LDLT in 10 of 11 PFIC1 recipients (91%), whereas the PFIC2 recipients showed no digestive symptoms. PFIC1 associated with severe liver steatosis and/or steatohepatitis may lead to cirrhosis over time, and to indications for retransplantation (1). However, liver steatosis and diarrhea may occur even after retransplantation (1). In LDLT, donor selection is very limited ethically, socially and medically. In Japan in particular, expansion of deceased-donor LT is not yet in sight, although the governmental legislation for organ transplantation was revised in 2009. There are no affirmations for repeated retransplantation that may collateralize the long-term outcomes of pediatric PFIC1 recipients. The outcomes of PFIC1 recipients after LDLT are still not sufficient, based on our data and a previous report (22). Our actual results for the early post-operative occurrences of steatosis and fibrosis oblige us to reconsider the timing of LDLT and to challenge some other therapies for PFIC1 patients. Previously, partial external biliary diversion (PEBD) has been documented as a surgical procedure for PFIC patients (23, 24). Some patients with PFIC1 or PFIC2 may also benefit from surgical biliary diversion (25–27). The criteria for identifying those PFIC1/2 patients who could benefit from UDCA or biliary diversion remain unclear (14). We clearly understand that LT represents the only alternative if these therapies fail (28). Although we do not have sufficient experience of PEBD for PFIC1, we now take this anticipatory surgery before LDLT into consideration, if the overall considerations including donor limitations and the patient status indicate the permissive possibility. We understand that PFIC1 patients who eventually have refractory cirrhosis will finally require LT, and we do not consider that PFIC1 contraindicates LDLT because not all of our PFIC1 recipients necessarily suffered graft losses after LDLT. Based on our results, LDLT accompanied by TEBD appears to be rather better than PEBD from the viewpoints of the etiology in PFIC1 and graft protection after LDLT.

Although PFIC1 and PFIC2 share similar laboratory findings, the initial evolution of cholestasis in PFIC2 patients is more severe than in the other PFIC types. Hepatocellular carcinoma may complicate the course. As patients with BSEP deficiency accompanied by biallelic truncating mutations have a considerable risk for hepatobiliary malignancy (15% of patients develop hepatocellular carcinoma or cholangiocarcinoma) (29, 30), close monitoring of malignancy in PFIC2 patients is justified. The histopathological findings reveal more perturbed liver architecture than PFIC1, with more pronounced lobular and portal fibrosis and inflammation. Hepatocellular necrosis and giant cell transformation are also much more pronounced in PFIC2 than in PFIC1. These differences between PFIC1 and PFIC2 probably reflect the severe lobular injury in PFIC2 (4, 9, 10). PFIC2 is caused by mutations in the ABCB11 gene (designated BSEP) (8). The BSEP gene encodes the ATP-dependent canalicular BSEP of the liver and is located on human chromosome 2. The mechanism is shown in Fig. 2C.

PFIC3 can be distinguished from the other types of PFIC because it rarely presents with cholestatic jaundice in the neonatal period, and instead occurs later in infancy and childhood and even in young adulthood. The evolution of the cholestasis is characterized as chronic icteric or anicteric. However, adolescent and young adult patients have cirrhotic symptoms owing to portal hypertension that may result in liver failure. PFIC3 is caused by genetic mutations in the ABCB4 gene (designated MDR3) located on chromosome 7. MDR3 is a phospholipid translocase involved in biliary phospholipid (phosphatidylcholine) excretion and is predominantly expressed in the canalicular membrane of hepatocytes (31). Cholestasis results from toxicity of the bile, in which detergent BSs are not inactivated by phospholipids, thus leading to bile canaliculi and biliary epithelium injuries. A schematic mechanism for PFIC3 is proposed in Fig. 2D.

The mechanism of PFIC1 is still unclear. To our knowledge, the downregulation of cystic fibrosis transmembrane conductance regulator in cholangiocytes has been reported in PFIC1, and this downregulation could contribute to the impairment of bile secretion and explain some of the extrahepatic features (32). The FIC1 gene is expressed in various organs, including the liver, pancreas, small intestine, and kidney, but is more highly expressed in the small intestine than in the liver (33). Therefore, enterohepatic cycling of BS should be considered in PFIC1, and this is a possible explanation of our experiences that PFIC1 recipients easily showed steatosis/steatohepatitis even after LDLT and that PFIC2 recipients showed no steatosis/steatohepatitis after LDLT.

Only one mutated allele or no mutation is rarely identified in a few PFIC patients (< 10%) (1). Mutations that may map to regulatory sequences of the genes are a possible explanation for these findings. A gene involved in the transcription of the PFIC genes (i.e., FXR) or in protein trafficking could also be involved (34, 35). It cannot be negated that other unidentified genes involved in bile formation may be responsible for the PFIC1/2/3 phenotypes. Furthermore, it can be

hypothesized that combined heterozygous mutations for MDR3 and BSEP lead to PFIC-like phenotypes (36). Another possible explanation is that the mutated protein may have a dominant-negative effect on the expression and/or function of the protein in a heterozygous state (37). Modifier genes and environmental influences could play roles in the expression of PFIC (3). The possibility of recurrence of PFIC after LT owing to alloimmunization of the recipient against the FIC1, BSEP or MDR3 proteins of the liver donor remains a theoretical matter of debate (1). It is hypothesized that PFIC patients with a severe mutation leading to the absence of the gene product would be immunologically naive for the FIC1, BSEP or MDR3 gene products. Moreover, alloimmunization necessarily occurs after LT. Although evidence regarding this possible hypothesis after LT has not been reported (28, 38), a case of a PFIC2 patient who experienced an unexplained severe bout of pure hepatocellular cholestasis resembling PFIC2 after deceased-donor LT has been reported (1). In the case of LDLT based on donor relationships with parents, it can be expected that the heterozygous status of the liver allograft leads to a predisposition for developing lithiasis or cholestasis favored by immunosuppressive drugs (39) that may interfere with canalicular protein function, as reported in a PFIC2 patient (1). We consider that this possibility is very rare as there is only one previous report (1), and we performed LDLT in which the donor origins were parents in 10 of 11 recipients (91%) without PFIC recurrences and a previous series of LDLT for PFIC is already documented (38). Our data and a review of the mechanisms by which previous papers have demonstrated that PFIC2 is indicated for LT, including LDLT, as a definitive therapy, similar to other diseases indicated for LT, and also that the clinical courses and outcomes after LDLT are still not sufficient for PFIC1 recipients owing to early post-operative steatosis/fibrosis. Although PFIC1 patients will require LT during the long-term progression of the disease, we suggest that LDLT for PFIC1 may be reconsidered especially with regard to the timing of LDLT under the current donor shortage. Moreover, the establishment of alternative and/or anticipatory strategies for LDLT induction is needed to improve the long-term prognosis of PFIC1.

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Authors' contributions

T. Hori wrote the paper and performed this research. M. Ueda, F. Oike, Y. Ogura, S. Sakamoto, M. Kasahara, K. Ogawa, Y. Yonekawa, K.I. Watanabe and H. Doi provided important opinions based on their specialized experiences. A. Miyagawa-Hayashino performed histopathological examinations. F. Chen, A.M.T. Baine, and L.B. Gardner helped to perform this research. Prof. S. Uemoto, Prof. Y. Takada, Prof. H. Egawa and Prof. T. Yorifuji designed this research. Prof. S. Uemoto and Prof. J.H. Nguyen supervised this research.

Ethical approval

The protocol of this study was approved by the Ethics Review Committee for Clinical Studies of Kyoto University Graduate School of Medicine.

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Living-donor liver transplantation for carbamoyl phosphate synthetase 1 deficiency

Kasahara M, Sakamoto S, Shigeta T, Fukuda A, Kosaki R, Nakazawa A, Uemoto S, Noda M, Naiki Y, Horikawa R. Living-donor liver transplantation for carbamoyl phosphate synthetase 1 deficiency. Pediatr Transplantation 2010: 14: 1036–1040. © 2010 John Wiley & Sons A/S.

Abstract: CPS1 is a mitochondrial matrix enzyme that catalyzes the first committed step of the urea cycle, the primary system for removing nitrogen produced by protein metabolism using *N*-acetylglutamate. Patients with CPS1 deficiency have severe hyperammonemia that results in serious neurologic sequelae and sometimes death. LT has been indicated for neonatal-onset CPS1 deficiency. This study retrospectively reviewed five children with a diagnosis of CPS1 deficiency who underwent LDLT from heterozygous donors. Between November 2005 and May 2010, 124 children underwent LDLT with an overall patient and graft survival of 91.0%. Five patients were indicated for LDLT because of CPS1 deficiency. All recipients achieved resolution of their metabolic derangement, without donor complication, with a normal feeding regimen without medication for their original metabolic liver disease. LDLT, even from heterozygous donors, appears to be a feasible option, associated with a better quality of life for treating patients with CPS1 deficiency. Long-term observation may therefore be necessary to collect sufficient data to confirm the efficacy of this treatment modality.

