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Patient Report

Neonatal intrahepatic cholestasis with hepatic siderosis and steatosis

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Abstract Neonatal intrahepatic cholestasis is a heterogeneous disease of undetermined cause. There is an unreported subset of idiopathic neonatal intrahepatic cholestasis with an unusual histological combination of hepatic siderosis and macrovesicular steatosis. The patients were a 34-day-old female and a 39-day-old male with normal birth weights. Their mothers had received oral iron supplement 4–6 weeks before delivery. The patients had obstructive jaundice noticed at the well-baby clinic at 1 month of life. They had high levels of serum galactose and tyrosine, hyperferritinemia. Urinary organic acid and bile acid analyses were negative, and galactose-1-phosphate uridyltransferase activity in red cells was normal. Liver biopsies showed diffuse iron deposits and macrovesicular fat. By substituting formula milk with lactose-free milk, the patients responded, and had normal biochemical tests within 5 months of life. Follow-up biopsies, at the age of 12 months, showed mild residual fibrosis without iron or fat deposits. They are both well at 3 and 6 years of age, respectively, without biochemical liver dysfunction and neurologic impairment. Prenatal iron-overload might contribute to the pathogenesis of the disease, but further studies are needed to confirm the assumption.

Key words fatty liver, hepatic siderosis, hyperferritinemia, neonatal intrahepatic cholestasis.

Neonatal intrahepatic cholestasis has multiple origins, and some forms remain idiopathic.[1] Recently, new diseases presenting clinically with neonatal intrahepatic cholestasis (i.e. bile acid metabolic errors and medium chain coenzyme-A dehydrogenase deficiency) have been disclosed.[2–4] However, many of the cases of idiopathic neonatal intrahepatic cholestasis (INIC) need further studies.

Fatty liver may be seen in patients with neonatal intrahepatic cholestasis of various origins, and metabolic diseases must be considered as one of the candidates. Iron deposits have been also documented in several metabolic diseases.[5–8] To our knowledge, however, the unusual histological combination of diffuse iron deposits and macrovesicular fat in the liver has never been described in INIC. We describe two such cases of what we consider to be a new subset of INIC.

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Methods

In the two patients the diagnosis of INIC was confirmed by the presence of cholestatic jaundice, and by eliminating known causative diseases (i.e. infectious hepatitis, metabolic and endocrine diseases, chemical hepatic injury, and biliary tract diseases). Total serum bile acid concentration was analyzed by an enzyme assay, and serum/urinary bile acid profiles were examined by gas chromatography and gas chromatography-mass spectrometry.[9,10] On ultrasound examination, the grading system reported by Needleman *et al.* was used to evaluate the severity of fatty liver.[11] Total activity of vitamin K dependent coagulant factors (II, VII and X) was tested by normotest (normal, > 60%).[12]

Table 1 Laboratory data

	Patient 1	Patient 2
Total bilirubin (mg/100 mL)	4.8	12.6
Direct bilirubin (mg/100 mL)	2.4	2.6
Total bile acids (μmol/L)*	134	120
AST (IU/L)	28	31
ALT (IU/L)	60	20
LDH (IU/L)	247	298
ALP (IU/L)	678	2230
γ-GTP (IU/L)	214	142
Triglyceride (mg/100 mL)	40	80
Total cholesterol (mg/100 mL)	194	195
Phospholipid (mg/100 mL)	234	314
Sodium (mEq/L)	139	138
Potassium (mEq/L)	4.8	5.6
Chloride (mEq/L)	102	108
Calcium (mg/100 mL)	10.2	6.2
Phosphate (mg/100 mL)	8.6	5.3
Vitamin E/total lipids (mg/g)†	0.85	1.04
Normotest		
Before vitamin K (%)	49	22
After vitamin K‡ (%)	87	47
PIVKA-II§ (μg/mL)	< 1.0	< 1.0
Alpha-fetoprotein (μg/mL)	5.1	16.4
Total protein (g/100 mL)	5.1	3.9
Albumin (g/100 mL)	3.6	2.6
Blood urea nitrogen (mg/100 mL)	8	13
Red blood cells ($\times 10^6$/mm^3)	3.50	3.57
Hemoglobin (g/100 mL)	10.5	11.0
Hematocrit (%)	32	33
Platelet ($\times 10^4$/mm^3)	48	35
White blood cells ($\times 10^3$/mm^3)	16.8	11.8
C-reactive protein (mg/100 mL)	< 0.25	< 0.25

*> 47 μmol/L; †> 0.6 mg/g; ‡after parental vitamin K supplement; §< 1.0 μg/mL.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, gamma-glutamyl transpeptidase.

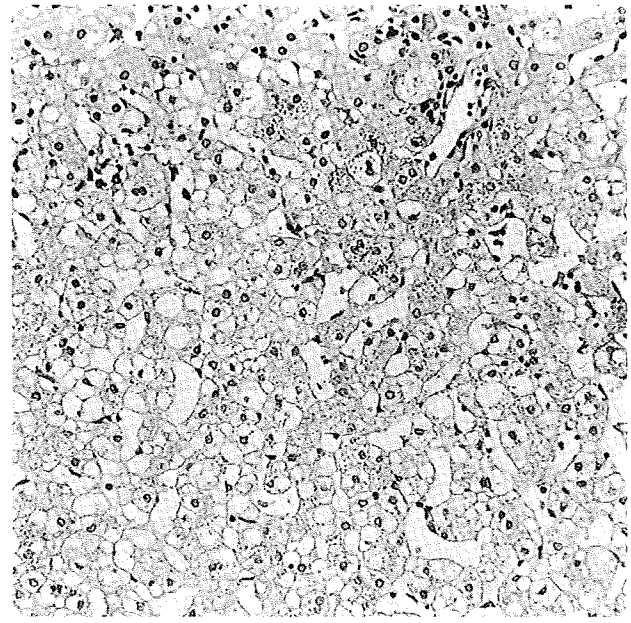

Fig. 1 The first liver biopsy specimen in patient 1 showing diffuse macrovesicular fat deposits (HE).

Patient 1

The first patient was female with a birthweight of 3370 g, born to a 28-year-old mother at full term. The family history for liver diseases was negative. Her parents were not related. The patient's sister, 3-years-old, had been fed with mixed milk in early infancy, and had a history of breast milk jaundice. The blood type of the patient and her mother were the same: A, Rh$^+$. At the age of 3 days, low activity of vitamin K dependent coagulant factors (16%, normal, > 40%; Hepaplastin Test,® Eisai Co., Tokyo, Japan) was noted in a screening examination. She received three serial oral supplements of vitamin K from the age of 3 to 8 days. The Hepaplastin test, however, did not normalize (< 39%) and she received phototherapy for 2 days because of neonatal jaundice. Neonatal screening for metabolic diseases (galactosemia, phenylketonuria, homocystinuria, maple syrup urine disease, and histidinemia, cretinism, and adrenal hyperplasia) was negative. Her mother had received 210 mg of oral iron preparation during the last 4 weeks of pregnancy, but took no medication after delivery. She had no alcohol intake. The parents had normal results of serum iron results. The patient had been fed breast milk alone until 8 days of life, but thereafter received formula milk. Jaundice, acholic stool and dark yellow urine were noticed at the well-baby clinic at 1 month of life, and she was referred to Akita University for further examinations at the age of 34 days. On admission, the patient looked well (bodyweight 4420 g, weight gain, 30 g/day) and normally developed. Facial appearance was normal. She had a normal size liver, and no splenic enlargement. Ophthalmological examination was negative.

Biochemical data on admission were as shown in Table 1. Serum total protein was 5.1 g/100 mL. Other major laboratory results were as follows: Coombs' tests (direct and indirect) negative; blood ammonia 64 μg/100 mL; fasting blood glucose 66 mg/100 mL; total iron binding capacity 157 μg/100 mL (controls, $n = 40$, 30–45 days of life, 255 ± 55); iron 105 μg/100 mL (94 ± 22); ferritin 1459 μg/100 mL (156 ± 67); urinalysis negative; fasting blood galactose 33 mg/100 mL (< 10); serum tyrosine 365 μmol/L (normal, < 148); methionine 49 μmol/L (< 54); and phenylalanine 131 μmol/L (< 104). Serum levels of branched-chain amino acids

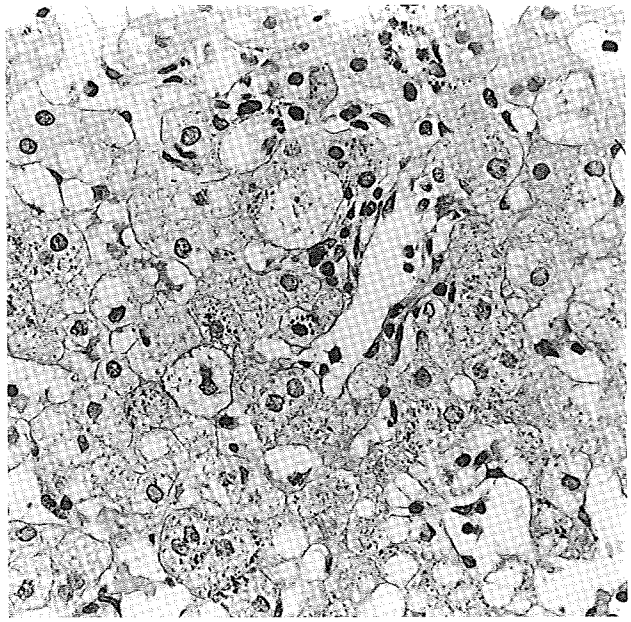

Fig. 2 The first liver biopsy specimen in patient 1 showing diffuse iron deposits in both hepatocytes and Kupffer cells (Prussian blue).

