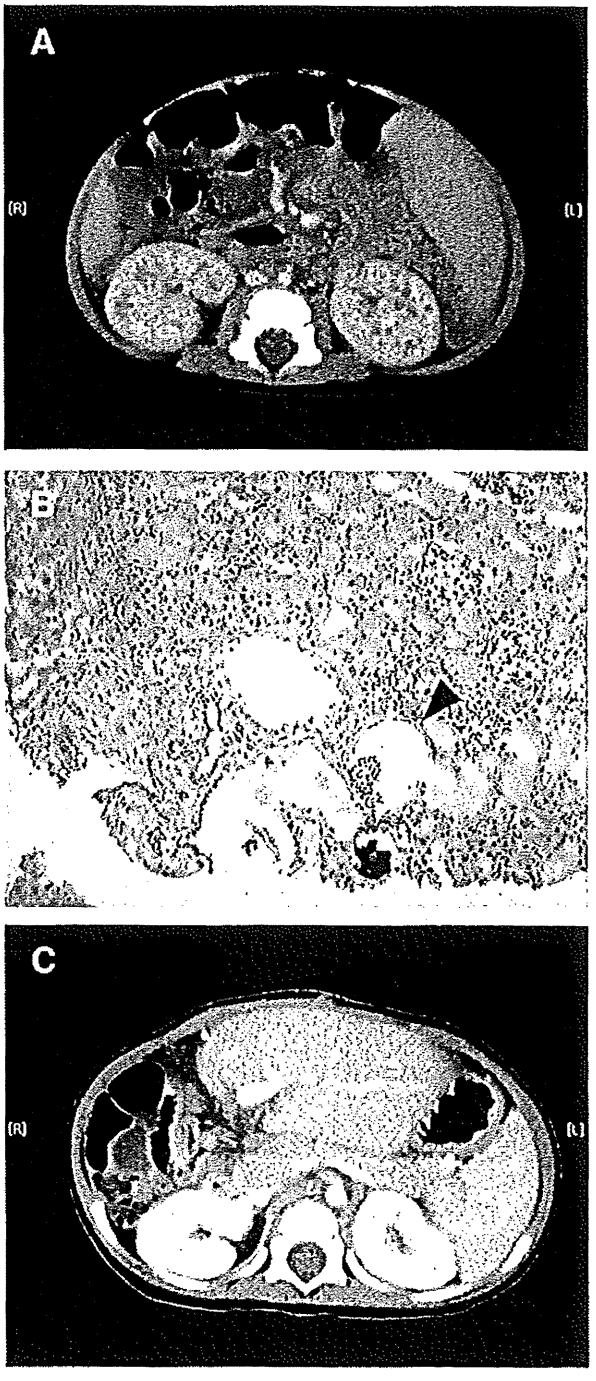

Figure 1. Polycystic kidneys in the patient with an oxysterol 7α-hydroxylase deficiency. (A) Bilateral polycystic kidneys were detected with contrast-enhanced computed tomography. (B) Microscopy of the kidneys revealed glomerular microcysts associated with atrophic glomeruli, cystic dilation of Bowman's capsule (black arrowhead), and renal tubular dilatation (white arrowhead; hematoxylin and eosin, original magnification ×400). (C) The resolution of the polycystic changes in the kidneys was demonstrated with contrast-enhanced computed tomography after LDLT.

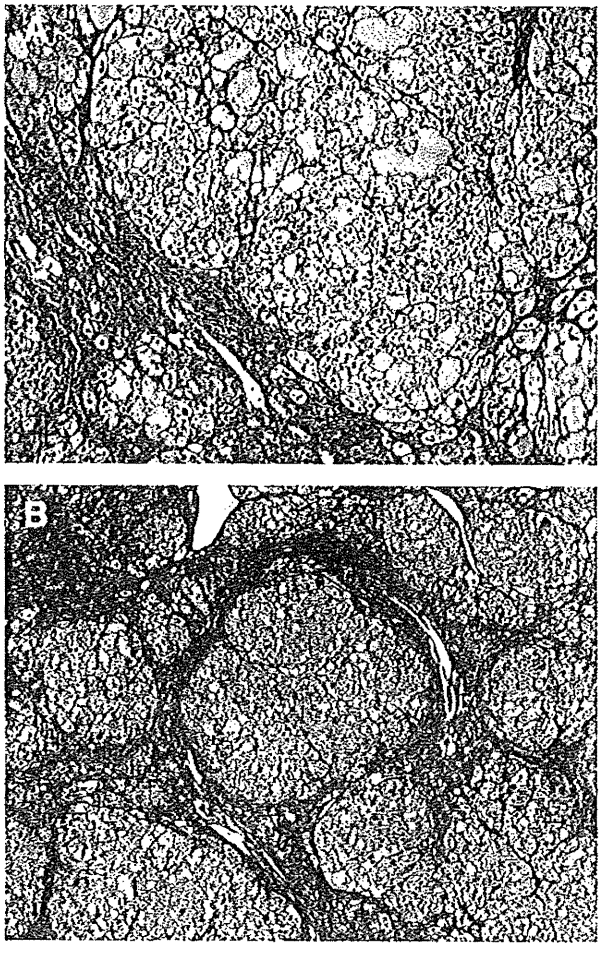

Figure 2. Hepatic pathology at the age of 8 months. A histological examination found features of neonatal hepatitis, which included giant cell transformation of hepatocytes and bridging fibrosis (hematoxylin and eosin, original magnification (A) ×100 or (B) ×40).

