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The role of genetic defects in carnitine-associated hepatic encephalopathy: a review of literature

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ABSTRACT

Hepatic encephalopathy (HE) is a serious neurological disorder characterized by brain dysfunction due to liver failure which occurs as a result of chronic or acute liver disease. HE can manifest with various neurological or psychiatric symptoms ranging from excessive sleepiness and sleep disorders to coma. HE is a serious disorder that in acute conditions can even lead to the death of the patient due to cerebral edema. Carnitine acts as a vital component in facilitating the transport of long-chain fatty acids into the mitochondria, thereby enabling their oxidation for the generation of energy. Carnitine additionally assumes a crucial role in the functionality of the brain. Carnitine deficiency is associated with various types of inherited disorders related to low levels of carnitine. A strong correlation exists between the insufficiency of carnitine and the occurrence of HE. If a deficiency of carnitine is identified through clinical symptoms or laboratory results in patients with liver dysfunction, treatment with carnitine replacement therapy is recommended. Thus, the administration of acetyl-L-carnitine in patients with HE can improve their mental and psychological conditions. In the present study, we provide an overview of the molecular and cellular mechanisms underlying HE. Our aim in this review has been genetic investigation of HE and genetic mutations to the causes of this neurological condition, which include carnitine deficiency, hyperammonemia, and etc. Finally, we discuss the genetic mutations that lead to carnitine deficiency as well as hyperammonemia and are associated with this neurological disease, together with the future treatment of this disease based on carnitine therapy. More studies soon will help early diagnosis (before poor prognosis) based on clinical observations, genetic tests, prenatal diagnosis, and new treatment strategies.

Keywords: Hepatic encephalopathy, Carnitine, Ammonia, Genetic, Treatment.

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Introduction

Hepatic encephalopathy (HE), which can appear as a variety of neurological or psychiatric disorders ranging from subclinical changes to coma, is generally characterized as brain dysfunction brought on by liver insufficiency and/or portal-systemic shunting. The underlying etiology of the liver illness is not taken into account in this definition of HE. However, the causes of chronic liver diseases (CLDs), such as viral hepatitis, alcoholism, non-alcoholic fatty liver disease, and primary biliary cholangitis, can all impact the brain via mechanisms different from those brought on by liver

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failure or dysfunction (1). The clinical symptoms of liver cirrhosis patients, especially those with liver encephalopathy, include excessive sleepiness during the day and sleep disorders in general. Hence, the disturbance in the quality of sleep causes a rapid increase in mortality among these patients. Treatment of patients with HE is still challenging and needs a better understanding of the underlying mechanisms (2).

Long-chain fatty acid transport into the mitochondrial matrix, where fatty acid oxidation occurs, is made possible by the amino acid derivative known as l- Carnitine (4-N-trimethylammonium-3-hydroxybutyric acid). Additionally, by producing acylcarnitines. l-carnitine defends the cell against the accumulation of acyl-CoA. L-carnitine is the active form of carnitine, and is made from the amino acids methionine and lysine. It is a compound with antioxidant activity (3, 4). Circulating carnitine is basically provided by animal-based nourishment products and, to a lesser degree, by endogenous biosynthesis within the liver and kidney (4). Thus, malnutrition and reduced food consumption cause carnitine deficiency. Carnitine deficiency disrupts essential liver metabolic mechanisms such as gluconeogenesis, albumin biosynthesis, fatty acid metabolism, and ammonia detoxification by the urea cycle and causes hyperammonemia plus hypoalbuminemia. Finally, these events will lead to HE (5). With the consumption of carnitine, the amount of extraintestinal ammonia production diminishes; as a result, it increases the detoxification power in liver cirrhosis patients (2). Although carnitine consumption has few side effects, some people may experience symptoms such as abdominal pain, nausea, and diarrhea (3).

Mitochondrial defects are very difficult to correct, though these diseases can be combated today with the development of antioxidant and autophagy drugs that destroy defective mitochondria. Although there are many problems related to the mechanisms and pathways of mtDNA transcription, the research conducted in relation to gene therapy, DNA editing, and prenatal diagnosis shows a bright future for the treatment of this genetic disease (6, 7). The aim of this study has been to examine the disease of HE considering the genetic pathway of carnitine as one of the factors causing this disorder. The combination of the treatments related to carnitine with some of the recent trends in gene therapy, personalized medicine, and gut-liver-brain axis opens interesting perspectives to improve the outcomes of HE treatment. Novel targeted genetic interventions and clinical trials for carnitine supplementation would be expected toward the development of more efficient and personalized therapies in HE patients.

An overview of hepatic encephalopathy causes; does carnitine deficiency have a prominent role?

One common neuropsychiatric complication of both acute and chronic liver disorders is HE. HE is recognized with the prominent symptoms of ataxia, asterixis, and impaired learning. HE-causing factors include toxins such as ammonia (a principal toxin in HE), DNA damage, chronic liver diseases (alcoholic and non-alcoholic fatty liver disease, primary biliary cholangitis, and viral hepatitis), and carnitine deficiency (1, 8, 9). Carnitine (β-hydroxy-γ-trimethylammonium butyrate) plays a vital role in energy metabolism as a hydrophilic quaternary amine. Carnitine causes β-oxidation by transferring long-chain fatty acids into mitochondria. In addition, carnitine functions as a scavenger by binding to acyl residues from the intermediate metabolism of amino acids and assisting in their removal (10). In many organic acidemias, this process is crucial for binding and eliminating aberrant organic acids as well as describes the secondary carnitine deficiency that may come about as a consequence of them (11). Also, the release of acyl-CoA from the mitochondria depends on carnitine. Thus, through the regulation of acyl-CoA and the pool of free CoA levels in the mitochondria, carnitine has a positive impact on gluconeogenesis, the glycolysis system, the urea cycle, and the tricarboxylic acid cycle in addition to fatty acid metabolism. Further, carnitine enhances oxidative stress, inflammation, and the performance of the biomembrane (5). Recent studies show that L-carnitine, which is synthesized from the combination of L-lysine and L-methionine in the brain, kidney, and liver, reduces serum ammonia levels in liver cirrhosis patients. So, it is used in the treatment of liver cirrhosis patients. Since one of the important complications of liver cirrhosis is HE, L-carnitine can be used to treat patients with HE (12). In this regard, a study was conducted on patients with different degrees of HE (from

minimal HE to coma). It was observed that patients who consumed L-carnitine or acetyl-L-carnitine significantly improved parameters related to HE (5). Carnitine deficiency usually occurs in the body in two ways, primary and secondary. However, sometimes it is difficult to distinguish between these two types. Primary carnitine deficiency is an uncommon autosomal recessive disease that occurs as a result of disruption of the carnitine transporter in the plasma membrane. Thus, carnitine cannot accumulate in the skeletal muscles and heart muscle; hence, the blood contains more carnitine, which the kidneys eliminate. Secondary carnitine deficiency occurs as a result of increased excretion of carnitine through the urine due to Fanconi syndrome, peritoneal dialysis, genetic defects, and many other reasons (13). If there is enough acetyl L-carnitine (the main carnitine ester) in the body, it reduces the amount of ammonia in the blood and brain through ureagenesis and removes the build-up of harmful fatty acyl-CoA metabolites. For this reason, acetyl L-carnitine is used to treat HE patients and reduce symptoms such as cognitive disorders and depression (14). The Functional Cycle of Carnitine Metabolism and Transport and Association with Hepatic Encephalopathy

