UK National Screening Committee

Screening for mitochondrial trifunctional protein disorders, including long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency

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

Version: FINAL

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The UK National Screening Committee secretariat is hosted by Public Health England.

About the UK National Screening Committee (UK NSC)

The UK NSC advises ministers and the NHS in the 4 UK countries about all aspects of population screening and supports implementation of screening programmes.
Conditions are reviewed against evidence review criteria according to the UK NSC's evidence review process.

Read a complete list of UK NSC recommendations.

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Plain English summary

Long-chain 3- hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency and Mitochondrial Trifunctional Protein (MTP) deficiency are rare conditions which stop the body from changing some fats to energy. The body struggles more after long periods without food or when under stress (for example during periods of illness).

Babies with LCHAD and MTP deficiencies can suffer from lack of energy, low blood sugar and feeding difficulties. They can also have developmental delay, liver disease, brain damage, enlarged heart muscle, and eye problems. Early death can occur. Dietary control, such as eating often and having a low fat, high carbohydrate diet, or taking special supplements helps with the management of LCHAD and MTP deficiencies. People with LCHAD or MTP deficiencies need support from experts in the conditions.

Screening and current UK NSC recommendations

Newborn screening may help to identify children with LCHAD or MTP deficiency. Dietary management could then begin earlier in the hope this may stop the baby from becoming very poorly.

The UK National Screening Committee reviewed the evidence on LCHAD and MTP deficiencies in 2014. They recommended that the NHS should not screen for these conditions. This was because a one-year study found no evidence that the test was effective at finding the conditions in babies with no symptoms.

The current review examines the evidence on:

- the frequency of the condition in the UK
- the links between genes and symptoms for people with LCHAD/MTP deficiency
- how good the test is at finding people with the condition
- the advantages of early treatment following screening versus later treatment following the onset of the illness

This review found that:

1. there were no studies from the UK on the number of people born with LCHAD/MTP deficiency. Two European studies supported the results from the most recent systematic review that approximately 0.67 per 100,000 newborns have the condition;
2. different outcomes do not seem to be linked to their specific mutation. But people with MTP deficiency may be more likely to be very ill from birth and people with LCHAD deficiency may be more likely to present later in infancy;
3. the evidence on the screening test indicates that false positives are common. There is also not enough information on babies who have had a negative screening test, to check if the test was right or wrong;
4. there is some evidence to suggest that people diagnosed before they have symptoms of LCHAD/MTP deficiency might have better outcomes than those treated once symptoms appear. But these studies are small and have considerable biases.

Recommendations

For these reasons, the review does not recommend screening for LCHAD or MTP deficiencies until we know more about:

- how many babies are born with LCHAD/MTP deficiency in the UK each year using medical record data rather than reviewing studies on screening
- the association between genetic mutations and symptoms
- how many babies who have a negative screening test go on to develop LCHAD/MTP deficiency
- whether in the UK babies treated following screening (before they had symptoms) went on to have better outcomes than those treated once they had developed symptoms.

Executive summary

Purpose of the review

The purpose of the review was to determine (1) the frequency of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) and mitochondrial trifunctional protein (MTP) deficiency in the UK, and to examine (2) the genotype and phenotype associations, (3) the test accuracy of acylcarnitine measurement in dried blood spots (DBS) using tandem mass spectrometry for LCHAD/MTP deficiency screening, and to determine (4) whether there are any advantages of early treatment following screening versus later treatment following the presentation of symptoms.

Background

LCHAD and MTP deficiency (LCHADD/MTPD) are rare fatty acid beta oxidation disorders (FAOD) caused by mutations in the genes coding for MTP. MTP is responsible for the beta oxidation of long-chain fatty acids to yield cellular energy in the form of adenosine triphosphate (ATP). The estimated prevalence of LCHAD/MTP deficiency is 1.02 per 100,000 births worldwide; there are an estimated 4.9 cases per year in England and Wales (from 729,674 births; 0.67 per 100,000) [1].

Babies with LCHAD/MTP deficiency can clinically present in 3 ways: (1) an early onset acute form, (2) an infant hepatic form, and (3) a later myopathic form which is slower to develop [2]. The early onset severe form may present as hypertrophic cardiomyopathy, hepatic encephalopathy or severe hypoketotic hypoglycaemia and can often lead to death [3, 4]. The infant hepatic form presents in infancy generally as hypoketic hypoglycaemia and/or hepatomegaly [4]. People with the later myopathic form tend to present with rhabdomyolysis, muscle pain or weakness, often following exercise or illness [5].

Screening of different acylcarnitines in newborn blood spots using tandem mass spectrometry (TMS) may help to identify cases before they become symptomatic and could be added to the UK newborn blood spot test.

Some study authors have suggested that starting treatment before the onset of symptoms might lead to better long-term outcomes than treatment following clinical diagnosis [5, 6].

Focus of the review

The aim of this report is to examine 4 key questions relating to the effectiveness and appropriateness of newborn screening using TMS for LCHADD/MTPD. Specific questions for the review are:

1. What is the birth prevalence of LCHAD/MTP deficiency in the UK?
   (UK NSC criterion 1: The epidemiology, incidence, prevalence and natural history of the condition should be understood)
2. What are the genotype-phenotype associations in LCHAD/MTP deficiency patients, including their clinical prognosis?
   (UK NSC criterion 1: The epidemiology, incidence, prevalence and natural history of the condition should be understood)
3. What is the test accuracy (sensitivity, specificity, and predictive values applicable to UK prevalence) of acylcarnitines measurement in dried blood spots (DBS) using TMS for LCHAD/MTP deficiency screening?
   (UK NSC Criterion 4: There should be a simple, safe, precise and validated screening test)
4. Does early treatment with dietary management following screening provide better long-term outcomes than later treatment after the presentation of symptoms?
   (UK NSC Criterion 9: There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care; UK NSC Criterion 11: There should be evidence from high quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity)

Methods

A rapid review approach was undertaken for key questions 1 and 2. Full systematic reviews were undertaken for key questions 3 and 4. Two searches were undertaken: a broad search for questions 1, 2 and 4 and a more targeted search for question 3. Key question 1 included studies published since 2013 to build upon understanding from a previous review,

and key question 2 was limited to studies published since 2000. No date limit was applied to key questions 3 or 4. For key questions 1 and 3 the population was general newborn infant populations, whilst for 2 and 4 the population was people with LCHAD or MTP deficiency. Searches were conducted in Web of Science (Core Collection), Medline (Ovid), Medline In-Process & Other Non-Indexed Citations (Ovid), Embase (Ovid) and the Cochrane Library. Reference lists of all included articles and relevant systematic reviews were screened.

Recommendation under review

The last UK NSC update review of screening for LCHAD and MTP deficiency was completed in May 2014 [7]. The review included results of the expanded newborn screening study, a health economic report, and a systematic review on the prevalence of the disorder [1, 8, 9]. The recommendation from the review was that additional focussed training should be provided to neonatal clinicians to raise awareness around the symptoms of LCHAD and MTP deficiency as clinical management was deemed more effective than systematic population screening. It was also recommended that a further review be undertaken in 2017/2018 [7].

Findings and gaps in the evidence of this review

Key question 1: What is the birth prevalence of LCHAD/MTP deficiency in the UK?

The reviewers found no studies of the birth prevalence of LCHAD/MTP in the UK published since 2013. Two studies were identified which investigated the incidence of LCHAD/MTP deficiency in Western-European countries [10, 11]. Nine further studies were identified from countries outside Western Europe (North America, Southeast Asia and the Middle East) [12-21]. The results from the Western-European studies found rates of 0.72 per 100,000 births across Portugal and Spain [10] and 0.79 per 100,000 births in Germany [11], supporting the findings of the most recent systematic review estimates of approximately 0.67 per 100,000 births [1].

Key question 2: What are the genotype-phenotype associations in LCHAD/MTP deficiency patients, including their clinical prognosis?

Sub-question: What is the incidence of asymptomatic and/or milder phenotype in the neonatal period?

Key question: Twenty-seven articles were included in this review [6, 22-47]. In these articles, 95 different genotypes and 76 different phenotypes were noted. Genotypes were grouped into 6 categories – LCHAD deficiency homozygous 1528G>C, heterozygous LCHAD deficiency 1528G>C, LCHAD deficiency mutations unspecified, MTP deficiency alpha subunit mutation, MTP deficiency beta subunit mutation, MTP deficiency subunit mutation unspecified. The most common genotype was the homozygous LCHAD deficiency mutation (157 people out of 301). There were wide variations between genotype category and symptoms/outcomes. This may be due to the very small number of patients included in each group (in some studies as low as one), or the type of mutation as opposed to the location of the mutation (e.g. deletions may lead to more severe phenotypes than missense mutations).

There may be some association between the genotype category and the presenting form of the disease: a greater proportion of people with MTP deficiency presented with the neonatal severe form or late onset myopathic forms of the disease compared to people with LCHAD deficiency (30/66 severe and 29/66 myopathic MTP deficiency compared to 4/49 severe and 6/49 myopathic in LCHAD deficiency group). There were more infant hepatic presentations in the LCHAD deficiency categories (38/49 LCHADD compared to 7/66 MTP deficiency). Further analysis is required to determine if type only or a combination of type and location of the genetic defect is linked to phenotypic presentation.

Sub-question: One paper was identified which reported on screening, number of cases and whether they went on to present with symptoms in the neonatal period [36]. The study screened round 1,200,000 infants and found 9 cases of LCHAD/MTP deficiency (7 LCHAD deficiency, 2 MTP deficiency). Of these 9 cases, one was still asymptomatic by age 3, although all 9 received treatment.

UK NSC criterion 1: Not met (met for Question 1 and not met for Question 2)

Key question 3: What is the test accuracy (sensitivity, specificity, and predictive values applicable to UK prevalence) of acylcarnitine

measurement in dried blood spots (DBS) using TMS for LCHAD/MTP deficiency screening?
Sub-question: Can the test distinguish between asymptomatic patients and those affected by milder forms of LCHAD and MTP deficiency?
Sub-question: Does the test detect other non-MTP conditions?

Key question: Ten articles, all reporting on cohort studies, were included in the review. Screening methods varied between studies, including the cut-off values that were used and how they were determined. Risk of bias was considered high in 2 or more domains in 9 out of 10 studies (90%). No study was at low risk of bias in all domains. The Flow and Timing domain was the most frequent source of bias, as none of the studies systematically followed up the babies that had screened negative.

There were significant concerns regarding the applicability of the studies to UK screening in 7 of the 10 studies (70%). The most frequent reason being that blood samples were taken before day 5 in 5 studies.

Twenty-three true positives and 40 false positives were identified from 3,951,358 newborns across all studies. The true positives comprised 11 babies with LCHAD deficiency, 2 with MTPD, and 10 where LCHAD and MTP deficiencies were not differentiated. One baby presented symptomatically before screening took place. The only available test accuracy metric was positive predictive value (PPV), which ranged from 0–100%. It was not possible to estimate sensitivity, specificity, or negative predictive value as newborns who screened negative were not systematically followed up. The included studies use a wide range of markers and thresholds. Test accuracy estimates differ greatly by study, with some suggesting good accuracy albeit on small numbers. However the results are not presented by marker so it is not possible to combine data from different studies, or determine which combination of markers and thresholds may yield good accuracy. The reviewers suggest collaboration between researchers to report scores on a range of relevant markers for both cases of LCHAD, cases of MTP, and in the unaffected population using consistent units.

Sub-question 1: There was no evidence to indicate that the screening test can distinguish between milder and asymptomatic types of LCHADD/MTPD.

Sub-question 2: There is some evidence that the primary markers for LCHAD/MTP deficiency may appear raised when newborns have other fatty acid oxidation disorders [48]. However, this appears to be in conjunction with higher rates of the primary markers for those conditions.

There is currently insufficient evidence about acylcarnitine measurement in DBS using TMS to screen for LCHAD/MTP deficiency from which to draw conclusions about its usefulness.

UK NSC criterion 4: Not met

Key question 4: Does early treatment with dietary management following screening provide better long-term outcomes than later treatment after the presentation of symptoms?

Eleven studies (reported in 13 papers) were identified for this review [6, 23, 26, 28, 31, 33, 38, 41, 49-53]. These comprised 3 related papers on a Swedish cohort [49-51], 2 European single country studies [31, 38], 2 European collaboration studies [41, 52], 4 single country non-European studies [23, 26, 28, 33], and 2 non-European collaboration studies [5, 6]. The number of LCHAD/MTP deficiency patients included per paper ranged from 5 to 59 [6, 26]. A total of 156 people with LCHAD deficiency, 18 people with MTP deficiency and 12 undifferentiated LCHAD/MTP deficiency (186 people in total) were included across all the studies. There was only one randomised controlled trial included within the review which had high applicability concerns, as the main focus of the trial was to determine the benefits of different drug treatments.

There is some evidence to suggest that early dietary management while asymptomatic may be associated with a reduction in heart [5, 26, 28, 31, 38, 52], visual [23, 31, 49, 50, 52], neurological [26, 49], motor and muscular problems [23, 26, 38, 49, 52]. It may also be associated with a reduction in hypoglycaemia [38, 50], failure to thrive [26], brain damage [51] acute metabolic encephalopathy [5] and developmental delay [5]. However, the majority of studies were small, and the methodological quality was moderate to weak in all studies. Furthermore, disease severity and age of participants within the early and late groups may have biased the results.

UK NSC criterion 9: Not met

UK NSC criterion 11: Not met

Recommendations on screening

The volume and quality of data identified in this review suggests that there is a lack of high-quality evidence about whether the benefits of screening for LCHAD/MTP deficiencies would outweigh the harms. Test accuracy estimates differ greatly by study, but heterogeneity in markers used prevented analysis of whether this is due to thresholds used, and lack of reporting of accuracy by marker preventing establishment of the optimal threshold. On this basis, the introduction of a screening programme in the UK is not currently recommended. Further research should be undertaken to explore the issues highlighted. The reviewers suggest collaboration between researchers to report scores on a range of relevant markers forboth cases of LCHAD, cases of MTP, and in the unaffected population using consistent units.

Limitations

For review questions 1 and 2, the reviewers used a rapid evidence approach (REA), meaning date limits were applied at sifting and only articles written in English were included, hence it is possible that relevant articles may have been missed. The reviewers were aware of 3 relevant articles which were not identified by the incidence question (question 1) search strategy; these were picked up by the test accuracy search. Sifting and data extraction for these questions were performed by one reviewer with a random 20% checked by a second reviewer. Therefore, there is a risk of error in excluding relevant studies and when extracting the data. For question 2, the inclusion criteria definitions were broad, which meant the inclusion of many small low quality patient case series.

A separate search was undertaken for question 3 as several papers on test accuracy do not specify LCHAD deficiency or MTP deficiency by any form of disease. Terms around *“inherited metabolic disease*”* and screening were added.

Evidence uncertainties

Further systematic reviews to evaluate the incidence of LCHAD/MTP deficiency in the UK would not be beneficial unless screening were in place. Instead, a study into existing UK health databases might provide useful information on the long-term prevalence of the disorders in the UK.

There is a lack of long-term follow up of screen negative cases. This is a particular issue when considering that the disease can present in young adults. Research projects using tandem mass spectrometry measurement of the acylcarnitines (C16OH, C16:1-OH, C18OH) with follow up of screen negative cases would be beneficial. Likewise, research reporting scores for a range of relevant markers for cases of LCHAD, cases of MTP, and in the general population, using consistent units would help determine the accuracy of the test.

There is considerable research available into the genotype-phenotype associations and outcomes following asymptomatic detection versus symptomatic detection. People with LCHAD deficiency seem to be more likely to have the infant hepatic form of the disease. The homozygous LCHAD deficiency group appears to be the largest (157 out of a total of 301 people identified. The remaining 144 people exhibited other 94 genotypes). This finding may have implications for the optimal point for these people to be treated. Likewise, it appears that treating people with LCHAD or MTP deficiencies whilst asymptomatic may have some benefit to their long-term outcomes. However, there is a lack of UK research in this area. Given the rarity of the diseases, a retrospective review of medical records identifying all people in the UK with LCHAD/MTP deficiency and their genotype, which prospectively follows their outcomes over time may be the most feasible approach to understanding genotype-phenotype associations and the relative benefits of early versus late treatment.

Introduction and approach

Background

Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) and mitochondrial trifunctional protein (MTP/TFP) disorders are rare fatty acid beta oxidation disorders (FAOD) caused by mutations in the genes coding for MTP. MTP is a multifunctional enzyme complex formed by 4 alpha and 4 beta subunits and is responsible for the beta oxidation of long-chain fatty acids to yield cellular energy in the form of adenosine triphosphate (ATP). In isolated LCHAD deficiency (LCHADD), mutations occur within the alpha subunit in the LCHAD enzyme, with normal activity in the other MTP enzymes. In MTP disorders, mutations result in deficient activities in the 2 other MTP enzymes (long-chain enoyl-CoA hydratase and long-chain 3-oxoacyl-CoA thiolase) [54]. This is depicted in Figure 1. Two genes, HADHA and HADHB, located in the same region of chromosome 2 encode for the 4 alpha and the 4 beta subunits respectively. Maternal and paternal carriers for these disorders have a mutation in the alpha or beta gene on one copy of chromosome 2, while the other copy of chromosome 2 harbours a normal (wild type) gene. Individuals who have the disorders have mutations in trifunctional protein genes on both copies of chromosome 2. The most frequently occurring form of these disorders is isolated LCHAD deficiency in which both chromosome 2 copies have the same missense mutation (G1528C) in the alpha subunit gene (the person is homozygous), causing incorporation of glutamine rather than glutamic acid into the active site of the LCHAD enzyme. In less common forms of LCHAD deficiency the person is heterozygous, carrying different alpha subunit mutations on each chromosome 2; in these individuals the G1528C mutation is usually present on one copy of chromosome 2. In one study, heterozygosity was found in 9/34 individuals tested, with the G1528C mutation accounting for 59 of 68 alleles [1].

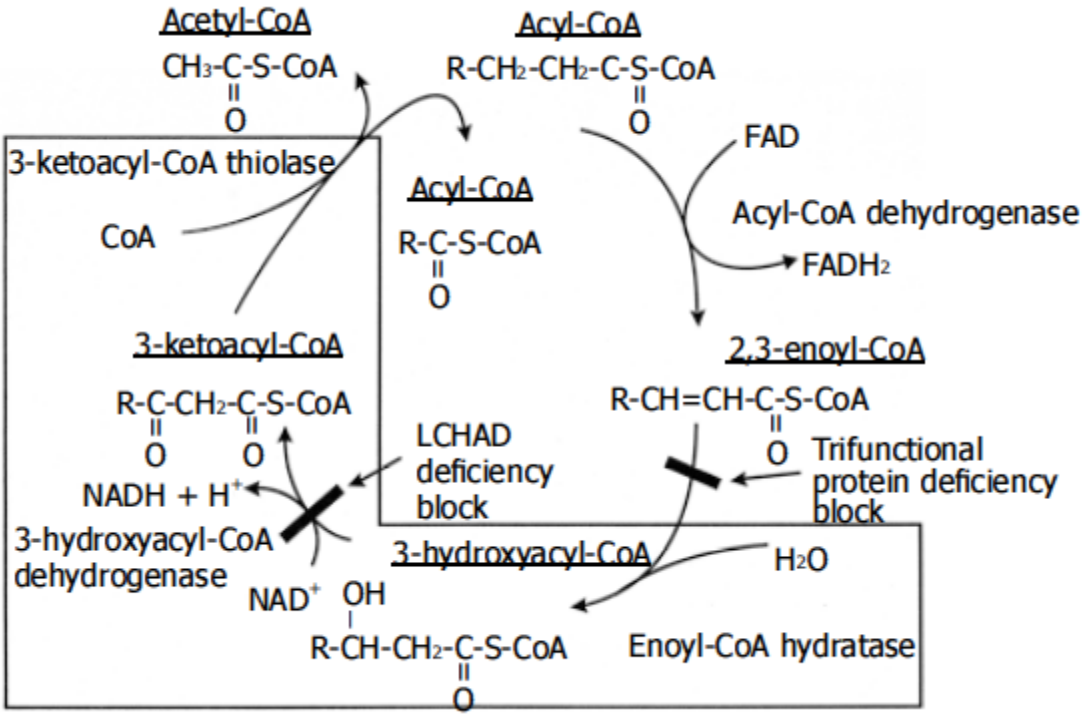

Figure 1 – Beta oxidation pathway showing where the LCHADD and MTP deficiency blocks occur in the beta oxidation sequence. LCHAD is within the MTP cycle, MTP deficiency inhibits all activity in the MTP [55]

MTP deficiency is rarer than LCHAD deficiency, with an estimated prevalence of 1 in 1,822,568 as opposed to 1 in 363,738 in the USA [17]. Mutations may occur in either HADHA or HADHB genes and may compromise the production of MTP protein precursors, the assembly of the octomer, the recognition and import of the octomer into the mitochondrion or its function within the mitochondrion. The estimated prevalence of LCHAD and MTP deficiencies is up to 1.02 per 100,000 births worldwide and there are estimated to be 0.67 cases in England and Wales per 100,000 [1]. Babies present with symptoms such as hypoketotic hypoglycaemia, failure to thrive, feeding difficulties, encephalopathy, liver disease, cardiomyopathy, hepatic symptoms, Reye syndrome, retinopathy and cardiac features, with 38% dying either before or within 3 months of diagnosis [56-58]. LCHAD and MTP deficiencies are treated through strict dietary management. This can include eating frequently, a low fat and high carbohydrate food plan, or taking supplements such as medium chain triglyceride (MCT) oil [58].

Both LCHAD and MTP deficiencies may develop at different ages based on different genotypes [2]. Early-onset severe cases are present from birth or a few days of life and often result in sudden infant death. A milder form is infant-onset which is induced by illness. These infants present with hypoketotic hypoglycaemia and lethargy when fasting. The third phenotype is a later onset form characterised by myopathy which is induced by exercise or illness. These people often survive to adolescence or adulthood.

Current policy context and previous reviews

The current recommendation not to screen for LCHAD deficiency was made in Spring 2014 [7]. This was based on the results of the expanded newborn screening study, a health economic report and a systematic review on the prevalence of the disorders [2, 8, 9]. The recommendation from the review was that additional focussed training should be provided to neonatal clinicians to raise awareness around the symptoms of LCHAD and MTP deficiencies as clinical management was deemed more effective than systematic population screening [7]. This decision was due to no asymptomatic cases being detected during the expanded blood spot screening study so that test accuracy had not been demonstrated. It was recommended that a further review be undertaken in 2017/2018.

Objectives

The objective of the review is to look for evidence to confirm or not the current recommendation not to screen for LCHAD and MTP deficiencies. The aim of this evidence review is to examine 4 key questions relating to the effectiveness and appropriateness of newborn screening using acylcarnitine measurement in dried blood spots and tandem mass spectrometry for LCHAD/MTP deficiency. The key questions for this review, the criteria they address and the number of studies included per question are provided in Table 1. 'Key questions for the evidence summary, and relationship to UK NSC screening criteria’

Table 1. Key questions for the evidence summary, and relationship to UK NSC screening criteria

	Criterion	Key questions	Studies Included
	THE CONDITION		
1	The condition should be an important health problem as judged by its frequency and/or severity. The epidemiology, incidence, prevalence and natural history of the condition should be understood, including development from latent to declared disease and/or there should be robust evidence about the association between the risk or disease marker and serious or treatable disease.	Question 1. What is the birth prevalence of LCHAD/MTP deficiency in the UK?	n=11 [10, 11, 13-21]
		Question 2. What are the genotype-phenotype associations in LCHAD/MTP deficiency patients, including their clinical prognosis? Sub-question: What is the incidence of asymptomatic and/or milder phenotype in the neonatal period?	n=27 [6, 22-47]
	THE TEST		
4	There should be a simple, safe, precise and validated screening test.	Question 3. What is the test accuracy (sensitivity, specificity, and predictive values applicable to UK prevalence) of acylcarnitines measurement in dried blood spots (DBS) using TMS for LCHAD/MTP deficiency screening?	n=10 [5, 9, 16, 18, 21, 36, 48, 59-61]
	THE INTERVENTION		
9	There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care. Evidence relating to wider benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme shouldn't be further considered.	Question 4. Does early treatment with dietary management following screening provide better long-term outcomes than later treatment after the presentation of symptoms?	n=13 [6, 23, 26, 28, 31, 33, 38, 41, 49-53]
	THE SCREENING PROGRAMME		
11	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	Question 4. Does early treatment with dietary management following screening provide better long-term outcomes than	n=13 [6, 23, 26, 28, 31, 33,

	Criterion	Key questions	Studies Included
	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.	later treatment after the presentation of symptoms?	38, 41, 49-53]

Methods

The current review was conducted by The University of Warwick, in keeping with the UK NSC evidence review process. Database searches were conducted on 23 April 2018 (questions 1, 2 and 4) and 19 June 2018 (question 3) to identify studies relevant to the questions detailed in Table 1. The reviewers used a rapid review approach for key questions 1 and 2 and a full systematic review approach for key questions 3 and 4. For this evidence summary the decision was made to use a systematic review approach for questions 3 and 4 looking at the test accuracy and early treatment respectively. The rationale for this decision was based on the conclusion reached by the UK NSC based on the outcomes of the expanded newborn screening study. No cases of asymptomatic LCHADD were identified by the screening evaluation. As such the feasibility of the test had not been demonstrated. Similarly, uncertainties remained regarding the prognosis for LCHADD and any potential benefit of early treatment following screening compared to later treatment after the presentation of symptoms. A systematic review approach including an attempt to statistical analysis was therefore identified as the best method to evaluate the quality of the evidence base with regard to question 3 and 4.

Identification and selection of studies

One systematic literature search was undertaken to cover review questions 1 (incidence), 2 (genotype/phenotype association) and 4 (treatment). A separate literature search was undertaken for key question 3 (screening test). Searches were conducted in MEDLINE (Ovid), EMBASE (Ovid), MEDLINE In-Process & Other Non-Indexed Citations

(Ovid), Web of Science (SCI-EXPANDED, SSCI and ESCI) and Cochrane Library (Cochrane reviews, other reviews, methods studies and technology assessments). No date limits were applied to the search, however date limits were applied for key questions 1 and 2 during sifting. Reference lists of all included articles were screened. The search strategies are presented in Appendix 1 - Search strategy tables 3-10. Eligibility criteria for each question are presented in Table 2 Inclusion and exclusion criteria for the key questions.

Review strategy

The following review process was followed:

1. For questions 1 and 2 each abstract was reviewed against the inclusion/exclusion criteria by one reviewer. Where there was insufficient information available in the title/abstract on which to make a decision, the article was retained to ensure that all potentially relevant studies were captured. A second reviewer independently assessed 20% of the titles and abstracts. Any disagreements were resolved by discussion until a consensus was reached, or with the involvement of a third reviewer. For questions 3 and 4 each abstract was reviewed independently against the inclusion/exclusion criteria by 2 reviewers. Where there was insufficient information available in the title/abstract on which to make a decision, the article was included at this stage to ensure that all potentially relevant studies were captured. Any disagreements were resolved by discussion until a consensus was met, with the involvement of a third reviewer if required.
2. Full-text articles required for the full-text review stage were acquired.
3. For questions 1 and 2 each full-text article was assessed against the inclusion/exclusion criteria by one reviewer, who determined whether the article was relevant to one or more of the review questions. A second reviewer independently assessed 20% of the full texts. Any disagreements were resolved by discussion until a consensus was reached, or with the involvement of a third reviewer. For questions 3 and 4 each full-text article was reviewed against the inclusion/exclusion criteria by 2 reviewers who determined whether the article was relevant to one or more of the review questions. Any disagreements were resolved by discussion

until a consensus was reached or with third reviewer involvement if required.

Eligibility criteria for each question are presented in Table 2 below.

Table 2 Inclusion and exclusion criteria for the key questions

Key question	Inclusion criteria							Exclusion criteria
	Population	**Target condition**	**Intervention**	**Reference Standard**	**Comparator**	**Outcome**	**Study type**	
1. What is the birth prevalence of LCHAD/MTP deficiency in the UK?	General newborn infant populations not at high risk of FAOD	The conditions under consideration are LCHADD and MTP	NA	No reference standard	No comparator	Birth prevalence of LCHADD	Any cross-sectional study, cohort study or report from the neonatal screening programmes	English language papers published before 2013 (this will be an update of a previous review that covered published literature before this date). Non-human studies, letters, editorials and communications, grey literature, non-systematic reviews and conference abstracts. Papers with no extractable data. Studies of fatty acid beta oxidation disorders where data from people with mitochondrial trifunctional protein disorders cannot be separated out from data on other fatty acid oxidation

								disorders (e.g. multiple acyl-CoA dehydrogenase and very long chain acyl-CoA dehydrogenase deficiencies). Studies where more than 10% of the sample do not meet our inclusion criteria.
2. What are the genotype-phenotype associations in LCHAD/MTP deficiency patients, including their clinical prognosis?	People with isolated LCHAD deficiency or MTP deficiency	The conditions under consideration are LCHAD deficiency and MTP deficiency	NA	No reference standard	NA	Any observable characteristic, e.g. timing of onset, health outcomes, mortality, cognition, motor function	Any cross-sectional study, cohort study or report from the neonatal screening programmes.	English language papers published before 2000. Non-human studies, letters, editorials and communications, grey literature, non-systematic reviews and conference abstracts. Papers with no extractable data. Studies of fatty acid beta oxidation disorders where data from people with mitochondrial trifunctional protein disorders cannot be separated out from data on other fatty acid oxidation disorders (e.g. multiple acyl-CoA dehydrogenase and very long chain acyl-CoA dehydrogenase deficiencies). Studies

								where more than 10% of the sample do not meet our inclusion criteria.
3. What is the test accuracy (sensitivity, specificity, and predictive values applicable to UK prevalence) of acylcarnitines measurement in dried blood spots (DBS) using TMS for LCHAD/MTP deficiency screening?	Neonatal or newborn infants	The conditions under consideration are LCHAD and MTP deficiencies	The index test is newborn screening for LCHADD and MTP using TMS measurement of acylcarnitines in dried blood spots	Urine testing for organic acids and blood acylcarnitines profiles, enzyme analysis in cultured fibroblasts or lymphocytes or mutation analysis	No comparator.	Sensitivity, specificity, predictive values	Cross-sectional test accuracy studies, case-control studies and cohort studies. If available, any randomised controlled trial of the screening pathway	Non-human studies, letters, editorials and communications, grey literature, non-systematic reviews and conference abstracts. Papers with no extractable data. Studies of fatty acid beta oxidation disorders where data from people with mitochondrial trifunctional protein disorders cannot be separated out from data on other fatty acid oxidation disorders (e.g. multiple acyl-CoA dehydrogenase and very long chain acyl-CoA dehydrogenase deficiencies). Studies where more than 10% of the sample do not meet our inclusion criteria.
4. Does early treatment with dietary management following	People with isolated LCHAD deficiency	The conditions under consideration are	Treatment with dietary restrictions and other nutritional	No reference standard	Treatment following the presentation of symptoms (either 1:	Any clinical outcome	Any study design in humans with comparative data	Non-human studies, letters, editorials and communications, grey literature, non-systematic reviews

screening provide better long-term outcomes than later treatment after the presentation of symptoms?	or MTP deficiency	LCHAD and MTP deficiencies	strategies (e.g. medium-chain triglyceride supplementation) following screening (universal newborn screening, cascade testing or incidental detection).	symptoms presenting after the screening period, or 2: symptoms presenting before the screening period)	and conference abstracts. Papers with no extractable data. Studies of fatty acid beta oxidation disorders where data from people with mitochondrial trifunctional protein disorders cannot be separated out from data on other fatty acid oxidation disorders (e.g. multiple acyl-CoA dehydrogenase and very long chain acyl-CoA dehydrogenase deficiencies). Studies where more than 10% of the sample do not meet our inclusion criteria.

Appraisal for quality/risk of bias tool

The following tools were used to assess the quality and risk of bias of each study included in the review:

- epidemiology studies: JBI Critical Appraisal Checklist for Studies Reporting Prevalence Data [62] was used for question 1 on prevalence
- cross sectional studies: JBI Checklist for Analytical Cross Sectional Studies [63] was used for question 2 on the genotype-phenotype association
- diagnostic accuracy studies: Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool modified [64] was used for question 3 on test accuracy
- cohort studies: Effective Public Health Practice Project (EPHPP) quality assessment tool [65] was used for question 4 on outcomes following asymptomatic or symptomatic detection

Quality assessment of all included studies was undertaken independently by 2 reviewers. Disagreements were resolved by consensus or through discussion with a third reviewer.

Methods of analysis/synthesis

For key questions 1–3, the reviewers conducted a narrative review. For question 4 the reviewers undertook a narrative review and followed this with statistical comparisons of (1) asymptomatic at detection vs symptomatic at detection, (2) screened vs unscreened, (3) asymptomatic screened vs symptomatic at screening vs late clinically diagnosed following symptoms, (4) LCHAD deficiency vs MTP deficiency.

Question level synthesis

Criterion 1 — Birth prevalence of LCHAD/MPT deficiency in the UK and genotype-phenotype associations in LCHAD/MTP deficiency patients

The condition should be an important health problem as judged by its frequency and/or severity. The epidemiology, incidence, prevalence and natural history of the condition should be understood, including development from latent to declared disease and/or there should be robust evidence about the association between the risk or disease marker and serious or treatable disease

Question 1 — What is the birth prevalence of LCHAD/MTP deficiency in the UK?

The UK NSC decision not to implement a national screening programme for LCHAD deficiency took into consideration the outcomes of the expanded newborn screening evaluation. Birth prevalence figures for LCHAD/MTP deficiency are variable and one of the aims of the study was to help determine disease incidence more closely in the screened areas. LCHADD was estimated to occur in the UK at a rate of 1:218,564 though the observed prevalence during the expanded newborn screening study was 1: 437,000 [9]. Given the rare nature of the condition, the aim of addressing this question in this review is to evaluate if more recent evidence has been published to more accurately determine prevalence of LCHAD/MTP deficiency in newborns in the UK.

Eligibility for inclusion in the review

Articles were included within this question if they gave the birth prevalence of LCHAD deficiency and/or MTP deficiency in the general newborn infant population not at high risk of fatty acid oxidation disorders (FAOD). Study types suitable for inclusion were cross-sectional studies, cohort studies or reports from neonatal screening programmes. This question was an update of the previous review, so searches were limited to English language papers published since 2013 [1]. Papers including non-human studies, letters, editorials and communications, grey

literature, non-systematic reviews and conference abstracts were excluded.

Description of the evidence

Appendix 2 contains a full PRISMA flow diagram (Figure 27), along with a table of the included publications (Table 11). Database searches yielded 7,483 unique results, of which 313 full texts were retrieved and sorted and 8 were judged to be relevant to this question. An additional 3 relevant articles were identified through the test accuracy search, so 11 articles were ultimately included in this review [10, 11, 13-21].

Characteristics of included studies

A study-level summary of data extracted from each included publication is presented in Appendix 3. 'Estimates of the incidence of LCHAD and MTP deficiency published up to 2013' are outlined in Table 14.

Details on studies included in this review are shown in Table 15 in Appendix 3. No studies were identified that were published after 2013 (the cut-off date from the previous review) which investigated the incidence of LCHAD/MTP deficiency in the UK. Two studies reported birth prevalence in Western-European countries [10, 11]. One of these studies was a comparison between Germany and countries in Southeast Asia [11].The remaining 9 studies reported on prevalence across Eastern Europe [16], North America [17], Southeast Asia [11, 14, 15, 18, 19, 21] and the Middle East [13, 20]. Nine of the 11 studies reported rates for either LCHAD deficiency alone or combined rates for LCHAD and MTP deficiencies; only one study reported on them as 2 separate conditions [17]. Data were collected between 1997 and 2015 across 9 studies, one study did not report the time period in which the data were collected [10]. Population sizes in the studies ranged from 2,440 [21] to 24,340,414 [17].

Discussion of findings

Quality appraisal of included studies

The 11 included studies [10, 11, 13-21] were quality appraised using the JBI Critical Appraisal Checklist for Studies Reporting Prevalence Data [62]. Details are shown below in Figure 2 and in Appendix 4 Table 21. For

4 out of 11 studies (36.4%) there were risks of bias on at least 2 items. Ten out of 11 studies (90.9%) were rated unclear on at least 2 items. Sample size was not adequate in 7 of the included studies [13-18, 20, 21]. These studies did not conduct a sample size calculation, and did not include enough people to find cases of LCHAD/MTP deficiency. Six of the studies did not adequately describe study participants and setting [15-18, 20, 21]. This lack of information meant it was unclear whether the population was comparable to the UK. Seven out of 11 (63.6%) studies were unclear in their reporting of the methods used for the identification of the condition and the reliability of the condition measurement [10, 11, 15-18, 21].

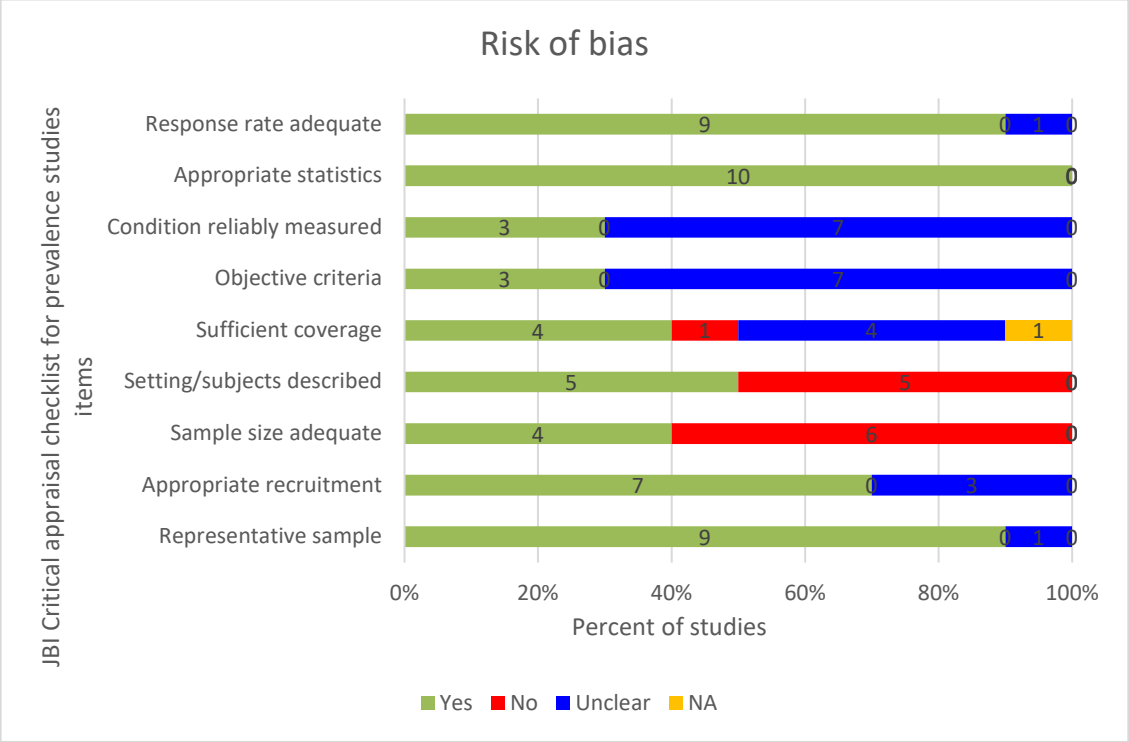

Figure 2 Risk of bias in included prevalence studies [62]
Yes means item was met; no means it was not met; NA, not applicable

Analysis of the evidence

One study reported rates for Iberia which included Portugal and every region in Spain [10]. In Iberia, the birth prevalence of LCHAD/MTP deficiency was 1:139,357 (from a cohort of 1,672,286 screened newborns). The period during which screening was undertaken was not

specified. Another study compared the prevalence of LCHAD/MTP deficiency in Germany to the rates in Southeast Asia [11]. In Germany, the birth prevalence was 1:127,000 (from a cohort of 7.51 million newborns from 2002 to 2015) compared to 1:840,000 and 1:1,148,000 in Japan and Korea.

