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Clinical manifestation and long-term outcome of citrin deficiency: Report from a nationwide study in Japan

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Abstract

Citrin deficiency is an autosomal recessive disorder caused by mutations in the *SLC25A13* gene. The disease can present with age-dependent clinical manifestations: neonatal intrahepatic cholestasis by citrin deficiency (NICCD), failure to thrive, and dyslipidemia by citrin deficiency (FTTDCD), and adult-onset type II citrullinemia (CTLN2). As a nationwide study to investigate the clinical manifestations, medical therapy, and long-term outcome in Japanese patients with citrin deficiency, we collected clinical data of 222 patients diagnosed and/or treated at various different institutions between January 2000 and December 2019. In the entire cohort, 218 patients were alive while 4 patients (1 FTTDCD and 3 CTLN2) had died. All patients <20 years were alive. Patients with citrin deficiency had an increased risk for low weight and length at birth, and CTLN2 patients had an increased risk for growth impairment during adolescence. Liver transplantation has been performed in only 4 patients (1 NICCD, 3 CTLN2) with a good response thereafter. This study reports the diagnosis and clinical course in a large cohort of patients with citrin deficiency and suggests that early intervention including a low carbohydrate diet and

MCT supplementation can be associated with improved clinical course and long-term outcome.

KEYWORDS
citrulline, Citrullinemia type 2, CTLN2, long-term survival, MCT, NICCD

1 | INTRODUCTION

Citrin is a Ca2+−binding aspartate/glutamate carrier located at the inner mitochondrial membrane, is mainly expressed in liver mitochondria, and is encoded by the *SLC25A13* gene. Citrin transfers cytosolic reducing equivalents produced during hepatic glycolysis into the mitochondria as part of the malate–aspartate reduced nicotinamide adenine dinucleotide (NADH) shuttle.[1] A defect in citrin causes an impaired aspartate–malate NADH shuttle function and results in decreased availability of cytosolic aspartate as one of the substrates of the urea cycle enzyme argininosuccinate synthetase.[2] Dysfunction of the aspartate–malate shuttle induces excessively increased cytosolic NADH and decreased mitochondrial NADH.[3] As a consequence of cytosolic NADH/NAD+ elevation, hepatic utilization of glucose in glycolysis and of lactate in gluconeogenesis is impaired.[4] At the same time, mitochondrial NADH/NAD+ reduction may disturb the beta-oxidation pathway, resulting in a severe energy deficit of hepatocytes in citrin deficiency and inadequate low blood ketone production during hypoglycemia.[5]

Citrin deficiency is an autosomal recessive disorder caused by mutations in the *SLC25A13* gene[1] that presents with age-dependent clinical manifestations: neonatal intrahepatic cholestasis by citrin deficiency (NICCD: OMIM 605814), failure to thrive and dyslipidemia by citrin deficiency (FTTDCD), and adult-onset type II citrullinemia (CTLN2: OMIM 603471).[6–8] Neonates or infants with citrin deficiency present with intrahepatic cholestasis and diverse metabolic abnormalities, including citrullinemia, hyperammonemia, hypergalactosemia, and hypoglycemia; this condition is referred to as NICCD. The clinical manifestations of NICCD improve often spontaneously at the end of infancy. After recovery from NICCD, some patients remain symptomatic with fatigue, recurrent episodes of hypoglycemia, failure to thrive, and dyslipidemia. They may prefer a lipid-rich and carbohydrate-restricted diet. This condition is characterized by failure to thrive and dyslipidemia; hence, classified as FTTDCD.[8] Following a silent remission period until after adolescence, less than 20% of patients develop a fatal metabolic disease, CTLN2, which is characterized by severe liver steatosis accompanied by hyperammonemia, cognitive impairment, and sudden episodes of unconsciousness due to brain edema.[9,10]

Citrin deficiency is a more prevalent condition in Asian regions, particularly in Japan.[11] The incidence is 1 per 17 000 births in Asia,[12] but much lower in Western countries. However, some patients may remain undiagnosed due to an unusual clinical course or due to an oligo- or even asymptomatic phenotype. There are several reports describing *SLC25A13* gene variants in patients with citrin deficiency, of which about 11 mutations are prevalent in Japanese patients.[12–16]

In recent years, several publications addressed the management of citrin deficiency.[17–20] However, the natural course of the disease under this management and the long-term outcome of patients with this condition have not been sufficiently ascertained. Therefore, we conducted a nationwide study to investigate clinical manifestations, medical therapy, and long-term outcome in Japanese patients with citrin deficiency. In this study, we present the results of this nationwide study and discuss the long-term outcome and medical management in this large patient cohort of Japanese origin.

2 | MATERIAL AND METHODS

2.1 | Study participants

In 2018, we invited 1009 medical institutions, including the respective departments of pediatrics, endocrinology and metabolism, neonatology, genetics, and transplant surgery, to participate in a questionnaire survey regarding patients with citrin deficiency (Data S1). Each institution was a medical center that serviced a local area in Japan and had approximately 300 beds, based on previous survey reports.[21] The questionnaire aimed to establish whether healthcare professionals had diagnosed or provided medical treatment to patients with citrin deficiency. Of the 1009 institutions, 731 (72%) responded, and 104 confirmed that patients with citrin deficiency had been diagnosed and/or treated at their facility. Thereafter, in 2019, we delivered a second questionnaire survey (Data S2) to these 104 institutions, of which 82 (79%) responded. Until the end of 2020, we hereby collected clinical data of 222 patients with citrin deficiency who had been diagnosed and/or treated in 104 institutions between January 2000 and December 2019. Data collection in this study was entirely retrospective.

2.2 | Diagnosis and data evaluation

The 222 patients with citrin deficiency were all diagnosed based on clinical manifestations, family history, metabolite analysis (blood amino acid analysis), and/or DNA analysis. Data of these 222 patients were analyzed in detail, in which a patient who visited several institutions was regarded as a single patient.

