New insights into carnitine-acylcarnitine translocase deficiency from twenty-three cases: management challenges and potential therapeutic approaches.

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Summary

Background

Carnitine acyl-carnitine translocase deficiency (CACTD) is a rare autosomal recessive disorder of mitochondrial long-chain fatty-acid transport. Most patients present in the first two days of life, with hypoketotic hypoglycaemia, hyperammonaemia, cardiomyopathy or arrhythmia, hepatomegaly and elevated liver enzymes.

Method

Multi-centre international retrospective chart review of clinical presentation, biochemistry, treatment modalities including diet, subsequent complications, and mode of death of all patients.

Results

Twenty-three patients from nine tertiary metabolic units were identified. Seven attenuated patients of Pakistani heritage, 6 of these homozygous c.82G>T, had later onset manifestations and long-term survival without chronic hyperammonemia. Of the sixteen classical cases, fifteen had cardiac involvement at presentation comprising cardiac arrhythmias (9/15), cardiac arrest (7/15) and cardiac hypertrophy (9/15). Where recorded, ammonia levels were elevated in all but one severe case (13/14 measured) and 14/16 had hypoglycaemia. Nine classical patients survived longer-term – most with feeding difficulties and cognitive delay. Hyperammonaemia appears refractory to ammonia scavenger treatment and carnitine, but responds well to high glucose delivery during acute metabolic crises. High-energy intake seems necessary to prevent decompensation. Anaplerosis utilizing therapeutic D,L-3-hydroxybutyrate, Triheptanoin and increased protein intake, appeared to improve chronic hyperammonemia and metabolic stability where trialled in individual cases.

Conclusion

CACTD is a rare disorder of fatty acid oxidation with a preponderance to severe cardiac dysfunction. Long-term survival is possible in classical early-onset cases with long-chain fat restriction, judicious use of glucose infusions, and medium chain triglyceride supplementation. Adjunctive therapies supporting anaplerosis may improve longer-term outcomes.

Short title – Treatment of CACTD

Synopsis CACTD is typically a rare severe fat oxidation disorder presenting with hyperammonaemia, hypoglycaemia and cardiac dysfunction, which can sometimes be treated with high glucose delivery and anaplerotic agents.

Contributions of Authors

Ryder B Study design, data collection, analyses, writing all drafts

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Competing Interests – Dr Inbar-Feigenberg received compassionate access to medication (Triheptanoin) from Ultragenyx for one patient. All other authors declare no conflict of interest.

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Ethical statement – Over arching ethics was performed at The Hospital for Sick Children, Toronto, Canada. Patients were consented to provide case report data per local practices.

Key words

Carnitine acyl-carnitine translocase deficiency, carnitine shuttle, hyperammonaemia, ketones, Triheptanoin, anaplerosis

INTRODUCTION

Carnitine-acylcarnitine translocase deficiency (CACTD) (OMIM#212138) is a rare autosomal recessive disorder of fatty acid oxidation (FAO) ¹. The incidence in Caucasian populations is estimated at 1:750,000-1:2,000,000 increasing to 1:60,000 in Hong Kong ². CACT is a component of the carnitine shuttle, essential for import of long chain fatty acids into the mitochondria matrix for beta-oxidation³. CACTD results in an inability to produce energy from long chain fatty acid metabolism and a proposed deficiency of free Coenzyme A (CoA) for essential reactions such as the tricarboxylic acid (TCA) cycle and oxidation of pyruvate. Symptoms of CACTD are purported to arise from the deficiency of acetyl CoA, and the toxic effects of accumulating long-chain acylcarnitine moieties ^{4,5}.

Clinically CACTD resembles the other severe long chain FAO disorders. Patients most commonly present in the first two days of life with hypoketotic hypoglycaemia, cardiomyopathy or arrhythmias, hepatomegaly with raised liver transaminases and elevated creatine kinase (CK). Hyperammonemia is a prominent feature and may lead to suspicion of a urea cycle disorder or organic acidemia. CACTD has the highest rate of cardiac arrhythmia and mortality amongst fatty acid oxidation disorders. ²

An acylcarnitine profile showing elevations most pronounced in C16, C18 and C18:1 acylcarnitine species suggests either CACT or CPTII deficiency (OMIM#608836). These disorders are indistinguishable on acylcarnitine profile therefore diagnosis is confirmed by

enzyme activity level or molecular analysis⁶. Urine organic acids may show a dicarboxylic aciduria (DCA) ⁵.

Treatment of CACTD like other severe long-chain FAO disorder's, consists generally of a diet normal in energy and protein, high in carbohydrate, low in long-chain triglycerides (LCT), and supplemented with ketogenic medium chain triglycerides (MCT)^{5,7,8}. Lipolysis is inhibited by avoidance of fasting and the use of high carbohydrate fluids during illness ^{3,9}. Accumulation of toxic long-chain acylcarnitines is limited by restriction of dietary long-chain fat to less than 10% of total caloric intake ^{1,8,10,11}. Carnitine is generally administered, doses ranging from 50-300mg/kg/day ^{1,4,8}.

Only 68 cases of CACTD have been reported in the literature. This report aims to further characterize this rare disease by describing the clinical, biochemical, molecular findings and treatment of 23 patients with CACTD.

METHOD

The study was approved by the Institutional Research Ethics Board of The Hospital for Sick Children, Toronto. Local consent processes were followed for review and publication of de-identified patient data. There were no exclusion criteria. This is a retrospective review of 23 cases of CACTD from metabolic centres in New Zealand, Australia, Canada, UK and Hong Kong (four previously reported). All patients with available data and a diagnosis of CACTD confirmed by CACT enzyme activity and/or molecular analysis were included in the study.

RESULTS

Individual case reports are available for all cases as online appendix 1.

Classical

16/23 cases had severe classical disease (Table 1) defined as a catastrophic collapse within 48 hours of birth (10-48 hrs). One was deemed classical despite presenting on day eight of life, being homozygous for variant c.199-10T>G which is associated with a severe phenotype in six patients in this cohort and other publications ^{2,10}. One was treated pre-symptomatically. Only 1 classical untreated case did not manifest hypoglycaemia.

