析を通じて、臨床応用可能なバイオマーカーの特定につなげる予定である。腎生検採取翌日の早朝第二尿を用いた尿中タンパク質の検討では、17 種類のタンパク質を同時に検討した。サスペンジョンビーズアレイテクノロジーは、複数種（理論上 100 種）のタンパク質に対する ELISA 反応を、FACS を応用して行う原理である（Fig. 5）。本症例は、キャッスルマン症候群疑いで腎生検を用いた精密診断のために入院された患者であることから、尿中への炎症性サイトカイン等の漏出を疑い、検討した。その結果、Fig. 18 にしめすように、3 種類のタンパク質のみが検出された。タンパク質そのものの安定性も考慮に入れる必要があるが、見いだされたタンパク質 N、O、P は何れも炎症反応に関連しており、本病態を反映することが示唆された。Fig. 15 の病理像においても、糸球体の肥大は軽度であり、近位尿細管刷子縁膜の軽度脱落という診断を受けている。最終的に IL-6 陰性であったことから、IgA 腎症と診断されたが、ヒト尿検体を用いた網羅的な ELISA 系は十分に機能しうることが示された。

ラットにおける単離近位尿細管を用いたマイクロアレイ解析の薬剤性腎障害マーカー探索という検討のため、先ずシスプラチン腎症について検討した。臨床に於いて、シスプラチンは多量の水分負荷（ハイドレーション、約 4L/24 時間）のもとに投与される。また、週 1 回のようなスケジュールも存在することから、臨床に即した軽度尿細管障害を念頭に、5mg/kg、腹腔内投与、2 日目で行った。その結果、5/6 腎摘出ラットでは全く検出されなかった炎症性遺伝子が数多く見いだされ、重金属による尿細管障害の特徴であることが示唆された（Fig. 17）。また、見いだされた遺伝子（i）について単離尿細管と whole kidney における発現変化の経時的な検討を行ったところ、単離近位尿細管ではシスプラチンの投与直後から上昇傾向にあるのに対して、whole kidney を用いた検討では一旦低下した後に上昇するというプロファイルを示した。一方、近位尿細管障害のマーカーである Kim-1 の発現量は、近位尿細管と whole kidney の何れにおいても上昇傾向を示した。これらの結果は、シスプラチンの作用機序に呼応して発現する遺伝子（i）は近位尿細管では、上昇傾向を示すものの、尿細管上皮細胞の毒性発現・障害はそれに引き続くために遺伝子(i)と Kim-1 の相違が認められたと考えられる。従って、薬剤性腎障害発見のためのバイオマーカーは、薬物個々の作用機序を反映したものが適していると考えられた。

驚いたことに、ラットシスプラチン腎症で見いだされた遺伝子（i）は、免疫疾患患者の尿中で見いだされたタンパク質（O）と一致することが判明した。本症例については、上皮細胞刷子縁膜の軽度脱落など、低用量シスプラチン腎症で認められた腎組織像と類似していることか

ら、見いだされた遺伝子（i）は、シスプラチン腎症の早期検出に有用な特異的尿中マーカーとなることが期待された。

E．結論

研究初年度では、ラット単離近位尿細管を用いたマイクロアレイ解析の確立と得られた結果の有用性を明らかにすることができた。また、メタボローム解析並びにプロテオーム解析の結果、バンコマイシン腎症及びシスプラチン腎症の検出に有望な尿中候補分子を見いだすことができた。また、慢性腎不全モデルラットを用いた系統的な解析から、代償性腎不全期における近位尿細管再生のマーカー遺伝子、末期腎不全期における mTOR 阻害薬の有用性を見いだすことができた。さらに、ヒト腎生検組織を用いた遺伝子発現解析系の確立に加えて、尿を用いた検討から軽度近位尿細管障害のマーカー分子の検出に成功した。特に、この分子は低用量シスプラチン腎症の尿中マーカーとしても有用であることが強く示唆された。従って、当初の計画を順調に達成することができ、次年度以降の継続にあたり研究計画の変更は不要であると結論するに至った。

