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Molecular and Clinical Analysis of Japanese Patients with Persistent Congenital Hyperinsulinism: Predominance of Paternally Inherited Monoallelic Mutations in the K ATP Channel Genes

Tohru Yorifuji, Rie Kawakita, Shizuyo Nagai, Akinori Sugimine, Hiraku Doi, Anryu Nomura, Michiya Masue, Hironori Nishibori, Akihiko Yoshizawa, Shinya Okamoto, Ryuichiro Doi, Shinji Uemoto and Hironori Nagasaka

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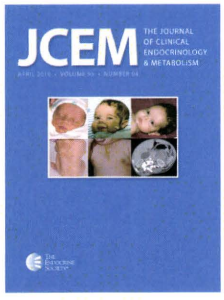

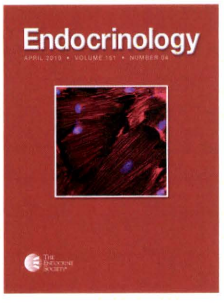

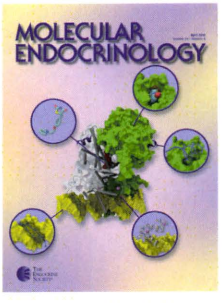

THE ENDOCRINE SOCIETY®

Brief Report—Endocrine Research

Molecular and Clinical Analysis of Japanese Patients with Persistent Congenital Hyperinsulinism: Predominance of Paternally Inherited Monoallelic Mutations in the K_{ATP} Channel Genes

Tohru Yorifuji, Rie Kawakita, Shizuyo Nagai, Akinori Sugimine, Hiraku Doi, Anryu Nomura, Michiya Masue, Hironori Nishibori, Akihiko Yoshizawa, Shinya Okamoto, Ryuichiro Doi, Shinji Uemoto, and Hironori Nagasaka

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Background: Preoperative identification of the focal form of congenital hyperinsulinism is important for avoiding unnecessary subtotal pancreatectomy. However, neither the incidence nor the histological spectrum of the disease is known for Japanese patients.

Aims: The aim of the study was to elucidate the molecular and histological spectrum of congenital hyperinsulinism in Japan.

Subjects: Thirty-six Japanese infants with persistent congenital hyperinsulinism were included in the study.

Methods: All exons of the ATP-sensitive potassium channel (K_{ATP} channel) genes (*KCNJ11* and *ABCC8*), the *GCK* gene, and exons 6 and 7 and 10–12 of the *GLUD1* gene were amplified from genomic DNA and directly sequenced. In patients with K_{ATP} channel mutations, the parental origin of each mutation was determined, and the results were compared with the histological findings of surgically treated patients. In one of the patients with scattered lesions, islets were sampled by laser capture microdissection for mutational analysis.

Results: Mutations were identified in 24 patients (66.7%): five in *GLUD1* and 19 in the K_{ATP} channel genes. Sixteen had a paternally derived, monoallelic K_{ATP} channel mutation predictive of the focal form. In 10 patients who underwent pancreatectomy, the molecular diagnosis correctly predicted the histology, more accurately than [18F]-3,4-dihydroxyphenylalanine positron emission tomography scans. Three patients showed focal lesions that occupied larger areas of the pancreas. Preferential loss of the maternal allele was observed in these islets.

Conclusion: The majority of the Japanese patients with K_{ATP} channel hyperinsulinism (84.2%) demonstrated paternally inherited monoallelic mutations that accurately predicted the presence of the focal form. (***J Clin Endocrinol Metab* 96: E141–E145, 2011**)

Persistent congenital hyperinsulinism is the main cause of prolonged hypoglycemia in infancy. The most common etiology is an inactivating mutation in one of two genes, *ABCC8* or *KCNJ11*, which code for the two subunits of the pancreatic ATP-sensitive potassium (K_{ATP}) channel. The second most common is an activating mu-

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Abbreviations: DOPA, 3,4-Dihydroxyphenylalanine; GCK, glucokinase; GLUD1, glutamate dehydrogenase; K_{ATP}, ATP-sensitive potassium channel; MLPA, multiple ligation-dependent probe amplification; PET, positron emission tomography.

tation in the glutamate dehydrogenase (*GLUD1*) gene, which is found in cases of hyperinsulinemia-hyperammonemia syndrome followed by an activating mutation in the glucokinase (*GCK*) gene with a much rare incidence (1).

Because severely affected infants often experience profound neurological sequelae (2, 3), appropriate management of hypoglycemia is critically important. Infants resistant to medical treatment usually undergo subtotal pancreatectomy. Although the procedure is often effective at controlling hypoglycemia, residual hypoglycemia is not uncommon, and many of the infants develop insulin-dependent diabetes mellitus postoperatively (1, 4).

Notably, the recognition of the focal form of persistent congenital hyperinsulinism has changed clinical practice because precise pre- and intraoperative identification of focal lesions allows us to perform a partial resection of the pancreas, leading to a complication-free cure (1, 5, 6).

Focal lesions are found in individuals with a paternally inherited, monoallelic K_{ATP} channel mutation (5–7). Subsequent somatic loss of the maternal allele (most likely caused by paternal isodisomy) leads to a loss of the activities of the K_{ATP} channel and the adjacent tumor suppressors (*H19* and *CDKN1C*) normally expressed by the maternal allele. These cells gain a growth advantage eventually forming a focal lesion of insulin-overproducing β-cells (8).

It has been reported that approximately 40% of patients with K_{ATP} channel hyperinsulinism have monoallelic mutations (9, 10) and that up to 40–60% of surgically treated patients have the focal form (1, 6, 7). However, to date, neither the incidence of focal lesions nor the clinical spectrum of persistent congenital hyperinsulinism has been reported for Asians.

In this study, we performed a comprehensive mutational analysis of Japanese patients with this disorder and correlated the results with the histology of surgically treated patients.

Subjects and Methods

Subjects

The study subjects were 36 Japanese infants with persistent congenital hyperinsulinism. The inclusion criteria were as follows: 1) a plasma insulin level of greater than 3 μU/ml in the presence of hypoglycemia [plasma glucose < 45 mg/dl (2.5 mmol/liter)], 2) hypoglycemia lasting beyond 3 months of age, and 3) the absence of insulinoma. The patients were born in 2005–2010 except for those with hyperinsulinemia-hyperammonemia syndrome who were recruited over a longer period (born in 1999–2009). For mutational analysis, written informed consent was obtained, and the study protocol was approved by the institutional review board.

Mutational analysis

Genomic DNA was extracted from peripheral blood leukocytes using a QIAmp DNA blood kit (QIAGEN, Hilden, Germany) as recommended by the supplier. Then all exons and the exon-intron boundaries of the *KCNJ11*, *ABCC8*, and *GCK* genes were amplified from genomic DNA. For the *GLUD1* gene, only exons 6 and 7 (the antenna domain) and exons 10–12 (the GTP binding domain) were amplified because previously reported mutations were exclusively found in these regions. The amplification conditions and the sequences of the primers are available as supplemental data, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. The amplified products were purified using the Wizard PCR Preps DNA purification system (Promega, Fitchburg, WI) and directly sequenced using the BigDye Terminator cycle sequencing kit (version 3.1; Applied Biosystems, Foster City, CA).

Deletion mutations that might not have been detected by the PCR-sequencing strategy described above were analyzed by multiple ligation-dependent probe amplification (MLPA) of all 39 exons of the *ABCC8* gene. The analyses were performed using SALSA MLPA kit P117 (MRC Holland, Amsterdam, The Netherlands) as recommended by the manufacturer.

[18F]-3,4-dihydroxyphenylalanine (DOPA) positron emission tomography (PET)

[18F]-DOPA PET studies were performed at the PET facility of Kizawa Memorial Hospital basically, as described by Ribeiro *et al.* (11). The scan results were fused with those of a computed tomography scan taken at the same time to localize the focal lesion more accurately.

Laser capture microdissection (LCM)

The scattered islets of patient 10 were sampled by LCM using the PixCell IIe LCM system (Arcturus, Mountain View, CA). DNA was extracted from the pooled islets using a FASTPURE DNA kit (Takara-bio, Ohtsu, Japan). DNA extracted from a normal pancreatic area on the same slide was used as the control.

Results

Patient profiles and mutations

The profiles of the patients and the results of the mutational analyses are listed in Table 1. In patients with elevated ammonia at the initial presentation, only patients 1–5 showed persistent hyperammonemia. Those five had mutations in *GLUD1*. Of the remaining 31 patients, mutations were identified in 19 (61.3%): 18 in *ABCC8*, one in *KCNJ11*, and none in *GCK*. No exonic deletions were identified by MLPA, and the four novel missense mutations were not found in 100 normal controls. p.R836X and p.R998X in *ABCC8* were identified in five and three unrelated patients, respectively, possibly representing relatively common mutations in Japanese.

