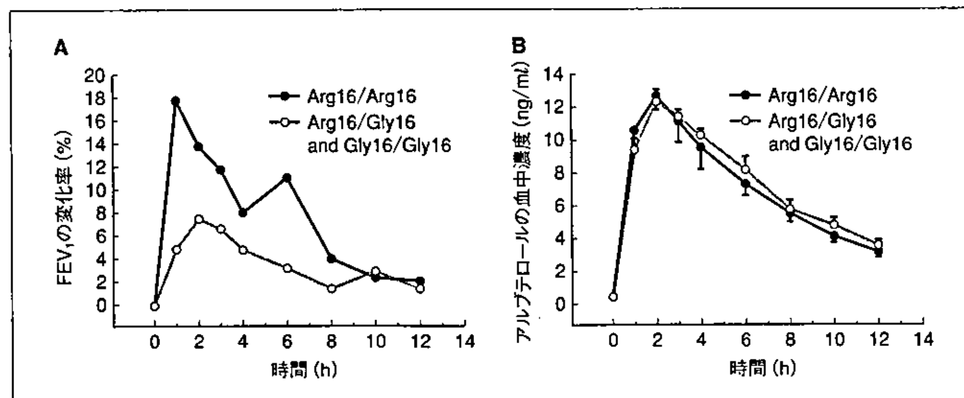

図4 β_2アドレナリンレセプター遺伝子多型とアルブテロールの効果（A）と血中濃度（B）との関連
（Lima JJ, et al : *Clin Pharmacol Ther* 65, 519-25, 1999[40] より）

かった薬剤も多い．これらについて K^+チャネルと複合体を形成すると考えられているKCNE2の変異が，クラリスロマイシンやスルファメトキサゾールが誘発するQT延長の原因となることが明らかとされており[41, 42]，特に後者の変異（Thr8Ala）は，1.6％の頻度であるため，投薬前の遺伝子型の確認により危険な副作用を回避できる可能性がある[41]．

また，直接の標的分子とは異なる遺伝子の多型と効果との相関がみられた事例も数多く報告されている．たとえばHMG-CoA還元酵素阻害薬プラバスタチンの効果が，CETP（cholesteryl ester transfer protein）のイントロンの多型（TaqIB）により決定され，高脂血症患者のうちB2/B2タイプ（16％）で，動脈硬化の進行抑制がみられないという報告[43]や，Alzheimer病治療薬や高脂血症薬の治療効果とアポリポタンパク質Eの多型との間に相関が認められるとする報告がある[44, 45]．

遺伝子情報に基づくテーラーメイド医療の実現可能性

本項ではごく一部しか事例を紹介できなかったが，現在ではSNP解析や発現解析において多くの手法が開発されており，高速化・大規模化が進んだため，薬の効果・副作用にかかわる因子やその多型に関する論文が続々と報告されている．また，薬の開発において最近は，分子標的治療を指向した薬剤が増加しており，標的分子の多型解析からレスポンダーをあらかじめ絞り込んだうえでの個別化医療が可能性をみせているし，代謝経路などに関しては，多型性のある1つの酵素のみを経由して排泄される薬剤は安全性の面から開発を嫌う傾向が出てきている．また一方で，QT延長や臓器毒性など薬物の種類を越えて共通の副作用を示すものに関しても徐々に明らかとなりつつあり，*in vitro* 実験で創薬段階初期のスクリーニングが可能になるものと思われる．クロザピンに関しては，4分子種の6種の変異から95％以上の精度でレスポンダーの予測が可能とされている[46]．

しかしながら，臨床研究の結果には互いに矛盾がみられるものも多い．その原因としては，試験そのものの規模，効果の指標，交絡要因の排除など試験デザインが，対象遺伝子の変異をみる十分な検出力をもっていないことや，単独のSNPにのみ焦点を当てて，ハプロタイプを考慮していないことに起因する見逃しなどがあるとされている．スタチン類に関しては，大規模多施設臨床試験の結果から，効果にかかわる遺伝子群の検討が数多く報告されている[47-49]

が，効果の指標（LDLの減少，血管壁の肥厚抑制，臨床イベントの減少）によって多型との関連が異なり，結果の解釈に注意を要する．また，複数の多型遺伝子のうち，特定の効果に対する特定の遺伝子の影響について，発現誘導などにつながるような環境要因の大きさと併せて寄与率を決定しておくことが必要となる．そのためには，小規模な検討では検体総数に限りがあり，特に頻度が低く多要因が絡む多型の場合には，影響がみづらいことが多い．倫理的問題も介在するであろうが，今後，多施設共同で試験を行い情報を共有することで，集団の規模を拡大し，多くの候補遺伝子についてハプロタイプ解析の結果と，臨床で重要視される治療指標との間の相関を取るような研究が必要であろう．その一方で，*in vitro*において適切な変異体のスクリーニング系を作成し，*in vivo*でみえた事象を説明可能にする取り組みが，将来の個別化医療に向けて必須になると思われる．

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トランスポーターの遺伝子多型と薬物動態の個人差

Impact of genetic polymorphisms of transporters on inter-individual variability of pharmacokinetics

前田和哉　杉山雄一
Kazuya MAEDA and Yuichi SUGIYAMA
東京大学大学院薬学系研究科分子薬物動態学教室

◎細胞の膜透過にかかわる薬物トランスポーターが多数同定され，多くの薬物の体内動態を決めるうえで重要な役割を果たしていることがわかってきた．トランスポーターの役割は，①肝や腎などで薬物の消失に関与し，物質の体内動態を決定すること，②血液脳関門のように体内動態に直接影響しないが，物質の内外移行に対する障壁となり，局所の物質の分布を決定することに大別される．いずれの場合も効果・副作用を規定する要因となりうることから，臨床におけるトランスポーターの重要性が明らかとされつつある．一方で近年，トランスポーターについて多くの遺伝子多型が同定され，*in vitro* 研究，臨床試験などにより臨床医療における意義が注目されている．本稿ではトランスポーターの遺伝子多型研究の現状について例をあげながら，これらの情報を臨床で活用可能な形にするための課題について述べてみたい．

Key word：SNPs(single nucleotide polymorphisms)，OATP2，MRP2，MDR1/P-gp，ハプロタイプ

一般に，同量の薬物を投与しても，その効果・副作用の出方は千差万別である．アメリカの統計では毎年200万人が薬の副作用で入院し，うち10万人が死亡しているという報告があり[1]，患者にとってむだな診療を減らす意味でも薬効・副作用の個人差の原因探求は急務とされている．薬物が薬効・副作用を発揮するまでの過程は，①投与された薬物が標的部位に到達する濃度を決定する薬物動態学(pharmacokinetics：PK)の領域と，②薬物が標的分子に結合した後の一連のシグナル伝達，転写制御などに関連する薬力学(pharmacodynamics：PD)の領域に大別される．近年，遺伝子多型の解析が進むにつれ，これらを支配する遺伝子についても一塩基置換(single nucleotide polymorphisms/SNPs)をはじめとする変異探索，臨床研究ならびに *in vitro* での変異体の機能解析が進みつつある．

PDにかかわる分子(レセプター，転写因子など)の認識性は比較的狭いことが多く，限定された薬物種でしか変異による影響がみられないケースが多いのに対し，PKを支配する分子〔代謝酵素(チトクロームP450など)，トランスポーターなど〕の基質認識性は広範であり，1つの分子の変異があらゆる薬物の体内分布，排泄を変動させる可能性があることに特徴がある．代謝酵素に関しては比較的早くから解析が進められ，テーラーメイド医療への基礎情報が蓄積しているのに対し，種々臓器の膜透過過程にかかわる薬物トランスポーターについても発見から日が浅いにもかかわらず事例が着実に集まりつつある．

図1には各種臓器に発現するおもなトランスポーターについて示した．トランスポーターの機能変動は肝や腎など血中からの薬物の消失にかかわる臓器で起こった場合，薬物の体内動態全体に影響を与える一方，血液脳関門などでは体内動態に直接影響を与えないが，物質の内外移行を制限するバリアー機能の変化に伴い，局所の薬物分布が変動し，ひいては薬効や副作用の増強・減弱に

