UK National Screening Committee

Screening for Very Long-Chain Acyl-Coenzyme A Dehydrogenase Deficiency

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

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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 very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD)

Key points

- **The clinical course VLCADD 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. This is additionally complicated by the fact that it can be difficult to distinguish heterozygous carriers from affected cases in which mutation in only one allele can be found**
- **Screening can identify heterozygotes, and the natural history of heterozygotes is not well understood**
- **Although there are accepted treatments, there is uncertainty over whether all cases identified through screening will require treatment**

Introduction

This review assesses newborn screening for very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD), 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. The first step of β-oxidation is performed by one of four different enzymes, depending on the length of the fatty acid's hydrocarbon chain:

- short-chain acyl-CoA dehydrogenase (for fatty acids with a hydrocarbon chain containing between four and six carbons)
- medium chain acyl-CoA dehydrogenase (for fatty acids with a hydrocarbon chain containing between six and ten carbons)
- long chain acyl-CoA dehydrogenase (for fatty acids with a hydrocarbon chain containing between ten and 14 carbons)
- very long chain acyl-CoA dehydrogenase (for fatty acids with a hydrocarbon chain containing between 14 and 20 carbons)

In VLCADD the β-oxidation of fatty acids with a hydrocarbon chain containing between 14 and 20 carbons is impaired.

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 VLCADD is not recommended in the UK.

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

This review

This review assesses newborn screening for VLCADD 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 VLCADD was 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

VLCADD 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 VLCADD in the UK were identified.

Table 1: Range of incidence

Condition	Lower end of reported range	Upper end of reported range
VLCADD	1:1,551,200 (Australia, unscreened)	1:31,500 (Victoria, Australia, screened)

VLCADD has a widely varying clinical course.

VLCADD has been divided into three phenotypes:

- A severe early onset form that presents between the first hours and the first months of life with cardiac arrhythmia, cardiomyopathy, and hepatopathy
- A hepatic or hypoketotic hypoglycaemic form that presents in early childhood with hypoketotic hypoglycaemia and hepatomegaly (often without cardiomyopathy)
- A late-onset form that presents in later childhood or adulthood with rhabdomyolsis, muscle cramps and/or pain, and/or exercise intolerance

It has also been suggested that screening is identifying cases that would remain asymptomatic, as the incidence of VLCADD has increased in places where screening has been introduced.

The distribution of phenotypes is unclear.

VLCADD is characterised by molecular heterogeneity, with many known pathological mutations. There is no prevalent mutation. Null mutations are associated with the severe, neonatal form of the disease. Missense mutations have been associated with all clinical phenotypes. Although VLCADD is a deficiency of the enzyme VLCAD, residual enzyme activity varies between cases. However there currently seems to be no accepted way of predicting disease severity on the basis of enzyme activity levels or from genotyping, especially if this reveals missense mutations in both alleles.

The epidemiology and natural history of VLCADD is not well understood.

The test

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

In VLCADD, the metabolic block causes long-chain C14–C18 acyl-CoAs to accumulate in mitochondria. To leave the mitochondria, they are reconverted into acylcarnitine esters, which can be assayed in blood. Raised levels of C14:0 (tetradecanoylcarnitine) and C14:1 (tetradecenoylcarnitine) acylcarnitines are the main screening markers for VLCADD.

Screening programmes that have screened for VLCADD 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)
VLCADD	C14:1 >0.35 to >0.75µmol/L C14: >0.76µmol/L	50% to 100%	99.96% to 100%	3.13% to 100%	>99.9% to 100%

Reasons for poor positive predictive value for VLCADD include the following:

- Healthy newborns may also have abnormally high acylcarnitine profiles suggestive of VLCADD in the first few days of life due to the stimulation of fatty acid oxidation during catabolism resulting in elevated levels of long chain acylcarnitines

- Screening may identify heterozygotes

In addition there is concern that cases of VLCADD may be missed by newborn screening as patients with VLCADD may present with normal acylcarnitine profiles during anabolic conditions (when fatty acid oxidation is not induced). Changes in metabolic state, from catabolism to anabolism, have been reported as leading to conflicting test results during the screening and diagnostic process.

As VLCADD is a recessive disorder, heterozygotes should be asymptomatic. However, individuals who are found to have a mutation in one allele only and who are therefore classified as heterozygotes may have a second mutation which has not been identified. The natural history of heterozygotes requires further study.

Treatment

The treatment for VLCADD is life-long dietary adaptation and supplementation, typically involving a low-fat diet, supplemented with medium-chain triglycerides (MCT) to avoid the metabolic block.

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

However, the evidence base for this approach 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, which individuals should receive treatment cannot be predicted.

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 VLCADD 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

Very long-chain Acyl-Coenzyme A Dehydrogenase Deficiency (VLCADD) is a rare autosomal recessive disorder caused by mutations in the gene that encodes the enzyme very long-chain acyl-CoA dehydrogenase (VLCAD), *ACADVL*.[1] As discussed in Criterion 2, estimates of incidence vary between 1:1,551,200 (Australia, in an unscreened population)[2] to 1:31,500 (Victoria, Australia, after screening was introduced)[3]. The incidence of VLCADD in the UK is unknown.

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. The first step of β-oxidation is performed by one of four different enzymes, depending on the length of the fatty acid's hydrocarbon chain:[1]

- short-chain acyl-CoA dehydrogenase (for fatty acids with a hydrocarbon chain containing between four and six carbons)
- medium chain acyl-CoA dehydrogenase (for fatty acids with a hydrocarbon chain containing between six and ten carbons)
- long chain acyl-CoA dehydrogenase (for fatty acids with a hydrocarbon chain containing between ten and 14 carbons)
- VLCAD (for fatty acids with a hydrocarbon chain containing between 14 and 20 carbons)

In VLCADD the β-oxidation of fatty acids with a hydrocarbon chain containing between 14 and 20 carbons is impaired, and the clinical symptoms of the disease reflect this.

The use of fatty acids to supply energy is crucial for several organs. The heart constantly utilises fatty acids as source of energy.[1] During fasting, the liver uses a break down product of β-oxidation, acyl CoA, to generate ketone bodies which are used by the brain as an energy source. Muscles also use fatty acids during prolonged exercise.[1] When fat cannot be utilised glucose is consumed without regeneration resulting in hypoglycaemia. In addition, fatty acids released from adipose tissue accumulate in the liver, skeletal muscle and heart resulting in hepatic steatosis and myopathy.

Therefore VLCADD can affect the heart, the liver, the ability to adapt to fasting, and the ability to generate energy for exercise.[1]

There are three phenotypes associated with VLCADD:[1]

- A severe early onset form that presents between the first hours and the first months of life with cardiac arrhythmia, cardiomyopathy, and hepatopathy
- A hepatic or hypoketotic hypoglycaemic form that presents in early childhood with hypoketotic hypoglycaemia and hepatomegaly (often without cardiomyopathy)
- A late-onset form that presents in later childhood or adulthood with rhabdomyolysis, muscle cramps and/or pain, and/or exercise intolerance

As discussed in Criterion 2, the proportion of patients with each of the phenotypes is unclear. VLCADD can be lethal, and one case series found that there is a high mortality rate without newborn screening in those with early onset disease and cardiomyopathy but the proportion of cases that could be expected to be lethal without treatment is unclear. Treatment is able to reverse some symptoms, but some occur despite treatment, for example exercise intolerance.

Criterion 1 uncertain: VLCADD is a rare disorder that has a varied clinical course. There are three phenotypes which are associated with VLCADD, and the distribution of cases between these three phenotypes is unclear. VLCADD can be lethal, but the proportion of cases that could be expected to be lethal without treatment is unclear. Therefore VLCADD is an important health problem for an uncertain proportion of a small number of people.

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

The 2004 HTA report assessed all defects of long-chain fatty acid catabolism together. It found that "no additional incidence data were available for defects of long-chain fatty acid catabolism within the UK. Owing to the lack of additional evidence, incidence data from the existing systematic reviews have been reported."[4]

"Pollitt and colleagues and Seymour and colleagues reported that the true incidence for these disorders was unknown and there was no secure data available on the prevalence. This is because these disorders have been identified relatively recently, clinical presentations are varied and non-specific, laboratory diagnosis is often difficult even in specialised centres, and there is limited information on the precise biochemical basis, natural history and prognosis of the disorders and the availability of effective treatments. Based on estimates from the number of cases clinically diagnosed in the UK (compared with similar disorders of established incidence), Pollitt and colleagues estimated the expected incidence as three cases per 100,000 births."[4]

Natural history

The clinical features of the three phenotypes are described by a case series published by Andresen et al. (1999).[6] This case series is outside of the search dates for the update review but was included as the three phenotypes had not been described in the HTA review. Andresen et al. (1999) described 55 unrelated patients representing all three phenotypes with the aim of correlating mutation type with clinical phenotype.[6] All the patients had experienced clinical symptoms. In this study:

- The severe childhood form presented before three days of life in 76% of cases, and before one year of life in all cases. The majority of patients had cardiomyopathy (92% of cases), hepatomegaly (80% of cases), and hypotonia (52% of cases). The severe form was lethal in 80% of cases.
- The mild childhood form presented before four years of age. The majority of patients had hepatomegaly (62% of cases), hypotonia (62%), and hypoketotic hypoglycaemia without cardiomyopathy as main presenting symptom (76%). No patients with this form died.
- The late onset form presented after 13 years of age, with rhabdomyolysis/myoglobinuria in all cases. No patients with this form died.

The clinical features of the patients are presented in detail in Table 3.

Table 3: Clinical and biochemical characteristics of 55 cases described in Andresen et al. (1999).[6] One patient has insufficient clinical and biochemical findings for categorization, and is excluded from the table.

Phenotype		Severe Childhood (n=25)	Mild Childhood (n=21)	Adult Onset (n=8)	Total (n=54)
Age at onset:	<3 days	76%	33%	0%	48%
	1 to 11 months	24%	29%	0%	22%
	1 to 4 years	0%	38%	0%	15%
	>13 years	0%	0%	100%	15%
Dead		80%	0%	0%	37%
>1 episode in the first 2 years of life or died in first episode		100%	29%	0%	57%
Cardiomyopathy		92%	19%	13%	52%
Hepatomegaly		80%	62%	0%	61%
Rhabdomyolysis/myoglobinuria		0%	14%	100%	20%
Hypotonia		52%	62%	13%	50%
Hypoketotic hypoglycaemia without cardiomyopathy as main presenting symptom		0%	76%	0%	30%

Extrapolating the data contained in this study to estimate the proportion of cases with each form, or other variables such as the proportion of cases that are lethal, is problematic. The inclusion criteria for cases was not reported and it is possible that the distribution of phenotypes is not representative. In addition, there is the possibility that mild, adult-onset forms may not receive a clinical diagnosis or be subject to a case report.

The distribution of phenotypes was also estimated in a cost-effectiveness analysis by Cipriano et al. (2007), discussed in Criterion 16.[7] This study estimated that 36% of cases were neonatal, classical, severe or early-onset, 64% 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 different case series of 30 patients.

Six additional studies reporting the clinical presentation and outcomes of at least four patients with VLCADD were identified in the update search.

