Fig 1. Concentration-dependent inhibition of calcineurin phosphatase activity in peripheral blood mononuclear cells (PBMCs) by tacrolimus and cyclosporine. Whole blood taken from a healthy volunteer was incubated with saline solution containing various concentrations of tacrolimus (*open circles*) or cyclosporine (*solid circles*) at 37°C with gentle shaking. After a 1-hour incubation, PBMCs were isolated from the blood samples by Ficoll-Hypaque gradient centrifugation (Ficoll-Paque Plus). Calcineurin phosphatase activity in PBMCs was measured by use of [γ–phosphorus 32] regulatory subunit type II (RII) phosphopeptide as a substrate. Data are shown as a percentage of the control activity measured in blood samples that had been treated with saline solution containing vehicle alone. Each *circle* represents the mean (±SD) of 3 independent experiments.

nitude of interindividual and residual variability in the error model was expressed as the percent coefficient of variation (% CV) and SD of enzyme activity (in picomoles per minute per milligram protein), respectively.

Clinical outcome analysis

Safety and efficacy of tacrolimus and cyclosporine were investigated in relation to blood drug concentrations and calcineurin phosphatase activity in PBMCs during the first 2 weeks after transplantation. Safety was evaluated by the adverse event of nephrotoxicity, which was defined as an initial increase in serum creatinine concentration of 0.5 mg/dL or greater above baseline. Efficacy was evaluated by acute rejection episode, which was defined as clinically and biochemically suspected rejections that were initially treated with steroid pulse therapy. To avoid the potential influence of preoperative renal dysfunction and mismatching of ABO blood group on these outcomes. we excluded 1 patient who had renal dialysis before the transplant in the cyclosporine

treatment arm and 10 patients receiving ABO-incompatible transplantation (9 in the tacrolimus group and 1 in the cyclosporine group). For patients who had an episode of nephrotoxicity or acute rejection, data regarding blood drug concentrations and calcineurin activity measured for 3 days immediately before the onset of each event were used to calculate the individual average values. For control patients without an episode of nephrotoxicity or acute rejection, data regarding blood drug concentrations and calcineurin activity available during 3 days immediately before the mean postoperative day of the onset of each event were used to calculate the individual average values.

Statistical analysis

Data were presented as mean ± SD or median and range, depending on data type. The differences in mean values between 2 groups were statistically examined with the unpaired t test. The statistical significance of differences in nonparametric values between 2 groups was analyzed with the Mann-Whitney U test or the Wilcoxon test. The Fisher exact probability test was used to compare the proportion of patients with a given characteristic between 2 groups. $P < .05$ was considered statistically significant. For the estimation of pharmacodynamic parameters, the statistical significance of the parameters was evaluated with the likelihood ratio test. A difference in the objective function (−2 log-likelihood difference [−2 LLD]) of more than 7.88, with 1 df, was considered statistically significant ($P < .005$).

RESULTS
In vitro effects of tacrolimus and cyclosporine on calcineurin phosphatase activity

Specific calcineurin phosphatase activity was measured in PBMCs from a healthy volunteer ($105 ± 7$ pmol · min^{-1} · mg protein^{-1} [mean ± SD], n = 3). Background activity resulting from protein phosphatase type 2C was almost negligible compared with basal calcineurin activity in the healthy volunteer ($4 ± 3$ pmol · min^{-1} · mg protein^{-1}, n = 3). No remarkable change in enzyme activity was observed for up to 48 hours when the blood samples were stored at room temperature after being collected. The values for inter-assay and intra-assay variability were 2.6% and 1.2% (% CV), respectively. Both tacrolimus and cyclosporine inhibited enzyme activity in a concentration–dependent manner under in vitro conditions (Fig 1). The IC$_{50}$ values for blood concentrations of tacrolimus and cyclosporine were calculated as $18.9 ± 4.6$ ng/mL and $181 ± 74$ ng/mL (mean ± SD), respectively. At high drug concentrations in whole blood (≥ 1000 ng/
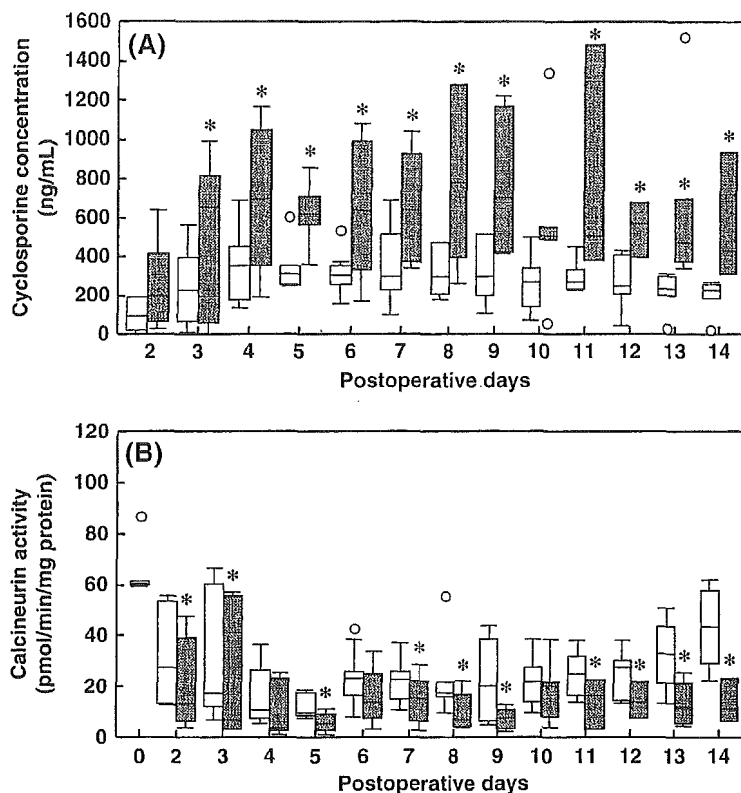
Fig 2. Blood cyclosporine concentration (**A**) and calcineurin phosphatase activity in PBMCs (**B**) during first 14 days after living-donor liver transplantation (N = 10). *Open* and *shaded boxes* represent data measured at the trough time point and at 2 hours after dosing of cyclosporine, respectively. Each *box plot* includes the median (*horizontal line*), length of the interquartile range r (*box*), whisker at greatest value lower than or equal to upper limit of interquartile range + 1.5r, whisker at lowest value greater than or equal to lower limit of interquartile range − 1.5r, and all outliers beyond the whiskers (*open circles*). *Asterisk*, $P < .05$, significantly different from data measured at the trough time point on the same postoperative day (Wilcoxon test).