Severe neonatal cardiac dysfunction was demonstrated in 2/16 at presentation, one with fractional shortening 11-16% (normal 28 – 45%) and another requiring inotrope support post cardiac arrest, until care was withdrawn. 4/16 had normal echocardiograms, 10/16 had arrhythmias at presentation, predominantly supraventricular and ventricular tachycardia, seven of these presenting with cardiac arrest requiring cardiopulmonary resuscitation (CPR) (Table 1). Of those with a normal echo at presentation all but one remained normal at follow up. Of eleven classical patients who survived beyond one year, , there was generally a trend for improvement: five had normal function, four had borderline to moderate LVH without cardiac dysfunction. One developed dilated cardiomyopathy at 3 years of age, and one had hypertrophic cardiomyopathy.

Hyperammonaemia was noted in 14/16 classical patients at presentation (range 50-1142 umol/, including one patient treated pre-symptomatically) and was not measured in the remaining two. All but one had chronic recurrent hyperammonemia despite intensive therapy. The patient that had no chronic hyperammonaemia (patient 10) took more total energy and protein than the other cases and used D,L-3-hydroxybutyrate during intercurrent illness (table 2). Seven patients had no sustained improvement on ammonia scavenging medications. Carglumic acid was

trialled in two without clear benefit. All responded well in the acute setting to high rates of dextrose administration (10-12 mg/kg/min). One patient had improvement in hyperammonemia after starting D,L-3-hydroxybutyrate and Triheptanoin (C7) soon thereafter.

Creatine Kinase levels were raised in 13/14 measured, up to >25,000 U/L 9/16 had evidence of acute renal injury at presentation, reflected in raised urea or creatinine. One had renal Fanconi syndrome in the neonatal period and two developed proximal renal tubular acidosis later in life. 3/16 developed unexplained chronic, severe diarrhoea improving with metronidazole in one patient. All surviving classical patients had liver echogenicity consistent with steatohepatitis or hepatomegaly. Two developed gallstones at 2 and 4 years of age and one had liver fibrosis at 10 years. No patients developed pancreatitis.

6/16 were deceased, two in the first week and the remainder aged 6 months-4 years. The surviving 10 classical patients range in age from 5 months to 11 years with normal neurocognitive outcome reported in 4/9 over one year of age. Formal neurocognitive assessments were only available in two patients.

Attenuated

7/23 cases were attenuated (Table 3), with confirmed homozygosity for variant c.82G> T (p.Gly28Cys), hereafter referred to as “attenuated variant”, in 6/7. These patients were all of Pakistani descent. 3/7 were treated pre-symptomatically. 4/7 presented clinically, between 9 days and 4 months of age.

Five had no evidence of cardiomyopathy on echocardiogram at presentation and remained normal at follow up, one had mild left ventricular hyper trabeculation and one presented at two months of age with asystolic arrest requiring CPR and echocardiogram findings of LVH

and impaired contractility. Longer term this patient had persistent hypertrophic cardiomyopathy. Only 1/7 attenuated case had hyperammonaemia at presentation and none had chronic hyperammonaemia.

All 7 attenuated patients are living. Five are felt to have normal neurocognitive function, without formal neurocognitive assessment, and two require some learning support in mainstream schooling.

DISCUSSION

Prior to this cohort of twenty-three patients just 68 CACTD cases have been reported, with few patients surviving into their second decade. Four patients in this cohort have been reported before¹²⁻¹⁴. The majority of cases present prior to a NBS result, often with ventricular arrhythmia and cardiorespiratory arrest. NBS may therefore expedite the diagnosis but does not generally allow pre-symptomatic treatment in classical cases.

Hyperammonaemia is both a presenting feature and chronic long-term management issue in classical CACTD^{1,5,15-17}, occurring in more than 50%¹¹. The hyperammonaemia is not well understood and there are sparse real time data during acute decompensation. Secondary hyperammonaemia may occur due to inhibition of ureagenesis by either toxic metabolites or substrate deficiency. Quantifiable urea cycle intermediates were not identified in the original report of CACTD¹, hence it was proposed that hyperammonaemia occurred due to insufficiency in the proximal part of the urea cycle. One of our cases had quantifiable orotate possibly indicating deficiency distal to carbamoyl-phosphate. Stanley et al concluded that

insufficient acetyl Co-A for synthesis of N-acetylglutamate was unlikely to be the cause of hyperammonaemia, given its persistence despite MCT supplementation. Consistent with this, is the inadequate response to carglumic acid reported in the literature and observed in our cohort (n=1). An improvement in hyperammonaemia was noted during a course of metronidazole for treatment of *Clostridium difficile* in two patients, leading to regular cycling of monthly metronidazole in patient 7. The presumptive mechanism would be clearance of bacteria which produce propionyl-CoA in the gut however a lack of response to carglumic acid would argue against the possibility of propionyl CoA inhibiting NAGS (OMIM #237310). The nitrogen efflux from mitochondria into the cytosol, to be incorporated into urea, is dependant on either glutamate or aspartate^{18,19}. Glutamate dehydrogenase oxidation of glutamate to 2-ketoglutarate and ammonia is favoured when cellular energy is low, GDH being activated to maintain energy generation through the oxidation of amino acids¹⁸. Of note, high mitochondrial GTP, ATP or palmitoyl-CoA concentrations would inhibit this process¹⁸. Aspartate efflux from the mitochondrion is also dependant on the TCA intermediate oxaloacetate¹⁹. Hence, in the setting of acute energy deficit, the provision of anaplerotic agents is likely to enhance ammonia incorporation into urea (Figure 1). It has been proposed that energy deficit may be part of the reason for hyperammonaemia in organic acidaemias although short chain fatty acid inhibition of NAGS also occurs²⁰. ATP-dependent cytosolic glutamine synthase (E.C. 6.3.1.2) is another major route of ammonia detoxification, producing glutamine from glutamate and ammonia²¹. The provision of glutamate or its precursors, is thought to stimulate GS, thereby enhancing ammonia removal by increased synthesis of glutamine. Hyperammonaemia in CACTD is not associated with elevated glutamine, perhaps due to GS downregulation at times of severe energy deficit.