Of the total 222 patients enrolled in this study, 59 patients with citrin deficiency had already been published before: 1 patient in,[22] 8 patients in,[23] and 52 patients in[24]; two patients were part of two independent publications.[23,24]

Patients with confirmed pathogenic *SLC25A13* variants on both alleles (74%, 164/222) were considered as being definitely diagnosed. Patients in whom only a single pathogenic variant was detected (8%, 18/222) and patients without any pathogenic *SLC25A13* variants (18%, 40/222) were only considered citrin deficient patients in the presence of typical manifestations such as elevated transaminases (>100 U/L) together with cholestasis and together with elevated amino acids concentrations including threonine (Thr), citrulline (Cit), and methionine (Met).

Physical manifestations were assessed and recorded by the clinicians who had diagnosed and/or treated the patients. Intellectual assessments were performed by clinical psychologists, pediatricians, and/or child psychiatrists using standardized tests, such as the Wechsler Preschool and Primary Scale of Intelligence (WPPSI), Wechsler Intelligence Scale for Children (WISC), Wechsler Adult Intelligence Scale (WAIS), Kaufman Assessment Battery for Children, Illinois Test of Psycholinguistic Abilities, and/or Enjoji Scale of Infant Analytical Development. An intelligence quotient (IQ) or developmental quotient (DQ) <70 in the follow-up evaluation tests was considered as impaired intelligence or development, respectively.

2.3 | Statistical analysis

Comparison of height and weight between groups was performed using Mann–Whitney U test in the Graphpad Prism 8 (GraphPad Software). Comparison in the plasma amino acids concentrations before and after undergoing medical treatment was performed using the Wilcoxon signed-rank test. p values of <0.05 were considered statistically significant.

3 | RESULTS

In this study, 222 patients with citrin deficiency were enrolled and divided for all further analysis into 3 groups, namely A. NICCD, B. post-NICCD including asymptomatic and FTTDCD, and C. CTLN2 according to their age of onset and/or age of diagnosis (Table 1). Moreover, we categorized NICCD and post-NICCD patients into 3 groups (0–3 years, 4–10 years, ≥11 years) according to their age in January 2021. Most patients were diagnosed based on clinical symptoms, but 49 patients (26%) in the NICCD group were detected based on elevated citrulline concentrations in dried blood spots as part of newborn screening (NBS) using tandem mass spectrometry.

3.1 | Survival and outcome

Within the entire cohort, 218 patients were alive, 3 patients were deceased from however mainly unrelated causes, and 1 patient was deceased from pancreatitis (Data S3). In detail, 1 male FTTDCD died during a traffic accident at the age of 22 years. The other 3 deceased patients were affected by CTLN2, including 1 male patient suffering from acute lymphocytic leukemia who died at the age of 79 years, 1 male patient who died from lung cancer at the age of 54 years, and 1 female patient who died from pancreatitis at the age of 54 years. Moreover, although the majority of CTLN2 patients (11/17) were normally employed with no significant disability (grade 0 or 1) in the modified Rankin Scale, two CTLN2 patients developed severe disability (grade 5).

3.2 | Clinical manifestation

Table 2 and Data S4 show the clinical manifestation in patients with citrin deficiency present at any time in the past or at the time the survey was conducted. All patients with NICCD (0–3 years, 4–10 years, and ≥11 years groups) had initially presented clinical signs and symptoms related to the disease and were accordingly diagnosed during infancy. In detail, many patients with NICCD had developed cholestasis, elevated transaminases, fatty liver, hyperlipidemia, hypoglycemia, and hypoproteinemia. Growth impairment was a significant complication although only a few patients had significant short stature (<−2.0 SD). However, most of these manifestations had already improved at the time the survey was conducted. Older patients with citrin deficiency have a risk of hepatoma or hepatocellular carcinoma.[19,20] Three patients with CTLN2 had developed liver tumor such as hepatoma or hepatocellular carcinoma. Clinical manifestations including poor weight gain, fatigue, anorexia, elevated transaminases, hyperlipidemia, hypoproteinemia, and fatty liver had been frequently developed in both NICCD (≥11 years) and CTLN2 group.

TABLE 1 Three pathogenic types in patients with citrin deficiency

Item	NICCD	Post-NICCD	CTLN2
Number of patients (Sex male/female)	192 (91/101)	13 (6/7)	17 (11/6)
Median age (IQR)	9 years 2 months (5 years 5 months–15 years 10 months)	18 years 6 months (10 years 9 months–23 years 10 months)	55 years 8 months (47 years 1 month–65 years 2 months)
Median age of onset (IQR)	1 month (2 weeks–2 months)	2 years 6 months (1 years 3 months–5 years 7 months)	40 years 5 months (24 years 5 months–53 years 8 months)
Median age at diagnosis (IQR)	2 months (1 month −5 months)	6 years 8 months (3 years 6 months–9 years 3 months)	48 years (33 years–61 years 1 month)
Diagnosis through NBS	49 (26%) (0–3 years: $N = 17$, 4–10 years: $N = 14$, ≥11 years: $N = 18$)		
Median birth length (cm), (IQR), males	47.8 (47.0–49.3)	47.0 (47.0–48.0)	
Median birth length (cm), (IQR), females	47.0 (45.8–48.7)	44.4 (44.2–44.6)	
Median birth weight (g), (IQR), males	2626 (2453–2760)	2595 (2500–2720)	
Median birth weight (g), (IQR), females	2530 (2298–2722)	2550 (2397–2710)	
Median adult height (cm), (IQR), males (≥ 17 years old)	172.1 (169.2–176.5) ($N = 10$)	176.7 (176.1–177.9) ($N = 3$)	168.3 (164.4–171.8) ($N = 8$)
Median adult height (cm), (IQR), females (≥ 17 years old)	156.8 (155.1–161.6) ($N = 11$)	153.2 (151.6–154.6) ($N = 3$)	151.2 (145.9–154.5) ($N = 6$)

Abbreviation: IQR, interquartile range.

There were no significant differences in height and body weight between male NICCD and post-NICCD ($p = 0.343$ and $p = 0.860$, respectively). The height in female post-NICCD was lower than those in female NICCD ($p = 0.021$). However, there was no significant difference in the body weight between female NICCD and post-NICCD ($p = 0.989$). Their height and weight at birth were shorter and lighter than those of normal Japanese newborns (height: 49.0 ± 1.9 cm in males and 48.5 ± 1.8 cm in females) (body weight: 3000 ± 400 g in males and 3000 ± 400 g in females).[24] There was no sufficient information available for the data at birth of the CTLN2 patients.

