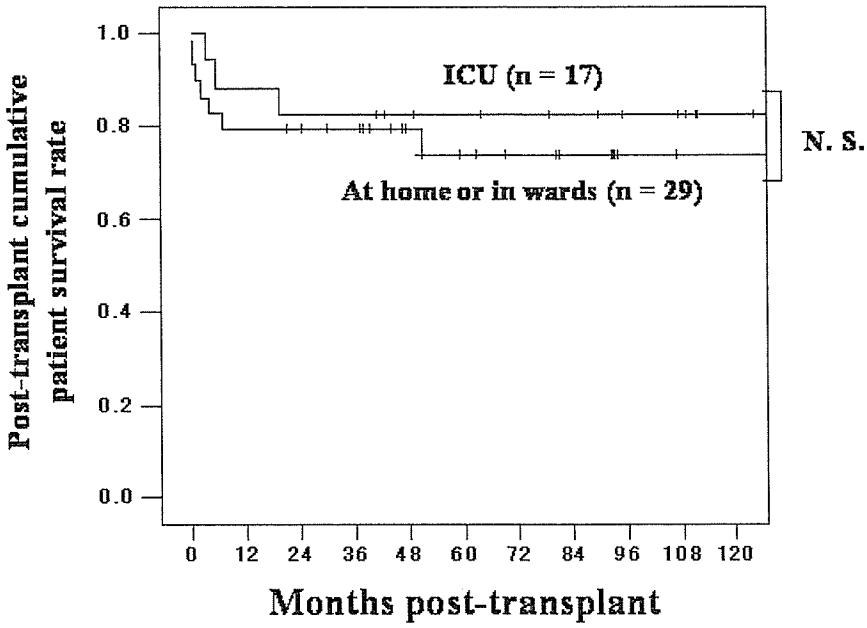

Figure 6: Comparison of post-transplant cumulative patient survival between patients who required the intensive care unit (ICU) stay in the pre-transplant period and those who did not. ICU stay in the pre-transplant period did not affect post-transplant patient survival.

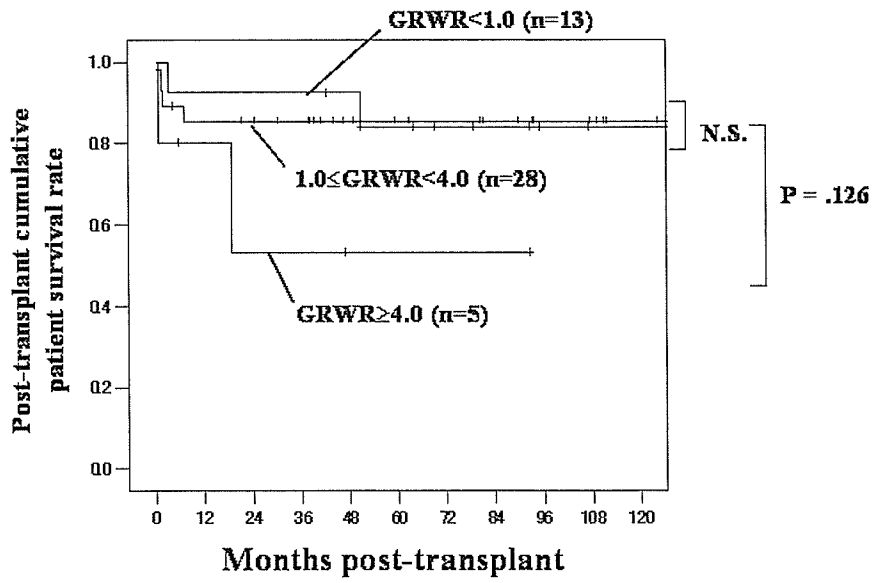

Figure 7: Correlation between post-transplant cumulative patient survival rates and graft-size matching evaluated by the graft-to-recipient weight ratio (GRWR). Although post-transplant cumulative patient survival rates were not different among patients with a graft-to-recipient weight ratio (GRWR) < 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$^{\Pi}$ (−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; Πstandard 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 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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Current Role of Liver Transplantation for the Treatment of Urea Cycle Disorders: A Review of the Worldwide English Literature and 13 Cases at Kyoto University

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To address the current role of liver transplantation (LT) for urea cycle disorders (UCDs), we reviewed the worldwide English literature on the outcomes of LT for UCD as well as 13 of our own cases of living donor liver transplantation (LDLT) for UCD. The total number of cases was 51, including our 13 cases. The overall cumulative patient survival rate is presumed to be more than 90% at 5 years. Most of the surviving patients under consideration are currently doing well with satisfactory quality of life. One advantage of LDLT over deceased donor liver transplantation (DDLT) is the opportunity to schedule surgery, which beneficially affects neurological consequences. Auxiliary partial orthotopic liver transplantation (APOLT) is no longer considered significant for the establishment of gene therapies or hepatocyte transplantation but plays a significant role in improving living liver donor safety; this is achieved by reducing the extent of the hepatectomy, which avoids right liver donation. Employing heterozygous carriers of the UCDs as donors in LDLT was generally acceptable. However, male hemizygotes with ornithine transcarbamylase deficiency (OTCD) must be excluded from donor candidacy because of the potential risk of sudden-onset fatal hyperammonemia. Given this possibility as well as the necessity of identifying heterozygotes for other disorders, enzymatic and/or genetic assays of the liver tissues in cases of UCDs are essential to elucidate the impact of using heterozygous carrier donors on the risk or safety of LDLT donor-recipient pairs. In conclusion, LT should be considered to be the definitive treatment for UCDs at this stage, although some issues remain unresolved. (*Liver Transpl 2005;11:1332-1342.*)

Urea cycle disorder (UCD) is one of the most common inborn errors of metabolism in the liver. Although no population studies have been performed, its prevalence is considered to be 1:30,000–46,000 live births.[1,2] Because the urea cycle is the final common pathway for the metabolism of waste nitrogen in humans, a defect of this pathway results in the accumulation of nitrogen as ammonia, glutamate, alanine, and intermediates prior to the metabolic block.[1,2] UCDs are caused by the following deficiencies in enzymes: carbamyl phosphate synthetase I deficiency (CPSID), ornithine transcarbamylase deficiency (OTCD), argininosuccinate synthetase deficiency (ASSD; neonatal onset form, citrullinemia type I [CTLN1]; adult onset form, citrullinemia type II [CTLN2]), argininosuccinate lyase deficiency (ASLD), arginase deficiency (argD), and *N*-acetylglutamate synthetase deficiency (NAGSD).[1,2] Clinical manifestations of these UCDs are determined principally but not only by serum concentrations of ammonia and glutamate with symptoms that range from mild cognitive deficit to deep coma and can range in severity from fatal neonatal hyperammonemia to asymptomatic adults. An approximate determination of which enzymes in the pathway are defective can be established based on quantitative serum amino

Abbreviations: LT, liver transplantation; UCDs, urea cycle disorders; LDLT, living donor liver transplantation; DDLT, deceased donor liver transplantation; APOLT, auxiliary partial orthotopic liver transplantation; CPSID, carbamyl phosphate synthetase I deficiency; OTCD, ornithine transcarbamylase deficiency; ASSD, argininosuccinate synthetase; CTLN1, citrullinemia type I; CTLN2, citrullinemia type II; ASLD, argininosuccinate lyase deficiency; argD, arginase deficiency; NAGSD, *N*-acetyl glutamate synthetase deficiency; QAAA, quantitative serum amino acid analysis; CT, computed tomography; GRWR, graft-to-recipient weight ratio; ACR, acute cellular rejection; PSP, portal steal phenomenon.

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acid analysis (QAAA) profiles, and an enzymatic assay of each enzyme using liver tissue extracted by needle biopsy can lead to a precise determination of the deficient enzyme. Furthermore, the genetic errors responsible for each enzyme deficiency have been almost entirely elucidated, and thus a genetic diagnosis will soon be established.[1,2] Because conservative medical treatments consisting mainly of protein-restricted diet and alternative pathway medication—both of which are intended to prevent an upsurge in serum ammonia—for these disorders have been refined by the recent precise biochemical and molecular recognition given to their pathophysiology, some affected patients have been able to survive for a long time with acceptable quality of life. However, conservative medical treatments are complicated and require close medical supervision to control the risk of severe hyperammonemic coma.[1-4]

Liver transplantation (LT) has played a significant role in the treatment of UCDs.[1-4] However, because of their rare occurrence, there have been no large series studies discussing the outcomes of LT in UCD patients. In this monograph, we examined the current role of LT for the treatment of UCDs by reviewing previously reported cases in the worldwide English literature as well as our single-center experience with living donor liver transplantation (LDLT) in 13 UCD patients.