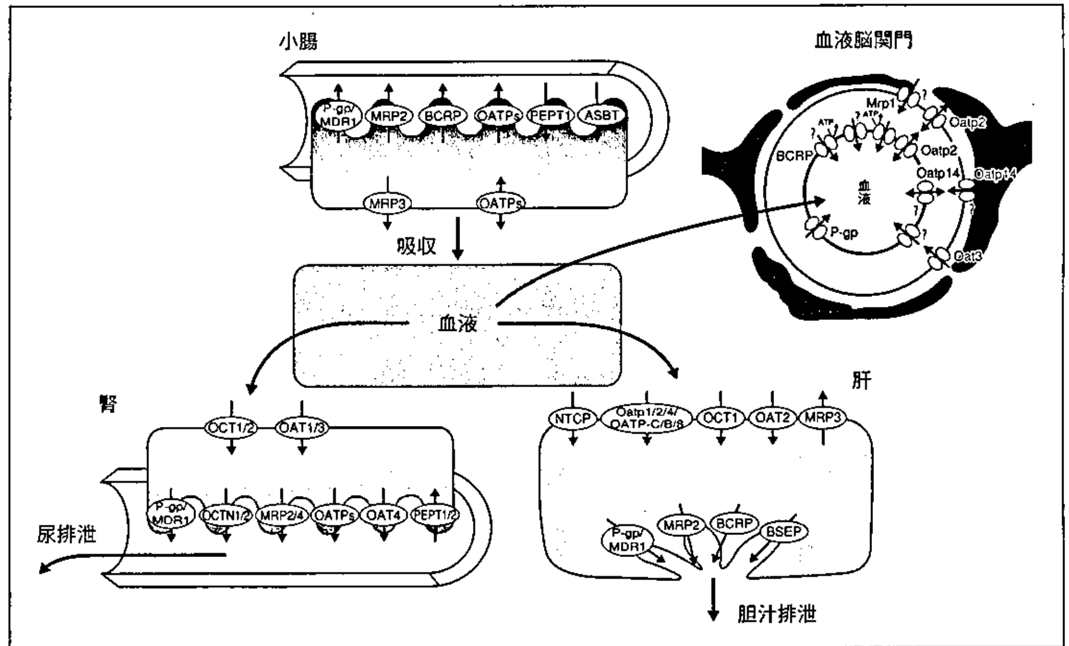

図 1　トランスポーターの全身分布(文献[31]より改変)

サイドメモ

ハプロタイプ解析の重要性

多くの研究では *in vitro*, *in vivo* 問わず，個々の SNPs に着目し，層別化・比較している．しかし，一部の複数の SNPs はたがいに高頻度でリンクして表れる連鎖不平衡(linkage disequilibrium)を示すことが明らかになってきた．それに伴い，たとえば 10 種類の SNPs があるとすれば，理論上 2^{10}種類のアレルが考えうるが，実際には上記で述べたリンクのため，数種類のアレルで集団の大部分を説明可能なケースがある．このアレル単位の SNPs の組合せパターンをハプロタイプとよび，2 本のアレルのハプロタイプ構成をディプロタイプとよんでいる．たとえば，β_2-アドレナリン受容体について，77 名の 13 カ所の SNPs が，12 種類のハプロタイプに場合分けされることが報告されており，mRNA レベルを層別化解析した結果，2 つのハプロタイプ間の比較で発現量の有意な差を見出したが，個々の SNPs に関してはいずれも有意差が見出せなかった事例がある[32]．また，*in vitro* 解析においても個々の SNPs が相反する結果を示す一方，両方の SNPs を合わせもった場合，片方の性質しかみえないケースが報告されており[33]，今後 *in vitro* 解析においてもハプロタイプを考慮した解析が必要である．

つながる．

本稿ではトランスポーターの遺伝子多型についての知見を紹介するとともに，今後の展望について論じる．

MDR1 の遺伝子多型

MDR1(multidrug resistance 1/P-glycoprotein)は 12 回膜貫通型の排出トランスポーターであり，2 つの ABC(ATP binding cassette)領域を介して，ATP の水解活性を駆動力とした輸送を担うトランスポーターである．肝の胆管側膜，腎や小腸の管腔側膜，血液脳関門の血液側膜など全身にわたって広範な発現が認められている．このトランスポーターの *in vivo* における重要性はおもにノックアウトマウスの解析を通じて明らかとなりつつあり，たとえば mdr1a$^{-/-}$マウスにおいて経口投与した paclitaxel の血漿 AUC の上昇[2]や，ivermectin や cyclosporin など MDR1 基質の脳移行の大幅な上昇[3,4]，ジゴキシンの胆汁排泄クリアランスの低下[5]などがあげられる．

MDR1 全長にわたる遺伝子多型解析は，Hoffmeyer らの 24 名の Caucasian を用いた研究による 15 カ所の変異の発見[6]を端緒として，現在では

0 カ所以上の変異が報告されるに至っている．う
5 coding 領域にはアミノ酸変異を伴う変異が 11
ヵ所，およびアミノ酸に影響を与えない silent
nutation が 4 カ所報告されている．とくに Hoff-
neyer らの報告で興味深いのは exon 26 の silent
nutation(C3435T)の変異型アレルを有するヒト
で，十二指腸における MDR1 の発現量に有意な低
下がみられ，それに伴いジゴキシン経口投与後の
血漿中 AUC の上昇がみられたことである[6]．本変
異は比較的頻度が高く，また人種差がみられ Afri-
ans で 10〜27%，Caucasians や Asians では 37〜
6%であることから，本変異の意義についての研
究が数多く報告されてきた．

これまでの C3435T に着目した臨床研究の結果
を表 1 にまとめたが，発現量，薬物動態ともに統
一した見解が得られていないのが現状である[7,8]．
また，薬効との関連では抗 HIV 治療薬を用いた治
療において治療開始 6 カ月後における CD4 細胞
の回復の程度を決める要因解析を行ったところ，
435 位が T/T の遺伝子型をもつことがよりよい
回復を決める一要因となっており，変異による
MDR1 の機能低下が細胞内からの治療薬の排出を
抑制し，より強い効果を発揮したことによるとい
う仮説が提唱されている[9]．また，病気との関連で
も腎上皮癌や潰瘍性大腸炎の発症リスクが，3435
位の変異で有意に高くなる傾向にあり，何らかの
異物解毒の機能低下が原因ではないかと考えられ
ている[10,11]．

3435 位の変異はアミノ酸変化を伴わないこと
から，機能変化を引き起こす原因のひとつの仮説
として別のアミノ酸変化を伴う変異との連鎖の可
能性が提唱され，連鎖不平衡(linkage disequilib-
rium：LD)解析も進められてきた．その結果，と
くに C1236T(slient)と G2677T/A(Ala893Ser/Thr)
との連鎖が強いことがわかり，2677/3435 の 2 種
および 3 種のハプロタイプに層別化した臨床試験
の結果，いずれも，T/T，T/T/T タイプのほうが
MDR1 の機能が弱いと思われる結果が得られてい
る[12-14]．

一方，*in vitro* での発現系を用いた解析の結果で
は Kim らは，アミノ酸変異を伴う 2677T の
MDR1 発現系において野生型と比較して単位蛋白
量当りのジゴキシンの細胞内蓄積が小さい(すな
わち MDR1 の機能が強い)と報告しているが[15]，
Morita や Kimchi-Sarfaty らは複数の薬物で 2677
位の変異は活性変化を引き起こさないと報告して
いる[16,17]ことから，アミノ酸変異自身が重要であ
るかは定かではない．また，coding 領域以外の，
たとえば転写を制御するような部位の変異との連
鎖も考えられ，さきの 3 カ所の LD block の統計的
解析から，周囲 40〜80 kb 以内に別の変異との連
鎖がある可能性も示唆されている[18]．

最近，5′上流域の 5 カ所の変異が 5 種類のハプ
ロタイプに分類され，うち 2 種類の保有者で有意
に大腸での MDR1 mRNA 発現量が低いことが明
らかとなり，この SNPs により周辺の配列に対し
て，何らかの転写因子と思われる蛋白質の結合能
が変化することが示された[19]．また，イントロン
にのみ 8 カ所の変異をもつハプロタイプで，有意
に SN-38(irinotecan の活性代謝物)の腎クリアラ
ンスが低下することが報告された[14]．残念ながら，
これらと 1236/2677/3435 位の変異には連鎖が認
められなかったが，他にも MDR1 の発現を変化さ
せる変異があることを示唆しており，さらなる検
証が求められる．