Ten studies were identified since 2013 from outside of the UK and Western Europe. These studies reported on the birth prevalence for Slovenia [16], USA by state [17], Taiwan [14], Singapore [15], Hong Kong [21], Japan [11], China [18], Malaysia [19], the United Arab Emirates [13] and Egypt [20]. In Slovenia, no reported cases were found in a cohort of 10,048 screened newborns born between 2013 and 2014, however in the same period in an unscreened cohort of 293,387 newborns the birth prevalence was found to be 1:293,897 [16]. Therrell et al. [17] reported 10-year birth prevalence data (January 2001 to December 2010) from each of 51 national partnering programmes (50 states and the District of Columbia) in the USA. Periods of screening ranged from 10 years (starting January 2001) in 6 programmes, to 17 months (starting July 2009) in one programme. LCHAD deficiency and MTP deficiency rates were reported separately, with an overall prevalence of 1:363,738 for LCHAD deficiency, ranging from 1:29,416 in Columbia to no cases detected in 21 of the 51 states. The overall prevalence for MTP deficiency was 1:1,822,568, ranging from no cases detected in 40 of the 51 states to 1:192,648 in Georgia. No cases of LCHADD/MTP deficiency were identified in the studies from Taiwan [14], Singapore [15], Japan [11], Hong Kong [21] or China [18].In Japan the birth prevalence from 1997–2005 was 1:840,000, while in Korea it was 1:1,148,000 between 2000 and 2015 [11]. The study in the United Arab Emirates from 2011–2014 reported birth rates for inborn errors of metabolism as a whole and the birth prevalence for LCHAD/MTP deficiency was calculated by reviewers to be 1:68,593 [13]. In Egypt, no cases of LCHAD deficiency were found within a one year period in a population of 25,276 newborns [20].

Quantity

Eleven studies were identified since the date of the last systematic review in 2013 [1]. Two were from Western European countries. Only 4 of the 11 studies involved sample sizes large enough to detect cases. This review builds on the 17 studies which found cases previously included in the most recent systematic review.

Quality

Potential sources of bias were common. In particular, studies did not provide sufficient information on the setting and subjects or statistical analysis of the results. Details regarding the measurement of LCHAD/MTP deficiency were not clearly provided.

Applicability

All studies reported figures for newborn populations comparable to the UK. No studies were reported for the UK since the previous estimates from the last systematic review [1]. This is to be expected as the conditions are not currently screened in the UK. There were 2 Western European studies [10, 11]. One study combined rates for Spain and Portugal and found a birth prevalence of 1:139,597 (0.72 per 100,000) [10]. The second compared Asian countries to rates reported in Germany [11]. The rates for Germany were 1:120,000 (0.79 per 100,000). Both of these studies show comparable rates to the estimated Western European birth prevalence from the previous review of 0.67 per 100,000 [1], though these figures suggest the disease may be slightly more prevalent.

The reported birth prevalence in screened populations for LCHADD/MTPD ranged from 1:68,593 in the United Arab Emirates to 1:363,738 for LCHADD and 1:1,822,568 for MTPD in the USA [13, 17]. Differing frequencies of LCHADD/MTPD between Europe, Southeast Asia, the Middle East, and North America may reflect inherent population differences. Therefore results from countries outside of Western Europe may not be applicable to the UK. The study from the United Arab Emirates reported that 81.5% of all cases of inborn errors of metabolism in the study resulted from consanguineous marriages [13]. Similar rates may be expected in other countries where consanguinity between parents is more common. The study in Egypt was over a very short time frame and with few participants, meaning detection of cases was unlikely due to the rarity of LCHAD/MTP deficiency [20]. In Southeast Asia, people with LCHAD deficiency were only identified in studies of more than 3 million people, and the birth prevalence rates were much lower (0.09-0.12 per 100,000) suggesting the genetic mutation might be less likely to occur in Asian ethnicities [11]. In the American states [17] LCHAD deficiency and MTP deficiency were estimated to be 1:363,738 and 1:1,822,568

respectively which is considerably lower than previously calculated UK birth prevalence (1:178,404; [1]). There were variations in the duration of screening across the different states and data missing due to the voluntary collaboration among the partnering programmes. Additional data by state are available in Appendix 3 Table 16. It is worth noting that in Western Europe LCHAD deficiency and MTP deficiency are screened for as one condition using the same acylcarnitines' profile. Differentiation between the 2 diseases occurs during confirmatory diagnostic testing such as enzyme or mutation analysis and therefore the prevalence of LCHAD might be lower.

Consistency

Our review found 2 studies undertaken in Western Europe [10, 11] and the results were close to the Western European estimation made in the most recent systematic review [1] of 0.67 cases per 100,000 (0.72 in this review).

Question 2 — What are the genotype-phenotype associations in LCHAD/MTP deficiency patients, including their clinical prognosis?

Sub-question: What is the incidence of asymptomatic and/or milder phenotype in the neonatal period?

The previous review identified a number of areas where the evidence base is still limited. There was insufficient information on the epidemiology and natural history of the defect, particularly in relation to the milder form. Crucially, uncertainties still remained with regard to the poor genotype-phenotype correlation and to the variability of prognosis.

The aim of addressing this question in this review update is to evaluate if more recent evidence has been published to more accurately determine the association between specific genotypes and the clinical symptoms in patients presenting with LCHAD/MTP deficiency.

Eligibility for inclusion in the review

Articles were included in this question if they provided details of the genotype mutation and reported on any observable characteristic, and if

the study population included people with isolated LCHAD deficiency or MTP deficiency. Study types suitable for inclusion were cross-sectional studies, cohort studies or reports from neonatal screening programmes. Searches were limited to English language papers published since 2000. Papers including non-human studies, letters, editorials and communications, grey literature, non-systematic reviews and conference abstracts were excluded.

Description of the evidence

Appendix 2 - Included and excluded studies contains a full PRISMA flow diagram (Figure 27) along with a table of the included publications and details of which questions these publications were identified as being relevant to (Table 11) All the publications excluded after review of full-text articles for question 2 as well as for question 1 and 4 are listed in Table 12 along with reasons for exclusion.

Database searches yielded 7,483 unique results, of which 7,170 were excluded at title/abstract assessment. The full texts of 313 articles were assessed and 27 were judged to be relevant to this question [6, 22-47]. No further articles were identified through hand searching reference lists of relevant systematic reviews or included studies.

Characteristics of included studies

A study-level summary of data extracted from each included publication is presented in Table 17 Appendix 3.

The 27 studies comprised one UK study [34] and 13 individual European studies [6, 12, 24, 27, 29, 31, 32, 36-38, 40, 43, 44, 66]. Within the 13 European studies, 3 were from Finland [31, 44, 47] and 3 were from Germany [36, 38, 40]. There were 3 studies which were collaborations between Germany and other countries such as USA, Israel, Canada, Switzerland, Austria and the Netherlands [39, 41, 42]. It was reported that there were 8 German patients who were the same across 2 of the studies [39, 42]. However it is also possible that there was crossover with the third paper and this has not been specified [39]. In total there were 6 individual studies from the USA [23, 26, 28, 30, 45, 46]. There was crossover from one patient within one of these studies and a patient from one of the studies from Germany [30, 40]. There were 4 studies from

Southeast Asia [22, 25, 33, 35]. Within these, 5 of the people included were the same across 2 studies [22, 35]. There were 13 cohort studies [6, 22, 24, 26, 31, 33, 36, 39, 41-44, 46, 47]. Ten of these were all patients reporting to one or more metabolic clinics in a particular country over a certain period of time [22, 24, 26, 31, 36, 43, 44, 46, 58]. The remaining 3 studies were cohorts which were picked due to a certain characteristic of the people included, specifically: heterogeneous mutations in the families [47], all with MTP deficiency beta subunit mutations [42] and all exhibiting the neuromyopathic phenotype [39]. Furthermore, there were 12 case series [23, 25, 27, 29, 30, 32, 34, 35, 37, 38, 40, 45], and one randomised controlled trial [28].

In total across all studies there were 211 LCHAD deficiency patients (a further 12 may be repeated across 2 studies but this was not clearly specified [31, 44]) and 90 MTP deficiency patients (14 the same [30, 39, 40, 42], a further 7 may have crossed over but this was unclear [58]).

Discussion of findings

Quality appraisal of included studies

The 27 included studies [6, 22-47] were quality appraised using the JBI Checklist for Cross Sectional Studies. There are no validated checklists for genetic association studies. Therefore, we used the JBI Checklist for Cross Sectional Studies as it allows the assessment of the key elements of bias that are commonly present in research. Results are presented below in Figure 3 and in Appendix 4 Table 22.

Nineteen out of 27 studies (70.4%) had risks of bias on at least 2 items. Of the remaining 8 studies, 2 or more items were rated as unclear or not applicable. The inclusion criteria were not clearly defined in 16 of the studies [25-27, 29, 30, 32-35, 37, 39, 40, 42, 44, 46, 58]. Most of the studies did not specify their criteria for including the participants within their study. This is particularly true for the small case series which were only reporting on a very small number of cases. In some cases these had been chosen because they were only a specific genotype or presenting with a specific outcome. Likewise, 16 of the 27 studies did not clearly describe the setting and the participants [6, 24, 28, 29, 32, 35, 38-40, 42-46, 58]. Often key information such as the demographics, which part of the country the participants were from, and time period were not reported.

Confounders were only clearly identified and reported in one study [41]. The remaining studies either did not report or did not appear to consider confounders such as dietary compliance. For 15 studies it was unclear whether the outcomes were measured in a valid and reliable way as studies did not mention how outcomes were determined [6, 24, 29, 32, 33, 35, 36, 38, 39, 42-44, 46, 58].

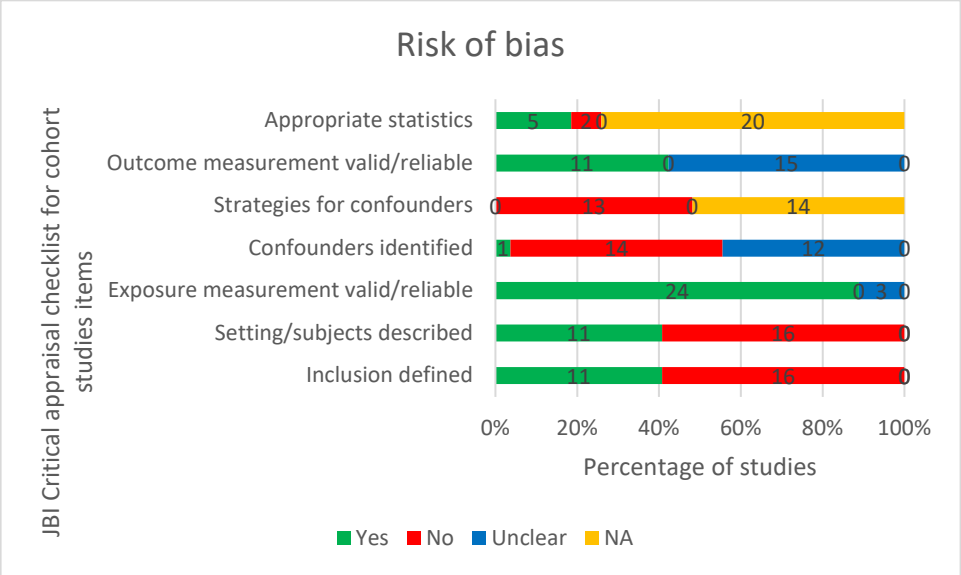

Figure 3. Risk of bias in included studies using JBI critical appraisal checklist for analytical cross sectional studies [63]

Yes means item was met; no means it was not met; NA, not applicable

Analysis of included studies

There were 95 genotypes among 301 people with LCHAD/MTP. Due to the large variation of genotypes, these were combined into 6 groups on the basis of their subunit (MTP deficiency alpha, beta or unspecified subunit) or their type of mutation (homozygous, heterozygous or unspecified LCHAD deficiency). There were 76 different phenotypes noted across the 27 studies. The most frequently reported phenotypes are noted in this report. There were 157 homozygous LCHAD deficiency patients and 45 heterozygous patients. The LCHAD deficiency mutation was unspecified in 9 patients. There were 24 MTPD patients with alpha subunit mutations and 51 with beta subunit mutations. The subunit mutation was unspecified in 15 patients. Overall there were 95 different

genotypes described across all the studies (29 different LCHAD deficiency mutations for LCHAD deficiency heterozygous cases, 40 different beta subunit mutations in MTP deficiency, 21 different alpha subunit mutations in MTP deficiency and 5 unspecified subunit mutations for MTP deficiency).

Full details on the range of phenotypes found from the studies are available in Appendix 5 Table 25. The most commonly reported phenotypes are reported below, with an accompanying forest plot. Effect size is the percent of people with that outcome. For this review it has been assumed that people with the 1528G>C mutation have LCHAD deficiency [26].

Mortality

Rates of mortality by genotype were reported in 17 studies [6, 22, 24, 27, 30-32, 34, 36-38, 40, 42, 44, 45, 47, 58]. Figure 4 shows the cases of mortality across the different genotype groups per study. Twelve studies reported mortality in the homozygous 1528G>C genotype [6, 24, 30-32, 34, 36, 38, 41, 44, 45, 47]. The range of death rates reported was 0–58% Time of death was reported in 5 of these studies and ranged from 3 days to 9 years, 7 months [6, 31, 32, 34, 58].

In the heterozygous LCHAD deficiency group, mortality was reported in 4 studies [6, 24, 32, 66] and ranged from 16.7% to 100% of cases. Age at time of death ranged from 7 days to 2 years and 6 months.

One study did not report the LCHAD deficiency mutation and reported mortality rates of 0 [36].

Nine studies reported rates of mortality for MTP deficiency beta subunit mutations [22, 24, 25, 27, 33, 35, 37, 38, 42]. The rates ranged from 0 to 100%. Age of death spanned from 12 hours to 4 months [37].

In the MTP deficiency alpha sub-group, mortality was reported in 6 studies [22, 24, 25, 30, 38, 40]. Mortality ranged from 0 to 100%. Age at time of death ranged from 2 to 48 days [25, 30].

Five studies reported mortality rates for MTP deficiency without specifying the subunit [22, 34, 36, 41, 45]. Mortality ranged between 0–100% and time of death from 2 days to 13 months [36, 58].

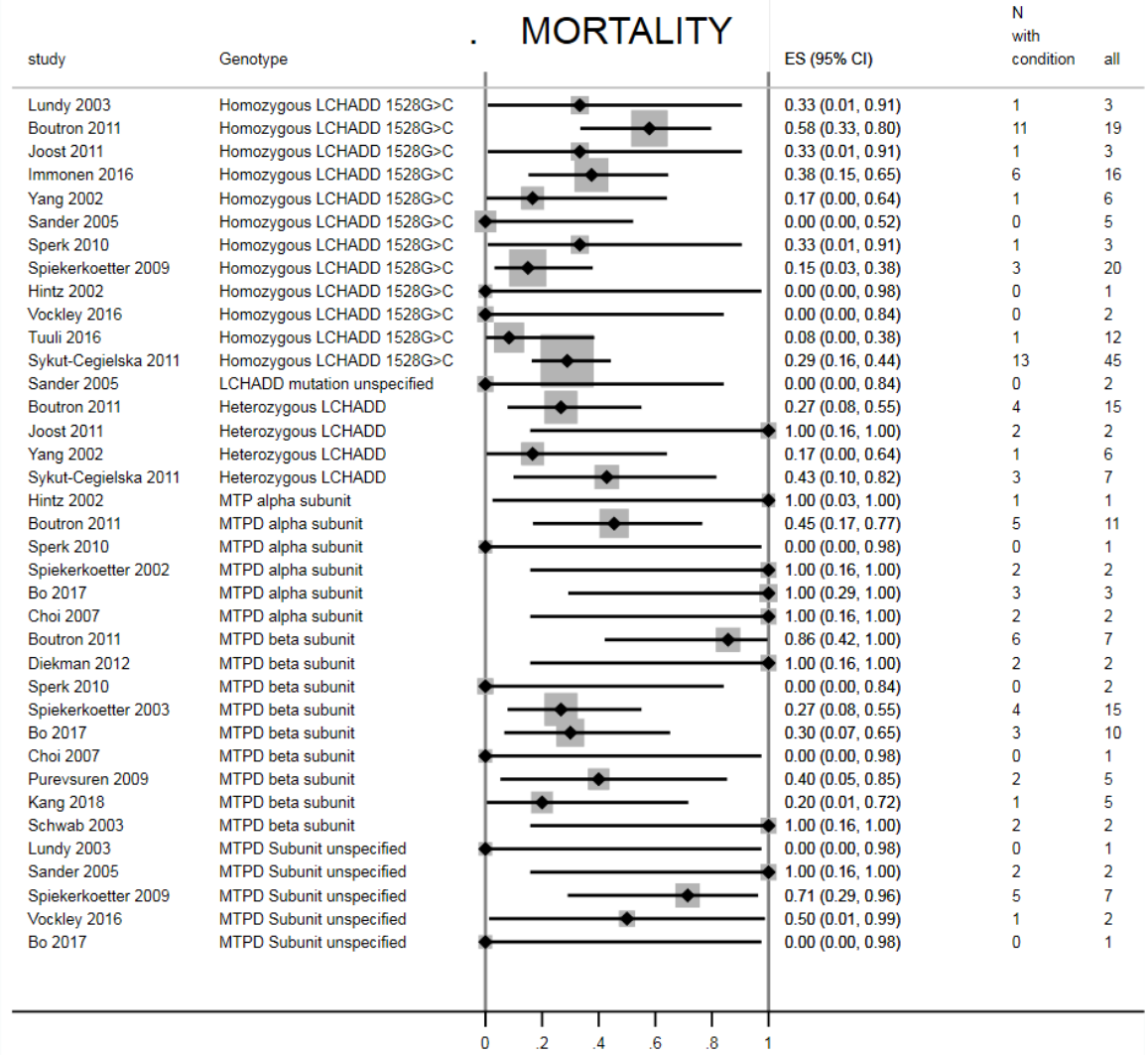

Figure 4– Forest plot showing the cases of mortality across the different genotype groups per study

Severity

Severity has been defined by 3 sub-groups: neonatal severe, infant hepatic and late onset neuromyopathic [2]. Nine studies classified genotype by severity [22, 24, 27, 35, 38-40, 42, 66].

Figure 5 shows the percentage of neonatal severe cases in each genotype group per study. The neonatal severe sub-group was identified in 5 studies [22, 27, 35, 38, 42]. In the homozygous LCHAD deficiency group, 33.3% of cases were classified as neonatal severe [38], compared to a range of 26.7% to 100% [22, 24, 27, 35, 38, 42] in the MTP deficiency beta subunit group and 45.5% to 100% in the MTP deficiency

alpha subunit group [22, 24, 40]. No studies reported neonatal severe cases for the other genotype groups.

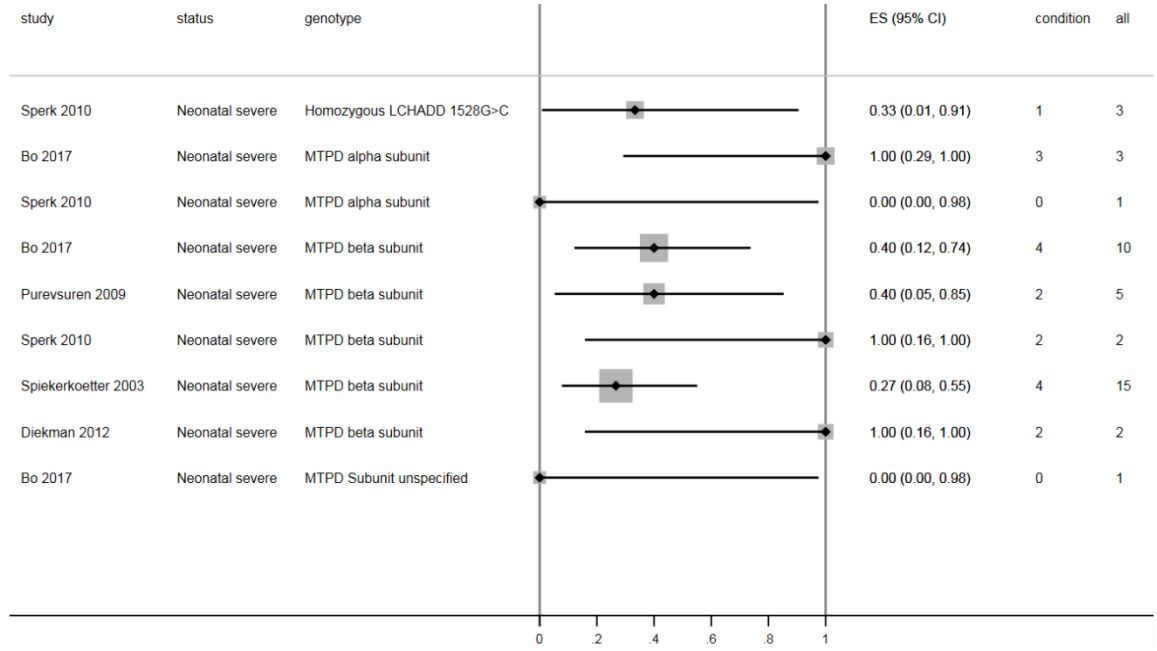

Figure 5 – Forest plot showing percentages of neonatal severe cases by genotype group

Infant hepatic cases

Figure 6 shows the percentage of infant hepatic cases by genotype group in each study. The infant hepatic sub-group was reported in six studies [22, 24, 35, 38, 42, 66]. There were more infant hepatic cases across all LCHAD deficiency genotypes compared to MTP deficiency. Between 33.3–100% of cases were classified as infant hepatic in the homozygous LCHADD group [24, 38, 66] and 50–87% of cases were classified as infant hepatic in the heterozygous LCHADD group [24, 47]. This compares to 13.3% to 40% in the MTP deficiency beta subunit group [22, 24, 35, 42] and 0% to 18.2% in the MTP deficiency alpha subunit group. No cases were reported in the unspecified LCHAD or MTP deficiency sub-groups.

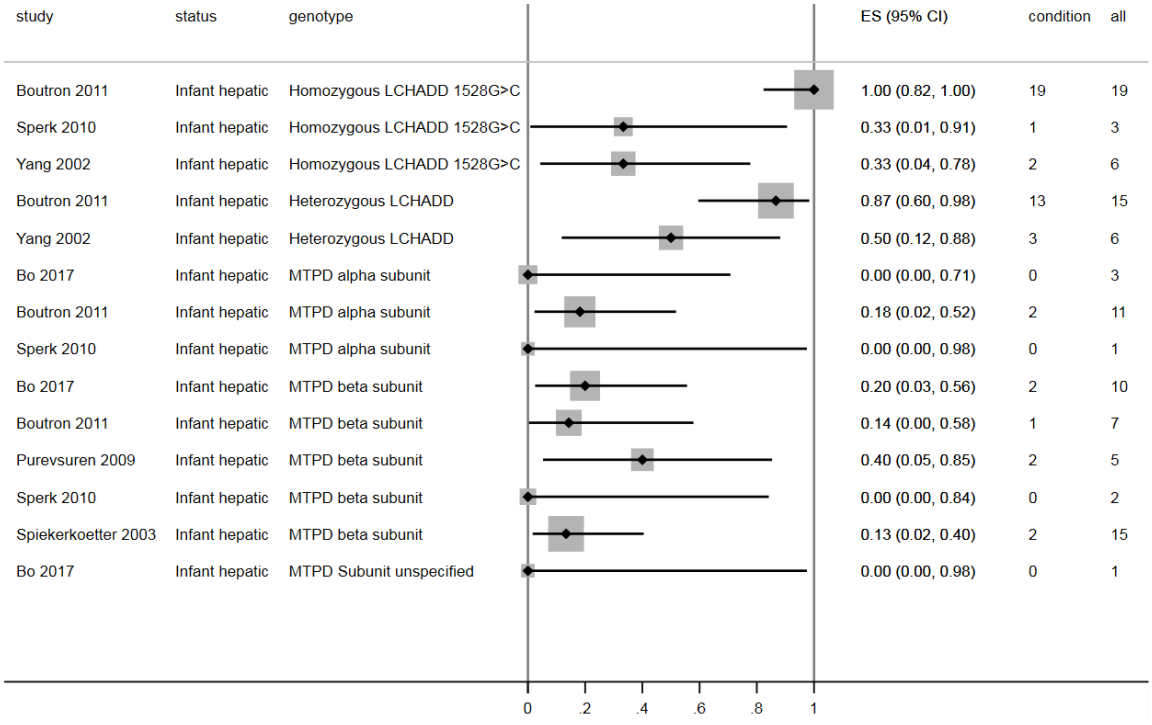

Figure 6 - Forest plot showing percentages of infant hepatic cases by genotype group

Neuromyopathic cases

Figure 7 shows the percentage of late onset neuromyopathic cases by genotype group in each study. Five studies reported the genotypes of late onset neuromyopathic cases [22, 24, 35, 39, 42]. The number of late onset neuromyopathic cases ranged more in MTP deficiency than LCHADD. Studies did not report late onset neuromyopathic cases in the homozygous LCHADD group and one study found 6.7% of the heterozygous LCHAD deficiency genotype was in the late onset neuromyopathic group [24]. In the MTP beta subunit group, 5 studies reported on severity in the neuromyopathic group. Severity ranged from 0–100%; one study each finding 0%, 20%, 40%, 60%, 100% out of 7, 5, 10, 15 and 11 people, respectively [22, 24, 35, 39, 42] compared to 36.4% in the MTP deficiency alpha subunit [24]. There was only one late onset neuromyopathic case in one study in the MTP deficiency subunit unspecified group [24].

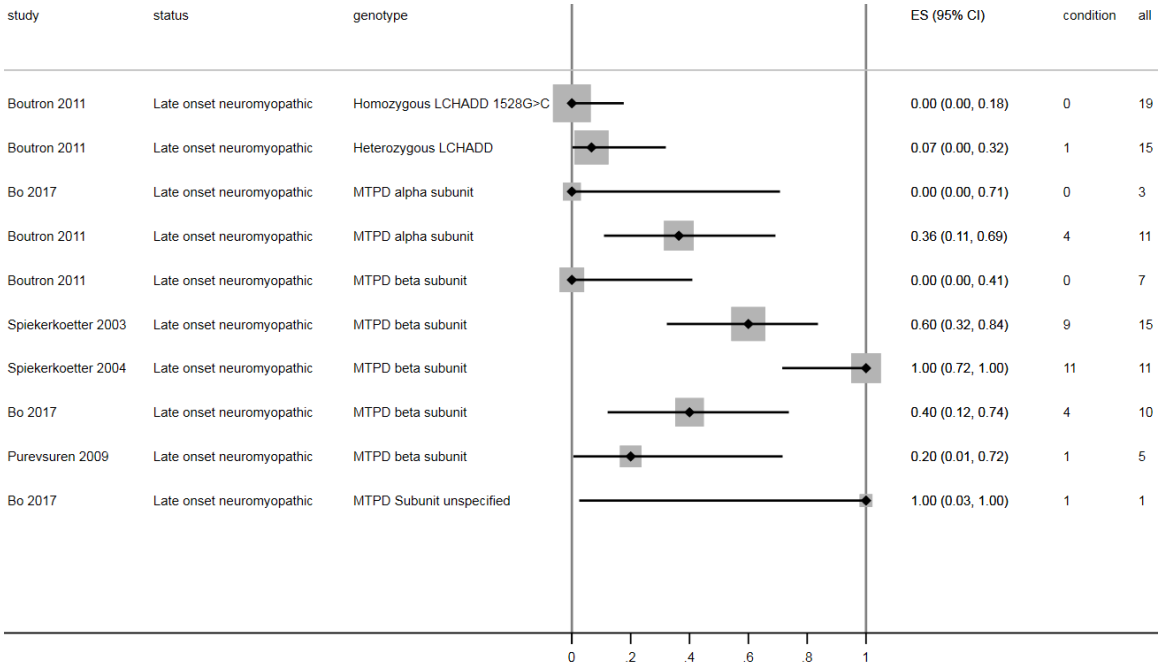

Figure 7 - Forest plot showing percentages of late onset neuromyopathic cases by genotype group

Cardiomyopathy

Figure 8 shows the percentage of cardiomyopathy cases per genotype group in each study. Eleven studies reported on cardiomyopathy [22, 27, 29, 31, 32, 35, 36, 38, 41, 43, 45].Of these, 8 were reporting on the homozygous 1528G>C LCHAD deficiency genotype [29, 31, 32, 36, 38, 41, 43, 45]. Rates of cardiomyopathy ranged between 0–100% across people with this genotype.

Two studies reported on the heterozygous LCHAD deficiency mutation [29, 32]. Cardiomyopathy was present in 0–100% of cases (1/2 in one and 2/2 in the final study).

There was one study which reported for LCHAD deficiency mutation unspecified group and found no people with cardiomyopathy [36].

Six studies reported cardiomyopathy rates for the MTP deficiency beta subunit mutation group [22, 27, 29, 35, 38, 42]. Rates of cardiomyopathy ranged from 0–100% (one study reported 0% out of one person, 3 studies

found rates of 20–40% out of 30 people, and 2 studies reported 100% out of 4 people).

Two studies reported on cardiomyopathy for people with MTPD alpha subunit mutations. One study reported a rate of cardiomyopathy of 0% (one case) and one reported a rate of 100% (2 people) [38, 40].

In the MTP deficiency unspecified subunit group there were 4 studies reporting on cardiomyopathy [22, 36, 41, 45]. Rates ranged from 0–57.1% at initial presentation (with 2 of the 3 studies finding no instances of cardiomyopathy out of 4 people). Only one of the studies reported on cardiomyopathy at initial presentation and the end of the study. This study found 0/2 cases at initial presentation but both cases had cardiomyopathy by the end of study.

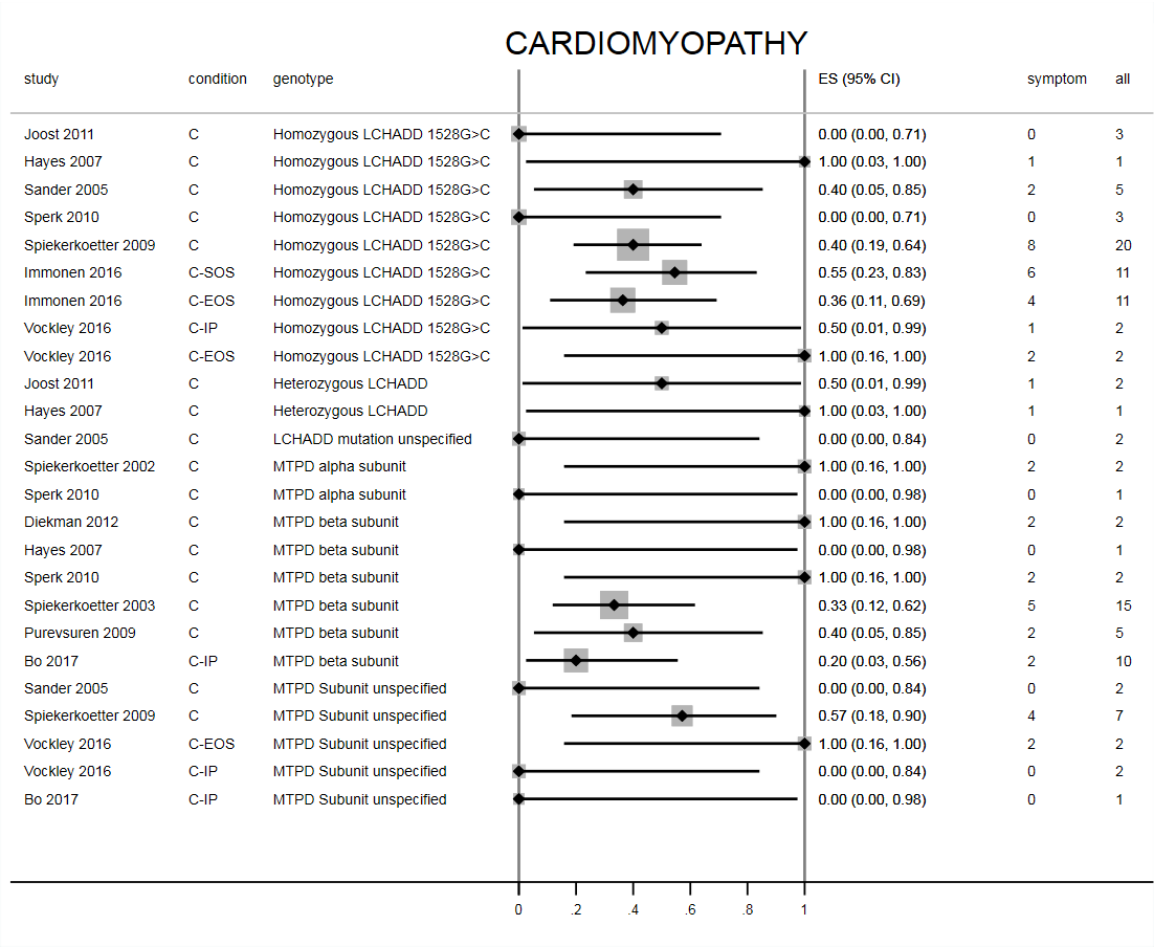

Figure 8 - Forest plot showing percentages of cardiomyopathy cases by genotype group

IP: initial presentation; SOS: start of study; EOS: end of study

Rhabdomyolysis

Figure 9 shows the percentages of rhabdomyolysis cases by genotype group in each study. There were 8 studies reporting on rhabdomyolysis [22, 23, 25, 29, 31, 33, 36, 45]. In the LCHAD deficiency homozygous groups rates of rhabdomyolysis ranged from 0–50% (2 studies found no cases of rhabdomyolysis out of 10 people, rates in the remaining 3 studies ranged from 20% to 50% out of 17 people) [23, 29, 31, 36, 45].

In the heterozygous LCHAD deficiency mutation group, the frequency of rhabdomyolysis ranged from 0 to 100% (0 in one study of 7 people, 1/1 in the other study) [23, 29].

Five studies reported the rates of rhabdomyolysis for people with MTP deficiency beta sub-group mutations; rates ranged from 20% to 100% [22, 23, 25, 29, 33].

No cases with an MTP deficiency alpha subunit mutation were reported to have this phenotype presentation [38].

Three studies reported rates of rhabdomyolysis in people with unspecified MTP deficiency mutation [22, 36, 45]. These ranged between 50–100%.

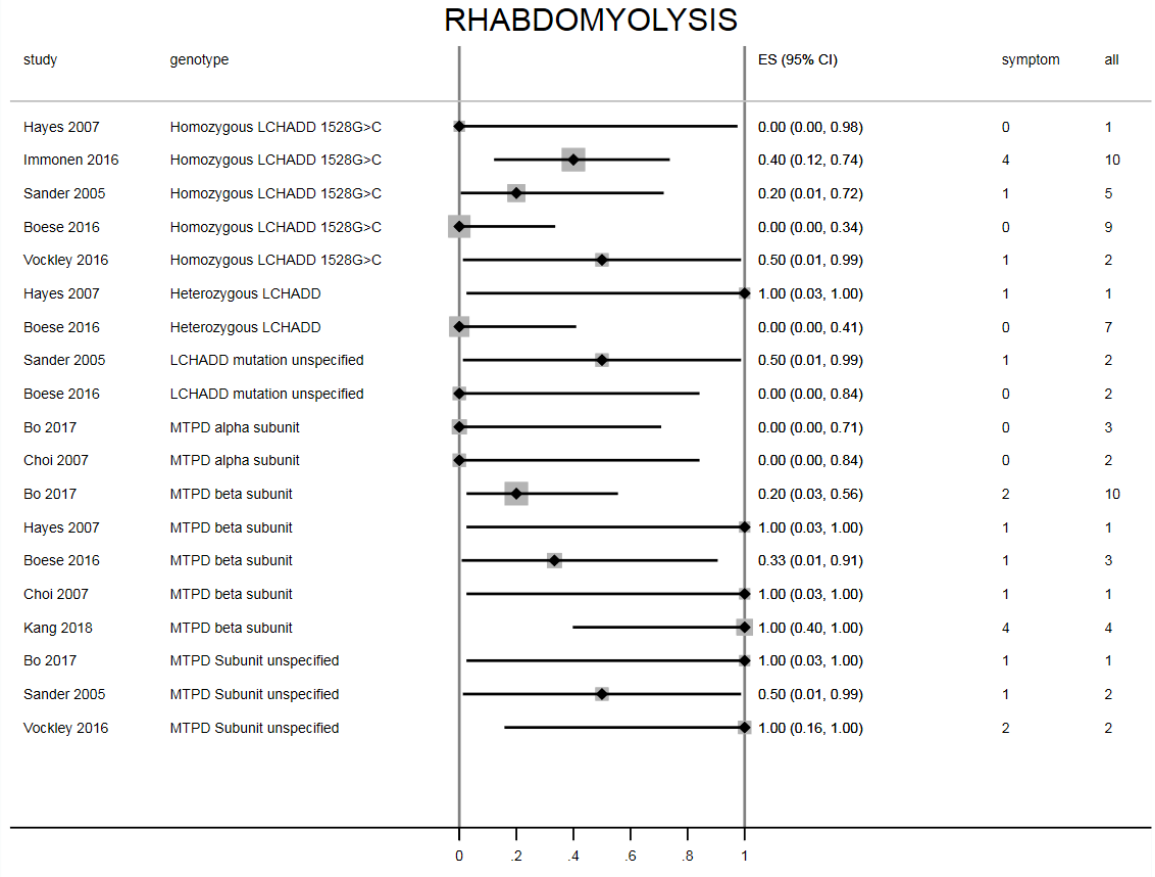

Figure 9 - Forest plot showing percentages of rhabdomyolysis cases by genotype group

Hypoglycaemia

Hypoglycaemia was reported in 16 studies [23, 25, 26, 29, 30, 32, 34-36, 38-43, 45]. Figure 10 shows the percentages of hypoglycaemia cases by genotype group in each study. Eleven of these studies reported on cases with the homozygous 1528G>C LCHAD deficiency mutation [23, 26, 29, 30, 32, 34, 36, 38, 41, 43, 45]. One looked at initial symptoms only and found no cases [45]. In the remaining studies, rates ranged from 40% to 100%.

In the 4 studies reporting hypoglycaemia for people with heterozygous LCHAD deficiency, rates ranged from 50% to 100% [23, 29, 32, 43].

Seven studies reported hypoglycaemia for MTP deficiency patients with beta subunit mutations [23, 25, 29, 35, 38, 39, 42]. This occurred in 0–100% of patients (0% only found in two studies of one person each, 100% found in one study of 2 people). The rates in the remaining studies were between 18.25% and 46.7% (out of 46 people).

Four studies reported hypoglycaemia for people with alpha subunit mutations [25, 30, 38, 40]. It occurred in 0–50% of people (0% in two studies of one person each, 50% in the remaining 2 studies involving 4 people).

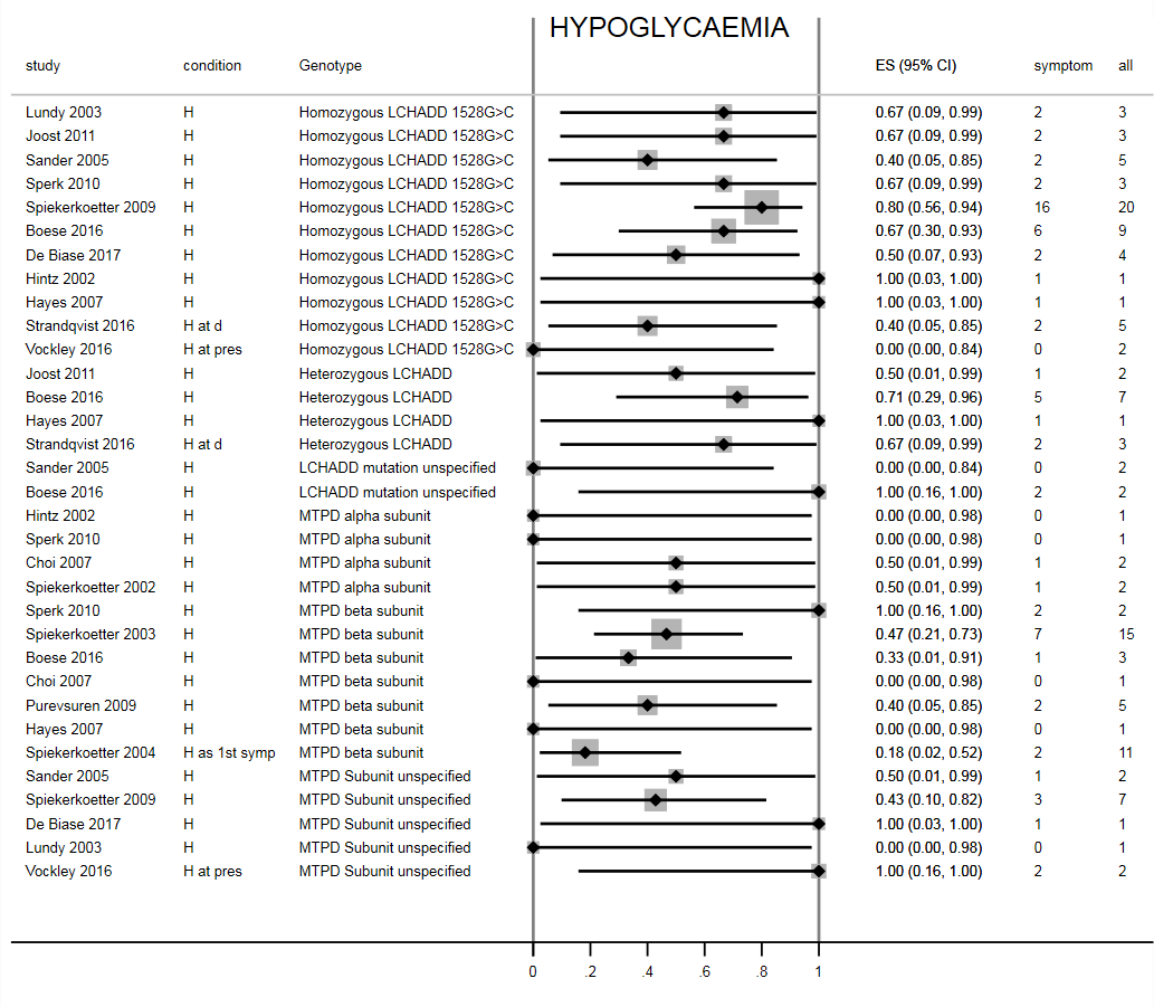

Figure 10 - Forest plot showing percentages of hypoglycaemia cases by genotype group

Hepatomegaly or hepatic failure

Figure 11 shows the percentages of hepatomegaly or hepatic failure by genotype group for each study. Hepatomegaly or hepatic failure was reported in 3 studies [29, 32, 34]. In the homozygous LCHAD deficiency group it ranged from 66.7% to 100% across 7 people. In the heterozygous LCHAD deficiency group it ranged between 0–100% across 2 studies of 3 people [29, 32]. One study reported on this outcome for the MTP deficiency beta subunit and found 0/1 cases reporting this outcome [29].