Patients and Methods

To the best of our knowledge, there have been 38 cases of LT for UCDs reported in the worldwide English literature (Table 1),[5-21] not including our own previously reported cases.[22-24] We reviewed these 38 cases in addition to our 13 LDLT cases (Table 2) to collect the following data: disease, age of onset, gender, time from onset to LT, disease severity, metabolic status, neurological status, timing of LT (elective or emergent), donor (deceased or living), graft type (whole or partial), auxiliary partial orthotopic liver transplantation (APOLT) or not, postoperative complications, survival outcomes, consequences of disease severity, metabolic status, and neurological status. Emergency transplantation was defined as LT that was performed under urgent conditions necessitating artificial ventilation because of severe hyperammonemic coma. With regard to our 13 LDLT cases, we further investigated the following variables: pretransplant status, donor, graft type, graft-to-recipient weight ratio (GRWR), and outcomes of LDLT, including postoperative complications, chronological changes in disease severity, metabolic status, neurological status, and quality of life. Disease severity, metabolic status, and neurological status were assessed by accepted grading scales essentially following Whitington et al.[3] with minor modifications, as shown in Table 3. Quality of life was also classified into 4 subgroups as shown in Table 3.

UCDs are inherited diseases; OTCD is inherited in an X-linked manner and the other 5 disorders in an autosomal recessive manner.[1,2,24] Thus, in cases of autosomal recessive disorders, the parents and offspring of an affected individual were heterozygotes; siblings had a 50% probability of being a heterozygote or a 25% probability of being latently diseased. Furthermore, females with OTCD can inherit causative genetic errors for OTCD from either parent.

With regard to our 13 LDLT cases, the parental donors of 6 girls with OTCD underwent a preoperative allopurinol loading test[25,26] to determine whether each donor was heterozygous. Preoperative donor needle biopsy of the liver used for enzymatic and/or genetic assays was performed when indicated. In addition, the following data were collected for all employed donors: age; relationship to recipient; mode of donor hepatectomy; resection volume (%) of donor hepatectomy calculated by the following formula: resection volume (%) = {(actual graft weight [g])/(total liver volume calculated with preoperative computed tomography [CT] volumetry [ml])} × 100 (%); and postoperative complications, including hyperammonemia. To evaluate the use of a heterozygote as a donor, mortality or morbidity related to the use of heterozygous donors were investigated. In our LDLT cases who were recipients of heterozygous livers, we examined whether hyperammonemia occurred without evidence of graft dysfunction. In addition, we asked both heterozygous donors and recipients of heterozygous livers about the presence of episodes suggestive of hyperammonemia. For previously reported cases, we investigated whether there was a description of mortalities or morbidities associated with the use of heterozygous donors.

With respect to our 13 LDLT cases, follow-up was continued until March 2005 or death for both donors and recipients.

Statistical analysis was conducted in a nonparametric manner using SPSS commercial statistic software (SPSS 12.0. for Windows; SPSS, Chicago, IL) when indicated. Numerical variables are shown as median (range). Survival was evaluated by the Kaplan-Meier life table analysis with the Breslow-Gehan-Wilcoxon test when indicated.

Results

Overall Cases

Patient Characteristics

The indications for LT were OTCD in 22 cases, CTLN2 in 20, CTLN1 in 4, CPSID in 4, and argD in 1 (Tables 1 and 2). The age of onset ranged from 0-62 years, with a median of 31.5 months. Two patients with OTCD were diagnosed prenatally by genetic assays.[21] The time from onset to LT ranged from 0.5-202 months, with a median of 9.0 months. Emergency transplantation was performed in 4 patients (case nos.

Table 1. Results of a Review of the Worldwide Literature Discussing the Outcomes of Liver Transplantation for the Urea Cycle Disorders

Case No.	Disease	Age of Onset	Gender	Age at LT	Donor	APOLT	Posttransplant Remaining Neurological Impairments	Survival Outcomes	Causes of Death	Reference No.
1	OTCD	0 yr 8 months	F	4 yr	Deceased	No	No	42 months, alive		(5)
2	CPSID	2 days	M	1 yr 8 months	Deceased	No	Yes	18 months, died	Pneumonia	(6)
3	OTCD	0 yr 0 months	M	1 yr 2 months	Deceased	Yes	Yes	7 months, died	Biliary stricture	(7)
4	CPSID	2 days	M	14 days	Deceased	No	Yes	40 months, alive		(8)
5	CTLN2	35 yr	M	35 yr 10 months	Deceased	No	No	34 months, alive		(8)
6	OTCD	0 yr 0 months		1 yr 9 months	Deceased	No	No	60 months, alive		(8)
7	OTCD			5 yr	Deceased	No	No	36 months, alive		(8)
8	OTCD			1 yr 8 months	Deceased	No		2 weeks, died	Hospital mortality	(8)
9	OTCD			2 yr 4 months	Deceased	No	Yes	18 months, alive		(8)
10	OTCD				Deceased	No				(9)
11	OTCD				Deceased	No				(9)
12	CTLN1				Deceased	No				(9)
13	OTCD	21 days	F	5 yr	Deceased	No	No	24 months, alive		(10)
14	OTCD	1 day	M	80 days	Deceased	No	No	6 months, alive		(10)
15	CTLN2	38 yr	M	39 yr	Deceased	No	No	12 months, alive		(11)
16	OTCD	2 days	M	0 yr 3 months	Deceased	No	No	63 months, alive		(12)
17	OTCD	0 yr 0 months	M	0 yr 7 months	Deceased	No	Yes	39 months, alive		(12)
18	OTCD	0 yr 0 months	M	40 days	Deceased	No	No	9 months, alive		(12)
19	CTLN	13 days	M	12 yr	Deceased	No	No	29 months, alive*		(13)
20	CTLN2	60 yr 0 months	F	60 yr 4 months	Living	No	No	13 months, alive		(14)
21	CTLN2	15 yr 6 months	M	16 y 0 m	Living	No	No	72 months, alive†		(15)
22	CTLN	10 yr 0 months	F	6 yr 0 months	Living	No	No	18 months, alive		(16)
23	ARD	0 yr 2 months	F	7 yr 0 months	Deceased	No	No	26 months, alive		(17)
24	CTLN2	12 yr	F	21 yr 0 months	Living	No	Yes	39 months, alive		(18)
25	CTLN2	61 yr 4 months	F	62 yr 0 months	Living	No	No	12 months, alive		(18)
26	CTLN2	25 yr 0 months	M	25 yr 7 months	Living	No	No	70 months, alive		(18)
27	CTLN2	44 yr	M	45 yr	Living	No	No	55 months, alive		(18)
28	CTLN2	23 yr	F	24 yr	Living	No	No	37 months, alive		(18)
29	CTLN2	17 yr	F	17 yr	Living	No	No	31 months, alive		(18)
30	CTLN2	21 yr	M	32 yr	Living	No	No	19 months, alive		(18)
31	CTLN1	0 yr 1 month	F	0 yr 10 months	Living	No	No	72 months, alive†		(19)
32	CTLN2	32 yr	M	32 yr	Living‡	Yes	No	24 months, alive		(20)
33	CTLN2	40 yr	F	42 yr	Living	Yes	No	22 months, alive		(20)
34	CPSID	0 yr 0 months	M	0 yr 5 months	Deceased	No	No	>30 months, alive		(21)
35	CPSID	0 yr 0 months	M	0 yr 3.5 months	Deceased	No	Yes	>30 months, alive§		(21)
36	OTCD	0 months |	M	0 yr 11 months	Deceased	No	No	>30 months, alive		(21)
37	OTCD	0 months |	M	0 yr 8 months	Deceased	No	No	>30 months, alive		(21)
38	OTCD	2 yr 6 months	F	2 yr 11 months	Deceased	No	No	>30 months, alive		(21)

Abbreviations: LT, liver transplantation; APOLT, auxiliary partial orthotopic liver transplantation; OTCD, ornithine transcarbamylase deficiency; CPSID, carbamyl phosphate synthetase I deficiency; CTLN2, type II citrullinemia; CTLN1, type I citrullinemia, ARD, acute respiratory disease.