Laforêt et al. (2009) described 13 adult patients with VLCADD in France who were diagnosed after first presenting with clinical symptoms before the age of 13.[8] To be included in the case series patients had to have survived until adulthood (although one patient was 12 years old), and to currently exhibit exercise intolerance and recurrent rhabdomyolysis episodes. Exercise intolerance and recurrent rhabdomyolysis episodes were triggered by strenuous exercise, fasting, cold or fever, and occurred even with treatment. One third of patients had had early-onset of symptoms in organs other than muscle.

Symptoms may have potentially been life-threatening, as patients from three families had eight siblings who died suddenly between two days and 20 months of age. Death was often triggered by diarrhoea or vomiting. However, most symptoms were reversible. For example, one patient presented with cardiac arrest at 36 hours of life. At 16 years of age this patient had normal echocardiography.

Clinical features and clinical course of the 13 patients in this study are shown in Table 4.

Table 4: Clinical features and clinical course in 13 patients with VLCADD described in Laforêt et al. (2009)[8]

	Sex	Age at onset (years)	Presenting symptoms	Age at diagnosis (years)	Current age (years)	Renal failure	Echocardiography	Treatment
1	F	Birth	Cardiac arrest Hypotonia Hepatomegaly	Birth	16	-	Normal	Carnitine, riboflavin, MCT diet
2	F	5	Exercise intolerance	0.2	12	-	Normal	Carnitine, riboflavin, MCT diet
3	M	0.5	Hypoglycaemia Hepatomegaly High creatine kinase level	23	23	-	Normal	-
4	M	1	Hypoglycaemia Hepatomegaly Exercise intolerance	12	21	-	Left ventricular hypertrophy	Carnitine, riboflavin, MCT diet
5	F	2.5	Reye syndrome Hepatomegaly	22	31	+	Septal hypokinesia	-
6	F	7	Exercise intolerance Myoglobinuria	17	25	-	Normal	Carnitine, MCT diet
7	M	11	Exercise intolerance Dizziness	11	Accidental death at 24 years of age	+	Normal	-
8	M	9	Exercise intolerance Myoglobinuria	21	29	-	Normal	Carnitine
9	F	9	Exercise intolerance	32	40	-	Normal	Carnitine
10	M	10	Myoglobinuria	40	42	+	Normal	Carnitine
11	F	10	Exercise intolerance Myoglobinuria	43	46	-	Normal	Coenzyme Q10
12	F	10	Exercise intolerance Myoglobinuria	36	39	-	Normal	Carnitine
13	M	13	Exercise intolerance	34	41	+	Normal	Riboflavin

MCT diet: diet with supplements of medium chain triglyceride (MCT) oil.

Spiekerkoetter et al. (2009) performed a retrospective analysis of 75 patients with long-chain fatty acid oxidation defects from 18 metabolic centres in Germany, Switzerland, Austria, and the Netherlands.[9] It was reported that no data are available about the correct number of patients followed at the respective institutions, some of whom might have died or have been lost during follow-up. Data were available for 30 of the 32 patients with VLCADD.

This study compared the clinical presentation of patients identified by screening with clinically diagnosed patients. The patients identified by screening had either been identified by newborn screening (18 patients, day of screening not reported) or via family screening after an index case had been diagnosed (either clinically or through newborn screening, two patients). Ten further patients had been clinically diagnosed.

Many patients identified by screening remained asymptomatic under preventative measures, and no patient identified by screening died. Three of the 20 patients identified by screening were symptomatic at the time of diagnosis. The remaining 17 were asymptomatic, and remained asymptomatic at follow-up to 7 years, presumably with treatment. The age of diagnosis in the clinically diagnosed patients varied between 1 day and 36 years (median 2.5 months). Two of the ten clinically diagnosed patients died, both of these patients were identified before the newborn screening era. The clinical features of the 30 patients with VLCADD are presented in Table 5.

Treatment reversed the initial clinical symptoms in patients with symptoms who survived. However, intermittent symptoms of skeletal myopathy such as muscle weakness, muscle pain and/or myoglobinuria occurred despite treatment in five of the 13 patients with symptoms (38%). These symptoms were triggered by intercurrent illnesses and following exercise.

Table 5: Clinical presentation of 30 patients with VLCADD reported in Spiekerkoetter et al. (2009)[9]

	Identified by screening (20/30)	**Clinically diagnosed (10/30)**
	Symptomatic at diagnosis 3/20	*Age at diagnosis* 1 day-36 years (median 2.5 months)
Cardiomyopathy	0/20	6/10
Arrhythmias	2/20 (one presented with QT syndrome)	3/10
Reye syndrome	0/20	1/10
Hypoglycaemia	1/20	6/10
Hepatopathy/-megaly	1/20	NR
Hypotonia/myopathy	1/20	4/10
Retinopathy	NR	0/10
Neuropathy	NR	0/10
Deceased (age)	0/20	2/10 (2 months, 3 months)

NR, not reported

Spiekerkoetter et al. (2003) described seven patients in Germany and the US with VLCADD who were identified by newborn screening (at two to four days of life) and one patient identified through family screening.[10] All identified individuals were treated with dietary modification as a preventative measure and remained asymptomatic during follow-up (to between 2 months and 3.5 years of age).

Boneh et al. (2005) described six patients in Victoria, Australia with VLCADD who were detected by newborn screening (at between 48 and 72 hours of life).[3] Five of the newborns were free of symptoms up to age of six months, but one newborn had hypoglycaemia and cardiovascular collapse without cardiomyopathy on day four of life. A high medium chain triglyceride formula with carnitine supplementation was initially prescribed for three of the patients with VLCADD. However, this practice was changed to free breastfeeding, monitoring carnitine levels and supplementing as required, and a 'sick-day' regime. Older children were put on a low fat diet. It was reported that there were no episodes of metabolic decompensation under this regime, even when babies were unwell, and growth and development were normal in all patients.

Schiff et al. (2013) described eight patients who were diagnosed with VLCADD after being identified by newborn screening (day of screening not reported) and one patient who did not undergo screening but was diagnosed after exhibiting clinical symptoms suggestive of VLCADD.[11] All patients were referred to a laboratory at the Children's Hospital of Pittsburgh, US. The clinical signs at diagnosis and at the last follow up are shown in Table 6. All patients identified by screening were asymptomatic at diagnosis. During follow-up, presumably with treatment, two patients experienced moderate elevation of creatine kinase during intercurrent infections. No other symptoms were described. The patient who was diagnosed after presenting with symptoms had severe hypertrophic cardiomyopathy, enlarged liver and episodes of hypoglycaemia when diagnosed at three months of age. This patient was normal at last follow-up at 16 years of age under treatment with triheptanoin.

Table 6: Clinical signs at diagnosis and at last follow-up of nine patients with VLCADD described by Schiff et al. (2013)[11].

Patient	Clinical signs at diagnosis	Clinical signs at last follow-up
1*	Asymptomatic	6 years old: normal
3*	Asymptomatic	2 years: moderate creatine kinase increase during intercurrent infections
4*	Asymptomatic	1 year: normal
5*	Asymptomatic	19 months: moderate creatine kinase increase during intercurrent infections
6*	Asymptomatic	6 months: normal
8*	Asymptomatic	18 months: normal
9*	Asymptomatic	18 months: normal
10*	Asymptomatic	6 years of age: lost to follow-up
11	Severe hypertrophic cardiomyopathy, enlarged liver, episodes of hypoglycaemia. Acylcarnitine profile diagnostic of VLCADD deficiency	16 1/3 years: normal (under triheptanoin)

*Patients identified by newborn screening.

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).[12] It can be presumed that after identification all children received treatment. 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 57% of the patients with VLCADD.

Of the 14 children with VLCADD (five girls and nine boys), 8 had developmental concerns (they received early intervention or special education services for motor, language or learning issues), while none were noted in the remaining six. Four children had speech concerns, seven had motor concerns and one child had cognitive delay. The characteristics of the 14 children with VLCADD are shown in Table 7.

Table 7: Developmental quotient (DQ) or intelligence quotient (IQ) and developmental concerns of 14 children with VLCADD identified through newborn screening reported in Waisbren et al. (2013)[12]

Case	Gender	Age at developmental and neuropsychological testing or when last seen	DQ/IQ†	Developmental concerns
1*	Male	3 years 6 months	115 (IQ)	None noted

2*	Male	3 years 6 months	115 (IQ)	None noted
3	Male	30 months	85 (DQ)	Speech/language and motor delays, autistic spectrum behaviour
4	Male	3 years 5 months	118 (IQ)	Mild articulation and fine motor weakness
5	Female	18 months	125 (DQ)	Motor delay
6	Female	11 months	NE	None noted
7	Male	4 years 4 months	120 (IQ)	Relative weakness in fine motor area
8	Female	13 months	NE	Speech/language delay
9	Female	12 months	115 (DQ)	None noted
10	Male	34 months	110 (IQ)	Relative weakness in fine motor area
11	Male	26 months	75 (DQ)‡	Speech/language and motor delays
12	Male	7 months	NE	None noted
13	Female	23 months	95 (DQ)	None noted
14	Male	6 years 3 months	110 (IQ)	Fine motor deficits, at risk for attention deficit and emotional problems

*twins
† most recent developmental quotient of intelligence quotient reported.
‡ cognitive delay (score >1 standard deviation below population norm)
NE, not evaluated

Four studies that reported on screening programmes also reported on clinical presentation and outcomes of patients with VLCADD identified by screening.

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.[13] The recommended time of sampling was between the third and the seventh day of life. One case of VLCADD was detected. This case was asymptomatic at diagnosis. Treatment started at 185 days of age and the patient remained asymptomatic over 35 months of follow-up.

Frazier et al. (2006) described newborn screening between 1997 and 2005 in North Carolina.[14] During this period 12 cases of VLCADD were identified by newborn screening. The mean age at the time of sampling was 39 hours. One infant with VLCADD died before diagnosis was made, at two days of age. One child with VLCADD died later in childhood (aged 15 months) from illnesses exacerbated by their disorder.

Lindner et al. (2011) described newborn screening for metabolic diseases in South-West Germany.[15] Over a period of ten years, six cases of VLCADD were identified by newborn screening (between day three and five of life for the first 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, one patient with VLCADD 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.[16] 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, three cases of VLCADD were identified. Two cases (66%) were asymptomatic at diagnosis. One child had hypoglycaemia and a possible seizure at nine days of age, on the day of reporting the positive screen result. This was successfully

treated, and the child has been well with normal growth and psychomotor development, as have the two other children.

These studies demonstrate the clinical course of VLCADD is varied, with three different phenotypes described.

The results of the studies identified suggest that:

- VLCADD can be lethal, and there is a high mortality rate without newborn screening in those with early onset disease and cardiomyopathy
- Symptoms are reversible, and patients that survive severe neonatal episodes go onto have a favourable clinical outcome
- Myopathy/rhabdomyolysis episodes can occur even with treatment
- Symptoms can be triggered by strenuous exercise, fasting, illness, cold or fever
- The majority of patients identified by newborn screening who are asymptomatic remain asymptomatic under preventative treatments
- Although designated as 'asymptomatic', a case series suggests that neuropsychological outcomes in patients with VLCADD should be monitored as over half of patients in one case series had developmental concerns.

