新たな倫理的問題が生じないよう、常にモニタリングを行い、必要に応じて意見交換を行う。

実験動物を用いる研究については、国立成育医療センター研究所動物実験指針に準拠して研究を実施する。特に、動物愛護と動物福祉の観点から実験動物使用は、目的に合致した最小限にとどめる。またその際、麻酔等手段により苦痛を与えない等の倫理的配慮をおこなう。実験者は、管理者と相互協力のもと適切な環境のもと飼育管理を行う。

C. 研究結果

昨年度に引き続き先天性代謝性肝疾患の患者さんから摘出されたレシピエント肝より、肝細胞を分離することに成功した。また得られた肝細胞を培養下で増殖させることにも成功した。具体的には肝移植の際に生じた余剰肝の供与を受けた。肝組織の詳細は以下の通りである。

Hep2013	女	胆道閉鎖症に対する生体肝移植。レシピエント肝。
Hep2014	女	プロピオン酸血症に対する生体肝移植。レシピエント肝。
Hep2015Rec	女	胆道閉鎖症に対する生体肝移植。レシピエント肝。
Hep2015Don	男	ドナー肝
Hep2016	女	胆道閉鎖症に対する生体肝移植。レシピエント肝。
Hep2017	女	糖原病1b に対する生体肝移植。レシピエント肝。
Hep2018	女	胆道閉鎖症に対する生体肝移植。レシピエント肝。
Hep2019	男	胆道閉鎖症に対する生体肝移植。レシピエント肝。
Hep2020	男	先天性肝線維症に対する生体肝移植。レシピエント肝。
Hep2021rec	男	糖原病 I 型に対する生体肝移植。レシピエント肝。
Hep2022don	男	ドナー肝
Hep2023don	男	ドナー肝
Hep2023rec	女	胆道閉鎖症に対する生体肝移植。レシピエント肝。
Hep2024don	女	ドナー肝

また、分離直後、細胞培養後の肝細胞について凍結保存を行った。

Hep2013	Hep2013rec_ppt（レシピエント肝実質細胞)
	Hep2013rec_sup1（レシピエント肝非実質細胞分画１）
	Hep2013rec_sup2（レシピエント肝非実質細胞分画２）
	Hep2013rec_sup3（レシピエント肝非実質細胞分画３）
Hep2015	Hep2015Don_ppt（ドナー肝実質細胞)
	Hep2015Don_sup1（ドナー肝非実質細胞分画１）
	Hep2015Don_sup2（ドナー肝非実質細胞分画２）
	Hep2015Don_sup3（ドナー肝非実質細胞分画３）
Hep2017	Hep2017sup1（レシピエント肝非実質細胞分画１）
	Hep2017sup2（レシピエント肝非実質細胞分画２）
Hep2018	Hep2018sup2+3（レシピエント肝非実質細胞分画）
Heo2019	Hep2019sup1+2（レシピエント肝非実質細胞分画）

さらに、*in vivo* における特性解析として、前述の初代培養肝細胞を免疫不全マウス（NOG マウス)の大腿四頭筋に移植することにより、各細胞株の分化能の評価を行った。その結果、移植後４週間目に摘出した大腿四頭筋において、抗ヒトアルブミン抗体陽性、抗ヒト肝細胞抗体（CK8/18）陽性、抗ヒトビメンチン抗体陽性を示す細胞を検出することに成功した。

初代培養細胞の多くは20継代から40継代程度の増殖能力を持つものの、継代を重ねるにつれて、増殖能、分化能が低下し、細胞死をむかえることが分かっている。研究に用いる場合、再現性のある結果が求められることから、細胞の性質を保持し、腫瘍化能を持たない寿命延長株が必要であると考え、CDK4、Cyclin D1、hTERT、これら３種類の遺伝子の導入を試みた。その結果、メチルマロン酸血症(MMA)のレシピエント肝細胞、およびCPS1 欠損症のレシピエント肝細胞の寿命延長株作製に成功した。

D. 考察

国立成育医療センターにてインフォームドコンセントが得られたヒト肝組織から、免疫不全マウス体内でアルブミン産生を伴うヒト肝細胞の分離と培養に成功した。また、本研究で凍結保存された細胞株は今後の研究活動を支えるための貴重な細胞ソースとなり得る。

希少疾患である有機酸代謝異常症、尿素サイクル異常症等の先天代謝異常症や遺伝性肝内胆汁うっ滞症では、生体試料の収集が困難と考えられている背景の中で、患者由来の組織より複数の細胞株を樹立できた本研究成果は、品質管理技術開発、合併症発症機序の解明を図る上で大きなアドバンテージと言える。