*The patient underwent retransplantation 17 months after the initial transplant because of secondary biliary cirrhosis due to biliary anastomotic stricture.

†Personal communication of 2005.2.

‡In this case, domino splitting liver harvested from a patient with familial amyloid polyneuropathy was used for the transplantation.

§The patient was listed for retransplantation because of secondary biliary cirrhosis due to biliary anastomotic stricture.

| These patients had the prenatal diagnosis by the genetic assessment.

Table 2. Thirteen Patients with the Urea Cycle Disorders Who Underwent Living Donor Liver Transplantation at Kyoto University

Case No.	Age at LDLT	Gender	Diagnosis	Time from Onset to LDLT (Months)	Donor	ABO- Blood Type Matching	APOLT	GRWR (%)	Survival Outcomes (Current Immunosuppression)	Pretransplant status (DS/MS/NS)	Status at the Latest Evaluation* (DS/MS/NS)	Quality of Life at the Latest Evaluation*
39	52 yr 7 months	F	CTLN2	202	Husband	Identical	Yes	0.84	92 months, alive (Tacrolimus alone)	4/3/1	0/0/0	Excellent
40	23 yr 6 months	M	CTLN2	10	Brother	Identical	Yes	0.78	77 months, alive (Tacrolimus alone)	4/4/4	0/0/0	Excellent
41	20 yr 3 months	M	CTLN2	38	Father	Compatible	Yes	1.21	1 month, died of sepsis	1/1/1		
42	30 yr 11 months	M	CTLN2 with HCC	15	Father	Compatible	No	1.55	29 months, died of brain metastases of HCC	3/3/0	0/0/0†	Excellent†
43	21 yr 8 months	F	CTLN2	4	Father	Identical	No	1.63	63 months, alive (Tacrolimus alone)	4/3/1	0/0/0	Excellent
44	18 yr 2 months	F	CTLN2	9	Mother	Identical	No	1.42	41 months, alive (Tacrolimus and mizoribine)	2/3/1	0/0/0	Good
45	39 yr 6 months	M	CTLN2	3	Wife	Identical	No	1.36	26 months, alive (Tacrolimus alone)	2/2/0	0/0/0	Excellent
46	2 yr 6 months	F	OTCD	3	Mother	Identical	No	2.67	121 months, alive (Tacrolimus alone)	4/4/4	0/0/0	Excellent
47	3 yr 0 months	F	OTCD	17	Father	Identical	Yes	2.08	118 months, alive (None)	4/4/4	0/0/0	Excellent
48	5 yr 9 months	F	OTCD	36	Father	Identical	Yes	1.34	103 months, alive (Tacrolimus alone)	4/3/1	0/0/0	Excellent
49	4 yr 10 months	F	OTCD	9	Mother	Identical	No	1.51	89 months, alive (Tacrolimus alone)	3/3/1	0/0/0	Excellent
50	7 yr 2 months	F	OTCD	86	Father	Identical	No	1.3	6 months, died in a traffic accident	4/3/1	0/0/0†	Good†
51	16 yr 2 months	F	OTCD	177	Father	Identical	No	0.94	60 months alive (Cyclosporin A alone)	4/3/2	0/0/0	Excellent

Abbreviations: LDLT, living donor liver transplantation; APOLT, auxiliary partial orthotopic liver transplantation; GRWR, graft-to-recipient weight ratio (%); DS, disease severity; MS, metabolic status; NS, neurological status; CTLN2, citrullinemia type II; HCC, hepatocellular carcinoma; OTCD, ornithine transcarbamylase deficiency.
*Assessed by grading scales or classified into subgroups as shown in Table 3.
†Evaluated at the outpatient clinic prior to death.

5, 40, 46, and 47). Whole liver deceased donor LT (DDLT) was performed in 20 cases, partial liver DDLT in 5, and LDLT in 26.

Surgical Outcomes

Among the 51 cases under consideration, there were only two hospital mortalities (case nos. 8 and 41) and 4 other deaths (case nos. 2, 3, 42, and 50). Two of the 4 deaths other than hospital mortalities arose from complications of LT or remaining neurological impairments (case nos. 2 and 3). The other two deaths (case nos. 42 and 50), both of which were among our cases, were unrelated to either the LDLT procedure or to the original UCD (Table 4). With respect to the cases taken from the literature, biliary complications were reported to have led to graft failure in three cases. As a result, case 3 died[7]; case 19 underwent a second LT 17 months after the first LT[13]; and case 35 had been placed on a waiting list for retransplantation.[21] Other than these three cases and the two cases of hospital mortality, no serious postoperative complications leading to mortalities or graft losses were observed. Based on our analysis, the cumulative posttransplant graft and patient survival rates were 93.7% and 93.7% at 1 year, and 88.9% and 91.3% at both 5 and 10 years, respectively (Fig. 1).

Metabolic and Neurological Outcomes After Liver Transplantation

Hyperammonemia, dietary restrictions, and the use of alternative pathway medications were completely eradicated by LT in all surviving patients, although neurological impairments remained in 7 (case nos. 2, 3, 4, 9, 17, 25, and 35) of the 47 patients in whom neurological status was evaluated. Six of these 7 (case nos. 2, 3, 4, 9, 17, and 35) received LT in their early infancy, at an age ranging from 0.5-28 months with a median of 10.5 months, and the remaining patient (case 25) was a 52-year-old adult. Among the 47 patients in whom neurological status was evaluated, 6 of the 21 patients who underwent DDLT showed remaining neurological impairments, compared with only one of 26 LDLT cases. In other words, neurological impairments were more likely to remain in pediatric cases (6 of 25 cases) than in adult cases (aged 12 years or more, 1 of 22 cases) and more likely to remain in patients who underwent DDLT than in those who underwent

Table 3. Grading Scales to Evaluate Disease Severity, Metabolic Status, and Neurological Status, and Classifications of Quality of Life

Severity of the Disease
Grade 4: many episodes of severe hyperammonemic coma, some with NH3* > 300 μmol/L
Grade 3: one to several episodes of hyperammonemic coma, no more than one with NH3* > 300 μmol/L
Grade 2: one to few episodes of hyperammonemic coma, none with NH3* > 300 μmol/L
Grade 1: only one episode of hyperammonemic coma, with NH3* < 300 μmol/L
Grade 0: no episodes of hyperammonemic coma, no NH3*> 100 μmol/L
Metabolic Status
Grade 4: no improvement, severe hyperammonemia, need for constant, full doses of medication
Grade 3: some improvement, moderate hyperammonemia, need for constant medication
Grade 2: major improvement, moderate hyperammonemia, need for some medication for control
Grade 1: almost complete correction, occasional hyperammonemia, with or without need for medication
Grade 0: complete correction, no hyperammonemia, no need for medication
Neurological Status
Grade 5: persistent coma or vegetative state
Grade 4: responds to noxious stimuli, but no social interaction, no ambulation, no communication
Grade 3: limited social interaction, no bipedal ambulation, limited communication through gestures
Grade 2: definite social interaction, fair ambulation, though possibly limited by spasticity
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 0: seems to be normal spectrum for social interaction, motor skills, language development and learning
Quality of Life
Excellent: receiving one or no immunosuppressive drugs and all the above grading scales corresponding to a score of 0
Good: receiving two or more immunosuppressive drugs and all the above corresponding to a score of 0
Fair: regardless of the number of immunosuppressive drugs each patient received, one or more of the above scales corresponding to a scale of 1
Poor: with any episodes of graft dysfunction to necessitate frequent or long hospital stay regardless of their causes and/or one or more of the above scales corresponding to a score of 2 or more

*Serum ammonia level.

LDLT; these differences did not reach the level of statistical significance.

Outcomes of Auxiliary Partial Orthotopic Liver Transplantation

Although APOLT is considered to be the preferred treatment for UCD,[7,20,22] it was performed in only 8 cases (case nos. 3, 32, 33, 39, 40, 41, 46, and 47). Of these 8 cases, a deceased donor was used in only 1 case (case 3, Table 1), and LDLT was performed in the other 7 cases (Tables 1 and 2). Comparison of posttransplant patient survival between the APOLT cases and the other 43 non-APOLT cases showed that the cumulative patient survival rates were 75.0% at each of 1, 5, and 10 years in the APOLT cases, and 95.0% at 1 year, 92.1% at 5 years, and 92.1% at 10 years in the non-APOLT cases (Fig. 2). There were no statistically significant differences in these rates between the two groups.