F．健康危険情報

特にない。

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H．知的財産権の出願・登録状況
（予定を含む。）

本研究で見いだしたバイオマーカー候補分子について、京都大学知財部の協力を得ながら順次特許出願をする予定である。

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分担研究報告書

腎組織標本の損傷スコア解析とヒト腎生検組織標本の収集

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研究要旨

腎生検による病理学的診断は、腎臓病患者に対する治療方針の選択に有用性が高い。一方、血清クレアチニン値が高い患者においては、検査そのものの危険性とのバランスから適応外となる。従って、薬剤性 AKI を呈したと考えられる患者由来の生検採取は困難であるが、免疫学的原疾患の腎臓病患者由来の生検組織を用いることによって、非特異的かつ原疾患由来の情報をあらかじめ整理することによって、トランスクリプトームで得られる情報の絞込に極めて重要かつ貴重な情報を提供しうると考えられる。本研究では、IgA 腎症などの確定診断のために腎生検採取の適応となった患者に協力を得て、トランスクリプトームに使用すると同時に、病理学的診断とスコア化を行い、データ解析の一助とすることを目的とした。研究初年度では、12 例の協力を得ることができた。

A. 研究目的

薬剤性腎障害には、患者個々の腎機能に応じた個別化投与設計による回避と並び早期の腎代替療法導入による増悪阻止という双方の適切な対応が求められる。Acute Kidney Injury (AKI)は、直近 48 時間以内における血清クレアチニンや尿量の変化を指標に分類されるが、AKI 発症から血清クレアチニン値上昇に要する時間には個体差が大きく、より迅速かつ的確に AKI を診断するためのバイオマーカーの特定と臨床応用が必要である。

本研究では、ヒト尿検体を中心としたプロテオミクス解析による薬剤性腎障害の非侵襲性マーカーを探索し、その臨床的重要性を明らかにすることを到達目標とする。分担研究者はヒト腎生検の病理組織標本を収集し、それを評価する定量的スコア化システムの構築および臨床的所見（糸球体濾過量、蛋白尿定量およびその分析、尿細管障害指標マーカーなど）を検討することによって、マーカー候補分子の妥当性および臨床的意義を評価す

る。

B. 研究方法

（1）腎生検の採取

確定診断のために腎生検採取の対象となった腎臓病患者のインフォームドコンセントに基づいて、採取した生検組織について、糸球体径、尿細管の肥大、繊維化、浮腫、上皮細胞の脱落等数値化を進める。

（2）倫理面への配慮

本研究は、ヘルシンキ宣言（1975 年、東京総会で修正）を尊重し計画されたものであり、対象患者個人の人権擁護を最優先する。すなわち、自由意志による同意が得られた場合にのみ実施対象とすること、同意した場合でも随時撤回できそれによる不利益を受けないこと、血液や組織由来の核酸が他の目的で使用されないこと、実施対象者の個人識別情報は連結不可能匿名化方式で厳重に管理保護されていること、遺伝子解析結果を含むすべての検査結果については守秘義務を守ること、研究成果の発表に際しては個人が特定できない方法でのみ行うこと、を遵守する。なお、本研究計画は、「腎疾患患者における薬物輸送体の発現量並びに遺伝子多型に関する臨床研究」（G-159、代表者 京都大学医学部附属病院教授・薬剤部長 乾 賢一、申請代表者 増田智先及び分担研究者 深津敦司は分担研究者として参画している）という題目で、京都大学医学研究科・医学部医の倫理委員会に審査を受け、研究科長より承認書が交付されており（平成17年7月6日付け）、平成17年6月29日に改正された「ヒトゲノム・遺伝子解析研究に関する倫理指針」（文部科学省、厚生労働省、経済産業省）を遵守するものである。

C. 研究結果

現時点で収集した腎生検組織の原疾患としては、IgA 腎症（59%）、腎硬化症（18%）、巣状糸球体硬化症（FGS、9%）、サルコイドーシス（9%）、Schonlein-Henoch 紫斑病（9%）などである。