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Key words: living donor liver transplantation – carbamoyl phosphate synthetase 1 deficiency – metabolic liver disease – liver transplantation

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CPS1 is a mitochondrial matrix enzyme that catalyzes the first committed step of the urea cycle, the primary system for removing nitrogen produced by protein metabolism using *N*-acetylglutamate. CPS1 deficiency is a rare autosomal recessive genetic disorder, which affects approximately one in 800 000 live births in Japan, and is characterized by episodes of life-threatening hyperammonemia in the neonatal period (1). Infants with a CPS1 deficiency usually appear normal at birth, but deteriorate within the first 48–78 h of life as ammonia accumulates in the body corresponding to the infant's increase in milk intake (2).

Abbreviations: CPS1, carbamoyl phosphate synthetase 1; DQ, developmental quotient; LDLT, living-donor liver transplantation; LT, liver transplantation; HDF, hemodiafiltration; HRLLS, hyper-reduced left lateral segment; LLS, left lateral segment.

The initial medical treatment of CPS1 deficiency-based hyperammonemia consists of protein restriction, and arginine, sodium benzoate, sodium phenylacetate/phenylbutyrate, and carnitine supplementation (3). These treatments, however, are not always sufficient for avoiding the accumulation of ammonia and recurrent hyperammonemia, which results in serious neurologic sequelae and can even lead to death (4). The prognosis of patients with neonatal-onset CPS1 deficiency is generally poor, with 66.7–76.9% of the patients reported to die from irreversible brain edema despite intensive medical treatment (1, 5).

LT may offer a complete cure for genetically acquired errors in the liver metabolism (6). Recent case studies have reported the benefits of LT in CPS1 deficiency, demonstrating that correcting hepatic enzyme deficiency by LT leads to clinical improvements, including a normal

feeding regimen, better quality of life, and fewer developmental delays, without the risk of recurrent hyperammonemia. The present report describes our recent experience with LDLT in five CPS1 deficiency patients.

Patients and methods

Between November 2005 and May 2010, 124 children underwent LDLT in our center, with an overall patient and graft survival of 91.0%. The diagnosis of CPS1 deficiency was made by amino acid analysis and/or mutation analysis of the peripheral blood lymphocytes in all five cases. Medical records were reviewed for the following: personal and family history, physical findings, laboratory data, histologic reports, surgical records, and special findings obtained by cardiologists, nephrologists, gastroenterologists, and radiologists. Developmental delay was measured using the DQ, which is normally used to express aspects of a child's development in a manner similar to the intelligent quotient (7). The DQ scores could be divided into the four degrees, given that normal development (≥70), mild delay (69–60), moderate delay (59–50), severe delay (≥50), according to the scale.

Five heterozygous donor candidates were evaluated by standard liver function tests, blood group combination, anatomic variation, and graft size matching, which showed normal liver function and serum ammonia/amino acid levels.

All patients underwent LDLT using a standard procedure. Veno-venous bypass was not used, because total clamping of the inferior vena cava could be avoided in all cases. Tacrolimus and low-dose steroids were used for initial immunosuppression. Tacrolimus administration was started on the day after transplantation. The target whole blood trough level of tacrolimus was 10–12 ng/mL for the first two wk, approximately 10 ng/mL for the following two wk and 8–10 ng/mL thereafter. Treatment with steroids was initiated at the time of graft reperfusion at a dose of 10 mg/kg, tapered from 1.0 to 0.3 mg/kg/day during the first month, and was withdrawn within the first three months. This study was approved by the institutional review board, and informed consent was obtained from all the cases.

Results

Table 1 shows a profile of the recipients involved in this study. The patients presented with irritability, a diminished oral intake, vomiting, and then somnolence/lethargy within the first 24–72 h of life. The diagnosis of CPS1 deficiency was initially made by amino acid analysis at initial serum and urine screening, because of elevated serum glutamine (909.3–3724.6 μM)/ornithine (115.8–664.5 μM), and decreased serum citrulline/urinary orotic acid (Table 2). The peak serum ammonia level in the patients ranged from 300 to 1445 μg/dL. The emergency pharmacologic management protocol for the patients in our center consisted of reversal of the catabolic state through caloric supplementation and pharmacologic scavenging of excess nitrogen. Treatment of the patients was pursued as quickly as possible (2). Three patients (cases #1, 2, 5) whose serum ammonia was > 500 μg/dL and/or whose ammonia level did not decrease after a high-calorie infusion and pharmaceutical treatment, received 1–3 courses of continuous veno-venous HDF. Case #3, who had a family history with a female child who died from recurrent hyperammonemia at four months of age, and who were

Table 1. Living donor LT for CPS1 deficiency

Case	Age	Sex	BW (kg)	Onset (days)	Peak NH3 (μg/dL)	Mutations	Hyperammonemic episode (HDF)	Neurological impairment	Donor	Graft type	DQ	Follow-up (yr)	Outcome
1	4 months	F	6.1	3	1412	fs514x/fs514s	5 (3)	Yes	Mother	HRLLS	56	2	Alive
2	6 months	F	6.9	2	1445	fs514x/R850H	3 (2)	Yes	Father	LLS	50	2.5	Alive
3*	8 months	F	8.0	1	300	fs514x/R233H	2 (0)	No	Mother	LLS	76	1.8	Alive
4	10 months	F	8.2	3	605	p668s/–	4 (0)	Yes	Mother	LLS	88	1	Alive
5	2 yr 7 months	F	14.0	2	1370	fs836x/R587H	2 (1)	Yes	Mother	LLS	50	4	Alive

*Prenatal diagnosis.

Table 2. Serum amino acid levels before and after LT

		Case 1		Case 2		Case 3		Case 4		Case 5	
	Reference range (μM)	Before	After	Before	After	Before	After	Before	After	Before	After
Glutamine	422.1–703.8	3274.6	697.6	992.8	773.1	1022.9	779.8	909.3	602.4	1526.0	523.6
Glycine	151.0–351.0	866.4	111.8	197.6	266.1	149.5	253.2	187.4	384.7	217.5	227.8
Alanine	208.7–522.7	2132.7	277.7	218.3	519.4	317.0	366.3	442.4	468.3	1396.7	366.5
Citrulline	17.1–42.6	Trace	Trace	Trace	Trace	9.2	4.2	7.9	8.1	Trace	Trace
Ornithine	31.3–104.7	155.8	17.7	664.5	82.9	147.0	55.2	115.8	41.1	138.9	61.5
Arginine	53.6–133.6	29.9	35.6	110.3	27.7	51.6	36.3	38.3	39.0	81.6	45.8

identified prenatally as being at risk for a CPS1 deficiency, chose to have their infant treated according to a diagnostic and therapeutic protocol (2).

Even after successful treatment for the first hyperammonemic coma, all patients again accumulated ammonia and experienced several episodes of hyperammonemia (mean: 3.2 ± 1.2 times), which necessitated intensive care treatment. Maintenance therapy for these five patients consisted of protein restriction (1.23 ± 0.20 g/kg/day natural protein), and supplementation of arginine (302.0 ± 148.1 mg/kg/day), sodium benzoate (222.0 ± 99.6 mg/kg/day), sodium phenylbutyrate (386.7 ± 46.2 mg/kg/day), and carnitine (45.0 ± 32.8 mg/kg/day). Four patients (except for case #3, who was diagnosed prenatally) showed low mental development. Phenobarbital as an anti-epileptic was administered to cases #1, 2, and 5. All of the patients had nasogastric tubes because of significant feeding disturbance. The main indication for LDLT was poor metabolic control in all the patients.

LDLTs were performed at four, six, eight, and 11 months, and two yr seven months of age, respectively. Because of the small body weight of the recipients, one HRLLS and four LLSs were used as liver grafts (8) (Table 1). The mean graft-to-recipient weight ratio was 3.05 ± 0.29%. The duration and blood loss of the recipient surgery ranged from 431 to 623 min and 236 to 712 g. Cold and warm ischemic times ranged from 26 to 91 min and 26 to 58 min, respectively.

All donors were uneventfully discharged from the hospital within seven 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.

