Citrin Deficiency

1

Yuan-Zong Song, Masahide Yazaki, and Takeyori Saheki

Keywords

CTLN2 (adult-onset type II citrullinemia) · NICCD (neonatal intrahepatic cholestasis caused by citrin deficiency) · FTTDCD (failure to thrive and dyslipidemia caused by citrin deficiency) · Aspartate-glutamate carrier · Malate-aspartate shuttle

Abbreviations

4-HPL	4-Hydroxyphenyllactate
4-HPPV	4-Hydroxyphenylpyruvate
ABC	ATP-binding cassette
ABCG5/8	ATP-binding cassette, subfamily G, member 5/8
AFP	α-Fetoprotein
Ag	Antigen
AGC	Aspartate-glutamate carrier
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ASS	Argininosuccinate synthetase
AST	Aspartate aminotransferase
BSEP	Bile salt export pump
CMV	Cytomegalovirus
CTLN1	Argininosuccinate synthetase deficiency or classical citrullinemia
CTLN2	Adult-onset type II citrullinemia
FTTDCD	Failure to thrive and dyslipidemia caused by citrin deficiency
GC-MS	Gas chromatography-mass spectrometry
GGT	γ-Glutamyltransferase
MCFA	Medium-chain fatty acid
MCT	Medium-chain triglyceride
MRD3	Multidrug-resistant protein 3
MS-MS	Tandem mass spectrometry
NICCD	Neonatal intrahepatic cholestasis caused by citrin deficiency
ORF	Open reading frame
TBA	Total bile acids
Tbil/Dbil	Total bilirubin/direct bilirubin

Y.-Z. Song
Department of Pediatrics, The First Affiliated Hospital, Jinan University, Guangzhou, Guangdong, China

M. Yazaki
Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan

Department of Clinical Laboratory Medicines, Shinshu University School of Health Sciences, Matsumoto, Japan
e-mail: mayazaki@shinshu-u.ac.jp

T. Saheki (✉)
Department of Hygiene and Health Promotion Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

T. Oohashi et al. (eds.), *Human Pathobiochemistry*, https://doi.org/10.1007/978-981-13-2977-7_1

1.1 Case Report

1.1.1 Patient 1

A 6-month-old male Chinese infant was referred to our hospital with jaundiced skin and sclera for nearly 6 months. Jaundice was observed on the third day after birth and then became gradually aggravated. At age 1.5 months, the infant underwent a physical examination at a local hospital that revealed a slightly enlarged liver 3 cm below the right subcostal margin. Subsequently, the baby was referred to several different hospitals in the local city, but the etiology of his jaundice remained unclear. At age 4 months, an anti-cytomegalovirus (CMV) IgM test at another local hospital was positive. Thus, CMV infection was diagnosed and intravenous ganciclovir was given for 1 month, but the therapeutic effect was unsatisfactory. At age 5 months, he was referred to a provincial hospital, where laboratory tests revealed abnormal liver function indices, increased levels of ammonia and lactate (Table 1.1), a reduced blood glucose level of 1.80 mmol/L (reference range 3.89–6.11 mmol/L), and positive CMV-Ag, CMV-IgM, and CMV-IgG. Once again, the infant was diagnosed with a CMV infection, and ganciclovir was given intravenously for an additional 20 days. As a result, CMV-Ag became negative, but the jaundice and abnormal liver function indices persisted. Therefore, the baby was referred to our hospital for further examination.

As the second baby of a non-consanguineous couple, the patient was born at the gestational age of 38 weeks after an uneventful pregnancy. The birth weight was 3.1 kg and the body length 50 cm. The baby was fed with breast milk. His

Table 1.1 Laboratory findings over time in the patient with NICCD

	Age at tests									
Indices (reference range)	5M	6M[a]	6.5M	7M	8M	11M	1Y4M	3Y8M	4Y6M	11Y7M
ALT (5–40 U/L)	96	42	26	46	39	23	27	19	14	13
AST (8–40 U/L)	200	140	77	147	74	44	47	36	35	21
GGT (7–50 U/L)	330	279	377	283	164	70	47	21	17	20
ALP (10–500 U/L)	437	399	300	350	385	216	194	247	300	485
TP (60–80 g/L)	56	72	78	52	69	73	64	72	70	67
Alb (35–55 g/L)	35	40	45	32	48	42	42	49	50	48
Glb (20–35 g/L)	21	32	33	21	22	31	21	23	20	19
Tbil(5.1–23.0 μmol/L)	94.8	107.4	50.2	43.7	12.2	2.3	3.0	7.2	11.0	8.7
DBil(0.6–6.8 μmol/L)	67.5	57.0	27.0	25.3	4.2	0.7	1.2	1.8	6.2	1.7
IBil(1.7–17.0 μmol/L)	27.3	50.4	23.2	18.4	8.0	1.6	1.8	5.4	4.8	7.0
TBA (0-10 μmol/L)	152	196	86	104	15	10	11	8	11	3
AFP (0-20 ng/mL)	–	320,000	140,000	19,000	450	10	–	–	–	–
TG(0.56–1.70 mmol/L)	–	2.82	1.63	–	–	–	–	2.64	0.96	1.12
Tchol(3.10–5.70 mmol/L)	–	6.76	4.03	–	–	–	–	3.94	4.74	5.00
Lactate (1.1–2.2 mmol/L)	2.70	1.54	–	–	–	2.11	–	–	–	–
Ammonia (<75 μg/dL)	95	113	73	94	67	57	–	–	–	–

[a]When the infant was referred to our hospital

Abbreviations: *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* γ-glutamyltranspeptidase, *ALP* alkaline phosphatase, *TP* total protein, *Alb* albumin, *Glb* globulin, *Tbil* total bilirubin, *Dbil* direct bilirubin, *Ibil* indirect bilirubin, *TBA* total bile acids, *TG* triglycerides, *Tchol* total cholesterol, *AFP* alpha-fetoprotein, Y and M represent year(s) and month(s) of age, respectively; –, not tested

elder brother was healthy. There was no family history of any genetic diseases.