Accepted Article

Aside from the response to glucose, hyperammonaemia in this cohort of patients appears to have responded to sodium D,L-3-hydroxybutyrate and an increase in dietary protein, which would provide anaplerosis^{13,22}. Triheptanoin is proposed to provide superior anaplerosis because heptanoyl CoA is metabolised to both acetyl- and propionyl-CoA as well as 4 and 5 carbon ketone bodies. The provision of both even and odd chain carbon intermediates to the TCA cycle is proposed to result in improved balance of the TCA cycle however the evidence for its use in CACTD remains limited. The safety and long-term efficacy of triheptanoin has been described in three infants with severe cardiogenic shock unresponsive to standard treatment^{22,23}. Within our cohort one patient was felt to be clinically more stable on triheptanoin, but this was introduced shortly after sodium D,L-3-hydroxybutyrate and liver enzymes, CK and ammonia levels, and cardiac function had normalised prior to the introduction of triheptanoin. Patient 1 developed raised CK levels after starting triheptanoin at 34 months of age therefore triheptanoin was stopped. He then developed dilated cardiomyopathy, dying of cardiac failure at 42 months of age.

Chronic hyperammonaemia was a prominent feature in 9 classical patients surviving infancy. High rates (10-12 mg/kg/min) of IV dextrose was the most effective acute management and a minimum dietary carbohydrate intake seemed necessary to prevent chronic recurrent hyperammonaemia^{8,24}. While a high dietary carbohydrate intake is effective, the cost longer term was excessive weight gain, hepatomegaly and steatohepatitis. The metabolic consequences of impaired long-chain fatty acid transport into the mitochondria, is enhanced de novo cytosolic lipogenesis²⁵. This leads to hepatic steatosis that is further exacerbated if chronically excessive carbohydrate or medium chain triglyceride is given^{26,27}. 10/14 severe patients surviving infancy had evidence of steatohepatitis, 2/14 with gallstones and 1/14

developed liver fibrosis by 10 years of age. The hyperammonaemia in this patient (Case 8) appeared less responsive to acute carbohydrate administration, possibly related to the reduction in GS expression in patients with liver cirrhosis ²³. This has led to consideration of therapeutic liver transplant.

Ammonia scavenging medications such as sodium benzoate and sodium phenylbutyrate have a limited effect ²⁸. When started concurrently with dextrose it is difficult to attribute effect, as hyperammonaemia generally responds promptly to high rates of intravenous glucose independent of medication use. ¹⁶ Within this cohort there was an inadequate response to ammonia scavengers and no response to protein restriction. In fact, perhaps via induction of urea cycle enzymes or by anaplerotic effect, greater metabolic stability (cases 10 and 12) and improved hyperammonemia (cases 8,10,12 and 16) was observed with higher protein intake (2 – 4 g/kg/day) with a reduction in number of hospital admissions ²⁹. Furthermore, complications related to steatosis are less likely with higher protein intake compared to high MCT or carbohydrate intake ³⁰.

This cohort highlights renal involvement in CACTD. Steatosis of the renal tubules, particularly proximal tubules, is found on post mortem ^{7,31-34}. All forms of renal involvement, as observed in this cohort, have been previously reported; renal tubulopathy, hyperkalaemia and oliguric renal failure with acute tubular necrosis ^{11,32,35}.

Reported GI disturbance consists of feeding difficulties, gastrostomy tube dependence, gastroesophageal reflux, impaired motility, recurrent vomiting and diarrhoea ^{1,4,16,36}. Within this cohort, feeding difficulties and vomiting were frequently observed. Unexplained severe chronic diarrhoea was noted in three, with improvement in diarrhoea and hyperammonemia

in one patient who received metronidazole. Prescribed feeding regimes with excessive oligosaccharides and MCT oil may contribute to osmotic diarrhoea and modify gut flora longer term. Pancreatitis has been observed in a case of CACTD (personal communication), but was not seen in our cohort.

CACTD is characterised by a high-esterified fraction of acylcarnitines (>90%, normal <30%) and extremely low free carnitine levels, often as low as 1-2 mmol/l¹. It has been proposed that high dose carnitine may worsen the accumulation of potentially cardiotoxic long-chain acylcarnitines³⁷. Cumulative experience in CACTD at L-carnitine doses ranging from 50-300mg/kg/day suggests supplementation does not lead to cardiotoxicity^{5,8,16,36}. Esterification with carnitine may be the only route of detoxification of toxic acyl-CoA intermediates⁸. It is possible that the acylcarnitines accumulating in CACTD may be less toxic outside the mitochondrial matrix⁷. Within this cohort, patients received up to 200mg/kg/day L-carnitine but generally did not achieve normal free carnitine levels. Accumulating acylcarnitines may inhibit reabsorption of free carnitine by the carnitine transporter, resulting in renal carnitine wastage¹.

The majority of reported patients receive q3-4 hourly diurnal feeds and continuous overnight feeds, to limit fasting and provide adequate calories. Clinical deterioration has been noted with fasting intervals greater than four hours in neonates with a severe presentation³⁸. The fasting limitations recommended for severe patients within this cohort ranged from three hours to eight hours. Clinical deterioration was observed in two classical patients when fasting intervals were extended to twelve and eight hours, with improvement when fasting duration was reduced. Based on these findings, extended fasts would not be recommended in classical CACTD but the optimal fasting duration at different ages has not been defined.

CACTD patients have been demonstrated to generate ketones on administration of MCT formula with only partial utilization of C10 MCT^{39,40}. Treatment with MCT formula aims to bypass the translocase defect however fatty acyl moieties of C10 length or longer account for 20-50% of total fatty acids in most commercially available MCT formulae and C10 fatty acids are also dependent on the carnitine shuttle. Approximately 40% of administered MCT could be inadequately oxidized in patients, so a formula containing less C10 and C12 MCT would therefore seem preferable. Some centres prefer a fat free formula with replenishment of MCT. Under-utilisation of C10 accounts for excessive energy requirement in CACTD. Generation of beta-hydroxybutyrate in CACTD on MCT loading can be less than 50% of that in VLCADD (MIM#201475)⁸. The exogenous ketone sodium D,L-3-hydroxybutyrate was given in 7 severe cases (patients 7-10, 12,13,16), as an assured source of ketones, with variable effect. Doses ranged from 300-900mg/kg/day. Patient 10 presented with a profound neonatal cardiac dysfunction, which appeared to respond dramatically to exogenous ketones, similar to that described in multiple acyl-CoA dehydrogenase deficiency⁴¹⁻⁴³. Patient 9 had improved metabolic stability after starting ketones and Triheptanoin (C7) at a similar time point, therefore it was difficult to know which intervention led to clinical improvement.