As a vital component for mitochondrial function, carnitine makes fatty acid transport easier (15). Carnitine temporarily binds to Acyl Coenzyme A (CoA) and Carnitine acyl transferases I and II are able to identify it (16-21). Carnitine dissociates from Acyl CoA within the mitochondrion and returns to the cytosol to start a new cycle (22, 23). Carnitine plays a role in mitochondrial metabolism, oxidative stress protection, neurohormone transcription regulation, neurotrophic factor, and apoptosis reduction in cell line cultures (24). Long-chain fatty acids go through processing by mitochondrial b-oxidation to produce ATP. Carnitine is required for Long-chain fatty acids initiating a outstanding journey through the intricate pathways of mitochondria's inner membrane, guided by the subtle orchestrations of cellular machinery and transport proteins, to fuel the fiery engines of energy production within the cellular realm (25). Enzyme-mediated processes transfer the acyl groups of long-chain fatty acids to carnitine. Acylcarnitine is susceptible to b-oxidation subsequent to getting translocated inside the mitochondrial matrix via acylcarnitine/carnitine translocase (16, 24, 26-29). Additionally, carnitine is essential for the maintenance of CoA-related compounds' homeostasis. Along with its main function according to the delivery of delivery of long-chain fatty acids within the internal mitochondrial membranes (30), carnitine eases the transportation of acylcarnitine esters that migrate from the intramitochondrial space into the cytosolic compartment (31-33). In addition, evidence indicates that peroxisomal b-oxidation relies on carnitine (34). Carnitine is also essential for the acyl-CoA transfer from the mitochondria. As a result, along with the metabolism of fatty acids, carnitine has an advantageous impact on gluconeogenesis, the tricarboxylic acid cycle, the urea cycle, the glycolysis system, plus the pool of free CoA accumulations in the mitochondria by modulation of acyl-CoA (35). The process of plasma membrane carnitine and mitochondrial fatty acid, translocation fatty acid absorption, and intramitochondrial fatty acid oxidation are additionally involved in energy production through extended fasting (36).

Carnitine palmitoyltransferase I (CPT-I), which is highly susceptible to malonyl-CoA and is situated in the outer layer of the membrane of mitochondria, as well as carnitine acylcarnitine translocase, which is a vital protein of the inner membrane, in addition to carnitine palmitoyltransferase II, positioned on the matrix-covered side of the inner mitochondrial membrane accomplish the transport system towards the mitochondrial matrix (15, 16, 37, 38). Carnitine-acylcarnitine translocase (CACT) then transports a particular of the products of this process, acylcarnitine, over the membrane that is found inside of the mitochondria. CPT II, which transfers the acyl group to catalyze the reversible reaction of carnitines to acyl-CoA, transfers the rest of the acyl of the acylcarnitine to return to coenzyme A over the inner membrane of the mitochondria. As a result, the generated acyl-CoA is subsequently ready for -oxidation (39). The carnitine produced in the subsequent stage returns to the mitochondrion's intermembranous region via the CACT and is accessible to supply fatty acid re-transport (40). Considering its potential contribution to energy production, carnitine can only be synthesized in a few tissues (25). As a result, carnitine is transported from the blood to various tissues via carrier-mediated routes (25, 41-46). To maintain concentrations of serum

carnitine, the intestinal epithelium and renal proximal tubules uptake carnitine from glomerular filtrate and foods, respectively (25, 41, 42, 46). Organic cation/carnitine transporter new type 2 (OCTN2) is responsible for the transportation of carnitine inside cells. It has also a special capacity to regulate the sodium-dependent transportation of dipolar ions such as carnitine and acylcarnitines (47).

Any disruptions to the functional cycle of carnitine can be attributed to a variety of factors, including genetic defects affecting the enzymes and transporters involved in synthesis, transportation, or utilization (15, 48). Primary carnitine deficiency, an uncommon autosomal recessive disorder caused by mutations in the SLC22A5 gene encoding OCTN2, is a notable example (49, 50). This condition makes difficult for the cells to uptake carnitine, which lowers intracellular levels and reduces fatty acid oxidation. As a result, when cases remains untreated, these individuals may develop hypoglycemia, cardiomyopathy, and muscle weakness, among other symptoms that might ultimately be fatal (51, 52).

Furthermore, it has been proposed that abnormalities in the metabolism of carnitine could play a role in the pathogenesis of HE, a neuropsychiatric disorder marked by altered consciousness and cognitive impairment brought on by liver dysfunction (53). HE is commonly associated with acute or chronic liver failure, where impaired hepatic function leads to the accumulation of toxic metabolites, including ammonia, in the bloodstream (54). Normally, the urea cycle in the liver transforms ammonia from amino acid metabolism into urea, which is then expelled through urine (55, 56). However, in liver failure, the impaired urea cycle function results in elevated ammonia levels, which can have neurotoxic effects on the brain, contributing to the development of HE (57).

The detoxification of ammonia and mitochondrial functions, in which carnitine is involved, are the underlying principles of the association between HE and carnitine metabolism (58, 59). Carnitine deficiency, whether primary or secondary to liver dysfunction, can impair the mitochondrial oxidation of fatty acids and disrupt the balance of acyl-CoA species, leading to the accumulation of toxic metabolites and oxidative stress within hepatocytes (60-62). Additionally, it participates in the urea cycle by aiding in the removal of acetyl CoA, which is required for the catabolism of fatty acids into urea in order to eliminate excess ammonia (63). Thus, carnitine deficiency in the context of liver failure can exacerbate ammonia-induced neurotoxicity and contribute to the pathogenesis of HE (64).

Besides its primary form, carnitine metabolism-related genetic diseases have also been connected to HE in the past (65). The deficiency of medium-chain acyl-CoA dehydrogenase (MCAD) or even deficiency of carnitine palmitoyltransferase II (CPT II) are examples of defects in fatty acid oxidation enzymes that result in accumulations of toxic acylcarnitines intermediate disrupting mitochondrial function and making them more vulnerable to metabolic decompensation and subsequent HE during periods of metabolic stress (66).

Furthermore, certain inborn metabolic disorders, such as organic acidemias or urea cycle disorders, might cause HE by indirectly altering carnitine metabolism (48, 67). These disorders are frequently caused by defects in enzyme systems involved in carnitine utilization and transport, as well as mitochondrial dysfunction (48, 68). Patients are susceptible to hepatic encephalopathy (HE) symptoms such as confusion, lethargy, and asterixis, particularly during metabolic crises or acute exacerbations of their underlying disease (52, 69).Thus, it is obvious that carnitine metabolism and the transport functional cycle are essential for maintaining cellular energy balance and mitochondrial function. When this cycle is disturbed, whether due to genetic abnormalities or liver disease, it can have serious consequences for metabolic health, making people more likely to develop hepatic encephalopathy. A complete understanding of how carnitine metabolism interacts with HE provides a foundation for better understanding the etiology of this condition and the identification of possible treatment targets.

