Table VI. Pharmacokinetic parameters of pravastatin after a single dose (10 mg) in various *OATP-C* genotypic patterns

Polymorphism					
130	*151*	*174*	*336*	*Genotype*	*No.*
Asn/Asn	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1a/*1a*	2
Asn/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1a/*1b*	4
Asp/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1b/*1b*	4
Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	9
Asp/Asp	Asn/Asn	Ala/Ala	Pro/Pro	*OATP-C*15/*15*	1
Asn/Asp	Asn/Ser	Val/Val	Pro/Pro	*OATP-C*1b/*16*	2
Asp/Asp	Asn/Asn	Val/Ala	Pro/Arg	Unidentiﬁed	1

AUC, Area under concentration-time curve; CL_t, total clearance; CL_{nr}, nonrenal clearance; k_e, terminal rate constant for elimination.
†Signiﬁcantly different from values in *OATP-C*1b/*1b* subjects as determined by Mann-Whitney *U* test ($P < .05$).

race and that most Japanese subjects who have a Val174Ala polymorphism also have an Asn130Asp polymorphism simultaneously.

Genetic variations have been examined in some transporter genes such as *MDR1* (multidrug resistance 1), *MRP1* (multidrug resistance protein 1), *MRP2* (multidrug resistance protein 2), and *OATP-8*. The numbers of nonsynonymous SNPs in *MDR1*, *MRP1*, *MRP2*, and *OATP-8* genes were reported to be 4, 4, 4, and 2, respectively, with allelic frequencies ranging from 21.8% to 49.0%, 1% to 7.3%, 1% to 12.5%, and 55% to 90%, respectively, among healthy Japanese subjects.[32-34] Although the data for these transporter genes are not sufﬁcient for conclusions to be made, it appears that nonsynonymous SNPs in the *OAT3* gene (only one nonsynonymous with an allelic frequency of <1% in this study) occur at a lower frequency than these genes, including *OATP-C*. These observations are consistent with a recent ﬁnding of a lower frequency of nonsynonymous SNPs in the *OCT2* (organic cation transporter 2) gene.[35] Similar to OAT3, the OCT2 protein is located on the basolateral membrane of the proximal tubule epithelium and is suggested to govern the entry of organic cations from the blood into the renal tubule, thereby controlling the ﬁrst step in renal secretion of organic cations. Thus, as has been suggested for OCT2, OAT3 may also be relatively intolerant of nonsynonymous changes.[35]

In vivo phenotypic data on the most common nonsynonymous variant revealed functional differences between subjects with the *OATP-C* reference and the variant. Subjects with the *OATP-C*15* allele had signiﬁcantly lower Cl_t and Cl_{nr} values than did those with a reference form of *OATP-C* (ie, **1a* and **1b* alleles), suggesting a functional consequence of the **15* allele in the pharmacokinetics of pravastatin. Hepatic clearance of pravastatin is rate-limited by uptake.[11,31] The hepatic uptake clearance of pravastatin determined in vivo by integration plot analysis was comparable to the blood ﬂow rate, suggesting high extraction in the liver during a single pass.[5] Thus low transport activity of OATP-C may lead to a reduction in hepatocellular uptake of pravastatin, resulting in lower total clearance. In addition, the possibility of an increasing F cannot be negated. Although the net in vivo effect of reduced transport activity on the overall pharmacokinetics of pravastatin remains unclear, these pharmacokinetic data from the subjects with the **15* allele are consistent with previous in vitro ﬁndings showing that the Ala174 variant (*OATP-C*5*) signiﬁcantly reduced estrone sulfate and estradiol-17β-D-glucuronide transport activity by using *OATP-C* variants generated in HeLa cells.[15] In vitro intrinsic clearance was markedly lower for the *OATP-C*5* variant than for the reference allele (*OATP-C*1b*), with a mean reduction rate of 75%.[15] These values were found to be very close to those in our healthy volunteers (35%-60%). It should be noted that Val174Ala is present in *OATP-C*5* and **15*, and the only difference between these 2 alleles is the presence of Asn130Asp in *OATP-C*15*. In contrast, the effect of the **1b* allele (Asp130) did not appear as evident as that of the **15* allele. The absence of functional changes in the **1b* allele is consistent with previous in vitro ﬁndings reported from 3 independent laboratories.[15-17] Nevertheless, caution must be taken with regard to the in vivo effects of Asn130Asp, because amino acid substitutions in extracellular loop 2 of the *OATP-C* are presumed to affect substrate speciﬁcity.[17] The Asn130Asp variant is located in extracellular loop 2.

One subject with the Arg336 variant had a high AUC value and low values for CL_t and CL_{nr} of pravastatin. Tirona et al[15] reported that all polymorphisms that localized to the putative transmembrane-spanning domain (MSD) were associated with a signiﬁcant reduc-

AUC (*ng · h/mL*)	CL_t ($L \cdot kg^{-1} \cdot h^{-1}$)	CL_r ($L \cdot kg^{-1} \cdot h^{-1}$)	CL_{nr} ($L \cdot kg^{-1} \cdot h^{-1}$)	CL_{sec} ($L \cdot kg^{-1} \cdot h^{-1}$)	k_e (h^{-1})
60.5	2.66	0.44	2.22	0.29	0.171
47.2 ± 27.4	1.95 ± 0.72	0.51 ± 0.12	1.45 ± 0.72	0.40 ± 0.12	0.279 ± 0.093
44.2 ± 6.38	2.39 ± 0.44	0.38 ± 0.03	2.01 ± 0.42	0.25 ± 0.03	0.312 ± 0.090
62.1 ± 21.8	1.57 ± 0.32†	0.46 ± 0.13	1.11 ± 0.34†	0.34 ± 0.12	0.255 ± 0.091
111.8	0.79	0.51	0.28	0.37	0.212
60.0	2.55	0.43	2.12	0.31	0.260
110.3	1.22	0.41	0.81	0.30	0.213

tion in transporting activity. The Pro336Arg variant is localized within MSD7.

As shown in Fig 1, mean serum concentration–time curves of pravastatin were different among the 3 genotypic groups. There was, however, a relatively large intergenotypic variability, particularly for the heterozygous carriers. These results indicate that unidentified factors may also contribute to the overall pharmacokinetics of pravastatin. Tamai et al[36] reported that pravastatin was weakly but significantly taken up by HEK293 cells transfected with *OATP-B*, another member of the *OATP* family expressed in human hepatocytes. In addition, some transporters may be involved in oral absorption of pravastatin.[37] These findings suggest that the multitransporter-mediated transport is involved in the distribution and disposition kinetics of pravastatin. A phenotyping probe that is highly specific for OATP-C would be extremely useful to further explore the genetics behind this variability in the pharmacokinetics of pravastatin.

Two polymorphisms in the OAT3 gene, T723A and Ala389Val, were unlikely to be associated with differences in either Cl_r or CL_{sec}. CL_r of pravastatin ranged from 0.38 to 0.51 $L \cdot kg^{-1} \cdot h^{-1}$ and was much higher than the glomerular filtration rate, indicating that tubular secretion is a predominant mechanism in renal excretion.[20] In addition, given that *OAT3* is predominantly expressed in the kidney,[21,22] both clearance values were used as phenotypic indexes in this study. The lack of change in CL_r and CL_{sec} of pravastatin was somewhat unexpected, because the less frequent nonsynonymous variants in *OCT2* were reported to result in significantly reduced and deleterious transport activities.[35] Both OAT3 and OCT2 are members of the SLC22 superfamily and have a similar localization in the proximal tubule epithelium but differ in their charge specificity; OCT2 transports positively charged compounds, whereas OAT3 transports anionic compounds. As described earlier, the allelic frequency of Ala389Val was extremely low among Japanese subjects. Thus, with these results taken into consideration, the 2 polymorphisms in the *OAT3* gene may not be a major determinant of the large interindividual variability in the pharmacokinetics of pravastatin.

In this study 23 healthy volunteers were not selected for a specific genotype. For a better understanding of the potential effects of genetic variation, a statistically significant number of subjects should be included in each genotype group for each gene. However, because of the low frequency of individuals who are homozygous for the variant allele in the Japanese population and because of various genotypic patterns across the 2 genes of interest, this aim could not be achieved. Obviously, the small number of subjects is a drawback in our study. Considerable variation in pharmacokinetic parameters among various genotype groups makes it difficult to attribute pharmacokinetic changes to one single allele. For instance, although differences in AUC values between **1a/*1a* and **1b/*1b* subjects were in the same range as those observed between **1b/*1b* and **1b/*15* subjects, significant changes in clearance values were only observed for the **1b/*1b-*1b/*15* comparison and not for the **1a/*1a-*1b/*1b* comparison. It is clear that these results should be confirmed in a population study involving larger numbers of subjects. Nevertheless, this report provides for the possibility that *OATP-C* gene polymorphism contributes to in vivo activity.

References

1. Evans WE, Relling M. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999;286:487-91.
2. Evans WE, Johnson JA. Pharmacogenomics: the inherited basis for individual differences in drug response. Annu Rev Genomics Hum Genet 2001;2:9-39.

3. Meyer UA, Zanger UM. Molecular mechanisms of genetic polymorphisms of drug metabolism. Annu Rev Pharmacol Toxicol 1997;37:269-96.
4. Rodrigues AD, Rushmore TH. Cytochrome P450 pharmacogenetics in drug development: in vitro studies and clinical consequences. Curr Drug Metab 2002;3:289-309.
5. Yamazaki M, Suzuki H, Sugiyama Y. Recent advances in carrier-mediated hepatic uptake and biliary excretion of xenobiotics. Pharm Res 1996;13:497-513.
6. Hatanaka T, Honda S, Sasaki S, Katayama K, Koizumi T. Pharmacokinetic and pharmacodynamic evaluation for tissue-selective inhibition of cholesterol synthesis by pravastatin. J Pharmacokinet Biopharm 1998;26:329-47.
7. Triscari J, O'Donnell D, Zinny M, Pan HY. Gastrointestinal absorption of pravastatin in healthy subjects. J Clin Pharmacol 1995;35:142-4.
8. Tamai I, Takanaga H, Maeda H, Ogihara T, Yoneda M, Tsuji A. Proton-cotransport of pravastatin across intestinal brush-border membrane. Pharm Res 1995;12:1727-32.
9. Yamazaki M, Suzuki H, Hanano M, Tokui T, Komai T, Sugiyama Y. Na^+-independent multispecific anion transporter mediates active transport of pravastatin into rat liver. Am J Physiol 1993;264:G36-44.
10. Tokui T, Nakai D, Nakagomi R, Yawo H, Abe T, Sugiyama Y. Pravastatin, an HMG-CoA reductase inhibitor, is transported by rat organic anion transporting polypeptide, oatp2. Pharm Res 1999;16:904-8.
11. Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, et al. A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. J Biol Chem 1999;274:37161-8.
12. Konig J, Cui Y, Nies AT, Keppler D. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. Am J Physiol Gastrointest Liver Physiol 2000;278:G156-64.
13. Cui Y, Konig J, Leier I, Buchholz U, Keppler D. Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. J Biol Chem 2000; 276:9626-30.
14. Abe T, Kakyo M, Tokui T, Nakagomi R, Nishio T, Nakai D, et al. Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. J Biol Chem 1999;274:17159-63.
15. Tirona RG, Leake BF, Merino G, Kim RB. Polymorphisms in OATP-C. Identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. J Biol Chem 2001; 276:35669-75.
16. Nozawa T, Nakajima M, Tamai I, Noda K, Nezu J, Sai Y, et al. Genetic polymorphisms of human organic anion transporter OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis. J Pharmacol Exp Ther 2001;302: 804-13.
17. Michalski C, Cui Y, Nies AT, Nuessler AK, Neuhaus P, Zanger UM, et al. A naturally occurring mutation in the SLC21A6 gene causing impaired membrane localization of the hepatocyte uptake transporter. J Biol Chem 2002; 277:43058-63.
18. Everett DW, Chando TJ, Didonato GC, Singhvi SM, Pan HY, Weinstein SH. Biotransformation of pravastatin sodium in human. Drug Metab Dispos 1991;19:740-8.
19. Arai M, Serizawa N, Terahara A. Pravastatin sodium (CS-415): a novel cholesterol-lowering agent which inhibits HMG-CoA reductase. Annu Rep Sankyo Res Lab 1988;40:1-38.
20. Singhvi SM, Pan HY, Morrison RA, Willard DA. Disposition of pravastatin sodium, a tissue-selective HMG-CoA reductase inhibitor, in healthy subjects. Br J Clin Pharmacol 1990;29:239-43.
21. Kusuhara H, Sekine T, Utsunomiya-Tate N, Tsuda M, Kojima R, Cha SH, et al. Molecular cloning and characterization of a new multispecific organic anion transporter from rat brain. J Biol Chem 1999;274:13675-80.
22. Cha SH, Sekine T, Fukushima JI, Kanai Y, Kobayashi Y, Goya T, et al. Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. Mol Pharmacol 2001;59:1277-86.
23. Hasegawa M, Kusuhara H, Sugiyama D, Ito K, Ueda S, Endou H, et al. Functional involvement of rat organic anion transporter 3 (rOAT3; Slc22a8) in the renal uptake of organic anions. J Pharmacol Exp Ther 2002;300:746-53.
24. Xie HG, Kim RB, Wood AJJ, Stein CM. Molecular basis of ethnic differences in drug disposition and response. Annu Rev Pharmacol Toxicol 2001;41:815-50.
25. Cepeda MS, Farrar JT, Roa JH, Boston R, Meng QC, Ruiz F, et al. Ethnicity influences morphine pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 2001;70:351-61.
26. Schaeffeler E, Eichelbaum M, Brinkmann U, Penger A, Asante-Poku S, Zanger UM, et al. Frequency of C3435T polymorphism of the MDR1 gene in African people. Lancet 2001;358:383-4.
27. Tamai I, Nezu J, Uchino H, Sai Y, Oku A, Shimane M, et al. Molecular identification and characterization of novel member of the human organic anion transporter (OATP) family. Biochem Biophys Res Commun 2000; 273:251-60.
28. Nebert DW. Suggestions for the nomenclature of human alleles: relevance to ecogenetics, pharmacogenetics and molecular epidemiology. Pharmacogenetics 2000;10: 279-90.
29. Ziegler K, Hummelsiep S. Hepatoselective carrier-mediated sodium-independent uptake of pravastatin and pravastatin-lactone. Biochim Biophys Acta 1993;1153:23-33.
30. Yamazaki M, Tokui T, Ishigami M, Sugiyama Y. Tissue-selective uptake of pravastatin in rats: contribution of a