Kyoto University Cases

Of our 13 cases, 5 patients underwent APOLT, using a left liver graft (segments II-IV according to Couinaud's Nomenclature for liver segmentations) or left lateral section liver graft (segments II-III); the remaining 8 patients underwent total hepatic replacement (Table 2). Serum ammonia levels fell to the normal range (11-35 μmol/L) within 4 days posttransplant in all patients. Several early postoperative complications were observed, most of which were managed with medication or surgical and/or radiological intervention, resulting in recovery in all patients but one (case 41), who died of sepsis following steroid pulse therapy for acute cellular rejection (ACR) diagnosed in the early postoperative period. Two other deaths (case nos. 42 and 50) were unrelated to the LDLT procedure (Table 2). With regard to long-term complications, late-onset ACR was observed in case nos. 39, 40, 47, and 48, and biliary anastomotic stricture was observed in case nos. 39 and 46. In case nos. 47 and 48, both of whom underwent APOLT, we observed the portal steal phenomenon (PSP), in which late-onset ACR was a trigger and the native liver remnant stole portal blood inflow from graft liver,[24] resulting in mild but refractory hyperammone-

Table 4. Characteristics of the 13 Employed Donors

Case	Recipient's Disease	Relationship with Recipient	Age	Heterozygote or Not	Mode of Donor Hepatectomy	Resection Volume (%) of Donor Hepatectomy*	Duration from Surgery
39	CTLN2	Husband	52	Nonheterozygote	Left hepatectomy†	33.2	92 months
40	CTLN2	Brother	24	Heterozygote or latently diseased	Left hepatectomy†	32.9	77 months
41	CTLN2	Father	54	Heterozygote	Left hepatectomy†	36.7	68 months
42	CTLN2	Father	59	Heterozygote	Right hepatectomy‡	53.2	65 months
43	CTLN2	Father	50	Heterozygote	Right hepatectomy‡	60.8	63 months
44	CTLN2	Mother	54	Heterozygote	Right hepatectomy‡	43.5	41 months,
45	CTLN2	Wife	38	Nonheterozygote	Right hepatectomy‡	64.4	26 months
46	OTCD	Mother	32	Heterozygote	Left lateral sectionectomy§	25.5	121 months
47	OTCD	Father	36	Nonhemizygote	Left lateral sectionectomy§	21.5	118 months
48	OTCD	Father	36	Nonhemizygote	Left lateral sectionectomy§	21.5	103 months
49	OTCD	Mother	35	Heterozygote	Left lateral sectionectomy§	22.1	89 months
50	OTCD	Father	29	Nonhemizygote	Left lateral sectionectomy§	24.1	63 months
51	OTCD	Father	44	Nonhemizygote	Left hepatectomy†	33.2	60 months

Abbreviations: CTLN2, citrullinemia type II; OTCD, ornithine transcarbamylase deficiency.
*Calculated from the following equation: {actual graft weight (g)}/{total liver volume calculated from preoperative CT volumetry (mL)} × 100 (%).
†Resection of segments II+III+IV according to Couinaud's nomenclature for liver segmentation.
‡Resection of segments V+VI+VII+VIII.
§Resection of segments II+III.

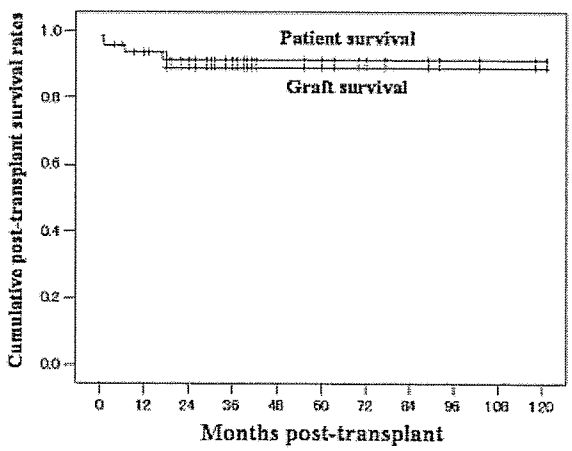

Figure 1. Cumulative posttransplant patient and graft survival rates in 51 patients with urea cycle disorders undergoing liver transplantation. Based on the present analysis, the cumulative posttransplant graft and patient survival rates were 93.7% and 93.7% at 1 year and 88.9% and 91.3% at both 5 and 10 years, respectively.

mia (100-200 μmol/L). In both cases, ligation of the right portal vein flowing into the native liver remnant successfully eradicated the PSP; ligation was performed at 26 months after LDLT in case 47 and at 16 months after LDLT in case 48. In case 48, however, a second PSP was brought on by abundant collateral vessels, which had developed around the previously ligatured right portal vein. As a result, case 48 underwent surgical removal of the native liver remnant at 64 months after LDLT.[24] These complications temporarily impaired the patients' quality of life, but all patients recovered after management with medications or surgical and/or radiological intervention. Consequently, all patients but case 41, in which LDLT resulted in hospital mortality, showed excellent or good quality of life at the latest evaluations (Table 2).

The postoperative observation period of the survivors ranged from 26-121 months, with a median of 77 months (Table 2). No surviving pediatric case has shown any evidence of problematic retardation in neurodevelopmental or physical growth, and all have been

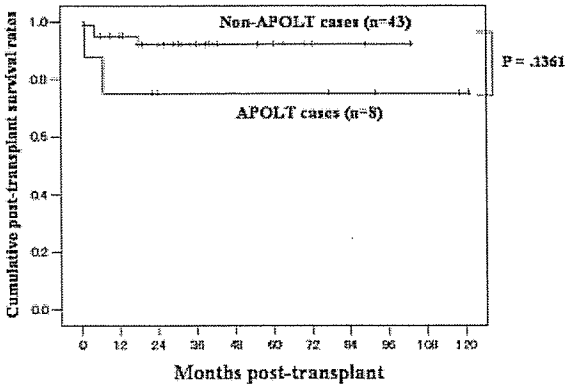

Figure 2. Comparison of cumulative posttransplant patient survival rates between auxiliary partial orthotopic liver transplantation (APOLT) cases and non-APOLT cases. The comparison of posttransplant patient survival between the 8 APOLT cases and the other 43 non-APOLT cases showed that the cumulative patient survival rates were 75.0% at each of 1, 5, and 10 years in the APOLT cases and 95.0% at 1 year, 92.1% at 5 years, and 92.1% at 10 years in the non-APOLT cases. There were no statistically significant differences in these rates between the two groups.

educated at ordinary schools. All surviving adult patients are currently doing well and leading socially normal daily lives. A comparison of cumulative posttransplant patient survival rates between the 13 UCD patients who underwent LDLT and 909 patients undergoing initial LDLT for other indications during the same study period at Kyoto University showed that these rates were better in the 13 UCD patients than in the other 909 patients, although these differences did not reach the level of statistical significance (Fig. 3).

Impact of Employing a Heterozygote as a Donor

Preoperative allopurinol loading testing for parental donors of girls with OTCD (case nos. 46-51) showed 4 fathers with no abnormal findings and two mothers with almost twice the normal upper values of urine orotic acid and orotidine peak levels after the loading. These results suggested that these two mothers were heterozygotes for OTCD. Thus, of 26 living donors, 14 parental and two offspring donors were heterozygous for the disorder in question, two sibling donors had a 50% probability of being heterozygous or a 25% probability to be latently diseased, and the other 8 donors were nonheterozygous.