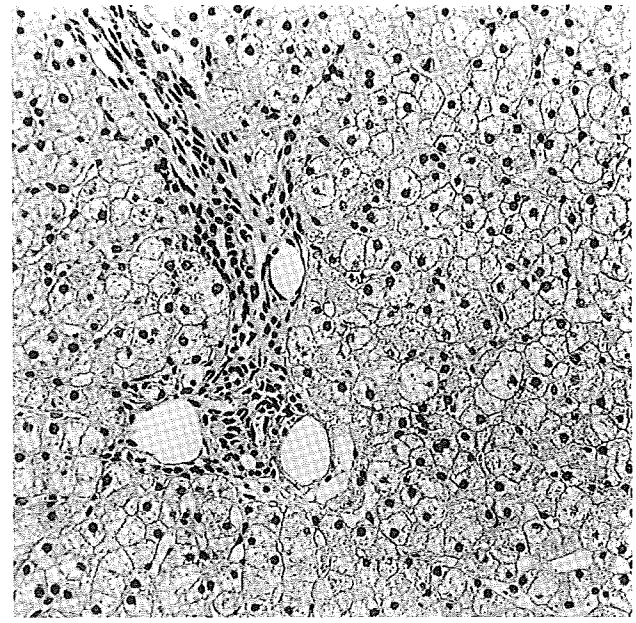

Fig. 3 The second liver biopsy specimen in patient 1 at 12 months of life. Mild portal fibrosis is seen (HE).

were within normal ranges. Blood gas analysis was normal, and blood lactate level was 18 mg/100 mL. Urinary organic acid analyses, including succinylacetone, were negative. Urinary bile acids consisted mainly of primary bile acids, cholic acid and chenodeoxycholic acid. Abnormal urinary bile acids, including long-chain bile acids, were not identified. Δ-4–3-oxosteroid-5-β bile acids were not significantly increased (< 5% of total bile acids). X-rays of the wrist joints showed no signs of vitamin D deficiency. Enzyme activities, galactokinase, galactose-1-phosphate uridyltransferase and uridine diphosphate galactose 4-epimerase in red blood cells were within normal ranges. Sweat chloride test was negative. Ultrasound tomography showed a diffuse mild fibro-fatty pattern with no abnormal portal vein flows. Computed tomography was negative. The patient had a human leukocyte antigen (HLA) typing of A2, A24, B46, B52. Liver biopsy at 48 days of life showed a diffuse macrovesicular fatty liver with iron deposits and mild extramedullary hematopoiesis, but minimal giant cell transformation. Portal fibrosis was slight and interlobular bile ducts appeared normal. Cellular infiltration in both portal and parenchymal areas was minimal (Figs 1,2).

After admission, formula milk was substituted with a lactose-free milk (Lactoless,® Meiji, Tokyo; 13%, 180 mL/kg per day). Abnormal biochemical conditions all gradually ameliorated. Direct hyperbilirubinemia had disappeared within 4 weeks, and complete normalization of all liver functions took 4 months. Percutaneous liver biopsy carried out at the age of 12 months showed mild residual fibrosis without fat or iron deposits (Fig. 3). She is now 6-years-old with normal liver function and normal neurologic development.

Patient 2

The second case was initially reported in 1997 as one of three cases of neonatal cholestasis associated with hypoproteinemia.[13] The male infant with a birthweight of 2700 g was born to a 23-year-old primigravida at full term. Galactosemia was observed by the Paigen method at neonatal mass screening, but galactose-1-phosphate uridyltransferase was proven to be normal by the Beutler method. The family history for liver diseases was negative, and his parents were not related. Blood types of the patient and his mother were the same: A, Rh^+. His mother had received 210 mg of oral iron supplement during the last 6 weeks of pregnancy, but had no alcohol intake. He had been fed solely by breast milk for the first 25 days of life, and thereafter breast feeding was supplemented with formula milk. At the age of 30 days, cholestatic jaundice was noticed, and he was referred to Akita University at the age of 39 days for further evaluation.

On admission the patient looked normally developed and well nourished (bodyweight 4040 g, weight gain 34 g/day). The patient had a normal size liver with a slightly firm consistency. On ophthalmological examination, cataract was not identified. Biochemical data on admission were as shown in Table 1. Serum total protein was 3.9 g/100 mL.

Other major laboratory results were as follows: Coombs' tests (direct and indirect) negative; blood ammonia 75 μg/100 mL; fasting blood glucose 75 mg/100 mL; total iron binding capacity 151 μg/100 mL (controls, $n = 40$, 30–45 days of life, 255 ± 55); iron 103 μg/100 mL (94 ± 22); ferritin 1791 μg/100 mL (156 ± 67); urinalysis negative; and fasting blood galactose 25 mg/100 mL (< 10). Serum tyrosine was increased at 335 μmol/L (< 148), and methionine was 60 μmol/L (< 59). Serum levels of phenylalanine, valine isoleucine and leucine were within their normal ranges. Urinary succinylacetone was negative. Urinary bile acids were mainly composed of primary bile acids, cholic acid and chenodeoxycholic acid. Abnormal urinary bile acids, including long-chain bile acids, were not identified. Δ-4–3-oxosteroid-5-β bile acids were not significantly increased (5.8% of total urinary bile acids). X-rays of the wrist joints showed no signs of vitamin D deficiency. Buccal mucosal and bone marrow biopsies were negative for iron deposits. The three enzymes involved in galactose metabolism in red blood cells were within normal ranges. Sweat chloride test was negative. Ultrasound tomography showed a diffuse mild fibro-fatty pattern without abnormal portosystemic shunts; computed tomography was negative. The patient had a HLA typing of A2, A24, B48, B62. Liver biopsy at 40 days of life showed diffuse macrovesicular fatty liver and hepatic siderosis, and extramedullary hematopoiesis, but minimal giant cell transformation. Portal fibrosis was mild, and the interlobular bile ducts appeared normal. Cellular infiltration in both the portal and parenchymal areas was minimal.

We substituted formula milk and breast milk with lactose-free milk (Lactoless®, 13%, 180 mL/kg per day) for the patient. Biochemical abnormalities quickly responded to this intervention and all ameliorated within 1 week, followed by complete normalization 3 months later. Thereafter, his clinical course was uneventful with normal physical and mental development. Percutaneous liver biopsy carried out at the age of 12 months showed mild residual fibrosis in the portal area without fat or iron deposits in the liver. He is now 3-years-old with normal biochemical liver function tests and neurological development.

Discussion

The two patients presented clinically with idiopathic neonatal hepatitis, high levels of blood galactose and serum tyrosine, and with an unusual histological combination; namely, hepatic steatosis and siderosis. Their mothers had received oral iron supplement before delivery and the patients had hyperferritinemia and hypotransferrinemia, from which the patients quickly recovered without the need for extensive treatment. These clinical, biochemical and histological features indicate an unreported subset of neonatal cholestasis.

Giant cell transformation, a non-specific finding, is seen in hepatic specimens from patients with a variety of types of neonatal cholestasis.[14,15] Some Japanese patients with INIC, however, have minimal giant cell transformation,[16,17] and some have significant fatty liver.[17] Diffuse fat associated with iron deposits in the liver is uncommon in neonatal cholestasis. Such a combination is seen in some metabolic diseases, including hereditary tyrosinemia, galactosemia, Zellweger syndrome, or neonatal hemochromatosis (NHC).[7,8]

The diagnosis of galactosemia, hereditary tyrosinemia or Zellweger syndrome was ruled out in the present cases based on clinical and biochemical examinations. NHC might be considered as a diagnosis for the present cases because it has features in common with neonatal intrahepatic cholestasis, but its clinical course is generally fatal, although there are reports of one survivor,[18] a successful orthotopic liver transplantation for one patient[19] and three others undergoing successful antioxidant therapy.[20] NHC has abnormal iron storage in multiple organs including the pancreas, heart, bone marrow and buccal small salivary glands. Case 2 had no iron deposits in the extrahepatic organs examined. A bile acid metabolic error in NHC has been reported, but the present case 2 had no significant increase of Δ-4–3-oxosteroid bile acids as reported in Δ-4–3-oxosteroid-5-β-reductase deficiency.[21] The two patients had no HLA typings, such as A3-B7 or A3-B14, as seen in primary hemochromatosis.

