JSSX Award
Essential Roles of Transporters in Absorption and Disposition of Endo/Xenobiotics

輸送体を基盤とする薬物動態と生理機構に関する研究

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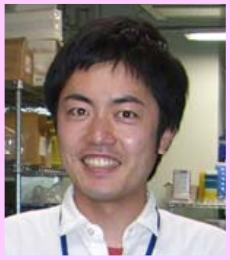

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Major Transporter-Related Research since 1982

1: Organic anion transporters (OATPs and MCTs):

1980'-: Hepatic disposition of drugs
1990'-: Intestinal absorption of drugs
2000'-: Hormone dependence of cancer cells
2010'-: Prostaglandins and pathophysiology.

2: Organic cation/carnitine transporters (OCTNs):

1997-: Finding of OCTN1 and OCTN2
1998-: Systemic carnitine deficiency
2000'-: Blood cell growth and differentiation
2000'-: Drug absorption and disposition (Lung, Kidney, Cancer *etc*.)

3: Peptide transporter (PEPT1):

1980'-: Intestinal absorption of β-lactam antibiotics
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2000'-: Oral and cancer delivery by PEPT1

4: Efflux transporters (MDR1)

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1990'-: P-gp as the blood-brain and intestinal-absorption barriers

5: Urate transporters (URAT1, GLUT9, BCRP)

2000'-: Drug-induced change of serum urate level
2010'-: Regulation mechanism and association with disease

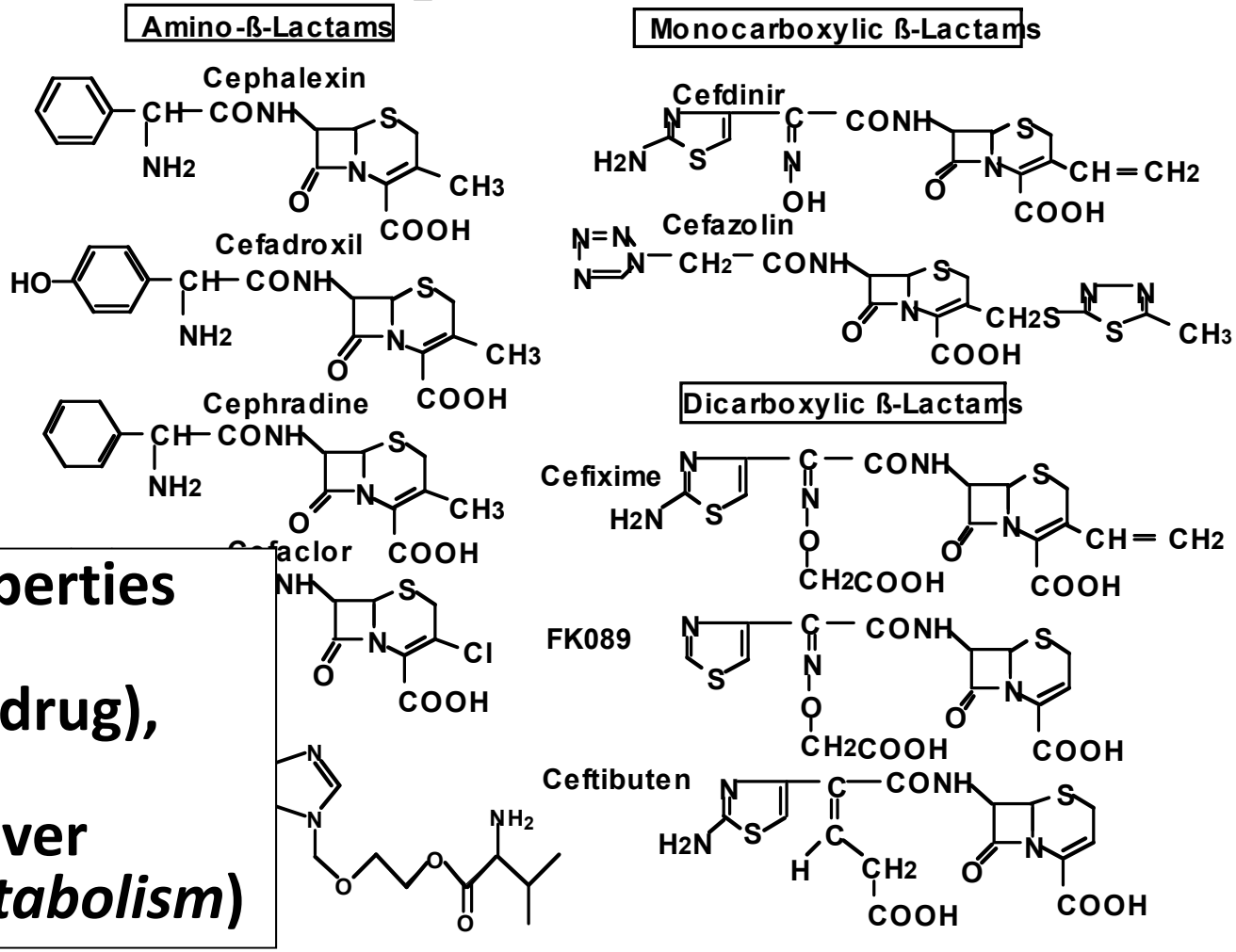
Physicochemical and PK Properties of ß-Lactam Antibiotics

Hydrophilic
Anionic or
Zwitterionic
pKa,
3-4 (acid)
6-8 (base)

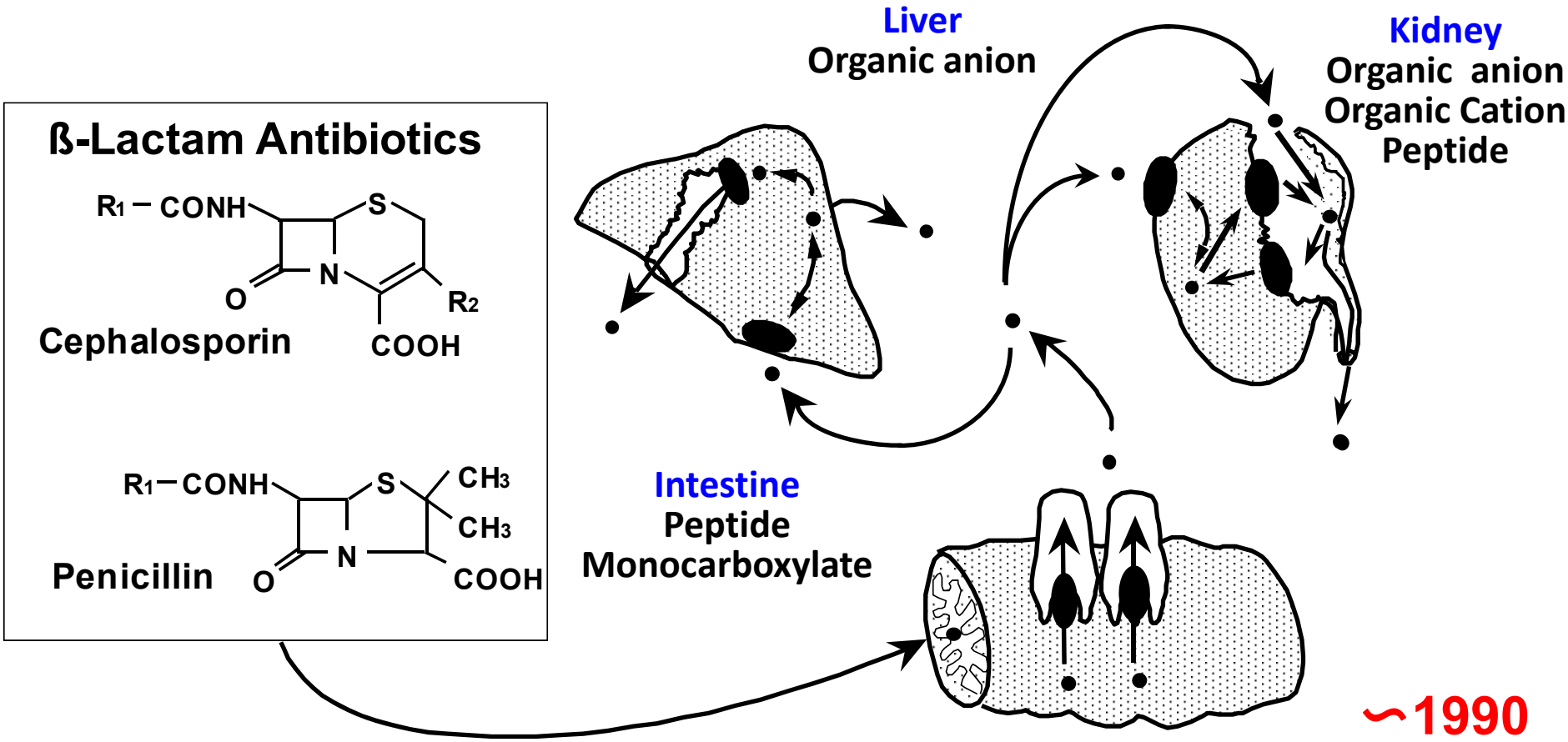
Variable PK properties
BA:0%～100%
(*NOT* prodrug),
Vd:0.2 L/kg BW
CL:Kidney and liver
(*NOT metabolism*)

Exhibit Tissue- and Compounds-Specific Membrane Permeability.

Carrier-Mediated Transport?



Ph.D. Thesis : Membrane Transport Mechanisms for ß-Lactam Antibiotics in Intestine, Liver and Kidney

