tacrolimus even at high blood drug concentrations in liver transplant patients, as well as in vitro experiments (Figs 1 and 4, *B*). Similar results were also obtained by our previous analysis, in which calcineurin activity in whole blood was measured after the administration of tacrolimus in rats.[12] These findings imply that tacrolimus may have previously unknown mechanism(s) of action for immunosuppression. Alternatively, the inhibition of immune function by tacrolimus might be mediated by selective inhibition of the calcineurin catalytic subunit isoform (α or β), which is more important for the activation of T lymphocytes.[26,27] Further studies are necessary to clarify whether calcineurin inhibition is critical for immunosuppression by tacrolimus.

Notably, cyclosporine-related nephrotoxicity was associated with high C_0 levels but not C_2 levels in liver transplant patients (Fig 5, *A*). In contrast, the calcineurin activity at the trough time point and at 2 hours after dosing of cyclosporine did not significantly differ between patients with and without nephrotoxicity (Fig 5, *A*). Therefore nephrotoxicity may be induced by extensive cyclosporine exposure as a result of increased C_0 levels, and calcineurin inhibition in PBMCs might not be associated with the adverse event. Patients taking cyclosporine with no rejection episode had high C_2 levels, and the C_0 levels were within the therapeutic range (Fig 5, *B*). On the other hand, the target C_2 levels were not achieved in the 2 patients with acute rejection (Fig 5, *B*). Furthermore, the mean calcineurin activity in patients receiving cyclosporine with no rejection episode was lower than 20 pmol $\cdot$ min^{-1} $\cdot$ mg protein^{-1}, whereas that in the 2 patients with acute rejection was higher than 20 pmol $\cdot$ min^{-1} $\cdot$ mg protein^{-1} (Fig 5, *B*). These results suggest that C_2 monitoring may be effective for the prevention of acute rejection and that the risk of nephrotoxicity will be reduced by C_0 monitoring in living-donor liver transplant patients treated with cyclosporine.

In this study tacrolimus-related nephrotoxicity occurred at high trough blood concentrations and at low calcineurin activity (Fig 6, *A*). On the other hand, patients taking tacrolimus with acute rejection had trough blood concentrations that were significantly lower than the concentrations in those with no rejection episode (Fig 6, *B*). Furthermore, we first found that high levels of calcineurin activity were related to acute rejection in living-donor liver transplant patients receiving tacrolimus (Fig 6, *B*). On the basis of our findings (Fig 6) and those of a previous study,[28] a target trough blood concentration of 10 ng/mL for tacrolimus may be safe and effective to reduce the risk of both nephrotoxicity and acute rejection in the initial period of living-donor liver transplantation. The trough monitoring of blood tacrolimus concentrations may be sufficient to predict overall calcineurin inhibition in PBMCs because a similar magnitude of calcineurin activity would be maintained during a dosing interval. In addition, the monitoring of calcineurin activity might have therapeutic potential to identify patients given tacrolimus in whom acute rejection subsequently occurs despite the trough blood concentrations being within the therapeutic range.

Sanquer et al[29] have recently reported that calcineurin phosphatase activity in mononuclear cells may be a functional index with which to predict acute graft-versus-host disease after allogenic stem-cell transplantation. In addition, the proportion of IL-2–producing $CD8^+$ T cells has been shown to be predictive of acute rejection after liver transplantation.[30] When these findings and our results are taken into consideration, pharmacodynamic assessment of calcineurin activity, as well as IL-2 production, in combination with classical therapeutic drug monitoring, may be useful for determining the individual therapeutic range of tacrolimus and cyclosporine in patients after living-donor liver transplantation. We have already reported a population pharmacokinetic model and parameters of tacrolimus in adult living-donor liver transplant patients.[31] Given that the population pharmacokinetic and pharmacodynamic models of tacrolimus can be combined, we could personalize the tacrolimus dosage required for adequate calcineurin inhibition by the Bayesian method.[32] However, the measurement of calcineurin activity in PBMCs is time-consuming and expensive and usually requires radioactive reagents, often precluding its routine clinical practice. Therefore an alternative method for calcineurin phosphatase assay should be developed that is more sensitive and feasible to perform compared with the HPLC method currently applied.[16]

In conclusion, we have demonstrated for the first time that inhibitory effects on calcineurin phosphatase activity in PBMCs differ between tacrolimus and cyclosporine in living-donor liver transplant patients. In addition, we have clarified that there is extensive interindividual variability in calcineurin activity for both drugs and that acute rejection is associated with increased calcineurin activity in patients given tacrolimus. Further prospective analysis in a large population should be performed to define the optimal therapeutic range of calcineurin activity to prevent acute rejections

in living-donor liver transplant patients receiving tacrolimus or cyclosporine.

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Living Donor Liver Transplantation for Pediatric Patients with Inheritable Metabolic Disorders

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Forty-six pediatric patients who underwent living donor liver transplantation (LDLT) using parental liver grafts for inheritable metabolic disorders (IMD) were evaluated to determine the outcomes of the surgery, decisive factors for post-transplant patient survival and the impact of using donors who were heterozygous for the particular disorder. Disorders included Wilson disease (WD, n = 21), ornithine transcarbamylase deficiency (OTCD, n = 6), tyrosinemia type I (TTI, n = 6), glycogen storage disease (GSD, n = 4), propionic acidemia (PPA, n = 3), methylmalonic acidemia (MMA, n = 2), Crigler-Najjar syndrome type I (CNSI, n = 2), bile acid synthetic defect (BASD, n = 1) and erythropoietic protoporphyria (EPP, n = 1). The post-transplant cumulative patient survival rates were 86.8 and 81.2% at 1 and 5 years, respectively. Post-transplant patient survival and recovery of the growth retardation were significantly better in the liver-oriented diseases (WD, OTCD, TTI, CNSI and BASD) than in the non-liver-oriented diseases (GSD, PPA, MMA and EPP) and pre-transplant growth retardation disadvantageously affected post-transplant outcomes. Although 40 of 46 donors were considered heterozygous for each disorder, neither mortality nor morbidity related to the heterozygosis has been observed. LDLT using parental donors can be recommended as an effective treatment for pediatric patients with IMD. In the non-liver-oriented diseases, however, satisfactory outcomes were not obtained by hepatic replacement alone.

Key words: Donor selection, heterozygous carrier, mode of inheritance

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Introduction

The use of liver transplantation (LT) has steadily increased, including for the treatment of some inborn metabolic deficiencies, irrespective of whether the liver is predominantly or only partly involved in disorder (1, 2). In some cases, however, there is a shortage of deceased donor organs and a living donor who is heterozygous for the disorder in question must be employed (3, 4). In pediatric cases of autosomal recessive disorder in particular, the donor is almost always a heterozygote because a parent is usually employed in such cases.

Between June 1990 and December 2003, 578 pediatric patients (aged less than 18 years) underwent initial living donor liver transplantation (LDLT) at Kyoto University Hospital. Of these 578, 46 underwent an LDLT using parental liver grafts for inheritable metabolic disorders (IMD). Although 24 of these cases have previously been reported (3–7), all were evaluated in the present study in order to determine their LDLT outcomes and decisive factors for post-transplant patient survival, and to clarify the impact of the use of heterozygous donors on both donors and recipients.

Patients and Methods

Forty-six pediatric patients with IMD indicated for LDLT at Kyoto University were examined in the present study. These included patients with Wilson disease (WD, n = 21; cirrhosis, 14; fulminant-type, 7), ornithine transcarbamylase deficiency (OTCD, n = 6), tyrosinemia type I (TTI, n = 6), glycogen storage disease (GSD, n = 4; type Ib, 1; type IV, 3), propionic acidemia (PPA, n = 3), Crigler-Najjar syndrome type I (CNSI, n = 2), methylmalonic acidemia (MMA, n = 2), bile acid synthetic defect of the liver (BASD, n = 1) and erythropoietic protoporphyria (EPP, n = 1) (Figure 1). Clinical records of these patients were reviewed to collect the following data: age at onset, gender, time from onset to LDLT, pre-transplant status (at home, in wards and in the intensive care unit (ICU)), the presence and degree of neurological impairments and growth retardation evaluated at the time of LDLT, ABO-blood-type matching, graft types, mode of operative procedure (auxiliary partial orthotopic liver transplantation (APOLT) or not), graft-to-recipient weight ratio (GRWR) calculated by the following formula: ((graft weight weighed after flushing the preservation solution (g)/ patient's body weight (g)) × 100 (%)), survival outcomes and neurological status, physical