mL), the inhibition of calcineurin activity by cyclosporine was almost complete, although the enzyme activity was only partially inhibited by tacrolimus (Fig 1).

Blood drug concentrations and calcineurin phosphatase activity after transplantation

Cyclosporine. Blood concentrations of cyclosporine gradually increased and showed substantial interindividual variability after liver transplantation (Fig 2, *A*). During the first 14 postoperative days, 42% of cyclosporine C_2 measurements were within the target range, although 36% of the C_0 measurements were greater than 300 ng/mL. By postoperative day 3, only 50% of patients had attained a C_0 level greater than 200 ng/mL, and the target C_2 level was achieved in 60% of patients. The median calcineurin activity at the trough time point

abruptly decreased to less than half of the median activity at baseline on postoperative days 2 and 3 (Fig 2, *B*). Furthermore, the enzyme activity at 2 hours after dosing of cyclosporine was significantly lower than that at the trough time point on the same postoperative day. Thereafter the C_0 level was maintained between 200 and 400 ng/mL, and the median C_2 level was within the range of 600 to 800 ng/mL and decreased to around 500 ng/mL by postoperative day 14. The median calcineurin activity at the trough time point remained low relative to the median baseline activity. About half of the measurements of calcineurin activity at the trough time point were lower than 20 pmol $\cdot$ min^{-1} $\cdot$ mg protein^{-1} within the first 2 weeks of transplantation. Furthermore, the enzyme activity at the trough time point was significantly suppressed at 2 hours after the administration on each postoperative day, except
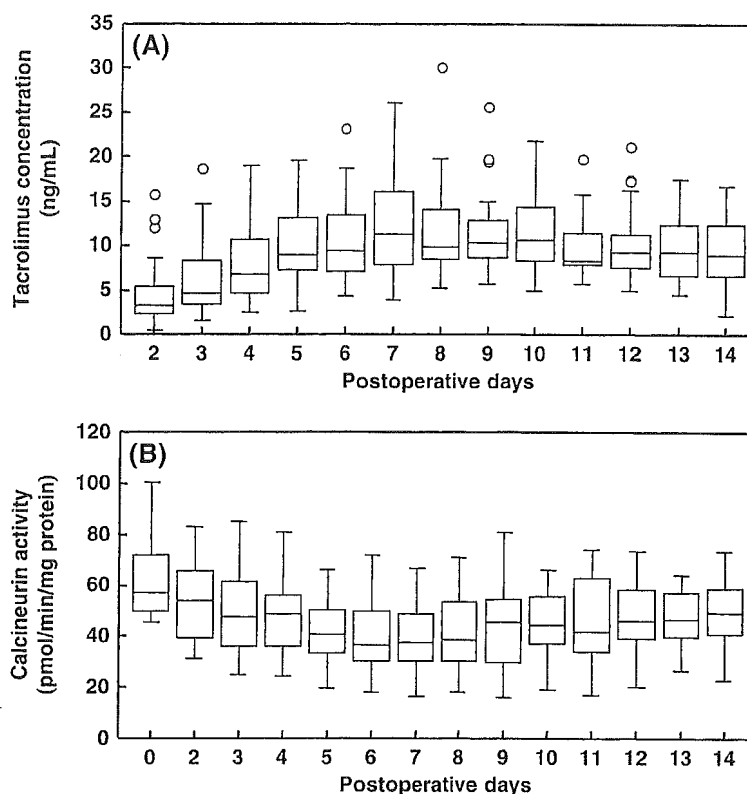
Fig 3. Blood tacrolimus concentration (**A**) and calcineurin phosphatase activity in PBMCs (**B**) during first 14 days after living-donor liver transplantation (N = 30). *Open boxes* represent data measured at the trough time point after dosing of tacrolimus. Each *box plot* includes the median (*horizontal line*), length of the interquartile range r (*box*), whisker at greatest value lower than or equal to upper limit of interquartile range + 1.5r, whisker at lowest value greater than or equal to lower limit of interquartile range − 1.5r, and all outliers beyond the whiskers (*open circles*).

for days 4, 6, and 10 of transplantation. Approximately 80% of the measurements of calcineurin activity at 2 hours after dosing fell below 20 pmol · min^{-1} · mg protein^{-1} during the first 14 days after liver transplantation. Moreover, the calcineurin activity at 2 hours after treatment was less variable than the corresponding blood cyclosporine concentrations.