The histopathologic examination of the explanted liver revealed microvesicular steatosis in all cases. All of the donors were discharged from the hospital within eight days of the operation and are currently doing well without any complications. The post-LDLT course was uneventful in cases #3 and #4. Case #2 showed biliary stricture, which was successfully managed with radiologic intervention. Cases #1 and #5 showed histologically proven acute cellular rejection episodes and were managed with steroid bolus injection. All children are currently doing well with a normal graft function at a follow-up of 1–4 yr after LDLT. Although the post-transplant DQ levels at the last time of follow-up, which were 56, 50, 74, 88, and 50, respectively, were not sufficient in our cases, there has been a marked improvement in the patients' quality of life after the successful LT. All patients achieved resolution of their metabolic derangement and were freed from the nasogastric tube and are now on a normal feeding regimen without any medication for the original metabolic liver disease.

Discussion

The aim of this study was to evaluate the outcome in patients who underwent LT for neonatal onset of CPS1 deficiency. As the liver is the only organ in which ammonia is significantly transformed to urea through the Krebs urea cycle, LT has been considered as a radical alternative therapy. It is not known how many liver transplants have been carried out for CPS1 deficiency, as The Urea Cycle Disorders Consortium is currently collecting data on CPS1 deficiency patients who have undergone LT (9).

To the best of our knowledge, there have been seven detailed cases of LT for neonatal onset of CPS1 deficiency reported worldwide in the English literature, not including the present cases (Table 3; 4, 5, 10–13). The median age of LT in these prior studies was 10 months (range, 14 days to six yr). Six of the seven patients were alive with excellent graft function at the time of publication. One patient with secondary biliary cirrhosis because of biliary anastomotic stricture was listed for re-transplantation. Five of the seven patients had neurologic impairment even

Table 3. Worldwide experience in LT for neonatal onset CPS1 deficiency

Case	Age (yr)	Sex	Donor	Onset	Peak NH3 (μg/dL)	Neurological impairment	Follow-up	Outcome	Reference
1	14 days	M	Deceased	2 days	514	Yes	37 months	Alive	5
2	3.5 months	M	Deceased	27 h	1431	Yes	>30 months	Alive (listed for ReTx)*	4
3	5 months	M	Deceased	30 days	979	No	>30 months	Alive	4
4	10 months	F	Living	1 days	589	No	2 yr 4 months	Alive	10
5	1 yr 2 months	M	Deceased	30 days	629	Yes	10 months	Alive	11
6	1 yr 8 months	M	Deceased	2 days	1800	Yes	18 months	Died (Pneumonia)	12
7	6 yr	–	Deceased	–	–	Yes	2 yr	Alive	13

*Secondary biliary cirrhosis because of biliary anastomotic stricture.

after successful LT. It has been reported that the extent of neurologic impairment in urea cycle disorders is strongly related to the degree and duration of the serum ammonia elevation (13). As a result of radical treatment with pharmacologic scavenging of excess nitrogen in early infancy, the prognosis of CPS1 deficiency has improved dramatically; however, 72.7% of the patients in these prior studies had already showed developmental delay at the time of LT (10, 14). For our study, we introduced continuous veno-venous HDF for the patients with neonatal onset of hyperammonemia if their serum ammonia level was continuously over 500 μg/dL and/or their ammonia level did not decrease with high-calorie infusion and pharmaceutical treatment. Prompt reduction of the serum ammonia level might be the most important contributor to patient survival and quality of life in neonatal onset of CPS1 deficiency.

In this study, no negative impacts of the use of heterozygous carriers as donors on either donors' or recipients' postoperative course have been observed to date. With respect to the use of heterozygous donors in our review of the patients with CPS1 deficiency, there were no descriptions of mortality or morbidity related to the use of heterozygous donors. Nevertheless, the advisability of using heterozygous carriers as donors should be considered uncertain in some urea cycle deficiencies. 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.

Even after successful treatment of severe hyperammonemia with pharmaceutical treatment with/without HDF, however, most of the surviving patients still require a considerable treatment regimen and may have handicaps that include impairment of development because of recurrent episodes of hyperammonemia (15). It has been reported that patients with neonatal onset of urea cycle disorders showed remarkable gains in their development after successful LT (13). Given the risk of continued neurologic compromise, the potential for improvement of development represents a major benefit of performing early LT. As such, we recommend early LT for the patients with neonatal onset CPS1 deficiency because it appears that LT can reduce the magnitude of progressive neurologic disability as a result of poor metabolic control.

The shortage of full-size grafts from pediatric donors once produced high waiting-list mortality in the pediatric population and prompted the identification of alternative graft sources for pediatric patients (16). To increase the supply of appropriate-sized organs for pediatric recipients, the techniques of reduced, split, and LDLT grafting were developed (17). Implantation of LLS grafts, however, can be a problem in small infants such as those in our series, because of a large-for-size graft. Hyper-reduced left lateral segmental LT has been recently introduced for small infants to mitigate the problem of large-for-size graft (8). The use of a hyper-reduced left lateral segmental graft was indicated if the graft-to-recipient weight ratio was estimated to be over 4.0% in preoperative CT volumetry. This procedure produced satisfactory results in our four-month-old patient. Tailoring the graft size according to infant size is a safe and useful alternative to pediatric LT. Although hepatocyte transplantation and gene therapy are promising new approaches for the treatment of neonatal-onset metabolic liver diseases, only limited success has been reported to date (18). Therefore, until the aforementioned technologies can be developed for wider application, LT is currently the only definitive therapy for these patients, which has been associated with significant improvement in patient outcomes. In the neonates with hyperammonemia from CPS1 deficiency, early aggressive pharmaceutical treatment with supportive HDF can minimize progressive neurologic disability and infant mortality, so that early LT can be performed with excellent patient and graft survival. Long-term observations may, however, be necessary to obtain sufficient data and establish a clear protocol for this treatment modality.

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■ 原 著

国立成育医療研究センターにおける小児生体肝移植の実態（第1報）
—小児肝移植のデータベース構築に向けて—

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Pediatric living donor liver transplantation: analysis of a single center experience of 100 Transplants

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【Summary】

【Background】 In Japan, about 130 pediatric liver transplants are performed every year. However, detailed nationwide data about risk factors and complications of pediatric liver transplantation have not been fully analyzed. The aim of this study was to present an overview of our experience and to clarify important parameters necessary for nationwide statistics of pediatric liver transplant in the future.

【Methods】 From November 2005 to August 2009, 100 living donor liver transplantations (LDLT) for children aged <18 years were performed in our institution. The recipients consisted of 46 boys and 54 girls aged between 1 month and 18 years (median age, 14 months). The analyzed parameters include primary disease (indication), preoperative status, model for end-stage liver disease (MELD)/pediatric end-stage liver disease (PELD) score, rejection, post-transplant complications, patient survival, duration of hospitalization, and cause of death. We also compared our data with nationwide statistics from 2006 to 2008.

【Results】 As for indications, cholestatic disease was significantly less frequent than nationwide statistics and there was a higher incidence of metabolic diseases and fulminant liver failures. Moreover, infants aged<12 months accounted for 46% of recipients in our institution. The overall 1-year and 3-year survival rates were 90% and 87.1%, respectively, with a mean follow-up duration of 14±27 months (range, 1-56 months). The risk factors of long-term hospitalization ≥60 days were a MELD/PELD score ≥20 and acute cellular rejection. In fulminant hepatic failure, acute cellular rejection developed more frequently ($p=0.02$)

【Conclusion】 We summarized our experience of 100 pediatric LDLT. A MELD/PELD score of 20 or more and acute cellular rejection were risk factors for long-term hospitalization, although they were not related to patient survival in pediatric LDLT.