No studies reported this outcome for LCHAD deficiency mutation unspecified or MTP deficiency alpha subunit mutation groups.

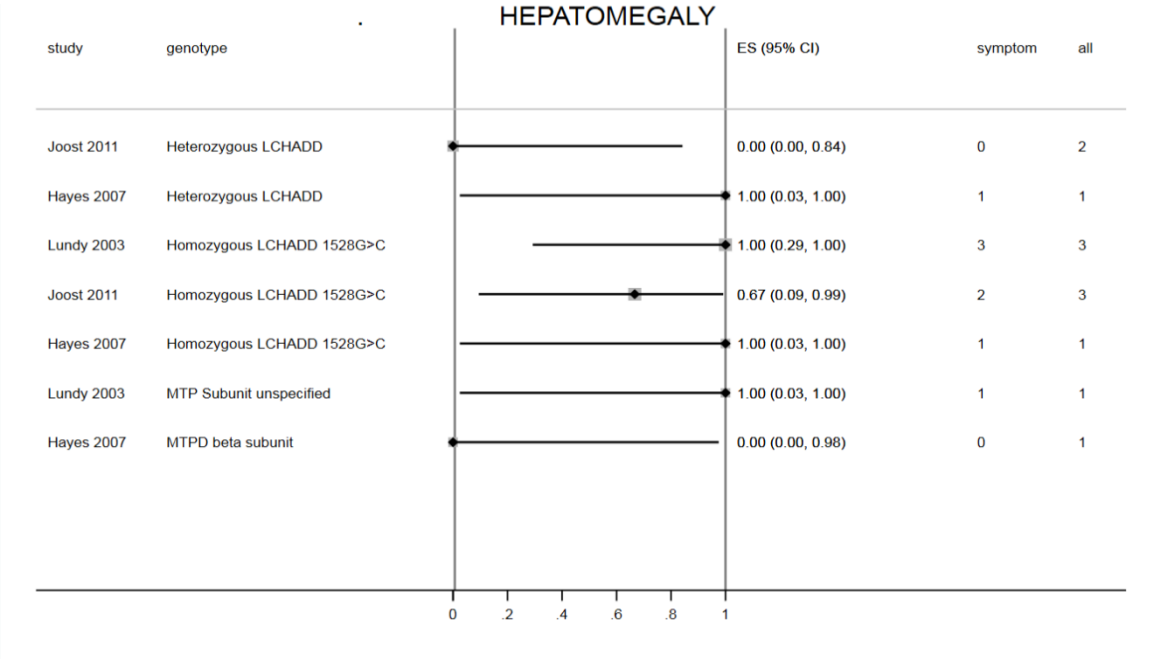

Figure 11- Forest plot showing percentages of hepatomegaly or hepatic failure cases by genotype group

Retinopathy

Figure 12 shows the percentages of retinopathy cases by genotype group per study. Retinopathy was reported in 5 studies [29, 31, 38, 46, 58]. All 5 reported on the LCHAD deficiency homozygous 1528G>C mutation and found it occurred in 0–100% of people (0% in 2 studies of 4 people, one study found a rate of 100% in one person, further studies ranged from 25–81.8% in 31 people). In the 2 studies reporting on retinopathy for the heterozygous LCHAD deficiency mutation, both studies reported that 100% of people had this outcome (2/2) [29, 46].

Two studies reported on retinopathy in people with MTP deficiency beta subunit mutations [29, 38]. Rates were 0% (0/2) and 100% (1/1). In the one study which reported on people with MTP deficiency alpha subunit mutations and retinopathy, no cases were found [38]. In the unspecified MTP deficiency subunit group, one study found 14.3% had retinopathy [41].

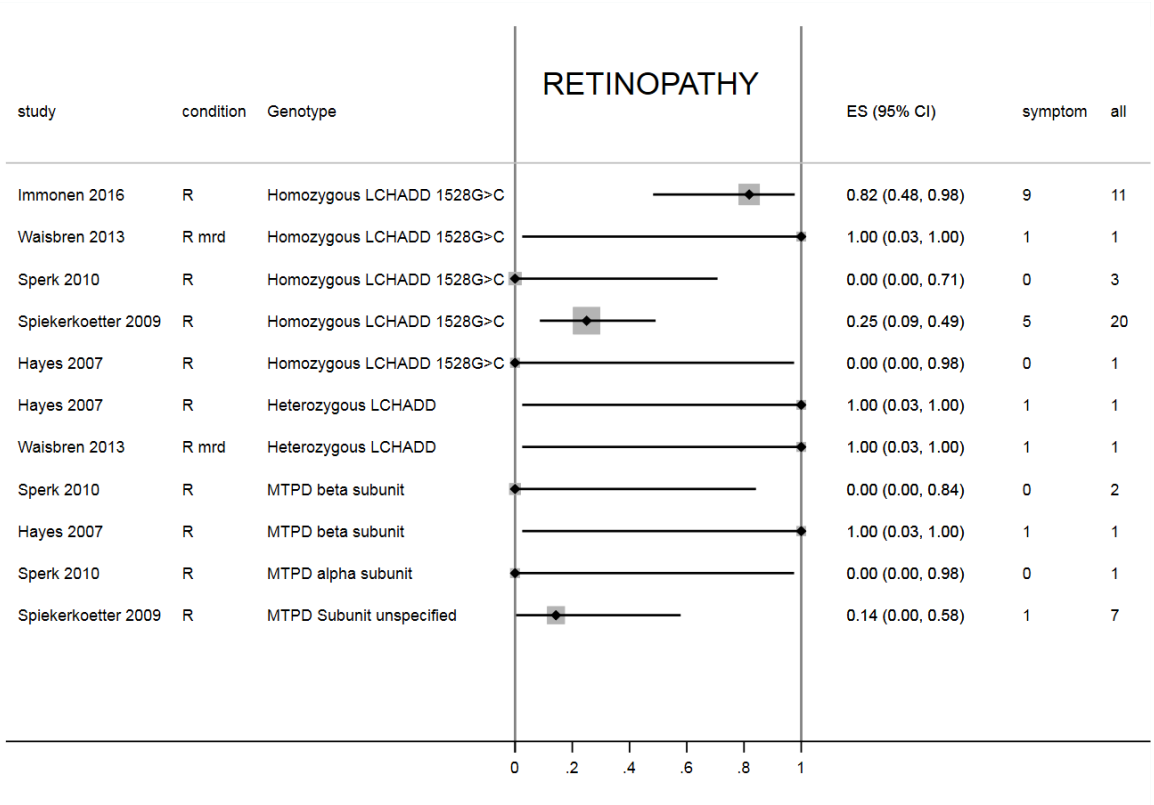

Figure 12 - Forest plot showing percentages of retinopathy cases by genotype group

Mrd: mild retinal defects

Sub-question: What is the incidence of asymptomatic and/or milder phenotype in the neonatal period?

There were no papers which directly addressed this question. Papers reporting on incidence only reported for the whole newborn population. They did not go on to do long term follow up or categorise the severity of the newborns in any way. However, one study was identified which showed the birth prevalence of LCHADD before newborn screening (1:225,000), with newborn screening (1:168,016) and in an unscreened population (1:117,396) [53]. The study was 9 years long (from 2002–2011) and included 586,979 people. The possible increase in prevalence after screening suggests the test could be detecting milder asymptomatic forms of the disease. However, it does not explain why the rate is higher in the unscreened population. The study stops in 2011 and does not provide the ages of the identified cases at this time or the follow up time. One paper was identified which reported on screening, number of cases and on whether they subsequently went on to present with symptoms in the neonatal period [36]. The study screened approximately 1,200,000

infants and found 9 cases of LCHAD/MTP deficiency (7 LCHAD deficiency, 2 MTP deficiency). Of these 9 cases only one was still asymptomatic by age 3; all 9 individuals were receiving treatment which is likely to have prevented symptoms.

Quantity

Overall, 27 studies were identified which described the genotype phenotype association [6, 22-47]. There were 95 different genetic mutations and 76 different possible phenotypes.

Quality

Studies had several methodological quality issues. There was a lack of information regarding inclusion criteria, settings, study subjects and the consideration of possible confounders (such as dietary adherence). It was also unclear from many of the studies whether valid and reliable tools had been used to determine the outcomes being measured.

Applicability

All studies reported the individual genotype mutation of LCHAD/MTP deficiency homozygous or subunit group. However, it is not known whether particular genetic mutations are more likely to occur in particular countries.

Consistency

Evidence from 27 studies has shown the large number of possible genetic mutations that can be associated with LCHAD or MTP deficiencies. Apart from the common homozygous LCHAD deficiency 1528G>C mutation group (157 people), almost every individual identified by the studies had a unique genetic mutation (144 people and 95 different genotypes). Grouping by genetic sub-group (LCHAD deficiency homozygous, LCHAD deficiency heterozygous, LCHAD deficiency unspecified, MTP deficiency alpha subunit mutation, MTP deficiency beta subunit mutation, MTP deficiency subunit unspecified) showed large differences in the number of patients presenting with each phenotype (most were 0–100% in each group). This may be largely due to the very small number of patients included in each group (in some studies as low as one). It may be that it

is an interaction between the type of mutation and location of the mutation which is responsible for the phenotypic presentation (e.g. deletions on particular genes may lead to more severe phenotypes than missense mutations). Environmental factors may also have mediating/moderating effects between genes and outcomes.

Severity was one of the few areas which showed more consistency across studies. There may be a greater proportion of people with MTP deficiency presenting with the neonatal severe form of the disease across both subunits (26.7–100%) compared to LCHAD deficiency (33–67%). However, the presence of very wide ranges limits our understanding of this phenomenon. There appear to be more infant hepatic presentations in the LCHAD deficiency group than the MTP deficiency group (33–100% compared to 13–40% respectively). There may be greater numbers of people with MTP deficiency presenting with late onset neuromyopathic symptoms than in LCHAD deficiency (0–100% compared to 6.7–15% respectively), but the broad range of figures on proportions in the MTP group prevent clear conclusions being drawn. However, the associated outcomes with these groups (death with neonatal severity, hepatic symptoms with hepatic infant group and neuromyopathic with the late onset group) do not reflect the same pattern across LCHAD and MTP deficiencies.

Summary of Findings Relevant to Criterion 1: Not met*

Question 1 – What is the birth prevalence of LCHAD/MTP deficiency in the UK

This element of criterion 1, addressed by question 1, is met.

No studies were found since the last review which provide the birth prevalence rates for the UK specifically. However, a full systematic review was undertaken in 2013 which reported on prevalence rates for comparable West European Countries and made UK estimates based on trial evidence. The results of this review support these findings. With no screening in place, no further reviews are likely to provide this information. However, information may be available from UK databases.

Question 2 – What are the genotype-phenotype associations in LCHAD/MTP deficiency patients, including their clinical prognosis?
Sub-question: What is the incidence of asymptomatic and/or milder phenotype in the neonatal period?

This element of criterion 1, addressed by question 2 and its sub-question, is not met.

Overall, 27 studies were identified which reported on the genotype-phenotype association in individual patients. There were 95 different genetic mutations and 76 different possible phenotypes. Grouping by homozygosity or subunit does not appear to map clearly to particular phenotypes. The specific presentation and pathway of the disease appears to vary greatly by individual and could be influenced by a number of other factors such as dietary compliance or the influence of

* **Met** -for example, this should be applied in circumstances in which there is a sufficient volume of evidence of sufficient quality to judge an outcome or effect which is unlikely to be changed by further research or systematic review.
Not Met - for example, this should be applied in circumstances where there is insufficient evidence to clearly judge an outcome or effect or where there is sufficient evidence of poor performance.
Uncertain -for example, this should be applied in circumstances in which the constraints of an evidence summary prevent a reliable answer to the question. An example of this may be when the need for a systematic review and meta-analysis is identified by the rapid review.

other health problems. Neonatal severe cases may be more common in MTP deficiency and infant hepatic cases in LCHAD deficiency, but firm conclusions on the subject cannot be drawn. Further analysis of the current evidence base to determine whether type rather than location of the defect is linked to phenotypic presentation is needed.

Due to a lack of evidence it is unclear if overdiagnosis is an issue. People who remain asymptomatic by the end of a study may have been overdiagnosed, they may go on to develop symptoms in later years, or they may remain asymptomatic.

Criterion 4 — Accuracy of acylcarnitines measurement in dried blood spots for LCHAD/MTP deficiency screening

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

Question 3 — What is the test accuracy (sensitivity, specificity, and predictive values applicable to UK prevalence) of acylcarnitines measurement in dried blood spots (DBS) using TMS for LCHAD/MTP deficiency screening?

Sub-questions: Can the test distinguish between asymptomatic patients and those affected by milder forms of LCHAD and MTP deficiency?
Does the test detect other non-MTP conditions?

The evidence generated by the expanded newborn screening study evaluation had some limitations, particularly in relation to the feasibility of the screening test. In particular, there was insufficient evidence that the screening test was effective at diagnosing LCHADD/MTP deficiency in babies that had no previous symptoms, with the only positive case being already treated at the point of screening.

The sub-questions were not addressed in previous reviews.

The aim of addressing this question and its sub-questions in this review is to evaluate multiple measures of test accuracy and to determine if evidence has been published indicating whether the screening test can distinguish between milder and more severe types of LCHAD/MTP deficiency or whether it can detect conditions other than MTP-related disorders.

Eligibility for inclusion in the review

Articles were included within this question if they:

- considered newborn screening for LCHAD deficiency or MTP deficiency in the general newborn population AND
- measured acylcarnitines in dried blood spots AND
- reported on (or it was possible to calculate) sensitivity, specificity and predictive values.

Reference standards considered to be appropriate were urine testing for organic acids and blood acylcarnitines profiles, enzyme analysis in cultured fibroblasts or lymphocytes or mutation analysis. Study types suitable for inclusion were cross-sectional test accuracy studies, case-control studies or cohort studies. If available, any randomised controlled trial of the screening pathway. Searches were not limited by date or language. Papers including non-human studies, letters, editorials and communications, grey literature, non-systematic reviews and conference abstracts were excluded. Studies of fatty acid beta oxidation disorders where data from newborns with mitochondrial trifunctional protein disorders could not be separated from data on other fatty acid oxidation disorders (e.g. multiple acyl-CoA dehydrogenase and very long chain acyl-CoA dehydrogenase deficiencies) were also excluded. Likewise, studies with no extractable data, or where more than 10% of the sample did not meet the inclusion criteria, were excluded.

Description of the evidence

Appendix 2 contains a full PRISMA flow diagram (Figure 28). The reviewers identified 1,194 unique records in electronic databases. 1,155 records were excluded at title/abstract sift, and 39 articles were kept for full text assessment. Of these, 28 were excluded [see Appendix 3 Table 15 Estimates of the incidence of LCHADD/MTPD (published since 2013)]
This left 11 publications. Two publications included an overlapping cohort [61, 67]. Only the data from the larger, more recent Lindner publication (which included all of the data from the Schulze publication) are reported.

Characteristics of included studies

The 10 included studies are summarised in Appendix 3 Table 18 and Table 19. These reported results from the newborn screening programme in China [18], Denmark and the Faroe Islands [5], Germany [36, 67], Slovenia [16], Spain [59] the UK [9], Hong Kong [21] and the USA [48, 60]. Sample sizes ranged from 10,048 [5] to 1,200,000 [36].

The TMS screening methods used differed between studies. For example, studies screened from day one up to day 28 of life [21, 60]. Also, different studies used different markers, the majority of studies used C16-OH or C16:1-OH or C18-OH, however 2 studies used C14OH [16,

67]. In one study the markers used to measure for LCHAD deficiency and MTP deficiency were unspecified [21].

There was some variation in the methods of diagnosis. Each study used comparable but unique cut-offs for each marker. In the majority of cases these cut-offs were modified during the course of the study based upon pilot results in their population, with the aim of reducing the number of false positives [9, 36, 60, 67]. In other cases, the timing of the cut-off decision was not reported [21, 59].

Discussion of findings

Quality appraisal of included studies

Quality appraisal (QUADAS-2) of the 10 included studies is summarised in Figure 13 and Figure 14, with further details provided in Appendix 4 Table 23. Risk of bias was considered high in 2 or more domains for 9 studies (90%) [5, 9, 16, 18, 21, 36, 48, 59, 60, 67]. No study was at low risk of bias in all 4 domains. The key risks of bias were that people who screened negative either did not receive the same reference standard as those who screened positive or were not actively followed up (Flow and Timing domain: 10/10 high-risk, 100%). Moreover, cut-off values were either not pre-specified or were changed during the study period (Index Test domain: 6/10 high-risk, 60%) [9, 16, 18, 21, 48, 60]. In one study, the screening test threshold was lowered after a baby presented symptomatically [9]. Unclear or incomplete reporting was common; the Patient Selection domain was rated as unclear in 7 out of 10 (70%) studies [16, 18, 21, 36, 48, 60, 67], and the Reference Standard domain was rated as unclear in 6 out of 10 (60%) studies [9, 16, 18, 36, 48, 60].

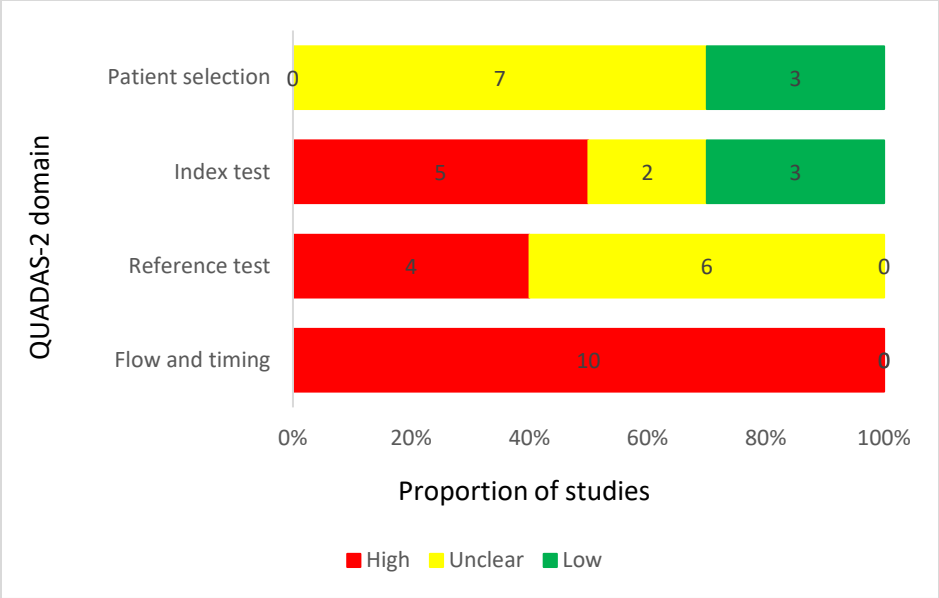

Figure 13. Risk of bias graph: review authors' judgements about each domain presented as percentages across included studies

There were significant concerns regarding the applicability of the studies to UK screening in 7 of the 10 studies (70%) in the Patient Selection domain [16, 21, 36, 48, 60, 67]. This was due to LCHAD/MTP deficiency being less common (1 in 300,000 births) than expected (0.67 in 100,000) compared to the UK population in one study [60], and blood samples being taken before day 5 in 5 studies [16, 21, 36, 48, 60, 67]. Studies show mixed results but suggest that concentrations of long-chain acylcarnitines C16, C16:1, C18, and C18:1 may vary with age at testing; countries not testing at approximately 5–8 days may need differing thresholds [68, 69]. Applicability concerns relating to the reference standard were unclear in 6 out of 10 studies (60%) because there was no system for following up those who screened negative [16, 18, 21, 36, 48, 60].

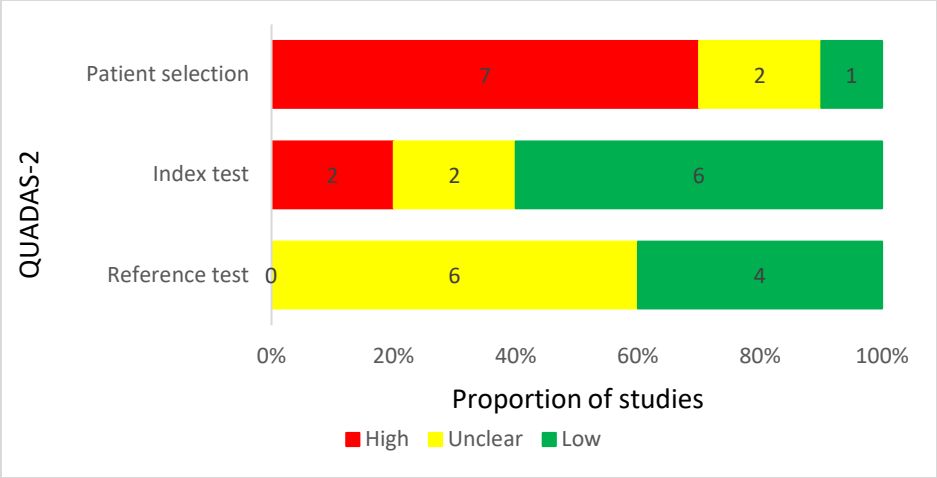

Figure 14 Applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies

Analysis of evidence

Across the 10 studies a total of 3,951,358 babies were screened for LCHAD/MTP deficiency. Within these, 23 true positives and 40 false positives were identified. The true positives included 11 babies with LCHAD deficiency, 2 babies with MTP deficiency, and 10 babies where LCHAD deficiency and MTP deficiency were not differentiated. Two cases of long-chain 3-ketoacyl-CoA thiolase (LCKAT) deficiency were detected incidentally.

Positive predictive values (PPV) ranged from 0–100%. Confidence intervals were very wide due to the small number of cases (23 in total, ranging from zero to 9 per study). PPV was reported as 33% (one true positive and 2 false positives from 436,969 babies screened) in one study [9], 47% (9 true positive and 10 false positives from 1,200,000 babies screened) in one study [36], and 100% in 4 studies (13 true positives from 2,037,824 babies screened) [5, 59, 60, 67]. Four studies reported 0% PPV (zero true positives and 28 false positives from 276,565 babies screened) [16, 18, 21, 48]. This is not surprising as the prevalence of the diseases is estimated to be 1.02 per 100,000, and the sample sizes in 3 of the 4 studies ranged from 2,440 to 100,077. The fourth study included 164,000 newborns but only used one marker to identify cases and therefore may have been less accurate [48]. Removing these studies gives a PPV that ranges from 33–100% (23 true positives and 12 false positives from 3,674,791 newborns). Test accuracy estimates differ

greatly by study, with some suggesting good accuracy albeit on small numbers (see Table 18, Appendix 3)[5, 9, 36, 59, 60, 67]. However the results are not presented by marker so it is not possible to combine data from different studies, or determine which combination of markers and thresholds may yield good accuracy The only study with cut-offs and markers comparable to the UK was the study by Bonham et al [9]. In this study PPV was reported to be 33% however the case identified clinically had already been detected clinically and was on treatment.
No other study reported if symptomatic babies were included in their screening populations. It was not possible to determine sensitivity, specificity, or negative predictive values because there was either no, or only partial, follow up of people who screened negative.

Sub-question: Can the test distinguish between asymptomatic patients and those affected by milder forms of LCHAD and MTP deficiency?

One of the 10 included studies addressed this sub-question. The study found no differences in acylcarnitine rates between patients with homozygous LCHADD compared to other mutations or MTP, neither did they find acylcarnitine levels correlated with clinical outcome [36]. Out of the 10 included studies for the main question, 2 studies identified 4 cases which were still asymptomatic at the end of the study [5, 36]. This may be indicative of overdiagnosis and suggest the test may detect cases which would not have gone on to ever be symptomatic. However it may also indicate that, if provided early enough, treatment can be effective in preventing or delaying the onset of symptoms. Likewise, as these studies did not include long-term follow up, it is possible that these very young babies went on to become symptomatic at a later date.

Sub-question: Does the test detect other non-MTP conditions?

One of the included studies detected non-MTP conditions using the marker for LCHADD [48]. The study used marker C16OH. Using this marker, 5 infants were flagged, of which 2 were confirmed to be cases of Carnitine Palmitoyltransferase (CPT) Type II and Very Long-Chain Acyl-CoA Dehydrogenase (VLCAD) deficiency. In both instances the newborns

also had higher rates for the markers associated with those specific diseases (C14:1 and C16).

Summary of Findings Relevant to Criterion 4: Not met*

Question 3 – What is the test accuracy (sensitivity, specificity, and predictive values applicable to UK prevalence) of acylcarnitines measurement in dried blood spots (DBS) using TMS for LCHAD/MTP deficiency screening?

Sub-questions:

- ***Can the test distinguish between asymptomatic patients and those affected by milder forms of LCHAD and MTP deficiency?***
- ***Does the test detect other non-MTP conditions?***

Ten studies reported on test accuracy of acylcarnitines measurement in dried blood spots (DBS) using TMS for LCHAD/MTP deficiency. There are some concerns regarding the applicability of the studies to the UK population; many of the studies included in the review screened on day 2 or 3 of life, as opposed to days 5–8 in the UK.

The only measure of test accuracy that was consistently reported (or where sufficient data were available to allow calculation) was PPV. Test accuracy estimates differ greatly by study, with some suggesting good accuracy albeit on small numbers, with PPV ranging from 0–100% (23 true positives and 40 false positives from 3,951,358 newborns). Heterogeneity in markers used prevented analysis of whether differences in test accuracy estimates are due to thresholds used, and lack of reporting of accuracy by marker preventing establishment of the optimal threshold. The only UK study identified a case who was already

* **Met** -for example, this should be applied in circumstances in which there is a sufficient volume of evidence of sufficient quality to judge an outcome or effect which is unlikely to be changed by further research or systematic review.
Not Met - for example, this should be applied in circumstances where there is insufficient evidence to clearly judge an outcome or effect or where there is sufficient evidence of poor performance.
Uncertain -for example, this should be applied in circumstances in which the constraints of an evidence summary prevent a reliable answer to the question. An example of this may be when the need for a systematic review and meta-analysis is identified by the rapid review.

being treated, so already detected clinically. All test accuracy data may have included those already symptomatic so may have overestimated accuracy to detect asymptomatic babies. PPV is related to the prevalence of a disease in the population and is not intrinsic to the test. Sensitivity, specificity, and negative predictive values could not be established due to a lack of systematic follow up of newborns who screened negative.

There is some evidence that the primary markers for LCHAD/MTP deficiency may appear raised when newborns have other fatty acid oxidation disorders [48]. However, this appears to be in conjunction with higher rates of the primary markers for those conditions.

There was no evidence to indicate whether the screening test can distinguish between milder and more severe types. There was also very little evidence to help determine whether the test is likely to detect milder forms.

Overall, measurement of acylcarnitines in dried blood spots using TMS for LCHAD/MTP deficiency screening results in a high number of false positives. There are significant concerns regarding risks of bias in the available studies, and, crucially, there is insufficient evidence to clearly judge test accuracy as none of the identified studies provided data on sensitivity, specificity, or negative predictive values. Further research should be undertaken to explore the issues highlighted. The reviewers suggest collaboration between researchers to report scores on a range of relevant markers for both cases of LCHAD, cases of MTP, and in the unaffected population using consistent units.

Overall based on current available evidence this criterion is not met.

Criterion 9 and 11 — Outcomes following early treatment compared to later treatment

9: There should be an effective intervention for patients identified through screening, with evidence that intervention at a pre-symptomatic phase leads to better outcomes for the screened individual compared with usual care. Evidence relating to wider benefits of screening, for example those relating to family members, should be taken into account where available. However, where there is no prospect of benefit for the individual screened then the screening programme should not be further considered.

11: 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” (such as Down’s syndrome or 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.

Question 4 — Does early treatment with dietary management following screening provide better long-term outcomes than later treatment after the presentation of symptoms?

This question has not been previously addressed. Given the uncertainties related to the variability of the prognosis, the aim of addressing this question in this review is to determine whether evidence has been published to indicate that early treatment following screening is more beneficial and leads to better long-term outcomes than later treatment, following the presentation of symptoms.

Eligibility for inclusion in the review

Articles were included in this question if they looked at people with isolated LCHAD deficiency or MTP deficiency. They were included if they compared treatment with dietary restrictions and other nutritional strategies (e.g. medium-chain triglyceride supplementation) following (1) pre-symptomatic detection (universal newborn screening, cascade testing or incidental detection) with (2) treatment following the presentation of symptoms (either before or after the screening period).

Study types suitable for inclusion were any study design in humans with comparative data. Searches were not limited by language or date. Papers including non-human studies, letters, editorials and communications, grey literature, non-systematic reviews and conference abstracts were excluded. Studies of fatty acid beta oxidation disorders where data from people with mitochondrial trifunctional protein disorders cannot be separated out from data on other fatty acid oxidation disorders (e.g. multiple acyl-CoA dehydrogenase and very long chain acyl-CoA dehydrogenase deficiencies) and studies where more than 10% of the sample do not meet our inclusion criteria were also excluded.

Description of the evidence

Appendix 2 contains a full PRISMA flow diagram (Figure 27) along with a table of the included publications and details of which questions these publications were identified as being relevant to (Table 11). Database searches yielded 7,483 results, of which 313 full texts were retrieved and sorted, and 12 were judged to be relevant to this question. An additional article was identified from the search for test accuracy, so 13 articles were ultimately included in this review [5, 6, 23, 26, 28, 31, 33, 38, 49-52, 58].

Characteristics of included studies

A study-level summary of data extracted from each included publication is presented in the summary and appraisal of individual studies Appendix 3 Table 20.

There were 13 papers reporting on 11 cohorts comparing early treatment in asymptomatic people detected through screening to people clinically diagnosed following the presentation of symptoms [5, 6, 23, 26, 28, 31, 33, 38, 49-52, 58]. These were comprised of 3 related papers on a Swedish cohort [49-51], 2 European single country studies [31, 38], 2 European collaboration studies [52, 58], 4 single country international studies [23, 26, 28, 33] and 2 international cohort studies [5, 6]. The number of LCHAD/MTP deficiency patients included per paper ranged from 5 people from a single clinic in Utah, USA to 59 from 2 centres in Poland and Denmark [6, 26]. A total of 156 people with LCHAD deficiency, 18 people with MTP deficiency and 12 people with

undifferentiated LCHAD/MTP deficiency were included across all the studies. In total there were 49 asymptomatic screened people, 16 symptomatic screened people and 119 clinically diagnosed symptomatic people included across all the studies.

Seven of the 11 included studies were retrospective cohort studies, using medical files and chart data. One study did not report the period of follow up [58]. The shortest study duration was 3 years (people with LCHAD/MTP deficiency up to 5 years of age) [38]. The longest period of follow period was up to 17 years [6]. Type of dietary management was not specified in 2 of the 7 retrospective cohort studies [5, 6]. In the remaining 5 studies all individuals received a dietary treatment of low fat intake and essential fatty acid supplementation. Whether patients were given docosahexaenoic acid (DHA) supplements, carnitine supplements and Triheptanoin treatment varied across studies and across patients within studies. Only one of the 7 studies reported on dietary compliance [26].

There were 3 prospective studies [5, 31, 49-51]. The first was the Swedish cohort study on people with LCHAD deficiency which reported on 10–12 people across the three papers, with a median follow up of 15 years. This study used retrospective medical records to identify people with LCHADD and then prospectively collected data on outcomes of interest. All received treatment of low fat intake and essential fatty acid supplementation and all but one received DHA. The second study also used retrospectively collected hospital records and collected prospective data on 16 people with LCHAD deficiency with follow up time up to 11 years [31]. All living patients were receiving a low-fat diet, MCT, essential fatty acids and DHA. The third prospective study was a case control study of 5 undifferentiated LCHAD/MTP deficiency patients, followed up to 109 months [5]. Type of dietary management was not reported. Dietary compliance was reported in 2 out of the 3 studies [31, 49-51].

The remaining study was a prospective randomised controlled trial including 12 people and testing a new treatment drug [28]. All had been treated with a low-fat diet and MCT oil, but 7/12 (58.3%) also received Triheptanoin and 5/12 (41.7%) received Trioctanoin to increase energy intake.

The definition of ‘early treatment’ and how groups were analysed differed between studies. In 8 of the 11 studies, ‘early’ was defined as screen

detected and asymptomatic whereas ‘late’ was defined as clinically detected and symptomatic (either before or after screening) [5, 6, 28, 31, 33, 38, 49-51]. Six of these 8 studies compared asymptomatic people detected through screening to symptomatic people clinically detected, and one of the studies compared asymptomatic people detected through screening to symptomatic people detected through screening [38]. The remaining 3 studies classified people as either screened (asymptomatic and symptomatic at screening) or unscreened clinically diagnosed [23, 26, 58].

Discussion of findings

Quality appraisal of included studies

The 11 included studies were quality appraised using the EPHPP quality assessment tool [65]. Quality appraisal is summarised in Figure 15, with further details provided in Appendix 4 Table 24. The overall methodological quality was moderate in 4 studies with one weak rated domain [5, 23, 33, 52]. The remaining 7 studies were all rated overall as weak, with 2 or more weak-rated domains.

All studies were of weak methodological quality in relation to confounding, since important factors (i.e. presenting form of LCHAD/MTP deficiency, genotype, compliance with treatment, co-treatment) were not controlled for in study design or analysis. Methodological quality was weak in relation to participant selection in 3 studies [26, 28, 41]. Data collection methods were of weak methodological quality in 6 of the 11 studies [6, 26, 28, 31, 38, 41].

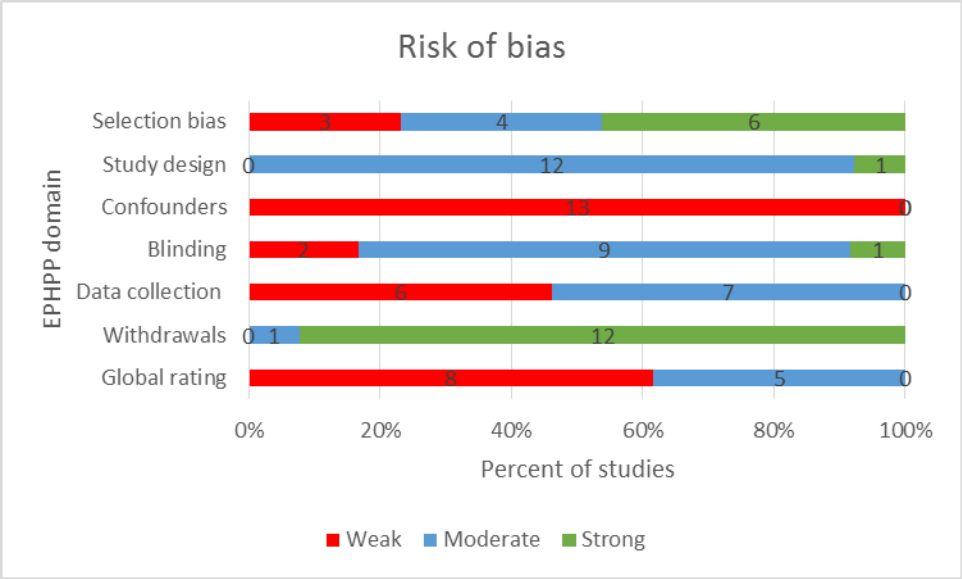

Figure 15 Risk of bias for question 4– authors judgements using the EPHPP tool [65]

Analysis of the evidence

Details on the evidence of outcomes following early versus late diagnosis are available in Appendix 3 Table 20. The most frequently reported types of outcome are described. Definitions of early versus late varied across the studies.

Mortality

Mortality was reported in 6 studies [5, 6, 31, 33, 38, 41]. In 4 of these studies, mortality rates were lower in the early group (0–20%, 1/26 total) than in the later groups (37.3–50%, 28/74 total) [31][38] [5] [6]). In the remaining 2 studies mortality was lower in the late group (0–33.3%, 2/9 total) than the early group (33.3–100%, 2/4 total) [33, 38]. The median age of mortality in the early group across the studies was 28 days (range 3 days–3 months) compared to 4 months (range 2 days–10 years 1 month) in the late group.

Heart related problems

Heart related problems were reported in 7 of the 11 studies [5, 26, 28, 31, 38, 52, 58]. The outcomes reported were cardiomyopathy, arrhythmias and cardiac complications. In all 7 studies there were fewer heart problems in the early treatment group (0–40%, 5/31 total patients in the

early treated group) compared to the later treatment group (25–100%, 20/32 total in the late treated group across the 7 studies). However, the only study to report the age of diagnosis of cardiomyopathy found the median age to be lower in the early treatment group (4 months) compared to the later treatment group [52].

Liver related problems

Liver related problems were reported in 2 studies [5, 52]. In both studies there were more instances of liver related problems in the later treatment group. In the first study there was one case out of 6 (16.7%) from the early group, whilst there were 4/8 (50%) cases in the later treatment group [52]. In the second study, none of the 3 people diagnosed from the early treatment group had liver related problems. Conversely, both patients in the late treatment group showed liver related problems (2/2, 100%) [5].

Reye syndrome

Reye syndrome was reported in one study [58]. There were somewhat fewer people with LCHAD/MTP deficiency who had this outcome in the early treatment group than the later treatment group [3/10 (30%) vs 6/17 (35.3%) respectively].

Visual problems

Problems related to vision were reported in 5 studies across 7 papers [23, 26, 31, 49, 52]. The studies reported on outcomes such as electroretinography (ERG) findings, best corrected visual acuity, ocular fundi findings and retinopathy. One study (across 2 papers) reported on ERG findings [49, 50]. One out of 2 (50%) individuals treated early had subnormal results and neither person had pathological results. Three out of 9 patients (33.3%) from the later treatment groups had subnormal results and 5 out of 9 patients (55.6%) had pathological findings. In the same study all early treatment patients had mild or no visual impairment (2/2), while in the later treatment groups one of 9 patients (11.1%) had moderate impairment and one out of 9 patients(11.1%) was blind. All people treated asymptomatically had either normal or subnormal (3/3) ocular fundi findings, and each patient in the later treatment group had either pathological or severely pathological findings (9/9) [50]. Three studies reported on retinopathy [26, 31, 52]. Two of the 3 studies found less retinopathy in the early treatment group (0–33.3%, 0/1 and 2/6) compared to 75–90% (6/8 and 9/10) showing mild to full retinopathy in the

late treatment group. One study found 100% retinopathy in the early treatment group, though this group only included one person. This compares to 75% retinopathy in the later treatment group (3 out of 4 patients).

Neurological problems

Neurological problems were reported in 4 studies [26, 31, 41, 50]. The reported outcomes were epilepsy, neuropathy and neurological symptoms. There were no instances of any neurological problems in any of the people who were treated early (n=15 across the studies) compared to problems in every late treatment group [10/36 (27.7%) ranging from 17.7% to 33.3%].

Motor and muscular problems

Muscular and motor problems were reported in six studies [23, 26, 38, 49, 52, 58]. The studies reported on psychomotor development, myopathy, episodes of rhabdomyolysis, and myoglobinuria. There were fewer motor and muscular problems in all early treatment groups compared to the late treatment groups across all studies (0–40%, 5 out of a total of 17 in the early group, compared to 25–82.4%, 21 out of a total of 38 in the late group).

