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A simple and rapid genetic test for Citrin deficiency

Shigeo Kure [1], Atsuo Kikuchi [1], Natsuko Arai-Ichinoi [1], Osamu Sakamoto [1], Yoichi Matsubara [2], Toshihiro Ohura [1) 3)]

1) Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan

2) Department of Medical Genetics. Tohoku University School of Medicine, Sendai, Japan

3) Division of Pediatrics, Sendai City Hospital, Sendai, Japan

[Background]

Citrin deficiency is an autosomal recessive disorder caused by mutations of the SLC25A13 gene and has two phenotypes: adult-onset type II citrullinemia (CTLN2) and neonatal hepatitis associated with intrahepatic cholestasis (NICCD). The clinical appearance of these diseases is variable, ranging from almost no symptoms to coma, brain edema, and severe liver failure that requires liver transplantation. Mutation analysis in SLC25A13 gene is important because of difficulties in the chemical diagnosis of citrin deficiency. Eleven prevalent *SLC25A13* mutations account for 95% of mutant alleles in Japanese patients with NICCD, which are favorable for genetic testing (Tabata et al. J Hum Genet, 53:534,2008). Development of a simple screening test for these mutations would be desired.

[Methods]

We employed real-time PCR to amplify seven genomic fragments, which contained mutated sites in Mutations I, II, III, IV, V, VI, and XIX in the real time PCR (LightCycler, Roche). Because Mutations VI, VII, VIII, IX, and XXI were clustered within 23 bases in exon 17 their mutation sites were contained in a single amplicon. The presence of the mutation in each amplicon was determined by the melting curve analysis with fluoresceinated oligonucleotide probes (HybProbe, Roche).

[Results]

All of the 11 mutations were successfully detected within an hour without false-positive results.

[Conclusion]

We have established the rapid and simple detection system of eleven prevalent *SLC25A13* mutations without the post PCR procedure. This simple test would facilitate the genetic diagnosis of citrin deficiency.

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Clinical presentations of patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) in Japan.

Ohura T[1)2)], Kobayashi K[3)], Tazawa Y[2)], Abukawa D[2)], Sakamoto O[2)], Saheki T[4)]

1) Dept. of Pediatrics, Sendai City Hospital, Sendai, Japan

2) Dept. of Pediatrics, Tohoku University School of Medicine, Sendai, Japan,

3) Dept. of Molecular Metabolism and Biochemical Genetics, Kagoshima University Graduate School of Medicine and Dental Sciences, Kagoshima, Japan

4) Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Japan

We clarified the clinical features of NICCD (neonatal intrahepatic cholestasis caused by citrin deficiency) by retrospective review of symptoms, management, and long-term outcome of 75 patients. The data were generated from questionnaires to pediatricians in charge of the patients. Thirty of the patients were referred to hospitals before 1 month of age because of positive results in newborn screening (hypergalactosemia, hypermethioninemia, and hyperphenylalaninemia). The other 45, the screen-negative patients, were referred to hospitals with suspected neonatal hepatitis or biliary atresia because of jaundice or discolored stool. Most of the screen-negative patients presented before 4 months of age, and 11 had failure to thrive. Laboratory data showed elevated serum bile acid concentrations, hypoproteinemia, low levels of vitamin K-dependent coagulation factors, and hypergalactosemia. Hypoglycemia was detected in 18 patients. Serum amino acid analyses showed significant elevation of citrulline and methionine concentrations. Most of the patients were given a lactose-free and/or medium chain triglyceride-enriched formula and fat-soluble vitamins. Symptoms resolved in all but two of the patients by 12 months of age. The two patients with unresolved symptoms suffered from progressive liver failure and underwent liver transplantation before their first birthday. Another patient developed citrullinemia type 2 (CTLN2) at age 16. It is important to recognize that NICCD is not always a benign condition.

To address whether citrin-deficient subjects demonstrate alterations in their nutrient intake, we further measured proportion of energy intake from carbohydrate, protein and fat in 7 children with citrin deficiency. The protein-fat-carbohydrate ratio (PFC ratio) of NICCD patients was 19% vs. 46% vs. 35%, which clearly shows a greater contribution of fat making up for the reduced carbohydrate proportion compared to that of the general Japanese population (15% vs. 28% vs. 57%). The results revealed that citrin-deficient patients had taken a lesser amount of carbohydrate from one year of age. The low-carbohydrate diet may be beneficial for citrin-deficient subjects. We propose that dietary intervention may be critically important in treating NICCD and CTLN2 patients.