Keywords: living donor liver transplantation (LDLT), pediatric patients

I. はじめに

生体肝移植が日本で最初に施行されたのは1989年11月であり[1]，それから2008年までの20年余りの間に小児の生体肝移植は約2,000例が施行されてきた[2-5]。日本肝移植研究会・肝移植症例登録報告によると，小児（18歳未満）の生体肝移植は，全国で年間120～140例前後が施行されている[2-5]。報告されている小児レシピエントの年齢は，原疾患・移植肝・累積生存率では「18歳未満」，性別・累積生存率では「0～9歳

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（2010・12・24受領；2011・3・10受理）

/10～19歳」，ABO血液型不適合群の累積生存率では「0～2歳/3～17歳」などと大まかに分類されている。小児の場合，対象疾患や予後などについて大人とは異なる特徴をもつと考えられるが，小児例について詳細に分類した全国データは存在しないのが現状である。

これまで，国立成育医療研究センター（以下，当センター）で施行された小児の生体肝移植症例について2008年[6]，2010年[7]に報告してきたが，今回は，より詳細にデータを解析し，日本肝移植研究会・肝移植症例登録報告（以下，全国統計）と比較しつつ，小児肝移植データベースの構築に向けた研究を行った。

II. 対象と方法

1. 対　象

2005年11月から2009年9月まで間に，当センターにおいて生体肝移植を施行されたレシピエント104例のうち，生体肝腎同時移植1例，および18歳以上の成人3例を除く，18歳未満の小児肝移植100例を対象とした。レシピエントは男児46例，女児54例で，年齢は生後1カ月～16歳，体重は2.8-63.8 kg，身長は50.0-174.0 cmであり，全症例とも初回移植である。また，使用するデータは2010年7月時点のものとし，1例のみ入院中であった。

2. 調査項目

1) レシピエント情報

(1) 移植前：生年月日，手術年月日，死亡年月日，年齢（満・月），性別，原疾患分類，原疾患，体重，身長，血液型，血液型適合度，術前状態（自宅，入院，ICU），既往手術回数，MELD（model for end-stage liver disease)/PELD（pediatric end-stage liver disease）スコア[8]（20未満，20以上），グラフト肝重量など

(2) 移植後：CIT（cold ischemic time），WIT（warm ischemic time），出血，手術時間，術後在院日数（60日未満，60日以上/90日未満，90日以上），合併症，拒絶反応，拒絶時期，免疫抑制剤，感染症，AST（aspartate aminotransferase），ALT（alanine aminotransferase），γ-GTP（gamma-glutamyl transpeptidase），T-Bil（total bilirubin），PT（prothrombin time）など

2) ドナー情報

続柄，体重，身長，血液型，年齢，graft segment，出血，手術時間，術後在院日数，合併症，AST，ALT，γ-GTP，T-Bilなど

3. 解析方法

調査項目について，Microsoft Excel 2007を用いて単純集計，クロス集計および母比率の検定[9]を行い，JMP 7を用いてPearsonのχ^2検定，Fisherの正確検定（片側検定，両側検定），累積生存率の検討を行った。各検定における有意水準αは0.05（あるいは0.01）とした。

全国統計との比較には，日本肝移植研究会・肝移植症例登録報告より，「レシピエントの原疾患（生体肝移植，初回移植)」，「生体肝移植におけるレシピエントとドナーのABO血液型適合度」の表を用い，必要に応じて2006～2008年の平均値を算出した[2,3]。

リスク因子に関しては，移植例全体および原疾患分類別（胆汁うっ滞性疾患，代謝性疾患，劇症肝炎，その他）に，予後に関連すると考えられる項目を検討した。また，本研究では術後在院日数60日以上を長期入院とし，リスク因子を検討した。

III. 結　　果

1. レシピエントの成績

当センターのレシピエントの成績を**表1**に示した。術前の患者重症度の指標となるMELD/PELDスコアの中央値は19であった。手術時間の中央値は9時間18分で，出血量の中央値は77.1 ml/kgであった。

2. 移植例数

当センターにおいては平均2.3例/月のペースで生体肝移植が施行されており，2006年は18例，2007年は22例，2008年は33例で，年々増加傾向にあった。全国の小児肝移植例数に占める割合も，2006年は15.1%（18/119例），2007年は17.9%（22/123例），2008

表1　レシピエントの成績

項目	Median	Range
MELD/PELDスコア	19	0-52
グラフト肝重量（g）	242.5	72-750
手術時間（min）	558	356-1558
出血量（ml/kg）	77.1	2.2-896.7
CIT（min）	37	10-164
WIT（min）	33	21-81
術後在院日数	49	9-404

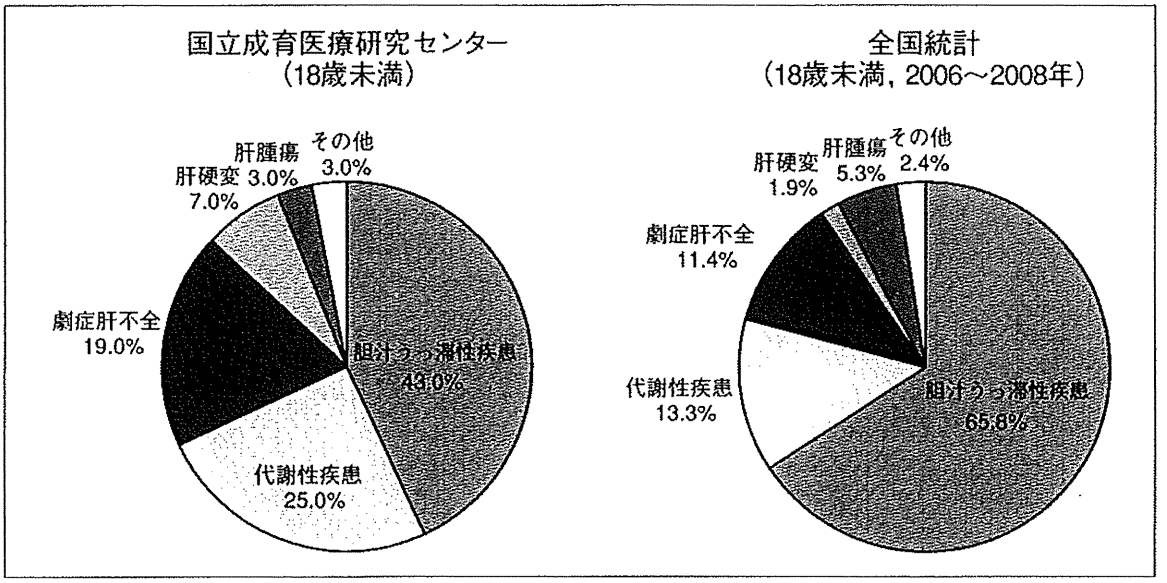

図1　レシピエントの原疾患分類

年は24.4%（33/135例）と徐々に増加してきている。

3. レシピエントの原疾患分類

当センターでは，胆汁うっ滞性疾患の割合が最も高く43.0%，次いで代謝性疾患が25.0%，劇症肝不全が19.0%であった（図1）。一方，全国統計（2006～2008年の平均値）[2-5]では，胆汁うっ滞性疾患の割合が最も高く65.8%，次いで代謝性疾患が，13.3%劇症肝不全が11.4%を占めている（図1）。頻度順は同じであるが，当センターでは全国統計と比較して，胆汁うっ滞性疾患の割合が低く，代謝性疾患や劇症肝不全の割合が高かった(有意水準αはそれぞれ0.01, 0.01, 0.05)。さらに，代謝性疾患の内訳を表2に示した。当センターの代謝性疾患に占める割合は，Wilson's diseaseが8.0%（2/25例），OTC deficiencyが16.0%（4/25例），glycogen storage diseaseが16.0%(4/25例)，methylmalonic acidemiaが40.0%（10/25例）であったが，全国統計[5]はそれぞれ，35.2%，16.4%，7.9%，11.5%であった。当センターは全国統計と比較して，methylmalonic acidemiaの割合が高く，Wilson's diseaseの割合が低いことが分かった（有意水準αはともに0.01)。

胆汁うっ滞性疾患のうち胆道閉鎖症の割合は，当センターでは90.7%（39/43例），全国統計では89.5%であり，劇症肝不全のうち原因不明の割合は，当センターでは89.5%（17/19例），全国統計では83.7%であり，いずれも有意な差は認められなかった。

表2　代謝性疾患の内訳

原疾患	症例数
Wilson's disease	2
OTC deficiency	4
Glycogen storage disease	4
Methylmalonic acidemia	10
Carbamyl phosphate synthase I deficiency	4
Propionic acidemia	1
合計	25

図2に全国の年間肝移植数（生体肝移植，初回移植）[2-5]に占める当センターの割合を示した。2006～2008年の平均値は，胆汁うっ滞性疾患が11.7%であるのに対し，代謝性疾患は38.0%，劇症肝不全は32.6%を占めていた。

4. レシピエントの移植時年齢

当センターのレシピエントの移植時年齢は，1歳未満が全体の46.0%を占めており，最頻値は8カ月，中央値は1歳2カ月であった（図3）。他の小児肝移植施設（2001年5月～2005年11月，75例）の報告[10]によると，対象者の年齢は7カ月～16歳，中央値が1歳6カ月であり，当センターの方が若年で移植を受け