Statistical analyses

Outcomes by comparison groups and analyses are presented in Appendix 5 Table 26. There were differences in the definition of ‘early treatment’ across studies. In the majority of cases ‘early’ was defined as screen detected and asymptomatic, whereas ‘late’ was defined as clinically detected and symptomatic. However, there is a sub-group of people who present with symptoms before screening. Studies varied in whether they included this group within an early “screened” group, or within a late “symptomatic at diagnosis” group. As this group include very severe cases, who appear more likely to have adverse outcomes such as early death, there are inherent biases on including them in either of the groups. Sub-group analyses were undertaken to examine differences between early and late treatment groups depending where this severe group were placed. Three analyses were undertaken: (1) asymptomatic vs symptomatically detected patients, (2) screen detection vs clinically detected, (3) asymptomatic at screening vs symptomatic at screening vs later clinically detected. We also compared outcomes between people

diagnosed with LCHAD deficiency vs those diagnosed with MTP deficiency as there is some evidence of differences in outcomes for the two conditions. Meta-analyses were not conducted due to variability in study methods. Chi-squared (or Fisher's exact) tests were conducted to examines differences in outcomes within individual studies.

1. Asymptomatic vs symptomatic detected patients

The majority of the included studies compared asymptomatic to symptomatic detection [5, 6, 26, 28, 33, 50]. This analysis is inherently biased in favour of early detection as asymptomatic cases tend to be milder. In addition, by including the severe cases who present within the first few days of life within the symptomatic group, the results are biased in favour of the asymptomatic group. This distinction does not represent a screening scenario. In a screening scenario both symptomatic and asymptomatic cases would be screened. By just looking at asymptomatic versus symptomatic cases it would inflate the benefit of screening.

One study reported post mortem cases and cases of unknown method of diagnosis within the symptomatic group. For the purpose of the analyses these cases were not included (n=7) [6]. While there was a trend towards better outcomes in the asymptomatic groups, no statistically significant group differences were noted for liver-, visual-, neurological-, motor or muscular problems, or any outcomes grouped in the "other" category ($p>0.05$, Fisher's exact test). The two areas in which some of the studies found significant results are discussed below.

Mortality

There were 6 studies comparing mortality between people who were asymptomatic and symptomatic at diagnosis [5, 6, 31, 33, 38, 41]. A total of 120 LCHADD/MTPD cases were included in the analyses. There was no significant difference in mortality rates in 5 of the 6 studies, as shown in Figure 16. In the remaining study a significantly fewer deaths were observed amongst individuals who were asymptomatic at screening (1/15, 6.7%) than those who were symptomatic at screening (19/44, 43.1%), $p=0.01$ [6].

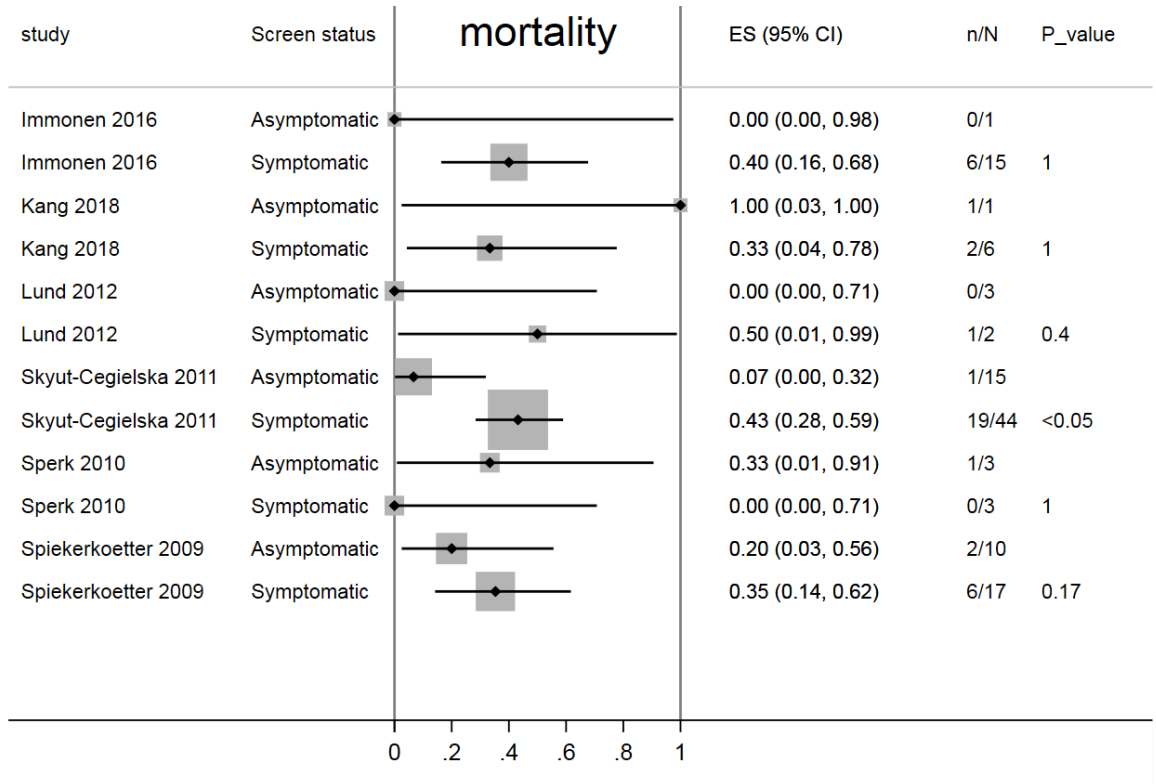

Figure 16– Forest plot showing percentage of deaths and p values across symptomatic and asymptomatic groups

Heart problems

There were 6 studies reporting on different types of heart problems across asymptomatic and symptomatic at diagnosis groups, 4 reported on cardiomyopathy [5, 31, 38, 52], one reported on arrhythmias [26] and one reported on general cardiac complications [28]. There were no statistically significant differences in the frequency of heart problems in 5 out of the 6 studies, as shown in Figure 17. In the remaining study, a significantly greater frequency of cardiac complications was observed in the symptomatic group (3/5, 60%) than the asymptomatic group (0/7, 0%), $p<0.05$ [28].

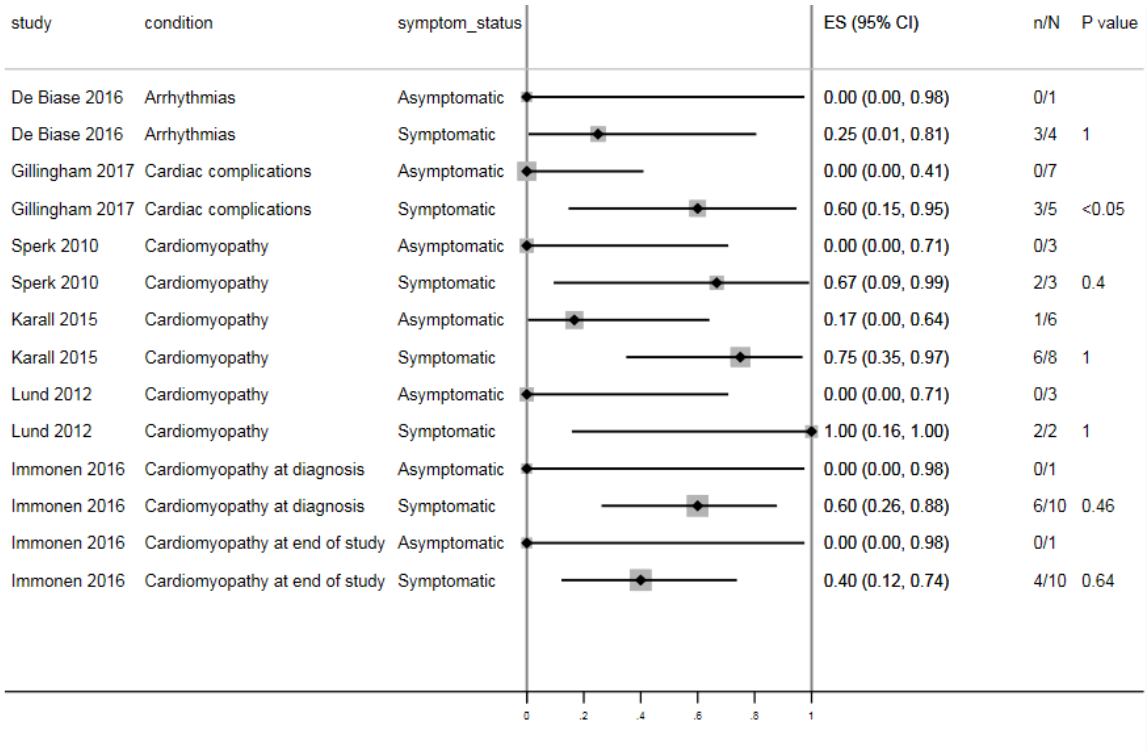

Figure 17 - Forest plot showing percentage of heart problems and p values across symptomatic and asymptomatic groups.

2. Screen detected vs Unscreened

In practice, symptomatic babies still get screened and are still treated at a young age. Further analyses were undertaken to see how grouping by screen detected and unscreened clinical detection affected outcomes. By analysing the groups according to whether they are screen detected or clinically detected there is bias. Including babies that are already symptomatic in the screen detected group biases against early detection, as these are the most unwell babies. However, other screen detected babies tend to be the least unwell, which biases in favour of early detection. Therefore, the overall direction of bias is unclear.

Mortality

Five studies reported on mortality [5, 6, 31, 33, 58]. A total of 105 LCHADD/MTPD cases overall were included in the analyses. Four of these studies did not find any significant differences between the screen detected and clinically detected groups (see Figure 18). In the final study,

there were significantly fewer deaths in the screened group (1/15, 6.7%) than the clinically detected group (13/37, 35.1%), p>0.05 (Fisher's exact) [7].

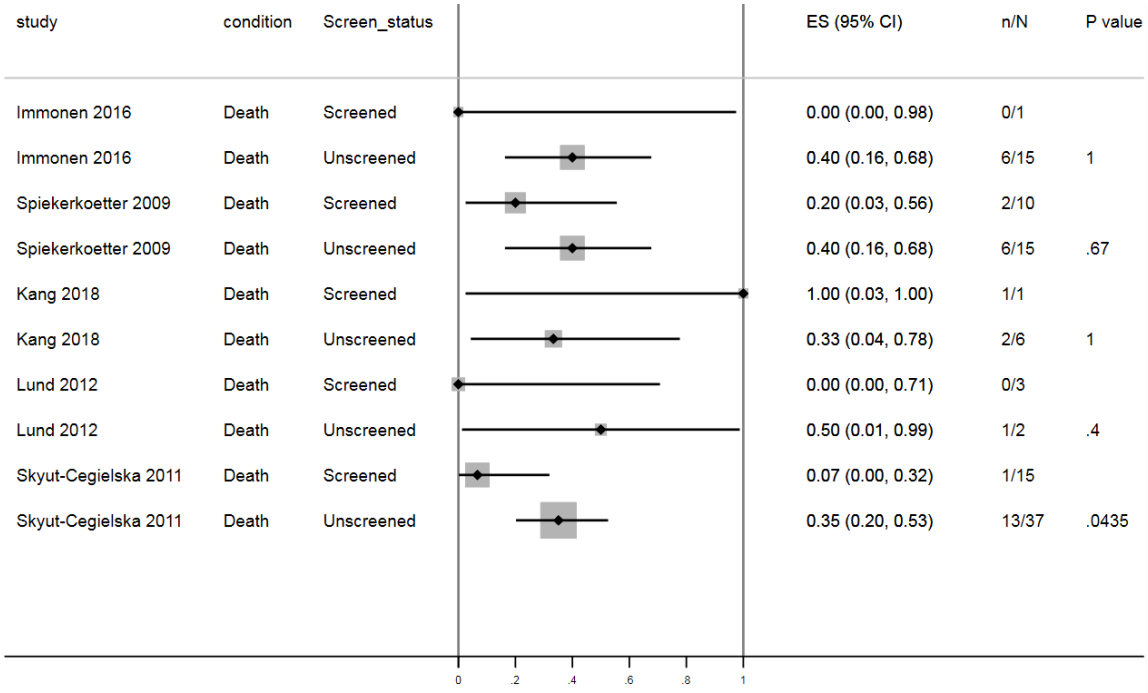

Figure 18 - Forest plot showing percentage of mortality and p values across studies for screen detected and unscreened clinically detected groups.

Heart problems

There were 6 studies reporting on different types of heart problems across screen detected and clinically detected groups: 4 reported on cardiomyopathy [6, 30, 37, 50], one reported on arrhythmias [25] and one reported on general cardiac complications [27]. In total 74 LCHAD/MTP deficiency patients were included in the analyses. Four of the 6 studies did not find any significant differences between these groups (see Figure 19). Significantly fewer cases of cardiomyopathy were reported in the screen detected group (2/9, 22.2%) compared to the clinically detected group (5/5, 100%), p=0.02 (Fisher's exact). A significant difference was also found between the screen detected and clinically detected groups for cardiac complications in one study (p<0.05), with none of the 7 patients in the screen detected group having cardiac complications, compared to 3 out of 5 patients (60%) in the clinically detected group [27].

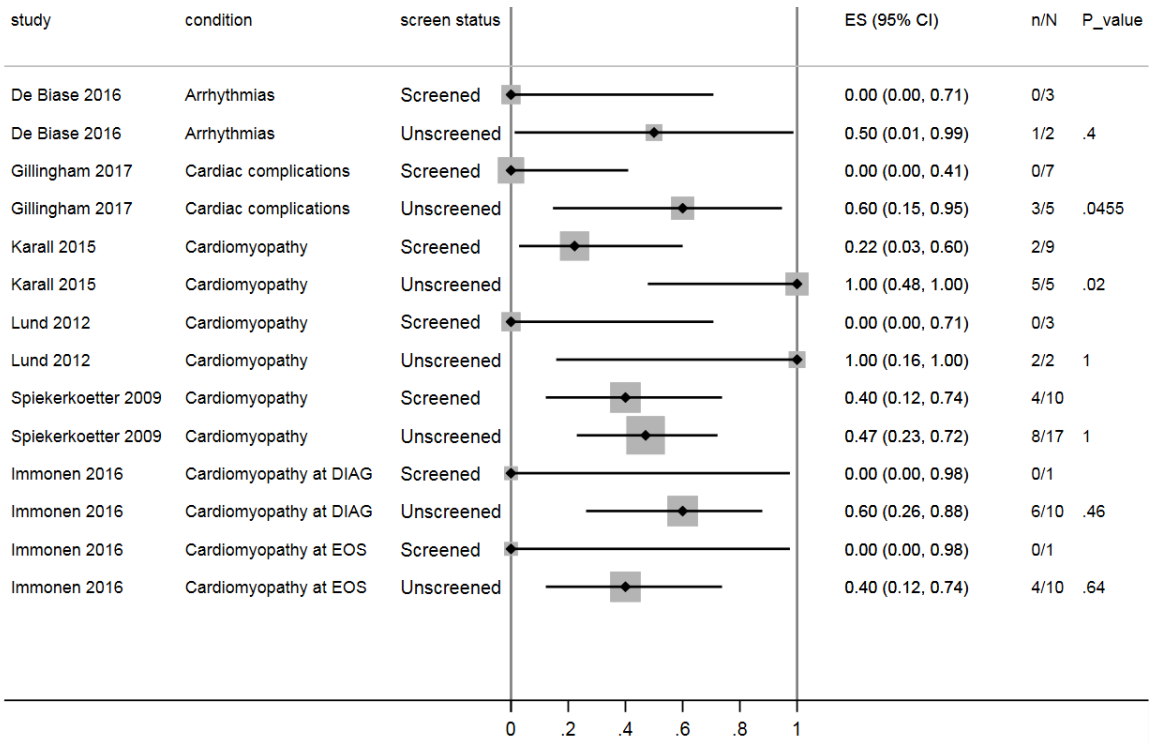

Figure 19- Forest plot showing percentage of heart problems and p values across studies for screen detected and unscreened clinically detected groups.

EOS: end of study; DIAG: at diagnosis

Liver problems

Three studies reported on liver problems [5, 52, 58]. Two of the studies did not find a significant difference between the screen detected and clinically detected groups (see Figure 20). In the remaining study, hepatopathy was significantly less common in the screen detected group (1/9, 11%) compared to the clinically detected group (4/5, 80%) [52].

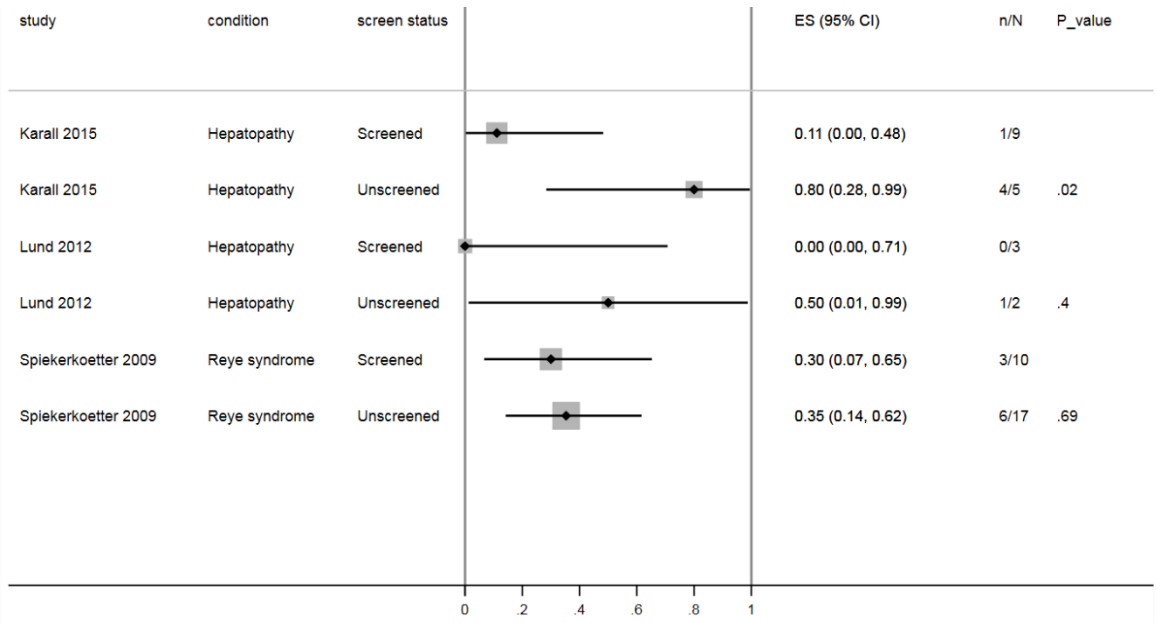

Figure 20- Forest plot showing percentage of liver problems and p values across studies for screen detected and unscreened clinically detected groups.

Visual problems

Six studies (from five cohorts) reported on eye problems [23, 26, 31, 49, 50, 52]. Five of the six did not find a significant difference between screen detected and clinically detected groups (see Figure 21). In one study, retinopathy was significantly less common amongst screen detected individuals (3/9, 100%) than clinically detected individuals (5/5, 100%), p=0.03 [52].

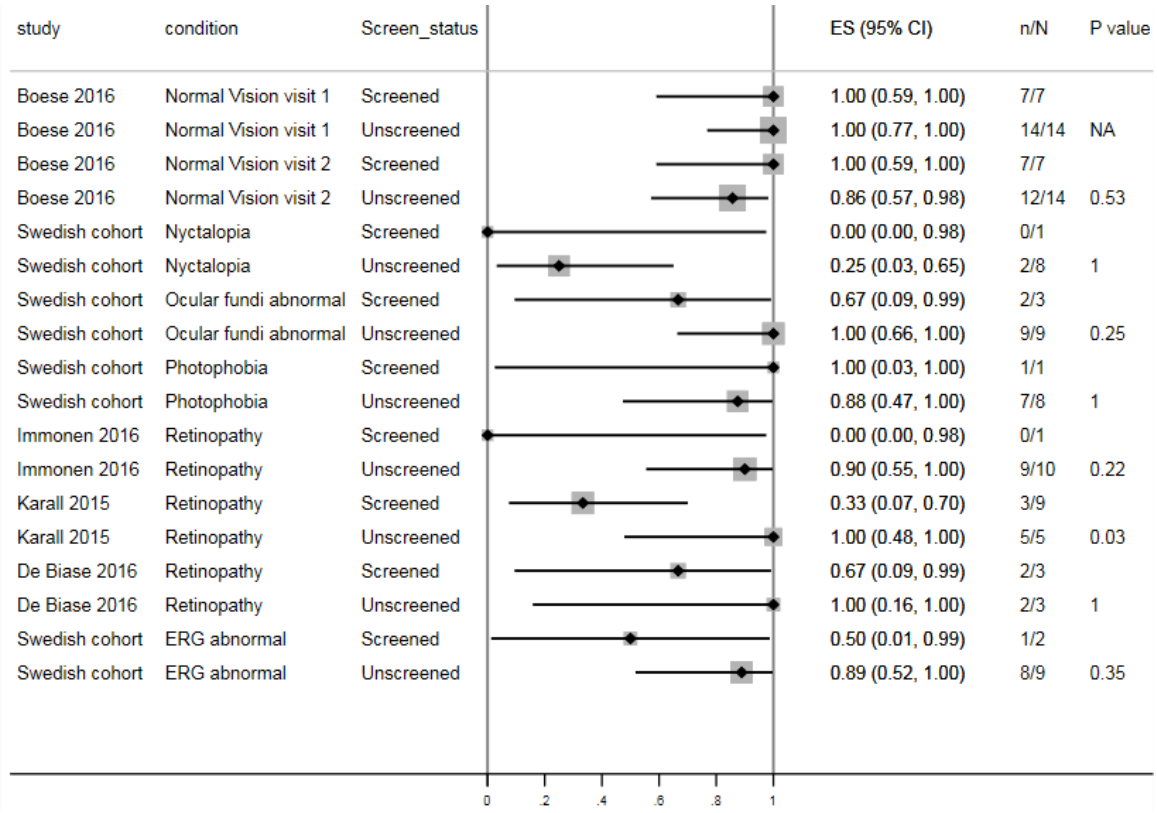

Figure 21- Forest plot showing percentage of visual problems and p values across studies for screen detected and unscreened clinically detected groups.

Motor and muscular problems

Four studies reported on motor and muscular problems [23, 26, 49, 58]. Three of these did not find a significant difference between screen detected and clinically detected groups (see figure 22). There were significantly fewer cases of hypotonia/myopathy in the screen detected group (4/10, 40%) compared to the clinically detected group (14/17, 82.4%) in the remaining study, p=0.03) [58].

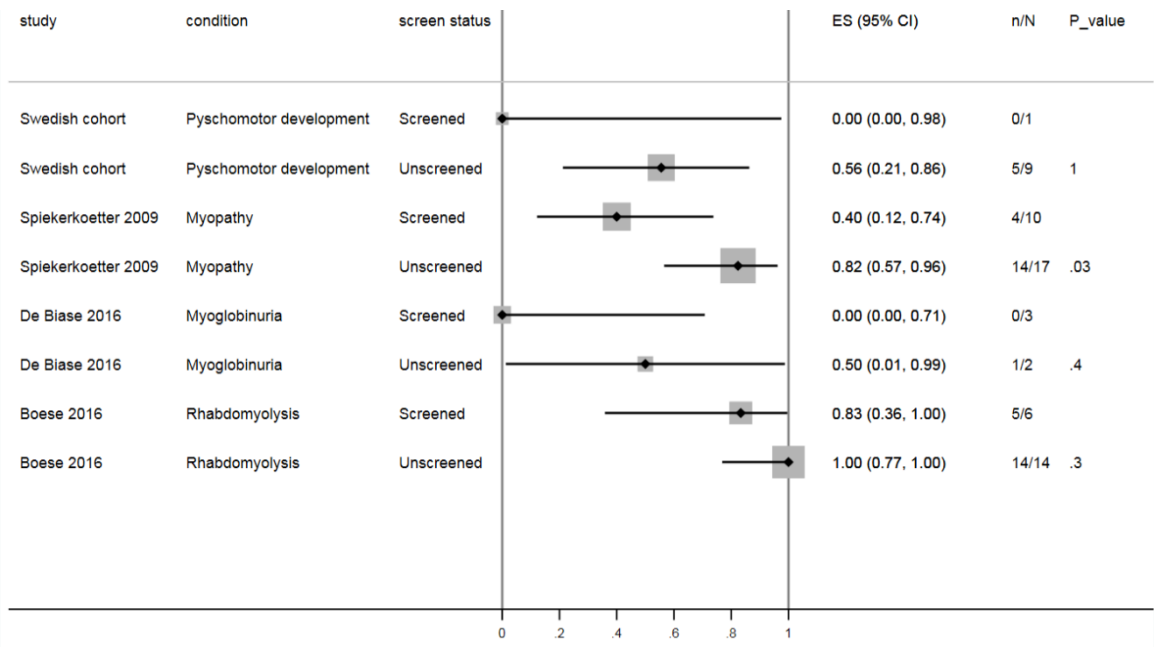

Figure 22- Forest plot showing percentage of motor and muscular problems and p values across studies for screen detected and unscreened clinically detected groups.

Hypoglycaemia

Two studies explored hypoglycaemia (not as a presenting symptom), and are presented in figure 23 [50, 58]. One of the 2 studies found a significant difference between screen detected and clinically detected groups (p=0.02) [58]. They found 4 out of 10 (40%) cases in the screen detected group compared to 15 out of 17 (88%) in the clinically detected group.

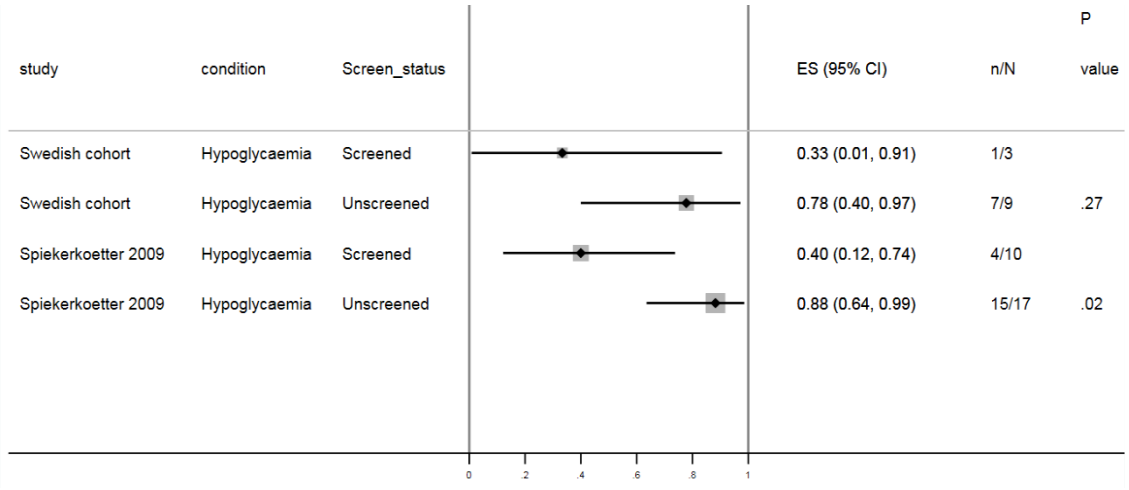

Figure 23 Forest plot showing percentage of hypoglycaemia and p values across studies for screen detected and unscreened clinically detected groups

3. *Asymptomatic screened, symptomatic screened and late clinically detected comparison*

In an attempt to control for the potential bias of the early presenting severe form of the diseases, the third analysis examined the 3 groups separately: asymptomatic at screening, symptomatic at screening and late clinically detected. There were 4 studies which provided these data [23, 26, 41, 52]. In one study the clinically detected group included false negative screened cases [52]. Chi-squared tests were undertaken to see if there was an overall significant difference between groups. If a significant difference was found, pairwise comparisons were conducted. Comparisons between asymptomatic at screening and late clinical detection are of most applicability to the decision of whether screening is appropriate, but is biased in favour of early detection. The reviewers found no significant differences in 3 of the studies, only one study reached significance [52]. No significant difference between groups was found for mortality, liver problems, neurological problems or muscular/motor problems.

Heart problems

Heart problems were reported between the 3 separate groups in 2 studies (see Figure 24) [26, 52]. No significant difference was reported for arrhythmias: asymptomatic screening group = 0/1 (0%), symptomatic

screening group = 0/2 (0%), late clinical detection group = 1/2 (50%) (p=1) [26]. There was a significant difference in the rates of cardiomyopathy in one study: 1/6 (16.7%) in the asymptomatic screened group, 1/3 (33.3%) in the symptomatic screened group, and 5/5 (100%) in the late clinically detected group, p<0.05. [52]. Pairwise analyses indicated significantly fewer cases of cardiomyopathy in the asymptomatic screened group compared to the late detected group, p=0.05. No other comparisons were significant.

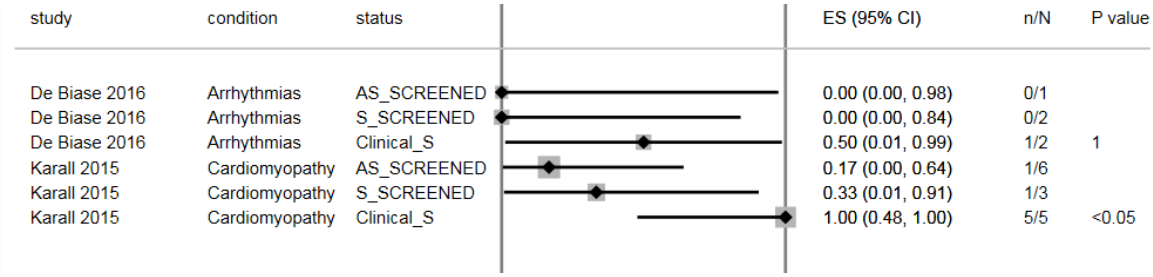

Figure 24- Forest plot showing percentage of heart problems and p values across studies for symptomatic screened, asymptomatic screened and clinically detected groups

Visual problems

Three studies reported visual problems across the 3 separate groups [23, 26, 52]. There was no significant difference in 2 studies (see Figure 25). One study found a significant difference between the groups in terms of retinopathy: asymptomatic screening group = 1/6 (16.7%), symptomatic screening group = 1/3 (33.3%), late clinical detection group = 4/5 (80%), p=0.05 [52]. No statistically significant differences were observed in pairwise comparison. This may be due to a lack of statistical power related to the small sample sizes.

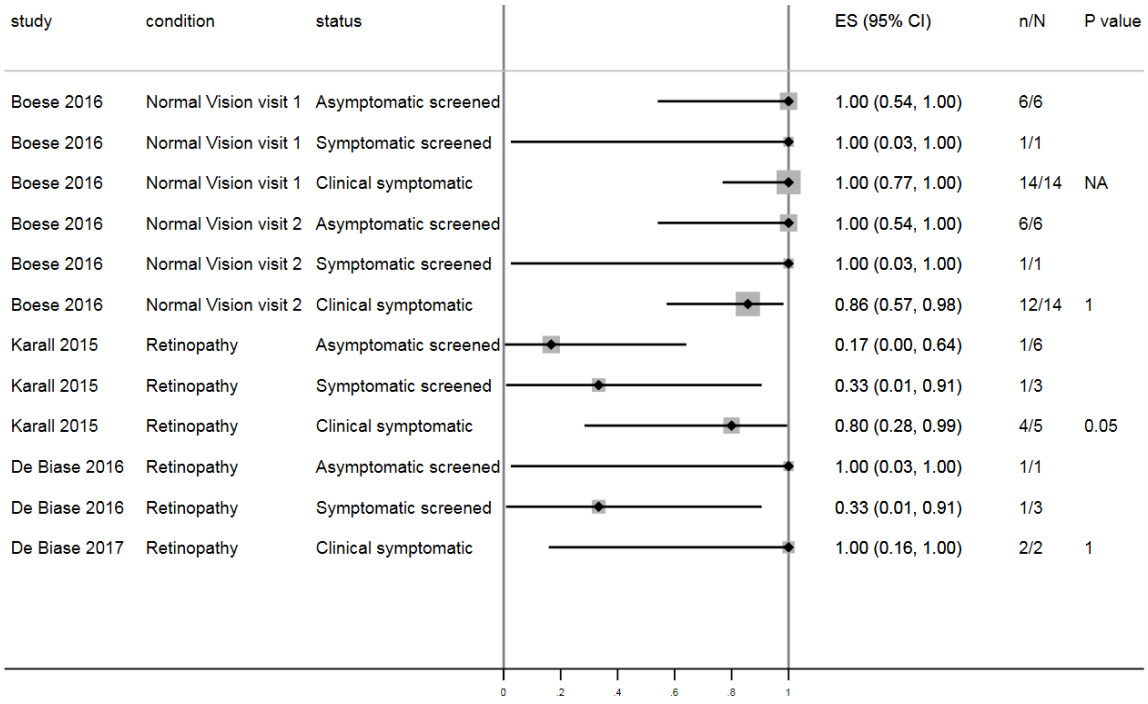

Figure 25 - Forest plot showing percentage of heart problems and p values across studies for symptomatic screened, asymptomatic screened and clinically detected groups

4. LCHADD vs MTPD

Across all 11 included studies, 5 included both LCHAD and MTP deficiency cases and reported them separately [23, 26, 28, 38, 58]. No significant differences were found between LCHAD deficiency and MTP deficiency for heart, liver, visual, neurological or motor/muscular outcomes. The results for mortality are discussed below.

Mortality

Out of the 5 studies reporting on both LCHAD deficiency and MTP deficiency cases as well as reporting outcomes separately, 2 reported mortality rates [38, 41]. One of the 2 studies did not show a significant difference [40] as shown in Figure 26. In the second study, the proportion of deaths was significantly lower in the LCHAD deficiency group (3/20, 15%) compared to the MTP deficiency group (5/7, 71.4%), p=0.01) [41].

There may be bias in the analyses as these included different forms of the disease: neonatal severe, infant hepatic and late onset neuromyopathic. Only one of the studies reported outcomes by both disease and severity [38]. When the analysis was stratified by disease type there were no significant differences for any outcome for LCHAD deficiency or MTP deficiency. However, this study only included 6 people.

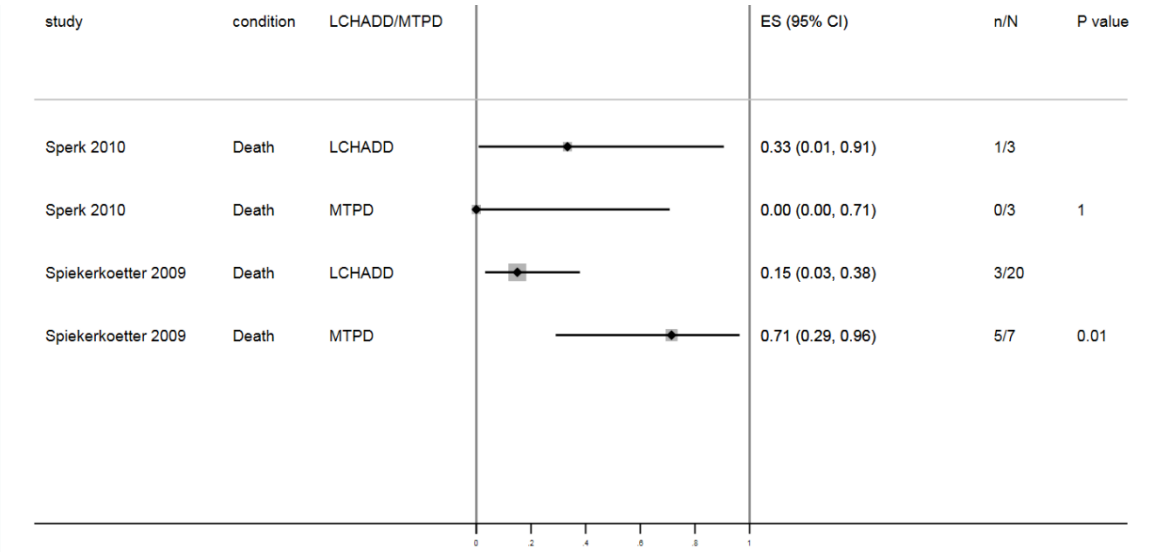

Figure 26- Forest plot showing percentage of mortality and p values across studies for people with LCHAD and MTP deficiency

Study evidence

Thirteen international articles were reported across 11 studies which were included in this review [5, 6, 23, 26, 28, 31, 33, 38, 49-52, 58]. There is some evidence to suggest that early dietary management may be associated with a reduction in heart [5, 26, 28, 31, 38, 52], visual [23, 31, 49, 50, 52], neurological [26, 49], motor and muscular problems [23, 26, 38, 49, 52]. It may also be associated with a reduction in hypoglycaemia [38, 50], failure to thrive [26], brain damage [51] acute metabolic encephalopathy [5] and developmental delay [5]. Few comparisons reached statistical significance [5, 26]. This is unsurprising given that the sample sizes were very small. Also, there were large variations in how studies defined ‘early’. It was unexpected that there were more studies showing a significant difference between screened and unscreened groups as opposed to asymptomatic and symptomatically diagnosed groups. This may be due to the age of the groups. Age at the time of the

study was reported in 8 out of the 11 studies [6, 23, 26, 28, 31, 33, 50, 52]. In 7 of those 8, the screened groups were considerably younger than the clinically diagnosed groups (median 2–10 years in the screened group compared to 19–22 years in the unscreened group) [6, 23, 26, 31, 33, 50, 52] so they may not have reached the same level of disease progression as those diagnosed clinically. Disease severity may bias the results but studies did not consistently report this.

Quantity

Thirteen international articles were reported across 11 studies which were included in this review. The number of LCHAD/MTP deficiency patients included in the analyses ranged from 5 [5, 26] to 59 [6]. In total there were 49 asymptomatic screened people, 16 symptomatic screened people and 119 clinically diagnosed symptomatic people included across all the studies. No studies reported on UK patients. This is unsurprising given that the criteria for inclusion was that there had to be a separate screened group, and the UK does not currently screen for LCHAD/MTP deficiency. However, 5 studies were reporting results for similar western European populations such as Sweden, Finland, Germany, Switzerland and Austria [31, 38, 49-52, 58]. The remaining 5 studies were from non-European countries. The 3 studies from Sweden reported on the same patient group and were classified here as the Swedish cohort study [49-51]. There was a high overlap between the 3 papers, with 10 of the 12 patients (83.3%) included in all 3. There may have been further overlap between German patients in the European collaboration study and the retrospective cohort study [41, 52]. However, it was not possible to clearly determine if this was the case. Sample size per treatment group was as small as one patient [26, 31, 33] up to 44 [6].

Quality

The methodological quality was moderate to weak in all 11 studies. The selection of people in the studies was weak in 3 of the studies [26, 28, 58]. Bar one study which did not report on treatment, all studies consistently gave patients a low fat diet with MCT supplementation, however additional supplements and medications varied between patients and studies. Likewise, only 4 studies reported on dietary compliance [23, 26, 31, 50]. Grouping unscreened patients according to age of presentation or age of treatment initiation might result in differences in

spectrum of disease between the groups. Initial symptoms are age-dependent and the clinical course differs according to age at onset of symptoms and form of LCHAD/MTP deficiency.

Applicability

All of the 11 included studies provided a comparison of outcomes of dietary management following asymptomatic detection (incidental, cascade testing or newborn screening) versus dietary treatment in a symptomatic population. Studies differed in whether they grouped in this manner or whether they grouped by screened vs unscreened. All studies reported whether the screened groups included symptomatic patients at the point of screening, but in one study it was not possible to identify which outcomes were for symptomatic screened patients, and which were for asymptomatic screened patients.

Consistency

There was a trend towards better outcomes in the ‘early’ identified group, however only occasionally did this reach statistical significance. However, where it did reach significance it was on the side of early detection. Likewise, there was a trend towards greater mortality in those with MTP deficiency than LCHAD deficiency; however this was only statistically significant in one study. There are issues surrounding the size of the studies which may have affected effect size. Unusually there were more instances of statistically significant differences between the screened and unscreened groups than the asymptomatic versus symptomatic groups. This may be explained by the younger age of the screened group in comparison to the unscreened group.

Summary of Findings Relevant to Criterion 9 and criterion 11: Not met*

Question 4 - Does early treatment with dietary management following screening provide better long-term outcomes than later treatment after the presentation of symptoms?

There is some evidence to suggest that early dietary management while asymptomatic may be associated with a reduction in heart [5, 26, 28, 31, 38, 52], visual [23, 31, 49, 50, 52], neurological [26, 49], motor and muscular problems [23, 26, 38, 49, 52]. It may also be associated with a reduction in hypoglycaemia [38, 50], failure to thrive [26], brain damage [51] acute metabolic encephalopathy [5] and developmental delay [5]. Follow up analyses comparing asymptomatic vs symptomatic detection, screen detection vs clinically detected, and asymptomatic screen detection vs symptomatic screen detected vs later clinical detection suggest a trend towards better outcomes following early detection. However, there are biases in all of the comparisons, which are mostly in the direction of overestimating any potential benefit of early detection, and the majority of studies are too small to show any statistically significant differences.

