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The therapeutic landscape of citrin deficiency

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Abstract

Citrin deficiency (CD) is a recessive, liver disease caused by sequence variants in the *SLC25A13* gene encoding a mitochondrial aspartate–glutamate transporter. CD manifests as different age-dependent phenotypes and affects crucial hepatic metabolic pathways including malate–aspartate-shuttle, glycolysis, gluconeogenesis, de novo lipogenesis and the tricarboxylic acid and urea cycles. Although the exact pathophysiology of CD remains unclear, impaired use of glucose and fatty acids as energy sources due to NADH shuttle defects and PPARα downregulation, respectively, indicates evident energy deficit in CD hepatocytes. The present review summarizes current trends on available and potential treatments for CD. Baseline recommendation for CD patients is dietary management, often already present as a self-selected food preference, that includes protein and fat-rich food, and avoidance of excess carbohydrates. At present, liver transplantation remains the sole curative option for severe CD cases. Our extensive literature review indicated medium-chain triglycerides (MCT) as the most widely used CD treatment in all age groups. MCT can effectively improve symptoms across disease phenotypes by rapidly supplying energy to the liver, restoring redox balance and inducing lipogenesis. In contrast, sodium pyruvate restored glycolysis and displayed initial preclinical promise, with however limited efficacy in adult CD patients. Ursodeoxycholic acid, nitrogen scavengers and L-arginine treatments effectively address specific pathophysiological aspects such as cholestasis and hyperammonemia and are commonly administered in combination with other drugs. Finally, future possibilities including restoring redox balance, amino acid supplementation, enhancing bioenergetics, improving ureagenesis and mRNA/DNA-based gene therapy are also discussed.

KEYWORDS

Citrin deficiency, malate-aspartate-shuttle, medium-chain triglycerides, SLC25A13, ursodeoxycholic acid

1 | INTRODUCTION

1.1 | General aspects and biochemistry

Citrin deficiency (CD) is a monogenic, autosomal recessive disease caused by mutations in the *SLC25A13* (or citrin, OMIM *603859) gene.[1] The disease affects primarily the malate–aspartate-shuttle in the liver, but consequently also impacts several other pathways including glycolysis, gluconeogenesis, de novo lipogenesis and the tricarboxylic acid (TCA) and urea cycles. CD is a global condition with high prevalence in East Asia including Japan, mainly due to several frequent mutations.[2,3] To date, more than 100 different citrin pathogenic mutations have been reported.[2] CD manifests as three different age-dependent phenotypes: neonatal intrahepatic cholestasis caused by CD (NICCD, OMIM #605814) in newborns or infants; followed by a generally asymptomatic, silent period, or by failure to thrive and dyslipidemia caused by CD (FTTDCD) in children; and finally the most severe form, citrullinemia type 2 (CTLN2, OMIM #603471) in adolescent or adult patients.[4–6]

Citrin is an aspartate–glutamate carrier localized at the inner mitochondrial membrane and is mainly expressed in the liver,[7] while the isoform aralar, encoded by the *SLC25A12* gene (OMIM *603667), is predominately expressed in skeletal muscle, the brain, kidneys, and the heart.[8] Citrin is a crucial component of the malate–aspartate shuttle that facilitates the transfer of reducing equivalents of NADH molecules from cytoplasm to mitochondria, a vital mechanism for maintaining the redox balance of both compartments (Figure 1). The loss of functional citrin disturbs the $NADH/NAD^+$ ratio, leading to reduced levels of NADH in mitochondria and lack of electron carrier molecules for ATP production.[2] In the cytosol, lack of citrin results in an increased $NADH/NAD^+$ ratio, thereby inhibiting glycolysis and gluconeogenesis. Given the importance of liver gluconeogenesis during fasting,[9] impaired gluconeogenesis can lead to hypoglycemia, which has been observed in NICCD and other CD phenotypes.[3,10–13]

Cytosolic aspartate, transported by citrin in exchange for glutamate, is utilized for the production of amino acids, including asparagine, for de novo nucleotide synthesis and in the urea cycle.[14,15] In this latter pathway, aspartate is one of the substrates of argininosuccinate synthetase (ASS), being essential for ammonia detoxification in the form of nontoxic urea. In adolescents and adults with CD, a so far unexplained liver-specific decrease in ASS without any abnormality in the *ASS1* gene or hepatic mRNA levels as well as a reduced cytosolic aspartate level impair ASS functionality, resulting in increased plasma citrulline and ammonia concentrations,[5,16,17] but this needs further investigations as some patients with adolescent and adult CD (AACD, a term suggested to replace the previous disease name "CTLN2"[18]) exhibited levels of ASS activity in liver tissue of more than 60% of controls (personal communication Prof. T. Saheki). Hyperammonemia is a main contributor to the most severe complication in these patients, which is the development of brain edema.[19]

Furthermore, CD is associated with chronic liver disease ranging from neonatal or infantile onset cholestasis to fatty liver, cirrhosis and in a few patients hepatocellular carcinoma.[3,17,20] Although the precise molecular mechanism underlying CD-associated liver disease has yet to be elucidated, recent studies showed downregulation of peroxisome proliferator-activated receptor alpha (PPARα), a protein that plays a crucial role in regulating lipid metabolism and energy homeostasis gene expression levels,[21–23] as a possible contributing factor. Specifically, PPARα downregulation impairs β-oxidation, resulting in reduced fatty acid breakdown and hepatic lipid accumulation.[21]

1.2 | The phenotypes of CD

Newborns and infants with citrin deficiency (NICCD) exhibit various clinical symptoms including low birth weight, jaundice, hepatomegaly, intrahepatic cholestasis, and in severe cases, fatty liver.[3,10,24] This clinical scenario is accompanied by alterations of the amino acid profile, galactosemia, hypoproteinemia and hypoglycemia, but only rarely hyperammonemia.[25,26] In most cases, symptoms resolve by 1 year of age, spontaneously or with dietary adjustments.[10] However, in few cases NICCD progressed into liver failure, requiring liver transplantation[10,24,27] or even resulting in fatal infantile liver cirrhosis.[6,28,29]

After resolution of NICCD, patients enter an intermediate, usually silent period, with sometimes mild, nonspecific symptoms such as abdominal pain and fatigue, and in more severe cases FTTDCD, characterized by growth impairment, recurrent hypoglycemia, hyperlipidemia, pancreatitis, gastrointestinal symptoms, fatty liver and fatigue.[5,6,30]

Finally, less than 20% of patients progress to the most severe form of the condition when they reach adolescent or adult age.[31] The reason why only a subset of CD patients are affected by AACD as the most severe form is still unclear, but its onset can be triggered by alcohol intake, excessive carbohydrate overload, fatigue, medication, infections and surgery.[21,32,33] Affected patients may develop a severe liver steatosis and cognitive impairment due to hyperammonemic encephalopathy.[17,19,32] In some adult patients, the disease rapidly progressed to fatal severe brain edema and acute liver failure, with liver