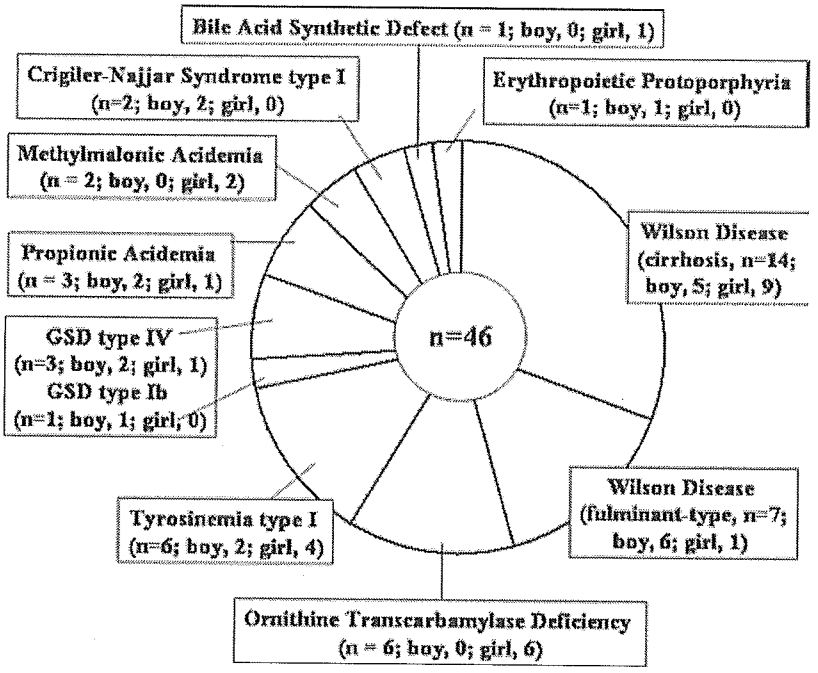

Figure 1: Indications for living donor liver transplantation of 46 pediatric patients with inheritable metabolic disorders at Kyoto University.

growth and quality of life at the latest evaluations. Neurological status was evaluated by a grading scale based on that of Whitington et al. (8) with minor modifications, as shown in Table 1. Physical growth was evaluated by comparing the weight and height of each patient with those in the standard growth curve and is expressed as a multiple of the standard deviation (SD) of the deviation from the standard curve. Growth data were classified into three subgroups, as shown in Table 1. Quality of life was classified into four subgroups also as shown in Table 1.

To clarify decisive factors for post-transplant patient survival, correlations among survival outcomes, whether each disorder predominantly involved the liver (liver-oriented disease group, LOD: WD, OTCD, TTI, CNSI and BASD; n = 36) or partly involved the liver (non-liver-oriented disease group, NLOD: GSD, PPA, MMA and EPP; n = 10), physical growth at the time of LDLT and graft-size matching evaluated by GRWR were investigated.

Whether or not each donor was a heterozygote for the recipient's disorder was determined by the mode of inheritance of each disorder (autosomal recessive inheritance for WD (3), TTI (9), GSD (10), PPA (4), MMA (4), CNSI (4) and BASD (11), autosomal dominant for EPP (12) and X-linked for OTCD (4)). In addition to our standard donor selection criteria, which have been described in detail elsewhere (13,14), some donors who were considered or suspected to be heterozygous carriers for their respective recipient's disorder underwent the following additional medical tests according to the disorder in question: for WD cases, assays for serum ceruloplasmin levels, urine copper excretion and the presence of Kayser-Fleischer corneal ring; for OTCD cases, quantitative serum amino acid analysis (QAAA) and allopurinol loading test (15,16); and for cases of PPA or MMA, serum propionic acid or methylmalonate level and the presence of metabolic acidosis confirmed by blood gas analysis. These additional tests were conducted periodically in the post-transplant period for each heterozygous carrier and each recipient of a heterozygous liver in order to study mortality or morbidity in relation to the use of heterozygous donors. Furthermore, in donor candidates for OTCD patients who showed abnormal findings in the QAAA and/or allopurinol loading test, genetic assay using peripheral blood leukocytes (17) was performed in order to confirm whether or not there were mutations in Xp21, where the ornithine transcarbamylase (OTC) gene lies. We performed genetic assay only for OTCD donors because the presentation of male hemizygotes or female heterozygotes for OTCD can range in severity from fatal neonatal hyperammonaemic coma to asymptomatic adults. Thus, we believe that such individuals require close medical vigilance for the onset of OTCD. With regard to the other autosomal recessive disorders, including the TTI, GSD, CNSI and BASD, no additional examination was performed. For all donors, the recipient's disorder, relationship of the donor to the recipient, donor age, mode of donor hepatectomy, resection rate of the donor hepatectomy calculated from the following equation: (actual graft weight weighed as stated above (g))/ (total liver volume calculated from preoperative computed tomography (CT) volumetry (mL) × 100(%) and immediate and long-term postoperative course were reviewed. In order to determine whether postoperative morbidities were related to the use of heterozygote donors, recipients of heterozygous livers were accompanied by their donors or other family members during follow-up and were asked about their pre-transplant symptoms. Heterozygous donors and other family members were also asked if they suffered symptoms similar to those of the recipients.

Follow-up was continued until January 2005 or death for both donors and recipients.

SPSS commercial statistics software was used for all statistical analyses (SPSS 12.0 for Windows; SPSS, Chicago, IL, USA). Survival was evaluated by the Kaplan-Meier life table analysis with the Breslow-Gehan-Wilcoxon test. Other variables were evaluated in a non-parametric manner. Values were shown as the median (range). The p-values of less than 0.05 were considered to be significant.

Results

Outcomes of LDLT

Seventeen of 46 patients were admitted to the ICU in the pre-transplant period: four of these 17 were admitted to

Table 1: Grading scale for evaluating neurological status and classification of physical growth and quality of life

Grading scale for evaluating neurological status
Grade 0: Seems to be normal spectrum for social interaction, motor skills, language development and learning
Grade 1: Good social interaction, full ambulation but perhaps partially impaired gross and fine motor skills, use of language, mildly delayed development, only modest learning deficits
Grade 2: Definite social interaction, fair ambulation, though possibly limited by spasticity
Grade 3: Limited social interaction, no bipedal ambulation, limited communication through gestures
Grade 4: Responds to noxious stimuli, but no social interaction, no ambulation, no communication
Grade 5: Persistent coma or vegetative state
Classification of physical growth
Normal: More than −1SD* in height
Slightly delayed: More than −2SD* and equal to or less than −1SD* in height
Delayed: Equal to or less than −2SD* in height
Classification of quality of life
Excellent: Neurological status corresponding to a score of 0 on the above scale, and receiving none of or one immunosuppressive drug and no metabolism correcting drugs
Good: Neurological status corresponding to a score of 0 on the above scale, and receiving 2 or 3 immunosuppressive drugs and/or metabolism correcting drugs
Fair: Neurological status corresponding to a score of 1 or 2 on the above scale, irrespective of any medication
Poor: Neurological status corresponding to a score of 3 or more, irrespective of any medication

*Physical growth was evaluated by comparing the weight and height of each patient with those in the standard growth curve, and was expressed as a multiple of the standard deviation (SD) of the deviation from the standard curve.

Table 2: Patients' characteristics

Patients' backgrounds	
Age at the onset (months)	48.6 (0–196)
Gender (Boy/ Girl)	21/ 25
Time from onset to LDLT* (months)	3.9 (0.3–181)
Age at LDLT* (months)	86.5 (1.4–199)
Pre-transplant status	
At home/ in wards/ in the ICU†	11/18/17
Pre-transplant status of physical growth‡	
Height	−0.35SD§ (−9.0SD§ to +3.4SD§)
Weight	−0.40SD§ (−9.0SD§ to +3.1SD§)
Delayed/slightly delayed/normal	16/2/28
Pre-transplant neurological status	
Grade 0/1/2/3/4/5	26/6/9/4/3/1
APOLT¶/total hepatic replacement	3/43
Donors for initial LDLT*	
Father/mother/stepfather	22/23/1
ABO blood type combination (Identical/compatible/incompatible)	26/13/7
Heterozygote/non-heterozygote	40/6
Graft liver (LLS**/LL††/RL‡‡)	25/17/4
GRWR§§ (%)	1.35 (0.61–9.68)

*Living donor liver transplantation; †intensive care unit; ‡represented in how far from the standard growth curve expressed as a multiple of the standard deviation; §standard deviation; evaluated by the grading scale as shown in Table 1; ¶auxiliary partial orthotopic liver transplantation; **left lateral section liver graft (segments II–III according to the Couinaud's nomenclature for liver segmentations); ††left liver graft (segments II–IV); ‡‡right liver graft (segments V–VIII); §§graft-to-recipient weight ratio.

the ICU for severe pre-transplant neurological impairments necessitating artificial ventilator support and the other 13 required intensive care due to severe worsening of their general condition arising from symptoms of hepatic failure other than neurological impairments (Table 2). The disorders of patients who required artificial ventilator support because of severe neurological impairments corresponding to a score of 4 or 5 on the grading scale described above were OTCD in two cases, fulminant-type WD in one and cirrhosis of WD in one. Marked pre-transplant growth retardation was observed in 16 patients; in 15 of these 16, disease onset was in early infancy. Seven of these 46 patients received ABO-incompatible liver grafts. There were 10 postoperative deaths during this study period. Six of the 10 deaths were hospital mortalities (defined as mortalities occurring during the recuperative hospital stay following the LDLT). The other four were observed during the long-term follow-up and two of these four deaths were unrelated to either the original diseases or the LDLT procedure (Table 3). Although the cause of mortality was related to biliary complications in three of the 10 patients who died (Table 3), three other patients suffering from biliary complications (anastomotic leakage in one patient and anastomotic stricture in 2) were managed with surgical and/or radiological intervention and achieved recovery. Several other postoperative surgical complications including hemoperitoneum in one patient, hepatic venous stenosis in two and portal venous stenosis in one were observed, but all of these patients also recovered after surgical and/or radiological intervention. A second LDLT was required for two patients. One of these cases was a 3-year and 8-month-old boy with GSD type IV (Table 3), who underwent initial LDLT using a maternal ABO-incompatible liver graft, which resulted in graft failure due to antibody-mediated rejection (18) arising from the ABO-incompatibility and was replaced by a paternal ABO-incompatible liver graft 6.2 months after the initial LDLT; unfortunately, the boy died of sepsis a month after the second LDLT. The other case was a 13-year-7-month-old girl who underwent an initial LDLT with a maternal ABO-compatible liver graft for cirrhosis due to WD; whereas this initial graft failed due to chronic portal vein thrombosis 126 months after the initial LDLT and was replaced by a paternal ABO-incompatible liver graft. The patient is currently doing well at 16.6 months after the second LDLT. Thirty-five of the 36 surviving patients currently show a normal neurological status

