Table 2. Patient Profiles During the Operation

Patient No.	Arterial Anastomosis		Hepatic Vein Anastomosis		Portal Vein Anastomosis		Warm Ischemic Time*	Operation Time
	Donor	Recipient	Donor	Recipient	Donor	Recipient		
1	LHA	PHA	LHV	Hepatic venous cloaca	LPV	PV trunk with interposed graft	56 min	14 hr 35 min
2	LHA	PHA	V2	Anatomical RHV	LPV	PV trunk with interposed graft	50 min	16 hr 22 min
			V3	Anatomical LHV+MHV				
3	LHA	RHA	LHV	Hepatic venous cloaca	LPV	PV trunk with branch patch method	35 min	10 hr 30 min
4	LHA	PHA	LHV	Hepatic venous cloaca	LPV	PV trunk	50 min	9 hr 36 min

Abbreviations: LHA, left hepatic artery; PHA, proper hepatic artery; RHA, right hepatic artery; V2, hepatic vein from segment 2; V3, hepatic vein from segment 3; LPV, left portal vein. All bile duct reconstruction procedures were performed using a Roux-en-Y choledochojejunostomy.
*Warm ischemic time was defined as the time from implantation of the liver graft to reperfusion of the portal flow.

Case 3

A 1-year 6-month-old girl with a history of BA presented with progressive hepatic failure. The patient had undergone a Kasai operation at the age of 8 months, at which time she was noted to have SI. LDLT was performed using a lateral segment graft from her mother. After the hepatectomy, the liver was transplanted in the usual manner, and a branch patch method was used for PV reconstruction. The posttransplant course was uneventful.

Case 4

A girl underwent a duodenocolonic dissociation (LADD) procedure and a duodenoduodenostomy for malrotation of the intestine and duodenal atresia at the age of 9 days, at which time she was noted to have SI and dextrocardia. At the age of 7 months, liver dysfunction and jaundice were pointed out, and she underwent exteriorization of the gall bladder, which was followed by a Kasai operation, because of persistent jaundice. Liver transplantation was scheduled for the deteriorating liver function at the age of 9 months. LDLT was performed using a reduced monosegmental graft from her mother. After a hepatectomy, the graft was placed in an LUQ. The retrohepatic IVC was absent, and the HV drained directly through the diaphragm into the right atrium. There was a discrepancy in diameter between the graft LHV and recipient HV, therefore, the anastomosis was performed end-to-end using an interrupted suture with 5-0 Prolene. Other reconstruction was performed in the usual manner. Her intensive care unit stay was extended by a respiratory disorder; however, there were no major complications during the postoperative course.

Discussion

Anatomical variations, especially vascular anomalies, increase the technical difficulties of liver transplantation and previously resulted in a high rate of mortality. Therefore, the technical aspects of performing liver transplantation in the present group of patients was more challenging than simply overcoming the problem of a mirror-image anatomy of the liver. Raynor et al.[4] was the first to report a successful DDLT procedure for a patient with SI, and later Farmer et al.[5] reported the results of DDLT for BA with SI in 6 patients. They concluded that the presence of SI requires technical modifications but does not significantly change the outcome.

There are only three reports of LDLT for these patients so far, despite the fact that the procedure has become a standard option for patients with end-stage liver disease.[6,8,9] The technical difficulties of performing LDLT for BA with SI have not been fully elucidated.

In the LDLT for the patient with an absence of the retrohepatic IVC, the direction and diameter of the graft HV and the recipient suprahepatic cava are important factors for successful HV reconstruction. We added two modifications to the usual end-to-end anas-

tomosis between the donor HV and recipient suprahepatic cava: (1) to prevent torsion and keep the direction of the HV anastomosis, the falciform ligament was fixed to the abdominal wall, and Doppler ultrasonography was used for real-time evaluation of the graft blood supply at each stage of the abdominal closure; and (2) to overcome the size discrepancy of anastomotic orifices, an interrupted suture method was used, which was the first time for us.

A preduodenal PV, which was the predominant anomaly in patients with SI, can be hazardous unless properly recognized during a hepatectomy. Most recent reports have noted that if a preduodenal PV is sufficiently mobilized and not injured, the anomaly rarely interferes with a standard end-to-end anastomosis.[4,5,8] In LDLT, PV reconstruction was also successfully performed with an end-to-end anastomosis, and preduodenal reconstruction was feasible and effective. Reconstruction of the PV in preduodenal position makes the PV straight and prevents kinking.[10] Since the graft from a living donor has a short PV and the recipient PV is quite sclerotic and small in BA cases, due to cholangitis or previous multiple laparotomies, vascular grafts for the PV reconstruction might be useful to overcome the problems. In addition, use of an interrupted suture for PV reconstruction in cases presenting a diameter-size discrepancy may contribute to preventing postoperative stenosis.

An aberrant HA supply is one of the most frequent anatomic variations seen in children with SI. Mattei et al.[6] found an aberrant HA supply, which included replaced and accessory HA branches, in 35% of patients with SI who had undergone liver transplantation. The majority of the variations reported were an HA originating from the superior mesenteric artery, a left HA originating from the left gastric artery, and a right HA originating from the celiac artery.[3,6,8] In our series, a proper HA was found arising directly from the supraceliac aorta in 3 of the 4 cases, however, we found only a single report of a patient with the same type of HA anomaly.[5] In LDLT, it is important to prevent damage when recipient HA dissection is performed during the hepatectomy because the artery of the hepatic graft is often short.

In LDLT for children, a lateral segment graft or left lobe graft is generally used.[7] However, in small infants, implantation of left lateral segment grafts can be a problem because of the large-for-size graft. To relieve the problem of large-for-size grafts in small infants, monosegmental LDLT and reduced monosegmental LDLT were recently performed by our group.[10] The indication for using these techniques was infants with an estimated graft-to-recipient weight ratio of greater than 4.0%. During those procedures, the segmental graft was safely placed in the left subphrenic space, and a suitable orientation was obtained for anastomosis of the hilar vessels. In these cases, a partial liver graft from a living-donor graft or split liver graft appears to be an optimal choice.

The present series represents the largest reported group of patients with BA complicated by SI who underwent successful LDLT. Complex anomalies associated with SI increase the technical difficulties of the operation. Additional caution is required when performing LDLT because a living-donor graft has short vessels and the availability of vascular grafts from the donor is limited. However, LDLT for BA complicated by SI can be managed successfully with technical modifications and scrupulous attention.

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Effect of intestinal CYP3A5 on postoperative tacrolimus trough levels in living-donor liver transplant recipients

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It has been reported that hepatic and intestinal cytochrome P450 (CYP) 3A4, CYP3A5 and P-glycoprotein affect the pharmacokinetics of tacrolimus, and that these proteins are associated with the large inter-individual variation in the pharmacokinetics of this drug. We previously showed that the concentration/dose ratio of tacrolimus tended to be lower in recipients of living-donor liver transplantation (LDLT) with a *CYP3A5*1/*1*-carrying graft. However, the effect of intestinal CYP3A5 remains to be elucidated. In the present study, we examined the *CYP3A5* genotype in both recipients and donors, and the effect of the recipients' polymorphism on the concentration/dose ratio of tacrolimus in patients after LDLT. The *CYP3A5*3* allele frequency was 80% in recipients and 77% in donors. The intestinal CYP3A5 mRNA expression level was significantly associated with genotype. The tacrolimus concentration/dose ratio was lower in recipients with the *CYP3A5*1/*1* and **1/*3* genotype (CYP3A5 expressors) compared to the *CYP3A5*3/*3* genotype (non-expressors). Amongst the combination of *CYP3A5* genotypes between the graft liver and the native intestine, CYP3A5 expressors in both the graft liver and the native intestine had the lowest concentration/dose ratio of tacrolimus during 35 days after LDLT. Patients with the intestinal *CYP3A5*1* genotype tended to require a higher dose of tacrolimus compared to the other group with the same hepatic *CYP3A5* genotype. These results indicate that intestinal CYP3A5, as well as hepatic CYP3A5, plays an important role in the first-pass effect of orally administered tacrolimus. *Pharmacogenetics and Genomics* 16:119–127 © 2006 Lippincott Williams & Wilkins.

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Introduction

Tacrolimus is widely used as a primary immunosuppressive agent in patients after solid organ transplantations. However, it is difficult to maintain an appropriate blood concentration of tacrolimus because of a narrow therapeutic index and remarkable intra- and inter-individual diversity [1,2]. Tacrolimus is metabolized by CYP3A4 and CYP3A5 in the liver and intestine, and subsequently pumped out by P-glycoprotein (Pgp), an ATP-dependent efflux pump, encoded by the multidrug resistance 1 (*MDR1/ABCB1*) gene [3–5]. Therefore, these proteins are considered to have a major influence on the first-pass effect in the liver and the intestine, and on the variations in tacrolimus pharmacokinetics. Previously, we have demonstrated that the enterocyte mRNA expression level of MDR1 was inversely correlated to the concentration/dose (C/D) ratio of tacrolimus immediately after living-donor liver transplantation (LDLT) [6].

Several single nucleotide polymorphisms (SNPs) in the genes encoding these proteins may have potential influences on the pharmacokinetics of tacrolimus. The *CYP3A5*1* (wild-type) allele achieved lower dose-normalized tacrolimus blood concentrations compared to *CYP3A5*3*(A6986G)/**3* homozygotes in renal transplant patients [7,8]. Therefore, *CYP3A5*1* or *CYP3A5*3* may be a key factor in the pharmacokinetics of tacrolimus. Because the genotype of *CYP3A5* in both the liver and the intestine of patients receiving other transplantations, including the kidney, heart, lung and stem cells is the same, the contribution of hepatic or intestinal CYP3A5 on the pharmacokinetics of tacrolimus is unclear. Previously, we found that recipients of a *CYP3A5*1/*1*-carrying graft tended to have a lower C/D ratio of tacrolimus than those with a *CYP3A5*3/*3*-carrying graft 3 weeks after LDLT, but the difference was not statistically significant [9]. Based on this background, intestinal CYP3A5 was hypothesized to be a factor affecting the postoperative variation in the pharmacokinetics of tacrolimus in patients receiving LDLT.

In the present study, we examined the *CYP3A5* genotype of both recipients and donors to clarify the role of hepatic

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and intestinal CYP3A5 in the pharmacokinetics of tacrolimus after LDLT with more subjects, focusing on the combination of *CYP3A5* genotype in the graft liver and the native intestine.