However, our knowledge of the natural history of VLCADD is based on a few studies describing small numbers of patients with VLCADD.

Aetiology

As mentioned above VLCADD is a rare autosomal recessive disorder caused by mutations in the gene that encodes the enzyme very long-chain acyl-CoA dehydrogenase (VLCAD), *ACADVL*.[1]

The chromosomal location of *ACADVL* is 17p11.2-p11.13.[17] The gene contains 20 exons and the cDNA coding region is 1968 base pairs long.[17] VLCADD is characterised by molecular heterogeneity, with hundreds of known pathological mutations.[1] There is no prevalent mutation.[17]

Based on the results of Andresen et al. (1999), Leslie et al. (2011) proposed that the severe, early onset form of the disease is associated with no residual enzyme activity, often resulting from null mutations, and milder childhood and adult forms are associated with residual enzyme activity, resulting from one or two missense mutations.[1] The original study investigated and characterised the mutations responsible for VLCADD in 55 unrelated patients by sequencing the protein-coding region of VLCAD.[6]

Mutations were identified in both alleles in 47 patients, but a mutation in only one allele was found in eight patients. Fifty eight mutations were identified, and only 18 of these were present in more than one patient.

Mutations were divided into null mutations and missense mutations and single-amino acid deletions. Mutations that would give rise to a protein lacking vital parts of the enzyme because of missplicing and/or direct introduction of a premature stop codon, and two mutations which had already been characterised and found to lead to an enzyme with no residual activity were classified as null mutations.

In the severe childhood disease phenotype, the majority (71%) of identified alleles were of the null type. In the milder childhood and adult disease phenotypes, the majority of alleles (82% and 93%, respectively) harboured missense mutations and single-amino acid deletions.

To make the correlation more precise, they then only considered patients with the same type of mutation in both alleles (i.e. two null mutations or two missense or single-amino acid deletion mutations). Fourteen of the 15 patients with two null mutations had the severe childhood phenotype, and only a single patient had the mild childhood phenotype. Only four of the 18 patients with two missense or single-amino acid deletion mutations had the severe childhood disease phenotype; nine patients had the mild childhood phenotype and five patients had the adult onset form of the disease. This information is summarised in Table 8 and further detail regarding the genotype-phenotype correlation is shown in Table 9.

Table 8: Genotype-phenotype correlation seen in Andresen et al. (1999).[6]

	Severe childhood	**Mild childhood**	**Adult Onset**
Two null mutations (n=15)	14 (93%)	1 (7%)	0
Two missense mutations or single amino acid deletions (n=18)	4 (22%)	9 (50%)	5 (28%)

Table 9: Genotype-phenotype correlation seen in Andresen et al. (1999) in more detail.[6]

		Percentage with genotype	
Variable		Two null mutations* (n=15)	Two missense mutations or single amino acid deletions (n=18)
Age at onset:	<3 days	80	44
	1 to 11 months	13	17
	1 to 4 years	7	11
	>13 years	0	28
Dead		73	17
>1 episode in the first 2 years of life or died in first episode		87	44
Cardiomyopathy		73	24
Hepatomegaly		80	29
Rhabdomyolysis/myoglobinuria		0	33
Hypotonia		40	29
Hypoketotic hypoglycaemia without cardiomyopathy as main presenting symptom		7	24

* Null mutations were mutations that would give rise to a protein lacking vital parts of the enzyme because of missplicing and/or direct introduction of a premature stop codon, or the missense mutations that cause the amino acid substitution R573W or deletion of K258 as these two mutations have already been characterised and found to lead to an enzyme with no residual activity.

However, as can be seen from this paper the correlation is not perfect and missense mutations have also been associated with severe clinical phenotypes. In this paper, although 56% of patients with the severe childhood phenotype had two null mutations, the remaining 44% did not (16% had two missense mutations or single amino acid deletions and 28% had one null mutation and one missense mutation/single amino acid deletion).

The amount of residual activity of VLCADD is hypothesized to determine the clinical phenotype.[18] However, as Andresen et al (1999) reports “residual VLCAD enzyme activities did

not differ significantly in cultured fibroblasts from patients with the different clinical phenotypes. This had been observed previously and probably occurs because the assays are undertaken for diagnostic purposes rather than for comparison of residual enzyme activities."[6]

The fact the mutations cannot always be identified in patients with VLCADD and that residual VLCAD activity varies between patients is exemplified by studies that have described the genotype and/or residual enzyme activity of patients. None of these papers correlated enzyme activity with clinical phenotype.

In Laforêt et al. (2009)(paper described in natural history section), sequence analysis of all 20 exons and exon/intron boundaries identified mutations in both alleles of *ACADVL* in 11 of the 13 clinically diagnosed patients (85%), or eight of the ten families studied, as some patients were siblings.[8] Mutations in only one allele were identified in the remaining two patients. The researchers speculate that these two patients might have unidentified mutations in the second allele located outside the examined regions, for example in regulatory domains or in intronic regions. VLCAD activity was assayed in 11 of the 13 patients by measuring palmitoyl-CoA oxidation in fibroblasts or lymphocytes. Enzyme activity ranged from between 4% and 42% of normal controls. Of note, the lowest VLCAD activity was seen in one of the patients in whom mutations in only one allele could be identified.

In Spiekerkoetter et al. (2003)(paper described in natural history section) eight asymptomatic patients with VLCADD identified by newborn or family screening were characterised.[10] Molecular genetic analysis was performed on seven patients, and missense mutations in both alleles were identified in all patients. VLCAD enzyme activity in lymphocytes was assayed in four patients, and found to be between 6% and 11% of normal.

In Boneh et al. (2005)(paper described in natural history section), to confirm the diagnosis of VLCADD all 20 exons of the VLCAD gene were sequenced in six patients identified by newborn screening.[3] In all six cases mutations were identified in both alleles of the gene.

Liebig et al. (2006) performed enzyme and molecular genetic analyses on 11 newborns who screened positive for VLCADD (elevated C14:1-carnitine, $\geq$0.25µmol/L on day 3 of life).[19] Enzyme activity was measured by assaying palmitoyl-CoA oxidation in lymphocytes, and all 20 exons of the VLCAD gene were sequenced. Palmitoyl-CoA oxidation ranged between undetectable and 12% of healthy controls in seven neonates, indicating VLCADD. In six of these seven neonates, disease causing mutations in both alleles were detected, but in one neonate only a single mutation was detected. The researchers suggest that the second mutation may be in a distant intronic regulatory control region that was not sequenced. In two neonates, palmitoyl-CoA oxidation was 48% and 44% of normal, which suggested that these neonates were heterozygotes, as known heterozygous controls had enzyme activities of 49% of normal. In one of these neonates a mutation in one allele was detected, but in the other neonate no mutations were detected. Two neonates had normal enzyme activity, and in both of these neonates both *ACADVL* alleles were normal.

Tajima et al. (2008) described enzyme activity in two newborns identified by newborn screening.[20] Palmitoyl-CoA oxidation in lymphocytes was 12.2% of normal in one patient and 11.5% in another. In both patients mutations in both alleles were identified. VLCAD activity in the parents of one patient was also assayed. VLCAD activity was 38.1% of normal in the father and 50.3% of normal in the mother, and heterozygous mutations were identified in both.

Ter Veld et al. (2009) developed a liquid chromatography tandem mass spectrometry (LC-tandem MS) method to determine VLCAD activity in lymphocytes in order to confirm a

diagnosis.[21] In this study they monitored VLCAD assay products (C16:1-CoA and C16:OH-CoA) from C16:0-CoA substrate from people identified by newborn screening and their parents and siblings. Whether the cases of VLCADD were symptomatic, and what their symptoms were, was not reported. VLCAD activity in controls (n=36) was 6.95±0.42mU/mg (range 1.95 to 11.91 mU/mg). Residual VLCAD activity in 4 patients with confirmed VLCADD was 0.13±0.07mU/mg (range 0.02 to 0.34 mU/mg) which was reportedly between 0.3% and 1.1% of normal. Heterozygous *ACADVL* mutation carriers showed residual VLCAD activities between 1.07 to 3.77 mU/mg, which was reportedly between 23.7% and 54.2% of normal (n=9). It should be noted that they used ferrocenium as an artificial oxidizing agent in the in vitro assay, and mutations that impair flavin adenine dinucleotide binding and/or electron transfer flavoprotein interaction may not be detected.

Spiekerkoetter et al. (2010) reported that palmitoyl-CoA oxidation in lymphocytes is assayed in all newborns identified with an acylcarnitine profile suggestive of VLCADD in Hamburg, Germany.[22] Those with enzyme activities <50% underwent molecular analysis of the VLCAD gene (*ACADVL*). The true positives in this study had palmitoyl-CoA oxidation in lymphocytes <5% mean activity of normal age-matched controls and mutations were identified in both alleles of *ACADVL*. Other newborns had palmitoyl-CoA oxidation between 10% and 30% of normal age-matched controls. All of these newborns were found to be heterozygotes. However, as the researchers state "in cases were only one heterozygous mutation is detected, diagnosis remains pending."

Hoffmann et al. (2012) reported VLCAD activity determination using palmitoyl-CoA oxidation in lymphocytes in 176 screen positives identified between April 2007 and December 2008.[23] This study reported that VLCAD residual activity less than 10% is diagnostic for VLCADD, but the clinical relevance of activities >10% is unclear. Six newborns had activities <10% (diagnostic for VLCADD), 34 had activities 10-50% (unknown significance) and 136 had activities >50%, and were categorised as false positives. In individuals with activities between 10-50%, sequence analysis of the VLCAD gene was performed. Five patients had mutations in both copies of VLCAD (activity 22% or less), 19 were heterozygous carriers (activities between 25% and 50%) and ten patients had no identifiable mutations (activities between 31% and 50%). Molecular and enzyme analysis was also performed in some heterozygous parents of children with mutations identified in both alleles. Enzyme activity varied between 32% and 75% of normal.

In Schiff et al. (2013)(paper described in the natural history section), genotyping, assays of VLCAD activity and immunoblotting, immunohistologic staining of VLCAD, prokaryotic mutagenesis and expression studies (for uncharacterised mutations) were performed to assess whether patients referred on the basis of newborn screening results or exhibition of clinical symptoms had VLCADD.[11] Nine cases of VLCADD were diagnosed. Mutations likely to be deleterious were identified in both alleles in eight patients, but in one patient only one mutated allele could be identified, even after testing for macro or micro gene deletions/duplications by microarray analysis. The researchers speculate that there could be a promoter mutation or a mutation leading to missplicing that is not identified by standard sequencing of exonic regions along with intron-exon boundaries. VLCAD activity in fibroblasts from patients diagnosed with VLCADD ranged from undetectable to 38% of normal.

Some studies have attempted to use different approaches to determine whether there is a correlation between genotype or enzyme activity and phenotype.