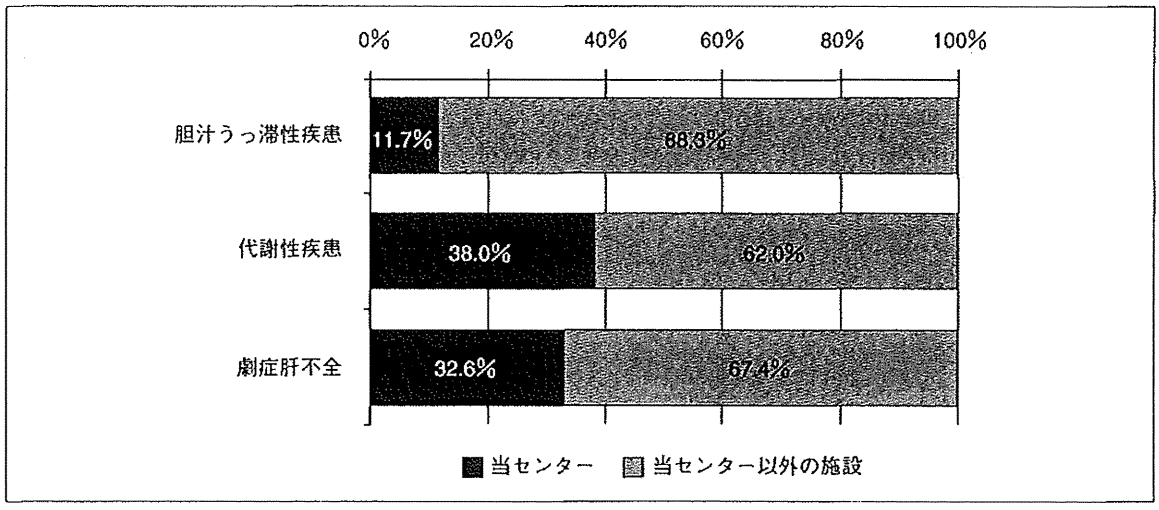

図2　全国に占める当センターの症例割合（2006～2008年）

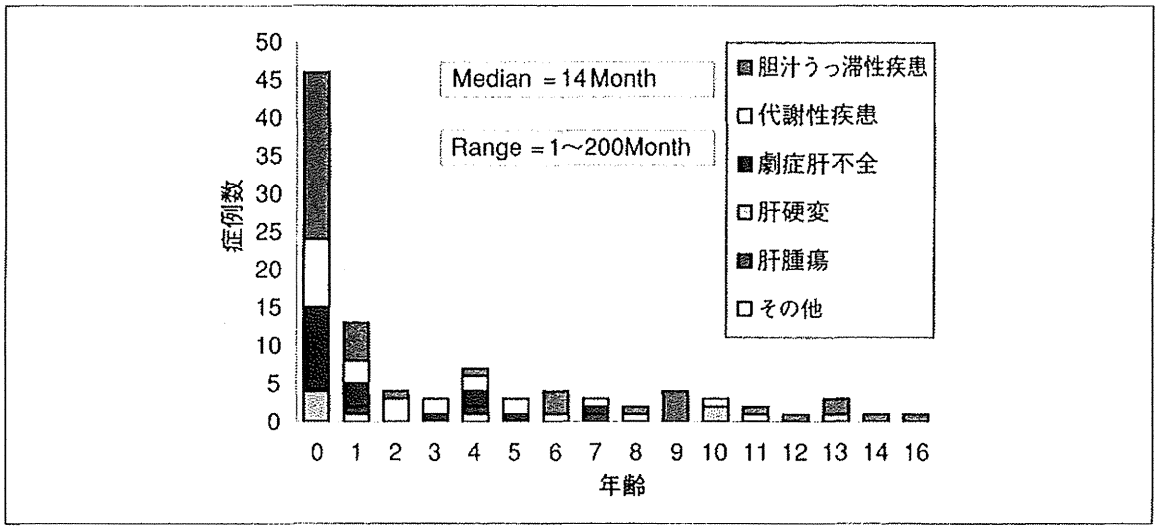

図3　レシピエントの移植時年齢（原疾患分類）

ている傾向にあった。

さらに，1歳未満の症例に限定して原疾患分類別にみると，胆汁うっ滞性疾患が47.8%（22例），劇症肝不全が23.9%（11例），代謝性疾患が19.6%（9例）を占めていた。1歳以上の症例と比較して，胆汁うっ滞性疾患が半数近くを占めていることは同様であるが，代謝性疾患と劇症肝不全の割合は逆転していた。特に劇症肝不全については，1歳未満が全体の57.9%（11/19例）を占めていることが分かった。この他，1歳以上では胆汁うっ滞性疾患が38.9%と少なく，代謝性疾患が29.6%と高かった（図4）が，有意な差はみられなかった（$p=0.29$）。また，1歳未満では，月齢8カ月に生体肝移植を施行されたレシピエントの割合が最も高く21.7%を占め，さらに，そのうち80.0%は胆汁うっ滞性疾患が占めていた。また，生後1カ月の移植例3例は，劇症肝不全のため緊急の肝移植を要した例であった。

5. ABO血液型適合度

レシピエントとドナーの間にABO血液型不適合が

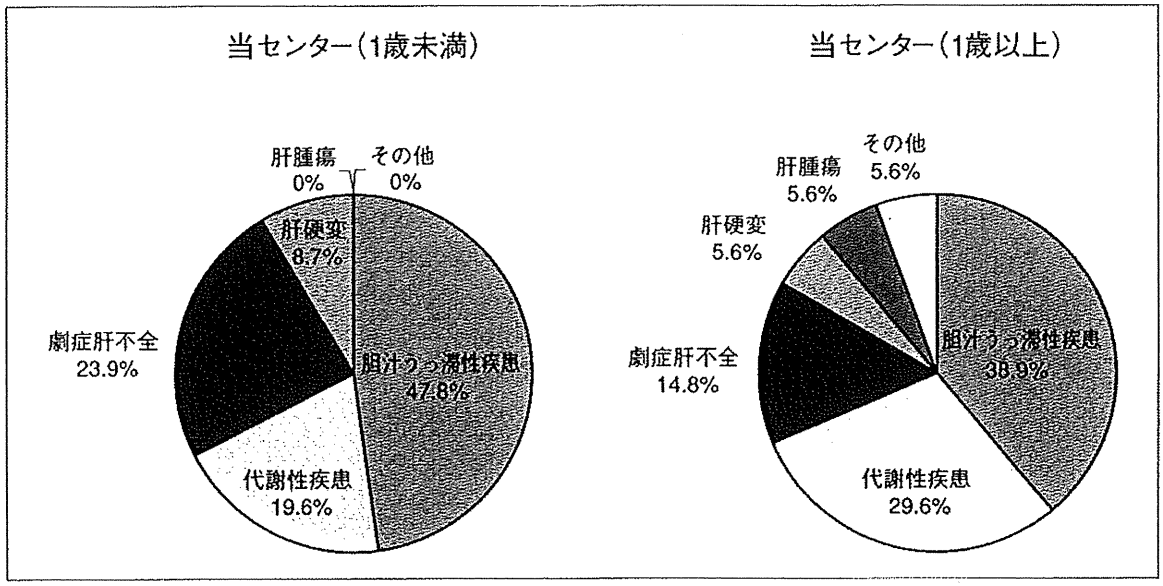

図4 レシピエントの原疾患分類（年齢別）

あった症例は，全体でみると14.0%であり，全国統計の12.5%[5]に近似していた。しかし，年齢を二分割して比較すると，不適合は1歳以上で5.6%，1歳未満で23.9%を占めており，1歳未満で有意に多いことが分かった（$p=0.01$）。

6. レシピエントの累積生存率

当センターのレシピエント全体の1年生存率は90.0%，3年生存率は87.1%であった（図5；平均追跡期間14±27カ月（1～56カ月））。全国統計の1年生存率は87.3%，3年生存率は85.7%であり[5]，当センターは全国統計よりやや高かった。

原疾患分類別に1年生存率，3年生存率をみると，胆汁うっ滞性疾患はともに88.4%，代謝性疾患はともに92.0%，劇症肝不全はともに89.5%であり，原疾患による生存率に有意差はみられなかった（図6，$p=0.62$）。全国統計（18歳以上も含む）の1年生存率，3年生存率は，胆汁うっ滞性疾患で87.0%，85.4%，代謝性疾患で89.2%，85.8%，劇症肝不全で75.0%，71.8%であり，有意差検定はできないが，代謝性疾患・劇症肝不全で当センターの成績が良好な傾向が認められた。

リスク因子について累積生存率を検討した。移植例全体でみると，年齢，性別，血液型適合度，術前状態，MELD/PELDスコア，合併症や拒絶反応，敗血症の有無はいずれも生存・死亡との関連はみられなかった（表3）。原疾患別に検討すると，胆汁うっ滞性疾患においてMELD/PELDスコアが有意に関連していた（図7）。1年生存率，3年生存率ともにMELD/PELDスコアが20未満の群で93.9%であるのに対し，20以上の群では70.0%であった（$p=0.03$）。