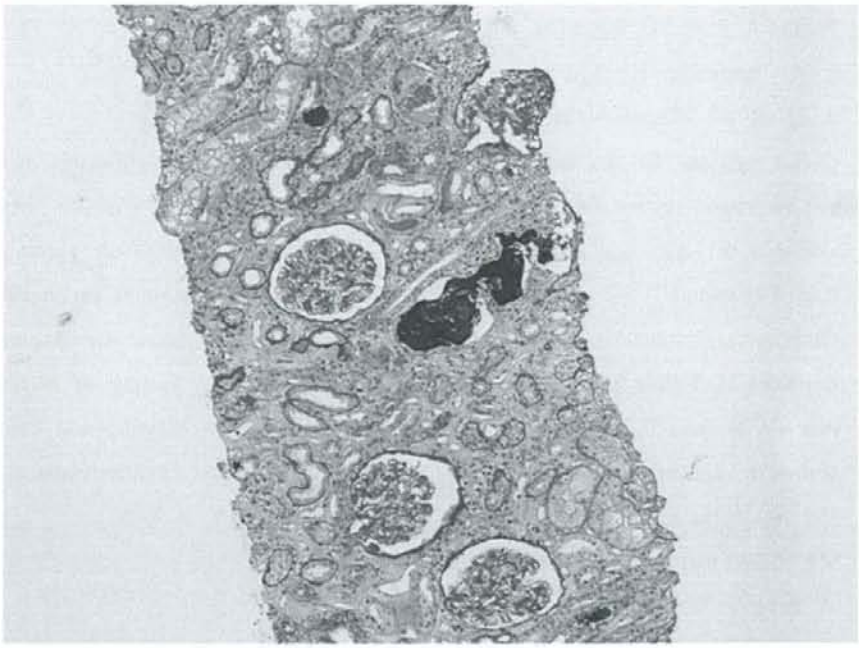
腎生検入院中にイヌリンクリアランスで GFR を測定し、尿タンパク量、尿中NAGなどを計測した。

病理組織標本の評価としては、以下のようなパラメーターを5段階で評価する半定量的システムを作成した。

1　糸球体病変
　糸球体径、半月体、メサンギウム細胞増殖、糸球体硬化面積
2　尿細管／間質病変
　間質病変（線維化および浮腫）の範囲　（障害された面積/標本面積）
　尿細管上皮細胞の障害度　壊死、拡張、細胞変性、線維化
　細胞浸潤

以下に収集した組織所見の一例を示す。

この症例は尿細管間質性腎炎の例である。写真に見られる通り糸球体にはあまり変化なく、間質障害が主体であり、かなりの部分が線維化に陥っていて尿細管の萎縮が著明である。



一部の線維化に陥っていない尿細管細胞も膨満しており障害がわかる。この症例は原因となる薬剤は判明しなかった。蛋白尿は0．3g/日で主に尿細管性と考えられる。障害された尿細管間質は標本の約70%である。Ccrは54ml/分で腎機能障害が見られる。NAG、β2MGとも高値で臨床的にも間質性腎炎と考えられた。

この症例の尿中での尿細管の障害の指標となるバイオマーカーを探索し、尿細管に発現する各種トランスポーターの発現と比較し尿中の物質から尿細管トランスポーターの機能が推測できないか調べる。

D.E. 現時点では未だ結論および考察をする段階ではない。本年度に収集したサンプルに関して来年度解析を行なう。

G. 研究発表

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H. 知的財産権の出願・登録状況
（予定を含む。）

本研究で見いだしたバイオマーカー候補分子について、京都大学知財部の協力を得ながら順次特許出願をする予定である。

III. 研究成果の刊行に関する一覧表

雑誌

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Kimura et al.	Transport of guanidine compounds by human organic cation transporters, hOCT1 and hOCT2.	Biochem Pharmacol	77: 8	1429-1436	2009
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Mima et al.	Acute renal failure after exercise in a Japanese sumo wrestler with renal hypouricemia.	Am J Med Sci	336: 6	512-514	2008
Tanaka et al.	Expression of BMP-7 and USAG-1 (a BMP antagonist) in kidney development and injury.	Kidney Int	73: 2	181-191	2008

IV. 研究成果の刊行物・別刷

Pharmaceutical Research (© 2009)
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Research Paper

MDR1 Haplotypes Conferring an Increased Expression of Intestinal CYP3A4 Rather than MDR1 in Female Living-Donor Liver Transplant Patients

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Purpose. This study investigated whether haplotypes in the multidrug resistance 1 (*MDR1*) gene had effects on mRNA expression levels of MDR1 and cytochrome *P*450 (CYP) 3A4, and on the pharmacokinetics of tacrolimus in living-donor liver transplant (LDLT) patients, considering the gender difference.

Methods. Haplotype analysis of *MDR1* with G2677T/A and C3435T was performed in 63 *de novo* Japanese LDLT patients (17 to 55 years; 44.4% women). The expression levels of MDR1 and CYP3A4 mRNAs in jejunal biopsy specimens were quantified by real-time PCR.