Iron stores in the liver are relatively high at birth, increase during the first 2 months of life, and then decrease rapidly during the first year of life. Simultaneously, visible iron in the neonatal liver increases at 3–4 weeks.[22] Serum ferritin values should vary correspondingly.[23] Bile acids enhance biliary iron excretion in iron-loaded rats.[24] Accordingly, abnormal bile acids or low/absence of primary bile acids may lead to cholestasis, resulting in secondary hemochromatosis. Iron deposits in the liver have been reported to be common in infants with subacute or chronic liver diseases of recognized etiology.[25] In neonatal cholestasis, biliary atresia and neonatal hepatitis, the parenchymal iron accumulation in the liver without fat accumulation has been reported.[16,25,26]

In a previous report, we presented three cases with hypoproteinemia associated with the unusual histologic combination of hepatic siderosis and steatosis, and speculated that iron-overload and/or malnutrition may have contributed to the pathogenesis of the disease.[13] In the present case 1, however, no hypoproteinemia was proven.

Many pregnant women are prescribed iron supplements. The present mothers had received oral iron preparations prior to their delivery. Excessive iron transport from mother to fetus does not normally occur and iron supplementation to

human mothers results in an increase of the maternal iron store, but essentially in a constant fetal iron store.[27,28] The placenta acts as a barrier against iron overload, but the barrier is permeable, as in the rare disease NHC. Actually, the iron concentration in the human fetal liver shows a striking interindividual variability.[29] Iron deposit induces lipid peroxidation, mitochondrial damage and calcium sequestration, resulting in hepatic cell necrosis, inflammatory cell infiltration, hyperferritinemia, accumulation of triglyceride in the liver, and finally fibrosis.[30] In adult hemochromatosis, fatty liver is an unusual finding, although hepatic steatosis is often observed in neonatal hemochromatosis.[8] According to this evidence, we hypothesize that prenatal iron overload can induce neonatal hepatic steatosis and siderosis in some babies, but further studies are needed to confirm this assumption.

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Idiopathic Neonatal Hepatitis Presenting as Neonatal Hepatic Siderosis and Steatosis

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Idiopathic neonatal hepatitis (INH) is a heterogenous disease of undetermined cause. We report a retrospective histologic reevaluation of INH. Sixty patients with INH were reviewed along with 32 biliary atresia (BA) patients. Histologic findings, iron and fat deposits, giant cell transformation, portal fibrosis, and bile duct proliferation were semiquantitatively graded from 0 to 4+. Significant histologic findings were defined as ≥2+. Frequencies of patients with significant histologic findings in the INH group were compared with those of the BA group. Among the patients with significant histologic findings, those in the INH group had significantly less iron deposits ($P < 0.01$), portal fibrosis ($P < 0.01$), and bile duct proliferation ($P < 0.01$) than those of the BA group. A combination of significant hepatic macrovesicular steatosis and siderosis was observed in 10 INH patients but not in any BA patient (10/60 vs 0/32, $P < 0.05$). Without extensive treatment, the 10 INH patients all recovered, and hepatic abnormalities normalized by the age of 12 months. In conclusion, the present study showed that the recognition of hepatic siderosis is helpful to distinguish BA from INH and that in a subset of INH patients hepatic macrovesicular steatosis and siderosis occurs.

KEY WORDS: neonatal hepatitis; biliary atresia; siderosis; steatosis.

Neonatal hepatitis in the newborn has multiple origins, including definable infections, anatomic variants, and metabolic errors. Idiopathic neonatal hepatitis (INH), however, is of unknown origin that remains to be determined (1, 2).

Clinicians continue to be challenged by cholestatic infants. The diagnosis of biliary atresia (BA) is a major task for pediatric hepatologist. Among many procedures, liver biopsy is still the most valuable procedure in making a diagnosis of BA and differentiating INH. Findings of bile duct proliferation and portal fibrosis have been reported to be useful for this purpose (3, 4).

Fatty liver in patients with neonatal hepatitis has various causes, including a variety of metabolic diseases (5, 6). Hepatic steatosis associated with siderosis has been also documented in some metabolic diseases (7, 8). To our knowledge, however, a histological combination of hepatic steatosis and siderosis in INH has not been reported.

In this study, we present a retrospective histologic reevaluation of INH in comparison with those of BA, particularly in terms of iron and fat deposits, and delineate an unusual subset associated with diffuse macrovesicular fat and iron accumulations in the liver in INH.

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MATERIALS AND METHODS

During the last 10 years (1986–1995), we evaluated 60 patients with INH, ranging in age from 34 to 126 days, who had undergone percutaneous liver biopsy. The clinical diagnosis of INH was confirmed by: (1) the presence of cholestatic jaundice (jaundice, acholic stool, and dark yellow urine), (2) the presence of a conjugated bilirubin fraction of ≥2 mg/100 ml or a total bilirubin of ≥20%, and (3) by eliminating known causes such as infectious hepatitis, metabolic diseases, chemical liver injury, and biliary tract diseases. For histological study, 32 BA patients ranging in age from 30 to 60 days were also examined.

Liver specimens obtained by percutaneous liver biopsy were routinely processed and stained with hematoxylin–eosin, Elastica–Masson, and Prussian blue stains. Histological findings were graded from 0 to 4+ by a semiquantitative method. The patient were classified as 0 in the absence of findings, and as 1+, 2+, 3+ and 4+ when the findings were slight, mild, moderate, and severe, respectively.

The histologic findings of fat accumulation were graded from 0 to 4+, according to the amount of fat deposits, ie, those without fat deposits were classified as 0, those with less than 10% hepatocytes as 1+, those with 10–25% hepatocytes as 2+, those with 25–50% hepatocytes as 3+, and those with more than 50% hepatocytes as 4+. Types of fat droplets in the liver were defined as macrovesicular when the fat globules were larger than the nuclei and as microvesicular when the fat globules were equal to or smaller than the nuclei. The histological findings of iron accumulation were graded: 0, none; 1+, fine granules only in a few of periportal cells; 2+, fine and coarse granules in periportal cells; 3+, many fine and coarse granules in one half to two thirds of the cells; 4+, many fine and coarse granules in two thirds to almost all cells. The histological findings of giant cell transformation were observed microscopically in a middle power field (×200) and graded: 0, none, 1+, less than 3 giant cells; 2+, less than 10 giant cells; 3+, less than 25 giant cells; and 4+, more than 25 giant cells. The histological findings of portal fibrosis were graded: 0, none; 1+, periportal expansion, minimal; 2+, periportal expansion, mild; 3+, periportal expansion, moderate; and 4+, periportal expansion, severe (bridging fibrosis or cirrhosis). The histological findings of bile duct proliferation were also graded: 0, none, 1+, mild bile duct proliferation in some portal tract; 2+, mild bile duct proliferation in each portal tract; 3+, moderate bile duct proliferation in each portal area; and 4+, severe bile duct proliferation in each portal area.

All specimens were examined and recorded by one of authors (Y.T.) who was not informed of the clinical findings. The reproducibility of this scoring system was assessed by two observers (Y.T. and F.N.). Intraobserver and interobserver variations were insignificant. Fat or iron accumulations, giant cell transformation, portal fibrosis, and bile duct proliferation were defined as significant when the grades were greater than 2+. Histologic abnormalities found in the INH patient group, as well as the frequencies of patients with significant histologic findings, were compared with those of BA.

Of 10 patients, all of normal birth weight and with significant hepatic steatosis and siderosis, two patients had no iron deposits in extrahepatic organs, buccal mucosa, and bone marrow, as proven by biopsies. Failure to thrive, that is, poor weight gain, was defined as body weight < 3 percentile for age.

Without extensive treatment, 58 INH patients including the 10 patients with significant hepatic steatosis and steatosis recovered, and hepatic abnormalities normalized by the age of 12 months. The two patients without fat and iron deposits had persistent liver dysfunction. Follow-up biopsies at 12 months of age in three of the 10 patients showed residual portal fibrosis without fat and iron deposits in two of these three.

Statistical analysis was based on chi-square test.

TABLE 1. SEMIQUANTITATIVE ANALYSIS OF HISTOLOGICAL FINDINGS IN PATIENTS WITH IDIOPATHIC NEONATAL HEPATITIS AND BILIARY ATRESIA

Histologic finding	*Grade* (N)				
	0	*1+*	*2+*	*3+*	*4+*
Idiopathic neonatal hepatitis (N = 60)					
Iron deposits	43	6	5	6	0
Fat deposits	37	10	6	6	1
Giant cell transformation	18	26	10	3	3
Portal fibrosis	10	25	22	2	1
Bile duct proliferation	54	3	2	1	0
Biliary atresia (N = 32)					
Iron deposits	6	8	10	6	2
Fat deposits	28	2	2	0	0
Giant cell transformation	13	8	7	2	2
Portal fibrosis	0	2	11	13	6
Bile duct proliferation	1	2	6	10	13

RESULTS

Iron deposits seen in 17 of the 60 INH patients (28%) were significant in 11 cases, and iron deposits present in 26 of 32 BA patients (81%) were significant in 18 cases. Fat deposits were found in 23 of the 60 INH patients (38%) including 10 with microvesicular fat deposits of grade 1+. Significant macrovesicular fat deposits were seen in 13 INH patients. Fat deposits were found in four of 32 BA patients (13%). All four patients had microvesicular deposits, less than grade 2+. Giant cell transformation was observed in 42 of the 60 INH patients (70%) and in 19 of the 32 BA patients (59%). Significant giant cell transformation was seen in 16 of the 60 INH patients and 11 of the 32 BA patients. Portal fibrosis was observed in 50 of the 60 INH patients (83%) and in all 32 BA patients (100%). Significant portal fibrosis was seen in 16 of the 60 INH patients and 11 of the 32 BA patients. Bile duct proliferation was observed in six of the 60 INH patients (10%) and in 31 of the 32 BA patients (96%). Significant bile duct proliferation was seen in three of the 60 INH patients and 29 of the 32 BA patients (Table 1).