Gobin-Limballe et al. (2007) investigated the effects of a drug called bezafibrate on the fatty acid oxidation capabilities in panel of cells from patients with VLCADD caused by missense mutations

to see whether this could be a way of predicting phenotypes and the functional consequence of genotypes.[24] Bezafibrate increases the expression of VLCAD. Cell lines were divided into groups based on the response to bezafibrate. Analysis of the fatty acid oxidation results in relation to patient phenotypes showed that 11 of the 12 (>90%) cell lines that did not significantly respond to bezafibrate came from patients with a severe (neonatal or infantile) presentation of the disease, and one cell line came from a patient with a myopathic phenotype who presented with severe muscular manifestations early in life. Nineteen of the twenty-one cell lines in which bezafibrate induced a significant increase in fatty acid oxidation originated from patients with the myopathic phenotype. On the basis of this result and other results of the study the substitutions P89S, V174M, K264E, V283A, R366H, K382Q, A416T, M437V and E504D were classified as mild and the substitutions N122D, G185S, G222R, T260M, R286G, G294E, G441D, G447R, R453Q, R469W, and R613W were classified as severe.

The possibility of predicting mutation effects using a 3D VLCAD model is also being evaluated by Gobin-Limballe et al. (2010).[25]

These studies demonstrate that.

- VLCADD is characterised by molecular heterogeneity, with hundreds of known pathological mutations.
- There is no prevalent mutation
- Null mutations are associated with the severe, neonatal form of the disease. Missense mutations have been associated with all clinical phenotypes, including the severe neonatal form
- Mutations in both alleles of *ACADVL* may not be detectable in patients with VLCADD
- Although VLCADD is caused by deficiency in VLCAD, residual enzyme activity varies between cases of VLCADD, including within cases with the same phenotype. Although heterozygous carriers may be expected to have VLCAD activity of approximately 50% of normal, publications reported enzyme activity lower than this in heterozygotes. VLCAD enzyme activity levels below which VLCADD can be diagnosed do not seem to be agreed upon or standardised
- No publications were identified that correlated residual enzyme activity with clinical phenotype
- Although work is ongoing in this area, there currently seems to be no accepted way of predicting phenotype

Latent period

The age of onset of symptoms in untreated VLCADD varies, as there are three phenotypes associated with VLCADD, meaning that the latent period will vary according to clinical phenotype. As described previously, in the Andresen et al. (1999) case series, the severe form presented before 3 days of life in 76% of cases, and before two years of life in all cases; the moderate form presented between 3 days of life and 4 years of age; and the late onset form presented after 13 years of age.[6]

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 10 to help determine whether screening programmes have been able to

identify cases of VLCADD before they became symptomatic. Approximately 10% of cases of VLCADD were symptomatic at diagnosis. However screening tends to take place earlier than current UK practice.

Table 10: Proportion of cases of VLCADD identified by newborn screening programmes that were symptomatic at diagnosis

Study	Age at screening	Proportion of cases that were symptomatic at diagnosis or who died before diagnosis	Age at onset of symptoms
Schulze et al. (2003)[13]	3 to 7 days	0/1	
Spiekerkoetter et al. (2003)[10]	2 to 4 days	0/7	
Wilcken et al. (2003)[26]	48 to 72 hours	0/2	
Frazier et al. (2006)[14]	39 hours	1/12	2 days
Boneh et al. (2005)[3]	48 to 72 hours	1/6	4 days
Spiekerkoetter et al. (2009)[9]	Not reported*	3/20	NR
Lindner et al. (2011)[15]	3 to 5 days (before 2002) 36 to 72 hours (after 2002)	0/6	
Lund et al. (2012)[16]	4 to 9 days (February 2002 to February 2009) 2 to 3 days (after February 2009)	1/3	9 days
Schiff et al. (2013)[11]	Not reported	0/8	
TOTAL		6/65 (9.2%)	

*this includes two cases identified by family screening
Abbreviations: NR, not reported

Detectable disease marker

VLCAD catalyses the initial step of mitochondrial β-oxidation of very long chain fatty acids.[1] In VLCADD long-chain C14–C18 acyl-CoAs accumulate in mitochondria before the β-oxidation block. To leave the mitochondria, they are reconverted into acylcarnitine esters, which can be assayed in blood collected as dried blood spots on Guthrie cards using tandem MS. Dried blood spots are already collected as part of the newborn screening programme.

C14:0 (tetradecanoylcarnitine) and C14:1 (tetradecenoylcarnitine) acylcarnitines are the main screening markers for VLCADD as stated by the National Academy of Clinical Biochemistry (NACB) practice guidelines and the New York Mid-Atlantic Consortium (NYMAC) for Genetic and Newborn Screening Services,[27,28] and C14:1 acylcarnitine serves as a disease-specific marker in many screening programmes (see Table 13), and is the acylcarnitine stated as being elevated in the confirmatory algorithm produced by the American College of Medical Genetics (ACMG).[29]

Levels of other acylcarnitines are also abnormal in VLCADD, and screening programmes have used levels or ratios of other acylcarnitines as secondary markers (see Table 13 and Table 14).

Levels of C14:1 are also elevated (median value in cases above the 99th cumulative percentile in the normal population) in glutaric acidemia type II and LCHAD/TFP deficiency.[5] Levels of C14 are also elevated in glutaric acidemia type II, carnitine palmitoyltransferase deficiency,

carnitine:acylcarnitine translocase deficiency and LCHAD/TFP deficiency.[5] However, screening programmes have managed to discriminate between these disorders, as no reports of false positives for VLCADD who actually had one of these other disorders were identified.

Incidence

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

The incidence of VLCADD in studies identified in the update search is shown in Table 11. In general the number of cases reported was small, making estimates of prevalence uncertain.

Incidences ranged from 1:1,551,200 (Australia, unscreened)[2] to 1:31,500 (Victoria, Australia, screened)[3].

Has incidence changed with screening?

Three studies reported the incidence of VLCADD before and after the introduction of screening.

Lund et al. (2012) compared the incidence of VLCADD in Denmark, the Faroe Islands and Greenland in the decade before newborn screening and the decade after the introduction of newborn screening.[16] In the decade before screening (01/01/1992 to 31/12/2001) the incidence of VLCADD was 1:674,754. After the introduction of screening (2002 to 2011) the incidence was 1:195,659.

Wilcken et al. (2009) reported that the incidence of VLCADD was 1:1,551,200 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.[2] In the screened cohort (born between 1998 and 2002), the incidence of VLCADD was 1:115,375.

Wilcken et al. (2003) compared rates of VLCADD 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 VLCADD varied: no cases were diagnosed between 1974 and 1978, 1978 and 1982), 1990 and 1994 or 1994 and 1998. However, between 1982 and 1986 one case was diagnosed (1:349,000) and between 1986 and 1990 two cases were diagnosed (1:180,000). During the period of newborn screening three cases were identified: two by screening and one after presenting clinically after being given a false negative result on newborn screening (1:120,667).

In all three studies the incidence of VLCADD increased with screening.

A number of researchers have suggested that screening detects cases that might have remained asymptomatic. This seems predominantly to be based on the finding that incidence has increased with screening.

Criterion 2 partially met.

Very long-chain Acyl-Coenzyme A Dehydrogenase Deficiency (VLCADD) is a rare autosomal recessive disorder caused by mutations in the gene that encodes the enzyme very long-chain acyl-CoA dehydrogenase (VLCAD), *ACADVL*. In VLCADD the β-oxidation of fatty acids with a hydrocarbon chain containing between 14 and 20 carbons is impaired, and the clinical symptoms of the disease reflects this. There are three phenotypes associated with VLCADD: a severe early onset form that presents between the first hours and the first months of life with cardiac arrhythmia, cardiomyopathy, and hepatopathy; a hepatic or hypoketotic hypoglycaemic form that presents in early childhood with hypoketotic hypoglycaemia and hepatomegaly (often without cardiomyopathy); and a late-onset form that presents in later

childhood or adulthood with rhabdomyolsis, muscle cramps and/or pain, and/or exercise intolerance. The proportion of patients with each of the phenotypes is unclear. VLCADD can be lethal, and from the results of one case series there is a high mortality rate without newborn screening in those with early onset disease and cardiomyopathy. Symptoms are reversible, and patients that survive severe neonatal episodes can go on to have a favourable clinical outcome. Myopathy/rhabdomyolysis episodes can occur even with treatment. Symptoms can be triggered by strenuous exercise, fasting, illness, cold or fever. The majority of patients identified by newborn screening who are asymptomatic remain asymptomatic under preventative treatments. Although designated as 'asymptomatic', a case series suggests that neuropsychological outcomes in patients with VLCADD should be monitored as over half of patients in one case series had developmental concerns.

VLCADD is characterised by molecular heterogeneity, with hundreds of known pathological mutations. There is no prevalent mutation. Null mutations are associated with the severe, neonatal form of the disease. Missense mutations have been associated with all clinical phenotypes. Although VLCADD is caused by deficiency in VLCAD, residual enzyme activity varies between cases. Although work is ongoing in this area, there currently seems to be no accepted way of predicting phenotype, especially if genotyping reveals missense mutations in both alleles.

Mutations in VLCAD lead to a metabolic block causing the accumulation of C14 to C18 acylcarnitines in the blood, which can be detected using tandem MS on dried blood spots on Guthrie cards. C14:0 (tetradecanoylcarnitine) and C14:1 (tetradecenoylcarnitine) acylcarnitines are the main screening markers for VLCADD.

VLCADD is a rare disease, with an estimated incidence between 1:31,500 (with screening) and 1:1,551,200 (without screening). However, the reported rates vary considerably between studies and the number of cases reported was small, making estimates of prevalence uncertain. The incidence of VLCADD has increased with screening. This finding seems to have led some researchers to speculate that screening is identifying cases that would remain asymptomatic. However, since identified cases receive preventative treatment it is difficult to assess this.

Table 11: Incidence/prevalence of VLCADD from studies that reported at least one case of VLCADD

Study	Country	Start date	End date	Number screened/ Number of births	Number of cases of VLCADD	Incidence	Screening programme in place?	Notes
Lund et al. (2012)[16]	Denmark, the Faroe Islands and Greenland	February 2002	April 2011	504,049	3	1:168,016	Yes	Incidence in screened infants
		February 2002	April 2011	82,930	0		No	Incidence in unscreened infants during the period with expanded newborn screening
		February 2002	April 2011	586,979	3	1:195,659	Among screened and unscreened	Combined incidence among screened and unscreened newborns during the period of expanded newborn screening
		01/01/1992	31/12/2001	674,754	1	1:674,754	No	Frequency in decade before expanded newborn screening (only clinically diagnosed)
Lindner et al. (2011)[15]	Germany	January 1999	30 June 2009	1,084,195	6	1:180,699	Yes	
Kasper et al. (2010)[30]	Austria	April 2002	December 2009	622,489	7	1:88,927	Yes	
Spiekerkoetter et al. (2010)[22]	Germany	NR	NR	90,338	2	1:45,169	Yes	
Wilcken et al. (2009)[2]	Australia	1994	1998	1,017,800	0		No	
		1998	2002	533,400	1	1:533,400	No	
		1994	2002	1,551,200	1	1:1,551,200	No	Unscreened (all)
		1998	2002	461,500	4	1:115,375	Yes	
la Marca et al.	Italy	January 2002	January 2008	160,000	1	1:160,000	Yes	

Study	Country	Start date	End date	Number screened/ Number of births	Number of cases of VLCADD	Incidence	Screening programme in place?	Notes
(2008)[31]								
Tajima et al. (2008)[20]	Selected areas of Japan	May 1997	December 2006	346,905	2	1:173,453	Yes	
Wilson et al. (2007)[32]	New Zealand	2004	2006	175,000	1	1:175,000	No	Clinical diagnosis
Boneh et al. (2006)[3]	Victoria, Australia	February 2002	April 2005	189,000	6	1:31,500	Yes	
Feuchtbaum et al. (2006)[33]	California	7 January 2002	13 June 2003	353,894	2 (1 detected by screening, one diagnosed prenatally)	1:177,000	Yes	
Frazier et al. (2006)[14]	North Carolina	28 July 1997	28 July 2005	944,078	12	1:79,000	Yes	
Comeau et al. (2004)[34]	New England	15 January 1999	31 January 2003	318,535	6 + 2 pending	1:39,817 to 1:53,089	Yes	
Wilken et al. (2003)[26]	New South Wales and the Australian Capital Territory	1974	1978	336,000	0		No	Number of births- no screening
		1978	1982	331,000	0		No	
		1982	1986	349,000	1	1:349,000	No	
		1986	1990	360,000	2	1:180,000	No	
		1990	1994	378,000	0		No	
		1994	1998	367,000	0		No	
		1998	2002	362,000	3	1:120,667	Yes	
Schulze et al. (2003)[13]	Germany	April 1998	September 2001	250,000	1 (+1 suspected†)	1:250,000	Yes	
Yoon et al. (2003)[35]	South Korea	April 2001	August 2003	37,817	1	1:37,817	Yes	Figures are for newborn screening only.