Materials and methods

Patients and clinical samples

Between October 2001 and December 2004, 204 recipients and 206 donors were enrolled in this study having first provided their written informed consent. Two recipients were excluded from this study, because we had no data on intestinal CYP3A5 genotype or the expression levels of mRNA. Recipients with end-stage liver disease underwent a LDLT at Kyoto University Hospital. The donor was a parent in 106 cases, a spouse in 42, an offspring in 31, a sibling in 21, a grandmother in two, an uncle in two, an aunt in one and a nephew in one. The demographics of the recipients and donors, including age, sex, body weight, graft-to-recipient body weight ratio (GRWR, %) and primary diseases, are listed in Table 1. Clinical samples of the upper jejunum were obtained from part of the Roux-en-Y limb for biliary reconstruction, and liver samples were obtained from part of the biopsy specimens for pathological testing of the graft at surgery (zero biopsy) [10]. 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.

Table 1 Recipient and donor demographics

	Recipient (n=204)	Donor (n=206)
Age		
Range (years)	0.25–70	18–64
Median (years)	19	37
Sex		
Male	110	117
Female	94	89
Body weight		
Range (kg)	3.7–99	
Median (kg)	43.5	
Graft-to-recipient body weight ratio (%)		
Range	0.49–4.95	
Median	1.38	
ABO blood group match		
Identical	125	
Compatible	41	
Incompatible	38	
Primary disease		
Biliary atresia	74	
Cirrhosis	73[a]	
Primary biliary cirrhosis	11	
Primary sclerosing cholangitis	6	
Fulminant hepatic failure	2	
Alagille sydrome	3	
Post living-donor liver transplantation	14	
Others	21[b]	

[a]Cirrhosis was induced by hepatitis C virus, hepatitis B virus, alcohol and unknown.
[b]The primary disease was Byler disease, Caroli disease, Wilson disease, biliary ectasia, citrullinemia, hepatoblastoma, hyperoxaluria, hypertyrosinemia, polycystic liver desease, portal vein thrombosis, propionic acidemia and systemic lupus erythematosus.

Isolation of genomic DNA and genotyping

Genomic DNA was extracted from a liver biopsy specimen of the graft and peripheral blood from recipients with a MagNAPure LC DNA Isolation kit I (Roche, Mannheim, Germany). Because the alleles of *CYP3A5*6* and *CYP3A5*7* have not been detected in the Japanese population or other Asian ethnic groups [11–13], we focused on the more frequent *CYP3A5*3* allele to examine a large inter-individual variation of tacrolimus pharmacokinetics in patients after liver transplantation. The polymorphism *CYP3A5*3* was examined according to the method of van Shaik *et al.* [14]. The polymerase chain reaction (PCR) process included initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and synthesis at 72°C for 30 s. The final extension was carried out for 10 min at 72°C.

Evaluation of intestinal expression levels of CYP3As and MDR1

Biopsy specimens from intestinal mucosa were homogenized in RLT buffer (Qiagen, Hilden, Germany) and total RNA was extracted with MagNAPure LC RNA Isolation kit II (Roche) and reverse transcribed as described previously [15]. The mRNA expression of CYP3A4, CYP3A5 and MDR1 was quantified by real-time PCR using an ABI prism 7700 sequence detector (Applied Biosystems, Foster, California, USA). The primer/probe sets for CYP3A4 and for CYP3A5 were as reported by Koch *et al.* [16]. Those for MDR1 and glyceraldehyde-3-phosphate dehydrogenase as an internal control were as described previously [15]. The mRNA quantification of the absolute amount for each gene was performed using the standard plasmid DNA as described previously [9,15]. Briefly, the amplified PCR fragments using specific primers for MDR1, CYP3A4 and CYP3A5 were ligated into the pCR-Script Cloning Vector (Stratagene, La Jolla, California, USA), the sequences were confirmed, and the purified plasmid DNA coding the PCR fragments for each gene was used as the standard plasmid DNA. The concentrations of the purified plasmid DNA were determined by ultraviolet spectrophotometry. The expression level of each gene in the intestine was calculated with the calibration curve by the serial diluted standard plasmid DNAs.

Measurement of tacrolimus concentrations

The blood concentration of tacrolimus was determined 12 h after the evening dosage using a semiautomated microparticle enzyme immunoassay (IMx, Dainabot Co., Ltd, Tokyo, Japan). The target whole-blood trough level was set between 5 and 15 ng/ml.

Statistical analysis

To improve normality, logarithmic transformations of the mRNA expression levels of CYP3A4, CYP3A5 and MDR1 were performed before the statistical analyses. The differences between groups were determined with the

Kruskal–Wallis test, followed by the Dunnet post-hoc test for multiple comparisons. These statistical analyses were performed with GraphPad PRISM, version 4 (GraphPad Software, Inc., California, USA). The Mann–Whitney *U*-test was used to compare the C/D ratio in between groups with a small GRWR (< 1.371%) or with a large GRWR (> 1.371%), and between each period in each group, and was performed using the statistical software package Stat View version 5.0 (Abacus Concepts, Berkeley, California, USA).

Results

Frequency and effect of *CYP3A5* polymorphism on intestinal expression levels of CYP3A4, CYP3A5 and MDR1

Table 2 shows the frequency of the *CYP3A5*3* SNP in recipients and donors. Because three recipients received a second LDLT during the period in this study, their polymorphism data were excluded from this allelic analysis. For the *CYP3A5*3* genotype, the **1* (A) and **3* (G) allele frequencies in recipients were 20% and 80%, respectively, and the **1* (A) and **3* (G) allele frequencies in donors were 23% and 77%, respectively. We measured the expression levels of CYP3A5 mRNA in the intestinal mucosa of recipients by real-time PCR ($n = 118$). One sample was excluded from the analyses for the mRNA expression of CYP3A5 in the intestine, because the expression level was undetectable. Figure 1 shows histograms of logarithmically-transformed mucosal expression levels of (a) CYP3A4, (b) CYP3A5 and (c) MDR1 mRNAs, respectively. The expression level of CYP3A5 mRNA in patients from whom clinical samples were obtained showed a weak bimodal pattern, whereas CYP3A4 and MDR1 showed unimodal patterns. Next, we investigated the effect of the polymorphism of *CYP3A5* on the mRNA expression levels of CYP3A4 and CYP3A5 (Fig. 2). The average CYP3A5 mRNA expression level in mucosa with the **1/*1*, **1/*3* and **3/*3* genotype was 0.861, 0.946 and 0.195 amol (10^{-18} mol)/μg total RNA, respectively. There was a significant difference between **1/*3* and **3/*3* ($P < 0.001$), but not between **1/*1* and **3/*3*. The CYP3A4 mRNA expression level did not differ significantly between *CYP3A5* genotypes (data not shown).

Relationship between the mRNA levels of CYP3A5 and CYP3A4 or MDR1

The expression levels of the intestinal CYP3A4 and MDR1 mRNAs, which act as absorptive barriers to tacrolimus ($n = 118$), were compared with that of CYP3A5 mRNA. There were significant but weak correlations between CYP3A5 and CYP3A4 (Fig. 3a) or MDR1 (Fig. 3b) with respect to mRNA expression levels ($r = 0.58$, $P < 0.0001$ and $r = 0.49$, $P < 0.0001$, respectively).

Influence of recipient genotype on the C/D ratio of tacrolimus during 5 weeks after transplantation

To evaluate whether the *CYP3A5* polymorphism in recipients is related to the pharmacokinetics of tacrolimus, we examined the effect of *CYP3A5* genotype on the tacrolimus C/D ratio during 5 weeks after surgery. Because four cases were treated with continuous haemodialysis filtration between postoperative days 1 and 3, these periods were excluded from analyses of genotype and phenotype. In addition, 89 cases were treated with temporary high-dose steroid injection to prevent acute cellular rejection. Days 1–8 during treatment were excluded because the systemic high-dose steroid injection could induce intestinal expression of CYP3A4 mRNA, but not that of MDR1 [17]. Moreover, the data on the concentration of tacrolimus during the first week after the operation was also excluded in the cases with a steroid-free protocol at reperfusion of the graft liver. As shown in Fig. 4, the C/D ratio of tacrolimus declined with time. For all periods, the patients with *CYP3A5*1/*1* had the lowest C/D ratio, but no significant difference was found between the *CYP3A5*1/*1* group and the *CYP3A5*1/*3* group. Furthermore, the median value of the C/D ratio of tacrolimus was 1.7- to 2.6-fold lower in the recipients with *CYP3A5*1/*1* than in the recipients with *CYP3A5*3/*3* during the initial 35 postoperative days (Fig. 4).

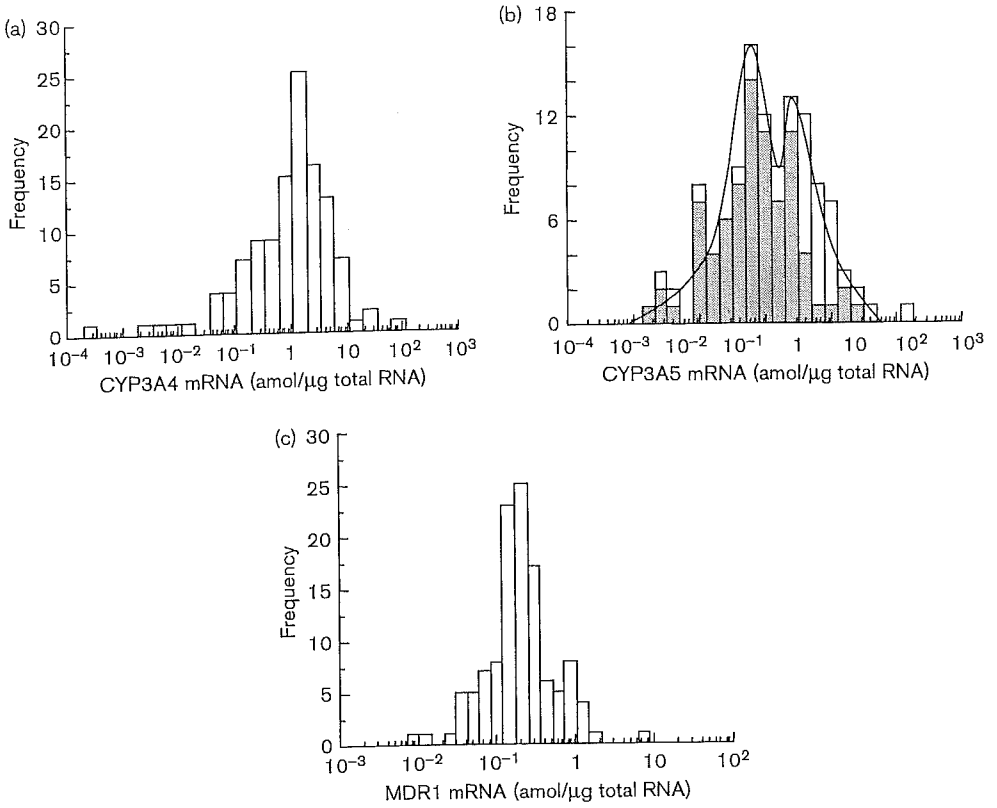
Influence of the combination of *CYP3A5* genotype in the graft liver and the native intestine on the C/D ratio