Results. Intestinal CYP3A4 mRNA expression levels (amol/μg total RNA) showed significantly higher values in women carrying the 2677TT-3435TT haplotype (median, 10.7; range, 5.92–15.2) than those with 2677GG-3435CC (3.03; range 1.38–4.68) and 2677GT-3435CT (median, 4.31; range, 0.07–9.42) (P=0.022), but not in men (P=0.81). However, *MDR1* haplotype did not influence mRNA expression levels of MDR1 nor the concentration/dose ratio [(ng/mL)/(mg/day)] of oral tacrolimus for the postoperative 7 days, irrespective of gender.

Conclusion. *MDR1* haplotype may have a minor association with the tacrolimus pharmacokinetics after LDLT, but could be a good predictor of the inter-individual variation of intestinal expression of CYP3A4 in women.

KEY WORDS: ABCB1; P-glycoprotein; small intestine; tacrolimus.

INTRODUCTION

P-glycoprotein (Pgp), encoded by the multidrug resistance 1 (*MDR1*, also known as *ABCB1*) gene, is expressed in several organs, including the intestine, liver, and kidney, and mediates the detoxification of numerous drugs (1–3). To date, more than 50 single nucleotide polymorphisms (SNPs) have been reported for the *MDR1* gene (4–6). Among them, the most frequently studied SNPs are the G2677T/A transversion (A893S) in exon 21 and the synonymous C3435T transition in exon 26. *MDR1* SNPs C3435T and G2677T/A have been shown to affect the expression of Pgp as well as cytochrome P450 (CYP) 3A4 (7,8). We previously demonstrated that 3435TT was associated with a lower expression of enterocyte CYP3A4 mRNA than 3435CC in living-donor liver transplant (LDLT) patients, mainly pediatric patients (7). On the other hand, Lamba *et al.* (8) found increased expression of enterocyte CYP3A4 in 2677TT genotype, although not significantly.

Conflicting results have also been reported for the association of *MDR1* SNPs with the pharmacokinetics of tacrolimus, a substrate of both Pgp and CYP3A4 (9–11). Kim *et al.* (12) showed that 3435TT had significantly higher dose-normalized tacrolimus concentrations than 3435CC in renal transplant patients. In contrast, others have shown that this SNP had no significant influence on tacrolimus dose requirements in renal transplant patients (11). Meanwhile, G2677T/A SNPs have been shown to influence blood concentrations of tacrolimus in pediatric heart transplant patients (10), but not in liver transplant patients (7).

Although there are discrepancies in these clinical findings, some studies have shown that G2677T/A and C3435T were linked at the *MDR1* gene (12,13), and it has been suggested that *MDR1* haplotype derived from G2677T/A and C3435T may be a more useful marker of Pgp activity than individual SNPs (14). Furthermore, the remarkable gender differences have been reported in the expression of CYPs and transporters (15,16).

In the present study, we examined whether *MDR1* haplotype derived from the G2677T/A and C3435T could affect the mRNA expression levels of MDR1 and CYP3A4 in the native intestine as well as the graft liver, and the pharmacokinetics of tacrolimus in LDLT patients, considering the influence of gender.

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ABBREVIATIONS: C/D, Concentration/dose; CYP, Cytochrome *P*450; LDLT, Living-donor liver transplantation; MDR, Multidrug resistance; Pgp, P-glycoprotein; SNP, Single nucleotide polymorphism.

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Table I. Characteristics of Patients

Variables	All	Men	Women
No. of LDLT patients, *n*	63	35	28
Age, years	43.3±10.5	45.2±9.3	40.9±11.7
Body weight, kg	62.3±12.4	68.3±10.8	54.7±10.0
ABO blood group match (identical/compatible/incompatible), n	41/ 6 / 16	25 / 6 / 4	16 / 0 / 12
Primary disease, *n*			
Cirrhosis	43	30	13
Primary biliary cirrhosis	8	1	7
Primary sclerosing cholangitis	3	3	0
Biliary atresia	3	0	3
Fulminant hepatic failure	1	0	1
Others[a]	5	1	4
No. of donors, *n*	63	33	30
Donor age, y	43.3±10.5	40.7±13.5	47.3±10.7

Data are expressed as number or mean±SD
[a] The primary disease was Budd–Chiari syndrome, nonalcoholic steatohepatitis, somatostatinoma, Wilson, or Caroli disease

MATERIALS AND METHODS

Patients and Clinical Samples

This study included 63 *de novo* LDLT patients (all Japanese), aged 17 to 55 years, who were treated with tacrolimus (Prograf®, Astellas Pharma, Tokyo, Japan), and their corresponding donors (Table I). Both the patients and their donors, having first provided written informed consent, were enrolled consecutively between April 2004 and December 2007.