Interestingly, of these patients with K_{ATP} channel mutations, only two had biallelic mutations, whereas the

TABLE 1. Profiles of the patients with mutations

Patient no.	Gender	Onset	Glucose (mg/dl) [mmol/liter]	Insulin (μU/ml) [pmol/liter]	Ammonia (μg/dl) [μmol/liter]	Mutation					Medical treatment
						Gene	cDNA	Protein	Previously reported?	Parental origin	
1	F	9 months	38 [2.1]	4.8 [33]	83 [49]	*GLUD1*	c.661C>T	p.R221C	yes	ND	F, D
2	M	7 months	30 [1.7]	3 [21]	132 [77]	*GLUD1*	c.797A>G	p.Y266C	yes	ND	F, D
3	F	3 months	29 [1.6]	4 [28]	246 [144]	*GLUD1*	c.1336G>A	p.G446S	Yes	ND	F, D
4	M	10 months	<45 [2.5]	7.7 [53]	154 [90]	*GLUD1*	c.1229A>G	p.N410S	No	ND	F, D
5	M	0 d	10 [0.6]	10 [69]	250 [147]	*GLUD1*	c.1229A>C	p.N410T	Yes	ND	F, D
6[a]	F	2 d	31 [1.7]	30.2 [210]	78 [46]	*ABCC8*	c.382G>A c.3748C>T	p.E128K p.R1250X	Yes, Yes	Biparental	
7	M	2 d	5 [0.3]	7.5 [52]	131 [77]	*ABCC8*	c.2506C>T c.4575_4587del13	p.R836X p.M1524Mfs1539X	Yes, No	Biparental	F, O
8	M	0 d	<45 [2.5]	11 [76]	58 [34]	*ABCC8*	c.4516G>A	p.E1506K	Yes	Mat	F, D
9[a]	F	1 month	<20 [1.1]	42.4 [294]	NA	*ABCC8*	c.2506C>T	p.R836X	Yes	Pat	
10[a]	M	2 d	10 [0.56]	23.5 [163]	NA	*ABCC8*	c.4412-13G>A	—	Yes	Pat	
11[a]	F	0 d	33 [1.8]	46.6 [324]	79 [46]	*ABCC8*	c.3745G>T	p.V1249F	No	Pat	
12[a]	F	3 months	20 [1.1]	5.16 [36]	78 [46]	*ABCC8*	c.2992C>T	p.R998X	Yes	Pat	
13[a]	F	0 d	23 [1.3]	101 [701]	45 [24]	*ABCC8*	c.4608 + 1G>A	—	No	Pat	
14[a]	M	0 d	22 [1.2]	22.7 [158]	75 [44]	*ABCC8*	c.2992C>T	p.R998X	Yes	Pat	
15[a]	M	5 months	33 [1.8]	5.42 [38]	NA	*ABCC8*	c.2992C>T	p.R998X	Yes	Pat	
16[a]	M	0 d	28 [1.6]	38.7 [269]	66 [39]	*ABCC8*	c.331G>A	p.G111R	Yes	Pat	
17	F	2 months	15 [0.8]	9.9 [69]	90 [53]	*ABCC8*	c.61_62insG	p.V21Gfs88X	No	Pat	F, O
18	M	0 d	19.6 [1.1]	44 [306]	79 [46]	*ABCC8*	c.2506C>T	p.R836X	Yes	Pat	F, O
19	F	7 months	35 [1.9]	11.2 [78]	97 [57]	*ABCC8*	c.2506C>T	p.R836X	Yes	Pat	F, O
20	M	4 months	<45 [2.5]	7.5 [52]	84 [49]	*ABCC8*	c.3928_3929insG	p.A1310Gfs1405X	No	Pat	F, O
21	M	2 d	38 [2.1]	3.4 [24]	91 [53]	*ABCC8*	c.4186G>T	p.D1396Y	No	Pat	F
22	F	0 d	9 [0.5]	22 [153]	NA	*ABCC8*	c.2506C>T	p.R836X	Yes	Pat	F, O
23	M	2 d	0 [0]	17.3 [120]	317 [186]	*ABCC8*	c.4412-13G>A	—	Yes	Pat	F, D
24[a]	M	0 d	33 [1.8]	21.9 [152]	75 [44]	*KCNJ11*	c.637G>A	p.A213T	No	Pat	

The clinical data are those at the initial presentation. Of the medically treated patients with monoallelic, paternally inherited K_{ATP} channel mutations (patients 17–23), none reported a family history of hypoglycemia. F, Frequent feeding; D, diazoxide; O, continuous sc injection of octreotide; M, male; F, female; Pat, paternal; Mat, maternal; NA, not available; ND, not determined.

[a] Patients who underwent surgery.

other 17 had monoallelic mutations. Furthermore, 16 of 17 of the mutations were of paternal origin. The single maternally inherited mutation was identical to a mutation previously reported by Huopio *et al.* (12) as a mutation causing hyperinsulinism in infancy and diabetes mellitus in adulthood. In fact, the mother of the patient developed diabetes at the age of 13 yr, and the maternal grandmother developed a mild form of diabetes during adulthood. Therefore, from the results of the mutational analyses, the incidence of a paternally inherited monoallelic mutation suggesting the presence of a focal lesion appears to be much higher in Japanese (84.2% of K_{ATP} channel hyperinsulinism cases).

Clinical studies and LCM studies

None of the patients with paternally inherited K_{ATP} channel mutations responded to diazoxide except for patient 23 who partially responded at the maximal dose of 2.5 mg/kg · d. Pancreatectomy was performed on 10 patients who were resistant to medical therapy, one with a biallelic *ABCC8* mutation (patient 6) and nine with monoallelic paternally inherited mutations, eight in *ABCC8* (patients 9–16), and one in *KCNJ11* (patient 24). [18F]-DOPA PET scans were performed in all patients preoperatively. The patient with the biallelic mutation (patient 6) showed typical diffuse uptake. Of the nine patients with monoallelic mutations, four showed a single focal uptake pattern (patients 9, 12, 15, and 16); two (patients 14 and 24) showed multifocal uptake; and the other three (patients 10, 11, and 13) showed irregular uptake throughout the pancreas, which was difficult to distinguish from that o diffuse lesions. The six patients with focal or multifocal uptake underwent partial resection of the pancreas. Histological examination revealed a single focal lesion in these patients. Five were almost completely cured, and one showed residual but milder hypoglycemia. Of the three patients who demonstrated irregular uptake during the PET study, two underwent subtotal pancreatectomy because their intraoperative findings did not rule out the presence of diffuse lesions. In one of these two patients (patient 13), postoperative histology revealed a large focal lesion in the tail and the body of the pancreas. In the other patient (patient 11), abnormal islets were found throughout the pancreas. The presence of normal islets in part of the pancreas suggested the diagnosis of a giant focal lesion. In the third patient (patient 10) with irregular [18F]-DOPA uptake (Fig. 1B), an arterial stimulation venous sampling study suggested the presence of a lesion in the body or the tail of the pancreas (Fig. 1A). Intraoperatively, no focal lesion could be identified by inspection or palpation. Although the margins of the lesion could not be clearly determined, partial resection was performed at 2.5 cm from the tail. This patient was also clinically cured after surgery. Postoperative histology revealed scattered, relatively large islets with a diameter of up to 700 μm clustered within the tail and the body. Each islet appeared to be separated by normal acinar cells, and no