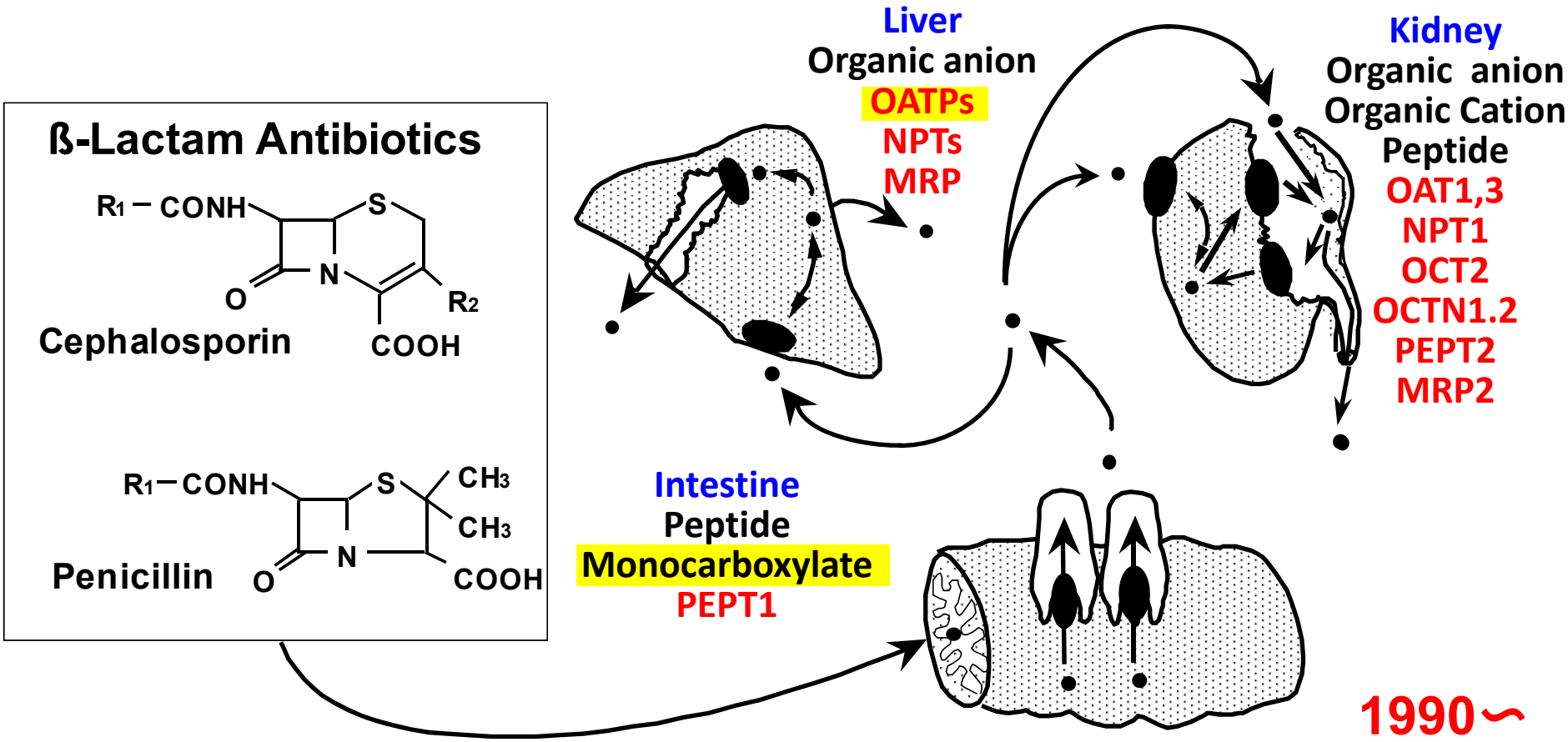


~1990

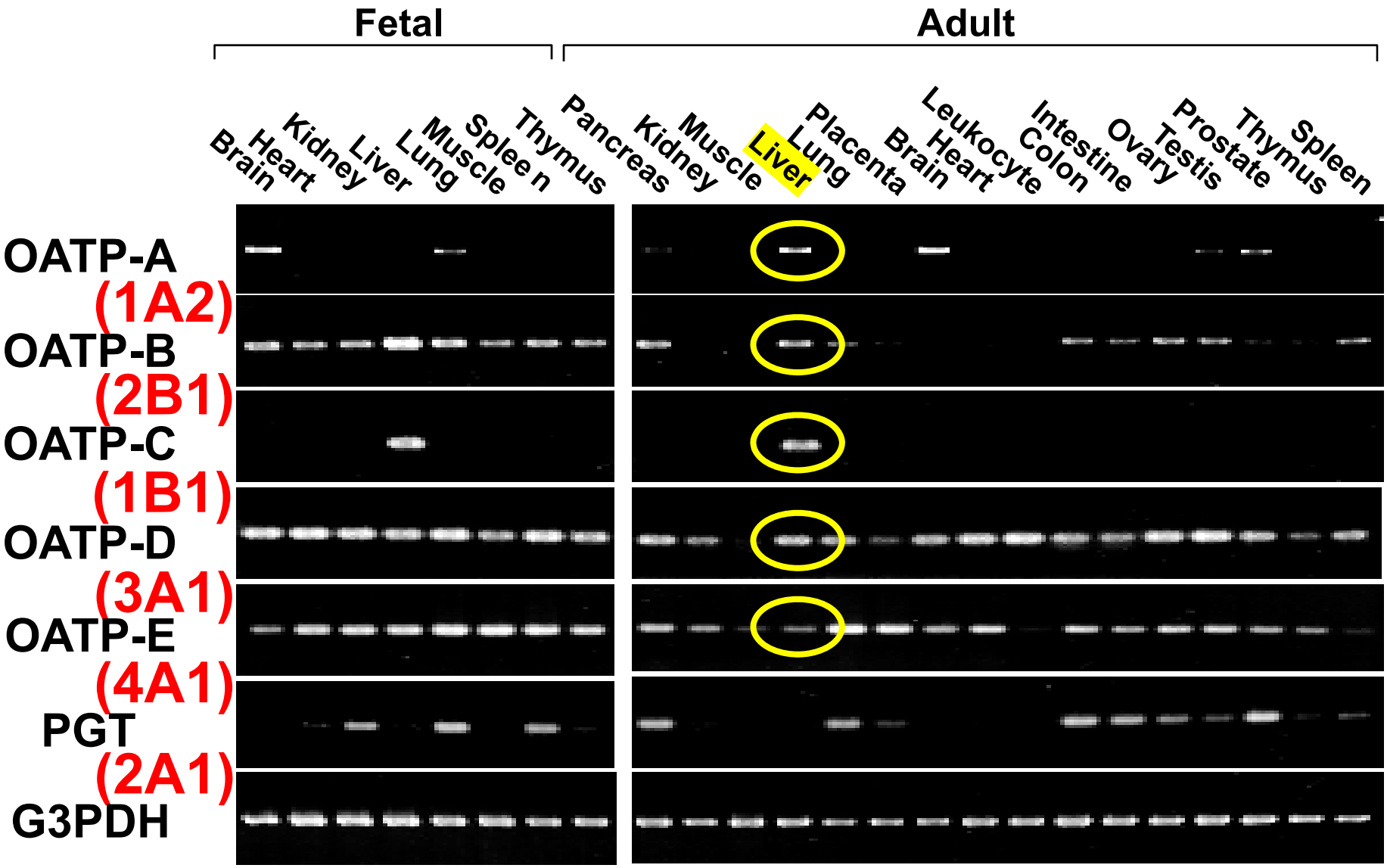
Transport studies by membrane physiological techniques, such as perfusion, tissues slices, isolated cells, membrane vesicles, *etc.*

⇨ **Many suggestion of carrier-mediated transport in intestinal absorption, and urinary and biliary excretions.**

Membrane Transport Mechanisms for ß-Lactam Antibiotics in Intestine, Liver and Kidney



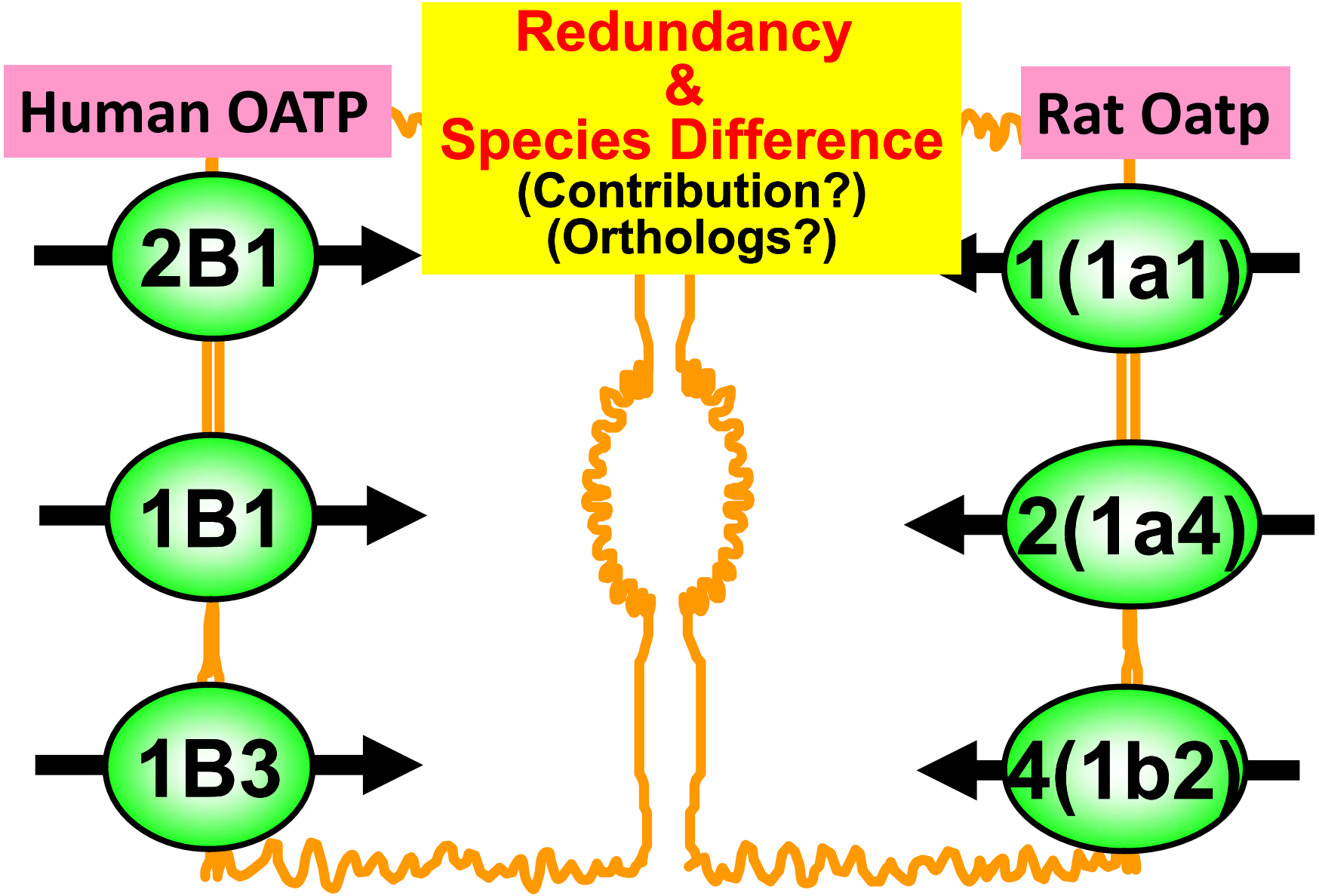
Molecular identification of involved transporters and analysis of their *in vivo* contribution, species difference and alteration of PK by genetic polymorphisms and DDI



Expression Profiles of Human OATPs by RT-PCR

Tamai et al., Biochem. Biophys. Res. Commun., 27:251-260, 2000

Identification of OATP/Oatp Transporters Responsible for Hepatic Uptake of β-Lactams in Human and Rats



Urinary and Biliary Excretion Profiles of β-Lactam Antibiotics in Humans and Rats

		Human			Rat		
	M.W.	Dose	X_{bile}	X_{urine}	Dose [a]	X_{bile}	X_{urine}
		mg/kg	*% of Dose*		*mg/kg*	*% of Dose*	
Cephalexin	347.4	250 [b]		90.0	20	9.3	74.0
Cefadroxil	381.4	500-1000 [b]		90.0	20	20.0	N.A.
Nafcillin	414.5	500 [b]	96.0	4~38	10	89.2	N.A.
Cefazolin	454.5	250-500 [d]		85.0	20	18.0	67.6
Cefotaxime	455.5	1000-2000 [c]		65.0	20	1.0	N.A.
Cefmetazole	471.5	2000 [c]		75.0	20	66.9	19.8
Cefditoren	506.6	200-400 [b]		20.0		N.A.	
Cefsulodin	532.6	1000 [b]		60.0		N.A.	
Ceftriaxone	554.6	2000 [c]		50.0	20	61.8	32.0
Cefoperazone	645.7	1000-2000 [c]		30.0	20	80.9	12.1

a) Intravenous administration
b) Oral administration
c) Intravenous administration
d) Intramuscular
N.A. : Not Available

Urinary and Biliary Excretion Profiles of β-Lactam Antibiotics in Humans and Rats