OATP2 の遺伝子多型

OATP2(organic anion transporting polypeptide 2/
OATP1B1/OATP-C)は肝の血管側膜上にのみ発現
が認められる 12 回膜貫通型の取込みトランス
ポーターであり，これまでの検討から非常に多様
な化合物を基質とすることが明らかとされてき
た．これらのなかには HMG-CoA 還元酵素阻害薬
(高脂血症薬)である pravastatin，抗結核薬 rifam-
picin，抗悪性腫瘍薬 methotrexate など臨床上重要
な薬物も多く含まれる[20]．

OATP2 の遺伝子多型に関しては Tirona らが，
71 名について 14 カ所のアミノ酸変化を伴う変異
を報告したのが最初である[21]．ここでも，たとえ
ば OATP2*1b(Asn130Asp)の頻度について，Euro-
pean-Americans では 0.30 に対し African-Ameri-
cans では 0.74 のようにいくつかの変異について
人種差がみられている．*In vitro* 実験の結果，うち
7 カ所の変異について，OATP ファミリートラン

表 1　MDR1 の C3435T 変異が薬物動態に与える影響

薬物名	評価パラメータ	被験者	reference
○T/T>C/C			
digoxin	血中濃度	Caucasian 21 名	Hoffmeyer, S. et al.：*Proc. Natl. Acad. Sci. USA*, **97**：3473, 2000
digoxin	AUC(0-4h), Cmax	Caucasian 24 名	Johne, A. et al.：*Clin. Pharmacol. Ther.*, **72**：584, 2002
digoxin	AUC	Caucasian＋African 32 名	Verstuyft, C. et al.：*Eur. J. Clin. Pharmacol.*, **58**：809, 2003
digoxin	Bioavailability	Japanese 15 名	Kurata, Y. et al.：*Clin. Pharmacol. Ther.*, **72**：209, 2002
cyclosporine	AUC(0-4, 0-12h), Cmax	Asian 14 名(心移植患者)	Chowbay, B. et al.：*Pharmacogenetics*, **13**：89, 2003
cyclosporine	AUC(0-4h)	Chinese 10 名(心移植患者)	Balram, C. et al.：*Br. J. Clin. Pharmacol.*, **56**：78, 2003
tacrolimus	血中濃度	180 名(腎移植患者)	Macphee, I. et al.：*Transplantation*, **74**：1486, 2002
tacrolimus	血中濃度	69 名(小児，心移植患者)	Zheng, H. et al.：*Am. J. Transplant.*, **3**：477, 2003
phenytoin	血中濃度	Turkish 96 名	Kerb, R. et al.：*Pharmacogenomics J.*, **1**：204, 2001
○T/T<C/C			
digoxin	AUC	Japanese 114 名	Sakaeda, T. et al.：*Pharm. Res.*, **18**：1400, 2001
digoxin	AUC	Japanese 117 名	Horinouchi, M. et al.：*Pharm. Res.*, **19**：1581, 2002
fexofenadine	AUC	Caucasian＋African-American 60 名	Kim, R. et al.：*Clin. Pharmacol. Ther.*, **70**：189, 2001
nelfinavir	血中濃度	Caucasian 123 名	Fellay, J. et al.：*Lancet*, **359**：30, 2002
cyclosporine	AUC(0-4h)	Caucasian＋African-American 10 名(腎移植患者)	Yates, C. et al.：*J. Clin. Pharmacol.*, **43**：555, 2003
○T/T=C/C			
digoxin	AUC(0-4h), Cmax	Caucasian 50 名	Gerloff, T. et al.：*Br. J. Clin. Pharmacol.*, **54**：610, 2002
fexofenadine	AUC	Caucasian 20 名	Drescher, S. et al.：*Br. J. Clin. Pharmacol.*, **53**：526, 2002
cyclosporine	トラフ濃度	Caucasian 124 名(腎移植患者)	von Ahsen, N. et al.：*Clin. Chem.*, **47**：1048, 2001
cyclosporine	AUC, Cmax	Caucasian＋African-American 14 名	Min, D. I. and Ellingrod, V. L.：*Ther. Drug Monit.*, **24**：400, 2002
tacrolimus	血中濃度	Caucasian＋African 81 名(腎移植患者)	Anglicheau, D. et al.：*J. Am. Soc. Nephrol.*, **14**：1889, 2003
tacrolimus	血中濃度	Japanese 69 名(肝移植患者)	Goto, M. et al.：*Pharmacogenetics*, **12**：451, 2002
nortriptyline	血中濃度	Caucasian 78 名	Roberts, R. L. et al.：*Pharmacogenomics J.*, **2**：191, 2002
talinolol	AUC	Caucasian 67 名	Siegmund, W. et al.：*Clin. Pharmacol. Ther.* **72**：572, 2002
loperamide	Cmax, AUC	Caucasian 16 名	Pauli-Magnus, C. et al.：*Clin. Pharmacol. Ther.*, **73**：72, 2003
dicloxacillin	血中濃度	17 名	Putnam, W. et al.：*Clin. Pharmacol. Ther.*, **73**：57, 2003
docetaxel	クリアランス	Asian 32 名	Goh, B. C. et al.：*J. Clin. Oncol.*, **20**：3683, 2002

過去，MDR1 C3435T について薬物動態が検討された報告を文献[7]に基づきまとめた．T/T(ホモ変異型)を C/C(野生型)と比較したときの評価パラメータの大小で分類した．

スポーターの代表的基質である estrone sulfate (E-sul)と estradiol-17β-glucuronide($E_2 17\beta G$)の輸送活性が有意に低下することが示される一方で，*10(Asp655Gly)のように，前者の輸送活性は低下するものの，後者の輸送活性は変化しないものなど，基質による活性変動の差がみられることを示唆する結果も観察されている[21]．これは後の Michalski らの報告で *1b において，sulfobromophthalein や $E_2 17\beta G$ の輸送が変わらないのに対し，taurocholate の輸送が有意に低下する事例[22]や，Tirona らの報告で，*1b について E-sul，$E_2 17\beta G$ の輸送は変わらないが rifampin の輸送は有意に低下する事例[23]もある．一方，5種のハプロタイプでは HeLa 細胞発現系において総蛋白量は差がないものの，細胞表面の発現量が低下しており，蛋白の maturation あるいは sorting の段階での欠陥が原因であろうと考えられる[21]．また，別の報告で T578G(Leu193Arg)も MDCKII 発現系において大部分が細胞内に観察されている[22]．*5 (Val174Ala)については，Tirona らは HeLa 一過的発現系で輸送活性低下，膜局在異常が起こるとしているが[21]，Nozawa らは，HEK293 発現系を用いて細胞膜表面の発現と，E-sul の輸送活性が変わらないことを示しており[24]，発現系による結果の相違の可能性があり，*in vitro* 実験の結果の解釈に注意を要することを示唆している．

臨床での薬物動態の変化について，Nishizato らは興味深い報告をしている[25]．日本人においては OATP2*15(Asn130Asp and Val174Ala)がアレル頻度約 15%で現れ，*5 はまったく観察されないことから，ハプロタイプ形成の人種差であろうと推察される．一方，日本人健常人にプラバスタチンを経口投与後の血漿中，尿中濃度推移を観察したところ，*15 保有者について *1b と比較して血漿中濃度推移の有意な増加，腎外クリアランス(ほぼ肝クリアランスを反映)の低下が観察された(図2)[25]．一方，当教室において，HEK293 細胞を用いて *1a，*1b，*5，*15 について *in vitro* 解析を行っており *15 では単位発現量当りの活性が著しく減少しており，その減少度合はさきの *in vivo* 試験のクリアランス低下の割合と定量的にある程度一致する結果を得ている(現在投稿中)．

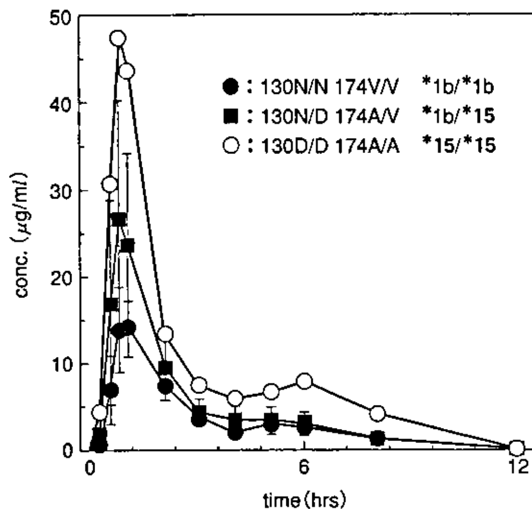

図 2　OATP2 の遺伝子型とプラバスタチンの血中濃度推移との関連[25]