As a feature of liver transplantation, there are some cases in which the genotype in the native intestine is different from that in the graft liver. Focusing on these combinations, we examined the influence of *CYP3A5* genotype in the graft liver and the native intestine on the postoperative tacrolimus C/D ratio. Among 183 patients for whom samples of the graft liver and peripheral blood were obtained to determine genotype, recipients were divided into four groups based on graft liver genotype and intestinal genotype, including *CYP3A5*1/*1* and *CYP3A5*1/*3* (**1* group, CYP3A5 expressors) or *CYP3A5*3/*3* (**3* group, non-expressors). Each group was identified as follows: both

Table 2 Allele frequency of *CYP3A5* polymorphism

Gene	Genotype	Recipient ($n=201$)		Donor ($n=206$)	
		n	Frequency (%)	*n*	Frequency (%)
CYP3A5	**1/*1* (AA)	9	4.5	12	5.8
	**1/*3* (AG)	63	31.3	70	34.0
	**3/*3* (GG)	129	64.2	124	60.2

Fig. 1



Histograms of intestinal mRNA expression level of (a) CYP3A4, (b) CYP3A5 and (c) MDR1. The data were logarithmically transformed to improve normality in 118 recipients of living-donor liver transplantation. (b) The patients were divided into two groups by *CYP3A5* genotypes: **1/*1* and **1/*3* (open column) and **3/*3* genotype (closed column).

the liver (donor) and the native intestine (recipient) carried a *CYP3A5*1* allele (Liver*1/Intestine*1), the liver and the native intestine carried the *CYP3A5*3/*3* and a *CYP3A5*1* allele, respectively (Liver*3/Intestine*1), the liver and the native intestine carried a *CYP3A5*1* allele and the *CYP3A5*3/*3*, respectively (Liver*1/Intestine*3) and both the liver and the intestine carried the *CYP3A5*3/*3* (Liver*3/Intestine*3). The group Liver*1/Intestine*1 exhibited a lower C/D ratio than any other group (Fig. 5). In the groups where the native intestine carried a *CYP3A5*1* allele, except for the period 2 weeks after surgery, there was a significant difference in the C/D ratio of tacrolimus between Liver*1/Intestine*1 and Liver*3/Intestine*1. In addition, when comparing the groups where the liver carried a *CYP3A5*1* allele, the median values of the C/D ratio of tacrolimus in Liver*1/Intestine*1 were significantly lower (1.6- to 2.5-fold) than those of Liver*1/Intestine*3.

Influence of GRWR on the C/D ratio

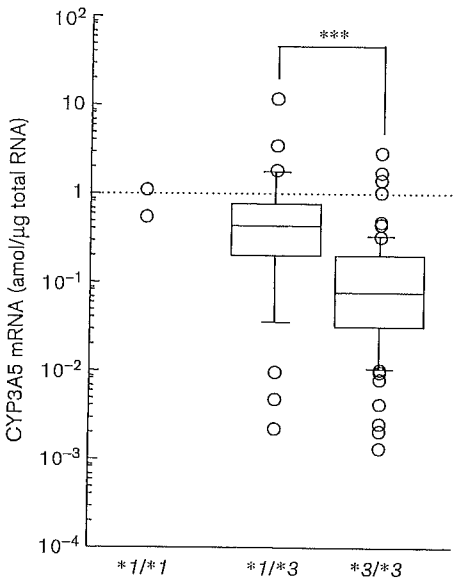
Previously, there have been reports that graft size affects patient survival and the C/D ratio of tacrolimus [6,18]. Next, we examined the influence of GRWR on the tacrolimus C/D ratio for 7 days after surgery in 183 patients (Fig. 6). After dividing the patients based on the median value of GRWR (1.371%), the C/D ratio of tacrolimus in the four groups was re-analysed. The C/D ratio of tacrolimus in patients receiving a relatively small graft showed large variations, and was significantly lower in Liver*1/Intestine*1 than in Liver*1/Intestine*3 or Liver*3/Intestine*3 (Fig. 6a). In recipients with a GRWR above 1.371%, the C/D ratio of tacrolimus was significantly lower in Liver*1/Intestine*1 compared to those in the other four groups (Fig. 6b). The C/D ratio of tacrolimus in each group with a small GRWR ($< 1.371\%$) was higher than that in the groups with a large GRWR ($> 1.371\%$).

Influence of time after surgery on the C/D ratio

Using the data on tacrolimus C/D ratio and *CYP3A5* genotype in Fig. 5, we re-analysed the influence of postoperative period on the tacrolimus pharmacokinetics in each group after LDLT. Comparing the C/D ratio of tacrolimus at 1–7 days after surgery with that at 15–21 days, 22–28 days or 29–35 days in each of the four

groups, there was a significant difference in Liver*1/Intestine*1 (15–21 days, $P = 0.0344$; 22–28 days, $P = 0.0023$; 29–35 days, $P = 0.0034$). In Liver*3/Intestine*3, there was a significant difference at 1–7 days and at 15–21 days or 22–28 days ($P = 0.0315$ and $P = 0.0040$, respectively), whereas there was no significant difference between that at 1–7 days and that at 29–35 days ($P = 0.1569$).

Fig. 2



Effect of *CYP3A5* polymorphism on its mRNA expression level in intestine. The mRNA expression level of CYP3A5 was determined using the real time polymerase chain reaction method in 118 intestinal mucosa. ***$P<0.001$, significant difference between groups.

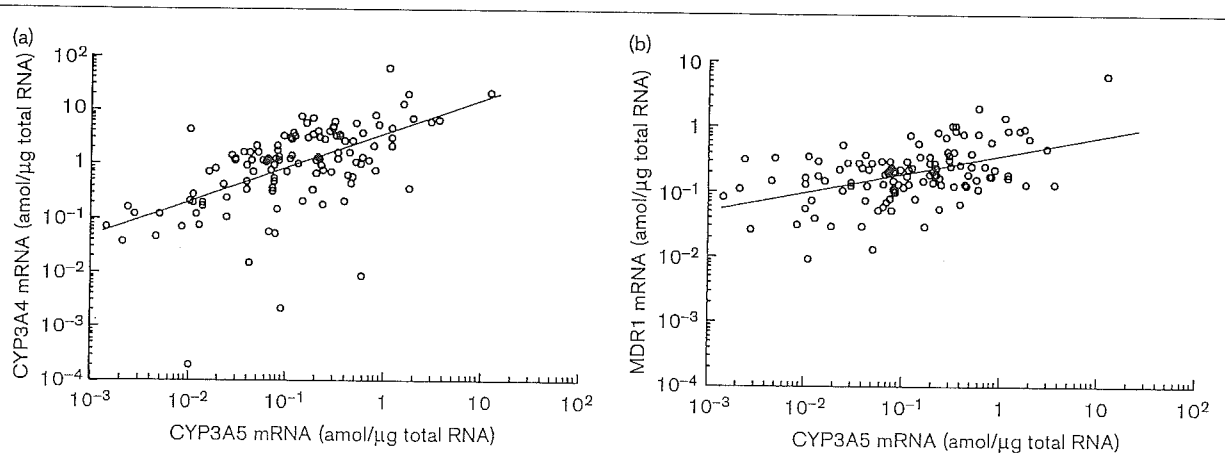
Discussion

Previously, we reported that LDLT-recipients with grafted livers carrying the *CYP3A5*1/*1* genotype required a high dose of tacrolimus to achieve the target concentration [9]. In the present study, we have investigated the genotype and mRNA expression level of CYP3A5 in the intestine in addition to the graft liver, and their effects on the C/D ratio of tacrolimus in an enlarged group of subjects.

We previously reported that 10 common SNPs, including 3435C/T and 2677G/TA, in the *MDR1* gene were unrelated to both the intestinal expression of MDR1 mRNA and the tacrolimus C/D ratio in the 46 LDLT recipients [19]. In addition, we found that the inverse correlation between the enterocyte expression level of MDR1 mRNA and tacrolimus C/D ratio in recipients of LDLT was limited during the first 7 postoperative days [6,20]. Therefore, we focused on whether the expression of CYP3A5 in the small intestine had an effect on the tacrolimus C/D ratio during the first month after LDLT. Because the trough concentrations of tacrolimus were well correlated with the area under the blood concentration–time curve (AUC) values [2] and multisampling would be difficult in LDLT recipients, including pediatric patients and infectious patients, due to ethical and economic limitations, we used the trough concentrations for analyses instead of the actual AUC values.