There is significant genetic heterogeneity in *SLC25A20*, and phenotype-genotype correlations are limited by numerous private mutations. The c.199-10T>G splice site variant is the most common mutation identified⁴⁴. This variant appears to be the reason for the increased incidence of CACTD in the Chinese population. The variant is associated with a severe presentation, frequently with cardiac arrest, within the first 3 days of life^{2,45}. 2/5 homozygous patients in this cohort died suddenly and unexpectedly of cardiac causes at 6 months and 3 years of age, while the other 3/5 homozygous are living, aged 5 months-4 years. One patient

heterozygous for this variant is alive with borderline LVH. Variant p.Gly28Cys, c.82G>T was identified in six patients in this cohort, and appears to be associated with Pakistani ethnicity and an attenuated phenotype^{11,31}; although was more severe in 2 compound heterozygotes in this cohort.

This retrospective case review faced several constraints. Data was incomplete for some cases; treatment, particularly dietetic management, naturally changed over time in response to the patient's clinical condition and some interventions were made concurrently. This prevented definitive conclusions being made regarding treatment effect. Trends for improvement are instead noted and the need for further research and improved collaborative registry based data collection highlighted.

Conclusion

This large cohort offers valuable insight into long-term management challenges experienced in CACTD, and potential therapeutic modalities requiring further investigation. Most patients have a severe classical course with onset of symptoms within the first 48 hours, and cases diagnosed through newborn screening remain rare. Despite early recognition and treatment, outcomes remain poor with high rates of sudden cardiac death and progressive neurocognitive disability in classical cases, potentially due to initial catastrophic presentation and recurrent hyperammonemia. The preponderance and pathogenesis of severe acute cardiac compromise and hyperammonemia in CACTD, relative to other FAODs, remains unexplained. The attenuated variant c.82G>T may still cause significant illness but there is later onset and the potential for better longer term outcome due to the absence of chronic hyperammonemia.

Accepted Article

Patients with CACTD seem to respond well to treatment with high rates of glucose infusion during acute decompensation. Early detection and standard treatment with high rates of IV dextrose and MCT feeds, may allow survival of the initial insult but long-term survival outcomes are sub-optimal using current treatment strategies. C10 MCT is probably underutilised leading to acetyl Co-A deficiency. Acute energy deficit may result in sudden, frequently cardiac death, which may occur in the context of prolonged fasting. For survivors within this cohort, there has been a trend for improvement with greater energy delivery from other sources (higher protein intake, D,L-3-hydroxybutyrate and Triheptanoin) but further research is required to define the role of these agents.

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Legends for Figures

Figure 1. Acute and chronic complications of CACTD

Number of cases with presenting features of classical (left bar) and attenuated (right bar) DD. Chronic complications in survivors over one year of age.

References

1. Stanley CA, Hale DE, Berry GT, Deleeuw S, Boxer J, Bonnefont JP. Brief report: a deficiency of carnitine-acylcarnitine translocase in the inner mitochondrial membrane. *N Engl J Med* 1992; **327**(1): 19-23. doi: 10.1056/NEJM199207023270104.
2. Yan HM, Hu H, Ahmed A, et al. Carnitine-acylcarnitine translocase deficiency with c.199-10 T>G and novel c.1A>G mutation: Two case reports and brief literature review. *Medicine (Baltimore)* 2017; **96**(45): e8549. doi: 10.1097/MD.00000000000008549.
3. Longo N, Amat di San Filippo C, Pasquali M. Disorders of carnitine transport and the carnitine cycle. *Am J Med Genet C Semin Med Genet* 2006; **142C**(2): 77-85. doi: 10.1002/ajmg.c.30087.
4. Roschinger W, Muntau AC, Duran M, et al. Carnitine-acylcarnitine translocase deficiency: metabolic consequences of an impaired mitochondrial carnitine cycle. *Clin Chim Acta* 2000; **298**(1-2): 55-68. doi: 10.1016/s0009-8981(00)00268-0.
5. Rubio-Gozalbo ME, Bakker JA, Waterham HR, Wanders RJ. Carnitine-acylcarnitine translocase deficiency, clinical, biochemical and genetic aspects. *Molecular aspects of medicine* 2004; **25**(5-6): 521-32.
6. Saudubray JM, Martin D, de Lonlay P, et al. Recognition and management of fatty acid oxidation defects: a series of 107 patients. *J Inherit Metab Dis* 1999; **22**(4): 488-502. doi: 10.1023/a:1005556207210.
7. Chalmers RA, Stanley CA, English N, Wigglesworth JS. Mitochondrial carnitine-acylcarnitine translocase deficiency presenting as sudden neonatal death. *J Pediatr* 1997; **131**(2): 220-5. doi: 10.1016/s0022-3476(97)70157-4.
8. Parini R, Invernizzi F, Menni F, et al. Medium-chain triglyceride loading test in carnitine-acylcarnitine translocase deficiency: insights on treatment. *J Inherit Metab Dis* 1999; **22**(6): 733-9. doi: 10.1023/a:1005548201355.
9. Arnold GL, Van Hove J, Freedenberg D, et al. A Delphi clinical practice protocol for the management of very long chain acyl-CoA dehydrogenase deficiency. *Molecular genetics and metabolism* 2009; **96**(3): 85-90.
10. Hsu BY, Iacobazzi V, Wang Z, et al. Aberrant mRNA splicing associated with coding region mutations in children with carnitine-acylcarnitine translocase deficiency. *Mol Genet Metab* 2001; **74**(1-2): 248-55. doi: 10.1006/mgme.2001.3235.
11. Vitoria I, Martin-Hernandez E, Pena-Quintana L, et al. Carnitine-acylcarnitine translocase deficiency: experience with four cases in Spain and review of the literature. *JIMD reports* 2015; **20**: 11-20.
12. Pierre G, Macdonald A, Gray G, Hendriksz C, Preece MA, Chakrapani A. Prospective treatment in carnitine-acylcarnitine translocase deficiency. *J Inherit Metab Dis* 2007; **30**(5): 815. doi: 10.1007/s10545-007-0518-x. Epub 2007 May 12.
13. Bhattacharya K, Matar W, Tolun A, et al. The use of Sodium DL-3-Hydroxybutyrate in severe acute neuro-metabolic compromise in patients with inherited ketone body synthetic disorders. *Orphanet Journal of Rare Disease* 2019.
14. Hammond J, Sim KG, Trenholm A, Stanley EL, Wilcken B. Sudden Infant Death in Infancy - Two NZ cases of Carnitine acylcarnitine translocase deficiency. *Journal of inherited metabolic disease* 1998; **21**(Suppl 2): 60.