OATP2 の基質薬物は広範であること，また *in vitro* 実験の結果から薬物により影響が異なる可能性があることなどから，今後いろいろな臨床事例が集積し，OATP2 の遺伝子多型の薬物動態における重要性が明らかになることが期待される．

MRP2 の遺伝子多型

MRP2(multidrug resistance associated protein 2)は 17 回膜貫通型の ABC トランスポーターであり，肝の胆管側や小腸や腎の管腔側などに発現がみられている．とくに肝においては OATP2 と並び基質認識性が広範で，かつかなりオーバーラップがみられることから，肝における経細胞輸送を担うトランスポーターとして重要であると考えられている．また，Mrp2 遺伝子欠損ラット(EHBR；Eisai Hyperbilirubinemic rats，TR^-rats)を用いた *in vivo*，CMV(canalicular membrane vesicle)実験の結果から，多くの薬物が Mrp2 を介して胆汁排泄されることが明らかとされている．また，ヒトにおいて MRP2 欠損は胆汁うっ滞を主症状とする Dubin-Johnson syndrome を引き起こす．

これまで本症の原因となる変異解析が進められ，非常に多くの変異が同定されている[26]．その多くはフレームシフト，ナンセンス変異のほか，ABC 領域付近の変異で ATP 水解活性に影響を与えるケースや，膜上に sorting されないケースであ

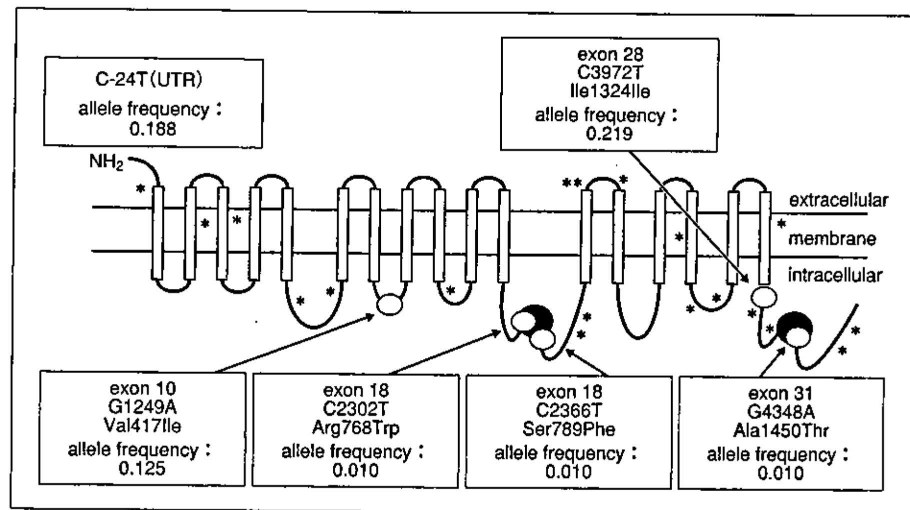

図 3　MRP2の構造とSNPs[26)]

○で示す個所は，文献[27)]で48人の日本人からみつかったSNPsの部位，頻度を示す．＊で示す個所は文献[28)]で72名の日本人由来のcell lineからみつかったSNPsの部位を示す．黒丸はABC(ATP binding cassette)領域を示す．

る．一方，健常人におけるSNPsについてItoらは48人の日本人健常人から6カ所のSNPsを見出し[27)]，また，Itodaらは72人の日本人からcell line化した細胞より27カ所のSNPsを発見している[28)](図3)．とくに日本人では5′上流域のC-24T，G1249T(Val417Ile)，C3972T(silent)の3カ所の頻度が比較的高く，アレル頻度で0.1〜0.2程度である．

当教室のHirouchiらはItoらの報告のうちアミノ酸が変化する4種類の変異に関してLLC-PK1発現細胞から調製した膜ベシクルにおける輸送活性を評価することで機能解析を試みた[29)]．その結果，C2366T(Ser789Phe)，G4348A(Ala1450Thr)の2つの変異に関しては，LLC-PK1細胞のapical側のみならず細胞内にも発現が認められた．さらに，輸送を観察した結果，前者は発現量当りの活性が野生型の1.4〜2.0倍程度になっているが，後者では$E_2 17\beta G$の輸送は非常に低下したものの，DNP-SGやleukotriene C_4といったグルタチオン抱合体では発現量当りの活性は変化がなかったことから，基質による違いが観察されているといえる．一方，頻度の高いG1249A(Val417Ile)は，局在，活性とも変化はみられなかった．今後，臨床での薬物動態に与えるこれらSNPsの影響が注目される．

おわりに

以上，トランスポーターのSNPsと機能との関連についていくつかの例を取り上げた．このほかにも肝や小腸，脳における排出トランスポーターBCRP(breast cancer resistance protein)や，有機カチオンの輸送に働くOCT1(organic cation transporter 1)，OCT2，OCTN2などについても，SNPsの*in vitro*機能解析が進められている[30)]．しかし，現時点ではヒトにおけるこれらSNPsの意義に関する臨床研究は数が少ない．*in vitro*解析の結果はときに実験条件により異なった結果を生む事例があるので，直接的にヒト*in vivo*における解析が必須となる．また，簡便にヒトにおけるトランスポーター機能を評価できるプローブ薬の探索も必要であると思われる．さらに，ヒトサンプルがようやく入手可能になったいま，ヒトサンプルをあらかじめgenotypingしておき，発現量や輸送活性をより直接的に比較することができることからも，ヒト組織バンクの充実も望まれるところである．一方で，これらSNPsの影響を*in vitro*実験の結果から予測する試みも創薬プロセスにおいて個人差を予測するうえで重要であり，そのためには単にSNPsによる機能変動の情報だけでなく，対象となるトランスポーター自身が体内動態にどの程度寄与しているかに関する情報も必要であり，各膜透

過プロセスにおける寄与率を評価できる系の確立も合わせて行うべきであろうと考えており，検討を進めている．そして，これらの統合された情報を利用して患者の遺伝子型に応じた投与設計が可能になることを期待している．

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●お知らせ●

■第2回世界薬学会議

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〒113-0033 東京都文京区本郷4-2-8　フローラビル7F
(財)日本学会事務センター内
TEL：03-3815-1681　FAX：03-3815-1691
E-mail：pswc2004@bcasj.or.jp

PHARMACOGENETICS AND GENOMICS

Polymorphisms of *OATP-C* (*SLC21A6*) and *OAT3* (*SLC22A8*) genes: Consequences for pravastatin pharmacokinetics

Objective: Our objective was to quantitate the contribution of the genetic polymorphisms of the genes for 2 human organic anion transporters—organic anion transporting polypeptide C (OATP-C) and organic anion transporter 3 (OAT3)—to the pharmacokinetics of pravastatin.
Methods: Genetic polymorphisms were screened by polymerase chain reaction–single-strand conformation polymorphism analysis, after sequencing with deoxyribonucleic acid obtained from 120 healthy volunteers. To examine whether polymorphisms in these 2 genes of interest alter transport activity, we conducted a clinical study (n = 23) with pravastatin as a selective probe drug.
Results: Among 120 healthy individuals, 5 nonsynonymous variants and 1 nonsynonymous variant were observed in the *OATP-C* and *OAT3* genes, respectively. The polymorphisms in the *OAT3* gene did not appear to be associated with changes in renal and tubular secretory clearance. In contrast, the *OATP-C* variants were associated with differences in the disposition kinetics of pravastatin. Subjects with the *OATP-C**15* allele (Asp130Ala174) had a reduced total and nonrenal clearance, as compared with those with the *OATP-C**1b* allele (Asp130Val174); nonrenal clearance values in **1b/*1b* (n = 4), **1b/*15* (n = 9), and **15/*15* (n = 1) subjects were $2.01 \pm 0.42\ L \cdot kg^{-1} \cdot h^{-1}$, $1.11 \pm 0.34\ L \cdot kg^{-1} \cdot h^{-1}$, and $0.29\ L \cdot kg^{-1} \cdot h^{-1}$, respectively, and the difference between **1b/*1b* and **1b/*15* subjects was significant ($P < .05$).
Conclusion: Certain commonly occurring single-nucleotide polymorphisms in *OATP-C*, such as T521C (Val174Ala), are likely to be associated with altered pharmacokinetics of pravastatin. Large clinical studies are needed to confirm these observations. (Clin Pharmacol Ther 2003;73:554-65.)