After enzymatic processing with ExoSAP-IT (USB Corp., Cleveland, OH), the direct sequencing of the amplified PCR products was undertaken with the DTCS quick-start kit (Beckman Coulter, Inc., Fullerton, CA) according to the manufacturer's protocol; the primers were the same as those used for PCR amplification. The sequencing reaction product was analyzed electrophoretically with the SEQ2000XL analyzer (Beckman Coulter, Inc., Brea, CA).

Two putative mutations were found in the patient. Subsequently, the patient's parents and 103 healthy individuals were screened for these 2 mutations by a direct sequence analysis or by the digestion of the appropriate PCR fragment with a restriction enzyme.

RESULTS

Hepatic and Renal Pathology

At the time of LT, the patient's liver weighed 541 g; it was atrophic and had irregular surface contours. A

TABLE 1. Initial, Pre-LT, and Post-LT Analyses of Bile Acids During UDCA Treatment

	Initial Analysis: 6 Months Old	Analysis Before LT: 8 Months Old	Analysis After LT: 9 Months Old	Analysis After LT: 28 Months Old
Serum (μmol/L)				
CA	ND	1.8	7.6	—
CDCA	1.1	9.5	2.1	—
Deoxycholic acid	0.2	ND	ND	—
UDCA	21.2	34.0	6.5	—
3β-Hydroxy-5-cholen-24-oic acid	7.3*	ND	ND	—
Polyhydroxylated bile acids	ND	0.1	ND	—
TBAs	29.8	45.4	16.2	—
Urine (μmol/mmol of creatinine)				
CA	3.5	5.5	0.2	0.2
CDCA	1.3	0.7	Trace	Trace
Deoxycholic acid	0.1	ND	ND	ND
Lithocholic acid	0.1	ND	ND	ND
UDCA	59.2	88.1	1.3	1.3
3β-Hydroxy-5-cholen-24-oic acid	41.7†	4.4‡	ND	ND
Polyhydroxylated bile acids	1.5	3.0	0.1	0.1
Allo-bile acids	0.1	0.1	ND	ND
Unsaturated ketonic bile acids	2.0	7.3	ND	ND
Other unsaturated bile acids	0.2	0.7	ND	ND
TBAs	109.7	109.8	1.6	1.6

NOTE: A dash indicates that the test was not performed.
*The percentage of TBAs was 24.5%, and the percentage with UDCA excluded from the denominator was 84.9%.
†The percentage of TBAs was 38.0%, and the percentage with UDCA excluded from the denominator was 82.9%.
‡The percentage of TBAs was 4.0%, and the percentage with UDCA excluded from the denominator was 20.3%.

liver biopsy sample showed changes consistent with micronodular cirrhosis; there were wide bands of fibrous tissue, marked lobular disarray, and frequent giant cell transformation (Fig. 2A,B). A renal biopsy sample revealed glomerular microcysts (Fig. 1B).

Clinical Course After LDLT

After an uneventful postoperative course, the patient was discharged on postoperative day 43; at this time, her therapy comprised oral tacrolimus, a corticosteroid, and UDCA. Subsequently, after 20 months of follow-up when the patient was 29 months old, her treatment consisted of only oral tacrolimus (0.07 mg/kg/day) and UDCA (5.8 mg/kg/day). At that time, the results of routine liver function tests were normal, and her growth and development were satisfactory (height = 89.1 cm, weight = 13.8 kg).

After LDLT, the renal cysts (Fig. 1C) and the rachitic bone lesions were resolved.

Biochemical Identification of an Inborn Error of Bile Acid Synthesis

An analysis of the bile acids present in her serum and urine during UDCA therapy when the patient was 6 months old detected large amounts of the rare bile acid 3β-hydroxy-5-cholen-24-oic acid (Table 1). In addition, small amounts of other uncommon bile acids, such as 3β-dihydroxy-Δ^5 bile acids, allo-bile acids, and 3-oxo-Δ^4 bile acids, were also detected in her urine. Common bile acids [eg, cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid, and lithocholic acid] were absent or were detected only in small amounts in her serum and urine. 3β-Hydroxy-5-cholen-24-oic acid accounted for 84.9% and 82.9% of TBAs (with UDCA excluded) in her serum and urine, respectively. In the absence of a standard sample for 3β-hydroxy-5-cholen-27-oic acid, this bile acid could not be accurately measured in samples from the patient.

The concentrations of common bile acids in her serum and urine at 8 months were higher than those at 6 months. The concentration of TBAs in her serum was also higher at 8 months. The concentration of 3β-hydroxy-5-cholen-24-oic acid in her urine was lower at 8 months, but the concentrations of other uncommon bile acids were higher (Table 1). After LDLT, the concentrations of bile acids in her serum and urine tended to normalize; in particular, uncommon bile acids could not be detected in her serum or urine (Table 1). The results of analyses of bile acids in specimens from the parents (before LDLT) are shown in Table 2.

Identification of Defects in the *CYP7B1* Gene

Two heterozygous mutations of the *CYP7B1* gene (but no mutations of the *CYP7A1* gene) were identified. One heterozygous mutation (R112X) was found in

TABLE 2. Analysis of the Parents' Bile Acids Before LDLT

	Father	Mother (Donor)
Serum (μmol/L)		
CA	—	ND
CDCA	—	0.5
Deoxycholic acid	—	0.4
Lithocholic acid	—	ND
UDCA	—	ND
3β-Hydroxy-5-cholen-24-oic acid	—	ND
TBAs	—	0.9
Urine (μmol/mmol of creatinine)		
CA	Trace	0.2
CDCA	Trace	Trace
Deoxycholic acid	Trace	Trace
Lithocholic acid	ND	ND
UDCA	Trace	Trace
3β-Hydroxy-5-cholen-24-oic acid	ND	ND
Polyhydroxylated bile acids	ND	Trace
Other unsaturated bile acids	ND	ND
TBAs	Trace	0.2

NOTE: A dash indicates that the test was not performed.

exon 3 at nucleotide 538; R112X is a previously reported C-to-T substitution that changes arginine (CGA) to a stop codon (TGA) at amino acid position 112.[3] The other heterozygous mutation (R417C), which has not been reported previously, is a C-to-T substitution in exon 6 at nucleotide 1453; it results in a substitution of cysteine (TGT) for arginine (CGT) at amino acid position 417. R112X was detected in the father but was absent in the mother and 103 healthy controls. R417C was detected in the mother but was absent in the father and 103 healthy controls.