Irrespective of their status as heterozygous or nonheterozygous, all donors in our LDLT cases (Table 4) fulfilled our standard donor selection criteria as described in detail elsewhere.[27,28] Concerning the heterozygote donors in our cases, three fathers (case nos. 41-43) and one mother (case 44) of CTLN2 patients showed neither elevation in plasma ammonia level nor abnormal QAAA profiles, and all were used as donors without further examinations. In case 40, because a 24-year-old brother of this CTLN2 patient was the only donor candidate, enzymatic assays using liver needle biopsy specimens were performed despite the donor's normal QAAA profiles and lack of elevation in serum ammonia level. The assays showed 30% of the normal value for argininosuccinate synthetase activity, suggesting that the brother was certainly either heterozygous or latently diseased. A genetic assay was not performed because the causative genetic errors of CTLN2 were not well understood at that time.[29] Because the recipient's condition necessitated emergency transplantation, the brother was used as a donor with a strict informed consent clearly stating the potential risks related to his heterozygosis or latent disease. We performed APOLT using a left liver graft in this case in order to avoid right liver donation so as to decrease the operative risks of the donor by reducing the

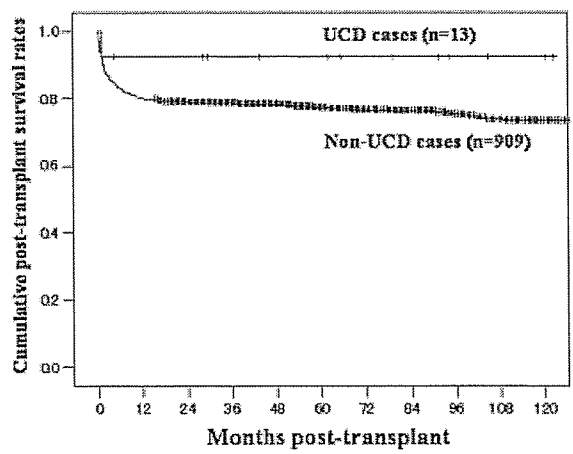

Figure 3. Cumulative posttransplant patient survival rates of 13 patients who underwent living donor liver transplantation (LDLT) and 909 patients who underwent LDLT for other indications during the same study period at Kyoto University. The comparison of cumulative posttransplant patient survival rates between the 13 UCD patients who underwent LDLT and 909 cases undergoing initial LDLT for other indications during the same study period showed that these rates were better in the 13 UCD cases than in the other 909 cases, although these differences did not reach the level of statistical significance. (Two deaths unrelated to LDLT procedures or the original UCD were excluded from the incidence of survival curve of the 13 UCD patients.)

extent of his hepatetomy.[20] Two mothers of girls with OTCD (case nos. 46 and 49), both of whom were determined to be heterozygous for OTCD as stated above, were further examined by enzymatic and genetic assays and proven to be heterozygous for mutations on Xp21, where the OTC gene lies,[1,2,24] but normal in OTC activity in the liver.

In case nos. 42-44, we performed right liver donation for heterozygous carriers; no major postoperative complications occurred in any donors, and all donors were uneventfully discharged from the hospital within 14 postoperative days. None of the donors showed consistent signs of hyperammonemia in the early postoperative period, and all have been doing well without any episodes suggestive of hyperammonemia. Furthermore, all recipients, including those who received heterozygous livers, have shown no episodes of either hyperammonemia without evidence of graft dysfunction or episodes suggestive of hyperammonemia.

With respect to the use of heterozygous donors in our review of the literature, there were no descriptions of mortality or morbidity related to the use of heterozygous donors.

Discussion

In the present metaanalysis, 40 of 51 patients are currently surviving with satisfactory quality of life obtained from the implementation of LT, and neurological impairments remain in 5 surviving patients. The cumulative patient survival rates are presumed to be more than 90% at 5 years posttransplantation. These outcomes are superior to those reported in cases of LT for other diseases.[30] Successful conservative treatment of severely affected UCD patients requires close medical supervision and may become complicated with a high number of medications and strictly restricted protein intake; nevertheless, anecdotal evidence suggests that these patients fare no better than those who undergo LT, and they are always accompanied by the fear of sudden fatal metabolic crisis.[1-4] Thus, LT should be more enterprisingly performed for cases of UCD because the results of the present study confirm that acceptable survival outcomes and quality of life for patients with UCD can be obtained through LT. In addition, a delay in LT for affected patients often leads to remaining neurological impairments, most notably in severely affected infants (case nos. 2, 3, 4, 9, 17, and 24). Furthermore, all individuals affected by UCD run the risk of severe hyperammonemic coma indicative of fatal metabolic crisis, which has been reported to be easily induced by slight stresses such as the common cold.[1-4] In neonatal cases in particular, hyperammonemia with serum levels of more than 300 μmol/L has been reported to easily lead to irreversible brain damage.[2,31] In the early days of both DDLT and LDLT, severely affected neonates rarely received LT due to their small body size and the scarcity of livers of appropriate size.[7,32] In modern times, however, splitting the liver has become a common procedure. In addition, monosegmental liver graft has been gaining wider acceptance even for premature neonates.[32] When DDLT is unavailable, LDLT is an ideal alternative to DDLT when a monosegmental graft is necessary because the donor hepatectomy is less invasive. At present, when the causative genetic errors of UCD have almost been clarified,[2] making prenatal diagnosis possible,[2,19,21,29] elective LDLT immediately after birth can be performed as occasions demand. For adult patients, right liver donation from a living donor has gained wider acceptance[33] and has almost resolved the small-for-size-graft problem.[34] If the extent of right hepatectomy for the donor exceeds 70% of the resection rate, APOLT can be an effective therapeutic option to avoid right liver donation and reduce the extent of donor hepatectomy. Living liver donor morbidity appears to have increased in recent years,[35,36] and this increase in morbidity has been attributed mainly to the wider acceptance of right liver donation.[35] APOLT using a left liver graft can correct UCDs by providing sufficient enzyme supplementation,[20,22] because most UCD livers are functionally normal other than the urea cycle.[1-4] APOLT has traditionally been preferred for the treatment of UCDs because the APOLT recipient will be released from life-long immunosuppressive therapy if gene therapies for the UCDs are established,[37] or if the graft liver is severely damaged, hepatocyte transplantation can be a successful alternative to hepatic retransplantation.[38] However, both of our first two APOLT cases (case nos. 47 and 48) suffered severe graft dysfunction caused by functional competition of portal blood inflow between the native liver remnant and the graft liver.[24] After these experiences, we have performed total portal diversion of the native liver remnant in all subsequent APOLT cases to prevent this functional competition.[39] This procedure benefits the graft liver but compromises the integrity of the native liver remnant, and thus these APOLT recipients would not benefit from gene therapies or hepatocyte transplantation even if these advanced therapies were clinically available. Furthermore, the postoperative morbidity rate of APOLT recipients was higher than that of non-APOLT

recipients,[39] and thus we have suspended our APOLT program over the last several years to reconsider the implications of applying APOLT to UCD patients. However, Yazaki et al.[20] report that partial portal diversion of the native liver remnant, in which only the right anterior branch of the portal vein was ligatured, successfully prevented functional competition of portal blood inflow between the graft and the native liver remnant in CTLN2 cases who underwent APOLT using a left liver graft. This refined procedure can lead not only to better living liver donor safety by avoiding the right liver donation but also to reapproval to perform APOLT in patients with noncirrhotic metabolic liver diseases, including UCDs, with the expectation of the establishment of gene therapies or hepatocyte transplantation because the integrity of the native liver remnant will be maintained with portal inflow supplied by the right posterior branch.

In the present study, no negative impacts of the use of heterozygous carriers as donors on either donors' or recipients' postoperative course have been observed to date. Nevertheless, the advisability of using heterozygous carriers as donors should be considered uncertain. Indeed, it was reported that a recipient of a liver harvested from an adult male deceased donor with unrecognized OTCD died as a result of severe hyperammonemia.[40] Male hemizygotes of OTCD can range in severity from fatal neonatal hyperammonemic coma to asymptomatic adults, whereas female asymptomatic heterozygotes of OTCD might be approved for donor candidacy according to the degree of X-inactivation in the liver because X-inactivation has been reported to be correlated with OTC activity only in the liver.[41] Based on these findings, we propose the following guidelines for the use of heterozygous carriers of UCDs as donors. In OTCD, preoperative enzymatic and genetic assay using liver tissue must be performed for all blood relative donor candidates to exclude male hemizygotes from donor candidacy; in addition, these male hemizygotes must be strictly followed up because of the potential risk of sudden metabolic crisis. Adult heterozygous females for OTCD will be employed as donors only if their liver OTC activity is normal. With regard to the other disorders, asymptomatic heterozygous carriers will be employed only if there are no other candidates. In such situations, liver tissue must be extracted for enzymatic and/or genetic analyses. A part of the tissue should be used to investigate the correlation between genetic errors and enzyme activities, and the remainder must be preserved for future analyses to precisely evaluate the impact of the use of heterozygous carriers for disorders on the risk and safety of both donors and recipients. It remains essential to conduct worldwide multicenter studies.