These manifestations are suggested to be frequently present in patients with citrin deficiency.

In order to check the rate of growth impairment due to the disease itself or its management, we collected the length at birth and during follow-up measurements (Figure 1). In patients ≥17 years of age, the height in male NICCD patients ($N = 10$) was higher than those in male CTLN2 ($N = 8$) although the difference was not significant ($p = 0.101$) (Table 1). In female patients ≥17 years of age, the height in NICCD patients ($N = 11$) was significantly higher than those in CTLN2 ($N = 6$) ($p = 0.014$) (Table 1).

3.3 | Molecular genetic background

Table 3 and Datas S5 and S6 list information concerning the molecular genetic background. Details to variants in *SLC25A13* gene were available for 186 patients (163 families), in whom 22 different variants were detected. Of those, six variants, namely c.1177+1G>A (34%, 112/326 alleles), c.852_855delTATG (28%, 91/326), c.1311+1G>A (8%, 26/326), c.674C>A (6%, 18/326), c.1750_1751 [insNM_138459.3:2672_24;1750+72_72_1751-4dup] (4%, 14/326), and c.1638_1660dup (3%, 9/326) were found on 83% of the alleles (270/326). Forty-eight patients (43 families) have homozygous mutations, 115 patients (105 families) have compound heterozygous mutations, and 17 patients (15 families) have heterozygous mutations found on only one allele. Four patients (4 families) without DNA analysis were family members of patients with known mutations. Twenty-two pathogenic variants included 8 nonsense variants (36%), 4 missense variants (18%), 5 splice donor/acceptor site variants (23%), 3 del/dup variants (14%), 1 silent variant (5%), and 1 intron variant (5%).

3.4 | Medical management

The dietary and medical management for all patients is summarized in Table 4 and Data S7. To highlight

TABLE 2 The clinical manifestation in NICCD patients ($N = 192$)

Manifestations/Age (Years)	0–3 ($N = 40$)	4–10 ($N = 74$)	≥11 ($N = 78$)	Total
Short stature (<−2.0 SD)	Present: 4 (10%) Previous: 4 (10%)	Present: 2 (3%) Previous: 12 (16%)	Present: 2 (3%) Previous: 5 (6%)	29 (15%)
Poor weight gain	Present: 0 (0%) Previous: 13 (33%)	Present: 0 (0%) Previous: 22 (30%)	Present: 2 (3%) Previous: 25 (32%)	62 (32%)
Hepatomegaly	Present: 2 (5%) Previous: 4 (10%)	Present: 0 (0%) Previous:17 (23%)	Present: 1 (1%) Previous:18 (23%)	42 (22%)
Spleen enlargement	Present: 0 (0%) Previous: 1 (3%)	Present: 0 (0%) Previous: 1 (1%)	Present: 0 (0%) Previous: 3 (4%)	5 (26%)
Hypoglycemia (<3.3 mmol/L)	Present: 0 (0%) Previous: 10 (25%)	Present: 2 (3%) Previous: 18 (24%)	Present: 0 (0%) Previous: 27 (35%)	57 (30%)
Hyperammonemia (>100 μM)	Present: 0 (0%) Previous: 7 (18%)	Present: 0 (0%) Previous: 12 (16%)	Present: 2 (3%) Previous: 0 (0%)	21 (11%)
Hyperammonemic coma	Present: 0 (0%) Previous: 0 (0%)	Present: 0 (0%) Previous: 1 (1%)	Present: 0 (0%) Previous: 0 (0%)	1 (0.5%)
Seizure	Present: 0 (0%) Previous: 2 (5%)	Present: 0 (0%) Previous: 0 (0%)	Present: 0 (0%) Previous: 8 (10%)	10 (5%)
Impaired intelligenc	Present: 0 (0%) Previous: 0 (0%)	Present: 0 (0%) Previous: 1 (1%)	Present: 4 (5%) Previous: 2 (3%)	7 (4%)
Abnormal brain MRI or CT	Present: 1 (3%) Previous: 0 (0%)	Present: 0 (0%) Previous: 0 (0%)	Present: 0 (0%) Previous: 3 (4%)	4 (2%)
Abnormal *electroencephalography*	Present: 0 (0%) Previous: 0 (0%)	Present: 0 (0%) Previous: 0 (0%)	Present: 0 (0%) Previous: 3 (4%)	3 (2%)
Fatigue	Present: 0 (0%) Previous: 0 (0%)	Present: 6 (8%) Previous: 4 (5%)	Present: 15 (18%) Previous: 15 (18%)	40 (21%)
Anorexia	Present: 0 (0%) Previous: 1 (3%)	Present: 1 (1%) Previous: 5 (7%)	Present: 6 (8%) Previous: 9 (12%)	22 (11%)
Nausea or vomiting	Present: 0 (0%) Previous: 1 (3%)	Present: 1 (1%) Previous: 0 (0%)	Present: 2 (3%) Previous: 14 (18%)	18 (9%)
Intractable diarrhea	Present: 0 (0%) Previous: 0 (0%)	Present: 0 (0%) Previous: 4 (5%)	Present: 0 (0%) Previous: 4 (5%)	8 (4%)
Elevated transaminases (>100 U/L)	Present: 3 (8%) Previous: 23 (58%)	Present: 0 (0%) Previous: 50 (67%)	Present: 1 (1%) Previous: 59 (76%)	136 (71%)
Cholestasis	Present: 32 (80%) Previous: 0 (0%)	Present: 0 (0%) Previous: 56 (76%)	Present: 0 (0%) Previous: 63 (81%)	151 (79%)
Hyperlipidemia	Present: 2 (5%) Previous: 4 (10%)	Present: 3 (4%) Previous: 11 (15%)	Present: 5 (6%) Previous: 21 (27%)	46 (24%)
Hypoproteinemia	Present: 0 (0%) Previous: 17 (43%)	Present: 0 (0%) Previous: 29 (39%)	Present: 0 (0%) Previous: 29 (37%)	75 (39%)
Fatty liver	Present: 2 (5%) Previous: 10 (25%)	Present: 4 (5%) Previous: 18 (24%)	Present: 7 (9%) Previous: 22 (28%)	63 (33%)
Leukopenia (WBC <4000/μl)	Present: 0 (0%) Previous: 1 (3%)	Present: 0 (0%) Previous: 0 (0%)	Present: 0 (0%) Previous: 2 (3%)	3 (2%)
Anemia (Hb <10 g/dl)	Present: 1 (3%) Previous: 10 (25%)	Present: 0 (0%) Previous: 13 (18%)	Present: 0 (0%) Previous: 9 (12%)	33 (17%)
Thrombocytopenia (Plt $<10 \times 10^4$/μl)	Present: 0 (0%) Previous: 1 (3%)	Present: 0 (0%) Previous: 1 (1%)	Present: 0 (0%) Previous: 2 (3%)	4 (2%)
Prolonged prothrombin time	Present: 0 (0%) Previous: 19 (48%)	Present: 0 (0%) Previous: 23 (31%)	Present: 0 (0%) Previous: 23 (30%)	65 (34%)