Clinical samples of the upper jejunum were obtained from a part of the Roux-en-Y limb for biliary reconstruction, and liver samples (2 mm cubic) were obtained from biopsy specimens for pathological testing of the graft at surgery (zero biopsy) (17). This study was conducted in accordance with the Declaration of Helsinki and its amendments, and was approved by *the Kyoto University Graduate School and Faculty of Medicine, Ethics Committee.*

Quantitation of mRNA Expression

Clinical samples of the upper jejunum and liver were immediately frozen in liquid nitrogen and stored at -80°C until used (18). The mRNA expression levels of CYP3A4 and MDR1 were quantified as described previously (7,18). Briefly, total RNA was isolated from biopsy specimens of the intestinal mucosa and graft liver, using MagNAPure LC RNA Isolation kit II (Roche, Mannheim, Germany), and was reverse-transcribed by Superscript II® reverse transcriptase (Invitrogen, Carlsbad, CA, USA) with random primers (100 ng/reaction) and digested by RNase H (Invitrogen). Real-time polymerase chain reaction (PCR) was performed using the ABI Prism 7700 Sequence Detector (Applied Biosystems, Foster, CA, USA).

Genotyping

Genomic DNA was extracted from peripheral blood of transplant patients or donors with Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA). Using this genomic DNA, the *MDR1* polymorphisms were detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method with specific primers (Table II), as described previously (7). *MDR1* haplotypes were analyzed using SNPAlyze ver. 5.0 (Dynacom, Chiba, Japan).

Dosage Regimen of Tacrolimus and Measurement of Tacrolimus Concentrations

The basic immunosuppressive regimen consisted of tacrolimus with low-dose steroids (18). Tacrolimus was administered orally at a dose of 0.075 mg/kg body weight every 12 h from the evening of postoperative day 1 (18,19). The target whole-blood trough concentration of tacrolimus was set at between 10 and 15 ng/mL during the first 2 weeks. Steroid treatment was started at graft reperfusion at a dose of 10 mg/kg, with a gradual reduction from 2 mg kg^{-1} day^{-1} to 0.3 mg kg^{-1} day^{-1} during the first 2 weeks after surgery. The dosage of tacrolimus was adjusted on the basis of whole-blood trough concentrations measured about 12 h after the evening dosage every day, by use of a semiautomated microparticle enzyme immunoassay (IMx®; Abbott, Tokyo, Japan) (19).

Statistical Analysis

Median values of the expression of MDR1 and CYP3A4 were compared among the haplotypes using the Kruskal–

Table II. Primer Sequences Used for Amplification of PCR Fragments

SNP	Primer sequence
Exon 21 G2677A	F: 5′-TGCAGGCTATAGGTTCCAGG-3′
	R: 5′-GTTTGACTCACCTTCCCAG-3′
Exon 21 G2677T	F: 5′-TGCAGGCTATAGGTTCCAGG-3′
	R: 5′-TTTAGTTTGACTCACCTTCCCG-3′
Exon 26 C3435T	F: 5′-TGTTTTCAGCTGCTTGATGG-3′
	R: 5′-AAGGCATGTATGTTGGCCTC-3′

Table III. Effects of *MDR1* Haplotypes (G2677T/A-C3435T) on the Expression of MDR1 and CYP3A4 in Native Intestine and Graft Liver

MDR1 haplotypes (G2677T/A-C3435T)	Native intestine (all recipients)			Graft liver (All donors)		
	n (%)	MDR1 mRNA	CYP3A4 mRNA	*n* (%)	MDR1 mRNA	CYP3A4 mRNA
GG-CC	6 (9.5)	0.23 (0.09–0.32)	3.8 (1.2–7.1)	10 (15.9)	0.84 (0.38–2.3)	70.6 (11.1–107.8)
GG-CT	2 (3.2)	0.09 (0.02–0.15)	3.3 (0.04–6.6)	1 (1.6)	2.9	129.6
GA-CC	9 (14.3)	0.19 (0.02–0.94)	4.8 (0.005–16.4)	7 (11.1)	0.79 (0.51–1.4)	56.7 (19.6–84.6)
GA-CT	1 (1.6)	0.27	4.0	3 (4.7)	0.42 (0.39–1.4)	54.1 (46.5–64.7)
GT-CC	2 (3.2)	0.33 (0.24–0.42)	14.7 (7.1–22.2)	3 (4.7)	0.97 (0.93–1.5)	132.0 (73.6–157.0)
GT-CT	24 (38.0)	0.24 (0.03–1.1)	4.1 (0.07–15.7)	18 (28.6)	0.89 (0.42–2.4)	55.5 (18.0–131.5)
GT-TT	1 (1.6)	0.05	2.4	1 (1.6)	1.1	40.3
AA-CC	1 (1.6)	0.12	0.93	1 (1.6)	0.60	31.6
AT-CC	0	–	–	2 (3.2)	0.53 (0.52–0.54)	35.5 (30.5–40.4)
AT-CT	5 (7.9)	0.12 (0.01–0.46)	4.3 (0.004–10.2)	4 (6.4)	0.92 (0.61–1.2)	64.7 (20.0–71.3)
TT-CT	1 (1.6)	0.84	22.7	3 (4.7)	0.88 (0.44–1.4)	70.5 (64.8–88.2)
TT-TT	10 (15.9)	0.29 (0.12–0.57)	6.3 (0.37–15.1)	10 (15.9)	0.96 (0.45–2.6)	43.9 (12.0–168.4)

