Transporter Pharmacogenomics

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Lilly
Answers That Matter.

Phamacogenomics in the News

Scientists expect that in the not-too-distant future patients will be tested routinely for a variety of genes that affect their response to drugs. The results should help doctors decide what and how much to prescribe, a major step forward in personalizing treatments for a range of ailments.

In the next three to five years, the cost of sequencing a person's genome will drop below $1,000 — less than the price of a colonoscopy, says National Institutes of Health director Francis Collins, who led the Human Genome Project to completion in 2003. Says Collins: "I think that will finally make pharmacogenomics" — the study of how variations in the human genome affect a person's responses to medications — "really practical.“

From: “Genetic testing may yield personalized health treatments”. USA Today, February 25, 2010

Definitions

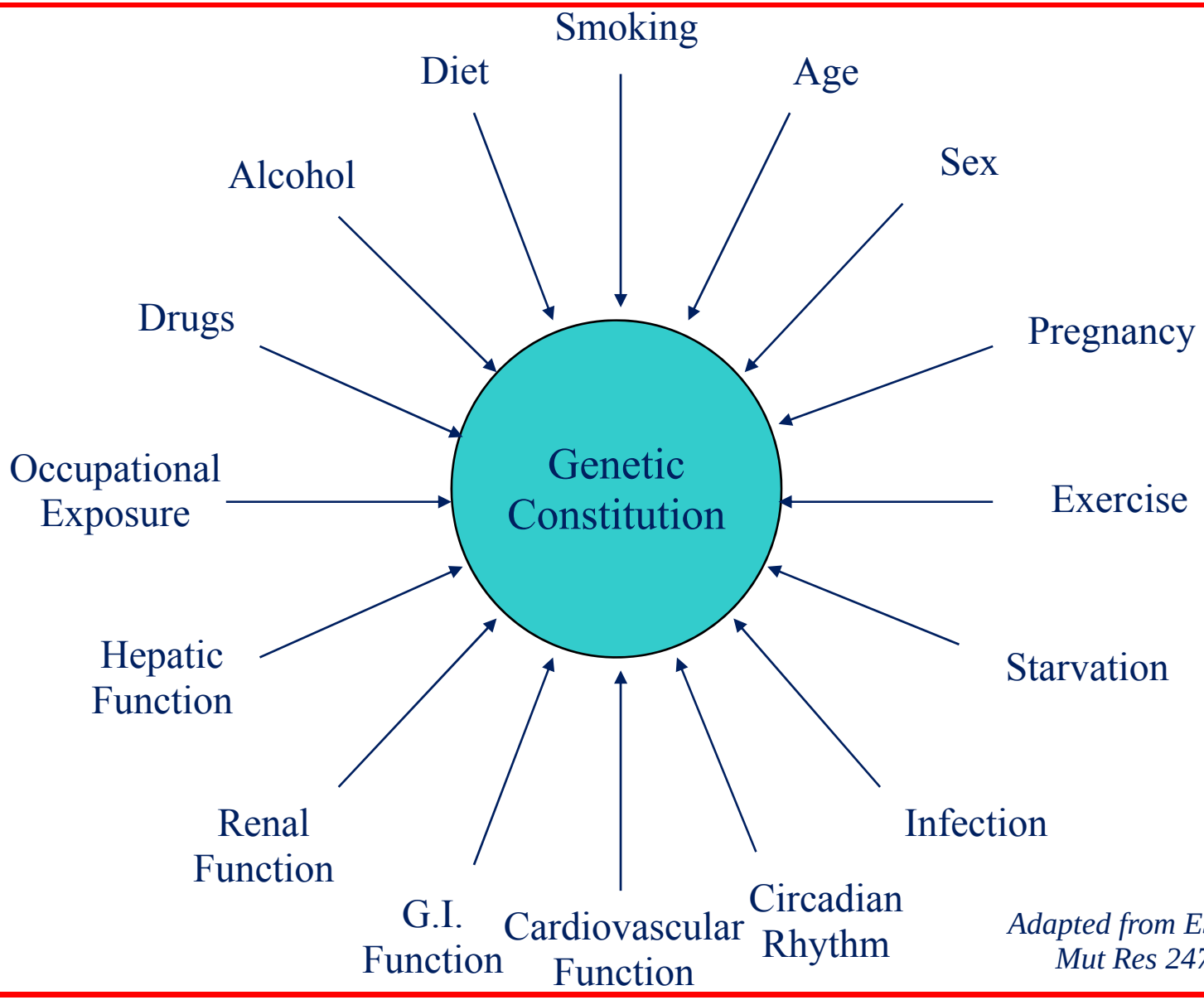
Single Nucleotide Polymorphism (SNP): change in the nucleotide sequence of a gene present at greater than 1%.

Haplotype: a fixed combination of SNPs that are inherited together.

Allele: Single copy of a gene (in most cases everybody has 2 alleles for a given gene).

Copy Number Variant (CNV): the number of copies of a particular gene varies from one individual to the next. It encompasses both the gain and loss of gene copies.

Sources of Interindividual Variability



Adapted from ES Vesell (1991) Mut Res 247: 214-257.

ADME Genes with Known Clinically Significant Polymorphisms

Cytochromes P450

CYP2B6, 2C9, 2C19, 2D6, 3A5

Non P450 Mediated Metabolism

Glucuronosyltransferases 1A1 (UGT1A1)

Sulfotransferases 1A1(SULT1A1)

N-Acetyltransferase 2 (NAT2)

Thiopurine S-Methyltransferase (TPMT)

Flavin Containing Monooxygenase 3 (FMO3)

Transporters

P-Glycoprotein (P-gp, MDR1, ABCB1)

Multidrug Resistance Transporter 2 (MRP2, ABCC2)

Breast Cancer Resistance Protein (BCRP, ABCG2)

Organic Anion Transporting Polypeptide 1B1 (OATP1B1, SLCO1B1)

Organic Cation Transporters 1 and 2 (OCT1 and 2)

Adapted from: Williams et al. (2008) "PhRMA White Paper on ADME Pharmacogenomics" J. Clin Pharm. 48:849-889.

When does ADME PGx Matter?

- Narrow Therapeutic Index
- Ethnic Differences
- Toxicity
- Pathways/Mechanism Analysis
- High variability
- Genotype influence of DDI potential

What are the Regulatory Implications?

Pharmacogenomic data is included in about 10% of drug labels in the US

- Phase 1 enzymes: CYP2C9, CYP2C19, CYP2D6
- Phase 2 enzymes: N-Acetyltransferase (NAT), Thiopurine Methyltransferase (TPMT), UDP-Glucuronosyltransferase 1A1 (UGT1A1)
- Transporters: not yet (OATP1B1 will likely be the first)

Both the EMEA and the FDA are expected to come out with Guidances in 2010 on PGx for ADME genes

(http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm)

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Genetic Classification of Transporters

Human Genome Project (HUGO)

Two Basic Catagories of Transporters

- ABC
- Solute (SLC)

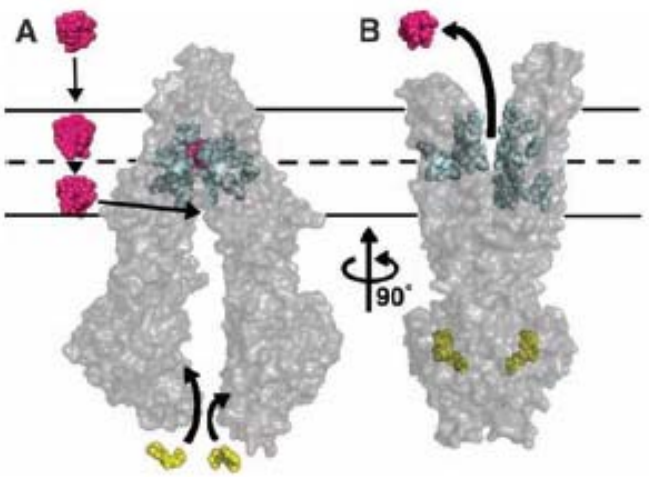
ABC Transporters

Contain an **A**TP **B**inding **C**assette

HUGO nomenclature: ABCletter#

Most ABC proteins are active transporters

Approximately 50 human genes



Aller et al, 2009, Science, 323:1718.

High clinical significance

- P-gp (ABCB1), BCRP (ABCG2), MRP's (ABCC#)