Tacrolimus. During the first 14 postoperative days, 72% of tacrolimus C_0 measurements were within the target range and only 10 measurements from 6 patients exceeded 20 ng/mL (Fig 3, *A*). The calcineurin activity gradually decreased according to the increase in trough blood concentrations immediately after liver transplantation (Fig 3, *B*). By postoperative day 7, 90% of patients had reached the target C_0 level. The calcineurin activity was lowest around 1 week after transplantation (median, 37.5 pmol · min^{-1} · mg protein^{-1} on day 7), although the enzyme activity between day 7 and the

other days (days 5, 6, and 8-12) was not statistically different. During postoperative days 8 to 14, most of the trough concentrations were controlled at between 5 and 15 ng/mL, and the median calcineurin activity was within the range of 40 to 50 pmol · min^{-1} · mg protein^{-1}. Moreover, only 9 measurements of the enzyme activity from 5 patients fell below 20 pmol · min^{-1} · mg protein^{-1} within the first 2 weeks of transplantation. Extensive interindividual variability was observed in the calcineurin activity, as well as in the blood drug concentrations, as demonstrated by the large differences between the minimal and maximal measurements on each postoperative day.

Relationship between blood drug concentrations and calcineurin phosphatase activity

Baseline characteristics did not differ between the tacrolimus and cyclosporine treatment groups except
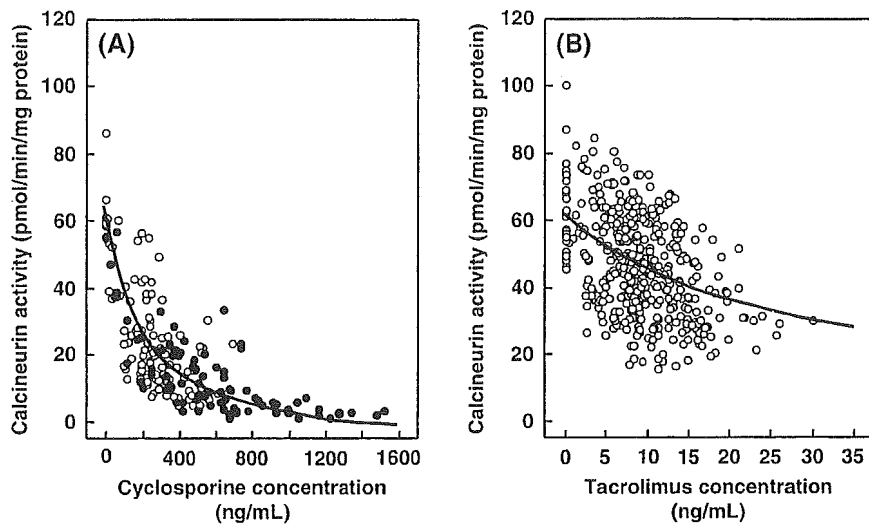
Fig 4. Relationship between calcineurin phosphatase activity in PBMCs and blood drug concentration of cyclosporine (**A**) (N = 10) or tacrolimus (**B**) (N = 30) in living-donor liver transplant patients. *Open* and *closed circles* represent data measured at the trough time point and at 2 hours after administration, respectively. The *bold lines* show the predicted calcineurin phosphatase activity versus blood drug concentration profile by use of the pharmacodynamic parameter mean estimates (shown in Table II) and the mean value of baseline calcineurin activity in each treatment arm.

Table II. Pharmacodynamic parameters of cyclosporine and tacrolimus in living-donor liver transplant patients

Parameter	Cyclosporine (N = 10)	Tacrolimus (N = 30)
Mean parameter and 95% CI		
E_{max} (pmol $\cdot$ min^{-1} $\cdot$ mg protein^{-1})	74.2 (63.6-84.8)	59.8 (49.5-70.1)
EC_{50} (ng/mL)	200 (127-274)	26.4 (15.7-37.1)
Interindividual and residual variability and 95% CI		
ω_{EC50} (%)	84.0 (21.3-117)	81.4 (49.8-104)
σ (pmol $\cdot$ min^{-1} $\cdot$ mg protein^{-1})	8.5 (5.9-10.4)	8.6 (7.7-9.5)

CI, Confidence interval; E_{max}, maximum effect; EC_{50}, blood concentration that yields half-maximal effect; ω_{EC50}, interindividual variability in EC_{50}; σ, residual variability.

for grafted liver weight (Table I). We used a simple E_{max} model to describe the concentration and response relationship of tacrolimus and cyclosporine. The final estimates of the pharmacodynamic parameters are shown in Table II. Because the interindividual variability for the E_{max} of the 2 drugs was not significantly different from 0, it was not incorporated into the final model. The calcineurin activity in patients receiving cyclosporine showed a steep decline according to the increase in blood drug concentrations and reached a plateau above a specific blood concentration, approximately 700 ng/mL (Fig 4, A). The population mean estimate of the EC_{50} for cyclosporine was 200 ng/mL (95% confidence interval [CI]. 127-274 ng/mL), and

large interindividual variability in the EC_{50} value was found (mean % CV, 84.0%). Although a marked variability in the calcineurin activity was observed around the EC_{50} value, the enzyme activity converged to a minimal level at high blood cyclosporine concentrations. On the other hand, tacrolimus showed less dynamic change in its effect on calcineurin activity with the increase of the blood drug concentration than did cyclosporine (Fig 4, B). The population mean estimate of the EC_{50} for tacrolimus was 26.4 ng/mL (95% CI, 15.7-37.1 ng/mL), and extensive interindividual variability in the EC_{50} value was again identified (mean % CV, 81.4%). In addition, remarkable variability in the level of calcineurin activity was evident within the