E. 結論

ヒト余剰肝（疾患肝、正常肝）から効率よく正常な機能を保持した肝細胞を分離、培養、凍結保存し、本事業にて利用できる基盤を整えた。

F. 健康危険情報

なし。

G. 研究発表

なし。

H. 知的財産権の出願・登録状況

なし。

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大浦敏博	シトリン欠損症	総編集　五十嵐隆 専門編集　高柳正樹	小児科臨床ピクシス23「見逃せない先天代謝異常」	中山書店
齋藤　昭彦	小児における抗菌薬治療	山口　徹 北原　光夫 福井　次矢	今日の治療指針201	医学書院
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Living-donor liver transplantation for carbamoyl phosphate synthetase 1 deficiency

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

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

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

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

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

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

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

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

Patients and methods

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

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

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

Results

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

Table 1. Living donor LT for CPS1 deficiency

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

*Prenatal diagnosis

Table 2. Serum amino acid levels before and after LT

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

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

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

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

All donors were uneventfully discharged from the hospital within seven postoperative days. None of the donors showed consistent signs of hyperammonemia in the early postoperative period, and all have been doing well without any episodes suggestive of hyperammonemia.

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

Discussion

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

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

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

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

*Secondary biliary cirrhosis because of biliary anastomotic stricture.

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

In this study, no negative impacts of the use of heterozygous carriers as donors on either donors' or recipients' postoperative course have been observed to date. With respect to the use of heterozygous donors in our review of the patients with CPS1 deficiency, there were no descriptions of mortality or morbidity related to the use of heterozygous donors. Nevertheless, the advisability of using heterozygous carriers as donors should be considered uncertain in some urea cycle deficiencies. With regard to the other disorders, asymptomatic heterozygous carriers will be employed only if there are no other candidates. In such situations, liver tissue must be extracted for enzymatic and/or genetic analyses. A part of the tissue should be used to investigate the correlation between genetic errors and enzyme activities, and the remainder must be preserved for future analyses to precisely evaluate the impact of the use of heterozygous carriers for disorders on the risk and safety of both donors and recipients. It remains essential to conduct worldwide multicenter studies.

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

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

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Original Article

Histological findings in the livers of patients with neonatal intrahepatic cholestasis caused by citrin deficiency

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Aim: To characterize the histological features of the livers of patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), we studied specimens from 30 patients diagnosed with NICCD by genetically analyzing the *SLC25A13* gene.

Methods: Liver biopsy specimens were subjected to hematoxylin–eosin, Azan, and Berlin-blue staining.

Results: Most specimens showed varying degrees of fibrosis. The degree of inflammation varied among the specimens, with half showing moderate or severe inflammatory changes. Fat deposition in hepatocytes was observed in almost all of the specimens, and severe fatty liver was noted in 20 (67%) of them. There was a mixture of two types of hepatocytes with macrovesicular or microvesicular fat droplets, and cholestasis was observed at a rate of 77%. Hemosiderin deposition, mostly mild and localized in periportal hepatocytes and macrophages in portal areas, was observed in 57% of the specimens.

Conclusion: A combination of mixed macrovesicular and microvesicular fatty hepatocytes and the above-described findings, such as fatty liver, cholestasis, necroinflammatory reaction and iron deposition, are almost never observed in other liver diseases in infants and adults. We believe that NICCD is a disease with characteristic hepatopathological features.

Key words: citrin, citrullinemia, fatty liver, fibrosis, neonatal intrahepatic cholestasis caused by citrin deficiency, *SLC25A13*.

INTRODUCTION

SAHEKI *ET AL.* reported that the enzyme abnormalities of citrullinemia can be classified as qualitative, type I and type III, or quantitative, type II.[1,2] The first, the classical form (CTLN1), is found in most patients with neonatal/infantile-onset citrullinemia, and was first described by McMurray *et al.*[3] In CTLN1, the enzyme defect is found in all tissues in which argininosuccinate synthetase (ASS) is expressed.[1,2,4] The second form, type II citrullinemia (CTLN2) is an adult- or late childhood-onset liver disease characterized by a liver-specific defect in ASS, and most of these patients have a fatty liver.[5] This enzyme abnormality is caused by a deficiency in citrin, a calcium-binding

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mitochondrial solute carrier protein which is encoded by the *SLC25A13* gene.[6]

Recently, several cases of *SLC25A13* mutations have been reported in early infancy with cholestatic liver disease.[7–13] Yamaguchi *et al.*[14] designated these findings as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). Citrin deficiency causes two age-dependent phenotypes, CTLN2 in adults and NICCD in infants.[15] Most NICCD patients showed hypoproteinemia, galactosemia, multiple aminoacidemia including citrullinemia, methionemia and tyrosinemia, cholestasis, and have a fatty liver.[7–13] Only a few papers have described the pathology of the NICCD[8,9,11,13] or CTLN2[5] liver.