specific carrier-mediated uptake system. Biopharm Drug Dispos 1996;17:775-89.
31. Yamazaki M, Akiyama S, Nishigaki R, Sugiyama Y. Uptake is the rate-limiting step in the overall hepatic elimination of pravastatin at steady-state in rats. Pharm Res 1996;13:1559-64.
32. Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. J Pharmacol Exp Ther 2001;297:1137-43.
33. Suzuki A, Tirona RG, Leake B, Echizen H, Takahashi H, Miyake F, et al. Polymorphisms in the digoxin uptake transporter OATP-8, among Japanese, African-, and European-American subjects [abstract]. Clin Pharmacol Ther 2002;71:P104.
34. Ito S, Ieiri I, Tanabe M, Sizuki A, Higuchi S, Otsubo K. Polymorphism of the ABC transporter genes, MDR1, MRP1 and MRP2/cMOAT, in healthy Japanese subjects. Pharmacogenetics 2001;11:175-84.
35. 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.
36. Tamai I, Nozawa T, Koshida M, Nezu J, Sai Y, Tsuji A. Functional characterization of human organic anion transporting polypeptide B (OATP-B) in comparison with liver-specific OATP-C. Pharm Res 2001;18:1262-9.
37. Tsuji A. Tissue selective drug delivery utilizing carrier-mediated transport systems. J Control Release 1999;62: 239-44.

CORRECTION

In "A comparison of three methods for predicting lithium doses in Chinese psychiatric patients" (Chang Y, Huang H, Chou M, Lin M. Clin Pharmacol Ther 2003;73:P88), the affiliation was printed incorrectly. It should have been Taipei Veterans General Hospital, Yu-Li Psychiatric Hospital, Taipei, Taiwan.

セミナー

トランスポーターの臨床的意義
遺伝子多型から見る薬物療法への寄与

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1 はじめに

薬理遺伝学や薬理ゲノミクスの台頭は薬物の個別適正化使用に大きなインパクトを与えている．その中では，薬物代謝酵素が代表として挙げられるが，吸収や分布，排泄過程に見る個人差の原因を薬物輸送タンパク(トランスポーター)遺伝子多型から解明する研究が精力的に進められている．本稿では，遺伝子多型を利用したトランスポーターの生体中での機能評価と相互作用をはじめとする薬物療法へのさまざまな関与について，現在までの知見を整理するとともに，この領域の研究が抱える問題点について考えてみたい．

2 トランスポーター遺伝子多型と機能評価

トランスポーター遺伝子多型の研究は(遺伝)疾患との関連が先行したため，薬効や体内動態との関連が注目されたのは，ここ2，3年と言える．表1には，多型の機能評価が加えられている主なトランスポーターを挙げた．発現細胞による輸送実験，諸臓器での発現量への影響といった *in vitro* 評価，健常成人による臨床試験，薬効や副作用，さらには疾患との関連(*in vivo*)について評価が加えられている．検討が加えられるトランスポーターは増えつつあるが，P-糖タンパク質(P-gp)をコードするMDR 1が先行し，情報量が最も多い．ここでは，MDR 1とOATP-Cを取り上げる．

1. *MDR1* 遺伝子

Mickley ら[1]が最初に2種類の変異を同定して以来，*MDR1* 遺伝子の広範囲にわたるスクリーニングが行われ，現在までに20種類以上の変異が確認されている.[2~4] 変異の特徴を挙げると，①変異の様式はすべてが1塩基置換(SNPs)で，代謝酵素などに見られる全領域の欠損や挿入などは報告されていない．②日本人100名の解析では，すべての検体において,必ずどこかに最低1か所の変異がみられる．

表1　遺伝子多型に基づいた機能評価が加えられている主なトランスポーター

トランスポーター	*In vitro* 評価	*In vivo* 評価
MDR 1 (multidrug resistance 1)	◎	◎
MRP 1 (multidrug resistance-associated protein 1)	◎	なし
MRP 2/cMOAT (multidrug resistance protein 2)	◎	◎
BCRP (breast cancer resistance protein)	◎	なし
OATP-C (organic anion transporting polypeptide-C)	◎	◎
OAT 3 (organic anion transporter-3)	なし	◎
OCT-1 (organic cation transporter-1)	◎	なし
OCT-2 (organic cation transporter-2)	◎	なし

本表には疾患との関連や動物実験，遺伝子改変による構造活性評価は含んでいない．

表2 *MDR1* 遺伝子多型のヒトでの機能評価

変異部位	基質薬物	対象	薬効，体内動態，副作用への影響	文献 No.
C 3435 T	ジゴキシン	健常成人	T/T>C/C(最高血中濃度)	2
C 3435 T	フェニトイン	健常成人	T/T>C/C(血中濃度)	5
C 3435 T G 2677 T	ジゴキシン	健常成人	M/M>W/M>W/W(吸収率) W/W>W/M>M/M(腎，尿細管分泌クリアランス)	6
C 3435 T	シクロスポリン	腎移植患者	T/T=T/C=C/C(トラフ濃度，拒絶反応の頻度)	7
C 3435 T	フェキソフェナジン	健常成人	T/T=C/C(血中濃度時間曲線下面積)	8
C 3435 T G 2677 T/A	タリノロール	健常成人	W/W=W/M=M/M(血中濃度時間曲線下面積)	9
C 3435 T	ドセタキセル	がん患者	T/T=T/C=C/C(全身クリアランス)	10
*2 haplotype	フェキソフェナジン	健常成人	*1/*1>*1/*2>*2/*2(血中濃度時間曲線下面積)	11
C 3435 T	ジゴキシン	健常成人	C/C>C/T=T/T(血中濃度時間曲線下面積)	12
C 3435 T	ノルトリプチリン	鬱患者	T/T>T/C>C/C(薬剤性低血圧)	13
C 3435 T	ネルフィナビル エファビレンツ	HIV-1 感染患者	T/T>C/T>C/C(CD 4 細胞数と免疫機能改善) C/C>C/T>T/T(トラフ濃度)	14
C 3435 T G 2677 T	ステロイド	小児心臓移植患者	W/W>W/M>M/M(免疫療法におけるステロイドの使用期間)	15

表中の遺伝子型の記載について，例えば，T/T, T/C, W/W, M/M はそれぞれ，チミンのホモ接合型，チミンとシトシンのヘテロ接合型，野生型(C 3435 T であれば，数値の前の塩基でシトシンを意味する．また，C 3435 T と G 2677 T のハプロタイプの場合は，シトシンとグアニンの組み合わせが野生型となる)のホモ接合型，変異(3435 位では，チミン，ハプロタイプの場合は両部位がチミンとなる)のホモ接合型を意味する．

それほど，変異の存在は珍しくない．③現在，多くの研究者が 3435 位のシトシンからチミンへの変異(C 3435 T)に注目した検討を加えているが，この変異はアミノ酸の置換を伴わない．④幾つかの変異がハプロタイプを構成している．すなわち，C 1236 T, G 2677 T/A，C 3435 T の 3 種類の変異を同時に保有する確立が高い．G 2677 T/A は 893 番目のアラニンがそれぞれスレオニン，セリンに変わる数少ないアミノ酸の置換を伴う変異である．

表 2 には，現在までに報告されている変異のヒトでの機能評価の一部をまとめた．10 種類程度の基質薬物による検討が報告され，いずれにおいても，変異のターゲットは C 1236 T，G 2677 T/A，C 3435 T である．体内動態に関するものが中心となっているが，免疫抑制剤と臓器移植後の拒絶反応，三環系抗うつ薬による副作用，HIV 治療薬による免疫機能改善など，薬効に対する影響も検討が加えられている．しかし，特に，体内動態への関与をみると，報告間で異なる知見が得られている．例えば，ジゴキシンを用いた検討では，変異による P-gp 機能(排出 (efflux))の低下を指摘する知見[2,6]がある一方で，機能亢進を指摘する知見[12]が報告されている．同様な現象はフェキソフェナジンでも生じている．ターゲットとしている変異箇所，すなわち，対象ボランティアの遺伝子背景(すべての報告に共通の変異は C 3435 T のみ)，評価に用いる速度論パラメータが報告間で厳密には異なっているものの，コンセンサスが得られていないのが現状と言える．この問題については，今後のトランスポーターのヒトでの機能を評価する上で重要な問題になるので，別項で考えてみたい．

2. *OATP-C* 遺伝子

OATP-C は主に肝臓に発現し，肝への薬物の取り込み(uptake)に働くトランスポーターとして知られている．現在までに 15 種類の変異が確認されている．その中で頻度，アミノ酸の置換から Asn 130 Asp, Val 174 Ala の 2 種類の変異が注目される．HMG-CoA 還元酵素阻害剤であるプラバスタチンは肝に選択的に取り込まれることで薬効を示すが，その取り込みに OATP-C が重要な働きをすると考えられる．130 D/D 174 V/V(Ⅰ群)，130 D/D 174 V/A(Ⅱ群)，130 D/D 174 A/A(Ⅲ群)の遺伝子型で層別した被検者にプラバスタチンを単回投与した際の血中濃度推移を図 1 に示した．Ⅱ群，Ⅲ群，すなわち 130 位と 174 位両部位に変異を有する被検者では，全身クリアランスの低下と血中濃度の上昇が認められている．特に，ホモ型変異保有者(Ⅲ群)の頻度は低いがクリアランスはⅠ群の 50% 程度となっ

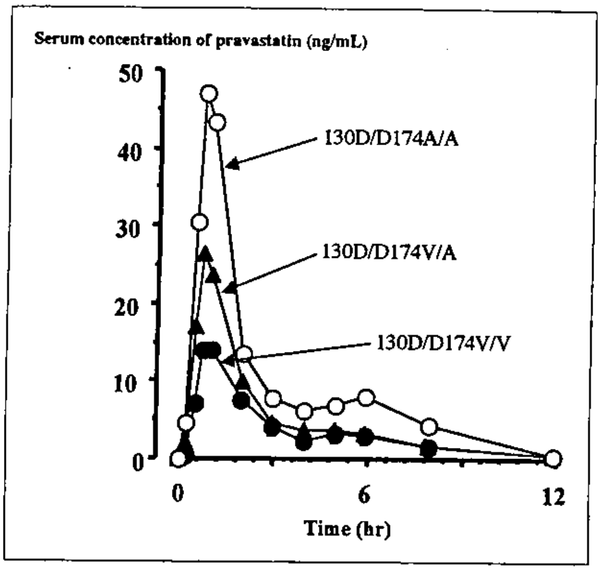

図1　OATP-C遺伝子型とプラバスタチン血中濃度推移

ている．174位の変異の寄与を強く示唆する結果であるが，被検者で見られた体内動態の変化は発現系を用いた *in vitro* 実験結果と良好な一致を見る．[16] *OATP-C* 遺伝子多型の機能評価は少なく，追試が必要であるが，130位の変異の基質特異性なども指摘されている．[17] スタチン系には，横紋筋融解症などの重篤な副作用が知られている．プラバスタチン以外のスタチンは肝代謝されるため，代謝物や筋肉側の要因を考慮する必要があるが，横紋筋融解症などの副作用は高い血中濃度の維持が原因の1つと指摘されていることから，これらの変異との関連が臨床的にも課題となる．