All patients with NICCD had presented clinical signs or symptoms related to the disease during infancy.
Present: clinical manifestations at the time when the survey was conducted; previous: clinical manifestations at any time in the past.

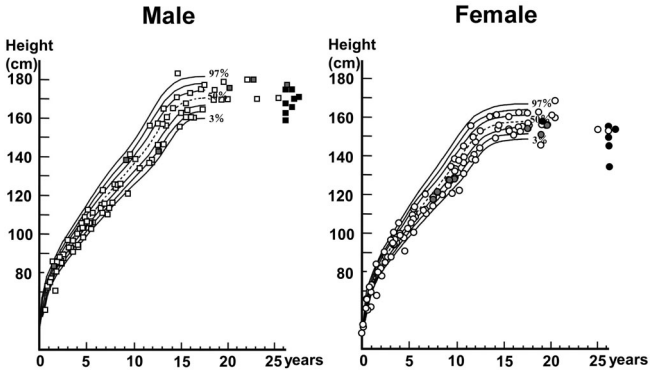

FIGURE 1 Cross-sectional data on the height in patients with citrin deficiency. Percentiles describe Japanese controls
Male patients
□: NICCD: $N = 88$, ■: FTTDCD: $N = 6$, ■: CTLN2: $N = 8$
Female patients
○: NICCD: $N = 97$, ●: FTTDCD: $N = 7$, ●: CTLN2: $N = 6$

differences during infancy and childhood, results are shown for NICCD and post-NICCD in the aforementioned three age groups. The most common dietary intervention in NICCD patients was the supplementation of medium-*chain triglycerides (MCT)* (used as oil or MCT formulas in 40% (16/40) or 90% (36/40) patients, respectively), while a carbohydrate-restricted diet, which does not include self-restricted diet, was prescribed by clinicians in only 15% (6/40) of patients in the 0–3 years old in the NICCD group. Fifteen percent (29/192) of patients with NICCD underwent carbohydrate restriction after 1 year of age though many patients with NICCD have chosen a self-restricted carbohydrate intake. Thirty-six percent (70/192) of patients with NICCD underwent MCT formulas or oil after 1 year of age. Drugs were rarely used in the present cohort, and especially standard drugs for hyperammonemia such as arginine, sodium benzoate, and sodium phenylbutyrate were not used at all in the NICCD and FTTDCD groups.

3.5 | Liver transplantation

A living donor liver transplantation (LDLT) was performed in only 4 patients (NICCD: 1 and CTLN2: 3). One male patient diagnosed with NICCD received LDLT at the age of 9 months when suffering from acute liver failure related to NICCD. Two male patients diagnosed with CTLN2 underwent LDLT at the age of 43 years and 37 years, respectively, to treat recurrent hyperammonemic attacks with concurrent signs of cerebral edema. One female CTLN2 patient underwent LDLT at the age of 12 years to improve her quality of life that was endangered due to frequent metabolic attacks induced by carbohydrates and sugar intake.

3.6 | Laboratory analyses

Table 5 and Data S8 show the plasma amino acid concentrations in patients with citrin deficiency before and during undergoing medical therapy. In the NICCD, plasma threonine (Thr), citrulline (Cit), methionine (Met), tyrosine (Tyr), ornithine (Orn), and arginine (Arg) concentrations at the time of diagnosis were significantly higher than those during treatment. Plasma glutamine (Gln), alanine (Ala), valine (Val), leucine (Leu), and isoleucine (Ile) concentrations were significantly lower than those during or after treatment. Moreover, even in patients diagnosed as CTLN2, increased plasma Thr and Cit concentrations before treatment were significantly lower following the start of treatment, and previously decreased plasma Gln and Ala concentrations were normalized after treatment.

4 | DISCUSSION

Citrin deficiency is a very rare condition in many parts of the world but is more prevalent in Asian regions, particularly in Japan.[11] The type of disease, clinical course, and outcome in citrin deficiency are mainly determined by the time of onset. As illustrated in this study, the severity of the disease can vary substantially and there is a broad clinical heterogeneity between the different forms of citrin deficiency and the various manifestations in children and adults. Based on the complex clinical picture, there is even the possibility that patients remain undiagnosed. In this study, we report the clinical situation from a large cohort managed in a single country and try to highlight the characteristics of this disease.