Table 3: Details of the 10 dead patients

Phase of mortality	Disease	Gender	Age at LDLT* (yr, mo)	Time from onset to LDLT* (months)	Cause of mortality	Duration of survival after LDLT* (months)
Hospital mortalities	Tyrosinemia type I	Girl	0y 4m	3.1	Severe graft congestion due to remarkable imbalance between body and graft sizes (GRWR† = 9.68%)	0.6
	GSD‡ type Ib	Boy	13y 2m	156	Systemic candidasis	1.4
	GSD‡ type IV	Boy	3y 8m	33.3	Antibody-mediated rejection due to the use of ABO-incompatible liver graft	7.2
	MMA§	Girl	1y 1m	12.3	Intra-abdominal infection due to major biliary anastomotic leakage	0.5
	MMA§	Girl	12y 2m	146	Aspergillosis	2.2
	Protoporphyria	Boy	15y 6m	84.3	Major biliary anastomotic leakage and candidasis	3.3
Late deaths	WD¶ (fulminant-type)	Boy	16y 6m	2.8	Chronic cholangitis due to biliary anastomotic stricture	50.7
	OTCD#	Girl	7y 2m	14.1	Died in a traffic accident	4.2
	Tyrosinemia type I	Girl	0y 3m	3.0	Died in a traffic accident	18.9
	BASD**	Girl	0y 9m	8.0	Hemolytic ureic syndrome caused by Escherichia coli infection	5.4

*Living donor liver transplantation; †graft-to-recipient weight ration; ‡glycogen storage disease; §methylmalonic acidemia; ¶Wilson disease; #ornithine transcarbamylase deficiency; **bile acid synthetic defect of the liver.

corresponding to a score of 0 on our grading scale. Only one patient, a 13-year-8-month-old boy with fulminant-type WD, in whom the neurological status just before LDLT corresponded to a score of 5 on our grading scale and in whom emergency LDLT using a liver graft from his stepfather was carried out, continues to show neurological impairments pertaining to a score of 3 on our grading scale at 63.7 months after LDLT. Taking these results together, the post-transplant cumulative patient survival rates were 86.9% at 1 year and 81.2% both at 5 and 10 years (Figure 2).

Decisive factors for post-transplant patient survival and evaluation of post-transplant physical growth and quality of life

Post-transplant cumulative patient survival rates were significantly better in the LOD group than in the NLOD group (Figure 3). Furthermore, post-transplant cumulative patient survival rates of patients with normal physical growth or slightly delayed physical growth at the time of LDLT were significantly higher than that of patients with delayed physical growth at the time of LDLT (Figure 4). In addition, physical growth, represented by the deviation from the standard growth curve at the time of LDLT, was significantly correlated with both the age of onset of each disorder and the time from onset to LDLT (Figure 5). Specifically, the earlier the age of onset or the longer the time from onset to LDLT in each patient, the worse the retardation of growth. An ICU-stay during the pre-transplant period did not affect post-transplant cumulative patient survival (Figure 6). Although graft-size matching was not significantly correlated with post-transplant cumulative patient survival rates, the post-transplant survival of patients with GRWR $\geq$ 4.0 tended to be worse than those of other patient groups (Figure 7). The age at onset of each disorder, time from onset to LDLT and physical growth evaluated at the time of LDLT were significantly younger, longer and more inhibited in the NLOD group than in the LOD group, respectively (Table 4). With regards to the 36 surviving patients, a comparison of physical growth and quality of life at the latest evaluations between patients with LOD and those with NLOD showed that physical growth was significantly better in the LOD group than in the NLOD group, whereas quality of life was similar between the two groups (Table 5). Concerning the quality of life, an excellent or good quality of life has been maintained in all surviving patients, irrespective of whether belonged to the LOD or NLOD group, with the single exception of a patient with fulminant-type WD who continues to show neurological impairments corresponding to a score of 3 on our grading scale, as stated above. With regard to the six patients in whom quality of life was determined to be not excellent but good (Table 5), all of these patients are still taking two or more immunosuppressive and/or metabolism correcting drugs. Two patients who underwent LDLT for WD developed de novo autoimmune hepatitis (19), at 18.6 months after LDLT and 87.6 months after LDLT and both of these patients are still receiving three immunosuppressive drugs (a calcineurin inhibitor (CI), azathiopurine and prednisolone), at 38.0 months and 89.6 months after LDLT, respectively. One patient who underwent LDLT for WD is still receiving CI and mycophenolate mofetil at 24.4 months after LDLT

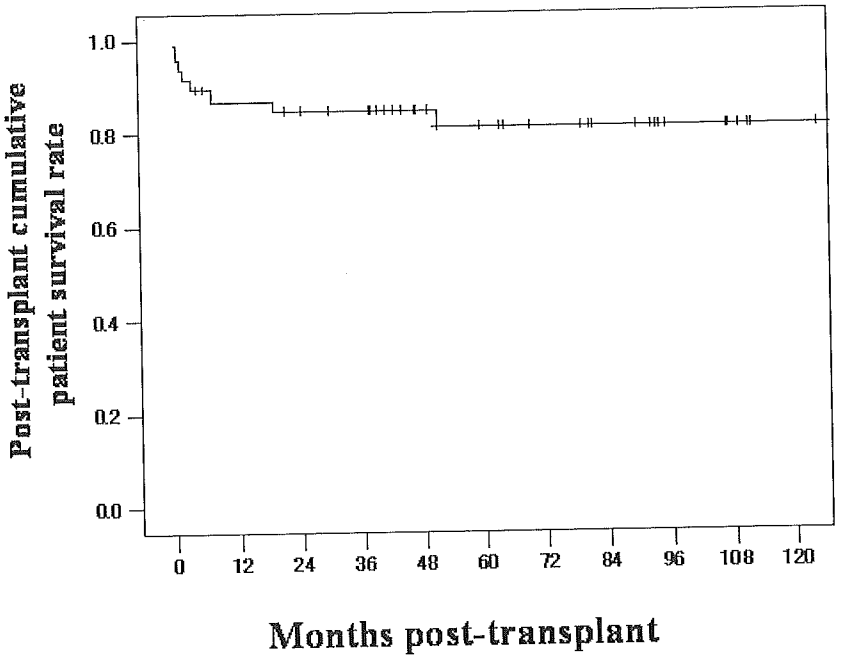

Figure 2: Cumulative post-transplant patient survival rates of living donor liver transplantation for 46 pediatric patients with inheritable metabolic disorders. Post-transplant survival of patients who underwent living donor liver transplantation for inheritable metabolic disorders at Kyoto University resulted in cumulative patient survival rates of 86.9% at 1 year and 81.2% both at 5 and 10 years.

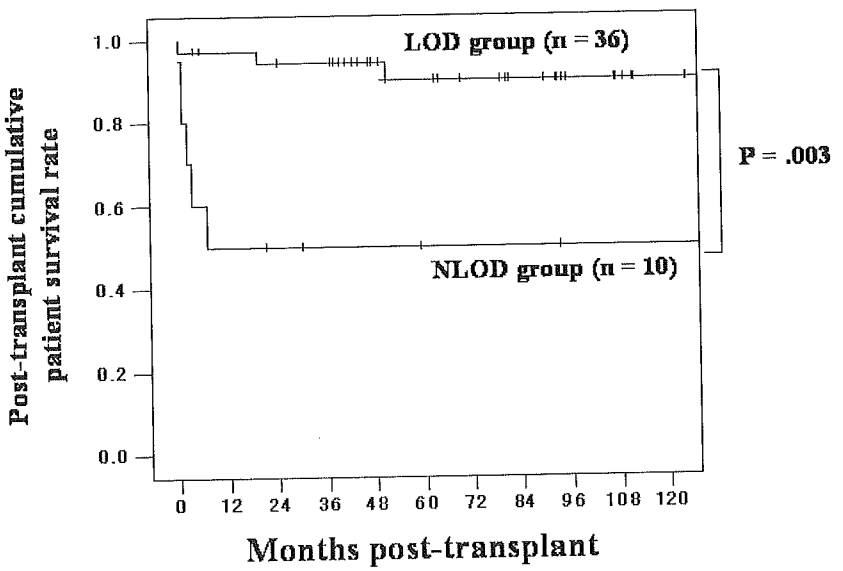

Figure 3: Comparison of post-transplant survival between liver-oriented diseases (LOD) and non-liver-oriented diseases (NLOD) groups. Post-transplant cumulative patient survival rate was significantly higher in patients with LOD than in those with NLOD.

because of mild but refractory acute cellular rejection. The other three patients, all of whom underwent LDLT for PPA, are still receiving CI and carnithine supplementation (6) at 59.3 months, 29.9 months and 21.2 months after LDLT, respectively.