3　トランスポーターと薬物相互作用

トランスポーターを介した薬物相互作用については，詳細な総説[18]があるので参照されたいが，ここでは臨床的に重要と考えられる点のみを取り上げる．

1．投与経路

PGPは広範囲な薬物を基質とすることから，誘導や阻害に基づく薬物相互作用を生じやすいとされる．キニジンやクラリスロマイシン併用によるジゴキシン血中濃度の上昇やリファンピシン併用によるジゴキシンやタリノロールの濃度低下は，それぞれ腎や腸管でのP-gpの阻害と誘導によるものと考えられる．また，シクロスポリンは強力なP-gpのモジュレーターであることから，エトポシドやドキソルビシンなどの基質薬物の輸送を阻害し，重篤な白血球減少や中枢性毒性を招く．ところが，最近の研究により，一部の相互作用が投与経路により左右されることが明らかとなりつつある．先に述べたジゴキシンとリファンピシン併用によるジゴキシン血中濃度の低下はジゴキシンを経口投与した時のみに生じ，静脈内投与時には生じない．また，併用による経口時AUCの低下は誘導された腸管のP-gp発現量と有意な相関が得られている．[19] 同様な現象はタリノロールとリファンピシン，[20] ジゴキシンとクラリスロマイシン[6]との相互作用で見られる．これらの知見は腸管に発現するP-gpが，どの程度作用を受ける薬物の吸収率に影響するかに左右されるものと考えられるが，その定量的予測の確立の必要性とともに臨床における相互作用を考える際には，頭の片隅に置く必要がある．

2．多型の関与

トランスポーターが関与する薬物相互作用を遺伝子多型から検討した研究は少なくMDR1を取り扱った数報に限られる．いずれも，ジゴキシンとレボチロキシン，[21] リファンピシン，[2] クラリスロマイシン[6]との相互作用である．*MDR1* 遺伝子型の違いで相互作用の程度に差が認められる結果が得られており，相互作用の個人差を考える新規のメカニズムとして注目される．しかし，多型の関与は先に述べた体内動態と同様に報告数が少ない上に，報告間で一致した知見が得られていないことから，更なる検討が必要と言える．

3．グレープフルーツジュース(GFJ)と薬物相互作用

GFJはテルフェナジン，サキナビル，トリアゾラム，シクロスポリンなどの多くの薬物の腸管でのチトクロームP4503Aによる代謝を阻害し，吸収率，血中濃度の上昇を招くことから要チェック食品である．最近，代謝に加え，トランスポーターとの興味ある相互作用が報告されている．フェキソフェナジンをGFJとともに服用すると，それまでの概念とは異なり，フェキソフェナジンの吸収率や血中濃度が低下する．[22,23]

P-gpとOATPはともに小腸の管腔側に発現するが，異なった輸送方向を示す．P-gpは細胞内に到

達した薬物を再度腸管へ排出する(吸収の低下に働く)のに対し，OATPsは逆に血液側に輸送する(吸収の増加に働く)．GFJは両輸送タンパクの機能を阻害するが，より低濃度で強力にOATPsの輸送を阻害することが*in vitro*実験で明らかとなった．吸収率や血中濃度の低下はGFJによるOATPsが担う吸収の阻害がその背景と考えられる．本現象に関与するOATPの同定など，多くの課題が残るものの，GFJを介した薬物相互作用には様々なメカニズムがその背景にあることに留意する必要がある．

4 ヒトにおけるトランスポーター研究が抱える問題点

ヒトにおけるトランスポーターの機能評価法の1つとして，遺伝子多型からのアプローチは有効と思われる．しかし，表2に示すように，P-gpについては一定の知見が得られていない．この原因を考察することは，今後の研究を展開する上で重要であろう．

a．基質特異性　先にGFJとフェキソフェナジンの相互作用を述べたが，この報告はフェキソフェナジンの吸収には，少なくとも2種類のトランスポーターの関与を考える必要があることを明確に示している．また，ジゴキシンもP-gpの他にOATP-8が輸送に関与していることが明らかとなっている．それぞれの輸送タンパクの体全体での寄与率などは不明である．同様なことは薬物代謝酵素でも言え，多型を有する代謝酵素のみで代謝される薬物ほど，その影響は著明に現れる．特異的な基質薬物を用いた評価が望まれる．

b．ハプロタイプ　冒頭でも述べたが*MDR1*遺伝子には多数の変異が見られ，そのうち数種類は同時に生じている．1種類の変異のみに注目するのではなく，同時に生じる変異のパターンによる評価が行われている．アロプテロールによる気管支拡張作用の個人差は，このパターンの評価により明らかとされた．[24] 一部の報告では，機能に重要なMDR1多型パターンが指摘されつつある．

c．その他の要因　ヒトDNA上に見られるCpG領域のシトシンは高度にメチル化されており，タンパクの発現に関与する．細胞やがん組織間にみられるP-gp発現量の違いがメチル化の違いによることを指摘する研究が多く報告されている．

5 おわりに

ノックアウトマウスや*in vitro*研究により，トランスポーターが薬物の体内動態や効果に大きく関与することが明らかとなりつつある．しかし，生体中での機能，さらには薬物療法への関与については，P-gpを除き，ほとんど検討が加えられていない．一方，*MDR1*遺伝子多型を通した機能評価からは，基質特異性などの問題点が指摘されるに至っている．加えて，薬物輸送では細胞や臓器の入口と出口で異なったトランスポーターの関与を考える必要がある．これらのことから，数種類のトランスポーターとその遺伝子の寄与を視野に入れた臨床試験等の展開が必要になることが予想されてくる．基礎研究で得られた数多くの知見を整理した効率的なヒトでの研究が望まれる．

参考文献

1) Mickley L. A. *et al., Blood,* 91, 1749-1759(1998).
2) Hoffmeyer S. *et al., Proc. Natl. Acad. Sci. U.S.A.,* 97, 3473-3478(2000).
3) Tanabe M. *et al., J. Pharmacol. Exp. Ther.,* 297, 1137-1143(2001).
4) Cascorbi I. *et al., Clin. Pharmacol. Ther.,* 69, 169-174(2001).
5) Kerb R. *et al., Pharmacogenomics J.,* 1, 204-210(2001).
6) Kurata Y. *et al., Clin. Pharmacol. Ther.,* 72, 209-219(2002).
7) von Ahsen N. *et al., Clin. Chem.,* 47, 1048-1052(2001).
8) Drescher S. *et al., Br. J. Clin. Pharmacol.,* 53, 526-534(2002).
9) Siegmund W. *et al., Clin. Pharmacol. Ther.,* 72, 572-583(2002).
10) Goh B. C. *et al., J. Clin. Oncol.,* 20, 3683-3690(2002).
11) Kim R. B. *et al., Clin. Pharmacol. Ther.,* 70, 189-199(2001).
12) Sakaeda T. *et al., Pharm. Res.,* 18, 1400-1404(2001).
13) Roberts R. L. *et al., Pharmacogenetic J.,* 2, 191-196(2002).
14) Fellay J. *et al., Lancet,* 359, 30-36(2002).
15) Zheng H. *et al., Human Immunol.,* 63, 765-770(2002).
16) Tirona R. G. *et al., J. Biol. Chem.,* 276, 35669-35675(2001).
17) Michalski C. *et al., J. Biol. Chem.,* 277, 43058-43063(2002).
18) Tsuji A., *Drug Metabol. Pharmacokin.,* 17, 253-274(2002).
19) Greiner B. *et al., J. Clin. Invest.,* 104, 147-153(1999).
20) Westphal K. *et al., Clin. Pharmacol. Ther.,* 68, 345-355(2000).
21) Siegmund W. *et al., Clin. Pharmacol. Ther.,* 72, 256-264(2002).
22) Banfield C. *et al., Clin. Pharmacokinet.,* 42, 311-318(2002).
23) Dresser G. K. *et al., Clin. Pharmacol. Ther.,* 71, 11-20(2002).
24) Drysdale C. M. *et al., Proc. Natl. Acad. Sci. U.S.A.,* 97, 10483-10488(2000).

Genetic Polymorphism of Organic Anion and Cation Transporters: Pharmacokinetic and Pharmacodynamic Consequences in Pharmacotherapy

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Abstract: It has been suggested that genetic polymorphisms in drug transporters as well as drug-metabolizing enzymes are associated with interindividual differences in drug disposition, efficacy, and toxicity and in disease. Organic anion and cation transporters are expressed in the selective or multiple tissues such as small intestine, liver and kidney, and mediate the transport of many clinically useful drugs. Polymorphisms of drug transporter genes have recently been identified and demonstrated to have functional significance for transporter activity and expression. For example, genetic variants in the OATP-C (SLC21A6) gene are associated with alterations in pravastatin and rifampin uptake into liver. In addition, homozygotes for OCTN2 (SLC22A5) mutant alleles cause systemic carnitine deficiency because of a disruption of carnitine reabsorption in the kidney. Since a growing number of preclinical and clinical studies have demonstrated that the polymorphisms of various drug transporter genes may be responsible for overall outcomes of pharmacokinetics and pharmacotherapy of certain drugs, further understanding of the physiology and biochemistry of drug transporters with respect to genetic variations will be important to establish individualized pharmacotherapy with clinically used drugs.

Key Words: Anion and cation transporters, Genetic polymorphisms, Clinically useful drugs, Pharmacokinetics, Pharmacodynamics

INTRODUCTION

Genetic polymorphisms in the genes encoding drug-metabolizing enzymes contribute to variations in the kinetic disposition and pharmacological effects of clinically useful drugs [Evans and Relling, 1999]. For example, cytochrome P450 (CYP) 2C9 is the key enzyme for the metabolism of phenytoin and warfarin, and at least three single-nucleotide polymorphisms (SNPs) in this enzyme are known to alter the pharmacokinetics of these drugs [Mamiya *et al.*, 1998; Ninomiya *et al.*, 2000; Scordo *et al.*, 2002; Takahashi *et al.*, 2003]. The variants of CYP2C9 (e.g., *CYP2C9*3*) are associated with large interindividual differences in the dose of warfarin required for appropriate anticoagulant efficiency, and an increased risk of major bleeding complications [Aithal *et al.*, 1998]. Also, the cure rate for *H.pylori* infection by proton pump inhibitors (omeprazol or rabeprazole) is significantly higher in poor metabolizers (PMs) than extensive metabolizers (EMs) of CYP2C19, another polymorphic CYP2C isoenzyme, because of high serum concentrations in PM patients [Furuta *et al.*, 1998; 2001]. In addition, many drugs interact with specific target proteins to exert pharmacological effects, such as receptors and channels. In schizophrenic treatment, genetic polymorphisms in neurotransmitter receptor-related genes determine the clinical response to clozapine [Arranz *et al.*, 2000].

Genetic variations in several drug transporters, which play an important role in drug absorption, distribution and elimination processes, have recently been reported. Among various drug transporters, P-glycoprotein, the MDR1 gene product, is one of the best studied and characterized; P-glycoprotein is an ATP-binding cassette transporter and functions as an energy-dependent efflux pump involved in drug disposition as well as multidrug-resistance. An SNP in exon 26 (C3435T) of MDR1 was found to be associated with reduced absorption and renal clearance of digoxin [Hoffmeyer *et al.*, 2000; Kurata *et al.*, 2002]. In addition to C3435T, G2677T determines success or failure in the antiviral treatment of HIV-1-infected patients [Fellay *et al.*, 2002] and chemotherapy for acute myeloid leukemia [Illmer *et al.*, 2002]. Mutations of multidrug resistance associated protein-2 (MRP2 / cMOAT) are known to cause a hyper-bilirubinemia (Dubin-Johnson syndrome) by reducing bilirubin excretion [Wada *et al.*, 1998; Toh *et al.*, 1999]. These observations clearly indicate that genetic variations in drug transporters as well as drug-metabolizing enzymes, receptors and targeting proteins lead to the interindividual difference in the efficacy and toxicity of drugs.

Organic anion and cation transporters are expressed in human tissues such as liver and kidney along the major routes of drug elimination, and thus play key roles in various pharmacokinetic stages. To date, physiological and functional properties of several organic anion transporters (OATs), organic anion transporting polypeptides (OATPs) and organic cation transporters (OCTs and OCTNs), isolated from human tissues, have been evaluated. More recently, genetic polymorphisms have been identified in genes encoding these transporters. In the present review, we summarize the current available data on the impact of genetic polymorphisms in organic anion and cation transporters on their pharmacokinetics and pharmacodynamics.

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HUMAN OATP / OAT FAMILY

1-1. Tissue Distribution and Functional Characterization

(a). OATP Family

OATP-A (SLC21A3/OATP/OATP1), initially isolated from human liver, is expressed in brain and liver [Kullak-Ublick *et al.*, 1995; Abe *et al.*, 1999; Tamai *et al.*, 2000]. A recent report has described that OATP-A protein is expressed in brain microvessels and capillary endothelial cells, and can regulate the influx of opioid peptides across the blood-brain barrier [Gao *et al.*, 2000]. The transport of thyroid hormones is also mediated by OATP-A [Fujiwara *et al.*, 2001; Kullak-Ublick *et al.*, 2001].