Although the differences did not reach the level of statistical significance, there was a trend in the present study for neurological deficits to persist in pediatric recipients as well as recipients of DDLT. We consider that an ability to schedule surgery, which is one of the biggest advantages of LDLT over DDLT, had a beneficial effect on the posttransplant neurological outcomes of LDLT recipients. In the past, LT has not been readily used for patients with UCDs. The management of patients with CPSID or OTCD who present in the newborn period is known to be difficult.[3] Thus, these patients must undergo LT immediately after the onset. Furthermore, patients in whom dietary restriction and alternative pathway medications are not very effective must be considered as potential candidates for LT. In other words, patients with UCDs in whom repeated hospitalizations as well as hemodialysis or peritoneal dialysis is required to control hyperammonemia should undergo LT as soon as possible. Especially in pediatric patients, long-term dietary restriction almost always leads to growth retardation, and the retardation of growth has been reported to disadvantageously affect the outcome of LT.[42] Therefore, earlier application of LT to pediatric patients with UCDs will be inevitable to prevent both growth retardation and neurological deficits. In addition, when LT is necessary for UCD patients, LDLT can be an important choice of treatment in order to avoid missing the optimal time range for LT.

In conclusion, LT should be considered to be the definitive treatment for UCDs, and thus more enterprising application of this procedure to UCD patients is acceptable. If DDLT is unavailable, the selection of living donors must be initiated immediately. However, the use of heterozygous carriers as LDLT donors for UCD has not yet been validated.

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Clinical Outcomes of Living Donor Liver Transplantation for Hepatitis C Virus (HCV)-Positive Patients

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Background. Whether hepatitis C virus recurrence occurs earlier and with greater severity for living donor liver transplantation (LDLT) than for deceased donor liver transplantation (DDLT) has recently become a subject of debate.
Methods. We retrospectively evaluated clinical outcomes for a cohort of 91 HCV-positive patients who underwent LDLT at Kyoto University with a median follow-up period of 25 months.
Results. Overall 5-year patient survival for HCV patients was similar to that for non-HCV patients (n=209) who underwent right-lobe LDLT at our institute (69% vs. 71%). Survival rate of patients without HCC (n=34) tended to be better than that of patients with HCC (n=57) (82% vs. 60%, $P=0.069$). According to annual liver biopsy, rate of fibrosis progression to stage 2 or more (representing significant fibrosis) was 39% at 2 years after LDLT. Univariate analysis showed that female recipient and male donor represented significant risk factors for significant fibrosis. Progression to severe recurrence (defined as the presence of liver cirrhosis (F4) in a liver biopsy and/or the development of clinical decompensation) was observed in five patients.
Conclusions. Postoperative patient survival was similar for HCV-positive and -negative recipients in our adult LDLT series. Rates of progression to severe disease due to HCV recurrence seemed comparable between our LDLT recipients and DDLT recipients described in the literature. Although longer-term follow-up is required, our results suggest that LDLT can produce acceptable outcomes also for patients suffering from HCV-related cirrhosis.

Keywords: Hepatitis C virus, Living donor, Recurrence.

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Chronic hepatitis C virus (HCV) has become a global epidemic, with an estimated 200 million people currently infected worldwide. Nowadays, HCV-related cirrhosis is the most common indication for liver transplantation. However, recurrence of HCV infection is universal and often occurs immediately after transplantation (*1*). The prevalence of chronic hepatitis C in HCV-positive liver transplant recipients is 70–90% after 1 year, and rate of fibrosis progression is accelerated so that 20–40% of patients progress to allograft cirrhosis within 5 years (*2–6*). As a result, graft and patient survival is significantly reduced for HCV-positive recipients compared with HCV-negative recipients (*6–7*).

In Japan, too, HCV-related cirrhosis and hepatocellular carcinoma (HCC) represent the most prevalent liver diseases, and living donor liver transplantation (LDLT) has become a treatment option for patients with these diseases. However, a warning was recently issued by some Western transplant centers that HCV recurrence may occur earlier and with greater severity, and graft loss caused by recurrent HCV may be more frequent for LDLT than for deceased donor liver transplantation (DDLT) (*8–11*). Some suggestions have been offered for mechanisms that could increase graft damage in HCV-infected LDLT recipients (*12*). First, because the right hepatic lobe graft undergoes intense regeneration immediately after LDLT, specific cellular changes occurring during this vigorous proliferative response may facilitate entry of HCV into hepatocytes or promote HCV replication. Second, since most living donors are primary relatives of the recipient, increased genetic similarity and a higher degree of HLA matching between donor and recipient compared with DDLT may affect the severity of recurrent HCV infection. Conversely, more recent studies have reported comparable results between LDLT and DDLT (*13–15*). Such discrepancies may be explained in part by the small numbers of LDLT patients included in these studies, or learning curve effects on recent data associated with increased experience (*16*).

This issue has attracted worldwide attention because, given the shortage of deceased donor organs, increasing numbers of patients are choosing to undergo LDLT. The matter is of critical importance in Japan and countries where almost all liver transplantations use living donor grafts. The present study retrospectively evaluated clinical outcomes for a comparatively large cohort of 91 patients who underwent LDLT for HCV-related cirrhosis at our institute. We investigated the frequency and severity of posttransplant recurrence of chronic HCV hepatitis and examined risk factors in order to clarify the role of LDLT in the treatment of patients with HCV cirrhosis.

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PATIENTS AND METHODS

Patients

Between March 1999 and April 2005, LDLT was performed at Kyoto University on 105 patients with HCV cirrhosis. Of these, 91 patients (61 men, 30 women) who had undergone LDLT by June 2004 and had been followed up for >12 months were included in this study (Table 1). Median age of subjects was 55 years (range, 30–69 years). Median model for end-stage liver disease (MELD) score was 16 (range, 4–33). HCV cirrhosis was accompanied by HCC in 57 patients (63%), including 25 who exceeded Milan criteria. Median MELD scores in groups with and without HCC were 15 (range, 4–33) and 18 (range, 9–33), respectively (P=0.015, Mann-Whitney U test).

All patients were positive for anti-HCV antibody before the operation. Preoperative HCV RNA load, measured using the polymerase chain reaction (PCR) method with an Amplicor HCV assay (Roche Molecular Systems, Pleasanton, CA), was obtained for 74 patients, with a median value of 260 kIU/ml (range: <0.5–2400). Patients treated during the early period, in whom viral load was measured only using DNA probe methods, were considered to lack relevant data. HCV genotype, determined using a system based on PCR with genotype-specific primers (*17*), was: 1b (n=52); 2a (n=6); 2b (n=3); others (n=2); not determined (low viral load, n=3); or not examined (n=25).

LDLT using a right-lobe graft was performed on all except two patients who received left lobe grafts. Operative procedures for donor and recipient surgery have been described elsewhere (*18*, *19*). Donors were 52 men and 39 women, with a median age of 40 years (range, 19–64 years). Relationship to the recipient was: child (n=36); spouse (n=34); sibling (n=17); parent (n=1); or other (n=3). ABO blood-type matching was incompatible in 15 cases.

After discharge, patients were scheduled for monthly visits to the outpatient clinic for the first year. Median duration of follow-up was 25 months (range, 1–72 months).