Impact of the use of heterozygous donor

In addition to the 46 donors for initial LDLT, two donors were employed for a second LDLT, as stated above. Both were fathers of patients with autosomal recessive disorders. A preoperative QAAA and allopurinol loading test were performed for the six parental donors of the girls with OTCD. The former analysis revealed normal QAAA profiles in all six parents. The latter test yielded no abnormal findings in the four fathers, but the two mothers had almost twice normal upper values of peak urine orotic acid and orothidine levels after the allopurinol loading. These results suggest that these four fathers were not hemizygotes for OTCD, whereas these two mothers were determined to be heterozygotes for OTCD. As a result, 42 of the 48 donors were heterozygous carriers for the patients' disorders and the other six were non-heterozygotes. No significant differences suggesting the deleterious effects of use of the heterozygous donors on donors' postoperative course were observed between the heterozygote donors and non-heterozygote donors (Table 6). One

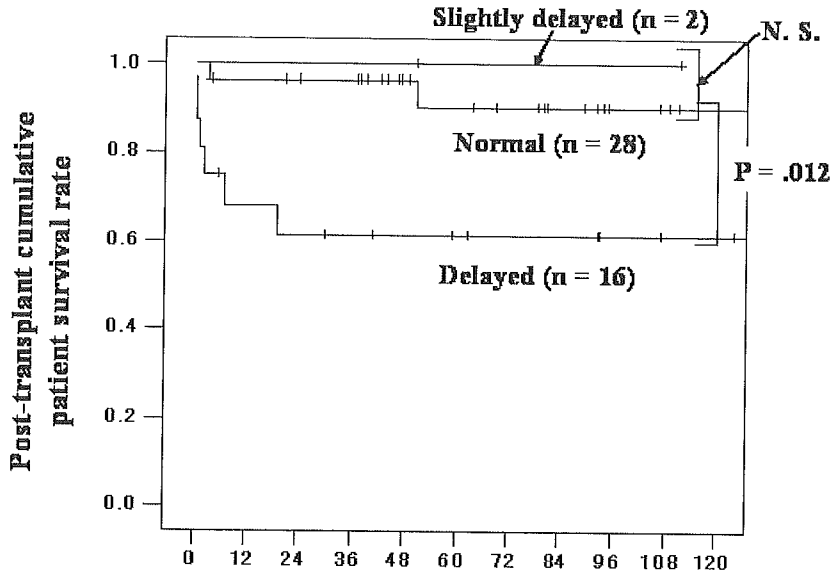

Figure 4: Comparison of post-transplant survival among three classifications of physical growth (normal, slightly delayed and delayed) at the time of living donor liver transplantation (LDLT). Post-transplant cumulative patient survival rates of patients with normal physical growth or slightly delayed physical growth at the time of LDLT were significantly higher than that of patients with delayed physical growth at the time of LDLT.

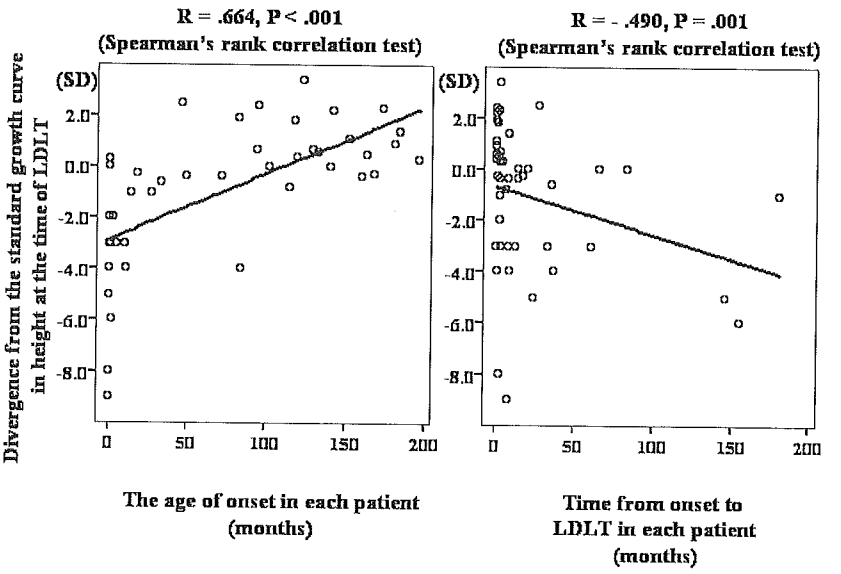

Figure 5: Correlation between physical growth and the age at onset of each disorder or time from onset to living donor liver transplantation (LDLT) in each patient. Physical growth represented in how far form the standard growth curve expressed as a multiple of the standard deviation (SD) at the time of LDLT was significantly correlated with both the age of onset of each disorder and the time from onset to LDLT. Namely, the earlier the age of onset in each patient was or the longer the time from onset to LDLT was, the worse the growth retardation was.

maternal donor, 37 years of age, of a patient who underwent LDLT for WD underwent right hepatectomy, for which resection rate was 61.2% and developed postoperative bile leakage from the cut surface of the liver remnant, which necessitated biliary decompression with the use of endoscopic retrograde nasal biliary drainage. Although the bile leakage was refractory and necessitated a prolonged hospital stay of 59 days before the donor was considered cured, the leakage did not lead to serious difficulties and the donor is currently doing well at 48.2 months after LDLT without any other complications. Two maternal donors of girls with OTCD, both of whom were determined to be heterozygous for OTCD as stated above, were genetically confirmed to have mutations in Xp21, where the OTC gene lies (4), but showed normal OTC activity in liver tissues extracted during donor surgery. No genetic assay was performed in the other 40 heterozygous donors, because the usefulness of genetic evaluations for disorders other than OTCD was considered uncertain at the time of LDLT. Regardless of whether or not they were heterozygotes, no major complications have been observed in any donors. All 48 donors are currently doing well.

Additional specific medical tests for heterozygous donors and recipients of heterozygous livers of the WD, OTCD, PPA and MMA cases have shown no problematic findings. Namely, all donors of WD cases have shown normal serum ceruloplasmin levels and undetectable levels of

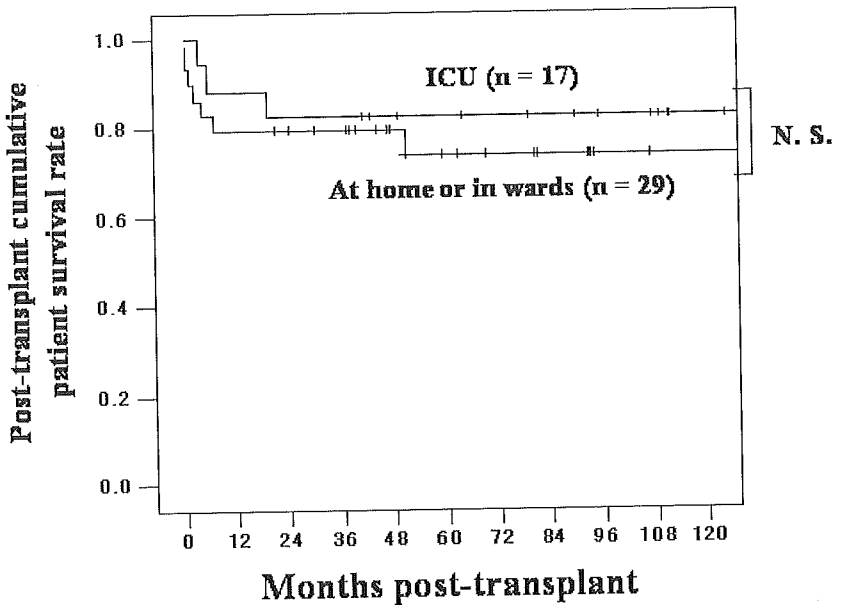

Figure 6: Comparison of post-transplant cumulative patient survival between patients who required the intensive care unit (ICU) stay in the pre-transplant period and those who did not. ICU stay in the pre-transplant period did not affect post-transplant patient survival.

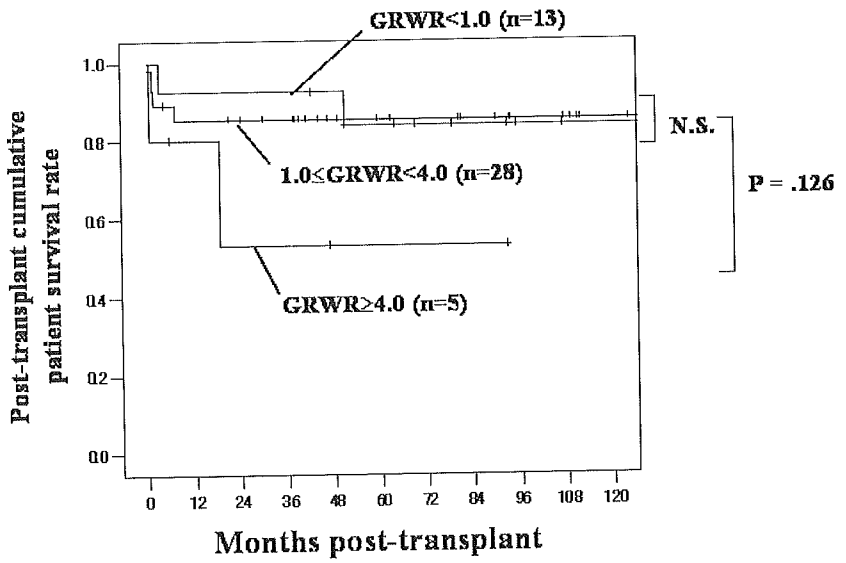

Figure 7: Correlation between post-transplant cumulative patient survival rates and graft-size matching evaluated by the graft-to-recipient weight ratio (GRWR). Although post-transplant cumulative patient survival rates were not different among patients with a graft-to-recipient weight ratio (GRWR) < 1.0, those with 1.0 ≤ GRWR < 4.0 and those with a GRWR ≥ 4.0, post-transplant cumulative patient survival rates tended to be worse in patients with a GRWR ≥ 4.0 than in other patient groups.