15. Lopriore E, Gemke RJ, Verhoeven NM, et al. Carnitine-acylcarnitine translocase deficiency: phenotype, residual enzyme activity and outcome. *Eur J Pediatr* 2001; **160**(2): 101-4. doi: 10.1007/s004310000644.
16. Costa C, Costa JM, Nuoffer JM, et al. Identification of the molecular defect in a severe case of carnitine-acylcarnitine carrier deficiency. *J Inherit Metab Dis* 1999; **22**(3): 267-70. doi: 10.1023/a:1005590223680.
17. Costa C, Costa JM, Slama A, et al. Mutational spectrum and DNA-based prenatal diagnosis in carnitine-acylcarnitine translocase deficiency. *Mol Genet Metab* 2003; **78**(1): 68-73. doi: 10.1016/s96-7192(02)00205-6.
18. Stanley CA. Hyperinsulinism/hyperammonemia syndrome: insights into the regulatory role of glutamate dehydrogenase in ammonia metabolism. *Mol Genet Metab* 2004; **81**(Suppl 1): S45-51. doi: 10.1016/j.ymgme.2003.10.013.
19. Saheki T, Kobayashi K, Iijima M, et al. Adult-onset type II citrullinemia and idiopathic neonatal hepatitis caused by citrin deficiency: involvement of the aspartate glutamate carrier for urea synthesis and maintenance of the urea cycle. *Mol Genet Metab* 2004; **81**(Suppl 1): S20-6. doi: 10.1016/j.ymgme.2004.01.006.
20. Haberle J, Chakrapani A, Ah Mew N, Longo N. Hyperammonaemia in classic organic acidaemias: a review of the literature and two case histories. *Orphanet J Rare Dis* 2018; **13**(1): 219. doi: 10.1186/s13023-018-0963-7.
21. Soria LR, Nitzahn M, De Angelis A, et al. Hepatic glutamine synthetase augmentation enhances ammonia detoxification. *J Inherit Metab Dis* 2019; **42**(6): 1128-35. doi: 10.1002/jimd.12070. Epub 2019 Mar 11.
22. Mahapatra S, Ananth A, Baugh N, Damian M, Enns GM. Triheptanoin: A Rescue Therapy for Cardiogenic Shock in Carnitine-acylcarnitine Translocase Deficiency. *JIMD reports* 2018; **39**:19-23.(doi): 10.1007/8904_2017_36. Epub Jul 9.
23. Vockley J, Charrow H, Ganesh J et al. Triheptanoin Treatment in Patients with Pediatric Cardiomyopathy Associated with Long Chain-Fatty Acid Oxidation Disorders. *Mol Genet Metab* 2016; **119** (3) 223-231. doi: 10.1016/j.ymgme.2016.08.008
24. Iacobazzi V, Invernizzi F, Baratta S, et al. Molecular and functional analysis of SLC25A20 mutations causing carnitine-acylcarnitine translocase deficiency. *Hum Mutat* 2004; **24**(4): 312-20. doi: 10.1002/humu.20085.
25. Giudetti AM, Stanca E, Siculella L, Gnoni GV, Damiano F. Nutritional and Hormonal Regulation of Citrate and Carnitine/Acylcarnitine Transporters: Two Mitochondrial Carriers Involved in Fatty Acid Metabolism. *International journal of molecular sciences* 2016; **17**(6).
26. Prentki M, Corkey BE, Madiraju SRM. Lipid-associated metabolic signalling networks in pancreatic beta cell function. *Diabetologia* 2019; **19**(10): 019-04976.
27. Tucci S, Flogel U, Hermann S, Sturm M, Schafers M, Spiekerkoetter U. Development and pathomechanisms of cardiomyopathy in very long-chain acyl-CoA dehydrogenase deficient (VLCAD(-/-)) mice. *Biochimica et biophysica acta* 2014; **1842**(5): 677-85.
28. Pande SV, Brivet M, Slama A, Demaugre F, Aufrant C, Saudubray JM. Carnitine-acylcarnitine translocase deficiency with severe hypoglycemia and auriculo ventricular block. Translocase assay in permeabilized fibroblasts. *J Clin Invest* 1993; **91**(3): 1247-52. doi: 10.1172/JCI116288.
29. Gillingham MB, Elizondo G, Behrend A, et al. Higher dietary protein intake preserves lean body mass, lowers liver lipid deposition, and maintains metabolic control in participants with long-chain fatty acid oxidation disorders. *J Inherit Metab Dis* 2019; **42**(5): 857-69. doi: 10.1002/jimd.12155. Epub 2019 Jul 24.