A rat intestinal cDNA expressing Slc22a5 or a carnitine transporter (CT1), also known as Octn2. CT1 encodes a protein that consists of 557 amino acids with 12 potential membrane-spanning domains (70). CT1 can regulate a high-affinity transportation of L-carnitine; additionally L-carnitine fulfils the role of a CT1 substrate with a high affinity (70, 71). In a study, CT1 only interacted with substances containing carnitine-like structures. CT1 was shown to be

significantly expressed in the liver, testis, kidney, and gut, where carnitine is actively transported (25).

The mitochondrial carnitine acyl-carnitine carrier (CAC) belongs to a member of the SLC25 gene family when it comes to classification. It includes 53 numbers of human solute transporters (72-74), and This 42 kb gene has 9 coding exons, corresponding to chromosome 3p21.31, which encodes the CAC, a 301 amino acid protein (75). The great majority of these are found in the inner membrane of the mitochondria. Up until now, just one member of the family had been discovered inside the peroxisomal membrane (75, 76). CAC is a member of the most thoroughly studied inner membrane of mitochondria membrane transport proteins. In addition to mechanistic, kinetic, and functional evidence, post-translational changes influencing CAC transport activity were also discovered (75). Carnitine deficiency causes a buildup of non-oxidized fatty acyl-coenzyme A molecules, limiting ammonia breakdown in the mitochondria. Encephalopathy can result from hyperammonemia (77, 78). L-carnitine as well as acetyl-L-carnitine supplementation significantly enhanced the markers linked to hepatic encephalopathy (65) (Figure 1).

Impact of genetic mutations on carnitine levels and function in hepatic encephalopathy

Carnitine biosynthesis and transport defects caused by genetic mutations have been identified as major

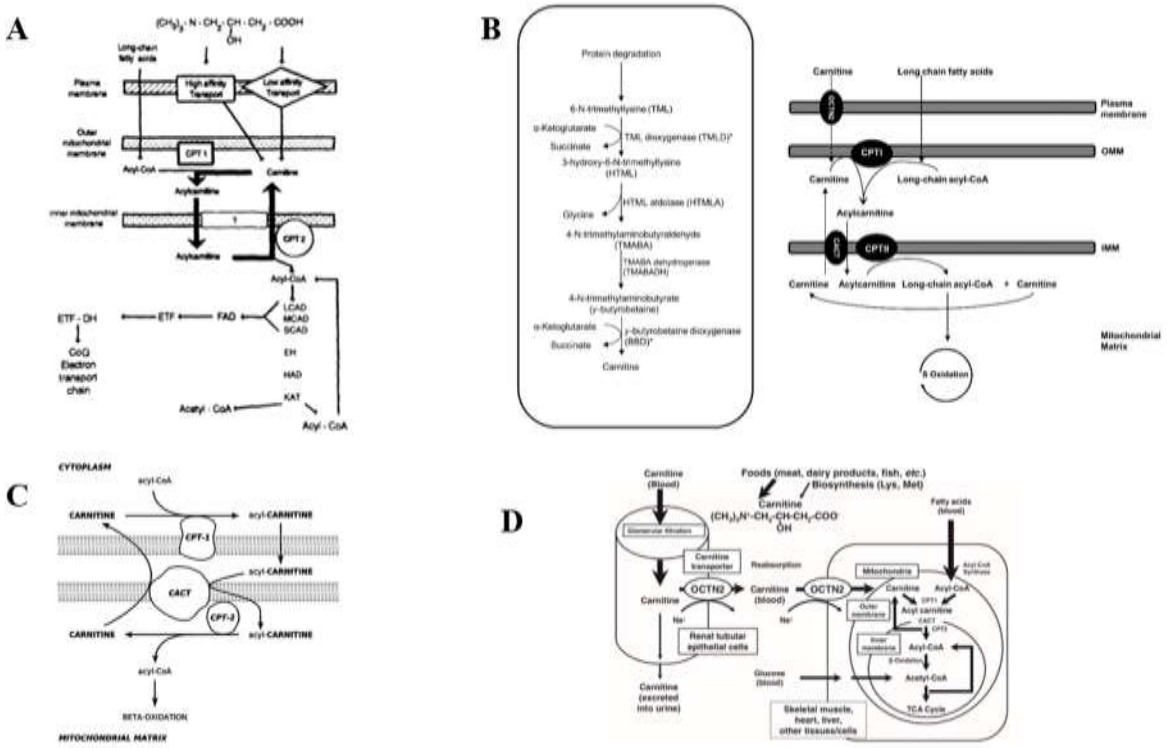

Figure 1. This figure depicts the carnitine shuttle's role in the pathway of mitochondrial β-oxidation. Panel A reveals the role of carnitine and the pathway of long-chain fatty acid (LCFA) oxidation within the mitochondria. Panel B provides a detailed view of carnitine biosynthesis, transport, and the mitochondrial carnitine-acylcarnitine cycle. It also notes that deficiencies in trimethyllysine dioxygenase (TMLD) and γ-butyrobetaine dioxygenase (BBD) have been reported and are denoted with asterisks. Panel C illustrates the process of carnitine transport from the cytosol to the mitochondrial matrix. In this process, carnitine binds cytosolic fatty acyl-CoA to form acylcarnitine, which is then transported into the mitochondrial matrix via the carnitine-acylcarnitine translocase (CACT) protein. Finally, Panel D provides a schematic illustration of the involvement of organic cation transporters (OCTNs) in carnitine disposition and the role of carnitine in beta-oxidation.

Note. A: Reprinted from "Carnitine Inborn Errors of Metabolism", by Almannai, Alfadhel and W. El-Hattab, M.A., M.A., A.W., 2019, Molecules, Volume 24 , no. 18: 3251, No special permission is required to reuse all or part of article published by MDPI, including figures and tables; B: Reprinted from "Hyperammonemic Encephalopathy Caused by Carnitine Deficiency", by Limketkai, B.N., Zucker, S.D., 2007, J GEN INTERN MED 23, p. 210–213, Order Number 501863403, Order Date Nov 23, 2023; C: Reprinted from "Pharmacological and pathophysiological roles of carnitine/organic cation transporters (OCTNs: SLC22A4, SLC22A5 and Slc22a21)", by Tamai, I. (2013). Biopharm. Drug Dispos, 34: 29-44. Copyright © 2012 John Wiley & Sons, Ltd, License Number 5674850134840, License date Nov 23, 2023.

causes of carnitine deficiencies in HE (79-81). The mutations are identified in genes named SLC22A4 (OCTN1) and SLC22A5 (OCTN2), which code for proteins that transport carnitine, resulting in aberrant liver uptake and accumulation (82-85). Reduced availability of carnitine in liver cells causes disordered mitochondrial β-oxidation of fatty acids, leading to energy deficits and metabolic abnormalities (86). Thus, an increased load of neurotoxic metabolites such as ammonia and short-chain fatty acids can result in cognitive impairment and impaired consciousness, which are hallmarks of HE (52, 69, 86, 87). Furthermore, clinical studies have revealed a clear association between lower levels of carnitines in serum and more severe symptoms, implying that it plays an important role in the prognosis or diagnosis of HE (88, 89). By understanding how these genes malfunction, resulting in abnormal levels of carnitines, it is possible to administer drugs specifically designed for individuals suffering from this condition, allowing them to regain normalcy and reduce their chances of developing hepatitis-related encephalopathy.