Data are expressed as median (range). We excluded one intestinal sample (GG-CC) from the analysis for undetectable values in the mRNA expression

Wallis test, followed by the Dunn *post hoc* test for multiple comparisons. Data are expressed as the median and range or mean±SD, depending on type. For all analyses, $P<0.05$ was considered statistically significant. All statistical analyses were conducted using GraphPad PRISM, version 4 (GraphPad Software, San Diego, CA, USA).

RESULTS

Frequencies of MDR1 SNPs

Allele frequencies of the 2677G, 2677T and 2677A were 43.1%, 43.1% and 13.8% in recipients, and 43.1%, 42.5% and 14.4% in donors, respectively. For C3435T, the frequencies of the 3435C and 3435T were 56.2% and 43.8% in recipients, and 58.7% and 41.3% in donors, respectively. The frequencies of genotypes in both recipients (G2677T/A, $P=0.41$; C3435T, $P=0.80$) and donors (G2677T/A, $P=0.54$; C3435T, $P=0.93$) complied with Hardy-Weinberg equilibrium. Construction of haplotypes, via estimation of maximization, resulted in 4 major (GT-CT, TT-TT, GA-CC, and GG-CC) and 8 minor haplotypes (GG-CT, GA-CC, GA-CT, GT-CC, GT-TT, AA-CC, AT-CT, and TT-CT) (Table III).

Effects of *MDR1* Haplotypes on the mRNA Expression Levels of MDR1 and CYP3A4

We evaluated the relationships between the *MDR1* haplotypes (G2677T/A-C3435T) and the mRNA expression levels (amol/μg total RNA) of MDR1 as well as CYP3A4 (Table III). We excluded one intestinal sample (GG-CC) from the analysis for undetectable values in the mRNA expression. The TT-TT haplotype tended to have a higher mRNA expression of intestinal CYP3A4, but did not affect the expression levels of intestinal MDR1. Stratified by gender, 2677TT-3435TT haplotype in the native intestine conferred a significantly higher CYP3A4 mRNA expression levels (median, 10.7; range, 5.92–15.2) than 2677GG-3435CC (3.03; range 1.38–

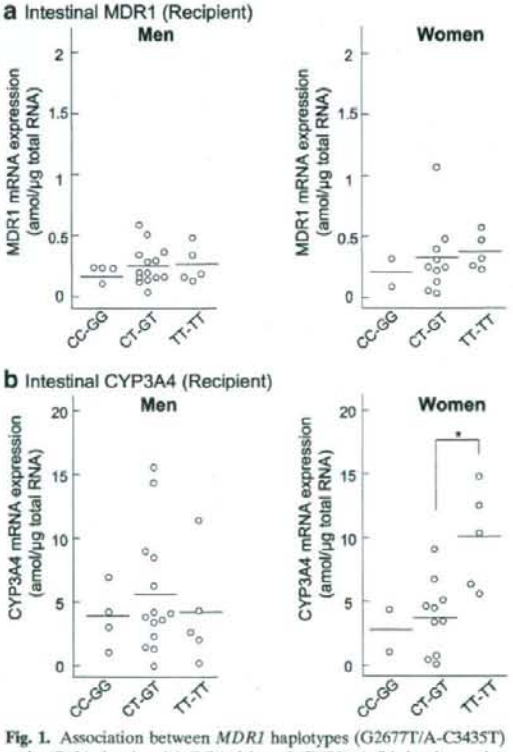

Fig. 1. Association between *MDR1* haplotypes (G2677T/A-C3435T) and mRNA levels of MDR1 (**a**) and CYP3A4 (**b**) in the native intestine (recipient). We excluded one intestinal sample (GG-CC) from the analysis for undetectable values in the mRNA expression. *$P<0.05$, significant difference between *MDR1* haplotype groups. The *bars* show the median mRNA expression levels in each haplotype.