Study	Country	Start date	End date	Number screened/ Number of births	Number of cases of VLCADD	Incidence	Screening programme in place?	Notes
Klose et al. (2002)[36]	Germany	1999	2000	844,575	2	1:422,288	No	
Zytkovicz et al. (2001)[37]	New England	1 February 1999	February 2001	164,000	1 presumptive case‡	1:164,000	Yes	

†Cases of 'disorder suspected' were judged positive on newborn screening and confirmed by recall, but a definite diagnosis remained questionable either because the diagnosis was difficult to achieve or because they were lost to follow-up.
‡As the child is clinically well the parents resisted further testing.
Abbreviations: NR, not reported

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.

Heterozygous carriers have been distinguished from cases of VLCADD on a number of bases, including genotype and VLCAD or fatty acid oxidation activity.

Carriers of VLCADD need to be differentiated from patients with VLCADD in whom mutation in only one allele can be found (see Criterion 2).

As VLCADD is a recessive disorder, heterozygotes are expected to remain asymptomatic.

Depending on the cut-off used, acylcarnitine screening can detect heterozygotes.[1] The distribution of acylcarnitines in heterozygotes is shown in Table 14 in Criterion 6.

Several studies previously described in Criterion 2 reported that newborn screening identified heterozygotes.

For example in Liebig et al. (2006) two heterozygotes (based on enzyme activity) were identified from eleven screen positives.[19] Spiekerkoetter et al. (2010) reported the identification of nine heterozygotes based on genotype from 18 screen positives who had enzyme activities less than 50% (the other screen positives had VLCAD activity of 50% or more).[22] Hoffmann et al. (2012) reported the identification of 19 heterozygotes based on genotype and VLCAD activity from 176 screen positives.[23] Lund et al. (2012) reported that seven of the nine newborns with false positive results identified by screening in Denmark, the Faroe Islands and Greenland were heterozygous for known mutations in *ACADVL*.[16] Schiff et al. (2013) described two heterozygous carriers who were identified via newborn screening.[11] They had had equivocal newborn screening test results and were found to have no abnormalities in VLCAD activity or antigen, but heterozygous mutations were identified. The two heterozygous carriers were asymptomatic at 1 year and 18 months follow-up, respectively.

The identification of heterozygous infants may lead to the identification of asymptomatic mothers. McGoey and Marble reported the case of an asymptomatic mother diagnosed with VLCADD after her newborn screened positive for VLCADD but was found to be a heterozygous carrier.[38]

Criterion 4 not met. Depending on the cut-off used, acylcarnitine screening can detect heterozygotes. No studies were identified that reported the natural history of heterozygotes. No papers were identified which explored the psychological implications of carrier status.

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

The 2004 HTA report found that "evidence is very limited regarding the sensitivity and specificity of neonatal screening for defects of long-chain fatty-acid catabolism using tandem MS."[4]

VLCADD can be screened for using tandem MS performed on acylcarnitines extracted from dried blood spots on Guthrie cards, which are already collected as part of the newborn screening programme.

VLCADD 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 VLCADD is screened for in 10 countries (Austria, the Flemish community of Belgium, Czech Republic, Denmark, Germany, Hungary, Netherlands, Portugal, Spain and Iceland, see Table 12) and some regions of Italy.[39]

Markers for VLCADD

As described in Criterion 2, C14:0 (tetradecanoylcarnitine) and C14:1 (tetradecenoylcarnitine) acylcarnitines are the screening markers for VLCADD as stated by the NACB practice guidelines and the NYMAC for Genetic and Newborn Screening Services,[27],[28] and C14:1 acylcarnitine serves as a disease-specific marker in many screening programmes (see Table 13), and is the acylcarnitine stated as being elevated in the confirmatory algorithm produced by the ACMG.[29]

Levels of other acylcarnitines are also abnormal in VLCADD, and screening programmes have used levels or ratios of other acylcarnitines as secondary markers (see Table 13 and Table 14).

Timing of the screening test

The timing of blood collection reported in publications describing screening programmes is shown in Table 13. The timing of the screening test in the 11 countries that reported screening for VLCADD 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 12.

For defects of long-chain fatty acid metabolism some researchers have recommended that newborn screening is performed between 36 and 72 hours of life to allow detection of the enzyme defect.[22] This is because patients with long-chain acylcarnitine defects may present with a normal acylcarnitine pattern during anabolic conditions when fatty acid oxidation is not induced. For example, this was observed in one of the patients with VLCADD described in Liebig et al. (2006)(described in Criterion 2).[19] In this patient neonatal screening on day three of life revealed elevated C14:1, but the confirmatory sample taken on between day five and seven was normal. In another study, Spiekerkoetter et al. (2010)(described in Criterion 2), of two patients with confirmed VLCADD, one patient had a normal acylcarnitine pattern on day five of life.[22] Boneh et al. (2006)(described in Criterion 2) reported that during three years in Victoria, Australia, six newborn babies were diagnosed with VLCADD, confirmed by mutation analysis.[3] Blood samples taken at 48-72 hours were abnormal whereas repeat samples at older age were normal in four babies. Urine analysis was normal in five out of five babies that had this tested.

Normalisation of acylcarnitines has been reported in other publications:

- A case report described an infant with VLCADD (diagnosed by mutation analysis and palmitoyl-CoA oxidation in lymphocytes) who had elevated C14:1-carnitine levels on day three of life (cut-off for abnormal values <3.1µmol/L) and normal levels on day seven.[40]
- Another study reported an infant who had VLCADD who had elevated newborn blood spot acylcarnitines at two days of age but normal confirmatory plasma samples after one week to 16 months.[41]

In addition, two false negatives who were missed due to normalisation of acylcarnitine profiles are described below. These results and the results of other studies have led to a debate over the value of obtaining a confirmatory repeat acylcarnitine profile (see Criterion 8).

However, it should be noted that in Lund et al. (2012)(discussed in Criterion 2), all nine false positives for VLCADD in Denmark, the Faroe Islands and Greenland occurred after the time interval for obtaining samples was changed from between four and nine days to between two and three days postpartum.[16] However, at the same time as changing the time interval for obtaining samples, the method of analysing samples was changed (from derivatized to underivatized, to allow the analysis of succinylacetone levels, the screening marker for tyrosinaemia type I) and the cut-offs for identifying VLCADD were lowered. This makes it difficult to determine what factor was responsible for the increased detection of false positives.

Table 12: Timing of blood spot sampling in member states of the European Union, candidate, potential candidate and European Free Trade Association countries that screen for VLCADD.[39]

Country	Timing of blood spot sampling
Austria	36-72 hours
Belgium (Flemish Community)	48-96 hours
Czech Republic	48-72 hours
Denmark	48-72 hours
Germany	48-96 hours
Hungary	48-72 hours
Italy*	48-96 hours
Netherlands	72-168 hours
Portugal	48-96 hours
Spain	48 hours -7 days
Iceland	48-72 hours

*varies by region

Sensitivity and specificity of screening for VLCADD

Features of newborn screening programmes that were described in studies identified in the update search are shown in Table 13. Several studies reported data which allowed the sensitivity and specificity of screening for VLCADD using tandem MS in newborn screening programmes to be calculated.

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. The determination of the accuracy of screening tests is also hampered by limitations in VLCADD diagnosis, as there seems to be no agreed cut-off for VLCAD enzyme activity for diagnosing VLCADD, and gene sequencing has not been able to identify mutations in both *ACADVL* alleles in clinically presenting cases (see Criteria 2 and 8). Spiekerkoetter et al. (2010) state that "in cases where only one heterozygous mutation is detected, diagnosis remains pending."[22]

Although C14:1 was used as the primary screening marker in the majority of programmes, it should also be noted that the cut-offs for screening were different and different programmes used different acylcarnitines as secondary markers.

The specificity of screening for VLCADD was found to be >99.99% and the sensitivity 100% in many newborn screening programmes. However, the positive predictive value varied between 3.13% and 100%. Low positive predictive values may partially be due to the identification of heterozygous carriers (see Criterion 4). For example, in Lund et al. (2012)(described in Criterion

2) seven of the nine newborns with false positive results identified by screening in Denmark, the Faroe Islands and Greenland were heterozygous for known mutations in *ACADVL*.[16]

Additionally, the acylcarnitine profile in healthy individuals can mimic that of VLCADD under catabolic conditions. The stimulation of fatty acid oxidation during catabolism results in elevated concentrations of long-chain acylcarnitines, and this typically happens during the first days after birth. For example, Spiekerkoetter et al. (2010)(described in Criterion 2) investigated 40 newborns with elevated C14:1 carnitine (higher than the cut-off point of 0.35µmol/l) or simultaneous elevation of two or more C-14-carntiine derivatives.[22] Only two cases VLCADD were definitively diagnosed. This led the researchers to conclude that healthy individuals with activated fatty acid oxidation may have false-positive results as acylcarnitine profiles from these individuals can mimic those of patients with VLCADD.

Cases missed by screening (false negatives)

Three reports of false negatives were identified who went onto present clinically. Two cases were fatal.

Wilcken et al. (2003)(described in Criterion 2) reported that during the first four years of screening (1998-2002; 362,000 infants) using tandem MS in New South Wales and the Australian Capital Territory, one false negative case of VLCADD presented clinically with hypoglycaemia at 12.0 months.[26] On newborn screening, at 48 to 72 hours, the concentration of C14:0 was 1.3µmol/l and the concentration of C14:1 was 2.0µmol/l (cut-off for both at the time 2.0µmol/l). In a repeat sample on day 14 C14:0 was 0.4µmol/l and C14:1 1.3µmol/l. Following this false negative case the cut-off levels were modified.