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Fatigue and quality of life in patients with citrin deficiency during adaptation and compensation stage

K. Kobayashi (The University of Tokyo Graduate School of Medicine), Y. Okano, K. Ihara, T. Ito, M. Yoshino, J, Watanabe, J, Kaji, T Ooura, M. Nagao, A. Noguchi, S. Mushiaki, N. Hohashi

Introduction

Citrin deficiency is reported to have a carrier prevalence of 1 in 70 in the Japanese population and homozygote prevalence is estimated to be 1 in 17,000. In other words, only about 20% of patients with a homozygous citrin deficient gene develop Adult-onset type II citrullinemia (CTLN2) in adulthood. Eighty per cent of patients won't develop CTLN2 but will maintain the silent stage condition.

Child and adolescent patients are thought to be in the adaptation and compensation stage, which is regarded as the silent stage. However, general fatigue, inappetence and disturbed growth in the adaptation and compensation stage patients are indicated in patients who will develop CTLN2 in adulthood as well as those who won't. Fatigue is a health problem that significantly impacts quality of life (QOL) and it is a frequent and ubiquitous complaint among patients with chronic disease.

The aims of this study are to describe fatigue and QOL in patients with citrin deficiency during adaptation and compensation, and to explore the relationship between fatigue and QOL.

Methods

The sample for this study comprised 53 outpatients with citrin deficiency (29 males and 24 females, average age 8.85 years) and 51 guardians. Patient fatigue was evaluated using self-reports and proxy-reports of the PedsQL Multidimensional Fatigue, and QOL was evaluated using the PedsQL Generic Core Scales.

Results

Regarding the PedsQL Multidimensional Fatigue Scale, the mean scores of patients and their guardians were significantly lower than those of healthy children and their parents: 46% patients were in the 25th percentile norm, and 33% were in 50th percentile norm. Concerning the PedsQL Generic Core Scales, no differences in mean scores were found between patients and healthy children: 32% of the patients were in the 25th percentile norm, and 35% were in 50th percentile norm. On the other hand, patients' guardians rated significantly lower than parents of healthy children.

When relationships between the PedsQL Multidimensional Fatigue Scale and the PedsQL Generic Core Scales were examined, significant correlations were found for both the patients and their guardians (Spearman's correlation $r = 0.54$ and 0.71, respectively).

When comparing the fatigue and QOL scores of patients and their guardians to examine the

agreement level between the patients' self-reports and the guardians' proxy-report, moderate to significant agreements were found. However, the guardians tended to rate their children's fatigue and QOL better than their children did.

Conclusion

This study described fatigue and QOL of patients with citrin deficiency during the adaptation and compensation stage for the first time. The results showed that patients had significantly worth fatigue scores than healthy children. It can therefore be concluded that even in the adaptation and compensation stage, children with citrin deficiency seem to have difficulty with fatigue and their QOL is affected.

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A rare manifestation of the patients with citrin deficiency: chronic pancreatitis and hepatic cancer

Shu-ichi Ikeda and Masahide Yazaki
Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan

[Introduction]

Adult patients with citrin deficiency usually develop hepatic encephalopathy with high plasma levels of citrulline and ammonia, but a few patients with this disorder seem to have had preceding pancreatitis or hepatic cancer. In this presentation we refer to these rare manifestations of the patients with citrin deficiency.

[Case Reports]

Three male patients experienced acute pancreatitis at ages 14, 21, 33 respectively. During the following 2 to 6 years they commonly experienced recurrent attacks of pancreatitis, resulting in spontaneous remission of this pancreatitis. Finally they developed hepatic encephalopathy at ages 25, 27, 35 respectively. CT revealed an atrophied pancreas with dotty calcification, and autopsy findings of two cases disclosed extensive and severe fibrosis with diffuse atrophy of the acinar cells, all of which were consistent with the histopathological findings of chronic pancreatitis. They had no history of alcoholism, and juvenile or young-age onset was characteristic for this form of chronic pancreatitis.