Energy derived from ATP hydrolysis

Good list of the human ABC proteins

- http://nutrigene.4t.com/humanabc.htm

Solute Transporters

Solute **C**arriers

HUGO nomenclature: SLC#letter#

≈360 solute transporters classified

48 families

Includes carriers from all tissues and organelles

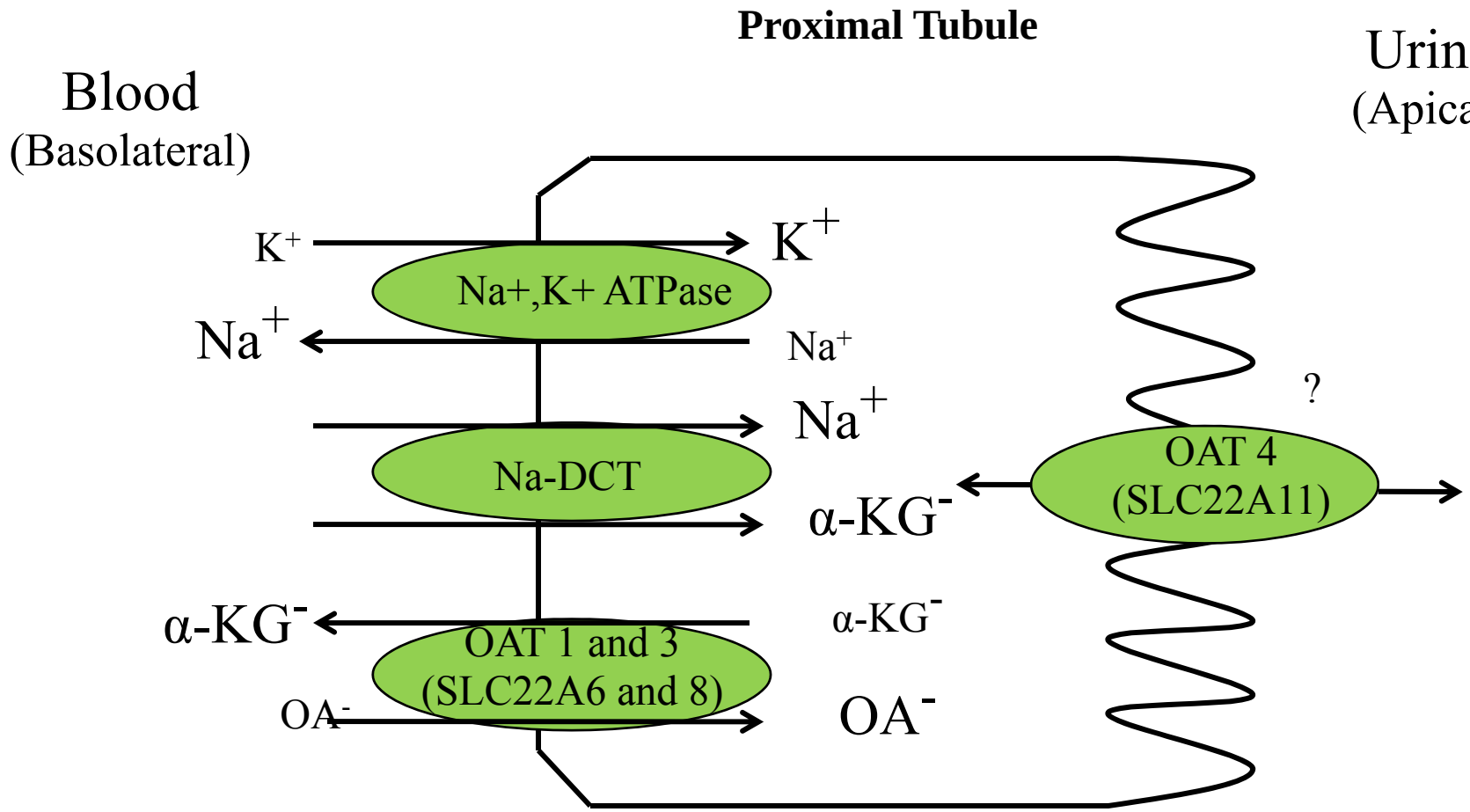
Rely on secondary energy source

Have both active and facilitated mechanisms

Good website for SLC transporters:

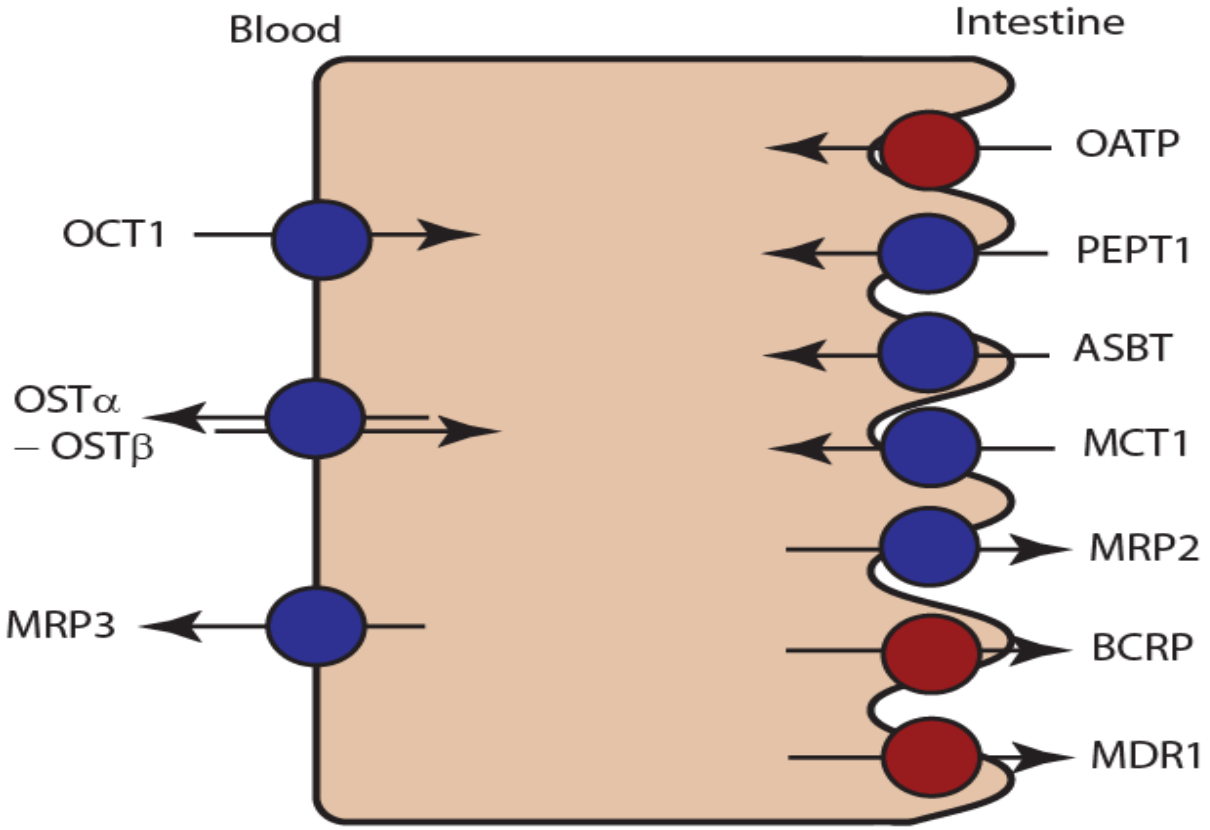
- http://www.bioparadigms.org/slc/menu.asp

Organic Anion Transporter Model



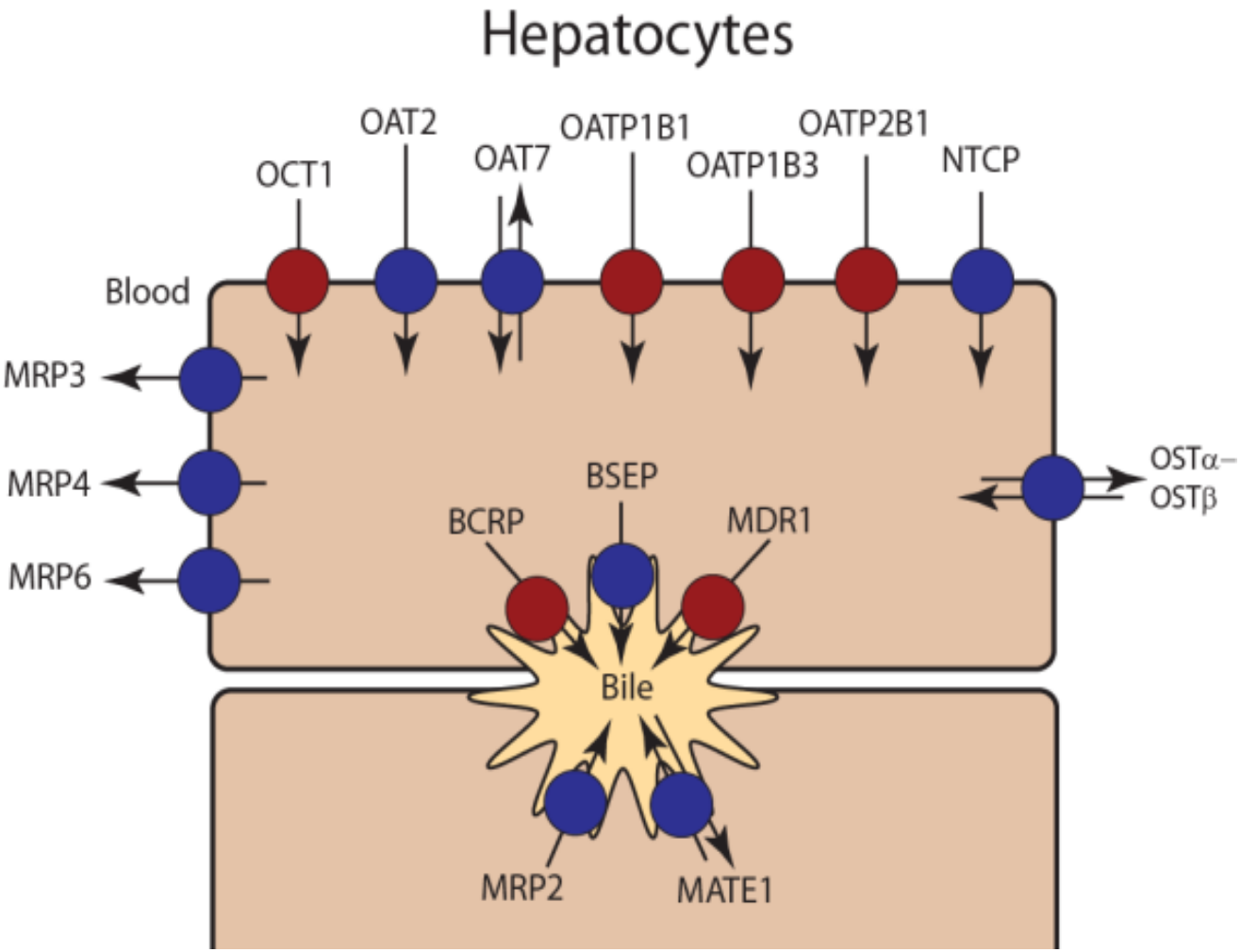
Na- DCT: sodium-dicarboxylate cotransporter;
α-KG: alpha-ketoglutarate;
OA-: organic anion