TABLE 1. Preoperative profile and clinical characteristics

Characteristic	Data
n	91
Recipient sex (male/female)	61/30
Median recipient age, years (range)	55 (30–67)
Child-Pugh grade (A/B/C)	2/26/63
Median MELD score (range)	16 (4–33)
Pretransplant HCC (yes/no)	57/34
HCV genotype (1b/2a/2b/others)	52/6/3/2
Median HCV-RNA, kIU/mL (range)	260 (<0.5–2400)
Pretransplant interferon therapy (yes/no)	30/61
Median donor age, years (range)	40 (19–64)
Donor gender (male/female)	52/39
Relation to recipient (related/unrelated)	57/34
ABO blood-type mismatch (yes/no)	15/76
HLA-A,B mismatch (≤2/≥3)	70/21
HLA-DR 2 mismatch (yes/no)	26/65
GRWR ≥1.0% (yes/no)	54/37
Immunosuppression (FK/CyA)	83/6
Steroid-free induction (yes/no)	19/72
Methylprednisolone boluses for rejection (yes/no)	32/59

Immunosuppression

The standard immunosuppression protocol comprised tacrolimus and low-dose steroid (*20*). The target whole-blood trough level for tacrolimus was 10–15 ng/ml during the first 2 weeks, approximately 10 ng/ml thereafter, and 5–8 ng/ml from the second month. Cyclosporine microemulsion was administered instead of tacrolimus for induction immunosuppression in six patients. Steroid therapy was initiated at a dose of 10 mg/kg before graft reperfusion, then tapered from 1 mg/kg/day on day 1 to 0.3 mg/kg/day until the end of the first month, followed by 0.1 mg/kg/day until the end of the third month. After this time, steroid administration was terminated. As an exception, 13 patients received steroid-free tacrolimus monotherapy as an induction procedure in an attempt to reduce HCC recurrence. In addition, four patients were assigned to a tacrolimus plus mycophenolate mofetil (MMF) (without steroid) group in a prospective comparative study started in March 2004 to evaluate the effects of steroid-free immunosuppression on recurrence of HCV. Another two patients transplanted with grafts from an identical twin did not receive any immunosuppressive treatment.

Patients who received ABO blood-type incompatible transplants were treated with preoperative plasma exchange or double-filtration plasmapheresis in order to reduce anti-A or B antibody titers. During the first 3 weeks postoperatively, prostaglandin E1 and additional steroids were administered via the portal vein or hepatic artery (*21*). Cyclophosphamide was also given intravenously for the first 2 weeks, and then orally.

Acute rejection episodes were documented by means of liver histology (*22*) and treated with methylprednisolone boluses if moderate or severe. OKT-3 was used for only one patient. MMF or azathioprine was added for patients who experienced refractory rejections or required reduction of tacrolimus dose due to adverse effects.

Antiviral Therapy

Prophylactic antiviral therapy for HCV was not administered. As a rule, antiviral treatment was used for patients with recurrent chronic hepatitis C. The treatment protocol consisted of interferon α2b ($3–6\times10^6$ units 3 times/week) plus ribavirin (400-800 mg/day orally for the first 6 months), followed by interferon monotherapy for 6 months.

Histological Assessment

A total of 398 liver biopsies were evaluated when patients displayed liver enzyme levels elevated more than two to three times the normal upper limit, or at yearly intervals when informed consent was obtained. Annual follow-up biopsies were obtained from 60 patients at 1 year after LDLT, 34 patients at 2 years, 14 patients at 3 years, 6 patients at 4 years, and 1 patient at 5 years. Biopsy specimens were evaluated by a single pathologist (H.H.) with extensive experience in the pa-

thology of liver transplantation. Necroinflammatory activity (A0-A4) and fibrosis stage (F0-F4) were assessed using the METAVIR score (*23*, *24*). Fibrosis of stage 2 or higher was defined as significant fibrosis and was used as one of the endpoints in this study. A stage score of 2 was considered easily separable from stage 1 as a dividing point, as stage 1 involves fibrosis confined to the portal tract.

Prognostic Factors for Patient Survival and HCV Recurrence

A total of 18 variables potentially associated with patient survival and HCV recurrence were evaluated. Pretransplantation variables included: recipient age; recipient gender; Child-Pugh grade; MELD score; presence of HCC; HCV genotype (1b vs. non-1b); HCV viral load; and history of previous antiviral treatment with interferon. Donor-related variables comprised: age; gender; relation to the recipient (related vs. unrelated); ABO-blood type and HLA compatibilities; graft-to-recipient body weight ratio (GRWR: $<1.0\%$ vs. $\geq 1.0\%$). Posttransplant variables were: induction immunotherapy (tacrolimus vs. cyclosporine, with or without steroid); and administration of steroid boluses.

Statistical Analysis

Overall survival, time to reach fibrosis of stage 2 or more according to liver biopsy (with time to last biopsy for all patients who did not reach fibrosis of stage 2), and time to severe HCV recurrence were evaluated. Severe HCV recurrence was defined as the presence of liver cirrhosis (F4) in a liver biopsy and/or the development of clinical decompensation secondary to liver diseases with portal hypertension (*11*). Cumulative probability curves of survival or HCV recurrence were calculated using the Kaplan-Meier method, and differences between these curves were compared using the log-rank test. The cutoff chosen for quantitative variables was the median, unless stated otherwise. Any variable identified as significant ($P<0.05$) in univariate analysis by log-rank testing was considered a candidate for multivariate analysis using Cox's proportional hazards regression model. Values of $P<0.05$ were considered statistically significant.

RESULTS

Patient Survival

As of the end of May 2005, 65 patients were still alive. One patient had received re-LDLT for graft cirrhosis due to HCV recurrence 31 months after first LDLT and has survived 14 months since then. Causes of death for the 26 patients were: sepsis (n=11); peritonitis (n=4); pneumonia (n=3); recurrent HCC (n=4); chronic rejection (n=2); veno-occlusive disease (n=1); and recurrent HCV (fibrosing cholestatic hepatitis (FCH), n=1). Overall patient survival rate at 5 years was 69%, similar to that of 209 non-HCV patients (71%) who underwent right-lobe LDLT at our institute between February 1998 and June 2004 (PBC/PSC, n=56; HBV cirrhosis, n=53; fulminant hepatitis, n=38; cholestatic disease, n=19; and others, n=43; Fig. 1). Among HCV-positive patients, 5-year survival rate tended to be better in patients without HCC (n=34) than in patients with HCC (n=57), although no significant difference was identified (82% vs. 60%, $P=0.069$; Fig. 2). None of the variables listed above as prognostic factors for patient survival and HCV recurrence displayed any significantly associations with patient survival.

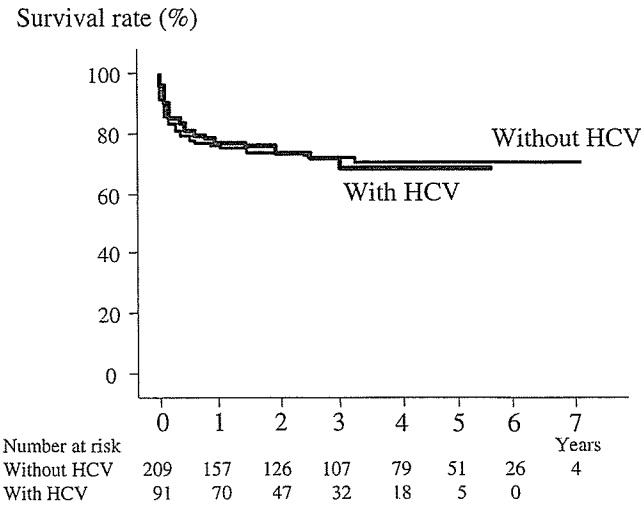

FIGURE 1. Patient survival after living donor liver transplantation (HCV vs. non-HCV). Overall patient survival rate for HCV-positive patients was 69% at 5 years, similar to that for non-HCV patients (71%, n=209) who underwent right-lobe LDLT at our institute between February 1998 and June 2004.

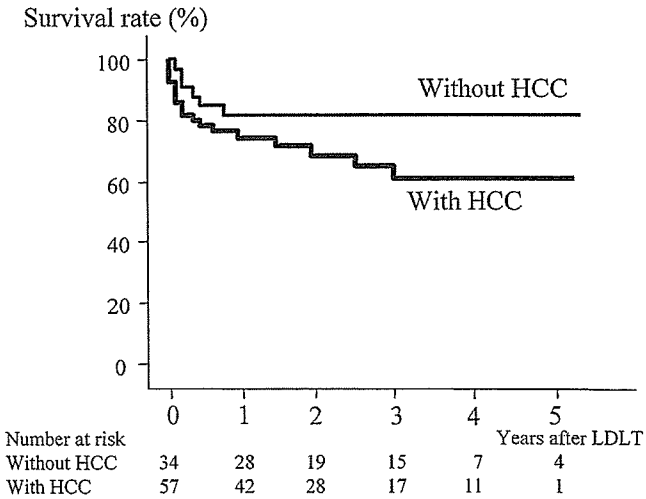

FIGURE 2. Patient survival for HCV patients (with vs. without HCC). Among HCV-positive patients, survival rate tended to be better for patients without HCC (n=34) than for patients with HCC (n=57), although no significant difference was found (82% vs. 60%, $P=0.069$).