Table III. Safety and efficacy of cyclosporine and tacrolimus in living-donor liver transplant patients

	Cyclosporine (n = 8)	Tacrolimus (n = 21)	P value*
Nephrotoxicity			
Incidence	4 (50%)	8 (38%)	NS
Time to event (d)	8 ± 2 (6-9)	8 ± 2 (6-11)	
Acute rejection			
Incidence	2 (25%)	5 (24%)	NS
Time to event (d)	14† (11-16)	9 ± 3 (6-13)	

Data are expressed as number of patients and percent or mean ± SD and range.
NS, Not significant.
*Statistical significance was examined with the Fisher exact probability test.
†The mean value alone is indicated.

therapeutic range (5-15 ng/mL). Even at trough blood concentrations higher than 20 ng/mL, the enzyme activity was not completely inhibited by tacrolimus. Residual variability in both treatment arms was relatively large compared with the assay variability.

To evaluate the significance of the E_{max} model for tacrolimus and cyclosporine, the EC_{50} value was set to nearly 0 (10^{-4}) or an infinite value (10^4). The value of −2 LLD increased by more than 7.88 in each hypothesis for both drugs. Therefore we confirmed that the estimated EC_{50} values were significantly different from 0 and the E_{max} model for tacrolimus and cyclosporine was significantly better in terms of goodness of fit compared with a linear model.

Clinical outcome and its relationship with pharmacokinetics and pharmacodynamics

Cyclosporine. After 11 patients were ruled out on the basis of the exclusion criteria, baseline characteristics were again similar in the tacrolimus and cyclosporine treatment groups, with the exception of the greater grafted liver weight in patients receiving tacrolimus. The incidence of nephrotoxicity or acute rejection was not significantly different between the 2 groups (Table III). The mean C_0 level, but not C_2 level, of cyclosporine in patients with nephrotoxicity was significantly higher than in those without this adverse event (Fig 5, A). The mean values for calcineurin activity at the trough time point and at 2 hours after dosing were comparable between the patients with and without nephrotoxicity (Fig 5, A). Only 2 patients had an acute rejection episode during cyclosporine therapy. In one patient the administration of cyclosporine was interrupted because of an infectious event on postoperative day 8. In the other patient difficulty in achieving adequate blood cyclosporine concentrations occurred as a result of poor absorption, and no remarkable calcineurin inhibition was observed in comparison with the baseline activity for the initial few days after trans-

plantation. The C_0 and C_2 levels from the 2 patients were both subtherapeutic, although most patients without acute rejection had high C_2 levels and C_0 levels within the therapeutic range (Fig 5, B). In addition, the mean calcineurin activity in patients with no rejection episode was lower than in the 2 patients who had acute rejection and remained below 20 pmol · min^{-1} · mg $protein^{-1}$ at both the trough time point and 2 hours after dosing (Fig 5, B).

Tacrolimus. The mean C_0 level of tacrolimus and the corresponding calcineurin activity in patients with nephrotoxicity were significantly different from those in patients without this adverse event (Fig 6, A). Five patients had an acute rejection episode during tacrolimus therapy. In 3 of these patients the individual C_0 levels were still within the therapeutic range (5-15 ng/mL). The mean C_0 level of tacrolimus in patients with acute rejection was significantly lower than in those without a rejection episode (Fig 6, B). Moreover, the mean calcineurin activity in patients who had acute rejection was significantly higher than in those with no rejection episode, whereas large interindividual variability was evident (Fig 6, B).

DISCUSSION

This is the first study to compare the inhibitory effects of tacrolimus and cyclosporine on calcineurin phosphatase activity in PBMCs in the setting of living-donor liver transplantation. Furthermore, we have investigated the clinical relevance of pharmacokinetics and pharmacodynamics of the 2 drugs in terms of nephrotoxicity and acute rejection during the first 2 weeks after liver transplantation.