30. Tucci S, Herebian D, Sturm M, Seibt A, Spiekerkoetter U. Tissue-specific strategies of the very-long chain acyl-CoA dehydrogenase-deficient (VLCAD-/-) mouse to compensate a defective fatty acid beta-oxidation. *PLoS one* 2012; **7**(9): e45429.
31. Morris AA, Olpin SE, Brivet M, Turnbull DM, Jones RA, Leonard JV. A patient with carnitine-acylcarnitine translocase deficiency with a mild phenotype. *J Pediatr* 1998; **132**(3 Pt 1): 514-6. doi: 10.1016/s0022-3476(98)70030-7.
32. Iacobazzi V, Pasquali M, Singh R, et al. Response to therapy in carnitine/acylcarnitine translocase (CACT) deficiency due to a novel missense mutation. *Am J Med Genet A* 2004; **126A**(2): 150-5. doi: 10.1002/ajmg.a.20573.
33. Korman SH, Pitt JJ, Boneh A, et al. A novel SLC25A20 splicing mutation in patients of different ethnic origin with neonatally lethal carnitine-acylcarnitine translocase (CACT) deficiency. *Mol Genet Metab* 2006; **89**(4): 332-8. doi: 10.1016/j.ymgme.2006.06.009. Epub Aug 17.
34. Vatanavicharn N, Yamada K, Aoyama Y, et al. Carnitine-acylcarnitine translocase deficiency: Two neonatal cases with common splicing mutation and in vitro bezafibrate response. *Brain & development* 2015; **37**(7): 698-703.
35. Fukushima T, Kaneoka H, Yasuno T, et al. Three novel mutations in the carnitine-acylcarnitine translocase (CACT) gene in patients with CACT deficiency and in healthy individuals. *J Hum Genet* 2013; **58**(12): 788-93. doi: 10.1038/jhg.2013.103. Epub Oct 3.
36. Al Aqeel AI, Rashid MS, Ruiters JP, Ijlst L, Wanders RJ. A novel molecular defect of the carnitine acylcarnitine translocase gene in a Saudi patient. *Clin Genet* 2003; **64**(2): 163-5. doi: 10.1034/j.1399-0004.2003.00117.x.
37. Bonnet D, Martin D, Pascale De L, et al. Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. *Circulation* 1999; **100**(22): 2248-53. doi: 10.1161/01.cir.100.22.2248.
38. Ogier de Baulny H, Slama A, Touati G, Turnbull DM, Pourfarzam M, Brivet M. Neonatal hyperammonemia caused by a defect of carnitine-acylcarnitine translocase. *J Pediatr* 1995; **127**(5): 723-8. doi: 10.1016/s0022-3476(95)70160-5.
39. Khabbush A, Orford M, Tsai YC, et al. Neuronal decanoic acid oxidation is markedly lower than that of octanoic acid: A mechanistic insight into the medium-chain triglyceride ketogenic diet. *Epilepsia* 2017; **58**(8): 1423-9. doi: 10.1111/epi.13833. Epub 2017 Jul 6.
40. Rogawski MA. A fatty acid in the MCT ketogenic diet for epilepsy treatment blocks AMPA receptors. *Brain* 2016; **139**(Pt 2): 306-9. doi: 10.1093/brain/awv369.
41. Van Hove JL, Grunewald S, Jaeken J, et al. D,L-3-hydroxybutyrate treatment of multiple acyl-CoA dehydrogenase deficiency (MADD). *Lancet (London, England)* 2003; **361**(9367): 1433-5.
42. Van Rijt WJ, Heiner-Fokkema MR, du Marchie Sarvaas GJ, et al. Favorable outcome after physiologic dose of sodium-D,L-3-hydroxybutyrate in severe MADD. *Pediatrics* 2014; **134**(4): e1224-8.
43. Gautschi M, Weisstanner C, Slotboom J, Nava E, Zürcher T, Nuoffer JM. Highly efficient ketone body treatment in multiple acyl-CoA dehydrogenase deficiency-related leukodystrophy. *Pediatr Res* 2015; **77**(1-1): 91-8. doi: 10.1038/pr.2014.154. Epub Oct 7.
44. Wang GL, Wang J, Douglas G, et al. Expanded molecular features of carnitine acylcarnitine translocase (CACT) deficiency by comprehensive molecular analysis. *Mol Genet Metab* 2011; **103**(4): 349-57. doi: 10.1016/j.ymgme.2011.05.001. Epub May 7.

45. Lee RS, Lam CW, Lai CK, et al. Carnitine-acylcarnitine translocase deficiency in three neonates presenting with rapid deterioration and cardiac arrest. *Hong Kong Med J* 2007; **13**(1): 66-8.

MCT procal, skimmed milk, and carbohydrate polymer, with walnut oil supplementation. He received continuous overnight gastrostomy feeds from 17 months of age. His diet provided 30 to 40% of total calories from fat with less than 10% from long chain fat. Energy intake was 83-116% of expected energy requirement (EER) but poor growth and worsening hyperammonemia was noted with energy intake less than 85% EER. Hyperammonemia improved but transaminases and hepatomegaly appeared to worsen with increased dietary carbohydrate. At 2 year and 10 months of age he started a transition to C7 oil and Tolerex (Nestlé, Vevey, Switzerland) low fat elemental formula. Despite no previous history of elevated Creatine Kinase (CK) levels, he developed increased Creatine Kinase up to 2841 U/L (<235) and worsening of transaminases. These improved with Portagen, MCT procal and skim milk feeds. Dilated cardiomyopathy was documented at 3 years and 3 months of age with Left Ventricular Ejection Fraction of 27% (severe dysfunction <30%). At 3 years and 6 months of age, he was admitted with intercurrent illness and died of cardiac failure.

Patient 2

A female infant of Indian ethnicity was born at term, after a pregnancy complicated by a mild increase in maternal GGT. Apgars were 9¹ and 10⁵ and growth parameters were between 2-9th percentile. She presented at 12 hours of age with poor feeding, hypothermia and hypoglycaemia, proceeding to a bradycardic arrest with blood glucose level of 0.5 mmol/L (2.6 – 5.0). Return of spontaneous circulation was achieved after 10 minutes of cardiopulmonary resuscitation however she developed encephalopathy and seizures. She received total body cooling for a total of 69 hours for presumed hypoxic encephalopathy and developed elevated liver transaminases with deranged coagulation, renal impairment and hyponatremia. Worsening metabolic acidosis on day 5 culminated in bradycardic arrest and resuscitation was unsuccessful. Biochemical test results were available post mortem. Plasma free carnitine was reduced 3.1 umol/L (4-33) with a raised acyl/free-carnitine ratio. Plasma acylcarnitine profile was suggestive of CPT II/CACT with an elevated C16 of 12.61 umol/L (<0.7). Molecular analysis of *SLC25A20* revealed compound heterozygous variants c.82G>T/c.706C>T.

