## Closing Remarks

11:00 **Reminiscence of Prof. Keiko Kobayashi –Discovery of citrin deficiency**
Takeyori Saheki (Kumamoto University, Japan)

11:15 **Closing Remarks**
Shu-ichi Ikeda (Shinshu University, Japan)

12:00 **Excursion ( including lunch)**
Bus tour to Kamikochi, Japan Alps National Park (http://www.kamikochi.or.jp/english/)
Please gather at lobby before GRANDE on 3F at 11:50.

19:00 **Farewell dinner (The Bright Garden Reception House)**
We will go to the Reception House by tour-bus directly from Kamikochi.

# Poster Presentation

P-1: **Prenatal diagnosis of citrin deficiency: SLC25A13 gene analysis in a family with fatal proband**
Xiao-Mei Tang (Jinan University, China)

P-2: **Identification of novel SLC25A13 splicing transcripts in human amniocytes: a transcriptome study**
Zhan-Hui Zhang (Jinan University, China)

P-3: **Variomics investigation of citrin deficiency: six-year experience in a Chinese Pediatric Center**
Xin-Jing Zhao (Jinan University, China)

P-4: **The characteristics of food intake in patients with type II citrullinemia**
Mio Nakamura (Shinshu University, Japan)

P-5: **Two patients with adult-onset type II citrullinemia (CTLN2) successfully treated by liver transplantation from cadaveric donors in Japan**
Akiyo Hineno (Shinshu University, Japan)

P-6: **Clinical pictures of aged CTLN2 patients with onset after 65 years**
Michiaki Kinoshita (Shinshu University, Japan)

P-7: **Risk of low protein diet therapy in CTLN2**
Kazuhiro Fukushima (Shinshu University, Japan)

P-8: **A case of adult-onset type II citrullinemia (CTLN2) associated with pregnancy and protein-restricted diet**
Yasuyuki Shimomura (Tsuyama Central Hospital, Japan)

P-9: **Two children of whom intrahepatic cholestases were possibly evoked by viral infection at the age of four months**
Shunsaku Kaji (Tsuyama Central Hospital, Japan)

# ABSTRACTS

**Plenary lecture**

## Overview of citrin deficiency: Pathophysiology and therapy for citrin deficiency based on the mouse model analysis

Takeyori Saheki
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Citrin deficiency is a newly-established disease entity that encompasses adult-onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestasis (NICCD), and results from mutations in the SLC25A13 gene that encodes citrin. It was first described in Japan, but is now known as a panethnic disease. Citrin is a liver-type aspartate (Asp)-glutamate (Glu) carrier in the inner mitochondrial membrane responsible for the electrogenic exchange of Asp for Glu and a H+ ion, and supplies Asp to synthesize protein, nucleotide and urea in cytosol. Furthermore, citrin is an important component of malate-Asp shuttle transporting NADH reducing equivalent from cytosol to mitochondria. One of the most prominent characteristics of citrin deficiency is the unique food preference; citrin deficiency subjects like protein-/fat-rich foods and dislike carbohydrate-rich foods. This food preference seems to be closely related to the pathogenesis. The conventional treatment procedures for hyperammonemia such as high-carbohydrate diets, and glucose and glycerol infusion are harmful for citrin deficiency. Especially, glycerol infusion for brain edema has caused deterioration of many CTLN2 patients. To elucidate the pathophysiology of citrin deficiency, and to develop therapeutics, we created a citrin deficiency model, citrin (Ctrn) and mitochondrial glycerol 3-phosphate dehydrogenase (mGPD) double knockout (KO) mouse. The resultant Ctrn/mGPD double KO mice showed citrullinemia, hypoglycemia, hyperammonemia which was enhanced by sucrose administration. The double KO mice disliked sucrose, and sucrose administration caused a dramatic increase in the hepatic glycerol 3-phosphate (G3P). Administration of ethanol or glycerol also dramatically increased the hepatic G3P of the double KO mice. We have found sodium pyruvate and some amino acids are effective for ameliorating pathophysiology of the double KO mice.