		Human			Rat		
	M.W.	Dose	X_{bile}	X_{urine}	Dose [a]	X_{bile}	X_{urine}
		mg/kg	*% of Dose*		*mg/kg*	*% of Dose*	
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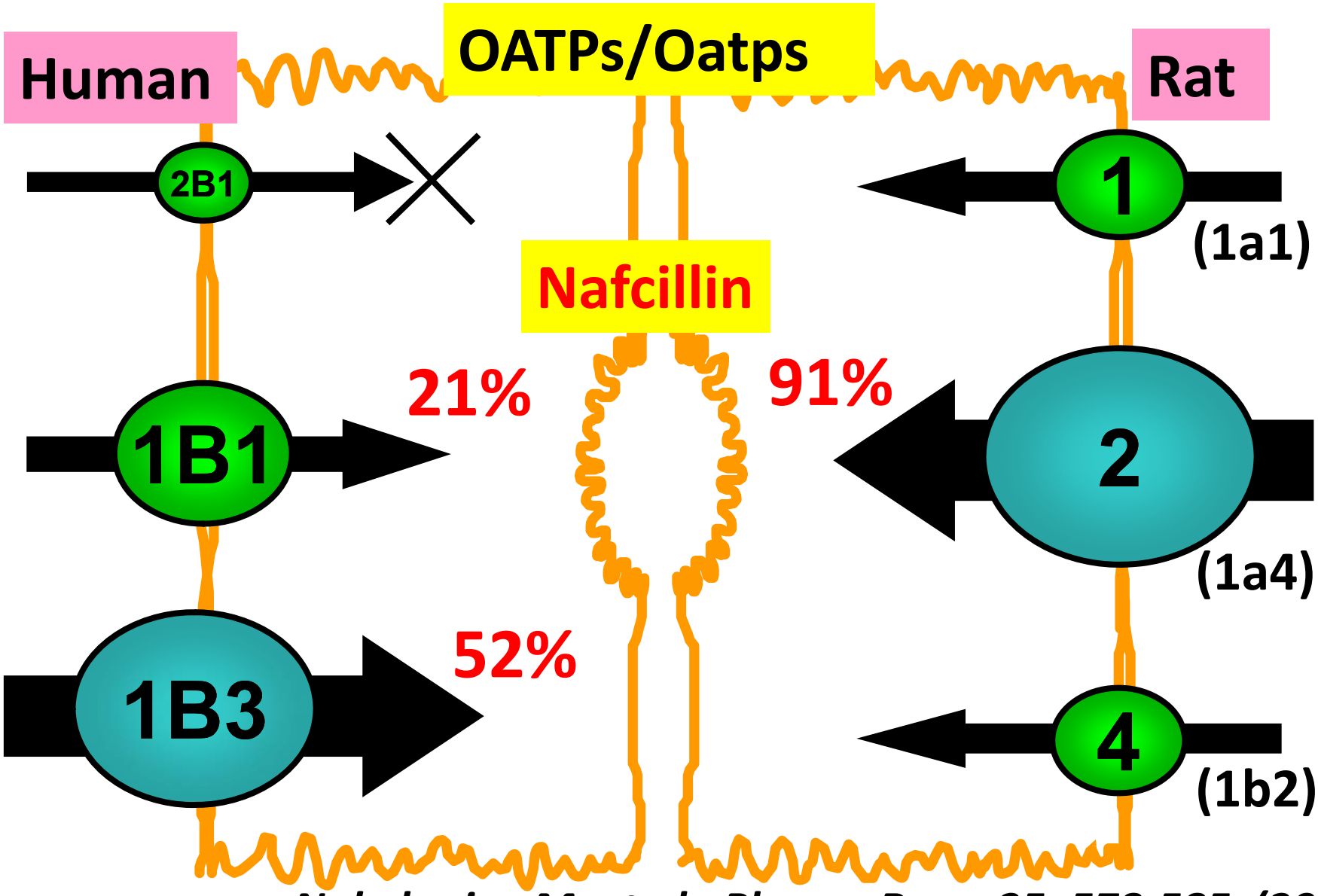
Cefo							N.A.
Cefr							19.8
Cefo							
Cefs							
Ceft							32.0
Cefoperazone	645.7	1000-2000 [c]		50.0	20	80.9	12.1

Functional Comparison OATPs and Oatps:

1: Kinetic Parameters
2: Relative Activity Factor (RAF) Analysis
3: Selective Inhibitors
4: *Vitro* and *Vivo correlation*

a) Intravenous administration
b) Oral administration
c) Intravenous administration
d) Intramuscular
N.A. : Not Available

Transporters Involved in Hepatic Uptake of Nafcillin in Human and Rat



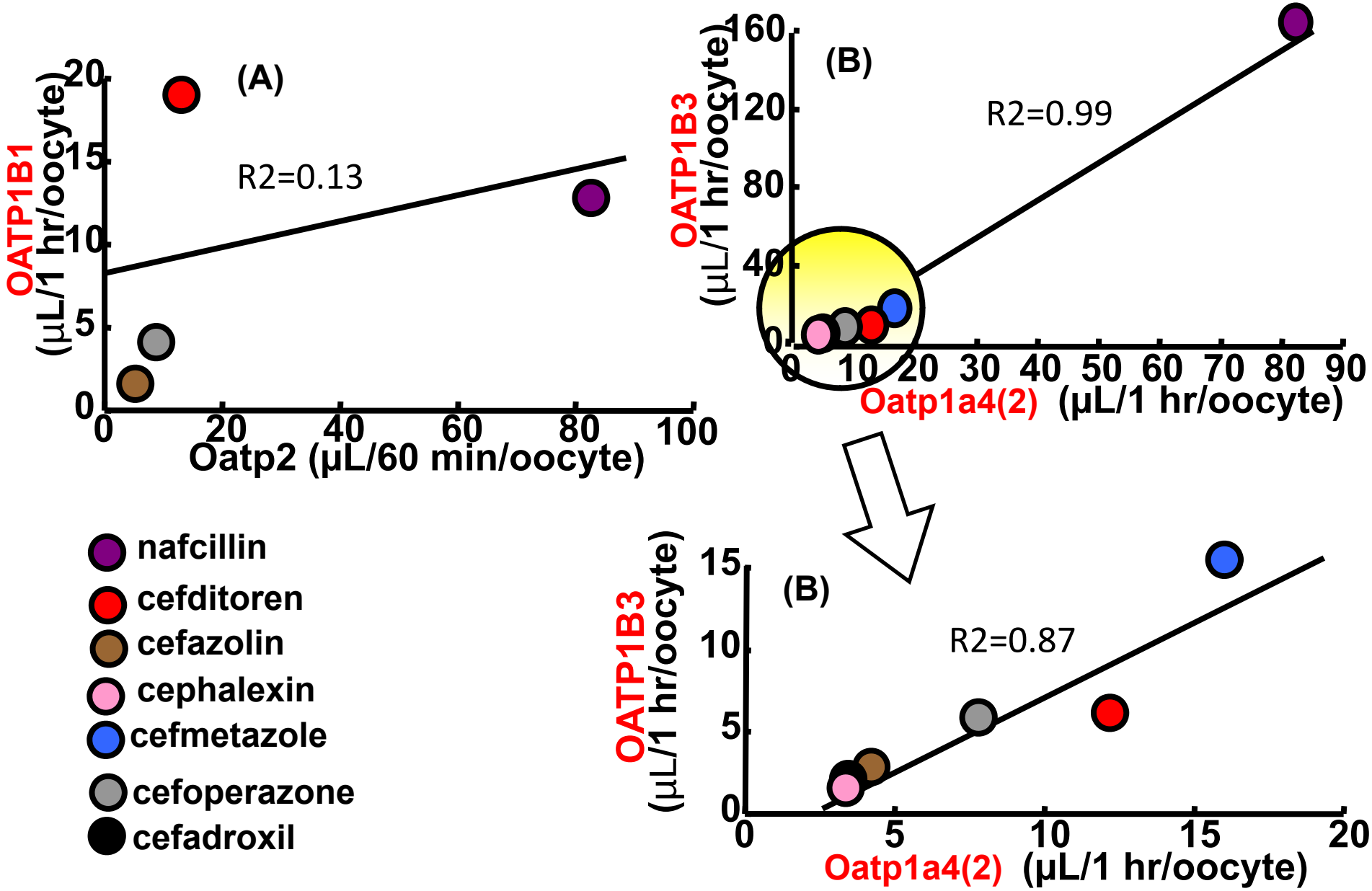
Nakakariya M. et al., Pharm. Res.., 25: 578-585 (2008)

Uptake of β-Lactam Antibiotics by *Xenopus* Oocytes Expressing OATPs/Oatps

β-Lactam Antibiotics	OATP-Mediated β-Lactam Uptake (nL/oocyte/120 min)					
	Human			Rat		
	1B1	1B3	2B1	1(1a1)	2(1a4)	4(1b2)
Cephalexin	×	1.3	×	1.1	2.0	0.8
Cefadroxil	×	2.0	×	0.3	1.5	0.3
Cefazolin	0.1	3.1	N.D.	1.5	7.8	0.8
Cefotaxime	0.2	0.2	N.D.	0.1	×	×
Cefmetazole	×	2.8	×	2.2	15.5	3.6
Ceditoren	10.2	3.3	N.D.	N.D.	2.6	N.D.
Cefsulodin	0.4	0.1	N.D.	×	7.1	×
Ceftriaxone	×	1.4	N.D.	1.3	×	0.6
Cefoperazone	48.4	48.4	×	1.1	4.7	×