Physical examination at admission revealed a body weight of 6.2 kg (−2.45SD), body length of 63.0 cm (−2.25SD), and head circumference of 40 cm (−2.77SD). His skin and sclera were mildly jaundiced. A chubby face was noticed. The lungs were clear on auscultation, and no abnormal cardiac sounds or murmurs were heard. The abdomen was distended, and the liver and spleen were palpable 5 cm and 2 cm below the right and left subcostal margins, respectively. His limb muscular tone was adequate, and the bilateral Babinski's, Brudzinski's, and Kernig's signs were all negative.

Liver function tests revealed increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltranspeptidase (GGT), alkaline phosphatase (ALP), total bile acids (TBA), and total/direct bilirubin (Tbil/Dbil), as well as decreased total protein and albumin. Blood ammonia, lactate, cholesterol, and triglyceride levels were also increased, and the serum alpha-fetoprotein (AFP) level reached 320,000 ng/mL, an extremely elevated level rarely seen in clinical practice (Table 1.1). A tandem mass spectrometry (MS-MS) analysis detected elevated citrulline, threonine, tyrosine, and methionine in dried blood samples. Large quantities of galactose, galactitol, galactonate, 4-hydroxyphenyllactate (4-HPL), and 4-hydroxyphenylpyruvate (4-HPPV) were detected in urine by gas chromatography-mass spectrometry (GC-MS). A *SLC25A13* gene analysis revealed biallelic mutations of c.851_854del4 and c.1638_1660dup (Fig. 1.1).

Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) was definitively diagnosed. Fat-soluble vitamins (A, D, E, and K) were given and breastfeeding was stopped. A lactose-free and medium-chain triglyceride (MCT)-enriched formula was introduced. As a result, his jaundice subsided, while the laboratory alterations recovered gradually. The patient has been followed up at our clinic for over 11 years. He has developed well with satisfactory anthropometric indices and social performance.

1.1.2 Patient 2

The patient was a 51-year-old Japanese male who was admitted to a local hospital due to consciousness disturbances. He had been well until he noticed a hand tremor when he climbed a mountain at age 51. After descending the mountain, he visited a neurosurgical clinic. His neurological examination was normal, and no abnormal findings were shown on a brain CT. Approximately a month later, his speech became rudimentary and garbled. The next morning, he was found lying unconscious on the floor of the entrance to his home, and he was immediately transferred to a local hospital. A brain CT was normal, and a laboratory examination revealed a highly elevated level of plasma ammonia (355 μg/dL, normal <70 μg/dL). He was thought to have hepatic encephalopathy and was treated with an infusion of branched amino acids followed by the hospital's low-protein diet (total calories 1600 kcal/day, protein 40 g/day) to reduce nitrogen sources. In addition, oral administration of lactulose and antibiotics (kanamycin) was started. After this treatment, the consciousness disturbances occurred frequently. An EEG recorded diffuse slow waves with occasional triphasic waves (Fig. 1.2A). Because his plasma levels of citrulline and arginine were elevated (408.9 nmol/mL, normal <40 nmol/mL and 186.4 nmol/mL, normal<120 nmol/mL, respectively), a urea cycle disorder was suspected, and he was admitted to Shinshu University Hospital. He did not have a family history suggestive of adult-onset type II citrullinemia (CTLN2). He had a food fondness for peanuts, milk, meat, and fish and had disliked sweets from childhood. On physical examination, he was very irritable and confused, and flapping tremor was noted in his hands. Icterus was not found and no hepatosplenomegaly was observed.

Laboratory data revealed a mild elevation of serum transaminases (AST 52 IU/L, normal <37 IU/L; ALT 96 IU/L, normal <45 IU/L). While serum GGT was moderately raised (138 IU/L, normal <50 IU/L), the levels of Tbil, albumin, and total cholesterol were within nor-