Intestinal Transporters



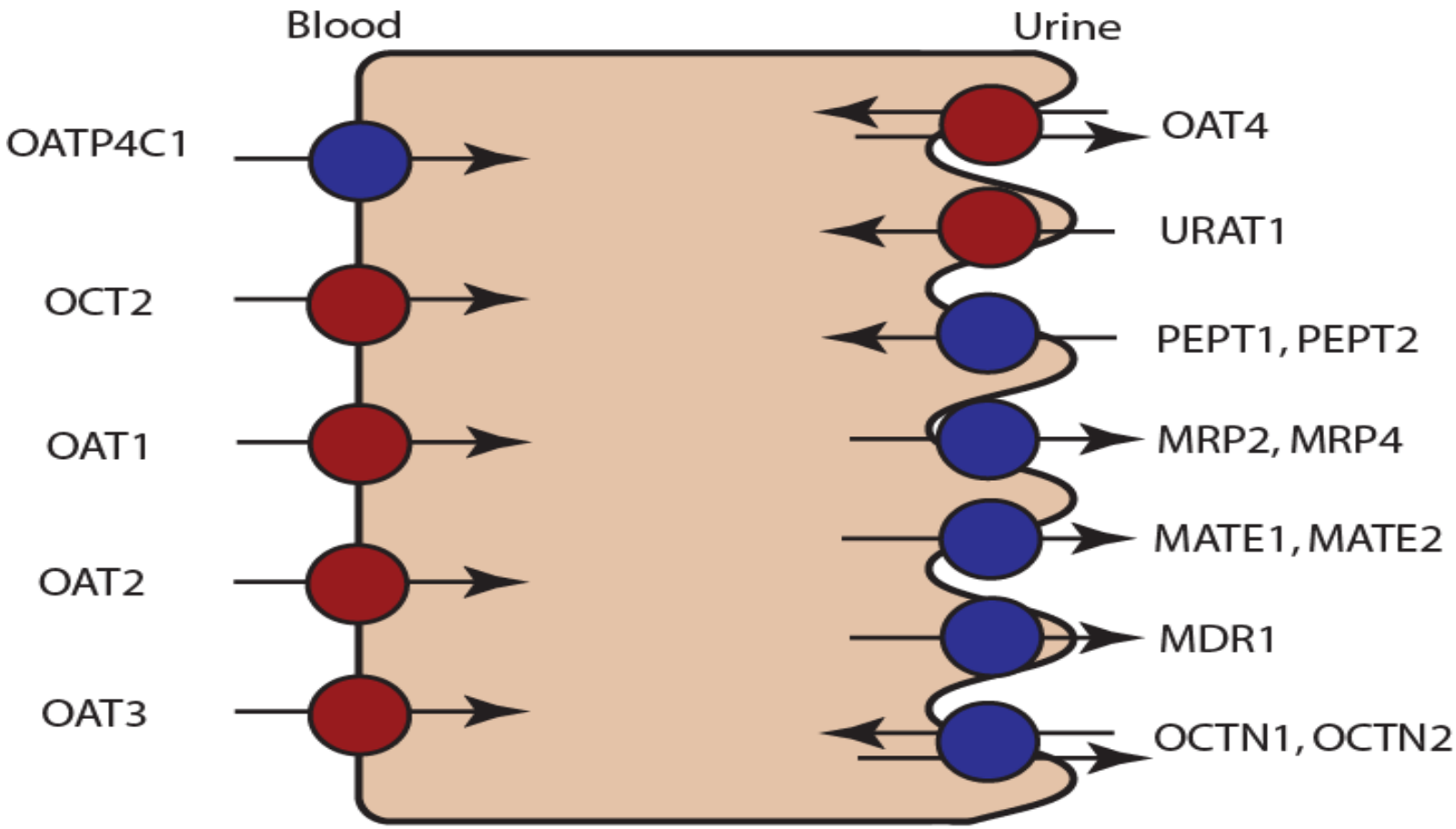
Giacomini et al. (2010) Nature Reviews Drug Discovery.

Liver Transporters



Giacomini et al. (2010) Nature Reviews Drug Discovery.

Transporters of the Renal Proximal Tubule



Giacomini et al. (2010) Nature Reviews Drug Discovery.

Drug Interactions Attributed to SLC22 Family Members

Implicated Transporter	Interacting Drug	Affected Drug	Clinical Pharmacokinetic Impact on Affected Drug
OATs	Probenecid	Cidofovir	CL_r ↓32%
	Probenecid	Furosemide	CL_r ↓66%
	Probenecid	Acyclovir	CL_r ↓ 32% and AUC ↑ 40%
OCTs	Cimetidine	Varenicline	AUC ↑ 29%
	Cimetidine	Pilsicainide	AUC ↑ 33%, CL_r ↓ 28%
	Cimetidine	Metformin	AUC ↑ 50%, CL_r ↓ 27%
	Cetirizine	Pilsicainide	CL_r ↓ 41%
	Cimetidine	Pindolol	CL_r ↓ approx 34%
	Cimetidine	Dofetilide	CL_r ↓ 33%

Adapted from: Giacomini et al. (2010) Nature Reviews Drug Discovery.

The Human Organic Anion Transporter Family

Name	Gene	Location	Mutation	Notes
OAT1	SLC22A6	Kidney (proximal tubules), placenta, brain	R50H	In Vitro Data Only, Present in Africans at approximately 4%. A highly conserved aa.
OAT2	SLC22A7	Liver (sinusoidal membrane), Kidney (proximal tubules)	No Information	
OAT3	SLC22A8	Kidney (proximal tubules), brain	No significant differences identified to date	Rare variants
OAT4	SLC22A11	Kidney (proximal tubules), placenta	rs11231809 rs4930423 rs1783811	Single study with Torsemide, did not repeat with Tenofovir

The Human Organic Cation Transporter Family

Name	Gene	Location	Mutation	Notes
OCT1	SLC22A1	Liver (sinusoidal membrane), Intestinal Epithelial (basolateral membrane.	Variety of reduced function, reduced expression and deletion variants	Metformin PK and efficacy links in small studies
OCT2	SLC22A2	Kidney (proximal tubules)	Reduced function variants	Metformin PK
OCT3	SLC22A3	Ubiquitous	Reduced expression variants	

Functional genomics of membrane transporters in human populations

Thomas J. Urban, Ronnie Sebro, Evan H. Hurowitz, Maya K. Leabman, Ilaria Badagnani, Leah L. Lagpacan, Neil Risch and Kathleen M. Giacomini

Genome Res. 2006 16: 223-230; originally published online Dec 14, 2005;

- 247-276 ethnically diverse DNA samples
- 11 SLC transporters
- 88 variants with amino acid changes
- For some variants they looked at multiple substrates

Allelic Frequency Differences Across Various Ethnic Groups

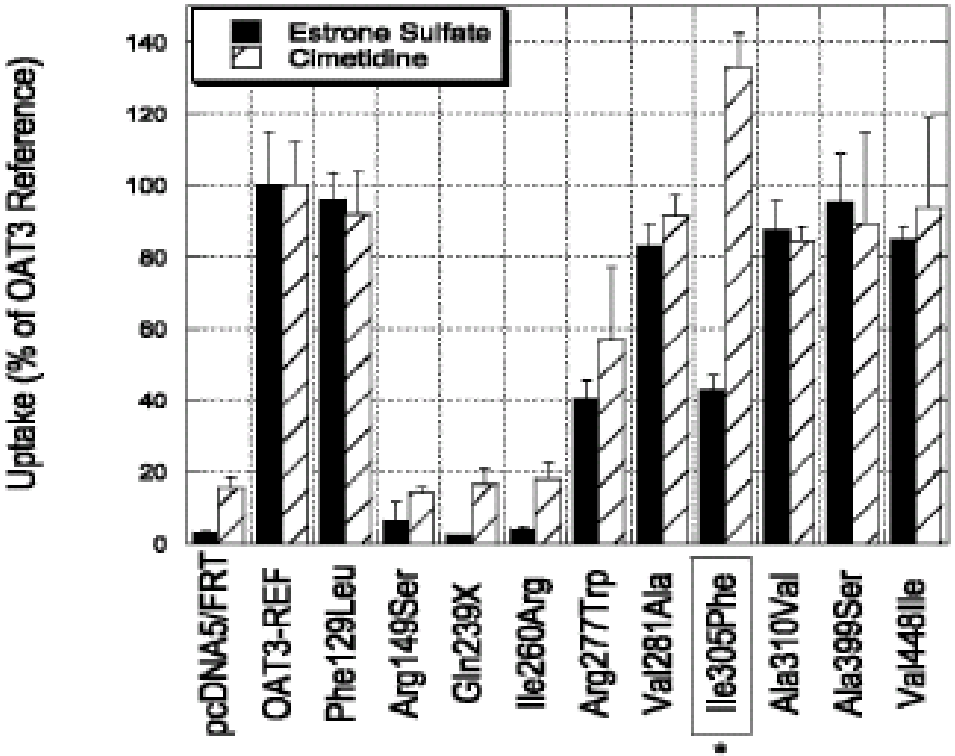
Table 2. Characteristics of variants that exhibit reduced function in biochemical assays

Variant	Total freq.[a]	AA freq.[b]	EA freq.	AS freq.	ME freq.	Grantham	EC/EU[c]	Loop/TMD[d]
OCT1-R61C	0.036	0.000	0.072	0.000	0.056	85	EC	L
OCT1-G220V	0.002	0.005	0.000	0.000	0.000	109	EC	T
OCT1-P341L	0.041	0.082	0.000	0.117	0.000	98	EU	L
OCT1-G401S	0.009	0.007	0.011	0.000	0.000	56	EC	L
OCT1-G465R	0.020	0.000	0.040	0.000	0.000	125	EC	L
OCT2-F45Ins	0.002	0.000	0.005	0.000	0.000	n/a	n/a	T
OCTN1-D165G	0.002	0.000	0.000	0.008	0.000	94	EC	L
OCTN1-M205I	0.002	0.006	0.000	0.000	0.000	10	EC	T
OCTN1-R282X	0.002	0.006	0.000	0.000	0.000	n/a	n/a	L
OCTN2-F17L	0.004	0.000	0.000	0.017	0.000	22	EC	L
OAT1-R454Q	0.002	0.006	0.000	0.000	0.000	43	EC	L
OAT3-R149S	0.004	0.000	0.006	0.008	0.000	177	EC	L
OAT3-Q239X	0.002	0.000	0.000	0.008	0.000	n/a	n/a	T
OAT3-I260R	0.002	0.000	0.000	0.008	0.000	97	EC	L
OAT3-R277W	0.002	0.007	0.000	0.000	0.000	101	EU	L
OAT3-I305F	0.009	0.000	0.000	0.035	0.011	21	EC	L
CNT1-V385Del	0.015	0.030	0.000	0.000	0.000	n/a	n/a	L
CNT1-S546P	0.003	0.005	0.000	0.000	0.000	74	EC	T
CNT3-G367R	0.002	0.000	0.000	0.008	0.000	125	EU	T
ENT2-D5Y	0.003	0.005	0.000	0.000	0.000	160	EU	L
ENT2-S184Del	0.003	0.000	0.005	0.000	0.000	n/a	n/a	L
ENT2-S282Del	0.003	0.005	0.000	0.000	0.000	n/a	n/a	L

[a]For OCT1, OCT2, CNT1, and ENT2 variants, total population includes 100 AA and 100 EA samples. For OCTN1, OCTN2, OAT1, OAT3, and CNT3, total population includes 80 AA, 80 EA, 60 AS, and 50 ME samples.
[b](AA) African American; (EA) European American; (AS) Asian American; (ME) Mexican American.
[c](EC) evolutionarily conserved; (EU) evolutionarily unconserved.
[d]Variant residue is located in predicted transmembrane domain (T) or loop region (L) of protein.

Urban TJ et al. (2006) Genome Research 16: 223-230.

Functional Characterization of Non-Synonymous Variants of OAT3 (SLC22A8) with Two Substrates



Urban TJ et al. (2006) Genome Research 16: 223-230.

Substrate Specificity of Variants in Nine SLC Transporters

Table 3. Number of variants in nine SLC transporters that change substrate specificity

Gene	Substrates tested (#)	Variants tested (#)	Number of variants with altered substrate specificity (%)
OCT2	6	4	3
	7	5	0
OCTN1	2	6	1
OCTN2	2	8	1
OAT3	2	10	1
CNT1	2	4	1
CNT2	2	3	0
	3	1	1
CNT3	2	7	0
	5	3	0
ENT1	2	1	0
	7	1	0
ENT2	4	5	0
	Total	58	8 (14%)

Urban TJ et al. (2006) Genome Research 16: 223-230.