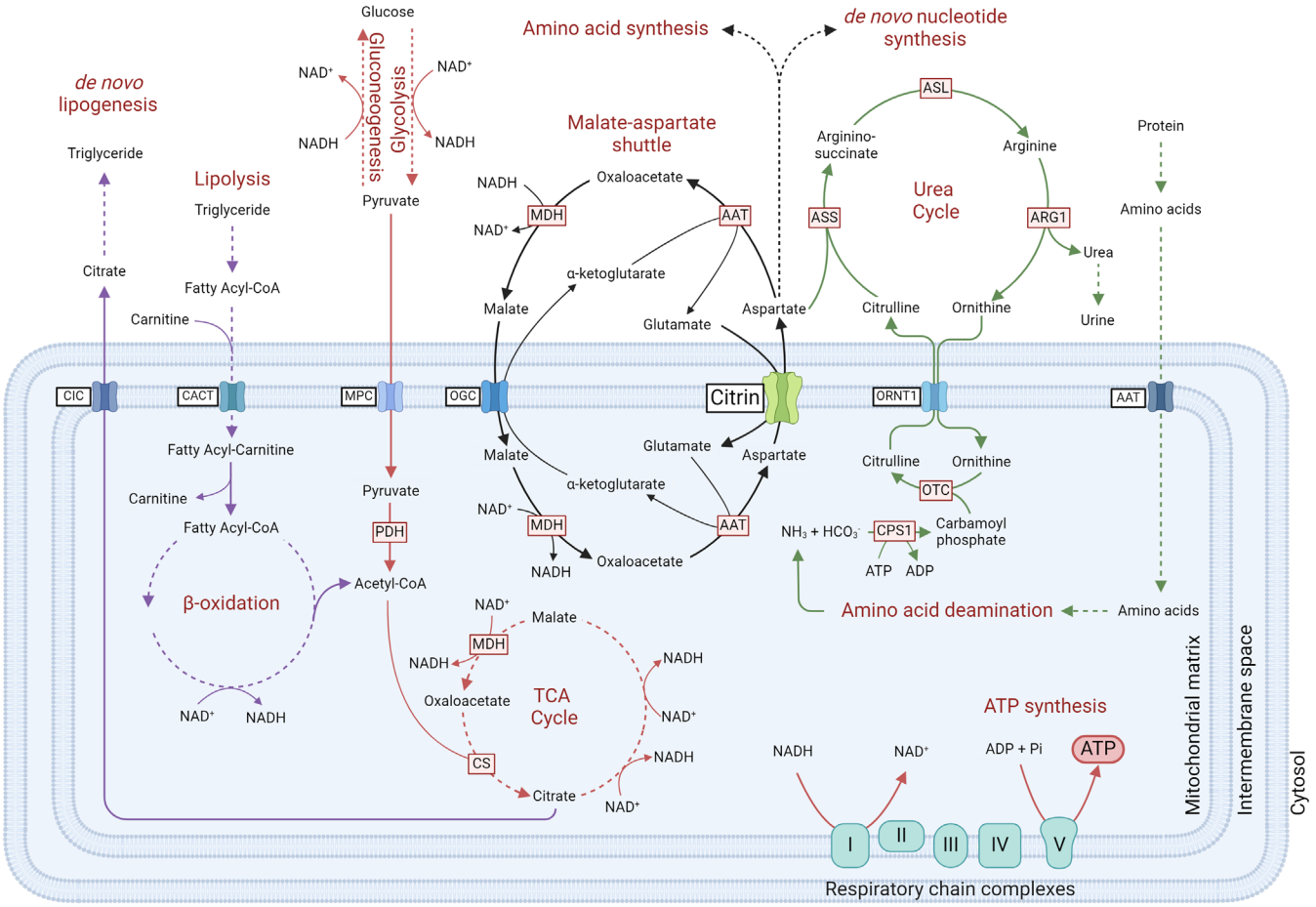

FIGURE 1 Representation of relevant metabolic pathways implicated in CD. Transport steps and biochemical pathways involved in glucose metabolism/TCA cycle (red), malate–aspartate shuttle, amino acid and nucleotide synthesis (black), protein degradation and urea cycle (green), lipid metabolism (purple) are schematically shown. Dashed lines indicate simplified reaction steps. Citrin is colored in green and other mitochondrial carriers, citrate carrier (CIC), carnitine-acylcarnitine translocase (CACT), mitochondrial pyruvate carrier (MPC), oxoglutarate carrier (OGC), ornithine transporter 1 (ORNT1) and amino acid transporter (AAT) are shown in shades of blue. Enzymes: pyruvate dehydrogenase (PDH), citrate synthase (CS), malate dehydrogenase (MDH), aspartate aminotransferase (AAT), carbamoyl phosphate synthetase 1 (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), and arginase 1 (ARG1) are highlighted in red. ATP – adenosine triphosphate, NAD^+ – nicotinamide adenine dinucleotide, TCA – tricarboxylic acid, NH_3 – ammonia, HCO^{3-} – bicarbonate ion. Created with BioRender.com.

transplantation currently being the only definitive therapy.[34,35]

A more detailed description of the clinical situation of this multifaceted condition including diagnostic aspects can be found in the preceding article in this Special Issue, dedicated to the "Clinical landscape of citrin deficiency".[18]

2 | OVERVIEW OF THE CURRENT TREATMENT LANDSCAPE

Current treatment for CD is characterized by various approaches described across different publications, but there is an absence of standardized treatment protocols and lack of preventive early interventions. The absence of a definitive cure for CD, except liver transplantation, which is limited and challenging, compounds the issue further. In recent years, clinical reports have suggested the benefit of therapies such as medium-chain triglyceride (MCT) oil, sodium pyruvate, arginine as well as dietary interventions[6,32,36–38] (Table 1). However, a consolidated resource is lacking, which would synthesize the existing clinical experiences, research findings, and emerging therapies.

In Figure 2, we provide an overview of the current therapies for CD as they are already practiced or just planned or discussed, analyze future and potential treatment options, and highlight existing challenges.

TABLE 1 Summary of clinical studies using MCT or sodium pyruvate as treatments for CD.

Publications	Patient information		Post-treatment outcome summary
	Cohort size; phenotype	Age range	
Case reports with MCT therapy			
Hayasaka et al.[43]	$N = 4$; NICCD	1.8–4 m.o.	• Resolution of jaundice and cholestasis • Normalization of most biochemical results • Worsening of LDH (3/4 patients)
Hayasaka et al.[42]	$N = 5$; AACD	38–62 y.o.	• Improvement or resolution in most biochemical result • Cit levels improved but not normalized (4/5 patients) • Normalized ammonia levels (4/5 patients) • Resolution of fatty liver (2/5 patients) • No HE relapse • Increase in body weight • Improvement in fatigue
Otsuka et al.[52]	$N = 2$; silent period	7 & 10 y.o.	• Improved OGTT • Lowered post-prandial blood ammonia and L/P ratios • Changes in food preference
Hayasaka et al.[38]	$N = 6$; AACD	48–68 y.o.	• Improvement or resolution in most biochemical results • All patients regained consciousness from HE • Improved but not normalized Cit levels (5/6 patients) • Normalized ammonia levels (4/6 patients) • One patient underwent liver transplant • One patient suffered HE attack during treatment • Improved liver steatosis and GS-positive hepatocyte distribution; increased ASS1-positive hepatocytes (one patient) • Increase in body weight
Watanabe et al.[51]	$N = 2$; AACD	48 & 61 y.o.	• Improvement or normalization in most biochemical results • Improved but not normalized Cit levels
Aoki et al.[40]	$N = 3$; NICCD	12–45 d.o.	• Improvement or normalization of most blood clotting factors • Normalization of blood coagulation potential
Case reports with sodium pyruvate therapy			
Mutoh et al.[37]	$N = 1$; AACD	13 y.o.	• Improvement or normalization in some biochemical results • Worsening of Arg & Gln levels • Persistent citrullinemia • Increase in body weight
Yazaki et al.[34]	$N = 2$; AACD	34 & 41 y.o.	• Limited efficacy • Relapse of HE attacks • Both patients eventually underwent liver transplant
Yazaki et al.[58]	$N = 1$; AACD	72 y.o.	• Limited efficacy in biochemical results • Increased body weight • Worsening of plasma Cit
Kogure et al.[44]	$N = 1$; AACD	68 y.o.	• Normalized ammonia and PSTI levels • Improved Cit levels but not normalized
Nagasaka et al.[45]	$N = 10$; silent period	3–13 y.o.	• Limited efficacy in normalizing TCA cycle metabolite levels • Further deviation of some metabolite levels from control values • No changes in plasma lactate, pyruvate and L/P ratios