Patient 3 (previously reported by Hammond et al as abstract in 1998)

A female infant was born to consanguineous Vietnamese parents at 36/40 after an uncomplicated pregnancy. Birth weight was 2775g. At 28 hours of age she developed hypoglycaemia and hypothermia, which persisted despite external warming and regular formula feeds. Hypoglycaemia and hypothermia resolved on intravenous 10% dextrose but recurred on attempted reintroduction of feeds. Initial metabolic screen showed mildly elevated ammonia and lactate with a respiratory alkalosis, severely reduced serum carnitine, raised acylcarnitine/free carnitine ratio and normal urine organic acids. A long chain fat disorder was suspected, regular feeds were ceased, and L-carnitine and IV 10% dextrose started at 7-9mg/kg/min. IV dextrose up to 13mg/kg/min was required to maintain blood sugar levels 4-6mmol/L. On return to full Pregestamil (53% MCT) feeds off dextrose, blood sugar levels were maintained but ammonia and lactate remained elevated. A repeat metabolic screen showed a compensated metabolic acidosis, dicarboxylic aciduria, and renal Fanconi's syndrome, which was treated with sodium bicarbonate 8.4% oral solution. Free carnitine remained low at 7umol/L, despite supplementation with L-carnitine 50mg/kg/day.

Figure 1.

Patient ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Age at onset	24 hours	12 hours	28 hours	24 hours	10 hours	Day 2	24 hrs	24 hours	15 hours	Day 2	Treated from birth	32 hours	36 hours	14.5 hours	Day 8	12 hours
Ethnicity	Chinese	Indian	Vietnamese	Caucasian (NZ)	Caucasian		Iranian	Chinese	Guyana/ Surinam	Caucasian (Aus)	Iranian	Irish	NZ Maori	Chinese	Chinese	Vietnamese
Lowest Glu (mmol/L)	1.1	0.5	LOW	LOW	1.4	1.5	<2.0	<0.5	No	1.9	No	LOW	<0.6	<1.1	2.3	0.6
Cardiac arrest	Yes	Yes	No	not documented	Yes	No	Yes	No	No	No	No	Yes	No	Yes	No	Yes
Presenting cardiac findings	RBBB, first-degree heart block. LVH	Bradycardic arrest (ROSC after 10 minutes)	Normal echo, ECG	AV block	VT and cardiac arrest requiring 45 mins CPR.	Severe hypertrophic cardiomyopathy	SVT, cardiac arrest, mild LVH visually (normal measurements)	Broad complex tachyarrhythmia atrial flutter; biventricular hypertrophy, troponin rise	Yes	Severe cardiac dysfunction (FS 16%),	SVT and ?VT. Normal echocardiogram	Out of hospital arrest. AV broad complex - (PEA)	Concentric LVH. Dysfunction with echogenic myocardium	Bradycardia, cardiomyopathy with thickened LV, RV and septum.	Hypertrophic cardiomyopathy, episodic bradycardia	VT arrest during intubation. Responded to defibrillation
Highest Ammonia	319	NP	Raised, level not documented	Not documented	590	195	50	190	1142	800	121	250	320	316	415	388
Other clinical features	Acidosis. Seizures, Acute renal injury	Acidosis. Encephalopathy, seizures, hypocalcaemia, renal impairment	Alkalosis and acidosis (renal tubular dysfunction) hypothermia	Apnoea, hypothermia acidosis.	Anuria, acidosis, Cerebral oedema	Elective NICU admission.	Acidosis, hypothermia, seizures elevated transaminases,	Seizures, elevated transaminases	Hypotonia, encephalopathy	Acidosis, Hypothermia, seizures, renal impairment	Elective treatment - sibling died	Acidosis, acute renal injury, mild coagulopathy		Seizures Acute renal failure and rhabdomyolysis	Elevated transaminases, Hypothermia, respiratory distress	Hypoglycaemic seizures
CK (U/L) (<300)	625		Raised	14,000		430	>25,000	1560	normal	762	190	23200	7440	480	1171	3836
Peak Lactate mmol/l	4.5	8.0	NR	Raised	5.1	5.6	6.0	6.9		4.0	NP	5		4.7	1.4	4.5
Free carn (MLN)	0.18	0.25	0.083	7 (day 10)	1.2	0.4	0.04	1		1	0.4	in range	1	1.1	0.5	1
C16 (MUN)	29	1	1.1	NA	10.1	C16 increased	1.1	1.7	12.6 (day 9)	1.2	3.35	7.8	1.7	14	18	3.3
Urine organic acids	Dicarboxylic aciduria. No ketones		Dicarboxylic aciduria, lactate, tyrosine metabolites	Lactate, C6, C8,C8:1, C10:1 dicarboxylic aciduria	No urine passed	Increased lactate, moderate dicarboxylic aciduria	Dicarboxylic aciduria with modest lactate elevation	Lactate slight increase and dicarboxylic acids slightly increased	Dicarboxylic aciduria	Dicarboxylic aciduria in absence of ketones.	Mild lactic aciduria. Trace hexanoylglycine suberylglycine	Mild elevation of 3-hydroxycarboxylic acids, with normal ketones	Significant dicarboxylic aciduria C6-C12	Dicarboxylic acids, tyrosine metabolites	Dicarboxylic acids	Mild to moderate dicarboxylic aciduria (C6>C8>C10) No other significant changes detected.
Enzyme activity	NP		0% CACT activity	CACT enzyme activity 4% of controls	NP	1% of controls: 0.0175(1.24) nmol/min/mg prot		NP	NP	CACT / CPT II fib ACP pattern (normal CPTII)	NP	NP		NP	NP	NP
SLC25A20 genotype	Homozygous c.199-10T>G	c.82G>T c.706C>T	Homozygous c.199-10T>G		c.646G>T Homozygous	NP	Homozygous c.67T>C	c.109C>T c.199-10T>G	Homozygous c.110G>C	c.50G>C c.326+1 delG	Homozygous c.417+1G>A	c.326+1 delG (Splice donor) ; c.691G>C	Homozygous c.804delG	Homozygous c.199-10T>G	Homozygous c.199-10T>G	Homozygous c.199-10T>G
Current Age / age & mode of death	3yrs ?cardiac arrest	7 days - cardiac arrest	6 months - broad complex VT	6 months - out of hospital sudden death	Care withdrawn on day 3 of life.	2 yrs 10 months - post-viral multi-organ failure	11 years	9 years	3 years	10 years	19 months	16 months	12 months	4 years	14 months	5 months