TABLE 2. FREQUENCIES OF PATIENTS WITH SIGNIFICANT HISTOLOGICAL FINDINGS IN PATIENTS WITH IDIOPATHIC NEONATAL HEPATITIS AND BILIARY ATRESIA

Histologic finding	*Idiopathic neonatal hepatitis (N = 60)*	*Biliary atresia (N = 32)*	P*
Iron deposits	11/60 (18%)	18/32 (56%)	<0.01
Fat deposits	13/60 (21%)	2/32 (6%)	NS
Giant cell transformation	16/60 (26%)	11/32 (34%)	NS
Portal fibrosis	25/60 (41%)	30/32 (87%)	<0.01
Bile duct proliferation	3/60 (5%)	29/32 (90%)	<0.01

* NS, not significant.

Among the patients with significant histologic findings, those in the INH group had significantly less iron deposits, portal fibrosis, and bile duct proliferation than those of the BA group (Table 2). A combination of significant hepatic macrovesicular steatosis and siderosis (Figure 1) was observed in 10 INH patients, but not in the BA group (10/60 vs 0/32, $P < 0.05$). In the 10 INH patients with significant hepatic steatosis and siderosis, mild fibrosis was observed (grades 1+ and 2+) without bile duct proliferation.

Thirty of the 32 BA patients (93%) had normal weight gain. Forty-two of the 60 INH patients (70%), including seven of the 10 INH patients with significant hepatic steatosis and siderosis, had normal weight gain.

DISCUSSION

Findings of bile duct proliferation and portal fibrosis have been reported to be valuable for the diagnosis of BA and the differential diagnosis of INH (3, 4). Moreover, the present study indicates that findings of significant hepatic iron deposits and the combination of significant hepatic macrovesicular steatosis and siderosis are helpful to distinguish BA from INH.

Giant cell transformation, a nonspecific finding, can be seen in hepatic specimens from patients with a variety of neonatal cholestasis (9, 10). We previously reported that Japanese patients with INH had minimal giant cell transformation (11). The present study showed the same result.

Hepatic steatosis associated with siderosis has been documented in some metabolic diseases, such as neonatal iron storage disease (NISD), Zellweger syndrome, hereditary tyrosinemia, and galactosemia (5, 6) but has rarely been reported in INH. NISD would naturally be considered as one possible diagnosis for the present 10 patients with the significant hepatic macrovesicular steatosis and siderosis. NISD has features in common with INH, the clinical onset is early in neonate, and the clinical course is uniformly fatal (8, 12). These clinical features are different from those of the present 10 patients. Furthermore, in NISD there is abnormal iron storage in extrahepatic organs, including the pancreas, bone marrow, and buccal mucosa (12). The present two cases examined had no extrahepatic iron deposits.

The present cases had diffuse iron and fat accumulations in the liver with accompanying mild fibrosis. Iron deposits induced lipid peroxidation and mitochondrial damage, resulting in hepatic cell necrosis, accumulation of triglyceride in the liver, and finally fibrosis (13). Iron overload to the liver may result in fat accumulations and fibrosis.

Iron deposits in the liver have been reported to be common in infants with subacute or chronic liver disease of recognized etiology (14). Bile acids enhance biliary iron excretion in iron-loaded rats (15). Accordingly, cholestasis may promote hepatic siderosis or delay the mobilization of hepatic iron stores into bile. In neonatal cholestasis, BA and INH, parenchymal iron accumulation in the liver has been reported. Significant iron deposits, however, are uncommon in INH patients (16, 17). In general, BA patients have normal weight gain while some INH patients, as shown in the present study, have difficulties in gaining weight. Malnutrition may lead to fatty liver; three of the 10 INH patients with hepatic siderosis and fatty liver failed to thrive, while the remaining seven patients had normal weight gain. The facts suggest that postnatal iron overload in the liver caused by cholestasis induces a histologic finding of hepatic siderosis, as in BA, which does not result in a histologic combination of hepatic siderosis and steatosis seen in the 10 INH patients. Other factors may also contribute to the pathogenesis of the disease.

NISD was generally thought of as a syndrome arising from severe fetal hepatic injury or from excess transplacental iron transport. Massive doses of iron sometimes cause death in experimented animals, but no iron-induced liver changes are seen in surviving animals (18). Excessive iron transport from the mother to the fetus does not normally occur, but the iron concentration in the human fetal liver shows a striking interindividual variability (19). Accordingly, we can not deny the possibility of prenatal iron overload as a cause contributing to the pathogenesis of the disease. The present cases with macrovesicular fat and iron accumulations in the liver may represent mild NISD, resulting from prenatal iron overload caused by excess transplacental iron transport or by nonsevere hepatic injury of unknown cause.

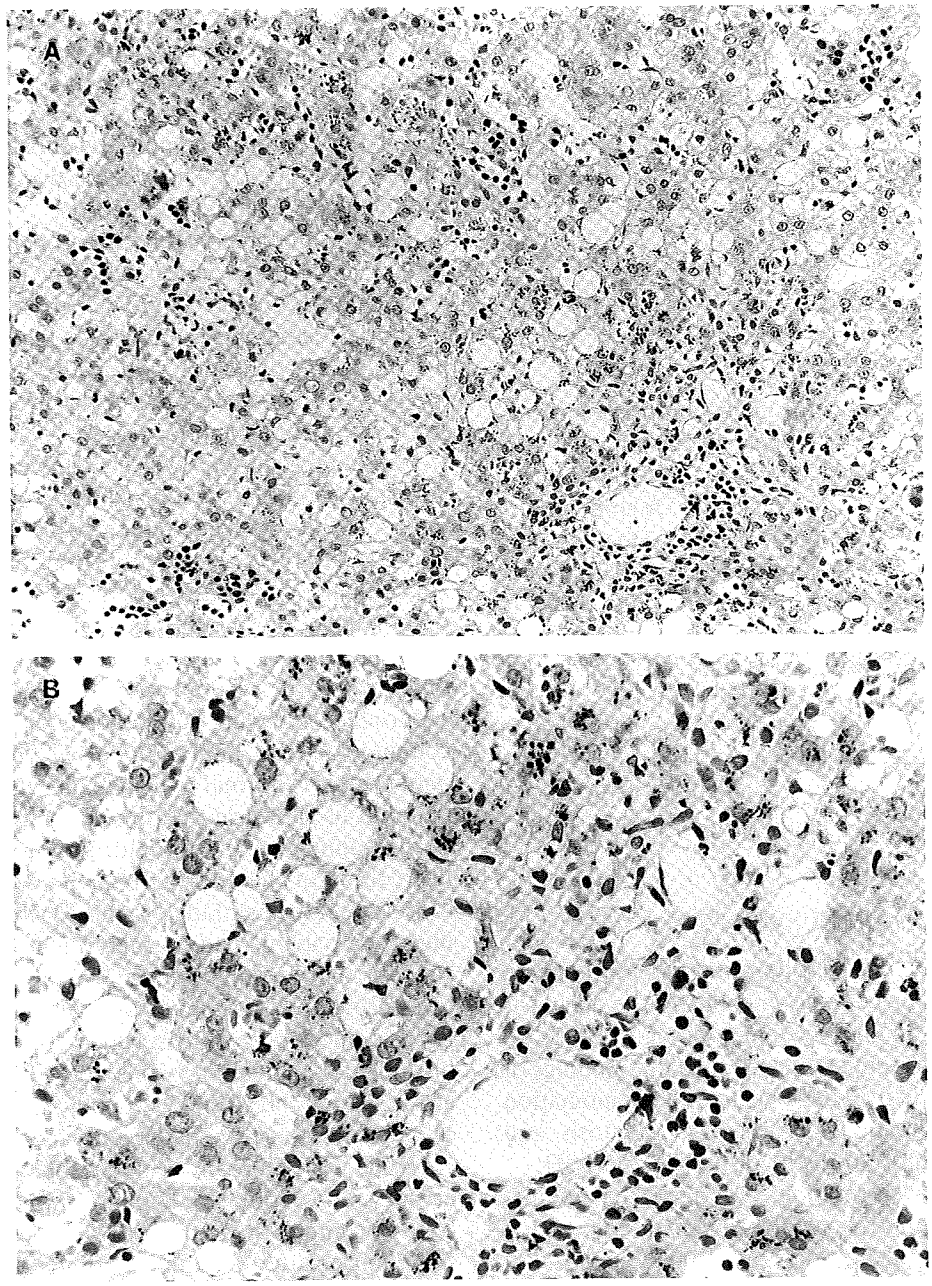

Fig 1. Histological findings in patient, 53 days old life: diffuse macrovesicular fat accumulations in the parenchyma and diffuse iron deposits in hepatic and Kupffer cells (Prussian blue stain; A: ×200, B: ×400).