OATP-C (SLC21A6/LST-1/OATP2) and OATP8 (SLC21A8) are selectively expressed in human liver and located on the basolateral membrane of hepatocytes [Abe *et al.*, 1999; Hsiang *et al.*, 1999; König *et al.*, 2000a, 2000b]. While OATP-C and OATP8 control the uptake of a broad range of substrates such as thyroid hormone [Abe *et al.*, 1999; Hsiang *et al.*, 1999; Kullak-Ublick *et al.*, 2001], methotrexate [Abe *et al.*, 2001; Tirona *et al.*, 2001]and rifampin [Tirona *et al.*, 2003], the uptake of unconjugated bilirubin is mediated by OATP-C but not OATP8 [Cui *et al.*, 2001]. Pravastatin [Hsiang *et al.*, 1999; Nakai *et al.*, 2001] and digoxin [Kullak-Ublick *et al.*, 2001] are reportedly transported by OATP-C and OATP8, respectively.

OATP-B (SLC21A9) has a broad tissue distribution when compared with other OATPs [Tamai *et al.*, 2000; Kullak-Ublick *et al.*, 2001]. OATP-B is localized to the basolateral side of human hepatocytes and facilitates substrate uptake from the portal circulation [Kullak-Ublick *et al.*, 2001]. However, the importance of OATP-B in the hepatic disposition of therapeutic drugs is currently unclear, since the substrate specificity of OATP-B is restricted compared with that of OATP-A, OATP-B and OATP-C [Kullak-Ublick *et al.*, 2001].

OATP-D (SLC21A11) and OATP-E (SLC21A12) are abundantly expressed in various peripheral tissues [Tamai *et al.*, 2000; Fujiwara *et al*, 2001]. However, little is known about the physiological and pharmacological functions of OATP-D with the exception of the tissue distribution of its mRNA. OATP-E contributes to the transport of thyroid hormone in peripheral tissues [Fujiwara *et al*, 2001]. OATP-E is also predominantly localized to the apical side of human placenta [Sato *et al.*, 2003]. In a recent study, OATP-F has been identified as high affinity thyroid hormone transporter, which is predominantly expressed in brain and testis [Pizzagalli *et al.*, 2002].

(b). OAT Family

Currently, four human organic anion transporters have been identified. OAT1 (SLC22A6) [Race *et al.*, 1999; Hosoyamada *et al.*, 1999; Sun *et al.*, 2001] and OAT3 (SLC22A7) [Race *et al.*, 1999; Cha *et al.*, 2001; Sun *et al.*, 2001] are mainly expressed in the kidney. OAT1 protein is localized on the basolateral membrane of the S2 segment of proximal tubules in the kidney [Hosoyamada *et al.*, 1999], whereas OAT3 protein is localized on the S1, S2 and S3 segments [Cha *et al.*, 2001]. OAT1, but not OAT3, exhibits the properties of an exchange-type transporter [Hosoyamada *et al.*, 1999; Cha *et al.*, 2001]. Although, in general, the OAT family is mainly expressed in the kidney, OAT2 is abundantly expressed in the liver (basolateral membrane) and, to a lesser extent, in the kidney [Simonson *et al.*, 1994; Sekine *et al.*, 1998]. OAT4 (SLC22A9), on the other hand, is expressed predominantly in the kidney and placenta [Cha *et al.*, 2000]. In the kidney, OAT4 protein is localized to the apical side of proximal tubules [Babu *et al.*, 2002a].

Previous studies report that the OAT family transports various clinically important drugs or endogenous anions. OAT1, OAT2, OAT3 and OAT4 transport organic anions such as probenecid [Hosoyamada *et al.*, 1999; Race at al., 1999; Cha *et al.*, 2000; Enomoto *et al.*, 2002], prostagrandin (PG) E_2 [Enomoto *et al.*, 2002] and $PGF_{2\alpha}$ [Enomoto *et al.*, 2002, Kimura *et al.*, 2002]. On the other hand, p-aminohippurate (PAH) is taken up via OAT1, OAT2, and OAT3 but not OAT4 [Hosoyamada *et al.*, 1999; Race at al., 1999; Cha *et al.*, 2000, 2001; Sun *et al.*, 2001].

In the human kidney, the OAT family seems to play important roles in the process of secreting or transporting various therapeutic drugs through the proximal tubules; OAT1, OAT2 and OAT3 mediate the uptake of organic anions into proximal tubular cells from the blood across the basolateral membrane, and then OAT4 regulates excretion into the proximal tubule fluid across the brush-border membrane. Methotrexate, an antitumor drug, is taken up via OAT1 and OAT3 at the basolateral membrane of proximal tubules and effluxed in the apical membrane of proximal tubules via OAT4 [Takeda *et al.*, 2002b]. Furosemide, a loop diuretic, is transported by OAT1 [Hosoyamada *et al.*, 1999; Race at al., 1999], OAT3 [Cha *et al.*, 2001] and OAT4 [Cha *et al.*, 2000]. Thus, furosemide that is taken up by OAT1 and OAT3 localized at the basolateral membrane in the proximal tubules exhibits a diuretic effect following action on the luminal side of the loop segment, and then excreted via OAT4 in the apical membrane. Other clinically important drugs including nonsteroidal anti-inflammatory drugs (NSAIDs) [Khamdang *et al.*, 2002], angiotensin II receptor antagonist [Race *et al.*, 1999], antiviral nucleotide analogs [Cihlar *et al.*, 1999; Ho *et al.*, 2000; Takeda *et al.*, 2002c], tetracycline [Babu *et al.*, 2002b] and cephalosporin antibiotics [Takeda *et al.*, 2002a] are also substrates for the OAT family (Table.1). Since NSAIDs such as ibuprofen and ketoprofen efficiently inhibit the transport of adefovir by OAT1 in the kidney at clinically relevant concentrations, but are not transported by OAT1 [Mulato *et al.*, 2000], it has been suggested that NSAIDs may reduce adefovir-induced nephrotoxicity [Apiwattanakul *et al.*, 1999; Mulato *et al.*, 2000].

1.2. Impact of OAPT-C Variants on Pharmacokinetics and Pharmacodynamics

1.2.1. Frequency and In Vitro Transport Activity of OATP-C Variants

Recently, a number of SNPs in the human OATP-C gene have been identified in different ethnic populations [Tirona *et al.*, 2001; Nozawa *et al.*, 2002; Nishizato *et al.*, 2003]. At least 17 non-synonymous variants and 16 allelic patterns

Table 1. Summary of organic anion and cation transporters.

Transporter	Gene symbol	Chr.	Tissue distribution		Therapeutic /physiological substrates [b]	Reference
			Expression	Polarity [a]		
OATP-A	SLC21A3	12	Brain, Liver	BL	fexofenadine, thyroid hormones	Kullak-Ublick *et al.*, 1995; 2001, Abe *et al.*, 1999; Cvetkovic *et al.*, 1999; Tamai *et al.*, 2000; Fujiwara *et al.*, 2001; Dresser *et al.* 2002
OATP-B	SLC21A9	11	Small intestine, Liver, Placenta	BL	pravastatin	Tamai *et al.*, 2000, 2001; Kullak-Ublick *et al.*, 2001
OATP-C	SLC21A6	12	Liver	BL	pravastatin, MTX, rifampin, thyroid hormones	Abe *et al.*, 1999; Hsiang *et al.*, 1999; König *et al.*, 2000a; Kullak-Ublick *et al.*, 2001; Nakai *et al.*, 2001; Tirona *et al.*, 2001, 2003
OATP-D	SLC21A11	15	Ubiqutous	?	?	Tamai *et al.*, 2000
OATP-E	SLC21A12	20	Ubiqutous	AP	thyroid hormones	Tamai *et al.*, 2000; Fujiwara *et al.*, 2001; Sato *et al.*, 2003
OATP-F	SLC21A14	12	Brain, Testis	?	thyroid hormones	Pizzagalli *et al.*, 2002
OATP8	SLC21A8	12	Liver	BL	digoxin, MTX, rifampin, thyroid hormones	König *et al.*, 2000b; Abe *et al.*, 2001: Kullak-Ublick *et al.*, 2001
OAT1	SLC22A6	11	Kidney	BL	MTX, probenecide, furosemide, benzylpenicillin, salicylate, indomethacin, losartan, tetracycline, oxytetracycline, minocycline, cephalosporin ab., acyclovir, ganciclovir, zidovudine, adefovir, cidofovir	Cihlar *et al.*, 1999; Race *et al.*, 1999; Hosoyamada *et al.*, 1999; Ho *et al.*, 2000; Sun *et al.*, 2001; Babu *et al.*, 2002b; Khamdang *et al.*, 2002; Takeda *et al.*, 2002a, 2002c
OAT2	SLC22A7	6	Kidney, Liver	BL	MTX, probenecid, $PGF_{2\alpha}$, tetracycline, oxytetracycline, minocycline, zidovudine	Simonson *et al.*, 1994; Sekine *et al.*, 1998; Babu *et al.*, 2002b; Enomoto *et al.*, 2002; Takeda *et al.*, 2002c
OAT3	SLC22A8	11	Kidney	BL	MTX, probenecide, furosemide, indomethacin, ibuprofen, diclofenac, cimetidine, quinidine, tetracycline, cephalosporin ab., valacyclovir, zidovudine	Race *et al.*, 1999; Cha *et al.*, 2001; Sun *et al.*, 2001; Babu *et al.*, 2002b; Khamdang *et al.*, 2002; Takeda *et al.*, 2002a, 2002c
OAT4	SLC22A9	11	Kidney, Placenta	AP	MTX, probenecid, furosemide, indomethacin, ibuprofen, diclofenac, piroxicam, tetracycline, cephalosporin ab., zidovudine	Cha *et al.*, 2000; Babu *et al.*, 2002a, 2002b; Enomoto *et al.*, 2002; Khamdang *et al.*, 2002; Takeda *et al.*, 2002a, 2002c
OCT1	SLC22A1	6	Liver	BL	acyclovir, ganciclovir	Gorboulev *et al.*, 1997; Zhang *et al.*, 1997; Motohashi *et al.*, 2002; Takeda *et al.*, 2002c
OCT2	SLC22A2	6	Kidney, Brain	BL	amantadine	Gorboulev *et al.*, 1997; Busch *et al.*, 1998; Motohashi *et al.*, 2002
OCT3	SLC22A3	6	Kidney, Liver, Placenta, Skeletal muscle	?	?	Wu *et al.*, 2000a
OCTN1	SLC22A4	5	Kidney, Bone marrow	AP	carnitine, verapamil, pyrilamine, quinidine	Tamai *et al.*, 1997; Yabuuchi *et al.*, 1999
OCTN2	SLC22A5	5	Kidney, Brain, Heart, Skeletal muscle, Placenta	AP	carnitine, verapamil, pyrilamine, quinidine, cephalosporin ab.	Wu *et al.*, 1998, 1999; Tamai *et al.*, 1998; Ohashi *et al.*, 1999, 2001; Ganapathy *et al.*, 2000

a) Basolateral; BL, Apical; AP.
b) MTX; Methotrexate, ab.; antibiotics, PG;Prostagrandin.

(haplotypes) have been found to date (Table 2 and 3). Among the 17 non-synonymous variants, N130D appeared in all ethnic populations. As shown in Table 2, G488A and E667G are more common mutations in African-Americans than other populations. In contrast, P155T and V174A are found at higher frequency in European-Americans. These results indicate that genotypic frequencies of OATP-C variants appeared to be dependent on race. However, the interethnic difference in haplotypes of the OATP-C allele has not been well documented.

In vitro experiments with cultured cells expressing the wild type and mutations revealed that the allelic variants of OATP-C*2, *3, *5, *6, *9, *12 and *13 significantly reduced estrone-3-sulfate and estradiol 17 β-D-glucuronide transport activity [Tirona *et al.*, 2001]. The F73L (*2 or *12), V82A (*3 or *13), V174A (*5), I353T (*6), G488A (*9) and L193R (unidentified allele) mutations decreased cellular surface membrane expression of OATP-C protein but did not change the expression of total cellular protein [Tirona *et al.*, 2001; Michalski *et al.*, 2002]. Interestingly, almost all these mutations localize to the putative transmembrane domains, suggesting an important role for these regions in the trafficking or sorting of mature transporter protein at the cellular membrane.

Rifampin, an antibiotic drug, is broadly used in the treatment of tuberculosis, and is a substrate of OATP-C [Vavricka *et al.*, 2002; Tirona *et al.*, 2003]. Using the recombinant vaccinia virus expression method, Tirona *et al.* have reported that rifampin transport is robustly reduced by the *1b, *2, *3, *5, *6, *7, *9, *12 and *13 allelic variants [Tirona *et al.*, 2003]. Interestingly, they also reported that OATP-C*1b (N130D) and *7 (N432D) did not affect the capacity for transporting estrone-3-sulfate and estradiol 17 β-D-glucuronide [Tirona *et al.*, 2001]. In contrast, uptake of cholyltaurine but not estradiol 17 β-D-glucuronide is found to be decreased by the OATP-C*1b allele [Michalski *et al.*, 2002]. Taken together, these results suggest that at least two variants, N130D and N432D located at putative N-

Table 2. Summary of Current Genetic Variants in OATP-C.