Abbreviations: α-KG, alpha-ketoglutarate; AACD, adolescent and adult citrin deficiency (formerly CTLN2); ACarn, acetylcarnitine; Ala, alanine; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; Amy, amylase; Arg, arginine; aPTT, activated partial thromboplastin time; ASS, argininosuccinate synthetase; AST, aspartate aminotransferase; BHB, β-hydroxybutyrate; BUN, blood urea nitrogen; Carn, carnitine; CD, citrin deficiency; ChE, cholinesterase; Citr, citrate; Cit, citrulline; Cr, creatinine; D. bil, direct bilirubin; FBG, fasting blood glucose; Fe, iron; FFA, free fatty acids; FI, fasting insulin: FIB, fibrinogen; FR, fischer ratio; Fum, fumarate; Gln, glutamine; GGT, gamma-glutamyl transpeptidase; Glu, glutamate; GS, glutamine synthetase; HE, hpatic encephalopathy; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HYA, hyaluronic acid; ICitr, isocitrate; L/P, lactate to pyruvate ratio; Lac, lactate; LDH, lactate dehydrogenase; Mal, malate; MCT, medium chain triglycerides; Met, methionine; Na+, sodium ions; NaPy, sodium pyruvate; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; NH3, ammonia; OGTT, oral glucose tolerance test; PSTI, pancreatic secretory trypsin inhibitor; PLT, platelet count; Pyr, pyruvate; RBC, red blood cell count; Suc, succinate; T/S, threonine to serine ratio; TG, triglycerides; T. bil, total bilirubin; T. chol, total cholesterol; TBA, total bile acid; TCA, tricarboxylic acid; Thr, threonine; TIBC, total iron-binding capacity; TP, total protein; Tyr, tyrosine; UA, uric acid; WBC, white blood cell count.