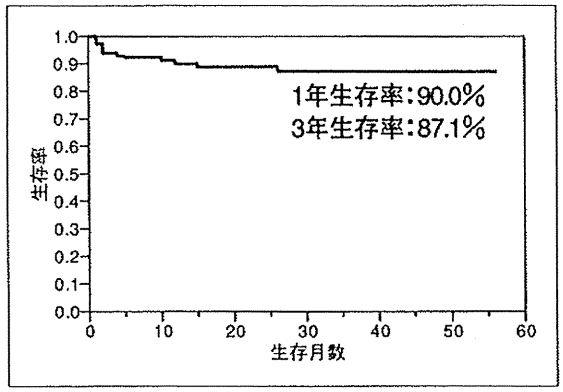

図5 累積生存率（移植例全体）

7. リスク因子の検討

移植例全体および原疾患分類別（胆汁うっ滞性疾患，代謝性疾患，劇症肝炎）に，生死，合併症・拒絶反応・敗血症の有無，術後在院日数（60日以上，90日以上）などの予後について，関連すると考えられる

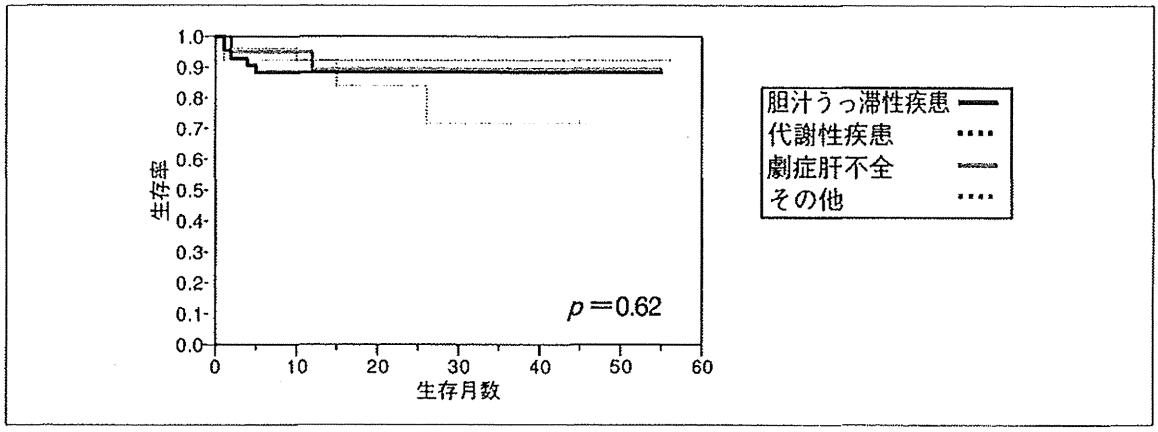

図6　累積生存率（原疾患分類）

表3　累積生存率の検討（*p* 値）

	全体 (n=100)	胆汁うっ滞性疾患 (n=43)	代謝性疾患 (n=24)	劇症肝不全 (n=19)
年齢（1歳未満，1歳以上）	0.70	0.68	0.70	0.78
性別（男児，女児）	0.78	0.63	0.20	0.46
血液型適合度（適合，不適合）	0.25	0.53	0.20	0.46
術前状態（自宅，入院，ICU）	0.66	0.10	0.41	—
MELD/PELD スコア（20未満，20以上）	0.10	*0.03*	—	—
合併症（有，無）	0.07	0.11	0.18	0.19
拒絶反応（有，無）	0.50	0.10	0.45	0.84
敗血症（有，無）	0.71	0.47	0.56	0.56

イタリック体：有意差あり

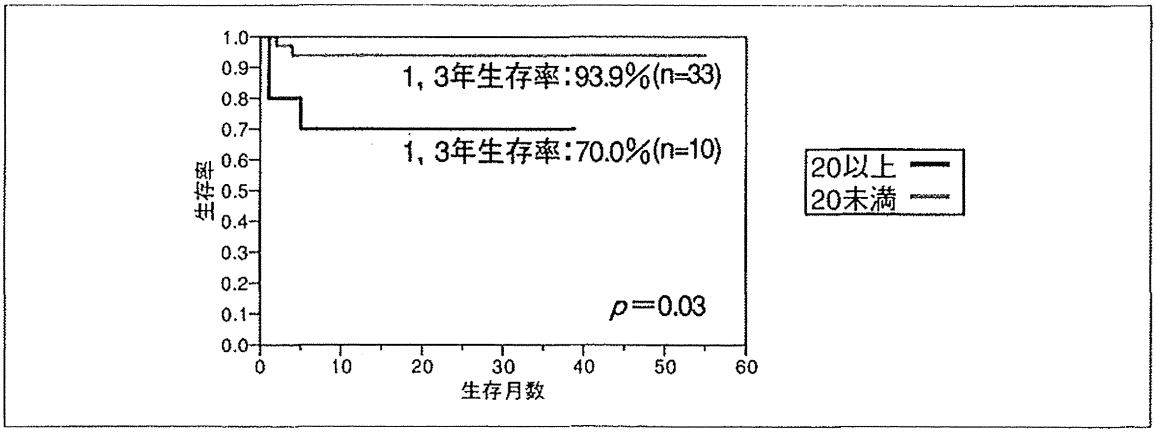

図7　MELD/PELD スコアによる累積生存率（胆汁うっ滞性疾患）

以下の因子を検討した。年齢，性別，血液型適合度，術前状態，MELD/PELD スコア合併症・拒絶反応・敗血症の有無について検討し，その結果を表4に示した。

特に，術後在院日数（60日以上）の長期入院40例について，いくつか有意な差がみられた。移植例全体

表4 リスク因子の検討（p 値）

移植例全体（n=100）	生死	合併症	拒絶反応	敗血症	術後在院日数(60)	術後在院日数(90)
年齢	0.79*	0.99*	0.22*	0.33*	0.45*	0.92*
性別	0.79*	0.51*	0.37*	0.78*	0.56*	0.59*
原疾患別分類	–	0.60*	0.29*	–	–	–
血液型適合度	0.63	0.93*	0.65*	1.00	0.17*	0.44
術前状態	–	0.50*	–	–	–	–
MELD/PELD スコア	0.14	0.54*	0.45*	0.07*	*0.02**	0.40*
合併症	*0.04***	–	0.81*	*0.02*	0.44*	*0.004*
拒絶反応	0.29	0.82*	–	0.50*	*0.02**	0.65*
敗血症	1.00	0.02	0.50*	–	0.25*	0.22

胆汁うっ滞（n=43）	生死	合併症	拒絶反応	敗血症	術後在院日数(60)	術後在院日数(90)
年齢	1.00	0.18	0.33*	1.00	0.42*	0.70
性別	0.63	0.72	0.72*	0.35	0.21*	0.66
血液型適合度	0.48	1.00	1.00	0.48	1.00	0.57
術前状態	–	–	–	–	–	–
MELD/PELD スコア	0.07	1.00	0.13	1.00	0.16	0.66
合併症	0.16	–	1.00	0.35	0.79*	0.08
拒絶反応	0.14	1.00	–	1.00	0.18*	1.00
敗血症	0.57	0.35	1.00	–	0.67	1.00

代謝性疾患（n=25）	生死	合併症	拒絶反応	敗血症	術後在院日数(60)	術後在院日数(90)
年齢	1.00	0.68	*0.03***	0.26	1.00	1.00
性別	1.00	0.41	0.66	*0.02*	0.68	0.11
血液型適合度	1.00	0.60	1.00	1.00	0.60	0.53
術前状態	–	–	–	–	–	–
合併症	1.00	–	1.00	0.51	1.00	0.10
拒絶反応	0.28	1.00	–	0.06	0.67	0.29
敗血症	1.00	0.51	0.06	–	0.57	0.52

劇症肝不全（n=19）	生死	合併症	拒絶反応	敗血症	術後在院日数(60)	術後在院日数(90)
年齢	1.00	1.00	0.66	1.00	0.64	0.59
性別	0.47	0.59	1.00	0.51	0.25	1.00
血液型適合度	0.47	0.61	1.00	1.00	0.61	0.53
術前状態	–	–	–	–	–	–
MELD/PELD スコア	–	–	–	–	–	–
合併症	0.48	–	0.66	0.51	0.32	0.59
拒絶反応	1.00	0.66	–	0.51	*0.04*	0.09
敗血症	1.00	0.51	0.51	–	1.00	1.00