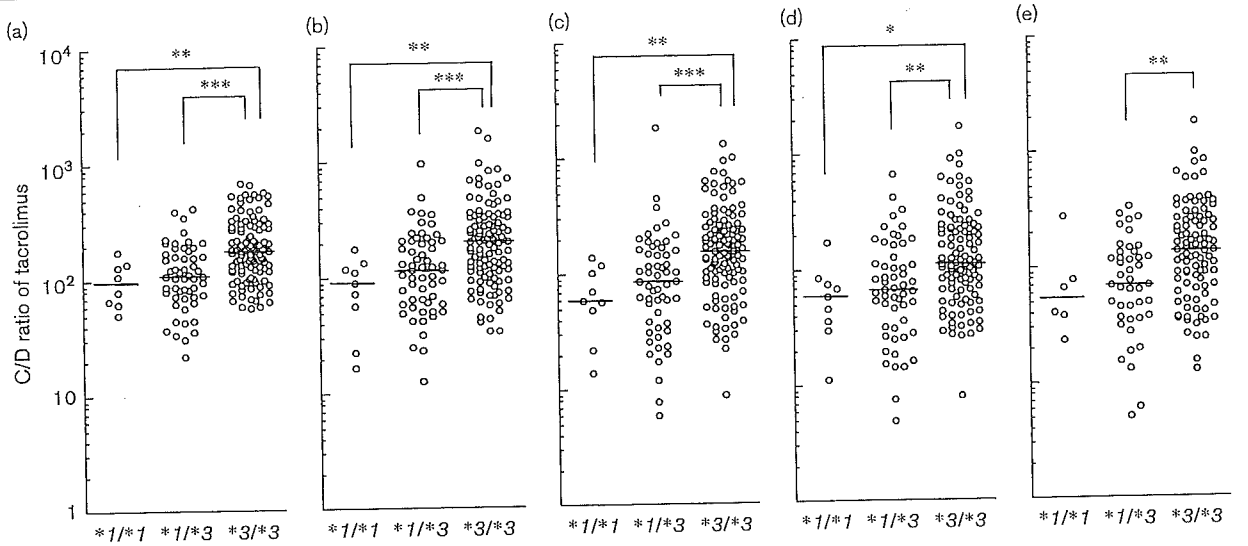
In this study, we demonstrated that the *CYP3A5*3/*3* genotype in recipients and donors was detectable in 64% and 60% of cases, respectively (Table 2). Our result was consistent with reports that the *CYP3A5*3/*3* genotype frequencies were 55.8–60.5% in the Japanese population [9,11,13,21,22]. The functional polymorphism *CYP3A5*3* was first identified by Kuehl *et al.* [23] and Hustert *et al.*

Fig. 3



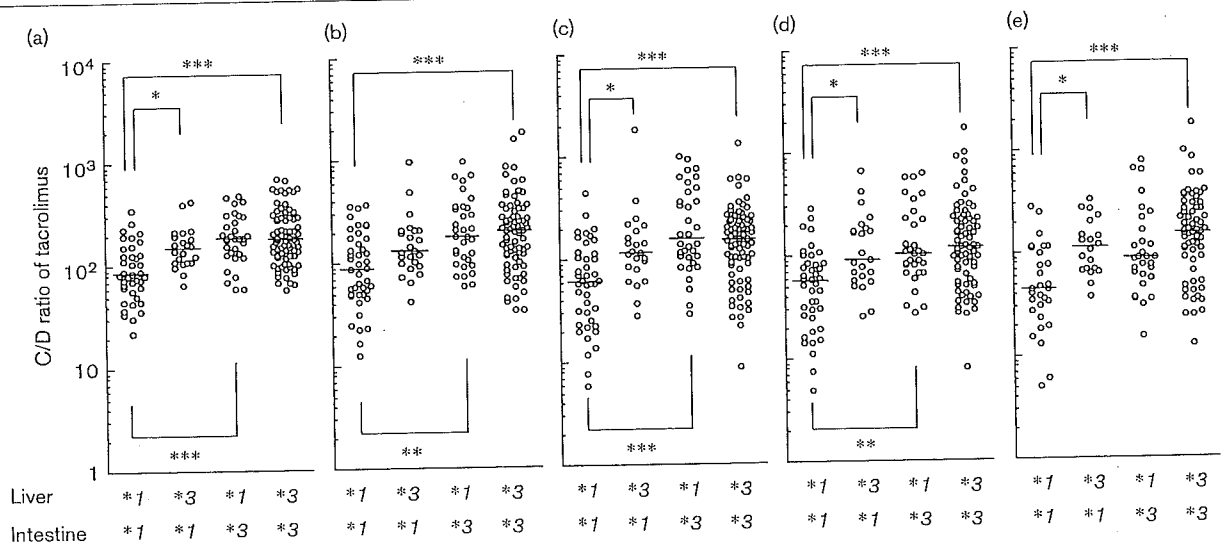
Enterocyte mRNA expression levels of (a) CYP3A4 and (b) MDR1 as a function of CYP3A5 mRNA level.

Fig. 4



The influence of intestinal *CYP3A5* polymorphism on the concentration/dose (C/D) ratio of tacrolimus in living-donor liver transplantation recipients during 5 weeks. The mean tacrolimus C/D ratio for the period (a) 1–7, (b) 8–14, (c) 15–21, (d) 22–28 and (e) 29–35 days post-transplantation was compared based on *CYP3A5* genotype. The bar shows the median of the tacrolimus C/D ratio in each group. $*P<0.05$, $**P<0.01$, $***P<0.001$, significant difference between groups.

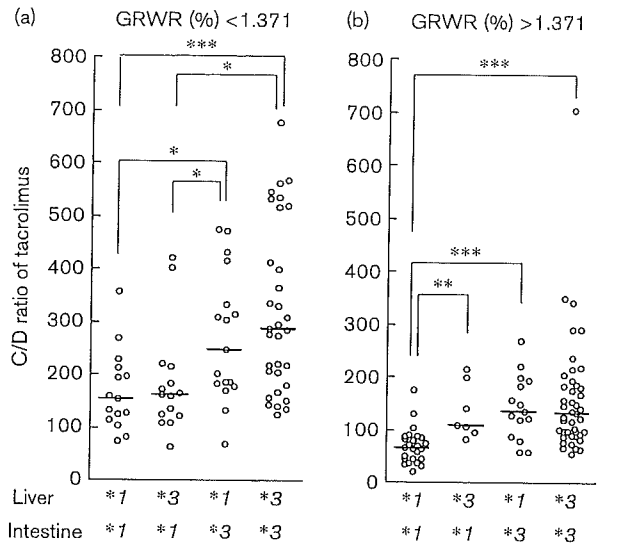
Fig. 5



The influence of graft liver and native intestinal *CYP3A5* genotype on the concentration/dose (C/D) ratio of tacrolimus in recipients after living-donor liver transplantation over 5 weeks. The cases in which *CYP3A5* genotype was determined in both donors and recipients were categorized into four groups based on graft genotype and intestinal genotype (**1, CYP3A5*1/*1* and *CYP3A5*1/*3*; **3, CYP3A5*3/*3*). The C/D ratio of tacrolimus is compared in each group for 5 weeks, over the period (a) 1–7, (b) 8–14, (c) 15–21, (d) 22–28 and (e) 29–35 days after transplantation. $*P<0.05$, $**P<0.01$, $***P<0.001$, significant difference between groups.

[24], who showed that the *CYP3A5*3/*3* genotype displayed sequence variability in intron 3 that caused a cryptic splice site and transcribed an extraordinarily spliced mRNA, and the truncated protein resulted in the absence of normal CYP3A5 protein from tissues. The mucosal expression level of CYP3A5 mRNA was affected by the *CYP3A5*3* genotype, and the histogram showed a weak bimodal pattern (Figs 1 and 2). These results were

Fig. 6



The effect of graft-to-recipient body weight ratio (GRWR) on the concentration/dose (C/D) ratio of tacrolimus in four groups. The cases ($n=183$) in which *CYP3A5* genotype was determined in both donors and recipients were categorized into four groups, as described in Fig. 5. The median value of GRWR was 1.371%. The C/D ratio of tacrolimus in the recipients with (a) GRWR < 1.371% and (b) GRWR > 1.371% is compared in each group for 7 days after living-donor liver transplantation. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significant difference between groups.

compatible with finding in the liver [9]. The present report demonstrates the effect of the *CYP3A5*3* genotype on the mucosal mRNA expression level of the gene product in end-stage liver failure.

Both Pgp and CYP3As are associated with the pharmacokinetics of tacrolimus in absorption and metabolism. Our previous report showed that there was no correlation between intestinal MDR1 and CYP3A4 in mRNA or protein levels in LDLT recipients [6]. The high-dose steroid injection induced intestinal expression of CYP3A4 mRNA, but not that of MDR1 in small-bowel transplant recipients [17]. In the present study, there was a significant but weak correlation between intestinal CYP3A5 and CYP3A4 or MDR1 in mRNA expression levels (Fig. 3). The preoperative status of many patients included cirrhosis and hyperbilirubinemia, and any medication inducible to these mRNAs was treated until surgery. Some inflammatory response or unknown mechanism(s) might regulate the expression of these mRNAs. Although these genes are close together on chromosome 7, the constitutive transcriptional regulation in the small intestine could be independent of each other.

Several studies have suggested that the *CYP3A5*3* polymorphism can explain the variability in the dose-adjusted concentration of tacrolimus in blood [7,21,23,25]. Genotyping of *CYP3A5*3* in recipients revealed that the C/D ratio of tacrolimus was significantly lower in patients with at least one *CYP3A5*1* allele (CYP3A5 expressors) than in patients with *CYP3A5*3/*3* (non-expressors) during 5 weeks after surgery (Fig. 4). This suggests that the effects of the *CYP3A5*1* genotype in recipients remain for at least 5 weeks after LDLT. To examine the association between genotype and phenotype more clearly, long-term follow-up in stable patients would be required. However, the episodes of acute cellular rejection occur more frequently during the first 6 postoperative weeks [26]. It is clinically significant to find that the *CYP3A5* genotype is one of the pharmacokinetic factors for tacrolimus therapy in the early period after LDLT.

In patients receiving liver transplantation, the genotypes of both the recipients themselves (intestine) and donors (liver) could be essential to the marked inter-individual variation in tacrolimus pharmacokinetics. In the present study, we classified the recipients into four groups by graft and intestinal genotypes to evaluate the contribution of intestinal CYP3A5 to tacrolimus pharmacokinetics. Immediately after LDLT, when the graft liver did not have a sufficient detoxifying function for ischemia and reperfusion at surgery, a greater accumulation of tacrolimus in the liver might cause saturation of liver metabolic capacity in the group where the intestine (*CYP3A5*3/*3*) had a low metabolic capacity, and therefore, the effect of liver genotype might be weak. However, the donor genotype would be significant when the recipient genotype of *CYP3A5* was **1*. By contrast, the recipient genotype was also significant when the donor genotype of *CYP3A5* was **1* (Fig. 5). These results suggest that both hepatic and intestinal genotypes of *CYP3A5* are important for the oral clearance of tacrolimus in liver transplant patients. It was reported that graft size had an influence on patient survival, graft survival and the dose of tacrolimus [6,18,27]. We evaluated the influence of GRWR on the C/D ratio (Fig. 6). The role of liver genotype is important in patients with high GRWR (> 1.371%), because there is a significance in the tacrolimus C/D ratio between Liver*1/Intestine*1 and Liver*3/Intestine*1 (Fig. 5b). By contrast, the tacrolimus C/D ratio in Liver*1/Intestine*3 was close to Liver*3/Intestine*3. Despite the relatively large graft liver, a non-linear effect might have occurred in the liver by the lowered metabolic activity in recipients with Intestine*3 (i.e. lowered intestinal first-pass effect). A high C/D ratio and large variation in the C/D ratio were found in each of the groups with a small GRWR (< 1.371%). Because GRWR was smaller in adult patients than in pediatric patients, the adult patients were speculated to show large variation in the C/D ratio of tacrolimus in each group immediately after LDLT. Moreover, in the group with a small GRWR, the C/D ratio in Liver*3/Intestine*1 was close to Liver*1/Intestine*1. Thus, the role of the hepatic *CYP3A5* genotype appeared to be weak. It could

be considered that intestinal metabolism is more important in a population with a small GRWR (Fig. 6). Patients carrying an intestinal *CYP3A5*1* allele tended to require a higher dose of tacrolimus compared to the other group carrying the same hepatic *CYP3A5* genotype (Liver*1/Intestine*1 versus Liver*1/Intestine*3 or Liver*3/Intestine*1 versus Liver*3/Intestine*3) (Figs 5 and 6). These findings strongly suggested that the intestinal absorptive barrier function, including CYP3A5-mediated metabolism for orally administered tacrolimus, was an important first-pass effect in patients after LDLT, if the hepatic content of CYP3A5 is small.