Table 4: Comparison of age at onset, time from onset to living donor liver transplantation (LDLT) and physical growth at the time of LDLT between patients with liver-oriented diseases (LOD) and those with non-liver-oriented diseases (NLOD)

	Patients with LOD* (n = 36)	Patients with NLOD† (n = 10)	P-value
Age at onset (months)	89.5 (0.1–196)	1.7 (0–102)	.003
Time from onset to LDLT‡ (months)	3.1 (0.3–181)	35.3 (3.6–156)	<.001
Physical growth evaluated at the time of LDLT‡			
Height§	$0SD^{\Pi}$ ($-9.0SD^{\Pi}$–$3.4SD^{\Pi}$)	$-3.0SD^{\Pi}$ ($-6.0SD^{\Pi}$–$0SD^{\Pi}$)	.001
Weight§	$-0.1SD^{\Pi}$ ($-6.0SD^{\Pi}$–$3.1SD^{\Pi}$)	$-2.0SD^{\Pi}$ ($-3.0SD^{\Pi}$ – $4SD^{\Pi}$)	.009
Normal/slightly delayed/delayed	26/2/8	2/0/8	.003

Table 5: Comparison of physical growth and quality of life at the latest evaluation between patients with LOD and those with NLOD in the 36 surviving patients.

	Patients with LOD* (n = 31)	Patients with NLOD† (n = 5)	P-value
Observation period (months)	78.7 (24.4–145.9)	59.3 (21.2–133.8)	.533
Physical growth at the latest evaluation			
Height§	0.70SD¤ (−2.0SD¤–3.9SD¤)	−2.0SD¤ (−3.0SD¤–0.6SD¤)	< .001
Weight§	0.10SD¤ (−2.0SD¤–2.2SD¤)	−0.8SD¤ (−2.0SD¤–2.0SD¤)	.040
Normal/slightly delayed/ delayed	28/2/1	1/1/3	.001
Quality of life: excellent/ good/fair/poor	27/3/0/1	2/3/0/0	.084

*Liver oriented diseases; †non-liver-oriented diseases; ‡living donor liver transplantation; §represented in how many far from the standard growth curve expressed as a multiple of the standard deviation; ¤standard deviation.
Numerical variables were evaluated by the Man-Whitney's U-test, and categorical variables were evaluated by the Fischer's exact probability test.

Table 6: Details of donors' characteristics

	Heterozygous donors (n = 42)	Non-heterozygous donors (n = 6)	P-value
Age at the time of LDLT* (years)	37 (23–53)	36 (27–44)	.338
Gender (male/female)	20/22	5/1	.114
Observation period (months)	89.1 (16.6–154.9)	87.8 (60.0–163.6)	.550
Mode of donor hepatectomy			
LLS†/LL‡/RL§	23/14/5	3/3/0	.834
Resection rate (%)	27.4 (16.3–69.5)	25.7 (21.5–34.3)	.820
Postoperative complications			
None/wound complications/bile leak	35/6/1	4/1/1	.265
Long-term complications	0	0	
Postoperative hospital stay (days)	9 (6–59)	9 (7–13)	.703

*Living donor liver transplantation; †left lateral sectionectomy (segments II–III according to the Couinaud's nomenclature for liver segmentations); ‡left hepatectomy (segments II–IV); §right hepatectomy (segments V–VIII).

urine copper excretion in all evaluations and were negative for Kayser-Fleischer corneal ring. The serum ceruloplasmin level was normalized immediately after LDLT and has been maintained in all patients with WD. Urine copper excretion decreased gradually after LDLT and was completely eradicated at around 12 months post-transplantation in all WD patients; accordingly, none of the patients with WD have received no chelator of copper after 12 months. Two patients with OTCD and their heterozygous-donor mothers have shown normal QAAA profiles and almost twice the upper normal values of urine orotic acid and orothidine after allopurinol loading in all annual evaluations. Both heterozygous-donor mothers of patients with OTCD have shown neither hyperammonemia nor any episodes suggestive of hyperammonemia. No episodes of heyperammonemia without evidence of graft dysfunction were observed in either of the recipients of heterozygous livers for OTCD. Donor and recipient pairs in three of the PPA cases and two donors in MMA cases showed no episode of metabolic acidosis and neither serum propionic acid nor methylmalonate was undetectable in any of the evaluations.

Of the 36 surviving patients, 32 were matched with heterozygous donors. None of these 32 has shown any evidence of recurrence of the original diseases and symptoms they suffered in the pre-transplant period. The 42 heterozygous donors also have shown no symptoms resembling those of the patients. Although eight of the 10 patients who died received heterozygous livers, their causes of death were considered to be unrelated to the heterozygosis (Table 3). Thus, neither mortality nor morbidity related to heterozygosis was observed in either donors or recipients.

Discussion

The present study corroborated that LDLT could provide acceptable survival outcomes and excellent quality of life for patients with IMD, although most donors in the present study were heterozygotes for their respective recipients' disorders and further demonstrated that growth retardation at the time of LDLT disadvantageously affected the outcomes of LDLT. Particularly in our patients with NLOD, the outcomes were unsatisfactory: five of 10 patients with NLOD died. These unsatisfactory outcomes for NLOD resulted from not only the growth retardation but also the fact that extrahepatic manifestations of these disorders disadvantageously affected the postoperative course of these patients. Recently, some therapeutic options for these extrahepatic manifestations of NLOD after LT have been reported to be efficacious (10,20–23). However, all of these

reported therapies were symptomatic treatments and the evidence of their efficacy seemed to be anecdotal. To achieve a satisfactory outcome in the treatment for NLOD, a breakthrough of some sort will be needed, such as development of a gene therapy (24–26) to eradicate the intrinsic underlying disorders. At this time, however, LT combined with these reported symptomatic therapies is the sole therapeutic procedure for NLOD patients with severe manifestations. Thus, to gain a better outcome, precise recognition of the optimal timing of LT is necessary. In the present study, we demonstrated that patients with growth retardation of less than −2SD in height showed significantly worse survival outcomes compared to those without growth retardation, irrespective of whether they had LOD or NLOD and growth retardation was significantly correlated with both the age of onset and the time from onset to LDLT. That is, LT must be conducted for patients with IMD before growth retardation reaches −2SD and thus in some patients with IMD, LT must be carried out in early infancy according to the disorders. At the beginning of LT as well as LDLT, infants who were unusually small missed the optimal timing for LT because their bodies were so small and thus there was a scarcity of appropriate sized livers (27,28). For the present, however, split liver graft has become a common procedure (27, 29) and monosegmental liver graft has been gaining wider acceptance even for premature neonates (28). Furthermore, we also demonstrated that the post-transplant survival of patients receiving grafts with a GRWR $>$ 4.0 tended to be worse than that of those with a GRWR $\leq$ 4.0, although the difference did not reach the level of statistical significance. Application of monosegmental grafts is also reasonable for eradicating these remarkable imbalances between body and graft sizes. In addition, as far as we were able to tell, the use of heterozygous donors has no negative impact on either donors or recipients. Hence, LDLT for pediatric patients with IMD using parental liver grafts could be an ideal treatment to prevent missing the optimal timing of LT, because one of the biggest advantages of LDLT over deceased donor LT is the ability to schedule surgery. Therefore, pediatric patients with these IMDs must always be managed with consideration for the optimal timing of LT. When growth retardation becomes apparent, LDLT must be carried out immediately if a deceased donor is unavailable.

On the other hand, living liver donor morbidity appears to have increased in recent years (30, 31). However, this increasing in morbidity has been attributed mainly to the wider acceptance of right liver donation (30). In the present study, right liver donation was employed in five cases, one of which showed biliary leakage necessitating a prolonged hospital stay even if it did not lead to serious difficulties, as stated above. In some pediatric LDLT cases, right liver donation is inevitable due to the patient's age at the onset of the disorder. For example, WD can range in age of onset from infancy to adulthood. Indeed, all five of the right liver donations in the present study were implemented for patients with WD. Conversely, however, all five of the present right liver donations were performed for heterozygous carrier donors and the bile leakage in a right liver living donor mentioned above was not considered to be related to the heterozygosis. Our results suggest that right liver donation for heterozygous carrier donors as well as for non-heterozygous donors under the standard donor selection criteria as described in detail elsewhere (13,14,30) can be performed safely, though it is true that right liver donation must be more carefully performed than other types of graft. Additionally, the present results may confirm that the use of heterozygous donors has no negative impact on either donors or recipients.