There was only one randomised controlled trial (RCT) included within the review and there were very high applicability concerns, as the main focus of the trial was to determine the benefits of different drug treatments.

Overall based on the quality and consistency of the available evidence, this criterion is not met.

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* **Met** -for example, this should be applied in circumstances in which there is a sufficient volume of evidence of sufficient quality to judge an outcome or effect which is unlikely to be changed by further research or systematic review.
Not Met - for example, this should be applied in circumstances where there is insufficient evidence to clearly judge an outcome or effect or where there is sufficient evidence of poor performance.
Uncertain -for example, this should be applied in circumstances in which the constraints of an evidence summary prevent a reliable answer to the question. An example of this may be when the need for a systematic review and meta-analysis is identified by the rapid review.

Review summary

Conclusions and implications for policy

In this report the reviewers examined 4 key questions relating to the effectiveness and appropriateness of newborn screening using TMS for LCHAD/MTP deficiency

What is the birth prevalence of LCHAD/MTP deficiency in the UK?

For question 1, no studies were found since the last review which provided birth prevalence for the UK specifically. However, a full systematic review was undertaken in 2013 which reported on prevalence rates for comparable Western European countries and made UK estimates based on evaluation evidence. The results of this review are in keeping with the evaluation estimates. With no screening in place, no further reviews are likely to provide this information. However, information may be sought from other UK sources.

What are the genotype-phenotype associations in LCHAD/MTP deficiency patients, including their clinical prognosis?
Sub-question: What is the incidence of asymptomatic and/or milder phenotype in the neonatal period?

For question 2, 27 studies were identified which reported on the genotype-phenotype association in individual patients. There were 95 different genetic mutations and 76different possible phenotypes. Grouping by homozygosity or subunit does not appear to map clearly to particular phenotypes. The specific presentation and pathway of the disease appears to vary greatly by individual and could be influenced by a number of other factors such as dietary compliance or the influence of other health problems. Neonatal severe cases may be more common in MTP deficiency and infant hepatic cases in LCHAD deficiency, but firm conclusions on this cannot be drawn based on the limitations of rapid review methodology. Further research to determine whether type rather than location of the defect is linked to phenotypic presentation is needed.

Due to a lack of evidence it is unclear if overdiagnosis is an issue. People who remain asymptomatic by the end of a study may have been overdiagnosed, or they may go on to develop symptoms in later years.

As the homozygous LCHAD deficiency group appears to be the largest (157 of a total of 301 people), this may have implications for the optimum point at which these individuals are treated.

What is the test accuracy (sensitivity, specificity, and predictive values applicable to UK prevalence) of acylcarnitine measurement in dried blood spots (DBS) using TMS for LCHAD/MTP deficiency screening?
Sub-question: Can the test distinguish between asymptomatic patients and those affected by milder forms of LCHAD and MTP deficiency?
Sub-question: Does the test detect other non-MTP conditions?

For question 3 the reviewers found 11 articles, giving details of 10 cohorts. There are some concerns regarding the applicability of the studies to the UK population; many of the studies included in the review screened on day 2 or 3 of life, as opposed to days 5–8 in the UK.

The only measure of test accuracy that was consistently reported (or where sufficient data were available to allow calculation) was PPV. PPV is related to the prevalence of a disease in the population and is not intrinsic to the test Sensitivity, specificity, and negative predictive values could not be established due to a lack of systematic follow up of newborns who screened negative. PPV ranged from 0–100% (23 true positives and 40 false positives from 3,951,358 newborns). Heterogeneity in markers used prevented analysis of whether this variation is due to thresholds used, and lack of reporting of accuracy by marker preventing establishment of the optimal threshold. .. All test accuracy data may have included those already symptomatic so may have overestimated the screening test’s ability to detect asymptomatic babies..

There is some evidence that the primary markers for LCHAD/MTP deficiency may appear raised when newborns have other fatty acid oxidation disorders [48]. However, this appears to be in conjunction with higher rates of the primary markers for those conditions.

There was no evidence to indicate whether the screening test can distinguish between milder and more severe types.

Limited data were available on asymptomatic/milder phenotype. In the one applicable study, only one out of 9 infants was still asymptomatic by age 3; all 9 individuals were receiving treatment which is likely to have prevented symptoms. Therefore, due to a lack of evidence it is unclear if overdiagnosis is an issue. People who remain asymptomatic by the end of a study may have been overdiagnosed, or they may go on to develop symptoms in later years."

Currently there is insufficient data regarding acylcarnitines measurement in dried blood spots using TMS for LCHAD/MTP deficiency screening to establish its accuracy, and there are significant concerns regarding risks of bias in the studies. Crucially, there is currently insufficient evidence to clearly judge test accuracy. There is a lack of long term follow up of screen negative cases. This is a particular issue when considering that the disease can present in young adults. On this basis, the introduction of a screening programme in the UK is not currently recommended. Further research should be undertaken to explore the issues highlighted. The reviewers suggest collaboration between researchers to report scores on a range of relevant markers for both cases of LCHAD, cases of MTP, and in the unaffected population using consistent units.

Does early treatment with dietary management following screening provide better long-term outcomes than later treatment after the presentation of symptoms?

For question 4 the reviewers found 11 studies across 13 papers [6, 23, 26, 28, 31, 33, 38, 41, 49-53]. There is some evidence to suggest that early dietary management while asymptomatic may be associated with a reduction in heart [5, 26, 28, 31, 38, 52], visual [23, 31, 49, 50, 52], neurological [26, 49], motor and muscular problems [23, 26, 38, 49, 52]. It may also be associated with a reduction in hypoglycaemia [38, 50], failure to thrive [26], brain damage [51] acute metabolic encephalopathy [5] and developmental delay [5]. However, the majority of studies are too small to show any significant effects, and evidence regarding these outcomes is subject to considerable bias. There is some evidence that treating people with LCHAD deficiency or MTP deficiency whilst asymptomatic is

associated with better long term outcomes. But the evidence for this is weak and subject to bias. There is a lack of UK research in this area.

Given the quality of the studies included within this review and the areas for further research which have been highlighted, at this point UK systematic population screening cannot be recommended. This review did not investigate the cost effectiveness of screening for LCHAD/MTP deficiency. Given that the treatment is dietary management which could be relatively cheap, this may be an important factor to consider.

Strengths and Limitations

The reviewers used a systematic approach to the design of our search strategies and to inclusion and exclusion and quality assessment. Also, this is the first review to explore the genotype-phenotype association across the studies. It has broadened our understanding of the plethora of mutations linked to these disorders and wide ranging observable characteristics people can present with.

Whilst undertaking the review, the reviewers noticed some key papers for the test accuracy question were not being picked up by the search strategy. Many of the studies were coded using the specific term “inborn errors of metabolism” with no reference to specific disorders. To ensure no studies were missed a new search was undertaken which included this key term and additional screening specific search terms. This means that the test accuracy search may include more recent papers than the search for the other 3 questions.

Questions 1 and 2 used a rapid evidence assessment approach (REA), meaning date limits were applied at sifting and only articles written in the English language were included; therefore it is possible that relevant articles may have been missed. The reviewers were aware of 3 articles which were missed for the incidence question that were picked up by the expanded test accuracy search. Sifting and data extraction for these questions were performed by one reviewer with a random 20% checked by a second reviewer. Therefore, there is a risk of error occurring in excluding studies and in extracting the data. However, the reviewers previously found this not to be the case in a similar review for rare diseases [70].

The large number of genotypes and phenotypes were unanticipated, in retrospect the reviewers should have been more specific with regards to the size of cohort studies included. By not defining cohort size, a large number of very small studies were included, which were of very low quality and did not provide any generalizable data.

It is unclear from the existing evidence whether asymptomatic cases may have been overdiagnosed and would never go on to develop symptoms. The cases mentioned may go on to develop symptoms in later years or they may be remaining asymptomatic because the treatment is slowing down the progression of the disease.

Appendix 1 - Search strategy

Electronic databases

Two separate searches were undertaken, one covered questions 1, 2 and 4 and the second search covered question 3. The search strategy included searches of the databases shown in Table 3 Summary of electronic database searches and dates for key questions 1, 2 and 4. MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print, Cochrane, Web of Science and Embase.

Table 3 Summary of electronic database searches and dates for key questions 1, 2 and 4

Database	Platform	Searched on date	Date range of search
MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print	Ovid SP	April 23rd 2018	1946 to April Week 2 2018
Embase	Ovid SP	April 23rd 2018	1974 to 2018 April 20
The Cochrane Library, including: - All Cochrane Reviews	Wiley Online	April 23rd 2018	1996 - HTA (Oct 2016), DARE (2015), NHS EED (Apr 2015), Methodology Register (July 2012), Database of systematic reviews April 2018
Web of Science	Clarivate	April 23rd 2018	1900 - 2018

Search Terms (Questions 1, 2 and 4)

Search terms included combinations of free text and subject headings (Medical Subject Headings [MeSH] for MEDLINE, and Emtree terms for Embase), grouped into the following categories:

- disease area: Mitochondrial trifunctional protein deficiency terms, LCHADD terms, HADH deficiency terms, Hydroxacyl and dehydrogenase combined with long chain, lipid metabolism inborn errors and fatty acid oxidation disorders.

Search terms for MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print and Embase are shown in Table 4 Search strategy for

MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print and Embase before deduplication

Search terms for the Cochrane Library databases are shown in Table 5 and for Web of Science in Table 6.

Table 4 Search strategy for MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print and Embase before deduplication

Term Group	#	Search terms	Results
Disease area	1	(mitochondrial trifunctional protein adj3 deficien*).ti,ab,kf	160
Disease area	2	mtp deficien*.ti,ab,kf.	135
Disease area	3	trifunctional protein deficien*.ti,ab,kf.	168
Disease area	4	(LCHAD or LCHADD).mp	518
Disease area	**5**	Long-Chain-3-Hydroxyacyl-CoA Dehydrogenase/	117
Disease area	6	3-Hydroxyacyl-CoA Dehydrogenase/df [Deficiency]	1534
Disease area	7	HADH Deficien*.mp.	7
Disease area	8	(Hydroxacyl and dehydrogenase).mp.	14
Disease area	9	long chain.mp.	61101
Disease area	10	8 and 9	3
Disease area	11	hydroxydicarboxylic acidur*.mp.	36
Disease area	12	Lipid Metabolism, Inborn Errors/	2755
Disease area	13	fatty acid oxidation disorder*.mp.	760
Disease area	14	Acyl-CoA Dehydrogenase/df [Deficiency]	2366
Disease area	15	1 or 2 or 3 or 4 or 5 or 6 or 7 or 10 or 11 or 12 or 13 or 14	7812

Table 5 Search strategy for the Cochrane Library Databases (Searched via the Wiley Online platform)

Term Group	#	Search terms	Results
Disease area	1	MTP deficien*	5
Disease area	2	Trifunctional protein near/3 deficien*	7
Disease area	3	Lchad or lchadd	9
Disease area	4	MeSH descriptor: [Long-Chain-3-Hydroxyacyl-CoA Dehydrogenase] explode all trees	1
Disease area	5	MeSH descriptor: [3-Hydroxyacyl CoA Dehydrogenases] explode all trees	86
Disease area	6	(Hydroxacyl and dehydrogenase) and long chain	0
Disease area	7	Hydroxydicarboxylic acidur*	1
Disease area	8	MeSH descriptor: [Acyl-CoA Dehydrogenase] explode all trees	13

Disease area	9	MeSH descriptor [Acyl-CoA Dehydrogenase, Long-Chain] explode all trees	2
Disease area	10	Mitochondrial trifunctional protein near/3 deficien*	0
Disease area	11	MeSH descriptor: [Lipid Metabolism, Inborn Errors] this term only	22
Disease area	12	Fatty acid oxidation disorder*	121
Disease area	13	#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or#11 or #12	240

Table 6 Search strategy for the Web of Science database

Term Group	#	Search terms	Results
Disease area	1	((("fatty acid oxidation disorder*" OR "LCHADD" OR "LCHAD" OR "Long-Chain-3-Hydroxyacyl-CoA Dehydrogenase" OR "HADH deficien*" OR ("hydroxyacyl" AND "dehydrogenase" AND "long chain")))) OR TS=(("hydroxydicarboxylic acidur*" or ("inborn errors" and "lipid metabolism"))) OR TS=("mtp deficien*" or ("mitochondrial protein" NEAR/3 deficien*) or ("trifunctional protein" near/3 deficien*))	911

Results were imported into EndNote and de-duplicated.

Table 7 Summary of electronic database searches and dates for key question 3

Database	Platform	Searched on date	Date range of search
MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print	Ovid SP	19th June 2018	1946 to June week 2
Embase	Ovid SP	19th June 2018	1974 to 2018 week 25
The Cochrane Library, including: - Cochrane Database of Systematic Reviews (CDSR) - Cochrane Central Register of Controlled Trials (CENTRAL) - Database of Abstracts of Reviews of Effects (DARE)	Wiley Online	19th June 2018	1996 - HTA (Oct 2016), DARE (2015), NHS EED (Apr 2015), Methodology Register (July 2012), Database of systematic reviews June 2018
Web of Science	Clarivate	19th June 2018	1900 - 2018

Search Terms (question 3)

Search terms included combinations of free text and subject headings (Medical Subject Headings [MeSH] for MEDLINE, and Emtree terms for Embase), grouped into the following categories:

- disease area: Mitochondrial trifunctional protein deficiency terms, LCHADD terms, HADH deficiency terms, Hydroxacyl and dehydrogenase combined with long chain, lipid metabolism inborn errors, inborn errors of metabolism and fatty acid oxidation disorder. Additional terms on inborn errors of metabolism, inborn metabolic disorder and inherited metabolic disease
- other term group: neonatal or newborn screening terms, mass spectrometry and tandem mass spectrometry terms, dried blood spot testing terms

Search terms for MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print and Embase are shown in Table 8. Search terms for the Cochrane Library databases are shown in Table 9 and Web of Science in Table 10

Table 8 Search strategy for MEDLINE, MEDLINE In-Process, MEDLINE Daily, Epub Ahead of Print and Embase before deduplication

Term Group	#	Search terms	Results
Disease area	1	(mitochondrial trifunctional protein adj3 deficien*).ti,ab,kf	163
Disease area	2	mtp deficien*.ti,ab,kf.	137
Disease area	3	trifunctional protein deficien*.ti,ab,kf.	169
Disease area	4	(LCHAD or LCHADD).mp	522
Disease area	**5**	long chain 3 hydroxyacyl coenzyme A dehydrogenase/	198
Disease area	6	3 hydroxyacyl coenzyme A dehydrogenase/	1538
Disease area	7	HADH Deficien*.mp.	7
Disease area	8	(Hydroxacyl and dehydrogenase).mp.	15
Disease area	9	long chain.mp.	61746
Disease area	10	8 and 9	3
Disease area	11	hydroxydicarboxylic acidur*.mp.	36
Disease area	12	Lipid Metabolism, Inborn Errors/	2776
Disease area	13	fatty acid oxidation disorder*.mp.	773
Disease area	14	Acyl-CoA Dehydrogenase/df [Deficiency]	2381
Disease area	15	Metabolism, Inborn Errors/	10175
Disease area	16	"inborn errors of metabolism".mp.	7278
Disease area	17	“inborn metabolic disorder*”.mp.	212

Disease area	18	Inherited metabolic disease*.mp.	2013
Disease area	19	1 or 2 or 3 or 4 or 5 or 8 or 9 or 10 or 11 or 12 or 13 or 16 or 17 or 18	35126
Other	20	neonatal screening.mp. or exp Neonatal Screening/	10526
Other	21	neonat* screening.mp.	10051
Other	22	newborn screening.mp	21485
Other	23	((neonat* or newborn*) adj5 screen*). mp.	39700
Other	24	20 or 21 or 22 or 23	39700
Other	25	exp Mass Spectrometry/ or exp Tandem Mass Spectrometry/ or tandem mass spectrometry.mp.	650977
Other	26	mass spectro*.mp.	715714
Other	27	tms.mp.	25891
Other	28	25 or 26 or 27	777839
Other	29	24 and 28	2915
Other	30	exp dried blood spot testing/	6823
Other	31	(blood spot* or dry blood spot*).mp.	17619
Other	32	30 or 31	30068
Other	33	29 or 32	31873
Other	34	19 and 33	1418

Table 9 Search strategy for the Cochrane Library Databases (Searched via the Wiley Online platform)

Term Group	#	Search terms	Results
Disease area	1	MTP deficien*	6
Disease area	2	Trifunctional protein near/3 deficien*	7
Disease area	3	Lchad or lchadd	9
Disease area	4	MeSH descriptor: [Long-Chain-3-Hydroxyacyl-CoA Dehydrogenase] explode all trees	1
Disease area	5	MeSH descriptor: [3-Hydroxyacyl CoA Dehydrogenases] explode all trees	86
Disease area	6	(Hydroxacyl and dehydrogenase) and long chain	0
Disease area	7	Hydroxydicarboxylic acidur*	1
Disease area	8	MeSH descriptor: [Acyl-CoA Dehydrogenase] explode all trees	13
Disease area	9	MeSH descriptor [Acyl-CoA Dehydrogenase, Long-Chain] explode all trees	2
Disease area	10	Mitochondrial trifunctional protein near/3 deficien*	0
Disease area	11	MeSH descriptor: [Lipid Metabolism, Inborn Errors] this term only	30
Disease area	12	Fatty acid oxidation disorder*	122
Disease area	13	MeSH descriptor: [metabolism, Inborn Errors] this term only	83

Disease area	14	“inborn errors of metabolism”	206
Disease area	15	“inborn metabolic disorder”	1
Disease area	16	“Inherited metabolic disease*”	317
Disease area	17	#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16	671
Other	18	MeSH descriptor: [Neonatal Screening] explode all trees	302
Other	19	Neonat* screening	1706
Other	20	Newborn screening	1606
Other	21	((newborn* or neonat*) near/5 screen*)	882
Other	22	#18 or #19 or #20 or #21	2238
Other	23	MeSH descriptor: [Mass Spectrometry] explode all trees	1493
Other	24	MeSH descriptor [Tandem Mass Spectrometry] explode all trees	619
Other	25	Tandem mass spectrometry	1982
Other	26	Mass spectro*	5420
Other	27	Tms	1468
Other	28	#23 or #24 or #25 or #26 or #27	4215
Other	29	#22 and #28	47
Other	30	MeSH descriptor: [Dried Blood Spot Testing] explode all trees	619
Other	31	Blood spot* or dreid blood spot* or dry blood spot*	1331
Other	32	#30 or #31	1331
Other	33	#29 or #32	1368
Other	34	#17 or #33	46

Table 10 Search strategy for Web of Science

Term Group	#	Search terms	Results
Disease area	1	((("fatty acid oxidation disorder*" OR "LCHADD" OR "LCHAD" OR "Long-Chain-3-Hydroxyacyl-CoA Dehydrogenase" OR "HADH deficien*" OR ("hydroxyacyl" AND "dehydrogenase" AND "long chain")))) OR TS=(("hydroxydicarboxylic acidur*" or ("inborn errors" and "lipid metabolism"))) OR TS=("mtp deficien*" or ("mitochondrial protein" NEAR/3 deficien*) or ("trifunctional protein" near/3 deficien*)) OR TS =(“inborn errors of metabolism” or “inborn metabolic disorder*” or “inherited metabolic disease*”) OR TS=(“inborn error*” NEAR/3metabolism)	5739
Other	2	TS=(“dry blood spot” or “dried blood spot*” or dbs)	15660

Other	3	TS=((“tandem mass spectro*” or tms or “mass spectro*”)) AND TS=(((newborn* or neonat*) near/5 screen*))	1280
Disease area	4	#3 OR #2	16324
Disease area	5	#4 AND #1	446

Results were imported into EndNote and de-duplicated.

Appendix 2 - Included and excluded studies

PRISMA flowchart

Figure 27 summarises the volume of publications included and excluded at each stage of the review for questions 1, 2 and 4. Fifty-one publications were ultimately judged to be relevant to one or more review questions and were considered for extraction. Publications that were included or excluded after the review of full-text articles are detailed below.

Figure 27 Summary of publications included and excluded at each stage of the review for questions 1, 2 and 4

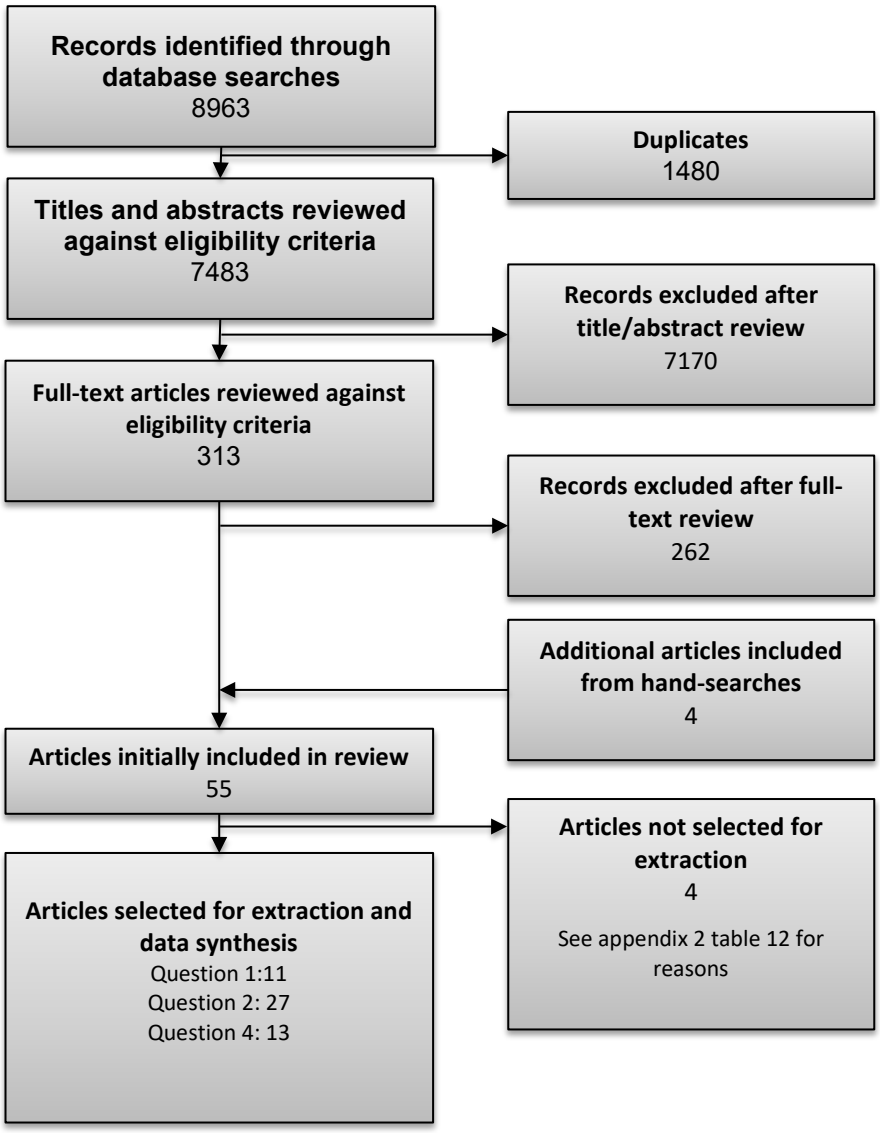
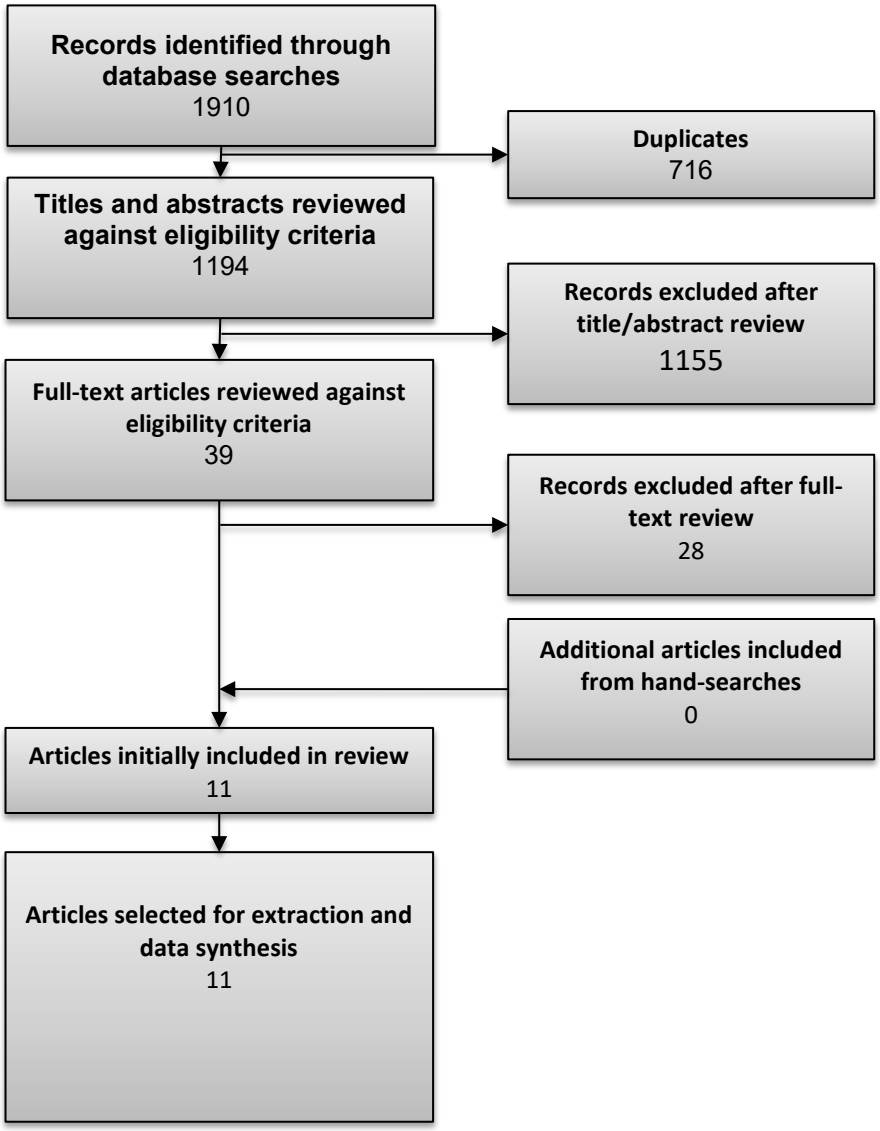


Figure 28 summarises the volume of publications included and excluded at each stage of the review for question 3. Thirty-nine publications were ultimately judged to be relevant to one or more review questions and were considered for extraction. Publications that were included or excluded after the review of full-text articles are detailed below in tables 11 and 12.

Figure 28 Summary of publications included and excluded at each stage of the review for question 3



The 11 publications included after review of full-texts for question 3 is summarised in Table 11 Summary of publications included after review of full-text articles, and the question(s) each publication was identified as being relevant to

Publications that were included or excluded after the review of full-text articles are detailed below in tables 11 and 13.

Table 11 Summary of publications included after review of full-text articles, and the question(s) each publication was identified as being relevant to

Study	Q1 Incidence	Q2 Genotype	Q3 Test Accuracy	Q4 Outcomes
Al-Jasmi 2016[13]	Y	-	-	-
Bo 2017 [22]	-	Y	-	-
Boese 2016 [23]	-	Y	-	Y
Bonham (ENBS trial) 2013 [9]	-	-	Y	-
Boutron 2011 [24]	-	Y	-	-
Chien 2013 [14]	Y	-	-	-
Choi 2007 [25]	-	Y	-	-
Couce 2011 [59]	-	-	Y	-
De Biase 2016 [26]	-	Y	-	Y
Diekman 2016 [27]	-	Y	-	-
Fahnehjelm 2008 [49]	-	-	-	Y
Fahnehjelm 2016 [50]	-	-	-	Y
Frazier 2006 [60]	-	-	Y	-
Gillingham 2017 [28]	-	Y	-	Y
Haglind 2013 [51]	-	-	-	Y
Hassan 2016 [20]	Y	-	-	-
Hayes 2007 [29]	-	Y	-	-
Hintz 2002 [30]	-	Y	-	-
Immonen 2016 [31]	-	Y	-	Y
Joost 2011 [32]	-	Y	-	-
Karall 2015 [52]	-	-	-	Y
Kang 2018 [33]	-	Y	-	Y
Lim 2014 [15]	Y	-	-	-
Lindner 2011 [67]	-	-	Y	-
Lund 2012 [5]	-	-	Y	Y
Lundy 2003 [34]	-	Y	-	-
Mak 2018 [21]	Y	-	Y	-

Purevsuren 2009 [35]	-	Y	-	-
Rocha 2014 [10]	Y	-	-	-
Sander 2005 [36]	-	Y	Y	-
Schulze 2003[61]	-	-	Y	-
Schwab 2003 [37]	-	Y	-	-
Shibata 2018 [11]	Y	-	-	-
Smon 2018 [16]	Y	-	Y	-
Sperk 2010 [38]	-	Y	-	Y
Spiekerkoetter 2002 [40]	-	Y	-	-
Spiekerkoetter 2003 [42]	-	Y	-	-
Spiekerkoetter 2004 [39]	-	Y	-	-
Spiekerkoetter 2009 [41]	-	Y	-	Y
Strandqvist 2015 [43]	-	Y	-	-
Sykut-Cegielska 2011 [6]	-	Y	-	Y
Therrell 2014 [17]	Y	-	-	-
Tuuli 2016 [44]	-	Y	-	-
Vockley 2016 [45]	-	Y	-	-
Waisbren 2013 [46]	-	Y		-
Yang 2002 [47]	-	Y	-	-
Yang 2018 [18]	Y	-	Y	-
Yunas 2016 [19]	Y	-	-	-
Zytkovicz 2001[48]	-	-	Y	-

Publications excluded after review of full-text articles

Of the 313 publications included after the review of titles and abstracts from the search for questions 1, 2 and 4, 263 were ultimately judged not to be relevant to this review. These publications, along with reasons for exclusion, are listed in Table 12 Publications excluded after review of full-text articles Q1, 2 and 4

Table 12 Publications excluded after review of full-text articles Q1, 2 and 4

Reference	Reason for exclusion
Adriaenssens, K. Van Sande, M. 1968, [Detection of metabolic diseases], Acta Neurologica et Psychiatrica Belgica	Review
Albasanz Gallan, J. L. [Genetics and metabolic disorders], Medicina Tropical	Review
Aleck, K. Partup, A. Shub, M. Harrison, H. Roe, C. 1997, Acute fatty liver of pregnancy (AFLP) is associated with fetal long chain 3-hydroxyacyl COA dehydrogenase (LCHAD) deficiency, American Journal of Human Genetics	Abstract only
Alonso, J. B. Gomez, R. G. Nieto, J. S. Martin, M. G. Lopez, V. M. N. Manso, G. M. Salinas, C. S. Cardona, A. L. U. 2015, Innate errors of metabolism in a pediatric hospital. Substantial differences between the extended neonatal pre- and post screening era. [Spanish], Revista Espanola de Pediatria	No LCHADD cases
Angdisen, J. Moore, V. D. Cline, J. M. Payne, R. M. Ibdah, J. A. 2005, Mitochondrial trifunctional protein defects: molecular basis and novel therapeutic approaches, Current Drug Targets - Immune Endocrine & Metabolic Disorders	Review
Arya, R. Candelier, C. K. 2001, Neonatal long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency (LCHAD) presenting as liver disease in the mother, Journal of Obstetrics and Gynaecology	Case study only 1 case
Autti-Ramo, I. Makela, M. Sintonen, H. Koskinen, H. Laajalahti, L. Halila, R. Kaariainen, H. Lapatto, R. Nanto-Salonen, K. Pulkki, K. Renlund, M. Salo, M. Tyni, T. 2005, Expanding screening for rare metabolic disease in the newborn: an analysis of costs, effect and ethical consequences for decision-making in Finland, Acta Paediatrica	Not relevant for questions 1,2 or 4
Babiker, O. Flanagan, S. E. Ellard, S. Girim, H. A. Hussain, K. Senniappan, S. 2015, Protein-induced hyperinsulinaemic hypoglycaemia due to a homozygous HADH mutation in three siblings of a Saudi family, Journal of Pediatric Endocrinology and Metabolism	Not LCHADD
Baily, M. A. Becker Jr, W. Hayes, M. Clayton, E. W. 2005, Exploring options for expanded newborn screening, Journal of Law, Medicine and Ethics	Review
Barnerias, C. Vianey-Saban, C. Brivet, M. Rabier, D. Touati, G. De Lonlay, P. Saudubray, J. M. 2005, TWO CASES OF PERIPHERAL NEUROPATHY REVEALING TRIFUNCTIONAL PROTEIN DEFICIENCY, Journal of Inherited Metabolic Disease	Abstract only
Bartlett, K. Eaton, S. J. Pourfarzam, M. 1997, New developments in neonatal screening, Archives of Disease in Childhood: Fetal and Neonatal Edition	Review
Bartoshesky, L. E. 2003, Newborn screening in Delaware, Delaware Medical Journal	Review
Baruteau, J. Sachs, P. Broue, P. Brivet, M. Abdoul, H. Vianey-Saban, C. Ogier de Baulny, H. 2013, Clinical and biological features at diagnosis in mitochondrial fatty acid beta-oxidation defects: a French pediatric study of 187 patients, Journal of Inherited Metabolic Disease	No information by patient

Reference	Reason for exclusion
Baumgartner, C. Baumgartner, D. 2006, Biomarker discovery, disease classification, and similarity query processing on high-throughput MS/MS data of inborn errors of metabolism, Journal of Biomolecular Screening	High risk population
Behrend, A. M. Harding, C. O. Shoemaker, J. D. Matern, D. Sahn, D. J. Elliot, D. L. Gillingham, M. B. 2012, Substrate oxidation and cardiac performance during exercise in disorders of long chain fatty acid oxidation, Molecular Genetics & Metabolism	Out of date limit for question 1, no relevant outcomes for question 2 and no early vs late treatment for question 4
Bergmann, S. R. Herrero, P. Sciacca, R. Hartman, J. J. Rubin, P. J. Hickey, K. T. Epstein, S. Kelly, D. P. 2001, Characterization of altered myocardial fatty acid metabolism in patients with inherited cardiomyopathy, Journal of Inherited Metabolic Disease	No age or method of diagnosis only current age and outcomes. No genotype data. Not relevant for incidence
Berry, S. A. Jurek, A. M. Anderson, C. Bentler, K. Region 4 Genetics Collaborative Priority, Workgroup, 2010, The inborn errors of metabolism information system: A project of the Region 4 Genetics Collaborative Priority 2 Workgroup, Genetics in Medicine	Not relevant
Bessey, A. Chilcott, J. Pandor, A. Paisley, S. 2014, The Cost-Effectiveness of Expanding the Nhs Newborn Bloodspot Screening Programme To Include Homocystinuria (Hcu), Maple Syrup Urine Disease (Msud), Glutaric Aciduria Type 1 (Ga1), Isovaleric Acidaemia (Iva), and Long-Chain Hydroxyacyl-Coa Dehydrogenase Deficiency (Lchadd), Value in Health	Abstract only
Bieneck, H. C. Ask, S. Halldin, M. Gardman, J. Nyberg, G. Alm, J. von Dobeln, U. Nordenstrom, A. 2008, Growth in 10 Swedish patients with long-chain 3OH-Acyl-CoA dehydrogenase (LCHAD) deficiency, Journal of Inherited Metabolic Disease	Abstract only
Bieneck, H. C. Nordenstrom, A. Halldin, M. Alm, J. Nemeth, A. Ask, S. Nyberg, G. Holmstrom, G. Tear, F. K. von Dobeln, U. 2007, Clinical follow-up of 10 children with long-chain 30H-acyl-CoA dehydrogenase (LCHAD) deficiency, Journal of Inherited Metabolic Disease	Abstract only
Boles, R. G. Buck, E. A. Blitzer, M. G. Platt, M. S. Gowan, T. M. Martin, S. K. Yoon, H. R. Madsen, J. A. Reyes-Mugica, M. Rinaldo, P. (1998). "Retrospective biochemical screening of fatty acid oxidation disorders in postmorterm livers of 418 cases of sudden death in the first year of life." Journal of Pediatrics 132(6): 924-933.	No genetic confirmatory testing which is only question it could be suitable for
Bonnet, D. Martin, D. Pascale De, Lonlay; Villain, E; Jouvet, P; Rabier, D; Brivet, M; Saudubray, J. M; 1999, "Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children." Circulation 100(22): 2248-2253.	No age or method of diagnosis or genotype data
Bonnet, D. Martin, D. PoggiTravert, F. Villain, E. Kachaner, J. Acar, P. Saudubray, J. M. 1997, "Arrhythmias and conduction defects as a presenting symptom of fatty-acid oxidation disorders in children." Circulation 96(8): 2437-2437.	Abstract only
Browning, M. F., Levy, H. L., Wilkins-Haug, L. E., Larson, C. and Shih, V. E. 2006, “Fetal fatty acid oxidation defects and maternal liver disease in pregnancy”. Obstetrics and Gynecology 107(1): 115-120	No relevant data on genotype-phenotype association. Maternal outcomes not foetal.