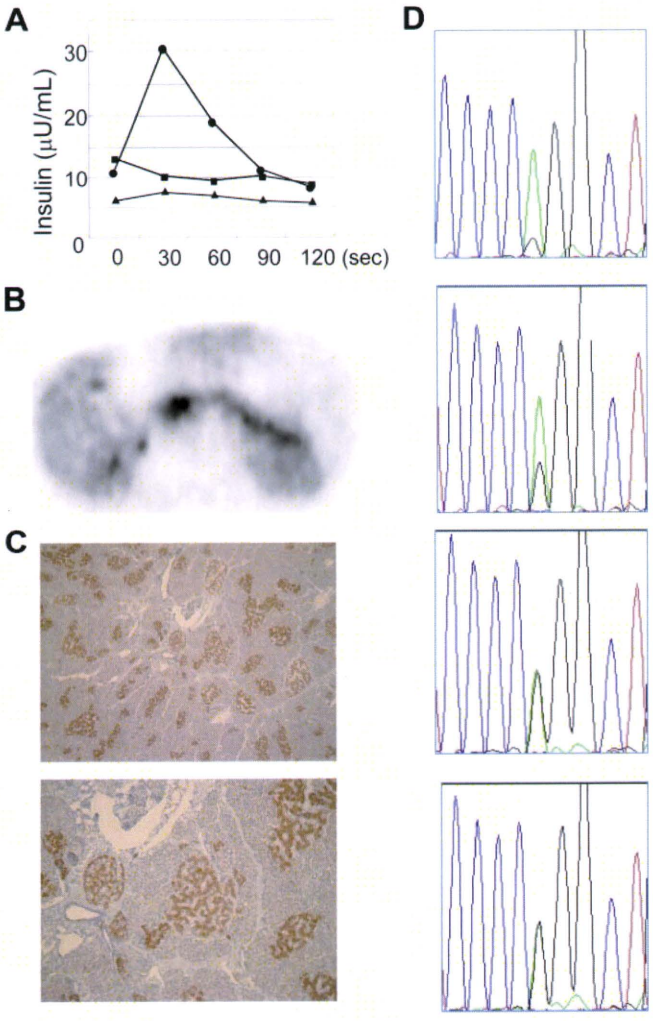

FIG. 1. Results of different diagnostic modalities in patient 10. A, Results of arterial stimulation venous sampling studies. The insulin concentration of the right hepatic vein was measured after the injection of calcium into the splenic (*filled circles*), gastroduodenal (*filled rectangles*), and superior mesenteric (*filled triangles*) arteries. An insulin response was observed only after stimulation of the splenic artery. B, A curved planar reconstruction of a [18F]-DOPA PET scan. The uptake in the head probably reflects an artifact. C, Chromogranin A staining of the resected pancreas showing the area in which abnormal islets were most densely distributed. Magnification, ×40 (*upper panel*), ×80 (*lower panel*). D, Mutational analysis of abnormal islet samples. The *upper two panels* show the results of two separate analyses of 30 (*upper panel*) and 40 (*lower panel*) islet samples. The *lower two panels* show the results of a similar analysis of an adjacent normal pancreatic area. The paternally inherited A allele (*green*) predominates in the abnormal islets, whereas the A and the wild-type G alleles (*black*) have similar intensities in the normal area of the pancreas.

single lesion composed of a solid β-cell cluster was identified by serial sections of the specimen (Fig. 1C). LCM was performed twice to collect samples from 30 and 40 of these islet clusters. Mutational analysis of the pooled DNA collected from these LCM samples revealed the predominance of the paternally inherited mutant allele within these scattered large islets compared with the surrounding normal pancreatic tissue (Fig. 1D).

Discussion

The most important finding of this study is the higher incidence of paternally inherited, monoallelic K_{ATP} channel mutations in Japanese patients with congenital hyperinsulinism ($P < 0.005$ by the sign test), which suggests that the majority of Japanese patients have the focal form. Although the number of patients is small, we believe our results represent the situation of the whole country for several reasons. First, a national survey in 2008–2009 conducted by the Ministry of Health, Labor, and Welfare of Japan estimated the incidence of persistent congenital hyperinsulinism as 1:35,400 births. Our study captured 23% of all cases during that period. Second, the patients were referred without geographical biases because ours is the only laboratory currently offering a comprehensive molecular diagnosis in Japan. Third, a previous report by Ohkubo *et al.* (13) also reported a high frequency (seven of 10) of monoallelic mutations in Japan. In contrast, patients with hyperinsulinism-hyperammonemia syndrome were collected somewhat arbitrarily over a longer period; therefore, the apparent higher incidence might not represent the actual incidence in Japan.

Conflicting results have been reported for the diabetogenesity of p.E1506K in *ABCC8* (12, 14, 15). The association might be a chance observation or might reflect a difference in the genetic background. If the association does exist, that might be due to the specific nature of the mutation, which confers the instability of the β-cells such as altered membrane potential of the cells.

Molecular diagnosis correctly predicted the histology in all patients who underwent pancreatectomy. On the contrary, the ability of [18F]-DOPA PET scans to identify focal lesions was inferior compared with the results of previous reports for other populations (16, 17). Histologically, at least two patients with ambiguous PET results had large focal lesions. The third patient (patient 10) appeared to have unusually scattered islets for a focal lesion. However, there remains the possibility that these islets are actually interconnected and represents a focal lesion with greater admixture of exocrine tissues. Although the number of patients was too small to draw a definite conclusion, larger lesions might be more common in the Japanese.

The reason that the incidence of the focal form of the disease is higher in Japanese is unclear. One possibility is that Japanese have a higher incidence of somatic isodisomy. If this occurred during the earlier stages of development, it would lead to the development of Beckwith-Wiedemann syndrome. However, the incidence of this syndrome caused by paternal isodisomy is not particularly higher in Japanese (18). Alternatively, cells with mutations common in Japanese might be more prone to develop into

a focal lesion, by either promoting a second hit of isodisomy or conferring a growth advantage after the disomic event. Further studies are necessary to address this question.

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Living-donor Liver Transplantation for Progressive Familial Intrahepatic Cholestasis

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Abstract
Background Progressive familial intrahepatic cholestasis (PFIC) results in liver cirrhosis during the disease course, although the etiology includes unknown mechanisms. Some PFIC patients require liver transplantation (LT).
Methods In this study, 11 patients with PFIC type 1 (PFIC1) and 3 patients with PFIC type 2 (PFIC2) who underwent living-donor LT (LDLT) were evaluated.
Results Digestive symptoms after LDLT were confirmed in 10 PFIC1 recipients (90.9%); 8 PFIC1 recipients showed steatosis after LDLT (72.7%), which began during the early postoperative period (71.5 ± 55.1 days). Seven of the eight steatosis-positive PFIC1 recipients (87.5%) showed a steatosis degree of ≥80%, which was complicated with steatohepatitis and resulted in fibrosis. Cirrhotic findings persisted in six PFIC1 recipients even after LDLT (54.5%), and three PFIC1 recipients finally died. The survival rates of the PFIC1 recipients at 5, 10, and 15 years were 90.9%, 72.7%, and 54.5%, respectively. In contrast, the PFIC2 recipients showed good courses and outcomes without any steatosis after LDLT.
Conclusions The clinical courses and outcomes after LDLT are still not sufficient in PFIC1 recipients owing to steatosis/steatohepatitis and subsequent fibrosis, in contrast to PFIC2 recipients. PFIC2 is good indication for LDLT. PFIC1 patients require LT during the disease course; therefore, we suggest that the therapeutic strategies for PFIC1 patients, including the timing of LDLT, under the donor limitation should be reconsidered. The establishment of more advanced treatments for PFIC1 patients is required to improve the long-term prognosis of these patients.

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Introduction

Progressive familial intrahepatic cholestasis (PFIC) refers to a heterogeneous group of autosomal recessive disorders of childhood that disrupt bile formation and present with cholestasis. PFIC is a rare disease, with an estimated incidence of 1 per 50,000–100,000 births [1]. Cholestasis of hepatocellular origin is the major sign in PFIC. The cholestasis appears within the first year of life and leads to death from liver failure at ages from infancy to adolescence [2, 3]. Although the etiology of PFIC still involves unknown mechanisms, the natural course of PFIC causes portal hypertension, liver failure, cirrhosis, carcinoma, and extrahepatic disorders.

PFIC is classified into three types as follows: (1) deficiency of familial intrahepatic cholestasis 1 (FIC1); (2) deficiency of bile salt export pump (BSEP); (3) deficiency of multidrug-resistant 3 (MDR3). Mutations in these genes

are related to the hepatocellular transport system involved in bile formation. The clinical, biochemical, radiological, and histological manifestations of each type have been described previously [1–11].

In PFIC type 1 (PFIC1) patients, cholestasis appears during the first months of life and causes recurrent episodes of jaundice that eventually become permanent. Severe pruritus is observed. The serum γ-glutamyltransferase (γ-GT) and cholesterol levels are normal, but the bile acid (BA) concentration is high. The hepatic histopathology is characterized by canalicular cholestasis and the absence of true ductular proliferation. PFIC1 is caused by mutations in the *ATP8B1* gene, which is designated FIC1 [6–12]. FIC1 is expressed in the liver, pancreas, small intestine, and kidney. The FIC1 protein is located on the canalicular membrane of hepatocytes [13–15]. FIC1 is more highly expressed in the small intestine than in the liver [12]. Taken together, these events lead to BA overload in hepatocytes, impaired bile secretion in cholangiocytes, and extrahepatic features in the intestine [1, 6, 14, 16]. Extrahepatic symptoms (persistent short stature, sensorineural deafness, watery diarrhea, pancreatitis, elevated sweat electrolyte concentration) have been confirmed in PFIC1 patients [9], and enterohepatic circulation should be considered in PFIC1.