Two patients were found to have hepatic cancer when both were referred to our hospital: one was a 40-year-old female who started to be suffered from disturbance of consciousness after the delivery of her first son. Her tumor was successfully removed, but the control of hepatic encephalopathy was very difficult and she followed an unfavorable outcome. The other was a 51-year-old man who was incidentally found to have a liver tumor and underwent the partial hepatectomy. After operation he became drowsy, showing an elevated plasma level of ammonia. One week later his condition was normalized. Although his hepatic encephalopathy could be controlled by the diet therapy with sodium pyruvate he died of the recurrence of liver cancer with extensive dissemination.

[Conclusion]

In considering the natural courses of the patients with citrin deficiency NICCD is an early manifestation and hepatic encephalopathy appears at a late stage. There is a long latent period between both disorders and during this latent period chronic pancreatitis and/or hepatic cancer may appear. Although the precise pathogenesis of chronic pancreatitis and hepatic cancer in the patients with citrin deficiency remains unclear, metabolic abnormalities due to citrin deficiency is surmised to play an important role in causing both disorders.

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Efficacy of MCT-milk in NICCD

Taisuke Takeda, Yoshiyuki Okano,Citrin deficiency working group
Department of Pediatrics, Osaka City University Graduate School of Medicine

Citrin deficiency is caused by mutation of SLC25A13 gene which encode aspirate glutamate carrier (AGC), citrin. NICCD (neonatal intrahepatic cholestasis caused by citrin deficiency) is one phenotype of citrin deficiency characterized by transient neonatal cholestasis and variable hepatic dysfunction. Its presentations are low birth weight, growth retardation, jaundice, acholic stool, hypoproteinemia, coagulopathy, hemolytic anemia, and hypoglycemia and usually disappers before1 year of age.

In some patients with NICCD, malabsorption of long-chain triglyceride and fat-soluble vitamin exist due to intrahepatic cholestasis. Some patients with NICCD have been treated with lactose-free formula, medium-chain triglycerides (MCT) formula, and/or fat-soluble vitamin supplementation. Even if the patients have not been treated with MCT/lactose-free formula, the symptoms are self-limiting between 6 to 12 months of age. However, some patients developed liver failure and underwent liver transplantation. Therefore, NICCD is not a safety disease. It is necessary to transfer the patients with NICCD as soon as possible to the silent stage, which is adaptation and compensation of metabolism, for better prognosis. The aim of our study is to clarify the efficacy of MCT-milk in NICCD.

We examined 25 patients from the questionnaire to pediatricians in charge of the patients.10 patients (MCT-group) had taken MCT milk, and 15 patients (non-MCT group) did not take MCT milk. We compared the body weight gain and laboratory data of these two groups. As for the result, MCT-group patients showed more rise in body weight SD score than non-MCT group. Improvement rate of laboratory data (T-bil, ALP, total bile acid) did not show any remarkable difference between the two groups. MCT-milk is effective in growth, and considered to be essential for NICCD.

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A therapy with medium-chain triglyceride (MCT)-supplemented formula in citrin deficiency

Kiyoshi Hayasaka
Department of Pediatrics, Yamagata University School of Medicine

Citrin plays a role in the transfer of NADH-reducing equivalent from cytosol to mitochondria as part of the malate-aspartate shuttle in liver. Citrin deficiency may cause an impairment of glycolysis due to an increase in the cytosolic NADH/NAD ratio leading to an energy shortage in the liver. Microvesicular fatty changes observed in the liver of NICCD are similar to those in the liver of Reye syndrome or hepatic mitochondrial DNA depletion syndrome, suggesting a low energy state of the liver. Mutations of the *SLC25A13* gene are responsible for neonatal intrahepatic cholestasis (NICCD) and adult-onset type II citrullinemia (CTLN2). We had a chance to treat four patients including three siblings with NICCD by lactose (galactose)-restricted and medium-chain triglyceride (MCT)-supplemented formula. This formula rapidly improved the clinical condition and laboratory findings. Early treatment was more effective and did not require long-term administration. Lactose (galactose)-restriction can avoid further increase in the cytosolic NADH/NAD ratio in the liver and MCT supplementation can provide energy to hepatic cells by producing an excess of acetyl-CoA in mitochondria.

Based on the clinical findings in NICCD, we extended our study to CTLN2 and will present a preliminary data. We administered MCT oil to two male patients (51-year-old and 62-year-old) with CTLN2. They had several episodes of hyperammonemic encephalopathy one month before. MCT oil was administered at every low-carbohydrate meal. They had no episode of hyperammonemic encephalopathy after the treatment. Postprandial blood ammonia concentration in one case decreased to normal level in a month. In the other, it decreased to less than 300 μg/dl in a month and then to less than 150 μg/dl after two months.