Ficicioglu et al. (2010) reported a case missed by screening that developed fatal hypoglycaemia when aged 9 months.[42] The newborn screening result on day two of life from this child was normal (C14:1 level of 0.58mmol/l, cut-off 0.75mmol/l; C14:0 level of 0.74mmol/l, cut-off 0.85mmol/l), and even when symptomatic the acylcarnitine profile was not suggestive of VLCADD. VLCADD was diagnosed after an affected sister was born, through analysis of fibroblast cultures from the original patient. Mutations in both copies of the VLCAD gene and reduced enzyme activity (11% compared with a normal control) were found.

Spiekerkoetter et al. (2012) reported a case whose newborn screening C14:1 levels were abnormal at 55 hours of life according to the cut-off values of the screening laboratory (patient value was 0.45µmol/l, cut-off was 0.36µmol/l).[43] A secondary screen was performed at eight days of life, which was normal, and due to this in combination with the fact that the elevation seen on the initial screen was mild VLCADD was excluded. The child presented with gastrointestinal infection with fever, vomiting and diarrhoea at 16 months of age. After 24 hours with nearly no food intake, the child became lethargic with rhythmic convulsions of legs and arms. The child was admitted to hospital. Cerebral oedema and cardiomyopathy developed within two to three days and the child died. Palmitoyl-CoA oxidation in lymphocytes revealed residual enzyme activity of 6% compared to normal controls, and sequencing identified mutations in both alleles of the VLCAD gene. This case demonstrates that mild elevations in acylcarnitines do not necessarily suggest mild VLCADD.

Criterion 5 partially met: there is a simple and safe screening test for VLCADD. Screening for VLCADD using tandem MS, to measure the levels of long chain acylcarnitines which accumulate due to the metabolic block, has been reported by programmes in several countries. C14:1 acylcarnitine is the disease specific acylcarnitine, and is the screening marker along with C14:0 recommended by US guidelines.

Patients with VLCADD may present with normal acylcarnitine profiles during anabolic conditions (when fatty acid oxidation is not induced) and therefore some researchers have recommended that specimen collection should occur between 36 and 72 hours of life. Specimen collection before 72 hours of life was reported from half of the countries that screened for VLCADD in the EUCERD survey, and in two thirds of publications on screening programmes identified from the update search. This is earlier than UK practice.

However, healthy newborns may also have abnormal acylcarnitine profiles in the first few days of life due to the stimulation of fatty acid oxidation during catabolism resulting in elevated levels of long chain acylcarnitines.

Therefore the metabolic state of infants may influence test results.

Screening may also identify heterozygotes. However, some of these heterozygotes are potentially affected cases in whom only mutation in one allele can been identified.

Despite this, in publications where it was possible to calculate the sensitivity and specificity of screening for VLCADD these were calculated to both be approximately 100%. However, there are important limitations to these studies, including the possibility that false negatives have been missed. In addition, some studies reported low positive predictive values.

Table 13: Features of newborn screening programmes that screen for VLCADD using tandem MS.

Study Location Period	Number of newborns screened	Age at blood collection and preterm protocol	Analytes and cut-offs (μmol/L unless noted)	True positives	False positives	True negatives	False negatives	Specificity	Sensitivity	Positive predictive value	Negative predictive value
Lund et al. (2012)[16] Denmark, the Faroe Islands and Greenland 01/02/2002 to 31/03/2011	504,049	2-3 days (4-9 days before 2009)	Primary marker C14:1>0.5U* Secondary markers C14:1/C0>0.02U or C14:1/C2>0.035 U (before 2009 Primary marker C14:1>1.0U Secondary markers C14:1/C0>0.034 U or C14:1/C2>0.069 U)	3	9†	504,037	0	>99.99%	100%	25%	100%
Kasper et al. (2010)[30] Austria April 2002 to December 2009	622,489	36-72 hours Preterm infants: additional samples at 14 days and	Increased C14:1 [primary analyte]; Increased C14; Increased C16 [Secondary analyte];	7	NR	NR	NR				

Study Location Period	Number of newborns screened	Age at blood collection and preterm protocol	Analytes and cut-offs (μmol/L unless noted)	True positives	False positives	True negatives	False negatives	Specificity	Sensitivity	Positive predictive value	Negative predictive value
		at discharge	Increased ratio of C14:1/C6; increased ratio of C14:1/C8 [Ratios] (exact cut-offs NR)								
Spiekerkoetter et al. (2010)[22] Germany 2 years (time period NR)	90,338	2-3 days	C14:1>0.35 or the simultaneous elevation of two or more C14-carnitine derivatives.	2	38#	NR	NR			5%	
la Marca et al. (2008)[31] Italy January 2002 to January 2008	160,000	48-72 hours Premature infants (<1.8kg): additional samples at 15 days and 30 days. Babies on parenteral nutrition: additional sample 48 hours after the end of	Primary marker C14:1>0.44 Secondary markers C14>0.57; C16>5.6; C18:1>2.43 Ratios C14:1/C4>1.42; C14:1/C5>3.69; C14:1/C8>3.51	1	NR	NR	NR				

Study Location Period	Number of newborns screened	Age at blood collection and preterm protocol	Analytes and cut-offs (µmol/L unless noted)	True positives	False positives	True negatives	False negatives	Specificity	Sensitivity	Positive predictive value	Negative predictive value
		parenteral nutrition Transfused newborns: additional sample 7 days after the end of transfusion									
Tajima et al. (2008)[20] Selected areas of Japan May 1997 to December 2006	346,905	5 days	C14:1>0.4	2	0	NR	NR			100%	
Feuchtbaum et al. (2006) [33] California 7 January 2002 to 13 June 2003	353,894	NR	NR	1	1	353,891	1‡	>99.99%	50%	50%	>99.99%
Frazier et al. (2006)[14] North Carolina 2003 to 2004 (although this study reported on the period between 1997 and 2005, the	239,415	At least 24 hours. The mean age at sampling was 39 hours.	Diagnostic cut-off C14:1>0.75 and C14:1/C12:1>3.0 (independent elevations not diagnostic [also ± C14, C16, C18:1)	5	1	239,409	0	>99.99%	100%	83.33%	100%

Study Location Period	Number of newborns screened	Age at blood collection and preterm protocol	Analytes and cut-offs (μmol/L unless noted)	True positives	False positives	True negatives	False negatives	Specificity	Sensitivity	Positive predictive value	Negative predictive value
number of false positives was only available for between 2003 and 2004)											
Schulze et al. (2003)[13] Germany April 1998 to September 2001	250,000	3-7 days Preterm infants: additional sample at 14 days	C14:1>0.43 or C14>0.76	1 (+ 1 suspected disorder)[a]	31	249,968	0	99.99%	100%	3.13%	100%
Zytkovicz et al. (2001)[37] New England February 1999 to February 2001	164,000	1-3 days NICU infants screened every 2 weeks and at discharge from NICU	C14:1/deuterium C16= 0.9	1 (presumed, refused diagnostic testing)	3	163,996	0	>99.99%	100%	25%	100%

*U, arbitrary units

†Nine false positive results were found after February 2nd 2009, when the time interval for obtaining samples was changed from 4-9 days to 2-3 days postpartum, the method of analysing samples was changed (from derivatized to underivatized) and the cut-offs for identifying VLCADD were lowered. Seven of the false positives were heterozygotes.

‡One case of VLCADD was diagnosed prenatally and was being treated, therefore, the deficiency was not detectable during the first month by screening

#True positives had palmitoyl-CoA oxidation <5% of mean activity of normal age matched controls and mutations were identified in both *ACADVL* alleles. Of the false positives, 18 had palmitoyl-CoA oxidation <50% of mean activity of normal age matched controls, and seven of these had activities between 10% and 30%. These seven were found to be heterozygotes, although the researchers go onto say that "in cases when only one heterozygous mutation is detected, diagnosis remains pending."
[a]Cases of 'disorder suspected' were judged positive on newborn screening and confirmed by recall, but a definite diagnosis remained questionable either because the diagnosis was difficult to achieve or because they were lost to follow-up.
Abbreviations: NICU, neonatal intensive care unit; 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

The 2004 HTA report found that "various thresholds were used to identify inborn errors of metabolism. Some studies used cut-off limits based on percentiles, whereas others used various concentrations of each compound or ratio as standard deviations above mean. Furthermore, infants and young children who are ill for any reason may have abnormal patterns of amino acids and acylcarnitines."[4]

"The variation in the age of sampling and the heterogeneity in the choice of metabolite, as well as in thresholds used to define a positive result, limits direct comparison of the discriminative power of tandem MS between studies."[4]

Distribution of acylcarnitines in the target population and cut-off levels for screening for VLCADD

As mentioned in Criterion 2, C14:0 (tetradecanoylcarnitine) and C14:1 (tetradecenoylcarnitine) acylcarnitines are the screening markers for VLCADD as stated by the NACB practice guidelines and the NYMAC for Genetic and Newborn Screening Services,[27],[28] and C14:1 acylcarnitine serves as a disease-specific marker in many screening programmes (see Table 13), and is the acylcarnitine stated as being elevated in the confirmatory algorithm produced by the ACMG.[29] Levels of these markers are elevated in other conditions, for example C14:1 is also elevated in glutaric acidemia type II and LCHAD/TFP deficiency.[5] C14 is also elevated in glutaric acidemia type II, carnitine palmitoyltransferase deficiency, carnitine:acylcarnitine translocase deficiency and LCHAD/TFP deficiency.[5]

In addition, as noted in Criterion 5, although C14:1 was used as the primary screening marker in the majority of programmes, the cut-offs for screening were different, and different programmes used different acylcarnitines as secondary markers (see Table 13).

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.[5] 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.[5] Levels and ratios of acylcarnitines indicative of VLCADD in the normal population and in 446 cases of VLCADD (and 92 VLCADD heterozygotes) are shown in Table 14. Markers that were considered indicative of VLCADD, because the median value in cases was above the 99th cumulative percentile in the normal population, were C12:1, C12, C14:2, C14:1, C14 and the ratios C14:1/C2, C14:1/C12:1 and C14:1/C16.[5]

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 VLCADD heterozygotes) that causes a particular acylcarnitine to be elevated to screen positive, rather than being specifically for VLCADD.

Recently, the Region 4 Genetics Collaborative 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.[44] 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.

Spiekerkoetter et al. (2010)(described in Criterion 2) used the VLCAD calculation tool on 40 screen positive patients (C14:1-carnitine higher than 0.35µmol/L or the simultaneous elevation of two or more C14-carnitine derivatives), two of whom were found to be true positives.[22] The VLCAD tool compiles a cumulative score comprised of the patient's concentrations for C14:2, C14:1 and C14-acyclcarnitines and the ratios in C14:1/C2, C14:1/C12:1 and C14:1/C16. A score >75 is suggestive for VLCADD. Two patients scored >75: a true positive and a healthy individual with palmitate oxidation activity of 112%. The other true positive scored 60. These results suggest that the tool requires further development.

Criterion 6 partially met. The cut-offs used in screening programmes that have screened for VLCADD have varied. The distribution of acylcarnitines in a UK population after specimen collection at day five of life is unknown, and therefore cut-offs cannot be set.

An international collaboration has published levels of acylcarnitines that were considered indicative of VLCADD (due to the median value in cases being above the 99th cumulative percentile in the normal population) in both the normal population and in cases of VLCADD. Acylcarnitines considered indicative of VLCADD included C14:1 and C14. 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. The cut-offs proposed by the international collaboration were based on screening for a panel of disorders.