Cholestasis with permanent jaundice is more severe in PFIC type 2 (PFIC2) patients than in those with the other PFIC types, although PFIC2 patients share similar laboratory findings with PFIC1 patients. The initial evolution of cholestasis appears during the first months of life and rapidly results in liver failure within the first few years of life. More severe pruritus is observed. The histopathological findings reveal more perturbed liver architecture than is seen in PFIC1, with more pronounced lobular and portal fibrosis and inflammation [2, 8, 9]. PFIC2 is caused by mutations in the *ABCB11* gene, which is designated BSEP [7, 17]. This gene encodes the ATP-dependent canalicular BSEP of the liver. The BSEP protein, which is expressed at the hepatocyte canalicular membrane, is the major exporter of primary BA against extreme concentration gradients. Mutations in this gene are responsible for decreased the secretion of bile salts (BSs), leading to decreased bile flow and accumulation of BSs inside the hepatocytes, which results in severe hepatocellular damage. Extrahepatic features have not been documented in PFIC2. However, hepatocellular carcinoma (HCC) and cholangiocarcinoma occurs at a considerable rate (15%) before 1 year of age [18, 19].

PFIC type 3 (PFIC3) patients show high γ-GT, normal cholesterol, and slightly elevated BA levels. PFIC3 can be distinguished from the other PFICs, because it rarely appears during the neonatal period but manifests during infancy, childhood, and even young adulthood [11, 20]. Pruritus is mild, and the evolution of cholestasis is chronic icteric or anicteric. Therapy with ursodeoxycholic acid (UDCA) may be especially effective for PFIC3 [1, 11, 21].

Regardless of the various types, PFIC patients develop hepatic failure and liver cirrhosis during the disease course. Therefore, it is currently justified that PFIC patients undergo liver transplantation (LT). Here, we present our results for PFIC patients after living-donor LT (LDLT) during two decades and discuss therapeutic strategies for PFIC patients.

Patients and methods

Patients

Since 1990, a total of 735 adult and 702 pediatric recipients underwent LT at Kyoto University Hospital. In all, 717 LDLT recipients whose ages at LDLT were <20 years were enrolled in this study. Among the LDLT recipients, 11 PFIC1 and 3 PFIC2 recipients were evaluated (Table 1); there were no LDLTs in PFIC3 patients. The Ethics Review Committee for Clinical Studies at Kyoto University Graduate School of Medicine approved the study protocol.

The 14 PFIC patients comprised five males and nine females, and their age range at LDLT was 0.6–18.2 years. The mean times from the diagnosis of PFIC to LDLT were 3.89 ± 5.63 years (range 0.21–16.3 years) for the PFIC1 recipients and 0.79 ± 0.75 years (range 0.12–1.60 years) for the PFIC2 recipients. The standard deviation (SD) values for height and body weight at LDLT were −4.5 ± 1.8 (range −7.5 to -1.1) and −2.1 ± 1.0 (range −3.5–0.3), respectively. Growth retardation was confirmed in all patients. One PFIC1 patient (case 5) had a past history of paroxysmal atrial fibrillation.

The serum total BA level was elevated to 439.1 ± 109.8 μmol/ml (range 299–600 μmol/ml), and the γ-GT level was normal at 16.6 ± 4.0 IU/L (range 12–26 IU/L). The mean Child-Pugh score was 7.9 ± 0.8 points (range 7–9 points). The mean score of the Model for End-stage Liver Disease (ages ≥12 years) or Pediatric End-stage Liver Disease (ages <12 years) was 12.3 ± 4.1 points (range 5–19 points). The preoperative statuses were 11 cases of at home and 3 cases of hospitalization. The United Network for Organ Sharing statuses were estimated to be 12 cases of status III and two cases of status IIB.

The donor relationships were 10 fathers, 3 mothers, and 1 grandmother. The mean donor age was 36.9 ± 7.1 years (range 28–47 years). The mean body mass index (BMI) in the donors was 22.3 ± 1.0 kg/m^2 (range 20.5–23.6 kg/m^2). One donor (case 8) was hepatitis B surface antibody (HBsAb)-positive. The ABO blood groups were

Table 1 Histopathological findings after LDLT

Case no.	Steatosis	Steatohepatitis	Fibrosis score (F)[a]	Other factors for fibrosis
PFIC type 1				
1	Severe	Yes	3	–
2	None	No	4	De novo AIH
3	Severe	Yes	4	–
4	None	No	0	–
5	Severe	Yes	3	–
6	Severe	Yes	3	–
7	Moderate	Yes	4	–
8	Severe	No	0	–
9	Severe	Yes	3	–
10	None	No	4	Chronic rejection
11	Severe	Yes	1	–
PFIC type 2				
12	None	Yes	0	–
13	None	Yes	0	–
14	None	Yes	0	–

AIH Autoimmune hepatitis, *LDLT* living-donor liver transplantation, *NASH* nonalcoholic steatohepatitis, *PFIC* progressive familial intrahepatic cholestasis

[a] The NASH score was used in steatosis-positive recipients. The Metavir score was used in steatosis-negative recipients

characterized as 11 cases identical, 2 cases compatible and 1 case incompatible (case 13). The results of lymphocyte crossmatches were negative.

Operation

There were 12 lateral-segment grafts and one case each of extended lateral-segment and left-lobe grafts. The mean graft/recipient weight ratio was 2.08 ± 0.91 (range 1.20–4.02). Histopathological analyses of biopsy specimens during the donor operation were performed in seven cases, and normal findings were confirmed. The mean operating time was 525.4 ± 57.4 min (range 402–636 min), and the mean blood loss was 949.3 ± 833.9 ml (range 105–2610 ml). The mean cold and warm ischemia times were 51.0 ± 29.4 min (range 15–99 min) and 35.9 ± 11.6 min (range 24–56 min), respectively. Biliary reconstruction at the initial LDLT was done by hepaticojejunostomy in 12 cases and by duct-to-duct reconstruction in 2 cases (cases 8 and 14). Histopathologically, cirrhosis without steatosis was confirmed in all of the native livers.

Immunosuppression

Immunosuppression after LDLT was started with tacrolimus and methylprednisolone. The trough level of tacrolimus was maintained at 8–15 ng/ml during the early postoperative period based on the clinical findings in each case. Methylprednisolone was given intravenously (1 mg/kg) once daily from postoperative day (POD) 1 to POD 3 followed by 0.5 mg/kg once daily for the next 3 days. On POD 7, methylprednisolone 0.3 mg/kg was given intravenously. Steroid administration was switched to oral prednisolone 0.3 mg/kg once daily on POD 8. Our regimens for ABO incompatibility were described previously [22, 23].

Histopathological analysis

In our institution, laboratory and ultrasonography (US) examinations are performed routinely after LDLT in all recipients. A liver needle biopsy (LNB) was performed if required based on the results of conventional liver function tests, findings of Doppler US, and consideration of the original diseases. All LNB specimens were strictly assessed by experienced pathologists.

All liver tissues were fixed in neutral-buffered formalin, embedded in paraffin, and sliced into 4 μm thick sections. The morphological characteristics were assessed after standard hematoxylin-eosin (H&E) staining, and hepatic fibrosis was reconfirmed by Masson trichrome and reticulin staining.

Posttransplant steatosis was evaluated as the percentage of hepatocytes involved in steatosis in the liver tissue [24]. Macrovesicular steatosis was graded semiquantitatively according to the percentage of involved hepatocytes as follows [24]: mild <30% of hepatocytes; moderate 30% to 60% of hepatocytes; severe >60% of hepatocytes. The diagnosis of steatohepatitis was defined according to any degree of steatosis, hepatocellular injury in the form of ballooning degeneration and/or Mallory's hyaline, mononuclear and polymorphonuclear infiltration, perisinusoidal fibrosis and portal/lobular inflammation. The fibrosis scores were strictly estimated based on the presence or absence of posttransplant steatosis. Estimation of the hepatic venous area is important at the early phase

of fibrosis progression in nonalcoholic steatohepatitis (NASH) [25], although the fibrosis in other types of hepatitis initially occurs in the periportal area. For assessing posttransplant fibrosis, we used the fibrosis scores in the NASH score for the PFIC1 recipients with steatosis and the Metavir score for the recipients without steatosis. The fibrosis scores in the recipients with steatosis were assigned as follows [25]: 1, perivenular fibrosis; 2, perivenular and periportal fibrosis; 3, bridging fibrosis; 4, cirrhosis. The fibrosis scores in the recipients without steatosis were assigned as follows [26]: 1, periportal fibrosis; 2, bridging fibrosis; 3, precirrhosis; 4, cirrhosis.