イタリック体：有意差あり，*：Pearson の χ^2 検定，**：Fisher の正確検定（片側検定），*印なし：Fisher の正確検定（両側検定）
年齢：（1歳未満・1歳以上），性別：（男児，女児），血液型適合度：（適合，不適合），術前状態：（自宅，入院，ICU），MELD/PELD スコア：（20未満，20以上），合併症：（有，無），拒絶反応：（有，無），敗血症：（有，無），術後在院日数（60）：（60日未満，60日以上），術後在院日数（90）：（90日未満，90日以上）

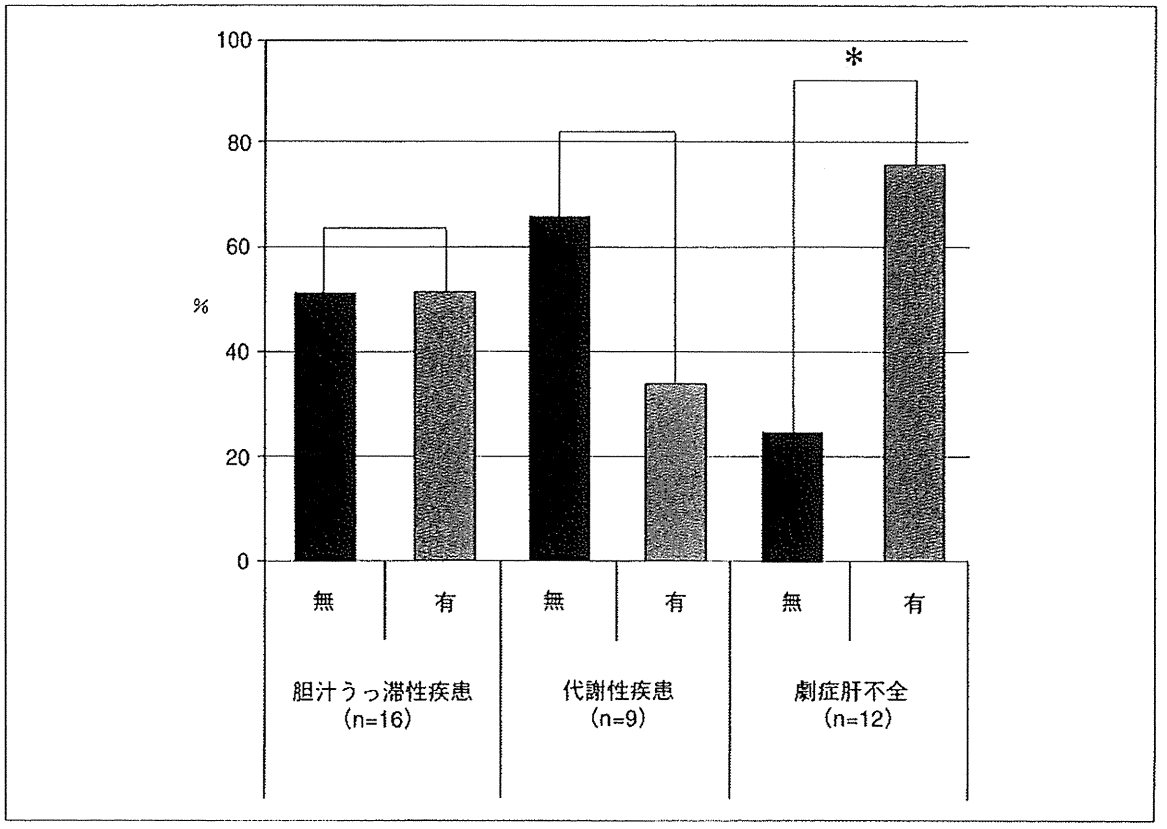

図 8　長期入院における拒絶反応の有無（原疾患分類別）

では，MELD/PELD スコアが 20 以上（21/34 例，$p=0.02$），拒絶反応あり（20/39 例，$p=0.02$）の症例が有意に多いことが分かった。原疾患分類別にみると，劇症肝不全において拒絶反応との関連がみられた。図 8 に，症例数の多い 3 疾患について拒絶反応の有無を示した。術後在院日数 60 日以上の例で，拒絶反応がみられた割合は，胆汁うっ滞性疾患 50.0%（8/16 例），代謝性疾患 33.3%（3/9 例），劇症肝不全 75.0%（9/12 例）であった。拒絶反応は，胆汁うっ滞性疾患や代謝性疾患では長期入院のリスク因子にならなかったが，劇症肝不全では有意に関係していることが分かった（$p=0.02$）。平均術後在院日数は，移植例全体で 59.1 日，胆汁うっ滞性疾患で 60.4 日，代謝性疾患で 56.9 日，劇症肝不全で 71.8 日であった。

IV. 考　　察

当センターの小児肝移植例の検討から，いくつかの知見が得られた。

18 歳未満のレシピエントにおいて，全国統計の原疾患分類では 65.8% を占める胆汁うっ滞性疾患が，当センターでは 43.0% と低かった。特に，1 歳以上 18 歳未満で 38.9% であったことが，当センターの胆汁うっ滞性疾患の割合を低くしたと考えられる。逆に，代謝性疾患および劇症肝不全の割合は，全国統計と比較して相対的に高かった。特に，1 歳未満では劇症肝不全が 23.9%，1 歳以上 18 歳未満では代謝性疾患が 29.6% と，それぞれ非常に高率であった。また，当センターは乳児例が 46.0% と約半数を占めていた。原疾患構成と併せて，当センターの特徴ではないかと推測される。

当センターのレシピエントの累積生存率は，1 年生存率が 90.0%，3 年生存率が 87.1% で，全国統計よりやや高かった。観察期間は短いが，一般に移植適応が困難であるとされている代謝性疾患や劇症肝不全が多いという当センターの原疾患構成を考慮すると，良好な成績を保持しているのではないかと考えられる。

さらに，長期入院のリスク因子を検討したところ，移植例全体では，MELD/PELD スコア（20 以上），拒

絶反応（あり）との関連が認められ，原疾患分類別にみると，劇症肝不全において拒絶反応との関連がみられた。劇症肝不全は他の疾患と比較して，平均術後在院日数が71.8日と最も長く，拒絶反応が原因で入院期間が延びているのではないかと推察される。劇症肝不全では，難治性拒絶反応が多いため[6]，入院期間短縮のためには早期拒絶反応の制御が重要であると考えられる。一方，代謝性肝疾患は肝移植後にも，栄養管理・投薬管理が必要な疾患が多く，これが入院期間の延長につながっている可能性がある[7]。

V. おわりに

近年，全国で施行される小児肝移植の約4分の1を当センターが占めているが，本研究でみられた乳児例，あるいは代謝性疾患や劇症肝不全のレシピエントが多い等の特徴は，当センターに独自のものである可能性があり，全国統計との比較は意義があったと考えられる。しかし，当センターの症例数では，集計にあたり項目数を増加させると，1項目あたりの症例数が少なくなり，正確な統計解析が不可能であった。より有用な結果を得るためには，ある程度症例数が必要であるため，単一センター報告には限界がある。

一方，当センターの集計結果と全国統計との比較にあたり，日本肝移植研究会の肝移植症例登録報告を引用したが，それだけでは比較可能な項目が少数であった。全国統計の集計に関しては，小児と大人の結果が一括して報告されているため，18歳未満のレシピエントをまとめて小児と定義していることはやむを得ない。しかしながら，本研究で，1歳未満と1歳以上では原疾患構成が異なるという結果も得られ，年齢の分類がより詳細であることが望まれる。

また，原疾患構成については，小児と大人では相違があり，大人ではあまりみられない小児特有の原疾患もある。全国統計[5]の累積人数では，代謝性疾患の「その他」の項目に分類されている小児の人数は，大人の5倍以上であり，小児の中でも他の原疾患の「その他」より2～7倍と多い。おそらく，代謝性疾患の「その他」にはcarbamyl phosphate synthase 1 deficiencyやASS欠損症，propionic acidemia等が含まれると考えられるが，現在，詳細は不明であり，このような原疾患項目の充実も望まれる。

さらに，小児は大人と異なり，肝移植による成長・発達への影響は大変重要な問題である。現在のところ，肝移植後のレシピエントの成長・発達については，いくつか報告がある[11,12]ものの，長期にわたる予後についてはほとんど知られていない。よって，心身の成長・発達に関する指標，例えば，身長，体重，座高，骨密度，甲状腺ホルモン，性ホルモン，IQ，DQ等が登録項目に追加され，データが集積されていくことを切望する。