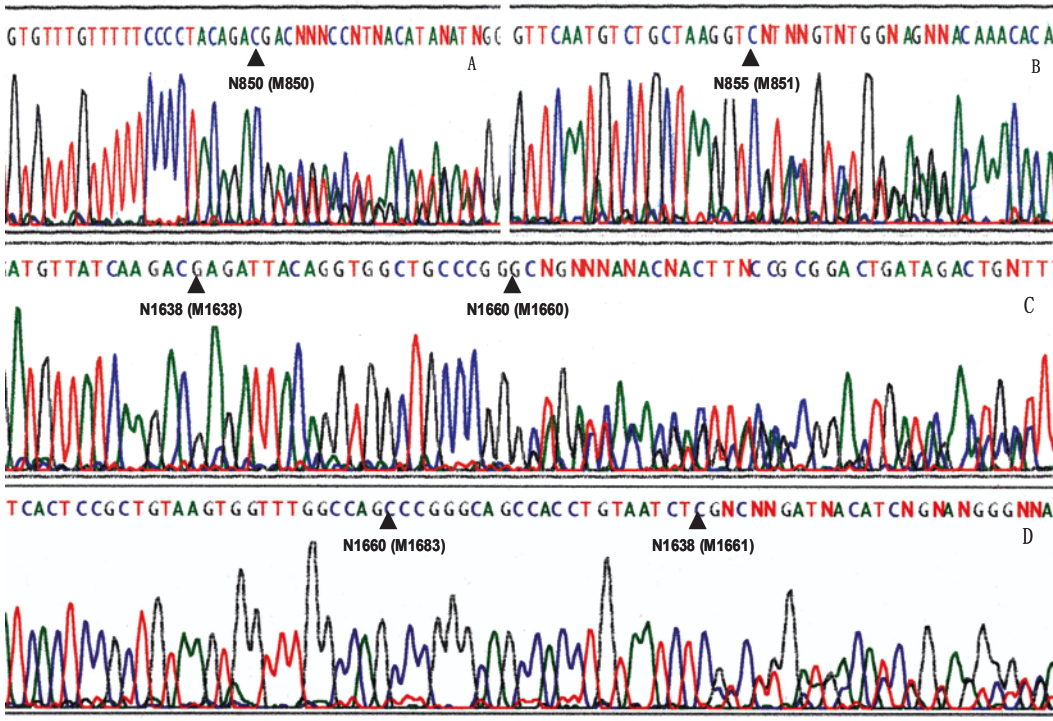

Fig. 1.1 Sanger sequencing results of exons 9 and 16 in the *SLC25A13* gene of the NICCD patient. Figures A and B represents the sequencing findings of exon 9, while figures C and D, of exon 16, in the coding and template strands of the *SLC25A13* gene, respectively. The digits in this figure denote the positions of the arrowhead-indicated bases within the normal (N) and mutated (M) *SLC25A13* alleles, respectively

mal values. There was no abnormality of coagulation function.

He was first thought to have hepatic encephalopathy. Laboratory data did not indicate the presence of hepatic failure. In addition, there was no serological evidence of hepatitis-related viral infection. The abdominal CT and MR images demonstrated neither liver cirrhosis nor extrahepatic portovenous shunt. Due to high plasma levels of citrulline and arginine, he was suspected to have CTLN2. DNA analysis of the *SLC25A13* gene, responsible for CTLN2, revealed that he was a compound heterozygote for the mutations of 851del 4 and IVS13+1 G > A, and a definite diagnosis of CTLN2 was made.

The patient began oral arginine (3 g/day) and a carbohydrate-restricted diet with a high fat content [total calories 1340 kcal/day, protein 50 g/day, carbohydrate 150 g/day, PFC (protein/fat/carbohydrate) ratio 15%:40%:45%]. His condition gradually ameliorated and his plasma ammonia level also decreased. After that, the daily dose of arginine was increased to 9 g/day, and total dietary calories were gradually increased to 1800 kcal in 3 months with the ratio of carbohydrate in the total dietary calories restricted to approximately 45% (Fukushima et al. 2010). After obtaining approval from the Institute Review Board of Shinshu University and written informed consent of the patient, oral intake of sodium pyruvate was added (6–9 g/day). Liver transplantation was not performed as there was no donor candidate for a live donor liver transplantation. At present, his condition has been stable for 8 years without receiving liver transplantation. An EEG recorded at age 59 years was normal, except for diffuse slow alpha waves (Fig. 1.2b).

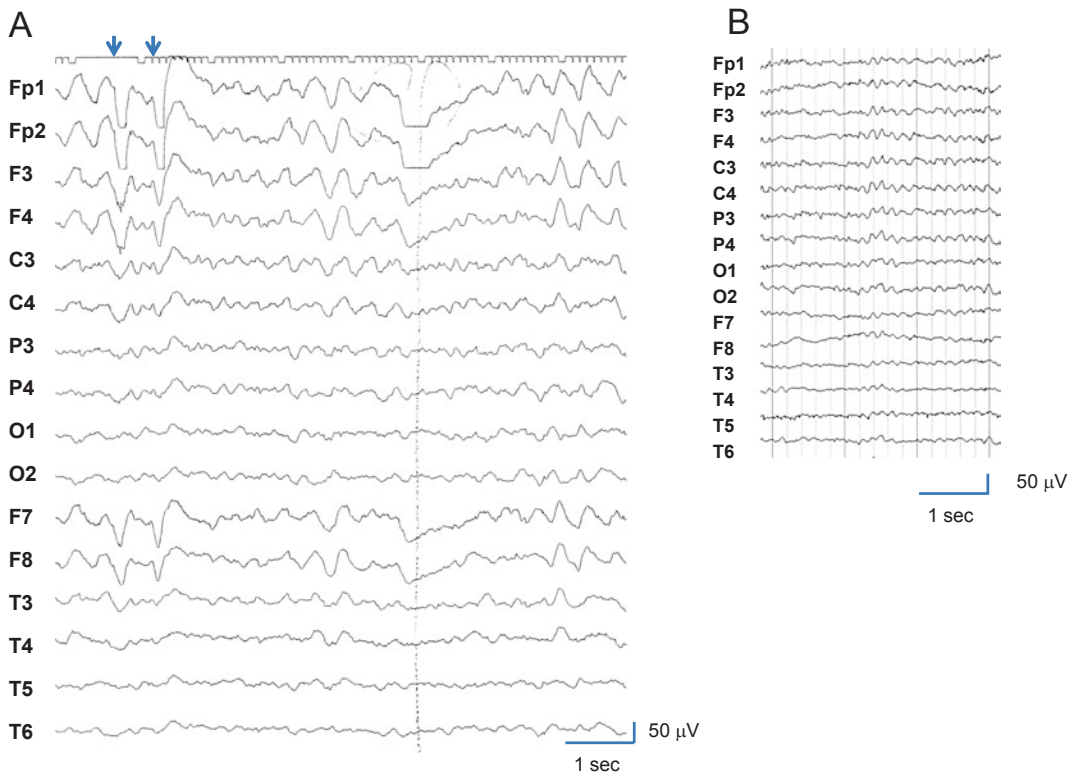

Fig. 1.2 EEG findings in the CTLN2 patient. (**a**) EEG recorded during treatment with a low-protein diet shows diffuse slow waves with occasional triphasic waves (arrows). (**b**) EEG at age 59 (7 years after starting sodium pyruvate therapy) appears almost normal, except for diffuse slow α-waves

1.2 Diagnosis

Patient 1 was a NICCD patient showing typical clinical and molecular manifestations. Prolonged jaundice and gradually aggravated hepatosplenomegaly in this patient suggested a chronic liver disease. Also, the growth retardation, as indicated by the body weight, body length, and head circumference at admission, suggested a long course of the disease. Unfortunately, none of these positive signs were pathognomonic. Moreover, although CMV infection did exist, this was not the major cause for this patient, as judged by the responses to ganciclovir treatment.

