Screening for Carnitine Transporter Deficiency

External review against programme appraisal criteria for the UK National Screening Committee (UK NSC)

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Bazian

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The UK NSC advises Ministers and the NHS in all four UK countries about all aspects of screening policy. Its policies are reviewed on a 3 yearly cycle. Current policies can be found in the policy database at http://www.screening.nhs.uk/policies and the policy review process is described in detail at http://www.screening.nhs.uk/policyreview

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Summary: Screening for Carnitine Transporter Deficiency (CTD)

Key points

- **The clinical course of CTD is variable, and there is no reliable way to predict phenotype/prognosis**
- **There is uncertainty over the accuracy of the screening test as most screening studies have not performed extensive follow-up, and therefore false-negatives could have been missed**
- **Screening can identify heterozygotes, and the natural history of heterozygotes is not well understood**
- **Although there is an accepted treatment, there is uncertainty over whether all cases identified through screening will require treatment**

Introduction

This review assesses newborn screening for carnitine transporter defect (CTD), a rare autosomal recessive disorder of fatty acid metabolism.

Fatty acids, a type of lipid, are molecules consisting of a hydrocarbon chain with a carboxyl group at the end. Fatty acids can be used to produce energy and other metabolites.

Fatty acids are broken down by a process called β-oxidation which occurs in the mitochondria of the cell. Before fatty acids can enter the mitochondria they are "activated" by the addition of Coenzyme A (CoA) to create a molecule known as acyl CoA. Acyl CoAs then need to be conjugated to carnitine before they can be transported into the mitochondrial matrix. Once inside the mitochondria the carnitine is removed. The acyl CoA can then undergo β-oxidation.

In CTD transport of carnitine into the cell is impaired. Without carnitine, fatty acids cannot enter mitochondria and therefore cannot be used to make energy.

When fat cannot be used to generate energy, glucose is consumed without regeneration resulting in hypoglycaemia. In addition, fat released from adipose tissue accumulates in the liver, skeletal muscle and heart resulting in hepatic steatosis and myopathy.

Current policy

Screening for CTD is not recommended in the UK.

Newborn screening for this condition is currently undertaken in the US. The European Union Committee of Experts on Rare Diseases (EUCERD) reported that screening for CTD is undertaken in seven member states of the European Union, candidate, potential candidate and European Free Trade Association countries.

This review

This review assesses newborn screening for CTD against the UK National Screening Committee (NSC) criteria for appraising the viability, effectiveness and appropriateness of a screening programme (National Screening Committee 2003).

The clinical and cost-effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry (MS) was last reviewed in 2004 by the Health Technology Assessment (HTA) NHS R&D HTA Programme. In this review screening for CTD was not considered. The literature search for the 2004 HTA was carried out in November 2001. Therefore the searches for this current review were carried out from January 2001.

The HTA review supported screening for phenylketonuria and medium-chain acyl-CoA dehydrogenase deficiency. However it found that robust clinical evidence was limited for many other inborn errors of metabolism that can be detected by tandem MS and that economic modelling using the available evidence did not support inclusion of other inherited metabolic diseases within a neonatal screening programme.

Summary of findings

Clinical course and epidemiology

CTD is a very rare, recessively inherited, disorder. In general the number of cases reported was small, making estimates of incidence uncertain. No studies reporting the incidence of CTD in the UK were identified.

Table 1: Range of incidence

Condition	Lower end of reported range	Upper end of reported range
CTD	1:775,600 (in Australia, without screening)	1:5,924 (in Denmark, the Faroe Islands and Greenland, without screening)*

*Faroe Islands have a high prevalence of CTD.

The age of onset of symptoms and the presenting features of CTD varies. Presenting features include cardiomyopathy, coma, hypoglycaemic encephalopathy, anaemia, motor delay, myopathy, hepatomegaly and/or failure to thrive. The introduction of newborn screening has led to the identification of mothers with CTD. The majority of these women were asymptomatic at diagnosis. This suggests that there could be a subtype of CTD that never presents clinically.

The distribution of phenotypes is unclear.

CTD is characterised by molecular heterogeneity, with many reported pathological mutations. There is no clear genotype/phenotype correlation or enzyme activity/phenotype correlation.

The epidemiology and natural history of CTD are not well understood.

The test

CTD can be screened for using tandem MS performed on carnitine and acylcarnitines extracted from dried blood spots on Guthrie cards. This method represents a simple and safe test.

Levels of free carnitine (C0) are low in CTD as the carnitine transporter also reabsorbs carnitine prior to its excretion in urine. Therefore, patients with CTD have increased urine carnitine levels and significantly decreased plasma carnitine levels.

Screening programmes that have screened for CTD have differed in the time of specimen collection, although it is generally earlier than in the UK. They have also varied in the cut-offs used. There is no clear cut-off for screening and no studies looking at this issue in a UK population.

Despite differences in some of the features of newborn screening programmes, in the majority of studies sensitivity and specificity for screening was reported to be high. However, positive predictive was generally low, although negative predictive values were high. However, it should be noted that there were limitations to the studies, including a lack of extensive follow-up to identify false negatives. Even if follow-up was performed, it is possible that mild or asymptomatic false negative cases would not be identified.

Table 2: Range of test characteristics

Condition	Cut offs reported	Sensitivity (range)	Specificity (range)	PPV (range)	NPV (range)
CTD	C0: <5.7 to <13µmol/L	45.45% to 100%	99.31% to >99.99%	0.48% to 33.33%	>99.9% to 100%

One of the reasons for poor positive predictive value for CTD was that screening can identify asymptomatic mothers with CTD. Because carnitine can be transported across the placenta, carnitine levels in the newborn period are strongly influenced by maternal C0 levels. Low carnitine levels may reflect a false-positive result in the infant (who is a heterozygous carrier), but a true-positive diagnosis of CTD in the mother. There has also been concern that a newborn with CTD may have normal carnitine levels due to transplacental transport of free carnitine from an unaffected mother, resulting in false negatives. Whether screening tests for CTD would be improved if undertaken once maternal C0 levels have less of an effect was not discussed in the papers identified by the literature search. It has also been reported that screening may identify heterozygotes without identifying affected individuals.

As CTD is a recessive disorder, heterozygotes should be asymptomatic. However, there has been the suggestion that heterozygotes may display some symptoms. The natural history of heterozygotes requires further study.

Treatment

The treatment for CTD is carnitine supplementation.

Treatment is reported to both prevent the primary manifestations of the disease and to reverse symptoms.

However, the evidence base is very limited.

It is possible that newborn screening is changing the distribution of phenotypes of the disorder, leading to the identification of more mild or asymptomatic cases in which treatment may not be required. As there is no reliable way to determine phenotype/prognosis, it cannot be predicted which individuals should receive treatment.

No RCTs of screening were identified in the update search.

Cost effectiveness

One cost-effectiveness study published in 2007 was identified. This study was done from a Canadian perspective and it is unclear how applicable this study would be to the UK. No UK based studies of cost-effectiveness were identified.

Implication for policy

Current policy on screening for CTD should be retained.

Implications for research

The following are required:

- The determination of the UK prevalence/incidence
- Follow-up studies of asymptomatic infants detected by screening
- Studies to determine whether phenotype or outcome can be predicted
- Studies of the natural history of heterozygotes
- Studies into the optimum timing of specimen collection
- Studies to determine optimal management and who needs treatment

Appraisal against UK NSC Criteria

These criteria are available online at http://www.screening.nhs.uk/criteria.

1. The condition should be an important health problem

Carnitine transporter deficiency (CTD), also known as systemic primary carnitine deficiency (CDSP) or carnitine uptake disorder (CUD), is an autosomal recessive disorder caused by mutations in *SLC22A5* (OCTN2), which encodes the carnitine transporter.

The carnitine transporter facilitates carnitine uptake through the cell membrane. Defects in the carnitine transporter lead to reduced levels of intracellular carnitine and reduced reabsorption of carnitine prior to its excretion in urine. In CTD patients have increased urine carnitine levels and significantly decreased plasma carnitine levels.

Carnitine is required for the transportation of long-chain fatty acids into the mitochondria, where they undergo β-oxidisation to produce energy. Without carnitine, fatty acids cannot enter mitochondria and therefore cannot be used to make energy.

The heart preferentially utilises fatty acids as a source of energy and, during periods of fasting, fatty acids are the predominant source of energy for other organs. When fat cannot be utilised glucose is consumed without regeneration resulting in hypoglycaemia. In addition, fat released from adipose tissue accumulates in the liver, skeletal muscle and heart resulting in hepatic steatosis and myopathy.

As described in Criterion 2, CTD can present at a range of ages with a wide variety of clinical phenotypes and clinical outcomes, from fatal to asymptomatic. There is currently no way of predicting phenotype or prognosis. Treatment with carnitine supplementation, can reverse symptoms, as well as prevent them.

Criterion 1 uncertain: CTD is a rare disorder that has a varied clinical course. CTD can be lethal, but the proportion of cases that could be expected to be lethal without treatment is unclear.

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage

Natural history

Several studies reporting the presentation and outcomes of at least four patients with CTD were identified in the update search.

Clinically presenting cases

Wang et al. (2001) described five patients from four families with CTD.[1] One patient was diagnosed after a sibling was diagnosed. Age of presentation varied between six months of age and three years of age. Symptoms included cardiomyopathy, hepatomegaly, hypoglycaemia, coma, muscle weakness and developmental delays. Where reported, there was a good response to carnitine supplementation.

Table 3: Details of the five patients reported in Wang et al. (2001)[1]

Patient	Clinical Symptoms	Therapy and	Carnitine	Mutations

		outcome	transport in fibroblasts (% of controls)	(protein)
268	Diagnosed with cardiomyopathy at 3 years of age after presenting with weakness and respiratory distress	Carnitine therapy, resolution of the clinical outcome (follow-up not reported)	<5%	Homozygous for a null mutation (133X/133X)
909	Presented at 2 years of age with cardiomyopathy	Carnitine therapy. Reversal of symptom. Currently developing and growing well at 12 years of age	<5%	Compound heterozygous for a null mutation and a missense mutation (133X/R19P)
Jer-T*	Presented at 2 years of age in a coma during an episode of gastroenteritis with vomiting and diarrhoea. Also had moderate hepatomegaly and hypoglycaemia.	Oral carnitine treatment (follow-up not reported)	<5%	Homozygous for a missense mutation (R339Q/R339Q)
Jer-H*	Diagnosed after sister (Jer-T) was diagnosed. Had weakness of the proximal limb girdle musculature and developmental delays.	Oral carnitine treatment. Marked improvement of muscle tone, general mode, alertness, activity and concentration span (follow-up not reported)	<5%	Homozygous for a missense mutation (R339Q/R339Q)
JGSQ	Presented at 6 months of age in a coma after a 2-day history of intermittent low-grade fever. Hepatomegaly, microvesicular steatosis and glycogen accumulation.	Carnitine supplements. No additional episodes of significant decompensation. At 4 years of age has normal growth and development.	<5%	Homozygous for a null mutation (Y4X/Y4X)

*sisters

Lamhonwah et al. (2002) described four families and five unrelated individuals with CTD, diagnosed based upon confirmatory fibroblast carnitine studies.[2] After diagnosis, the patients were treated with carnitine. Table 4 describes the age of onset, presenting feature(s), biochemical abnormalities and Table 5 describes the clinical outcome of the patients. The age of presentation varied between eight days and 60 months. Presenting features included cardiomyopathy, coma, hypoglycaemic encephalopathy, anaemia, motor delay, myopathy, hepatomegaly and/or failure to thrive. All patients had a beneficial response to carnitine therapy.