In the group of the youngest patients, those affected by NICCD, most were diagnosed based on their clinical manifestation and the diagnosis was usually confirmed by gene analysis. Medical therapy started in this group generally within 6 months of life. Only 26% of NICCD patients in this study were detected by NBS using tandem mass spectrometry to measure amino acids including Cit, Met, Tyr, Arg, Val, and Leu + Ile in DBS. According to previous reports, up to 50% of patients with NICCD may be detected by NBS.[25] The nationwide NBS for inherited amino acid disorders including both types of citrullinemia was started in Japan in 2014 though this NBS had been performed in some areas already before. Even after January 2014, only 31% of NICCD patients (23/75) in this study were detected through NBS. Of the total 23 patients, 4 patients presented with hypoglycemia (17%), 4 patients with hyperammonemia (17%), 13 patients with elevated transaminases (57%), 20 patients with cholestasis (87%), 2 patients with hyperlipidemia (9%), 10 patients with

TABLE 3 Pathogenic *SLC25A13* variants in patients with citrin deficiency in Japan

Variant No.	Nucleic acid	Amino acid	Location	ClinVar	PolyPhen-2	Frequency (%)			
						Total	NICCD	FTTDCD	CTLN2
1	c.15G>A	p.K5=	Exon 1	Pathogenic	–	1/326 (0.3)	1/284 (0.4)	0/24	0/26
2	c.46G>T	p.E16*	Exon 2	NR	–	1/326 (0.3)	1/284 (0.4)	0/24	0/26
3	c.615+1G>C	p.A206Lfs*7	Intron 6	Pathogenic	–	1/326 (0.3)	1/284 (0.4)	0/24	0/26
4	c.674C>A	p.S225*	Exon 7	Pathogenic	–	18/326 (5.5)	16/284 (5.6)	0/24	2/26 (7.7)
5	c.847G>T	p.G283*	Exon 8	NR	–	1/326 (0.3)	1/284 (0.4)	0/24	0/26
6	c.852_855delTATG	p.M285Pfs*2	Exon 9	Pathogenic	–	91/326 (27.9)	76/284 (26.8)	9/24 (37.5)	6/26 (23.1)
7	c.955C>T	p.R319*	Exon 10	Pathogenic	–	1/326 (0.3)	1/284 (0.4)	0/24	0/26
8	c.1018+1G>A	–	Intron 10	NR	–	10/326 (3.1)	8/284 (2.8)	2/24 (8.3)	2/26 (7.7)
9	c.1078C>T	p.R360*	Exon 11	Pathogenic/ likely pathogenic	–	2/326 (0.6)	2/284 (0.7)	0/24	0/26
10	c.1177+1G>A	p.A340_R392del	Intron 11	Pathogenic	–	112/326 (34.3)	107/284 (37.7)	8/24 (33.3)	4/26 (15.4)
11	c.1230+1G>A	–	Intron 12	NR	–	2/326 (0.6)	2/284 (0.7)	0/24	0/26
12	c.1311+1G>A	p.V411_C437del	Intron 13	Pathogenic	–	26/326 (8.0)	21/284 (7.4)	2/24 (8.3)	3/26 (11.5)
13	c.1478A>G	p.D493G	Exon 15	NR	Probably damaging (1.000)	1/326 (0.3)	0/284	0/24	1/26 (3.8)
14	c.1511A>G	p.Y504C	Exon 15	NR	Probably damaging (1.000)	1/326 (0.3)	1/284 (0.4)	0/24	0/26
15	c.1592G>A	p.G531D	Exon 16	Pathogenic	Probably damaging (1.000)	4/326 (1.2)	2/284 (0.7)	0/24	2/26 (7.7)
16	c.1638_1660dup	p.A554Gfs*17	Exon 16	Pathogenic	–	9/326 (2.8)	9/284 (3.2)	0/24	0/26
17	c.1645C>T	p.Q549*	Exon 16	NR	–	1/326 (0.3)	0/284	0/24	1/26 (3.8)
18	c.1750_1751[insNM_ 138459.3:2672_24;1750+ 72_72_1751–4dup]	p.A584Vfs*2	Intron 16	Pathogenic	–	14/326 (4.3)	11/284 (3.9)	1/24 (4.2)	3/26 (11.5)
19	c.1793 T>G	p.L598R	Exon 17	NR	Possibly damaging (0.573)	1/326 (0.3)	1/284 (0.4)	0/24	0/26
20	c.1799dupA	p.Y600*	Exon 17	Pathogenic	–	4/326 (1.2)	4/284 (1.4)	0/24	0/26
21	c.1801G>T	p.E601*	Exon 17	Pathogenic	–	6/326 (1.8)	4/284 (1.4)	1/24 (4.2)	1/26 (3.8)
22	c.1813C>T	p.R605*	Exon 17	Pathogenic	–	3/326 (0.9)	1/284 (0.4)	1/24 (4.2)	1/26 (3.8)

Abbreviation: NR, not registered.

TABLE 4 Medical therapy in patients with citrin deficiency

Groups	NICCD			Post-NICCD			CTLN2
Treatment/Age	**0–3 (*N* = 40)**	**4–10 (*N* = 74)**	**≥11 (*N* = 78)**	**0–3 (*N* = 1)**	**4–10 (*N* = 4)**	**≥11 (*N* = 8)**	**≥11 (*N* = 17)**
Carbohydrate restriction	6 (15%)	14 (19%)	15 (19%)	1	0 (0%)	1 (13%)	8 (47%)
MCT oil	16 (40%)	33 (45%)	32 (41%)	1	2 (50%)	3 (38%)	9 (53%)
MCT formula	36 (90%)	50 (68%)	37 (47%)	1	1 (25%)	1 (13%)	0 (0%)
Galactose free formula	4 (10%)	17 (23%)	22 (28%)	0	0 (0%)	0 (0%)	0 (0%)
Vitamin A	13 (33%)	29 (39%)	29 (37%)	0	1 (25%)	1 (13%)	1 (6%)
Vitamin D	21 (53%)	33 (45%)	36 (46%)	0	1 (25%)	1 (13%)	1 (6%)
Vitamin E	21 (53%)	33 (45%)	32 (40%)	0	1 (25%)	1 (13%)	1 (6%)
Vitamin K	23 (58%)	40 (54%)	40 (51%)	0	1 (25%)	1 (13%)	1 (6%)
Ursodeoxycholic acid	21 (53%)	27 (37%)	29 (37%)	0	1 (25%)	0 (0%)	4 (24%)
Sodium pyruvate	0 (0%)	0 (0%)	8 (10%)	0	0 (0%)	1 (13%)	2 (12%)
Arginine	0 (0%)	0 (0%))	4 (5%)	0	0 (0%)	1 (13%)	5 (29%)
Essential amino acids	1 (3%)	0 (0%)	0 (0%)	0	0 (0%)	0 (0%)	5 (29%)
L-carnitine	1 (3%)	3 (4%)	3 (4%)	0	0 (0%)	0 (0%)	3 (18%)
Sodium benzoate	0 (0%)	0 (0%)	0 (0%)	0	0 (0%)	0 (0%)	1 (6%)
Sodium phenylbutyrate	0 (0%)	0 (0%)	0 (0%)	0	0 (0%)	0 (0%)	2 (12%)