Cholestasis is a salient biochemical feature in NICCD. In this patient, the elevated GGT, TBA, and Tbil/Dbil all indicated a cholestatic liver disease. Reduced plasma levels of total protein and albumin, as well as increased blood ammonia, lactate, cholesterol, and triglyceride, as shown in Table 1.1, are common in NICCD subjects. The serum AFP level is usually dramatically increased. Once again, these biochemical alterations are neither specific for NICCD nor reliable for the definite diagnosis of such patients. There are no well-recognized clinical or biochemical diagnostic criteria for NICCD patients thus far.

Metabolome investigation often provides valuable clues for NICCD diagnosis. On GC-MS urinary analysis, coexistence of the markers for galactosemia (galactose, galactitol, and galactonate) and tyrosinemia (4-HPL and 4-HPP) is quite common in such cases. On MS-MS analysis of blood samples, typical NICCD patients have elevated citrulline, methionine, threonine, tyrosine, lysine, arginine, and ornithine, along with raised long-chain acylcarnitine levels. These metabolome alterations are usually transient, and

not specific, either. Some NICCD patients have no such changes, especially in those who received dietary therapy, while some patients with other inborn errors of metabolism, such as galactosemia or mitochondrial DNA depletion syndrome, exhibit similar metabolome changes sometimes.

Thus far, *SLC25A13* gene and/or its expression product analysis has been taken as the reliable diagnostic tools for NICCD. Usually, the identification of biallelic *SLC25A13* mutations concludes a NICCD diagnosis (Lin et al. 2016).

In the diagnosis of CTLN2 (patient 2), detection of raised plasma ammonia level may be the first key to suspect CTLN2. Therefore, plasma ammonia should always be checked for patients with disturbed consciousness. However, as plasma ammonia level is not always high in CTLN2 patients, ammonia level should be measured several times, even if the first evaluation of ammonia shows no abnormality. An elevation of ammonia tends to be observed after a meal. Also, plasma amino acids assay including citrulline and arginine are mandatory. By abdominal US, CT, or MRI, the presence of other causes of hyperammonemia should be carefully checked (e.g., liver cirrhosis and portovenous shunt). The unique food preference is also a good clue to suspect citrin deficiency. The confirmation of diagnosis is made with a DNA analysis of *SLC25A13* (Kobayashi et al. 1999; Kikuchi et al. 2012; Lin et al. 2016).

1.3 Biochemical Perspectives

1.3.1 Historical Perspectives and Clinical Phenotypes

In 1981, Saheki et al. described two types of citrullinemia, qualitative and quantitative. The former is classical citrullinemia or CTLN1, caused by mutations in argininosuccinate synthetase (ASS) that catalyzes formation of argininosuccinate from citrulline and aspartate. The latter is named adult-onset type II citrullinemia or now CTLN2, in which ASS protein with normal enzymatic properties is liver specifically decreased. The gene (*SLC25A13*) causative for CTLN2 was described by Kobayashi et al. in 1999 and found to encode a liver-type mitochondrial aspartate-glutamate carrier, designated citrin. The human gene *SLC25A13* is localized at chromosome 7q21.3, which consists of 18 exons and encodes a 3.4 kb transcript with a predicted open reading frame (ORF) of 2025 bp. Citrin, as liver-type aspartate-glutamate carrier isoform 2 (AGC2), functions to export aspartate from the mitochondrial matrix in exchange for cytosolic glutamate and H^+(Palmieri et al. 2001), playing important roles in supplying aspartate to the cytosol for protein, nucleotide, and urea syntheses, and as a member of the malate-aspartate shuttle. Biallelic *SLC25A13* mutations result in citrin deficiency, a disease entity with three age-dependent clinical phenotypes, i.e., NICCD (OMIM#605814) in neonates or infants, CTLN2 (OMIM#603471) in adolescents/adults, and failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD) between NICCD and CTLN2 stages (Kobayashi et al. 2014). In addition, it is well-known that nonalcoholic fatty liver disease (NAFLD) including steatohepatitis (NASH) is frequently seen in citrin-deficient patients (Komatsu et al. 2008). Also, approximately 10–20% of citrin-deficient patients accompany pancreatitis or hepatoma in the pre-CTLN2 stage (Ikeda et al. 2001; Soeda et al. 2008). Hence, careful differential diagnosis may be also needed for patients with juvenile-onset, nonalcoholic pancreatitis or hepatic cancer unassociated with hepatitis virus infection. However, precise clinical pictures of citrin-deficient patients, especially in the pre-CTLN2 stage, remain obscure. While this disease is distributed worldwide, citrin deficiency is relatively more common among East Asian populations; however, some cases in western countries have been reported.

1.3.2 Structure and Function of Citrin

Citrin (AGC2) consists of 675 amino acid residues with a molecular weight of approximately 74 kD. The carboxy-terminal portion demonstrates similarity (20–30% amino acid identity) with proteins of the mitochondrial solute-carrier

family (Kobayashi et al. 1999). The amino-terminal portion contains eight EF-hand domains that are conserved in calcium-binding proteins (Thangaratnarajah et al. 2014). The overall structure of citrin is most similar to that of the isoform, aralar (77.8% identity), which is encoded by the gene *SLC25A12*. AGC catalyzes unidirectional transport of glutamate and H^+ from cytosol to mitochondria and aspartate from mitochondria to cytosol under in vivo conditions. Properties, such as calcium activation and unidirectionality, indicate the metabolic significance of both AGCs.