Yohei Nishizato, BS, Ichiro Ieiri, PhD, Hiroshi Suzuki, PhD, Miyuki Kimura, PhD, Kiyoshi Kawabata, PhD, Takeshi Hirota, MS, Hiroshi Takane, PhD, Shin Irie, MD, Hiroyuki Kusuhara, PhD, Yoko Urasaki, PhD, Akinori Urae, MD, Shun Higuchi, PhD, Kenji Otsubo, PhD, and Yuichi Sugiyama, PhD *Fukuoka, Yonago, and Tokyo, Japan*

From the Division of Pharmaceutical Sciences, Department of Clinical Pharmacokinetics, Graduate School, Kyushu University, Fukuoka; Department of Hospital Pharmacy, Faculty of Medicine, Tottori University, Yonago; Graduate School of Pharmaceutical Sciences, Tokyo University, Tokyo; Kyushu Pharmacology Research Clinic, Fukuoka; and Drug Metabolism and Pharmacokinetics Research Laboratories, Sankyo Co, Ltd, Tokyo.

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Reprint requests: Ichiro Ieiri, PhD, Department of Hospital Pharmacy, Faculty of Medicine, Tottori University, Nishi-machi 36-1, Yonago 683-8504, Japan.
E-mail: *ieiri-ttr@umin.ac.jp*

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Recent clinical studies indicate that the large interindividual variability in drug responses occurs as a result of molecular alterations to various proteins such as drug-metabolizing enzymes, drug targets and receptors, and drug transporters. Most of the studies on molecular alterations performed to date have focused on the impact of genetic variation on the expression and function of these proteins.[1,2] Although genetic polymorphisms of hepatic metabolizing enzymes involved in phase I (eg, oxidative and hydrolytic) and phase II (eg, glucuronidation, sulfate conjugation, and acetylation) reactions have been intensively investigated,[3,4] little is known about the role of genetic variations in the transporters that act in the liver and kidney.

Pravastatin, one of the 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors (statins), is widely used in the treatment of hypercholesterolemia. Cumulative in vivo and in vitro studies have revealed that various active transport mechanisms are involved in the disposition kinetics of pravastatin.[5] Pravastatin is rapidly absorbed from the upper region of the small intestine, probably via a proton-gradient–dependent carrier-mediated mechanism,[6-8] and then taken up efficiently from the circulation by the liver through organic anion transporting polypeptide C (OATP-C), a sodium-independent bile acid transporter.[9,10] Human OATP-C (gene *SLC21A6*), also known as liver-specific transporter 1 (LST-1) or OATP2, is expressed at the basolateral membrane of human hepatocytes responsible for the hepatocellular uptake of a variety of endogenous and foreign chemicals.[11-14] Recently, a number of single-nucleotide polymorphisms (SNPs) have been identified in the human *OATP-C* gene.[15-17] Some of these SNPs have been found to be associated with an altered in vitro transport capability.[15-17] Tirona et al[15] performed experiments with human *OATP-C*–transfected HeLa cells and indicated that T217C (*OATP-C*2*), T521C (*OATP-C*5*), T1058C (*OATP-C*6*), G1463C (*OATP-C*9*), and A1964G (*OATP-C*10*) variants were associated with significantly reduced estrone sulfate or estradiol-17β-D-glucuronide transport activities in comparison with activities of the *OATP-C*1a* allele (originally deposited OATP-C complementary deoxyribonucleic acid [cDNA] sequences); transport activities ranged from 7% to 53% of the value for the reference allele. In contrast, Nozawa et al,[16] using expression systems with human embryonic kidney (HEK293) cells, found that the T521C (*OATP-C*5*) allele did not alter the uptake of tritium-labeled estrone-3-sulfate. Although the cell type used in each study was different, the contribution of polymorphisms of the human *OATP-C* gene to the transport activities has remained questionable. To date, no study has addressed the genotype-phenotype relationship in light of *OATP-C* in humans.

The isomer RMS-416 (3′α-isopravastatin), which was found in the largest quantities in plasma, urine, and feces,[18] is produced by chemical degradation in the stomach rather than by cytochrome P450–dependent metabolism in the liver because pravastatin is chemically unstable under acidic conditions.[7,19] Although other metabolites have been identified, none accounts for more than 1% of the dose.[18] The chemical degradation of pravastatin is thought to contribute to its low bioavailability (ie, 18%).[20] Pravastatin and RMS-416 are cleared through both hepatic and renal routes, and tubular secretion is a predominant mechanism of renal excretion.[20] Human organic anion transporter 3 (OAT3, gene *SLC22A8*), a member of the SLC22 superfamily, is predominantly expressed in the kidney and localized on the basolateral membrane of the proximal tubules.[21,22] Recently, Hasegawa et al[23] indicated that pravastatin appeared to be a relatively specific substrate of OAT3 with the use of rat Oat3–expressing LLC-PK1 cells. Because uptake from blood through the basolateral membrane of the epithelial cells in the proximal tubules is the first step in tubular secretion, OAT3 may also contribute to the urinary excretion of pravastatin in humans.

In view of the pharmacokinetic properties, at least 2 genes (ie, *OATP-C* and *OAT3*) are of interest as candidates that may lead to large interindividual variability in the pharmacokinetics and clinical outcome of pravastatin therapy. This study was designed to evaluate the functional significance of genetic polymorphisms of the human *OATP-C* and *OAT3* genes with regard to the disposition kinetics of pravastatin. In addition, interracial differences in the frequency of polymorphisms in drug metabolic enzymes and transporters are reported to be associated with ethnic differences in pharmacokinetics and pharmacodynamics of certain drugs.[24-26] Therefore, before functional characterization, we intended to assess the genetic structure of the 2 genes by using 120 genome deoxyribonucleic acid (DNA) samples obtained from Japanese subjects and to compare the allelic frequency between Japanese and other racial populations.

METHODS

Identification of variants in OATP-C and OAT3 genes. Genomic DNA was isolated from blood samples with use of the Toyobo blood kit on a Toyobo HMX-2000 robot (Toyobo, Osaka, Japan). Blood samples were obtained from 120 unrelated healthy Japanese

volunteers residing in Fukuoka who were judged to be healthy on the basis of medical history, physical examination, and laboratory test results. The subjects were carefully interviewed and considered to be of identical ethnicity by lineage and birth. The protocol was approved by the Tottori University Ethics Committee, and informed consent was obtained. The primer design was based on published sequences (GenBank/European Molecular Biology Laboratory [EMBL] accession Nos. AB026257 and AJ132573[12,27] for the *OATP-C* gene and No. AB042505 for the *OAT3* gene), and some primer sets for the *OATP-C* gene were obtained from a work by Tirona et al.[15] These primers created appropriately sized fragments (approximately 350 base pairs) for the screening of polymorphisms by subsequent single-strand conformation polymorphism (SSCP) analysis. After polymerase chain reaction (PCR), SSCP analysis was performed with the GenePhor system (Amersham Pharmacia Biotech AB, Uppsala, Sweden) as recommended by the manufacturer. The PCR product (6 μL) was mixed with 3 μL of 20-mmol/L ethylenediaminetetraacetic acid, 95% formamide, and 0.05% bromphenol blue, and this mixture was heated at 95°C for 5 minutes and then quick-chilled in an ice-water bath. The resulting single-stranded DNA (5 μL) was then loaded on a 12.5% polyacrylamide gel (GeneGel excel 12.5/24 kit; Amersham Pharmacia Biotech AB). Electrophoresis was carried out at 450 V of constant power at 15°C for 2 to 5 hours, depending on the fragment size. After electrophoresis, gels were stained by an automated gel stainer with PlusOne (Amersham Pharmacia Biotech AB).

DNA sequence. PCR products were sequenced either directly or after subcloning on an ABI 3100 automatic sequencer (Applied Biosystems, Foster City, Calif) by a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). If the direct sequencing was incomplete, each amplified PCR product was subcloned into the pGEM vector (Promega, Madison, Wis) and transformed into competent JM109 cells (Promega). Before sequencing, reaction mixtures were purified with a DyeEx Spin kit (QIAGEN GmbH, Hilden, Germany). The sequencing primers were those used in the PCR amplifications. The sequences of both strands were analyzed for products from at least 2 independent PCR amplifications to ensure that the identified polymorphisms were not PCR-based artifacts.