The researchers state that cases 9, 10 and 11 highlight the importance of early treatment. These three cases were siblings, and had the same genotype and phenotype. Because of the prior diagnosis of his two older siblings, the youngest infant (case 11) was diagnosed and treated from birth with high-dose carnitine supplementation and has no clinical symptoms.

Table 4: Clinical, biochemical and molecular features of cases described in Lamhonwah et al. (2002)[2]

Patient	Mutation in *SLC22A5* (allele 1/ allele 2)	% of control carnitine uptake in fibroblasts*	Gender	Age at presen-tation (months)	Presenting feature	Cardio-myopathy	Myopathy or motor delay	Hypo-glycaemic encephalo-pathy	Hypo-tonia	Failure to thrive	Plasma carnitine total/free (μM)		Beneficial response to carnitine therapy
											Pre-therapy	Post-therapy	
1 (F11)	67-69 deletion/ 14344G>A	2.0	F	1	Cardiac[a]	Dilatative	+	-	+	+	19/ 15	25-60/ 17-33	C/F/M
2 (F12	17081 del C/ 17081 del C	1.1	F	1	Hypotonia	Hypertrophic	+	+	+	-	0	5	C/E/M
3 (L32)	22521 C>T/ 22521 C>T	2.8	F	6	Coma[b], cardiac[a], anaemia, motor delay[c], failure to thrive	Hypertrophic	+	+	+	+	0.78/ 0.72	10-36/ 5-28	C/E/F/H/ M
4 (E1136)	14196 C>T/ 22521 C>T	20.5	F	11	Coma[b], hepatomegaly	Hypertrophic	NR	+	NR	NR	9.2/ 3.2	NR	C
5 (E1137)	20876-7 ins A/ 20876-7 ins A	4.8	M	60	Coma[b]	Dilatative	-	+	NR	NR	10.8/ 10.4	NR	C
6 (E1245)	95 A>G/ 95 A>G	20.6	NR	2	Coma[b], hypotonia, hepatomegaly	Left ventricular hypertrophy	NR	+	+	-	NR	NR	Yes
7 (E1936)	265 dup 11/ 22605 C>G	5.9	M	1	Coma[b]	Hypertrophic	NR	+	NR	NR	Very low	NR	Yes
8 (E1808)	22611 C>T/ 22611 C>T	7.1	M	27	Coma[d]	NR	NR	NR	NR	NR	Low	15.4/ 2.7	Yes
9 (P1)	19015 del A/ 22521 C>T	16	M	36	Cardiac[a], anaemia, hypotonia, motor delay[c], hepatomegaly	Hypertrophic	+	-	+	-	2/ 1	22-76/ 14-55	C/M
10 (P2)	19015 del A/ 22521 C>T	16	F	8 days	Cardiac[a],	Hypertrophic	+	-	+	+	4/ 0	16-50/ 13-40	C/F
11 (P3)	19015 del A/ 22521 C>T	16	M	Carnitine therapy at	None	None	-	-	-	-	2/ 0	4/ 2 at 12	Carnitine therapy at

				birth								hours post dose	birth

***% of control carnitine uptake at 5µM carnitine in fibroblasts.**
[a]Cardiac, cardiomyopathy
[b]Coma, hypoglycaemic encephalopathy
[c]Motor delay, myopathy and/or motor delay
[d]No apparent hypoglycaemia
Abbreviations: NR, not reported; M, male; F, female; C, cardiomyopathy; E, hypoglycaemic encephalopathy; F, failure to thrive; H, hypotonia, M, myopathy/motor delay

Table 5: Clinical outcome (with treatment) of cases described in Lamhonwah et al. (2002)[2]

Patient	Clinical outcome
1 (F11)	Alive at 19.5 years; asymptomatic, bright; normal left ventricular function; mild left ventricular dilatation
2 (F12	Alive at 15.7 years; asymptomatic, bright
3 (L32)	Alive at 2.5 years; borderline hypotonia
4 (E1136)	Alive at 11 years
5 (E1137)	Alive at 19 years; recovering cardiac function
6 (E1245)	Alive at 6.5 years; left ventricular hypertrophy on electrocardiogram
7 (E1936)	Alive at 4.5 years; well
8 (E1808)	Alive at 11 years; normal cardiac function
9 (P1)	Alive at 10 years; muscle cramps; mild developmental delay
10 (P2)	Alive at 4 years; well
11 (P3)	Alive at 3 years; well

Melegh et al. (2004) described two Hungarian Romany (Gypsy) children with CTD who presented with cardiomyopathy and decreased carnitine levels (see Table 6).[3] Both children were found to be homozygous for a deletion in *SLC22A5* that results in a frameshift at R282D that leads to a premature stop codon (V295X). Carnitine treatment resulted in dramatic improvement of the cardiac symptoms. Analysis of the two children's families identified three children who had died suddenly who were also found to be homozygous for this mutation. Two of these children died after contracting an upper respiratory tract infection.

Table 6: Clinical description of the two Hungarian Romany (Gypsy) children with CTD and their siblings who died suddenly who were found to be homozygous for the V295X mutation. There were other family members who died suddenly described in this paper, but are not reported here as the diagnosis of CTD was not confirmed.

Patient	Description
III/7 (proband)	Baby was apathetic. At 3 months of age cardiomyopathy was seen on chest radiography, electrocardiogram revealed high T waves, and ultrasound showed non-obstructive cardiomyopathy with shortening fraction of 30%. Hepatomegaly with moderate increase of liver enzymes was found. Two months after the introduction of carnitine treatment the electrocardiogram abnormality disappeared, the shortening fraction was 33.5% and the liver enzymes were in the normal range. Apathy and spontaneous activity improved.
III/5 (cousin of III/7)	Died aged 6 months after hospitalization for a mild upper respiratory tract infection.
III/8 (sibling of II/7)	Died aged 2 years 9 months after hospitalization for an upper respiratory tract infection.
II/3 (proband)	Diagnosed at 5 years of age. Presented with growth retardation, slight exercise intolerance, recurrent infections, and a mild cardiac decompensation. Hypertrophic cardiomyopathy was detected by echocardiography and radiography. After carnitine therapy was started there was a dramatic improvement in clinical symptoms, and electrocardiogram and ultrasound findings. Significant weight gain was observed and the infection tendency almost disappeared.
II/1 (sibling of II/3)	Died suddenly aged 6 years.

Amat di San Filippo et al. (2006) described eight patients with CTD (see Table 7).[4] One patient had been identified by newborn screening. For the clinically presenting cases, the age of onset of symptoms varied between seven weeks and two and a half years of age. In some cases, symptoms were triggered by infection or illness. Symptoms included cardiomegaly, cardiomyopathy, heart failure, lethargy, hypotonia, irritability, hepatomegaly, and failure to thrive. Where this was reported, response to carnitine therapy was good.

Table 7: Patients described in Amat di San Filippo et al. (2006)[4]

Patient	Mutation in OCTN2 (allele 1/ allele 2)	% of control carnitine uptake in fibroblasts*	Gender	Age of onset of symptoms and clinical symptoms	Outcome
PCD22	T440M/ A142S;R488H	<10%	F	Laboured respirations at 6 months of age. Cardiomegaly identified at 1 year of age. Started on	Alive at 24 years of age

				carnitine supplements at 18 months of age.	
PCD23	Y4X/ Y4X	<10%	F	Lethargy, hypotonia, irritability, and hepatomegaly at 9 months of age.	NR
PCD24	W283R/ W283R	<10%	F	Cardiomyopathy diagnosed following breathing problems at 18 months of age. Heart failure precipitated by respiratory infection at 21 months of age. Started on carnitine supplements.	Alive at 11 years of age
PCD25	P78fsX129/ P78fsX129	<10%	M	Severe metabolic deterioration following gastroenteritis at 5 months of age. Started on carnitine supplements.	Well (age at follow-up NR)
PCD26	P398L/ S280F	<10%	F	None- identified via newborn screening. Started on carnitine supplements.	Asymptomatic (age at follow-up NR)
PCD27	R282Q/ R282Q	<10%	F	Failure to thrive, gastroesophageal reflux, and hypotonia at 7 weeks of age. Started on carnitine supplements.	Normalisation of growth parameters at 5 months of age. At 9 months of age, developmental evaluation indicated a 3 month delay. She started talking at 1year of age. She has continued to grow and is showing catch-up development (age at follow-up NR)
PCD28	F22del/ F22del	<10%	M	Nonresponsive after 1 week of viral illness at 2.5 years of age. Started on carnitine supplements.	Responded well to carnitine supplements. No other details reported.
PCD29	W256X/ T468R	<10%	F	NR	Alive at 33 years of age. She is overweight and had weakness of one leg with muscle aches when she walks.

***exact residual activities were not reported**
Abbreviations: NR, not reported

Lund et al. (2007) described eight patients with CTD from three families who were identified clinically or by family screening after a proband presented clinically in the Faroe Islands (see Table 8).[5] All of the patients were homozygous for the 95 A>G mutation in *SLC22A5*, which leads

to a N32S substitution in OCTN2. The natural history of patients varied, ranging from patients who had died from their disease to asymptomatic individuals, despite the fact that all of the patients were homozygous for the same mutation in *SLC22A5*. All symptomatic patients responded favourably to supplementation with L-carnitine, but only if treated early.

Table 8: Description of eight patients with CTD diagnosed after clinical presentation or family screening reported in Lund et al. (2007).[5]

CTD family	Clinical/paraclinical details	Clinical status at last follow-up (period of follow-up)	Plasma free/ total carnitine at presentation and last follow-up*	Carnitine dose at initial/last follow-up (mg/kg/day)
1- Proband	Hypoglycaemia, hepatomegaly and hypotonia at 4 months. Normal ECHO. Asymptomatic on L-carnitine 120mg/kg/day until 4 years when muscular weakness was noted. L-carnitine was increased to 200mg/kg/day with no effect. Cardiac and intellectual functions normal. Can walk and run short distances.	Slight muscular weakness (11 years)	Initial 2.5/2.5 Last 34/41	120/200
1- Younger sister	Laryngitis at age 3 years with respiratory failure, development of cerebral palsy and 4 months later encephalopathy with cerebral oedema and death. Diagnosed retrospectively.	Dead	Not available	Not applicable
2- Proband	Febrile illness with encephalopathy, seizures, cardiac failure and death at 14 months. Diagnosed retrospectively.	Dead	Not available	Not applicable
2- Half-brother	Asymptomatic/clinically normal with normal ECHO when investigated at 7 days because of positive family history.	Normal (7 years)	Initial 2.9/6.7 Last 43/57	100/140
3-Proband	Failure to thrive at 5 months. Normal development on L-carnitine.	Normal (3 years)	Initial N/A Last 47/57	180/150
3- Brother	Diagnosed at 4 years because of positive family history. Slow weight gain in early life. Now hypermobile, otherwise normal.	Normal (3 years)	Initial 1.7/2.6 Last 42/55	105/125
3- Mother	Diagnosed at 29 years because of positive family history. Reduced physical strength in childhood/adolescence. ECHO normal, 2 normal pregnancies.	Normal (3 years)	Initial 3.5/4.4 Last 40/53	110
3- Mother's brother	Diagnosed at 19 years because of positive family history. Encephalitis-like episode at 2 years of age, recovered fully. Complained of fatigue. ECHO normal. Less tired after supplementation with L-carnitine.	Normal (3 years)	Initial 2.1/2.4 Last 24/34	175

* Reference range (μmol/L): free carnitine 19-60; total carnitine 30-73

Yamak et al. (2007) described four patients from two families who all had dilated cardiomyopathy as the only clinical phenotype associated with CTD.[6] All four patients were homozygous for the R254X mutation.