1.3.3 Metabolic Functions of Citrin

Citrin plays at least three important roles in metabolism.

1. Citrin supplies aspartate from mitochondria to cytosol, where aspartate is needed for the synthesis of protein, nucleotides, and urea.
2. Citrin plays a role in transport of cytosolic NADH-reducing equivalent and energy metabolism as a member of the malate-aspartate shuttle.
3. Citrin is essential for gluconeogenesis from lactate and some other reduced substrates, such as glycerol and sorbitol. Citrin plays a role in maintaining stoichiometry of cytosolic NADH between production and consumption.

The transport of NADH-reducing equivalent from the cytosol to mitochondria, or the NADH shuttle, is mediated mainly by two systems, the malate-aspartate and glycerophosphate shuttles. The former is distributed in most tissues, while the latter is predominant in the skeletal muscles, brown adipose tissue, and brain. In the human liver, glycerophosphate shuttle activity is much less than that of the malate-aspartate shuttle, while the two shuttles are almost equally active in the rodent liver. This is why there were virtually no symptoms of human citrin deficiency in *Slc25a13* gene defect or citrin-KO mice.

1.3.4 Pathophysiology of Citrin Deficiency

The increased cytosolic NADH/NAD^+ ratio in hepatocytes, caused by the cytosolic metabolism of glucose, glycerol, and ethanol, all known precipitating factors of CTLN2, has been well-recognized as a key biochemical alteration. The high cytosolic NADH inhibits glycolysis at the step of glyceraldehyde 3-phosphate dehydrogenase, leading to a low supply of pyruvate into the mitochondria. This, together with no NADH-reducing equivalent from the cytosol to mitochondria, reduces the cytosolic and mitochondrial energy production from carbohydrates. Moreover, in citrin-deficient individuals, the mRNAs encoding enzymes/proteins involved in fatty acid oxidation and fatty acid transport are markedly suppressed, and the expression of peroxisome proliferator-activated receptorα (PPARα), a master regulator of hepatic lipid metabolism, is significantly suppressed (Komatsu et al. 2015). Serum concentrations of ketone bodies are also decreased in these patients (Imamura et al. 2003), suggesting reduced mitochondrial β-oxidation activity. All these findings suggest energy deficiency.

Fatty liver has been found in most citrin deficiency patients including NICCD and CTLN2 (Komatsu et al. 2008). It is most likely because, as stated above, the expression of PPARα is significantly suppressed, although the expression of hepatic genes associated with lipogenesis and triglyceride hydrolysis is not changed. It is also possible that the citrate-pyruvate shuttle, which is originally involved in fatty acid synthesis and induces a decrease in cytosolic NADH, may be activated, resulting in the stimulation of fatty acid synthesis.

1.3.5 Hyperammonemia in CTLN2 Is Different from Other Urea Cycle Enzyme Defects

It should be noted that the mechanism of hyperammonemia in citrin deficiency is different from other hyperammonemia caused by urea cycle

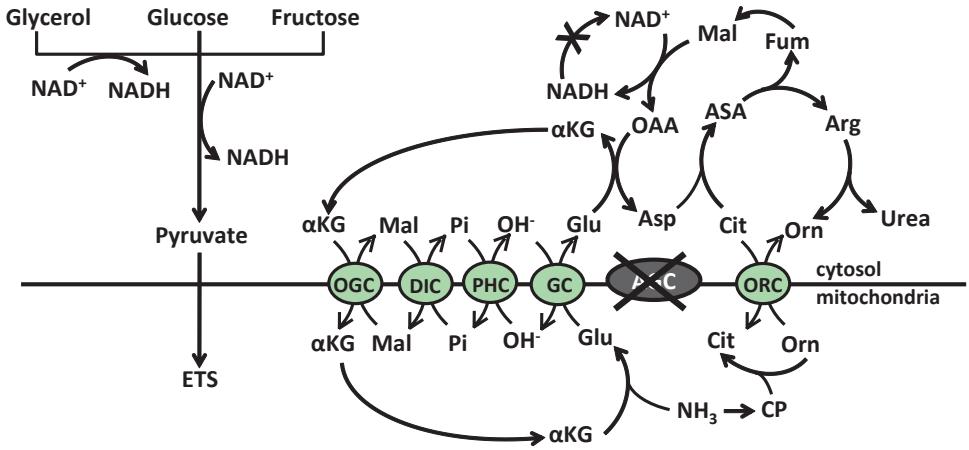

Fig. 1.3 Effect of carbohydrate metabolism on ureagenesis under citrin deficiency. *Arg* arginine, *Asp* aspartate, *ASA* argininosuccinate, *Cit* citrulline, *CP* carbomoylphosphate, *Fum* fumarate, *μKG* α-ketoglutarate ; *Mal* malate, *Pi* inorganic phosphate, *OAA* oxaloacetate, *Orn* ornithine, *AGC2* aspartate-glutamate carrier 2 or citrin, *DIC* dicarboxylate carrier, *ETS* electron transport system, *GC* glutamate carrier, *OGC* α-ketoglutarate malate carrier, *ORC* ornithine carrier, *PHC* phosphate carrier, *ASL* argininosuccinate lyase, *ASS* argininosuccinate synthetase, *AST* aspartate aminotransferase, *CPS1* carbamoylphosphate synthetase 1, *OCT* ornithine carbamoyltransferase, *MDH* malate dehydrogenase

enzyme deficiencies or conventional hyperammonemia. The intake of protein is harmful to patients with conventional hyperammonemia, while carbohydrate intake is harmful for citrin deficiency. The mechanism of hyperammonemia in citrin deficiency is as follows.