Role of OCT1 Reduced Function Variants on Metformin Plasma Concentrations

Plasma pharmcokinetics of metformin after oral administration (after second dose of 1,000 mg)

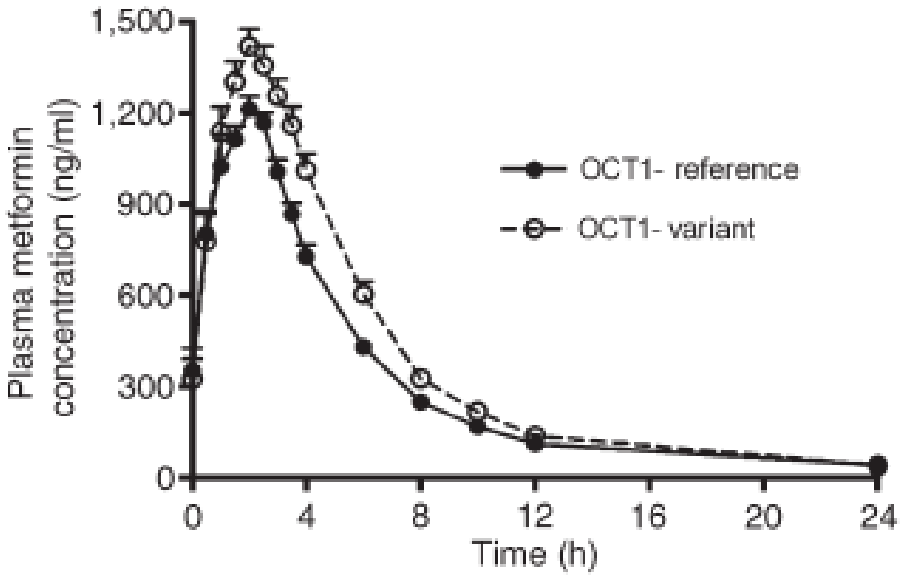


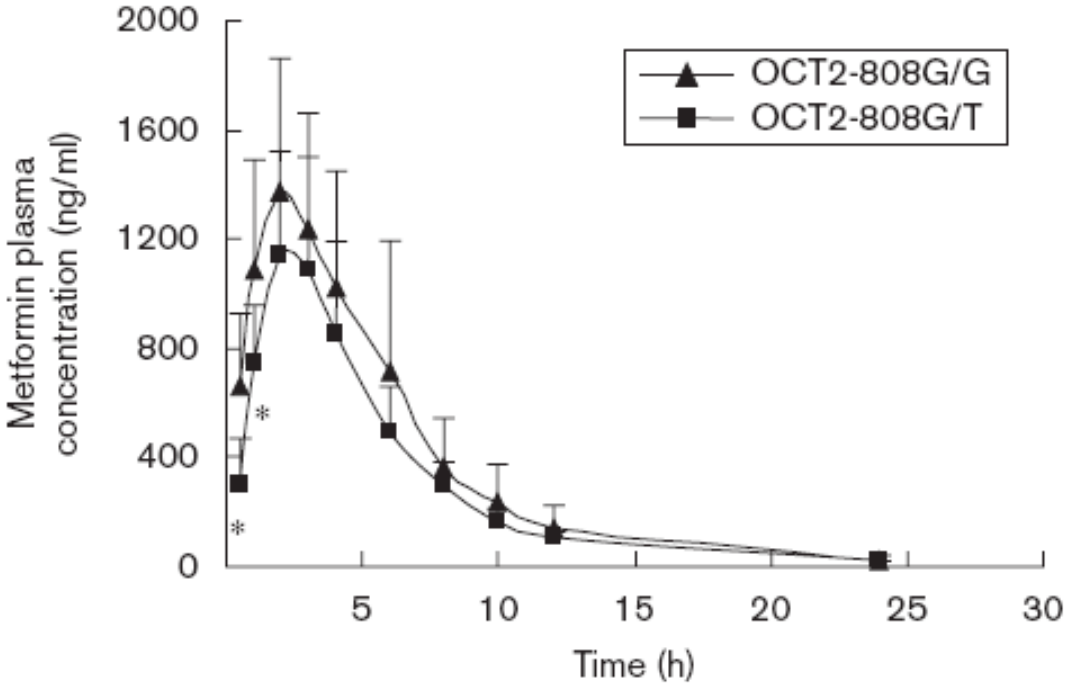
Table 3 Metformin pharmacokinetic parameters from healthy individuals who only carry OCT1-reference alleles (OCT1-reference) and those who carry an OCT1 variant allele

	OCT1-reference (n=8)[a]		OCT1-variant (n=12)[a]	
	Mean	SD	Mean	SD
$T_{1/2}$ (h)	7.3	2.3	5.8	1.2
T_{max} (h)	1.9	0.52	2.2	0.72
C_{max} (μg/ml)	1.3	0.10	1.5*	0.19
AUC_A (h μg/l)	7,700	970	9,200**	1,200
AUC_B (h μg/l)	4,500	1,200	6,900*	1,600
V/F (l)	2,600	1,800	1,200**	400
CL/F (l/h)	240	73	150*	37
CL_R (l/h)	40	16	38	21
$f_{e,u}$ (%)	19	8.8	28	16

Variants are a combination of R61C (n=4), G401S (n=3), 420del (n=1), 420del and G465R (n=3), G174S and 420del (n=1)

Shu et al. CLIN PHARM & THER. 83: 2, 2008.

Effect of OCT2 Genetic Variation on Metformin Pharmacokinetics



The mean metformin plasma concentration–time curves in individuals with organic cation transporter 2 (OCT2) genetic variants. A single oral dose of 850 mg metformin HCl tablet was administered to individuals homozygous for the reference allele (808G/G, $n=14$, triangles) or those heterozygous for the variant allele (808G/T, $n=9$, squares). Blood was drawn at the indicated time points. Significant differences in metformin plasma concentrations between two genotypes are indicated by asterisks. Data represent mean ± SD.

Chen et al. (2009) Pharmacogenetics and Genomics 19:7, 497-504.

Effect of OCT2 Genetic Variation on Metformin Pharmacokinetics

Table 1 **Summary of metformin pharmacokinetic parameters in individuals homozygous for the OCT2 reference allele (808G/G) and heterozygous for the variant allele (808G/T)**

	OCT2-808G/G ($n=14$)	OCT2-808G/T ($n=9$)	P value
T_{max} (h)	2.0 (2.0–6.0)	2.0 (1.0–3.0)	0.425
C_{max} (ng/ml)	1456 ± 511	1170 ± 340	0.171
AUC_{last} (ng·h/ml)	8670 ± 3436	6485 ± 2215	0.106
AUC_{inf} (ng·h/ml)	8821 ± 3489	6604 ± 2195	0.105
V/F (l)	445 ± 206	485 ± 232	0.667
CL/F (ml/min)	1464 ± 603	1912 ± 894	0.164
$t_{1/2}$ (h)	3.54 ± 0.75	2.95 ± 0.60	0.059
CL_R (ml/min)	441 ± 108	614 ± 158	0.005
CL_{CR} (ml/min)	102 ± 23	94 ± 26	0.468
$SrCL_R$ (ml/min)	340 ± 97	519 ± 148	0.002

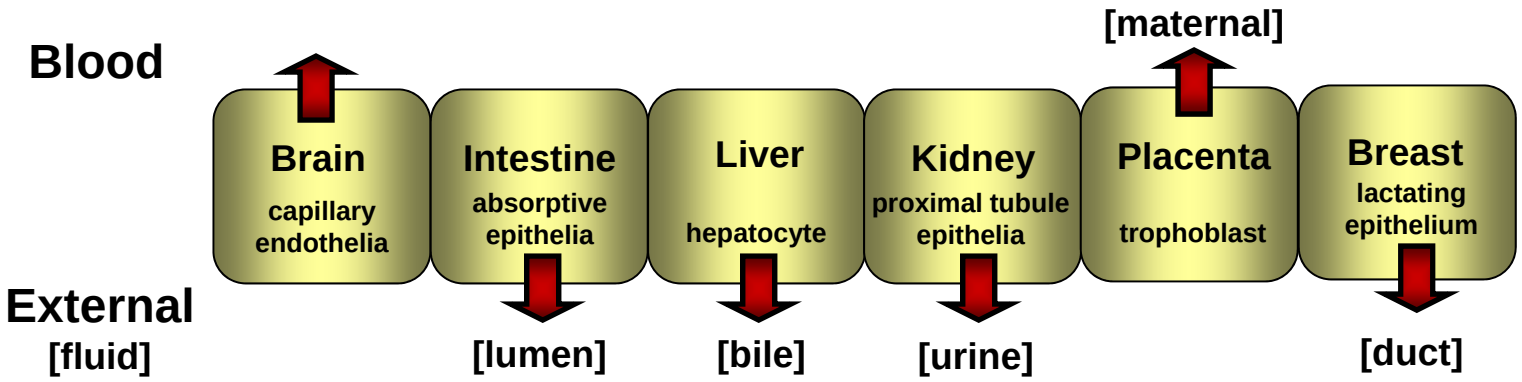
Data were obtained after oral administration of 850 mg of metformin HCl tablet. Median (range) used for T_{max} and arithmetic mean (SD) used for other parameters.

AUC_{inf}, area under the plasma concentration versus time curve from 0 to infinity; AUC_{last}, area under the plasma concentration versus time curve from 0 to last time point; C_{max}, maximal plasma concentration; CL/F, apparent plasma clearance; CL_R, renal clearance; CL_{CR}, creatinine renal clearance; OCT2, organic cation transporter 2; $SrCL_R$, renal clearance by secretion; T_{max}, time of maximal plasma concentration; $t_{1/2}$, plasma terminal elimination half-life; V/F, apparent oral volume of distribution.

Chen et al. (2009) Pharmacogenetics and Genomics 19:7, 497-504.