Allele	Localization		Effect	Allele Frequency %						
	Genetic	Cellular [a]		E-A [b] (N=42) Tirona *et al.*, 2001	A-A [b] (N=22) Tirona *et al.*, 2001	A-A [c] (N=104-150)	CA [b] (N=81) Michalski *et al.*, 2002	CA [c] (N=150)	Jpn [b] (N=267) Nozawa *et al.*, 2002	Jpn [b] (N=120) Nishizato *et al.*, 2003
T217C	Exon 2	TM 2	F73L	2	0	n.d.	n.d.	n.d.	n.d.	0
T245C	Exon 3	TM 2	V82A	2	0	n.d.	n.d.	n.d.	n.d.	0
A388G	Exon 4	EL 2	N130D	30	74	77	n.d.	46	53.7	63
A452G	Exon 4	EL2	N151S	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4
G455A	Exon 4	EL 2	R152K	0	0	n.d.	n.d.	n.d.	0	0
C463A	Exon 4	EL 2	P155T	16	2	n.d.	n.d.	n.d.	n.d.	0
A467G	Exon 4	EL 2	E156G	2	0	n.d.	n.d.	n.d.	n.d.	0
T521C	Exon 5	TM 4	V174A	14	2	1	n.d.	12	0.7	16
T578G	Exon 5	TM 4	L193R	n.d.	n.d.	n.d.	< 0.3	n.d.	n.d.	0
G721A	Exon 6	EL 3	D241N	0	0	n.d.	n.d.	n.d.	0	0
C1007G	Exon 8	TM 7	P336R	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1
T1058C	Exon 8	TM 7	I353T	2	0	n.d.	n.d.	n.d.	n.d.	0
A1294G	Exon 9	EL 5	N432D	1	0	n.d.	n.d.	n.d.	n.d.	0
A1385G	Exon 10	EL 5	D462G	1	0	n.d.	n.d.	n.d.	n.d.	0
G1454T	Exon 10	EL 5	C485F	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1
G1463C	Exon 10	EL 5	G488A	0	9	n.d.	n.d.	n.d.	n.d.	0
A1964G	Exon 14	C-terminus	D655G	2	0	n.d.	n.d.	n.d.	n.d.	0
A2000G	Exon 14	C-terminus	E667G	2	34	n.d.	n.d.	n.d.	n.d.	0

a) TM: Transmembrane, EL: Extracellular loop, n.d.; not determined
b) E-A; European-American, A-A; African-American, CA; Caucasian, Jpn; Japanese
c) our recent unpublished data.

Table 3. The Influence of OATP-C Variants on Expression or Transport Activity.

Haplotype	Variant	Functional consequence		Reference
		Transport activity [a]	Protein expression	
*1b	N130D	rifampin↓	–	Tirona *et al.*, 2003
*1c	R152K	–	–	
*2	F73L	estrone-sulfate↓, estradiol-Glu↓, rifampin↓	total cellular and membrane expression↓	Tirona *et al.*, 2001, 2003
*3	V82A, E156G	estrone-sulfate↓, estradiol-Glu↓, rifampin↓	membrane↓ total cellular expression→	Tirona *et al.*, 2001, 2003
*4	P155T	–	–	
*5	V174A	1) estrone-sulfate↓, estradiol-Glu↓, rifampin↓ 2) estrone sulfate→	1) membrane expression_, total cellular expression→ 2) protein expression→	1) Tirona *et al.*, 2001, 2003 2) Nozawa *et al.*, 2002
*6	I353T	estrone-sulfate↓, estradiol-Glu↓, rifampin↓	membrane expression↓, total cellular expression→	Tirona *et al.*, 2001, 2003
*7	N432D	rifampin↓	–	Tirona *et al.*, 2003
*8	D462G	–	–	
*9	G488A	estrone-sulfate↓, estradiol-Glu↓, rifampin↓	membrane expression↓, total cellular expression→	Tirona *et al.*, 2001, 2003
*10	D655G	estrone-sulfate↓	–	Tirona *et al.*, 2001
*11	E667G	–	–	
*12	F73L, D655G	estrone-sulfate↓, rifampin↓	–	Tirona *et al.*, 2001, 2003
*13	V82A, E156G, E667G	estrone-sulfate↓, rifampin↓	–	Tirona *et al.*, 2001, 2003
*14	N130D, P155T	–	–	
*15	N130D, V174A	n.d.	n.d.	
unidentified	N130D, P155T, L193R	estradiol-Glu↓↓	retained intracellularly	Michalski *et al.*, 2002

a) Estrone sulfate; Estrone-3-sulfate, Estradiol-Glu; Estradiol 17β-D-Glucuronide
—; unchanged when compared to OATP-C*1a, n.d.; not determined

glycosylation sites in the predicted extracellular loops (2 and 5, respectively) [Abe *et al.*, 1999], exhibit an altered transport capability dependent on substrate.

1.2.2. Influence of OATP-C Polymorphisms on Drug Disposition

Human OATP-C is predominantly expressed on the basolateral membrane in hepatocytes [Abe *et al.*, 1999; Hsiang *et al.*, 1999; König *et al.*, 2000a]. It has been reported that the hepatocellular uptake of bilirubin and its glucronide conjugates is mediated via OATP-C [Cui *et al.*, 2001]. Thus, it seems likely that OATP-C plays an important role in the hepatocellular elimination, metabolism and conjugation of clinical drugs as well as various endogenous compounds.

Pravastatin, one of the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), is widely used in the treatment of hypercholesterolaemia. Recently, we have investigated the contribution of the polymorphism of the OATP-C gene to the pharmacokinetics of pravastatin, a substrate for OATP-C [Nishizato *et al.*, 2003]. Among 23 healthy Japanese volunteers, four non-synonymous variants (N130D, N151S, V174A and P336A) and more than five haplotypes (OATP-C*1a, *1b, *15, *16, and unidentified) were observed in the OATP-C gene. Subjects with the *15 allele (D130A174) had a reduced total and non-renal clearance compared to those with the *1b allele; non-renal clearance in *1b/*1b (n = 4), *1b/*15 (n = 9), and *15/*15 (n = 1) subjects was 2.01±0.42, 1.11±0.34 and 0.29 (L/hr/kg), respectively. These results suggest that the OATP-C*15 allele is likely to be associated with altered pharmacokinetics of pravastatin. As the hepatic clearance of pravastatin is rate limited by uptake [Hsiang *et al.*, 1999], low transport activity of OATP-C may lead to a reduction of hepatocellular uptake of pravastatin, resulting in lower total clearance. The target organ of pravastatin is the liver. In addition, although pravastatin is generally well tolerated in patients with hypercholesterolaemia, it is not free of serious adverse effects, such as skeletal muscle abnormalities (e.g., benign myalgias and rhabdomyolysis). The mechanism of statin-induced rhabdomyolysis is not clearly known, though high serum concentrations of pravastatin have been linked to it. Since certain variants of OATP-C may reduce hepatoselective uptake of pravastatin and be associated with high serum concentrations, patients who carry the mutated alleles would theoretically be expected to have both a loss of pharmacological effects and an increased risk of adverse events. These theoretical but important hypotheses should be tested further.

1.2.3. Influence of OATP-C Polymorphisms on Drug Interaction

Drug-drug interactions are clinically important because of alterations in the metabolism and elimination of certain drugs. Rifampin is well known as an inducer of drug metabolizing enzymes and drug transporters such as CYP2A6, 2B6, 2C8, 2C9, 3A4, 3A5, 3A7, UDP-glucuronosyltransferase-1A (UGT1A) [Rae *et al.*, 2001], P-glycoprotein [Greiner *et al.*, 1999; Westphal *et al.*, 2000], and MRP2/cMOAT [Formm *et al.*, 2000]. As described above, Tirona *et al.* demonstrated that the human liver-specific transporter OATP-C mediates the hepatocellular uptake of rifampin and that several naturally occurring OATP-C variants were found to have markedly reduced rifampin transport activity. Furthermore, they indicated that expression of OATP-C enhances rifampin-mediated pregnane X receptor (PXR) activation as a result of increased intracellular substrate retention. The above-mentioned induction of drug-metabolizing enzymes and drug transporters by rifampin is known to depend upon the activation of the adapted nuclear receptor PXR [Lehmann *et al.*, 1998; Geick *et al.*, 2001]. Although there is no clinical evidence to support this hypothesis, certain genetic variants of OATP-C would be expected to contribute to rifampin-mediated drug interactions.

2. HUMAN OCT / OCTN FAMILY

2.1. Tissue Distribution and Functional Characterization

(a). OCT Family

The mRNA transcript of human OCT1 is predominantly expressed in the liver, and weakly expressed in kidney, whereas OCT1 protein is detected only in the liver, not in the kidney [Gorboulev *et al.*, 1997; Zhang *et al.*, 1997, Motohashi *et al.*, 2002]. Rat Oct1 is localized at the sinusoidal membrane of hepatocytes in the liver [Meyer-Wentrup *et al.*, 1998]. In Oct1 knockout mice, the accumulation of substrate drugs (metformin, TEA, metaiodobenzylguanidine and MPP+) in liver is diminished compared to that in wild-type mice [Jonker *et al.*, 2001; Wang *et al.*, 2002]. Therefore, it seems that OCT1 plays a predominant role in the transport of substrates across the sinusoidal membrane of human liver. On the other hand, human OCT2 mRNA and protein are expressed in the kidney [Gorboulev *et al.*, 1997; Motohashi *et al.*, 2002], and localized mainly at the basolateral membrane in the distal tubules [Gorboulev *et al.*, 1997]. However, a recent report suggests that OCT2 protein is localized to the basolateral membrane in the proximal tubules like OAT1, OAT2 and OAT3 but not in the glomeruli, distal tubules or collection ducts [Motohashi *et al.*, 2002]. Therefore, OCT2 likely plays a more important role in the renal disposition of organic cations in human kidney than other members of the OCT family. The mRNA of OCT3 is broadly expressed in human tissues including the liver, kidney and placenta [Wu *et al.*, 2000a]. In mouse kidney, Oct3 mRNA is expressed in proximal and distal convoluted tubules, but not in the glomerulus [Wu *et al.*, 2000a]. However, little is known about the intracellular localization of OCT3 as well as OCT1 in human tissues.

Aciclovir and ganciclovir, antiviral drugs, are predominantly eliminated from kidney, with approximately 63 % and 91 % of the administered dose recovered unchanged in the urine, respectively [Morse *et al.*, 1993]. In a current study using cells stably expressing human organic anion and cation transporters, these drugs were transported by OAT1 and OCT1 [Takeda *et al.*, 2002c]. However, these drugs might be mainly excreted through a renal mechanism via OAT1 transport, since OAT1 protein but not OCT1 protein is expressed in the kidney [Motohashi *et al.*, 2002]. Although NSAIDs are not transported by OCT1 and OCT2, the transporting of an organic cationic substrate via OCT1 and OCT2 is inhibited by some NSAIDs [Khamdang *et al.*, 2002].

(b). OCTN Family

OCTN1 and OCTN2 transport organic cations such as TEA in a pH-dependent or an Na^+-independent manner [Yabuuchi *et al.*, 1999; Wu *et al.*, 1999]. On the other hand, OCTN2 mediates carnitine transport in a Na^+-dependent manner [Tamai *et al.*, 1998]. OCTN1 and OCTN2 have similar substrate specificity. In human tissues, OCTN1 mRNA is expressed predominantly in kidney, trachea, bone marrow and fetal liver [Tamai *et al.*, 1997]. The expression of OCTN2 mRNA in human tissues is detected strongly in kidney, placenta, heart and skeletal muscle, and weakly in brain, lung and liver [Wu *et al.*, 1998, Tamai *et al.*, 1998]. In the kidney, rat Octn1 and Octn2 mRNA are expressed in the absorptive cells in both proximal and distal tubules [Wu *et al.*, 1999, 2000b]. Although the functional characterization of OCTNs in human body has not been well documented, human OCTNs may influence the disposition of various drugs in the kidney. Subcellular localization and *in vitro* functional characterization suggest that OCTN1 contributes to the active secretion of organic cations across the renal brush-border membrane [Tamai *et al.*, 1997], and OCTN2 exhibits properties as an exchange transporter in Na^+-dependent carnitine reabsorption in the kidney and Na^+-independent secretion of organic cations [Ohashi *et al.*, 2001]. Some cephalosporin (β-lactam) antibiotics such as cephaloridine, cefoselis, cefepime and cefluprenam are substrates for OCTN2 [Ganapathy *et al.*, 2000].