Urea can be synthesized from ammonia in the absence of citrin (Fig. 1.3). Glutamate formed from ammonia leaves the mitochondria via a glutamate carrier and is converted to aspartate in the cytosol, and urea is formed via argininosuccinate and arginine. In this metabolic pathway, oxaloacetate should be formed from fumarate via malate, the reaction in which NADH is formed. The NADH formed should be oxidized back to NAD^+. Under active metabolism from glucose, fructose, glycerol, and ethanol, NADH is formed. If the NADH is not oxidized back to NAD^+, oxaloacetate and aspartate cannot be formed, resulting in the inhibition of the ASS reaction, leading to citrullinemia and finally hyperammonemia.

Active carbohydrate metabolism inhibits urea synthesis, and an increase in NADH inhibits glycolysis at the step of glyceraldehyde-3-phosphate dehydrogenase, resulting in an energy deficit. It should be also noted that hyperammonemia in citrin deficiency is observed after eating or in the afternoon to evening, but not in the morning or after fasting.

Citrin-deficient subjects dislike carbohydrates, such as cooked rice and sweets, but enjoy protein- and fat-rich diets. This peculiar food preference could be related to the abovementioned pathophysiology and is important for diagnosis and treatment (Saheki et al. 2008).

At the end stage of CTLN2, hepatic ASS is decreased to less than 10% of the control on average, although the mechanism is not known. This decrease in hepatic ASS makes the intake of protein difficult as in CTLN1, rendering the treatment of CTLN2 more difficult.

1.3.6 Possible Mechanisms Related to Aberrant Metabolisms Under Citrin Deficiency

Cholestasis is defined as an impairment of bile secretion and flow followed by a lack of bile in the intestine and accumulation of potentially toxic cholephiles (substances that are soluble in or extracted by bile acid) in the liver and the systemic circulation. The canalicular bile secretion

involves many energy-dependent molecular processes. As examples, the cross-canalicular excretion of bile acids, phospholipids, and cholesterol, the three major organic solutes in bile, is mediated by the ATP-binding cassette (ABC) transporters BSEP, MDR3, and ABCG5/8, respectively. Since citrin deficiency results in energy-lacking hepatocytes, it is not surprising for NICCD patients to have intrahepatic cholestasis that presents as late-onset/prolonged jaundice, hepatosplenomegaly, and/or abnormal biochemical changes including elevated aminotransferases, Tbil, Dbil, and TBA levels.

Dramatically elevated AFP with reduced albumin is quite common in NICCD patients. AFP and albumin have highly homologous and conserved primary structures, and their genes belong to the same family on human chromosome 4. AFP decreases dramatically and immediately after birth, resulting in the substitution of AFP by albumin. Since the expression pattern of the proteins mirrors development and maturation of the liver, the delayed switch of AFP to albumin may reflect the immature liver development in NICCD, although other factors, such as hepatocellular destruction and regeneration, are also likely to contribute to low albumin and high AFP.

Coexistence of the markers for galactosemia (galactose, galactitol, and galactonate) and tyrosinemia (4-HPL and 4-HPP) is quite common in urine samples of NICCD subjects, suggesting secondary defects in galactose and tyrosine metabolisms. UDP-galactose epimerase, one of the enzymes of galactose metabolism, has been reported to be inhibited by NADH as a competitor of NAD^+ bound to the enzyme. NAD^+ plays a role in the enzyme mechanism, although the enzyme is not oxidoreductase. This inhibition by NADH may be the cause of galactosemia in NICCD.

In blood samples of NICCD infants, in addition to elevated long-chain acylcarnitines, the levels of some amino acids (including citrulline, ornithine, arginine, threonine, methionine, and tyrosine) are also increased. Deficiency of citrin affects the liver cytosol concentration of aspartate for ASS, leading to an accumulation of citrulline, an upstream substrate in the same cycle. An elevated level of arginine, a downstream product of ASS in the urea cycle, can be explained by the fact that arginine is mainly synthesized in the kidney and small intestine (during neonatal period) from citrulline formed in the small intestine, and the accumulation of citrulline in NICCD accelerates this process. The increased threonine might reflect an inhibited degradation of this amino acid via threonine dehydrogenase, a biochemical reaction giving rise to NADH. The elevated long-chain acylcarnitine levels might result from the impaired mitochondrial β-oxidation of fatty acids.

1.3.7 Molecular Perspectives

Analysis of the *SLC25A13* gene and/or its expression product is a reliable diagnostic tool for NICCD. To date, 106 pathogenic *SLC25A13* mutations/variations have been reported (Lin et al. 2016), most of which are point mutations or short insertions/deletions. Among the *SLC25A13* mutations detected in Chinese NICCD patients, c.851_854del, c.1638_1660dup, c.615+5G>A, and IVS16ins3kb account for approximately 85% of all mutated alleles. In Japan, 11 mutations including c.851_854del, c.1019_1177del, c.1231_1311del, and c.675C > A account for approximately 95% (Kikuchi et al. 2012). *SLC25A13* cDNA cloning analysis with human peripheral blood lymphocytes (PBLs) may be a less invasive and more feasible diagnostic tool in cases where no positive results are available with conventional methods, such as polymerase chain reaction (PCR), long and accurate PCR (LA-PCR), PCR-restriction fragment length polymorphism (PCR-RFLP), and Sanger sequencing. In such cases, detection of citrin protein in biopsied liver specimens or cultured skin fibroblasts using Western blotting is an alternative diagnostic tool. In addition, both fresh and expanded PBLs have been tried as the protein source for Western blotting analysis of citrin molecules. Figure 1.4 illustrates a representative molecular diagnostic approach for NICCD using Western blotting to detect citrin protein in mitochondrial proteins extracted from cultured PBLs.