The pathogenesis of citrin deficiency is likely an energy shortage of the liver due to impaired glycolysis. MCT therapy is likely effective in citrin deficiency and its effect should be confirmed in further cases.

O-16

Liver Transplantation for Citrullinemia Type II Patients - Shinshu University Experience

Shin-ichi Miyagawa, Toshihiko Ikegami, Shiu-ichi Ikeda*
First Department of Surgery, * Third Department of Internal Medicine
Shinshu University, School of Medicine, Masumoto, Japan

In citrullinemia type II (CTLN2), the most successful therapy to date has been liver transplantation, which prevents episodic hyperammonemic crises, corrects the metabolic disturbances, and eliminates preferences for protein-rich foods. Auxiliary partial orthotopic liver transplantation (APOLT) was initially introduced as a temporary or permanent support for patients with potentially reversible fulminant hepatic failure and its indications have been extended to congenital metabolic disorders of the liver including citrin deficiency.

Patients: We have performed liver transplantation for 16 CTLN2 patients since 1995. Fourteen patients underwent living donor liver transplantation (LDLT) using left liver graft from living donor and 2 underwent deceased donor liver transplantation. In 14 LDLTs, 5 patients underwent permanent APOLT because of both small ratio of graft volume/ standard liver volume (GV/ SLV) less than 35% and another additional reason of an expectation that genetic therapies for citrin deficiency will become available through portal blood flow in future. In APOLT patients, right liver without the middle hepatic vein was preserved and left liver graft with middle hepatic vein was implanted orthotopically. Immediately after anastomosis of graft left portal vein with recipient's left portal vein, recipient's right portal vein was ligated alone in the earlier 4 patients and was ligated and transected in the latest 1 patient.

Results: All patients survived now and 5-year, 10-year, 15-year survival rates are 100%. In16 LDLTs, postoperative complications were hepatic artery thrombosis in 1 patient, intestinal obstruction in 1, biliary stenosis in 3, and portal blood flow steel to the recipient's remnant native liver in 3, which is a characteristic of APOLT. Because of hyperammonia crises associated with portal flow steel, the remnant native liver was removed in 2 portal vein ligated patients. The latest right portal vein transected patient has biliary stricture and has repeated intrabiliary hemorrhage related to portal blood flow steel through remarkablely developed collateral portal branches around bile duct connecting to intrahepatic portal vein in the recipient's remnant native liver at present 4 years after LDLT. The patient is now under treatment, although re-transplantation using whole liver is the best choice. Accordingly, in 5 patients with planned permanent APOLT, 2 patients needed removal of recipient's remnant native liver and one is suffering from portal blood flow steel probably due to recipient's remnant native liver. All except for this patients are well-lived without neuropsychiatric symptoms associated with hyperammonia due to citrin deficiency after liver transplantation.

Conclusions: Liver transplantation could provide good prognosis to citrullinemia type II patients. Although APOLT contributes to donor pool expansion in LDLT, permanent APOLT has a potential risk of portal blood flow steel, even if right portal vein is transected. Considering liver transplantation as the most successful therapy for CTLN2, temporal APOLT might be a better option for patients with small liver graft in LDLT than permanent one.

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Sodium-pyruvate therapy for citrin-deficient patients in the adaptation period

Yoshiyuki Okano, Citrin deficiency working group
Department of Pediatrics, Osaka City University Graduate School of Medicine

Adult-onset type II citrullinemia (CTLN2) is characterized by frequent attacks of hyperammonemia, liver steatosis, mental derangement, sudden unconsciousness, and ultimately death within a few years of onset. Kobayashi et al. found CTLN2 is caused by mutations of the SLC25A13 gene on chromosome 7q21.3, encoding a calcium-binding mitochondrial solute carrier protein, designated citrin. Infants carrying mutations in the SLC25A13 gene exhibit neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), including hypertyrosinemia, hypergalactosemia, hypoproteinemia, and hypoglycemia. The symptoms in almost all the patients are self-limiting between 6 to 12 months of age. However, some patients with this disorder exhibit severe hepatic dysfunction requiring liver transplantation.