2.2. Impact of OCT1 Variants on Pharmacokinetics and Pharmacodynamics

2.2.1. Frequency and In Vitro Transport Activity of OCT1 Variants

The human OCT1 contains 554 amino acids and is predicted to have 12 putative transmembrane domains (TMs) with the extracellular localization of the large hydrophilic oop between TM1 and 2 [Zhang *et al.*, 1997]. A current eport has identified several synonymous and non-ynonymous variants in the human OCT1 gene [Kerb *et al.*, :002]. By systematic screening of the OCT1 gene in a ıealthy Caucasian population, 10 mutations were detected in he coding region, of which 8 variants caused non-synony-ıous amino acid changes (Table 4). Among those, R61C, '160L, and M429del are often observed in the Caucasian ·opulation, with the respective allele frequencies of 9.1, 22, nd 16%, respectively. In a functional characterization using (enopus oocytes with OCT1 point mutations, three variants R61C, C88R, and G401S) reduced the transport activity ompared with the wild-type. Interestingly, C88R and ;401S are found to exhibit altered substrate selectivity. The :61C and C88R variants localize to the large extracellular ɔop, and G401S variant occurs in the intracellular loop etween TM 8 and 9 that is highly conserved in major :ansport proteins [Zhang *et al.*, 1997].

.2.2. Influence of OCT1 Polymorphisms on Drug)isposition

In full Oct1-deficient mice, the accumulation in liver and xcretion into small intestine of the model agent TEA are ɔund to be reduced compared with the wild-type mice, ecause Oct1 knockout mice exhibit a reduced hepatic uptake nd subsequently direct intestinal excretion of the cationic ubstrate [Jonker *et al.*, 2001]. Interestingly, the hepatic ptake of metformin after intravenous injection is dramatically ɛduced in Oct1 gene-knockout mice [Wang *et al.*, 2002]. ⁄letformin belongs to the family of biguanide anti-diabetic rugs, and leads to a reduction of gluconeogenesis through n inhibition of complex I of the mitochondrial respiratory chain in hepatocytes [Owen *et al.*, 2000; Hundal *et al.*, 2000]. In humans, metformin is extensively eliminated from kidney via glomerular filtration and tubular secretion and recovered in urine at approximately 80 % of the administered dosage [Sirtori *et al.*, 1978; Tucker *et al.*, 1981]. However, since the liver, which expresses human OCT1 protein, is one of the main therapeutic targets of metformin, patients with polymorphisms involved with functional change in the OCT1 gene might exhibit a decrease or lack of pharmacological efficacy for the drug. At the moment, little is known about the specific substrates for OCT1 among therapeutic drugs except antiviral agents [Takeda *et al.*, 2002c].

2.3. Impact of OCT2 Variants on Pharmacokinetics and Pharmacodynamics

2.3.1. Frequency and In Vitro Transport Activity of OCT2 Variants

Members of the organic cation transporter family share a predicted 12 TM structure with a large extracellular loop between TM1 and 2. Genetic variations of human OCT2 have been recently identified in ethnically diverse populations [Leabman *et al.*, 2002]. Eight variable sites including non-synonymous amino acid changes occur in the coding region that distributes throughout the loops and transmembrane domains (Table 5). *In vitro* studies using oocytes expressing various variants showed that M165I and R400C have significantly reduced uptake activity for the prototypical organic cation MPP+ compared with the OCT2 wild-type, although these variants are present only in the African-American population. A single-nucleotide insertion at position 134 leads to a prematurely terminated protein that might abolish the transporter function. Furthermore, K432Q,

Table 4. Summary of OCT1 Gene Polymorphisms in Caucasians [Kerb *et al.*, 2002].

Allele	Localization		Effect	Transport activity [b)]	Genotype Frequency % (N=217-243)		
	Genetic	Cellular [a)]			Wt/Wt	Wt/Mut	Mut/Mut
G-1795A	Promoter			n.d.	74.5	23.7	1.8
G-1685A	Promoter			n.d.	98.1	1.9	0
G-1672C	Promoter			n.d.	98.1	1.9	0
C181T	Exon 1	EL 1	R61C	MPP+↓	83.2	15.6	1.2
T262C	Exon 1	EL 1	C88R	MPP+↓, TEA↓, serotonin ↓	98.8	1.2	0
C8237G	Exon 2	TM 2	F160L	–	61.4	34	4.6
G17857A	Exon 7	IL 4	G401S	MPP+↓, TEA↓, serotonin ↓	93.5	6.5	0
A17878G	Exon 7	TM 9	M408V	n.d.	17.7	45.2	35.1
G17897C	Exon 7	TM 9	G414A	n.d.	99.6	0.4	0
17914 ATG del	Exon 7	TM 9	M420del	–	71.1	26.3	2.6
G32870A	Exon 9	IL 5	G465R	n.d.	97	3	0

) TM: Transmembrane, EL: Extracellular loop, IL; Intracellular loop
) MPP+; 1-methyl-4-phenylpyridinium, TEA; Tetraethylammonium.
–; unchange when compared to wild type of OCT1, n.d.; not determined

present in both the African-American and Mexican-American population, has an increased affinity for MPP+. These variants may be involved in the altered transport function of organic cations, but occur at low frequency. In contrast, the high-frequency variant, A270S (haplotypes *3D and *3E), is less sensitive to hydrophobic inhibitors [Leabman *et al.*, 2002].

2.3.2. Influence of OCT2 Polymorphisms on Drug Disposition

At the moment, it is not clear whether the polymorphisms in the OCT2 gene are associated with interindividual differences of drug disposition. In addition to the kidney, OCT2 is expressed in neurons of widespread brain regions including the hippocampus and various subcortical nuclei [Gorboulev *et al.*, 1997; Busch *et al.*, 1998]. Monoamine neurotransmitters such as dopamine, serotonin, norepinephrine, histamine and choline are transported by OCT2 [Busch *et al.*, 1998, Sweet *et al.*, 2001]. Interestingly, amantadine and memantine, anti-Parkinsonian drugs, are substrates and competitive inhibitors of OCT2 [Busch *et al.*, 1998]. It has been suggested that amantadine exhibits a pharmacological effect through increased extracellular concentrations of neurotransmitters by inhibition of dopamine uptake via OCT2.

2.4. Impact of OCTN2 Variants on Pharmacokinetics and/or Pharmacodynamics

2.4.1. Polymorphisms of OCTN2 and Systemic Carnitine Deficiency (SCD)

Primary systemic carnitine deficiency (SCD; MIM 212140) is a rare autosomal recessive disorder caused by defective carnitine transport and characterized by hypoketotic hypoglycemia, cardiomyopathy and myopathy. Carnitine is essential factor for the transfer of long-chain fatty acids from cytosol to mitochondrial matrix where β-oxidation takes place for the production of ATP.

Recently, it has been clarified that SCD is caused by mutations in human OCTN2 which undergoes sodium-dependent carnitine transport in heart, skeletal and renal tissues for β-oxidation. All SCD patients are hetero- or homozygous for various OCTN2 mutant alleles creating a disruption of carnitine transport. The mutations in the OCTN2 gene involved with the functional deficiency of carnitine transport are shown in Table 6. Notably, the mutations causing a missense or a frameshift (a partial nucleotide deletion or insertion) creating a truncated protein have been reported in numerous papers. OCTN2 encodes a polypeptide of 557 amino acids with twelve putative transmembrane domains [Tamai *et al.*, 1998; Wu *et al.*, 1998]. The single missense mutation, C844T in exon 5, converts the arginine at amino acid position 282 into a stop codon (R282X), leading to the production of a truncated protein (by 275 amino acids) compared with the wild-type [Vaz *et al.*,1999; Wang *et al.*, 1999]. Wang *et al.* [1999] have reported an SCD patient who is a compound heterozygote for a paternal 1-bp insertion in exon 7 and a maternal 1-bp deletion in exon 8. The paternal mutation changes the codon for the tyrosine at amino acid position 401 to a STOP codon (Y401X). Also, the maternal mutation causes a frameshift starting at the codon for the glycine at position 435 and then creates a stop codon at position 458 (458X), resulting in the production of premature truncated protein. On the other hand, the 113-bp deletion including the initial ATG codon in exon 1 shifts the next available ATG codon at amino acid position 177 (Nezu *et al.*, 1999).

Table 5. Summary of OCT2 Gene Polymorphisms [Leabman *et al.*, 2002].

Allele	Localization		Effect	Transport activity [b]	Allele frequency %				
	Genetic	Cellular [a]			CA [c] (N=200)	A-A [c] (N=200)	A-S [c] (N=60)	ME [c] (N=20)	PA [c] (N=14)
134 Ins A	Exon 1	TM 1	45	prematured protein (47 AA)	0.5	0	0	0	0
C160T	Exon 1	EL 1	P54S	n.d.	0	0.5	0	0	0
T481C	Exon 2	TM 2	F161L	n.d.	0.5	0	0	0	0
A493G	Exon 2	TM 2	M165V	n.d.	0	0.5	0	0	0
G495A	Exon 2	TM 2	M165I	MPP+ ↓	0	1	0	0	0
G808T	Exon 4	TM 6	A270S	sensitive to TBA inhibition↓	15.7	11	8.6	15	7
C890G	Exon 5	IL 4	A297G	n.d.	0.5	0	0	0	0
C1198T	Exon 7	IL 5	R400C	MPP+ ↓ sensitive to TBA inhibition↑	0	1.5	0	0	0
A1294C	Exon 8	EL 5	K432Q	affinity for MPP+ ↑ sensitive to TBA inhibition↑	0	1	0	5	0

a) TM; Transmembrane, EL: Extracellular loop, IL; Intracellular loop,
b) MPP+; 1-methyl-4-phenylpyridinium, TBA; Tetrabutylammonium.
c) CA; Caucasian, A-A; African-American, A-S; Asian-American, ME; Mexican-American, PA; Pacific Islander
Ins; Insertion, AA; Amino acid, n.d.; not determined

Table 6. Summary of OCTN2 Gene Polymorphisms.

Position [a)]	Localization			Effect	Transport function		Reference
	Genetic	Cellular [b)]			Carnitine transport	TEA transport	
113-bp Del	Exon 1			shift ATG at codon 177	n.d.	n.d.	Nezu *et al.*, 1999
226 Ins C	Exon 1			truncating	n.d.	n.d.	Nezu *et al.*, 1999
255-22851 Del (255-1649 Del)	Exon 1-8	TM 11		truncating	n.d.	n.d.	Lamhonwah and Tein, 1998
15591 Ins 19-bp (874 Ins 19 -bp)	Exon 4	TM 4		truncating	n.d.	n.d.	Lamhonwah and Tein, 1998
15592-17283 Del (875-1046 Del)	Exon 4-5	TM 4-6		truncating	n.d.	n.d.	Lamhonwah and Tein, 1998
G 8639 A	Exon 2	EL1	W132X	truncating	no activity	n.d.	Koizumi *et al.*, 1999; Tang *et al.*, 1999; Nezu *et al.*, 1999
C 14417 T	Exon 2	IL 2/3 [#]	R169W		no activity	n.d.	Wang *et al.*, 2000c
A 14447 T	Exon 3	TM 3	M179L		activity↓	n.d.	Koizumi *et al.*, 1999
A 14544 G	Exon 3	TM 4	Y211C		no activity	–	Seth *et al.*, 1999; Vaz *et al.*, 1999
G 15663 T	Exon 4	TM 5	G242V		no activity	n.d.	Wang *et al.*, 2000c
C 17302 T	Exon 5	IL 6/7	R282X	mRNA level↓	no activity	n.d.	Vaz *et al.*,1999; Wang *et al.*, 1999
G 17307 T	Exon 5	IL 6/7	W283C		no activity	n.d.	Koizumi *et al.*, 1999
	Exon 5	IL 6/7	W283R		no activity	n.d.	Mayatepek *et al.*, 2000
C 17360 A	Exon 5	IL 6/7	A301D		no activity	n.d.	Wang *et al.*, 2000c
T 19278 C	Exon 6	TM 7	W351R		no activity	n.d.	Wang *et al.*, 2000c
	Exon 7	TM 7	M352R	protein level→	no activity	no activity	Seth *et al.*, 1999; Wu *et al.*, 1999
21098 Ins A	Exon 7	TM 9	Y401X	mRNA level↓	no activity	n.d.	Wang *et al.*,1999
G 22759 T	Exon 8	IL 10/11	V446F		no activity	n.d.	Mayatepek *et al.*, 2000
G 22777 A	Exon 8	IL 10/11	E452K		no activity	n.d.	Wang *et al.*, 2000a, 2000b
22727 Del G	Exon 8	IL 10/11	458 X	mRNA level↓	no activity	n.d.	Wang *et al.*,1999
C 22823 G	Exon 8	TM 11	S467C		no activity	-	Koizumi *et al.*, 1999; Ohashi *et al.*, 2002
C 22856 T	Exon 8	TM 11	P478L	protein level→	no activity	activity↑	Seth *et al.*, 1999; Tang *et al.*, 1999; Wu *et al.*, 1999
G 23934 A	Intron 8			truncating	n.d.	n.d.	Nezu *et al.*, 1999

a) Position of nucleotide substitution, accession number AB016625 (NM_00306) Ins; Insertion, Del; Deletion
b) TM: Transmembrane, EL: Extracellular loop, IL; Intracellular loop.
\# Location in the intracellular loop between transmembrane domains 2 and 3
—; unchanged when compared to wild type of OCTN2, n.d.; not determined

Moreover, previous studies have shown OCTN2 mutations causing a production of truncated protein in SCD patients (Table 6). These mutations lead to the loss of several putative transmembrane domains. The lack of several predicted transmembrane domains would likely result in the production of OCTN2 protein that either is rapidly degraded or not functional.