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特集 肝・消化管疾患の新しい臨床

Ⅰ．肝疾患

NICCD（neonatal intrahepatic cholestasis caused by citrin deficiency）の病態と診断

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Key Words
シトリン欠損症
新生児肝内胆汁うっ滞
脂肪肝
シトルリン血症
新生児マス・スクリーニング

要旨

シトリン（肝型aspartate-glutamate carrier:AGC）は，ミトコンドリア内膜に局在する膜貫通輸送体の一つである．このシトリン欠損による新生児肝内胆汁うっ滞（neonatal intrahepatic cholestasis caused by citrin deficiency:NICCD）の変異ホモ接合体の頻度は1/19,000（日本）であり，常染色体の遺伝性疾患としては高頻度である．NICCDは，閉塞性黄疸，新生児マス・スクリーニングが発見の契機となるが，その特徴は，脂肪肝，多種高アミノ酸血症，低蛋白血症である．NICCDの治療は新生児肝炎の治療に準ずるが，大部分の症例は1歳前に改善し，乳児期以降は，見かけ上は健康な時期（適応・代償期）を過ごす．NICCDのフォロー・アップで重要なのは，肝硬変合併の有無と脳症合併時の治療である．

はじめに

近年まで，わが国における肝障害を伴う代謝性疾患としては，ガラクトース血症，遺伝性チロシン血症（Ⅰ型），糖原病，Wilson病，胆汁酸代謝異常，肥満に合併する脂肪肝（あるいは脂肪性肝炎）などが重要な疾患としてあげられてきた．欧米で報告されるalpha-1-antitrypsin欠乏症，膵線維嚢胞症（cystic fibrosis of the pancreas），遺伝性果糖不耐症（furuclose intolerance），Niemann-Pick病（type C），arginase deficiency（argininemia），UDP-galactose-4'-epimerase deficiency，fructose-1,6-diphosphatase deficency，Zellweger症候群，neonatal adrenal leukodystrophy，新生児ヘモクロマトーシス，脂肪酸代謝異常，Wolman disease，コレステロールエステル蓄積症，β-lipoprotein deficiencyなどのわが国での例はきわめてまれ，あるいは報告例は認めていなかった．

さてシトルリン血症（CTLN1）を含む尿素サイクル代謝異常では，肝腫大，脂肪肝，肝機能異常，肝線維化（肝硬変）などが報告されてきたが，arginase deficiencyを例外として，胆汁うっ滞合併の報告はなかった．しかし，シトリン欠損による新生児肝内胆汁うっ滞（neonatal intrahepatic cholestasis caused by citrin deficiency:NICCD）の発見により，新生児・乳児期に胆汁うっ滞を合併する代表的疾患として位置づけられた．本稿では，NICCDの基礎と臨床を概説する[1)~14)]．

シトルリン血症の分類

ASS遺伝子異常（古典的型シトルリン血症，CTLN1:OMIM #215700）とSLC25A13遺伝子異常に分類され，後者には，成人発症Ⅱ型シトルリン血症（CTLN2:OMIM #603471）とシトリ

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表1 *ASS* および *SLC25A13* 遺伝子異常の臨床像

	発症時期	遺伝子異常	責任遺伝子	そのほか
CTLN1	新生児・乳児期	*ASS*	*9q34*	全身のASS*蛋白質低下
CTLN2	成人期	*SLC25A13*	*7q21.3*	肝臓特異的ASS蛋白質低下**
NICCD	新生児・乳児期	*SLC25A13*	*7q21.3*	

* ASS, argininosuccinate synthetase
** 肝ASS mRNAおよびASS遺伝子の異常はない

ン欠損による新生児肝内胆汁うっ滞（NICCD: OMIM #605814）に分類される（**表1**）.

このほか，シトルリン血症は，ASA尿症（argininosuccinate lyase欠損症），リジン尿性蛋白質不耐症（lysinuricprotein intolerance），ピルビン酸カルボキシラーゼ欠損症状などでも認められる.

SLC25A13変異と頻度[11) 13)]

これまで，CTLN2約140例，NICCD約150例の解析により，27種のSLC25A13遺伝子の変異が同定されている．現在のところ，大部分の症例は日本人であるが，台湾，中国，ベトナム，イスラエルなどでも発見され，シトリン欠損症が日本に限定されることなく広く分布していることが示されている.

12種の変異に関してヘテロ接合体の検索が行われ，日本人では1/69の頻度で保因者が発見された．この割合から計算される変異ホモ接合体（両alleleに変異遺伝子をもつhomozygoteならびにcompound heterozygote）の頻度は1/19,00となり，常染色体の遺伝性疾患としては高頻度であることが判明した．日本以外の変異ホモ接合体の頻度は，台湾1/13,000，中国1/24,000，韓国1/49,000である．日本人症例では5種の変異が比較的高頻度に認められ，とくに［I］：851del4および［II］：IVS11＋1G＞Aが高頻度に発見されている.

しかし，①家系内にCTLN2未発症の変異ホモ接合体がいる，②高齢（79歳）発症のCTLN2の患者さんがいる，③NICCDでは男女差がないが，CTLN2では男性例が多い，④CTLN2の患者さんの中にはてんかんなどの精神科疾患，膵炎，肝がん，高脂血症などの疾患として診断され，治療を受けている可能性がある，⑤タンデムマスを用いた新生児スクリーニングでは，NICCDは1/34,000の頻度で発見されているが，1991年の全国調査によるCTLN2の調査では1/230,000，血族結婚率の計算では1/100,000と推測される.このことから，変異ホモ接合体のほとんどはNICCDを経験すると考えられるが，CTLN2の発症とその遺伝子的背景あるいは環境因子などは不明のままである.

シトルリンの機能[11) 13)]

シトリン（肝型AGC）は，ミトコンドリア内膜に局在する膜貫通輸送体の一つである（aspartate-glutamate carrier，以下AGCと略す）．肝臓におけるシトリンの役割は以下である（**図1**）.

①アンモニアからの尿素合成において，ミトコンドリアから細胞質へaspartate（Asp）を供給する．アンモニアはミトコンドリア内でglutamate（Glu）を経てAspとなり，Aspはミトコンドリア膜のAGCを介してGluと交換されて細胞質に輸送され，細胞質でASS反応に供給される．通常，AspはGluからアミノ基転移酵素により簡単に合成される非必須アミノ酸であるが，Aspの炭素骨格であるオキザロ酢酸（oxaloacetate，以下OAAと略す）は，主としてミトコンドリアで生成されるので，Aspの合成も主としてミトコンドリアであると考えられる．尿素合成以外に，蛋白質合成，ピリミジンヌク

レオチド合成にもミトコンドリアから細胞質へのAspの輸送が重要である．②乳酸からの糖新生では，細胞質でのNADH・NAD＋の収支からAGCを必要とする．つまり，細胞質のNADH収支を保つために，ミトコンドリアで生じるOAAはAspとなり，AGCにより細胞質へ輸送されることが重要である．③リンゴ酸・アスパラギン酸（malate-aspartate, MA）シャトルの一員として，細胞質で生じたNADH還元当量をミトコンドリアに輸送する．好気性解糖では，pyruvate（Pyr）はミトコンドリアに入るので，細胞質で生成されたNADH還元当量はNADHシャトルのいずれかでミトコンドリアに輸送される必要がある（図2）．

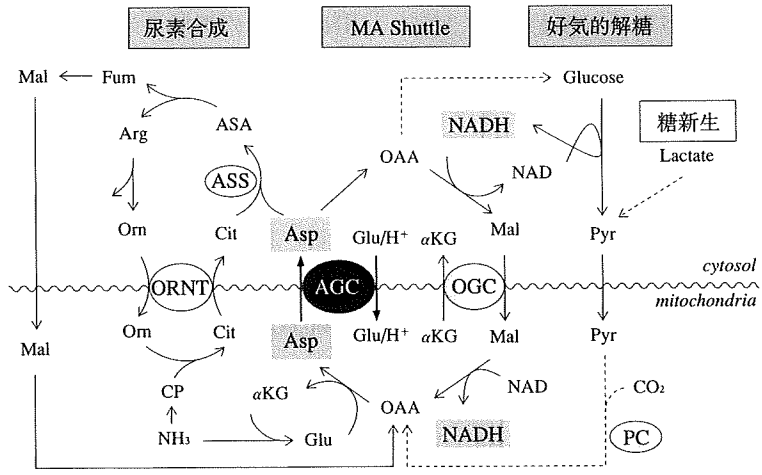

図1　シトリン（肝型AGC）の役割（文献13）より引用）

AGC：aspartate（Asp）glutamate（Glu）carrier，MA shuttle：malate aspartate suttle，OAA：oxaloacetate，Mal：malate，α KG：α-ketoglutarate，OGC：oxoglutarate carrier，CP：carbamoyl phosphate，ORNT：ornithine（Orn）transporter，ASA：argininosuccinate，Fum：fumarate，PC：pyruvate（Pyr）carboxylase