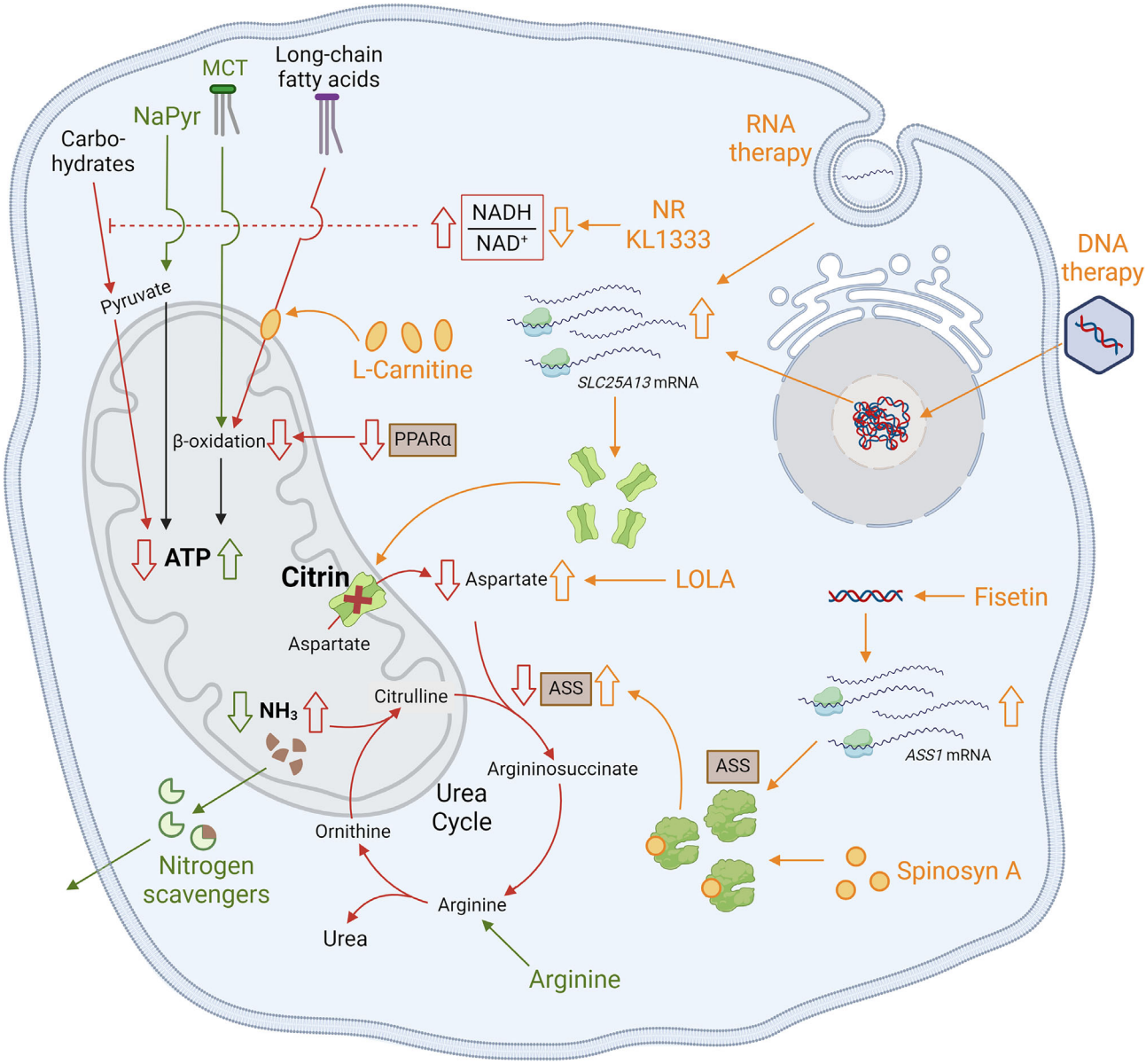

FIGURE 2 Overview of the current and prospective treatment approaches for CD. Red color indicates pathways and processes implicated in CD manifestation. Current and prospective treatment options and their effects are marked in green and yellow, respectively. In CD, an elevated NADH/NAD^+ ratio inhibits carbohydrate metabolism and energy production. For addressing this energy shortage, medium chain triglycerides (MCT) are used to stimulate beta-oxidation, serving as an alternative source for adenosine triphosphate (ATP) production. Sodium pyruvate (NaPyr) addresses redox imbalance by stimulating gluconeogenesis and acting as an energy reservoir. In CD, urea cycle activity is diminished resulting in ammonium accumulation. To manage hyperammonemia, nitrogen scavengers eliminate excess ammonium, while arginine boosts ammonium detoxification via the urea cycle. Accordingly, L-aspartate-L-ornithine (LOLA) supplementation elevates aspartate levels, stimulating ureagenesis and aspartate-dependent pathways. Peroxisome proliferator-activated receptor alpha (PPARα) downregulation in CD impairs β-oxidation and transport of long-chain fatty acids across the mitochondrial membrane, for which L-carnitine supplementation could aid β-oxidation restoration. Compounds like nicotinamide riboside and KL1333 aim to increase the nicotinamide adenine dinucleotide (NAD) production, restoring redox balance and increasing energy production. Simultaneously, to enhance reduced argininosuccinate synthetase (ASS) content, frequently observed in CD patients, natural occurring molecules including fisetin and spinosyn A can be employed. Finally, emerging genetic methodologies such as DNA and RNA therapy aim to target the underlying genetic defect or induce transient endogenous citrin protein formation, respectively. Created with BioRender.com.

2.1 | Dietary management

The dietary management of classical urea cycle disorders (UCD) such as citrullinemia type 1 (ASS deficiency) is characterized by protein restriction and high carbohydrate intake to provide sufficient energy.[47] In contrast, the hallmark of CD patients, even without clinical intervention, is a strong dietary preference for food rich in

protein and fat, and low in carbohydrates.[30,48] The recommended diet in CD comprises intake of 15%–25% protein, 40%–50% fat, and 30%–40% carbohydrates as excess carbohydrates can lead to manifestation of symptoms and metabolic decompensation.[33,49]

The reason why CD patients cannot tolerate high amounts of carbohydrates is hypothesized to be due to the accumulation of cytosolic NADH as a result of impairments in the malate–aspartate shuttle. Hereby, redox-sensitive processes such as glycolysis, gluconeogenesis and ureagenesis are affected, explaining the occurrence of carbohydrate aversion, hypoglycemia, and elevated blood ammonia.[15,21,50] A linear relationship between plasma ammonia and postprandial glucose levels in CD patients has been shown, and it was hypothesized that intake of carbohydrates may lead to an increase in cytosolic NADH/NAD^+ ratio, which may contribute to impaired ureagenesis and hyperammonemia.[5] Unlike carbohydrates, the utilization of protein and fat as energy sources is possible in CD as these metabolites do not contribute to the cytosolic buildup of NADH.[32]

Although dietary management has been reported to be effective as a baseline therapy, it still cannot address all the symptoms associated with CD. For instance, in a cohort of 32 CD patients (10 asymptomatic, 22 FTTDCD) who either self-selected or were prescribed a low carbohydrate, high protein/fat diet, about half exhibited slow growth and patients with FTTDCD also experienced abdominal pain and/or hypoglycemia.[30] Furthermore, the inadequacy of dietary management is exemplified in adult CD patients, who typically show the strongest dietary preferences but still may exhibit severe symptoms such as hyperammonemia, hepatic encephalopathy (HE), low body mass index (BMI), and significant biochemical derangements.[34,39] This necessitates alternative or additional therapeutic interventions such as anaplerotic therapies or other medications, which are described in the following sections.

2.2 | Medium chain triglycerides (MCT)

An emerging concept that explains the pathophysiology of CD is the chronic energy deficit in the liver due to the impairment in the malate–aspartate shuttle.[32,50] Many metabolic processes affected in CD require ATP, and it has been postulated that insufficient ATP levels are a main determinant of the disease.[38,50] MCT can rapidly supply energy to the liver, hereby treating the underlying energy shortage, hence MCT was suggested to have the potential to benefit all CD phenotypes.[38] Additionally, it is thought that MCT may promote the malate-citrate shuttle to improve the hepatic cytosolic NADH/NAD^+ ratio.[32,50]

On this basis, there has been continued interest in MCT as a potential therapy to treat the underlying energy deficit associated with CD. Therefore, herein we review the available literature to provide an overview on the usage and efficacy of MCT to treat CD (Table 2).

A total of 151 publications that specifically mention the use of MCT to treat any form of CD were identified of which six manuscripts were included in the below literature review.[38,40,42,43,51,52] The included studies were all case reports and originated from Japan, with however low homogeneity concerning CD phenotypes, MCT dosage and duration, and outcome measures. Therefore, a meta-analysis on the identified studies was not possible and instead the data was summarized and described together.

2.2.1 | Effects of MCT in NICCD

MCT remains to be the most widely administered supplement for NICCD. A recent nationwide study in Japan showed that 36 out of 40 NICCD patients were treated with MCT.[3] Considering that NICCD patients often present with cholestasis, MCT may be particularly effective as a nutritional supplement compared to other forms of fat since it is easily digested under cholestatic conditions to provide energy to the liver.

A single study in four NICCD infants showed that treatment with lactose-restricted formula supplemented with MCT normalized most abnormal laboratory findings along with increased body weights and the resolution of cholestasis by the end of the treatment period in all patients (Table S1).[43] Patients 1 to 3 (all siblings), were given MCT-containing formula, while patient 4 was given a lactose-free formula mixed with MCT oil due to complications associated with galactosemia.[43]

The second included study described three NICCD patients who presented with coagulopathies. One patient received a lactose-free formula supplemented with MCT oil while the remaining two patients received MCT formulas. It should be noted, however, that details of MCT dosage were not provided. The authors reported that all three patients saw improvements in their coagulation factors after receiving nutritional therapy, with most laboratory values returning to normal ranges.[40]

Collectively, these preliminary results suggest that MCT treatment can significantly improve and normalize most laboratory parameters and resolve clinical complications associated with NICCD.

2.2.2 | Effects of MCT in the silent period

MCT treatment was examined in two siblings in the silent period who frequently experienced hypoglycemic

TABLE 2 Summary of biochemical findings in CD patients from clinical reports using MCT as a treatment.