Using blood samples from a healthy volunteer, we first examined the in vitro concentration-dependent inhibition of calcineurin activity by tacrolimus and cyclosporine in PBMCs (Fig 1). The mean IC_{50} value of cyclosporine was comparable to that in 5 healthy volunteers reported by Caruso et al.[19] The mean IC_{50} value
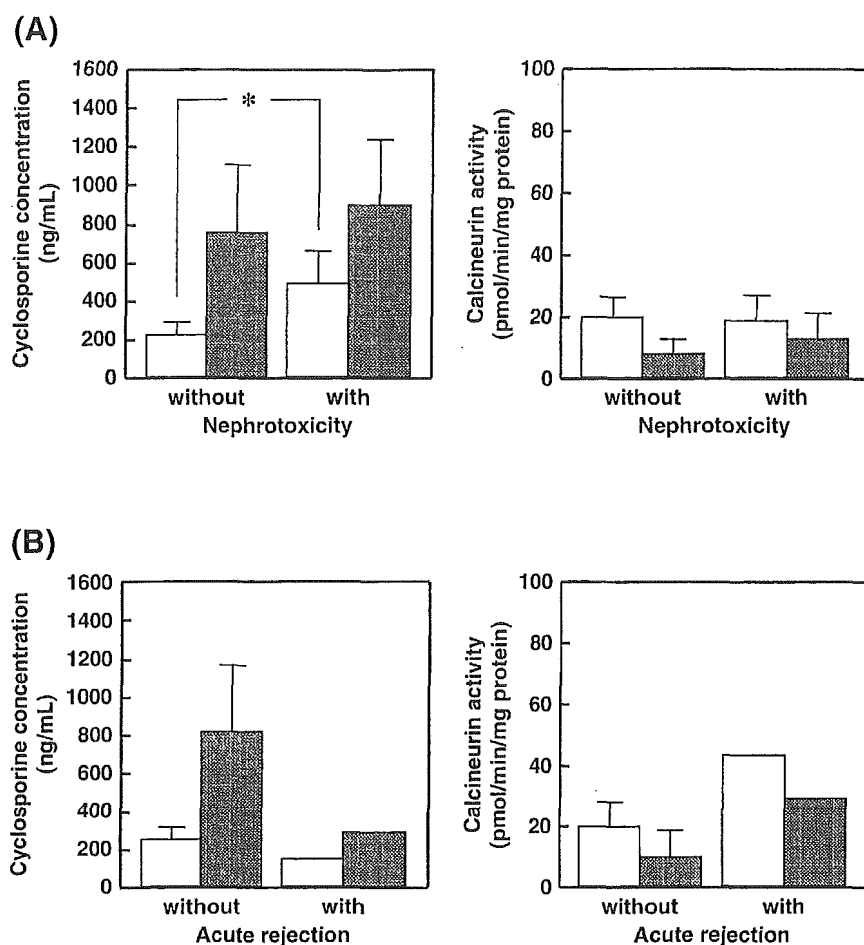
Fig 5. Blood cyclosporine concentration and calcineurin phosphatase activity in PBMCs in living-donor liver transplant patients (n = 8) with and without nephrotoxicity (**A**) or acute rejection (**B**). *Open* and *shaded columns* represent data measured at the trough time point and at 2 hours after dosing, respectively. Each column indicates the mean ± SD. For data from 2 patients with acute rejection, the mean value alone is indicated by each column. *Asterisk*, $P < .05$, significantly different from the mean value in patients without nephrotoxicity (unpaired t test).

of tacrolimus was approximately 10 times smaller than that of cyclosporine, indicating that tacrolimus is more potent than cyclosporine in calcineurin inhibition. However, at higher blood drug concentrations (≥ 1000 ng/mL), the inhibition by tacrolimus was incomplete whereas that by cyclosporine was almost complete. It has been reported that calcineurin inhibition by tacrolimus can be increased by the addition of exogenous FK506-binding protein.[20] A possible explanation for the incomplete inhibition by tacrolimus is that FK506-binding protein in PBMCs may be limiting.

In patients treated with tacrolimus, the calcineurin activity gradually decreased according to the increase

in the trough blood concentration after liver transplantation (Fig 3). On the other hand, the enzyme activity abruptly decreased in the cyclosporine treatment group immediately after transplantation and tended overall to be lower than that in the tacrolimus treatment group (Fig 2). These findings may be supported by the observations from in vitro experiments that cyclosporine could reduce the enzyme activity more effectively than tacrolimus within the respective therapeutic ranges (Fig 1). The population mean estimates of EC_{50} for tacrolimus and cyclosporine in liver transplant patients agreed well with the respective IC_{50} values obtained in a healthy volunteer (Table II). Furthermore, the mean
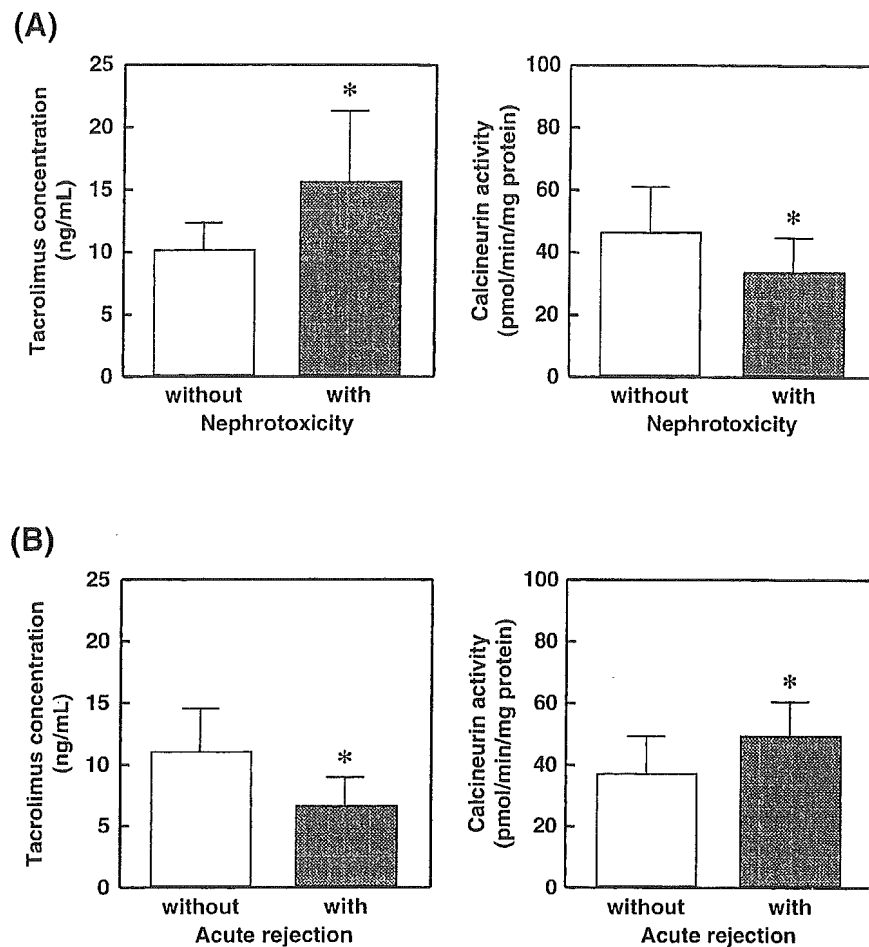
Fig 6. Blood tacrolimus concentration and calcineurin phosphatase activity in PBMCs in living-donor liver transplant patients (n = 21) with and without nephrotoxicity (**A**) or acute rejection (**B**). Each column indicates the mean ± SD of data measured at the trough time point. *Asterisk, P < .05*, significantly different from the mean value in patients without the given event (unpaired *t* test).