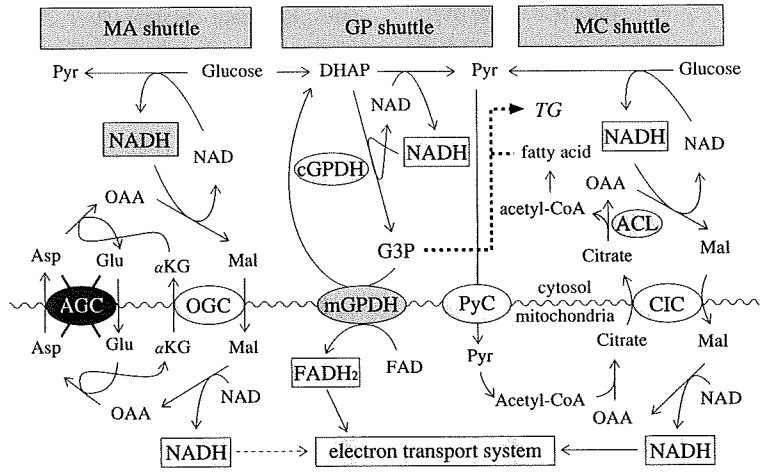

図2　NADHシャトル（文献13）より引用）

GP shuttle：glycerophosphate shuttle，c/mGPDH：cytosol/mitochondrial GP dehydrogenase，DHAP：dihydroxyacetone phosphate，G3P：glycerol 3 phosphate，MC shuttle：malate citrate shuttle，PyC：pyruvate carrier，CIC：citrate carrier，ACL：ATP citrate lyase，TG：triglyceride

肝臓においてもっとも重要なNADHシャトルはMAシャトルである．ヒト肝臓ではmitochondorial glycerophosphate dehydrogenase（mGPDH）の活性が低いので，glycerophosphate（GP）シャトルは，ほとんど機能していないと考えられている．Mlate-citrate（MC）シャトルが作動すると，アセチルCoAが細胞質に蓄積し，脂肪酸の合成が亢進する．

シトリン欠損の病態[11)13)]

シトリン欠損症では，生後1歳までにNICCDの症状を示すが，その後は見かけ上，健康な時期（適応・代償期）を過ごすが，代償機能が低下してCTLN2として発症する．シトリン欠損症に見られる多彩な症状は，肝AGCの機能不全から説明できる．

1．適応・代償機構

肝型AGCが欠損すると，MAシャトルが障害され，NADHが細胞質に蓄積するが，シトリン欠損症では，その代償機能としてGPシャトルやMCシャトルが代償機能として作動し，細胞質に蓄積したNADH還元当量を処理している可能性が考えられている．本来，脂肪酸合成系として機能するMCシャトルが働くと細胞質のNADHを減少させるが，結果として細胞質のアセチルCoAの蓄積を招き，脂肪酸の合成が促進し，脂肪酸分解が抑制される．

一方，GPシャトルはミトコンドリアと細胞質に局在するglycerophosphate dehydrogenase（mGPDHとcGPDH）で構成されるが，mGPDH活性が低いと，グリセロール3リン酸が（G3P）が供給され，中性脂肪の合成が促進する．

以上が，シトリン欠損症で見られる脂肪肝や高脂血症の病因であると推定されている．

2．細胞質へのaspartateの供給

通常では非必須アミノ酸であるAspが，シトリン欠損症では必須アミノ酸に近い状態となり，Asp減少がアミノ酸のアンバランスをきたし，蛋白質合成や核酸合成を障害している可能性がある（NICCDでは低出生時体重，低蛋白血症，体重増加不良が観察されている）．また，シトリン欠損症では，エネルギー源として糖質が使えないために，アミノ酸からAspやOAAを供給するとともに，エネルギーを確保していると推測されている．

NICCDでみられる一過性のシトルリン血症は，AGCの機能不全によりAspが供給されず，ASSのもう一つの基質であるcitrulline（Cit）が代謝されないためと推定される．細胞質のAsp濃度減少がASS蛋白質の安定性低下につながる可能性もあるが，CTLN2の肝特異的ASS蛋白質低下の機序は不明である．

3．細胞質へのNADHの蓄積

好気性解糖では，ピルビン酸（Pyr）がミトコンドリアに入るために，Pyrを乳酸へ変換し，細胞質に生じるNADHをNAD^+にリサイクルできない．そのため，細胞は細胞質のNADH還元当量をミトコンドリアに輸送するシステム（NADHシャトル）をもっている．

さて，還元基質からの糖新生においてAGCは重要である．たとえば，乳酸からの糖新生では，細胞質でのNADH・NAD＋の収支を保つために，ミトコンドリア内で生成されるOAAはAspとして輸送される．一方，グリセロールやソルビトールなどの還元基質からの糖新生，あるいは細胞質でAspを生成するために必要なOAAの産生のためにも，NADHシャトルが必要である．

NICCDで観察されるガラクトース血症は，ガラクトース代謝関連酵素に異常を認めないことから，細胞質で上昇したNADHがガラクトース代謝系酵素の一つであるUDP-galactose epimeraseを阻害する結果と考えられている．低血糖は，乳酸やアミノ酸からの糖新生障害が推定されている．また，*SLC25A13*遺伝子異常の多くの症例が糖質を嫌い，アルコールを飲めず，

飲酒後発症する理由は，細胞質NADHの蓄積に起因すると考えられる．

4．代償期の症状と食嗜好

CTLN2肝移植症例の調査では，4～5歳ころから40％前後の症例で多彩な症状が経験されているが，最大の特徴は，特異な食嗜好（糖質は嫌う，蛋白質・脂肪を好む）である．この極端な偏食は，細胞質にNADHを産生させる糖質を嫌い，細胞質のAspを増加させ，同時にNADHの再酸化を促進する蛋白質性食品の摂取を身体が要求していると推察されている．

SLC25A13 遺伝子異常の臨床

1．成人発症Ⅱ型シトルリン血症（CTLN2）[11) 13)]

1）臨床像

小林らの解析によるCTLN2（170例）の特徴を示す（**表2**）．発症は11～79歳，20～40歳の男性例が多い．

多彩な精神神経症状のため，精神的疾患（うつ病，統合失調症）や急性アルコール中毒と診断されている場合がある．とくに，夜間の不眠，不穏，もうろう状態などを繰り返し，次第に意識レベルの低下が進行する．90％の症例では，body mass index（BMI）20以下，40％では17以下で「やせ型」の症例が多い．症例の多くは，糖質（米飯，ジュースなど）を嫌い，蛋白

表2　CTLN2の臨床像

意識障害：失見当識/異常行動/けいれんやてんかん様発作
胃腸障害
全身倦怠感
脂肪肝（線維化は認めるが肝硬変はまれ）
肝がん
高脂血症
膵炎
スリムな骨格
特異な食嗜好

	血漿アミノ酸	
	Cit	Arg
	nmol/ml	
control	20～40	80～130
CTLN1	2500±1040	58±31
CTLN2	520±290	230±170

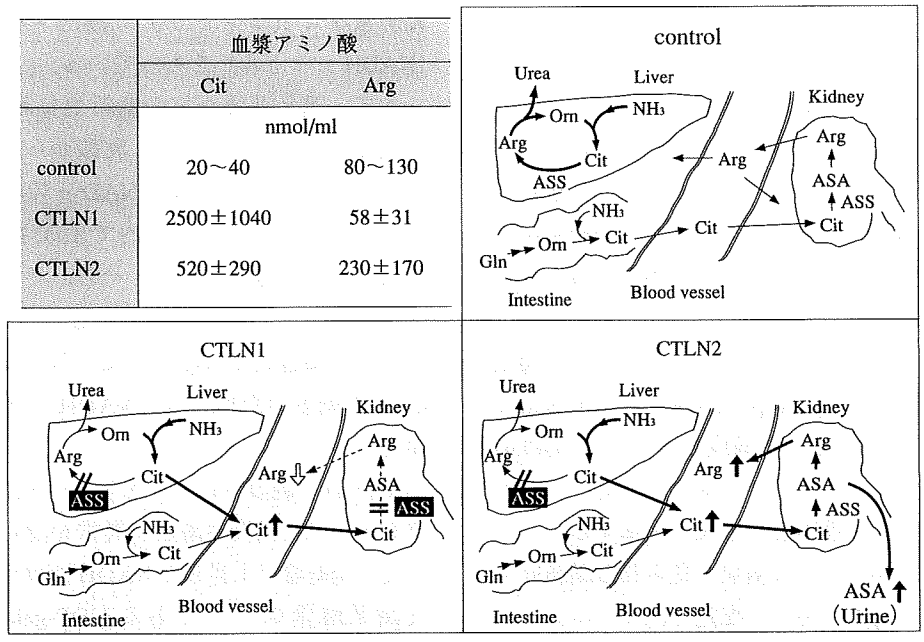

図3　CTLN1とCTLN2の血漿アミノ酸による鑑別とその代謝的基盤（文献11）より引用）

CTLN1：classical citrullinemia, CTLN2：adult-onset type Ⅱ citrullinemia, ASS：argininosuccinate synthetase, Cit：citrulline, Arg：arginine, Orn：ornithine, ASA：argininosuccinate, Gln：glutamine．正常における血中Citは小腸に由来し（右上），シトルリン血症では肝臓からの漏出で増加する．CTLN1での腎ASS異常は血中Argの低下をまねく（左下）が，CTLN2では腎ASSが正常であるので，血中Citの増加，血漿Arg値の上昇と尿中へのASAの排泄増加をまねく