Table 9: Four patients from two families who all had dilated cardiomyopathy as the only clinical phenotype associated with CTD as described in Yamak et al. (2007)[6]

Patient	Clinical symptoms	Treatment and outcome	Mutation
1*	Dilated cardiomyopathy (age at diagnosis not reported)	150mg/kg/day oral L-carnitine. Complete resolution of dilated cardiomyopathy and resolution of the left ventricular systolic dysfunction and mitral insufficiency (follow-up not reported)	Homozygous for the null mutation R254X
2*	Dilated cardiomyopathy (age at diagnosis not reported)	150mg/kg/day oral L-carnitine. Complete resolution of dilated cardiomyopathy and resolution of the left ventricular systolic dysfunction and mitral insufficiency (follow-up not reported)	Homozygous for the null mutation R254X
3*	Dilated cardiomyopathy (age at diagnosis not reported)	150mg/kg/day oral L-carnitine. Complete resolution of dilated cardiomyopathy and resolution of the left ventricular systolic dysfunction and mitral insufficiency (follow-up not reported)	Homozygous for the null mutation R254X
4	Dilated cardiomyopathy (age at diagnosis not reported)	150mg/kg/day oral L-carnitine. Normal cardiac function at 6 years of age	Homozygous for the null mutation R254X

*sisters

Lee et al. (2010) described eight patients with CTD that presented clinically in Taiwan (see Table 10).[7] The clinically diagnosed cases presented at between 6 months and 20 years with cardiomyopathy, hyperammonaemia and/or Reye-like syndrome.

Table 10: Age, gender and symptoms of cases of CTD described in Lee et al. (2010).[7]

Case	OCTN2 mutations allele1/allele2	Age at diagnosis	Gender	Symptoms
Clinically presenting case 1	R254X/IVS3 + 1G>A	20 years	Male	Cardiomyopathy, hyperammonaemia, Reye-like syndrome
Clinically presenting case 2	R254X/Y378X	6 months	Female	Reye-like syndrome
Clinically presenting case 3	R254X/R254X	2 years 5 months	Male	Cardiomyopathy, hyperammonaemia, Reye-like syndrome, metabolic acidosis
Clinically presenting case 4	R254X/R254X	1 year 2 months	Male	Cardiomyopathy, hyperammonaemia, hypoglycaemia, Reye-like syndrome, metabolic acidosis
Clinically presenting case 5	R254X/R254X	1 year 7 months	Female	Reye-like syndrome
Clinically presenting case 6	ND/ND	6 months	Male	Cardiomyopathy, hyperammonaemia, hypoglycaemia, Reye-like syndrome, metabolic acidosis
Clinically presenting case 7	F17L/R471C	5 years 3months	Female	Hyperammonaemia, hypoglycaemia, Reye-like syndrome

Clinically presenting case 8	G234R/R254X	8 years 7 months	Female	Hyperammonaemia, hypoglycaemia, Reye-like syndrome

ND not detected/mutation not found

Kilic et al. (2012) described eight Turkish patients with CTD (see Table 11).[8] Six patients (patients 1, 2, 3, 4, 5 and 7) presented with signs and symptoms of heart failure, cardiomyopathy and low carnitine levels, in addition to symptoms of malaise, easy fatigability, anorexia (loss of appetite) and anaemia (in five patients) at between one month and 54 months of age. One of the patients (patient 4) also presented with facial dysmorphia, microcephaly, and developmental delay. Two asymptomatic siblings were also diagnosed with CTD (patients 6 and 8) Echocardiography revealed left ventricular dilation in one of the asymptomatic siblings (patient 6), and the other patient had difficulty in walking and climbing stairs (patient 8). All patients were supplemented with L-carnitine. The clinical features of the patients, before and after one year of L-carnitine supplementation, are also shown in Table 11. L-carnitine supplementation improved clinical symptoms in all symptomatic patients: brain natriuretic peptide levels, which reflect the degree of heart failure, decreased and ejection fractions increased.

Table 11: Clinical and biochemical features of eight Turkish patients with CTD reported in Kilic et al. (2012)[8]

Patient	Mutation in OCTN2 allele 1/ allele 2	Age (months)/ Gender	Age at onset/ age at diagnosis (months)	Cardiomyopathy type	Plasma free carnitine levels (before and after treatment, μmol/L)	BNP levels (before and after treatment pg/ml)	Ejection Fraction % (before and after treatment)	Mental retardation (mild)
1	T337Pfs12X/ T337Pfs12X (null)	69/ Female	54/59	Dilated cardiomyopathy	1.36/10.17	986/50	37/69	-
2	G411V/ G411V (missense)	57/ Male	1/9	Hypertrophic cardiomyopathy	3.96/9.26	-/-	49/79	+
3	R282X/ R282X (null)	77/ Female	6/10	Dilated cardiomyopathy	1.19/19.89	175/33.5	46/85	-
4	R289X/ R289X (null)	130/ Male	15/119	Dilated cardiomyopathy	2.22/24.81	134/60	34/72	+
5†	G152R / G152R (missense)	145/ Female	4/124	Dilated cardiomyopathy	1.37/15.74	340/12.6	22/67	-
6†	G152R/ G152R (missense)	101/ Female	75/75	Dilated cardiomyopathy	2.75/19.12	21.7/35	63/63	-
7‡	R254X/ R254X (null)	104/ Female	24/89	Dilated cardiomyopathy	1.45/16	986/13.5	37/71	-
8‡	R254X/ R254X (null)	82/ Male	66/67	Normal	6.76/17.94	28.3/90	76/76	+

Abbreviations: fs, frameshift; BNP brain natriuretic peptide

†, ‡ siblings

Cases identified by newborn screening

Free (C0) carnitine is the screening marker for CTD. Several countries screen for CTD by assaying levels of C0 in the newborn bloodspot using tandem mass spectrometry (MS).

Schulze et al. (2003) reported on the newborn screening programme in Germany between April 1998 and September 2001, during which time 250,000 babies were screened.[9] The recommended time of sampling was between the third and the seventh day of life. One case of CTD was detected. This case was asymptomatic at diagnosis. Treatment started at 60 days of age and the patient remained asymptomatic over 15 months of follow-up.

Lund et al. (2007) also described three patients with CTD from two families who were diagnosed by prospective neonatal tandem MS screening, or by family screening clinically after a proband was identified in the Faroe Islands (see Table 12).[5] All of the patients apart from one were homozygous for the 95 A>G mutation in *SLC22A5*, which leads to a N32S substitution in OCTN2. The other child was heterozygous for the mutation (other mutation unknown). All the patients were asymptomatic and remained asymptomatic.

Table 12: Description of 3 patients with CTD identified via newborn screening or family screening after newborn screening as reported in Lund et al. (2007).[5]

CTD family	Clinical/paraclinical details	Clinical status at last follow-up (period of follow-up)	Plasma free/ total carnitine at presentation and last follow-up*	Carnitine dose at initial/last follow-up (mg/kg/day)
4- Proband	Asymptomatic when diagnosed via newborn screening.	Normal (0.5 years)	Initial 2.4/2.9 Last 24/-	100
4- Sister	Asymptomatic when diagnosed at 4 years because of positive family history, remains asymptomatic.	Normal (0.5 years)	Initial 3.5/- Last 31/-	100
5- Proband†	Identified by newborn screening. Clinical details not reported	Not reported	Not reported	Not reported

* Reference range (μmol/L): free carnitine 19-60; total carnitine 30-73

† heterozygous for the 95A>G mutation

Lee et al. (2010) also described five newborns who were diagnosed with CTD by newborn screening in Taiwan (bloodspots obtained 48 hours after birth), in addition to the eight clinically detected cases.[7] Symptoms and age at diagnosis are detailed in Table 13. Newborns identified by screening were asymptomatic at diagnosis, in contrast to the clinically diagnosed cases who presented with cardiomyopathy, hyperammonaemia or Reye-like syndrome. The newborn screening identified cases one, two and five received carnitine supplementation immediately after diagnosis, and a trial discontinuation at three months of age saw free carnitine levels drop rapidly. Case three did not take carnitine but was reported to be well. Case four had irregular medication before 18 months of age and had several episodes of Reye-like syndrome.

Table 13: Age, gender and symptoms of cases of CTD described in Lee et al. (2010).[7]

Case	OCTN2 mutations allele1/allele2	Age at diagnosis	Gender	Symptoms
Newborn screening 1	R254X/S362L	78 days	Female	Asymptomatic

Newborn screening 2	R254X/P143L	21 days	Female	Asymptomatic
Newborn screening 3	R254X/ND	19 days	Male	Asymptomatic
Newborn screening 4	ND/ND	18 days	Female	Asymptomatic
Newborn screening 5	ND/ND	30 days	Female	Asymptomatic

ND not detected/mutation not found

Couce et al. (2011) reported on the newborn screening programme in Galicia, Spain between July 2000 and July 2010.[10] One case of CTD was identified by newborn screening (samples collected between five and eight days of life up to and including 2002, and on three days of life thereafter). This case was asymptomatic at diagnosis, and was free of symptoms at follow-up (length of follow up not reported).

Lindner et al. (2011) described newborn screening for metabolic diseases in South-West Germany.[11] Over a period of ten years, three cases of CTD were identified by newborn screening (between day three and five of life for one portion of the study, and then between 36 and 72 hours), and were treated and monitored. None of the neonates were symptomatic at diagnosis. During follow-up of patients with metabolic diseases for an average of 3.3 years, no patients with CTD were reported to have experienced metabolic decompensations.

Lund et al. (2012) described the clinical status of children with true positive results identified by expanded newborn screening in Denmark, the Faroe Islands and Greenland between February 2002 and March 2011.[12] Between February 2002 and February 2009 the recommended age for obtaining a screening sample was four to nine days after birth, and the median age of children screened was five days. After February 2009 the recommended age for obtaining a screening sample was two to three days, and the median age of children screened was two and a half days. From the 504,049 newborns screened five newborns with CTD were identified. Four children were asymptomatic at diagnosis. Delayed confirmatory testing caused the late start of treatment (at 18 months) in one case of CTD and this child had failure to thrive and motor delay, but she was well at last follow-up, at age five. All children with CTD were asymptomatic at the last follow-up.

Waisbren et al. (2013) described the neuropsychological outcomes of children diagnosed with fatty acid oxidation disorders through newborn screening in Massachusetts, US over a period of 12.5 years (day of screening not reported).[13] The aim of this study was to determine whether fatty acid oxidation disorders were associated with developmental delays and neuropsychological impairments. Developmental concerns were noted in 29% of the patients with CTD.

During the period of the study seven children were diagnosed with CTD (five girls, two boys). All children with CTD were treated with L-carnitine. Two children had developmental concerns, (they received early intervention or special education services for motor, language or learning issues): one had speech concerns and both had motor concerns. Three children had IQ/DQ measurements, none of whom had cognitive delay (defined as IQ score >1 standard deviation below population norm). The characteristics of the seven children with CTD are shown in Table 14. This retrospective study concluded that screening for developmental delays, neuropsychological deficits and behavioural problems in children with fatty acid oxidation disorders is warranted.