Haplotype analysis and nomenclature. In addition to the unphased SNP analysis, we performed haplotype analysis for the 3 major polymorphisms, Asn130Asp, Asn151Ser, and Val174Ala, by 2 approaches: (1) Haplotypes in individuals who were homozygous at all SNP sites or heterozygous at no more than one of the variable sites were assigned directly from the results of unphased SNP analysis; (2) haplotypes in the remaining subjects (eg, multiply heterozygous) were determined by a combination of long PCR and subcloning. First, the association between 2 polymorphisms at positions 130 and 174 was determined from the long PCR-restriction fragment length polymorphism according to the method of Nozawa et al.[16] Second, PCR products of exon 4 were subcloned into the pGEM vector to determine the association between Asn130Asp and Asn151Ser. After transformation, a single colony representing the sequence from 1 chromosome was chosen for sequencing. The difference between the unphased genotypic result and the cloned sequence thus provided for the phased genotype for both chromosomes. However, haplotypes in individuals who had either a Pro336Arg (n = 3) or Cys485Phe (n = 1) polymorphism could not be determined because of the long genomic distance from other SNP sites (eg, Val174Ala).

On the basis of the proposed nomenclature for polymorphisms[28] and previous findings,[15,16] in this study we used the following nomenclature: *OATP-C*1a* for no polymorphisms at all SNP positions (GenBank/EMBL accession Nos. AB026257 and AJ132573[12,27]),[15] *OATP-C*1b* for Asp130 (accession No. AF205071[11]),[15] *OATP-C*5* for Ala174,[15] and *OATP-C*15* for Asp130Ala174.[16]

Pravastatin pharmacokinetics in healthy subjects. After approval by the Ethics Review Board of Kyushu Pharmacology Research Clinic, 23 healthy male volunteers (age, 21-40 years; weight, 52.4-97.8 kg) gave written informed consent to participate in the study. These 23 participants were recruited from a population of 120 Japanese volunteers. None had taken any drugs for at least 1 week before the study. Each subject was physically normal and had no antecedent history of significant medical illness or hypersensitivity to any drugs, and each had a body mass index of between 18 and 30 kg/m^2. The subjects' health status was again judged to be normal on the basis of a physical examination with blood chemical screening, a complete blood cell count, and urinalysis before the study.

The participants came to the clinic after an overnight fast. After urination, each volunteer received a single oral dose of 10 mg of pravastatin (Mevalotin; Sankyo Co Ltd, Tokyo, Japan) with 150 mL of water. The participants were required to remain in a supine position until 4 hours after dosing, when a standardized light lunch was served. Serial blood samples were collected from an indwelling venous catheter immedi-

ately before and at 0.25, 0.5, 0.75, 1, 2, 4, 5, 6, 8, 12, and 24 hours after pravastatin administration. Urine samples were collected for 24 hours, and the amount and pH of urine were measured. Serum was separated by centrifugation and stored, as urine, at −80°C until quantitative analysis. For each volunteer, creatinine clearance (CL_{cr}) was determined by standard methods, with the ratio of the creatinine concentration in urine to the serum creatinine concentration multiplied by the 24-hour urine volume.

Assay of pravastatin and RMS-416. Pravastatin and its degradation product, RMS-416, were measured in serum and urine by HPLC–triple-quadrupole mass spectrometry. One milliliter of distilled water and 100 µL of internal standard (R-122798, 800 ng/mL in water; synthesized by Sankyo Co Ltd) were added to 1 mL of serum or 0.5 mL of urine, and this mixture was adjusted to pH 6.0 with 0.1-mol/L phosphate buffer (pH 4.0). The mixture was applied to a Bond Elut cartridge (Varian, Inc, Harbor City, Calif) and then washed 2 times with 3.0 mL of distilled water. After removal of the water from the column under vacuum conditions, the analytes and standards were eluted with 2.0 mL of acetonitrile, and the eluate was evaporated to dryness under nitrogen gas at 40°C. The residue was reconstituted with acetonitrile (120 µL) and ultrasonicated for 3 minutes. After 10-mmol/L ammonium acetate (180 µL) was added to the solution, 20-µL aliquots were injected into the liquid chromatography–mass spectrometry system. Separation by HPLC was conducted by use of a Waters 2690 Separations Module (Waters Chromatography, Milford, Mass) with an Inertsil octadecylsilane C18 column (150 × 4.6 mm; 5 µm) (GL Sciences Inc, Tokyo, Japan). Acetonitrile/water/ammonium acetate/formic acid/tetraethylammonium acetate (400/600/0.77/0.2/0.6 [vol/vol/wt/vol/vol]) was used as the mobile phase. The flow rate was 1.0 mL/min, and the autosampler chamber was kept at 6°C. Mass spectra were determined with a TSQ API-II tandem mass spectrometer (Thermo Finnigan, San Jose, Calif) system in the negative ion–detecting mode at the atmospheric pressure–chemical ionization interface. The vaporizer was operated at a temperature of 520°C, with the heated capillary temperature set at 240°C. The samples were ionized by reacting with solvent-reactant ions produced by the corona discharge (5 µA) in the chemical ionization mode. The pressure for the nitrogen sheath gas was 80 psi, and the auxiliary gas was not used. The precursor ions of pravastatin ($[M\text{-}H]^-$) at a mass-to-charge ratio (*m/z*) 423.1, RMS-416 $[M\text{-}H]^-$ at *m/z* 423.1, and R-122798 $[M\text{-}H]^-$ at *m/z* 409.1 were admitted to the first quadrupole (Q1). After the collision-induced fragmentation in the second quadrupole (Q2), the product ions of pravastatin at *m/z* 321.2, RMS-416 at *m/z* 321.2, and R-122798 at *m/z* 321.2 were monitored in the third quadrupole (Q3). The collision-offset energy was optimized at 20 eV for pravastatin and RMS-416 and at 25 eV for R-122798. The daughter scan width was set at 1.0 amu, and the total scan time was 0.6 second. The peak area ratio of each compound to the corresponding internal standard was computed with LC_{QUAN} software (Thermo Finnigan). The calibration curve was constructed with the use of weighted ($1/x^2$) linear regression plotting the spiked plasma concentrations against the measured peak area ratios. The calibration curves were linear over the standard concentration range of 0.05 ng/mL to 100 ng/mL for serum and 10 ng/mL to 4 µg/mL for urine standards.

Pharmacokinetic analysis and statistical analysis. The following parameters were estimated for pravastatin and its degradation product, RMS-416: area under the concentration-time curve from 0 to 24 hours [AUC(0-24)] and terminal rate constant for elimination (k_e). AUC(0-24) was calculated by standard noncompartmental methods, and k_e was determined by log-linear regression. Presystemic conversion of pravastatin to RMS-416 occurs in the stomach, before the compound reaches the upper region of the small intestine, the major absorption site. As the conversion rate has been shown to depend on the individual intragastric pH value, apparent bioavailability (F) was calculated as follows: $F = AUC(0\text{-}24)_{pravastatin}/[AUC(0\text{-}24)_{pravastatin} + AUC(0\text{-}24)_{RMS\text{-}416}]$. We, therefore, calculated the apparent oral clearance (CL_t) of pravastatin as follows: $CL_t = [Dose \times F/AUC(0\text{-}24)]$. The renal clearance ($CL_r$) was calculated as $CL_r = Ae(0\text{-}24)/AUC(0\text{-}24)$, in which Ae(0-24) represents the amount of pravastatin excreted in urine from 0 to 24 hours. The apparent tubular secretory clearance (CL_{sec}) was estimated as $CL_{sec} = CL_r - CL_{cr}$. The nonrenal clearance (CL_{nr}) was calculated as $CL_{nr} = CL - CL_r$. All pharmacokinetic data are given as mean ± SD. The statistical differences between various group parameters were determined with either the Mann-Whitney *U* test or ANOVA (with the Tukey-Kramer multiple comparisons test), as appropriate. $P < .05$ was taken to be the minimum level of statistical significance.