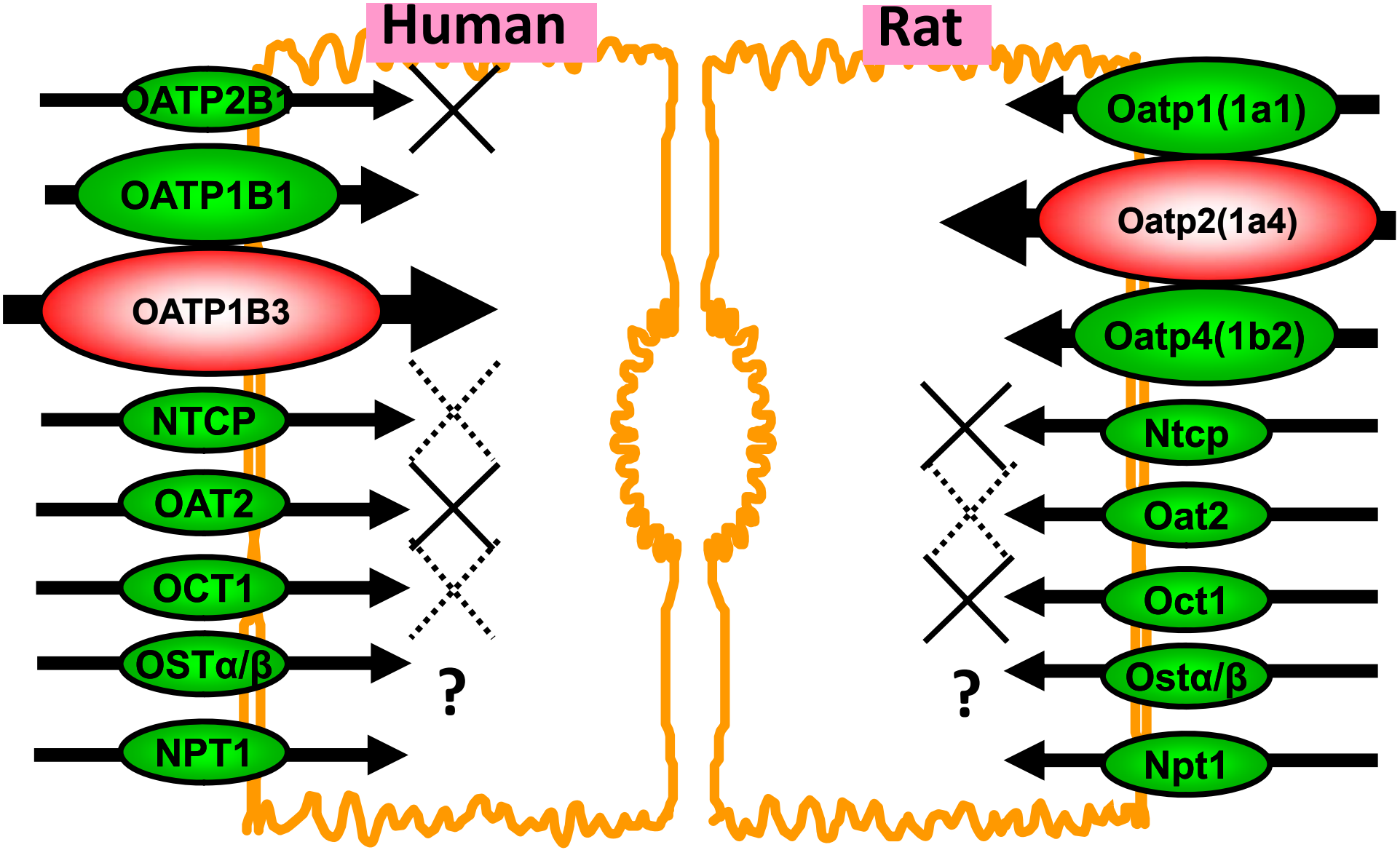
β-Lactam Antibiotics : 5 mM

× : Not Transported
N.D. : Not Determined



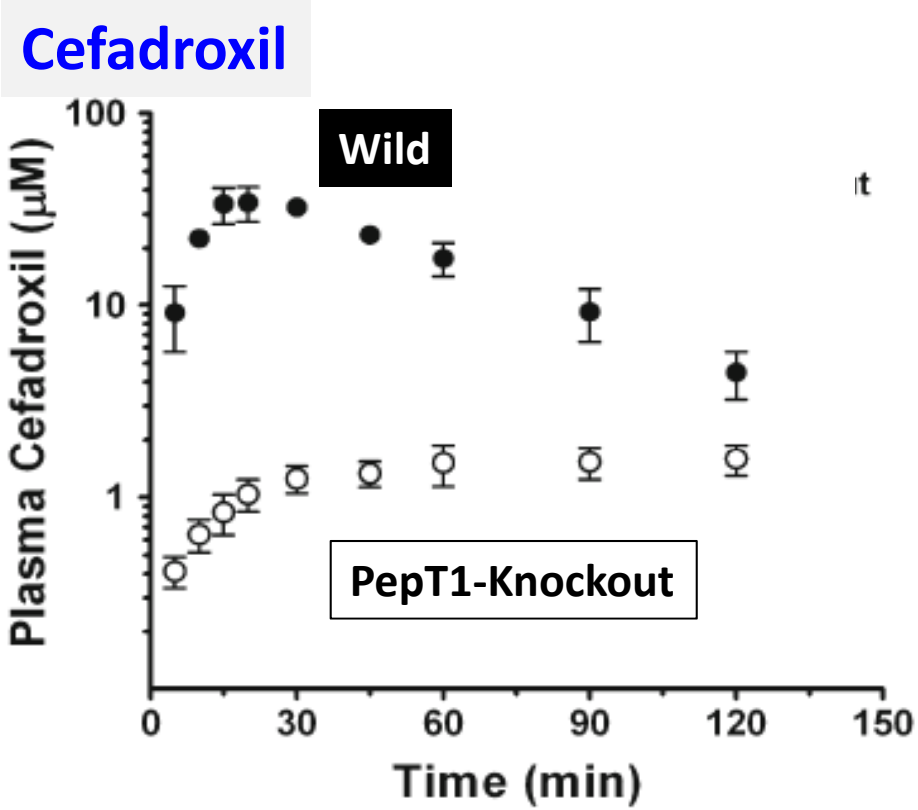
Comparison of *Vmax*/*Km* Values between OATP1B1 or OATP1B3 *vs.* Oatp1a4(2)

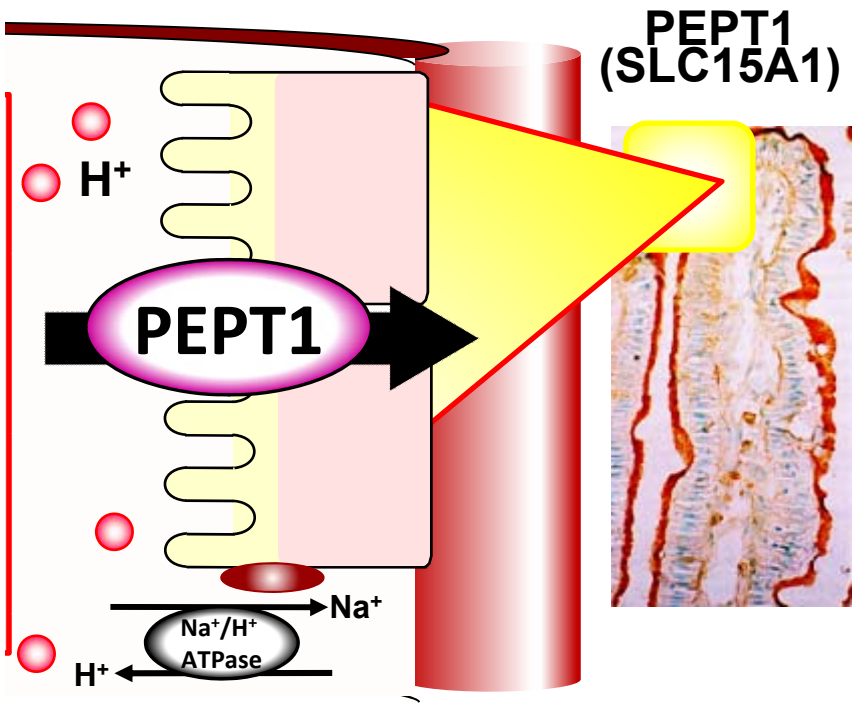
Transporters Involved in Hepatic Uptake of β-Lactam Antibiotics in Human and Rat



Nakakariya M. et al., Drug Metab. Pharmacokinet., 23: 347-355 (2008)

Peptide Transporter PEPT1 Responsible for Intestinal Absorption of β-Lactam Antibiotics





Decreased Cefadroxil Concn in PepT1-knockout Mice

Smith DE. et al.,
Pharm. Res., 30:1017-1025 (2013)

Apical Membrane Expression of PEPT1

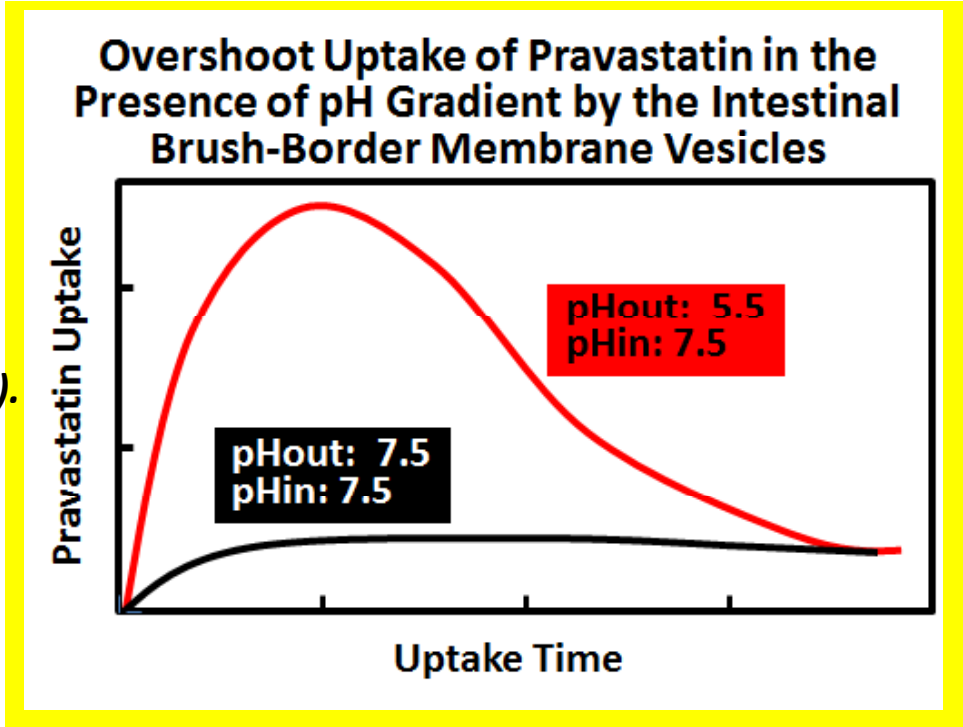
Sai Y. et al.,
FEBS Lett., 392: 25-29 (1996)

Question in 1990's :
Does pH-Dependent Transporter Contribute to Intestinal Absorption of Organic Anions such as Pravastatin?

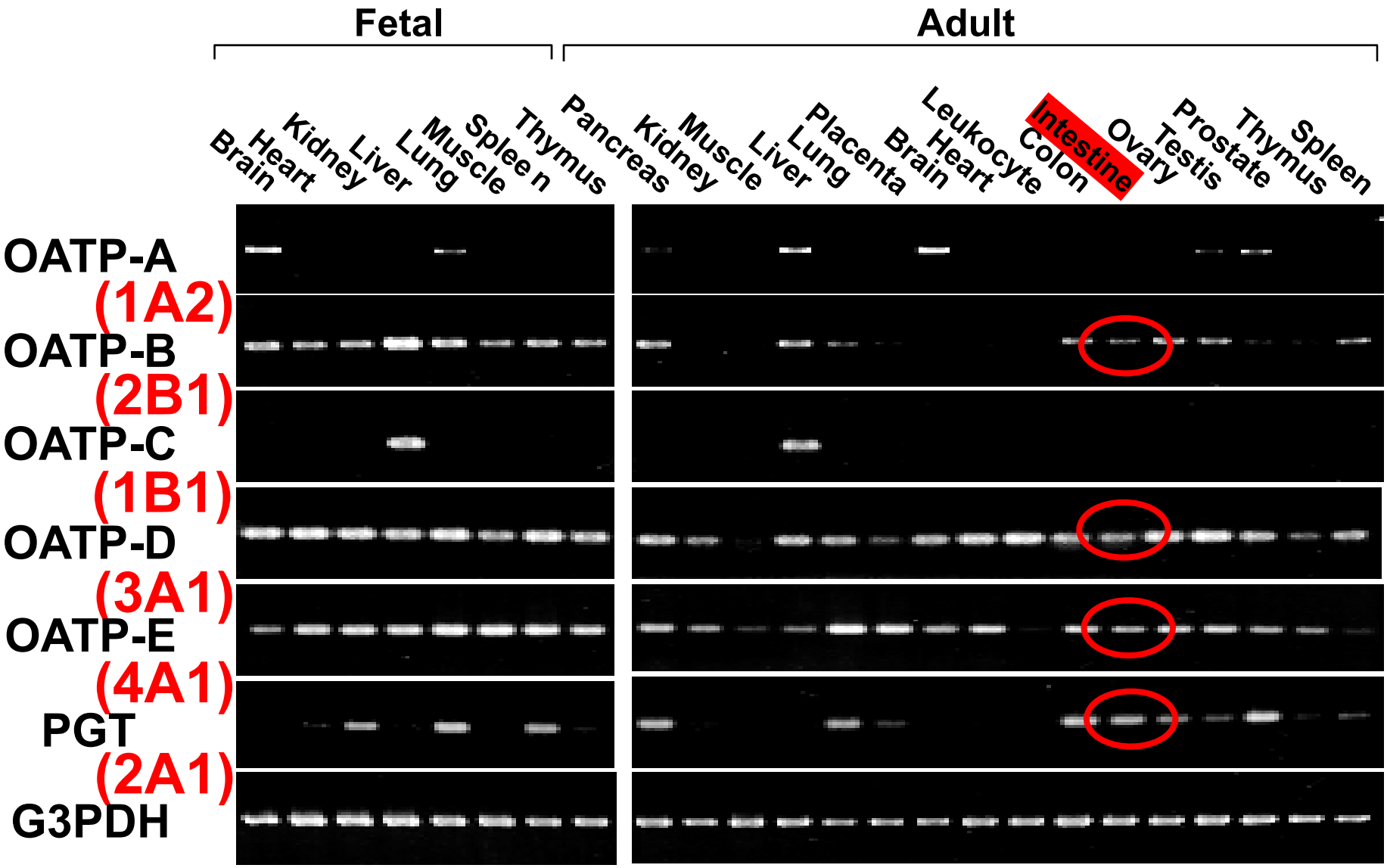
Proton-cotransport of pravastatin across intestinal brush-border membrane.
Tamai I, Takanaga H, Maeda H, Ogihara T, Yoneda M, Tsuji A.
Pharm. Res. 12: 1727-1732 (1995).