Reference	Reason for exclusion
Bursle, C., Weintraub, R., Ward, C., Justo, R., Cardinal, J. and Coman, D. (2017) Mitochondrial Trifunctional Protein Deficiency: Severe Cardiomyopathy and Cardiac Transplantation. Jimd Reports 10:10.	Only 2 cases and one case was never formally diagnosed so no comparator group
Chakrapani, A. Olpin, S. Cleary, M. Walter, J. H. Wraith, J. E. Besley, G. T. (2000). "Trifunctional protein deficiency: three families with significant maternal hepatic dysfunction in pregnancy not associated with E474Q mutation." Journal of Inherited Metabolic Disease 23(8): 826-834.	Only relevant for question 4 and no screened group
Chen, C. H. Chi, C. C. Shu, N. Y. 1991, Recurrent acute encephalopathy due to fatty acid metabolic defect--report of two cases, Zhonghua yi xue za zhi = Chinese medical journal; Free China ed	Both incidental cases and no genetics information
Chrastina, P. Kostalova, E. Paulova, M. Varholakova, L. Stastna, S. Elleder, M. Zeman, J. (2008). "LCHAD deficiency - The most frequent fatty acid oxidation disorder in newborn screening in the Czech Republic." Journal of Inherited Metabolic Disease 31: 29-29.	Abstract only
Cipriano, L. E. Rupar, C. A. Zaric, (2007) The cost-effectiveness of expanding newborn screening for up to 21 inherited metabolic disorders using tandem mass spectrometry: results from a decision-analytic model (Structured abstract). Value in Health 10, 83-97	Review
Ciske, J. B. Hoffman, G. Hanson, K. Annable, K. M. Wolff, J. Litsheim, T. Laessig, R. Aronson, R. (2000). "Newborn screening in Wisconsin: program overview and test addition." WMJ 99(2): 38-42.	No data. Mentions pilot test and 0 cases LCHADD but no methods and do not know total numbers
Coates, P. M. (1998). "Fatty acid metabolism in mitochondria: defects and genetics." Biofactors 7(3): 201-202.	Review
Crocker, A. C. (1976). "Inborn errors of lipid metabolism: early identification." Clinics in Perinatology 3(1): 99-113.	Review
Crouch, W. H., Jr. and C. M. Evanhoe (1967). "Inborn errors of metabolism." Pediatric Clinics of North America 14(1): 269-282.	Review
den Boer, M. E. Dionisi-Vici, C. Chakrapani, A. van Thuijl, A. O. Wanders, R. J. Wijburg, F. A. (2003). "Mitochondrial trifunctional protein deficiency: a severe fatty acid oxidation disorder with cardiac and neurologic involvement." Journal of Pediatrics 142(6): 684-689.	Do not have genotype and outcome or age at diagnosis and outcome, just severity
den Boer, M. E. Ijlst, L. Wijburg, F. A. Oostheim, W. van Werkhoven, M. A. van Pampus, M. G. Heymans, H. S. Wanders, R. J. (2000). "Heterozygosity for the common LCHAD mutation (1528g>C) is not a major cause of HELLP syndrome and the prevalence of the mutation in the Dutch population is low." Pediatric Research 48(2): 151-154.	Not direct genome and outcome data and published before 2013 so can't be in for incidence
den Boer, M. E. Wanders, R. J. Morris, A. A. I. Jlst L; Heymans, H. S. Wijburg, F. A. (2002). "Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: clinical presentation and follow-up of 50 patients." Pediatrics 109(1): 99-104.	Patients are not grouped by age of diagnosis or by genotype
Dereddy, N. R. Kronn, D. Krishnan, U. Dereddy, N. R., et al. (2009). "Defects in long chain fatty acid oxidation presenting as severe cardiomyopathy and cardiogenic shock in infancy." Cardiology in the Young 19(5): 540-542.	No screened vs unscreened group and only know genotype of one of the cases

Reference	Reason for exclusion
Ding, J. H. Yang, B. Z. Nada, M. A. Roe, C. R. (1996). "Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: The major disease-causing mutation and diagnosis." Pediatric Research 39(4): 851-851.	Genome question but before 2000
Dionisivici, C. Garavaglia, B. Burlina, A. Bertini, E. Wanders, R. J. A. Hashimoto, T. Sabetta, G. Invernizzi, F. Taroni, F. Didonato, S. 1995, "MITOCHONDRIAL TRIFUNCTIONAL PROTEIN-DEFICIENCY - CLINICAL AND BIOCHEMICAL FINDINGS IN POOR PATIENTS." Annals of Neurology 38(3): 544-544.	Withdrawn. 4 cases all late, no genotype info
Divry, P. Vianey-Saban, C. Mathieu, M. (1999). "Determination of total fatty acids in plasma: cis-5-tetradecenoic acid (C14:1 omega-9) in the diagnosis of long-chain fatty acid oxidation defects." Journal of Inherited Metabolic Disease 22(3): 286-288.	No age at diagnosis data or genotype
Djouadi, F. Habarou, F. Le Bachelier, C. Ferdinandusse, S. Schlemmer, D. Benoist, J. F. Boutron, A. Andresen, B. S. Visser, G. de Lonlay, P. Olpin, S. Fukao, T. Yamaguchi, S. Strauss, A. W. Wanders, R. J. Bastin, J. (2016). "Mitochondrial trifunctional protein deficiency in human cultured fibroblasts: effects of bezafibrate." Journal of Inherited Metabolic Disease 39(1): 47-58.	Have genotype but no phenotype info
Dogan, E. Uysal, S. Ozturk, Y. Arslan, N. (2017). "Selective screening for inborn errors of metabolism: A report of six years experience." Iranian Journal of Pediatrics 27 (5) (no pagination)(e11323).	High risk group, no LCHADD, no genotyping
Domingo, S. J. L. Koninckx, C. R. Serra, J. D. Calvete, J. F. Tomas, M. L. C. Gomez, A. G. Rubio, A. (1995). "Long-chain acyl coenzyme A dehydrogenase deficiency: A new case, DEFIENCIA DE ACIL COA DESHIDROGENASA DE CADENA LARGA. CASO CLINICO." Anales Espanoles de Pediatria 42(6): 456-458.	Single case
Duran, M. Deklerk, J. B. C. Pollthe, B. T. Wanders, R. J. A. Huymans, J. G. M. (1991). "LONG-CHAIN 3-HYDROXYACYL-COA DEHYDROGENASE-DEFICIENCY - PLASMA AND URINE ORGANIC-ACIDS." American Journal of Human Genetics 49(4): 53-53.	All diagnosed through cascade testing. No genotype info
Estrella, J. Wilcken, B. Carpenter, K. Bhattacharya, K. Tchan, M. Wiley, V. (2014). "Expanded newborn screening in New South Wales: missed cases." Journal of Inherited Metabolic Disease 37(6): 881-887.	False positive rates are a literature review. No data on LCHADD, no incidence data for LCHADD
Feuchtbaum, L., Lorey, F., Faulkner, L., Sherwin, J., Currier, R., Bhandal, A. and Cunningham, G. 2006. California's experience implementing a pilot newborn supplemental screening program using tandem mass spectrometry. Pediatrics 117(5): S261-S269	Only applicable for question 1 and outside date limit
Fincke, M. L. (1965). "Inborn Errors of Metabolism." Journal of the American Dietetic Association 46: 280-284.	Review
Finsterer, J. and S. Zarrouk-Mahjoub (2017). "Trifunctional Protein Deficiency Due to HADHB Mutations Is a Multisystem, beta-Oxidation Disorder." Archives of Iranian Medicine 20(12): 767-769.	Letter
Francis, D. E. (1979). "Inborn errors of metabolism: the need for sugar." Journal of Human Nutrition 33(2): 146-154.	Abstract only
Francois, J. (1975). "Ocular manifestations of inborn errors of carbohydrate and lipid metabolism." Bibliotheca Ophthalmologica: Supplementa ad Ophthalmologica(84): I-VII, 1-175.	Review
Frazier, D. M., Millington, D. S., McCandless, S. E., Koeberl, D. D., Weavil, S. D., Chaing, S. H. and Muenzer, J. (2006). The tandem mass spectrometry newborn screening experience in North Carolina: 1997-2005. Journal of Inherited Metabolic Disease 29(!):76-85.	Only applicable for question 1 and outside date limit

Reference	Reason for exclusion
Fukushima, K., et al. (2004). "Lack of common mutation in the alfa-subunit of the mitochondrial trifunctional protein and the polymorphism of CYP2E1 in three Japanese women with acute fatty liver of pregnancy/HELLP syndrome." Hepatology Research 30(4): 226-231.	Outcomes for mothers not newborns
Gillingham, M. (2006) Nutritional therapy and clinical outcomes in children with LCHAD deficiency. Inborn error review series (dietary management of inborn errors) 16, 6	No full text available
Gillingham, M. Van Calcar, S. Ney, D. Wolff, J. Harding, C. (1999). "Dietary management of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD). A case report and survey." Journal of Inherited Metabolic Disease 22(2): 123-131.	Single case. Compared briefly to previous case but no genotyping and no late group
Gillingham, M. B. Connor, W. E. Matern, D. Rinaldo, P. Burlingame, T. Meeuws, K. Harding, C. O.Gillingham, M. B., et al. (2003). "Optimal dietary therapy of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency." Molecular Genetics & Metabolism 79(2): 114-123.	Treatment only. No mapping of cases and outcomes just dosage
Gillingham, M. B., Weleber, R. G., Neuringer, M., Connor, W. E., Mills, M., van Calcar, S., Ver Hoeve, J., Wolff, J. and Harding, C. O. (2009) Effect of Feeding, Exercise and Genotype on Plasma 3-Hydroxyacylcarnitines in Children with Lchad Deficiency. Topics in Clinical Nutrition 24(4):359-365	Not relevant
Gillingham, M. B., et al. (2015). "Odd or even? Results from a randomized trial of triheptanoin compared to MCT in patients with long-chain fatty acid oxidation disorders." Molecular Genetics and Metabolism 114(3): 320-321.	Abstract
Gillingham, M. B., Weleber, R. G., Neuringer, M., Connor, W. E., Mills, M., van Calcar, S., Ver Hoeve, J., Wolff, J. and Harding, C. O. (2005) Effect of optimal dietary therapy upon visual function in children with long-chain 3-hydroxyacyl CoA dehydrogenase and trifunctional protein deficiency. Molecular Genetics & Metabolism 86 (1-2):124-33.	Effect of DHA supplement not genotype. No groups for question 4.
GILLINGHAM, M. B., JORDAN, J., STADLER, D. & HARDING, C. O. 2005. Effects of increased dietary protein on energy balance and metabolic control in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency. Molecular Genetics and Metabolism, 84, 220-220.	Abstract
GILLINGHAM, M. B., MATERN, D. & HARDING, C. O. 2009. Effect of Feeding, Exercise and Genotype on Plasma 3-Hydroxyacylcarnitines in Children with Lchad Deficiency. Topics in Clinical Nutrition, 24, 359-365.	No outcomes
GILLINGHAM, M. B., PURNELL, J. Q., JORDAN, J., STADLER, D., HAQQ, A. M. & HARDING, C. O. 2007. Effects of higher dietary protein intake on energy balance and metabolic control in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. Molecular Genetics & Metabolism, 90, 64-9.	No outcomes
GILLINGHAM, M. B., SCOTT, B., ELLIOTT, D. & HARDING, C. O. 2006. Metabolic control during exercise with and without medium-chain triglycerides (MCT) in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. Molecular Genetics & Metabolism, 89, 58-63.	No outcomes
GLASGOW, J. F. T., MOORE, R., ROBINSON, P. H. & MCKIERNAN, P. J. 1992. The phenylpropionic acid load test: Experience with 72 children at-risk for beta-oxidation disorders. Irish Journal of Medical Science, 161, 586-588.	Not relevant
GOKMEN-OZEL, H., DALY, A., DAVIES, P., CHAHAL, S. & MACDONALD, A. 2010. Errors in emergency feeds in inherited metabolic disorders: A randomised controlled trial of three preparation methods. Archives of Disease in Childhood, 95, 776-780.	Not relevant
GOLBAHAR, J., AL-JISHI, E. A., ALTAYAB, D. D., CARREON, E., BAKHIET, M. & ALKHAYYAT, H. 2013. Selective newborn screening of inborn errors of amino acids, organic acids and fatty acids metabolism in the Kingdom of Bahrain. Molecular Genetics & Metabolism, 110, 98-101.	Did not screen for LCHADD

Reference	Reason for exclusion
GREENBERG, C. R., MHANNI, A. A., CORKERY, T., SALTEL-OLSON, J., MALLORY, C. & SEARGEANT, L. 2007. Whole blood palmitate oxidation as a screening test for fatty acid oxidation disorders: A five-year experience. Journal of Inherited Metabolic Disease, 30, 47-47.	Abstract
GREGERSEN, N., ANDRESEN, B. S. & BROSS, P. 2000. Prevalent mutations in fatty acid oxidation disorders: diagnostic considerations. European Journal of Pediatrics, 159 Suppl 3, S213-8.	Review
GREGERSEN, N., ANDRESEN, B. S., CORYDON, M. J., CORYDON, T. J., OLSEN, R. K., BOLUND, L. & BROSS, P. 2001. Mutation analysis in mitochondrial fatty acid oxidation defects: Exemplified by acyl-CoA dehydrogenase deficiencies, with special focus on genotype-phenotype relationship. Human Mutation, 18, 169-89.	Review
GREGERSEN, N. & OLSEN, R. K. 2010. Disease mechanisms and protein structures in fatty acid oxidation defects. Journal of Inherited Metabolic Disease, 33, 547-53.	Review
Griffin, A. C., Strauss, A. W., Bennett, M. J. and Ernst, L. M. 2012. Mutations in long-chain 3-hydroxyacyl coenzyme a dehydrogenase are associated with placental maternal floor infarction/massive perivillous fibrin deposition. Pediatric & Developmental Pathology 15(5):368-74.	Wrong patient group – all cases of premature fetal demise
GU, X. F., HAN, L. S., GAO, X. L., YAN, Y. L., YE, J. & QIU, W. J. 2004. A pilot study of selective screening for high risk children with inborn error of metabolism using tandem mass spectrometry in China. [Chinese]. *Zhonghua er ke za zhi,* Chinese journal of pediatrics. 42, 401-404.	High risk group
HAGENBUCHNER, J., SCHOLL-BUERGI, S., KARALL, D. & AUSSERLECHNER, M. J. 2018. Very long-/ and long Chain-3-Hydroxy Acyl CoA Dehydrogenase Deficiency correlates with deregulation of the mitochondrial fusion/fission machinery. *Scientific Reports,* 8, 3254.	Cell study
HAGENFELDT, L., VENIZELOS, N. & VON DOBELN, U. 1995. Clinical and biochemical presentation of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Journal of Inherited Metabolic Disease,* 18, 245-8.	Review. Incidence data before 2013
HAGLIND, C. B., NORDENSTROM, A., ASK, S., VON DOBELN, U., GUSTAFSSON, J. & STENLID, M. H. 2015. Erratum to: increased and early lipolysis in children with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency during fast.[Erratum for J Inherit Metab Dis. 2015 Mar;38(2):315-22; PMID: 25141826]. *Journal of Inherited Metabolic Disease,* 38, 377.	Erratum
HAGLIND, C. B., NORDENSTROM, A., ASK, S., VON DOBELN, U., GUSTAFSSON, J. & STENLID, M. H. 2015. Increased and early lipolysis in children with long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency during fast. *Journal of Inherited Metabolic Disease,* 38, 315-322.	Not relevant
HAGLIND, C. B., STENLID, M. H., ASK, S., ALM, J., NEMETH, A., DOBELN, U. & NORDENSTROM, A. 2013. Growth in Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency. *Jimd Reports,* 8, 81-90.	All early. No genotype information
HALE, D. E., BATSHAW, M. L. & COATES, P. M. 1985. Long-chain acyl coenzyme A dehydrogenase deficiency: An inherited cause of nonketotic hypoglycemia. *Pediatric Research,* 19, 666-671.	Review
HALE, D. E., STANLEY, C. A. & COATES, P. M. 1990. Genetic defects of acyl-CoA dehydrogenases: studies using an electron transfer flavoprotein reduction assay. *Progress in clinical and biological research,* 321, 333-348.	Review
HALE, D. E., STANLEY, C. A. & COATES, P. M. 1990. The long-chain acyl-CoA dehydrogenase deficiency. *Progress in clinical and biological research,* 321, 303-311.	Review
HALE, D. E., THORPE, C., BRAAT, K., WRIGHT, J. H., ROE, C. R., COATES, P. M., HASHIMOTO, T. & GLASGOW, A. M. 1990. The L-3-hydroxyacyl-CoA dehydrogenase deficiency. *Progress in clinical and biological research,* 321, 503-510.	Single case
HALL, P. L., MARQUARDT, G., MCHUGH, D. M. S., CURRIER, R. J., TANG, H., STOWAY, S. D. & RINALDO, P. 2014. Postanalytical tools improve performance of newborn screening by tandem mass spectrometry. *Genetics in Medicine,* 16, 889-895.	No LCHADD

Reference	Reason for exclusion
HAN, L., HAN, F., YE, J., QIU, W., ZHANG, H., GAO, X., WANG, Y., JI, W. & GU, X. 2015. Spectrum analysis of common inherited metabolic diseases in Chinese patients screened and diagnosed by tandem mass spectrometry. *Journal of Clinical Laboratory Analysis,* 29, 162-8.	High risk group and no LCHADD
HAN, L. S., YE, J., QIU, W. J., GAO, X. L., WANG, Y. & GU, X. F. 2007. Selective screening for inborn errors of metabolism on clinical patients using tandem mass spectrometry in China: a four-year report. *Journal of Inherited Metabolic Disease,* 30, 507-14.	High risk group and no LCHADD
HAN, L. S., YE, J., QIU, W. J., GAO, X. L., WANG, Y., ZHANG, Y. J. & GU, X. F. 2007. Application of tandem mass spectrometry on the diagnosis of fatty acid oxidation disorders. [Chinese]. *Chinese Journal of Medical Genetics,* 24, 692-695.	High risk
HARDING, C. O., GILLINGHAM, M. B., VAN CALCAR, S. C., WOLFF, J. A., VERHOEVE, J. N. & MILLS, M. D. 1999. Docosahexaenoic acid and retinal function in children with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Journal of Inherited Metabolic Disease,* 22, 276-80.	No outcome data
HARDING, C. O., SCOTT, B. & GILLINGHAM, M. B. 2005. MCT supplementation immediately prior to exercise improves exercise tolerance in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency. *Molecular Genetics and Metabolism,* 84, 222-222.	Abstract only
HARZER, K. 1979. [Prenatal diagnosis of incurable familial metabolic diseases. Prenatal diagnosis of disorders of lipid metabolism]. *Medizinische Welt,* 30, 1810-6.	Review. Too early for genotype
HE, M., PEI, Z., MOHSEN, A. W., WATKINS, P., MURDOCH, G., VAN VELDHOVEN, P. P., ENSENAUER, R. & VOCKLEY, J. 2011. Identification and characterization of new long chain Acyl-CoA dehydrogenases. *Molecular Genetics and Metabolism,* 102, 418-429.	Not relevant
HEALTH QUALITY, O. 2003. Neonatal screening of inborn errors of metabolism using tandem mass spectrometry: an evidence-based analysis. *Ontario Health Technology Assessment Series,* 3, 1-36.	Review and not LCHADD
HINTON, C. F., MAI, C. T., NABUKERA, S. K., BOTTO, L. D., FEUCHTBAUM, L., ROMITTI, P. A., WANG, Y., PIPER, K. N. & OLNEY, R. S. 2014. Developing a public health-tracking system for follow-up of newborn screening metabolic conditions: a four-state pilot project structure and initial findings. *Genetics in Medicine,* 16, 484-90.	Before 2013
HOFFMANN, G. F., VON KRIES, R., KLOSE, D., LINDNER, M., SCHULZE, A., MUNTAU, A. C., ROSCHINGER, W., LIEBL, B., MAYATEPEK, E. & ROSCHER, A. A. 2004. Frequencies of inherited organic acidurias and disorders of mitochondrial fatty acid transport and oxidation in Germany. *European Journal of Pediatrics,* 163, 76-80.	No LCHADD. Incidence is before 2013. No genotype. No TA data
Huang, X. W., Yang, J. B., Tong, F., Yang, R. L., Mao, H. Q., Zhou, X. L., Huang, X. L., Yang, L. L., Huang, C. G. and Zhao, Z. Y. (2011). Screening for neonatal inborn errors of metabolism by electrospray ionization-tandem mass spectrometry and follow-up. Zhonghua Erke Zazhi 49(10):765-70	Only applicable for question 1 and outside date limit
HUSSA, C., FICICIOGLU, C., VERONA, M., GANESH, J., PAYAN, I., PATANO, J., LIEBHART, R. & YUDKOFF, M. 2006. Is breastfeeding an option in the dietary management of long chain fatty acid oxidation disorders? Our experience with seven patients. *Journal of Inherited Metabolic Disease,* 29, 113-113.	Abstract
IBARRA-GONZALEZ, I., FERNANDEZ-LAINEZ, C., BELMONT-MARTINEZ, L., GUILLEN-LOPEZ, S., MONROY-SANTOYO, S. & VELA-AMIEVA, M. 2014. Characterization of inborn errors of intermediary metabolism in mexican patients. [Spanish]. *Anales de Pediatria,* 80, 310-316.	High risk, no genotype information
IBDAH, I., SIMS, H., GIBSON, B., PIZZURRO, M., TREEM, W., BENNETT, M. & STRAUSS, A. 1996. The molecular basis of long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. *Faseb Journal,* 10, 2195-2195.	Abstract
IBDAH, J. A., ISAACS, J., TREEM, W., BENNETT, M. & STRAUSS, A. W. 1996. The molecular basis of acute fatty liver of pregnancy associated with pediatric long chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Hepatology,* 24, 300-300.	Abstract

Reference	Reason for exclusion
IBDAH, J. A., ISAACS, J., TREEM, W. & STRAUSS, A. W. 1996. Acute fatty liver of pregnancy and maternal long chain 3-hydroxyacyl-CoA dehydrogenase. *Gastroenterology,* 110, A1215-A1215.	Abstract
IBDAH, J. A., TEIN, I., DIONISI-VICI, C., BENNETT, M. J., L, I. J., GIBSON, B., WANDERS, R. J. & STRAUSS, A. W. 1998. Mild trifunctional protein deficiency is associated with progressive neuropathy and myopathy and suggests a novel genotype-phenotype correlation. *Journal of Clinical Investigation,* 102, 1193-9.	Before 2000 for genotype
IBDAH, J. A., YANG, Z. & BENNETT, M. J. 2000. Liver disease in pregnancy and fetal fatty acid oxidation defects. *Molecular Genetics & Metabolism,* 71, 182-9.	Review
Ibdah, J. A., Zhao, Y., Viola, J., Gibson, B., Bennett, M. J. and Strauss, A. W. 2001. Molecular prenatal diagnosis in families with fetal mitochondrial trifunctional protein mutations. Journal of Pediatrics 138(3):396-9	No outcomes in current patient group
IJLST, L., OOSTHEIM, W., RUITER, J. P. N. & WANDERS, R. J. A. 1997. Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: Identification of two new mutations. *Journal of Inherited Metabolic Disease,* 20, 420-422.	No phenotypes and before 2000
IJLST, L., RUITER, J. P. N., VREIJLING, J. & WANDERS, R. J. A. 1996. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: A new method to identify the G1528C mutation in genomic DNA showing its high frequency (90%) and identification of a new mutation (T2198C). *Journal of Inherited Metabolic Disease,* 19, 165-168.	Single case
IJLST, L., USKIKUBO, S., KAMIJO, T., HASHIMOTO, T., RUITER, J. P., DE KLERK, J. B. & WANDERS, R. J. 1995. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: high frequency of the G1528C mutation with no apparent correlation with the clinical phenotype. *Journal of Inherited Metabolic Disease,* 18, 241-4.	Genotype but before 2000
INSINGA, R. P., LAESSIG, R. H. & HOFFMAN, G. L. 2002. Newborn screening with tandem mass spectrometry: examining its cost-effectiveness in the Wisconsin newborn screening panel (Structured abstract). *Journal of Pediatrics* [Online], 141. Available: http://cochranelibrary-wiley.com/o/cochrane/cleed/articles/NHSEED-22002001875/frame.html	Not LCHADD
ITO, T. 2015. Mass Screening for Inborn Errors of Metabolism. [Japanese]. *Rinsho byori,* The Japanese journal of clinical pathology. 63, 441-449.	Review
IWANCZAK, F. & SMIGIEL, R. 2004. The most common genetic inherited defects of the protein and fat metabolism in children. [Polish]. *Gastroenterologia Polska,* 11, 375-383.	Review
JACKSON, S., BARTLETT, K., LAND, J., MOXON, E. R., POLLITT, R. J., LEONARD, J. V. & TURNBULL, D. M. 1991. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatric Research,* 29, 406-411.	Before 2000 for genotype, no screened cases
JIANG, M., LIU, L., MEI, H., LI, X., CHENG, J. & CAI, Y. 2015. Detection of inborn errors of metabolism using GC-MS: Over 3 years of experience in southern China. *Journal of Pediatric Endocrinology and Metabolism,* 28, 375-380.	High risk
JOHNSON, D. W. & TRINH, M. U. 2003. Analysis of isomeric long-chain hydroxy fatty acids by tandem mass spectrometry: application to the diagnosis of long-chain 3-hydroxyacyl CoA dehydrogenase deficiency. *Rapid Communications in Mass Spectrometry,* 17, 171-5.	Not relevant
KAMIJO, T., WANDERS, R. J., SAUDUBRAY, J. M., AOYAMA, T., KOMIYAMA, A. & HASHIMOTO, T. 1994. Mitochondrial trifunctional protein deficiency. Catalytic heterogeneity of the mutant enzyme in two patients. *Journal of Clinical Investigation,* 93, 1740-7.	Animal study
KAUR, M. & BIRLA, S. 2008. Threshold challenge in India-newborn screening for aminoacids, organic acids and fatty acid oxidation disorders. *Journal of Inherited Metabolic Disease,* 31, 150-150.	Abstract
KELLY, D. P., MENDELSOHN, N. J., SOBEL, B. E. & BERGMANN, S. R. 1993. Detection and assessment by positron emission tomography of a genetically determined defect in myocardial fatty acid utilization (long-chain acyl-CoA dehydrogenase deficiency). *American Journal of Cardiology,* 71, 738-744.	All late and no genotype info
KEPPEN, L. D. & RANDALL, B. 1999. Inborn defects of fatty acid oxidation: a preventable cause of SIDS. *South Dakota journal of medicine,* 52, 187-188; discussion 188-189.	Single case and not LCHADD

Reference	Reason for exclusion
KIMURA, M. & YAMAGUCHI, S. 2001. Trifunctional protein deficiency and long-chain-3-hydroxy-acyl CoA dehydrogenase deficiency. [Japanese]. *Ryoikibetsu shokogun shirizu*, 77-79.	No LCHADD
KLOSE, D. A., KOLKER, S., HEINRICH, B., PRIETSCH, V., MAYATEPEK, E., VON KRIES, R. & HOFFMANN, G. F. 2002. Incidence and short-term outcome of children with symptomatic presentation of organic acid and fatty acid oxidation disorders in Germany. *Pediatrics,* 110, 1204-11.	Do not know age or method of diagnosis for LCHADD cases or genotype
KOBAYASHI, H., HASEGAWA, Y., ENDO, M., PUREVSUREN, J. & YAMAGUCHI, S. 2007. A retrospective ESI-MS/MS analysis of newborn blood spots from 18 symptomatic patients with organic acid and fatty acid oxidation disorders diagnosed either in infancy or in childhood. *Journal of Inherited Metabolic Disease,* 30, 606.	Abstract
KONG, X. F., ZHANG, X. X., YU, Y. Y., SHI, Q., LA, D. D., ZHU-GE, C. D., DENG, L., GONG, Q. M., SHEN, B. Y., PENG, C. H. & LI, H. W. 2007. No mutation was found in the alpha-subunit of the mitochondrial tri-functional protein in one patient with severe acute fatty liver of pregnancy and her relatives. *Journal of Gastroenterology & Hepatology,* 22, 2107-11.	One case
KORENKE, G. C., MARQUARDT, I., MOTZ, R., VOGES, A., WANDERS, R. J. A., STEUERWALD, U. & SANDER, J. 2005. Long-chain hydroxyacyl-CoA dehydrogenase deficiency-LCHAD defect. Two-year follow-up of two patients. [German]. *Monatsschrift fur Kinderheilkunde,* 153, 657-663.	Both screened and no genotype information
L, I. J., RUITER, J. P., HOOVERS, J. M., JAKOBS, M. E. & WANDERS, R. J. 1996. Common missense mutation G1528C in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. Characterization and expression of the mutant protein, mutation analysis on genomic DNA and chromosomal localization of the mitochondrial trifunctional protein alpha subunit gene. *Journal of Clinical Investigation,* 98, 1028-33.	Yeast cell study
L, I. J., WANDERS, R. J., USHIKUBO, S., KAMIJO, T. & HASHIMOTO, T. 1994. Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of the major disease-causing mutation in the alpha-subunit of the mitochondrial trifunctional protein. *Biochimica et Biophysica Acta,* 1215, 347-50.	Genotype but before 2000
LABARTHE, F. 2008. New therapeutic approaches in mitochondrial fatty acid oxidation disorders. [French]. *Archives de Pediatrie,* 15, 608-610.	Review
LABARTHE, F., BENOIST, J. F., BRIVET, M., VIANEY-SABAN, C., DESPERT, F. & DE BAULNY, H. O. 2005. PARTIAL HYPOPARATHYROIDISM ASSOCIATED WITH A MITOCHONDRIAL TRIFUNCTIONAL PROTEIN DEFICIENCY. *Journal of Inherited Metabolic Disease,* 28, 105-105.	Abstract
LANDAU, Y. E., LICHTER-KONECKI, U. & LEVY, H. L. 2014. Genomics in newborn screening. *Journal of Pediatrics,* 164, 14-19.	Review
LANDAU, Y. E., WAISBREN, S. E., CHAN, L. M. & LEVY, H. L. 2017. Long-term outcome of expanded newborn screening at Boston children's hospital: benefits and challenges in defining true disease. *Journal of Inherited Metabolic Disease,* 40, 209-218.	No outcomes
LANTHALER, B., WIESER, S., DEUTSCHMANN, A., SCHOSSIG, A., FAUTH, C., ZSCHOCKE, J. & WITSCH-BAUMGARTNER, M. 2014. Genotype-based databases for variants causing rare diseases. *Gene,* 550, 136-40.	Not LCHADD
LAW, E. L. K. 2010. Diagnosis of fatty acid oxidation disorders by mass spectrometry. *Clinica Chimica Acta,* 411, 906-906.	Abstract
LI, F., YANG, Z., ZHANG, A., SUN, X., WANG, J. & MENG, R. 2015. [The changes of LCHAD in preeclampsia with different clinical features and the correlation with NADPH P47-phox, p38MAPK-alpha, COX-2 and serum FFA and TG]. *Chung-Hua Fu Chan Ko Tsa Chih [Chinese Journal of Obstetrics & Gynecology],* 50, 92-100.	No outcomes
Lim, J. S., Tan, E. S., John, C. M., Poh, S., Yeo, S. J., Ang, J. S., Adakalaisamy, P., Rozalli, R. A., Hart, C., Tan, E. T., Ranieri, E., Rajadurai, V. S., Cleary, M. A. and Goh, D. L. (2014). Inborn Error of Metabolism (IEM) screening in Singapore by electrospray ionization-tandem mass spectrometry (ESI/MS/MS): An 8 year journey from pilot to current program. Molecular Genetics & Metabolism 113(1-2):53-61	Only applicable for question 1 and out of date range

Reference	Reason for exclusion
LINDNER, M., HOFFMANN, G. F. & MATERN, D. 2010. Newborn screening for disorders of fatty-acid oxidation: experience and recommendations from an expert meeting. *Journal of Inherited Metabolic Disease,* 33, 521-6.	No results, too early for incidence
LOEBER, J. G. 2007. Neonatal screening in Europe; the situation in 2004.[Erratum appears in J Inherit Metab Dis. 2008 Jun;31(3):469]. *Journal of Inherited Metabolic Disease,* 30, 430-8.	Review and no LCHADD
LUCAS, T. G., HENRIQUES, B. J., RODRIGUES, J. V., BROSS, P., GREGERSEN, N. & GOMES, C. M. 2011. Cofactors and metabolites as potential stabilizers of mitochondrial acyl-CoA dehydrogenases. *Biochimica et Biophysica Acta - Molecular Basis of Disease,* 1812, 1658-1663.	Not relevant
LUKACS, Z. 2009. Newborn screening in Germany, Austria and Switzerland : CCCurrent status. [German]. *Monatsschrift fur Kinderheilkunde,* 157, 1209-1214.	Review
LUND, A. M., DIXON, M. A., VREKEN, P., LEONARD, J. V. & MORRIS, A. A. M. 2003. What is the role of medium-chain triglycerides in the management of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency? *Journal of Inherited Metabolic Disease,* 26, 353-360.	Not relevant
LUND, A. M. & LEONARD, J. V. 2001. Feeding difficulties in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Archives of Disease in Childhood,* 85, 487-488.	Not relevant
LUND, A. M., SKOVBY, F., VESTERGAARD, H., CHRISTENSEN, M. & CHRISTENSEN, E. 2010. Clinical and biochemical monitoring of patients with fatty acid oxidation disorders. *Journal of Inherited Metabolic Disease,* 33, 495-500.	Review
LUNDEMOSE, J. B., KOLVRAA, S., GREGERSEN, N., CHRISTENSEN, E. & GREGERSEN, M. 1997. Fatty acid oxidation disorders as primary cause of sudden and unexpected death in infants and young children: an investigation performed on cultured fibroblasts from 79 children who died aged between 0-4 years. *Molecular Pathology,* 50, 212-7.	Not LCHADD
MACDONALD, A., WEBSTER, R., WHITLOCK, M., GERRARD, A., DALY, A., PREECE, M. A., EVANS, S., ASHMORE, C., CHAKRAPANI, A., VIJAY, S. & SANTRA, S. 2018. The safety of Lipistart, a medium-chain triglyceride based formula, in the dietary treatment of long-chain fatty acid disorders: a phase I study. *Journal of Pediatric Endocrinology & Metabolism,* 31, 297-304.	One case
MALVAGIA, S., HAYNES, C. A., GRISOTTO, L., OMBRONE, D., FUNGHINI, S., MORETTI, E., MCGREEVY, K. S., BIGGERI, A., GUERRINI, R., YAHYAOUI, R., GARG, U., SEETERLIN, M., CHACE, D., DE JESUS, V. R. & LA MARCA, G. 2015. Heptadecanoylcarnitine (C17) a novel candidate biomarker for newborn screening of propionic and methylmalonic acidemias. *Clinica Chimica Acta,* 450, 342-8.	Not standard screening population
MARIN-GARCIA, J. & GOLDENTHAL, M. J. 2002. Fatty acid metabolism in cardiac failure: Biochemical, genetic and cellular analysis. *Cardiovascular Research,* 54, 516-527.	Review
MARQUARDT, G., CURRIER, R., MCHUGH, D. M., GAVRILOV, D., MAGERA, M. J., MATERN, D., OGLESBEE, D., RAYMOND, K., RINALDO, P., SMITH, E. H., TORTORELLI, S., TURGEON, C. T., LOREY, F., WILCKEN, B., WILEY, V., GREED, L. C., LEWIS, B., BOEMER, F., SCHOOS, R., MARIE, S., VINCENT, M. F., SICA, Y. C., DOMINGOS, M. T., AL-THIHLI, K., SINCLAIR, G., AL-DIRBASHI, O. Y., CHAKRABORTY, P., DYMERSKI, M., PORTER, C., MANNING, A., SEASHORE, M. R., QUESADA, J., REUBEN, A., CHRASTINA, P., HORNIK, P., ATEF MANDOUR, I., ATTY SHARAF, S. A., BODAMER, O., DY, B., TORRES, J., ZORI, R., CHEILLAN, D., VIANEY-SABAN, C., LUDVIGSON, D., STEMBRIDGE, A., BONHAM, J., DOWNING, M., DOTSIKAS, Y., LOUKAS, Y. L., PAPAKONSTANTINOU, V., ZACHARIOUDAKIS, G. S., BARATH, A., KARG, E., FRANZSON, L., JONSSON, J. J., BREEN, N. N., LESKO, B. G., BERBERICH, S. L., TURNER, K., RUOPPOLO, M., SCOLAMIERO, E., ANTONOZZI, I., CARDUCCI, C., CARUSO, U., CASSANELLO, M., LA MARCA, G., PASQUINI, E., DI GANGI, I. M., GIORDANO, G., CAMILOT, M., TEOFOLI, F., MANOS, S. M., PETERSON, C. K., MAYFIELD GIBSON, S. K., SEVIER, D. W., LEE, S. Y., PARK, H. D., KHNEISSER, I., BROWNING, P., GULAMALI-MAJID, F., WATSON, M. S., EATON, R. B., SAHAI, I., RUIZ, C., TORRES, R., SEETERLIN, M. A., STANLEY, E. L., HIETALA, A., MCCANN, M., CAMPBELL, C., HOPKINS, P. V., DE SAIN-VAN	Not relevant

Reference	Reason for exclusion
DER VELDEN, M. G., ELVERS, B., MORRISSEY, M. A., SUNNY, S., KNOLL, D., WEBSTER, D., FRAZIER, D. M., MCCLURE, J. D., SESSER, D. E., et al. 2012. Enhanced interpretation of newborn screening results without analyte cutoff values. *Genetics in Medicine,* 14, 648-55.	
MARSDEN, D. 2003. Expanded newborn screening by tandem mass spectrometry: the Massachusetts and New England experience. *Southeast Asian Journal of Tropical Medicine & Public Health,* 34 Suppl 3, 111-4.	One case
MARTINEZ-QUINTANA, E., PENA-QUINTANA, L., ARTILES-VIZCAINO, J. A. & RODRIGUEZ-GONZALEZ, F. 2009. Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency and cardiogenic shock. *International Journal of Cardiology,* 136, e1-2.	Letter
MARTINS, E., COSTA, A., SILVA, E., MEDINA, M., CARDOSO, M. L., VIANEY-SABAN, C., DIVRY, P. & VILARINHO, L. 1996. Lethal dilated cardiomyopathy due to long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Journal of Inherited Metabolic Disease,* 19, 373-374.	One case
MATERN, D., CUTHBERT, C. D., TORTORELLI, S., CHACE, D. H., HAHN, S. & RINALDO, P. 2004. The diagnosis of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency by newborn screening using tandem mass spectrometry. *Pediatric Research,* 55, 272A-272A.	Abstract
MATERN, D., STRAUSS, A. W., HILLMAN, S. L., MAYATEPEK, E., MILLINGTON, D. S. & TREFZ, F. K. 1999. Diagnosis of mitochondrial trifunctional protein deficiency in a blood spot from the newborn screening card by tandem mass spectrometry and DNA analysis. *Pediatric Research,* 46, 45-9.	Genotype but before 2000
MATSUBARA, Y. 1995. Hepatic mitochondrial fatty acid oxidation disorders. [Japanese]. *Ryoikibetsu shokogun shirizu*, 312-316.	Review
MATTHEWS, R. P., RUSSO, P., BERRY, G. T., PICCOLI, D. A. & RAND, E. B. 2002. Biliary atresia associated with a fatty acid oxidation defect. *Journal of Pediatric Gastroenterology and Nutrition,* 35, 624-628.	One case
MCCOIN, C. S., PICCOLO, B. D., KNOTTS, T. A., MATERN, D., VOCKLEY, J., GILLINGHAM, M. B. & ADAMS, S. H. 2016. Unique plasma metabolomic signatures of individuals with inherited disorders of long-chain fatty acid oxidation. *Journal of Inherited Metabolic Disease,* 39, 399-408.	Do not know age or method of diagnosis and no genetics information
MCHUGH, D. M. S., CAMERON, C. A., ABDENUR, J. E., ABDULRAHMAN, M., ADAIR, O., AL NUAIMI, S. A., AHLMAN, H., ALLEN, J. J., ANTONOZZI, I., ARCHER, S., AU, S., AURAY-BLAIS, C., BAKER, M., BAMFORTH, F., BECKMANN, K., PINO, G. B., BERBERICH, S. L., BINARD, R., BOEMER, F., BONHAM, J., BREEN, N. N., BRYANT, S. C., CAGGANA, M., CALDWELL, S. G., CAMILOT, M., CAMPBELL, C., CARDUCCI, C., CARIAPPA, R., CARLISLE, C., CARUSO, U., CASSANELLO, M., CASTILLA, A. M., RAMOS, D. E. C., CHAKRABORTY, P., CHANDRASEKAR, R., RAMOS, A. C., CHEILLAN, D., CHIEN, Y. H., CHILDS, T. A., CHRASTINA, P., SICA, Y. C., COCHO DE JUAN, J. A., COLANDRE, M. E., ESPINOZA, V. C., CORSO, G., CURRIER, R., CYR, D., CZUCZY, N., D'APOLITO, O., DAVIS, T., DE SAIN-VAN DER VELDEN, M. G., PECELLIN, C. D., DI GANGI, I. M., DI STEFANO, C. M., DOTSIKAS, Y., DOWNING, M., DOWNS, S. M., DY, B., DYMERSKI, M., RUEDA, I., ELVERS, B., EATON, R., ECKERD, B. M., EL MOUGY, F., EROH, S., ESPADA, M., EVANS, C., FAWBUSH, S., FIJOLEK, K. F., FISHER, L., FRANZSON, L., FRAZIER, D. M., GARCIA, L. R. C., BERMEJO, M. S. G. V., GAVRILOV, D., GERACE, R., GIORDANO, G., IRAZABAL, Y. G., GREED, L. C., GRIER, R., GRYCKI, E., GU, X., GULAMALI-MAJID, F., HAGAR, A. F., HAN, L., HANNON, W. H., HASLIP, C., HASSAN, F. A., HE, M., HIETALA, A., HIMSTEDT, L., HOFFMAN, G. L., HOFFMAN, W., HOGGATT, P., HOPKINS, P. V., HOUGAARD, D. M., HUGHES, K., HUNT, P. R., HWU, W. L., HYNES, J., et al. 2011. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: A worldwide collaborative project. *Genetics in Medicine,* 13, 230-254.	Not relevant
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MILLINGTON, D. S., TERADA, N., CHACE, D. H., CHEN, Y. T., DING, J. H., KODO, N. & ROE, C. R. 1992. The role of tandem mass spectrometry in the diagnosis of fatty acid oxidation disorders. *Progress in Clinical & Biological Research,* 375, 339-54.	Tested on people disease already known for
MOORE, S. J., HAITES, N. E., BROOM, I., WHITE, I., COLEMAN, R. J., POURFARZAM, M. & MORRIS, A. A. M. 1998. Acylcarnitine analysis in the investigation of myopathy. *Journal of Inherited Metabolic Disease,* 21, 427-428.	Single case
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NADLER, H. L. 1972. Allotransplantation for the treatment of inborn errors of metabolism. *Annals of Internal Medicine,* 77, 314-6.	Review
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NAIKI, M., OCHI, N., KATO, Y. S., PUREVSUREN, J., YAMADA, K., KIMURA, R., FUKUSHI, D., HARA, S., YAMADA, Y., KUMAGAI, T., YAMAGUCHI, S. & WAKAMATSU, N. 2014. Mutations in HADHB, which encodes the beta-subunit of mitochondrial trifunctional protein, cause infantile onset hypoparathyroidism and peripheral polyneuropathy. *American Journal of Medical Genetics. Part A,* 164A, 1180-7.	Not LCHADD
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Olpin, S. E., Clark, S., Andresen, B. S., Bischoff, C., Olsen, R. K., Gregersen, N., Chakrapani, A., Downing, M., Manning, N. J., Sharrard, M., Bonham, J. R., Muntoni, F., Turnbull, D. N. and Pourfarzam, M. 2005. Biochemical, clinical	Exclude by date, no genotype-phenotype association data and no