Carnitine deficiency prevents mitochondrial oxidation of fatty acids towards carbon dioxide throughout every tissue as well as ketones within the liver, resulting in lipid buildup in the cytosol (90, 91). Carnitine deficiency due to mutations impairs energy generation from long-chain fatty acids, particularly during fasting or stressful situations (90). Since skeletal and especially cardiac muscles rely on fatty acid oxidation for the majority of their energy, carnitine deficiency is expected to have the greatest impact on these tissues (30, 91). There are two types of primary carnitine deficiency syndromes: muscular and systemic. Through the study by Zhang et al. (92) in 2010, it was demonstrated that the myopathic form has significantly lower skeletal muscle carnitine concentrations but normal plasma plus liver levels, without evidence of renal carnitine leakage (92). Among two different studies conducted by Di Mauro et al.(93) and Pons et al. (94), the plasma acylcarnitine levels were normal, while there was no evidence of organic aciduria (93, 94). Although oral L-carnitine medication was beneficial in some patients, it did not restore muscular carnitine reserves (93, 94). Carnitine uptake was normal according cultured myoblasts of the individual suffering muscular carnitine insufficiency through a study performed by Mesmer et al. (95). The general therapy of carnitine insufficiency is based on the principles of avoiding fasting and excessive exercise, as well as dietary recommendations based on etiology. Carnitine deficit due to inadequate dietary intake, increased needs, excess losses, decreased synthesis, or (occasionally) enzyme deficiencies can be addressed by administering L-carnitine 25 mg/kg orally every 6 hours until the daily carnitine intake for adults is 150–500 mmol/day (96, 97).

Primary systemic carnitine deficiency (PSCD)

The start of clinical symptoms in PSCD spans from one month to seven years, through a variety of various presentation styles including progressive cardiomyopathy, myopathy as well as hypoketotic hypoglycemia encephalopathy. Every type of manifestation is possible depending on circumstances (98-100). Progressive cardiomyopathy seems the most frequent type and mainly affects elderly people. Myopathy, which manifests as hypotonia or gradually progressing proximal weakness, is frequently accompanied by encephalopathy or cardiomyopathy. Infants are more likely to develop acute encephalopathy linked with hypoketotic hypoglycemia. In contrast to myotonic primary carnitine deficit, the other type, the systemic carnitine deficiency, can be defined by reduced plasma carnitine concentrations (101-104). Carnitine levels are significantly lower in all burdened tissues (skeletal muscle, heart, liver) (101-106). Due to an issue with the renal carnitine transporter, people who suffer from systemic carnitine deficiency also have an important leak of carnitine from the kidneys (102-104, 107).

PSCD is caused by SLC22A5 gene mutations, which primarily encode the organic cation transporter 2 (OCTN2). The mutations clustering in exons 1 and 4, the c.760C>T (p. R254X), furthermore missense mutations c.34G>A [p.Gly12Ser] (108, 109), in addition to nonsense mutations such as Arg282ter nonsense (110). Additional compound heterozygous mutations include SLC22A5 mutations in c.1195C>T (inherited from his father) and c.517delC (inherited from his mother) (111), and other different mutations by various mechanisms such as c.288delG, c.495C>A, c.774_775insTCG, c.824+1G>A, and c.1418G> (112), .51C > G

(p.Phe17Leu) and c.760C > T (p.Arg254Ter) (113). Likewise, other types of mutations include substitution of asparagine 57, 64, and 91 with glutamine, protein kinase C-dependent (Ser-164, Ser-225, Ser-280, Ser-322 and Ser-323), protein kinase A-dependent phosphorylation (Ser-402), of arginine 169 with glutamine, proline or tryptophan, T219K and S225L (114), c.95A>G (p.N32S) mutation in SLC22A5 (115). (Table 1). Carnitine therapy for PSCDS substantially alleviated symptoms, though without fully replenishing tissue carnitine stores. This discovery generated the hypothesis that carnitine supplementation causes a transitory increase in plasma carnitine levels, enabling the restoration of function in the low-affinity transporter responsible for carnitine uptake (116). The cornerstone of treating primary carnitine insufficiency is the lifetime prescription of high-dose oral L-carnitine, often ranging from 100 to 200 mg/kg per day, split into three doses (82, 96). Maintenance treatment with L-carnitine can elevate plasma levels, where the dose is titrated based on both plasma levels and the response (96). The dose needs to be adjusted based on the amounts of free carnitine in the plasma. Carnitine is generally tolerated and causes not many side effects. High doses may cause diarrhea and stomach discomfort. The metabolism of bacteria in the colon can create trimethylamine, which smells fishy. This side effect could be mitigated by lowering the carnitine dose; alternatively, a course of treatment of oral metronidazole or otherwise probiotics may be recommended (80, 82, 96). Primary carnitine deficiency has favorable results and a fair prognosis if affected patients continue to take carnitine supplements (96).

Secondary carnitine deficiency

Secondary carnitine deficiency, also known as carnitine insufficiency, has been linked to a variety of inherited and acquired disorders, which is characterized by low tissue or plasma carnitine levels. Secondary carnitine deficiency is most commonly caused by metabolic diseases caused by improper oxidation of acyl-CoA metabolites inside the mitochondria (117, 118). Management strategies include preventing fasting, consuming food regularly, and administering nocturnal corn starch (96, 119). Dietary treatment should prioritize carbohydrates and medium-chain triglycerides (which require no carnitine shuttle) while limiting long-chain fatty acids (119). L-carnitine therapy is contentious in mitochondrial fatty acid oxidation diseases (120).

Mitochondrial carnitine-acylcarnitine cycle disorders

Carnitine is crucial for transporting fatty acids through the mitochondrial matrix to provide β-oxidation (121). Fatty acids become stimulated by long-chain acyl-CoA synthetase when they enter cells, producing them. Distinct-sized fatty acids have distinct long-chain acyl-CoA synthetases (121, 122). The mitochondrial carnitine–acylcarnitine cycle transports long-chain acyl-CoAs to the mitochondrial matrix after activation to penetrate the inner membrane of the mitochondria permeability barrier. CACT transports acylcarnitines inside the mitochondrial matrix in the second stage. Carnitine palmitoyltransferase II (CPT II) inside of the inner mitochondrial membrane transforms acylcarnitines to acyl-CoA as well as carnitine in the final step (123). These disorders include CAC deficiency and CPT 2 Deficiency (48) (Table 1).

CAC deficiency

CAC deficiency has been defined as a severe autosomal recessive and nonpopulation-specific disease with a male-to-female ratio of one (124). The heart, skeletal muscles, liver, and brain suffer the most. The disorder causes life-threatening comas during fasting (because of hypoglycemia, as the liver is unable to generate ketone bodies by fat even muscles using glucose), muscle weakness, cardiomyopathy, cardiac arrhythmia, in addition to abnormal liver function (125). Vomiting, lethargy, weakness, hypotonia, seizures, heart failure, respiratory distress, and hepatomegaly are other symptoms. In addition to hypoglycemia, metabolic changes in blood consist of acidosis, dicarboxylic aciduria, hypoketosis, hyperammonemia, increased long-chain acylcarnitines, reduced free carnitine, occasional hypocalcemia, as well as mildly increased liver enzyme and creatine kinase levels (125). Mutations in C.576G>A, c.106-2a>t, and c.576G>A (126), A homozygous C558T transition (127). The treatment involves avoiding meals and fasting frequently. The diet should be carbohydrate-rich and low in fat. MCT should account for the majority of the daily fat consumption. Carnitine is widely used, though it is controversial. Concerns have been raised regarding the potential toxicity of acylcarnitine buildup in long chain fatty acid oxidation diseases (96).