hypoproteinemia (43%), 5 patients with fatty liver (22%), and 13 patients with prolonged PT (57%), respectively. However, when Shigetomi et al. retrospectively surveyed Cit values in the NBS of 13 patients with NICCD, only one patient exceeded the citrulline cutoff concentration at 74.5 μmol/L, while the other 12 patients presented Cit concentrations below the cutoff.[22] Zhang et al. could detect only 4 patients with citrin deficiency when studying the NBS from 146, 152 newborns.[26] Therefore, in line with the findings from our study, the present NBS approach can detect only part of NICCD patients, highlighting the need for a high awareness in healthcare professionals to diagnose patients based on their symptoms such as cholestasis at early infancy. In plasma amino acids of patients with NICCD, Cit, Met, Tyr, and Arg concentrations were elevated, and Val, Leu, and Ile concentrations were lowered (Table 5 and Data S8). To improve the detection accuracy in the NBS, the ratio of total increased amino acids values (Cit + Met+Tyr + Arg)/total decreased amino acids values (Val + Leu + Ile) measured in DBS may improve the detection rate. Moreover, the total bile acids (T-BA) concentrations in DBS may be a helpful additional parameter for detecting patients with citrin deficiency.[25] The combination of the ratio of Cit + Met+Tyr + Arg/Val + Ile + Leu and T-BA concentrations in the DBS may increase the sensitivity of NBS for citrin deficiency. Moreover, these aforementioned amino acids and T-BA concentrations at age 1 month are considered to discriminate between patients with and without citrin deficiency.[25]

The best method to confirm the diagnosis is gene analysis, particularly since the genetic background in Japanese patients is well described.[12–16] According to these previous reports, there are a few prevalent variants in the *SLC25A13* gene in Japanese patients, namely, c.852_855delTATG, c.1177+1G>A, c.1638_1660dup, c.674C>A, c.1311+1G>A, and c.1750_1751[insNM_138459.3:2672_24;1750+72_72_1751-4-dup] mutations in *SLC25A13* gene was 33.2%, 37.6%, 3.4%, 5.3%, 8.2%, and 4.6%, respectively.[16,27] These 6 mutations were accounted for 92.3% in the total alleles[16] and were the most common mutations in this study also.

The aforementioned six prevalent mutations in Japanese patients with citrin deficiency are largely contributing to the relatively high incidence of the disease, and lead to increased awareness and understanding of the disease in many clinicians, which may contribute to the improved outcome. There are some reports about clinical manifestations in patients with c.1177+1G>A or c.852_855delTATG mutations.[10,28–31] In homozygous c.1177+1G>A or homozygous c.852_855delTATG mutations, there were phenotype–genotype correlations because low height and weight at birth, elevated transaminases (>100 U/L), cholestasis, hypoproteinemia, and prolonged prothrombin time were frequently reported (Data S9). Moreover, even in compound heterozygous genotypes including c.1177+1G>A or c.852_855delTATG mutations, there were likely to be phenotype–genotype correlations (Data S9). However, among siblings of the

TABLE 5 The plasma amino acids analysis in patients with citrin deficiency before and after undergoing medical therapy

(A) NICCD (0–3 years) ($N = 32$)

	Thr	Glu	Gln	Gly	Ala	Cit	Met	Tyr	Val	Leu	Ile	Orn	Lys	Arg
Before	Median: 572.7 (IQR:402.0–850.7)	58.5 (46.6–74.7)	274.7 (190.2–396.6)	192.9 (160.4–211.1)	207.8 (162.4–229.8)	266.6 (139–562.7)	86.5 (66.7–147.0)	99.1 (67.0–216.2)	141.5 (121.1–182.3)	88.0 (67.3–104.6)	42.6 (35.3–54.0)	115.8 (98.8–174.2)	254.9 (181.7–289.3)	206.5 (126.0–261.4)
After	120.0 (120.4–156.1)	56.8 (36.2–95.9)	524.9 (493.7–549.2)	160.1 (147.6–172.1)	284.2 (248.9–315.8)	32.3 (28.9–34.2)	27.1 (20.4–35.3)	74.1 (64.8–105.5)	248.0 (224.9–288.5)	131.6 (107.9–147.6)	73.9 (58.7–88.8)	61.3 (45.5–69.4)	187.0 (172.6–210.7)	79.0 (69.5–93.5)
p value	<0.001***	0.849	<0.001***	0.114	0.002**	<0.001***	0.004**	0.068	<0.001***	0.002**	<0.001***	<0.001***	0.019*	<0.001***
Reference levels	66.5–188.9	12.6–62.5	422.1–703.8	151.0–351.0	208.7–522.7	17.1–42.6	18.9–40.5	40.4–90.3	147.8–307.0	76.6–171.3	43.0–112.8	31.3–104.7	108.7–242.2	53.6–133.6

(B) NICCD (4–10 years) ($N = 70$)

	Thr	Glu	Gln	Gly	Ala	Cit	Met	Tyr	Val	Leu	Ile	Orn	Lys	Arg
Before	475.7 (293.2–649.3)	56.3 (40.4–85.1)	272.1 (232.4–370.5)	175.5 (147.9–223.8)	220.9 (166.1–268.7)	240.1 (119.4–418.0)	70.8 (46.9–148.5)	137.8 (83.9–198.4)	153.3 (126.6–190/6)	100.1 (76.2–132.3)	54.6 (40.3–76.5)	137.3 (105.2–176.9)	298.9 (232.9–416.3)	196.9 (114.2–285.1)
After	118.8 (99.1–135.8)	44.3 (33.1–58.8)	522.0 (449.6–551.6)	151.8 (140.9–180.9)	267.8 (228.1–324.2)	28.1 (24.0–34.3)	29.5 (23.2–36.8)	88.7 (72.0–108.7)	297.4 (221.5–344.8)	161.5 (132.0–205.6)	91.9 (69.0–112.8)	64.0 (51.2–78.4)	204.5 (169.6–239.7)	77.2 (60.9–93.6)
p value	<0.001***	0.192	0.002**	0.037*	0.021*	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