Breast Cancer Resistance Protein, BCRP, ABCG2

ATP-**B**inding **C**assette Superfamily
ABCG Sub-Family
ABCG2 (aka MXR, WHITE2, ABCP, BMDP, ABC15)



655 amino acids (72 kD)
6 transmembrane domains
one Walker motif (a half transporter)

ABCG2 Substrates

- Natural Substrates

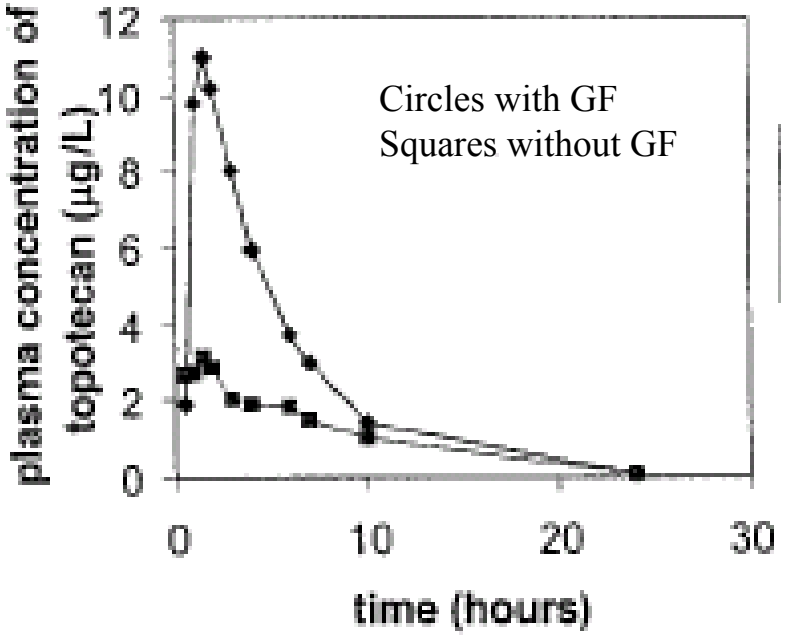
  Porphyrins; PhIP; Folic acid & polyglutamate forms; Flavonoids; Sulfated conjugates of steroids

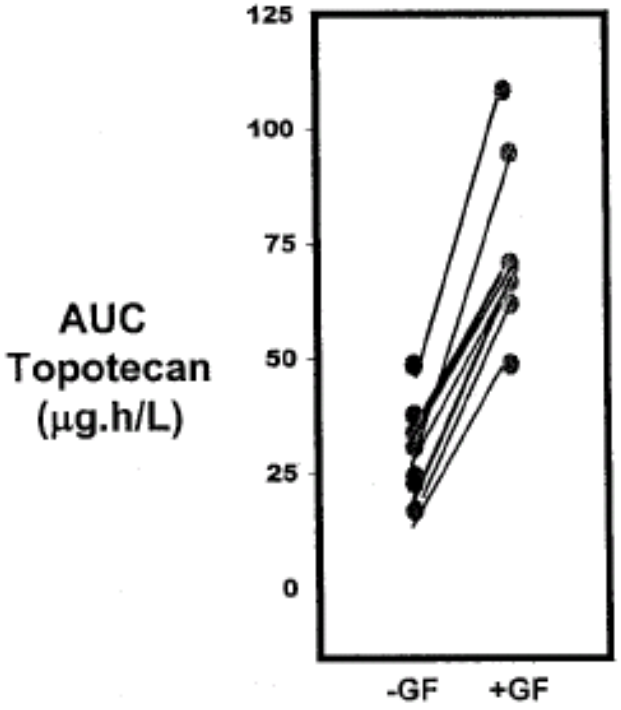
- Drugs Effluxed by ABCG2

  Mitoxantrone; Topotecan; Methotrexate; Prazosin; Gleevec; Omeprazole; Pantoprazole; Lansoprazole Labeprazole

  Sulfated conjugates of xenobiotics

Effect of GF120918 on Topotecan Oral Exposure in Patients

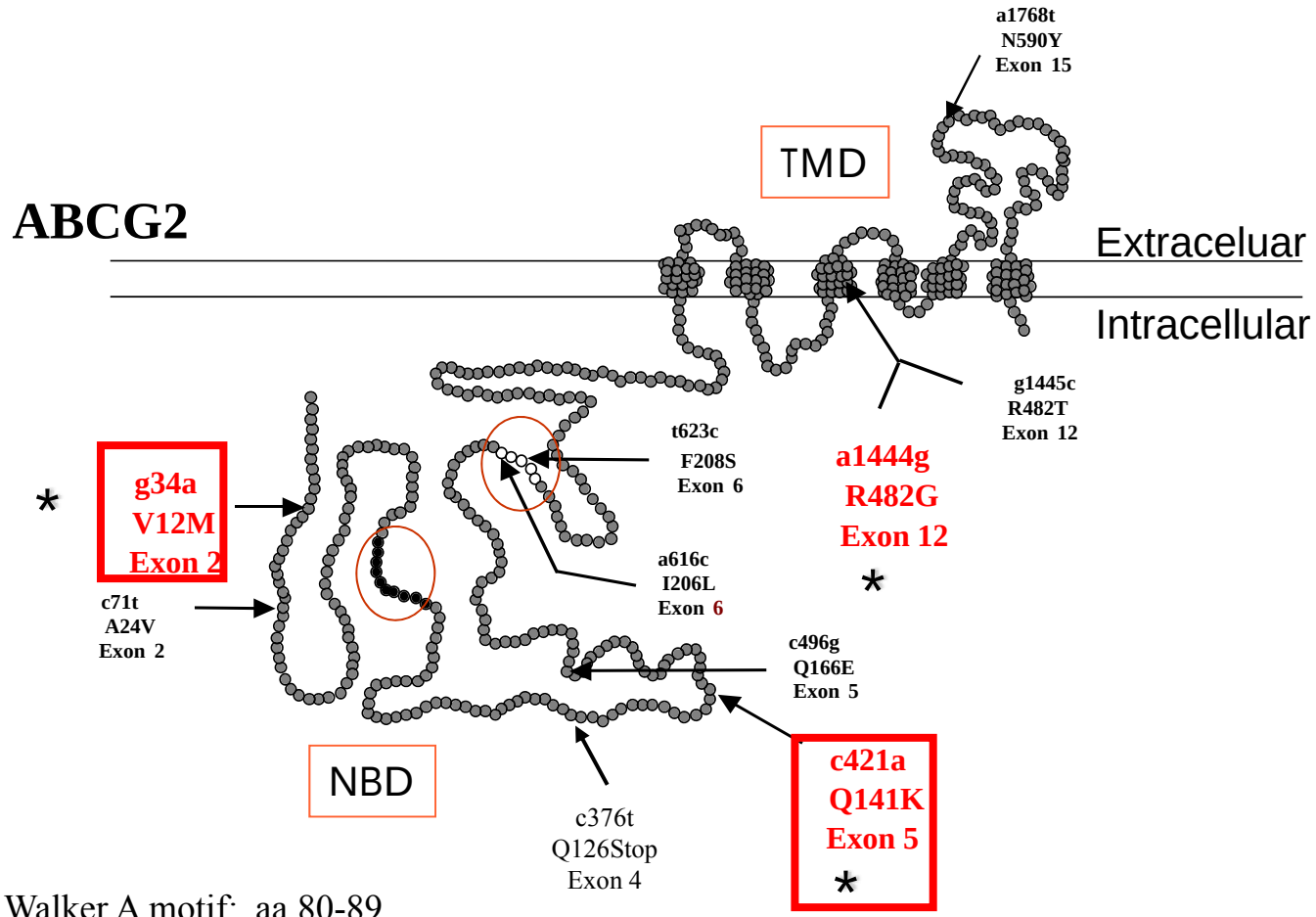




1.0 mg/m^2 topotecan with or without 1,000 mg oral GF

Kruijtzer et al., J. Clin. Onc., 2002.

Human ABCG2 Coding Region Variants

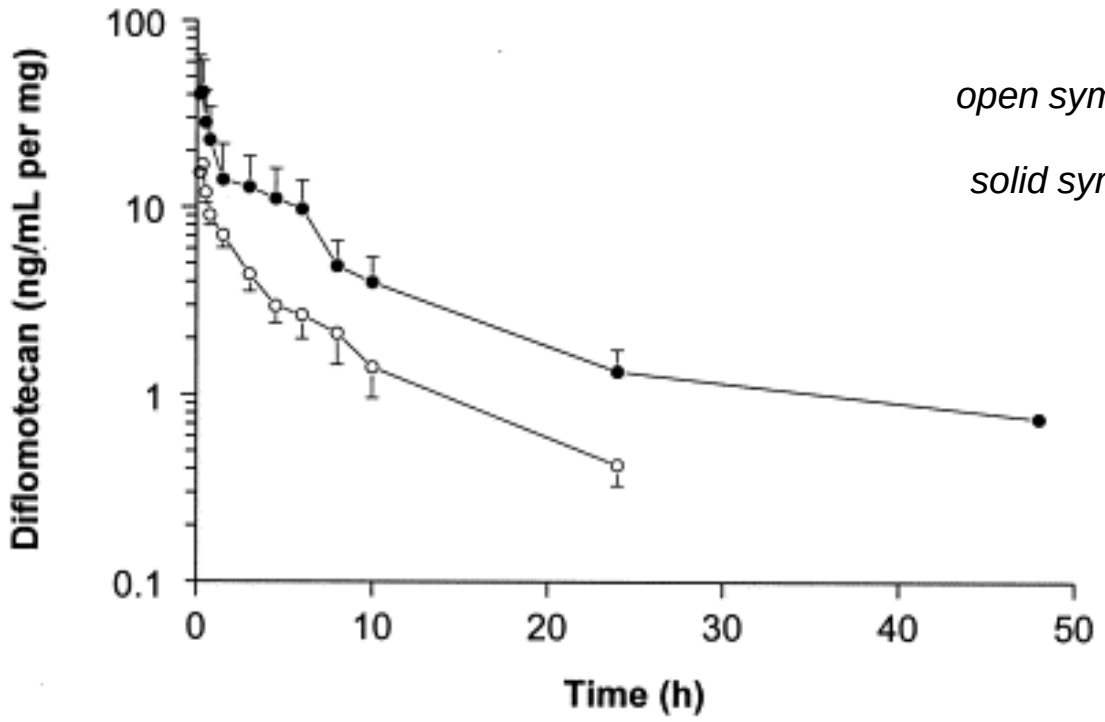


- ● Walker A motif: aa 80-89
- ○ Walker B motif: aa 206-210
- * Variants with in vitro activity differences

TMD: TransMembrane Domain
NBD: Nucleotide Binding Domain

Zamber, Pharmacogenetics, 2003

Plasma Concentration–Time Profiles of Diflomotecan as a Function of BCRP (*ABCG2)* 421C>A (Q141K) Genotype

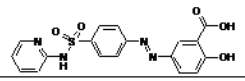
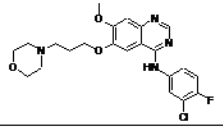
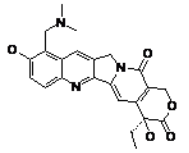
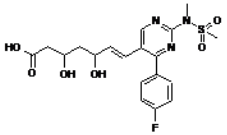
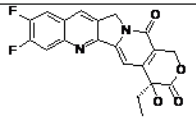
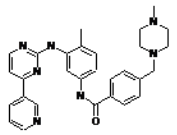
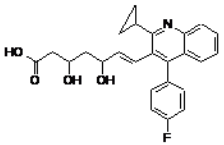


open symbols: homozygous wild-type patients [n = 15]
solid symbols: heterozygous variant patients [n = 5]

