.66	0.63
(95% CI)		(0.34–1.05)	(0.42–1.05)	(0.42–0.94)
C_{max} (ng/ml)	343 ± 127	224 ± 139	179 ± 42.0[c]	204 ± 104
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(95% CI)		(0.25–1.37)	(0.32–0.91)	(0.32–1.00)
t_{max} (h)	1.5 (1.5–2.5)	1.5 (1.0–3.0)	1.8 (1.5–2.5)	1.5 (1.0–3.0)
$t_{1/2}$ (h)	3.2 ± 0.6	3.0 ± 0.4	3.9 ± 1.1	3.4 ± 0.9
Ratio vs. CC genotype		0.94	1.19	1.05
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CL/F/weight (l/h/kg)	0.6 ± 0.2	1.0 ± 0.4	0.8 ± 0.2	0.9 ± 0.3
Ratio vs. CC genotype		1.61	1.42	1.52
(95% CI)		(0.92–2.80)	(0.84–2.41)	(1.00–2.32)

[c]P<0.05 (compared with CC group).

Inconsistent effects of c.1457C>T polymorphism of SLCO2B1 on fexofenadine pharmacokinetics after 60mg oral administration.

	n	C_{max} (ng ml^{-1})	p Values	$t_{1/2}$ (h)	p Values	AUC_{0-24} (ng h l^{-1})	p Values
S-Fexofenadine							
SLCO2B1							
*1/*1	14	111 (27–186)	0.931	2.6 (2.0–4.9)	0.12	446 (112–643)	0.031
*1/*3+*3/*3	10	113 (53–152)		3.6 (1.8–7.7)		675 (298–1123)	
R-Fexofenadine							
SLCO2B1							
*1/*1	14	148 (40–269)	0.183	3.3 (2.5–5.7)	0.134	764 (241–1113)	0.212
*1/*3+*3/*3	10	133 (61–179)		4 (2.5–6.2)		916 (496–1366)	

ITC

Pharmacogenet Genomics. 2011,21(2):84-93.

Pharmacogenetics of *SLCO2B1*

rs number	Nucleotide change	Amino acid variation	Allelic frequency			
			Caucasian	Asian	Mexican	African-American
Rs56837383	C.43C>T	P15S	0	0	0	1.6
Rs148248368	C.277C>A	P115S	NA	NA	NA	NA
Rs35199625	C.601G>A	V201M	0	4.2	1.2	0.8
Rs12422149	C.935G>A	R312Q	8.5	37.5	29.5	10.5
Rs1621378	C.1175C>T	T392I	NA	NA	NA	NA
Rs111782322	C.1174G>A	G414S	NA	NA	NA	NA
Rs2306168	C.1475C>T	S486F	3.9	19.2	9.1	34.1
Rs140407559	C.1526G>A	R509H	NA	NA	NA	NA
Rs143480565	C.1624G>A	V542M	NA	NA	NA	NA
Rs145875125	C.1638C>A	N546K	0	0	0	0.8
Rs149242910	C.1642G>A	V548M	NA	NA	NA	NA
Rs149765874	C.2071G>A	V691I	NA	NA	NA	NA

NA, not available.

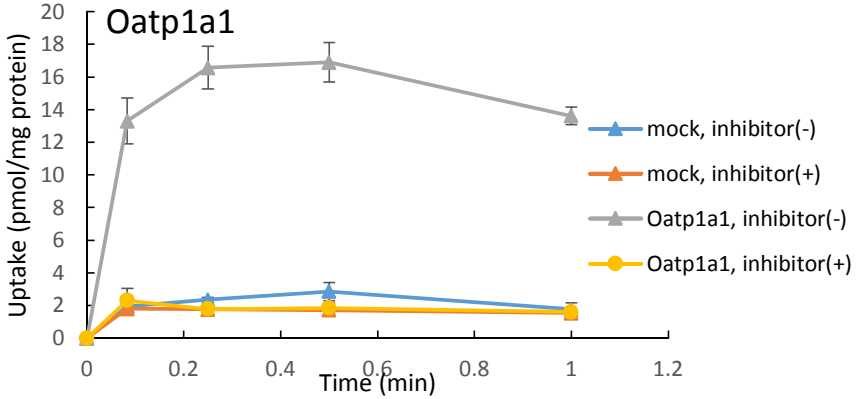
Conclusions

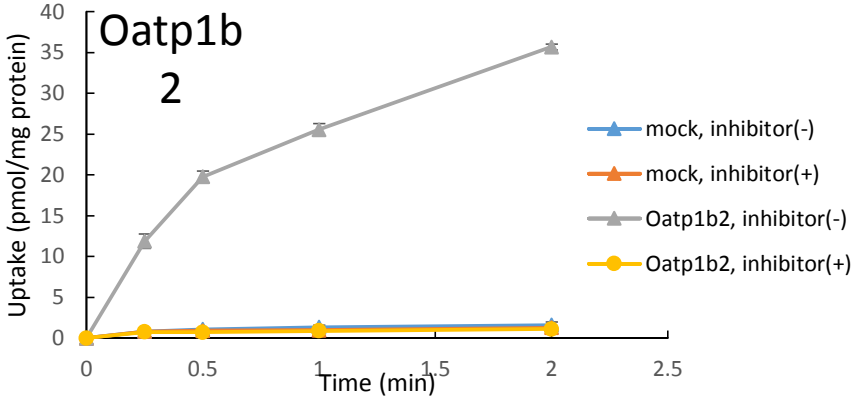
- ✓ This is the first report of successful prediction of *in vivo* hepatobiliary clearance and hepatic concentrations of a drug from studies in SCRH and transporter-expressing cell lines.

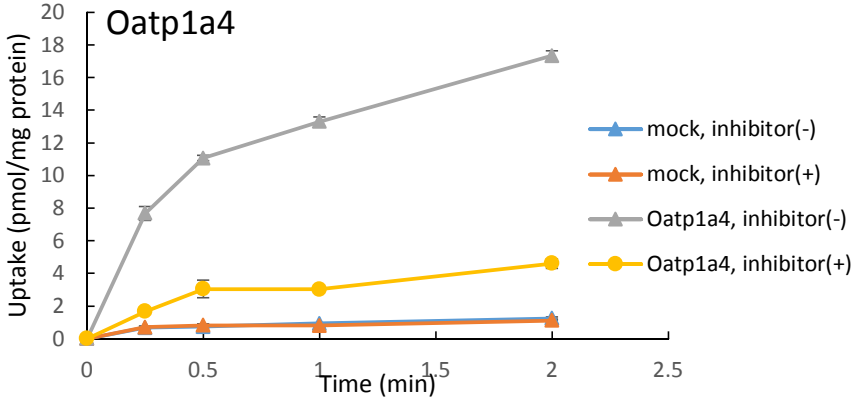
- ✓ The under-prediction of $CL_{s,uptake}$ of RSV is due to the lower expression of Oatps in SCRH vs. liver tissue.

- ✓ Transporter expression should be measured in *in vitro* systems used to predict *in vivo* hepatobiliary clearance of drugs.

RSV uptake into CHO-rOatp1a1, HEK293-rOatp1a4, and HEK293-rOatp1b2 cells







RSV concentration: 0.5 µM (hot + cold)
Pre-incubation: 10 min
Inhibitor: rifamycin SV (100 µM)

Hepatic RSV concentrations were predicted well