(C) NICCD (≥11 years) ($N = 62$)

	Thr	Glu	Gln	Gly	Ala	Cit	Met	Tyr	Val	Leu	Ile	Orn	Lys	Arg
Before	391.6 (252.4–610.6)	63.5 (37.9–98.3)	256.3 (209.0–355.9)	158.8 (135.2–223.3)	206.8 (145.7–252.6)	257.1 (96.6–431.9)	97.5 (43.4–193.1)	135.1 (85.7–236.8)	150.8 (111.4–201.8)	85.1 (67.6–114.8)	49.1 (38.1–65.4)	127.4 (92.3–177.2)	275.5 (212.7–400.5)	134.4 (79.8–238.3)
After	142.7 (122.4–172.4)	39.2 (26.6–64.4)	536.9 (473.9–592.0)	166.7 (141.8–195.8)	313.1 (289.9–365.1)	30.6 (24.5–38.9)	31.0 (26.4–39.0)	85.3 (68.6–99.6)	314.4 (266.2–380.0)	170.1 (129.5–193.0)	91.0 (68.7–108.4)	69.8 (53.4–82.2)	244.2 (208.5–269.5)	89.6 (77.0–113.7)
p value	<0.001***	0.058	<0.001***	0.274	<0.001***	<0.001***	<0.001***	0.002**	<0.001***	<0.001***	<0.001***	<0.001***	0.270	0.005**

(D) Post–NICCD ($N = 10$)

	Thr	Glu	Gln	Gly	Ala	Cit	Met	Tyr	Val	Leu	Ile	Orn	Lys	Arg
Before	135.4 (117.4–271.0)	57.3 (47.6–66.8)	390.4 (271.6–462.4)	153.7 (133.8–175.7)	287.3 (202.6–313.6)	59.2 (21.7–96.0)	34.7 (21.0–54.1)	92.4 (54.5–110.2)	193.9 (150.9–318.0)	115.6 (86.2–163.8)	73.0 (50.5–87.1)	78.5 (64.4–120.2)	206.2 (124.3–281.1)	82.3 (42.5–135.6)
After	141.2 (134.5–167.0)	40.8 (27.6–51.7)	539.9 (488.5–565.2)	181.2 (172.7–188.8)	363.1 (313.5–416.1)	29.9 (23.9–31.8)	35.6 (33.1–37.3)	93.6 (71.1–107.9)	341.1 (304.0–408.0)	177.8 (169.8–190.8)	96.4 (94.6–105.8)	72.3 (61.8–80.1)	221.5 (199.5–331.7)	83.1 (67.2–111.7)
p value	0.382	0.455	0.021*	0.054	0.064	0.140	0.562	0.470	0.083	0.022*	0.038*	0.475	0.379	0.723

(E) CTLN2 ($N = 12$)

	Thr	Glu	Gln	Gly	Ala	Cit	Met	Tyr	Val	Leu	Ile	Orn	Lys	Arg
Before	192.2 (130.0–237.1)	61.2 (39.2–77.2)	428.9 (317.4–556.3)	148.0 (138.1–159.7)	197.2 (183.5–240.2)	383.1 (141.3–493.7)	29.6 (28.1–40.0)	78.5 (45.4–96.4)	164.9 (124.5–225.3)	81.6 (71.8–989.9)	41.9 (37.0–65.9)	82.9 (73.7–103.6)	262.6 (148.8–409.5)	214.7 (170.6–259.7)

(Continues)

TABLE 5 (Continued)

(E) CTLN2 ($N = 12$)	Thr	Glu	Gln	Gly	Ala	Cit	Met	Tyr	Val	Leu	Ile	Orn	Lys	Arg
After	116.9 (92.1–142.5)	46.1 (39.0–54.3)	603.7 (541.6–634.4)	134.4 (117.5–161.1)	250.0 (204.1–292.8)	168.0 (62.1–306.3)	25.3 (20.0–33.7)	71.4 (56.3–83.0)	157.7 (143.7–214.1)	85.8 (73.5–114.6)	44.1 (37.5–61.8)	108.5 (68.6–139.1)	191.6 (144.2–215.9)	173.3 (84.8–179.5)
p value	0.018*	0.088	0.025*	0.226	0.038*	0.028*	0.139	0.110	0.161	0.124	0.161	0.124	0.167	0.260

Abbreviations: Thr, *threonine*; Glu, glutamic acid; Gln, glutamine; Gly, glycine; Ala, alanine; Cit, citrulline; Val, valine; Leu, leucine; Iso, isoleucine; Orn, ornithine; Lys, lysine; Arg, arginine; IQR, *interquartile range*.

same family, there were only weak phenotype–genotype correlations because these siblings did not always present the same clinical manifestations including elevated transaminases, cholestasis, and hypoglycemia. Interestingly, the height and body weight at birth and the result of NBS were different between the same family members with the disease.