Table 14: Neuropsychological outcomes for seven children with CTD (all treated with L-carnitine) identified through newborn screening reported in Waisbren et al. (2013)[13]

Case	Gender	Age at developmental and neuropsychological testing or when last seen	DQ/IQ†	Developmental concerns
1	Female	3 years 2months	NE	None noted
2	Female	15 months	120 (DQ)	Motor delay
3	Male	6 months	NE	None noted
4	Male	3 years 5months	105 (IQ)	Speech/language and motor delays
5	Female	22 months	NE	None noted
6	Female	4 years 2months	113 (IQ)	None noted
7	Female	4 years 2months	NE	None noted

† Most recent developmental quotient or intelligence quotient reported.
DQ, developmental quotient; IQ, intelligence quotient; NE not evaluated

Mothers identified by newborn screening

Carnitine levels in the newborn period are strongly influenced by maternal C0 levels, as carnitine can be transported through the placenta.[14] Low carnitine levels may reflect a false-positive result in the infant but a true-positive diagnosis of CTD in the mother.[14] There has also been concern that a newborn with CTD may have normal carnitine levels due to transplacental transport of free carnitine from an unaffected mother, resulting in false negatives.[7]

Several studies reported on the identification of mothers via newborn screening. The majority of these women were asymptomatic at diagnosis.

La Marca et al. (2008) reported that newborn screening of 160,000 infants (time of sampling 48 to 72 hours) in Tuscany led to the identification of one maternal case of CTD.[15]

Vijay et al. (2006) reported the identification of four mothers as the result of two screening programmes: umbilical cord screening at Bradford Royal Infirmary between February 2003 and August 2005 and heel-prick screening collected at 7 days from all infants from the West Yorkshire newborn screening region (March 2004 to August 2005).[16] Some infants would have been part of both screening programmes. Four infants were identified who had cord blood or newborn sample carnitine concentrations less than 2μmol/L, and repeat samples less than 3μmol/L, and were provisionally diagnosed with CTD, and treated with oral L-carnitine at a dose of 100mg/kg per day. Blood carnitine levels rose to within the normal range, which is unusual, as normally carnitine levels remain low despite supplementation in cases of CTD due to persistent renal loss. Mothers were therefore investigated. All four mothers were found to have free carnitine less <1.2μmol/L. All were previously healthy, although two complained of occasional fatigue. Electrocardiograms were normal, with no evidence of cardiomyopathy. Fibroblast studies of fatty acid oxidation and carnitine uptake confirmed CTD. One infant was also found to have CTD.

Schimmenti et al. (2007) reported on six women diagnosed with CTD though their unaffected infants having low free carnitine levels on newborn screening (in Minnesota [3], Germany [1], California [1], and South Carolina [1]).[17] Three women were asymptomatic, two women had decreased stamina, easy fatigability with exercise, and in one case fasting intolerance. None had heart anomalies. One mother was symptomatic, with ventricular tachycardia. All symptoms, including cardiac function, improved with carnitine therapy.

El-Hattab et al. (2010) described five families in which low free carnitine levels in the infants' newborn screening led to the diagnosis of maternal CTD.[18] Three mothers were asymptomatic, one had decreased stamina during pregnancy and one had mild fatigability and developed preeclampsia. Only one infant was found to have CTD.

Lee et al. (2010) also reported that seven mothers were diagnosed with CTD by newborn screening in Taiwan.[7] Symptoms and age at diagnosis are detailed in Table 15. The majority of mothers were asymptomatic, but one mother had cardiomyopathy and heart size decreased after three months carnitine supplementation, and one mother reported being more energetic with carnitine supplementation.

Table 15: Age, gender and symptoms of cases of CTD described in Lee et al. (2010).[7]

Case	OCTN2 mutations allele1/allele2	Age at diagnosis	Gender	Symptoms
Maternal 1	S467C/Y396X	26 years	Female	Asymptomatic
Maternal 2	R254X/S467C	32 years	F	Asymptomatic
Maternal 3	R282Q/S467C/S467C	34 years	F	Cardiomyopathy
Maternal 4	F17L/F17L	31 years	F	Asymptomatic
Maternal 5	F17L/S467C	24 years	F	Asymptomatic
Maternal 6	R254X/ND	31 years	F	Less energetic
Maternal 7	R254X/S467C	33 years	F	Asymptomatic

ND not detected/mutation not found

Sarafoglou et al. (2010) reported an asymptomatic mother identified through newborn screening of her child.[19]

Newborn screening also identified eight mothers with CTD in Denmark, the Faroe Islands and Greenland between February 2002 and March 2011 (Lund et al. [2012][12]). Prior to the diagnoses these women had no symptoms or signs of CTD, and these women were asymptomatic at last follow-up. An adult brother of a mother with CTD had hypertrophic cardiomyopathy with arrhythmia, and he was subsequently found to also have CTD.

Waisbren et al. (2013) reported that in Massachusetts, from 1999, newborn screening results in five children with normal laboratory values on confirmatory testing led to the identification of their mothers' CTD.[13] One mother was found to be a carrier and experienced chronic fatigue until beginning carnitine treatment. The other mothers, who were found to have CTD, were asymptomatic. All were treated with carnitine and achieved near normal metabolic status.

Distribution of phenotypes

The only study which estimated the distribution of phenotypes was a cost-effectiveness analysis by Cipriano et al. (2007), discussed in Criterion 16.[20] This study estimated that 50% of cases were neonatal, classical, severe or early-onset, 50% of cases were later-onset, chronic or mild, and 0% of cases were mild variations that would not be detected or treated without newborn screening. This distribution was based on the results of a review of mitochondrial fatty acid oxidation disorders, and it is unclear whether this reflects the true distribution of phenotypes.

Summary

- Untreated CTD can be lethal.

- The age of onset of symptoms and the presenting features of CTD varies. Presenting features include cardiomyopathy, coma, hypoglycaemic encephalopathy, anaemia, motor delay, myopathy, hepatomegaly and/or failure to thrive.
- Symptoms can be improved/reversed with carnitine supplementation, and patients who are diagnosed go on to have favourable clinical outcomes.
- Cases identified by newborn screening have been asymptomatic at diagnosis, and after prompt diagnosis have remained asymptomatic with carnitine supplementation.
- Newborn screening has identified maternal cases of CTD. The majority of these cases are asymptomatic. The identification of these cases suggests that there is a form of CTD that does not present clinically. However, newborn screening may identify newborns with this form.
- Knowledge of CTD is based on a few studies describing small numbers of patients with CTD.

Genotype or biochemical phenotype correlation with clinical phenotype

A number of studies have investigated whether clinical phenotype can be predicted from genotype or residual carnitine transport activity (biochemical phenotype).

There does not seem to be either a genotype-phenotype correlation or an enzyme activity-phenotype correlation.

Wang et al. (2001) described five patients from four families (described under natural history, see Table 3.[1] The phenotype and age of onset was similar for patients 268, 909, Jer-T, JGSQ, despite the fact that patient Jer-T was homozygous for a missense mutation, patient 909 was compound heterozygous for a missense and null mutation, and patients 268 and JGSQ were homozygous for null mutations. In addition, two sisters with the same mutation (Jer-T and Jer-H; R399Q) had different symptoms: one presented early in life with hypoglycaemic coma and one with developmental delays.[1]

Lamhonwah et al. (2002) (described under natural history, see Table 4) reported there was no clear correlation between residual carnitine uptake and severity of clinical presentation or age of onset, and that a larger series of cases would be required to draw conclusions regarding the severity of the biochemical phenotype arising from specific mutations.[2]

In Lund et al. (2007) (described under natural history, see Table 8 and Table 12) the natural history of patients varied, ranging from patients who had died from their disease to asymptomatic individuals, despite the fact that ten of the patients were homozygous for the same missense mutation in *SLC22A5*.[5]

No Genotype-phenotype relationship was reported in Kilic et al. (2012) (described in the natural history section, see Table 11).[8] Siblings with the same mutation had different ages of onset of symptoms and different disease progressions. For example patients 6 and 8 in this case series were asymptomatic (although echocardiography revealed left ventricular dilation in one patient) when identified by family screening. By the age that they were diagnosed, their siblings, carrying the same mutation, had clinical symptoms

However, some mutations have been linked to specific phenotypes by some authors. Yamak et al. (2007) described four patients from two families who all had dilated cardiomyopathy as the only clinical phenotype associated with CTD (see the natural history section, Table 9).[6] All four

patients were homozygous for the R254X mutation. The authors report that all cases with the R254X mutation that have been reported have dilated cardiomyopathy.

Rose et al. (2012)[21] tried to look at the differences between mothers identified by newborn screening and patients who presented clinically.

They analysed cell lines from 14 mothers identified by newborn screening and 14 symptomatic patients who presented between five months and six years of age with cardiomyopathy (n=7), hypoglycaemia (n=4) and Reye syndrome (n=3).

Carnitine transport in fibroblasts from the asymptomatic mothers and symptomatic patients were assayed and compared with carnitine transport in fibroblasts from six controls. On average, carnitine transport was lowest in symptomatic patients (2.9% of the control levels), and was significantly lower than carnitine transport in controls and in mothers identified by newborn screening ($p<0.01$). Carnitine transport was also significantly lower in mothers identified by newborn screening compared to controls (7.4% of the control levels, $p<0.01$). However, analysis of individual patients found that carnitine transport was higher in some symptomatic patients than in some mothers identified by newborn screening.

Mutation analysis revealed that there was an increased frequency of nonsense mutations in symptomatic patients compared to mothers identified by newborn screening (two sided $p=0.0008$ with Fisher's exact test). None of the mothers identified by newborn screening had null mutations in both alleles. See Table 16.

Table 16: Mutations in alleles from symptomatic cases and mothers identified by newborn screening

	Nonsense/ Frameshift	Missense/ in frame deletion	Splicing/ others*	Total
Symptomatic	16	12	0	28
Mothers identified by newborn screening	2	20	6	28
Total	18	32	6	28

*This includes mutations which were not identified. Mutations in only one allele could be found in three mothers identified by newborn screening, but these patients' cells had markedly reduced carnitine transport.

At least some of the missense mutations identified in the mothers identified by newborn screening had reportedly been identified in symptomatic patients. The researchers explored the effect of the missense mutations identified in symptomatic patients and mothers identified by newborn screening on carnitine transport by testing the carnitine transport of recombinant OCTN2. No significant difference was observed in the average transport activity measured in cells expressing missense mutations identified in symptomatic patients versus mothers identified by newborn screening. This led the researchers to conclude that the reduced carnitine transport in fibroblasts from symptomatic women must be due to the increased frequency of nonsense/frameshift mutations rather than the type of missense mutation.

Summary

- No clear genotype/phenotype correlation. Patients with the same mutations from the same family can have different presentations
- Environmental factors or other genes may play a role.
- No clear enzyme activity/phenotype correlation

- There is a suggestion that mothers identified by newborn screening have higher carnitine transport on average and do not have null mutations in both alleles.