In each group, the C/D ratio of tacrolimus tended to decline at 5 weeks after LDLT (Figs 4 and 5). Previously, we demonstrated that the clearance value in a population pharmacokinetic model increased linearly with time up to 30 days after LDLT [28], and that the C/D ratio of tacrolimus decreased with time irrespective of *CYP3A5* genotype in grafted liver after LDLT [9]. Moreover, the graft livers were reported to show a substantial increase in volume 1 month after LDLT [29]. Although the medications, liver functions, renal functions, intestinal mobility and other factors change with time after LDLT, these parameters may affect regeneration of the grafted liver including its detoxifying function. Therefore, the reduction of C/D ratio with time might result from the regeneration of the grafted partial liver.

The genotype of genes coding for drug metabolizing enzymes in the liver and intestine will always be identical in all patients, except those who have undergone liver or intestinal transplantation. In the present study, the patients expressing CYP3A5 protein or not in both tissues demonstrated results corresponding to previous reports, where the expressors had a lower C/D ratio of tacrolimus than the non-expressors (Fig. 5). In the present study, an important role for intestinal CYP3A5 in the pharmacokinetics of tacrolimus was also suggested by comparing the genotype combinations between the graft liver and the native intestine (Figs 5 and 6). Therefore, in previous reports, the effect of *CYP3A5* genotype on tacrolimus pharmacokinetics in recipients except in liver transplantation would include the effect of CYP3A5 not only in the liver, but also in the intestine.

In conclusion, we have revealed that *CYP3A5* genotype is associated with the expression level of CYP3A5 mRNA in the intestine as well as the liver, and with the postoperative tacrolimus C/D ratio in patients after LDLT. These results indicate that intestinal CYP3A5 plays an important role in the first-pass effect of orally administered tacrolimus.

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Intestinal MDR1/ABCB1 level at surgery as a risk factor of acute cellular rejection in living-donor liver transplant patients

Background: Although the prevention of immunologic reactions with sufficient immunosuppression prolongs graft and patient survival rates, the large interindividual variation in tacrolimus pharmacokinetics interferes with treatment. In this study we have examined whether intestinal MDR1 (ABCB1) is a potential biomarker predicting the occurrence of acute cellular rejection, as well as a factor to predict absorption of tacrolimus, after living-donor liver transplantation.

Methods: By use of tissue specimens of intestinal mucosa (n = 164) obtained at surgery, the messenger ribonucleic acid (mRNA) expression of intestinal MDR1 and cytochrome P450 (CYP) 3A4 was quantified.

Results: The probability of acute cellular rejection during the first 10 days after surgery was significantly associated with the average trough concentration of tacrolimus between postoperative days 2 and 4 (45.1% for <7 ng/mL versus 22.9% for >7 ng/mL, $P = .0040$). High levels of MDR1 were associated with an episode of acute cellular rejection before postoperative day 10 (odds ratio, 2.306 [95% confidence interval, 1.058-5.028]) and with a poor survival rate during the first postoperative year (odds ratio, 7.413 [95% confidence interval, 1.567-36.073]). The mRNA expression level of MDR1 was inversely correlated with the tacrolimus concentration–oral dose ratio during the initial 4 days after surgery in patients with a graft-to-recipient weight ratio greater than 1.5 ($r = -0.6798$, $P < .0001$) and those with a graft-to-recipient weight ratio of less than 1.5 ($r = -0.7180$, $P < .0001$).

Conclusion: The enterocyte MDR1 mRNA level was suggested to be a risk factor for acute cellular rejection and death after surgery. Therefore obtaining a sufficient tacrolimus blood level via this molecular information–based initial dosage adjustment may enable the episode of acute cellular rejection after liver transplantation to be reduced. (Clin Pharmacol Ther 2006;79:90–102.)

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Living-donor liver transplantation and subsequent immunosuppressive therapy are well acknowledged to provide excellent results and are usually used in coordination with a cadaveric organ transplant program.[1,2] In countries where cadaveric donors are limited, living-donor liver transplantation is often the only treatment option for patients with end-stage liver disease.[3] Because loss of the graft liver will lead to death, postoperative immunosuppressive therapy is essential to protect the grafted liver from immunologic reactions. As acute cellular rejection occurs mostly within 6 weeks of a transplant,[4] high-dose steroid pulse therapy or anti-CD3 monoclonal antibody treatment is required to save the graft liver.[5,6] Subclinical rejection, where cytologic or histologic signs of rejection exist in the absence of clinical dysfunction of the graft, is particularly frequent (incidence of around 59%) between days 5 and 14 after

liver transplantation.[6] Twenty-five percent of patients with subclinical rejection are treated with high-dose steroid injections. Therefore early exposure to immunosuppressants could reduce the frequency of acute cellular rejection including subclinical rejection. However, these antirejection treatments lead to overimmunosuppression and an infectious state, which are closely associated with death.[7] Although there is a need to protect patients from opportunistic infections including enterobacterium, Epstein-Barr virus, cytomegalovirus, and herpes simplex virus after antirejection treatment, some anti-infectious treatments with antibiotics, antifungal agents, and antiviral drugs are accompanied by drug-induced hepatic and renal dysfunction.[8,9] In addition, high-dose steroid injections should be avoided in patients carrying the hepatitis B or C virus, because steroidal drugs allow the amplification of these viruses in the graft liver and accelerate the recurrence of virus-related hepatitis and cirrhosis.[10,11] Therefore acute cellular rejection should be avoided to prevent further complications, especially immediately after transplantation.

The calcineurin inhibitor tacrolimus (FK-506) has been used as a primary immunosuppressive agent in orthotopic liver transplantation.[12] Therapeutic drug monitoring has facilitated maintaining the blood concentration of tacrolimus within a narrow therapeutic range (between 10 and 20 ng/mL) to prevent side effects such as nephrotoxicity, neurotoxicity, and life-threatening infection.[13,14] However, the bioavailability of orally administered tacrolimus is variable, ranging from 4% to 89% (with a mean value of about 25%),[15,16] and a dosage regimen for the drug immediately after transplantation has yet to be established. This can be attributed to several factors, including poor absorption or extensive first-pass metabolism in the intestine and liver. Therefore a rational dosage regimen for tacrolimus should be determined as early as possible, focusing on its pharmacokinetic interindividual variability.

Tacrolimus is principally metabolized by cytochrome P450 (CYP) 3A subfamilies in the liver. The contribution of active secretion by P-glycoprotein (the product of the *MDR1/ABCB1* gene) and the metabolism by CYP3A expressed in enterocytes are acknowledged as factors influencing the bioavailability of tacrolimus.[17] We reported that the intraindividual variation in the concentration/dose (C/D) ratio of tacrolimus was closely related to the variation in the enterocyte messenger ribonucleic acid (mRNA) expression level of MDR1, but not CYP3A4, in recipients of living-donor small-bowel transplantation.[18,19] Similar results were obtained in patients after living-donor liver transplantation during the initial 7 days after surgery.[20,21] Because of the small number of cases in our past reports, we could not analyze the relationship between the intestinal mRNA expression level of MDR1 and endpoints such as acute cellular rejection. It is necessary to clarify the clinical significance of the intestinal expression level of MDR1 in patients after living-donor liver transplantation to establish the clinical usefulness of adjusting the initial dosage of tacrolimus.

In this study we examined whether the intestinal expression level of MDR1 mRNA could be a molecular marker for acute cellular rejection episodes in patients after living-donor liver transplantation with more enrolled patients, as well as the potential contribution of the molecular information to initial dose setting.

METHODS

Patients and mucosal specimens. The study included 164 patients, having first provided written informed consent, who were enrolled consecutively between November 1998 and December 2004 in whom tissue specimens had been obtained at surgery. The donor was a parent in 119 cases, a spouse in 14, a sibling in 12, an offspring in 12, a grandmother in 3, an uncle in 2, an aunt in 1, and a father-in-law in 1. The demographics of the recipients are listed in Table I. The clinical samples of the upper jejunum were obtained from a part of the Roux-en-Y limb for biliary reconstruction or from a part of the mucosal specimen around the bile drainage tube at living-donor liver transplantation.[22] This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Ethics Committee of Kyoto University, Kyoto, Japan; each adult patient and each parent of small children provided written informed consent.

Dosage regimen of tacrolimus, analysis of blood samples, and criteria for acute cellular rejection. The basic immunosuppression regimen consisted of tacrolimus with low-dose steroids.[23] To cover the immediate postoperative period (the day of living-donor liver transplantation, day 0, and postoperative day 1), induction of immunosuppressive therapy was started from the day before the operation, except in cases of hepatic encephalopathy and severe infection. Tacrolimus was administered orally at a dose of 0.075 mg/kg body weight every 12 hours from the evening of day 1.[13,23] The target for the post-transplantation whole-blood trough concentration of tacrolimus was 10 to 12 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 $mg \cdot kg^{-1} \cdot d^{-1}$ to 0.3 $mg \cdot kg^{-1} \cdot d^{-1}$ during the first 2 weeks after surgery.