Evaluation of Liver Histology

During the first year after LDLT, necroinflammatory changes suggesting recurrent hepatitis C were observed in 50 patients: A1 in 32 patients; A2 in 17 patients; and A3 in one patient. Afterwards, the percentage of patients who received biopsy among those alive at yearly intervals was as follows: 1 year, 86% (60/70); 2 years, 72% (34/47); 3 years, 44% (14/32); 4 years, 33% (6/18); and 5 years, 20% (1/5). Ten patients who were alive for >1 year (range, 22–72 months) without any

evidence of progressive liver disease never underwent any biopsy at 1 year or later. Significant fibrosis (stage 2 or more) was identified in 23 patients, including 3 patients who developed to fibrosis of stage 4 within 1 year. Excluding 19 patients who died within 1 year without identified fibrosis and the 10 patients alive without biopsy for >1 year, cumulative probability of significant fibrosis was 19% at 1 year after LDLT, 39% at 2 years, and 58% at 3 years. Follow-up was censored at the time of last biopsy for all patients who did not reach fibrosis of stage 2.

The results from univariate analysis of risk factors for significant fibrosis are summarized in Table 2. Female recipient and male donor were significantly associated with development of significant fibrosis. Analysis of quantitative variables, donor age and GRWR, demonstrated that rate of significant fibrosis was not significantly different even when cutoff levels were changed. Multivariate analysis with Cox's hazards model showed that neither female recipient nor male donor represented independent risk factors for significant fibrosis (data not shown).

Severe Recurrence of HCV

FCH was diagnosed in two patients, one of whom died of liver failure 7 months after LDLT. The other patient suffered from FCH 2 months after LDLT, but recovered from cholestasis and was still alive after 28 months. Final liver biopsies of both patients showed fibrosis of stage 4. Another three patients also developed fibrosis of stage 4 during follow-up. One patient whose liver biopsy led to a diagnosis of recurring chronic hepatitis with F3 fibrosis 10 month after LDLT also suffered from stenosis of duct-to-duct biliary anastomosis. This patient underwent hepaticojejunostomy, but died of fungal pneumonia 1 month later. Liver histology at autopsy revealed F4 fibrosis. Another patient received re-LDLT for recurrent decompensated cirrhosis, as described above. The other patient who developed to biopsy-proven stage 4 fibrosis at 50 months was alive without decompensation as of 63 months after LDLT. In total, severe recurrence (progression of biopsy-proven cirrhosis and/or occurrence of clinical decompensastion) was diagnosed in five patients, and cumulative probability of severe recurrence was 8% at 2 years. Of the five patients presenting with severe recurrence, three were female and all had received the liver graft from a male donor.

TABLE 2. Risk factors associated with fibrosis of stage 2 or higher

Factors	n	Recurrence rate (number of patients at risk) 1 year	2 years	3 years	*P* value
Total[a]	62	19% (50)	39% (14)	58% (5)	
Recipient sex					0.006
Male	40	10% (36)	27% (10)	48% (4)	
Female	22	36% (14)	60% (4)	70% (1)	
Donor sex					0.047
Male	36	25% (28)	49% (6)	59% (1)	
Female	26	12% (22)	26% (8)	47% (4)	

[a] The 19 patients who died within 1 year without identified fibrosis and the 10 patients alive without biopsy for >1 year were excluded.

DISCUSSION

In the present study, only one patient had died of recurrent HCV as of the time of writing, and the majority of posttransplant deaths were attributable to postoperative complications occurring within a few months after LDLT. Infectious complications such as sepsis, pneumonia and peritonitis represented the most common causes of early mortality, as was the case in HCV-negative recipients. One-year mortality rates were 23% and 25%, respectively. Currently, overall 5-year patient survival rate for HCV-positive patients appears similar to that for non-HCV patients in our adult LDLT series (69% vs. 71%; Fig. 1). Of the 91 patients, HCC was present in 57 (63%), including 25 patients who exceeded the Milan criteria. Four patients died of recurrent HCC after LDLT, and the survival rate tended to be lower for patients with HCC than for patients without HCC (82% vs. 74% at 1 year, and 82% vs. 60% at 5 years; Fig. 2). Only one patient in this cohort had to undergo re-transplantation, and 5-year graft survival rates were 68% for all patients and 82% for patients without HCC. These results are comparable to the reported DDLT outcomes in the UNOS database: patient and graft survival rates of HCV-positive patients (n=3955) at 2 years were 81% and 75%, respectively (*13*); and rates for HCV-positive but HCC-negative patients (n=5640) at 5 years were 74.6% and 69.9%, respectively (*7*).

Progression of fibrosis due to recurrent chronic hepatitis is key to determining graft prognosis after liver transplantation for HCV-positive recipients. In the present study, progression of fibrosis in the liver biopsy was assessed and fibrosis to stage 2 or more was defined as significant fibrosis. The probability of progression to significant fibrosis was 39% at 2 years after LDLT. Several risk factors associated with posttransplant recurrence of hepatitis C have been identified (*5, 25–27*). These include pretransplant viral load, genotype 1b, donor age and graft steatosis, recipient age, race, gender, coexistence of HCC, and rejection treatment using bolus steroid or antilymphocyte preparations. Among the 18 potential variables examined in our study, univariate analysis identified female recipient and male donor as closely related to significant fibrosis. However, multivariate analysis showed that neither variable represented a significant independent risk factors. Actually, some correlation among these two variables was noted. Of the 30 female recipients, 24 had received a liver graft from a male donor (son or husband). An association between female gender of the recipient and severity of recurrent HCV has been demonstrated in previous studies (*6, 7*). However, no previous reports have implicated gender of the donor as an involved factor. Although difficulty exists in determining which is the predominant factor, the combination of male donor and female recipient may exert a negative impact on HCV recurrence.

Rapid proliferation of hepatocytes during postoperative graft regeneration may contribute to a higher rates of both HCV replication and severe recurrence in LDLT (*11*). This seems to imply a higher risk of recurrence in cases involving smaller grafts, which are supposed to undergo regeneration at a higher rate. Our study, however, showed that progression of significant fibrosis was similar for patients who received grafts with GRWRs of <1.0% or ≥1.0%. This result is supported by a recent report (*28*) showing that liver

regeneration following partial liver transplant does not increase the risk of HCV recurrence. Likewise, neither the relationship between donor and recipient nor degree of HLA matching seemed to influence recurrence. The results of our study thus do not support the hypothesis that these factors may exert negative effects on HCV recurrence in LDLT patients.

Due to the small number of patients treated using DDLT in Japan, HCV recurrence rates could not be compared between DDLT and LDLT. In previous studies on HCV recurrence after DDLT (*1, 13, 25, 29, 30*), histologically diagnosed recurrence of chronic HCV occurred in 65–90% of HCV-positive DDLT recipients during the first 2 years. However, a lack of uniform definitions for recurrent HCV, even when histological liver biopsy findings are used as criteria, has been indicated as one reason for the difficulties in comparing studies on HCV recurrence (*31*). Recently, a report from Spain demonstrated that severe recurrence of hepatitis C, defined as the development of cirrhosis or clinically decompensated liver disease, is more frequent in LDLT recipients (*11*). According to this report, the 2-year probability of developing severe recurrence was 45% after LDLT, compared to 22% after DDLT ($P=0.019$). When the same definitions were applied, rate of severe recurrence was only 8% at 2 years in our study. Arguably as many as 19 patients (21%) died within 1 year before developing HCV recurrence in our series. However, considering that the probability of either death or severe recurrence was 29% at 2 years, the results for our LDLT series were not likely to be greatly inferior to other reported cases.

In conclusion, postoperative patient survival was similar for HCV-positive and -negative recipients in our adult LDLT series. Rate of recurrence for chronic HCV and prevalence of progression to severe disease for our LDLT recipients appeared comparable to those for DDLT reported in the literature. Although these results need to be confirmed with a longer follow-up period, the present findings suggest that LDLT can produce acceptable outcomes for patients suffering from end-stage liver disease due to chronic HCV.

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