RESULTS

Identification of variants in OATP-C and OAT3 genes. For identification of polymorphisms, SSCP analysis of all 14 exons of *OATP-C* and of all 10 exons of *OAT3* except exon 1 was performed with DNA obtained from 120 unrelated subjects. In the *OATP-C*

Table I. Polymorphisms of *OATP-C* and *OAT3* genes in a Japanese population (N = 120)

Gene and location	*Position*	*Allele*	*Nucleotide sequence*	*Amino acid substitution*	*Allele frequency*	*Genotype*	*Frequency*
OATP-C							
Exon 4	388	A*	tatcAattc	Asn130	0.371	A/A	0.125
		G	tatcGattc	Asp130	0.629	A/G	0.492
						G/G	0.383
Exon 4	452	A*	ctcaAtaga	Asn151	0.963	A/A	0.933
		G	ctcaGtaga	Ser151	0.037	A/G	0.058
						G/G	0.008
Exon 5	521	T*	tatgTgttc	Val174	0.842	T/T	0.692
		C	tatgCgttc	Ala174	0.158	T/C	0.300
						C/C	0.008
Exon 5	571	T*	accaTtggg	Synonymous	0.642	T/T	0.408
		C	accaCtggg		0.358	T/C	0.467
						C/C	0.125
Exon 5	597	C*	atttCgcta	Synonymous	0.570	C/C	0.342
		T	atttTgcta		0.430	C/T	0.458
						T/T	0.200
Exon 8	1007	C*	aatcCcctg	Pro336	0.988	C/C	0.975
		G	aatcGcctg	Arg336	0.012	C/G	0.025
						G/G	0.000
Intron 9	−50†	TTT	tTTTtcttc	—	0.992	TTT/TTT	0.992
		Deletion	t—tcttc	—	0.008	TTT/–	0.008
						–/–	0.000
Exon 10	1454	G*	ccctGtcta	Cys485	0.992	G/G	0.992
		T	ccctTtcta	Phe485	0.008	G/T	0.008
						T/T	0.000
Intron 10	−12‡	A*	atacAcaac	—	0.992	A/A	0.992
		G	atacGcaac	—	0.008	A/G	0.008
						G/G	0.000
Intron 13	+50§	T*	taatTccta	—	0.992	T/T	0.992
		G	taatGccta	—	0.008	T/G	0.008
						G/G	0.000
Intron 13	+78‖	A*	tataAtaat	—	0.992	A/A	0.992
		C	tataCtaat	—	0.008	A/C	0.008
						C/C	0.000
Exon 14	2040	C*	ttgtCcctt	Synonymous	0.992	C/C	0.992
		A	ttgtAcctt		0.008	C/A	0.008
						A/A	0.000
OAT3							
Exon 2	153	G*	gcccGcccc	Synonymous	0.925	G/G	0.858
		A	gcccAcccc		0.075	G/A	0.133
						A/A	0.008
Intron 3	+79¶	G*	acccGcaaa	—	0.683	G/G	0.475
		C	acccCcaaa	—	0.317	G/C	0.417
						C/C	0.108
Exon 5	723	T*	taacTgtgt	Synonymous	0.725	T/T	0.517
		A	taacAgtgt		0.275	T/A	0.417
						A/A	0.017
Exon 8	1166	C*	gctgCcctg	Ala389	0.992	C/C	0.983
		T	gctgTcctg	Val389	0.008	C/T	0.017
						T/T	0.000
Intron 9	+81#	G*	cccaGggga	—	0.892	G/G	0.808
		A	cccaAggga	—	0.108	G/A	0.167
						A/A	0.025

OATP-C, Organic anion transporting polypeptide C gene; *OAT3*, organic anion transporter 3 gene.
*Reference allele; GenBank/EMBL accession No. AB026257 and No. AJ132573[12,27] for the *OATP-C* gene and No. AB042505 for the *OAT3* gene.
†A (t—tcttc) deletion 50 bases upstream from the 5' boundary of exon 10.
‡An adenine-to-guanine transition 12 bases upstream from the 5' boundary of exon 11.
§A thymine-to-guanine transition 50 bases downstream from the 3' boundary of exon 13.
‖An adenine-to-cytosine transition 78 bases downstream from the 3' boundary of exon 13.
¶A guanine-to-cytosine transition 79 bases downstream from the 3' boundary of exon 3.
#A guanine-to-adenine transition 81 bases downstream from the 3' boundary of exon 9.

Table II. Haplotypes of *OATP-C* gene in 120 Japanese subjects

Allele	*Frequency (%) (n = 240)*	*Genotype*	*Frequency (No.) (n = 120)*
*OATP-C*1a*	32.5	*OATP-C*1a/*1a*	13 (10.8%)
*OATP-C*1b*	45.8	*OATP-C*1a/*1b*	37 (30.8%)
*OATP-C*5*	0.0	*OATP-C*1a/*15*	14 (11.7%)
*OATP-C*15*	15.0	*OATP-C*1a/*16*	1 (0.8%)
*OATP-C*16*†	3.8	*OATP-C*1b/*1b*	26 (21.7%)
Unidentified alleles‡	3.3	*OATP-C*1b/*15*	17 (14.2%)
		*OATP-C*1b/*16*	4 (3.3%)
		*OATP-C*15/*15*	1 (0.8%)
		*OATP-C*15/*16*	2 (1.7%)
		*OATP-C*16/*16*	1 (0.8%)
		Unidentified	4 (3.3%)

†A novel haplotype.
‡Haplotypes in individuals who had either Pro336Arg (n = 3) or Cys485Phe (n = 1) polymorphism could not be determined in this study.

Table III. Allelic frequencies of *OATP-C* variants among different ethnic populations

Variant and location	*Japanese subjects* (n = 120)*	*Japanese subjects† (n = 267)*	*European American subjects‡ (n = 49)*	*African American subjects‡ (n = 44)*
Phe73→Leu (exon 2)§	0.00	—	0.02 (0.01-0.03)	0.00
Val82→Ala (exon 3)§	0.00	—	0.02 (0.01-0.03)	0.00
Asn130→Asp (exon 4)§	0.63 (0.60-0.66)	0.60 (0.59-0.63)	0.30 (0.25-0.35)	0.74 (0.67-0.81)
Asn151→Ser (exon 4)‖	0.04 (0.03-0.05)	—	0.00	0.00
Pro155→Thr (exon 4)§	0.00	—	0.16 (0.13-0.20)	0.02 (0-0.04)
Glu156→Gly (exon 4)§	0.00	—	0.02 (0.01-0.03)	0.00
Val174→Ala (exon 5)§	0.16 (0.13-0.18)	0.11 (0.10-0.12)	0.14 (0.10-0.18)	0.02 (0-0.04)
Pro336→Arg (exon 8)‖	0.01 (0.01-0.02)	—	0.00	0.00
Ile353→Thr (exon 8)§	0.00	—	0.02 (0.01-0.03)	0.00
Asn→432Asp (exon 9)§	0.00	—	0.01 (0-0.02)	0.00
Asp462→Gly (exon 10)§	0.00	—	0.01 (0-0.02)	0.00
Cys485→Phe (exon 10)‖	0.01 (0.01-0.02)	—	0.00	0.00
Gly488→Ala (exon 10)§	0.00	—	0.00	0.09 (0.05-0.13)
Asp655→Gly (exon 14)§	0.00	—	0.02 (0.01-0.03)	0.00
Glu667→Gly (exon 14)§	0.00	—	0.02 (0.01-0.03)	0.34 (0.27-0.41)

Values in parentheses indicate 95% confidence intervals which we calculated on the basis of values from the original studies.[15,16] Dashes indicate data were not reported.
*Data from this study.
†Data from Nozawa et al.[16]
‡Data from Tirona et al.[15]
§These variants were first identified by Tirona et al.[15]
‖These variants were newly identified in this study.

gene, 12 polymorphisms were detected by SSCP analysis and identified by subsequent sequencing (Table I). Of these, 5 polymorphisms resulted in the following amino acid substitutions: A388G (Asn130Asp), A452G (Asn151Ser), T521C (Val174Ala), C1007G (Pro336Arg), and G1454T (Cys485Phe). In the *OAT3* gene, 5 SNPs were identified, and a cytosine-to-thymine transversion at position 1166 (C1166T) in exon 8 was associated with an amino acid substitution from Ala to Val at codon 389 with an allelic frequency of 0.008 (Table I).

On the basis of haplotype analysis, at least 4 haplotypes were observed (Table II). In this study we found a novel allele-possessing polymorphism of Asn151Ser on one allele. The allele was designated as *OATP-C*16*. The allelic frequencies of *OATP-C*1a*, *OATP-C*1b*, *OATP-C*5*, *OATP-C*15*, and *OATP-C*16* were 32.5%, 45.8%, 0%, 15.0%, and 3.8%, respectively, in 120 healthy Japanese subjects.

Comparisons of genotypic frequencies among different racial populations. Unfortunately, the genetic polymorphism of *OAT3* has not been well documented.