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and molecular findings in LCHAD and general mitochondrial trifunctional protein deficiency. Journal of Inherited Metabolic Disease 28(4):533-44.	comparison of screened vs unscreened
ORII, K. E., AOYAMA, T., WAKUI, K., FUKUSHIMA, Y., MIYAJIMA, H., YAMAGUCHI, S., ORII, T., KONDO, N. & HASHIMOTO, T. 1997. Genomic and mutational analysis of the mitochondrial trifunctional protein beta-subunit (HADHB) gene in patients with trifunctional protein deficiency. *Human Molecular Genetics,* 6, 1215-24.	Genotype but before 2000
OUNAP, K., KAHRE, T., ROOMETS, E., ZORDANIA, R., LAHT, T. M. & WANDERS, R. J. A. 2005. SCREENING FOR THE COMMON LCHAD MUTATION G1528C IN ESTONIAN POPULATION. *Journal of Inherited Metabolic Disease,* 28, 104-104.	Abstract
OURA, T. 1969. [Inborn errors of metabolism, associated with ocular symptoms]. *Nippon Ganka Kiyo - Folia Ophthalmologica Japonica - Bulletin of Japanese Ophthalmology,* 20, 749-58.	Review
OURA, T. & KOZAKI, M. 1969. [Congenital metabolic disorders with eye manifestations]. *Ganka - Ophthalmology,* 11, 872-83.	Review
OZASA, H. & TANAKA, K. 1988. Short chain and long chain acyl-CoA dehydrogenase deficiencies. [Japanese]. *Tanpakushitsu kakusan koso,* Protein, nucleic acid, enzyme. 33, 564-567.	Too early for relevant question
PANDOR, A., EASTHAM, J., CHILCOTT, J., PAISLEY, S. & BEVERLEY, C. 2006. Economics of tandem mass spectrometry screening of neonatal inherited disorders. *International Journal of Technology Assessment in Health Care,* 22, 321-6.	Review and not LCHADD
PARINI, R., GARAVAGLIA, B., SAUDUBRAY, J. M., BARDELLI, P., MELOTTI, D., ZECCA, G. & DI DONATO, S. 1991. Clinical diagnosis of long-chain acyl-coenzyme A-dehydrogenase deficiency: use of stress and fat-loading tests. *Journal of Pediatrics,* 119, 77-80.	Single case
PENG, M., LIU, L., JIANG, M., LIANG, C., ZHAO, X., CAI, Y., SHENG, H., OU, Z. & LUO, H. 2013. Measurement of free carnitine and acylcarnitines in plasma by HILIC-ESI-MS/MS without derivatization. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences,* 932, 12-8.	Not relevant
PICHLER, K., MICHEL, M., ZLAMY, M., SCHOLL-BUERGI, S., RALSER, E., JORG-STRELLER, M. & KARALL, D. 2017. Breast milk feeding in infants with inherited metabolic disorders other than phenylketonuria - a 10-year single-center experience. *Journal of Perinatal Medicine,* 45, 375-382.	No late cases and no genotype info
PIEKUTOWSKA-ABRAMCZUK, D., OLSEN, R. K., WIERZBA, J., POPOWSKA, E., JURKIEWICZ, D., CIARA, E., OLTARZEWSKI, M., GRADOWSKA, W., SYKUT-CEGIELSKA, J., KRAJEWSKA-WALASEK, M., ANDRESEN, B. S., GREGERSEN, N. & PRONICKA, E. 2010. A comprehensive HADHA c.1528G>C frequency study reveals high prevalence of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency in Poland. *Journal of Inherited Metabolic Disease,* 33 Suppl 3, S373-7.	Too early for incidence, no phenotype info for genotypes
POLLITT, R. J. 1993. Neonatal screening. *Journal of Clinical Pathology,* 46, 497-499.	Review
POLLITT, R. J. 1995. Disorders of mitochondrial long-chain fatty acid oxidation. *Journal of Inherited Metabolic Disease,* 18, 473-90.	Review
PONS, R., ROIG, M., RIUDOR, E., RIBES, A., BRIONES, P., ORTIGOSA, L., BALDELLOU, A., GIL-GIBERNAU, J., OLESTI, M., NAVARRO, C. & WANDERS, R. J. A. 1996. The clinical spectrum of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Pediatric Neurology,* 14, 236-243.	No cases diagnosed not by screening and date of the paper is too early for the genotype question
POTTER, B. K., LITTLE, J., CHAKRABORTY, P., KRONICK, J. B., EVANS, J., FREI, J., SUTHERLAND, S. C., WILSON, K. & WILSON, B. J. 2012. Variability in the clinical management of fatty acid oxidation disorders: results of a survey of Canadian metabolic physicians. *Journal of Inherited Metabolic Disease,* 35, 115-23.	Not relevant
POWELL, C. K., ISAACS, J. D., SIMS, H. F. & STRAUSS, A. W. 1995. MOLECULAR CHARACTERIZATION OF FETAL LONG-CHAIN 3-HYDROXYACYL-COA DEHYDROGENASE-DEFICIENCY ASSOCIATED WITH ACUTE FATTY LIVER OF PREGNANCY. *Pediatric Research,* 37, A151-A151.	Abstract

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POWELL, C. K., SIMS, H. F., BRACKETT, J. C. & STRAUSS, A. W. 1994. COMPLETE CHARACTERIZATION OF THE HUMAN CARDIAC LONG-CHAIN 3-HYDROXYACYL-COA DEHYDROGENASE CDNA AND GENE. *Circulation,* 90, 245-245.	Abstract
PRIMASSIN, S. & SPIEKERKOETTER, U. 2010. ESI-MS/MS measurement of free carnitine and its precursor gamma-butyrobetaine in plasma and dried blood spots from patients with organic acidurias and fatty acid oxidation disorders. *Molecular Genetics & Metabolism,* 101, 141-5.	Not study on the whole population. No genotype of outcome data
PRZYREMBEL, H., JAKOBS, C., L, I. J., DE KLERK, J. B. C. & WANDERS, R. J. A. 1991. Long-chain 3-Hydroxyacyl-CoA dehydrogenase deficiency. *Journal of Inherited Metabolic Disease,* 14, 674-680.	One case
QUINTANA, E. M., QUINTANA, L. P. & GONZALEZ, F. R. 2007. Long-chain 3-hydroxyacyl-coenzyme a dehydrogenase deficiency and cardiomyopathy. *Revista Espanola De Cardiologia,* 60, 1332-1334.	Letter
REY, J. 1972. [Hereditary digestive enzyme defects]. *Medecine et Chirurgie Digestives,* 1, 41-4 contd.	Review
RICE, G. M. & STEINER, R. D. 2016. Inborn errors of metabolism (metabolic disorders). *Pediatrics in Review,* 37, 3-17.	Review
ROE, C. R. & BRUNENGRABER, H. 2015. Anaplerotic treatment of long-chain fat oxidation disorders with triheptanoin: Review of 15 years Experience. *Molecular Genetics & Metabolism,* 116, 260-8.	Do not know method of diagnosis
ROE, C. R., ROE, D. S., WALLACE, M. & GARRITSON, B. 2007. Choice of oils for essential fat supplements can enhance production of abnormal metabolites in fat oxidation disorders. *Molecular Genetics & Metabolism,* 92, 346-50.	Cell study
ROOMETS, E., KIVELA, T. & TYNI, T. 2013. Early dietary therapy in preventing progression of retinopathy in long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency caused by the homozygous G1528C mutation. *Acta Ophthalmologica,* 91.	Abstract
SAKAKIDA, H. 1972. [Clinical practice of diet therapy in various diseases. 4. Abnormal metabolic regulation and its therapy by diet. (2) Lipid metabolism disorders and others]. *Kangogaku Zasshi - Japanese Journal of Nursing,* 36, 1056-9.	Review
SAUDUBRAY, J. M., MARTIN, D., DE LONLAY, P., TOUATI, G., POGGI-TRAVERT, F., BONNET, D., JOUVET, P., BOUTRON, M., SLAMA, A., VIANEY-SABAN, C., BONNEFONT, J. P., RABIER, D., KAMOUN, P. & BRIVET, M. 1999. Recognition and management of fatty acid oxidation defects: a series of 107 patients. *Journal of Inherited Metabolic Disease,* 22, 488-502.	Do not know age of patients or genotype
SAUDUBRAY, J. M., MARTIN, D., POGGI-TRAVERT, F., BILLETTE DE VILLEMEUR, T., SPADA, M., BARTULI, A., JOUVET, P., BRIVET, M., SLAMA, A., VIANEY-LIAUD, C., DEMAUGRE, F., BONNEFONT, J. P., RABIER, D., CHARPENTIER, C. & KAMOUN, P. 1997. Clinical presentations of inherited mitochondrial fatty acid oxidation disorders: An update. *International Pediatrics,* 12, 34-40.	Do not know age of diagnosis or genotype
SCHAEFER, J., JACKSON, S., DICK, D. J. & TURNBULL, D. M. 1996. Trifunctional enzyme deficiency: adult presentation of a usually fatal beta-oxidation defect. *Annals of Neurology,* 40, 597-602.	Genotype question and before 2000
SCHRIJVER-WIELING, I., VAN RENS, G. H., WITTEBOL-POST, D., SMEITINK, J. A., DE JAGER, J. P., DE KLERK, H. B. & VAN LITH, G. H. 1997. Retinal dystrophy in long chain 3-hydroxy-acyl-coA dehydrogenase deficiency. *British Journal of Ophthalmology,* 81, 291-4.	Genotype question and before 2000
SERRANO-AGUILAR, P., CASTILLA-RODRIGUEZ, I., VALLEJO-TORRES, L., VALCARCEL-NAZCO, C. & GARCIA-PEREZ, L. 2015. Neonatal screening in Spain and cost-effectiveness. *Expert Opinion on Orphan Drugs,* 3, 971-974.	Not relevant
SHAWKY, R. M., ABD-ELKHALEK, H. S. & ELAKHDAR, S. E. 2015. Selective screening in neonates suspected to have inborn errors of metabolism. *Egyptian Journal of Medical Human Genetics,* 16, 165-171.	Not LCHADD/MPTD
SHIGEMATSU, Y., HIRANO, S., HATA, I., TANAKA, Y., SUDO, M., TAJIMA, T., SAKURA, N., YAMAGUCHI, S. & TAKAYANAGI, M. 2003. Selective screening for fatty acid oxidation disorders by tandem mass spectrometry: difficulties in practical discrimination. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences,* 792, 63-72.	No LCHADD

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SIDDIQ, S., WILSON, B. J., GRAHAM, I. D., LAMOUREUX, M., KHANGURA, S. D., TINGLEY, K., TESSIER, L., CHAKRABORTY, P., COYLE, D., DYACK, S., GILLIS, J., GREENBERG, C., HAYEEMS, R. Z., JAIN-GHAI, S., KRONICK, J. B., LABERGE, A. M., LITTLE, J., MITCHELL, J. J., PRASAD, C., SIRIWARDENA, K., SPARKES, R., SPEECHLEY, K. N., STOCKLER, S., TRAKADIS, Y., WAFA, S., WALIA, J., WILSON, K., YUSKIV, N., POTTER, B. K. & CANADIAN INHERITED METABOLIC DISEASES RESEARCH, N. 2016. Experiences of caregivers of children with inherited metabolic diseases: a qualitative study. *Orphanet Journal Of Rare Diseases,* 11, 168.	Not relevant
SIMS, H. F., BRACKETT, J. C., POWELL, C. K., TREEM, W. R., HALE, D. E., BENNETT, M. J., GIBSON, B., SHAPIRO, S. & STRAUSS, A. W. 1995. The molecular basis of pediatric long chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with maternal acute fatty liver of pregnancy. *Proceedings of the National Academy of Sciences of the United States of America,* 92, 841-5.	Animal study
SKLADAL, D., SASS, J. O., GEIGER, H., GEIGER, R., MANN, C., VREKEN, P., WANDERS, R. J. & TRAWOGER, R. 2000. Complications in early diagnosis and treatment of two infants with long-chain fatty acid beta-oxidation defects. *Journal of Pediatric Gastroenterology & Nutrition,* 31, 448-52.	One case
SOLIS, J. O. & SINGH, R. H. 2002. Management of fatty acid oxidation disorders: a survey of current treatment strategies. *Journal of the American Dietetic Association,* 102, 1800-3.	Not relevant
SPIEKERKOETTER, U. 2010. Mitochondrial fatty acid oxidation disorders: clinical presentation of long-chain fatty acid oxidation defects before and after newborn screening. *Journal of Inherited Metabolic Disease,* 33, 527-32.	Review
SPIEKERKOETTER, U., KHUCHUA, Z., YUE, Z., BENNETT, M. J. & STRAUSS, A. W. 2004. General mitochondrial trifunctional protein (TFP) deficiency as a result of either alpha- or beta-subunit mutations exhibits similar phenotypes because mutations in either subunit alter TFP complex expression and subunit turnover. *Pediatric Research,* 55, 190-6.	No outcomes/phenotypes
SPIEKERKOETTER, U., LINDNER, M., SANTER, R., GROTZKE, M., BAUMGARTNER, M. R., BOEHLES, H., DAS, A., HAASE, C., HENNERMANN, J. B., KARALL, D., DE KLERK, H., KNERR, I., KOCH, H. G., PLECKO, B., ROSCHINGER, W., SCHWAB, K. O., SCHEIBLE, D., WIJBURG, F. A., ZSCHOCKE, J., MAYATEPEK, E. & WENDEL, U. 2009. Treatment recommendations in long-chain fatty acid oxidation defects: consensus from a workshop. *Journal of Inherited Metabolic Disease,* 32, 498-505.	Review
SPIEKERKOETTER, U. D., SUN, B., KHUCHUA, Z., BENNETT, M. J. & STRAUSS, A. W. 2002. Molecular and phenotypic heterogeneity in mitochondrial trifunctional protein deficiency due to beta-subunit mutations. *Pediatric Research,* 51, 226A-226A.	Abstract
STRAUSS, A. W., SPIEKERKOETTER, U., DING, L., TOKUNAGA, C., ZYKOVITZ, T., MARSDEN, D., RINALDO, P. & BENNETT, M. 2004. The changing spectrum of fatty acid oxidation disorders post-newborn screening. *Molecular Genetics and Metabolism,* 81, 156-157.	One case
SUN, W., WANG, Y., YANG, Y., WANG, J., CAO, Y., LUO, F., LU, W., PENG, Y., YAO, H. & QIU, P. 2011. The screening of inborn errors of metabolism in sick Chinese infants by tandem mass spectrometry and gas chromatography/mass spectrometry. *Clinica Chimica Acta,* 412, 1270-4.	High risk group and no LCHADD
SYKUT-CEGIELSKA, J., POHORECKA, M., TAYBERT, J., GRADOWSKA, W., OLSEN, R. K. J. & ANDRESEN, B. S. 2007. Intrauterine growth retardation in patients with LCHAD deficiency. *Journal of Inherited Metabolic Disease,* 30, 50-50.	Abstract
TAKAHASHI, T., YAMADA, K., KOBAYASHI, H., HASEGAWA, Y., TAKETANI, T., FUKUDA, S. & YAMAGUCHI, S. 2015. Metabolic disease in 10 patients with sudden unexpected death in infancy or acute life-threatening events. *Pediatrics International,* 57, 348-53.	One case
TAKUSA, Y. & YAMAGUCHI, S. 1998. Mitochondrial trifunctional protein (TP) deficiency. [Japanese]. *Ryoikibetsu shokogun shirizu*, 422-425.	Review

Reference	Reason for exclusion
TAL, G., PITT, J., MORRISY, S., TZANAKOS, N. & BONEH, A. 2015. An audit of newborn screening procedure: impact on infants presenting clinically before results are available. *Molecular Genetics & Metabolism,* 114, 403-8.	No LCHADD
TAMAOKI, Y., KIMURA, M., HASEGAWA, Y., IGA, M., INOUE, M. & YAMAGUCHI, S. 2002. A survey of Japanese patients with mitochondrial fatty acid beta-oxidation and related disorders as detected from 1985 to 2000. *Brain & Development,* 24, 675-80.	Does not include age at diagnosis or genotype details
TAUBENSLAG, L. 1972. [Congenital defects of lipid metabolism]. *Archivos Argentinos de Pediatria,* 70, 13-4.	Review
TEAR, F. K., HOLMSTROM, G. & YING, L. 2008. Ocular characteristics in 10 children with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: a cross-sectional study with long-term follow-up (vol 86, pg 329, 2008). *Acta Ophthalmologica,* 86, 466-466.	Erratum
TERRONE, G., RUOPPOLO, M., BRUNETTI-PIERRI, N., COZZOLINO, C., SCOLAMIERO, E., PARENTI, G., ROMANO, A., ANDRIA, G., SALVATORE, F. & FRISSO, G. 2014. Child neurology: Recurrent rhabdomyolysis due to a fatty acid oxidation disorder. *Neurology,* 82, e1-4.	Single case
THOMASON, M. J., LORD, J., BAIN, M. D., CHALMERS, R. A., LITTLEJOHNS, P., ADDISON, G. M., WILCOX, A. H. & SEYMOUR, C. A. 1998. A systematic review of evidence for the appropriateness of neonatal screening programmes for inborn errors of metabolism. *Journal of Public Health Medicine,* 20, 331-343.	Review
TREEM, W. R., SHOUP, M. E., HALE, D. E., BENNETT, M. J., RINALDO, P., MILLINGTON, D. S., STANLEY, C. A., RIELY, C. A. & HYAMS, J. S. 1996. Acute fatty liver of pregnancy, hemolysis, elevated liver enzymes, and low platelets syndrome, and long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *American Journal of Gastroenterology,* 91, 2293-300.	Genome study before 2000
TREEM, W. R., STANLEY, C. A., HALE, D. E., LEOPOLD, H. B. & HYAMS, J. S. 1991. Hypoglycemia, hypotonia, and cardiomyopathy: The evolving clinical picture of long-chain acyl-CoA dehydrogenase deficiency. *Pediatrics,* 87, 328-333.	Single case
TREEM, W. R., WITZLEBEN, C. A., PICCOLI, D. A., STANLEY, C. A., HALE, D. E., COATES, P. M. & WATKINS, J. B. 1986. Medium-chain and long-chain acyl CoA dehydrogenase deficiency: clinical, pathologic and ultrastructural differentiation from Reye's syndrome. *Hepatology,* 6, 1270-8.	No age of diagnosis/method and too early for genotype
TURAKA, K., BRYAN, J. S., GORDON, A. J., KWONG, H. M., JR., REDDY, R., TSIPURSKY, M. & SELL, C. H. 2012. Clinical and image-guided chorioretinal findings in long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Journal of Pediatric Endocrinology & Metabolism,* 25, 565-7.	Abstract
TURNBULL, D. M., SHEPHERD, I. M. & AYNSLEY-GREEN, A. 1988. Inherited defects of mitochondrial fatty acid oxidation. *Biochemical Society transactions,* 16, 424-427.	Review
TYNI, T., EKHOLM, E. & PIHKO, H. 1998. Pregnancy complications are frequent in long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *American Journal of Obstetrics and Gynecology,* 178, 603-608.	Too early for genotype question
TYNI, T., IMMONEN, T., LINDAHL, P., MAJANDER, A. & KIVELA, T. 2012. Refined staging for chorioretinopathy in long-chain 3-hydroxyacyl coenzyme A dehydrogenase deficiency. *Ophthalmic Research,* 48, 75-81.	Do not know age of diagnosis
TYNI, T., KIVELA, T., LAPPI, M., SUMMANEN, P., NIKOSKELAINEN, E. & PIHKO, H. 1998. Ophthalmologic findings in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency caused by the G1528C mutation - A new type of hereditary metabolic chorioretinopathy. *Ophthalmology,* 105, 810-824.	Genotype paper before 2000
TYNI, T., PALOTIE, A., VIINIKKA, L., VALANNE, L., SALO, M. K., VON DOBELN, U., JACKSON, S., WANDERS, R., VENIZELOS, N. & PIHKO, H. 1997. Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency with the G1528C mutation: clinical presentation of thirteen patients. *Journal of Pediatrics,* 130, 67-76.	Genotype paper before 2000
TYNI, T. & PIHKO, H. 1997. Clinical outcomes in long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency - Reply. *Journal of Pediatrics,* 131, 938-+.	Reply
TYNI, T., RAPOLA, J., PAETAU, A., PALOTIE, A. & PIHKO, H. 1997. Pathology of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency caused by the G1528C mutation. *Pediatric Pathology & Laboratory Medicine,* 17, 427-47.	All screened late and genotype before 2000

Reference	Reason for exclusion
USHIKUBO, S., AOYAMA, T., KAMIJO, T., WANDERS, R. J., RINALDO, P., VOCKLEY, J. & HASHIMOTO, T. 1996. Molecular characterization of mitochondrial trifunctional protein deficiency: formation of the enzyme complex is important for stabilization of both alpha- and beta-subunits. *American Journal of Human Genetics,* 58, 979-88.	Genome study before 2000
UUSIMAA, J., VAINIONPAA, L., SIMILA, S., MIETTINEN, R. & NUUTINEN, M. 1997. L-3-Hydroxyacyl-CoA dehydrogenase deficiency: Two cases with pigmentary retinopathy. *Journal of Inherited Metabolic Disease,* 20, 848-850.	Two cases both late and too early for genotype study
VALAYANNOPOULOS, V., BARNERIAS, C., ROMANO, S., BRIVET, M., VIANNEY-SABAN, C., DESGUERRE, I., TOUATI, G., SAUDUBRAY, J. M. & DE LONLAY, P. 2006. Peripheral neuropathy as a presenting symptom in mitochondrial trifunctional protein (MTP) deficiency. *Journal of Inherited Metabolic Disease,* 29, 56-56.	Abstract
VAN GRUNSVEN, E. G., VAN ROERMUND, C. W. T., DENIS, S. & WANDERS, R. J. A. 1997. Complementation analysis of fibroblasts from peroxisomal fatty acid oxidation deficient patients shows high frequency of bifunctional enzyme deficiency plus intragenic complementation: Unequivocal evidence for differential defects in the same enzyme protein. *Biochemical and Biophysical Research Communications,* 235, 176-179.	Not screening, no genotype or outcomes
VAN HOVE, J. L. K., KAHLER, S. G., FEEZOR, M. D., RAMAKRISHNA, J. P., HART, P., TREEM, W. R., SHEN, J. J., MATERN, D. & MILLINGTON, D. S. 2000. Acylcarnitines in plasma and blood spots of patients with long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Journal of Inherited Metabolic Disease,* 23, 571-582.	No outcomes
VAN MALDERGEM, L., TUERLINCKX, D., WANDERS, R. J., VIANEY-SABAN, C., VAN HOOF, F., MARTIN, J. J., FOURNEAU, C., GILLEROT, Y. & BACHY, A. 2000. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and early-onset liver cirrhosis in two siblings. *European Journal of Pediatrics,* 159, 108-12.	Do not have info on genotype for all patients
VENTURA, F. V., RUITER, J. P. N., IJLST, L., DE ALMEIDA, I. T. & WANDERS, R. J. A. 1998. Lactic acidosis in long-chain fatty acid beta-oxidation disorders. *Journal of Inherited Metabolic Disease,* 21, 645-654.	Cell study
VIANEY-LIAUD, C., DIVRY, P., GREGERSEN, N. & MATHIEU, M. 1987. The inborn errors of mitochondrial fatty acid oxidation. *Journal of Inherited Metabolic Disease,* 10 Suppl 1, 159-200.	Review
VICI, C. D., BERTINI, E., BURLINA, A., GARAVAGLIA, B., HALE, D. E., BARTULI, A., MAZZIOTTA, M. R. M., SABATELLI, M. & SABETTA, G. 1990. NEUROMUSCULAR INVOLVEMENT IN 2 UNRELATED CHILDREN WITH LONG-CHAIN 3-HYDROXYACYL-COA DEHYDROGENASE (LCHAD) DEFICIENCY. *Pediatric Research,* 28, 305-305.	Abstract
VILASECA, M. A., GOMEZ-LOPEZ, L., LAMBRUSCHINI, N., GUTIERREZ, A., GARCIA, R., MEAVILLA, S., MORENO, J. & ARTUCH, R. 2011. Long-chain polyunsaturated fatty acid concentration in patients with inborn errors of metabolism. *Nutricion Hospitalaria,* 26, 128-136.	No LCHADD
VIST, G. E., FRONSDAL, K. B., JOHANSEN, M., HOFMANN, B. & FRETHEIM, A. 2007. *Knowledge Centre for the Health Services at The Norwegian Institute of Public Health (NIPH),* NIPH Systematic Reviews, Executive Summaries.	Review
VOCKLEY, J., BURTON, B., BERRY, G. T., LONGO, N., PHILLIPS, J., SANCHEZ-VALLE, A., TANPAIBOON, P., GRUNEWALD, S., MURPHY, E., BOWDEN, A., CHEN, W. C., MU, Y. M., CATALDO, J., MARSDEN, D. & KAKKIS, E. 2018. RESULTS FROM A 78-WEEK SINGLE-ARM, OPEN-LABEL PHASE 2 STUDY TO EVALUATE UX007 IN PEDIATRIC AND ADULT PATIENTS WITH MODERATE TO SEVERE LONG-CHAIN FATTY ACID OXIDATION DISORDERS (LC-FAOD). *Molecular Genetics and Metabolism,* 123, 274-275.	Abstract
VOCKLEY, J., BURTON, B., BERRY, G. T., LONGO, N., PHILLIPS, J., SANCHEZ-VALLE, A., TANPAIBOON, P., GRUNEWALD, S., MURPHY, E., HUMPHREY, R., MAYHEW, J., BOWDEN, A., ZHANG, L., CATALDO, J., MARSDEN, D. L. & KAKKIS, E. 2017. UX007 for the treatment of long chain-fatty acid oxidation disorders: Safety and efficacy in children and adults following 24weeks of treatment. *Molecular Genetics & Metabolism,* 120, 370-377.	No age at diagnosis, canott separate from other conditions, no genotype info
VOCKLEY, J., MARSDEN, D., MCCRACKEN, E., DEWARD, S., BARONE, A., HSU, K. & KAKKIS, E. 2015. Long-term major clinical outcomes in patients with long chain fatty acid oxidation disorders before and after transition to triheptanoin treatment--A retrospective chart review. *Molecular Genetics & Metabolism,* 116, 53-60.	Do not know age at diagnosis or genotype

Reference	Reason for exclusion
VREKEN, P., VAN LINT, A. E., BOOTSMA, A. H., OVERMARS, H., WANDERS, R. J. & VAN GENNIP, A. H. 1999. Quantitative plasma acylcarnitine analysis using electrospray tandem mass spectrometry for the diagnosis of organic acidaemias and fatty acid oxidation defects. *Journal of Inherited Metabolic Disease,* 22, 302-6.	High risk group only
WAISBREN, S. E., HE, J. & MCCARTER, R. 2015. Assessing Psychological Functioning in Metabolic Disorders: Validation of the Adaptive Behavior Assessment System, Second Edition (ABAS-II), and the Behavior Rating Inventory of Executive Function (BRIEF) for Identification of Individuals at Risk. *Jimd Reports,* 21, 35-43.	Do not know age or method of diagnosis or genotype
WAJNER, M., COELHO DDE, M., INGRASSIA, R., DE OLIVEIRA, A. B., BUSANELLO, E. N., RAYMOND, K., FLORES PIRES, R., DE SOUZA, C. F., GIUGLIANI, R. & VARGAS, C. R. 2009. Selective screening for organic acidemias by urine organic acid GC-MS analysis in Brazil: fifteen-year experience. *Clinica Chimica Acta,* 400, 77-81.	Urine analysis
WANDERS, R. J., IJLST, L., DURAN, M., JAKOBS, C., DE KLERK, J. B., PRZYREMBEL, H., ROCCHICCIOLI, F. & AUBOURG, P. 1991. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: different clinical expression in three unrelated patients. *Journal of Inherited Metabolic Disease,* 14, 325-8.	Both late cases, no genotype info
WANDERS, R. J., VREKEN, P., DEN BOER, M. E., WIJBURG, F. A., VAN GENNIP, A. H. & L, I. J. 1999. Disorders of mitochondrial fatty acyl-CoA beta-oxidation. *Journal of Inherited Metabolic Disease,* 22, 442-87.	Review
WANDERS, R. J. A., IJLST, L., VANGENNIP, A. H., JAKOBS, C., DEJAGER, J. P., DORLAND, L., VANSPRANG, F. J. & DURAN, M. 1990. LONG-CHAIN 3-HYDROXYACYL-COA DEHYDROGENASE-DEFICIENCY - IDENTIFICATION OF A NEW INBORN ERROR OF MITOCHONDRIAL FATTY-ACID BETA-OXIDATION. *Journal of Inherited Metabolic Disease,* 13, 311-314.	Case study
WASANT, P., SVASTI, J., SRISOMSAP, C., LIAMMONGKOLKUL, S., NAYLOR, E. W. & MATSUMOTO, I. 1999. Inherited metabolic disorders in Thailand--Siriraj experience. *Southeast Asian Journal of Tropical Medicine & Public Health,* 30 Suppl 2, 124-37.	No LCHADD – case study
WILCKEN, B. 2010. Expanded newborn screening: reducing harm, assessing benefit. *Journal of Inherited Metabolic Disease,* 33, S205-10.	Review
WILCKEN, B. 2010. Fatty acid oxidation disorders: outcome and long-term prognosis. *Journal of Inherited Metabolic Disease,* 33, 501-6.	Review
WILCKEN, B., LEUNG, K. C., HAMMOND, J., KAMATH, R. & LEONARD, J. V. 1993. Pregnancy and fetal long-chain 3-hydroxyacyl coenzyme A dehydrogenase deficiency. *Lancet,* 341, 407-8.	All early. Too early for genotype
WILCOX, R. L., NELSON, C. C., STENZEL, P. & STEINER, R. D. 2002. Postmortem screening for fatty acid oxidation disorders by analysis of Guthrie cards with tandem mass spectrometry in sudden unexpected death in infancy. *Journal of Pediatrics,* 141, 833-6.	No LCHADD
WILEY, V., CARPENTER, K., BAYLISS, U. & WILCKEN, B. 2003. Newborn screening--is it really that simple? *Southeast Asian Journal of Tropical Medicine & Public Health,* 34 Suppl 3, 107-10.	No results for LCHADD
WILSON, C., KNOLL, D., DE HORA, M., KYLE, C., GLAMUZINA, E. & WEBSTER, D. 2017. The Risk of Fatty Acid Oxidation Disorders and Organic Acidemias in Children with Normal Newborn Screening. *Jimd Reports,* 35, 53-58.	Not LCHADD
XIE, L. J., ZHU, J. X., ZHU, X. D., LI, H. J., HAN, L. S. & GU, X. F. 2008. Combined use of tandem mass spectrometry with urine gas chromatography/mass spectrometry is useful for diagnosis of inborn errors of metabolism in children. [Chinese]. *Chinese Journal of Contemporary Pediatrics,* 10, 31-34.	High risk group
YAMADA, K., HASEGAWA, Y., YOSHIKAWA, Y., TAKAHASHI, T., KOBAYASHI, H., MUSHIMOTO, Y., PUREVSUREN, J. & YAMAGUCHI, S. 2013. Clinical study of organic acidemias and fatty acid oxidation disorders detected in adults. [Japanese]. *Clinical Neurology,* 53, 191-195.	Not LCHADD
YAMAGUCHI, S. 2001. Acyl-CoA dehydrogenase deficiency (very-long-chain, long-chain, medium-chain, short-chain), mitochondrial. [Japanese]. *Ryoikibetsu shokogun shirizu*, 144-147.	Review

Reference	Reason for exclusion
YAMAGUCHI, S. 2002. [Enzymes of mitochondrial beta-oxidation]. *Nippon Rinsho - Japanese Journal of Clinical Medicine,* 60 Suppl 4, 88-93.	Review
YAMAGUCHI, S. 2002. Mitochondrial 3-hydroxyacyl-CoA dehydrogenase (SCHAD, LCHAD). [Japanese]. *Nippon rinsho,* Japanese journal of clinical medicine. 60 Suppl 4, 101-104.	Review
YAMAMOTO, T., MISHIMA, H., MIZUKAMI, H., FUKAHORI, Y., UMEHARA, T., MURASE, T., KOBAYASHI, M., MORI, S., NAGAI, T., FUKUNAGA, T., YAMAGUCHI, S., YOSHIURA, K.-I. & IKEMATSU, K. 2015. Metabolic autopsy with next generation sequencing in sudden unexpected death in infancy: Postmortem diagnosis of fatty acid oxidation disorders. *Molecular Genetics and Metabolism Reports,* 5, 26-32.	Not relevant
YANG, Z., LANTZ, P. E. & IBDAH, J. A. 2007. Post-mortem analysis for two prevalent beta-oxidation mutations in sudden infant death. *Pediatrics International,* 49, 883-7.	One case
ZHENG, J., ZHANG, Y., HONG, F., YANG, J., TONG, F., MAO, H., HUANG, X., ZHOU, X., YANG, R., ZHAO, Z. & HUANG, X. 2017. [Screening for fatty acid oxidation disorders of newborns in Zhejiang province:prevalence, outcome and follow-up]. *Zhejiang da Xue Xue Bao Yi Xue Ban/Journal of Zhejiang University Medical Sciences,* 46, 248-255.	No LCHADD
ZHU, J. & YANG, Z. 2006. Study of the inborn errors of mitochondrial fatty acid beta-oxidation deficiency. [Chinese]. *Beijing da xue xue bao,* Yi xue ban = Journal of Peking University. Health sciences. 38, 214-217.	Review
ZHU, J. M., YANG, Z., YU, M., WANG, R., YE, R. H., YANG, H. X., ZHAI, G. R. & WANG, Q. 2005. Screening for the G1528C mutation in long chain fatty acid oxidation enzyme in Han nationality in Beijing population. [Chinese]. *Beijing da xue xue bao,* Yi xue ban = Journal of Peking University. Health sciences. 37, 72-74.	No LCHADD

Of the 39 publications included after the review of titles and abstracts from the search for question 3, 28 were ultimately judged not to be relevant to this review. These publications, along with reasons for exclusion, are listed in Table 13.

Table 13 Publications excluded after review of full-text articles Q3

Reference	Reason for exclusion
Abdel-Hamid, M.;Tisocki, K.; Sharaf, L.;Ramadan, D. 2007, Development, validation and application of tandem mass spectrometry for screening of inborn metabolic disorders in Kuwaiti infants. Medical Principles & Practice 16(3):215-21	No test accuracy data
Alfadhel, M; Al Othaim, A; Al Saif, S; Al Mutairi, F; Alsayed, M; Rahbeeni, Z; Alzaidan, H; Alowain, M; Al-Hassnan, Z; Saeedi, M; Aljohery, S; Alasmari, A; Faqeih, E; Alwakeel, M; AlMashary, M; Almohameed, S; Alzahrani, M; Migdad, A; Al-Dirbashi, O. Y; Rashed, M; Alamoudi, M; Jacob, M; Alahaidib, L; El-Badaoui, F; Saadallah, A; Alsulaiman, A; Eyaid, W; Al-Odaib, A. 2017, Expanded Newborn Screening Program in Saudi Arabia: Incidence of screened disorders, Journal of Paediatrics & Child Health	no LCHADD screened
Alratrout, R; Alsadah, Z; Ansari, N. 2017, The frequency of inherited metabolic and endocrine disorders in the eastern and north-western Jawf provinces of Saudi Arabia: Four years data from the newborn screening department, ministry of health, Dammam, Current Pediatric Research	Did not screen for LCHADD
Cantu-Reyna, C; Zepeda, L. M; Montemayor, R; Benavides, S; Gonzalez, H. J; Vazquez-Cantu, M; Cruz-Camin, H. 2016, Incidence of inborn errors of metabolism by expanded newborn screening in a Mexican hospital, Journal of Inborn Errors of Metabolism and Screening	LCHADD screened but no cases
Chong, S. C; Law, L. K; Hui, J; Lai, C. Y; Leung, T. Y; Yuen, Y. P. 2017, Expanded newborn metabolic screening programme in Hong Kong: a three-year journey, Hong Kong Medical Journal	Screened but no cases found
Chrastina, P.; St'astna, S.; Myskova, H.; Kosarova, M.; Elleder, M.; Zeman, J. 2005, Newborn screening of inherited metabolic disorders by tandem mass spectrometry. [Czech]. Klinicka Biochemie a Metabolismus 13 (2):77-80	Paper could not be located
Estrella, J., Wilcken, B., Carpenter, K., Bhattacharya, K., Tchan, M. and Wiley, V. 2014, Expanded newborn screening in New South Wales: missed cases. Journal of Inherited Metabolic Disease. 37(6):881-7	Not test accuracy
Feuchtbaum, L; Lorey, F; Faulkner, L; Sherwin, J; Currier, R; Bhandal, A; Cunningham, G. 2006, California's experience implementing a pilot newborn supplemental screening program using tandem mass spectrometry, Pediatrics	No test accuracy data
Filiano, J. J; Bellimer, S. G; Kunz, P. L. 2002, Tandem mass spectrometry and newborn screening: pilot data and review, Pediatric Neurology	Cost paper and did not include LCHADD
Fleischman, A; Thompson, J D; Glass, M. 2014, Systematic Data Collection to Inform Policy Decisions: Integration of the Region 4 Stork (R4S) Collaborative Newborn Screening Database to Improve MS/MS Newborn Screening in Washington State, Jimd Reports	Not LCHADD
Guo, K; Zhou, X; Chen, X; Wu, Y; Liu, C; Kong, Q. 2018, Expanded newborn screening for inborn errors of metabolism and genetic characteristics in a Chinese population, Frontiers in Genetics	Did not screen for LCHADD
Hannon, H; Lim, T; Adam, B; Therrell, B. 2003, Outcomes from tandem mass spectrometry (MS/MS) workshops in the United States and the performance evaluation of MS/MS laboratories, Southeast Asian Journal of Tropical Medicine & Public Health	Not population testing

Reference	Reason for exclusion
Harms, E; Olgemoller, B. 2011, Neonatal Screening for Metabolic and Endocrine Disorders. Deutsches Arzteblatt International 108(1-2):11-21	Not test accuracy
Hassan, F. A.; El-Mougy, F.; Sharaf, S. A.; Mandour, I.; Morgan, M. F.; Selim, L. A.; Hassan, S. A.; Salem, F.; Oraby, A.; Girgis, M. Y.; Mahmoud, I. G.; El-Badawy, A.; El-Nekhely, I.; Moharam, N.; Mehaney, D. A.; Elmonem, M. A. 2016, Inborn errors of metabolism detectable by tandem mass spectrometry in Egypt: The first newborn screening pilot study. Journal of Medical Screening 23(3):124-9	Not test accuracy
Huang, H. P; Chu, K. L; Chien, Y. H; Wei, M. L; Wu, S. T; Wang, S. F; Hwu, W. L. 2006, Tandem mass neonatal screening in Taiwan--report from one center, Journal of the Formosan Medical Association	Did not test for LCHADD
Johnson, A. W; Mills, K; Clayton, P. T. 1996, The use of automated electrospray ionization tandem MS for the diagnosis of inborn errors of metabolism from dried blood spots, Biochemical Society Transactions	Conference abstract
la Marca, G; Malvagia, S; Casetta, B; Pasquini, E; Donati, M. A; Zammarchi, E. 2008, Progress in expanded newborn screening for metabolic conditions by LC-MS/MS in Tuscany: update on methods to reduce false tests, Journal of Inherited Metabolic Disease	No cases
Lee, H. C; Mak, C. M; Lam, C. W; Yuen, Y. P; Chan, A. O; Shek, C. C; Siu, T. S; Lai, C. K; Ching, C. K; Siu, W. K; Chen, S. P; Law, C. Y; Tai, H. L; Tam, S; Chan, A. Y. 2011, Analysis of inborn errors of metabolism: disease spectrum for expanded newborn screening in Hong Kong, Chinese Medical Journal	No cases and not clear that they screened for LCHADD
Naylor, E. W; Chace, D. H. 1999, Automated tandem mass spectrometry for mass newborn screening for disorders in fatty acid, organic acid, and amino acid metabolism, Journal of Child Neurology	Did not screen for LCHADD
Scolamiero, E; Cozzolino, C; Albano, L; Ansalone, A; Caterino, M; Corbo, G; di Girolamo, M. G; Di Stefano, C; Durante, A; Franzese, G; Franzese, I; Gallo, G; Giliberti, P; Ingenito, L; Ippolito, G; Malamisura, B; Mazzeo, P; Norma, A; Ombrone, D; Parenti, G; Pellecchia, S; Pecce, R; Pierucci, I; Romanelli, R; Rossi, A; Siano, M; Stoduto, T; Villani, G. R; Andria, G; Salvatore, F; Frisso, G; Ruoppolo, M. 2015, Targeted metabolomics in the expanded newborn screening for inborn errors of metabolism, Molecular Biosystems	No mention of screening for LCHADD
Shibata, N; Hasegawa, Y; Yamada, K; Kobayashi, H; Purevsuren, J; Yang, Y; Dung, V. C; Khanh, N. N; Verma, I. C; Bijarnia-Mahay, S; Lee, D. H; Niu, D. M; Hoffmann, G. F; Shigematsu, Y; Fukao, T; Fukuda, S; Taketani, T; Yamaguchi, S. 2018, Diversity in the incidence and spectrum of organic acidemias, fatty acid oxidation disorders, and amino acid disorders in Asian countries: Selective screening vs. expanded newborn screening, Molecular Genetics and Metabolism Reports	Include for incidence but no test accuracy data
Schulze, A.; Lindner, M.; Kohlmuller, D.; Olgemoller, K.; Mayatepek, E.; Hoffmann, G. F. 2003, Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. Pediatrics 111(6):1399-406	Not test accuracy
Scolamiero, E.; Cozzolino, C.; Albano, L.; Ansalone, A.; Caterino, M.; Corbo, G.; di Girolamo, M. G.; Di Stefano, C.; Durante, A.; Franzese, G.; Franzese, I.; Gallo, G.; Giliberti, P.; Ingenito, L.; Ippolito, G.; Malamisura, B.; Mazzeo, P. Norma, A.; Ombrone, D.; Parenti, G.; Pellecchia, S.; Pecce, R.; Pierucci, I.; Romanelli, R.; Rossi, A.; Siano, M.; Stoduto, T.; Villani, G. R.; Andria, G.; Salvatore, F.; Frisso, G.; Ruoppolo, M. 2015, Targeted metabolomics in the expanded newborn screening for inborn errors of metabolism. Molecular Biosystems 11(6):1525-35	Not test accuracy
Shibata, N.; Hasegawa, Y.; Yamada, K.; Kobayashi, H.; Purevsuren, J.; Yang, Y.; Dung, V. C.; Khanh, N. N.; Verma, I. C.; Bijarnia-Mahay, S.; Lee, D. H.; Niu, D. M.; Hoffmann, G. F.; Shigematsu, Y.; Fukao, T.; Fukuda, S.; Taketani, T.; Yamaguchi, S. 2018, Diversity in the incidence and spectrum of organic acidemias, fatty acid oxidation disorders, and amino acid disorders in Asian countries: Selective screening vs. expanded newborn screening. Molecular Genetics and Metabolism Reports 16:5-10	No test accuracy data