2.4.2. Influence of OCTN2 Polymorphisms on Drug Disposition

Several drugs such as verapamil, quinidine and some cephalosporin antibiotics are recognized by human OCTN2 [Ohashi *et al.*, 1999; Ganapathy *et al.*, 2000]. Most of the mutations in the OCTN2 gene are identified in SCD patients

(Table 6). Heterozygotes for the OCTN2 mutations (e.g., W132X, S467C, W283C and M179L) with impaired carnitine transport are also identified in healthy subjects who are characterized by low levels of serum carnitine [Koizumi *et al.*, 1999]. It is considered that the truncating mutations containing a deletion of the coding region or change of the conserved splice acceptor site in the OCTN2 gene fail to transport organic cations as well as carnitine. However, the S467C mutant of OCTN2 elicits a loss of carnitine transport without interference in OCTN2-mediated TEA transport [Ohashi *et al.*, 2002]. Similar results are reported in P478L and Y211F [Seth *et al.*, 1999]. Carnitine has an anionic and cationic moiety within the molecule and exists as a zwitterion under physiological conditions. OCTN2 recognized both anionic and cationic charges, and transported carnitine or organic cations in an Na^+-dependent or Na^+-independent manner. Mutations in S467C and P478L are located in transmembrane domain 11 which is the anion recognition site and is closely related to the Na^+-binding site on OCTN2 protein [Seth *et al.*, 1999; Ohashi *et al.*, 2002]. On the other hand, E452K is located in the intracellular loop between predicted transmembrane domains 10 and 11 [Wang *et al.*, 2000b]. Therefore, these variants might influence the pharmacokinetic or pharmacodynamic properties of zwitter-ionic drugs but not cationic drugs. In future studies, it will be important to clarify whether these mutations in the OCTN2 gene influence the transport function of organic cations.

3. FUTURE OF DRUG TRANSPORT RESEARCH IN HUMANS

Drug transporters are widely distributed in human tissues and have an important role in regulating the absorption, distribution, and excretion of many clinically useful drugs. Although it is obvious that additional studies with regard not only to drug transporters but also drug metabolizing enzymes and functional target proteins (receptors and converting enzymes) are necessary to facilitate the translation of pharmacogenomics into clinical practice (i.e., tailoring drug therapy), some critical issues must be considered.

In order to establish a genotype-phenotype correlation, a specific probe drug(s) for each objective protein is essential. In drug transport studies, digoxin and fexofenadine are often used as probe drugs for P-glycoprotein. However, recent studies have clearly indicated that these drugs are dual substrates for P-glycoprotein and other polymorphic drug transporters: digoxin for OATP-8 and fexofenadine for OATP-A [Suzuki *et al.*, 2002; Dresser *et al*, 2002]. Thus, the contribution of at least two transporters with regard to genetic variations needs to be considered in order to describe the pharmacokinetics, and then the clinical outcome of each drug treatment more accurately. The involvement of multi transporters in the pharmacokinetics of these drugs is one possible reason for the controversial and conflicting findings on genotype-phenotype correlations of P-glycoprotein (MDR1) [Kim, 2002]. Nevertheless, it is clear that the identification of specific probe drugs for each drug transporter is required.

Most drug effects are determined by the interplay of several proteins (gene products) that regulate the pharmacokinetics and pharmacodynamics of medications. The synergistic action of intestinal CYP3A and P-glycoprotein is a typical interplay for detoxication [Zhang and Benet, 2001]. The majority of initial data on the importance of polymorphisms with regard to drug transporters are focused on a single SNP or a single gene. Currently, strategies for genotype-phenotype studies have been extended from a single SNP analysis to haplotype analysis [Tang *et al.*, 2002; 12:437-50; Nishizato *et al.*, 2003]. In addition, candidate-gene(s) analysis based on the knowledge of the mechanism of drug action and pathways of metabolism and disposition has been introduced with the development of powerful molecular diagnostic methods. We believe these evolving strategies will lead to the accurate elucidation of genetically determined drug responses.

ACKNOWLEDGEMENTS

This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, and Science of Japan and from the Ministry of Health, Labor and Welfare of Japan.

ABBREVIATIONS

CYP	=	Cytochrome P450
SNP	=	Single-nucleotide polymorphism
PM	=	Poor metabolizer
EM	=	Extensive metabolizer
MRP2	=	Multidrug resistance associated protein-2
OAT	=	Organic anion transporter
OATP	=	Organic anion transporting polypeptide
OCT	=	Organic cation transporter
PG	=	Prostagrandin
PAH	=	p-aminohippurate
NSAID	=	Nonsteroidal anti-inflammatory drug
UGT1A	=	UDP-glucuronosyltransferase-1A
PXR	=	Pregnane X receptor
TEA	=	Tetraethylammonium
MPP+	=	1-methyl-4-phenylpyridinium
TM	=	Transmembrane
SCD	=	Primary systemic carnitine deficiency

REFERENCES

Abe, T., Kakyo, M., Tokui, T., Nakagomi, R., Nishio, T., Nakai, D., Nomura, H., Unno, M., Suzuki, M., Naitoh, T., Matsuno, S., Yawo, H. (1999) Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *J. Biol. Chem.*, **274**, 17159-63

Abe, T., Unno, M., Onogawa, T., Tokui, T., Kondo, T.N., Nakagomi, R., Adachi, H., Fujiwara, K., Okabe, M., Suzuki, T., Nunoki, K., Sato, E., Kakyo, M., Nishio, T., Sugita, J., Asano, N., Tanemoto, M., Seki, M., Date, F., Ono, K., Kondo, Y., Shiiba, K., Suzuki, M., Ohtani, H., Shimosegawa, T., Iinuma, K., Nagura, H., Ito, S., Matsuno, S. (2001) LST-2, a human liver-specific organic anion transporter, determines methotrexate sensitivity in gastrointestinal cancers. *Gastroenterology*, **120**, 1689-99

Aithal, G.P., Day, C.P., Kesteven, P.J.L., Daly, A.K. (1998) Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet*, **353**, 717-9

Apiwattanakul, N., Sekine, T., Chairoungdua, A., Kanai, Y., Nakajima, N., Sophasan, S., Endou, H. (1999) Transport properties of nonsteroidal anti-inflammatory drugs by organic anion transporter 1 expressed in Xenopus laevis oocytes. *Mol. Pharmacol.*, **55**, 847-54

Arranz, M.J., Munro, J., Birkett, J., Bolonna, A., Mancama, D., Sodhi, M., Lesch, K.P., Meyer, J.F.W., Sham, P., Collier, D.A., Murray, R.M., Kerwin, R.W. (2000) Pharmacogenetic prediction of clozapine response. *Lancet*, **355**, 1615-6

Babu, E., Takeda, M., Narikawa, S., Kobayashi, Y., Enomoto, A., Tojo, A., Cha, S.H., Sekine, T., Sakthisekaran, D., Endou, H. (2002a) Role of human organic anion transporter 4 in the transport of ochratoxin A. *Biochemica. Et. Biophysica. Acta.*, **1590**, 64-75

Babu, E., Takeda, M., Narikawa, S., Kobayashi, Y., Yamamoto, T., Cha, S.H., Sekine, T., Sakthisekaran, D., Endou, H. (2002b) Human organic anion transporters mediate the transport of tetracycline. *Jpn. J. Pharmacol.*, **88**, 69-76

Busch, A.E., Karbach, U., Miska, D., Gorboulev, V., Akhoundova, A., Volk, C., Arndt, P., Ulzheimer, J.C., Sonders, M.S., Baumann, C., Waldegger, S., Lang, F., Koepsell, H. (1998) Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. *Mol. Pharmacol.*, **54**, 342-52

Cha, S.H., Sekine, T., Fukushima, J., Kanai, Y., Kobayashi, Y., Goya, T., Endou, H. (2001) Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. *Am. Soc. Pharmacol. Exp. Ther.*, **59**, 1277-86

Cha, S.H., Sekine, T., Kusuhara, H., Yu, E., Kim, J.Y., Kim, D.K., Sugiyama, Y., Kanai, Y., Endou, H. (2000) Molecular cloning and characterization of multispecific organic anion transporter 4 expressed in the placenta. *J. Biol. Chem.*, **275**, 4507-12

Cihlar, T., Lin, D.C., Pritchard, J.B., Fuller, M.D., Mendel, D.B., Sweet, D.H. (1999) The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. *Mol. Pharmacol.*, **56**, 570-80

Cui, Y., König, J., Leier, I., Buchholz, U., Keppler, D. (2001) Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. *J. Biol. Chem.*, **276**, 9626-30

Cvetkovic, M., Leake, B., Fromm, M.F., Wilkinson, G.R., Kim, R.B. (1999) OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab. Dispos.*, **27**, 866-71

Dresser, G.K., Bailey, D.G., Leake, B.F., Schwarz, U.I., Dawson, P.A., Freeman, D.J., Kim, R.B. (2002) Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin. Pharmacol. Ther.*, **71**, 11-20

Enomoto, A., Takeda, M., Shimada, M., Narikawa, S., Kobayashi, Y., Kobayashi, Y., Yamamoto, T., Sekine, T., Cha, S.H., Niwa, T., Endou, H. (2002) Interaction of human organic anion transporter 2 and 4 with organic anion transport inhibitors. *J. Pharmacol. Exp. Ther.*, **301**, 797-802

Evans, W.E., Relling, M.V. (1999) Pharmacogenomics: translating functional genomics into rational therapeutics. *Science*, **286**, 487-91

Fellay, J., Marzolini, C., Meaden, E.R., Back, D.J., Buclin, T., Chave, J.P., Decosterd, L.A., Furrer, H., Opravil, M., Pantaleo, G., Retelska, D., Ruiz, I., Schinkel, A.H., Vernazza, P., Eap, C.B., Telenti, A. (2002) Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet*, **359**, 30-6

Formm, M.F., Kauffmann, H.M., Fritz, P., Burk, O., Kroemer, H.K., Warzok, R.W., Eichelbaum, M., Siegmund W, Schrenk D (2000) The effect of rifampin treatment on intestinal expression of human MRP transporters. *Am. J. Pathol.*, **157**, 1575-80

Fujiwara, K., Adachi, H., Nishio, T., Unno, M., Tokui, T., Okabe, M., Onogawa, T., Suzuki, T., Asano, N., Tanemoto, M., Seki, M., Shiiba, K., Suzuki, M., Kondo, Y., Nunoki, K., Shimosegawa, T., Iinuma, K., Ito, S., Matsuno, S., Abe, T. (2001) Identification of thyroid hormone transporters in human: different molecules are involved in a tissue-specific manner. *Enterology*, **142**, 2005-12

Furuta, T., Ohashi, K., Kamata, T., Takashima, M., Kosuge, K., Kawasaki, T., Hanai, H., Kubota, T., Ishizaki, T., Kaneko, E. (1998) Effect of genetic differences in omeprazole metabolism on cure rates for *Helicobacter pylori* infection and peptic ulcer. *Ann. Intern. Med.*, **129**, 1027-30

Furuta, T., Shirai, N., Takashima, M., Xiao, F., Hanai, H., Nakagawa, K., Sugimura, H., Ohashi, K., Ishizaki, T. (2001) Effects of genotypic differences in CYP2C19 status on cure rates for *Helicobacter pylori* infection by dual therapy with rabeprazole plus amoxicillin. *Pharmacogenetics*, **11**, 341-8

Ganapathy, M.E., Huang, W., Rajan, D.P., Carter, A.L., Sugawara, M., Iseki, K., Leibach, F.H., Ganapathy, V. (2000) β–lactam antibiotics as substrates for OCTN2, an organic cation/carnitine transporter. *J. Biol. Chem.*, **275**, 1699-1707

Gao, B., Hagenbuch, B., Kullak-Ublick, G.A., Benke, D., Aguzzi, A., Meier, P.J. (2000) Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J. Pharmacol. Exp. Ther.*, **294**, 73-9

Geick, A., Eichelbaum, M., Burk, O. (2001) Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J. Biol. Chem.*, **276**, 14581-7

Gorboulev, V., Ulzheimer, J.C., Akhoundova, A., Ulzheimer-Teuber, I., Karbach, U., Quester, S., Baumann, C., Lang, F., Busch, A.E., Koepsell, (1997) Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol.*, **16**, 871-81

Greiner, B., Eichelbaum, M., Fritz, P., Kreichgauer, H.P., von Richter, O., Zundler, J., Kroemer, H.K. (1999) The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J. Clin. Invest.*, **104**, 145-53