Therefore, the present study was designed to clarify the histological findings of the NICCD liver.

METHODS

Patients

WE STUDIED THE liver histological findings of 30 patients aged 2.9 ± 1.7 months with a range of 1–7 months consisting of 17 men and 13 women who had been diagnosed with NICCD with *SLC25A13* mutations by genetic analysis including five patients who were documented in previous reports.[7–11] Moreover, mutations in *SLC25A13* were detected in both alleles of 29 patients and in a single allele of one patient. Mutation detection and DNA diagnosis of the *SLC25A13* gene were performed as previously described ([6,14,16] and T. Saheki et al., 2006, unpublished data). Moreover, we examined biochemical data within 1 week before or after liver biopsy for 30 patients with NICCD.

Methods

Liver biopsy specimens from 30 patients diagnosed with NICCD were subjected to hematoxylin–eosin, Azan, and Berlin-blue staining. The grading of fibrosis and inflammation was based on Ludwig's Classification with slight modifications (Table 1).[17] The other histopathological features were graded as none, mild, moderate and severe, and scored as 0, 1, 2 and 3, respectively.

Grading was independently performed by three pathologists, and the grade for each specimen was determined by consensus between two or three of them.

Relationship between age and histological findings

To clarify the relationship between age and the histological findings, the cases were divided into three groups according to their ages: group A, less than 2 months old; group B, 3–4 months old; and group C, more than 5 months old. The average of the grading score of the histological findings for each group was then obtained.

Table 1 Histological classification of liver biopsy

Stage of fibrosis	
Stage 0	No portal fibrosis
Stage 1	Mild to moderate fibrous expansion of portal tract
Stage 2	Bridging fibrosis between portal tracts without lobular distortion
Stage 3	Bridging fibrosis between portal tracts with lobular distortion
Stage 4	Liver cirrhosis
Grade of inflammation	
Grade 0	None (0)
Grade 1	Mild (1–3)
Grade 2	Moderate (4–6)
Grade 3	Severe (≥7)

Parentheses indicate scores derived by Ludwig's scoring system.

Statistical analysis

The data regarding the relationship between age and histological findings were analyzed using the Mantel–Haenszel linear trend test. *P*-values less than 0.05 were regarded as statistically significant.

RESULTS

Patients

THE PROGNOSIS OF almost NICCD patients at 1 year of age was fairly well. However, some NICCD patients had developed progressive liver failure by then. For example, two patients developed liver failure by 6 months (patient 28) and 7 months (patient 30)[10] of age and one patient (patient 9) developed behavioral aberrations, which included shouting and episodes of violence, by 16 years of age.[9,18] Two patients, one with liver failure[10] and one with mental derangement,[9,18] received a living-related liver transplant. Therefore, the outcomes of the NICCD patients were not always favorable. We obtained four sets of follow-up liver biopsy specimens from patients 8, 9, 13 and 18 (data not shown).

From the clinical laboratory data, serum levels of citrulline, α-fetoprotein, ferritin and pancreatic secretory trypsin inhibitor (PSTI) were noted to have generally increased (Table 2). We also detected high serum levels of total and direct bilirubin, aspartate (AST) and/or alanine aminotransferase (ALT), total bile acids and

Table 2 Biochemical data on liver biopsy in the 30 patients with neonatal intrahepatic cholestasis caused by citrin deficiency

Patient No.	1	2	3	4	5	6	7	8	9	10	11
Age (months)/sex	1/M	1/M	1/M	1/M	1/F	1/F	2/M	2/M	2/M	2/M	2/M
Total/direct bilirubin (mg/dL)	9.0/3.4	12.6/2.6	3.3/2.2	10.4/5.8	5.6/1.9	3.3/0.7	6.2/3.8	9.9/5.4	7.6/3.3	6.6/2.6	3.6/1.6
AST/ALT (IU/L)	96/38	31/20	115/61	121/24	62/41	43/21	112/28	109/50	41/20	100/30	190/53
Total bile acids (μM)	250	120	513	298	210	52	323	331	n.d.	240	212
γ-GTP (IU/L)	206	142	131	251	186	148	142	408	130	n.d.	125
Total cholesterol (mg/dL)	212	195	n.d.	181	161	158	175	206	133	n.d.	196
Total protein/albumin (g/dL)	4.9/3.2	3.9/2.6	5.3/4.0	4.5/3.0	5.1/3.5	4.4/3.3	4.7/2.6	-/-	3.6/1.9	-/-	4.7/2.8
Citrulline (nmol/mL)	4.3	n.d.	85.0	n.d.	40.5	149.0	74.3	12.6	n.d.	117.0	211.0
α-Fetoprotein (ng/mL)	n.d.	n.d.	n.d.	200 700	n.d.	n.d.	n.d.	n.d.	29 600	n.d.	n.d.
PSTI (ng/mL)	n.d.	n.d.	n.d.	91.0	n.d.	40.0	24.0	n.d.	n.d.	n.d.	110.0
Ferritin (ng/mL)	447	n.d.	n.d	2656	n.d.	117	502	1830	n.d.	n.d.	n.d.
Prothrombin activity (%)	75	26	93	55	37	88	70	37	9	n.d.	76
Mutation type	V/XIX	I/II	I/I	I/II	II/II	I/V	II/V	II/V	II/II	I/V	I/V