EC_{50} value for cyclosporine was reasonably consistent with the IC_{50} values reported in kidney transplant patients.[14,19] Large interindividual variability in the EC_{50} value was demonstrated in patients receiving tacrolimus and cyclosporine (Table II). The variability could be explained by the difference in drug concentrations in PBMCs or the difference in function or content of proteins relating to the pharmacodynamics, such as immunophilins and calcineurin. We have reported that intestinal P-glycoprotein plays an important role in limiting oral absorption of tacrolimus and cyclosporine from the gut lumen after living-donor liver transplantation.[21,22] Because P-glycoprotein is also expressed in PBMCs. it may contribute to the difference in drug distribution into the cells.[23]

Clearly, the concentration and response relationship differed between tacrolimus and cyclosporine in liver transplant patients, suggesting that their pharmacodynamic properties for calcineurin inhibition are not identical in vivo (Fig 4). Cyclosporine produced a steep decline in calcineurin activity and exerted no additional effect at blood concentrations above a certain threshold (approximately 700 ng/mL) (Fig 4, A). These results of the effects of cyclosporine were similar to those in a study that measured the inhibition of stimulated interleukin 2 (IL-2) production in whole blood by cyclosporine.[24] Although tacrolimus was demonstrated to completely suppress lymphocyte proliferation and IL-2 production in human mixed lymphocyte reactions,[25] the calcineurin activity was only partially inhibited by

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

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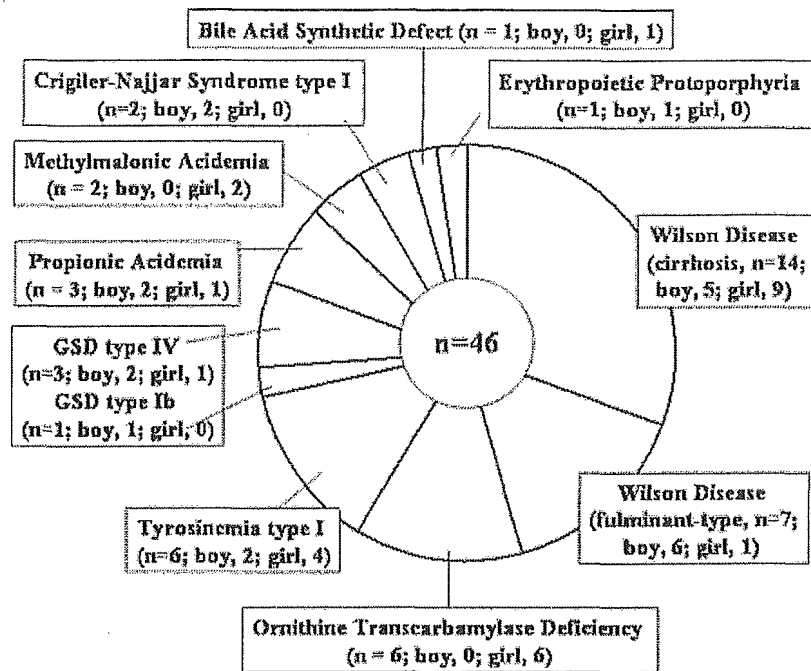
Figure 1: Indications for living donor liver transplantation of 46 pediatric patients with inheritable metabolic disorders at Kyoto University.

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	26/13/7
(Identical/compatible/incompatible)	
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 complica-