質や脂肪に富む食品（魚ピーナッツ，大豆，卵，乳製品，魚肉類など）を好む．健常者における炭水化物：脂質：蛋白質の摂取エネルギー比は，年齢や性別に関係なくほぼ60:25:15であるが，シトリン欠損症（CTLN2未発症，4～33歳）では，ほぼ35:45:20である．

「患者と家族の会」が発足し，患者さん側からの貴重な情報がよせられている．「食べ物の好き嫌いが激しくて，なんてわがままな娘なの」と思っていたけれど，「娘の超偏食は，病気の身体が要求するものだったのね」．「学校給食で，すべてを食べるように強要されて，食後の嘔吐，眠気，倦怠感などで苦しんだ経験がある」．以上のエピソードは高アンモニア血症予防のための食行動，高アンモニア血症に由来する症状と考えられる．少年たちの「むかつく，きれる，あばれる」などの問題行動の診断にさいしても参考とすべきであろう．

2）検査所見

特徴的な検査所見は，夕方から夜間に顕著となる高アンモニア血症である．血漿アミノ酸では，シトルリンとアルギニンが高値を示し，アルギニンが低値を示すCTLN1との鑑別が可能である．CTLN1に比較すると，CTLN2のシトルリンのレベルは軽い．このほか，スレオニン/セリン比の上昇，分岐アミノ酸低下によるFisher比の低下などがある．

肝機能検査では，軽度の障害を示す．このほか，血清pancreatic secretory trypsin inhibitor（PSTI）の上昇が多くの症例で観察されている．PSTIの上昇は肝臓における*PSTI*遺伝子の発現亢進によるが，その機序は不明である．しかし，PSTIの上昇はCTLN2発症前から認められるので，診断に有用とされている．

おもな死因は脳浮腫であり，病理学的には激しい破壊性の病変（類嚢胞脳）が認められる．

抗ASS抗体を用いた免疫組織学的解析では，特異な肝組織内分布（肝小葉内での不均一なASS塊状分布）が認められる．発症後の予後不良を示唆すると考えられている．

表3　NICCDの臨床像および検査所見

肝組織像（脂肪肝，胆汁うっ滞，ヘモジデリン沈着，肝線維化）
比較的軽度な黄疸
高度な胆汁うっ滞（ビタミンE欠乏，ビタミンK欠乏）
軽度な肝脾腫
低出生体重児/体重増加不良*
高胆汁酸血症
高α-fetoprotein血症
多種高アミノ酸血症（シトルリン，メチオニン，チロシン，スレオニン，ほか）
低蛋白血症*
高ガラクトース血症/白内障
高アンモニア血症
低血糖
そのほか（筋緊張低下，けいれん，皮下出血，浮腫，腹水，腹部膨満，精神発達遅延，高乳酸血症）

* 栄養状態がよいように見えるが非顕性の浮腫を合併している症例がある

3）治療（肝移植）

CTLN2は，発症後急速な経過で死に至ることが多い，予後不良の疾患とされてきた．しかし，肝特異的なASS活性低下であることから，不可逆的な脳の器質的障害合併前の肝移植の治療成果が期待され，1988年以降，35例（生体部分肝移植33例）で施行されている．

2．シトリン欠損による新生児肝内胆汁うっ滞（NICCD）[1)～10) 12) 14)]

1）臨床像

2001年，NICCDが成人期発症状シトルリン血症Ⅱ型（CTLN2）と同一の遺伝子（SLC25A13）変異による疾患であることが明らかにされ[3) 4)]，現在まで約150例前後の症例が診断されている．NICCDは，①閉塞性黄疸，②新生児マス・スクリーニング（ガラクトース，メチオニン，フェニルアラニン）がおのおの半数での発見の契機となる．NICCDの臨床像および検査所見の特徴は，**表3**のように概略される．

肝硬変を伴う肝不全例を例外として，浮腫，腹水，高アンモニア血症，低血糖はまれである．NICCDの臨床像で重要なのは，シトルリン血症

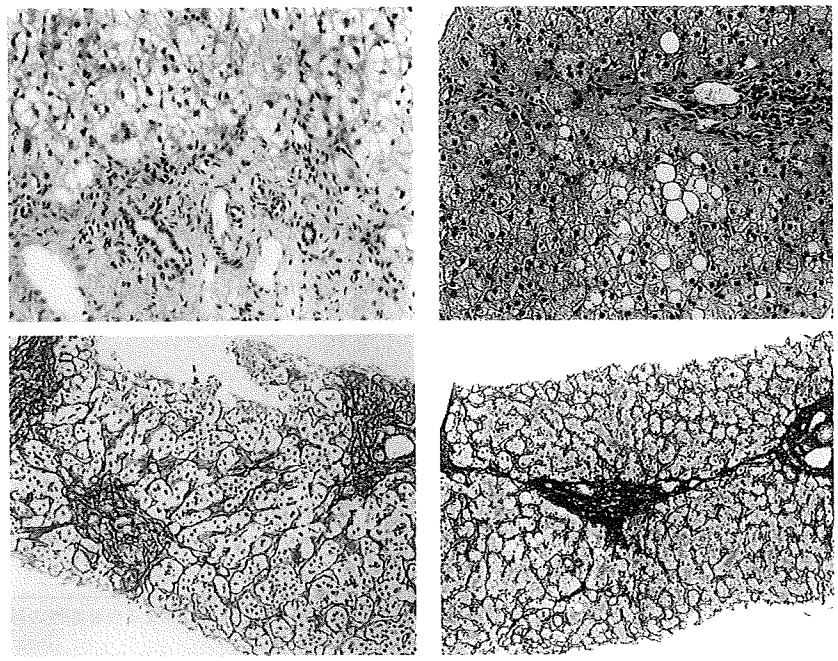

図4 肝組織像

左上下段は生後5カ月時，右上下段は1歳時の肝組織像を示す（×200）．上段はHE染色像，下段はAg染色像．5カ月では門脈域の線維性拡大と門脈周辺部の脂肪沈着が観察される．1歳時では，脂肪沈着は門脈域のごく周辺部に限定されるが門脈域間の線維性架橋が観察される（症例M.R.）

を認めないことがある（母乳栄養児），肝硬変（肝不全）合併例を例外として，明らかな高アンモニア血症はまれである点である．

①肝組織像：一般的に，脂肪肝，脂肪性肝炎，脂肪性肝線維症，まれに肝硬変の組織像を示す（**図4**）．脂肪肝，肝線維化は1歳頃までに鎮静化するが，一部では，門脈域周辺の脂肪沈着や門脈領域の架橋形成などが観察される．脂肪肝の再燃は，乳児期および幼児期以降でも確認されている．この場合には，血清トランスアミラーゼ（AST，ALT）値の上昇を伴う．非代償性肝硬変は4例で報告され，いずれも生体肝移植が適応されている．4例中3例は1歳以下の症例である．1例は，浮腫および腹水を合併したNICCDの症例であるが，その後の経過中に肝硬変と高アンモニア血症を合併し，16歳で肝移植を受けている[5]．

②シトルリン血症を含む多種高アミノ酸血症：新生児・乳児期の胆汁うっ滞性疾患では，

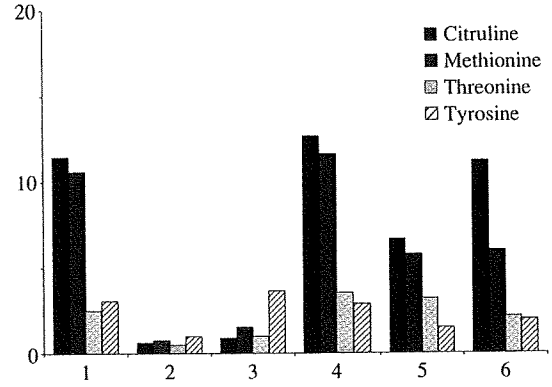

図5 血漿アミノ酸プロフィル

症例1と4～6で多種高アミノ酸血症が観察される．Y軸は各アミノ酸（citrulline, methionine, threonine, tyrosine）正常上限値の倍数値