12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2/M	2/F	2/F	2/F	2/F	3/M	3/M	3/M	3/F	3/F	4/M	4/M	4/F	4/F	5/M
10.2/3.9	11.1/3.6	13.0/8.5	6.9/2.7	5.3/2.4	6.1/3.5	6.0/3.8	9.6/2.7	8.8/3.2	12.0/2.6	5.1/2.5	6.7/3.7	5.4/3.5	6.2/2.4	15.0/10.1
106/22	86/23	133/45	78/25	74/44	98/36	232/48	85/44	95/39	75/19	95/90	295/105	208/100	83/24	146/66
240	320	172	290	143	302	269	205	389	157	283	172	253	127	355
213	132	78	209	160	124	249	n.d	149	198	145	270	132	90	129
n.d.	n.d.	204	232	n.d.	194	n.d.	140	223	256	128	169	n.d.	n.d.	n.d.
4.9/3.7	4.0/3.5	3.8/2.6	4.1/2.7	n.d.	5.3/3.9	n.d.	5.7/3.8	5.1/3.1	4.8/3.0	4.2/3.5	4.8/3.1	5.5/3.5	n.d.	4.0/2.8
242.0	478.0	581.0	n.d.	291.7	839.1	208.0	n.d.	32.2	392.0	675.0	524.0	27.5	28.4	5.8
n.d.	n.d.	87 000	n.d.	91 940	n.d.	n.d.	n.d.	n.d.	n.d.	75 300	n.d	n.d.	n.d.	10 578
n.d.	24.0	n.d.	n.d.	57.0	n.d.	n.d.	n.d	62.0	12.9	12.5	n.d	n.d.	n.d.	188.0
743	n.d.	775	n.d.	1651	n.d.	n.d.	n.d.	n.d.	200	n.d.	n.d.	503	n.d.	n.d.
87	n.d.	n.d.	25	51	43	n.d.	66	50	75	29	39	69	15	15
IV/IV	II/II	II/V	II/II	II/II	II/III	I/I	II/II	VI/VI	I/II	I/-	I/II	VIII/X	IV/VI	II/V

27	28	29	30	Mean ± SD	Range	Normal range
5/F	6/M	6/F	7/F			
5.8/3.4	5.5/3.9	6.2/2.0	5.9/2.9	7.6 ± 3.0/3.6 ± 2.0 (n = 30)	3.3–15.0/0.7–10.1	0.2–1.1/0.0–0.4
260/169	123/87	127/38	191/67	120.3 ± 63.7/49.2 ± 33.3 (n = 30)	31–295/20–169	6–40/5–40
213	n.d	150	168	241.3 ± 96.1 (n = 28)	52–513	5–25
67	149	65	292	168.6 ± 75.0 (n = 28)	65–408	5–32
n.d.	148	n.d.	168	183.1 ± 34.8 (n = 19)	133–256	130–220
6.4/4.7	4.5/3.0	4.6/2.7	6.0/3.2	4.7 ± 0.7/3.2 ± 0.6 (n = 25)	3.6–6.4/1.9–4.7	6.5–8.3/3.7–5.2
48.2	11.0	41.3	86.8	179.1 ± 199.2 (n = 25)	4.3–291.7	17–43
n.d.	11 000	329 000	207 000	115 790.9 ± 108 111.0 (n = 9)	11 000–329 000	<10 000
n.d.	n.d.	21.9	n.d	58.5 ± 53.6 (n = 11)	12.5–188.0	22–46
n.d.	n.d	n.d.	197	874.6 ± 816.3 (n = 11)	117–2656	12–80
88	9	41	29	51.3 ± 26.0 (n = 25)	9–93	70–140
I/II	I/II	I/II	I/II			

AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; PSTI, pancreatic secretory trypsin inhibiter; M, male; F, female; n.d., not done; I, 851del4; II, IVS11 + 1G > A; III, 1638ins23; IV, S225X; V, IVS13 + 1G > A; VI, 1800ins1; VIII, E601X; X, IVS6 + 5G > A; XIXIVS16ins3kb; -, unknown; SD, standard deviation.