Table I. Demographic characteristics of recipients (N = 164)

Age (y)	0.3-67 (median, 3.2)
Adults (≥15 y) (n = 54)	15.0-67 (median, 46)
Children (<15 years) (n = 110)	0.3-13.7 (median, 1.2)
Body weight (kg)	4.3-92.1 (median, 13.5)
Graft-to-recipient weight ratio (%)	0.63-5.6 (median, 1.84)
Gender (male/female)	70/94
Graft lobe (left/right)	113/51
ABO blood group match (identical/compatible/incompatible)	99/37/28
Preoperative condition (home-bound/hospitalized/intensive care unit–bound)	71/85/8
Primary disease*	
Biliary atresia	89 (21)
Cirrhosis	
Hepatitis B virus	9 (1)
Hepatitis C virus	12 (3)
Primary biliary cirrhosis	7 (2)
Unknown	3 (0)
After liver transplantation	11 (3)
Primary sclerosing cholangitis	8 (4)
Fulminant hepatic failure	4 (2)
Other†	21 (6)

*The number of patients with acute cellular rejection episodes during the initial 10 days after surgery is denoted in parentheses.
†The primary disease was Byler disease in 4 cases (2), Alagille syndrome in 3 (0), Wilson disease in 2 (2), hepatoblastoma in 3 (0), polycystic liver disease in 2 (1), biliary dilation in 2 (0), multiple hepatocellular carcinoma in 1 (0), citrullinemia in 1 (1), hypertyrosinemia in 1 (0), Budd-Chiari syndrome in 1 (0), and portal vein deficiency in 1 (0). The number of patients with acute cellular rejection episodes during the initial 10 days after surgery is denoted in parentheses.

The dosage of tacrolimus was adjusted on the basis of whole-blood trough concentrations measured about 12 hours after the evening dosage every day, by use of a semiautomated microparticle enzyme immunoassay (IMX; Dainabot, Tokyo, Japan).[24]

Acute cellular rejection was principally diagnosed with liver biopsy specimens, and the histologic diagnosis was performed according to criteria based on the Banff schema.[25] All episodes of rejection were treated with a high-dose steroid bolus injection.

Evaluation of intestinal expression levels of MDR1 and CYP3A4. Biopsy specimens from intestinal mucosa were homogenized in RLT buffer (Qiagen, Hilden, Germany), and total RNA was isolated with MagNA-Pure LC RNA Isolation kit II (Roche) and reverse-transcribed as described previously.[26] The isolated total RNA (500 ng/40 μL reaction mixture) was reverse-transcribed by Superscript II reverse transcriptase (Invitrogen, Carlsbad, Calif) with random primers (100 ng/reaction) and digested by RNase H (Invitrogen). After dilution of the single-stranded deoxyribonucleic acid (DNA) mixture with 60 μL of sterile water (final volume, 100 μL), 5-μL aliquots were used for a subsequent real-time polymerase chain reaction (PCR) (final volume, 20 μL) with an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster, Calif). The primer/probe set used for glyceraldehyde 3-phosphate dehydrogenase, as an internal control, was predeveloped TaqMan Assay Reagents (Applied Biosystems), and the reaction was performed according to the manufacturer's instructions. The primer/probe set specific for MDR1 and CYP3A4 was as described previously.[26] Each PCR fragment of the target sequences was generated with specific primer/probe sets as described, ligated into the pCR-Script Cloning Vector (Stratagene, La Jolla, Calif), and confirmed to have the exact sequences of the cloned amplicons by the chain-termination method by use of a fluorescence 373A DNA sequencer (Applied Biosystems). After measurement of the concentrations of the purified plasmid DNA by spectrophotometry, the corresponding concentrations (in moles per microliter) were calculated and serial dilutions of respective plasmid DNA were used as standards for calibration curves. The starting mRNA concentration of MDR1 or CYP3A4 was established by determining the fractional PCR threshold cycle number at which a fluorescence signal generated during the replication process passed above a threshold value. The initial amount of target mRNA in each sample was estimated from the experimental fractional PCR threshold cycle value with a standard curve generated by use of known amounts of standard plasmid DNA.

Statistical analyses. Normally distributed values were presented as mean ± SD. Values that were not

normally distributed were presented as the median and range. Logarithmic transformation of the mRNA levels of MDR1 and CYP3A4 was performed to improve normality before statistical analyses were performed. The nonpaired Student *t* test was used to compare groups with respect to normally distributed variables. If different variances between 2 samples were found with the F test, an unpaired *t* test with Welch correction was performed. The Mann-Whitney *U* test was used to compare groups without normality. The calculated mRNA expression levels of MDR1 and CYP3A4 in each intestinal specimen were categorized as high or low, if the quantified value for the mRNA in question exceeded or fell below the median value for all specimens, respectively. Statistical tests were 2-tailed, and significance was defined as $P < .05$.

The outcome measure studied was immunologic events and survival, defined as the time from living-donor liver transplantation to the first episode of acute cellular rejection during the initial 10 days after surgery and to death during the first year after surgery, respectively. The patients without complications until at least postoperative day 10 were categorized as the event-free group. The patients who were diagnosed with acute cellular rejection by liver biopsy before postoperative day 10 were categorized as the acute cellular rejection group. The probability analysis was performed according to the method of Kaplan and Meier, and the outcome was compared among the subgroups by use of a 2-tailed log-rank test for univariate comparisons. An odds ratio was calculated for the risk. Statistical analyses were performed by use of the statistical software package StatView (version 5.0; Abacus Concepts, Berkeley, Calif).

RESULTS

Patients. Table I shows the demographics and primary diseases of living-donor liver transplant recipients whose mucosal samples we studied. Of the recipients who had acute cellular rejection, 28, 11, and 3 had an ABO blood type that was identical, compatible, and incompatible with that of their donor, respectively. Moreover, 18, 21, and 3 recipients with acute cellular rejection were home-bound, hospitalized, and intensive care unit (ICU)–bound, respectively, before surgery. Of the recipients with acute cellular rejection, 29 and 13 had a graft from the left lobe and right lobe, respectively. Steroid-pulse therapy was used in 32 patients without acute cellular rejection during the first 10 days after surgery, and 11 post–liver transplant patients were treated with immunosuppressants until immediately before the second transplantation. Therefore these 43 patients were excluded from the analyses for the probability of acute cellular rejection but not from the analyses on gene expression and tacrolimus pharmacokinetics, and the analyses for acute cellular rejection were performed with the findings of the other 121 recipients, including 82 event-free patients and 39 acute cellular rejection patients. The survival analysis was performed with these 121 recipients, including 13 patients who died within 1 year after transplantation.

Acute cellular rejection and postoperative tacrolimus trough level. By comparing the daily trough concentration of tacrolimus between the event-free group (n = 82) and the acute cellular rejection group (n = 39), it was found that the trough concentration at postoperative days 3 ($P = .0075$) and 4 ($P = .0022$) was significantly lower in the acute cellular rejection group (Fig 1). These results suggest that the blood level of tacrolimus immediately after living-donor liver transplantation was associated with the occurrence of acute cellular rejection until postoperative day 10. Then, we examined the relationship between the average trough concentration of tacrolimus between postoperative days 2 and 4 and the complications of patients, because the tacrolimus was usually administered to recipients in the ICU during the first 3 days after liver transplantation. At first, we categorized the patients by the average trough concentrations of tacrolimus between postoperative days 2 and 4. Because a low dosage of tacrolimus was administered to patients at risk of infection or renal impairment from the preoperative status to avoid any further deterioration in condition, the categorization was started from 5 ng/mL, which is considered the lower limit of the initial average concentration of tacrolimus. As shown in Fig 2, the frequency of acute cellular rejection tended to be high in patients with relatively lower tacrolimus blood levels, between 5 and 7 ng/mL. The other complications frequently occurred in the patients whose average tacrolimus trough levels were below 5 ng/mL. The frequency of acute cellular rejection compared with the event-free group tended to be lower in the patients whose average tacrolimus trough levels were maintained above 7 ng/mL. Next, we examined the probability of acute cellular rejection in the recipients dividing the average trough concentration of tacrolimus at 7 ng/mL between postoperative day 2 and 4 (Fig 3). Kaplan-Meier analysis demonstrated that the average trough concentration of tacrolimus immediately after living-donor liver transplantation was significantly associated with acute cellular rejection ($P = .0040$). The resultant odds ratio was 2.772 (95% confidence interval [CI], 1.265-6.075) for the patients whose mean trough level of tacrolimus was

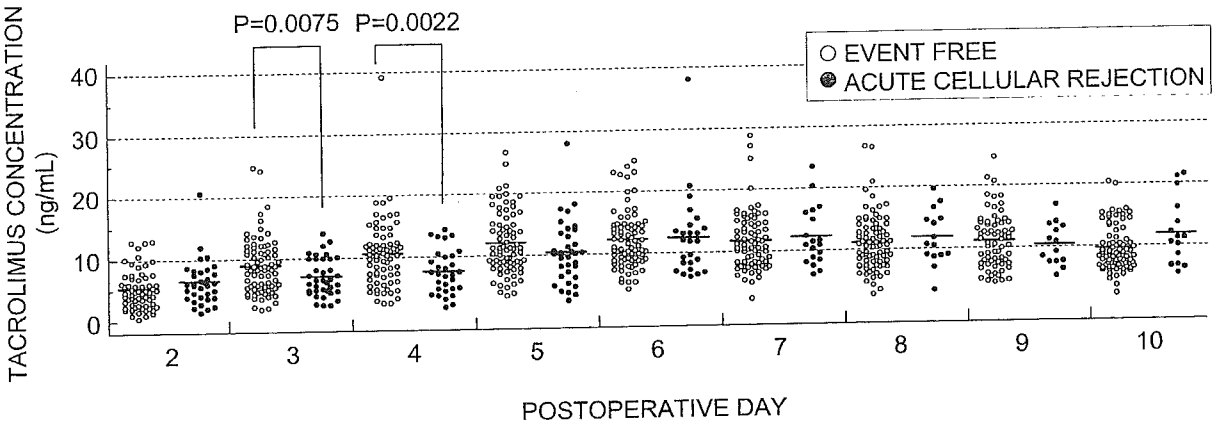

Fig 1. Daily trough levels of tacrolimus in living-donor liver transplant patients. Trough concentrations of tacrolimus in 121 patients receiving de novo living-donor liver transplants are illustrated. The patients are divided into 2 groups: event-free (*open circles*) and acute cellular rejection (*solid circles*). A statistical analysis was performed with the unpaired *t* test after Welch correction. *P* values of less than .05 are shown.