Table 1. Detailed examination of the general types of carnitine deficiency including the main group Primary carnitine deficiency syndromes (PCD) including two subgroups of diseases I. Primary systemic carnitine deficiency (PSCD) and II. Primary myopathic carnitine deficiency and the main group of Mitochondrial carnitine-acylcarnitine cycle disorders include subgroups of diseases I. CACT deficiency, I. CPT I deficiency and I. CPT II deficiency by separating enzymes, Carnitine Levels Changes (measurable forms of carnitine in different body tissues such as blood, muscles, liver, heart and skeletal), metabolic changes (such as hyperammonia, hypoketotic hypoglycemia, acidosis, aciduria and hypocalcemia and etc.), various forms of Genetic Mutations (by gene, chromosome, codon, amino acid, etc.), the main gene involved in the disease, clinical manifestations, and diagnosis with free carnitine and long-chain acylcarnitines (particularly C16 and C18:1) and C0/C16+C18 ratio, TML/γ, TML, butyrobetaine ratio, HTML and available treatments.

Syndrome	Enzymes	Carnitine Levels Changes	Metabolic Alteration	Genetic Mutations	Gene	Clinical Manifestations	Diagnostic	Treatment	References
Primary carnitine deficiency syndromes (PCD)									
Primary systemic carnitine deficiency (PSCD)	OCTN2 carnitine transporter	Reduced plasma carnitine, Lower, Reduced carnitine levels in burdened tissues (skeletal muscle, heart, liver), Renal carnitine leak	Hypoketotic hypoglycemia encephalopathy, Fasting-induced metabolic decompensations	Exons 1 and 4, the c.760C>T (p. R254X), c.34G>A [p.Gly12Ser], Arg282ter,c.1195C>T, c.517delC, c.288delG, c.495C>A, c.774_775insTCG, c.824+1G>A, c.1418G>, .51C > G (p.Phe17Leu), c.760C > T (p.Arg254Ter), .51C > G (p.Phe17Leu) c.760C > T (p.Arg254Ter), asparagine 57, 64, and 91 with glutamine, protein kinase C-dependent (Ser-164, Ser-225, Ser-280, Ser-322 and Ser-323), protein kinase A-dependent phosphorylation (Ser-402), of arginine 169 with glutamine, proline or tryptophan, T219K and S225L, c.95A>G (p.N32S) mutation in SLC22A5	*SLC22A5*	Myopathy, Progressive cardiomyopathy, Hypoketotic hypoglycemia encephalopathy, Fasting-induced metabolic decompensations accompanied by concurrent diseases, Asymptomatic	Low free carnitine	Carnitine supplementation	(80, 82, 84, 108-114, 189-195)
Primary myopathic carnitine deficiency	OCTN2 carnitine transporter	Lower skeletal muscle carnitine, Normal plasma and liver carnitine levels, Normal plasma acylcarnitine levels, No renal carnitine leak	No evidence of organic aciduria	Same as PSCD	*SLC22A5*	Skeletal or cardiac myopathy, Easy fatigability	Same as PSCD	Oral L-carnitine medication	(92-94)
Mitochondrial carnitine–acylcarnitine cycle disorders									
CACT deficiency	CACT	Reduced free carnitine, Occasional, Increased long-chain acylcarnitines	Hypoglycemia, Acidosis, Dicarboxylic aciduria, Hypoketosis, Hyperammonemia, hypocalcemia, Mildly increased liver enzyme, Creatine kinase levels	C.576G>A, c.106-2a>t, and c.576G>A, A homozygous C558T transition	*SLC25A20*	Life-threatening comas, Muscle weakness, Cardiomyopathy, Cardiac Arrhythmia, Abnormal liver function, Vomiting, Lethargy, weakness, Hypotonia, Seizures, Heart failure, Respiratory distress, Hepatomegaly	Increased long-chain acylcarnitines (particularly C16 and C18:1) Low free carnitine	High carbohydrate diet and low-fat Frequent feeding, avoidance of fasting Medium-chain triglycerides (MCT) Carnitine supplementation	(119, 125-127, 196-199)
CPT II deficiency	*CPT2*	Low total and free carnitine levels, High acylcarnitine: free carnitine ratios, Low free carnitine	Hypoketotic hypoglycemia	Exon 4, S113L, P50H, F448L, M214T, Y479F), S113L, p.F383Y, R503C, R124Stop	*CPT2*	Neonatal form: Liver failure, Hypoketotic hypoglycemia, Arrhythmias, Seizures, Cardiomyopathy, Dysmorphic features, Brain malformations, Renal Infantile form: Hepatomegaly, Hypoketotic, hypoglycemia, Liver failure, Arrhythmias, Cardiomyopathy Myopathic form: recurrent attacks of rhabdomyolysis	Low free carnitine Increased long-chain acylcarnitines (particularly C16 and C18:1)	High carbohydrate diet and low-fat Frequent feeding, avoidance of fasting MCT	(119, 134-138, 200-206)

**: means that there has been no evidence-based study so far.*
Abbreviations: OCTN2: Organic cation/carnitine transporter 2, SLC22A5: solute carrier family 22 member 5, CACT: Carnitine-acylcarnitine translocase, MCT: Medium-chain triglycerides, CPT I: Carnitine palmitoyltransferase I, CPT II: Carnitine palmitoyltransferase II, TM: trimethyl-lysine, TMLD: trimethyllysine dioxygenase, TMLHE: trimethyllysine hydroxylase deficiency, HTML: 3-hydroxy-trimethyl-lysine, BBD: butyrobetaine dioxygenase, BBOX1: gamma butyrobetaine hydroxylase

CPT 2 deficiency

Muscular CPT deficiency is the most prevalent and the most benign type of carnitine deficiency (128). The inactivation of inner mitochondrial CPT I1 has been demonstrated to be the underlying reason (129). A common symptom experienced by adults with the disease is episodes of weakness in the muscles brought on by intense and prolonged physical exertion, fever, or cold. Regardless of the significant muscular presentation, there is deficiency of an enzyme that is not limited to just muscle, though it might potentially be seen in various tissues (129). The signs of cardiomyopathy and hypoketotic hypoglycemia within severe infantile hepatomuscular type of CPT I1 deficiency imply pathologic involvement of additional organs (130). The CPT I1 gene is found on chromosomes 1 in humans (131). A single C →T transition was discovered in a patient who suffered from the severe infantile type of CPT I1 deficiency (Sl) (131), which leads to the substitution of an arginine+cysteine (129). This mutation appears to have no impact on the processing or synthesis of CPT 11, and that was found in normal size and levels in fibroblasts (129). The transfection of mutant CPT I1 cDNA toward cos-1 cells greatly lowered CPT I1 activity. Through a different severe CPT I1 deficiency individual, the reduction of protein biosynthesis was declared (132), and it was demonstrated that mutations in exon 4(133), S113L, P50H, and F448L and two novel mutations (M214T and Y479F) (134), S113L (135), p.F383Y (136), R503C (137), R124Stop (138) can cause CPT II. Regarding CACT deficiency, the treatment principles are the same as those that were mentioned earlier for CACT deficiency. It is important to avoid performing prolonged exercise as well as other known triggers (139, 140).