Table IV. Genetic background of healthy volunteers

	OATP-C					*OAT3*	
Subject No.	*Asn130Asp*	*Asn151Ser*	*Val174Ala*	*Pro336Arg*	*Genotype*	*T723A*	*Ala389Val*
1	Asn/Asp	Asn/Ser	Val/Val	Pro/Pro	*OATP-C*1b/*16*	T/A	C/C
2	Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	A/A	C/C
3	Asn/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1b/*16*	T/T	C/C
4	Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	A/A	C/C
5	Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	T/A	C/C
6	Asp/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1b/*1b*	T/A	C/C
7	Asn/Asn	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1a/*1a*	T/T	C/C
8	Asp/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1b/*1b*	T/T	C/C
9	Asp/Asp	Asn/Asn	Ala/Ala	Pro/Pro	*OATP-C*15/*15*	T/T	C/C
10	Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	T/T	C/C
11	Asp/Asp	Asn/Asn	Val/Ala	Pro/Arg	Unidentified	T/A	C/C
12	Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	T/A	C/C
13	Asn/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1a/*1b*	T/A	C/C
14	Asn/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1a/*1b*	T/T	C/C
15	Asn/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1a/*1b*	T/A	C/C
16	Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	T/T	C/C
17	Asp/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1b/*1b*	A/A	C/C
18	Asp/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1b/*1b*	T/T	C/T
19	Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	T/A	C/C
20	Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	T/A	C/C
21	Asn/Asn	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1a/*1a*	T/T	C/C
22	Asp/Asp	Asn/Asn	Val/Ala	Pro/Pro	*OATP-C*1b/*15*	T/T	C/C
23	Asn/Asp	Asn/Asn	Val/Val	Pro/Pro	*OATP-C*1a/*1b*	T/T	C/C

Table V. Genotype in *OAT3* gene and phenotypic indexes

Genotype pattern	*No.*	CL_r $(L \cdot kg^{-1} \cdot h^{-1})$	CL_{sec} $(L \cdot kg^{-1} \cdot h^{-1})$
No mutation	10	0.44 ± 0.14	0.32 ± 0.10
T723A	12	0.45 ± 0.10	0.33 ± 0.11
Ala389Val	1	0.40	0.26

CL_r, Renal clearance; CL_{sec}, tubular secretory clearance.

The allelic frequency of *OATP-C* variants in different ethnic groups is summarized in Table III. Asn130Asp (63%), Val174Ala (16%), and Asn151Ser (4%) variants were found at a relatively high incidence in this study. Current and previous findings reported by Tirona et al[15] indicate that the Asn130Asp variant was more common in Japanese and African American subjects than in European American subjects ($P < .05$); however, Japanese and European American subjects had a significantly higher frequency of the Val174Ala polymorphism than was found in African American subjects ($P < .05$). An Asn151Ser variant was observed only in Japanese subjects; however, Pro155Thr (16% in European American subjects) and Glu667Gly (34% in African American subjects) were not observed in Japanese subjects. These results indicate that genotypic frequencies of *OATP-C* variants appeared to be dependent on race, as has been previously reported.[15]

Pharmacokinetics of pravastatin and polymorphisms of the OATP-C and OAT3 genes. In the *OAT3* gene, 2 polymorphisms, the synonymous T723A (n = 12) and the nonsynonymous Ala389Val (n = 1), were observed in our 23 healthy volunteers (Table IV). As shown in Table V, there were no remarkable differences in the mean CL_r and CL_{sec} of pravastatin among the 3 genotypic groups.

In the *OATP-C* gene, 7 genotypes (ie, allelic patterns) were observed in our 23 healthy volunteers (Table VI). The mean (±SD) CL_t rates of pravastatin in *OATP-C*1a/*1a*, **1a/*1b*, and **1b/*1b* subjects were 2.66 $L \cdot kg^{-1} \cdot h^{-1}$, 1.95 ± 0.72 $L \cdot kg^{-1} \cdot h^{-1}$, and 2.39 ± 0.44 $L \cdot kg^{-1} \cdot h^{-1}$, respectively (Table VI). Similar to the CL_t, mean CL_{nr} did not differ among the 3 genotypic groups. In contrast, the AUC of pravastatin was increased in subjects with the *OATP-C*15* allele (Table VI). The CL_t and CL_{nr} values were significantly

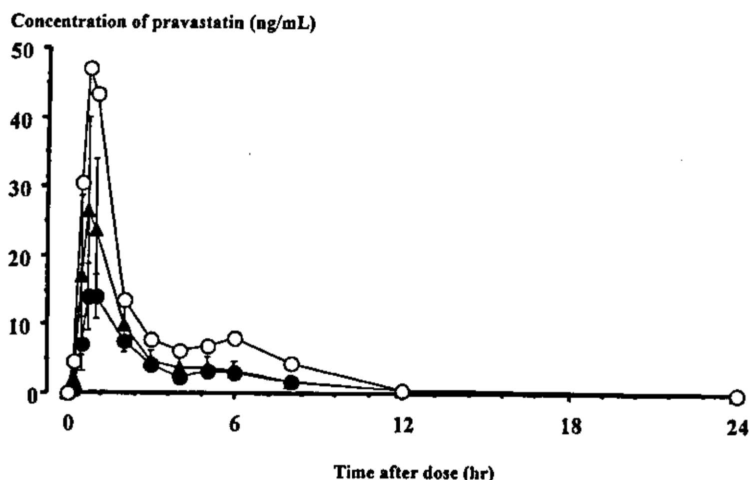

Fig 1. Mean serum concentration over time after a single oral pravastatin dose of 10 mg in 3 organic anion transporting polypeptide C (OATP-C) genotypic groups. *Solid circles, OATP-C*1b/*b* subjects (n = 4); *triangles, *1b/*15* subjects (n = 9); *open circles, *15/*15* subject (n = 1).

lower ($P < .05$) in heterozygotes for the **15* allele (**1b/*15*) compared with homozygotes for the **1b* allele (**1b/*1b*). The subject with the **15/*15* genotype (ie, homozygote for the **15* allele) had the highest AUC value and the lowest CL_t and CL_{nr} values among all study volunteers. Mean serum concentration–time curves of pravastatin in the 3 genotypic groups with regard to the **15* allele are shown in Fig 1.

Close examination of the data in Table VI reveals that CL_r and apparent CL_{sec} values in subjects with the **15* allele were opposite those observed for CL_t and CL_{nr}. Although the difference did not reach the level of significance, both CL_t and CL_{nr} tended to be greater in subjects with the **15* allele. These results suggest that extrarenal (hepatic) clearance is the major determinant for overall clearance of pravastatin. In addition to those with the **15* allele, the subject with the Pro336Arg polymorphism had a relatively high AUC (110.3 ng · h/mL) and low CL_t (1.22 L · kg^{-1} · h^{-1}) and CL_{nr} (0.81 L · kg^{-1} · h^{-1}) clearance values.

DISCUSSION

Recently, various organic anion transporters such as OATPs and OATs have been identified. Cumulative in vitro and in vivo studies indicate that OATP-C and OAT3 are responsible for the hepatic and renal uptake of organic anions, respectively.[6,10,29-31] Thus the hepatoselective distribution and subsequent disposition kinetics of pravastatin found in animal studies are, at least partially, believed to result from transporter-mediated active transport. However, there are no data from human studies on the impact of these polymorphisms on the pharmacokinetics of pravastatin.

Before the functional characterization of the 2 genes of interest, we analyzed genetic polymorphisms in a Japanese population and compared allelic frequencies among different ethnic groups. In our systematic screening for genetic polymorphisms in the human *OATP-C* gene, 3 nonsynonymous variants (Asn151Ser, Pro336Arg, and Cys485Phe) were newly observed. Thus at least 17 nonsynonymous variants have been found to date in the human *OATP-C* gene. Among the nonsynonymous polymorphisms, Asn130Asp and Val174Ala appeared commonly in Japanese subjects, and allelic frequencies of these polymorphisms in this study were in keeping with those of a previous report.[16] These 2 polymorphisms are widespread not only in Japanese subjects but also in white subjects,[15] with total frequencies of between 30% and 60% and between 14% and 16%, respectively (Table III).

It is interesting that, on the basis of haplotype analysis, the *OATP-C*5* allele, which was observed in 14% of European American subjects,[15] was not observed in this study, despite the fact that a Val174Ala polymorphism was present at comparable frequency in the 2 racial populations. In addition, by contrast to the initial haplotype analysis by Tirona et al,[15] in which the *OATP-C*15* allele was absent in European American subjects, the *OATP-C*15* allele was common in Japanese subjects. A Val174Ala polymorphism existed in both **5* and **15* alleles. These results indicate that the frequency of the *OATP-C*15* allele is dependent on