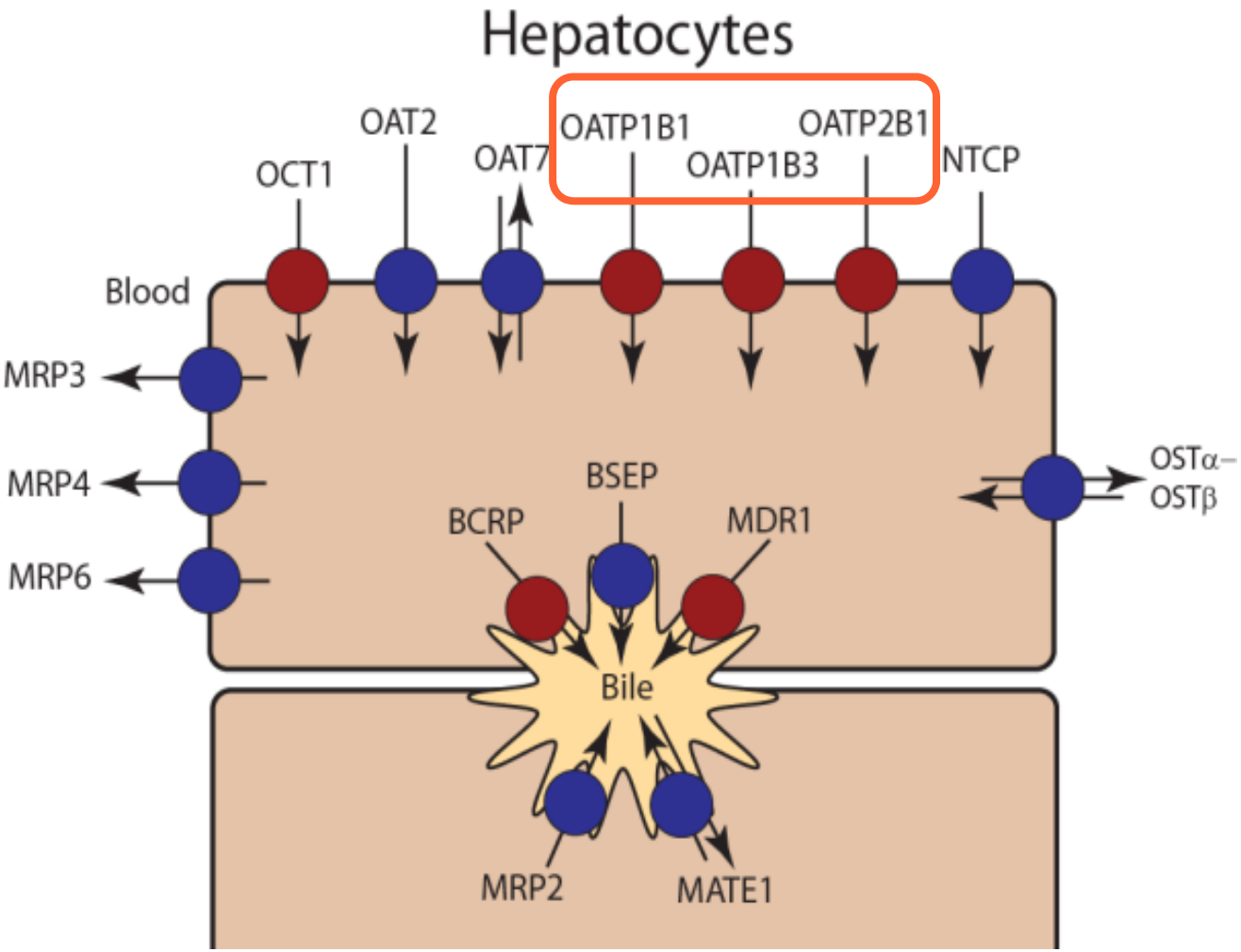
Dose of 0.1-0.27 mg. Data are presented as dose-normalized (ie, observed concentration/dose in milligrams) mean values (*symbols*) ± SE (*error bars*) with interpolated concentration-time points (*solid lines*).

Alex Sparreboom et al., Clin. Pharm Ther. Vol 76, July 2004, pp 38-44.

Clinical Studies of ABCG2 Genomics

Drug	Structure	Dose, Route	# Patients	Ethnic Group, Gender	Result	Reference
Sulfasalazine		2000 mg po	37*	Japanese Male	1.7-3.5X increase in AUC, Cmax	Yamasaki et al (2008) Clin Pharmacol Ther, ePub
Sulfasalazine		1000 mg po	17*	Caucasian Both	1.7-2.4X increase in AUC, Cmax	Urquhart et al (2008) Pharmacogen & Genomics, ePub
Sulfasalazine		500 mg po	36*	Chinese Both	No effect on AUC, Cmax	Adkison et al (2008) ASCPT mtg poster
Gefitinib (IRESSA)		250 mg po	124^	Caucasian Both	44% with mutation had diarrhea vs. 12% with WT	Cusatis et al (2007) JNCI 98(23):1739
Topotecan		<2.5 mg po, iv	18^	Caucasian Both	1.35X increase in oral bioavailability	Sparreboom et al (2005) Canc Biol Ther 4:650
Rosuvastatin		20 mg po	14*	Chinese Both	1.8X increase in AUC and Cmax	Zhang et al (2006) Clin Chim Acta 373:99
Diflomotecan		<0.5 mg po, iv	22^	Caucasian Both	3X increase in AUC and Cmax for iv only	Sparreboom et al (2004) Clin Pharmacol Ther 76:38
Imatinib (GLEEVEC)		100-1000 mg po	82^	Caucasian Both	No difference	Gardner et al (2006) Clin Pharmacol Ther 80:192
Pitavastatin		2 mg po	38*	Japanese Male	No difference	Ieiri et al (2007) Clin Pharmacol Ther. 82:541

Liver Transporters



Giacomini et al. (2010) Nature Reviews Drug Discovery.

Some Xenobiotics/Natural Products Known to Interact with OATP1B1 (SLCO1B1)

Pravastatin (S,I)

Cerivastatin (S, I)

Rosuvastatin (S,I)

Atorvastatin (S,I)

Fluvastatin (S)

Simvastatin (S,I)

Repaglinide (S, I)

Atrasentan (S)

Rosiglitazone (I)

Fexofenadine (S)

Estrone Sulfate (S,I)

Estradiol 17ß-D-glucuronide (S,I)

Bile Acids (S,I)

Bromosulfophthalein (BSP) (S,I)

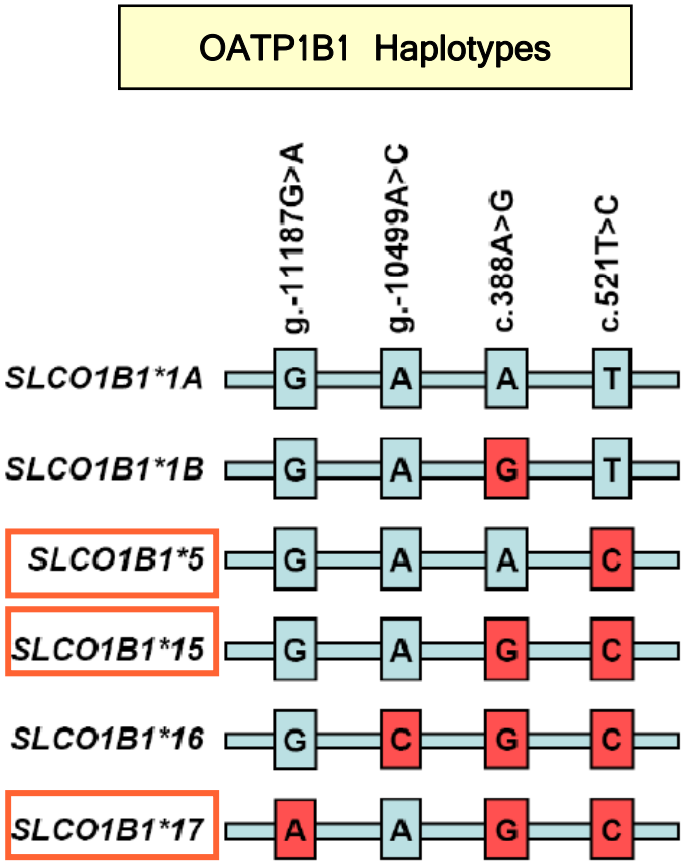
Rifampin (S, I)

Cyclosporin A (I)

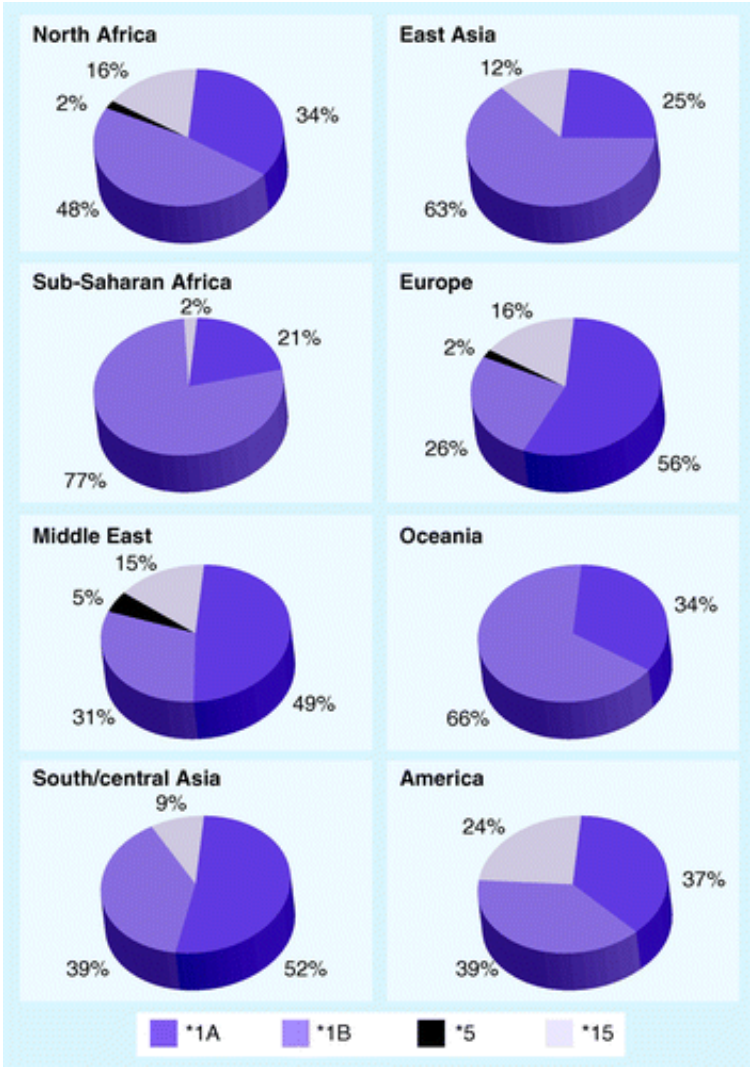
Gemfibrozil (I)