**O-2**

## Sustaining hypercitrullinemia, hypercholesterolemia and augmented oxidative stress in Japanese children with aspartate/glutamate carrier isoform 2-citrin- deficiency even during the silent period

Hironori Nagasaka[a], Yoshiyuki Okano[b], Hirokazu Tsukahara[c], Yosuke Shigematsu[c], Toru Momoi[d], Junko Yorifuji[d], Takashi Miida[e], Toshihiro Ohura[f], Keiko Kobayashi[g], Takeyori Saheki[h], Kenichi Hirano[i], Masaki Takayanagi[a], Tohru Yorifuji[d]

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Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) shows diverse metabolic abnormalities such as urea cycle dysfunction together with citrullinemia, galactosemia, and suppressed gluconeogenesis. Such abnormalities apparently resolve during the first year of life. However, metabolic profiles of the silent period remain unknown. We analyzed oxidative stress markers and profiles of amino acids, carbohydrates, and lipids in 20 asymptomatic children with aspartate/glutamate carrier isoform 2-citrin-deficiency aged 1–10 years, for whom tests showed normal liver function. Despite normal plasma ammonia levels, the affected children showed higher blood levels of ornithine ($p<0.001$) and citrulline ($p<0.01$)—amino acids involved in the urea cycle—than healthy children. Blood levels of nitrite/nitrate, metabolites of nitric oxide (NO), and asymmetric dimethylarginine inhibiting NO production from arginine were not different between these two groups. Blood glucose, galactose, pyruvate, and lactate levels after 4–5 h fasting were not different between these groups, but the affected group showed a significantly higher lactate-to-pyruvate ratio. Low-density and high-density lipoprotein cholesterol levels in the affected group were 1.5 times higher than those in the controls. Plasma oxidized low-density lipoprotein apparently increased in the affected children; their levels of urinary oxidative stress markers such as 8-hydroxy-2'-deoxyguanosine and acrolein-lysine were significantly higher than those in the controls. Results of this study showed, even during the silent period, sustained hypercitrullinemia, hypercholesterolemia, and augmented oxidative stress in children with citrin deficiency.

**O-4**

## Analysis of hepatic metabolism in CTLN2 model mouse by using perfusion system

Mitsuaki Moriyama[1], Miharu Ushikai[2], Eishi Kuroda[2], Katsura Takano[1], Yoichi Nakamura[1], Keiko Kobayashi[2], Takeyori Saheki[3,4]

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In humans, the mutations of *SLC25A13*, the gene encoding citrin, cause both adult-onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestasis, referred as human citrin deficiency. Citrin, a liver-type mitochondrial aspartate (Asp)-glutamate (Glu) carrier, plays significant roles in (i) exchanging mitochondrial Asp for cytosolic Glu and a proton, which is important in transfering cytosolic NADH reducing equivalents into mitochondria for the generation of ATP by oxidative phosphorylation via the malate-Asp shuttle; a process essential for aerobic glycolysis, (ii) supplying Asp for the formation of argininosuccinate during urea synthesis and (iii) exporting Asp as a substrate for phosphoenolpyruvate formation and the creation of glucose from 3-carbon precursors such as lactate (Lac). To understand the pathophysiology of CTLN2, we investigated hepatic metabolism of wild-type, citrin-knockout (KO), mitochondrial glycerol-3-phosphate dehydrogenase (mGPD)-KO, and citrin/mGPD double-KO mice using liver perfusion system. Gluconeogenesis from Lac was dramatically decreased in the livers of citrin-KO and citrin/mGPD double-KO mice, however, when pyruvate was used as a substrate, no difference in the rate of gluconeogenesis was seen among four genotypes. The deficits in ureogenesis from ammonia in the liver of citrin/mGPD double-KO mice was accompanied by an increase in the perfusate lactate-to-pyruvate (L/P) ratio, which is similar to citrin-KO mice. The effect of high glucose load on ureogenesis was also investigated, since CTLN2 patients dislike carbohydrate-rich diet. Infusion of 25 mM glucose in the perfusate partially inhibited the ureogenesis from ammonia in the liver of citrin/mGPD double-KO mice. These results suggest that the severe metabolic disturbances present in citrin/mGPD double-KO mice, the model of citrin deficiency. We also discuss the alterations of the hepatic amino acid levels and tricarboxylic acid cycle intermediates.