Statistical analysis

The survival rates were calculated by the Kaplan–Meier method, with a log-rank test. Statistical analyses were performed using SPSS Software Version 16.0 (SPSS, Chicago, IL, USA).

Results

Clinical course after LDLT

The mean hospital stay after LDLT was 70.7 ± 42.8 days (range 29–189 days). Viral infections and rejection, mainly during the early postoperative period, remain major complications [27]. Epstein-Barr virus and cytomegalovirus infections were detected after LDLT in 6 of 14 PFIC recipients (cases 5, 6, 8, 11–13) and were successfully treated. In all, 7 of the 14 PFIC recipients showed acute cellular rejection (ACR) after LDLT (cases 2, 4, 6, 11–14). Venous and biliary complications remain important [28, 29], and three recipients had stenosis of the hepatic vein or bile duct after LDLT (cases 1, 5, 7). These complications were successfully treated by interventional radiology or reconstruction as soon as possible after their detection.

Digestive symptoms after LDLT were confirmed in 10 of 11 PFIC1 recipients (90.9%) but were not encountered in any of the PFIC2 recipients. Cirrhotic findings including esophageal varix and splenomegaly (the longest diameter was >15 cm on imaging studies) even after LDLT were confirmed in 6 of the 11 PFIC1 recipients (54.5%). These PFIC1 recipients (cases 2, 3, 5, 7, 8, 10) underwent endoscopic or surgical therapy for esophageal varix and splenomegaly, including endoscopic injection sclerotherapy, endoscopic variceal ligation, and splenectomy. One PFIC1 recipient (case 2) suffered from de novo autoimmune hepatitis (AIH) and has been closely followed. Among the PFIC2 recipients, one recipient (case 14) received steroid pulse therapy and muromonab-CD3 therapy for refractory ACR during the early postoperative period, and the therapy was successful. The complications after LDLT are summarized in Table 2.

Histopathological findings after LDLT

Most PFIC patients underwent LNBs at intervals of 1–2 years after LDLT and histopathological follow-up according to these LNBs, although our institution does not employ a protocol biopsy. The mean number of LNBs after LDLT was 8.3 ± 5.1 times/recipient (range 3–23 times/recipient). The histopathological findings are summarized in Table 1.

Steatosis and steatohepatitis in the transplanted liver allografts

In all, 8 of 11 PFIC1 recipients exhibited steatosis after LDLT (72.7%); no steatosis was detected in the remaining 3 PFIC1 recipients. The changes in the degree of steatosis after LDLT in each case are shown in Fig. 1. Steatosis after LDLT in the steatosis-positive PFIC1 recipients seemed to begin during the early postoperative period, as the mean time to the initial confirmation of any steatosis was 71.5 ± 55.1 days after LDLT (range 21–191 days). Seven of the eight steatosis-positive PFIC1 recipients (87.5%) had ≥80% steatosis. The mean postoperative day for the steatosis to reach its peak among the steatosis-positive recipients was 229.6 ± 253.7 days (range 21–736 days). Seven of the eight steatosis-positive PFIC1 recipients had the complication of steatohepatitis (87.5%). In contrast, the PFIC2 recipients did not show any steatosis (Fig. 1).

Hepatic fibrosis in the transplanted allografts

Altogether, 9 of the 11 PFIC1 recipients exhibited fibrosis after LDLT, whereas it was not detected in the remaining 2 PFIC1 recipients. Two of the nine fibrosis-positive PFIC1 recipients (cases 2, 10) exhibited fibrosis without steatosis for other reasons (de novo AIH and chronic rejection, respectively). Only one PFIC1 recipient (case 4) had no steatosis or fibrosis, and another PFIC1 recipient (case 8) had steatosis but no fibrosis (F). Seven of the eight steatosis-positive PFIC1 recipients (87.5%) had F scores of ≥3, although one case stayed at F = 1 (case 11). The mean postoperative day for the F score to reach its peak among the eight steatosis-positive PFIC1 recipients was 1342.7 ± 1168.9 days (range 34–3254 days). The changes in the fibrosis scores after LDLT in each case are shown in Fig. 2. The initial confirmation of any fibrosis after

Table 2 Clinical courses and outcomes after LDLT

Case no.	Digestive symptoms	Complications (POD—treatment)	Outcome (POD)
1	Yes	Biliary stenosis (POD 3962—IVR)	Alive (6884)
2	Yes	ACR (moderate, PODs 100 and 2592—SPT)	Alive (6604)
		De novo AIH (POD 913—steroid)	
		Esophageal varices (POD 2546—EVL)	
3	Yes	Esophageal varices (POD 1624—EVL, EIS)	Dead (5032)
		Splenomegaly (POD 2595—splenectomy)	
		Rupture of splenic artery (POD 5032—hemostasis)	
4	Yes	Intraperitoneal bleeding (PODs 4 and 5—hemostasis)	Alive (5605)
		ACR (mild, PODs 13 and 2595—SPT)	
		Bad compliance of medicine and alcohol drinking	
5	Yes	EBV infection (POD 21—acyclovir)	Dead (4671)
		Bad compliance of medicine	
		Esophageal varices (POD 3529—EVL)	
		Splenomegaly (POD 3864—splenectomy)	
		Fatal dysrhythmia, myocarditis after re-LDLT on POD 4646 (POD 4671)	
		Biliary stenosis (POD 124—reconstruction)	
6	Yes	Cytomegalovirus infection (POD 136—ganciclovir)	Alive (4295)
		ACR (moderate, POD 140—SPT)	
		Intraperitoneal bleeding (POD 2—hemostasis)	
7	Yes	Stenosis of hepatic vein (POD 191—IVR)	Alive (4065)
		Splenomegaly (POD 1806—splenectomy)	
		Biliary stenosis (POD 1836—IVR)	
		Biliary stenosis (POD 48—reconstruction)	
8	No	Cytomegalovirus infection (POD 65—ganciclovir)	Alive (3384)
		Esophageal varices (POD 720—EIS)	
9	Yes	–	Alive (3265)
10	Yes	Chronic rejection (POD 182—Re-LDLT on POD 1393)	Dead (2005)
		Arterioportal shunt (POD 1825—Re-LDLT on POD 1986)	
		Rupture of esophageal varices (POD 2004—hemostasis)	
11	11	Cytomegalovirus infection (POD 33—ganciclovir)	Alive (2028)
		ACR (mild, POD 23—SPT)	
12	No	ACR (mild, POD 13—SPT)	Alive (2453)
		EBV infection (POD 27—acyclovir)	
		Cytomegalovirus infection (POD 34—ganciclovir)	
13	No	ACR (moderate, POD 14—SPT)	Alive (1601)
		EBV and EBV hepatitis (POD 34—acyclovir)	
		Cytomegalovirus infection (POD 103—ganciclovir)	
14	No	Refractory ACR (severe, PODs 7, 14, and 24—SPT and muromonab-CD3)	Alive (500)

The postoperative days (PODs) are shown as the days after the initial LDLT

ACR Acute cellular rejection, *EBV* Epstein-Barr virus, *EIS* endoscopic injection sclerotherapy, *EVL* endoscopic variceal ligation, *IVR* interventional radiology, *LNB* liver needle biopsy, *SPT* steroid pulse therapy

LDLT in the eight steatosis-positive PFIC1 recipients was 327.8 ± 353.4 days (range 34–932 days). As an example, the histopathological findings in case 6 are shown in Fig. 3. In contrast, the PFIC2 recipients did not exhibit any fibrosis (Fig. 2), although one recipient (case 14) temporarily showed an F score of 1 at PODs 39 and 47 owing to refractory ACR that was successfully treated.