Ho, E.S., Lin, D.C., Mendel, D.B., Cihlar, T. (2000) Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. *J. Am. Soc. Nephrol.*, **11**, 383-93

Hoffmeyer, S., Burk, O., von Richter, O., Arnold, H.P., Brockmöller, J., Johne, A., Cascorbi, I., Gerloff, T., Roots, I., Eichelbaum, M., Brinkmann, U. (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc. Natl. Acad. Sci. USA*, **97**, 3473-3478

Hosoyamada, M., Sekine, T., Kanai, Y., Endou, H. (1999) Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am. J. Physiol.*, **276**, F122-8

Hsiang, B., Zhu, Y., Wang, Z., Wu, Y., Sasseville, V., Yang, W.P., Kirchgessner, T.G. (1999) A novel human hepatic organic anion transporting polypeptide (OATP2). *J. Biol. Chem.*, **274**, 37161-8

Illmer, T., Schuler, U.S., Thiede, C., Schwarz, U.I., Kim, R.B., Gotthard, S., Freund, D., Schkel, U., Ehninger, G., Schaich, M. (2002) MDR1 gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Res.*, **62**, 4955-62

Jonker, J.W., Wagenaar, E., Mol, C.A., Buitelaar, M., Koepsell, H., Smit, J.W., Schinkel, A.H. (2001) 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.*, **21**, 5471-5477

Kerb, R., Aynacioglu, A.S., Brockmoller, J., Schlagenhaufer, R., Bauer, S., Szekeres, T., Hamwi, A., Fritzer-Szekeres, M., Baumgartner, C., Ongen, H.Z., Guzelbey, P., Roots, I., Brinkmann, U. (2001) The predictive value of MDR1, CYP2C9 and CYP2C19 polymorphisms for phenytoin plasma levels. *Pharmacogenomics J.*, **1**, 204-10

Kerb, R., Brinkmann, U., Chatskaia, N., Gorbunov, D., Gorboulev, V., Mornhinweg, E., Keil, A., Eichelbaum, M., Koepsell, H. (2002) Identification of genetic variations of the human organic cation transporter hOCT1 and their functional consequences. *Pharmacogenetics*, **12**, 591-5

Khamdang, S., Takeda, M., Noshiro, R., Narikawa, S., Enomoto, A., Anzai, N., Piyachaturawat, P., Endou, H. (2002) Interactions of human organic anion transporters and human organic cation transporters with nonsteroidal antiinflammatory drugs. *J. Pharmacol. Exp. Ther.*, **303**, 534-9

Kim, R.B. (2002) MDR1 single nucleotide polymorphisms: multiplicity of haplotypes and functional consequences. *Pharmacogenetics*, **12**, 425-7

Kimura, H., Takeda, M., Narikawa, S., Enomoto, A., Ichida, K., Endou, H. (2002) Human organic anion transports and human organic cation transporters mediate renal transport of prostaglandins. *J. Pharmacol. Exp. Ther.*, **302**, 293-298

Koizumi, A., Nozaki, J., Ohura, T., Kayo, T., Wada, Y., Nezu, J., Ohashi, R., Tamai, I., Shoji, Y., Takada, G., Kibira, S., Matsuishi, T., Tsuji, A. (1999) Genetic epidemiology of the carnitine transporter

OCTN2 gene in a Japanese population and phenotypic characterization in Japanese pedigrees with primary systemic carnitine deficiency. *Hum. Mol. Genet.*, **8**, 2247-54

König, J., Cui, Y., Nies, A.T., Keppler, D. (2000a) A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **278**, G156-64

König, J., Cui, Y., Nies, A.T., Keppler, D. (2000b) Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J. Biol. Chem.*, **275**, 23161-68

Kullak-Ublick, G.A., Hagenbuch, B., Stieger, B., Schteingart, C.D., Hofmann, A.F., Wolkoff, A.W., Meier PL (1995) Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. *Gastroenterology*, **109**, 1274-82

Kullak-Ublick, G.A., Ismair, M.G., Stieger, B., Landmann, L., Huber, R., Pizzagalli, F., Fattinger, K., Meier, P.J., Hagenbuch, B. (2001) Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology*, **120**, 525-33

Kurata, Y., Ieiri, I., Kimura, M., Morita, T., Irie, S., Urae, A., Ohdo, S., Ohtani, H., Sawada, Y., Higuchi, S., Otsubo, K. (2002) Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. *Clin. Pharmacol. Ther.*, **72**, 209-19

Lamhonwah, A.M., Tein, I. (1998) Carnitine uptake defect: frameshift mutations in the human plasmalemmal carnitine transporter gene. *Biochem. Biophys. Res. Commun.*, **252**, 396-401

Leabman, M.K., Huang, C.C., Kawamoto, M., Johns, S.J., Stryke, D., Ferrin, T.E., DeYoung, J., Taylor, T., Clark, A.G., Herskowitz, I., Giacomini, K.M. (2002) Polymorphisms in a human kidney xenobiotic transporter, OCT2, exhibit altered function. *Pharmacogenetics*, **12**, 395-405

Lehmann, J.M., McKee, D.D., Watson, M.A., Willson, T.M., Moore, J.T., Kliewer, S.A. (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest.*, **102**, 1016-23

Mamiya, K., Ieiri, I., Shimamoto, J., Yukawa, E., Imai, J., Ninomiya, H., Yamada, H., Otsubo, K., Higuchi, S., Tashiro, N. (1998) The effects of genetic polymorphisms of CYP2C9 and CYP2C19 on phenytoin metabolism in Japanese adult patients with epilepsy: studies in stereoselective hydroxylation and population pharmacokinetics. *Epilepsia*, **39**, 1317-23

Mayatepek, E., Nezu, J., Tamai, I., Oku, A., Katsura, M., Shimane, M., Tsuji, A. (2000) Two novel missense mutations of the OCTN2 gene (W283R and V446F) in a patient with primary systemic carnitine deficiency. *Hum. Mutant.*, **15**, 118

Meyer-Wentrup, F., Karbach, U., Gorboulev, V., Arndt, P., Koepsell, H. (1998) Membrane localization of the electrogenic cation transporter rOCT1 in rat liver. *Biochem. Biophys. Res. Commun.*, **248**, 673-78

Michalski, C., Cui, Y., Nies, A.T., Nuessler, A.K., Neuhaus, P., Zanger, U.M., Klein, K., Eichelbaum, M., Keppler, D., König, J. (2002) A naturally occurring mutation in the SLC21A6 gene causing impaired membrane localization of the hepatocyte uptake transporter. *J. Biol. Chem.*, **277**, 43053-43063

Morse, G.D., Shelton, M.J., O'Donnell, A.M. (1993) Comparative pharmacokinetics of antiviral nucleoside analogues. *Clin. Pharmacokinet.*, **24**, 101-23

Motohashi, H., Sakurai, Y., Saito, H., Masuda, S., Urakami, Y., Goto, M., Fukatsu, A., Ogawa, O., Inui, K. (2002) Gene expression levels and immunolocalization of organic ion transporters in the human kidney. *J. Am. Soc. Nephrol.*, **13**, 866-874

Mulato, A.S., Ho, E.S., Cihlar, T. (2000) Nonsteroidal anti-inflammatory drugs efficiently reduce the transport and cytotoxicity of adefovir mediated by the human renal organic anion transporter 1. *J. Pharmacol. Exp. Ther.*, **295**, 10-15

Nakai, D., Nakagomi, R., Furuta, Y., Tokui, T., Abe, T., Ikeda, T., Nishimura, K. (2001) Human liver-specific organic anion transporter, LST-1, mediates uptake of pravastatin by human hepatocytes. *J. Pharmacol. Exp. Ther.*, **297**, 861-7

Nezu, J., Tamai, I., Oku, A., Ohashi, R., Yabuuchi, H., Hashimoto, N., Nikaido, H., Sai, Y., Koizumi, A., Shoji, Y., Takada, G., Matsuishi, T., Yoshino, M., Kato, H., Ohura, T., Tsujimoto, G., Hayakawa, J., Shimane, M., Tsuji, A. (1999) Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. *Nat. Genet.*, **21**, 91-94

Ninomiya, H., Mamiya, K., Matsuo, S., Ieiri, I., Higuchi, S., Tashiro, N. (2000) Genetic polymorphism of the CYP2C subfamily and extenssive serum phenytoin concentration with central nervous system intoxication. *Ther. Drug. Monit.*, **22**, 230-2

Nishizato, Y., Ieiri, I., Suzuki, H., Kimura, M., Kawabata, K., Hirota, T., Takane, H., Irie, S., Kusuhara, H., Urasaki, Y., Urae, A., Higuch, S., Otsubo, K., Sugiyama, Y. (2003) Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: Consequences for pravastatin pharmacokinetics. *Clin. Pharmacol. Ther.*, In press

Nozawa, T., Nakajima, M., Tamai, I., Noda, K., Nezu, J., Sai, Y., Tsuji, A., Yokoi, T. (2002) Genetic polymorphisms of human organic anion transporters OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis. *J. Pharmacol. Exp. Ther.*, **302**, 804-813

Ohashi, R., Tamai, I., Yabuuchi, H., Nezu, J., Oku, A., Sai, Y., Shimane, M., Tsuji, A. (1999) Na^+-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. *J. Pharmacol. Exp. Ther.*, **291**, 778-84

Ohashi, R., Tamai, I., Nezu, J., Nikaido, H., Hashimoto, N., Oku, A., Sai, Y., Shimane, M., Tsuji, A. (2001) Molecular and physiological evidence for multifunctionality of carnitine/organic cation transporter OCTN2. *Mol. Pharmacol.*, **59**, 358-66

Ohashi, R., Tamai, I., Inano, A., Katsura, M., Sai, Y., Nezu, J., Tsuji, A. (2002) Studies on functional sites of organic cation/carnitine transporter OCTN2 (SLC22A5) using a Ser467Cys mutant protein. *J. Pharmacol. Exp. Ther.*, **302**, 1286-94

Pizzagalli, F., Hagenbuch, B., Stieger, B., Klenk, U., Folkers, G., Meier, P.J. (2002) Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. *Mol. Pharmacol.*, **16**, 2283-96

Race, J.E., Grassl, S.M., Williams, W.J., Holtzman, E.J. (1999) Molecular cloning and characterization of two novel human renal organic anion transporters (hOAT1 and hOAT3). *Biochem. Biophys. Res. Commun.*, **255**, 508-14

Rae, J.M., Johnson, M.D., Lippman, M.E., Flockhart, D.A. (2001) Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. *J. Pharmacol. Exp. Ther.*, **299**, 849-57

Sato, K., Sugawara, J., Sato, T., Mizutamari, H., Suzuki, T., Ito, A., Mikkaichi, T., Onogawa, T., Tanemoto, M., Unno, M., Abe, T., Okamura, K. (2003) Expression of organic anion transporting polypeptide E (OATP-E) in human placenta. *Placenta*, **24**, 144-8

Scordo, M.G., Pengo, V., Spina, E., Dahl, M.L., Gusella, M., Padrini, R. (2002) Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. *Clin. Pharmacol. Ther.*, **72**, 702-10

Sekine, T., Cha, S.H., Tsuda, M., Apiwattanakul, N., Nakajima, N., Kanai, Y., Endou, H. (1998) Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. *FEBS Lett.*, **429**, 179-82

Seth, P., Wu, X., Huang, W., Leibach, F.H., Ganapathy, V. (1999) Mutations in novel organic cation transporter (OCTN2), an organic cation/carnitine transporter, with differential effects on the organic cation transport function and the carnitine transport function. *J. Biol. Chem.*, **274**, 33388-92

Simonson, G.D., Vincent, A.C., Roberg, K.J., Huang, Y., Iwanij, V. (1994) Molecular cloning and characterization of a novel liver-specific transport protein. *J. Cell Sci.*, **107**, 1065-72

Sirtori, C.R., Franceschini, G., Galli-Kienle, M., Cighetti, G., Galli, G., Bondioli, A., Conti, F. (1978) Disposition of metformin (*N'*, *N'*-dimethylbiguanide) in man. *Clin. Pharmacol. Ther.*, **24**, 683-93.

Sun, W., Wu, R.R., van Poelje, P.D., Erion, M.D. (2001) Isolation of a family of organic anion transporters from human liver and kidney. *Biochem. Biophys. Res. Commun.*, **283**, 417-22

Suzuki, A., Tirona, R.G., Leake, B., Echizen, H., Takahashi, H., Miyake, F., Kim, R.B. (2002) Polymorphisms in the digoxin uptake transporter OATP-8, among Japanese, African- and European-American subjects [abstract]. *Clin. Pharmacol. Ther.*, **71**, P104

Sweet, D.H., Miller, D.S., Pritchard, J.B. (2001) Ventricular choline transport. *J. Biol. Chem.*, **276**, 41611-9

Takahashi, H., Wilkinson, G.R., Caraco, Y., Muszkat, M., Kim, R.B., Kashima, T., Kimura, S., Echizen, H. (2003) Population differences in S-warfarin metabolism between CYP2C9 genotype-matched