Although we did not perform any preoperative genetic assays for possible heterozygous carriers in the present study, genetic and enzymatic assays of OTC using liver tissue must be included hereafter in the parental donor selection criteria for females affected with OTCD. Male hemizygotes of OTCD can range in severity from fatal neonatal hyperammonaemic coma to asymptomatic adults. Indeed, it was reported that the recipient of a liver harvested from an adult male deceased donor who had unrecognized OTCD died as a result of hyperammonemia (32). Therefore, a genetic assay is necessary to exclude male hemizygotes from blood relative donor candidates for females with OTCD, and if male hemizygotes for OTCD are identified, they must be strictly followed-up, because such individuals may themselves be candidates for LT due to their risk of developing sudden hyperammonaemic coma. On the other hand, female heterozygotes for OTCD may be used as donors only if an enzymatic assay using liver tissue shows normal OTC activity, because normal OTC activity in female heterozygotes for OTCD suggests that there is considerable degree of X-inactivation in the liver (17). With regard to disorders other than OTCD, we believe that preoperative genetic assays are not essential, because the results of the present study suggest that the use of heterozygous donors has no negative impact on either donors or recipients. However, we also recognize that the use of heterozygous carrier donors has not yet been fully verified to have no negative impact on outcomes of LDLT, and further studies including more cases and more prolonged observation periods are required. Enzymatic and/or genetic assays using liver tissue of both donors and recipients with the use of heterozygotes as donors to better understand the pathophysiology of these IMDs may help us to definitively determine whether or not the use of heterozygous donors has any negative impact. Thus, extraction of liver tissue for these assays should be mandatory. A part of the liver tissue should be used to examine the correlation between currently known genetic mutations and the clinical manifestations of these IMDs. The remainder of the liver tissue must be preserved for more advanced analyses in the future.

In conclusion, our results indicate that LDLT for pediatric patients with IMD using parental donors can be

recommended as an effective treatment for pediatric patients with IMD. However, in the case of patients with NLOD, some optional treatments may be necessary to achieve a better outcome of LDLT.

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Living Donor Liver Transplantation for Noncirrhotic Inheritable Metabolic Liver Diseases: Impact of the Use of Heterozygous Donors

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Background. In living donor liver transplantation (LDLT), the liver donor is almost always a blood relative; therefore, the donor is sometimes a heterozygous carrier of inheritable diseases. The use of such carriers as donors has not been validated. The aim of the present study was to evaluate the outcome of LDLT for noncirrhotic inheritable metabolic liver disease (NCIMLD) to clarify the effects of using a heterozygous carrier as a donor.

Methods. Between June 1990 and December 2003, 21 patients with NCIMLD underwent LDLT at our institution. The indications for LDLT included type II citrullinemia (n = 7), ornithine transcarbamylase deficiency (n = 6), propionic acidemia (n = 3), Crigler-Najjar syndrome type I (n = 2), methylmalonic acidemia (n = 2), and familial amyloid polyneuropathy (n = 1). Of these 21 recipients, six underwent auxiliary partial orthotopic liver transplantation.

Results. The cumulative survival rate of the recipients was 85.7% at both 1 and 5 years after operation. All surviving recipients are currently doing well without sequelae of the original diseases, including neurological impairments or physical growth retardation. Twelve of the 21 donors were considered to be heterozygous carriers based on the modes of inheritance of the recipients' diseases and preoperative donor medical examinations. All donors were uneventfully discharged from the hospital and have been doing well since discharge. No mortality or morbidity related to the use of heterozygous donors was observed in donors or recipients.

Conclusions. Our results suggest that the use of heterozygous donors in LDLT for NCIMLD has no negative impact on either donors or recipients, although some issues remain unsolved and should be evaluated in further studies.

Keywords: Liver transplantation, Organ donation, Noncirrhotic metabolic liver disease, Donor selection, Inherited diseases.

(*Transplantation* 2005;80: 623–628)

Liver transplantation has become a well-recognized therapy for hepatic failure resulting from acute or chronic liver disease. It also plays a role in the treatment of certain inborn errors of metabolism that do not directly injure the liver (*1,2*). Due to the unavailability of deceased donors, living donors have been employed as a major organ resource for liver transplantation in Japan (*3,4*). In living donor liver transplantation (LDLT), the donor is almost always a blood relative of the patient. Because most inborn errors of metabolism are inherited, an obligate heterozygous carrier of the recipient's disorder has sometimes been used as a liver donor (*5–8*). For example, in the case of autosomal recessive disorders, the recipient may gain only half of normal enzyme activity when a parent is used as the donor. In this situation, it is difficult to conclude that a heterozygous liver can correct the disorder and that there will be no relapse of the original disease in the long-term postoperative course. Furthermore, the immediate and long-term risks of heterozygous carrier donors have not yet been fully clarified (*5–8*).

From 1990 to 2003, 21 patients underwent LDLT for noncirrhotic inheritable metabolic liver diseases (NCIMLD) at our institution. Although three of these cases have previously been reported (*9–11*), all were evaluated in the present study in order to determine their LDLT outcomes and to clarify the impact of the use of heterozygous donors on the postoperative course of both donors and recipients.

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PATIENTS AND METHODS

Twenty-one patients with NCIMLD indicated for LDLT included type II citrullinemia (CTLN2, n = 7), ornithine transcarbamylase deficiency (OTCD, n = 6), propionic acidemia (PPA, n = 3), Crigler Najjar syndrome type I (CNSI, n = 2), methylmalonic acidemia (MMA, n = 2), and familial amyloid polyneuropathy (FAP, n = 1) (Table 1). All were evaluated based on the mode of operative procedure (auxiliary partial orthotopic liver transplantation [APOLT] or not) (*9,12–14*), graft-to-recipient-weight ratio (GRWR), liver donor (blood relative or not), and the immediate and long-term postoperative course.

All of the 21 donors fulfilled our standard donor selection criteria as described in detail elsewhere (*15,16*). Of these 21, 12 donors were considered to be heterozygous carriers for respective recipient's disorder based on the modes of inheritance of the disorders (Table 2): autosomal recessive inheritance for CTLN2 (*1,2,5*), PPA (*10,17*), and CNSI (*1,18*); multifactorial autosomal recessive inheritance for MMA (*19,20*); autosomal dominant inheritance for FAP (*20*); and X-linked inheritance for OTCD (*1, 2, 21*). Parents with autosomal recessive disorders and mothers with X-linked inheritance disorders were considered to be heterozygous carriers (*5–8, 17, 22–24, 25*). Furthermore, the father of an affected patent with

TABLE 1. Patient characteristics

Case	Age (years)	Sex	Diagnosis	Timing of LDLT	APOLT	ABO-blood type	Donor	Outcomes
1	52	F	Citrullinemia type II	Elective	Yes	Identical	Husband	83 months, alive
2	23	M	Citrullinemia type II	Emergent	Yes	Identical	Brother	68 months, alive
3	20	M	Citrullinemia type II	Elective	Yes	Compatible	Father	1 month, died
4	30	M	Citrullinemia type II	Elective	No	Compatible	Father	30 months, with multiple HCC died of recurrent HCC
5	21	F	Citrullinemia type II	Elective	No	Identical	Father	54 months, alive
6	18	F	Citrullinemia type II	Elective	No	Identical	Mother	32 months, alive
7	39	M	Citrullinemia type II	Elective	No	Identical	Wife	17 months, alive
8	2	F	OTCD	Emergent	No	Identical	Mother	112 months, alive
9	3	F	OTCD	Emergent	Yes	Identical	Father	109 months, alive
10	5	F	OTCD	Elective	Yes	Identical	Father	94 months, alive
11	5	F	OTCD	Elective	No	Identical	Mother	80 months, alive
12	7	F	OTCD	Elective	No	Compatible	Father	4 months, died in traffic accident
13	16	F	OTCD	Elective	No	Identical	Father	51 months, alive
14	2	F	Propionic acidemia	Elective	No	Incompatible	Mother	59 months, alive
15	5	M	Propionic acidemia	Elective	No	Identical	Father	30 months, alive
16	1	M	Propionic acidemia	Elective	No	Identical	Father	21 months, alive
17	5	M	Crigler-Najjar type I	Elective	Yes	Compatible	Mother	81 months, alive
18	4 months	M	Crigler-Najjar type I	Elective	No	Compatible	Mother	47 months, alive
19	1	F	Methylmalonic acidemia	Elective	No	Compatible	Father	15 days, died of metabolic stroke
20	12	F	Methylmalonic acidemia	Elective	No	Compatible	Father	2 months, died of aspergillosis
21	58	M	FAP	Elective	No	Identical	Brother	58 months, alive

LDLT, living donor liver transplantation; APOLT, auxiliary partial orthotopic liver transplantation; HCC, hepatocellular carcinoma; OTD, ornithine transcarbamylase deficiency; FAP, familial amyloid polyneuropathy.

an X-linked inheritance disorder has a probability, albeit an extremely low one, of being heterozygous (*25*).

The mothers of the two CNSI cases (Cases 17 and 18) received no specific medical tests because no problem was found in the results of their routine medical examinations, including serum direct and indirect bilirubin levels. Because of surgical risks and the uncertainty of its significance, preoperative donor liver biopsy for use in enzymatic or genetic assays was performed in only one donor. In Case 2 (CTLN2), an enzymatic assay related to a urea cycle disorder (UCD) using a liver needle biopsy specimen was performed for a brother of the recipient, the only donor candidate, and showed 30% of the normal value for argininosuccinate synthetase activity (*5–7*) despite normal quantitative plasma amino acid analysis (QAAA) and normal plasma ammonia level. No genetic assay was performed because the causative genetic errors of the disease were not well understood at that time (*26*). Although the brother may have been latently diseased, we considered him a heterozygous carrier based on the results of his QAAA and employed him as a liver donor with strict informed consent. For all donors, we reviewed the recipient's disease, donor relationship to the recipient, donor age, the mode of donor hepatectomy, immediate and long-term postoperative course, and resection rate of the donor hepatectomy calculated from the following equation: [{actual graft weight (g)}/{total liver volume calculated from preoperative computed tomography volumetry (ml)}] × 100%.