Most of the patients with citrin deficiency revealed specific food preferences with an aversion to high carbohydrate foods and a preference for high-protein or high-fat foods.[23] For patients with citrin deficiency, protein- and fat-rich diet with restricted carbohydrate including a protein:fat:carbohydrate ratio being 15%–25%:40%–50%:30%–40% along with the appropriate energy intake is recommended (ratio in healthy controls is 10%–15%:25%–35%:50%–60%).[32] This food prevalence is important in disease management. Some recent studies have demonstrated the effectiveness of MCT oil in addition to the diet.[18] The use of lactose-free formula should be considered in patients with hypergalactosemia. MCT formula or oil may be reduced following the improvement of liver function and cholestasis. There are patients that had previously undergone MCT formula or/and lactose intake restriction but have stopped these dietary interventions at the time of investigation (Data S7). The use of MCT formula for NICCD patients may improve thriving and alleviate cholestasis (Table 2).[17]

MCT are absorbed mainly as medium-chain fatty acids and metabolized to acetyl-CoA by β-oxidation, enhance tricarboxylic acid cycle activity, and increase adenosine triphosphate concentrations in hepatocytes.[5,33,34] Moreover, MCT supplementation can promote lipogenesis, resulting in improvement of NADH/NAD+ ratio through the malate-citrate shuttle and reduction of oxidative stress.[33,34] Therefore, MCT supplement may improve the aberrant metabolic state resulting from dysfunction of aspartate–malate shuttle. Only 37 percent (83/222) of patients with citrin deficiency in this study are receiving MCT oil or MCT formulas. Based on the biochemical rationale, it may advisable that patients continue receiving MCT supplements even after the improvement of their clinical status.

Moreover, some studies have demonstrated the effectiveness of arginine and of sodium pyruvate in addition to the diet and MCT supplementation.[18] Lipid-soluble vitamins including retinol palmitate, alfacalcidol, and tocopherol acetate[6,7,35] and ursodeoxycholic acid[32] may be used for patients with prolonged and/or persistent cholestasis.

Living donor liver transplantation (LDLT) was performed in only 4 patients (NICCD: 1 and CTLN2: 3). Usually, unmanageable severe liver failure is seen as the main indication for liver transplantation in citrin deficiency, and there are only a few reports about this

procedure in this condition.[28,36] With the improved awareness of clinicians, and the advanced medical therapies for this condition, it can be hoped that more patients are diagnosed and treatment is started early enough to prevent the progression of liver cirrhosis and liver failure, which then would probably result in a further reduced incidence of severe liver disease.

One interesting aspect of citrin deficiency is the development of length and weight. In our study, most patients were born full-term but presented decreased length and weight compared to the Japanese standard full term. Numakura et al.[24] reported lower birth weight (2.6 ± 0.3 kg) and shorter length (47.7 ± 2.2 cm) in the NICCD patients compared to those of Japanese standard subjects (3.0 ± 0.4 kg and 48.8 ± 1.9 cm, respectively). This growth failure was suggested to be due to the impairment of lipogenesis.[24] While our study results were similar to those of the aforementioned study, the findings presented here suggest the final height in the NICCD patients to be the same as in healthy Japanese controls. Although growth impairment in patients with citrin deficiency is likely to persist during adolescence, this report suggests that early detection and intervention might improve the growth impairment in patients with citrin deficiency because the height in NICCD patients ≥17 years of age was greater than those in CTLN2 patients of the same age.

Moreover, the overall survival of patients with citrin deficiency in this study was exceptionally good, with only a single CTLN2 patient who died from pancreatitis.[37,38] This is one of the complication in patients with CTLN2 since Komatsu et al. reported that 5 patients with citrin deficiency (CTLN2) (5/19, 26%) had developed pancreatitis.[37] In our study, a total of 4 CTLN2 patients (4/17, 24%) have developed pancreatitis, showing a similar prevalence as in the report by Komatsu.[37] We believe that older patients with unexplained cirrhosis, liver failure, or pancreatitis may in fact remain undiagnosed for their citrin deficiency because this study included only a surprisingly low number of deceased patients with citrin deficiency.

There are some limitations because this study is a cross-sectional cohort observational study. The clinical guidelines for citrin deficiency in Japan were published in 2015, possibly leading to a change in management of those patients based on improved diagnostic methods and overall medical treatment. Moreover, not all patients enrolled in this study might have undergone the same management modalities. Further, not in all patients, the *SLC25A13* genotype could be elucidated. As well, patients were included in this study at different stages of their dietary and medical treatment, which likely influenced the data on clinical manifestations and amino acids analyses. Moreover, we acquired the data of many patients diagnosed with NICCD but only a limited number of those diagnosed with CTLN2, who were not affected by NICCD as infants. Therefore, we could not sufficiently explore the specific aspect of adult citrin deficiency.

This study did not demonstrate a causal relationship between early therapeutic interventions including dietary treatment and an improved clinical course and long-term outcome in NICCD. Rather, this study suggests the possibility that dietary intervention may alleviate the clinical manifestation and improve the long-term outcome in NICCD as a result. It may be suggested that medical management can contribute to preventing long-term complications including liver tumors, liver cirrhosis, and pancreatitis because these complications presented in patients with CTLN2 were not developed in older patients with NICCD. The impact of medical management including dietary therapy on these long-term complications need, however, to be carefully evaluated in future studies.

In conclusion, we present here the clinical manifestation and long-term outcome in patients with citrin deficiency through a nationwide study in Japan. This study suggests the association of early intervention including a low carbohydrate diet and MCT supplementation with an improved clinical course and long-term outcome in patients with citrin deficiency. Nevertheless, we recommend to regularly follow up NICCD patients long-term and continue efforts to better understand the progress of disease and the impact of medical management, especially in CTLN2.

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CONFLICT OF INTEREST

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AUTHORS' CONTRIBUTIONS

Jun Kido and Kimitoshi Nakamura were responsible for the design of the research. Jun Kido, Toju Tanaka, Masayoshi Nagao, Takaaki Sawada, Yoichi Wada, Chikahiko Numakura, Kei Murayama, Yoriko Watanabe, Kanako Kojima-Ishii, Hideo Sasai, Kiyotaka Kosugiyama contributed to practicing medicine and data collection from patients with citrin deficiency. Jun Kido, Keishin Sugawara, and Kimitoshi Nakamura checked and analyzed the data. Jun Kido and Johannes Häberle wrote the manuscript. Jun Kido and Kimitoshi Nakamura supervised this study. All authors read and approved the final manuscript for submission. All authors have agreed to both be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work.

DATA AVAILABILITY STATEMENT

Data archiving is not mandated but data will be made available on reasonable request.

ETHICS STATEMENT

This study was approved by the ethical committee of the Faculty of Life Science, Kumamoto University (Ethics. No.1660). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients or their legal guardians for being included in the study.

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