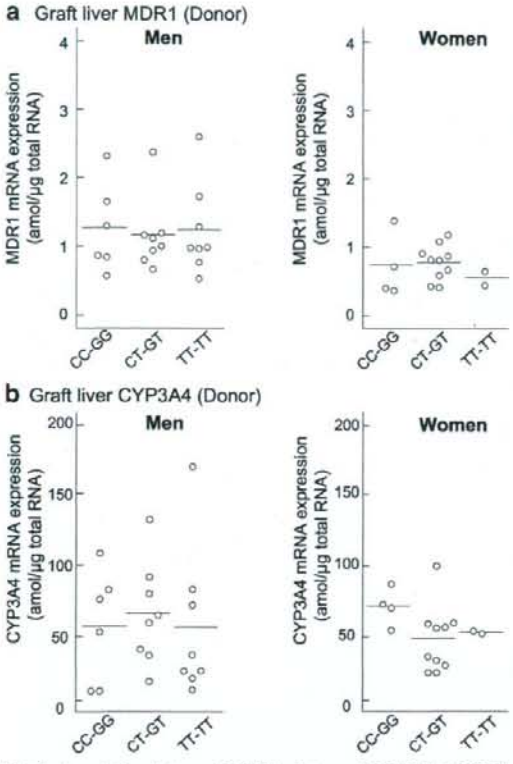

Fig. 2. Association between *MDR1* haplotypes (G2677T/A-C3435T) and mRNA levels of MDR1 (**a**) and CYP3A4 (**b**) in the graft liver (donor). The *bars* show the median mRNA expression levels in each haplotype.

4.68) and 2677GT-3435CT (median, 4.31; range, 0.07–9.42) haplotypes in women (P=0.022), but not in men (P=0.81) (Fig. 1 b). There were no significant differences in the mRNA expression levels of intestinal MDR1 among *MDR1* haplotypes (Fig. 1 a). In addition, we found no significant association between *MDR1* haplotypes and the expression of MDR1 and CYP3A4 in the graft liver, irrespective of gender (Fig. 2).

Effects of *MDR1* Haplotypes on the Pharmacokinetics of Tacrolimus

Next, to assess whether the *MDR1* haplotypes affect the pharmacokinetics of tacrolimus, we examined the concentration/dose (C/D) ratio of tacrolimus in LDLT patients for the first week after the surgery (Table IV). There was no significant difference in the C/D ratio of tacrolimus among the *MDR1* haplotypes.

DISCUSSION

Previously, we analyzed 10 common SNPs, including G2677T/A and C3435T in the *MDR1* gene in 46 LDLT recipients aged 0.6 to 59.6 years, and found that individual SNPs did not relate to either the intestinal expression of MDR1 mRNA or the C/D ratio of tacrolimus (7). In the present study, we restricted analysis to subjects aged 17 to 55 years in a larger cohort, and focused on *MDR1* haplotypes for the most common SNPs, G2677T/A and C3435T, in the mRNA expression of MDR1 as well as CYP3A4, and the pharmacokinetics of tacrolimus in 63 LDLT recipients, with consideration of the gender difference. Our results revealed that 2677TT-3435TT haplotype had significantly higher levels of intestinal CYP3A4 mRNA than those with 2677GT-3435CT haplotype in women (P=0.022), but not in men (P=0.81). To our knowledge, this is the first study to reveal the female-specific effect of *MDR1* haplotype on the intestinal expression of CYP3A4 mRNA.

To date, more than 30 allelic variants in the *CYP3A4* gene have been identified, but low variant frequencies, often combined with a lack of functional consequences, indicate their limited contribution to the large inter-individual variations in CYP3A4 expression (20,21). Hirota *et al.* (22) demonstrated using liver tissues from eight Caucasians that the whole CYP3A4 gene was sequenced, but none of the SNPs in the 5'-flanking region and 3'-UTR of the *CYP3A4* gene was associated with differences in total CYP3A4 mRNA levels and testosterone 6β-hydroxylation capability. Therefore, some genetic markers in addition to SNPs in the *CYP3A4* gene could be useful to explore the large inter-individual variation of CYP3A4 content, including its enzymatic capacity.