Detectable disease marker

CTD can be screened for by tandem MS on dried blood spots already collected as part of newborn screening. In CTD defects in the carnitine transporter lead to reduced levels of plasma carnitine. As discussed in Criteria 5 and 6, the National Academy of Clinical Biochemistry (NACB) practice guidelines, the New York Mid-Atlantic Consortium (NYMAC) for Genetic and Newborn Screening Services and the American College of Medical Genetics (ACMG) diagnostic algorithm all state that low levels of free (C0) carnitine is the marker for CTD.[22-24] Levels of other acylcarnitines (fatty acid esters of carnitine, which are formed so that fatty acids can enter mitochondria) are also abnormal in CTD, and screening programmes have used levels of other acylcarnitines as secondary markers (see Table 21).

Levels of free carnitine (C0) are also lower than normal (median values in cases below the 1st percentile in the normal population) in maternal 3-methylcrotonyl-CoA carboxylase deficiency, maternal medium-chain acyl-CoA dehydrogenase deficiency, and maternal glutaric acidaemia type I, as well as maternal cases of CTD.[25]

Summary

- Free carnitine (C0) is the marker for CTD

Latent period

The age of onset of symptoms of CTD varies.

In the reports of clinically presenting patients included in this update review, the earliest age of symptom onset was eight days.

To see whether newborn screening programmes have been able to identify cases of CTD before they become symptomatic, newborn cases identified by newborn screening have been reviewed to see if they were symptomatic at the time of diagnosis. .Many of the studies which reported whether cases identified by newborn screening were symptomatic at diagnosis have already been described in the natural history section, but are summarised in Table 17. Only one child of the 26 children was symptomatic at the time of diagnosis. In this case it was reported that delayed confirmatory testing caused late start of treatment (at 18 months), and this child had failure to thrive and motor delay.

Table 17: Proportion of cases of CTD identified by newborn screening programmes that were symptomatic at diagnosis (cases of maternal CTD were not considered)

Study	Age at screening	Proportion of newborn cases that were symptomatic at diagnosis or who died before diagnosis	Age at onset of symptoms
Schulze et al. (2003)[9]	3 to 7 days	0/1	
Wilcken et al. (2003)[26]	48 to 72 hours	0/3	
Hoffmann et al. (2004)[27]	NR	0/1	
Amat di San	NR	0/1	

Filippo et al. (2006)[4]			
Lund et al. (2007)[5]	NR	0/2	
Lee et al. (2010)[7]	48 hours	0/5	
Couce et al. (2011)[10]	5 to 8 days (before 2003) 3 days (after 2003)	0/1	
Lindner et al. (2011)[11]	3 to 5 days (before 2002) 36 to 72 hours (after 2002)	0/3	
Lund et al. (2012)[12]	4 to 9 days (February 2002 to February 2009) 2 to 3 days (after February 2009)	1/5	Unclear*
TOTAL		1/26	

*Delayed confirmatory testing caused late start of treatment (at 18 months), and this child had failure to thrive and motor delay.

Summary

- Reports from newborn screening programmes suggest that CTD can be detected before the onset of symptoms.

Incidence

No studies reporting the incidence of CTD in the UK were identified in the update search.

The results of studies reporting the incidence of CTD in other countries are shown in Table 18. CTD is also screened for by several newborn screening programmes, and the results of the screening programmes can be used to estimate incidence. Newborn incidence ranged from 1:5,924 (in Denmark, the Faroe Islands and Greenland, without screening)[12] to 1:775,600 (in Australia, without screening)[28].

Has incidence changed with screening?

Lund et al. (2012) reported the incidence of CTD in Denmark, the Faroe Islands and Greenland.[12] Five newborns were identified by screening (1:100,809). There were six cases missed by screening. Among the unscreened cohort, 14 cases were identified (1:5,924). However, 13 of these cases were diagnosed by "population screening" and one because of hypoglycaemia.

Wilcken et al. (2009) reported that the incidence of CTD was 1:775,600 in an Australian cohort of unscreened infants born between 1994 and 2002 who were either born before the introduction of screening or were born in a region that had not yet introduced screening.[28] In the screened cohort (born between 1998 and 2002), the incidence of CTD was 1:153,833.

Hoffmann et al. (2004) compared the frequency of ten potentially treatable conditions, including CTD, presenting with clinical symptoms and/or referred for metabolic testing in one area of Germany, with the frequency of the disorders in a cohort of newborns who were screened in Bavaria and Baden Württemberg between January 1999 and December 2000.[27] No cases of CTD presented clinically during this period (from 844,575), but one case was diagnosed by tandem MS screening (from 382,247).

Wilcken et al. (2003) compared rates of CTD diagnoses after the introduction of tandem MS (1998 to 2002) with historical rates in New South Wales and the Australian Capital Territory.[26] The historical incidence of CTD varied: no cases were diagnosed between 1974 and 1978, 1978 and 1982 or 1982 and 1986. However, between 1990 and 1994 one case was diagnosed

(1:378,000) and between 1986 and 1990 and 1994 and 1998 two cases were diagnosed (1:180,000 and 1:183,500, respectively). Screening identified three cases (1:120,667).

Summary

- The reported incidence of CTD varies. It is unclear if newborn screening has increased the incidence of CTD (which might have suggested that newborn screening is either identifying asymptomatic cases which would never have presented clinically, cases which die without diagnosis or both).

Criterion 2 partially met. The age of onset of symptoms and the presenting features of CTD varies. Presenting features include cardiomyopathy, coma, hypoglycaemic encephalopathy, anaemia, motor delay, myopathy, hepatomegaly and/or failure to thrive. CTD can be lethal, but symptoms can be improved/reversed with carnitine supplementation, and patients who are diagnosed go on to have favourable clinical outcomes. There is no clear genotype/phenotype correlation or enzyme activity/phenotype correlation.

CTD can be screened for by tandem MS on dried blood spots already collected as part of newborn screening. Low levels of free carnitine (C0) is the marker for CTD. Cases identified by newborn screening have been asymptomatic at diagnosis, and have remained asymptomatic with carnitine supplementation. However, carnitine levels in the newborn period are strongly influenced by maternal C0 levels, as carnitine can be transported through the placenta. Newborn screening has identified maternal cases of CTD. The majority of these cases are asymptomatic. The identification of these cases suggests that there is a form of CTD that does not present clinically.

The lack of clear genotype/phenotype or enzyme activity/phenotype correlation means that there is currently no way of predicting the phenotype/prognosis of cases identified by newborn screening, and there is the possibility that at least some cases might have a form of CTD that would never present clinically.

The reported incidence of CTD varies. It is unclear if newborn screening has increased the incidence of CTD (which might have suggested that newborn screening is either identifying asymptomatic cases which would never have presented clinically, cases which die without diagnosis, or both). The incidence of CTD in the UK has not been described, and it is difficult to estimate the distribution of phenotypes.

Table 18: Incidence of newborn CTD

Study	Country	Start date	End date	Number screened/ Number of births	Number of cases of CTD	Incidence	Screening programme in place?	Notes
Wang et al. (2013)[29]	Taiwan	6 months		30,237	1	1:30,000 or greater	Yes	
Couce et al. (2011) [10]	Spain	July 2000	July 2010	210,165	1	1:210,165	Yes	
Lund et al. (2012)[12]	Denmark, the Faroe Islands and Greenland	February 2002	April 2011	504,049	5	1:100,809	Yes	Incidence in screened infants. During this period eight maternal cases of CTD were also identified via newborn screening
		February 2002	April 2011	82,930	14†	1:5,924	No	Incidence in unscreened infants during the period with expanded newborn screening
		February 2002	April 2011	586,979	25	1:23,479	Among screened and unscreened (including 6 false negatives)	Combined incidence among screened and unscreened newborns during the period of expanded newborn screening
		01/01/1992	31/12/2001	674,754		N/A	No	Frequency in

Study	Country	Start date	End date	Number screened/ Number of births	Number of cases of CTD	Incidence	Screening programme in place?	Notes
								decade before expanded newborn screening (only clinically diagnosed)
Lindner et al. (2011)[11]	Germany	January 1999	April 2005	583,553	3 + 1 lost to follow-up before diagnosed	1:194,518	Yes	Incidence calculation based on 3 confirmed cases
Kasper et al. (2010)[30]	Austria	April 2002	December 2009	622,489	2	1:311,245	Yes	
Lee et al. (2010)[7]	Taiwan	January 2007	July 2009	202,076	3	1:67,000	Yes	During this period 6 maternal cases were identified
Wilcken et al. (2009)[28]	Australia	1994	1998	1,017,800	2	1:508,900	No	
		1998	2002	533,400	0		No	
		1994	2002	1,551,200	2	1:775,600	No	Unscreened (all)
		1998	2002	461,500	3	1:153,833	Yes	
la Marca et al. (2008)[15]	Tuscany, Italy	January 2002	January 2008	160,000	0		Yes	During this period 1 maternal case was identified by screening
Hoffmann et al. (2004)[27]	Germany (Bavaria, Baden Wurternberg)	January 1999	December 2000	382,247	1	1:382,247	Yes	
Wilken et al. (2003)[26]	New South Wales and the Australian	1974	1978	336,000	0		No	
		1978	1982	331,000	0		No	
		1982	1986	349,000	0		No	

Study	Country	Start date	End date	Number screened/ Number of births	Number of cases of CTD	Incidence	Screening programme in place?	Notes
	Capital territory	1986	1990	360,000	2	1:180,000	No	
		1990	1994	378,000	1	1:378,000	No	
		1994	1998	367,000	2	1:183,500	No	
		1998	2002	362,000	3	1:120,667	Yes	
Schulze et al. (2003)[9]	Germany	April 1998	September 2001	250,000	1 (+2 suspected*)	1:250,000	Yes	

†Of the 14 unscreened cases, 13 were diagnosed by population screening and one because of hypoglycaemia.
*These infants were judged positive on newborn screening and confirmed by recall, but a definite diagnosis remained questionable either because the diagnosis is difficult to achieve or because they were lost to follow-up.

3. All the cost-effective primary prevention interventions should have been implemented as far as practicable

Not applicable

4. If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.

Newborn screening for CTD can identify asymptomatic homozygous mothers and heterozygous infants. This may in turn lead to the identification of other homozygous and heterozygous family members through cascade testing. Screening can also identify carriers (heterozygous) without identifying a homozygote. For example, Wang et al. (2013) reported that during newborn screening in Taiwan, 209 infants were given false positive results, 11 of which were heterozygotes for the pR254X mutation.[29] Lund et al. (2012) (described in Criterion 2) stated that during biochemical screening of 504,049 infants in Denmark, the Faroe Islands and Greenland, 28 newborns had false positive results, four of which had been born by *SLC22A5* heterozygous mothers and/or were themselves heterozygous.[12]

As CTD is a recessive disorder, it is expected that heterozygotes will be asymptomatic.

It has been suggested that heterozygotes may be at risk of cardiomyopathy and other symptoms.However, Amat di San Filippo et al. (2008) found that heterozygosity for CTD is not more frequent in patients with unselected types of cardiomyopathy, suggesting that it is unlikely to be an important cause of cardiomyopathy in humans.[31] This was based on an analysis of the frequency of mutations in the *SLC22A5* gene in 324 patients with cardiomyopathy that, when expressed in recombinant OCTN2, affected carnitine transport. Two variants affecting carnitine transport were identified in patients with cardiomyopathy (2/648 alleles). This was compared with the frequency of variants reported in another publication that had looked at the frequency of variants in 270 unrelated healthy persons in the San Francisco Bay Area as part of the Studies of Pharmacogenetics in Ethnically Diverse Populations (SOPHIE). This publication identified two variants that affected recombinant OCTN2 carnitine transport in the Amat di San Filippo et al. test system. These variants were found a total of three times (3/540 alleles). The frequency of variants that affected OCTN2 transport was not significantly different in this healthy population compared to the frequency in patients with cardiomyopathy.