The previously cited nucleotide numbers indicating the positions of the individual mutations are based on GenBank accession number NM_004820.

DISCUSSION

Our patient manifested clinical and laboratory features that are associated with an oxysterol 7α-hydroxylase deficiency: progressive jaundice beginning soon after birth, hepatomegaly, conjugated hyperbilirubinemia unaccompanied by pruritus, an absence of normal TBA concentrations in serum (measured enzymatically with 3α-hydroxysteroid dehydrogenase), normal serum γ-glutamyltransferase levels, and progressive intrahepatic cholestasis associated with severe hepatic fibrosis.[2,3] High levels of 3β-monohydroxy-Δ^5 bile acids in her serum and urine and a compound heterozygous mutation in the *CYP7B1* gene were detected. She underwent LDLT, after which the features of cholestatic liver disease were resolved.

Macroscopically, the surface of the excised liver was dark brown and was characterized by many large nodules. The patient's liver was found to have the following microscopic features: giant cell transformation of hepatocytes (Fig. 2A); consistent and prominent portal zone inflammation; periportal fibrosis, which had progressed to micronodular cirrhosis associated with bile ductular proliferation by 8 months (Fig. 2B); and bile plugs in a few cholangioles and hepatocytes but not in interlobular ducts. These findings agreed with those reviewed by Bove et al.[8] Interestingly, polycystic changes in the kidneys were demonstrated by computed tomography; such changes also occur in patients with Zellweger syndrome.[9] Her renal function was normal despite the cystic renal lesions (Fig. 1A). A microscopic examination of her kidneys revealed glomerular microcysts associated with atrophic glomeruli, cystic dilation of Bowman's capsule, and renal tubular dilation (Fig. 1B). The cause of these renal changes has not been established; they may have arisen because of renal toxicity induced by certain bile acids (especially monohydroxy bile acids such as 3β-hydroxy-5-cholen-24-oic acid).

After LDLT, the levels of uncommon toxic bile acids such as 3β-monohydroxy-Δ^5 bile acids decreased, presumably because of the increased activity of oxysterol 7α-hydroxylase, and the polycystic changes in the kidneys were resolved.

The level of 3β-hydroxy-5-cholen-24-oic acid as a percentage of TBAs and the absolute concentration of this bile acid in her serum and urine decreased between the first and second analyses of the bile acids (the interval was only 2 months; see Table 1). The changes indicated that the main pathway for bile acid metabolism had changed from the acidic pathway to the classic pathway; the activity of cholesterol 7α hydroxylase gradually increased from the late neonatal period to late infancy, even though the C_{27} bile acid present in patients with this disease, a 3β-monohydroxy-Δ^5-C_{27} bile acid,[2] is a high-affinity ligand for farnesoid X receptor (FXR; Fig. 3).[10] We suggest that the level of activity of cholesterol 7α-hydroxylase in this patient (ie, a low level or none) is consistent with previous physiological observations establishing that the activity of cholesterol 7α-hydroxylase is low or absent in the fetal and early neonatal periods.[11,12]

Progressive liver disease in patients with this condition may be exacerbated by the accumulation of 3β-hydroxy-5-cholen-24-oic acid; our patient had developed cirrhosis by the age of 8 months. Clearly, there is a need for a novel treatment that promotes the excretion of toxic monohydroxy bile acids such as 3β-monohydroxy-Δ^5 bile acids. Currently, orthotopic LT is the only therapeutic option. Any new treatment ideally would prevent the development of cirrhosis after the diagnosis is made in the early neonatal period. An early diagnosis would be facilitated by screening for inborn errors of bile acid synthesis: urine samples would be subjected to liquid secondary ionization mass spectrometry.[13]

Primary bile acid therapy with CA, CDCA, or both is effective in the treatment of inborn errors of bile acid synthesis.[14,15] In our patient, however, *CYP7A1* enzyme activity in the classic pathway would have