Participation of a proton-cotransporter, MCT1, in the intestinal transport of monocarboxylic acids.
Tamai I, Takanaga H, Maeda H, Sai Y, Ogihara T, Higashida H, Tsuji A.
Biochem. Biophys. Res. Commun., 214:482-489 (1995).

Intestinal brush-border membrane transport of monocarboxylic acids mediated by proton-coupledtransport and anion antiport mechanisms.
Tamai I, Takanaga H, Maeda H, Yabuuchi H, Sai Y, Suzuki Y, Tsuji A.
J. Pharm. Pharmacol., 49:108-112 (1997).



Although MCT1 (SLC16A1) transported anionic compounds such as benzoic acid, but not clinically used drugs.



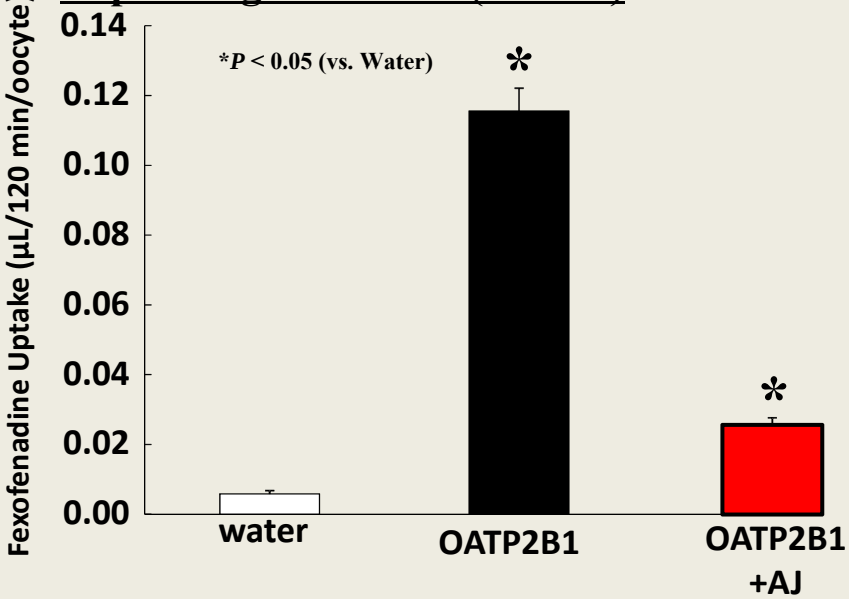
Expression Profiles of Human OATPs by RT-PCR

Tamai et al., Biochem. Biophys. Res. Commun., 27:251-260, 2000

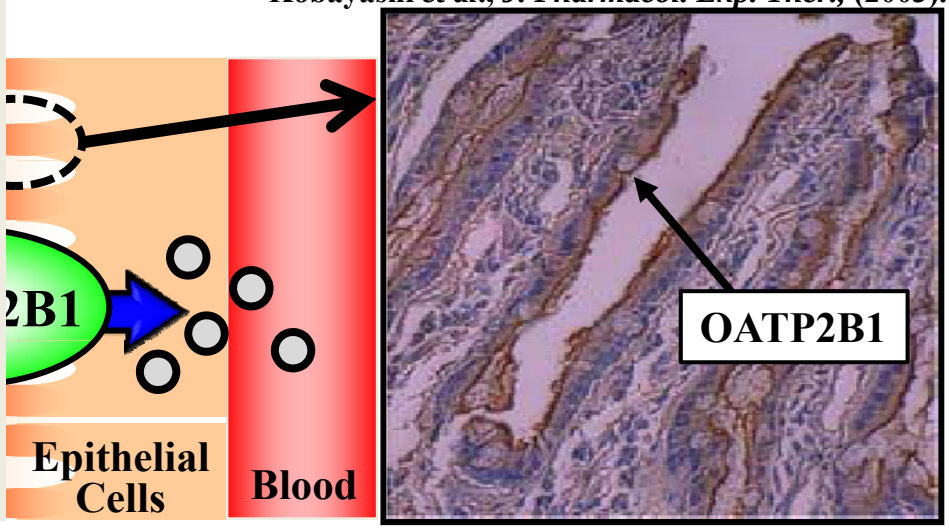
cess of Drug Intestinal Absorption

ting Polypeptide (OATP) 2B1

◆ **Fexofenadine Uptake by *Xenopus* Oocytes Expressing OATP2B1 (*in vitro*)**

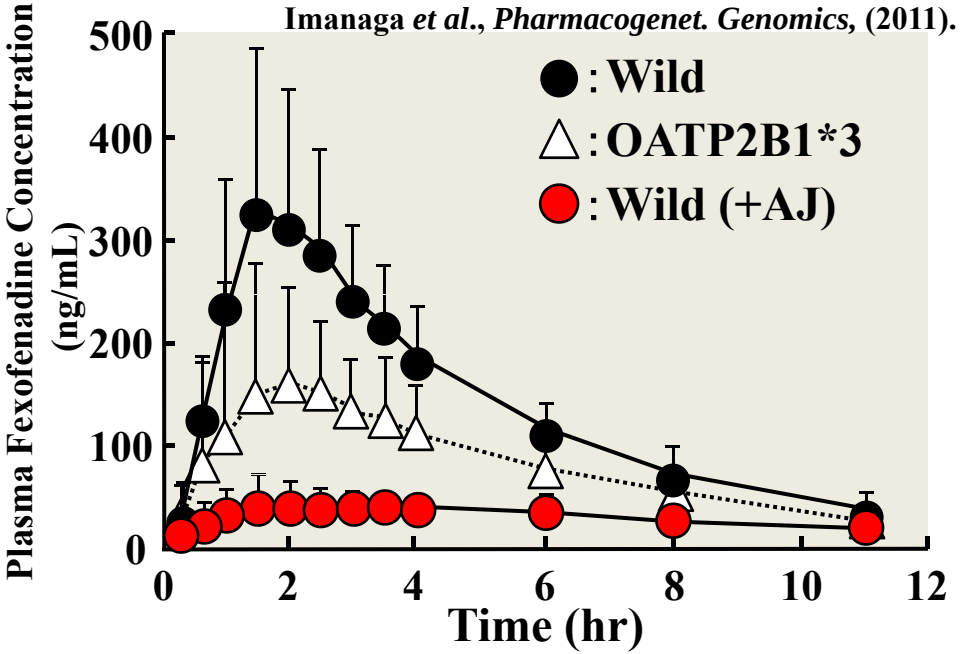


Kobayashi *et al.*, *J. Pharmacol. Exp. Ther.*, (2003).



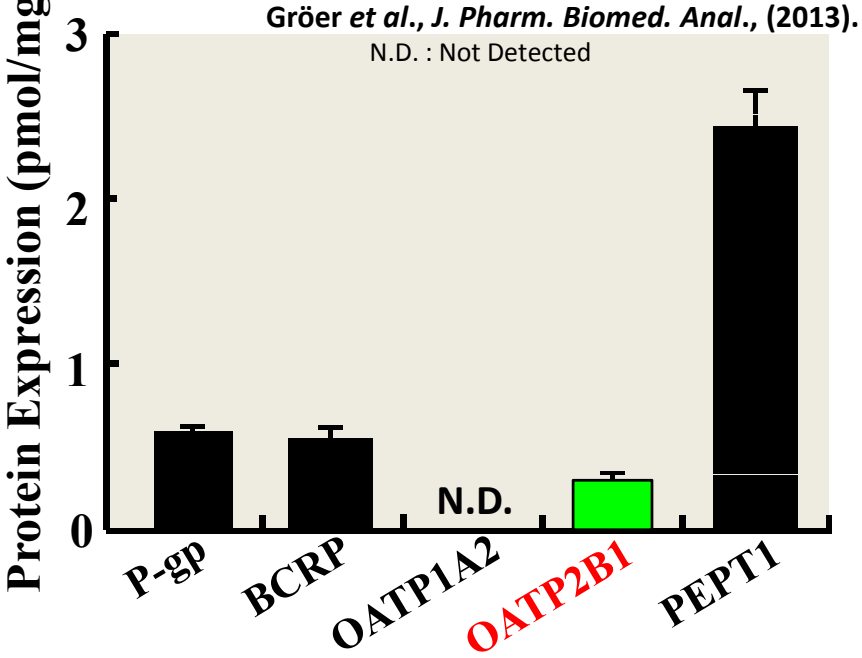
◆ **SNP: c.1457C > T (486 S>F, OATP2B1*3)**

Imanaga *et al.*, *Pharmacogenet. Genomics*, (2011).