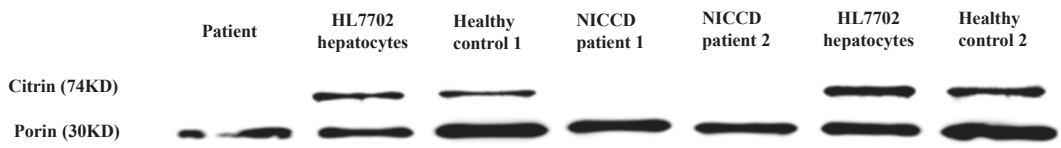

Fig. 1.4 Western blotting analysis of mitochondrial proteins. Citrin signal was detected in the cultured PBLs of two healthy controls and the HL7702 hepatocyte line, but absent in the cultured PBLs of this patient as well as two NICCD patients diagnosed previously

1.4 Treatment

1.4.1 Treatment of NICCD

To our knowledge, no specific medication has been developed to treat NICCD. Fat-soluble vitamins and zinc supplementation might be beneficial since these nutrients are usually deficient in NICCD infants. Notably, galactose-free and MCT-enriched formulas have been reported to be clinically and biochemically effective for NICCD patients in an increasing number of clinical cases.

Breast milk or common formula contains high carbohydrate in the form of lactose. In the gut, this carbohydrate is digested into galactose and glucose, which are then absorbed into the blood to serve as major substrates for energy production and fuel storage in human neonates or infants, and galactose is a well-known precursor of glucose. In the cytosol of hepatocytes of NICCD patients, the catabolic metabolism of glucose (glycolysis) produces NADH, and the resultant elevated NADH/NAD^+ ratio inhibits glycolysis, causing an energy shortage and leading to cholestasis and liver damage in NICCD patients. In addition, the energy shortage might distort the mitochondrial structures in citrin-deficient hepatocytes, thus impairing the mitochondrial β-oxidation, giving rise to lipid accumulation and resulting in fatty liver, and aggravating the energy shortage. Moreover, as previously stated, secondary galactosemia due to citrin deficiency might also be involved in NICCD pathogenesis, leading to accumulation of a large quantity of galactitol and galactonate. Galactitol has been suggested to be a substrate that causes jaundice, hepatosplenomegaly, hepatocellular insufficiency, and cataracts.

Energy shortage in the liver caused by an impairment of glycolysis due to an increased NADH/NAD^+ ratio has been proposed as an important pathophysiology of citrin deficiency. MCT is quickly hydrolyzed and absorbed mainly as medium-chain fatty acids (MCFA). The absorption of MCFA is not bile acid-dependent, which might reduce the burden of the liver to synthesize and excrete bile salt into the gut. Moreover, MCFA can be better absorbed and transported via the portal vein into the liver and is mainly metabolized to acetyl-CoA along with $FADH_2$ and NADH, which could supply more such substrates to hepatic cells as energy sources. Also, it is important to note that MCT administration is known to stimulate de novo hepatic lipogenesis, which consumes cytosolic NADH to produce NAD^+, thus decreasing the cytosolic NADH/NAD^+ ratio.

Taking all these factors together, it is not surprising that NICCD infants respond well to galactose-free and MCT-enriched therapeutic formulas.

1.4.2 Treatment of CTLN2

1.4.2.1 Liver Transplantation

To replace citrin and support (compensate for) citrin function are of utmost importance in the therapy for patients with CTLN2. Hence, liver transplantation is one of the most promising therapies for CTLN2 patients (Ikeda et al. 2001). After liver transplantation, abnormal laboratory findings including plasma ammonia and citrulline can be rapidly normalized. Also, neuropsychotic symptoms are completely absent after liver translation. However, the shortage of donors is a serious challenge, especially in Japan, and other therapeutic options should be developed.

1.4.2.2 Nonsurgical Treatment

In citrin deficiency, the cytosolic ratio of NADH/NAD^+ in the hepatocytes dramatically increases after glucose metabolism (Saheki and Kobayashi 2002). Most likely to evade this condition, citrin-deficient patients are assumed to avoid carbohydrates by their unique food preferences. The PFC ratios in the diet calories of patients with citrin deficiency are different from those in healthy individuals, and the energy ratio of carbohydrates in citrin-deficient patients (approximately 40%) is much lower than in healthy individuals (54–58%) (Saheki et al. 2008; Nakamura et al. 2011). Therefore, a carbohydrate-restricted diet with an energy ratio of approximately 40% carbohydrate is effective for CTLN2 patients (Imamura et al. 2003; Fukushima et al. 2010; Nakamura et al. 2011). In a hospital protein-restrictive diet for liver disorders (40 g/day), the carbohydrate ratio exceeds 70%; therefore, this diet should be avoided in CTLN2 patients (Fukushima et al. 2010). At Shinshu University Hospital, a carbohydrate-restriction diet (with a PFC ratio 15%:45%:40%, total 1720 kcal) has been recently served as a basic hospital diet for CTLN2 patients (Nakamura et al. 2011).

Several previous studies have reported the therapeutic efficacy of oral intake of MCT in CTLN2 patients (Hayasaka et al. 2014) to compensate for the shortage of energy in the hepatocytes caused by citrin deficiency.

Like patient 2, the efficacy of oral intake of sodium pyruvate has been described in other CTLN2 patients. Sodium pyruvate was first tried in patients with mitochondrial disorders (Tanaka et al. 2007). This chemical is presumed to play an important role in decreasing the cytosolic NADH/NAD^+ ratio (Moriyama et al. 2006). Long-term therapeutic efficacy of sodium pyruvate in CTLN2 patients is still being examined. Approximately 60% patients treated with sodium pyruvate have had a favorable response so far (Mutoh et al. 2008; Yazaki et al. 2013).

As for other therapeutic options, the efficacy of arginine granule has been described (Imamura et al. 2003). To reduce production of ammonia by intestinal bacteria, oral administration of lactulose or antibiotics may be also effective; however, this treatment remains controversial.

1.4.2.3 Unfavorable Treatments

High-carbohydrate consumption and drinking alcohol should be avoided. As demonstrated by patient 2, a hospital diet for liver disease (protein-restriction diet) may also be risky for CTLN2 patients because of the high-carbohydrate content (Fukushima et al. 2010).