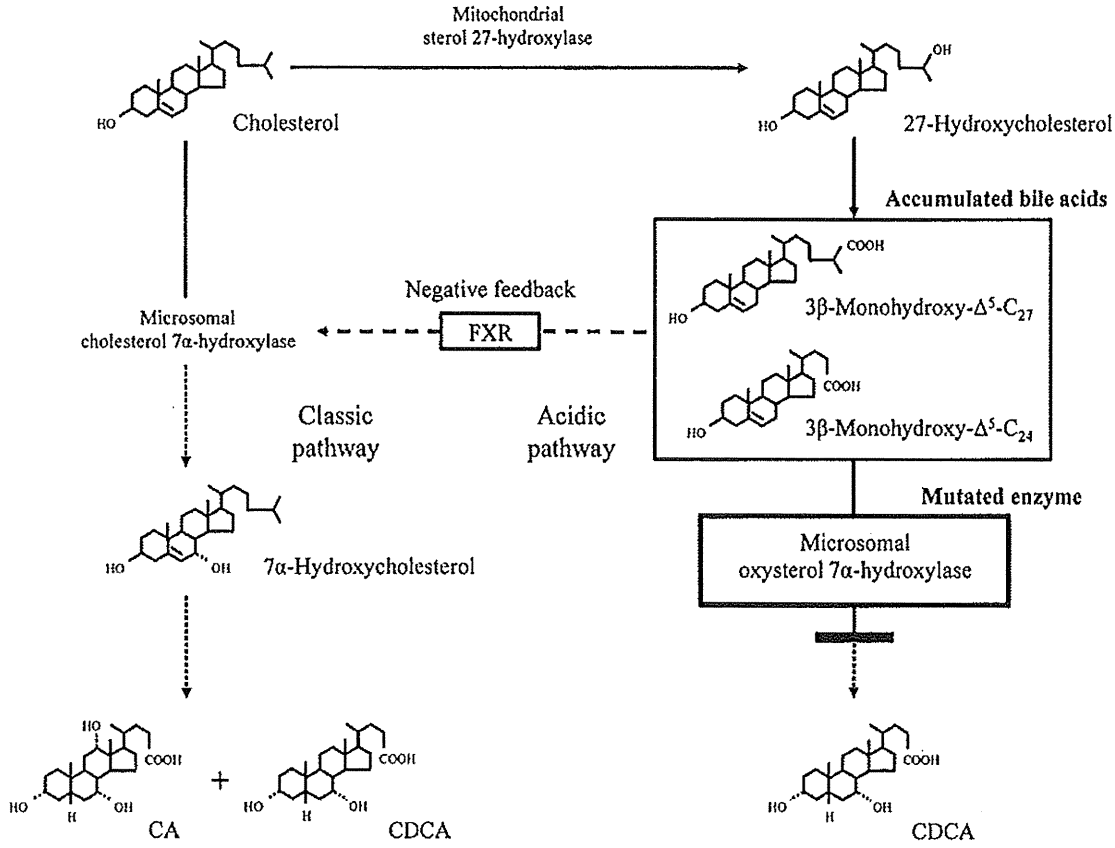

Figure 3. Flow chart showing the effects of an oxysterol 7α-hydroxylase deficiency. The reduced synthesis of primary bile acids from cholesterol in the classic pathway is associated with the increased synthesis of 3β-monohydroxy-Δ^5 bile acids in the acidic pathway. The 3β-monohydroxy-Δ^5 bile acids (especially 3β-monohydroxy-Δ^5-C_{27} acid), which have a high affinity for FXR, lead to reduced cholesterol 7α-hydroxylase activity. However, the activity of cholesterol 7α-hydroxylase may increase from the late neonatal period to late infancy when the main pathway of bile acid synthesis changes from the acidic pathway to the classic pathway.

been suppressed via FXR by therapeutic doses of primary bile acids; consequently, toxic intermediates of bile acid synthesis such as 3β-monohydroxy-Δ^5-C_{27} bile acid in the acidic pathway would have accumulated. We suggest that this patient may have benefited at an early stage from mild suppression of *CYP7A1* induced by low-dose primary bile acid therapy. Because our patient was diagnosed at a late stage, primary bile acid therapy was not attempted.

The human *CYP7B1* gene contains 6 coding exons that correspond to 506 amino acids; so far, 2 distinct mutations that result in an oxysterol 7α-hydroxylase deficiency have been reported.[2,3] Our patient had 2 heterozygous mutations, R112X and R417C, in the *CYP7B1* gene. The previously reported R112X heterozygous nonsense mutation in exon 3 was identified in the father but not in the mother or in control subjects. The novel R417C heterozygous missense mutation in exon 6 was identified in the mother but not in the father or in control subjects. The patient received 1 allele containing the R112X mutation from the father and another allele containing R417C from the mother. Thus, the patient was a compound heterozygote for the *CYP7B1* gene.

Screening for the potentially informative mutation R417C was undertaken for 103 healthy individuals, but this mutation was absent in all of them. Moreover, the R417C mutation was predicted to probably have an adverse effect (score = 1.000) by an analysis with Polymorphism Phenotyping version 2.[16] Accordingly, we believe that the R417C mutation may have contributed to a loss of function of oxysterol 7α-hydroxylase in our patient.

LT is an established treatment for patients with heritable metabolic disorders. For our patient, we obtained an allograft from her mother. After LDLT, all abnormal effects of chronic cholestatic liver disease were gradually resolved. When an appropriate parent is available as the donor, LDLT represents an effective treatment option for pediatric patients with heritable metabolic disorders.[17] In pediatric patients with autosomal recessive disorders, the parent who serves as the donor is almost always a heterozygote. Morioka et al.[17] confirmed that transplantation from

heterozygous donors does not have a negative impact on either the donor or the recipient. However, when the effects of the disease are less hepatospecific than the effects in our patient, additional treatment may be necessary to optimize the outcome after LDLT.

In conclusion, we have reported the first Japanese patient with an oxysterol 7α-hydroxylase deficiency and compound heterozygous mutations (R112X and R417C) in the *CYP7B1* gene. LT with an allograft obtained from a heterozygous living donor was followed by the resolution of the manifestations of the disease.