Previous studies reported that *MDR1* genotypes were associated with mRNA expression levels of enterocyte CYP3A4 (7,8). For example, Lamba *et al.* (8) found that carriers of variant alleles of *MDR1* gene tended to have

Table IV. Effects of intestinal *MDR1* Haplotypes (G2677T/A-C3435T) on the C/D Ratio of Tacrolimus in LDLT Patients for the Period of 1–7 Days Post Transplantation

	All		Men		Women	
MDR1 haplotypes (G2677T/A-C3435T)	*n*	C/D ratio of tacrolimus [(ng/mL)/(mg/day)]	*n*	C/D ratio of tacrolimus [(ng/mL)/(mg/day)]	*n*	C/D ratio of tacrolimus [(ng/mL)/(mg/day)]
GG-CC	7	2.0 (1.5–10.8)	4	1.7 (1.5–10.8)	3	3.7 (1.8–6.9)
GT-CT	24	4.2 (1.3–18.7)	14	3.6 (1.3–17.6)	10	4.7 (2.3–18.7)
TT-TT	10	3.9 (2.1–12.6)	5	2.5 (2.1–4.3)	5	8.6 (3.4–12.6)
P-value		0.37		0.51		0.36

Data are expressed as median (range)

increased expression of enterocyte CYP3A4, in the analysis of three separate cohorts (n=20, 27, and 10, respectively). However, their intestinal study populations were too small to detect differences among the *MDR1* polymorphisms by gender. Because the remarkable gender differences have been reported in the expression of CYPs and transporters (15,16), we hypothesized that the expression of CYP3A4 associated with *MDR1* genotypes would differ by gender. In the present study, we showed that *MDR1* G2677T/A-C3435T haplotypes significantly influenced the intestinal expression in women (Fig. 1). These results suggest that the *MDR1* haplotype may be a useful predictor of the inter-individual variability of the intestinal expression of CYP3A4 and the extent of some CYP3A4-mediated drug interactions in women.

The exact mechanism by which *MDR1* G2677T/A-C3435T haplotypes influence CYP3A4 expression in women remains unknown. In our results, we observed no significant difference in MDR1 mRNA expression among *MDR1* G2677T/A-C3435T haplotypes (Fig. 1 a), but it has been shown that "silent" polymorphisms (in particular, C3435T) in the *MDR1* gene can alter Pgp conformation and substrate specificity, especially when no change in MDR1 mRNA and protein levels has been reported (23). Furthermore, *in vitro* studies showed that G2677T/A-C3435T haplotypes can reduce the activity of Pgp (14). Therefore, reduced function of Pgp could possibly lead to high intracellular concentrations of endogenous regulators such as sex-steroid hormones, regulating the expression of CYP3A4 (24–27). In contrast, we found no significant differences in the hepatic expression of CYP3A4 among *MDR1* haplotypes for G2677T/A-C3435T in women. These opposing effects of the same haplotypes on hepatic and intestinal mRNA expressions of CYP3A4 in women could be partly due to differences in the underlying mechanism between the two types of organ.

Furthermore, we found that intestinal *MDR1* haplotypes had no effects on the C/D ratio of tacrolimus in LDLT patients (Table IV). Some studies have reported that 2677T or 3435T alleles affected the pharmacokinetics of tacrolimus in Caucasians (9,28,29), while others demonstrated contrary results in Asians (7,30). Similar discrepancies have been observed for digoxin (31). These might reflect disparities in different frequencies of *MDR1* haplotypes in different ethnicities (32,33). The 2677TT-3435TT haplotype is found in 42% of Caucasians and 8% of African-Americans (34), while 15.9% in the present Japanese population (Table III). Based on the present results and these previous findings, the *MDR1* haplotype is suggested to have a minor effect on the pharmacokinetics of tacrolimus in Asians compared with Caucasians.

CONCLUSION

In conclusion, the *MDR1* haplotype derived from G2677T/A and C3435T was significantly associated with intestinal CYP3A4 mRNA expression in women, but not in men, suggesting that it could be a good marker to predict the basal mRNA level of intestinal CYP3A4 in women. However, this effect was not observed for the pharmacokinetics of tacrolimus in LDLT patients. Therefore, extensive clinical pharmacokinetic studies are necessary to elucidate this effect on other drugs which are CYP3A4 substrates, in consideration of gender-differences in pharmacokinetics.

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