以上のように，小児の肝移植全国登録に対して，登録項目の充実や詳細な集計結果の公表が期待される。本研究の報告によって，多くの先生方に現状を知っていただくとともに，さまざまなご意見を賜り，今後，全国的なデータベースのよりよい構築につなげていくことを目指したいと考えている。

なお，本研究の一部は第28回日本肝移植研究会および第46回日本移植学会で発表した。

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ORIGINAL ARTICLE

Successful Heterozygous Living Donor Liver Transplantation for an Oxysterol 7α-Hydroxylase Deficiency in a Japanese Patient

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Only 2 patients with an oxysterol 7α-hydroxylase deficiency caused by mutations of the cytochrome P450 7B1 (*CYP7B1*) gene have been reported; for both, the outcome was fatal. We describe the clinical and laboratory features, the hepatic and renal histological findings, and the results of bile acid and *CYP7B1* gene analyses for a third patient. This Japanese infant presented with progressive cholestatic liver disease and underwent successful heterozygous living donor liver transplantation. Sources of relevant data included medical records, hepatic and renal histopathological findings, gas chromatography/mass spectrometry analyses of bile acids in serum and urine samples, and analyses of the *CYP7B1* gene in the DNA of peripheral blood lymphocytes. Large excesses of 3β-hydroxy-5-cholen-24-oic acid were detected in the patient's serum and urine. Cirrhosis and polycystic changes in the kidneys were documented. The demonstration of compound heterozygous mutations (R112X/R417C) of the *CYP7B1* gene led to the diagnosis of an oxysterol 7α-hydroxylase deficiency. After liver transplantation with an allograft from a heterozygous living donor (the patient's mother), the features of decompensated hepatocellular failure abated, and the renal abnormalities were resolved. In conclusion, we report the first Japanese patient with an oxysterol 7α-hydroxylase deficiency associated with compound heterozygous mutations of the *CYP7B1* gene; in this patient, liver transplantation with an allograft from a parental donor was effective. *Liver Transpl 17: 1059-1065, 2011.* © 2011 AASLD.

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Nine inborn errors of bile acid synthesis are categorized as inherited metabolic liver diseases.[1] One of these inborn errors of bile acid synthesis, an oxysterol 7α-hydroxylase deficiency, was first described by Setchell et al.[2] and is due to autosomal recessive inheritance. The gene encoding oxysterol 7α-hydroxylase, cytochrome P450 7B1 (*CYP7B1*), is located on chromosome 8q21.3. This rare inborn error of bile acid synthesis responds poorly to bile acid therapy because the progression to cirrhosis is rapid and occurs at an early age. So far, only 2 patients with an oxysterol 7α-hydroxylase deficiency and an associated mutation of the *CYP7B1* gene have been reported; both died in infancy either before or after liver transplantation (LT).[2,3]

We report the first successful use of LT in the management of an oxysterol 7α-hydroxylase deficiency associated with a mutation of the *CYP7B1* gene; we

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; CYP7A1, cytochrome P450 7A1; CYP7B1, cytochrome P450 7B1; FXR, farnesoid X receptor; LDLT, living donor liver transplantation; LT, liver transplantation; ND, not detected; PCR, polymerase chain reaction; TBA, total bile acid; UDCA, ursodeoxycholic acid.

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used an allograft from a heterozygous living donor. We describe the clinical and laboratory features of our patient together with the hepatic and renal histopathological findings and the results of bile acid and *CYP7B1* gene analyses.

PATIENTS AND METHODS

Clinical Course and Laboratory Findings

A female Japanese infant with a birth weight of 3004 g was delivered by spontaneous vaginal delivery without complications at a gestational age of 39 weeks. The mother was a primigravida, and the pregnancy was uneventful. The parents were not consanguineous; both were healthy without evidence of liver disease. In particular, her mother had no pruritus, jaundice, or abnormal results for routine tests of liver function during her pregnancy.

The infant developed progressive jaundice by the age of 5 months. At 6 months, she was referred to Juntendo University Hospital with jaundice and hepatic dysfunction.

According to a physical examination, her growth and development were within normal limits (height = 66.1 cm, weight = 7.8 kg). No dysmorphic features were present. Jaundice and hepatosplenomegaly were noted. No abnormal neurological signs were elicited. Her stool was yellowish. The initial results of the laboratory tests included the following: a serum aspartate aminotransferase level of 803 U/L (normal < 37 U/L), an alanine aminotransferase level of 345 U/L (normal < 43 U/L), an alkaline phosphatase level of 4334 U/L (normal = 110-348 U/L), a total bilirubin level of 13.1 mg/dL (normal < 1.2 mg/dL), a direct bilirubin level of 7.7 mg/dL (normal < 0.3 mg/dL), an albumin level of 3.1 g/dL (normal = 4.0-5.2 g/dL), a prothrombin time of 26.0 seconds (normal = 11.1-15.1 seconds), and a blood ammonia level of 95 μg/dL (normal < 70 μg/dL). The serum 25-hydroxy vitamin D level was <5 ng/mL (normal = 7-41 ng/mL). The levels of other vitamins, including vitamins A, E, and K, were not assayed. The serum γ-glutamyltransferase level was 36 U/L (normal < 75 U/L), the total cholesterol level was 171 mg/dL (normal = 150-219 mg/dL), and the serum total bile acid (TBA) level was 6.5 μmol/L (normal < 10 μmol/L). The results of a complete blood count were within normal limits. Specific liver diseases such as autoimmune hepatitis and chronic viral hepatitis and other metabolic defects were excluded by the appropriate investigations. Abdominal ultrasonography revealed a visible gallbladder and hepatosplenomegaly; no choledochal cysts, no dilation of bile ducts, and no ascites were demonstrated. Contrast-enhanced computed tomography showed polycystic changes in the kidneys (Fig. 1A). There was radiological evidence of rickets. Serial technetium-99m diisopropyl iminodiacetic acid cholescintigraphy showed that the tracer had entered the intestine.

The patient's initial management included the administration of medium-chain triglycerides, ursodeoxycholic acid (UDCA; 16 mg/kg/day), fat-soluble vitamins, and infusions of fresh frozen plasma. After 61 days of these treatments, her liver function tests had deteriorated further (aspartate aminotransferase level = 568 U/L, alanine aminotransferase level = 232 U/L, alkaline phosphatase level = 2205 U/L, total bilirubin level = 14.3 mg/dL, direct bilirubin level = 9.1 mg/dL, albumin level = 3.7 g/dL, prothrombin time = 20.9 seconds, γ-glutamyltransferase level = 24 U/L, and TBA level = 54.4 μmol/L). At the age of 8 months, the patient developed decompensated hepatocellular failure, and she was referred to the National Center for Child Health and Development for an LT assessment. The patient underwent liver biopsy and kidney biopsy. Living donor liver transplantation (LDLT) was performed; her mother was the donor.

Progressive familial intrahepatic cholestasis types 1 and 2, which are characterized by low serum γ-glutamyltransferase levels, were excluded for our patient because her serum TBA levels were not elevated before her treatment with UDCA.

Qualitative and Quantitative Analyses of Bile Acids

Serum and urine samples were collected and stored at −25°C until they were analyzed. The concentrations of individual bile acids in her urine were corrected for the creatinine concentration and were expressed as micromoles per millimole of creatinine.

After the synthesis of positive control samples for rare bile acids that occur in patients with inborn errors of bile acid synthesis (eg, 3β-hydroxy-Δ^5 bile acid,[4] 3-oxo-Δ^4 bile acid,[5] and allo-bile acids[5]), we analyzed the bile acids in her serum and urine with gas chromatography/mass spectrometry and with selected ion monitoring of characteristic fragments of methyl ester/dimethylethylsilyl ether/methoxime bile acid derivatives, as described previously.[5] The samples were prepared for gas chromatography/mass spectrometry analysis by enzymatic hydrolysis (30 U of cholylglycine hydrolase) and solvolysis (150 U of sulfatase; Sigma Chemical, St. Louis, MO). *N*-Acetylglucosamine was not used.

Genetic Analysis

Informed parental consent was obtained, and analyses of the cholesterol 7α-hydroxylase gene [cytochrome P450 7A1 (*CYP7A1*)] and the *CYP7B1* gene were undertaken with the DNA of peripheral blood lymphocytes from the patient, the patient's parents, and 103 healthy controls. Polymerase chain reaction (PCR) primers were designed to amplify fragments containing the exon coding regions of the *CYP7A1* and *CYP7B1* genes.[6,7] DNA fragments, which included all coding regions of the *CYP7A1* and *CYP7B1* genes, were amplified with PCR.