tions (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

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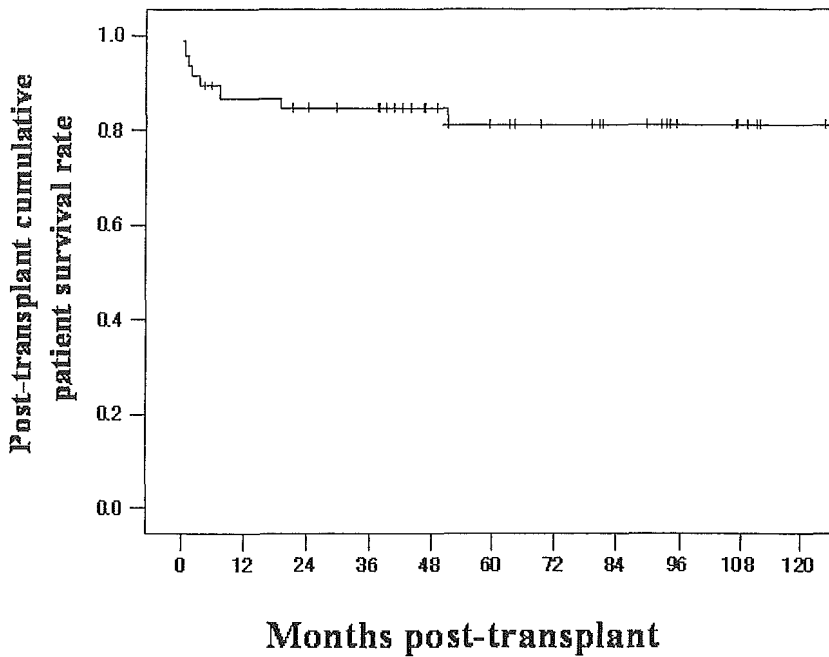
Figure 2: Cumulative post-transplant patient survival rates of living donor liver transplantation for 46 pediatric patients with inheritable metabolic disorders. Post-transplant survival of patients who underwent living donor liver transplantation for inheritable metabolic disorders at Kyoto University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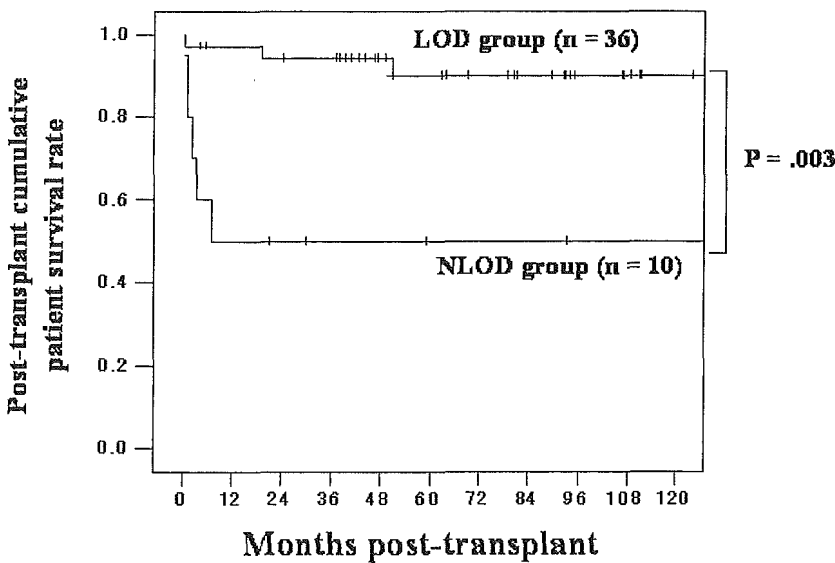

Figure 3: Comparison of post-transplant survival between liver-oriented diseases (LOD) and non-liver-oriented diseases (NLOD) groups. Post-transplant cumulative patient survival rate was significantly higher in patients with LOD than in those with NLOD.