Mortality and morbidity were studied in relation to the use of heterozygous donors, and some recipients of heterozygous livers and some heterozygous donors also underwent specific medical tests in addition to routine checkups, depending on the recipient's disease. Plasma ammonia level measurement was included in the immediate and long-term postoperative routine medical checkups in cases of UCD; QAAA was performed preoperatively (normal profile, normal serum ammonia level in donors), 1, 3, 6, 12 months after LDLT and annually thereafter in cases of CTLN2; an allopurinol loading test (*27,28*) was performed preoperatively and annually after the LDLT in cases of OTCD; and in cases of PPA, plasma propionic acid level measurement was carried out preoperatively (undetectable in donors), blood gas analysis (BGA) was conducted preoperatively to confirm whether metabolic acidosis was present (normal values in both pH and base excess in donors), and routine postoperative blood tests included both plasma propionic acid measurement and BGA. No specific tests were conducted in the CNSI cases. To determine whether postoperative morbidities were related to the use of heterozygote donors, recipients of heterozygous livers were accompanied by their donors or other family members during follow-up and were asked about their preoperative symptoms. Heterozygous donors and other family members were also asked if they suffered symptoms similar to those of the recipients.

Follow-up was continued until April 2004 or death for both donors and recipients.

SPSS commercial statistics software was used for all statistical analyses (SPSS 12.0 for Windows, Chicago, IL), and P values of <0.05 were considered to be significant.

RESULTS

Outcome of LDLT

The patients' characteristics are summarized in Table 1. Cases 2, 8 and 9 underwent emergency LDLT for life-threatening hyperammonemia, and all three required preoperative apheresis therapy including plasmapheresis and continuous

TABLE 2. Characteristics of the 12 heterozygote donors

Case	Recipient's diseases	Relation to recipient	Age (years)	Mode of donor hepatectomy	Resection rate of donor hepatectomy[a]	GRWR (%)[b]	Duration from surgery (months)
2	Citrullinemia type II	Brother	24	Left hepatectomy[c]	32.9	0.78	68
3	Citrullinemia type II	Father	54	Left hepatectomy[c]	36.7	1.21	58
4	Citrullinemia type II	Father	59	Right hepatectomy[d]	53.2	1.55	56
5	Citrullinemia type II	Father	50	Right hepatectomy[d]	60.8	1.69	54
6	Citrullinemia type II	Mother	54	Right hepatectomy[d]	43.5	1.42	32
8	OTCD	Mother	32	Left lateral segmentectomy[e]	25.5	2.08	112
11	OTCD	Mother	35	Left lateral segmentectomy[e]	22.1	1.51	80
14	Propionic academia	Mother	40	Left lateral segmentectomy[e]	22.9	2.72	59
15	Propionic academia	Father	37	Left lateral segmentectomy[e]	19.3	1.27	30
16	Propionic academia	Father	31	Left lateral segmentectomy[e]	22.4	3.37	21
17	Crigler-Najjar type I	Mother	42	Left lateral segmentectomy[e]	20.8	1.23	81
18	Crigler-Najjar type I	Mother	33	Left lateral segmentectomy[e]	30.9	4.93	47

[a] Calculated by: {[actual graft weight (g)]/[total liver volume calculated from preoperative CT volumetry (ml)]} × 100%.
[b] Graft-to-recipient weight ratio calculated by {[actual graft weight (g)]/ [recipient's body weight (g)]}× 100%.
[c] Resection of segments II + III + IV according to Couinaud's nomenclature for liver segmentation.
[d] Resection of segments V + VI + VII + VIII.
[e] Resection of segments II + III.
OTCD, ornithine transcarbamylase deficiency.

hemodiafiltration immediately prior to surgery. The other 18 patients underwent LDLT electively and required no apheresis therapy.

APOLT was performed in six recipients, in whom a left liver (segments II-IV according to Couinaud's nomenclature for liver segmentation) or left lateral segment liver (segments II-III) graft was implanted orthotopically following native left hepatectomy with caudate lobe resection (segments I-IV). In Case 1, APOLT was implemented in order to intend to compensate for the relatively small graft (GRWR was 0.84% in this case) (*29*); it was also implemented in expectation of the future establishment of gene therapies (*30*) or hepatocyte transplantation (*31*) because our institution did not have a right liver donation program from living donors at that time (*32*). In Case 2, the donor had a 100% probability of being a heterozygous carrier or even latently diseased. Thus we applied APOLT to this case to avoid right liver donation; this decreased the operative risks to the donor by reducing the extent of donor hepatectomy (*33*). As for the other four cases, we applied APOLT to these cases with the expectation of future establishment of gene therapies or hepatocyte transplantation. There was no statistical difference between the 5-year survival rates of the APOLT group (83.3%) and the non-APOLT group (78%).

There were five postoperative deaths among recipients, three of which were related to the LDLT procedure. Case 3 died of sepsis, Case 19 of metabolic stroke (*23*), and Case 20 of pulmonary aspergillosis. The other two patients died of brain metastases from hepatocellular carcinoma (Case 4) and a traffic accident (Case 12). The overall cumulative survival rate of all 21 patients was 80.0% at 1 year after operation and 75.6% at 5 years (Kaplan-Meier life table analysis). Excluding the deaths unrelated to LDLT, the cumulative survival rates were 85.7% at both 1 year and 5 years.

Impact of Employing a Potential Obligate Carrier as a Donor

The donors were the father in 11 cases, the mother in 6, a brother in 2, and a spouse in 2 cases; thus a total of 19 patients received grafts from a blood relative (Table 1), 12 of whom (5 fathers, 6 mothers, and 1 brother) were considered heterozygous donors based on the mode of inheritance of the disorder in question (Table 2). Four fathers of patients with OTCD (Cases 9, 10, 12, and 13) also had various probabilities of being heterozygous. However, their results in the allopurinol loading test showed no abnormal findings, and thus we did not consider them heterozygotes. As stated above, additional preoperative medical tests for heterozygote donors showed no unusual findings for either the CTLN2 cases or the PPA cases. Two mothers of OTCD patients proved to be partially deficient in ornithine transcarbamylase based on the results of the allopurinol loading test, in which peak values of urine orotic acid and orotidine were almost twice normal upper values after allopurinol loading (*27,28*), despite normal plasma ammonia levels. Nevertheless, both were used as liver donors because there were no other available donors. Both underwent enzymatic and genetic assays using liver tissue extracted during donor surgery and were proven heterozygous for mutations at Xp21, where the ornithine transcarbamylase gene is located (*22,25*), even though their OTC activity was normal in the liver tissue and neither showed relevant symp-

toms either preoperatively or early in the postoperative period (*2,5,22*). The mothers of the two CNSI patients also showed no symptoms related to their respective heterozygosis. Enzymatic or genetic assays using liver tissue extracted during donor surgery were performed only in Cases 2, 8 and 11, because the significance of these investigations was considered uncertain at that time.

The age of the donors at LDLT, the mode of donor hepatectomy, and resection rate of donor hepatectomy are shown in Tables 2 and 3 for heterozygous and nonheterozygous): donors, respectively. There were no statistical differences in these variables between the two groups, and there were no major complications in any donors. All 21 donors were uneventfully discharged from the hospital within 14 postoperative days; they returned to their preoperative normal daily lives within 2 months of surgery and are currently doing well. Furthermore, there have been no cases to date of any symptoms possibly arising from the heterozygosis, including episodes of hyperammonemia in the cases of UCD, metabolic acidosis in the cases of PPA, or jaundice in the cases of CNSI.

Of the 12 patients who were matched with heterozygous donors, none has shown any evidence of recurrence of the original disease. The 10 survivors of these 12 recipients have not required any dietary restriction during long-term follow-up, although the three PPA patients still receive carnitine supplementation (*10,17*). No statistical difference was found between the survival rates of recipients of heterozygous livers and nonheterozygous livers, with 82.5% survival in the former and 77.8% in the latter at 5 years after operation. Although episodes of hyperammonemia secondary to graft dysfunction due to rejection were observed in Cases 1, 2, 9 and 10 (all of whom underwent APOLT), there have been no episodes of complications arising from the use of a heterozygous donor.

Heterozygous donors to CTLN2 recipients have shown neither abnormal profiles in QAAA nor any episodes suggestive of hyperammonemia or actual hyperammonemia to date. CTLN2 recipients of heterozygous livers have shown no abnormal QAAA profiles and have suffered no episodes of hyperammonemia except Case 2, whose QAAA profile included high levels of citrulline and glutamate during several episodes of hyperammonemia secondary to graft dysfunction due to rejection.

Annual allopurinol loading tests of OTCD Cases 8 and 11, who received heterozygous livers, have shown peak values of urine orotic acid and orotidine excretion of 1.5- to 3-times normal upper values in both donors and recipients. However, there have been neither episodes of hyperammonemia nor episodes suggestive of hyperammonemia in donors or recipients.

Finally, there have been no episodes of metabolic acidosis in PPA recipients or their donors, and no episodes of jaundice have been observed in the CNSI cases. Thus, neither mortalities nor morbidities related to heterozygosis have been observed in either donors or recipients. Regardless of whether or not heterozygous donors were used, no surviving pediatric recipients have shown any problematic retardation in physical growth or neurological impairments. All surviving adult recipients are currently enjoying their normal daily lives as before the onset of the original disease. In addition, all of surviving patients and their families including donors currently declare their well-being and their feeling to be cured.