Table 14: Acylcarnitine levels and ratios in the normal population and affected cases. Levels in heterozygotes are shown in brackets.

CV coefficient of variation (standard deviation/mean)
*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
VLCADD	$C_{12:1}$	58	0.010	88%	0.063	33%	0.27	31%	242 (59)	0.035 (0.15)	0.34 (0.43)	1.3 (0.94)
(heterozygotes)	C_{12}	71	0.040	39%	0.14	36%	0.41	36%	287 (68)	0.096 (0.28)	0.62 (0.58)	2.4 (1.4)
	$C_{14:2}$	68	0.010	82%	0.036	44%	0.090	43%	275 (63)	0.042 (0.061)	0.24 (0.12)	1.3 (0.27)
	$C_{14:1}$	94	0.030	34%	0.12	28%	0.37	18%	438 (91)	0.41 (0.49)	1.8 (0.86)	10 (1.7)
	C_{14}	90	0.071	34%	0.23	17%	0.50	17%	403 (88)	0.24 (0.36)	1.3 (0.73)	7.4 (1.8)
	$C_{14:1}/C_2$		NR		NR		NR		329 (87)	0.016 (0.015)	0.089 (0.030)	0.71 (0.062)
	$C_{14:1}/C_{12:1}$		NR		NR		NR		242 (59)	1.1 (0.93)	5.2 (2.0)	38 (4.4)
	$C_{14:1}/C_{16}$		NR		NR		NR		400 (87)	0.059 (0.12)	0.41 (0.24)	1.8 (0.50)

NR, not reported

7. The test should be acceptable to the population

Tandem MS is performed on 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 VLCADD were identified in the update search. Acceptability is important in this instance due to the potential of the test to identify heterozygous infants (see Criterion 4).

Summary: Criterion 7 uncertain. No studies exploring the acceptability of the test for VLCADD 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

VLCADD is screened for in the US. The ACMG, NYMAC and NACB have developed guidelines and algorithms for the confirmation of screen-positives.

The ACMG, NYMAC and NACB recommend that plasma acylcarnitines are assayed.[27-29] If the plasma acylcarnitines fit the VLCADD profile then the disorder is confirmed. However, the ACMG and NYMAC differ from the NACB as they recommend performing further diagnostic tests in individuals without diagnostic acylcarnitine profiles. The recommendation to perform further diagnostic tests may be due to reports identified in the update search of normalisation of acylcarnitine profiles in patients with VLCADD under anabolic conditions (see Criterion 5 for a further discussion of normalisation of acylcarnitine profiles).[3,19,22,40,41,43]

NYMAC and the NACB also recommend measurement of urine organic acids.[27,28]

Further diagnostic tests include gene sequencing (recommended in all screen positives by NYMAC[28]) and assays of fatty acid β-oxidation or VLCAD enzyme activity.

As VLCADD is a recessive disorder, mutations in both alleles are expected. However, as described in Criterion 2 mutations in both alleles have not been identified in all patients with VLCADD. NYMAC report that if no mutations are detected the disease is unlikely (as long as metabolites normal and not consanguineous).[28] If two known or likely pathological mutations, one in each allele, are detected the diagnosis of VLCADD is confirmed. However, if only one known or likely pathological mutation is detected an enzyme assay or functional study is required.[28]

The ACMG state that if the fatty acid oxidation probe assay shows accumulation of C16 acylcarnitine and lower levels of C14 and C12, severe VLCADD is diagnosed.[29] If the fatty acid oxidation probe assay shows higher levels of C12 acylcarnitine relative to C10 and C14, mild VLCADD is diagnosed (it is not reported what this is based on). If fatty acid oxidation is normal then the case is designated a false positive.

None of the guidelines report cut-offs for VLCAD activity which is diagnostic for VLCADD.

Diagnostic follow-up tests for VLCADD reported in newborn screening programmes are shown in Table 15.

Table 15: Diagnostic follow-up in newborn screening programmes. Cut-offs were not reported.

Study **Location** **Period**	**Follow-up diagnostic tests**
Lund et al. (2012)[16] Denmark, the Faroe Islands and Greenland 01/02/2002 to 31/03/2011	Urine organic acids, plasma acylcarnitines, DNA
Lindner et al. 2011[15] Germany 1 January 1999 to 30 June 2009	Characteristic acylcarnitine profile in plasma/DBS and/or enzyme activity in lymphocytes or fibroblasts and/or informative genotype.
Kasper et al. (2010)[30] Austria April 2002 to December 2009	Urine organic acids, enzyme analyses in fibroblasts and/or lymphocytes, and genetic testing.
Tajima et al. (2008)[20] Selected areas of Japan May 1997 to December 2006	C14:1 acylcarnitine levels in serum at two weeks of life
Frazier et al. (2006)[14] North Carolina July 1997 to July 2005	Urine organic acids and plasma acylcarnitine profile. Enzyme and mutation analyses were done whenever the specific tests were available and were approved by third-party reimbursers
Schulze et al. (2003)[13] Germany April 1998 to September 2001	Enzyme activity in fibroblasts/lymphocytes (cut-off not reported)

As described in Criterion 2, although there is some genotype-phenotype correlation, it is difficult to predict phenotype of VLCADD based on genotype, and no publications were identified which correlated residual VLCAD activity with clinical phenotype. Although work is on-going in this area, there currently seems to be no accepted way of predicting phenotype, especially if genotyping reveals missense mutations in both alleles.

Criterion 8 partially met: There are recommendations for the diagnostic investigation of patients who screen positive for VLCADD. However, cut-offs for many of the diagnostic tests have not been reported. As described in Criterion 2, although there is some genotype-phenotype correlation, it is difficult to predict phenotype of VLCADD based on genotype, and no publications were identified which correlated residual VLCAD activity with clinical phenotype. Although work is on-going in this area, there currently seems to be no accepted way of predicting phenotype, especially if genotyping reveals missense mutations in both alleles.

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 2004 HTA report stated that,

> "no additional evidence was available on the effectiveness of treatments for defects of long-chain fatty acid catabolism". Pollitt and colleagues reported that normally a carbohydrate-rich diet is prescribed, with restriction of long-chain fats and their replacement by medium-chain triglycerides for patients with defects of long-chain fatty acid catabolism. Similar findings were reported by Seymour and colleagues. This strict dietary regimen has been shown to be highly effective in a number of patients; however… there was no long-term experience of outcome for disorders of long-chain fatty acid oxidation."[4]

A low-fat diet, supplemented with medium-chain triglycerides (MCT), can be used to avoid the metabolic block in VLCADD and to prevent the primary manifestations of the disease. However, no studies evaluating the effectiveness of the treatment, or showing that early treatment leads to significantly better outcomes than late treatment were identified in the update search. Two publications reporting consensus recommendations were identified, and a publication reporting the treatment and outcomes of 27 patients with VLCADD.

Arnold et al. (2009) reported a Delphi clinical practice protocol for the management of VLCADD.[45] A Delphi clinical practice protocol was developed as the researchers state that "there is a paucity of data regarding which infants are at risk for neonatal or childhood symptoms, the extent to which symptoms can be prevented by early diagnosis or treatment, or which treatments are likely to be most efficacious." The prevention of primary manifestations centred on the prevention of catabolic fasting stress (recommendation grade D- case report and expert opinion based). Consensus feeding recommendations are shown in Table 16. Categorisation of asymptomatic children identified by newborn screening into different phenotypes seemed to be on the basis of DNA or probe/enzyme studies.

Table 16: Consensus feeding recommendations from Arnold et al. (2009)[45]

Phenotype	*Age range*	*Feeding*	*Status*	*Recommendation*
Severe infantile cardiomyopathy	*<12 months*	*Breast-fed*	*Asymptomatic*	*No consensus*
			Symptomatic	*Maximally MCT-enriched formula*
		Bottle fed	*Asymptomatic*	*Change from standard formula*
			Symptomatic	*Maximally MCT-enriched formula*
	>12 months	*Not applicable*	*Asymptomatic*	*LCT restriction, MCT supplementation*
			Symptomatic	*10% calories LCT, 20% MCT*

Fasting intolerance or myopathy	*<12 months*	*Breast-fed*	*Asymptomatic*	*Continue breast-feeding*
			Symptomatic	*Supplement with MCT*
		Bottle-fed	*Asymptomatic/ symptomatic*	*No consensus*
	>12 months	*Not applicable*	*Asymptomatic*	*Dietary modification recommended "Heart-healthy" diet plus MCT*
Fasting intolerance	*>12 months*	*Not applicable*	*Symptomatic*	*Dietary modification recommended "Heart-healthy" diet plus MCT*

MCT, medium-chain triglycerides; LCT, long-chain triglycerides

Spiekerkoetter et al. (2009) reported consensus treatment recommendations for VLCADD based on the expert opinion of metabolic specialists from 18 metabolic centres in Europe.[46] In addition to avoiding fasting, it was recommended that symptomatic patients follow a long-chain fat-restricted and fat modified diet (modified by MCT supplementation). Patients with exercise-induced muscle pain and weakness may benefit from increased MCT (or carbohydrate) supply just prior to more extensive exercise (for example in a dose of 0.25 to 0.5g MCT/kg). For asymptomatic patients (for example those identified by newborn screening), current recommendations are to mildly reduce the fat content of the diet to 30% to 40% of total energy. L-carnitine supplementation for VLCADD is reportedly controversial; there is no published evidence that carnitine is beneficial. No placebo controlled randomised controlled trials of carnitine supplementation for inborn errors of metabolism were identified in a 2012 Cochrane review.[47] The recommendations for fat and carbohydrate intake are summarised in

Table 17.

Table 17: Recommendations for fat and carbohydrate intake for patients with VLCADD from Spiekerkoetter et al. (2009)[46]

VLCADD variant	Age	Fat	Carbohydrates	Energy
Asymptomatic (and CK, AST and ALT values within normal limits)	0-4 months	Half breast milk/infant formula. Half special low-fat formula • Monogen, SHS. Amounts per 100ml: LCT 0.21g, MCT 1.89g Or • Basic-f, Milupa + 2.0g MCT oil per 100ml. Amounts per 100ml: LCT 0.06g, MCT 2.00g	No additional carbohydrates (unless clinically indicated)	No additional energy (according to D-A-CH recommendations)
	After 4 months (introduction of solid food)	Fat reduction to 30-40% of total energy (low values of D-A-CH nutrition recommendations); 10-15% of energy from fat should be from MCT		
Symptomatic	0-4 months	No breast milk or infant formula milk. 100% special low-fat formula • Monogen, SHS. Amounts per 100ml: LCT 0.21g,	No additional carbohydrates (unless clinically indicated)	No additional energy (according to D-A-CH recommendations)

		MCT 1.89g + essential fatty acids (3.5g/day) Or • Basic-f, Milupa + 2.0g MCT oil per 100ml. Amounts per 100ml: LCT 0.06g, MCT 2.00g + essential fatty acids (3.5g/day)		
	After 4 months (introduction of solid food)	Fat reduction to 25-30 (-40)%; 20% of energy from fat should be from MCT; 3-4% of energy from fat should be from essential fatty acids (according to D-A-CH nutrition recommendations)		

D-A-CH, German Austrian Swiss dietary recommendations; MCT, medium-chain triglycerides; LCT, long-chain triglycerides; CK, creatine kinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Monogen, SHS and Basic-f, Milupa, + MCT oil are MCT-containing special formulas.