**O-6**

## 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)]

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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.

**O-9**

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

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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.

**O-10**

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

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**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.

**O-13**

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

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**[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.

**O-14**

**Efficacy of MCT-milk in NICCD**

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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.

O-15

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

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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

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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.

**O-17**

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

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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.

**O-18**

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

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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〕　分担研究報告

厚生労働科学研究費補助金（難治性疾患克服研究事業）
シトリン欠損症の実態調査と診断方法および治療法の開発
（分担）研究報告書

# シトリン欠損症モデル（citrin/mGPD double-KO）マウスを用いた病態解析と新規治療法の開発

研究分担者　佐伯　武頼　熊本大学生命資源研究・支援センター特任教授

**研究要旨**：シトリン欠損症モデル（Ctrn/mGPD double-KO）マウスを用い、(1) CTLN2 発症要因となる糖（ショ糖）、エタノール、グリセロールの自由摂取による摂取量を測定した結果、double-KO マウスは高濃度溶液をいずれも好まないこと、また、それらの高濃度溶液の経口強制投与によって肝内代謝物の glycerol-3-phosphate が共通に上昇することを見出した。さらに (2) 高糖質食（AIN-93M）投与によって double-KO マウスの体重は顕著に減少すること、この現象は摂食量の減少によること、カゼイン、成分アミノ酸、ピルビン酸ナトリウム、中鎖トリグリセリド（MCT）の添加によって摂取量の回復と体重の増加が得られることを見出した。

## A．研究目的

本分担研究では、分担者らが新たに開発したシトリン欠損症モデルマウスを用い、シトリン欠損症の病態を解明し、内科的治療法を開発・確立することである。シトリン欠損症は、佐伯と小林らが、成人発症II型シトルリン血症（CTLN2）の病因遺伝子、SLC25A13、を同定したことから始まる（Kobayashi eet al. Nat Genet 1999;22:159-163）。SLC25A13にコードされるミトコンドリア膜輸送体をシトリン（citrin; Ctrn）と命名した。Ctrnはその後、aspartate（Asp）glutamate(Glu) carrier（AGC)であることも判明した（Palmieri et al. EMBO J 2002;20:5060-5069）。Ctrnの欠損はCTLN2の病因遺伝子であるだけでなく、新生児期の肝内胆汁うっ滞症(intrahepatic cholesta-sis caused by citrin deficiency; NICCD)をも引き起こすことがわかり、シトリン欠損症という疾患概念を打ち立てることができた（Saheki & Kobayashi J Hum Genet 2002;47:333-341）。

シトリン欠損症における特徴的な現象のひとつは、患者の特異な食癖である。すなわち、患者は糖質を嫌い、たんぱく質・脂質に富む食物を好む（Saheki et al. J Inherit Metab Dis 2008; 31:386-394）。また、嫌っている糖質の過剰投与は高アンモニア血症を引き起こす。さらに、アルコール摂取や高アンモニア血症治療薬のグリセオール（10%グリセロールと5%フルクトースを含む）は発症誘引または患者に不可逆的変化をもたらし早期の死亡に導いて来た（Yazaki et al.

Int Med:2005;44:188-195）。

本研究では分担者らが確立したシトリン欠損症モデルであるCtrn/mGPD double-KOマウスを用い、食餌を通常の固形食であるCE-2から、CE-2より高糖質食の成分粉餌食であるAIN-93Mをdouble-KOマウスに投与した時に観察される体重減少と食餌摂取量の変化について検討した結果を示し、それらの変化を解消する効果を持つ物質について検討した結果、ならびにそれらの物質が肝内代謝物濃度にどのような効果を持つかを比較検討した結果を報告する。

B. 研究方法

1) マウス

実験には、４種のマウスを使用した。すなわち、野生型（wt)、シトリン欠損（Slc25a13ノックアウト；Ctrn-KO）、ミトコンドリア局在グリセロリン酸脱水素子酵素欠損（mGPD-KO）、 およびCtrn/mGPD double-KOマウスを作成し、生後９０日齢~１６０日齢で実験に用いた。通常は、げっ歯類用固形試料CE-2（Clea Japan)を投与し、水は自由摂取とした。