	Biochemical findings[a]			
Patient age	**Improved but not normalized**	**Normalized**	**Unchanged**	**Worsened**
Hayasaka et al.[43] (Phenotype: NICCD)				
1.8 m.o		Cit, Thr, ALB, T. bil, D. bil, ALP, GGT, ChE, TBA	Met, Tyr, AST, ALT	LDH
2 m.o		Cit, Thr, Met, Arg, ALB, T. bil, D. bil, AST, ALT, ALP, GGT, ChE, TBA	Tyr	LDH
2.8 m.o		Cit, Thr, Met, Arg, ALB, T. bil, D. bil, AST, ALP, GGT, ChE, TBA, PSTI	Tyr, ALT	LDH
4 m.o		Cit, Thr, Met, ALB, T. bil, D. bil, AST, ALT, GGT, ChE, TBA	NH_3, Tyr, Arg, LDH, ALP	
Hayasaka et al.[42] (Phenotype: AACD)				
38 y.o	NH_3, Cit, PSTI	Glu	Gln, FR, ALB, T. bil, AST, ALT, LDH, ALP, ChE, T. chol, TG, BUN, Fe, TIBC	GGT, HYA
41 y.o	Cit, GGT	NH_3, T. bil	Glu, Gln, FR, ALB, ALT, LDH, ChE, TG, BUN, Fe, TIBC	AST, ALP, T. chol, HYA
53 y.o	Cit, Glu	NH_3, Gln, FR	ALB, T. bil, AST, ALT, LDH, ALP, GGT, ChE, BUN	T. chol, PSTI
53 y.o	Cit, GGT	NH_3, FR, ALT, PSTI	Glu, Gln, ALB, T. bil, AST, LDH, ALP, ChE, T.chol, BUN	TG
62 y.o	PSTI, HYA	NH_3, Cit, Gln, FR, AST, ALT, ALP, GGT, ChE, TG, Fe, TIBC	Glu, ALB, T. bil, LDH, T. chol, BUN	
Otsuka et al.[52] (Phenotype: silent period)				
7 y.o	Reference ranges not provided			
10 y.o				
Hayasaka et al.[38] (Phenotype: AACD)				
48 y.o	Cit, Arg, FR	NH_3, ALB, ALT	Gln	
54 y.o	Cit, FR	NH_3, Arg, ALT	Gln, ALB, ChE	
60 y.o	Cit, ChE	Arg, FR, ALB	Gln, ALT	NH_3
62 y.o	Cit, FR		NH_3, Gln, ALB, ALT, ChE	Arg
67 y.o	Cit	NH_3, Gln, ALB, ChE	FR, ALT	Arg
68 y.o		NH_3, Cit, Gln, FR, ALT, ChE	Arg, ALB	
Watanabe et al.[51] (Phenotype: AACD)				
48 y.o	Cit, FR	NH_3, ALB, AST, ALT, GGT, TG	Gln, ALP, ChE	
61 y.o	Cit, ChE	NH_3, Gln, ALB, AST, ALP, GGT	FR, ALT	
Aoki et al.[40] (Phenotype: NICCD)				
12 d.o	Antithrombin	aPTT, FIB		
28 d.o	Antithrombin, Factors II, IX	FIB, Factors V, VII, VIII, X, XI	aPTT, Factor XII	
45 d.o	Antithrombin, aPTT	FIB, Factors II, V, VII, VIII, IX, X, XI	Factor XII	

Abbreviations: α-KG, alpha-ketoglutarate; AACD, adolescent and adult citrin deficiency (formerly CTLN2); ACarn, acetylcarnitine; Ala, alanine; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; Amy, amylase; Arg, arginine; aPTT, activated partial thromboplastin time; ASS, argininosuccinate synthetase; AST, aspartate aminotransferase; BHB, β-hydroxybutyrate; BUN, blood urea nitrogen; Carn, carnitine; CD, citrin deficiency; ChE, cholinesterase; Citr, citrate; Cit, citrulline; Cr, creatinine; D. bil, direct bilirubin; FBG, fasting blood glucose; Fe, iron; FFA, free fatty acids; FI, fasting insulin: FIB, fibrinogen; FR, fischer ratio; Fum, fumarate; Gln, glutamine; GGT, gamma-glutamyl transpeptidase; Glu, glutamate; GS, glutamine synthetase; HE, hpatic encephalopathy; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HYA, hyaluronic acid; ICitr, isocitrate; L/P, lactate to pyruvate ratio; Lac, lactate; LDH, lactate dehydrogenase; Mal, malate; MCT, medium chain triglycerides; Met, methionine; Na+, sodium ions; NaPy, sodium pyruvate; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; NH3, ammonia; OGTT, oral glucose tolerance test; PSTI, pancreatic secretory trypsin inhibitor; PLT, platelet count; Pyr, pyruvate; RBC, red blood cell count; Suc, succinate; T/S, threonine to serine ratio; TG, triglycerides; T. bil, total bilirubin; T. chol, total cholesterol; TBA, total bile acid; TCA, tricarboxylic acid; Thr, threonine; TIBC, total iron-binding capacity; TP, total protein; Tyr, tyrosine; UA, uric acid; WBC, white blood cell count.

[a]Based on reference ranges provided in citations.

attacks and complained of fatigue. After 4 months of MCT treatment, fatigue and fasting blood glucose levels in both siblings improved and no hypoglycemia was noted after an oral glucose tolerance test (OGTT).[52] One patient (brother) exhibited elevations in postprandial blood ammonia levels that were normalized after MCT therapy. Postprandial lactate-to-pyruvate (L/P) ratios of both siblings were elevated before treatment but normalized after MCT therapy.[52] Taken together, these findings suggest that MCT may also have potential in improving clinical symptoms associated with the silent period or FTTDCD, but it should be noted that there are no detailed data on the long-term use of MCT in these CD phenotypes.

2.2.3 | Effects of MCT in adolescent and adult CD

In a cohort of 11 adult CD patients, nine patients who initially presented with HE, hyperammonemia, and disturbed consciousness recovered from encephalopathy after receiving MCT therapy, with five patients regaining consciousness within 1 to 3 days.[38,42,51] Of this cohort, five were also reported to be free of encephalopathic relapse, one chose to undergo liver transplantation,[38] and one experienced a relapse in HE although this was attributed to work-related stress[38] (Table S2). Most biochemical parameters, including transaminases, improved in all 11 patients after MCT treatment. Ammonia levels were normalized in nine out of 11 patients after treatment, but were not normalized in the remaining two patients, who had considerably higher baseline ammonia levels. While plasma citrulline levels decreased in all 11 patients following MCT treatment, they did not fully normalize in most patients (9/11) but remained elevated, possibly due to a decrease in the amount of ASS protein and enzymatic activity.[38]

Fatty liver, commonly observed in CD, is postulated to be a consequence of hepatic PPARα downregulation.[17,53,54] MCT supplementation in animals has indicated both beneficial and harmful outcomes on liver steatosis.[55,56] Yet, successful resolution of fatty liver was observed in 2 CD patients under long-term MCT treatment.[42] The authors postulate that MCT can promote lipogenesis through the malate-citrate shuttle, leading to PPARα upregulation.[50]

2.2.4 | Discussion and conclusions concerning MCT as treatment for CD

To the best of our knowledge, this work represents the first literature review of the potential efficacy of MCT with the present results suggesting improvements in clinical symptoms in all CD phenotypes. However, it is important to note that the above reports are based on a relatively small sample size without controls, and some biochemical parameters did not significantly improve following MCT treatment. It is also unclear whether MCT alone is effective in treating or even preventing onset of pathology in adolescent and adult CD patients. While optimal doses for MCT remain to be established for each phenotype, a recent publication has provided suggested dosages based on expert opinion.[41] Given the biochemical rationale of MCT and its potential efficacy for CD, further studies are urgently needed to systematically investigate its use in CD and to identify any potential long-term side effects. Finally, the obvious inability of MCT to cure all metabolic abnormalities in CD highlights the need to consider additional modalities such as those discussed in the following sections.