Treatment for PFIC recipients after LDLT

All the PFIC1 recipients received UDCA therapy. Therapy with a BA adsorptive resin for PFIC1 recipients has been introduced in our institution [18], and 7 of 11 PFIC1 patients (cases 1, 2, 5–7, 9, 11) received this treatment combined with supplementations of pancreatic enzymes,

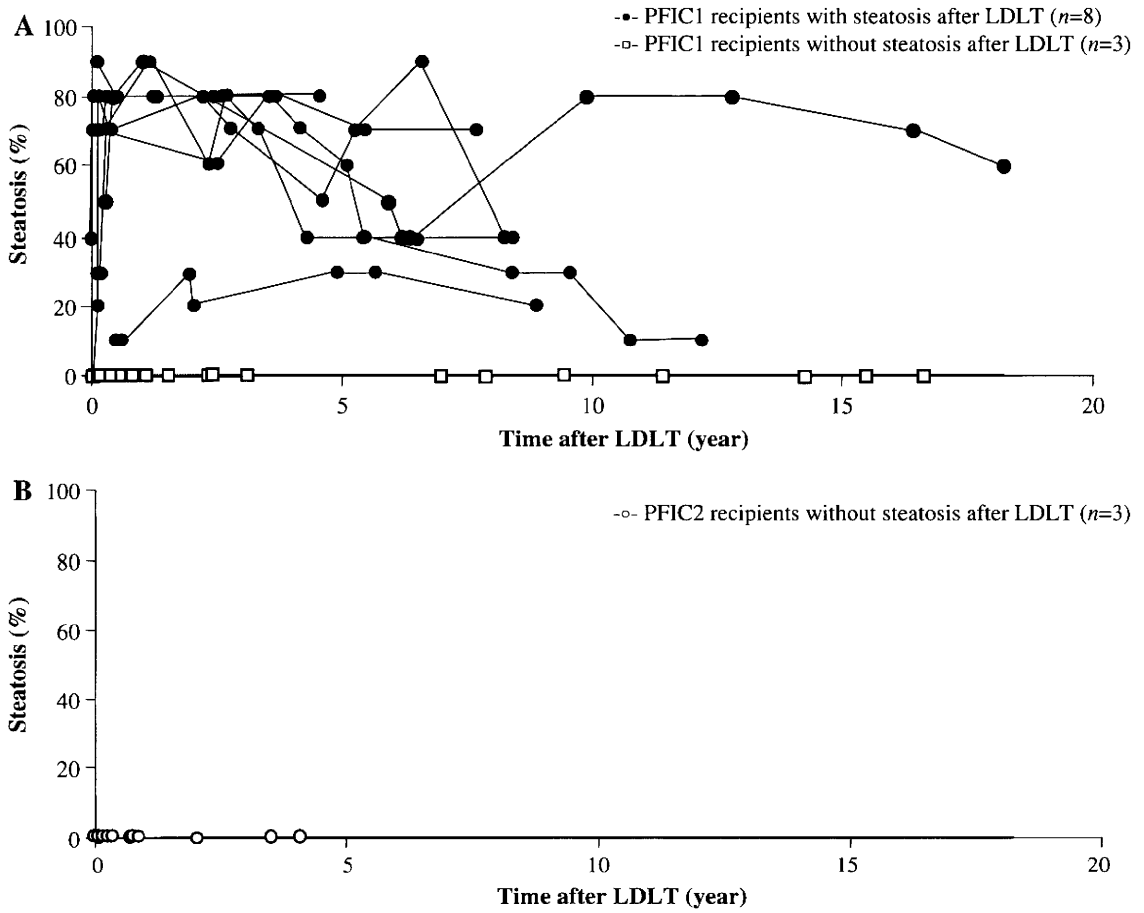

Fig. 1 Time course of steatosis in allografts after living donor liver transplantation (LDLT). **a** Temporal changes in the degree of steatosis after LDLT in progressive familial intrahepatic cholestasis type 1 (*PFIC1*) recipients. Eight PFIC1 recipients presented with steatosis after LDLT, and three PFIC1 recipients did not. Seven of the eight steatosis-positive recipients had the complication of steatohepatitis. *Filled circles* and *open squares* represent the degree of steatosis in PFIC1 recipients with and without steatosis after LDLT, respectively. **b** Temporal changes in the degree of steatosis after LDLT in PFIC type 2 (*PFIC2*) recipients. None of the three PFIC2 patients presented with steatosis after LDLT. *Open circles* represent the degree of steatosis in the PFIC2 recipients

protease inhibitors, bicarbonate, and fat-soluble vitamins. Positive or subtle effects against digestive symptoms were confirmed in all cases, although the symptoms persisted. Regarding the degree of steatosis and the fibrosis scores in the six steatosis-positive PFIC1 recipients who received these combined therapies (cases 1, 5–7, 9, 11), all of the recipients showed temporary responses to these treatments. However, in the histopathological findings of the latest LNBs, the degree of steatosis and the fibrosis scores for these six patients persisted at 46.7% ± 28.0% (range 10–80%) and 3.0 ± 1.1 (range 1–4), respectively. No specific treatment against steatosis were necessary in the three PFIC2 recipients.

Outcomes and survival rates after LDLT in the PFIC1, PFIC2, and other recipients

The mean observation periods were 11.9 ± 4.5 years for the PFIC1 recipients and 4.2 ± 2.7 years for the PFIC2 recipients. In all, 3 of the 11 PFIC1 recipients died, whereas all three PFIC2 recipients survived (Table 2). It should be noted that all three PFIC1 recipients with poor outcomes also had cirrhotic findings even after LDLT. One PFIC1 recipient (case 3) died after rupture of the splenic artery at POD 5032. Another PFIC1 recipient (case 5) underwent retransplantation on POD 4646 owing to graft loss but died from cardiac failure 25 days after the retransplantation. The third PFIC1 recipient (case 10) suffered from chronic rejection at 6 months after the LDLT and underwent retransplantation on POD 1393. Thereafter, an arterioportal shunt after the retransplantation caused graft loss, and yet another retransplantation was performed on POD 1986 after the initial LDLT. However, the esophageal varix ruptured on POD 2005 after the initial LDLT. The survival rates of the PFIC1 recipients at 5, 10, and 15 years after LDLT were 90.9%, 72.7%, and 54.5%, respectively. All three PFIC2 recipients survived. The survival rates of the other 703 recipients at 5, 10, 15, and