The period between NICCD and CTLN2 does not remarkably show clinical symptoms and has been thought as the silent stage by adaptation and compensation of metabolism. However, even if in the silent stage, the patients with citrin deficiency have sometimes showed inappetence, general fatigue, disturbance in growth, abdominal discomfort, hyperlipidemia, and pancreatitis. We have revealed a strong feeling of fatigue and a decline of the QOL in patients in the adaptation period.

We have examined effects of sodium pyruvate on the patients with citrin deficiency in the adaptation period. Enrolled patients did not show intrahepatic cholestasis, impairment of hepatic function test, and hyperammonemia. Sodium pyruvate (100-300 mg/kg/day) has been taken twice or three times a day after meals. As for the preliminary results of treatments during 6-12 months, 1) The patients did not improve their fatigue and QOL by PedsQL tests. 2) Lac/Pyr ratio was decreased. 3) The oxidative stress status in the patients was improved after sodium pyruvate therapy. 4) The patients were able to eat carbohydrates such as the rice and noodles which they could not take before the treatment. We need long term observations and more patients in the adaptation period for the study on the effects of the pyruvate sodium.

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Therapeutic approaches for patients with adult onset type II citrullinemia (CTLN2) - low carbohydrate diet and oral administration of sodium pyruvate

Masahide Yazaki [1)], Kazuhiro Fukushima[1)], Michiharu Komatsu[2)], Shu-ichi Ikeda[1)], Takeyori Saheki[3)]

1)Department of Medicine(Neurology and Rheumatology) , Shinshu University School of Medicine

2)Department of Medicine(Gastroenterology) , Shinshu University School of Medicine

3) Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Japan

Adult-onset type II citrullinemia (CTLN2) is an autosomal recessive disease characterized by highly elevated plasma levels of citrulline and ammonia due to the urea cycle dysfunction associated with citrin deficiency. Patients with CTLN2 present various neurological symptoms with hyperammonemia that closely resemble those of hepatic encephalopathy. Since 1990, twenty-nine CTLN2 patients have been admitted and treated in Shinshu University Hospital. Of 30 patients, fifteen patients received liver transplantation (LT). After LT, neurological symptoms soon disappeared and all had returned to their previous social lives. Among the 15 patients who have not undergone LT, six died of intractable encephalopathy or development of hepatic cancer. Recently, eleven patients have been treated with oral intake of sodium pyruvate and low carbohydrate diet. One patient stopped taking the sodium pyruvate in a few days because of nausea. Of 10 patients, seven patients have had relatively good clinical courses (ranged from 0.5 to 4 years) with decrease in frequency of encephalopathy. However, three patients underwent LT during one month to one year after starting of sodium pyruvate therapy because frequency of attacks of encephalopathy did not decrease. Hepatic steatosis markedly ameliorated after the treatment with sodium pyruvate in 4 patients. Our observation indicates that liver transplantation is a very promising therapy but other therapeutic approaches should be also established since all patients do not always receive LT because of shortage of donor. The therapy with low carbohydrate diet and sodium pyruvate may cure many patients with CTLN2.

〔II〕　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

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〔III〕　研究成果の刊行物・別刷

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Citrin Deficiency

Includes: Citrullinemia Type II, Failure to Thrive and Dyslipidemia Caused by Citrin Deficiency, Neonatal Intrahepatic Cholestasis Caused by Citrin Deficiency

Keiko Kobayashi, PhD
Department of Molecular Metabolism and Biochemical Genetics
Kagoshima University Graduate School of Medical and Dental Sciences
Kagoshima, Japan

Takeyori Saheki, MD, PhD
Institute of Resource Development and Analysis
Kumamoto University
Kumamoto, Japan
takesah@kumamoto-u.ac.jp

Yuan-Zong Song, MD, PhD
Department of Pediatrics
The First Affiliated Hospital
Jinan University
Guangzhou, China
songyuanzong@hotmail.com

Initial Posting: September 16, 2005; Last Update: January 5, 2012.

Summary

Disease characteristics. Citrin deficiency can manifest in newborns as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), in older children as failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD), and in adults as recurrent hyperammonemia with neuropsychiatric symptoms in citrullinemia type II (CTLN2). Often citrin deficiency is characterized by fondness for protein-rich and/or lipid-rich foods and aversion to carbohydrate-rich foods.