DISCUSSION

Even when the recipient's disease is known to be inheritable, a blood relative who is heterozygous for the disorder must sometimes be used as a liver donor for LDLT in Japan or other countries where the deceased donors are usually unavailable. It is therefore extremely important to understand the risks of LDLT with heterozygous donors. In the present study, no negative impact was found on the immediate or long-term postoperative courses of either donors or recipients. Although 3 of the 12 heterozygous donors underwent right hepatectomy, which is considered to induce a considerable regenerative process in the donor remnant liver, they showed no serious difficulties either early postoperatively or during long-term follow-up. Regardless of the use of heterozygous or nonheterozygous donors, LDLT produced acceptable survival outcomes with a cumulative survival rate of 85.7% at 5 years after operation and excellent quality of life for all NCIMLD cases. We therefore believe that more extensive use of LDLT is acceptable in the treatment of NCIMLD.

On the other hand, the onset mechanisms of most cases of NCIMLD are not yet completely understood, and some acquired genetic mutations in heterozygous liver may cause a recurrence or new onset of the original disease (*6-8,10,18,19,21,25,26,34,35*). For example, in a study by Saheki and Kobayashi, some CTLN2 cases were caused by the additional effects of genetic or environmental modifiers in the heterozygous carriers (*35*). In addition, it was reported that a recipient of a liver harvested from an adult male deceased donor who had unrecognized OTCD could die as a result of hyperammonemia (*36*). In the present study, liver tissues were not extracted during donor surgery for cryopreservation and future examination, and certain types of study are therefore not possible with the present group of subjects. Most of the genetic mutations of these inborn errors of metabolism are already known and their roles have been clarified (*8,17,18,20–22,25,26,34–36*), even if not completely understood. Because the number of our cases was small and the diseases varied, further follow-up and more studies are necessary to confirm the efficacy and safety of LDLT with heterozygous livers. Therefore, in cases of LDLT for these inborn errors of metabolism with the use of heterozygotes as donors, hereafter the liver tissue must be extracted from both donors and recipients in order to elucidate the impact that the use of the heterozygotes as donors would have on the risk or safety of both donors and recipients. This is because enzymatic and genetic assays using a part of the liver tissue should be mandatory for the recognition of correlations between enzymatic and genetic variations. Moreover, the remainder of the liver tissue must be preserved for more advanced analyses in the future.

Most NCIMLDs arise from enzyme deficiencies, and enzyme supplementation can sometimes correct the disorders (*9,12–14*). APOLT has thus been widely indicated, and indeed, 6 of the 21 patients in the present study underwent APOLT. APOLT recipients will be released from life-long immunosuppressive therapy and maintained with their own liver when gene therapies for these disorders become clini-

TABLE 3. Characteristics of the nine nonheterozygote donors

Case	Recipient's diseases	Relation to recipient	Age (years)	Mode of donor hepatectomy	Resection rate of donor hepatectomy[a]	GRWR (%)[b]	Duration from surgery (months)
1	Citrullinemia type II	Husband	52	Left hepatectomy[c]	33.2	0.84	83
7	Citrullinemia type II	Wife	38	Right hepatectomy[d]	64.4	1.36	17
9	OTCD	Father	36	Left lateral segmentectomy[e]	21.5	2.08	109
10	OTCD	Father	36	Left lateral segmentectomy[e]	21.5	1.34	94
12	OTCD	Father	29	Left lateral segmentectomy[e]	24.1	1.36	54
13	OTCD	Father	44	Left hepatectomy[c]	33.2	0.94	51
19	Methylmalonic academia	Father	37	Left lateral segmentectomy[e]	24.1	3.97	115
20	Methylmalonic acidemia	Father	46	Left hepatectomy[c]	23.6	1.01	66
21	FAP	Brother	66	Left hepatectomy[c]	43.5	0.99	58

[a] Calculated by: {{actual graft weight (g)}/{total liver volume calculated from preoperative CT volumetry (ml)}} × 100%.
[b] Graft-to-recipient weight ratio calculated by {{actual graft weight (g)}/{recipient's body weight (g)}}× 100%.
[c] Resection of segments II + III + IV according to Couinaud's nomenclature for liver segmentation.
[d] Resection of segments V + VI + VII + VIII.
[e] Resection of segments II + III.
OTCD, ornithine transcarbamylase deficiency; FAP, familial amyloid polyneuropathy.

cally available. Furthermore, hepatocyte transplantation could be an alternative to hepatic retransplantation when the graft liver is severely damaged; however, clinical success with these therapies has not yet been reported and probably will not be popularized in the near future (*22,30,31*). Furthermore, the postoperative course of APOLT patients tends to be complicated, as shown in Cases 1, 2, 9, and 10. Until gene therapy or hepatocyte transplantation for these disorders has been established, application of APOLT should be limited to particular cases, such as cases with small-for-size graft (*29*), or cases in which right liver donation threatens donor safety because of an extreme volume imbalance between the right and left hepatic lobes (*32*).

In conclusion, LDLT provides acceptable survival outcomes and excellent quality of life for NCIMLD patients even in cases of a heterozygous carrier donor. The results of the present study suggest that the use of a heterozygous carrier as a liver donor has no negative impact on either the donors or the recipients in LDLT for NCIMLD. However, to confirm our results, enzymatic and genetic assays, using liver tissue extracted from both donor and recipient pairs of LDLT with the use of heterozygotes as donors for these inborn errors of metabolism, should hereafter be mandatory and the remainder of the liver tissue must be preserved for more advanced analyses in the future.

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CD36 (Nak^a) Sensitization with Platelet-Transfusion Refractoriness in a Liver Transplant Recipient

We report here an unusual case of a liver transplantation followed by anti-CD36 (GP IV, Nak^a) sensitization, probably from transfusion, and involving platelet-transfusion refractoriness (PTR). A 47-year-old Japanese man with hepatitis B virus-related liver cirrhosis received a liver transplant in 2001 from his 52-year-old brother with one human leukocyte antigen (HLA) locus mismatch (Table 1). After the transplantation, the patient's platelets dropped to 2×10^{10}/L, and platelets were transfused frequently to keep the count at an acceptable level. The number of platelets fluctuated between 2 and 4×10^{10}/L with a transient rise to 10×10^{10}/L after transfusion, indicating a poor response to transfusions of random-donor platelets. No obvious bleeding episodes were observed. Anti-CD36 iso-antibody, which was negative before and 5 days after the transplantation, was first detected after 8 days. Platelets from 21 donors had been transfused by day 8. The recipient was administered immunosuppressants, and tacrolimus (4 mg/day) was changed to prednisolone (5 mg/day) on day 7 because of liver dysfunction. The titer of the antibody, which was ×16 on day 8, went up to ×128 on day 22. No anti-HLA or anti-human platelet antigen (HPA) was detected throughout the period. The recipient and the liver donor were negative for CD36 antigen. The patient was transfused effectively with platelets from CD36 negative, but HLA nonselected, donors. After day 99 when the titer went down to ×16, the platelet count persisted at approximately 4 to 5×10^{10}/L without frequent transfusions. There was no clear evidence of sepsis or rejection during the clinical course. The titer of anti-CD36 declined gradually after CD36-negative transfusion and disappeared on day 232. Nevertheless, the low platelet count was not resolved completely. The patient died of multi-organ failure on day 377.

TABLE 1. Inspection results of the recipient and donor

	Recipient	Donor
Blood type (Rh)	A (+)	O (+)
HLA-A	24, 26	26, −
-B	35, −	35, −
-C	Cw9, −or10	Cw9, −or10
-DR	4, 11	4, 11
HBs-Ag	+	−
HBs-Ab	−	+
HBe-Ag	−	−
HBe-Ab	+	−
HBc-Ab	+	+
HCV-Ab	−	−
CD36 on platelets	−	−

HLA, human leukocyte antigen; HCV, hepatitis C virus.

Alloimmune thrombocytopenias in adults are classified into posttransfusion purpura, passive alloimmune thrombocytopenia, transplantation-associated alloimmune thrombocytopenia, and PTR. Transplanted solid organs contain passenger lymphocytes that can transmit autoimmune disease or initiate alloimmune disorders in recipients. Alloimmune thrombocytopenia is an uncommon, but not rare, cause of thrombocytopenia. Alloantibodies against platelet-specific alloantigens, in particular HPA-1a among white populations, can cause severe thrombocytopenia in recipients.

PTR usually results from various antiplatelet antibodies related to frequent platelet transfusions (*1*). Antibodies to HLA are the most common cause, and platelet-specific allo- or iso-antibodies, such as anti-CD36, can also give rise to PTR in combination with anti-HLAs. CD36 is localized on platelet GP IV (*2*), which is an 88 kDa glycoprotein expressed on platelets and monocytes/macrophages. There are two types of CD36 deficiencies. In type I, CD36 is absent from both platelets and monocytes, whereas it is absent only from platelets in type II deficiency (*3*). It has been reported that 5% to 10% of Asians and approximately 2.4% of African Americans have platelets lacking the major membrane GP IV that carries CD36 (*4*). In the Japanese population, the frequencies of type I and type II deficiencies are 0.54% and 4.0%, respectively (*3*). Although individuals with CD36 deficiency are apparently healthy with no obvious bleeding tendency, type I deficiency may potentially result in the production of anti-CD36 after transfusion or pregnancy (*3*). In our case, only anti-CD36 was produced with no anti-HPA or anti-HLA. Although it was a very rare case (*5*), anti-CD36 antibody in the recipient that was caused by frequently transfused CD36-positive platelets of random-donors resulted in PTR. As far as we know, this is the first case of PTR caused by anti-CD36 in a liver transplant recipient.

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