Other studies described in Criterion 2 have reported that heterozygotes have displayed symptoms. Sarafoglou et al. (2010) suggest that heterozygous individuals may become symptomatic, for example under oxidative stress.[19] They reported on three generations of a family: an asymptomatic heterozygous infant identified through newborn screening, an asymptomatic mother with CTD (identified through newborn screening of the child) and the heterozygous maternal grandparents who reported some cardiac symptoms, which overlap with CTD and that improved with L-carnitine supplementation. Waisbren et al. (2013) reported that one mother identified by newborn screening to be a carrier experienced chronic fatigue until beginning carnitine treatment.[13] Vijay et al. (2006) reported on one infant who was categorised as a carrier for CTD who had deterioration of appetite and weight without carnitine supplementation.[16]

No studies were identified exploring the psychological implications arising from the identification of heterozygosity.

Criterion 4 not met. Screening can detect heterozygotes. The natural history of heterozygotes is not well understood.

5. There should be a simple, safe, precise and validated screening test

CTD can be screened for using tandem MS performed on carnitine and acylcarnitines extracted from dried blood spots on Guthrie cards.

CTD is part of the core panel of conditions that are screened for in the US.

A 2012 report on the practices of newborn screening for rare disorders in member states of the European Union, candidate, potential candidate and European Free Trade Association countries found that CTD is screened for in six countries (Austria, Denmark, Hungary, Portugal, Spain and Iceland) and some regions of Italy (see Table 19).[32]

Markers for CTD

The National Academy of Clinical Biochemistry (NACB) practice guidelines, the New York Mid-Atlantic Consortium (NYMAC) for Genetic and Newborn Screening Services and the American College of Medical Genetics (ACMG) diagnostic algorithm all state that low levels of free (C0) carnitine is the marker for CTD.[22-24] Levels of other acylcarnitines (fatty acid esters of carnitine, which are formed so that fatty acids can enter mitochondria) are also abnormal in CTD, and screening programmes have used levels of other acylcarnitines as secondary markers (see Table 21 and Table 22).

Timing of the screening test

The timing of blood collection reported in publications describing screening programmes is shown in Table 21. The timing of the screening test in the seven countries that reported screening for CTD in the 2012 report on the practices of newborn screening for rare disorders in member states of the European Union, candidate, potential candidate and European Free Trade Association countries is shown in Table 19.

Table 19: Timing of blood spot sampling in member states of the European Union, candidate, potential candidate and European Free Trade Association countries that screen for CTD.[32]

Country	Sample time
Austria	36-72 hours
Denmark	48-72 hours
Hungary	48-72 hours
Italy*	48-96 hours
Portugal	48-96 hours
Spain	48 hours – 7 days
Iceland	48-72 hours

*varies by region

Wilcken et al (2001) looked at carnitine levels in the neonatal population at different ages in Australia (see Table 20).[33] They concluded that "there is not a sufficient change in whole blood free carnitine levels from day 2 to day 8 that would dictate different action levels for newborn screening dependent on day of screening."

Table 20: Free carnitine levels (μmol/L whole blood) in the neonatal population, measured by tandem mass spectroscopy

Neonate	Number	Free	Free	Number (%)	Number	Number

class		carnitine median	carnitine 5th and 85th centiles	<10μmol/L	<7μmol/L	<5μmol/L
Age 2 days	13,311	26.6	14.0-52.0	98 (0.73%)	4	0
Age 3 days	79,422	26.8	14.1-51.8	500 (0.63%)	45	3
Age 4 days	51,248	27.2	14.1-52.3	330 (0.64%)	30	2
Age 5-8 days	5,546	27.5	14.3-49.6	41 (0/74%)	7	1
Birth weight <2,000g	3,264	30.5	14.0-58.6	48 (1.47%)	17	2

No publications were identified in the update search which explored whether the timing of the test could be modified so that maternal carnitine levels do not influence the outcome of the test.

Prematurity

Premature infants may have low plasma carnitine concentrations due to a lack of carnitine placental transfer in the third trimester and decreased tissue stores. In addition, immature renal tubular function could lead to increased renal carnitine elimination.[34] The protocol for screening preterm infants (where reported) for individual screening programmes is also shown in Table 21.

In Lund et al. (2012), six of the 36 false positives were born prematurely.[12]

Sensitivity and specificity of screening for CTD

Studies that reported data which allowed the sensitivity and specificity of screening for CTD using tandem MS in newborn screening programmes to be calculated are shown in Table 21. Maternal cases of CTD identified by newborn screening were considered to be false positives.

However, there are many limitations to these studies. In many cases no false negatives were reported, but extensive follow-up to ascertain whether there were false negatives was not performed. Even if follow-up was performed, it is possible that mild or asymptomatic false negative cases would not be identified.

Although C0 was used as the primary screening marker, it should also be noted that the cut-offs for screening were different, and different programmes used different panels of acylcarnitines as secondary markers.

Specificity ranged between 99% and >99.99%; and sensitivity ranged between 68% and 100%. Positive predictive value in most studies was low, ranging between 0.48% and 33.33%.

Cases missed by screening

Lund et al. (2012) reported that six newborns had false negative screening results with the screening algorithm that was active at the time they were born.[12] Five of these would have had positive results using the currently active algorithm.

Lund et al. (2007) reported that two of five probands were missed by newborn screening.[5]

Criterion 5 partly met: there is a simple and safe screening test for CTD. CTD can be screened for by tandem MS on the dried blood spot already collected as part of the newborn screening programme. In publications where it was possible to calculate the sensitivity and specificity of screening for CTD these were found to be high, although the positive predictive value of

screening varied between 0.48% and 33.33%. However, there are important limitations to these studies, including the possibility that false negatives have been missed. No studies were identified in the update search which explored whether the timing of the test could be modified so that maternal carnitine levels do not influence the outcome of the test.

Table 21: Features of newborn screening programmes for CTD. Newborn screening results which led to the identification of mothers were counted as false positives.

Study Location Period	Number of newborns screened	Age at blood collection and preterm protocol	Analytes and cut-offs (μmol/L)	True positives	False positives	True negatives	False negatives	Specificity	Sensitivity	Positive predictive value	Negative predictive value
Schulze et al. (2003)[9] Germany April 1998 to September 2001	250,000	3-7 days Premature infants: additional sample a 14 days	C0<10; total acylcarnitines (C3 to C18)<5	1 (+2 with suspected disorderwho were either difficult to diagnose or lost to follow-up)	86	249,913	0	99.97%	100%	1.15%	100%
Frazier et al. (2006)[35] North Carolina 2003 to 2004	239,415	At least 24 hours. The mean age at sampling was 39 hours	C0≤13 (borderline cut-off)	0	0	239,415	0				100%

Study Location Period	Number of newborns screened	Age at blood collection and preterm protocol	Analytes and cut-offs (μmol/L)	True positives	False positives	True negatives	False negatives	Specificity	Sensitivity	Positive predictive value	Negative predictive value
la Marca et al. (2008)[15] Tuscany, Italy January 2002 to January 2008	160,000	48-72 hours Premature infants (<1.8kg): additional samples at 15 and 30 days Babies on parenteral nutrition: additional sample 48 hours after the ending of parenteral nutrition Transfused newborns: additional sample 7 days after the end of transfusion	C0<8 decreased total acylcarnitines	0	NR	NR	NR				

Study **Location** **Period**	**Number of newborns screened**	**Age at blood collection and preterm protocol**	**Analytes and cut-offs (μmol/L)**	**True positives**	**False positives**	**True negatives**	**False negatives**	**Specificity**	**Sensitivity**	**Positive predictive value**	**Negative predictive value**
Kasper et al. (2010)[30] Austria April 2002 to December 2009	622,489	36-72 hours	Decreased free carnitine (C0); decreased total acylcarnitines (C3 to C18)	2	NR	NR	NR				
Lee et al. (2010)[7] Taiwan January 2007 to July 2009§	202,076	48 hours of life, 24 hours of feeding Babies who were premature, given a special formula or had poor oral intake were rescreened.	C0 <8 (January 2008- July 2009) <6.44 (June 2007-December 2007) <10.95 (January 2007-May 2007)	3	110 (counting the six maternal cases as false positives)	201,963	0	99.95%	100%	2.65%	100%
Couce et al. (2011)[10] Spain July 2000	210,165	3 days (since 2002, before samples	NR specifically. Cut off for C0<9.5	1	Unclear. 2 false positive cases with	210,162	0	>99.99%	100%	33.33%	100%

Study Location Period	Number of newborns screened	Age at blood collection and preterm protocol	Analytes and cut-offs (μmol/L)	True positives	False positives	True negatives	False negatives	Specificity	Sensitivity	Positive predictive value	Negative predictive value
to July 2010		collected between the 5th and 8th day)			abnormal carnitine (have assumed that these patients screened false positive for CTD)						
Lund et al. (2012)[12] Denmark, the Faroe Islands and Greenland February 2002 to April 2011	504,049	2-3 days (2009-2011) 4-9 days (trial period 2002-2009) Preterm infants: repeated screen at 32 weeks gestational age or when oral feeding had been	C0<5.7 Secondary analyte: C5<0.43 Before 2006, low carnitine was screened for (not formally CTD). The cut-offs used were C0<7.8 (primary) and C2<6.6 (secondary)	5	36 (counting the 8 maternal cases as false positives)	504,002	6	>99.99%	45.45%	12.20%	>99.99%

Study Location Period	Number of newborns screened	Age at blood collection and preterm protocol	Analytes and cut-offs (μmol/L)	True positives	False positives	True negatives	False negatives	Specificity	Sensitivity	Positive predictive value	Negative predictive value
		established									
Wang et al. (2013)[29] Taiwan 6 months	30,237	NR	C0<12.0*	1	209	NR	NR			0.48%	

*If the cut-off was set at <6μM there were four false positives, and the true positive case was missed
†False positive results were from newborns in which CTD could not be confirmed in the child or the mother
‡The results from 6 newborns were false negative with the screening algorithm that was active at the time they were born. One false negative would have been missed with the currently active algorithm
§304,536 infants were screened between 2001 and 2005 when the cut-off for C0 was <2.6μmol/L. No cases of CTD were identified. A further 88,200 infants were screened in 2006 when the cut off for C0 was <2.86μmol/L. One newborn case of CTD was identified. Between January 2007 and May 2007 31,329 infants were screened and the cut off was set at <10.95μmol/L. No cases of CTD were identified. Between June 2007 and December 2007 the cut-off was set at <6.44μmol/L, and 1 newborn case plus 1 maternal case was identified from the 59,785 infants screened. Between January 2008 and July 2009 the cut-off was set at <8μmol/L, and 2 newborn cases plus 5 maternal cases were identified from the 110,962 infants screened. The number of false positives was available only for the period of 2007 to July 2009.
§§101 low carnitine, 12 rescreened. After recall 15 re-tested.
NR not reported

6. The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed

Distribution of carnitine and acylcarnitine levels in the target population and cut-off levels for screening for CTD

As mentioned in Criterion 2, low C0 (free carnitine) is the primary screening marker for CTD. However, levels of free carnitine (C0) are also lower than normal (median values in cases below the 1st percentile in the normal population) in maternal 3-methylcrotonyl-CoA carboxylase deficiency, maternal medium-chain acyl-CoA dehydrogenase deficiency, and maternal glutaric acidaemia type I, as well as maternal cases of CTD.[25]

In addition, as noted in Criterion 5, although C0 was used as the primary screening marker, the cut-offs for screening were different, and different programmes used different panels of acylcarnitines as secondary markers (see Table 21).