2.3 | Sodium pyruvate

Sodium pyruvate (NaPy) has been suggested as a treatment for CD due to its potential ability to alleviate redox imbalance by stimulating gluconeogenesis and to serve as an energy source.[37,57] Initial preclinical studies on the citrin-KO mouse model demonstrated that liver perfusion of NaPy was effective in improving hepatic L/P ratios in a dose dependent manner and enhanced ureagenesis from exogenously administered ammonia but without reducing elevated plasma citrulline levels.[57]

The first clinical study followed 2 years later when NaPy was administered to a 13-year-old patient who presented with low BMI, fatigue, and postprandial lethargy without hyperammonemia. Laboratory findings revealed significantly elevated plasma citrulline, pancreatic secretory trypsin inhibitor (PSTI), HDL-cholesterol, and Thr/Ser ratio. NaPy therapy was initiated at 177 mg/kg/day with arginine and the patient was monitored periodically for up to 3 years.[37] Improvements in BMI, food intake, general fatigue, and postprandial lethargy were noted within 6 months of therapy, however, NaPy had limited efficacy at improving biochemical parameters. PSTI levels were normalized in the patient, but abnormal plasma amino acids (Cit, Arg, Gln, Thr/Ser ratio), and HDL-cholesterol levels persisted. The authors concluded that NaPy therapy was effective in the patient.[37]

A further survey of the current literature on the specific use of NaPy in CD identified four case reports in adult CD patients, one nonrandomized study in a cohort of 10 children in the silent period (Table 3) and no studies of NaPy use in NICCD patients.[34,37,44,45,58]

Reports on the effectiveness of NaPy in adult CD patients provided mixed results. In two patients, NaPy

TABLE 3 Summary of biochemical findings in CD patients from clinical reports using sodium pyruvate as a treatment.

		Biochemical findings[a]			
Patient age (years)	**NaPy dose (g/day)/ duration (days)**	**Improved but not normalized**	**Normalized**	**Unchanged**	**Worsened**
Mutoh et al.[37] (Phenotype: AACD)					
13	6/1095	T/S	TG, PSTI	T. bil, AST, ALT, Amy, T. chol, HDL-C, BUN, UA, NH_3, TP, Lac, Pyr, Na^+, Cit, Ala, FR	Arg, Gln
Yazaki et al.[34] (Phenotype: AACD)					
34	3–15/77	Exact values not provided (results in graph format)			
41	3/23				
Yazaki et al.[58] (Phenotype: AACD)					
72	3–9/121	ALB, Arg, FR	RBC, Hb, TP, T. chol, ALP, GGT, Amy, PSTI	WBC, PLT, Cr, TG, ALT, LDH, T. bil, prothrombin time, activated partial prothromboplasmin time, NH_3	BUN, AST, Cit
Kogure et al.[44] (Phenotype: AACD)					
68	4.5–8/1825	Cit	NH_3, PSTI		
Nagasaka et al.[45] (Phenotype: silent period)[b]					
3–13	300[c]/91–182		Carn	Citr, ICitr, α-KG, ACarn, BHB, FFA, FBG, HbA1c, Lac, Pyr, L/P	Suc, Fum, Mal, FI

Abbreviations: α-KG, alpha-ketoglutarate; AACD, adolescent and adult citrin deficiency (formerly CTLN2); ACarn, acetylcarnitine; Ala, alanine; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; Amy, amylase; Arg, arginine; aPTT, activated partial thromboplastin time; ASS, argininosuccinate synthetase; AST, aspartate aminotransferase; BHB, β-hydroxybutyrate; BUN, blood urea nitrogen; Carn, carnitine; CD, citrin deficiency; ChE, cholinesterase; Citr, citrate; Cit, citrulline; Cr, creatinine; D. bil, direct bilirubin; FBG, fasting blood glucose; Fe, iron; FFA, free fatty acids; FI, fasting insulin: FIB, fibrinogen; FR, fischer ratio; Fum, fumarate; Gln, glutamine; GGT, gamma-glutamyl transpeptidase; Glu, glutamate; GS, glutamine synthetase; HE, hpatic encephalopathy; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HYA, hyaluronic acid; ICitr, isocitrate; L/P, lactate to pyruvate ratio; Lac, lactate; LDH, lactate dehydrogenase; Mal, malate; MCT, medium chain triglycerides; Met, methionine; Na+, sodium ions; NaPy, sodium pyruvate; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; NH3, ammonia; OGTT, oral glucose tolerance test; PSTI, pancreatic secretory trypsin inhibitor; PLT, platelet count; Pyr, pyruvate; RBC, red blood cell count; Suc, succinate; T/S, threonine to serine ratio; TG, triglycerides; T. bil, total bilirubin; T. chol, total cholesterol; TBA, total bile acid; TCA, tricarboxylic acid; Thr, threonine; TIBC, total iron-binding capacity; TP, total protein; Tyr, tyrosine; UA, uric acid; WBC, white blood cell count.

[a]Based on reference ranges provided in citations.

[b]Reference ranges not provided. Data are compared against control values obtained from healthy volunteers.

[c]Units in mg/kg/day.

treatment in conjunction with dietary management was not effective in reducing the frequency of encephalopathic attacks nor did it improve hyperammonemia. Eventually, both patients underwent successful liver transplantation.[34] In another report, a 73-year-old CD patient was treated with NaPy for 4 months and while PSTI and Fischer ratios were normalized, the majority of laboratory findings were not improved or even worsened.[58] Long-term NaPy treatment in another adult CD patient normalized blood ammonia levels and there was no recurrence of encephalopathy although plasma citrulline and PSTI levels remained elevated.[44]

A nonrandomized clinical study investigating the effects of NaPy therapy on TCA metabolites in 10 CD children showed no corrections in metabolite profiles including plasma L/P ratios after 3 to 6 months of therapy.[45] As the clinical symptoms of the patients were not detailed, it is unknown whether NaPy led to any functional improvements.

Although studies are limited, the collective findings suggest limited efficacy of NaPy in CD. Most biochemical results were not significantly improved by NaPy and recurrent encephalopathic attacks remained.[34,58] While initial preclinical findings were promising,[57] the limited efficacy seen in clinical studies could be due to poor oral bioavailability of NaPy. Because in mouse studies NaPy was administered through liver perfusion techniques,[57] the contributions of the human GI tract, where NaPy is potentially decarboxylated, were not accounted for.[46] In healthy adults, orally ingested NaPy even at a relatively high dose of 25 g failed to increase whole blood or plasma pyruvate concentrations, which may be due to rapid intestinal decarboxylation of NaPy, likely through fermentation by gut bacteria,[46] and/or uptake and metabolism by skeletal muscle.[59] The development of new formulations of pyruvate with enhanced oral bioavailability may be helpful in this regard.

Although both MCT and NaPy aim to address the same fundamental defects associated with the condition, current findings from limited studies suggest that MCT may be more effective. Another important practical consideration worth mentioning is that NaPy is not an approved drug, while MCT is a widely available nutraceutical and therefore easily accessible.

2.4 | Ursodeoxycholic acid (UDCA)

Cholestasis is a commonly observed symptom associated with NICCD[10] and while the cause of this is not fully understood, it has been suggested that the impaired energy production caused by CD leads to a reduction in the production and secretion of bile acids.[33] UDCA is a mainstay treatment for cholestatic liver diseases including cholestasis associated with NICCD by improving bile acid biosynthesis and bile flow.[3,10,60,61] UDCA has also been reported to have properties that may protect hepatocytes against bile acid-induced cytotoxicity.[62] Although mainly indicated for NICCD, the use of UDCA in post-NICCD patients has also been reported in the literature,[3,52,63] but there is a lack of systematic studies into the use of UDCA in CD.