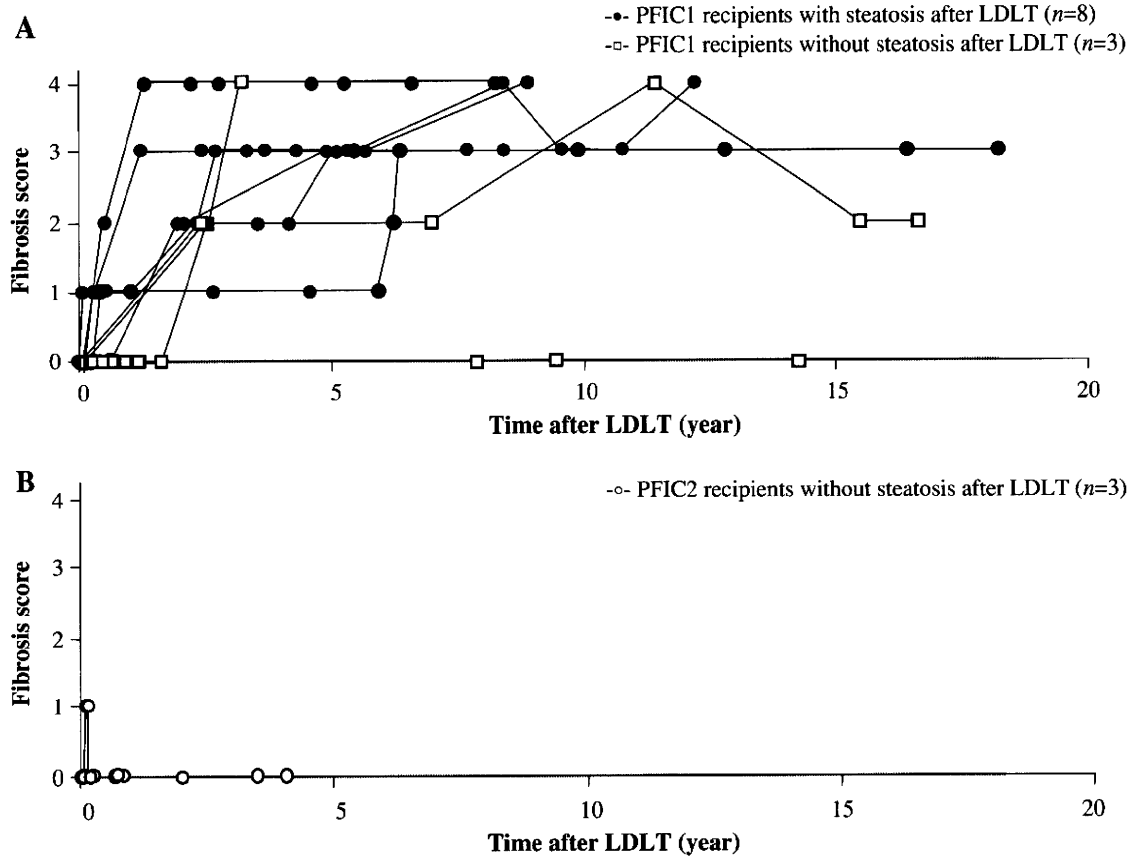

Fig. 2 Time course of fibrosis in allografts after LDLT. **a** Temporal changes in the scores for hepatic fibrosis after LDLT in PFIC1 recipients. Eight PFIC1 recipients with steatosis after LDLT subsequently developed positive fibrosis, and seven of these eight recipients had fibrosis (F) scores of ≥3. Among the three PFIC1 recipients without steatosis after LDLT, one recipient (case 4) showed no fibrosis, and two recipients (cases 2 and 10) had F scores of 4 due to reasons other than steatosis [de novo autoimmune hepatitis (AIH) and chronic rejection, respectively]. *Filled circles* and *open squares* represent the scores for hepatic fibrosis in the PFIC1 recipients with and without steatosis after LDLT, respectively. **b** Temporal changes in the scores for hepatic fibrosis after LDLT in PFIC2 recipients. All three PFIC2 patients had F scores of 0, although one recipient (case 14) temporarily had an F score of 1 at postoperative days (PODs) 39 and 47 owing to refractory acute cellular rejection (ACR), which was successfully treated. *Open circles* represent the scores for hepatic fibrosis after LDLT in the PFIC2 recipients

20 years after LDLT were 83.3%, 79.9%, 77.4%, and 76.5%, respectively.

Discussion

Although our PFIC1 recipients who received UDCA therapy showed only temporary effects and their steatosis and fibrosis persisted, therapy with UDCA (20–30 mg/kg/day) is considered for the initial therapeutic management of PFIC, especially in PFIC3 patients [1, 11, 21], although this therapy cannot be stopped in female patients during pregnancy [30]. We have no experience of LDLT for PFIC3 patients. However, some previous reports have documented PFIC3 recipients who underwent LDLT [31, 32], and LT was required at a mean age of 7.5 years in those patients [1]. We understand that even PFIC3 recipients require LT owing to resultant cirrhosis [33]. In our institution, the PFIC2 recipients maintained good graft conditions and showed excellent outcomes. We suggest that early LDLT may have a sufficient advantage for PFIC2 patients.

Steatosis is categorized in nonalcoholic fatty liver disease [25, 34]. Although steatosis itself is considered to be nonprogressive, steatosis with a developed fibroinflammatory counterpart can develop into cirrhosis [35, 36]. Continuous fat accumulation in hepatic cells is an initial step in the processes that result in necroinflammation and fibrosis in steatohepatitis [25, 37, 38]. Currently, oxidant stress, free fatty acids, lipid peroxidation products, and ATP depletion are focused on as factors that may induce cell injury and subsequent fibrosis in the fatty liver [39, 40]. Our results demonstrated that PFIC1 patients may have persistent steatosis progression even during the early

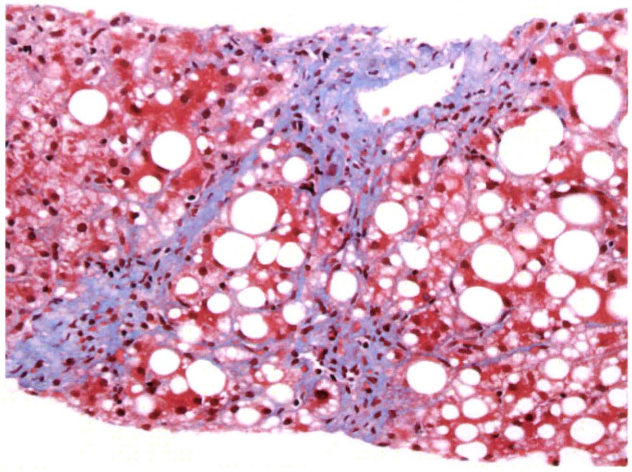

Fig. 3 Histopathological findings of steatosis and subsequent fibrosis after LDLT. A representative section from case 6 shows fibrosis with an F score of 3 at POD 468. In this case, 70% steatosis complicated by severe steatohepatitis was confirmed at POD 69 by hematoxylin-eosin staining (not shown), and the degree of steatosis worsened to 80% at POD 138. Subsequently, this case resulted in hepatic fibrosis with an F score of 3. (Masson trichrome and reticulin)

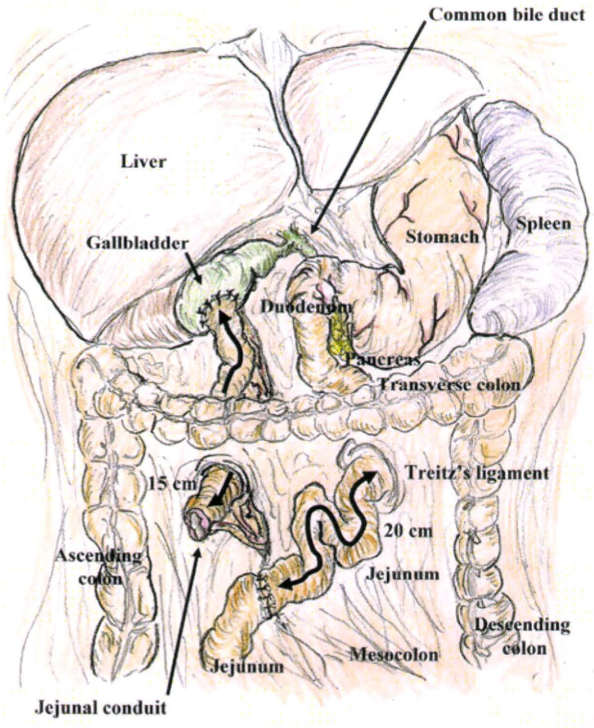

Fig. 4 Surgical technique for partial external biliary diversion (PEBD) in our institution. PEBD was performed as a cholecystojejunocutaneostomy. An isolated jejunal interposition 15 cm in length was made with the proper mesentery at a point 20 cm distant from Treitz's ligament. Next, the proximal side of this interposition was anastomosed to the body of the gallbladder in a side-to-end manner. The jejunal interposition was placed between the gallbladder and the skin; and end-stoma was made in the right lower quadrant of the abdominal wall

postoperative period after LDLT and that steatohepatitis after LDLT can be associated with subsequent fibrosis and allograft failure.

The extrahepatic features in PFIC1 patients do not improve or may be aggravated after LT [1, 9]. Chronic diarrhea may become intractable when biliary BS secretion is restored after LT [6, 9, 16], although diarrhea may be favorably managed by certain medications [9, 16]. Similar to these previous reports, our results confirmed digestive symptoms after LDLT in PFIC1 recipients but not in PFIC2 recipients. The clinical courses of our PFIC1 recipients were not satisfactory, and some of our PFIC1 recipients suffered from cirrhotic findings even a long time after the LDLT. The hyperdynamic state in cirrhotic recipients cannot be restored immediately, even after normalization of the portal pressure by LDLT [41–43]. We suggest that continuous graft damage including fibrosis in the PFIC1 recipients disturbed the restoration of their peculiar hemodynamics and that the persistence of these systemic hemodynamics may have resulted in fatal complications, such as rupture of dilated vessels, even a long time after the LDLT.

The outcomes of LDLT in our PFIC1 recipients are still not sufficient, nor were they in a previous report [44]. Donor selection for LDLT is limited ethically, socially, and medically, although repeated retransplantation can augment the long-term survival of pediatric PFIC1 patients. Our findings for the early postoperative occurrence of steatosis and fibrosis oblige us to reconsider the timing of LDLT and to challenge some other therapies for PFIC1 patients. Partial external biliary diversion (PEBD) has been documented as a possibility for PFIC patients [45, 46]. Some patients with PFIC may benefit from PEBD [47], although its effects remain controversial [45, 46]. The criteria for identifying PFIC patients who could benefit from UDCA or PEBD are unclear [48], although nasobiliary drainage and gene mutations are reported to select potential responders to PEBD [48]. LT represents the only alternative if these therapies fail [49].