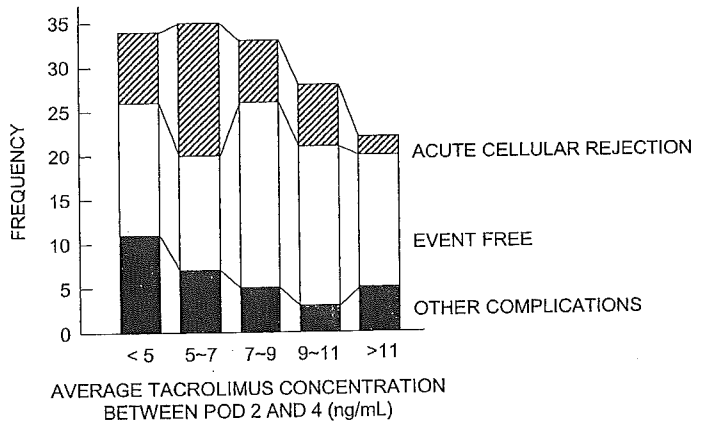

Fig 2. Frequency of complications after living-donor liver transplantation with respect to tacrolimus trough level between postoperative days (POD) 2 and 4. Frequencies of an event-free clinical course, acute cellular rejection, and the need for high-dose steroid treatment for other complications are shown as *open, hatched,* and *solid columns*, respectively. The patients were classified on the basis of the average trough concentration of tacrolimus between postoperative days 2 and 4.

below 7 ng/mL between postoperative days 2 and 4 (Table II).

Association between intestinal mRNA level of MDR1 or CYP3A4 and acute cellular rejection. We previously reported that patients with high levels of enterocyte MDR1, but not CYP3A4, required about 2-fold higher oral dosages of tacrolimus than patients with low levels of MDR1.[20] On the basis of the previous findings, we have re-examined the expression profile of the intestinal mRNA level of MDR1 and CYP3A4 to re-evaluate the influences of these factors on the risk for acute cellular rejection, as well as the interindividual variation of postoperative tacrolimus pharmacokinetics. In Fig 4, *A* and *B*, the logarithmically transformed distribution of the intestinal expression level of MDR1 and CYP3A4 at living-donor liver transplantation is shown. The median value of MDR1 and CYP3A4 was 0.242 amol/μg (range, 0.01-6.51

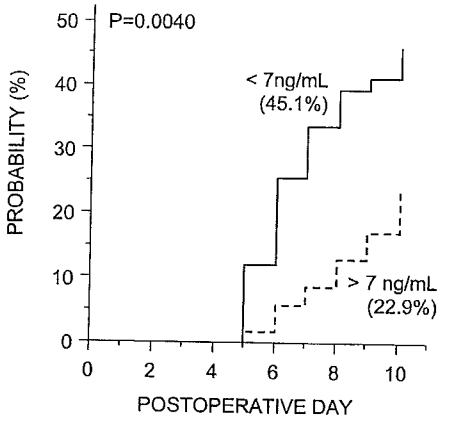

Fig 3. Probability of acute cellular rejection episodes in initial 10 days after living-donor liver transplantation. Kaplan-Meier curves show the probability of acute cellular rejection with respect to the average trough concentration of tacrolimus between postoperative days 2 and 4 (<7 ng/mL or >7 ng/mL). *P* values were determined with the log-rank test.

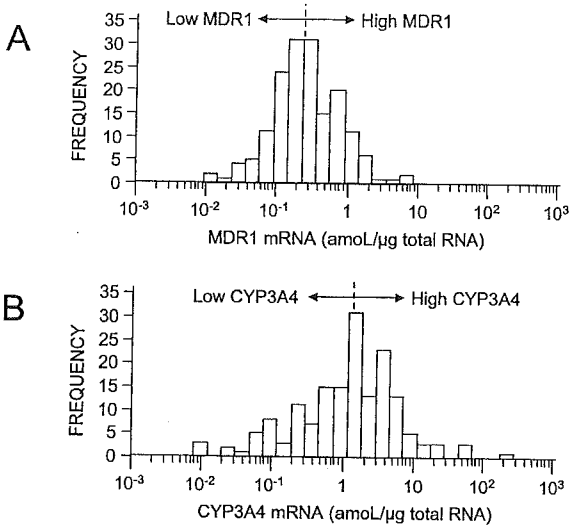

Fig 4. Histograms of messenger ribonucleic acid (mRNA) expression of intestinal MDR1 and CYP3A4 at living-donor liver transplantation. Distribution of mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in intestinal mucosa, both logarithmically transformed to improve normality, are illustrated as histograms for 164 recipients after living-donor liver transplantation. The *dotted lines* denote the median value. RNA, Ribonucleic acid.

amol/μg) of total RNA and 1.278 amol/μg (range, 0.002-185.5 amol/μg) of total RNA, respectively. After dividing the samples by each median value, we examined the probability of acute cellular rejection based on the expression of MDR1 or CYP3A4 (high or low). As illustrated in Fig 5, *A*, a high level of intestinal MDR1 expression was associated with the probability of acute cellular rejection (42.1% in high-MDR1 group versus 23.4% in low-MDR1 group, $P = .0265$). The resultant odds ratio was 2.376 (95% CI, 1.087-5.191) for the patients with a high level of intestinal MDR1 mRNA at living-donor liver transplantation (Table II). However, there was no significant association between the intestinal CYP3A4 mRNA level and the probability of acute cellular rejection ($P = .9211$) (Fig 5, *B*). The odds ratio showed that a high level of CYP3A4 mRNA at liver transplantation was not a risk factor for the occurrence of postoperative acute cellular rejection (Table II). Moreover, the mRNA expression level of mucosal MDR1 in the patients with acute cellular rejection was weakly but significantly higher compared with those in the event-free group ($P = .0476$) (Fig 5, *C*).

Furthermore, the impact of mRNA expression levels of absorptive barriers on patient survival was also examined. According to the method of Kaplan-Meier and subsequent log-rank statistics, the high-level expression of both MDR1 mRNA (Fig 6, *A*) and CYP3A4 (Fig 6, *B*) was significantly associated with patient survival. The odds ratio of the intestinal expression level of MDR1 mRNA at surgery was 7.413 (95% CI, 1.567-36.073), whereas that of CYP3A4 was 3.590 (95% CI, 0.936-13.769).

Table II. Risk factors associated with acute cellular rejection until postoperative day 10

Factors	*Odds ratio*	*95% CI*
Mean trough level of tacrolimus <7 ng/mL between postoperative days 2 and 4	2.772	1.265-6.075
High level of intestinal MDR1 mRNA at surgery	2.376	1.087-5.191
High level of intestinal CYP3A4 mRNA at surgery	1.026	0.485-2.168

CI, Confidence interval; mRNA, messenger ribonucleic acid.

Dosage adjustment based on expression level of intestinal MDR1. To obtain more information about the effect of the intestinal expression level of MDR1 on the pharmacokinetics of tacrolimus, as well as the risk of acute cellular rejection, we compared the daily oral dosage and trough level of tacrolimus between the high- and low-MDR1 groups (Fig 7). The oral dosages

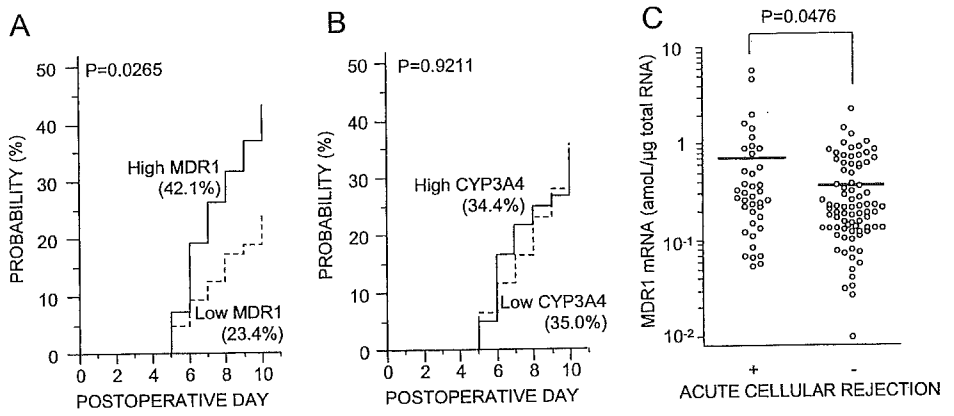

Fig 5. mRNA expression levels of MDR1 and CYP3A4 and acute cellular rejection episodes in 121 recipients of living-donor liver transplantation. The mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in mucosa derived from living-donor liver transplant recipients were determined by a real-time polymerase chain reaction (PCR) analysis, as described in the Methods section. High and low indicate whether the expression level of MDR1 mRNA and CYP3A4 mRNA in individual mucosa was higher or lower than the median value for all intestinal samples, respectively. *P* values were determined with the log-rank test. **C**, The mRNA expression levels of MDR1 in mucosa were shown with (+) or without (−) acute cellular rejection during 10 days postoperatively. The *P* value was determined with the Mann-Whitney *U* test.