Genetic polymorphisms and susceptibility of carnitine to hepatic encephalopathy

The cellular mechanism and molecular pathogenesis of HE remains largely elusive and not fully understood. The activation of microglia cells by glutamine, ammonia, dysfunction of astrocytes, and neurotoxic agents leads to inflammatory signaling, disruption of brain homeostasis, neurodegeneration, and the onset of HE (141). Three main known factors contributing to HE are elevated levels of ammonia in the blood, systemic inflammation, and oxidative stress caused by multiple genetic alterations in the glutaminase gene (142). Neuroinflammation, permeabilization of the blood-brain barrier (BBB), swelling of astrocytes, elevated intracranial pressure, and cerebral herniation represent the principal pathological cerebral observations in more severe instances (143, 144).

The data documented in the one of studies provide evidence supporting the hypothesis that a genetic factor has an influence on the emergence of overt HE. This is indicated by the association between glutaminase activity and HE, the fluctuating prevalence of overt HE in cirrhosis patients, and the impact of particular polymorphisms in the glutaminase gene on protein activity (145, 146). An initial observation of an association between a microsatellite, which is a repetitive sequence of base pairs, in the promoter sequence of the GLS gene and the occurrence of HE was initially reported by Romero Gomes and colleagues in a cohort study (147). A genetic marker in the glutaminase gene's promoter region is associated with the advancement of HE in patients with severe liver dysfunction and when the long allele is present, it leads to a notable increase in enzyme activity. This genetic marker may help identify patients at risk for overt HE so that they can be monitored (147).

The intestinal phosphate-activated glutaminase (PAG), in individuals suffering from chronic liver disease, has been documented to be four times higher compared to those without this medical condition. This increased activity has been linked to the presence of HE (148). In a study conducted in 2023, researchers merged two transcriptomic data sets from brain tissues of patients with cirrhosis and HE. Through the utilization of an integrative bioinformatics approach, the study not only investigated DEGs, but also provided novel insights into the pathophysiology of HE. Further, the study identified potential therapeutic options for the treatment of HE. A combined analysis of the GSE57193 and GSE41919 data sets revealed upregulation of 274 genes and downregulation of 183 genes. Through the use of protein-protein interaction network analysis, a group of 12 hub genes were identified. These hub genes, namely EGFR, AQP4, BDNF, ERBB2, NTSR2, NTRK2, GFAP, PAX6, SLC1A2, RHOC, RHO, and PXN, were identified as important genes within the genetic network (149).

EGFR activation in astrocytes exposed to ammonia as a model for HE causes swelling of astrocytes (150). In the HE mice that are induced by azoxymethane, the activation of EGFR via p38 MAPK/NFκB pathway may play a role in the disruption the BBB and the advancement of cerebral edema (151). Mitochondrial disorders have a notable influence on a frequency of 1 in 5,000 live births and are intrinsically associated with the occurrence of multiple organ failure. The protein encoded by MICOS13 is an integral component of the mitochondrial contact site and cristae organizing system (MICOS) (152, 153). Varieties in MICOS13 induce mitochondrial HE (154). Every documented MICOS13 splicing variety resulted in frameshifts and the incorporation of untimely stop codons. In a study, a novel variation in MICOS13 was discovered in a patient with liver failure, cerebellar atrophy (HE), microcephaly, and pulmonary edema related to deficiencies in mitochondrial complex and depletion of mtDNA. Their findings strongly suggest a connection between MICOS13 and mtDNA maintenance in mitochondrial DNA depletion syndrome (MTDPS) (153).

Numerous studies have been conducted to clarify the molecular landmarks in the etiology of HE. As previously emphasized, the skeletal musculature possesses the ability to eliminate ammonia through the functioning of glutamine synthetase (GS). In typical situations, the significance of GS activity is minimal; however, in the context of HE, the expression and functionality of its corresponding gene are enhanced (155).

Molecular mediators play an important role in HE; increased gene expression of GS in the skeletal muscle is a molecular mediator in HE. Altered gene expression of MAO-A and Aquaporin IV also play a role. Additionally, elevated cerebral mRNA levels of eNOS in ALF and the Glutamate-NO-cGMP pathway contribute are involved along with affected gene expression of PTBR and the Neurosteroid system (pregnenolone, THDOC etc). Lastly, GABA-A receptor/ion channel GLUT-1, GLT-1, and GFAP in ALF are molecular mediators in this regard (156) (Fig. 2). Clinical Implications of Genetic Findings in Carnitine-Associated Hepatic Encephalopathy

The cellular mechanism and molecular pathogenesis of HE remain largely elusive and not fully understood. The activation of microglia cells by glutamine, ammonia, dysfunction of astrocytes, and neurotoxic agents lead to inflammatory signaling, disruption of brain homeostasis, neurodegeneration, and the onset of HE (141). Three main known factors contributing to HE are elevated levels of ammonia in the blood, systemic inflammation, and oxidative stress caused by multiple genetic alterations in the glutaminase gene (142). Neuroinflammation, permeabilization of the BBB, swelling of astrocytes, elevated intracranial pressure, and cerebral herniation represent the principal pathological cerebral observations in more severe instances (143, 144).

In clinical trials, acetyl-L-carnitine showed promise in lowering ammonia levels and improving cognitive abilities in patients with HE (157, 158). The main reason for using carnitine supplements is that acetyl-L-carnitine reduces ammonia in blood and brain by crossing the BBB (159-162). It boosts acetylcholine esterase synthesis, potentially aiding dementia treatment (163). Acetyl-L-carnitine is believed to benefit HE symptoms by providing neuronal energy and diminishing ammonia levels through urea synthesis (164). Also, the result of a previous study demonstrated that the administration of L-carnitine helped reduce the impact of NH4Cl on astrocytes. This suggests that supplementing with L-CA can result in an antioxidant effect in astrocytes experiencing hyperammonemia (165).