An international collaboration, the Region 4 Genetics Collaborative, has collected data on levels of individual markers and ratios in the normal population and in true positives in order to improve cut-off values for amino acids and acylcarnitines that can be detected by tandem MS.[25] The age of specimen collection was 24-48 hours in 57% of participating sites, 3 days in 34% of sites and 5 days in 9% of sites.[25] Levels and ratios of acylcarnitines indicative of CTD in the normal population and in 196 cases of CTD (and 89 maternal cases of CTD) are shown in Table 22. Markers that were considered indicative of CTD, because the median value in cases was below the 1st cumulative percentile in the normal population, were C0, C2, C3, C16, C18:1, C18 and the ratios all acylcarnitines/Citrulline and C3/Methionine.[25]

This collaboration also identified ideal cut-off ranges (where cut-offs for screening programmes should lie). However, these cut-offs were designed to identify patients with any condition (including maternal cases of CTD) that causes a particular marker to be decreased/ elevated to screen positive, rather than being specifically for CTD.

Recently, this collaboration has reported the development of multivariate pattern-recognition software designed to convert metabolic profiles into a composite score driven by the degree of overlap between the normal population and the disease range.[36] The software has been developed using the tandem MS profiles of 12,077 patients affected with 60 metabolic disorders and 644 heterozygote carriers for 12 conditions. The authors report that, as of 15 December 2011, a total of 90 active tools were available, 37 of which were applicable to the differential diagnosis of two or more conditions. The authors report that an “all conditions” tool, designed to evaluate a full amino acid and acylcarnitine profile to suggest any possible diagnosis is soon to be released. The tools are intended to generate a score that drives the interpretation and resolution of cases with potentially abnormal tandem MS results.

Criterion 6 partially met. Free carnitine (C0) is currently the primary analyte used in newborn screening programmes. The distribution of C0 and other acylcarnitines in the unaffected population and patients with CTD has been published by an international collaboration. However, an agreed cut-off level for free carnitine has not been agreed upon, and newborn screening programmes have used a range of cut-offs.

Table 22: Acylcarnitine and carnitine levels and ratios in the normal population and affected newborn cases. Levels in affected maternal cases are shown in brackets.[25]

CV coefficient of variation (standard deviation/mean); NR not reported; ACs/Cit: (C0+C2+C3+C16+C18:1)/Cit ratio
*Number of sites
**The number of cases for each condition is also provided. Differences between analyte counts related to the same condition reflect the past and current testing panels of the participating sites.

		Normal population (μmol/L)							Affected cases (μmol/L apart from ratios)			
			1 percentile		50 percentile		99 percentile			1 percentile	50 percentile	99 percentile
	Marker	N*	Value	CV	Value	CV	Value	CV	N **	Value	Value	Value
Carnitine transporter deficiency (maternal)	C_0	93	11	28%	24	25%	59	26%	193 (86)	1.9 (0.53)	6.2 (5.1)	17 (11)
	C_2	76	10	32%	23	19%	52	17%	179 (69)	2.9 (2.7)	10 (9.8)	30 (22)
	C_3	93	0.57	28%	1.75	20%	4.74	20%	173 (71)	0.079 (0.18)	0.45 (0.54)	1.3 (1.7)
	C_{16}	93	0.80	41%	2.8	18%	6.0	15%	176 (71)	0.15 (0.30)	0.88 (0.99)	2.4 (2.0)
	$C_{18:1}$	84	0.49	22%	1.2	15%	2.5	12%	150 (62)	0.053 (0.053)	0.37 (0.38)	1.6 (0.71)
	C_{18}	83	0.31	29%	0.81	16%	1.7	15%	129 (56)	0.051 (0.067)	0.27 (0.25)	0.94 (0.49)
	ACs/Cit		NR		NR		NR		116 (50)	0.51 (0.70)	1.6 (1.6)	4.5 (3.5)
	C_3/Met		NR		NR		NR		158 (67)	0.003 (0.007)	0.021 (0.021)	0.088 (0.065)

NR not reported

7. The test should be acceptable to the population

Tandem MS is performed on carnitine and acylcarnitines extracted from dried blood spots on Guthrie cards, which are already collected as part of the newborn screening programme. No studies exploring the acceptability of the test in the context of CTD were identified in the update search. Acceptability is important in this instance due to the potential of the test to identify heterozygous infants and asymptomatic homozygous mothers (see Criterion 4).

Criterion 7 uncertain. No studies exploring the acceptability of the test for CTD were identified in the update search.

8. There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals

CTD is screened for in the US. The ACMG, New York Mid-Atlantic Consortium (NYMAC) for Newborn Screening Services and the National Academy of Clinical Biochemistry (NACB) have issued guidelines on the diagnostic investigation of people who screen positive for CTD.

The ACMG, NYMAC and NACB recommend that free and total carnitine in plasma and urine are assayed.[22-24] If the plasma C0 is low and plasma total carnitine is normal/low, CTD is confirmed. The diagnosis can be confirmed by enzyme assay of OCTN2 (carnitine transport) in fibroblasts and/or mutation analysis. Maternal testing should also be considered.

It should be noted that it is unclear whether cut-offs for carnitine transport diagnostic for CTD are agreed upon, and that several studies reported in Criterion 2 did not manage to identify mutations in both alleles of *SLC22A5.*

Diagnostic follow-up in newborn screening programmes

Diagnostic follow-up tests for CTD reported in newborn screening programmes are shown in Table 23.

Table 23: Diagnostic follow-up

Study **Location** **Period**	**Follow-up/Diagnostic testing**	**Follow-up/Diagnostic testing of mothers**
Lund et al. (2012)[12] Denmark, the Faroe Islands and Greenland February 2002 to April 2011	Urine organic acids, plasma acylcarnitines, DNA, carnitine transport in fibroblasts.	All mothers of newborns with positive screening results for CTD were investigated at confirmatory testing for this disorder
Lindner et al. (2011)[11] Germany January 1999 to April 2005	Pathological tubular carnitine reabsorption, fibroblast transport studies.	NR
Kasper et al. (2010)[30] Austria	Urine organic acids, enzyme analyses in fibroblasts and/or lymphocytes, genetic testing	NR

April 2002 to December 2009		
Lee et al. (2010)[7] Taiwan January 2007 to July 2009§	DBS free carnitine, liver enzymes, blood glucose, ammonium, urine ketone, electrocardiography, urine organic acid analysis, molecular analysis	DBS free carnitine measurements and molecular analyses were also done for mothers
Frazier et al. (2006)[35] North Carolina July 1997 to July 2005	Urine organic acids, plasma acylcarnitine profile. Enzyme and mutation analyses were done whenever the specific tests were available and were approved by third-party reimbursers	NR
Schulze et al. (2003)[9] Germany April 1998 to September 2001	Carnitine uptake in fibroblasts, mutational analyses, decreased tubular carnitine reabsorption	NR

NR not reported

As described in Criterion 2 there is no clear genotype/phenotype or enzyme activity/phenotype correlation, so there is no way of predicting the phenotype/prognosis of cases identified by newborn screening.

Criterion 8 uncertain: There are recommendations for the diagnostic investigation of patients who screen positive for CTD. CTD may be diagnosed by the measurement of plasma carnitine, molecular genetic testing of the *SLC22A5* gene or by assessing carnitine transport in fibroblasts. However, it was unclear whether cut-offs for diagnosis have been agreed upon. In addition, as there is no clear genotype/phenotype or enzyme activity/phenotype correlation there is no way of predicting the phenotype/prognosis of cases identified by newborn screening.

9. If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested, should be clearly set out

Not applicable

10. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment

The mainstay of treatment of CTD is avoidance of fasting and lifelong L-carnitine supplementation.

L-Carnitine is used to treat a number of different contexts for inborn errors of metabolism. However, the evidence base for its use is acknowledged to be limited. No placebo controlled randomised controlled trials of carnitine supplementation for inborn errors of metabolism were identified by a 2012 Cochrane review. The authors pointed out that this does not mean that carnitine is ineffective or should not be used, rather, that given the lack of evidence both on the effectiveness and safety of carnitine and on the necessary dose and frequency to be prescribed,

the current prescribing practice should continue to be observed and monitored with care until further evidence is available.[37]

The symptoms of CTD, for example cardiomyopathy, failure to thrive, hypoglycaemic encephalopathy, hypotonia and myopathy/motor delay, can be reversed with carnitine supplementation, as described in Criterion 2 (see Table 3 to Table 11).

Criterion 10 uncertain: L-carnitine supplementation is the established treatment for CTD. L-carnitine supplementation can prevent symptoms and reverse symptoms of CTD. No studies were identified that compared the outcomes of screen detected cases with clinically detected cases. However, screen detected cases receiving treatment have remained asymptomatic, and no cases of fatal CTD have been reported in screen identified patients, although it should be noted that there is no way of determining the phenotype/prognosis of screen-detected cases without treatment, and it is possible that at least some of these cases would have remained asymptomatic without treatment.

11. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered

The ACMG and NYMAC recommend that maternal testing for CTD should be considered if a newborn screens positive for CTD.[23,24] It has been reported that even asymptomatic adult patients with CTD should receive treatment to prevent the possibility of decompensation during intercurrent illness or stress,[34] and the asymptomatic mothers identified by screening programmes have been given carnitine supplementation (see Criterion 2). However, it is unclear whether these patients need carnitine supplementation.

Criterion 11 uncertain: it is uncertain whether asymptomatic adult patients with CTD identified through newborn screening or cascade testing require carnitine supplementation. At the moment there is no way of determining the phenotype/prognosis of screen-detected cases, and there is a possibility that some screen-detected cases would remain asymptomatic without treatment.

12. Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme

Not assessed

13. There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (eg. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened

The 2004 HTA report stated that "RCTs of screening for rare disorders are difficult because of the enormous numbers that would be needed for adequate power."[38]

No RCTs of screening for CTD were identified.

Criterion 13 not met. No RCTs of screening for CTD were identified.

14. There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public

Not assessed

15. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment)

No studies reporting harms associated with the test, diagnostic procedure or treatment were identified.

16. The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (ie. value for money). Assessment against this criteria should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource

One study which assessed the cost-effectiveness of screening for CTD was identified in the update search.