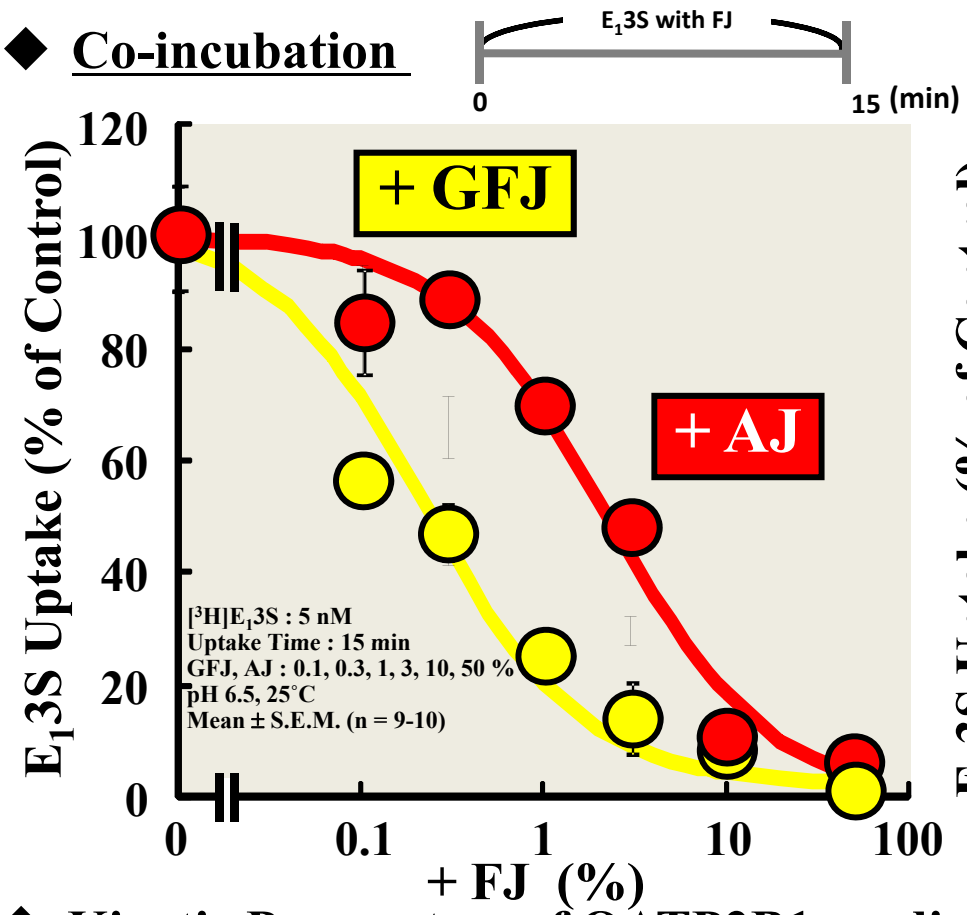
◆ **Human Jejunal Tissue**

Gröer *et al.*, *J. Pharm. Biomed. Anal.*, (2013).

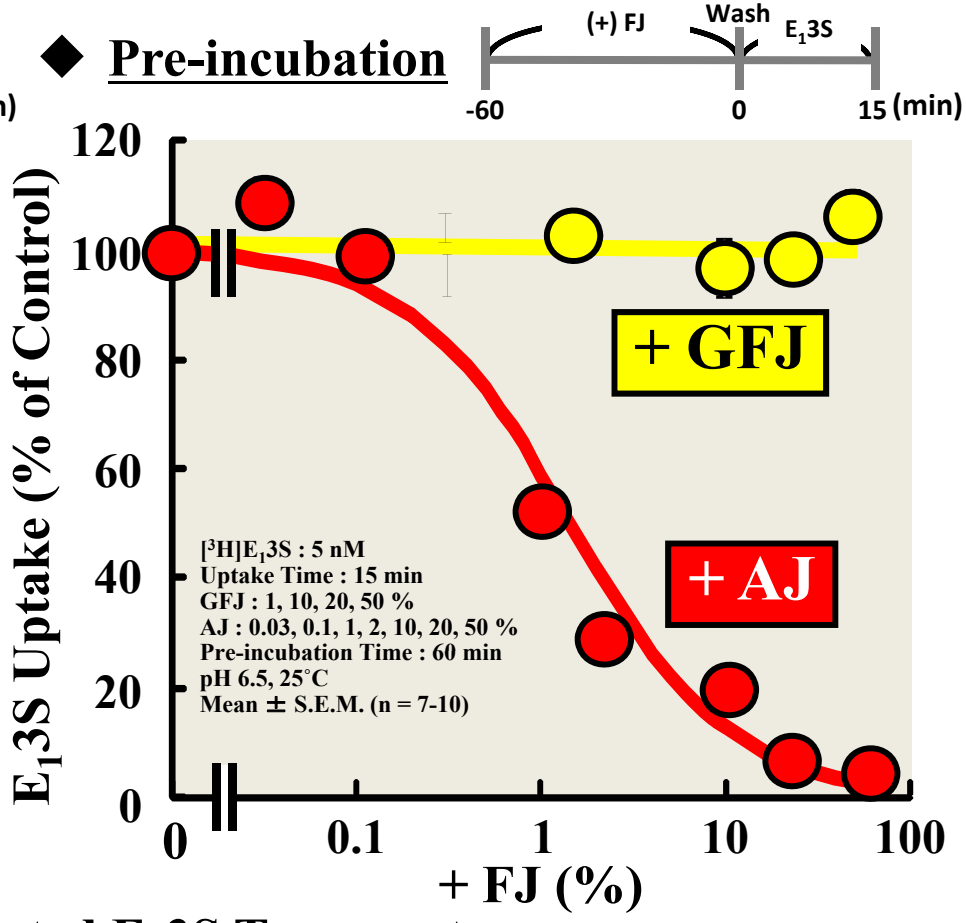


Inhibitory Effect of Fruit Juice (FJ) on OATP2B1

◆ Co-incubation



◆ Pre-incubation

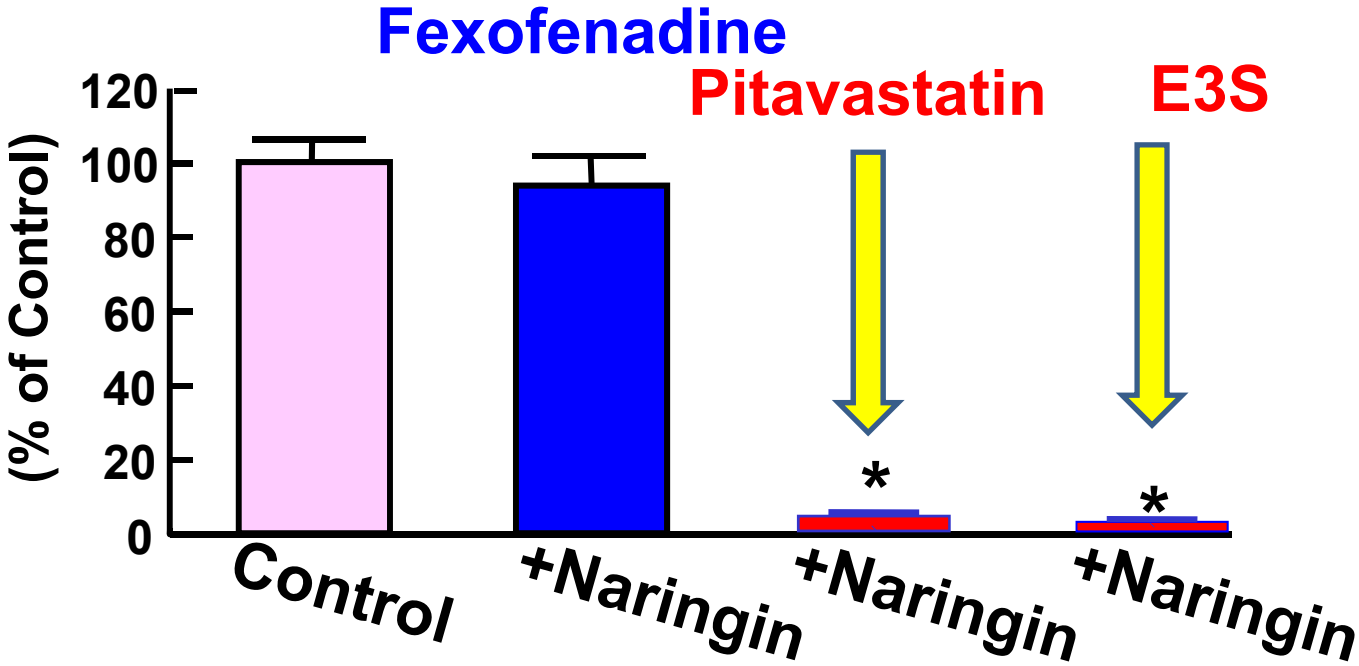


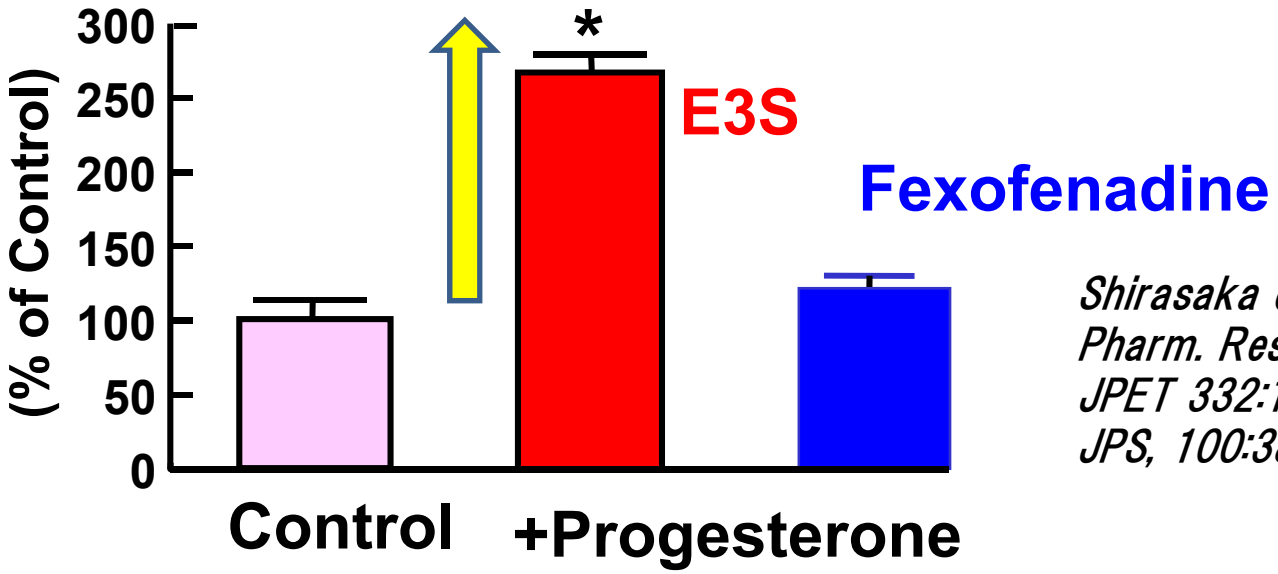
◆ Kinetic Parameters of OATP2B1-mediated $E_1$3S Transport

Condition of Administration	K_m (μM)		V_{max} (pmol/min/oocyte)		V_{max}/K_m (μL/min/oocyte)
Control	0.550 ±0.150		0.288 ±0.067		0.524
Co-incubation with AJ	1.32 ±0.25	×2.4 ↑	0.429 ±0.007	×1.5 →	0.325 ↓
Pre-incubation with AJ	0.506 ±0.109	×0.9 →	0.132 ±0.027	×0.5 ↓	0.261 ↓