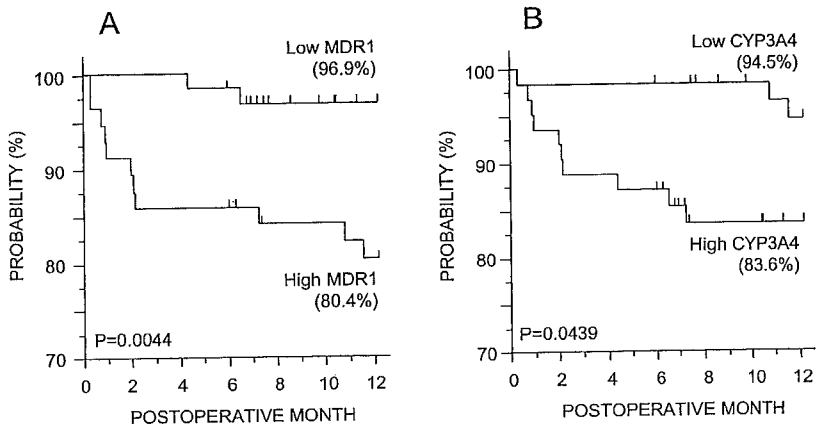

Fig 6. mRNA expression levels of MDR1 and CYP3A4 and cumulative survival rate in 121 recipients of living-donor liver transplantation. The mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in mucosa derived from living-donor liver transplant recipients were determined by a real-time PCR analysis, as described in the Methods section. High and low indicate whether the expression level of MDR1 mRNA and CYP3A4 mRNA in individual mucosa was higher or lower than the median value for all intestinal samples, respectively. *P* values were determined with the log-rank test. *Tick marks* indicate the length of follow-up of individual patients who survived.

of tacrolimus were significantly higher in patients categorized in the high-MDR1 group than those in the low-MDR1 group from postoperative day 3 (Fig 7, *A*). However, the daily trough levels of tacrolimus between postoperative days 2 and 10 were comparable between the 2 groups (Fig 7, *B*). The odds ratio was 2.283 (95% CI, 1.058-4.926) for patients whose average trough concentration of tacrolimus between postoperative days

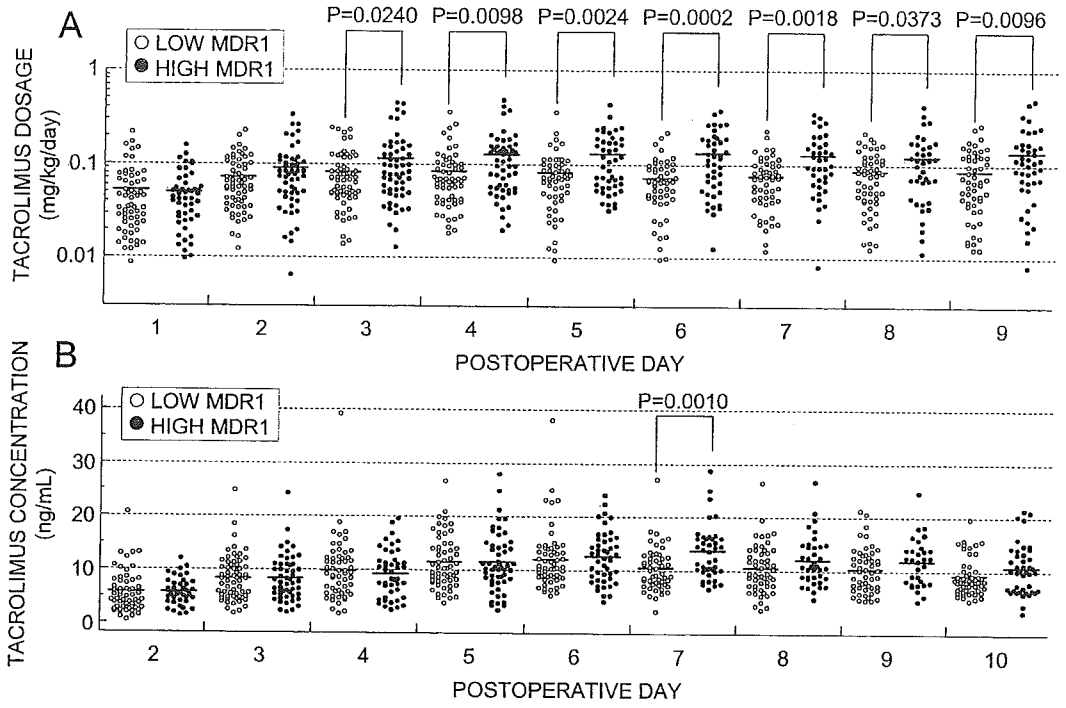

Fig 7. Postoperative oral dosage and trough concentration of tacrolimus in recipients after living-donor liver transplantation. Daily oral dosages (**A**) and trough concentrations (**B**) of tacrolimus for 121 patients receiving de novo living-donor liver transplants are illustrated. The patients are divided into 2 groups: low MDR1 (*open circles*) and high MDR1 (*solid circles*). Low MDR1 and high MDR1 indicate whether the expression level of MDR1 mRNA in individual mucosa was lower or higher than the median value for all intestinal samples. Statistical analysis was performed by use of the unpaired t test after Welch correction. P values of less than .05 were shown.

2 and 4 was under 7 ng/mL and 2.306 (95% CI, 1.058-5.028) for patients whose intestinal expression level of MDR1 at surgery was greater than 0.242 (Table II).

Correlation between mRNA level of MDR1 and tacrolimus C/D ratio. In this study the average trough concentration of tacrolimus immediately after living-donor liver transplantation and the intestinal expression level of MDR1 were identified as factors useful for predicting the risk of acute cellular rejection immediately after transplantation (Figs 3 and 5 and Table II). If the mRNA level of MDR1 at operation is a potential pharmacokinetic factor, control of the tacrolimus blood concentration will be easier, and the frequency of episodes of acute cellular rejection may be reduced. On the basis of this hypothesis, we performed a correlation analysis of the molecular data on intestinal absorptive barriers and tacrolimus pharmacokinetics to examine whether the MDR1 mRNA level at operation could be a pharmacokinetic factor for individualized initial dosage adjustment. As shown in Fig 8, the mRNA expression level of MDR1 ($r = -0.5672$, $P < .0001$), but not of CYP3A4 ($r = -0.0490$, $P = .5466$), was inversely correlated with the C/D ratio of tacrolimus between postoperative days 2 and 4. Although the mass of graft liver from the living donor was limited in the adult patients, the graft liver was relatively sufficient or large in the pediatric patients. Therefore it is also important to evaluate the engrafted liver mass as the graft-to-recipient weight ratio (Graft liver mass [in kilograms]/Recipient body weight [in kilograms] at surgery × 100 [percent]).[2] Furthermore, when the patients were divided into 2 groups based on the graft-to-recipient weight ratio (1.5) (Fig 9, *A*), the coefficient of the correlation between the intestinal mRNA level of MDR1 and tacrolimus C/D ratio improved to −0.6798 ($P < .0001$) and −0.7180 (P

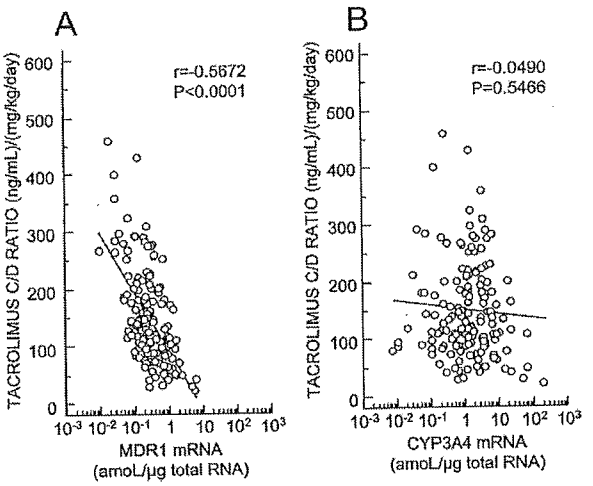

Fig 8. Concentration/dose (C/D) ratio as a function of mRNA expression levels of MDR1 and CYP3A4 in 164 recipients of living-donor liver transplantation. The average C/D ratio in the first 4 days after surgery is compared with the logarithmically transformed mRNA levels of MDR1 (**A**) and CYP3A4 (**B**).

< .0001) for the patients whose graft-to-recipient weight ratio was over 1.5 and those whose ratio was under 1.5, respectively (Fig 9, *B* and *C*). However, the coefficient of the correlation between the intestinal MDR1 mRNA level at surgery and the tacrolimus C/D ratio after postoperative day 5 gradually decreased (Table III).

DISCUSSION

Acute cellular rejection, an early complication of orthotopic liver transplantation, occurs in more than 50% of patients[27-29] and can be diagnosed only by means of a liver biopsy.[25] Although it has minimal impact on either death or late graft function, acute cellular rejection should be avoided to reduce the adverse effects of antirejection treatment. The use of high-dose steroids is the first step in both the induction of immunosuppression and the treatment of acute cellular rejection despite side effects such as osteoporosis, recurrent viral hepatitis, and infections.[30-32] The direct association between acute cellular rejection and patient mortality rate is weak,[7] but this episode would be a trigger for other severe complications such as infections, drug-induced renal injury and neurotoxicity, and recurrence of hepatitis with viral amplification in patients receiving the antirejection treatment. In our study the patients categorized in the high-MDR1 group showed a higher frequency of acute cellular rejection until postoperative day 10 and poor survival within 1 year after surgery (Figs 5 and 6). Therefore the individual patients' clinical history should be explored because "acute cellular rejection" might be hidden behind the diagnosis at death. The incidence of acute cellular rejection in our series was 25.6% (42/164 cases) until postoperative day 10 and 32.2% overall (39/121 cases, excluding 32 patients treated with high-dose steroids for other reasons and 11 cases of post-transplant graft liver failure) (Table I). Of 13 patients who died within 1 year after transplantation, 11 were categorized in the high-MDR1 group. The mortality rate of high-MDR1 patients who had acute cellular rejection early on was 25% (6/24 cases), whereas all 15 patients in the low-MDR1 group were alive despite an episode of acute cellular rejection. In addition, the mortality rate of event-free patients was 15% (5/33 cases) in the high-MDR1 group and 4% (2/49 cases) in the low-MDR1 group. Focusing on the patients with acute cellular rejection during the first 10 days after surgery, the high-level expression of intestinal MDR1 was suggested to be associated with poor survival by χ^2 statistics (6/24 cases in high-MDR1 group versus 0/15 cases in low-MDR1 group, $P = .0352$). Although our results were derived from a relatively small number of cases, the intestinal expression level of MDR1 at surgery could be a prognostic factor in patients with acute cellular rejection at an early phase. If the occurrence of acute cellular rejection can be avoided in the high-MDR1 patients, the mortality rate may be decreased to a level comparable to that in the low-MDR1 group. To reduce the frequency of acute cellular rejection early on, the average trough concentration of tacrolimus during the initial 4 days after surgery should be kept above 7 ng/mL, with an initial dosage adjustment that takes into consideration the intestinal expression level of MDR1 at surgery. In addition, extensive exposure to tacrolimus at an early phase may reduce the mortality rate of patients categorized in the high-MDR1 group.

The intestinal adenosine triphosphate–driven efflux pump MDR1 is considered to play an important role in drug pharmacokinetics.[33] This drug transporter prevents the luminal entry of orally administered drugs such as tacrolimus, cyclosporine (INN, ciclosporin), and sirolimus at apical membranes. Since the report by Hoffmeyer et al,[34] several single-nucleotide polymorphisms (SNPs) in *MDR1* affecting expression or function (or both) have been reported. Notably, C3435T and G2677T/A are detected at a relatively high frequency and have been examined for influences on the drug pharmacokinetics and expression level of the gene product.[34,35] We previously found that these SNPs did