メチオニン，チロシンが高値を示すことが知られていた．NICCDでは，シトルリン，メチオニン，チロシン，スレオニンフェニルアラニンなどの多種高アミノ酸血症が特徴的所見であるが，必発ではない．混合栄養や母乳栄養児では認めないことがある（**図5**）．

その理由の一つとして，人工乳の蛋白濃度は母乳の2倍前後に調整され，蛋白負荷が強化された状態にあるためと考えられている[2) 6) 10)]．

③低蛋白血症：臨床的に新生児肝炎と診断された60例中57例の血中蛋白レベルは4.9 g/100 m*l*以上であった．しかし，そのほかの3例は，臨床的に浮腫・腹水が観察されないが，高度な低蛋白血症（3.2～4.1 g/100 m*l*），および胆汁うっ滞，脂肪肝，ヘモジデリン沈着などの特異な肝組織像を示し，未知の代謝性疾患が疑われていたが，最終的にNICCDと診断された．一般的にNICCDでは，低蛋白血症が高頻度に認められる．われわれの症例では，14例中半数が5.0 g/100 m*l*以下であった[1)]．浮腫や腹水を合併し，肝移植が実施された症例では，低蛋白血症（3.7～4.1 g/100 m*l*）が認められている[5)]．

④高ガラクトース血症・白内障：新生児マス・スクリーニングあるいは新生児乳児早期の胆汁うっ滞性疾患では，高ガラクトース血症を認めることがあるが，その一部にNICCDが潜んでいたと考えられる．NICCDの一部では高ガラクトース血症が認められるが，ガラクトース代謝関連3酵素活性に異常はないが[4)]，細胞質内のNADHの異常産生のため，UDP-galactose-4'-epimerase活性が抑制される可能性が指摘されている．現在まで，3例の白内障合併例が報告されている．

⑤低血糖：われわれの14症例の経験では，低血糖の経験はないが，非徴候性および徴候性低血糖の症例がおのおの1例報告されている（43 mg/100 m*l*，40 mg/100 m*l*）．詳細は不明であるが，前者では新鮮凍結血漿が使用され，後者では浮腫が認められ，ともに肝不全が背景にあると考えられる．したがって，NICCDで低血糖を認める場合には，広範な肝細胞障害があることを疑うことが重要である．

⑥高アンモニア血症：新生児の尿素サイクルは未成熟であり，一過性の高アンモニア血症を認めることがある．また，乳児期では腸内細菌叢が未発達なため，腸管のアンモニア産生が少ない．しかし，NICCDの尿素サイクルに関連する酵素活性は正常であり，非代償性の肝不全がある場合を例外として，明らかな高アンモニア血症は認めない．

NICCDでは，新生児マス・スクリーニング発見例では軽度の高アンモニア血症が半数以上で指摘されている（100～200 μg/100 m*l*）．その他の5症例でも軽度の高アンモニア血症が報告されているが，いずれも低出生体重児である（102～196 μg/100 m*l*）．以上から，軽度の高アンモニア血症の成因は，AGCの機能低下およびアンモニア処理機構の未成熟性と考えている．

⑦胆汁うっ滞：NICCDでは，血中直接型ビリルビンに比較し，高度な胆汁うっ滞が認められる（**表4**）[12)]．その機序としては，NICCDでは，生理的胆汁うっ滞を背景とし，NADHのミトコンドリア内への輸送が障害され，エネルギー産生を低下し，ATP依存性胆汁酸排泄機構に影響を与える結果，高度な胆汁うっ滞が成立する．さらに脂肪吸収障害（体重増加不良）により，

表4　NICCD，新生児肝炎，胆道閉鎖の胆汁うっ滞の比較

	NICCD n＝14	新生児肝炎 n＝14	胆道閉鎖 n＝14
総ビリルビン　mg/100 m*l*	7.0（2.7）	8.3（2.3）	9.3（2.5）*
直接ビリルビン　mg/100 m*l*	3.4（1.4）	5.4（2.2）*	6.2（2.1）*
総胆汁酸　μmol/*l*	229（59）	150（50）*	121（38）*
総胆汁酸/直接ビリルビン　Ratio	77（35）	32（17）*	21（10）*
GGTP　IU/*l*	181（26）	73（28）*	256（46）

* NICCDに対して有意差のあるもの

表5　NICCDの尿中胆汁酸

	NICCD n＝3	新生児肝炎 n＝3	胆汁性肝硬変 n＝3
総胆汁酸　μmol/mmolCr	11.3～45.1	8.4～94.8	14.2～55.7
C/CDC	0.11～0.51	4.17～23.3	0.10～1.29
総3-oxo-Δ⁴胆汁酸　(%)	3.6～7.1	6.7～23.6	31.8～68.3

表6　*SLC25A13*遺伝子異常の乳児期以降の臨床像

非肥満児の脂肪肝/脂肪性肝炎
（線維化は認めるが肝硬変はまれ）
ケトン血性（あるいはケトン産生不良の）低血糖
無熱性けいれん
てんかん
精神運動発達遅延
高グリセライド血症
意識障害：失見当識/異常行動/けいれんや
てんかん様発作
全身倦怠感
高脂血症
小柄でスリムな骨格
特異な食嗜好

表7　シトリン欠損症の治療：問題のある治療および可能性のある治療（文献13）より引用）

避けるべき治療法*
アルコール（禁忌）
高糖質・高カロリー（問題あり）
Glycerol（危険）
効果が期待できる治療法
Sodium benzoate or phenylacetate：アンモニア・アミノ酸代謝の促進
Arginine：尿素合成促進
Pyruvate：細胞質NADHの酸化とOAAの供給
Aspartate or asparagines：細胞質のAsp供給？

＊還元・酸化ストレスの危険性がある．発作（高アンモニア血症）の誘発，代謝障害（高脂血症，脂肪肝）を増悪し，予後不良を導く可能性がある

エネルギー代謝がさらに障害される悪循環が成立しているものと考えている．

この他，NICCDの尿中胆汁酸分析では，特異な所見が観察されている（**表5**）[12]．尿中胆汁酸レベルは他の胆汁うっ滞性疾患と同レベルであるが，①コール酸/ケノデオキシコール酸比（C/CDC）は低値，②総3-oxo-Δ⁴胆汁酸レベルは正常．以上の所見は，胆汁酸代謝の未熟成を示唆し，NICCDで観察される高度な胆汁うっ滞の原因の一つと考えられている．

⑧その他の臨床像：CTLN2発症患者さんの幼児期以降の臨床像は先に示したが（**表2**），NICCDの子どもたちの経過観察から得られた乳児期以降の臨床像を次に示す（**表6**）．

⑨治療と経過：NICCDの治療は新生児肝炎の治療に準ずる．利胆薬（ウルソ－デオキシコール酸），脂溶性ビタミン，中鎖脂肪酸（MCT）ミルクの投与が基本であるが，ガラクトース血症のある場合には，ガラクトース除去ミルクを投与する．

大部分の症例は1歳前に改善する．一般的に，多種高アミン酸血症，直接ビリルビン，総胆汁酸，GGTP，トランスアミラーゼ（AST，ALT）値の異常は，この順序で改善し消失する．無治療で自然に回復することもあるが，黄疸がないだけで治癒と判断すると，脂肪性肝炎は残存したまま，肝病変が進行する可能性があるので注意が必要である．

NICCDのフォロー・アップで重要なのは，①アルコールの禁忌，②脳症合併時の治療法である．高アンモニア血症だけでなく，インフルエンザ脳症＊，Reye症候群などのあらゆる脳症合併時に注意が必要である．理由は，高濃度の糖液の補液やグリセオール（10%グリセロールと5%フルクトース）の使用は避けるべきだと考えられているからである．高濃度の糖液は，細胞質内のNADHが蓄積し，脂肪酸，中性脂肪の産生を亢進させ，高アンモニア血症をひきおこす．グリセロールはglycerol-3-phosphateに変

換され，細胞質のNADHを大量に生成し尿素合成を阻害する．フルクトースと同様に，一方的にリン酸化を受け，ATPレベルが低下する可能性がある．これらが重複し，急激な肝障害をひきおこす危険があると考えられる．現在，アルギニンやピルビン酸による内科的治療が検討されている（表7）[11)13)]．

＊ 子どものインフルエンザ脳症では，脳圧亢進の治療としてD-マンニトールが推奨され，注意書きに「低血糖の時，グリセオールの使用で症状の悪化をみることがある．」と記載されている．

NICCDで発症した子どもが，「将来CTLN2として発症するか」は大きな問題である．現在までところ，非代償性の肝硬変に進行し，高アンモニア血症を合併し，肝移植を受けた症例を除けばCTLN2の発症の報告はない．CTLN2では肝硬変の合併はまれであることから，合併例は，NICCD後の壊死性肝硬変あるいは持続性・反復性脂肪性肝炎からの進行例の可能性があると考えている．したがって，NICCDの経過観察のポイントは，第一に「進行性肝病変が潜在していないか」である．

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