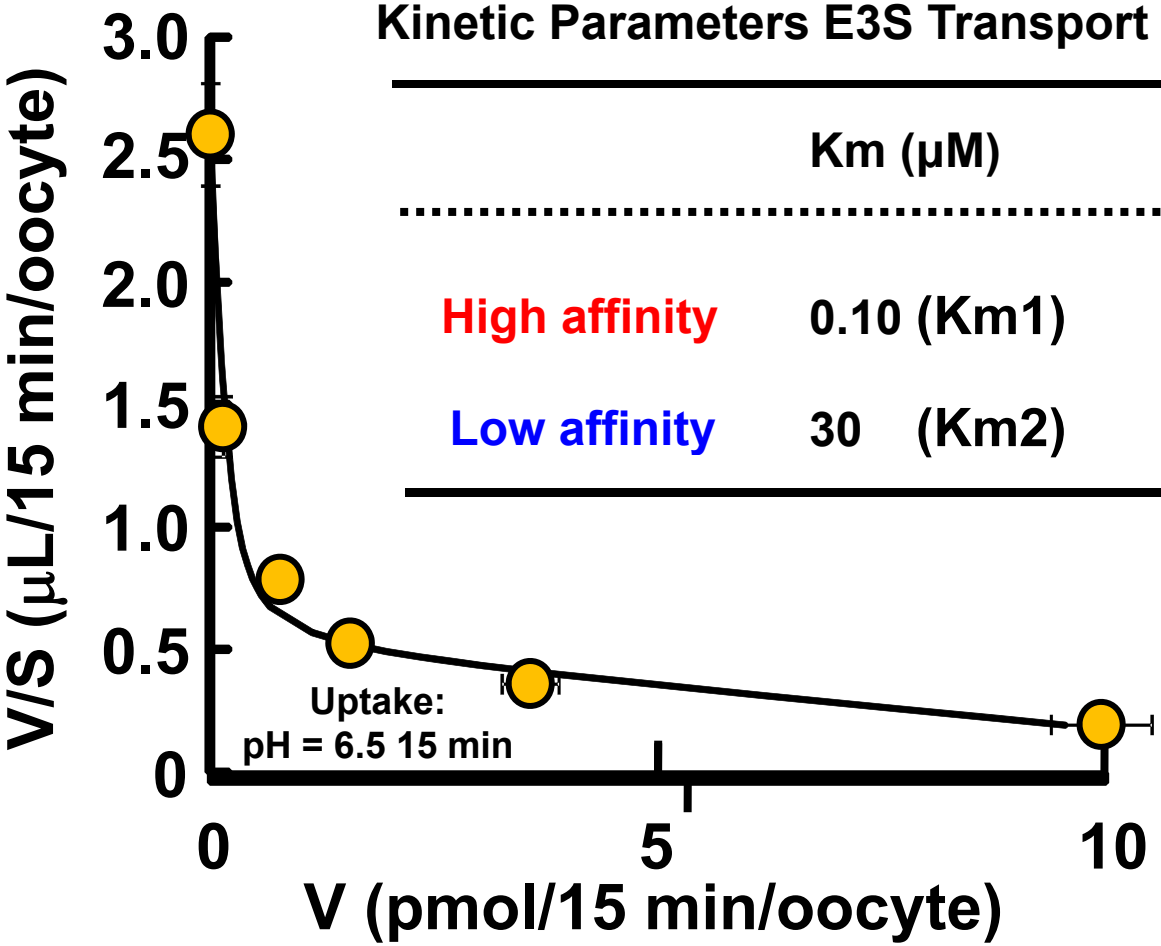
Shirasaka *et al.*, *DMD (2013)* *and* *JPS* (2013).

Substrate-Dependent Inhibiton by Naringin and Stimulation by Pogesterone on OATP2B1 Expressed in *Xenopus* Oocytes





Shirasaka et al.,
Pharm. Res., 26: 560-567 (2009),
JPET 332:181-189(2010),
JPS, 100:3843-3853 (2011)



Kinetic Parameters E3S Transport by OATP2B1

	Km (µM)	Vmax (pmol/min)
High affinity	0.10 (Km1)	0.014 (Vmax1)
Low affinity	30 (Km2)	1.0 (Vmax2)

Estron-3-sulfate

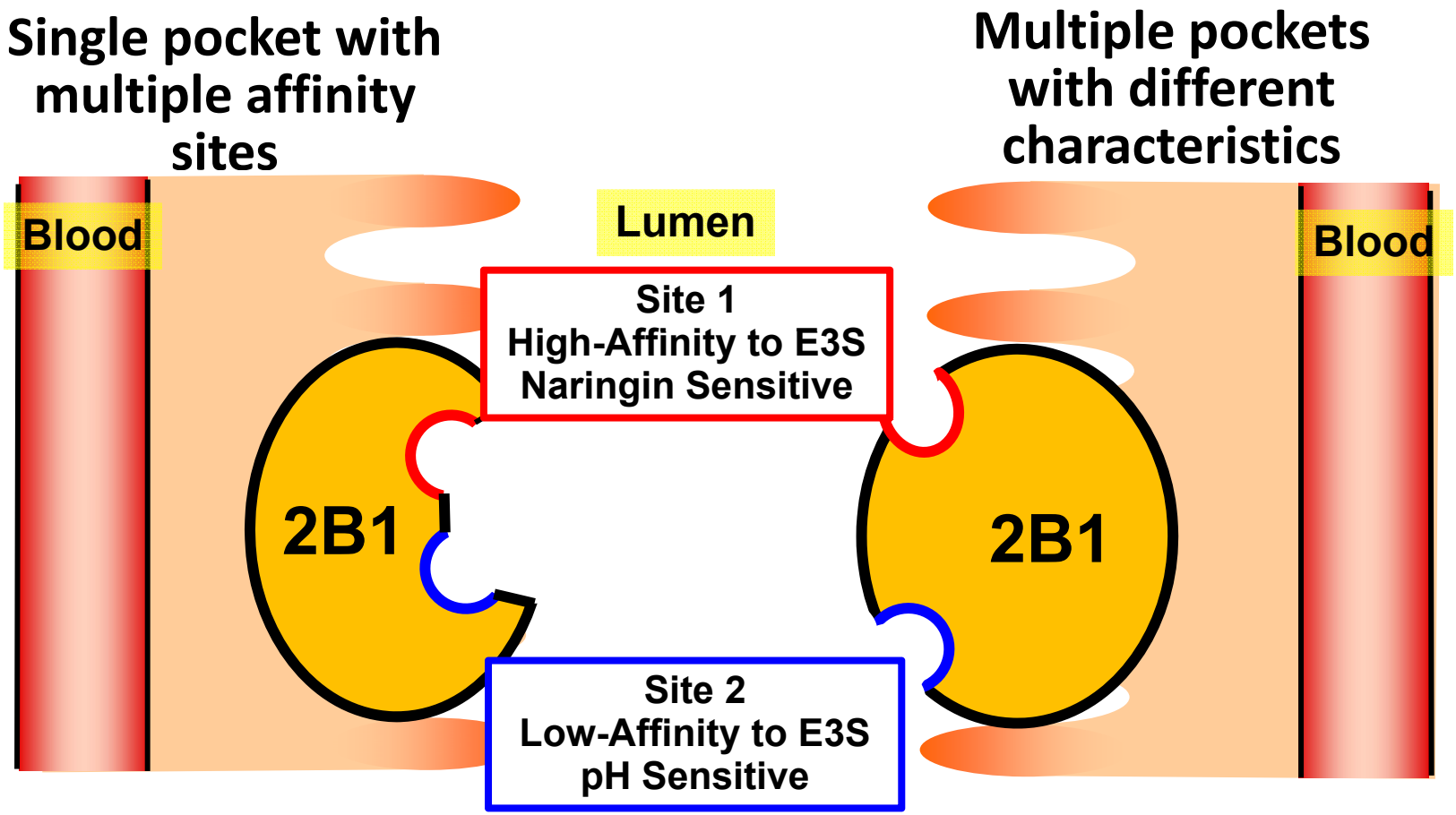
Affinity	0.004 µM	50 µM
High	80%	2%
Low	20%	98%

Concentration Dependence of E3S Uptake by OATP2B1 Expressed in Oocytes

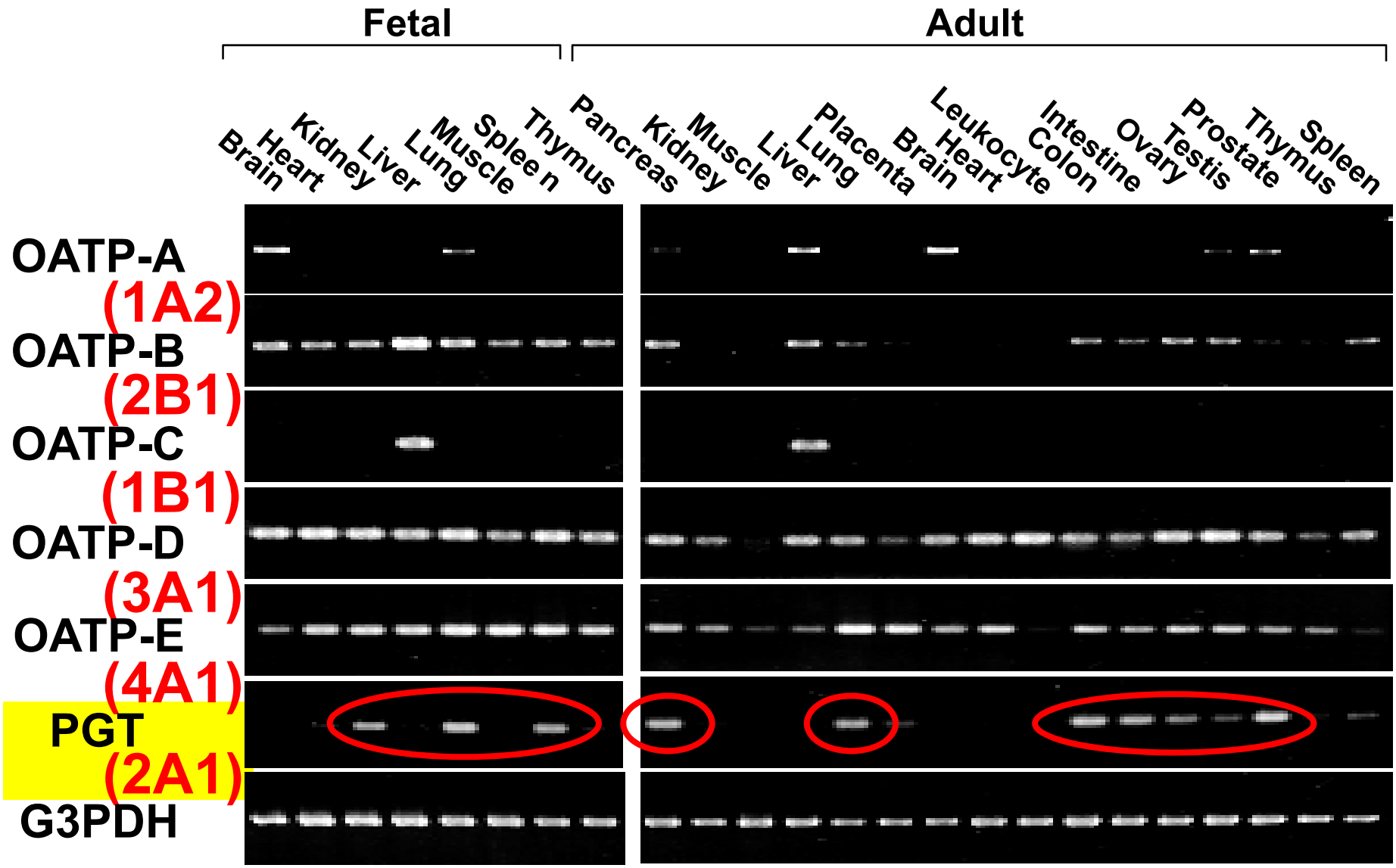
Drug Metab. Pharmacokinet., 27: 360-364 (2012)