Table 1 – Presenting features of patients with early severe classical-onset CACTD. All except patient 15 presented < 48 hours of life. Patients 7 -16 still survive. VT – ventricular tachycardia. SVT – supraventricular tachycardia. PEA – Pulseless electrical activity. RBBB – right bundle branch block. LVH – left ventricular hypertrophy. Free carn (Free carnitine - MLN – multiple of lower normal reference range); C16 (MUN – multiples of upper normal reference range). Glu – Blood Glucose level (normal range typically 2.6 – 5.0 mmols/L) – LOW represents value below detection limit. Lactate normal range typically <2.0 mmols/L. Ammonia – normal range typically < 80 umol/L. NZ – New Zealand; Aus – Australia

Patient ID	1	6	7	8	9	10	11	12	13	14	15	16
Energy Intake kcal/kg/day	68	105	53		90	90	87	66	98			98
Total Energy Intake (%EER)	82		112			104	92		111	130	115	102
Total fat (% of total energy)	34	7	25	29	31	19	20	25	23	30	21-24	22
LCT (% of total energy)	9	5	4	5	9	8	3	4	4	4	<10	3.5
MCT (% of total energy)	25	2	21	24	C7 24	11	C8 (17)	21	19	26		18.5
Protein g/kg	2.5	3.0	1.4	1.2	2.0	3.0	1.7	2.4	2.8			3.1
Protein (% of total energy)	15	14	11	10	9	16	8	11	12	10	11	9.8
% calories from CHO	51	78	64	60	60	65	72	64	64	60	65-67	69.3
Maximum fasting interval		3 hrs	2.5 hrs	Reached 11 hrs; reduced back to 4 hrs	6hrs CNPF	4 hrs	3 hrs	4 hrs	4 hrs (reduced from 8 hrs)			4 hrs
Supplements	Walnut oil, C7	Walnut oil	Flax seed oil, multivitamins, essential amino acids, ketones	Beneprotein, Ketones	Ketones, C7	Ketones	PUFA	Ketones	Beneprotein Ketones	Walnut oil		Ketones
L-carnitine (mg/kg/day)	up to 125	Yes	Yes	100 (re-started 10 yrs)	100	100	22	100	100 (poor compliance)	130-150	130	50

Table 2 - Dietary Treatment of severe early onset CACT deficiency in patients that survived neonatal period. EER – estimated energy requirement.

MCT – medium chain triglyceride. LCT – long chain triglyceride. CHO – carbohydrate. PUFA –long chain poly-unsaturated fatty acids. C7 – Triheptanoin.

CNPF – continuous nocturnal pump feeds.

Patient identifier	17	18	19	20	21	22	23
Age at onset	NBS +ve (day 9)	2 months	Prospective Rx	Day 9	Prospective Rx	1 month	4 months
Hypoglycemia	No	Yes	No	Yes	No	No	Yes
Presenting cardiac findings	Normal echo, ECG	Asystolic arrest requiring CPR for 2 minutes LVH, Impaired contractility.	Small ASD only	Mild left branch pulmonary stenosis	Normal	Normal	Mild left ventricular hypertrabeculation
Other clinical features		Seizures	Nil	Nil	Nil	Nil	Unresponsive after missing night feed - glucose <0.6 mmol/L
Free carnitine (MLN)	0.47	0.67	NP	1.2	0.84	0.04	0.8
C16-acylcarnitine (MUL)	13	C16 increased		C16 increased			1.23
Urine organic acids	Normal at 9 days of age.	4-hydroxyphenyllactate & 4-hydroxyphenylpyruvate (Liver dysfunction)	Grossly increased adipate, suberate & sebacate – but on MCT supplementation	Benzoate & succinate increased (specimen deterioration)	NA	DCA present, minimal 3 hydroxy-n-butyrate	Normal
Enzyme activity	Normal VLCAD activity	CACT enzyme activity 4%:	NP	NP	NP	Normal CPT1 and CPT2	NP
Beta oxidation	FAO flux 12%	Defective oxidation of oleate, Myristate and Myr/Oleate ratio	Defective oxidation of myristate and oleate	NP	NP	NP	NP
Abdominal ultrasound	Normal	Normal	Liver echogenicity. No gallstones.	Normal	Normal	Normal	Mild increase in echogenicity

Neuroimaging	MRI/MRS normal.	NA	NA	NA	NA	NP	NP
Other	Normal growth and development. Diabetes insipidus.	Spastic quadriplegia, microcephaly, severe learning difficulties, oral diet	Normal development. Overweight.	Normal growth & development but learning support at school	Normal growth & development	Normal. Not compliant with diet	
Total Energy Intake (%EER)	66	105	117	98	98		
Total fat (% Energy from fat)	30	7	22	20	29		
LCT (% Energy from LCT)	13	5	2	4	6		
MCT g (% calories from MCT)	17	2	20	18	23		
Protein g/kg/day	1.1	2	3	2.3	2.3		
Protein (% Energy from protein)	16	13	13	13	13		
% calories from CHO	54%	80%	65%	65%	58%		
Maximum fasting interval	12	6 hours (on profile)	13 hours (on profile)	5 hours (on profile)	4 hours (on profile)		
Supplements	Walnut oil, flax oil	Walnut oil	Non compliant (EFA)	Non compliant (EFA)	NA		
L-carnitine	Yes	Yes	Yes	Yes	Yes	No	Yes
Current age (years)	10	20	16	10	5	25	8

Table 3 Features of later onset CACT deficiency – all of Pakistani heritage. Genetic studies performed in all cases except case 22 and were homozygous c.82G>T (p.Gly28Cys). Diet data were unreliable for case 22 and 23. NP – not performed. NA – not applicable. MLN – multiples of lower limit of normal. MUL – multiples of upper limit of normal. EER – estimated energy requirement. MCT – medium chain triglyceride. LCT – long chain triglyceride. CHO – carbohydrate. EFA – essential fatty acid supplement - Arachidonic Acid and Docosahexaenoic Acid.