2.5 | Nitrogen scavengers

Nitrogen scavengers such as benzoate, phenylacetate, and phenylbutyrate are first line therapies in the management of hyperammonemia in UCDs.[47] This class of drugs bypass the urea cycle to provide an alternative pathway for nitrogen excretion by conjugating with nitrogen bearing amino acids such as glycine or glutamine and are later excreted in urine.[3,47,64,65] Nitrogen scavengers are most often used for adult CD patients to manage elevated ammonia levels,[3,66] but there are no systematic studies in CD available.

2.6 | L-arginine

L-arginine is an intermediary metabolite of the urea cycle used to clinically manage hyperammonemia in UCDs (except for arginase 1 deficiency) and is often administered together with nitrogen scavengers.[65,67] L-arginine lowers ammonia levels by enhancing ureagenesis.[68] Its use and effectiveness in CD were first reported in a 37-year-old patient where arginine granules were effective in lowering and maintaining normal blood ammonia levels. When arginine therapy was temporarily discontinued in this patient, ammonia levels increased significantly, and the patient fell into a coma.[36] However, in contrast to most UCDs (except for arginase 1 deficiency), plasma arginine levels in CD are often elevated, questioning the role of arginine supplementation in this condition. There are no systematic studies available, but a thorough biochemical study would be of interest to understand the mechanism of action of arginine therapy in CD.

2.7 | Liver transplantation

At present, liver transplantation remains the only curative treatment for CD and is typically performed in adult patients with severe symptoms who are unresponsive to other treatments,[15,19,69] and rarely in NICCD

patients.[24,27] Post-transplantation, laboratory results and clinical symptoms of patients were reported to normalize. Interestingly, the unique food preferences of patients also disappeared after liver transplantation and they no longer require diet restrictions.[34,69–71] These drastic improvements also highlight that CD primarily affects the liver. Despite these positive outcomes, significant drawbacks concerning liver transplantation remain such as lifelong immunosuppression and associated risks, high costs, and shortage of suitable donors.

2.8 | General discussion on the current treatment landscape

The present overview demonstrates that research on existing therapies for CD remains at a relatively nascent stage. In order to advance the establishment and development of effective therapies for CD, several outstanding issues need to be addressed first.

Efficiently characterizing and organizing large patient cohorts with CD is vital for expediting therapy evaluation and development, as it facilitates insights into the disease's history, streamlines patient recruitment, and reduces study costs.

Furthermore, identification of CD-specific biomarkers and establishment of informative functional tests in patients are needed to reliably assess the efficacy of therapies in clinical trials. Current biochemical parameters such as plasma amino acids, liver function markers, and ammonia levels are nonspecific for CD and may fluctuate greatly depending on the time of sampling. A potential functional test for CD is to examine the ureagenesis function of patients across phenotypes, as this may be a good indicator of disease severity and progression and whether patients are at risk of developing the adult form of the disease. Recently, new methods have been developed that use low concentrations of stable isotope tracer coupled with more robust analytical methods that may be considered.[72]

Finally, there is a need to develop suitable preclinical models of CD to facilitate preclinical studies on potential therapies. To date, only two animal models exist for CD, the citrin-KO mouse that fails to display features of human CD, and the citrin/mitochondrial glycerol-3-phosphate dehydrogenase (mGPD) double-KO mouse that demonstrates several features of the disease.[73,74] Also, mice express much higher hepatic levels of the citrin protein isoform, aralar, which may functionally compensate for the loss of citrin.[75] The development of other animal models, such as rat models, may be considered as rats are genetically and metabolically closer to humans compared to mice.[76] Cellular models of CD are limited and include a citrin-KO cellular model in the HepG2 hepatoblastoma cell line[77] as well as hepatocyte-like cells derived from CD-induced pluripotent stem cells.[22] However, it has been shown that HepG2 cells lack the expression of several urea cycle enzymes, resulting in limitations for studies of the urea cycle,[78,79] while iPSC-derived hepatocytes are generally immature and more closely resemble fetal rather than adult hepatocytes.[80]

3 | FUTURE THERAPEUTIC OUTLOOK

3.1 | Restoring the redox balance

In vivo and in vitro models of CD demonstrate a disrupted NADH/NAD^+ ratio as a result of impaired malate–aspartate shuttle activity.[73,74,77] This imbalance causes impairment of glycolysis and gluconeogenesis in the cytosol, while the shortage of NADH in mitochondria impairs the function of the TCA cycle and hence ATP production.

Redox balance can be restored by increasing the amount of NAD^+ in the cytosol, either by providing a precursor molecule such as nicotinamide riboside (NR)[77] or by pharmacologically stimulating NAD^+ synthesis.[81] The latter approach has been pursued using KL1333, a compound able to modulate NAD(P)H:quinone oxidoreductase towards enhanced NADH to NAD^+ conversion.[81] KL1333 treatment was able to restore NAD^+ and ATP levels and improve mitochondrial biogenesis of skin fibroblasts from a patient with mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes.[81]

Another study focused on HepG2 citrin-knockout (KO) cells for assessing the potential effect of NR treatment. These cells exhibit several characteristics of CD, including increased ammonia accumulation, higher cytosolic NADH/NAD^+ ratio and impaired glycolysis.[77] HepG2 citrin-KO cells supplemented with NR successfully reduced the cytosolic NADH/NAD^+ ratio, enhanced glycolysis and fatty acid oxidation, but did not improve ammonia detoxification,[77] highlighting the need for combination treatment for additionally targeting ammonia-related CD manifestations. Taken together, these results highlight the importance and ability of NADH/NAD^+ rebalancing approaches in establishing new treatments for CD.

3.2 | Aspartate and other amino acids supplementation

The most serious consequence of adult CD is a condition resembling HE,[44] and it is speculated that in addition to

the energy deficit in liver cells, insufficient cytosolic aspartate levels hamper urea cycle functionality. Therefore, restoration of aspartate availability could rationally aid ureagenesis function.

Amino acid supplementation (as well as MCT and NaPy) for restoring urea cycle function has been tested on citrin/mGPD double-KO mice with induced hyperammonemia.[73,82] Combinations of either ornithine and alanine or ornithine and aspartate were equally effective in decreasing blood ammonia to levels similar to control animals. Furthermore, both treatment combinations increased hepatic aspartate and decreased hepatic citrulline concentrations by ASS protein activation.[82]

For ameliorating HE and hyperammonemia in patients not affected by CD, supplementation with L-ornithine-L-aspartate (LOLA) has been proposed and tested in a double-blind randomized controlled trial as well as a randomized clinical trial.[83–85] Oral administration of LOLA to patients with stable, overt, chronic encephalopathy was beneficial at improving the extent and preventing further episodes of HE and in reducing blood ammonia concentrations.[83,84]

Overall, LOLA and other amino acids supplementation show some promise in mitigating ammonia-induced brain edema and have already undergone clinical trials for different liver conditions,[86] but further investigations of LOLA and other amino acids on broader aspects of CD are required.