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Preoperative Dialysis for Liver Transplantation in Methylmalonic Acidemia

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Abstract: Dialysis immediately before liver transplantation for patients with methylmalonic academia (MMA) with the mut0 mutation is considered to be necessary to reduce plasma methylmalonic acid (MMA) levels and prevent metabolic decompensation for a successful surgical outcome; however, this has not yet been conclusively confirmed. Ten pediatric patients underwent living donor liver transplantation at the National Center for Child Health and Development, Tokyo, Japan. Seven patients received dialysis immediately before surgery, but the three most recent patients did not receive dialysis. We monitored plasma MMA levels and evaluated metabolic status during the perioperative period. Plasma MMA levels of patients who received preoperative dialysis were significantly decreased. However, lactic acidosis developed in two patients during surgery. One of the patients who had decreased renal function suffered from severe lactic acidosis after the transplantation and died on post operative day 44. In the three patients who did not receive preoperative dialysis, high plasma MMA levels persisted, but they did not develop metabolic decompensation. Their plasma MMA levels gradually decreased after transplantation. Our results indicated that reducing MMA with preoperative dialysis does not decrease the risk of metabolic decompensation. We will need to evaluate whether preoperative dialysis is necessary for the success of surgery with more cases in the future. Adequate perioperative glucose infusion and careful lactate monitoring are pivotal for success. **Key Words:** Lactic acidosis, Liver transplantation, Methylmalonic acidemia, Preoperative dialysis, Renal insufficiency.

Methylmalonic acidemia is a rare autosomal recessive genetic disorder characterized by a complete or partial deficiency of methylmalonyl-CoA mutase or by defects in the synthesis of adenosylcobalamin. The estimated frequency in Japan is 1:80 000. Most (~90%) neonates with the mut0 (complete deficiency of methylmalonyl-CoA mutase) develop clinical symptoms, such as vomiting, hypotonia, lethargy and convulsions, and >50% of them die before reaching 10 years of age. Developmental delay and chronic renal failure are inevitable in the other 50%. Such patients require a severely protein-restricted diet and their quality of life is clearly reduced.

Liver transplantation can decrease plasma and urine methylmalonic acid (MMA) levels, reduce the frequency of metabolic decompensation, alleviate protein restriction and lead to an improved quality of life (1). To our knowledge, there have been 25 patients with methylmalonic acidemia who have undergone liver transplantation (2–15). Some authors advocated that preoperative dialysis immediately before liver transplantation is necessary to reduce plasma MMA levels and to prevent metabolic decompensation (5,7,11–13). However, there have been no reports of the necessity of preoperative dialysis, and whether it is essential remains unknown. Between November 2005 and June 2010, 10 pediatric patients with mut0 methylmalonic acidemia underwent living donor liver

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TABLE 1. *Preoperative characteristics of 10 patients with methylmalonic acidemia who underwent living donor liver transplantation*

Patient number	Age at onset	Age at LT (mo)	Gender	Weight at LT (kg)	History of blood purification therapy	24 h Ccr (mL/min/ 1.73 m²)	Serum cystatin C (mg/L)	Donor	Preoperative hemodialysis
1	1 day	58	Male	15.0	None	136.2	Not done	Father	Yes
2	3 day	90	Female	24.6	PD	158.9	0.94	Father	Yes
3	0 day	63	Male	15.2	PE	136.2	0.78	Father	Yes
4	9 day	7	Female	8.4	None	94.0	0.88	Father	Yes
5	45 days	31	Male	11.8	PD	138.1	0.86	Father	Yes
6	7 months	52	Female	20.0	BE-PD	65.9	1.60	Father	Yes
7	3 days	12	Female	8.9	BE	159.9	0.82	Father	Yes
8	14 days	18	Male	11.5	CHD	115.9	1.01	Father	No
9	2 months	65	Male	17.7	None	96.5	1.29	Mother	No
10	3 days	9	Male	9.3	HD	124.7	1.01	Father	No

24 h Ccr, 24-hour creatinine clearance; BE, blood exchange; CHD, continuous hemodialysis; LT, liver transplantation; PD, peritoneal dialysis; PE, plasma exchange.

transplantation at our center. Seven patients who have already been described (2) received dialysis immediately before surgery; however, as 6th and 7th patients developed lactic acidosis during surgery in spite of reducing MMA by preoperative dialysis, we tried liver transplantation without preoparative dialysis for the most recent three patients. In this study, we monitored plasma MMA levels during the perioperative period in all of the patients and evaluated perioperative metabolic status. We also discuss the necessity for preoperative dialysis.

PATIENTS AND METHODS

Table 1 shows the characteristics of the 10 patients (6 boys and 4 girls; median age, 41.5 months; range, 7–90 months; median weight, 13.4 kg; range, 8.4–24.6 kg) who underwent living donor liver transplantation in the National Center for Child Health and Development, Tokyo, Japan, between November 2005 and June 2010. This center is the largest pediatric liver transplantation center in Japan and transplant surgeons, pediatric nephrologists, pediatric surgeons and specialists in pediatric metabolic disease are regularly available. So patients from all parts of the country who need liver transplantation are referred here.

Gene analysis confirmed that all of the patients had the mut0 mutation accompanied by a history of recurrent metabolic decompensation episodes, mild to moderate developmental delay and required tube feeding to provide enough energy to avert aggravating catabolism. Patient 6 had developed chronic renal insufficiency due to the disease by the time of liver transplantation. No patients received chronic renal replacement therapy before liver transplantation. Patients 1 to 7 underwent preoperative dialysis, whereas patients 8 to 10 did not.