S – Substrate, I – Inhibitor
Bolded – Clinical Inhibitors

OATP1B1 Population Genetics

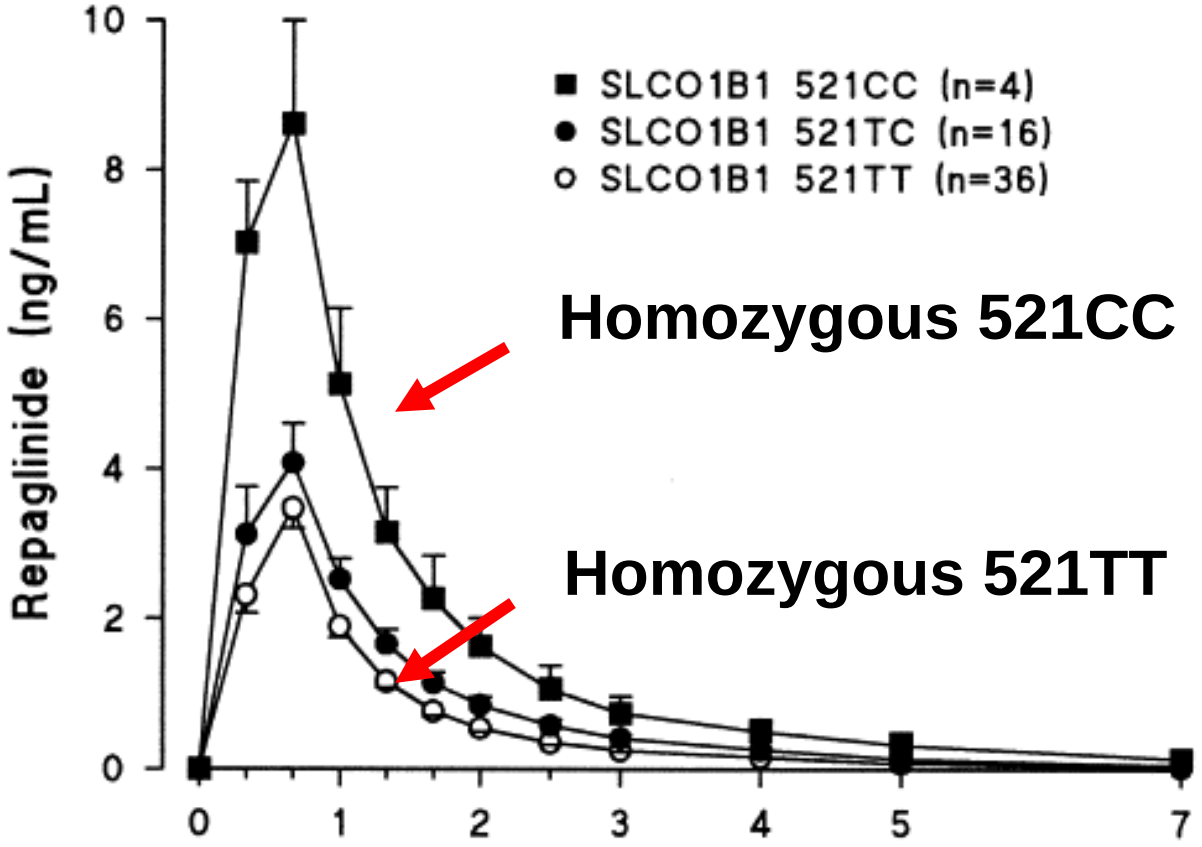


Pharmacogenomics (2007) 8(7):787-802



Pharmacogenomics (2008) 9(1):19-33

Individuals with Polymorphisms of OATP1B1 Have Higher Plasma Levels of Repaglinide

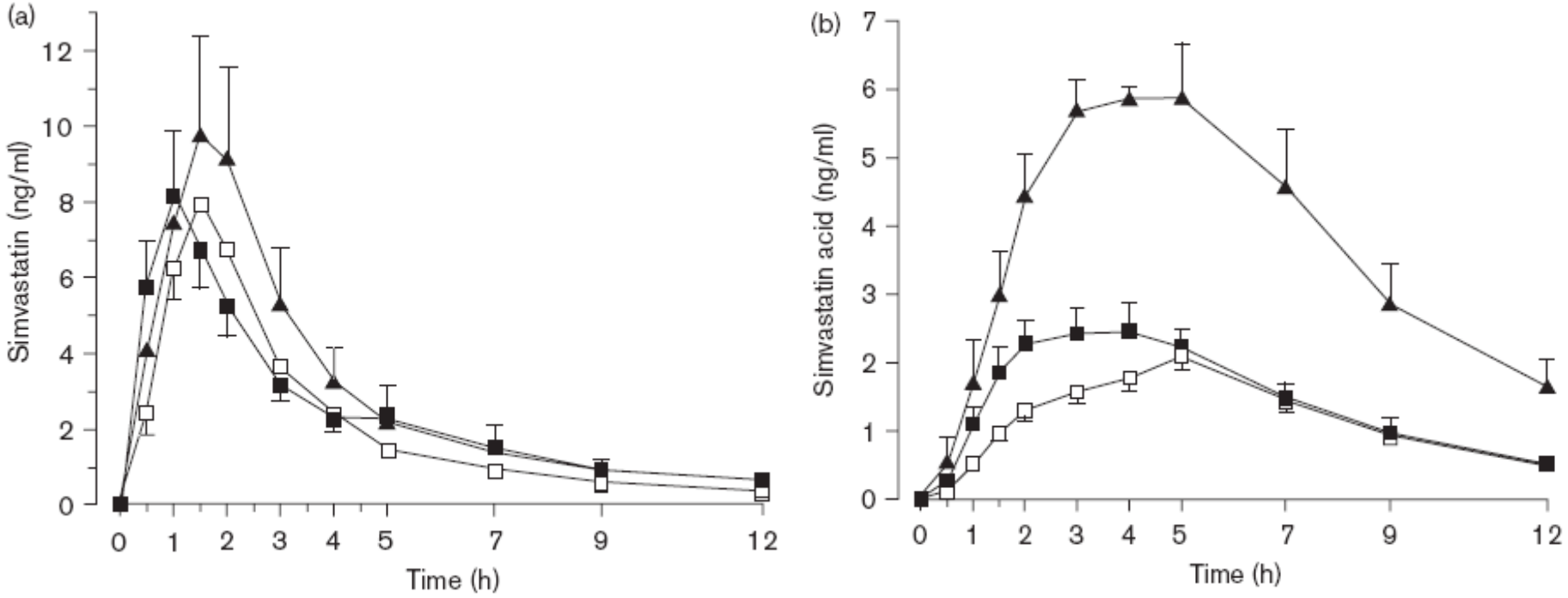


Niemi M. et al, Clin Pharmacol Ther 2005; 77:468-78

Relevance of OATP1B1 Genetic Variants

Drug	Allele (n)	PK Change	Ref
Pravastatin	*1b/*1b (4): *1b/*15 (9): *15/*15 (n=1)	Clnr decrease in *15/*15 AUC increase in *15/*15	Nishizato et al, 2003
Atorvastatin/ Pravastatin	1628>G (<1% in Jap)	2/ 7 patients with myopathy had mutation	Morimoto et al, 2004
Fexofenadine	*1a/*1a (10): *1a/*5 (8): *5/*5 (2)	Increase AUC with *5/*5 Decrease CLnr with *5/*5	Miemi et al (2005b)
Repaglinide	*1a/*1a(36): *1a/*5 (16): *5/*5 (4)	Increase AUC with *5/*5 Increase Cmax with *5/*5	Niemi et al, (2005a)
Pitavastatin	Grp 1: *1a/*1a (1) Grp 2: *1a/*1b (8): *1b/*1b (4) Grp 3: *1a/*15 (5): *1b/*15 (6)	AUC: Grp1<Grp2,Grp3 Cmax: Grp1<Grp2<Grp3	Chung et al, 2005
Rosuvastatin	A388G T521C	AUC: TT<TC<CC Cmax: TT<TC,CC	Lee et al, 2005b
Simvastatin	521CC (n=4) 521TC (n=11) 521TT (n=16)	AUC: TT<TC<CC Cmax: TT<TC<CC Tmax: TT>TC> CC	Pasanen et al., 2006
Irinotecan	Grp 1: *1a/*1a (6) Grp 2: *1a/*1b (23): *1b/*1b (31) Grp3: *1b/*15 (10): *15/*15 (1)	Irinotecan: Cmax, AUC: Grp1<Grp2<Grp3 CL: Gr1>Grp2>Grp3 SN38: Cmax, AUC: Grp1<Grp2<Grp3 SN38G: Cmax, AUC: Grp1>Grp2>Grp3	Xiang et al. 2006