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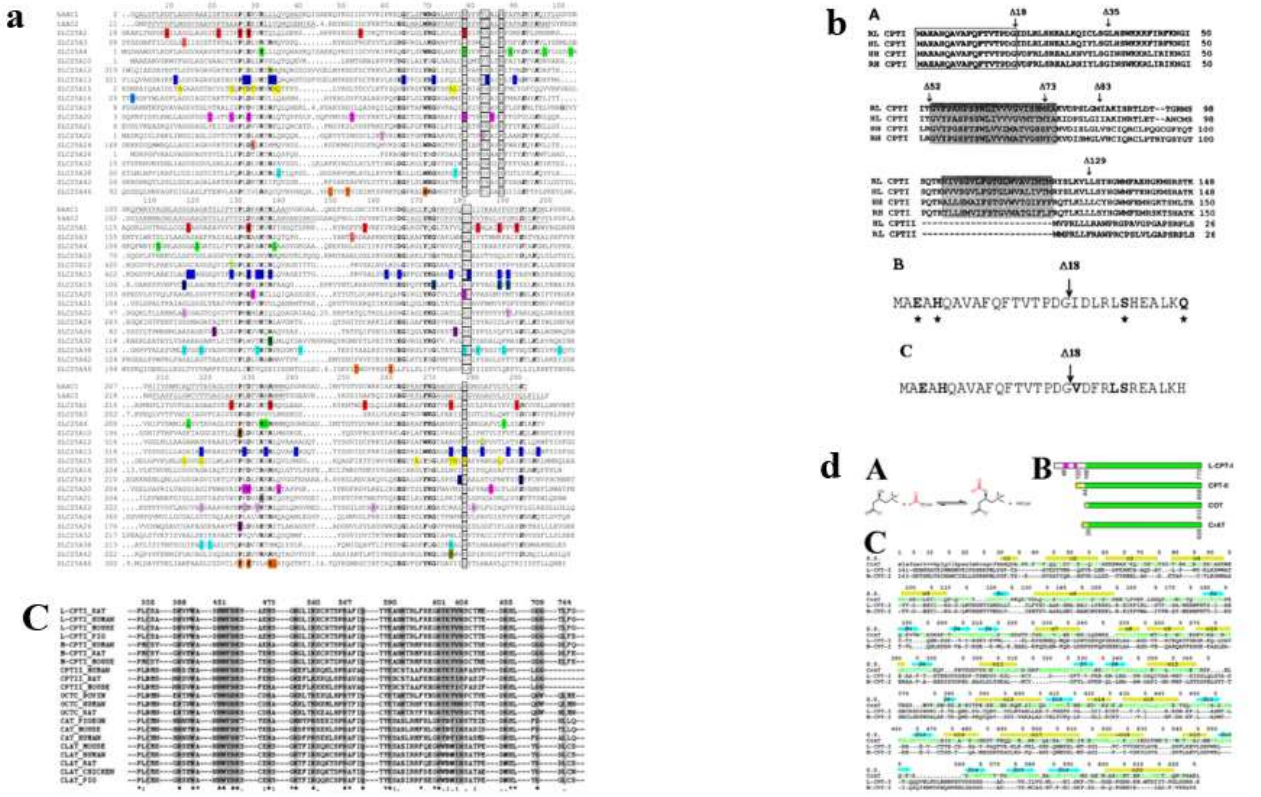

Figure 2. A comparison of the amino acid sequences of mitochondrial carriers is shown in this figure. The alignment highlights the locations of mutations that cause diseases. The shaded areas in (a) depict the positions of two membrane-spanning domains in CPTIs, which are present in both human and rat liver CPTs; (b) displays the sequence of the first 150 N-terminal residues, with arrows indicating the positions of deletion mutants. Finally, (c) shows the first 30 N-terminal residues of rat L-CPTI and human heart M-CPTI. The text describes the sequence alignment of specific portions of the C-terminal region present in various acyltransferases, with a focus on carnitine acyltransferases. The reaction catalyzed by the carnitine acyltransferases is presented in (A), while (B) presents the domain organization of L-CPT-I, CPT-II, CrOT, and CrAT, where the catalytic domains are highlighted in green, the two transmembrane segments of L-CPT-I in magenta, and the mitochondrial targeting sequences of CPT-II and CrAT in yellow. Finally, (C) outlines a sequence alignment of mouse CrAT and human liver- and muscle-type carnitine palmitoyltransferase I (L-CPT-I and M-CPT-I).

Note. A: Reprinted from "Diseases Caused by Mutations in Mitochondrial Carrier Genes SLC25: A Review", by Palmieri F, Scarcia P, Monné M., Biomolecules. 2020 Apr 23;10(4):655, Volume 10, No special permission is required to reuse all or part of article published by MDPI, including figures and tables; B: Reprinted from "Structure-Function Studies with the Mitochondrial Carnitine Palmitoyltransferases I and II.", by Woldegiorgis, G., Dai, J. & Arvidson, D., 2005, Monatsh. Chem. 136, 1325–1340, Order Number 501863409, Order Date Nov 23, 2023. C: Reprinted from "Structure and Function of Carnitine Acyltransferases", by JOGL, G., HSIAO, Y.-S. and TONG, L., 2004, Annals of the New York Academy of Sciences, 1033: 17-29, License Number 5674850528574 License date Nov 23, 2023.

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Therapeutic options of carnitine-associated hepatic encephalopathy

For better treatment of HE patients, these patients are classified based on 4 factors: underlying disease, degree of disease manifestation (confusion, changes in sleep and wakefulness patterns, incoherent speech, behavioral changes, lethargy and coma, (exacerbating factors, and period) epizootically, two or six months or less). Typically, three types of underlying diseases are seen in these patients: acute liver failure, cirrhosis as a result of portal hypertension, and portal shunt (166). By examining the pathology of liver cirrhosis, which causes HE, it can be understood that one of the causes of this disease is the lack of carnitine, which is mostly received naturally through food. Carnitine is present throughout the cell membrane as a part of the shuttle mechanism, in which long-chain fatty acids are converted into L-acetylcarnitine, and carnitine acetyltransferase and carnitine are involved in the following process: acetyl coenzyme A + carnitine = acetylcarnitine + coenzyme A. This reaction regulates the concentration of coenzyme A and acetyl CoA inside the cell (167). Acetyl-L-carnitine is a short-chain carnitine ester produced in cells such as peroxisomes and mitochondria, which plays a role in the transfer of acetyl-moieties in the membrane of these organelles. Therefore, the administration of acetyl-L-carnitine for patients with HE can improve mental and neurological activities, such as focusing on short-term memory, computational abilities, scanning, and visual tracking. Basically, acetyl-L-carnitine reduces ammonia through ureagenesis and prevents the side effects of ammonia (14) (Figure 3).

There is evidence that L-Carnitine lowers ammonia levels in blood and the brain as well as improves HE (168-171). The acyl-coenzyme A that L-carnitine transfers to the mitochondria is activated by the tricarboxylic cycle where ureagenesis is induced, which reduces the levels of ammonia (172). Oral L-carnitine supplementation was found to be beneficial in lowering blood ammonia concentrations and improving HE in patients with mild to moderate HE (168, 170, 173, 174). In addition, it was discovered that oral L-carnitine diminished blood ammonia levels in HCC patients (175). Hence, even in patients with cirrhosis who also have HCC, oral L-carnitine supplementation may be effective at improving and preventing HE (176). These results implied that L-carnitine had a direct and potent effect on HE. Furthermore, no significant side effects were noted in these investigations after taking L-carnitine, independent of the method of administration (168-171, 173, 174). New studies suggest that around 25% of the carnitine required by the body is produced in different organs; among the organs, the role of the liver in carnitine production is more important. Therefore, liver cirrhosis patients with liver cell dysfunction have a higher chance of carnitine deficiency (5). The studies of Abbasnezhad et al. on