1）AIN-93M投与実験

AIN-93Mはアメリカ栄養協会推奨の成熟げっ歯類に充分の栄養を含む栄養成分が明確な粉餌食である。CE-2とAIN-94Mの大きな違いは、CE-2の糖質50%、たんぱく質25.1%であるのに対し、AIN-93Mの糖質は72.1%、たんぱく質14%と糖質とたんぱく質含量にある。

CE-2からAIN-93Mに切り替え1週間飼育しその間の食餌摂取量と体重を測定した。その後、AIN-93Mにカゼイン、アラニン（Ala）、グルタミン酸ナトリウム（Glu-Na）、ピルビン酸ナトリウム(Pyr-Na)、中鎖トリグリセリド（MCT）を含む各種脂質を加え（添加量分のコーンスターチを減じ、総量を合わせた）、1日食餌摂取量と体重を記録した。

2）ショ糖(5g/kg)による肝代謝異常に対する各種物質の投与効果の比較

25%ショ糖液に、ピルビン酸ナトリウム（20mmol/kg; 2.2g/kg)、Ala (20mmol/kg; 1.8g/kg)、Na-Glu (10mmol/kg; 1.7g/kg) 、MCT (0.25g/kg)を混じ、 gastric tubeで強制的に経口投与し、1時間後に頚椎脱臼し、すばやく肝臓の一部を取り出し、freeze-clampで急速に肝臓代謝を止めた。凍結肝臓は液体窒素存在下に粉末化し、3% perchloric acidで除蛋白後、肝抽出液を得た。肝抽出液は、1M 重炭酸カリウムで中和し、測定に用いた。

3）測定

Glycerol-3-phosphate (G3P)、citrate、malateなどは酵素法で定量した。アミノ酸は、樹脂で精製後、化学修飾するEZ:faast アミノ酸分析キット(Phenomenex Ltd., Los Angeles, USA)を用いて修飾後、UPLC/MSを用い、分離・同定・定量した。

4) 統計処理

体重変化は対応のあるt検定でおこなった。食餌間の多検体の検定にはANOVA解析を用いた。

C. 研究結果

1）AIN-93Mの体重に対する効果と摂取量ならびにカゼイン添加の効果

AIN93Mは成熟げっ歯類には充分な栄養素を含むとアメリカ栄養協会によって推奨されている成分の明らかな粉餌食である。CE-2を投与されていたマウスにAIN-93Mを投与すると、体重が減少した。この現象は食餌摂取量の低下に起因する。事実、対照マウスのmGPD-KOマウスをpair-fed法で飼育するとdouble-KOマウスとほぼ同じレベルまで体重が低下した。前述のようにCE-2とAIN-94Mの大きな違いは、CE-2、糖質50%、たんぱく質25.1%であるのに対し、AIN-93Mの糖質は72.1%、たんぱく質14%と糖質とたんぱく質含量にある。そこで、AIN-93Mにカゼインを8%加え、総たんぱく質含量22%にすると摂取量が増え、体重も増加した（図1）。16%カゼイン添加（総た

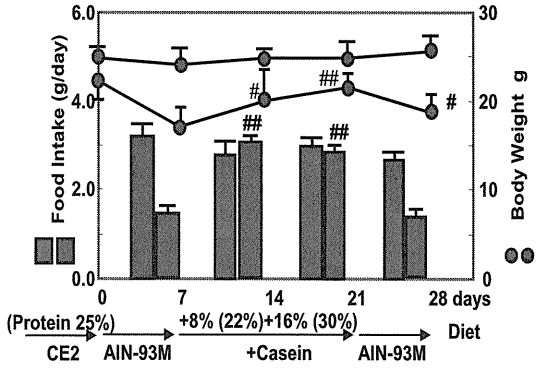


図１．CE-2からAIN-93Mへの食餌変換に伴う摂食量と体重変化、ならびにカゼインの効果青はmGPD-KO赤はdouble-KOマウスを示す。有意差#p<0.05; ##p<0.01はAIN-93Mとの比較を示す。