3.3 | Enhancing bioenergetics

Contributing to the energy deficit in CD,[32] citrin deficient hepatocytes struggle to utilize glucose due to impaired glycolysis or fatty acids due to reduced β-oxidation rates attributed to PPARα downregulation. Additionally, it has been proposed that fatty liver observed in some cases of CD is the result of decreased β-oxidation without the corresponding decrease in lipogenesis.[77] Therefore, stimulating β-oxidation using e.g. L-carnitine could increase mitochondrial NADH and ATP supply as well as reduce the buildup of excess hepatic fatty acids. L-carnitine is a natural constituent of human cells[87] and plays an important role in fatty acid metabolism.[88] It acts as an obligatory cofactor for fatty acid oxidation and facilitates long-chain fatty acid transport across the mitochondrial membranes, thereby promoting β-oxidation and ATP generation.[88,89] Supplementation of L-carnitine in the context of pulmonary arterial hypertension led to augmentation of fatty acid oxidation and lipolysis, and reduction of lipid accumulation in vitro.[90] Nevertheless, future clinical studies to rectify bioenergetic deficits in CD with compounds such as L-carnitine would be needed for improving our understanding of the disease and its management.

3.4 | Enhancing argininosuccinate synthetase and other urea cycle enzymes

Hyperammonemic episodes in CD are generally attributed to the lack of aspartate and, at least in the majority but not all of the respective patients, decreased ASS activity.[11,17,91] While cytosolic aspartate deficiency is a direct consequence of a defective citrin protein, the reason for decreased ASS function remains unclear, and requires additional studies for a correct and complete understanding of the origin of hyperammonemia in CD. Several FDA-approved small molecules are shown to upregulate ASS expression or enhance its activity, namely fisetin and spinosyn A.[92] Specifically for fisetin, which is a flavonoid molecule commonly found in fruits and vegetables, it has been proposed to upregulate both ASS and argininosuccinate lyase (ASL) protein expression in various cancer cell lines and in vivo in mice enterocytes.[92] Furthermore, one study has demonstrated that spinosyn A, component of the commonly-used pesticide spinosad with low toxicity in mammals,[93–95] and its derivative can specifically bind to ASS protein and dose-dependently enhance its catalytic activity.[95] Therefore, possible analogous effects mediated by fisetin or spinosyn A could potentially restore urea cycle function in CD hepatocytes both in vitro and in vivo. While these molecules have not yet been tested in relevant CD models, they present an intriguing possibility for alleviating hyperammonemia in CD patients.

3.5 | Gene therapy

Gene therapy has been shown to represent a valuable and promising approach for treating various diseases including inherited metabolic liver disorders such as Wilson's disease and UCDs, e.g. ornithine transcarbamylase (OTC) and ASS deficiency.[96–98] By directly targeting the underlying genetic abnormality, gene therapy has the potential to provide long-lasting or even permanent solutions to conditions without previous successful treatment options. Gene therapy holds tremendous potential for treating metabolic liver diseases including CD, since it may be performed in a carefully planned approach during the silent period, hereby preventing progression of liver disease and the onset of adolescent or adult CD. Below, we describe established or currently planned approaches towards this goal.

3.6 | mRNA-based gene therapy approaches

mRNA-based therapies have emerged as a recent and innovative approach in gene therapy that showed promising results in the treatment of inborn errors of liver metabolism, including UCDs.[99–103] mRNA molecules, commonly delivered intravenously via lipid nanoparticles are largely taken up by the hepatocytes, where they use the cell's own translational and transport machinery, bypassing the need for exogenous enzyme production.[99,104] Furthermore, mRNA therapy does not integrate into the genome, mitigating the risk of insertional mutagenesis, however, due to their short lifespan in cells, regular mRNA infusions are required.[100,101]

Notably, a breakthrough in mRNA therapy for CD was achieved using codon-optimized mRNA encoding the human citrin protein, which was encapsulated in lipid nanoparticles that were administered intravenously to citrin-KO and double KO (ctrn/mGPD-dKO) mice.[73,74,105] The goal of this study was to increase hepatic citrin levels and restore metabolic balance. Since the majority of disease-causing citrin mutations lead to a complete absence of citrin protein,[2,106] even a partial restoration of citrin protein can result in clinical improvement. This was in fact achieved by restoration of citrin levels to 2% to 5% of wild-type within 24 h post-injection in the citrin-KO mice, when plasma citrulline and ammonia were effectively reduced to near physiological levels.[105]

Further validation on the safety of this approach and its effect on the different symptoms of CD such as on fatty acid accumulation or on ASS protein levels and activity are yet to be determined but mRNA therapy holds promise for alleviating the disease.

3.7 | DNA-based gene therapy approaches

In DNA gene therapy, a functional copy of the defective gene is introduced into the patient's cells, allowing for the correction or modification of genetic defects at the DNA level. Correction of UCDs via liver-directed gene therapy has been reported in mouse models of OTC,[107] ASS1,[97] ASL,[108] CPS1[109] and ARG1 deficiency.[110] While gene therapy faces challenges related to immune responses triggered by the recognition of an exogenous sequence as a foreign entity, an approach that provides cells with a similar sequence to the endogenous one may help circumvent these immune responses. In the case of CD, aralar, which shares approximately 80% identity with citrin and exhibits similar transport properties, emerges as a suitable candidate for gene therapy due to its expression in various cell types, including certain liver cells, namely Kupffer cells.[111]

Recently, the possibility of introducing an exogenous aralar sequence in the hepatocytes of citrin-KO mice was explored as a potential therapeutic strategy.[74] The introduction of an exogenous aralar sequence substantially restored the function of the malate–aspartate shuttle in CD liver mitochondria and balanced NADH/NAD^+ levels in hepatocytes isolated from citrin-KO mice.[75] Furthermore, it has been reported that citrin is around 8 times more abundant than aralar in mice liver while in human liver citrin is 400 times more abundant,[75] suggesting that aralar therapy could be clinically beneficial. It is important to note that overexpression of aspartate/glutamate carriers (AGCs) has been connected with different cancer types, including hepatocellular carcinoma,[112,113] therefore, providing exogenous AGC to patients has to be finely tuned. It is also unclear whether there will be potential immunological responses against wild-type citrin protein given citrin's intracellular localization in the inner mitochondrial membrane and the fact that its isoform aralar is being endogenously expressed.[75] Hence, gene editing of the defective citrin gene should also be attempted. While the effect of exogenous citrin or aralar expression on hallmarks of CD such as hyperammonemia and fatty liver is yet to be determined, gene therapy holds potential as the future of CD treatment.

4 | CONCLUSIONS

Since the discovery of *SLC25A13* as the causative gene for CD 25 years ago, advancement in the understanding of CD and developments in dietary and therapeutic interventions have enhanced the quality of life in affected patients. Among these therapies, the present review highlights MCT as the most promising therapeutic candidate for all CD phenotypes given its biochemical rationale. However, only few additional effective therapeutic options exist for CD and there is still no definite and curative treatment besides liver transplantation. Large opportunities remain in the research and development of new effective therapies, from small molecules that can alleviate the impact of the condition to a potential cure such as gene therapies to express a functional copy of the citrin protein. There is also the need to sufficiently characterize and organize large patient cohorts for natural history studies and clinical trials, to establish effective biomarkers and functional tests, as well as to generate suitable preclinical models in order to advance the development of treatments for CD.

AUTHOR CONTRIBUTIONS

Toni Vuković and Li Eon Kuek contributed equally to this manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

This study is a review paper, which does not require approval by any Institutional Review Board (IRB).

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