Except for patient 2, who started dialysis 2 days before transplantation, the remaining 6 patients began dialysis on the day before transplantation (Table 2). Patients 1 and 2 underwent continuous hemodialysis, but MMA was not sufficiently removed in patient 2. Thus, patients 3 to 7 underwent continuous hemodiafiltration (CHDF). Dialysis was administered under the control of mechanical ventilation in the pediatric intensive care unit because body movement interrupts the dialysis and they need sedation

TABLE 2. *Mode of preoperative dialysis for seven patients*

Patient number	Mode of dialysis	Duration of dialysis (h)	Area of hemofilter membrane (m²)	QB/QD/QS (mL/min)/(mL/h)/(mL/h)	Anticoagulant
1	CHD	10	0.6	50/800/0	Heparin
2	CHD	40	1.0	100/2000/0	NM
3	CHDF	17	0.6	50/1000/1000	NM
4	CHDF	19	0.3	30/1000/500	NM
5	CHDF	18	0.6	30/1000/300	NM
6	CHDF	18	0.6	50/1000/600	NM
7	CHDF	7	0.3	40/1000/600	NM

CHD, continuous hemodialysis; CHDF, continuous hemodiafiltration; NM, nafamostat mesilate; QB, blood flow rate; QD, dialysate flow rate; QS, substitution flow rate.

TABLE 3. *Peri- and postoperative plasma methylmalonic academia levels (normal range, 0.35 ± 0.22 nmol/mL)*

Patient number	Admission to hospital	Just before dialysis	Just after dialysis	Just before surgery	Anhepatic time	After reperfusion	Just after operation	POD 1	POD 7	POD 30
1	268.0	92.2	58.5	70.8	62.0	64.0	47.8	37.5	37.7	99.4
2	47.0	40.0	33.6	50.7	44.7	48.2	48.8	60.9	65.1	59.2
3	143.0	31.7	32.9	25.9	32.9	38.1	39.6			36.4
4	39.0	14.4	13.4	12.3	19.0	26.8	38.4	37.0	28.1	29.3
5	375.0	137.3	87.1	86.4	114.6	106.4	100.2	190.5	109.6	87.8
6	1970.0	357.0	140.5	251.0	199.0	181.5	146.4	329.0	176.9	232.0
7	166.0	107.3	38.7	38.7	46.3	35.2	31.5	44.4	22.7	13.8
8	278.0	NA	NA	342.0	298.0	337.0	251.0	175.8	77.7	59.6
9	702.0	NA	NA	302.0	303.0	230.0	191.0	117.5	147.9	124.4
10	255.0	NA	NA	160.0	147.0	119.0	88.0	129.0	33.8	8.5

NA, not applicable; POD, post operative day.

during dialysis. Central catheters were inserted into the right internal jugular veins of all patients to access blood. These procedures were approved by the institutional review board, and informed consent was obtained in all the cases.

We examined perioperative plasma MMA levels and blood lactate levels in all patients. The Wilcoxon signed-rank test was used to compare plasma MMA levels before and after preoperative dialysis. The results were analyzed with JMP ver. 8.0 (SAS Institute Japan Ltd, Tokyo, Japan). Statistical significance was established at $P < 0.05$.

RESULTS

Table 3 shows peri- and postoperative plasma MMA levels (normal range, 0.35 ± 0.22 nmol/mL) of the 10 patients. MMA levels at the time of hospital admission were very high, possibly because most of the patients lived far from the hospital and might have been stressed from transfer. The plasma MMA levels of seven patients who underwent preoperative dialysis were significantly decreased (pre vs. post: 111.4 ± 117.1 vs. 57.8 ± 43.3 nmol/mL; $P = 0.02$) and these levels remained suppressed, except in patient 6. In particular, very high plasma MMA levels before dialysis (patients 5, 6, and 7) remarkably decreased. Patient 6 who had decreased renal function suffered from severe uncontrollable lactic acidosis and acute rejection of the graft after the transplantation, and she required CHDF. She continued to have high plasma MMA levels until she died of sepsis on post operative day 44. High plasma MMA levels persisted during the operations in the three patients who did not undergo preoperative dialysis. However, their plasma MMA levels gradually decreased after liver transplantation.

Table 4 shows perioperative blood lactate levels. Relatively high blood lactate levels persisted during surgery. Patients 6 and 7 developed lactic acidosis despite preoperative dialysis. Lactate levels in patient 6 became greatly elevated (11.8 mmol/L, normal range 0–2) and prominent metabolic acidosis required the administration of a large amount of sodium bicarbonate during surgery. There were no particular problems in surgeries. Although patients 8, 9 and 10 did not undergo preoperative dialysis and high plasma MMA levels persisted (>100 nmol/mL), they did not develop lactic acidosis (lactate levels, 1.4–4.7 mmol/L). These three patients successfully completed liver transplantation without any adverse events. All patients were

TABLE 4. *Perioperative blood lactate levels (mmol/L)*

Patient number	Just before dialysis	Just after dialysis	Just before surgery	Anhepatic time	After reperfusion	Just after operation
1	3.1	2.9	3.6	5.5	4.2	3.5
2	3.9	3.6	4.1	4.1	3.3	4
3	2.6	2.5	3.2	2.7	2.7	3.3
4	1.3	1.1	1.8	2.6	2.5	1.8
5	1.4	2.4	2.2	2.1	1.5	1.9
6	3.6	5.5	9.7	11.8	10.9	6.6
7	1.1	2.1	3.6	6.2	7.9	7.9
8	NA	NA	3.6	4.7	2	1.4
9	NA	NA	5	4.2	2.7	1.9
10	NA	NA	2.1	2.2	1.5	1.5

NA, not applicable.

TABLE 5. *Perioperative blood glucose levels (mg/dL)*

Patient number	Just before dialysis	Just after dialysis	Just before surgery	Anhepalic time	After reperfusion	Just after operation
1	115	119	94	156	136	148
2	89	136	140	159	144	126
3	126	127	151	83	153	130
4	159	116	74	115	142	224
5	104	153	144	94	95	120
6	82	138	93	139	168	169
7	161	317	275	289	279	184
8	NA	NA	179	117	170	140
9	NA	NA	113	96	112	148
10	NA	NA	123	231	154	228

NA, not applicable.

infused with hyperalimentation to avoid catabolic aggravation during surgery and relatively high blood glucose levels persisted during surgery (Table 5). Patient 7 developed severe hyperglycemia that required insulin during the surgery.