Hypothesis:

Multiple Binding Sites/Pockets on OATP2B1 with Different Substrate/Modulator Sensitivity



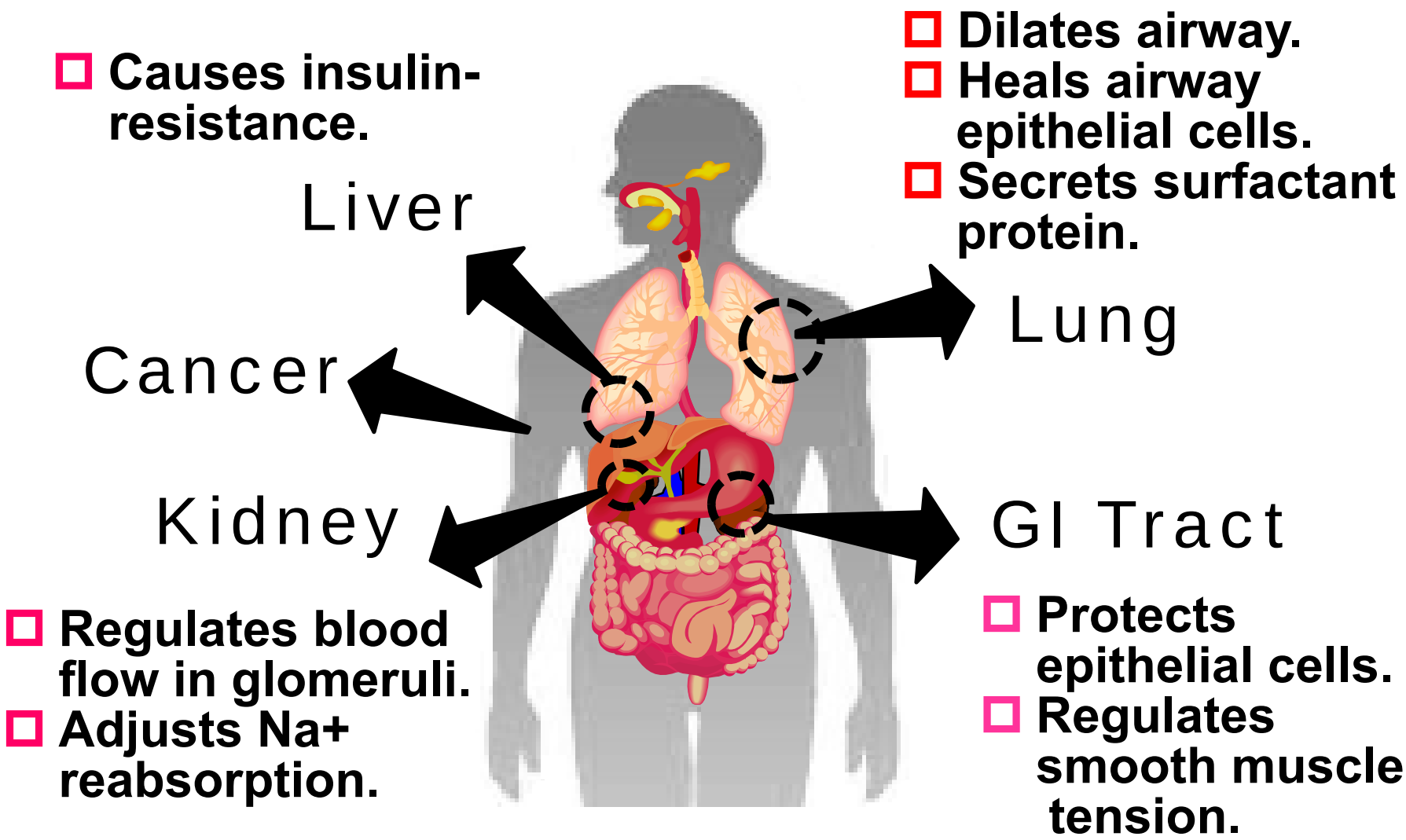
Drug Metab. Pharmacokinet., 27: 360-364 (2012)



Tamai et al., Biochem. Biophys. Res. Commun., 27:251-260, 2000

Expression Profiles of Human OATPs by RT-PCR

Diverse Biological Activities of PGE_2



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2000'-: Hormone dependence of cancer cells
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2000'-: OCTNs and drug delivery and disposition (Cancer, Lung *etc*.)

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2000'-: Drug-induced change of serum urate level
2010'-: Regulation mechanism of urate

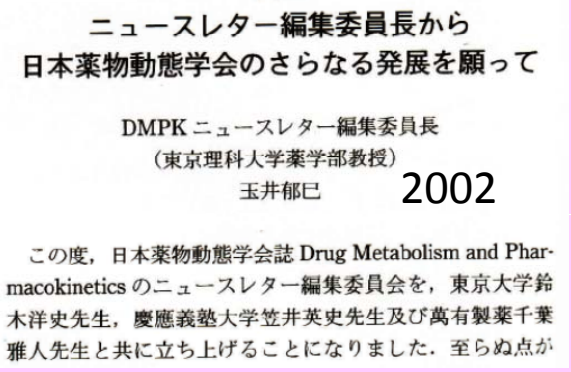
Thank JSSX, because I got a lot of ---

- Help and encouragement from JSSX people.
- Information through Annual Meeting, WS & SC, and DMPK.
- Stimulation and encouragement by JSSX leadership.

2006-2009: Chair of JSSX Committee of International Affairs.
Encouragement by Dr. Yuichi Sugiyama (Riken) to make relationship with researchers outside Japan. Organization of AP-ISSX and involvement in ISSX leadership.

2002 - 2004: DMPK News Letter:

- Suzuki H. (U Tokyo)
- Chiba M. (Taiho Pharm.)
- Kasai H. (Certara)



展望

ニュースレター編集委員長から
日本薬物動態学会のさらなる発展を願って

DMPK ニュースレター編集委員長
（東京理科大学薬学部教授）
玉井郁巳

2002

この度，日本薬物動態学会誌 Drug Metabolism and Pharmacokinetics のニュースレター編集委員会を，東京大学鈴木洋史先生，慶應義塾大学笠井英史先生及び萬有製薬千葉雅人先生と共に立ち上げることになりました．至らぬ点が

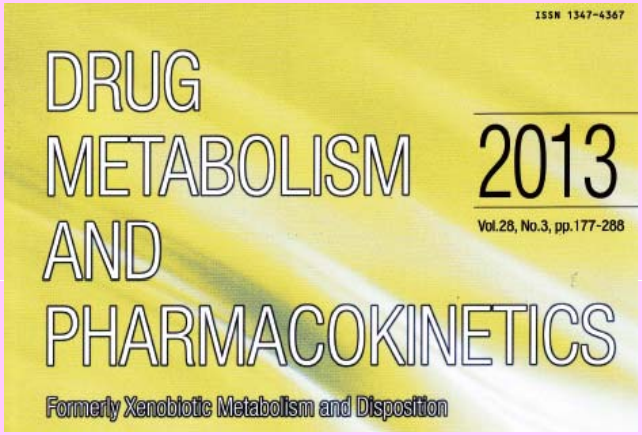
2006: 「創薬動態」from JSSX

- Suzuki H.
- Chiba M.
- Kasai H.
- Hisaka A.

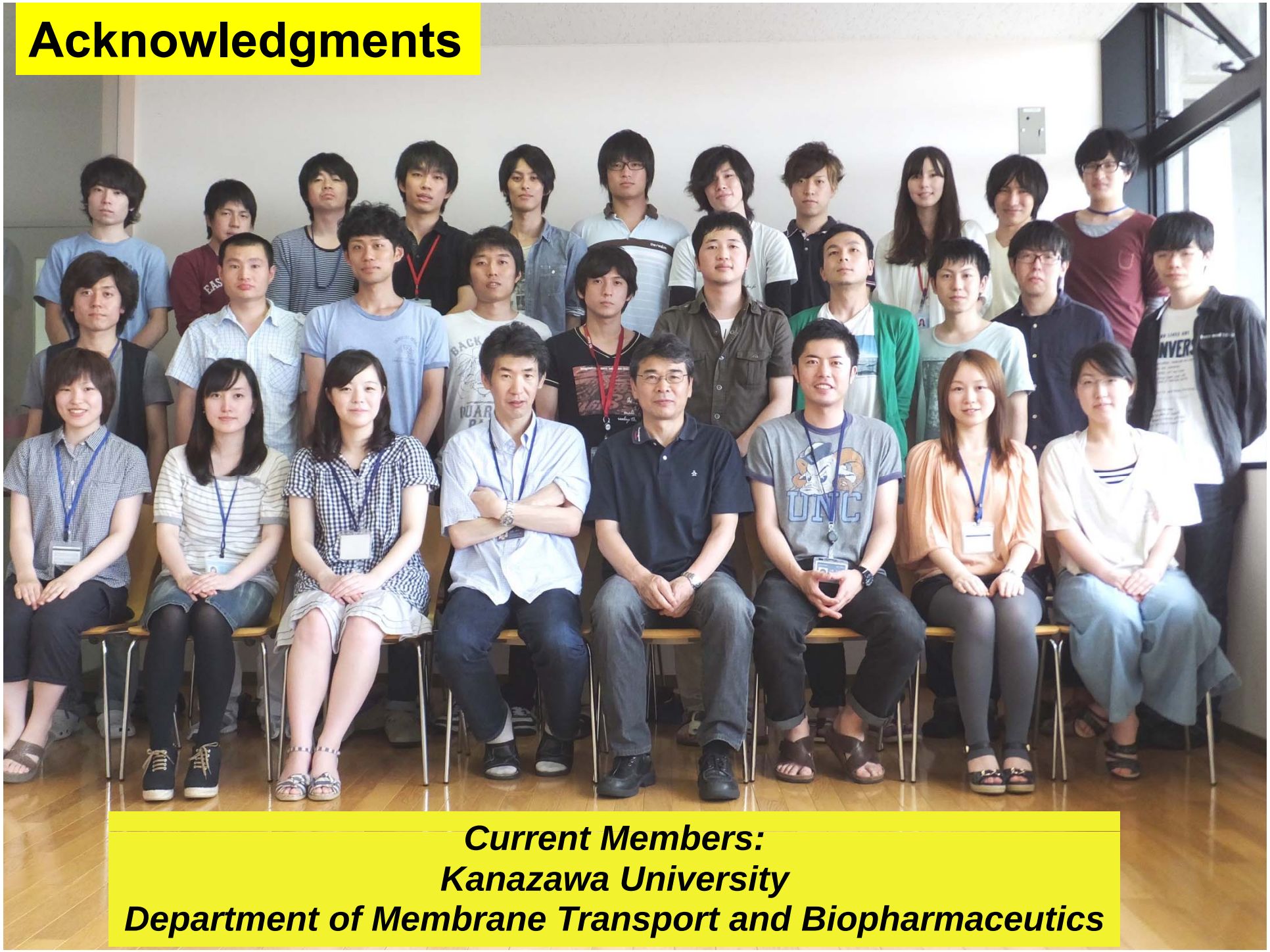
& JSSX Members

2010 – 2013: EIC, DMPK

AEs:
- Chung SJ.
- Deguchi Y.
- Hashimoto Y.
- Hosokawa M.
- Huang JD.
- Izumi T.
- Kato Y.
- Kawai R.
- Ozawa S.
- Tanigawa T.
- Tohkin M.
- Yamada H.



Acknowledgments



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