NICCD: Children younger than age one year have growth retardation with transient intrahepatic cholestasis, hepatomegaly, diffuse fatty liver and parenchymal cellular infiltration associated with hepatic fibrosis, variable liver dysfunction, hypoproteinemia, decreased coagulation factors, hemolytic anemia, and/or hypoglycemia. Although NICCD is generally not severe and symptoms often resolve by age one year with appropriate treatment, some infants succumb to infection and liver cirrhosis and others require liver transplantation.

FTTDCD: Around age one to two years, many children with citrin deficiency develop the food preferences mentioned. Some have growth retardation, hypoglycemia, and fatigue as well as hyperlipidemia, pancreatitis, fatty liver, and hepatoma. One or more decades later, some individuals with NICCD or FTTDCD develop CTLN2.

CTLN2: Onset is sudden and usually between ages 11 and 79 years. Manifestations are recurrent hyperammonemia with neuropsychiatric symptoms including nocturnal delirium, aggression, irritability, hyperactivity, delusions, disorientation, restlessness, drowsiness, loss of memory, flapping tremor, convulsive seizures, and coma; death can result from brain edema. Symptoms are often provoked by alcohol and sugar intake, medication, and/or surgery. Affected individuals may or may not have a prior history of NICCD or FTTDCD.

Diagnosis/testing. The diagnosis of citrin deficiency is suspected from clinical and biochemical findings (in general, increased blood or plasma concentration of ammonia, plasma or serum concentration of citrulline and arginine, plasma or serum threonine-to-serine ratio, and serum concentration of pancreatic secretory trypsin inhibitor [PSTI]). Identification of biallelic mutations in *SLC25A13*, the only gene in which mutations are known to cause citrin deficiency, confirms the diagnosis.

Management. *Treatment of manifestations:* NICCD: Supplement diet with fat-soluble vitamins and use of lactose-free formula (in those with galactosemia) or formulas containing medium-chain triglycerides. FTTDCD: In addition to dietary treatment, administration of sodium pyruvate may improve growth. CTLN2: Liver transplantation prevents hyperammonemic crises, corrects metabolic disturbances, and eliminates preferences for protein-rich foods; arginine decreases blood ammonia concentration and lessens hypertriglyceridemia by reducing calorie/carbohydrate intake and increasing protein intake. Arginine and sodium pyruvate may effectively treat hyperammonemia and fatty liver, thereby delaying the need for liver transplantation.

Prevention of primary manifestations: Lipid and protein-rich low-carbohydrate diet.

Surveillance: Periodic measurement of plasma concentration ammonia and citrulline, PSTI for all phenotypes associated with citrin deficiency. Follow up of children who have had NICCD for the laboratory and physical findings of FTTDCD.

Agents/circumstances to avoid: Low-protein high-carbohydrate diets; glycerol and fructose infusions for brain edema; alcohol; acetaminophen and rabeprozole.

Evaluation of relatives at risk: It is appropriate to identify affected sibs of a proband so that appropriate dietary management can be instituted before symptoms occur.

Genetic counseling. Citrin deficiency is inherited in an autosomal recessive manner. When both parents are carriers, each sib of an affected individual has, at conception, a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. When one parent is a carrier and the other parent has two mutated *SLC25A13* alleles, each sib of an affected individual has, at conception, a 50% chance of being affected and a 50% chance of being an asymptomatic carrier. Carrier testing for at-risk relatives and prenatal testing for pregnancies at increased risk are possible if the disease-causing mutations in the family are known.

Diagnosis

Clinical Diagnosis

Citrin deficiency has two distinct well-recognized phenotypes: neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) and citrullinemia type II (CTLN2) (see Figure 1) [Saheki & Kobayashi 2002, Yamaguchi et al 2002, Kobayashi & Saheki 2004, Saheki & Kobayashi 2005, Kobayashi et al 2006]. Failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD was recently proposed as a novel intermediate phenotype [Song et al 2011].

- **Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD)** characterized by transient neonatal cholestasis and variable hepatic dysfunction
- **Failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD)** characterized by post-NICCD growth retardation before CTLN2 onset and abnormalities of serum lipid concentrations, including triglycerides, total cholesterol, and HDL-cholesterol. Clinical diagnosis of citrin deficiency during this stage is difficult in the absence of a history of unique food preferences or without molecular testing.
- **Citrullinemia type II (CTLN2)** characterized by childhood- to adult-onset, recurring episodes of hyperammonemia and associated neuropsychiatric symptoms