Cipriano et al. (2007) performed a cost effectiveness analysis from a societal perspective of replacing screening for phenylketonuria using the Guthrie bacterial inhibition assay with expanded screening for up to 21 inherited metabolic disorders, including CTD, using tandem MS in Ontario, Canada.[20] Each metabolic disorder was assessed independently in addition to being assessed as a bundle. Using a decision analytic model, with life years saved as the outcome, the analysis considered:

- the incidence and the severity of the conditions, and the health benefits of screening/an early diagnosis. The incidence of CTD was estimated at 1:120,667 based on the incidence of CTD after the introduction of screening in New South Wales and the Australian Capital territory reported in Wilcken et al. (2003)[26] (see Criterion 2, Table 18). The distribution of phenotypes was also estimated: 50% of cases were estimated to have neonatal, classical, severe or early-onset form of CTD, 50% of cases were estimated to have later-onset, chronic or mild form CTD, and 0% of cases were estimated to have mild variations that would not be detected or treated without newborn screening. This distribution was based on the results of a review of mitochondrial fatty acid disorders. This distribution is inconsistent with the finding that asymptomatic mothers have been identified by newborn screening (see Criterion 2). Life expectancy with the neonatal, classical, severe or early-onset form of CTD was assumed to be the same as carnitine palmitoyl transferase II deficiency (CPT II), and estimated to be 25 years with an early diagnosis and 14 years with a clinical diagnosis. Life expectancy with the later-onset, chronic or mild form of the disease was estimated to be 50 years with an early diagnosis

compared with 35 years with a clinical diagnosis (based on expert opinion combined with literature reports).

- the sensitivity, specificity and positive predictive rate of the test. The average positive predictive rate was estimated at 20%. For fatty acid β-oxidation disorders the sensitivity was estimated at 100%, the specificity 99.95%.
- the start-up costs of tandem MS screening (including capital equipment required to start an tandem MS facility, training costs, computers and analysis software, and maintenance)
- the cost of confirmatory testing
- the cost of treatment, hospitalisation, social services and education. For example L-carnitine [100mg/kg/day] was assumed to cost 7,565 Canadian dollars per year.

The incremental cost and the incremental cost-effectiveness ratios (ICERs) for screening for CTD by itself are shown in Table 24.

Table 24: Incremental cost effectiveness of each disease evaluated independently and a breakdown of incremental costs, savings and life years gained per patient screened. All costs given in 2004 Canadian dollars. From Cipriano et al. (2007).[20]

Disease	Incremental cost ($) (including start-up*)	Incremental cost ($) (excluding start-up)	Incremental life years gained ($) ($x10^{-5}$)	ICER† ($) (including start-up)	ICER ($) (excluding start up)	Order of cost-effectiveness‡
Carnitine transporter defect (CTD)	19.89	1.52	3.59	554,039	42,340	7

*Programme start-up and base operation costs (whether screening for one or more diseases) is $18.37 per infant

† The incremental cost-effectiveness ratio (ICER) describes the incremental cost required to acquire the benefit of one additional life-year. It is calculated by dividing the total incremental cost by the incremental life years gained

‡Position relative to the 21 inherited metabolic disorders considered

Criterion 16 uncertain. One cost-effectiveness study published in 2007 was identified. The incremental cost per additional life year was $42,340 Canadian dollars (excluding start up, as tandem MS is currently used to screen for other newborn conditions). This study was done from a Canadian perspective and it is unclear how applicable this study would be to the UK. No UK based studies of cost-effectiveness were identified.

17. All other options for managing the condition should have been considered (eg. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available

Not assessed

18. There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards

Not assessed

19. Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme

Not assessed

20. Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice

Not assessed

21. Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public

Not assessed

22. If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members

Not applicable

Conclusions

The condition

Carnitine transporter deficiency (CTD), also known as systemic primary carnitine deficiency (CDSP) or carnitine uptake disorder (CUD), is a rare autosomal recessive disorder.

The age of onset of symptoms and the presenting features of CTD varies. Presenting features include cardiomyopathy, coma, hypoglycaemic encephalopathy, anaemia, motor delay, myopathy, hepatomegaly and/or failure to thrive. CTD can be lethal, but symptoms are reversible with carnitine supplementation, and patients who are diagnosed go on to have favourable clinical outcomes. There is no clear genotype/phenotype correlation or enzyme activity/phenotype correlation, and the proportion of cases that could be expected to be lethal without treatment is unclear.

Carnitine levels in the newborn period are strongly influenced by maternal C0 levels, as carnitine can be transported through the placenta. Newborn screening has identified maternal cases of CTD. The majority of these cases are asymptomatic. The identification of these cases suggests that there is a form of CTD that does not present clinically.

Newborn cases identified by newborn screening have been asymptomatic at diagnosis, and have remained asymptomatic with carnitine supplementation. However, as newborn screening has identified asymptomatic mothers suggesting that there is an asymptomatic form of the condition, and as there is no way to predict prognosis of cases due to the lack of genotype/phenotype correlation or enzyme activity/phenotype correlation, it is unclear how many of these cases could have remained asymptomatic without treatment.

The reported incidence of CTD varies. It is unclear if newborn screening has increased the incidence of CTD (which might have suggested that newborn screening is either identifying asymptomatic cases which would never have presented clinically or identifying cases which die without diagnosis, or both). The incidence of CTD in the UK has not been described, and it is difficult to estimate the distribution of phenotypes.

The screening test

There is a simple and safe screening test. CTD can be screened for by tandem MS on dried blood spots already collected as part of newborn screening. Low levels of free carnitine (C0) in blood is the marker for CTD. However, cut-offs for screening have not been agreed upon. Screening programmes have also collected specimens at different times, and no studies were identified in the update search which explored whether the timing of the test could be modified so that maternal carnitine levels do not influence the outcome of the test

Screening can detect heterozygotes, and asymptomatic maternal cases.

Despite this the reported sensitivity and specificity of the test is high, although the positive predictive value of the test is low and varies between 0.48% and 33.33%. However, it should be noted that there were important limitations to these studies. In many cases no false negatives were reported, but extensive follow-up to ascertain whether there were false negatives was not performed. Even if follow-up was performed, it is possible that mild or asymptomatic false negative cases would not be identified.

Diagnostic Confirmation

There are recommendations for the diagnostic investigation of patients who screen positive for CTD. CTD may be diagnosed by the measurement of plasma carnitine, molecular genetic testing of the *SLC22A5* gene or by assessing carnitine transport in fibroblasts. However, it was unclear whether cut-offs for diagnosis have been agreed upon.

There is currently no way to predict phenotype/prognosis of cases identified by newborn screening due to the lack of genotype/phenotype correlation or enzyme activity/phenotype correlation.

Treatment

The avoidance of fasting and L-carnitine supplementation is the established treatment for CTD. L-carnitine supplementation can prevent symptoms and reverse symptoms of CTD. No studies were identified that compared the outcomes of screen detected cases with clinically detected cases. However, screen detected cases have remained asymptomatic following treatment, and no cases of fatal CTD have been reported in screen identified patients undergoing treatment.

It is uncertain whether asymptomatic adult patients with CTD identified through newborn screening or cascade testing require carnitine supplementation. Some cases identified by newborn screening may also not require treatment, but as mentioned previously there is currently no way to predict phenotype/prognosis.

Implications for policy

- Current policy on screening for CTD should be retained.

Implications for research

Further research is required into:

- The UK prevalence/incidence of CTD
- Follow-up studies of asymptomatic infants and mothers detected by screening
- Whether phenotype/prognosis can be predicted
- The natural history of heterozygotes
- The timing of newborn specimen collection to avoid maternal carnitine levels influencing test results

Methodology

The draft update report was prepared by Bazian Ltd., and then adapted in line with comments from the National Screening Committee.

Search strategy

BACKGROUND: Screening for the fatty acid oxidation disorders very long chain acyl CoA dehydrogenase deficiency, carnitine uptake defect and trifunctional protein deficiency has not been assessed individually. However, a 2004 Health Technology Assessment (full reference below) did recommend against screening for a collection of long chain fatty acid disorders associated with individual enzyme deficiencies.

Pandor A, Eastham J, Beverley C, et al. Clinical effectiveness and cost-effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry: a systematic review. Health Technology Assessment 2004; 8(12)

The literature search for the 2004 HTA was carried out in November 2001. Therefore the searches for this current review were carried out from January 2001 to ensure no relevant publications were missed.

SOURCES SEARCHED: Medline, Embase, and the Cochrane Library.

DATES OF SEARCH: January 2001 – June 2013

SEARCH STRATEGY:

1. vlcad.tw. (178)

2. (very long chain adj3 dehydrogenase deficien$).tw. (114)

3. trifunctional protein deficien$.tw. (92)

4. carnitine uptake defect.tw. (9)

5. carnitine deficien$.tw. (946)

6. (carnitine adj (transporter or uptake) adj deficien$).tw. (16)

7. 1 or 2 or 3 or 4 or 5 or 6 (1271)

8. neonatal screening/ (6737)

9. ((neonat$ or newborn$) adj2 screen$).tw. (7135)

10. 8 or 9 (10229)

11. mass screening/ (78162)

12. exp Infant, Newborn/ (480411)

13. 11 and 12 (4629)

14. 10 or 13 (13866)

15. Lipid Metabolism, Inborn Errors/ (2292)

16. fatty acid oxidation disorders.tw. (173)

17. 15 or 16 (2390)

18. 14 and 17 (148)

19. 7 or 18 (1388)

20. 19 (1388)

21. limit 20 to yr="2001 -Current" (661)

Similar searches were also carried out in Embase and the Cochrane Library.

All searches carried out on 3 June 2013

Medline	**661**
Embase	**1009**
Cochrane Library	**307**
Total	**1977**

Inclusions and exclusions

The above search strategies retrieved 1977 references in total. After duplicate references were removed a total of 1325 potentially relevant references were left. The title and abstracts of the remaining citations were scanned for relevance to screening for the three fatty acid oxidation disorders: very long chain acyl CoA dehydrogenase deficiency, carnitine uptake deficiency and trifunctional protein deficiency, focussing on the following:

- the natural history of all three conditions
- presentation
- incidence and prevalence
- the test
- the treatment
- screening for fatty acid oxidation disorders in general

284 references were deemed to be relevant

The final set of references was then passed to the expert reviewer for further appraisal and possible inclusion in the review.

Systematic reviews and meta-analyses	**3**
Structured abstracts	**1**
Non-systematic reviews	**23**

The condition
• Epidemiology (4)
• Carnitine uptake defect (12)
• Trifunctional protein deficiency (2)
• Very long chain acyl CoA dehydrogenase deficiency (5)
• Very long chain acyl CoA dehydrogenase deficiency and carnitine uptake defect (2)
• Case studies
  • Carnitine uptake defect (15)
  • Trifunctional protein deficiency (10)
  • Very long chain acyl coA dehydrogenase deficiency (11)
• Genetics (15)
• Presentation/diagnosis case studies
  • Carnitine uptake defect (19)
  • Trifunctional protein deficiency (6)
  • Very long chain acyl CoA dehydrogenase deficiency(28)
	142

• Very long chain acyl CoA dehydrogenase deficiency and trifunctional protein deficiency (1) • Postmortem identification (12)	
The test	**23**
The treatment • Treatment/managaement strategies (13) • Carnitine uptake defect (3) • Trifunctional protein deficiency (4) • Very long chain acyl CoA dehydrogenase deficiency (3) • Case studies • Carnitine uptake defect (2) • Very long chain acly CoA dehydrogenase deficiency (5)	**30**
The screening programme • Screening for inborn errors (41) • Screening for fatty acid oxidation disorders (4) • Screening for specific fatty acid oxidation disorders (11) • Follow-up of screening (6)	**62**
Total	**284**

Quality

Studies not in English, conference abstracts, non-systematic reviews, editorials, other opinion pieces, and those with nonhuman data were excluded. Case series and experimental studies of fewer than four patients with CTD were excluded except where they reported cases missed by screening or symptomatic heterozygotes. Additional relevant references identified during the preparation of the report were also included.

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