We previously reported the risk of encephalopathy by glycerol infusion, which is a hyperosmotic diuretic solution consisting of 10% glycerol and 5% fructose used mainly to treat brain edema (Yazaki et al. 2005). Since then, its administration has been prohibited for CTLN2 patients in Japan. Instead, D-mannitol is recommended for brain edema in CTLN2 patients. Although a detailed pathophysiology is not available, glycerol metabolism seems to further facilitate cytosolic NADH accumulation (Yazaki et al. 2005). Most likely due to a similar mechanism, the infusion of hyperalimentation fluid containing high levels of glucose is also risky for CTLN2 patients.

End-of-Chapter Questions

1. Citrin deficiency has different clinical presentations among patients of different ages. What are the three age-dependent phenotypes?
2. NICCD patients usually have colorful but nonpathognomonic clinical and laboratory presentations. What are the major clinical and laboratory features for this phenotype?
3. Why do NICCD patients respond well to galactose-free and MCT-enriched formulas?
4. Why is a conventional hospital diet for liver disease or an intravenous infusion of glycerol solution risky for CTLN2 patients?
5. Why does carbohydrate intake result in hyperammonemia in citrin deficiency?

References

Fukushima K, Yazaki M, Nakamura M et al (2010) Conventional diet therapy for hyperammonemia is risky in the treatment of hepatic encephalopathy associated with citrin deficiency. Intern Med 49:1–20

Hayasaka K, Numakura C, Toyota K et al (2014) Medium-chain triglyceride supplementation under a low-carbohydrate formula is a promising therapy for adult-onset type II citrullinemia. Mol Genet Metab Rep 1:42–50

Ikeda S, Yazaki M, Takei Y et al (2001) Type II (adult onset) citrullinemia: clinical pictures and the therapeutic effect of liver transplantation. J Neurol Neurosug Psychiatr 71:663–670

Imamura Y, Kobayashi K, Shibatou T et al (2003) Effectiveness of carbohydrate-restrictive diet and arginine granules therapy for adult-onset type II citrullinemia: a case report of siblings showing homozygous *SLC25A13* mutation with and without the disease. Hepatol Res 26:68–72

Kikuchi A, Arai-Ichinoi N, Sakamoto O et al (2012) Simple and rapid genetic testing for citrin deficiency by. Screening 11 prevalent mutations in *SLC25A13*. Mol Genet Metab 105:553–558

Kobayashi K, Sinasac DS, Iijima M et al (1999) The gene mutated in adult-onset type II citrullinaemia encodes a putative mitochondrial carrier protein. Nat Genet 22:159–163

Kobayashi K, Saheki T, Song YZ (2014) Citrin deficiency. In: Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP (eds) Gene reviews [Internet]. University of Washington, Seattle; 1993–2005 Sept 16

Komatsu M, Yazaki M, Tanaka N et al (2008) Citrin deficiency as a cause of chronic liver disorder mimicking non-alcoholic fatty liver disease. J Hepatol 49:810–820

Komatsu M, Kimura T, Yazaki M et al (2015) Steatogenesis in adult-onset type II citrullinemia is associated with down-regulation of PPARα. Biochim Biophys Acta 1852:473–481

Lin WX, Zeng HS, Zhang ZH et al (2016) Molecular diagnosis of pediatric patients with citrin deficiency in China: *SLC25A13* mutation spectrum and the geographic distribution. Sci Rep 6:29732

Moriyama M, Li MX, Kobayashi K et al (2006) Pyruvate ameliorates the defect in ureogenesis from ammonia in citrin-deficient mice. J Hepatol 44:930–938

Mutoh K, Kurokawa K, Kobayashi K, Saheki T (2008) Treatment of a citrin-deficient patient at the early stage of adult-onset type II citrullinaemia with arginine and sodium pyruvate. J Inherit Metab Dis 31(Suppl 2):S343–S347

Nakamura M, Yazaki M, Kobayashi Y et al (2011) The characteristics of food intake in patients with type II citrullinemia. J Nutr Sci Vitaminol 57:239–245

Palmieri L, Pardo B, Lasorsa FM et al (2001) Citrin and aralar 1 are Ca^{2+}-stimulated aspartate/glutamate transporters in mitochondria. EMBO J 20:5060–5069

Saheki T, Kobayashi K (2002) Mitochondrial aspartate glutamate carrier (citrin) deficiency as the cause of adult-onset type II citrullinemia (CTLN2) and idiopathic neonatal hepatitis (NICCD). J Hum Genet 47:333–341

Saheki T, Kobayashi K, Terashi M et al (2008) Reduced carbohydrate intake in citrin-deficient subjects. J Inherit Metab Dis 31:386–394

Soeda J, Yazaki M, Nakata T et al (2008) Primary liver carcinoma exhibiting dual hepatocellular-biliary epithelial differentiations associated with citrin deficiency: a case report and review of the literature. J Clin Gastroenterol 42:855–860

Tanaka M, Nishigaki Y, Fuku N et al (2007) Therapeutic potential of pyruvate therapy for mitochondrial diseases. Mitochondrion 7:399–401

Thangaratnarajah C, Ruprecht JJ, Kunji ER (2014) Calcium-induced conformational changes of the regulatory domain of human mitochondrial aspartate/glutamate carriers. Nat Commun 5:5491

Yazaki M, Takei Y, Kobayashi K et al (2005) Risk of worsened encephalopathy after intravenous glycerol therapy in patients with adult-onset type II citrullinemia (CTLN2). Intern Med 44:188–195

Yazaki M, Kinoshita M, Ogawa S et al (2013) A 73-year-old patient with adult-onset type II citrullinemia successfully treated by sodium pyruvate and arginine. Clin Neurol Neurosurg 115:1542–1545