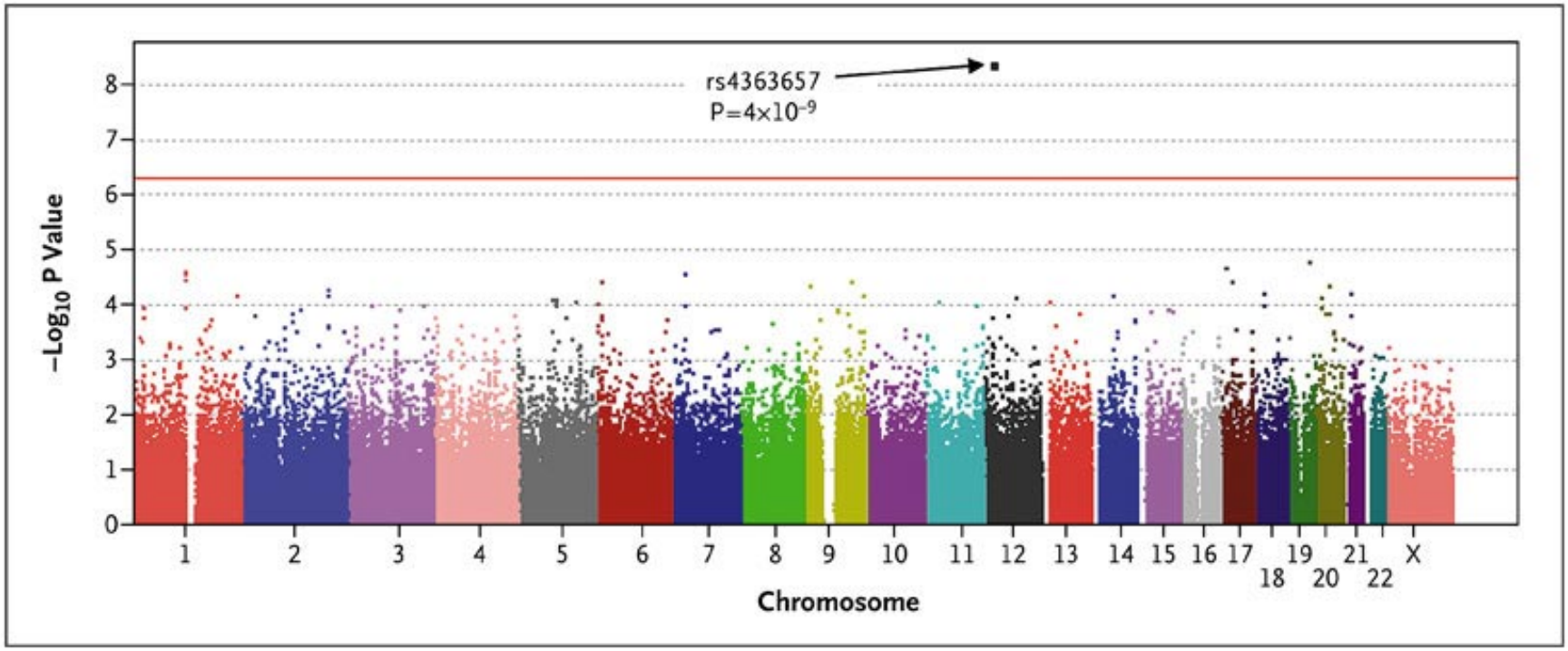
OATP1B1 Genotypes Impact the Pharmacokinetics of Simvastatin



521CC – Solid Triangle
521TC – Solid Squares
521TT – Open Squares

Pasanen et al (2006) Pharmacogenetics and Genomics16:873–879

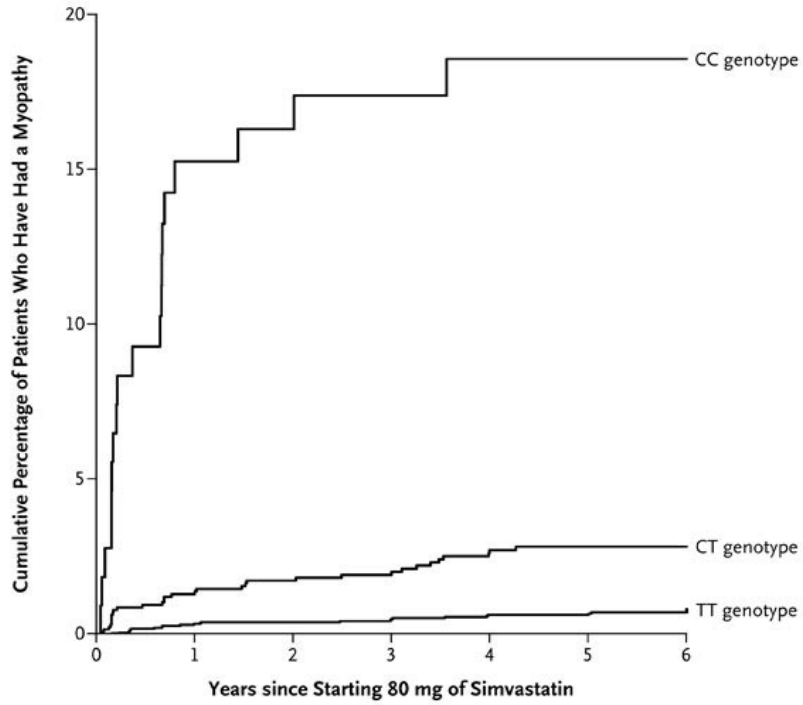
OATP1B1 Variants and Statin-induced Myopathy



- Genome Wide Association Study (GWAS) using approximately 300,000 markers
- 12,000 participants; all were taking simvastatin (80 mg) daily
- 85 subjects with definite or incipient myopathy and 90 controls
- No associations between myopathy and SNPs in any other region yielded an uncorrected P value of $< 10^{-5}$

NEJM (2008) 359(8):789-799

Cumulative Risk of Myopathy: Simvastatin (80 mg)



Genotype	Population Frequency	Cumulative No. and Percentages with Myopathy							
		Year 1				Year 5			
				Attributable to gentoype				Attributable to gentoype	
		no.	%	*no.*	*% of total*	*no.*	%	*no.*	*% of total*
TT	0.730	12	0.34	0	0	21	0.63	0	0
CT	0.249	17	1.38	12.8	75	32	2.83	24.9	78
CC	0.021	16	15.25	15.6	98	19	18.55	18.4	97
All genotypes	1.000	45	0.91	28.4	63	72	1.56	43.3	60

521CC homozygotes:
- 18% cumulative risk
- Myopathy occurred primarily during the first year

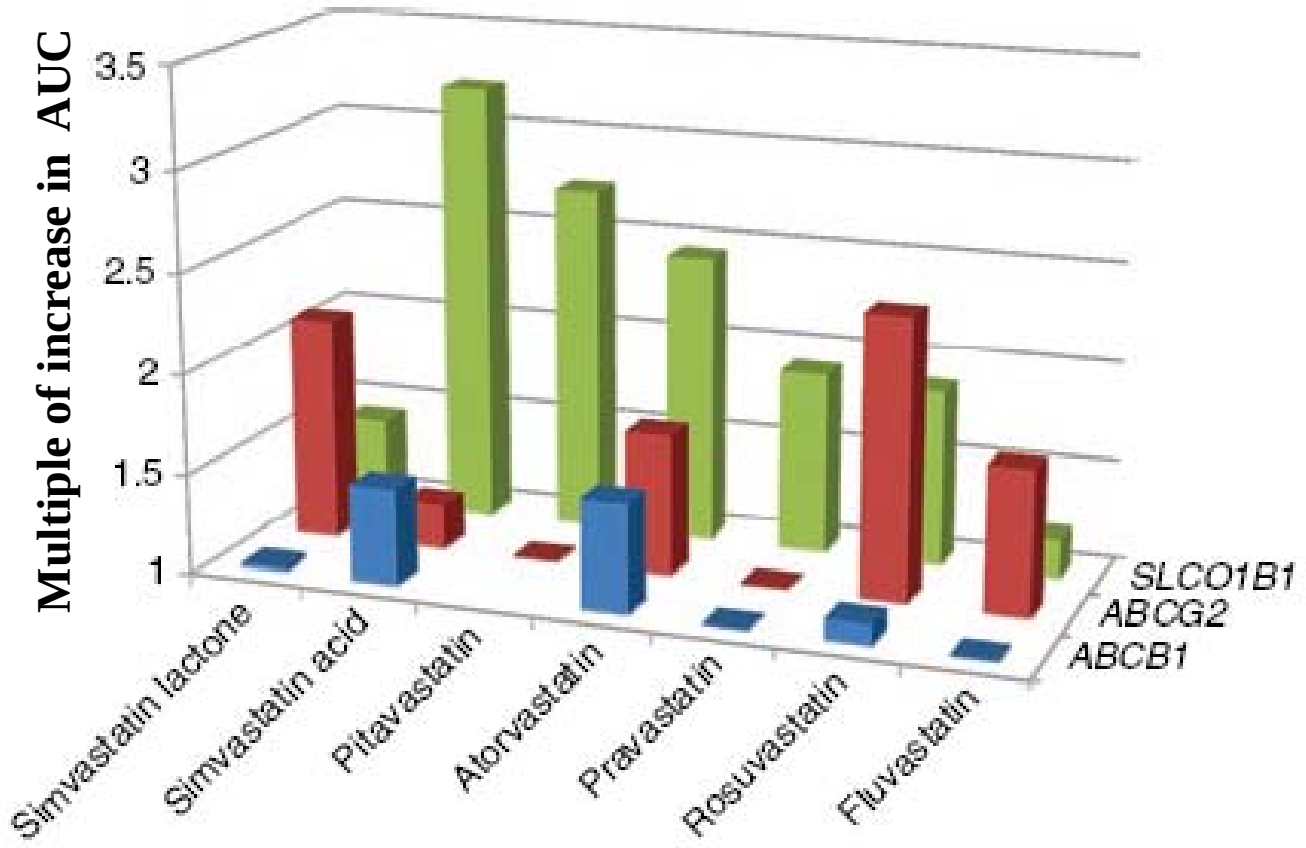
521CT heterozygotes:
- 3% cumulative risk

521TT homozygotes:
- 0.6% cumulative risk

Overall, more than 60% of myopathy cases were associated with the 521T>C variant in OATP1B1.

NEJM (2008) 359(8):789-799

Effects of SLCO1B1, ABCG2, and ABCB1 Genotypes on the Systemic Exposure of Various Statins.



Genotypes used for comparison to reference genotypes:
OATP1B1: 521CC
ABCG2: 421AA
ABCB1: 1236TT, 2677TT, 3435TT

Niemi (2010) Clin Pharm Ther 87:1, 130-133.

Transporters with Clinically Relevant Genetic Polymorphisms

Transporter	Substrates	Organ	Functional Effect
OATP1B1 (OATPC, SLCO1B1)	statins*, repaglinide*, valsartan, olmesartan*	Hepatocytes (sinusoidal)	Decreased Affinity
OCT2 (SCL22A2)	metformin*, procainamide, ranitidine, amantadine, oxaliplatin, varenicline*	Kidney proximal tubule, neurons	Decreased Affinity, Increased Activity, Known Substrate Differences
OCT1 (SLC22A1)	metformin*, Oxaliplatin	Hepatocytes (sinusoidal), intestinal Enterocytes	Decreased Affinity
BCRP (MXR, ABCG2)	Mitoxantrone, methotrexate, topotecan, imatinib, irinotecan, statins*, sulphate conjugates, porphyrins	Intestinal enterocytes, hepatocytes (canalicular), kidney proximal tubule, brain endothelia, placenta, stem cells, mammary glands (lactating)	Decreased Expression, Known Substrate Differences
MRP2 (cMOAT, ABCC2)	Glutathione and glucuronide conjugates, methotrexate	Hepatocytes (canalicular), kidney (proximal tubule), enterocytes (luminal)	Increased Activity or Decreased Activity
MDR1 (P-gp, ABCB1)	Digoxin*, loperamide*, berberine, irinotecan, doxorubicin, vinblastine, paclitaxel, fexofenadine	Intestinal enterocytes, kidney proximal tubule, hepatocytes (canalicular), brain endothelia	Unclear

Acknowledgements

Mengmeng Wang

Steve Wrighton

Tom Raub

Steve Hall