The patients remained under observation for various periods ranging from 19 to 53 months after transplantation. All patients except for patient 6 remained alive, free of metabolic decompensation requiring hospital administration and could ingest food by mouth. Tube feeding was no longer required and the parents all agreed that the quality of life of these children had improved as a result of transplantation.

DISCUSSION

A total of 25 patients with methylmalonic acidemia have undergone liver transplantation (2–15). Sixteen patients underwent preoperative dialysis immediately before liver transplantation to remove MMA, although the other nine patients did not undergo preoperative dialysis. Twenty-one patients successfully completed liver transplantation; four patients (three of them received preoperative dialysis and one of them did not receive preoperative dialysis) died after the operation. Nagarajan et al. (11) advocated that hemodialysis is desirable a few hours before combined liver-kidney transplantation to reduce serum MMA levels and to avoid immediate toxicity to the transplanted kidney. Hsui et al. (13) also performed dialysis immediately before transplantation and suggested that it is necessary for a successful surgical outcome. The purposes of preoperative dialysis are to reduce MMA levels to maintain an optimal environment for the graft and to prevent lactic acidosis and metabolic decompensation during surgery. Seven of our patients underwent preoperative dialysis in accordance with these reports. However, the real necessity for this procedure remains to be demonstrated. Therefore, we evaluated the necessity of preoperative dialysis by monitoring plasma MMA levels and metabolic status during the perioperative period.

Because the molecular weight of MMA is 118 Daltons, we postulated that it could be easily removed with continuous hemodialysis. We applied this procedure to patients 1 and 2, but plasma MMA levels were not sufficiently removed in patient 2. Therefore, we applied CHDF to patients 3 to 7. Plasma MMA levels were significantly decreased with CHDF, but we could not prevent lactic acidosis during surgery in patients 6 and 7. Patient 6 developed severe lactic acidosis again after transplantation and died of sepsis on postoperative day 44. We speculate that preexisting renal insufficiency caused by the disease is an important risk factor for a poor outcome because control of the acid-base balance could be difficult under conditions of reduced renal function. Lactic acidosis is likely to occur during the anhepatic phase because the liver plays an important role in lactate metabolism. In contrast, high plasma MMA levels persisted during the operation in three patients who did not receive preoperative dialysis, but they did not develop metabolic decompensation during the perioperative period. They successfully completed the operations and their plasma MMA levels gradually decreased after transplantations. Our findings indicate that reducing MMA levels with preoperative dialysis does not decrease the risk of metabolic decompensation and it is not pivotal for the success of liver transplantation. However, the number of cases was small and we have not conducted a prospective randomized controlled study.

The adverse effects of dialysis include the need for mechanical ventilation in the pediatric intensive care unit, central blood access from the day before surgery and complications such as hypoproteinemia due to dialysis and infection. It was necessary for us to

perform continuous hemodialysis overnight to remove MMA sufficiently, so we needed mechanical ventilation. These factors might stress the patients, aggravate disease activity and result in metabolic decompensation. Sufficient hyperalimentation is necessary for patients with methylmalonic acidemia to prevent catabolism, but excessive hyperalimentation often leads to hyperglycemia due to mitochondrial dysfunction, which in turn might cause lactic acidosis between removal of the diseased liver and transplantation. Thus, transfusion must be controlled with properly adjusted glucose doses and blood glucose levels should be carefully monitored. Patients who have already developed renal insufficiency are at very high risk for liver transplantation and such patients might warrant another strategy, such as prior kidney transplantation or combined liver-kidney transplantation.

CONCLUSION

Reducing methylmalonic acid levels with preoperative dialysis cannot prevent lactic acidosis during surgery and we did not find any merits to carrying out hemodialysis. This study result should be supported with more cases in future. Adequate glucose infusion and careful perioperative lactate monitoring are pivotal for success. Those patients who have already developed renal insufficiency are at very high risk for liver transplantation.

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別紙様式

高チロシン血症を示す新生児における最終診断への診断プロトコールと治療指針の作成に関する研究班

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VI. 遺伝性高チロシン血症の診断治療指針

遺伝性高チロシン血症の診断治療指針

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1．はじめに

遺伝性高チロシン血症は 3 つの病型に分類されている。遺伝形式はいずれも常染色体劣性である。

（1）遺伝性高チロシン血症 I 型:フマリルアセト酢酸ヒドラーゼの欠損によって発症する。

（2）遺伝性高チロシン血症 II 型：細胞質チロシンアミノ基転移酵素の欠損によって発症する。

（3）遺伝性高チロシン血症 III 型：4-ヒドロキシフェニルピルビン酸酸化酵素が欠損することで発症する。またホーキンシン尿症も同じ酵素異常により発症する常染色体優性遺伝性疾患である。

2．臨床症状

（1）遺伝性高チロシン血症 I 型では、進行する肝障害と腎尿細管障害が特徴的である。生後数週から始まる肝腫大、発育不良、下痢、嘔吐、黄疸などが見られる。重症例では肝不全へ進行し、無治療であれば生後 2~3 ヶ月で死亡する。生後数ヶ月から 1 年程度で肝障害を発症する亜急性型や、肝障害の進行は緩やかであるが最終的には肝硬変、肝不全に至る慢性型も存在する。肝臓癌を発生する症例も多く、多発性腫瘍も報告されている。腎臓では尿細管機能障害によって、低リン血症性くる病、ビタミン D 抵抗性くる病などが認められる。