Reports of treatment

Spiekerkoetter et al. (2009)(described in Criterion 2) reported the actual treatment of 27 of the patients with VLCADD.[9] Treatment was not reported separately for those identified by screening and those that presented clinically. In 25 patients (of the 27 with available data) long-chain triglyceride (fat) intake was restricted, with the maximum amount given ranging from 0.8 to 2.1g/kg/day. Twenty one (of 27) received MCT supplementation, at between 0.7 and 3.3g/kg/day. Data on treatment when aged under 4 months were available for ten asymptomatic infants. Three of these patients received MCT-containing formula plus essential fatty acids; six received breast milk/infant formula and MCT-containing formula: 50-50%; and one received fat free and infant formula: 50-50%. Data were available for the treatment of five symptomatic infants when they were aged under 4 months. Four of these patients received MCT-containing formula plus essential fatty acids; one received breast milk/infant formula and MCT-containing formula: 50-50%. The number of patients who received supplementation of essential fatty acids with a modified diet and addition carbohydrates or tube feeding to delay lipolysis is shown in Table 18. Twenty eight of 52 (54%) patients with long-chain fatty acid disorders (information not available specifically for VLCADD) received L-carnitine. No patient with VLCADD received supplementation with docosahexaenoic acid (DHA, a fatty acid).

Table 18: The number of patients with VLCADD who received supplementation of essential fatty acids with a modified diet and additional carbohydrates or tube feeding to delay lipolysis

		Number of patients
Supplementation of essential fatty acids	Data available	25/32
	Essential fatty acids supplemented	20/25
	As breast milk or infant formula	4/20
	As walnut, soy or wheatgerm oil	12/20
	As other oil	4/20
	Amount (g/kg/day)	0.5-1 (mean 0.65)
Additional carbohydrates	Data available	22/32
	Took Additional carbohydrates	9/22
	As glucose polymer	4/22
	As uncooked cornstarch	5/22
	Continuous nasogastric tube feeding overnight	3/22

Compliance and outcome

Spiekerkoetter et al. (2009)(described in Criterion 2) also reported the compliance and outcomes of the 27 the patients with VLCADD with treatment data.[9]

Adherence, as estimated by the attending physician, was considered optimal in 20/27 (74%) and suboptimal in 5/27 (20%) of patients with VLCADD. Whether there were differences in the interventions in patients with optimal or suboptimal compliance was not reported.

Despite highly rated compliance, five people with VLCADD presented with intermittent symptoms of skeletal myopathy such as muscle weakness, muscle pain and/or myoglobinuria. These five patients were either clinically diagnosed or had symptoms when they were diagnosed by newborn screening. All five patients had their symptoms during intercurrent illness or following exercise. It was not reported whether adherence was optimal or suboptimal in these patients.

Criterion 10 uncertain: A low-fat diet, supplemented with medium-chain triglycerides (MCT), is recommended to avoid the metabolic block in VLCADD and to prevent the primary manifestations of the disease. Diet modification was reported to be effective in the previous HTA report, and treatment was reported to reverse symptoms in studies included in Criterion 2. In addition, with preventive treatment the majority of patients identified by newborn screening remained asymptomatic. However, no studies evaluating the effectiveness of the treatment, or showing that early treatment leads to significantly better outcomes than late treatment were identified in the update search. Two publications reporting consensus recommendations were identified. One of these suggested that patients with different forms of the condition may need different treatments. However, this was based on weak evidence. A publication reporting the treatment and outcomes of 27 patients with VLCADD was also identified, which showed that symptoms can develop even with treatment, as seen in the discussion of natural history in Criterion 2.

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

Criterion 11 not met: It has been suggested that patients with different forms of the condition may need different treatments, and that some patients may not need any intrusive diet at all. However, as discussed earlier there currently seems to be no accepted way of predicting phenotype, especially if genotyping reveals missense mutations in both alleles.

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."[4]

No RCTs of screening for VLCADD were identified.

Criterion 13 not met. No RCTs of screening for VLCADD 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

The 2004 HTA review found that with the exception of phenylketonuria and MCADD that "economic modelling using the available evidence does not support including other inherited metabolic diseases within a neonatal screening programme at present."[4]

One study which assessed the cost-effectiveness of screening for VLCADD 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 VLCADD, using tandem MS in Ontario, Canada.[7] 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 VLCADD was estimated at 1:120,667 based on the incidence of VLCADD 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 11). The distribution of phenotypes was also estimated: 36% of cases were estimated to have the neonatal, classical, severe or early-onset form of VLCADD and 64% of cases were estimated to have later-onset, chronic or mild form VLCADD, 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 case-series of 30 patients, and differs from the distribution reported in other case series (see Criterion 2). Life expectancy with the neonatal, classical, severe or early-onset form of VLCADD was assumed to be the same as with LCHADD, and estimated to be 25 years with an early diagnosis and 12 years with a clinical diagnosis; and the life expectancy with the later-onset, chronic or mild form VLCADD with an early diagnosis was estimated to be 35 years or 20 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 at 99.95%.
- the start-up costs of tandem MS screening (including capital equipment required to start a 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 and MCT (120ml/day) was assumed to cost 4,380 Canadian dollars per year.

The incremental cost and the incremental cost-effectiveness ratios (ICERs) for screening for VLCADD individually are shown in Table 19.

Table 19: 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[7]

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‡
VLCADD	19.97	1.60	4.32	462,269	37,037	6

*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 $37,037 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

Very long-chain Acyl-Coenzyme A Dehydrogenase Deficiency (VLCADD) is a rare autosomal recessive disorder. The estimated incidence of VLCADD is between 1:31,500 (with screening) and 1:1,551,200 (without screening). However, the reported rates vary considerably between studies and the number of cases reported was small, making estimates of prevalence uncertain. No studies were identified that reported the incidence of VLCADD in the UK.

There are three phenotypes associated with VLCADD but the proportion of patients with each of the phenotypes is unclear.

VLCADD can be lethal, with evidence of a high mortality rate without newborn screening in those with early onset disease and cardiomyopathy.However, it should be noted that knowledge of the natural history of VLCADD is limited and based on a few studies describing small numbers of patients with VLCADD. Key areas of uncertainty relate to the correlation between genotype and biochemical and clinical phenotype and between enzyme activity levels and disease severity. Screening may also identify heterozygotes. No studies were identified that reported the natural history of heterozygotes. No papers were identified which explored the psychological implications of carrier status.

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The incidence of VLCADD has increased with screening. This finding together with the observation that the majority of patients identified by newborn screening are asymptomatic seems to have led some researchers to speculate that screening is identifying cases that would remain asymptomatic. However, since there is currently no accepted way of predicting phenotype, and identified cases receive preventative treatment, it is difficult to assess this.

The screening test

There is a simple and safe screening test. Mutations in VLCAD lead to a metabolic block causing the accumulation of C14 to C18 acylcarnitines in the blood, which can be detected using tandem MS on dried blood spots on Guthrie cards. C14:0 (tetradecanoylcarnitine) and C14:1 (tetradecenoylcarnitine) acylcarnitines are the main screening markers for VLCADD.

C14:1 acylcarnitine is the disease specific acylcarnitine, and is the screening marker along with C14:0 recommended by US guidelines. Levels of other acylcarnitines are also abnormal in VLCADD.

Metabolic status can affect screening results. Patients with VLCADD may present with normal acylcarnitine profiles during anabolic conditions (when fatty acid oxidation is not induced) and therefore some researchers have recommended that specimen collection should occur between 36 and 72 hours of life when catabolism is likely to occur. However, healthy newborns may also have abnormal acylcarnitine profiles in the first few days of life due to the stimulation of fatty acid oxidation during catabolism resulting in elevated levels of long chain acylcarnitines. False positive screening and false negative results from the screening and diagnostic process have been reported in some studies.

An upper limit of 72 hours for specimen collection was reported from five of the countries that screened for VLCADD in the EUCERD survey, and an upper limit of 96 hours was reported in four.

This is earlier than UK practice. In two thirds of publications on screening programmes identified from the update search the upper limit was 72 hours.

Cut offs for screening vary and have been the subject of a large study. No cut off has been identified for the UK population.

Despite this, in publications where it was possible to calculate the sensitivity and specificity of screening for VLCADD these were calculated to both be approximately 100%. It should be noted that these values are based on studies where extensive follow-up for 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. Low positive predictive values were reported in some studies.

Diagnostic Confirmation

There are recommendations for the diagnostic investigation of patients who screen positive for VLCADD. VLCADD was commonly diagnosed by genotyping and by assaying residual VLCAD activity.

Hundreds of pathological mutations have been identified in *ACADVL*, and there is no prevalent mutation. In some cases of VLCADD mutations in only one allele have been identified, and it has been presumed that there is a mutation in the other allele which was not detected by the approach used. Although VLCADD is caused by deficiency in VLCAD, residual enzyme activity varies between cases, and there appears to some overlap between heterozygotes and patients with VLCADD. Residual VLCAD activity cut-offs that were diagnostic for VLCADD were not identified. It therefore appears that it might be difficult to distinguish heterozygotes from cases where mutation in only one allele is identified.

In addition, there currently seems to be no accepted way of predicting phenotype, although null mutations have been associated with the severe, neonatal form of the disease, whilst missense mutations have been associated with all clinical phenotypes. No publications were identified which correlated residual VLCAD activity with clinical phenotype or outcome.

The treatment

A low-fat diet, supplemented with medium-chain triglycerides (MCT), can be used to avoid the metabolic block in VLCADD and to prevent the primary manifestations of the disease. Diet modification was reported to be effective in the previous HTA report.

However, no studies evaluating the effectiveness of the treatment, or showing that early treatment leads to significantly better outcomes than late treatment were identified in the update search. Studies of patients with VLCADD have shown that symptoms are reversible, and patients that survive severe neonatal episodes can go onto have a favourable clinical outcome. Myopathy/rhabdomyolysis episodes can occur even with treatment.

Two publications reporting consensus recommendations were identified. One of these suggested that patients with different forms of the condition may need different treatments. However, there currently seems to be no accepted way of predicting phenotype.

Implications for policy

- Current policy on screening for VLCADD should be retained.

Implications for research

- Outcome studies, especially of asymptomatic infants detected by screening.
- Studies to determine whether phenotype or outcome can be predicted
- Studies to determine the optimal management of patients with VLCADD, and who needs treatment
- Studies of the natural history of heterozygotes to determine whether they are at risk of disease, especially those heterozygotes with VLCAD activities similar to cases of VLCADD

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)
  • Very long chain acyl CoA dehydrogenase deficiency and trifunctional protein deficiency (1)
• Postmortem identification (12)
	142

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)	**62**

• Screening for specific fatty acid oxidation disorders (11) • Follow-up of screening (6)	
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 VLCADD were excluded except where they reported cases missed by screening, or maternal cases identified via newborn screening. Additional relevant references identified during the preparation of the report were also included.

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