Testing

Table 1. Biochemical Findings in Citrin Deficiency by Phenotype

Phenotype (Age)	Blood or Plasma Concentration of Ammonia (µmol/L)	Plasma or Serum Concentration of Citrulline (C) [1]	Plasma or Serum Concentration of Arginine (A) (µmol/L)	Plasma or Serum Threonine-to-Serine Ratio	Serum Concentration of Pancreatic Secretory Trypsin Inhibitor (PSTI) [2] (ng/mL)
Control	18-47 [3]	17-43 [3]	54-130 [3]	1.10	4.6-20 [3]
NICCD (0-6 months)	60	300	205	2.29	30
FTTDCD (>1 to 11 years)	Normal, or slightly elevated	Normal, or slightly elevated	Usually normal	Unknown	Unknown
CTLN2 (11-79 years)	152	418	198	2.32	71

Kobayashi et al [2006]

1. Citrullinemia, which can be detected on newborn screening, is the earliest identifiable biochemical abnormality of NICCD [Tamamori et al 2004].

2. Because the serum PSTI concentration is high in some individuals with NICCD [Tamamori et al 2002] and also in individuals before the onset of CTLN2 [Tsuboi et al 2001], the measurement of serum PSTI concentration may be useful in presymptomatic diagnosis of CTLN2.

3. Range

In addition to the findings in Table 1, the following are observed in citrin deficiency:

NICCD

- **Plasma concentration of galactose, methionine, and/or phenylalanine** is elevated in newborn screening blood spots in approximately 40% of children with NICCD [Ohura et al 2003, Ohura et al 2007].

- **Plasma concentrations of threonine, methionine, and tyrosine** are elevated (see Table 2).

Table 2. Plasma Concentrations of Threonine, Methionine, and Tyrosine at Age 0-6 Months in NICCD

Amino Acid	Median (25%-75% Range) (µmol/L)	Control Range (µmol/L)
Threonine	496 (291-741)	67-190
Methionine	124 (53-337)	19-40
Tyrosine	178 (99-275)	40-90

Kobayashi et al [2006]

- **Plasma concentration of bilirubin, bile acids, and alpha-fetoprotein** are elevated (see Table 3).

Table 3. Measurements of Hepatic Cell Function at Age 0-6 Months in NICCD

Assayed Item	Median (25%-75% range) (mg/dL)	Control Range (mg/dL)
TB in NICCD	4.9 (2.8-8.0)	0.2-1.0
TB in CTLN2	0.8 (0.52-1.1)	
DB in NICCD	2.5 (1.5-3.7)	0-0.4
DB in CTLN2	0.3 (0.2-0.4)	
TB/DB ratio in NICCD	0.55 (0.41-0.66)	—
TBA	239 (172-293)	5-25
AFP	91,900 (33,200-174,700)	260-6,400 [1, 2] 2-55 [2, 3]

Kobayashi et al [2006]

TB= total bilirubin

DB= direct bilirubin

TBA= total bile acids

AFP= α-fetoprotein

1. 0-1 month

2. Tamamori et al [2002]

3. >1 month

FTTDCD

- **Dyslipidemia** manifests as abnormal levels of triglyceride and cholesterol (including total-, HDL- and LDL-cholesterol) [Song et al 2009a, Song et al 2011].
- **Other abnormal laboratory findings** include increased lactate to pyruvate ratio, elevated cholesterol, and higher levels of urinary oxidative stress markers [Kobayashi & Saheki 2004, Saheki & Kobayashi 2005, Kobayashi et al 2006, Nagasaka et al 2009, Lee et al 2010].

CTLN2

- **Pancreatic secretory trypsin inhibitor (PSTI)** concentration is increased in the liver [Kobayashi et al 1997] (see Table 1). Note: PSTI mRNA is increased 30-140 fold in the liver of individuals with CTLN2
- **Fischer ratio** (branched-chain amino acids [BCAAs] Val+Leu+Ile / aromatic amino acids [AAAs] Tyr+Phe) in the plasma or serum is decreased from ~3.4 to ~2 as a result of decreased BCAA.
- **Liver-specific argininosuccinate synthetase (ASS) enzyme activity** is decreased to approximately 10% that of controls (secondary effect of mutations) [Yasuda et al 2000].
- **Plasma α-fetoprotein concentration** is normal in almost all individuals with CTLN2 [Kobayashi et al 1997], except some individuals with CTLN2 associated with hepatoma [Hagiwara et al 2003].

Both NICCD and CTLN2