After our experience with the 11 PFIC1 recipients described here, in 2009 we introduced PEBD as an anticipatory surgery before LDLT in a female PFIC1 patient aged 1.8 years (Fig. 4). We are closely following this case, and her clinical symptoms, which include itching, bad temper, agrypnia, and digestive symptoms. They fortunately diminished during the first year after PEBD. The histopathological findings in follow-up LNBs revealed that the liver damage has not progressed based on the intraoperative LNB findings. Although we have not had sufficient experiences of PEBD for PFIC1, we now consider this anticipatory surgery before LDLT if the overall considerations, including the donor limitation and patient status,

indicate its possibility. We do not believe that PFIC1 contraindicates LDLT because not all of our PFIC1 recipients necessarily suffered graft losses after LDLT. However, we hope that optimal control by PEBD and possible procrastination with a stable status until LDLT may contribute to the long-term quality of life in PFIC1 patients under the donor limitation situation. On the other hand, we performed total external biliary diversion (TEBD) in one PFIC1 recipient at retransplantation (case 10), although we had no experience with TEBD at the initial LDLT. We cannot confirm the effects of LDLT accompanied by TEBD because this recipient suffered graft loss owing to an arterioportal shunt after the retransplantation.

Only one mutated allele or no mutation is identified in a few PFIC patients (<10%) [1]. Mutations that may map to regulatory sequences of the genes is a possible explanation for this observation. A gene related to the transcription of PFIC genes or protein trafficking could also be involved [50]. It cannot be negated that other unidentified genes involved in bile formation may be responsible for the PFIC phenotypes. The mutated protein may have a dominant-negative effect on the expression and/or function of the protein in a heterozygous state [51]. Modifier genes and environmental influences could play roles in the expression of PFIC [52]. The possibility of PFIC recurrence after LT owing to alloimmunization of the recipient against the FIC1, BSEP, and MDR3 proteins of the donor remains a theoretical matter of debate. 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, and MDR3 gene products [1]. In LDLT based on donor relationships with parents, it can be expected that the heterozygous status of the liver allograft will lead to a predisposition for developing lithiasis or cholestasis favored by immunosuppressive drugs that may interfere with canalicular protein function [53]. We think that this possibility is rare because we performed LDLT in which the donor origins were parents in 10 of 11 cases without PFIC recurrences, and this possible hypothesis was not reported in previous series [49].

Some investigators have documented that more advanced strategies, including cell transplantation, gene therapy, or specific targeted pharmacotherapy, may represent alternative therapies for all PFIC types in the future [48]. Our own results and a review of the mechanisms in previous articles have demonstrated that LT, including LDLT, may have advantages in PFIC2 patients as a definitive therapy and that the clinical courses and outcomes after LDLT are still not sufficient in PFIC1 patients owing to postoperative steatosis/fibrosis. As PFIC1 patients do require LT during the disease course, we suggest that the therapeutic strategies for PFIC1 patients, including the timing of LDLT under the donor limitation, should be reconsidered. The LDLT should not be performed in PFIC1 patients until effective interventions can be made to correct the metabolic defects, although PFIC2 is good indication for LDLT. The establishment of more advanced treatments for PFIC1 patients is required to improve the long-term prognosis.

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Conflict of interest None of the authors has a conflict of interest.

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ORIGINAL ARTICLE

Prescription trends for treatment of paediatric gastroenteritis at a Japanese hospital between 1997 and 2007

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SUMMARY

Objective: **We aimed to investigate recent trends in prescriptions for the treatment of paediatric gastroenteritis in Japan over a 10-year period (1997–2007).**

Methods: **In this retrospective cohort study, we collected data for 2295 prescriptions for 1241 putative cases of paediatric gastroenteritis, which were treated between 1997 and 2007 at Hamamatsu University Hospital, Hamamatsu, Japan.**

Results: **The most frequently prescribed drugs were probiotics (n = 621), followed by anti-emetics (n = 474). In most years between 1997 and 2007, more cases were treated with probiotics than with any other drug type (30·6–63·3% of cases), with the percentage increasing between 2005 and 2007. In contrast, the frequencies of anti-emetic and antipyretic prescriptions remained fairly stable, and prescriptions for antibiotics decreased slightly over the study period. Anti-emetics were commonly used in this hospital.**

Conclusion: **Although experimental evidence upon which to base recommendations is lacking, Japanese evidence-based guidelines are critical for improving the quality of treatment of paediatric gastroenteritis.**

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***Keywords*: children, database, gastroenteritis, guideline, prescription**

INTRODUCTION

The symptoms of infectious gastroenteritis generally include diarrhoea, vomiting and fever. Among children, the major cause of gastroenteritis is viral infection, with viruses such as rotaviruses, enteroviruses, adenoviruses and noroviruses having been implicated in gastroenteritis outbreaks. Worldwide, viral diarrhoeal disease is a leading cause of paediatric morbidity and mortality, with 1·5 billion episodes and 1·5–2·5 million deaths estimated to occur annually among children aged <5 years (1, 2).

The World Health Organization; the European Society of Paediatric Gastroenterology, Hepatology and Nutrition; and the US Centers for Disease Control and Prevention have all issued guidelines for the treatment of children with gastroenteritis (1995, 2001 and 2003, respectively) (3–5). All of these guidelines recommend that even in the absence of signs of dehydration, oral rehydration treatment should be administered, but drugs (e.g. antidiarrhoeal agents, anti-emetics and antibiotics) should not. However, previous studies in the US, Italy and France have revealed that, in many cases, paediatric patients are in fact often being administered such drugs, especially anti-emetics (6–9). Treatment of vomiting in children using anti-emetics remains a controversial issue.

In Japan, infectious gastroenteritis is common among children, with approximately 900 000 to one million episodes being reported annually (10). The Japanese Ministry of Health, Labour and Welfare

(MHLW) has issued a short document on the management of noroviruses, which recommends that antidiarrhoeal agents not be administered, to avoid prolonging the infection (11). To date, no official guidelines on the drug treatment of gastroenteritis have been issued in Japan. Furthermore, there are no data available on the usage of anti-emetics for the treatment of paediatric gastroenteritis in Japan (particularly with respect to formulation). To better understand recent prescription trends with respect to treatment of gastroenteritis among children in Japan, in this study, we gathered data on prescriptions for paediatric gastroenteritis patients treated at Hamamatsu University Hospital between 1997 and 2007.

METHODS

Data for this retrospective cohort study were obtained using the drug order entry system at the Department of Hospital Pharmacy, Hamamatsu University Hospital, Hamamatsu, Japan. The database is based on the physician order entry system providing electronic information on each patient's characteristics including date on visit, birth date, gender, diagnostic code, prescription drugs and related clinical departments. There were 607 beds, and the number of outpatients was 1119/day from April 2007 to 31 March 2008 at Hamamatsu University Hospital.

The study population is consisted of inpatients and outpatients who had been diagnosed with infectious gastroenteritis between 1 January 1997 and 31 December 2007 according to the International Classification of Diseases, Tenth Revision (ICD-10) (12). For inclusion, patients needed to have been aged between 6 months and 6 years at the time a prescription was written.

Age and sex data were collected for each patient. For each prescription, we ascertained the drug name, dosage and clinical department where the patient was when the drug was prescribed. The medicines prescribed were then appropriately categorized using the drug tariff code issued by MHLW.

The study design was approved by the ethical review boards of Kyoto University School of Medicine (No. E-383) and Hamamatsu University School of Medicine (No. 19-144).

RESULTS

Patients and prescriptions

We analysed data for a total of 1241 putative cases of gastroenteritis diagnosed, receiving at least one medication between 1997 and 2007. The median age of patients was 2·1 years, and 56·7% were male (Table 1).

A total of 2295 prescriptions were filled for patients diagnosed with paediatric gastroenteritis during the study period. The 10 most frequently prescribed medication types were probiotics (n = 621 prescriptions), anti-emetics (n = 474), antibiotics (n = 206), antipyretics (n = 180), expectorants (n = 127), antihistamines (n = 109), antitussives (n = 106), antidiarrhoeals (n = 90), antipruritics (n = 55) and bronchodilators (n = 52) (Table 2). Of these, 91·5% were prescribed by staff in the paediatrics department, and 4·3%, 1·4% and 1·0% by staff in the emergency, paediatric surgery and otorhinolaryngology departments.

Changes in prescription trends

For our analysis of changes in prescription trends over the study period, we selected probiotics, anti-emetics, antibiotics, antipyretics and antidiarrhoeal agents for further study, because they were the most common agents directed at the diarrhoea itself or its symptoms. In almost every year between 1997 and 2007, more cases were treated with probiotics than with any other drug type (30·6–63·3% of cases), with the proportion tending

Table 1. Data on the study population and prescriptions

Patients	
Total number of putative cases	1241
Male sex (%)	56·7
Median age (years)	2·1
Prescriptions	
Total number of prescriptions	2295
Median number of medicines co-prescribed	3 (range: 1–10)
Source of prescription (%)	
Paediatrics	91·5
Emergency	4·3
Paediatric surgery	1·4
Otorhinolaryngology	1·0
Other department	1·8