（2）遺伝性高チロシン血症 II 型では、I 型や III 型より血中チロシン値が高い。II 型の皮膚病変はチロシンの針状結晶が析出することによって出現し、手掌･足底に限局した過剰角化、びらんを生じる。また角膜においてもびらん・潰瘍が生じる。血中チロシン濃度が特に高い一部の症例では精神発達の遅れを認めることがある。

（3）III 型の症状は I 型、II 型よりも軽度であり、無症状の症例も存在する。これまでに失調、痙攣、軽度の精神発達遅延などが報告されている。

3．診断指針

上記の臨床症状を呈する患者では、血中アミノ酸分析やタンデムマス検査によって血中チロシン値を測定することがまず必要である。高チロシン血症を呈する患者では遺伝性高チロシン血症 I 型、II 型、III 型以外に、他の原因による血中チロシン値の高値を鑑別する。

（1）遺伝性高チロシン血症 I 型の診断では肝障害の有無が重要である。肝機能障害の結果、血清トランスアミナーゼの上昇や凝固因子の合成低下などを認める。腎尿細管機能障害に

より低リン酸血症、糖尿、蛋白尿などが認められる。また、血清中αフェトタンパクの増加が特徴的である。確定診断のためには、尿有機酸分析をおこないチロシン代謝産物である4-ヒドロキシフェニルピルビン酸、4-ヒドロキシフェニル乳酸、4-ヒドロキシフェニルピルビン酢酸などの増加と、サクシニルアセトンの増加を明らかにする。尿中サクシニルアセトンの増加は診断的な価値が高い。また、酵素診断は肝細胞、培養皮膚線維芽細胞を検体として、フマリルアセト酢酸ヒドラーゼ活性を測定する。

（2）遺伝性高チロシン血症II型では皮膚の過剰角化・びらんや角膜のびらん・潰瘍から本症を疑われる。血中アミノ酸分析では血中チロシンは20mg/dl以上と極めて高値である。尿有機酸分析では4-ヒドロキシフェニルピルビン酸、4-ヒドロキシフェニル乳酸、4-ヒドロキシフェニルピルビン酢酸が大量に見出される。細胞質チロシンアミノ基転移酵素活性の測定には肝生検が必要である。

（3）遺伝性高チロシン血症III型では臨床症状は特徴的ではない。血中アミノ酸ではチロシンが約20mg/dl程度まで増加し、尿中へ4-ヒドロキシフェニルピルビン酸およびその酸化物が大量に検出される。確定診断では肝 4-ヒドロキシフェニルピルビン酸酸化酵素を測定する。III型の軽症型であるホーキンシン尿症は尿中ホーキンシンを検出することで診断される。

図1　遺伝性高チロシン血症の代謝障害部位

フェニルアラニン

↓　フェニルアラニン水酸化酵素

チロシン

↓　チロシンアミノ基転移酵素（**高チロシン血症II型**）

4－ヒドロキシフェニルピルビン酸

↓　4－ヒドロキシフェニルピルビン酸酸化酵素（**高チロシン血症III型、ホーキンシン尿症**）

ホモゲンチジン酸

↓　ホモゲンチジン酸酸化酵素

マレイルアセト酢酸

↓　マレイルアセト酢酸イソメラーゼ

フマリルアセト酢酸

↓　フマリルアセト酢酸分解酵素（**高チロシン血症I型**）

フマル酸　＋　アセト酢酸

表１　高チロシン血症の分類

病型	遺伝性	血漿中のチロシン上昇	酵素欠損	主な症状
遺伝性高チロシン血症Ｉ型	常劣	軽度	フマリルアセト酢酸分解酵素	肝細胞障害　尿細管障害 低血糖　ガラクトース代謝異常 神経症状　肝細胞癌
遺伝性高チロシン血症II型	常劣	高度	チロシンアミノ基転移酵素	精神発達遅延　皮膚の異常角化 角膜びらん　潰瘍
遺伝性高チロシン血症III型	常劣	中等度	4ヒドロキシフェニルピルビン酸酸化酵素	失調　けいれん 軽度の精神発達遅延
ホーキンシン尿症	常優	一過性	4ヒドロキシフェニルピルビン酸酸化酵素	一過性発育遅延 食欲不振
肝障害に伴う高チロシン血症	原疾患による	さまざま		原疾患による
新生児一過性高チロシン血症	なし	さまざま		無症状または不活発

５．治療指針

チロシン高値の患者ではＩ型、II型、III型とその他の原因による高チロシン血症の鑑別を対症療法と同時に行う。新生児期には臓器障害がなければ基本的には経過観察する。

（１）Ｉ型では肝障害の進行を早期に防止することが重要であり、ニチシノン (NTBC: 2-(2-nitro-4-trifluoromethyl-benzoyl)-1,3-cyclo- hexanedione)を使用し、低フェニルアラニン・低チロシン食を併用する。治療の効果判定には肝機能検査と血清αフェトタンパク値の測定が有用である。NTBC を使用しない例では肝不全に至ることが多く、肝移植が行われる。NTBC は国内では入手困難であり、個人輸入が必要となる。

（２）II型では低フェニルアラニン・低チロシン食をおこない、血液中のチロシン値を低下させる血中チロシン値を 10mg/dl 以下に保つ。

（3）III 型では II 型と同様に、低フェニルアラニン・低チロシン食による食事療法を行う。

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難治性疾患克服研究事業　研究症例分野

「高チロシン血症を示す新生児における最終診断への診断プロトコールと

治療指針の作成に関する研究」研究班