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
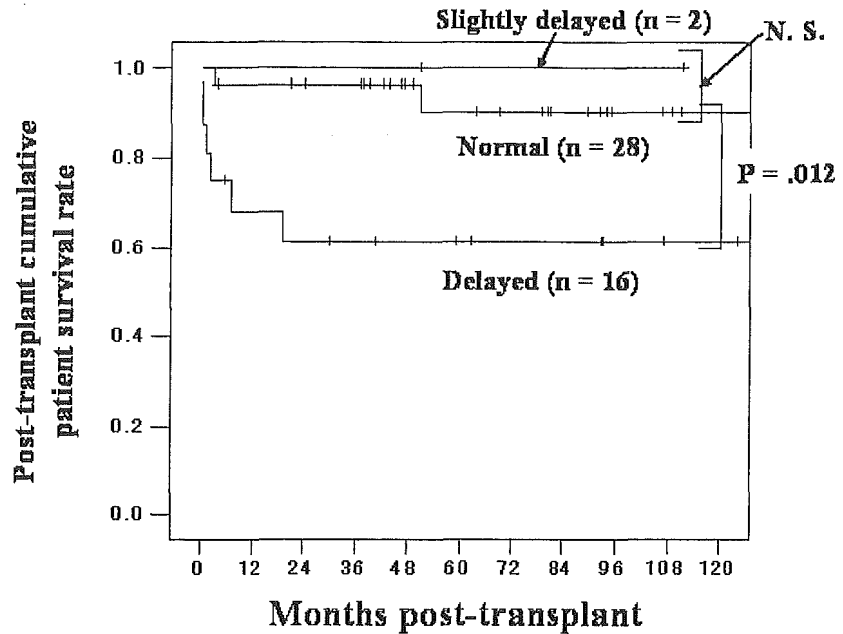
Figure 4: Comparison of post-transplant survival among three classifications of physical growth (normal, slightly delayed and delayed) at the time of living donor liver transplantation (LDLT). Post-transplant cumulative patient survival rates of patients with normal physical growth or slightly delayed physical growth at the time of LDLT were significantly higher than that of patients with delayed physical growth at the time of LDLT.
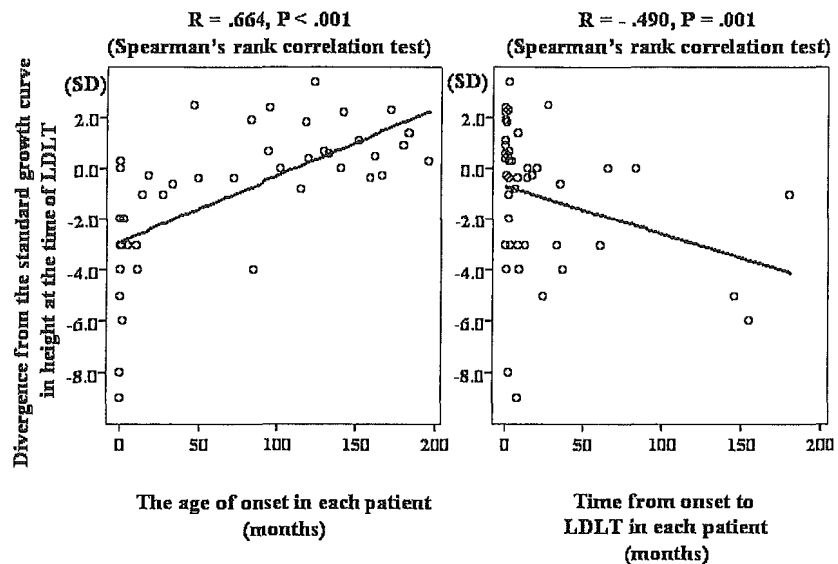
Figure 5: Correlation between physical growth and the age at onset of each disorder or time from onset to living donor liver transplantation (LDLT) in each patient. Physical growth represented in how far form the standard growth curve expressed as a multiple of the standard deviation (SD) at the time 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
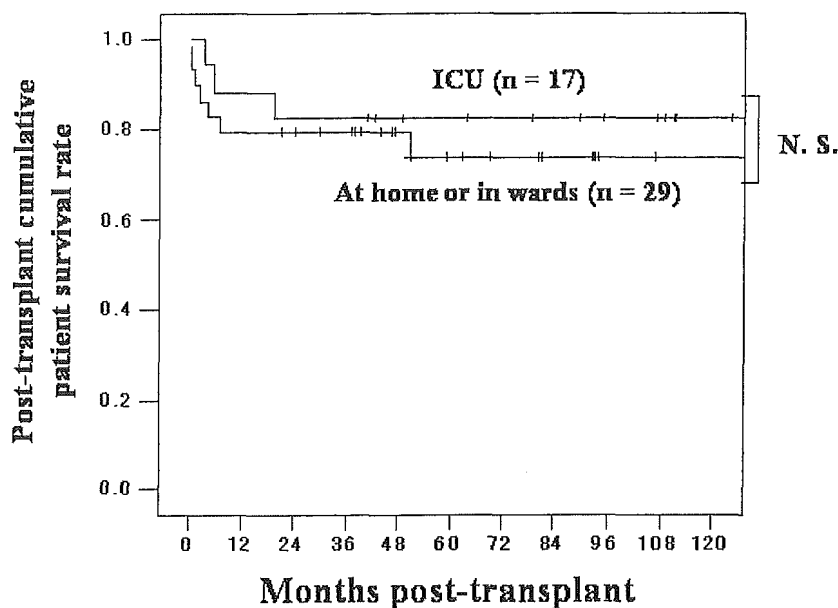
Figure 6: Comparison of post-transplant cumulative patient survival between patients who required the intensive care unit (ICU) stay in the pre-transplant period and those who did not. ICU stay in the pre-transplant period did not affect post-transplant patient survival.
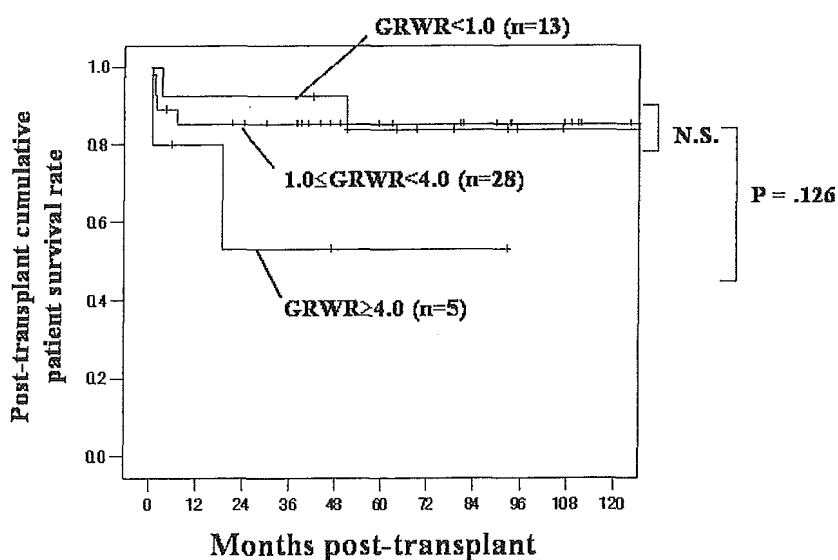

Figure 7: Correlation between post-transplant cumulative patient survival rates and graft-size matching evaluated by the graft-to-recipient weight ratio (GRWR). Although post-transplant cumulative patient survival rates were not different among patients with a graft-to-recipient weight ratio (GRWR) < 1.0, those with $1.0 \leq$ GRWR < 4.0 and those with a GRWR $\geq$ 4.0, post-transplant cumulative patient survival rates tended to be worse in patients with a GRWR $\geq$ 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[i] (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$^\Pi$ (−2.0SD$^\Pi$−3.9SD$^\Pi$)	−2.0SD$^\Pi$ (−3.0SD$^\Pi$−0.6SD$^\Pi$)	< .001
Weight[§]	0.10SD$^\Pi$ (−2.0SD$^\Pi$−2.2SD$^\Pi$)	−0.8SD$^\Pi$ (−2.0SD$^\Pi$−2.0SD$^\Pi$)	.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; $^\Pi$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 ≤ 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 pa-

tients 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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