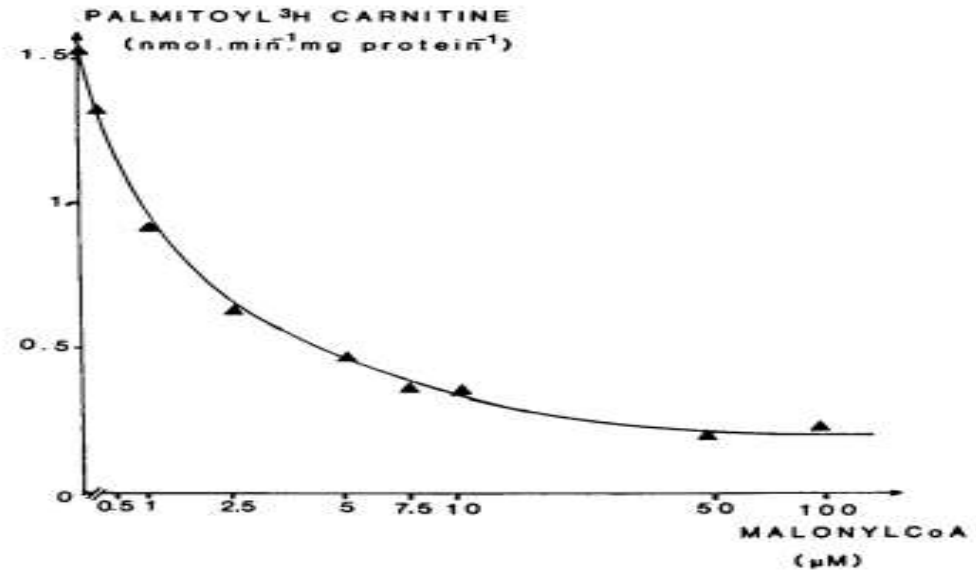

Figure 3. In this figure, the impact of malonyl CoA on CPT (carnitine palmitoyltransferase) activity is illustrated using assay A. The experiment involved homogenized fibroblasts that were preincubated with and without malonyl CoA for a period of 2.5 minutes. The reaction was then initiated by adding L-~Hcarnitine to the mixture, allowing the researchers to observe and measure the effect of malonyl CoA on CPT activity.

Note. Reprinted from "Hepatic and muscular presentations of carnitine palmitoyl transferase deficiency", by Demaugre, F., Bonnefont, J.P., Mitchell, G., Nguyen-Hoang, N., Pelet, A., Rimoldi, M., Donato, S.D. and Saudubray, J.M., 1988, two distinct entities. Pediatric research, 24(3), pp.308-311. Order Number 501863405, Order Date Nov 23, 2023.

liver cirrhosis patients revealed that the use of L-carnitine in these patients lowered the ammonia level, the bilirubin level, the blood level of aspartate aminotransferase without a significant effect on alanine aminotransferase, increased albumin, and eventually caused a decline in blood urea nitrogen and creatinine. Therefore, the treatment of liver cirrhosis through L-carnitine can help prevent HE complications (177).

Besides removing the precipitating factors, including infection and variceal hemorrhage, nonabsorbable disaccharides such as lactulose or non-absorbable antibiotics are also used to treat OHE (178, 179). Drug therapies are thought of as first-line therapies, with liver transplantation coming in second, if required (178, 179). Lactulose, a type of non-absorbable disaccharide, lowers blood ammonia levels by lowering the quantity of ammoniagenic bacteria and by converting ammonia into a nonabsorbable ammonium nitrate. Current meta-analyses have demonstrated that these treatments have a positive impact on HE, with a risk ratio (RR) of 0.58, as well as contribute to increased survival, with a RR of 0.59 (180, 181). The f nonabsorbable antibiotics like rifaximin are also thought to be effective in patients with HE (182, 183), and are considered to be an alternative to first line therapy for individuals with OHE (178).

Branching-chain amino acid (BCAA) supplementation has been shown to have therapeutic effects in individuals with OHE (RR, 0.73), according to a recent meta-analysis of 16 randomized controlled studies (184). Meanwhile, there have been negative reports about the consequences of BCAA supplementation, such as increased ammonia production and cataplerosis (185). Furthermore, BCAA supplementation was shown to have differing effects on disturbed consciousness depending on liver function (186). Despite the lack of major adverse effects, BCAA supplementation can be effective on treating patients with HE. However, further research is needed to establish its effectiveness (187).

A study in 2013 on 1012 genes of brain cells of liver cirrhosis patients with and without HE indicated that the level of gene expression in the cerebral cortex of cirrhotic patients with HE changed by about 1.5%. These genes are actually related to oxidative stress genes, microglia activation, receptor signaling, inflammatory pathways, cell proliferation and

apoptosis, etc. (188). Some research shows that a genetic network is probably related to HE disease; the examples include epidermal growth factor receptor (EGFR), rb-b2 receptor tyrosine kinase 2 (ERBB2), brain-derived neurotrophic factor (BDNF), glial fibrillary acidic protein (GFAP), solute carrier family 1 member 2 (SLC1A2), aquaporin 4 (AQP4), neurotrophic receptor tyrosine kinase 2 (NTRK2), Ras homolog family member C (RHOC), neurotensin receptor 2 (NTSR2), rhodopsin (RHO), paxillin (PXN) and paired box6 (PAX6). Although the exact genetic mechanism of HE is not known, future research can find new ways to treat these patients (149).

Conclusion

In general, HE and its etiology were reviewed. According to the latest research, the pathogenesis of HE, which is based on the factors of hyperammonemia, oxidative stress by altered glutaminase gene expression and inflammation, were discussed in this study, along with the effective genes discovered in this disease. By analyzing the pathophysiology of liver cirrhosis, a condition that leads to HE, one can comprehend that the deficiency of carnitine is one of the contributing factors to this disorder. Carnitine as a major cellular factor to prevent hyperammonemia was discussed in detail. Genetic disorders that lead to a decline in the amount of carnitine in the body or its dysfunction include Primary systemic carnitine deficiency, Secondary carnitine deficiency, CAC deficiency, CPT I Deficiency, and CPT 2 Deficiency. These disorders manifest with different types of symptoms. The use of L-carnitine supplement increases the detoxification power caused by the increase of ammonia in cirrhotic patients. The utilization of acetyl-L-carnitine for individuals suffering from HE has the potential to enhance cognitive and neurological functions. There are also genetic defects in the ammonia detoxification path, which leads to hyperammonemia, which is of different types such as mitochondrial, cytosolic, etc. They can be mentioned as genetic defects in mitochondrial enzymes such as carbamoylphosphate synthetase 1 deficiency, N-acetylglutamate synthase deficiency, and ornithine transcarbamylase deficiency. The importance of diagnosing genetic defects related to prenatal and maternal cases was discussed. Despite its rarity, carnitine acylcarnitine translocase deficiency is a genetic defect that due to various reasons (including death in infancy) should be diagnosed quickly with genetic tests. The novelty of this paper can be summarized as follows: Detailed molecular and genetic insight into the pathophysiology of this disease as well as a set of new therapeutic strategies related to targeting genetic pathways, which will be further explored in the future; different treatment options based on carnitine in HE. Further comprehensive research is still needed for quick diagnosis and effective treatments to become commonplace and implemented. It is also better to conduct future research on the removal of ammonia or its non-production to prevent toxicity; one of the most important ways of which is to remove ammonia by glutamine synthetase around the portal vein.

Conflict of interests

There is no conflict of interest for authors of this article.

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