Reference	Reason for exclusion
Tal, G; Pitt, J; Morrisy, S; Tzanakos, N; Boneh, A. 2015, An audit of newborn screening procedure: impact on infants presenting clinically before results are available, Molecular Genetics & Metabolism	Did not screen for LCHADD
Vilarinho, L.; Rocha, H; Sousa, C.;Marcao, A; Fonseca, H; Bogas, M; Osorio, R. V. 2010. Four years of expanded newborn screening in Portugal with tandem mass spectrometry. Journal of Inherited Metabolic Disease 33(supp3):S133-8.	Not test accuracy
Wilcken, B; Wiley, V; Hammond, J; Carpenter, K. 2003, Screening newborns for inborn errors of metabolism by tandem mass spectrometry	No cases
Yunus, Z. M; Rahman, S. A; Choy, Y. S; Keng, W. T; Ngu, L. H. 2016, Pilot study of newborn screening of inborn error of metabolism using tandem mass spectrometry in Malaysia: outcome and challenges, Journal of Pediatric Endocrinology & Metabolism	Not test accuracy

Appendix 3 - Summary and appraisal of individual studies

Table 14. Estimates of the incidence of LCHADD and MTPD published up to 2013 [1]

Reference	Country and time period	Time period	Screened or clinical detection	Population size	Number of identified cases	Birth Prevalence	
						One in	Per 100,000
EUROPE							
Kasper et al	Austria	Apr 2002 – Dec 2009	Screened	622,489	9	69,165	1.45
Lund et al	Denmark, Faroe Islands, Greenland	2002-2011	Screened	504,049	3	168,016	0.6
Burgard et al	Hungary	2008-2009	Screened	22,661	*1*	22,661	4.41
Lindner et al	Germany, Baden-Wurttemberg	Jan 1999-Apr 2005	Screened	1,084,195	6	180,699	0.55
Roscher et al	Germany, Bavaria	Jan 1999-Jul 2001	Screened	307,676	1	307,676	0.33
Sander et al	Germany, Hanover	1999-2005	Screened	1,200,000	11	109,091	0.92
Sykut-Cegielska et al	Poland	2001-2009	Screened	658,492	11	59,863	1.67
Sykut-Cegielska et al	Poland	2001-2009	Clinical	3,348,000	29	115,448	0.87
Vilarinho et al	Portugal	2005-2009	Screened	316,243	3	105,414	0.95
NORTH AMERICA							
Chace et al	Pennsylvania & North carolina	Not presented	Screened	1,100,000	2	550,000	0.18
Frazier et al	North Carolina	1999-2005	Screened	749,695	3	249,898	0.40
AUSTRALASIA							
Wilcken et al	New South Wales	April 1974-Mar 1994	Clinical	1,754,000	5	350,800	0.29
Wilcken et al	Australia, New South Wales	April 1994-2002	Clinical	1,551,200	3	517,067	0.19
Wiley et al	New South Wales	April 1998-Dec 2013	Screened	1,065,713	2	532,857	0.19
SOUTHEAST ASIA							
Yoon et al	Korea	April 2001-Mar 2004	Screened	79,179	3	26,393	3.79
MIDDLE EAST							
Abdel-Hamid et al	Kuwait	May 2004 – Mar 2006	Screened	1,158	3	386	259.09

Table 15 Estimates of the incidence of LCHADD/MTPD (published since 2013)

Reference	Country	Condition	Time period	Population size	Number of identified cases	Birth Prevalence	
						One in	Per 100,000
EUROPE							
*Rocha et al [10]	Iberia (Portugal and Spain)	LCHADD/MTPD	Unclear	1,672,286	12	139,357	0.72
Shibata et al [11]	Germany	LCHADD/MTPD	2002 - 2015	~7,510,000	NR	127,000	0.79
Smon et al Screened [16]	Slovenia	LCHADD/MTPD	2013-2014	10,048	0	*NA*	NA
Unscreened	Slovenia	LCHADD/MTPD	2013-2014	293,387	1	293,897	0.34
NORTH AMERICA							
*Therrell et al [17]	USA	LCHADD	Jan 1 2001 – Dec 31 2010	24,370,414	67	363,738	0.27
	USA	MTPD	Jan 1 2001 – Dec 31 2010	23,693,387	12	1,822,568	0.05
SOUTHEAST ASIA							
Chien et al [14]	Taiwan	LCHADD/MTPD	Jan 1 2003 – Dec 31 2012	790,569	0	NA	NA
Lim et al [15]	Singapore	LCHADD/MTPD	Jul 2006 – Apr 2014	177,267	0	NA	NA
Shibata et al [11]	Japan	LCHADD/MTPD	1997-2015	3,360,000	NR	840,000	0.12
	Korea	LCHADD/MTPD	2000-2015	3,440,000	NR	1,148,000	0.09
Mak et al [21]	Hong Kong	LCHADD	Oct 1 2012 – Aug 31 2014	2440	0	NA	NA
Yang et al [18]	Jining City, China	LCHADD/MTPD	2014-2015	100,077	0	NA	NA
Yunas et al [19]	Malaysia	LCHADD/MTPD	Jun 2006-Dec 2008	21,417	0	NA	NA
MIDDLE EAST							
Al-Jasmi et al [13]	United Arab Emirates	LCHADD/MTPD	2011-2014	68,593	1	68593	1.46
Hassan et al [20]	Egypt	LCHADD	Jan-Nov 2008	25,276	0	NA	NA

LCHADD, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; NA, not applicable; NR, not reported; MTPD, Mitochondrial Trifunctional Protein Deficiency
*Additional data by area provided in appendices, table 16.

Table 16 Additional data for Therrell et al [17]. Question 1 Incidence

	LCHADD			MTPD		
State	**Cases**	**Births**	**Date calc**	**Cases**	**Births**	**Date calc**
Connecticut	0	273,897	May 1 2004	0	288,079	Unclear
Maine	0	135,961	Jan 2001	0	32,918	July 1 2008
Massachusetts	4	785,593	Jan 2001	1	785,593	Jan 2001
New Hampshire	1	47,020	July 1 2007	0	47,020	July 1 2007
Rhode Island	1	56,665	July 1 2006	0	56,665	July 1 2006
Vermont	0	48,316	Jan 1 2003	0	29,832	Jan 1 2006
Delaware	0	97,626	Jan 1 2003	0	48,743	Jan 1 2007
District of Columbia	1	29,416	Jan 1 2006	0	29,416	Jan 1 2006 (no data 2008-2010)
Maryland	1	509,796	Jan 1 2004	0	509,796	Jan 1 2004
New Jersey	2	656,334	Jan 1 2005	1	656,334	Jan 1 2005
New York	3	1,755,287	Jan 1 2004	0	1,755,287	Jan 1 2004
Pennsylvania	1	215,616	Jul 1 2009	0	215,616	Jul 1 2009
Virginia	0	504,757	Mar 1 2006	0	504,757	Mar 1 2006
West Virginia	0	40,279	Feb 1 2009	0	40,279	Feb 1 2009
Alabama	0	228,879	Apr 16 2007	1	228,879	Apr 16 2007
Florida	3	1,145,134	Jan 1 2006	1	1,145,134	Jan 1 2006
Georgia	3	577,945	Jan 1 2007	3	577,945	Jan 1 2007
Louisiana	1	322,531	Jan 1 2006	0	322,531	Jan 1 2006
Mississippi	0	322,489	Jun 1 2003	0	322,489	Jun 1 2003
North Carolina	3	1,245,716	Jan 2001	2	1,245,716	Jan 2001
South Carolina	3	358,022	Nov 1 2004	0	359,022	Nov 1 2004
Tennessee	2	615,964	Jan 1 2004	0	615,964	Jan 1 2004
Illinois	3	1,567,305	Jan 1 2002	0	1,567,305	Jan 1 2002
Indiana	0	704,310	Jan 1 2003	0	704,310	Jan 1 2003
Kentucky	0	273,250	Jan 1 2006	0	273,250	Jan 1 2006
Michigan	1	690,037	Jan 2001	0	690,037	Apr 18 2005
Minnesota	4	705,026	Jan 2001	1	637,598	Jan 2002
Ohio	2	1,035,764	Jan 1 2004	0	1,035,764	Jan 1 2004
Wisconsin	0	695,148	Jan 2001	1	381,129	Aug 1 2005
Arkanasas	0	96,056	Jul 1 2008	0	96,056	Jul 1 2008
Iowa	0	316,368	Jan 1 2003	0	316,368	Jan 1 2003
Kansas	0	105,394	Jul 1 2008	0	105,394	Jul 1 2008
Missouri	4	483,977	Jan 1 2005	0	483,977	Jan 1 2005

	LCHADD			MTPD		
State	**Cases**	**Births**	**Date calc**	**Cases**	**Births**	**Date calc**
Nebraska	1	200,373	Jul 1 2003	0	200,373	Jul 1 2003
North Dakota	1	64,625	Aug 1 2004	0	64,625	Aug 1 2004
Oklahoma	0	119,427	Oct 1 2008	0	119,427	Oct 1 2008
South Dakota	0	97,956	Jan 1 2003	0	97,956	Jan 1 2003
Arizona	2	419,677	Aug 31 2006	0	419,677	Aug 31 2006
Colorado	1	313,189	Jul 1 2006	0	313,189	Jul 1 2006
Montana	0	59,836	Jul 1 2004	0	59,836	Jul 1 2004 (no data 2007-8)
Nevada	0	297,539	Jan 1 2003	0	297,539	Jan 1 2003
New Mexico	0	114,820	Jan 1 2007	0	114,820	Jan 1 2007
Texas	7	1,661,279	Dec 6 2006	0	1,661,279	Dec 6 2006
Utah	1	276,174	Jan 1 2006	1	276,174	Jan 1 2006
Wyoming	0	32,458	Jul 1 2006	0	32,458	Jul 1 2006
Alaska	0	86,374	Jan 1 2003	0	86,374	Jan 1 2003
California	6	2,997,046	Jul 11 2005	2	2,997,046	Jul 11 2005
Hawaii	0	149,783	Jan 1 2003	0	149,783	Jan 1 2003
Idaho	1	184,644	Jan 1 2003	0	184,644	Jan 1 2003
Oregon	4	428,110	Jan 1 2002	0	288,758	Jan 1 2005

Table 17. Characteristics of included studies – Question 2 Genotype/Phenotype association

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
UK study					
Lundy 2003[34]	Case series Follow up period: Up to 3 years Study setting: NR, UK Number of centres: NR	N=4 3 LCHADD patients and 1 MTPD patient All patients which had acute respiratory distress syndrome	LCHADD: all homozygous 1528G>C MTPD: Mutation and subunit not specified		Maternal phenotype Death Age at time of death ARDS Hypoglycaemia Encephalopathy Jaundice Hepatomegaly Cardiac, renal and hepatic failure Bilateral tensions pneumothoraces Hypotonia Tachypnoea
European studies					
Boutron 2011[24]	Cohort study Time period: 1989-2010 Study setting: NR, France Number of centres: NR	N=52 34 LCHADD patients and 18 MTPD Study reports cases by HADHA and HADHB mutation only. Specified here as LCHADD or MTPD by the presence of the 1528G>C mutation. Alpha subunit cases here classified as MTPD may include LKAT cases.	19 homozygous 1528G>C LCHADD 15 compound heterozygous LCHADD 18 MTPD: 11 in the alpha subunit, 7 in the beta subunit	Fibroblast cultures, transcriptomic analyses, mutation analysis by sequencing and in silico analysis of missense mutations. Fibroblast cultures not undertaken/results not provided for all cases	Severity Maternal phenotype Death

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Diekman 2012[27]	Case series Follow up period and time period: NR Study setting: NR, Netherlands Number of centres: NR	N=2 Both MTPD patients	Both had HADHB mutations	NBS testing followed by enzymatic analysis and DNA mutation analysis	Maternal phenotype Death Age at time of death Necrotizing enterocolitis Cardiomyopathy Organ failure Respiratory failure
Joost 2011[32]	Expanded screening prevalence study with a case series of positive cases Time period: 2004-2010 Study setting: Tartu University Hospital, Tallinn Children's Hospital, Estonia Number of centres: 2	N=5 All LCHADD patients	3 homozygous 1528G>C patients 2 compound heterozygous LCHADD	DNA screening of 1040 newborns 2004-2010, molecular testing of 102 symptomatic patients from 2004-2007 and 425 from 2008-2010.	Maternal phenotype Death Age at time of death Muscular hypotonia Cardiomyopathy Hypoglycaemia Liver steatosis Hepatic dysfunction/hepatomegaly
Hayes 2007[29]	Case series Follow up period: Up to 15 years Study setting: Children's University Hospital Dublin Number of centres: 1	N=3 2 LCHADD patients, 1 MTPD	1 homozygous LCHADD 1528G>C 1 heterozygous LCHADD mutation not reported 1 MTPD mutation in the HADHB subunit	Clinical presentations. Tested with combinations of urine, enzyme and mutation analysis 1 case was a false negative NBS screen	Maternal phenotype Hypotonia Hepatomegaly/hepatic failure Hypoketotic hypoglycaemia Hypertrophic cardiomyopathy/cardiac failure Pigmentary retinopathy Rhabdomyolysis Learning difficulties Peripheral motor and sensory neuropathy Fatigability

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Schwab 2003[37]	Case series Study duration/follow up: NR Study setting: Germany Number of centres: NR	N=2 Both MTPD patients 2 siblings – 4 previous siblings had died in Egypt	MTPD beta subunit mutations	Cascade/prenatal testing. Molecular genetic analysis and immunoblot analysis	Death Age at time of death
Strandqvist 2015[43]	Prospective cohort study with retrospective data collection Follow up time: Up to 21 years Study setting: Karolinska University Hospital and Uppsala University, Finland Number of centres: 2	N=8 All LCHADD 10 invited, 8 participated. Cross over with the patients in studies by Fahnehjelm et al 2008, 2016 and Haglind 2013 – included for question 4 on outcomes.	5 LCHADD homozygous 1528G>C 3 compound heterozygous LCHADD	1 cascade tested, 7 clinically diagnosed Mutation analysis not specified	Epilepsy Symptoms at diagnosis: Lethargy Acidosis, Hypotonia, Cardiomyopathy Hypoglycaemia, Seizures, Elevated liver enzymes/liver enlargement, Neonatal hypothermia, Diarrhoea, Apnoea, Renal-liver-and heart failure, Intraventricular/intraparenchymal haemorrhage grades III-IV, Periventricular leukomalacia, failure to thrive, asymptomatic. Neurological outcomes – not by patient so not reported in this review

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Sykut-Cegielska, 2011[6]	Retrospective cohort Follow-up time: Up to 17 years. 1992-2009 Study setting: Children's memorial health institute (CMHI) and Institute of Mother and Child (IMC) Warsaw, Poland with contribution of molecular study in Aarhus University Hospital, Skejby, Denmark. Number of centres: 2	N = 54 All LCHADD	45 LCHADD homozygous 1528G>C 7 compound heterozygous LCHADD 7 LCHADD mutation not analysed or unclear 2nd mutation	15 cascade, NBS or incidentally screened. 44 diagnosed clinically Molecular analysis and enzymatic assays	Death Age at time of death
Finnish Cohort studies					
Immonen 2016[31]	Prospective cohort with retrospective data from hospital records. Comparison with historical cohort (not reported in the paper) Study duration: 1997-2010. Patients aged 1-11 years Study setting: University hospitals, Finland Number of centres: NR	N=16 All LCHADD patients Patients reported in previous studies which were excluded in this review (Tyni et al, 1997, 1998)	All homozygous 1528G>C	2 clinical cases diagnosed post-mortem. 1 diagnosed prenatally the rest clinically Mutation analysis not specified	Death Age at time of death In patients surviving >6m after diagnosis: Cardiomyopathy Neuropathy Retinopathy IQ Episodes of rhabdomyolysis Obesity

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Tuuli 2016[44]	Prospective cohort with retrospective data from hospital records. Study duration: 1976-2014 Patients aged 5-49 years Study setting: Helsinki university hospital Number of centres: 1	N=12 3 additional patients were approached but declined to take part in the study All LCHADD patients Not specified but likely to cross over with Immonen 2016	All homozygous 1528G>C	Mutation analysis from DNA extracted from blood or cultured fibroblasts	Death Polyneuropathy (via various measurements)
Yang 2002[66]	Cohort study Study duration: NR Setting and centres: NR, Finland	N=12 All LCHADD patients. Data extracted from this study alone. 11 families and 12 patients within our inclusion time period and reported on in the paper 24 cases reported in Ibdah 1999 not included	6 LCHADD homozygous E474Q 6 heterozygous LCHADD E474Q	Analysed child and the mother using enzymatic assays and mutation analysis (molecular prenatal diagnosis)	Phenotype Maternal phenotype Death
German studies					
Sander 2005[36]	Screening programme results, followed by cohort study of identified cases Study duration: 1999-2005 Study setting: Screening laboratory, Hannover, Germany.	N=9 Initially screened 1,200,000 neonates. Identified 11 cases. 2 removed from our review as they were LKAT cases LCHADD n=7 MTPD n-2	5 homozygous LCHADD 1528G>C 2 LCHADD mutation unspecified 2 MTPD subunit unspecified	NBS screening (method and test accuracy details included for question 3) Enzyme analysis in cultured fibroblasts or lymphocytes and mutation analysis	Maternal phenotype Death Age at death Hypoglycaemia Cardiomyopathy Rhabdomyolysis Motor retardation

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Sperk 2010[38]	Case series (6 cases) – clinical histories obtained from referring physicians Maximum follow up until age 5 years Study duration: 3 years Study setting: University Childrens hospital, Dusseldorf, Germany Number of centres:1	N=6 LCHADD n = 3 MTPD n= 3	3 homozygous LCHADD 1528G>C 3 MTPD: 1 in alpha subunit 2 in beta subunit	Patients were all screened in accredited German screening laboratories according to the screening guidelines. Further molecular or enzymatic diagnostic work-up	Severity Death Cardiomyopathy Myopathy Hypoglycaemia
Spiekerkoetter 2002[40]	Case series Time period/follow up: NR Study setting and centres: NR	N=2 Both MTPD 1 of the patients reported in Hintz 2002	Both alpha subunit mutations	Clinical and family history obtained from referring physicians. Children then had enzyme analysis in fibroblasts, sequencing analysis and microsatellite repeat analysis	Severity Maternal phenotype Death Age at time of death Cardiomyopathy Hypoglycaemia Lactic acidosis
Germany and international cohort studies					
Spiekerkoetter 2003[42]	Cohort study Follow up period: Age to age 22 years Study setting: Vanderbilt University Medical Centre or Washington University Number of centres: 2	N=15 All MTPD From 13 families	All beta subunit mutations	Documented MTPD cases analysed with fibroblasts, western blot analysis and genetic analysis	Severity Maternal phenotype Death Age at time of death Hypoketotic hypoglycaemia Cardiomyopathy Skeletal myopathy Neuropathy Peripheral neuropathy

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Spiekerkoetter 2004[39]	Retrospective cohort study using patients from 4 previous studies Follow up period: Up to 25 years – from diagnosis to follow up 12 years Study setting: Unclear; USA, Germany, Israel, Canada	N=11 All MTPD All with neuromyopathic phenotype Patients 1-8 from Spiekerkoetter 2003, 9 and 10 from Ibdah 1999 and 11 from Tein 1995. Latter two studies excluded here due to date of study	8 cases with beta subunit mutations 3 cases with alpha subunit mutations	10/11 confirmed by enzyme analysis. All 11 had mutation analysis	Severity First myoglobinuria Median (range) Respiratory failure median (range) First symptom Hypoglycaemia Hypotonia Motor delay Muscle weakness Leg weakness Respiratory failure Lethargy Progressive weakness Episodic severe weakness Symptoms induced by exercise Symptoms induced by illness Peripheral neuropathy Respiratory failure Foot deformities Improved with therapy
Spiekerkoetter 2009[41]	Retrospective cohort. Questionnaires sent to metabolic centres Follow-up time: NR Study setting: Metabolic Centres, Germany/Switzerland/Austria /the Netherlands Number of centres: 18	N = 37 20 LCHADD, 7 MTPD Possibly some patients previously reported in Spiekerkoetter 2004 but not specified	20 LCHADD homozygous 1528G>C mutation 7 MTPD mutations subunit not specified	Unclear	Severity Maternal phenotype Death Age of death Cardiomyopathy Arrhythmias Reye Syndrome Hypoglycaemia Hypotonia/myopathy Retinopathy Neuropathy
USA studies					

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Boese 2016[23]	Retrospective case series Tine period: 20/9/1994-18/8/2015. Follow up period median 5.6 years (0.3-20.2y) Study setting: Oregon health and science University (OHSU) Casey Eye Institute Number of centres: 1	N=21 18 LCHADD, 3 MTPD	9 homozygous LCHADD 1528G>C mutation 7 compound heterozygous LCHADD 2 LCHADD 2nd mutation not identified 3 MTPD beta subunit	A combination of clinical findings, acylcarnitine analysis, or enzymatic assays in cultured skin fibroblasts. All underwent molecular testing	Clinical presentation – hypoketotic hypoglycaemia, cardiorespiratory arrest, rhabdomyolysis or asymptomatic History >1 episode of rhabdomyolysis Measurements for chorioretinopathy
De Biase 2017[26]	Retrospective cohort (Retrospective data collected via chart review) Average follow-up time: nearly 10 years (9.2± 5.9 years) Study setting: Metabolic Clinic University of Utah, USA Number of centres: 1	N=5 4 LCHADD patients and 1 MTPD patient	4 homozygous LCHADD 1528G>C 1 MTPD subunit unspecified	NBS or clinically diagnosed. Genotyping method unspecified	Maternal phenotype Hypoglycaemia at diagnosis Muscle pain Failure to thrive Retinitis pigmentosa
Gillingham 2017[28]	Randomised double blind parallel RCT (retrospective data collected on time of diagnosis) Follow-up time NR Study setting: Oregon Health and Science University and University of Pittsburgh, USA Number of centres: 2	N = 12 10 LCHADD patients and 2 MTPD patients	6 homozygous LCHADD 1528G>C 4 heterozygous LCHADD 2 heterozygous MTPD. Subunit unspecified	NBS or clinical Genotyping method not specified	Cardiac complications

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Hintz 2002[30]	Case series Time period: NR Study setting and number of centres: NR; USA	N=2 1 LCHADD, 1 MTPD	1 homozygous LCHADD 1528G>C 1 MTPD alpha subunit mutation	Clinically with early neonatal presentations Urine organic acid analysis, plasma acylcarnitine analysis, molecular genetic analysis. Fibroblast culture for enzyme analysis planned but not performed in one case	Death Age at time of death Presented with: Hypoglycaemia Hyperkalaemia hypocalcaemia Seizure activity Renal failure Neurodevelopmentally normal
Vocley 2016[45]	Case series Time period: Unclear, starting September 2015 Setting and centres: NR	N=4 10 cases included in the study, only 4 relevant to these conditions 2 LCHADD, 2TFPD All patients being treated with Triheptanoin	2 homozygous LCHADD 1528G>C 2 MTPD subunit unspecified	NR	Maternal phenotype Death Age at time of death Initial presentation – Hypoglycaemia, Cardiomyopathy, Heart failure, biventricular dysfunction, Rhabdomyolysis Cardiomyopathy Age of cardiomyopathy presentation
Waisbren 2013[46]	Cohort study Follow up period: 12.5 years Study setting: Metabolic centre, Massachusetts, USA Number of centres: 1	N=2 85 children with FAODs, only with included in this review with the relevant condition Both LCHADD	2 homozygous LCHADD 1528G>C	NBS screening	Developmental delay (IQ<85) Speech delay Muscle pain Mild retinal function deficits
Southeast Asian Studies					

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Bo 2017[22]	Cohort study Study duration/follow up period: NR Study setting: Shimame University; Japan Number of centres: 1	N=14 5 cases reported from Purevsuren 2009, 4 cases in case reports (Kobayashi 2015, Naiki 2014 and Yagi 2011) plus 5 new cases described All MTPD	3 HADHA mutations 10 HADHB mutations 1 subunit unspecified	Physician questionnaires Genomic DNA using DNA Micro Kit. Western blot analysis and enzyme assay using fibroblasts	Severity Maternal phenotype Death Age at time of death Symptoms at onset – Hypotonia, Coma, Cardiac features, Respiratory failure, Cardiomyopathy, Cardiopulmonary arrest, Seizures, Lactic acidosis, Lethargy, Rhabdomyolysis, Fatigue Myalgia Current outcome – rhabdomyolysis, mental retardation, recurrently Hypotonia, developmental delay, wheel-chair mobile, artificial ventilation, hypoparathyroidism, peripheral neuropathy
Choi 2007[25]	Case series Study duration/follow up period: NR Study setting: NR, Seoul, Korea Number of centres: NR	N=4 All MTPD	2 alpha subunit mutations 1 beta subunit mutation 1 subunit unspecified	TMS result, urine analysis and DNA mutation analysis from peripheral blood leukocytes	Death Age at time of death Respiratory arrest Metabolic acidosis Hypoglycaemia Cardiac failure Hepatic dysfunction Myoglobinuria Acute renal failure Rhabdomyolysis Pericardial effusion Lethargy Elevated liver enzymes

Study	Study design	Participants	Genotypes reported	Diagnosis/ Genotyping	Outcomes reported
Purevsuren 2009[35]	Case series Study duration/follow up period: NR Study setting: NR; Japan Number of centres: NR	N=5 All MTPD	All beta subunit mutations	Enzyme assay, western blot analysis, mutations of HADHA and HADHB genes analysis	Death Age at time of death Age at onset Other outcomes – clinical course: Cardiomyopathy Hypoglycaemia Hyperammonemia Metabolic acidosis Liver dysfunction Lactic acidemia Hypotonia Developmental delay Muscle pain
Kang, 2018[33]	Retrospective cohort Follow-up time: ~10 years Time period: May 2002 – February 2016 Study setting: Department of Medical Genetics, Asan Medical Center Children's Hospital, Seoul Korea Number of centres: NR	N=7 22 included in their study but only 7 with relevant conditions LCHADD/MTPD not differentiated in the study but by mutations all are suggestive of MTPD	2 alpha subunit mutation 5 beta subunit mutations	Plasma acylcarnitines analysis, molecular analysis	Death Age at time of death In surviving patients: recurrent rhabdomyolysis, sensorimotor polyneuropathy, difficulty walking/running/climbing

NR, not reported

Table 18. Characteristics of included test accuracy studies

Study	Number screened	Marker(s)	Cut off(s)	2x2 table				Test accuracy			
				TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)*	NPV (95% CI)
Assessed test accuracy, extractable data											
Bonham 2013[9]	437,187	Primary: C16OH Secondary: C16-OH; C16:1-OH; C18-OH	>0.12; >0.15 – lowered from 0.2	1[b]	2	NR	NR	NA	NA	0.33 (0.02, 0.87)	NA
Couce 2011[59]	210,165	C16OH, C18-10H, C18OH	0.79/0.68uM, 0.97/0.51uM, 1.4/0.7uM	2[a]	0	NR	NR	NA	NA	1 (0.52, 1)	NA
Frazier 2006[60]	239,415	C16-OH, C18:1 , C18:1-OH	>0.18[c] >4.08[d] >0.14[d]	2[a]	0	NR	NR	NA	NA	1 (0.20, 1)	NA
Lindner 2011[67]	1,084,195	Primary: C14OH Secondary (and/or): C16:1OH; C16OH; C18:1OH; C18OH	>0.12; umol/L >0.22; >0.20; >0.12; >0.11	6 (5 NBS, 1 cascade testing)[b]	0	NR	NR	NA	NA	1 (0.52, 1)	NA
Lund 2012[5]	504,049	Primary: C16OH Secondary: C18:1OH	>0.12U; >0.1U	3[b]	0	NR	NR	NA	NA	1 (0.31, 1)	NA
Mak 2018 [21]	2440	Unclear	Unclear	0	2	NR	NR	NA	NA	0 (0,0.82)	NA
Sander 2005[36]	1,200,000	C16-OH, C18:1-OH, C14:1, C14-OH	>0.35 µmol/L >0.2 µmol/L >0.08 µmol/L >0.06 µmol/L	9 (7 LCHADD, 2 MTP)	10	NR	NR	NA	NA	0.47 (0.25, 0.71)	NA
Smon 2018[16]	10,048	C16:1OH, C16OH, C18:1OH, C16 OH/16	99.9th percentiles NR	0	8	NR	NR	NA	NA	0 (0, 0.40)	NA

Yang 2018[18]	100,077	LCHADD: C16OH MTPD C18OH and C18:1OH:	>0.04 µmol/L; >0.03 µmol/L; >0.05 µmol/L	0	13 (4 LCHAD, 9 TPD)	NR	NR	NA	NA	0 (0, 0.28)	NA
Zytkovicz 2001[48]	164,000	C16OH	0.1	0	5	NR	NR	NA	NA	0 (0, 0.54)	NA

*=calculated by report authors, [a] LCHAD, [b] LCHAD/MTP, [c] single cut-off, [d] independent elevation not diagnostic; NA = not applicable, NBS = newborn blood spot, NR = not reported

Table 19. Baseline study characteristics of included test accuracy studies

Study	Country, time period	Study design	Source and type of material	Age at specimen collection	Method of extraction and derivatisation	Type of MS/MS conditions	Analytes and cut-off	Re-testing of positive samples and reference standard
UK and Europe								
Bonham 2013 (ENBS trial) [9]	United Kingdom 16th July 2012 – 19th July 2013	Prospective expanded newborn screening study in 437,187 neonates 6 centres	DBS, Guthrie filter paper	5-8 days as per UK regulations	Not reported but general UK guidelines are: Internal standards (IS) of stable isotopes of Phe, Tyr, Leu, Met, C8, C5, C5-DC are prepared in a suitable solvent (e.g. 80% methanol) and are used to elute Phe, Tyr, Leu, Met, C8, C10, C5, C5-DC from a punched dried blood spot disc in multiwell plates. MS/MS sampling can be direct from the original plate (with blood spots in situ) or the eluates may be transferred to a fresh plate before sampling	NR – UK methods	Primary: C16OH Secondary: C16-OH; C16:1-OH; C18-OH >0.12; >0.15 – lowered from 0.2	Referred directly to specialist metabolic physician. Reference standard: blood, urine and DNA for mutation analysis

Study	Country, time period	Study design	Source and type of material	Age at specimen collection	Method of extraction and derivatisation	Type of MS/MS conditions	Analytes and cut-off	Re-testing of positive samples and reference standard
Couce 2011 [59]	Galicia, Spain July 1st 2000 – July 1st 2010	Propsective newborn screening study in 210,165 neonates. Cohort of positive cases then followed prospectively. Mean follow up of 69 months	DBS. Whatman 903 paper	5-8 days up to 2002. 2003-2010 on day 3	NR	MS/MS Applied Biosystems Sciex 2000 apparatus	C16OH, C18-10H, C18OH 0.79/0.68uM, 0.97/0.51uM, 1.4/0.7uM Not reported if cut off was pre-specified	Positive result repeat tested. High results referred to clinical unit if borderline second sample requested by NBS lab, if second result also high then referred. Reference test: Enzyme and/or molecular studies
Lindner 2011 [67]	South West Germany April 1998-September 2001 Lindner January 1st 1999 - 30th June 2009	Prospective newborn screening programme in 250,000 neonates up to 2001. Crossover with Lindner 1,084, 195	DBS, Schleicher ~& Schuell 2992 up to 1999, then Schleocher and Schuell 903 since 2000. One 3mm punch	3rd and 7th day of life. 5th day median up to 2001 Up to 2005 day 3-5 2005 onwards 36-72 hours	DBS into single well of 96 well microtiter filter plate, 100 uL methanol stock solution containing internal standards. Unclear whether standards made in house or purchased. Centrifuged after 20 mins and eluate evaporated – reconstituted in 60 uL on 3 N HCl/butanol. Sealed and incubated at 65 degrees 15 mins. Dried and again reconstituted in 100 uL solvent of acetonitrile/water/formic acid. Acylcarnitines measured by positive precursor ion scan of m/z 85	API 365 triple quadruple TMS with an ion spray device 2.5 min run time	C14OH and/or C16:1OH; C16OH; C18:1OH; C18OH >0.12; >0.22; >0.20; >0.12; >0.11 umol/L Cut-off now pre-specified: 99.5th percentile based on data collected from 10,000 healthy neonates	Repeat analysis. Only TP if both samples positive. Any discrepancy a third test done and interpreted by an experienced metabolic disease specialist. Positive cases repeated DBS or referral. Urine sample for gas chromatography/MS analysis requested Reference standard: Enzyme activity in fibroblasts/lymphocytes 2003 onwards – acylcarnitine profile in plasma, DBS and or genotype and or enzyme activity

Study	Country, time period	Study design	Source and type of material	Age at specimen collection	Method of extraction and derivatisation	Type of MS/MS conditions	Analytes and cut-off	Re-testing of positive samples and reference standard
Lund [5]	Denmark, Faroe Islands and Greenland Feb 1st 2002 – Mar 31st 2011 (trial 2002 – 2009)	Prospective trial screening programme and implemented programme from 2009 in 586,979. Affected children compared to a historic cohort diagnosed clinically. 2-109 month follow up	DBS, Schleicher and Schuell 903 filter paper up to 2010, then replaced by Ahlstrom 226 filter paper	4-9 days after birth (median 5 days). After Feb 2009 2-3 days and median age 2.5 days.	Protocol developed at Staten's Serum Institut, Copenhagen.	SciEx API2000 up to 30th June 2003. 2003 – Feb 2nd 2009 PerkinElmer Neogram AA and acylcarnitines TMS Kit. Feb 2009 – PerkinElmer NeoBase non-derivatized MS/MS kit. Waters Micromass Quattro micro TMS	Primary: C16OH Secondary: C18:1OH >0.12U; >0.1U Cut off pre-specified	Positive samples re-analaysed. Centre for IMD Copenhagen University Hospital contacted for confirmatory tests. Reference standard: Urine organic acids, plasma acylcarnitines, molecular-genetic analyses
Sander [36]	Germany 1999 – 2005	Prospective screening programme in 1,200,000 neonates. Cohort of 11 cases followed up	DBS, S&S 2992 filter paper (Schleicher & Schüll) 3.2mm	97.5% by day 5. Some several days later. Recommend 36-72 hours	According to standard methods. Extracted 200uL methanol containing deuterated internal standards. After evaporation of the extracts, acylcarnitines and amino acids were butylated using 50uL/HCl at 65 degrees 15 mins. Material solved in methanol. Quantification comparing signals with internal standards	3 TMS (TMS quarto LC, Micromass, Manchester). Hydroxyaclcarnitines measured in MRM mode. Acylcarnitines and free carnitine in full scan MCA mode	C16-OH, C18:1-OH, C14:1, C14-OH Threshold not pre-specified - Chosen after a patient with known MTP and 5000 normal controls	Repeated analyses of fresh dried blood spots Reference standard: Enzyme analysis in cultured fibroblasts or lymphocytes, mutation analysis
Smon [16]	Slovenia 2013-2014	Retrospective pilot study of blood spots in 10048 newborns	DBS, One 3mm disk	48-72 hours Analysed 6-11 months later	Analytes were extracted using the extraction buffer with added internal standards. Analytes were derivatized to butyric esters, the derivatization reagent was evaporated and samples were	PerkinElmer 200 HPLC system coupled to AB Sciex 3200 QTRAP (AB Sciex, Singapore)	C16:1OH, C16OH, C18:1OH, C16 OH/16 Cut-offs at 99.9th centiles	113 with the highest probability of IEM were retested Reference standard: NGS, organic acids in urine, additional

Study	Country, time period	Study design	Source and type of material	Age at specimen collection	Method of extraction and derivatisation	Type of MS/MS conditions	Analytes and cut-off	Re-testing of positive samples and reference standard
					analysed after reconstitution in the reconstitution buffer	using chromosystems' kit amino acids and acylcarnitines from dried blood (Gruefelfing, Germany)	Set after the completion of the study	acylcarnitine test using DBS
USA								
Frazier [60]	North Carolina, USA 28th July 1997 – 28th July 2005	Prospective newborn screening programme in 944,078 neonates. 239,415 included in the analysis 2 centres	DBS; Scleicher and Schuell 903 filter paper	At least 24 hours. Mean 39 hours	Used Millington et al 1990 extraction method. 1/8 inch pa-per punch, 96-well Evergreen polystyrene microtitre plates. Used methanol with deuterated internal standards. KIT method using internal standards from VU University medical centre amsterdam Covered and rotated at 200rpm room temp 30 mins Set on a heat block 45-55 degrees 30 mins Derivatized 100ul of 3 mol/L HCl in n-butanol and incubating 30 mins 60 degrees – evaporated butanolic HCl for 20 mins. Dried at room temperature 5mins Reconstituted 100 ul 80% acetonitrile, incubated 15 mins.	Micromass Ltd/Water Corp Quattro LC TMS Gilson instruments 215 Liquid Handler autosampler and Hewlett Packard/Agilent Technologies series 1100 Isocratic HPLC pump 2 mins run time	C16-OH, C18:1, C18:1-OH >0.18, >4.08, >0.14 Latter two cut-offs were independent elevation not diagnostic. Cut-off decided in January 2003	Repeat punching from the same card. If results were borderline a request sent for a repeat sample. If above the diagnostic cut-off sent to a metabolic specialist. Reference standard: Urine organic acids and a plasma acylcarnitine profile. Enzyme and mutation analyses done whenever the tests were available and approved by 3rd party reimbursers

Study	Country, time period	Study design	Source and type of material	Age at specimen collection	Method of extraction and derivatisation	Type of MS/MS conditions	Analytes and cut-off	Re-testing of positive samples and reference standard
Zytkovicz [48]	New England, USA 1991-2001	Prospective screening programme in 160,000 neonates	S&S Grade 903 filter paper (Schleicher and Schuell)	1-3 days after birth	Butanolic-HCl plus 55mL of acetyl chloride to 500mL butanol. Internal standards purchased from HJ ten Brink. Single 3.2mm DBS placed in a polypropylene microtiter plate. Methanolic internal standard solution added manually of with Labsystemns Multidrop Dispensor. Microplate shaken for 20 min extraction. Methanol extract then manually transferred to a second polypropylene microtiter plate and dried with a hot air blower. 65 degree forced air oven for 15 mins	1100 Hewlett Packard HPLC pump. Model 215 Gilson autosampler sent sample to MS/MS. Micromass Quattro LC triple-quadrupole TMS Flow of ~95 uL/min Injection – to – injection time ~1.9 mins Analyzed with multiple reaction monitoring	C16OH/d-C16 Cut-offs: 0.1	Positive samples re-punched the DBS and reanalysing sample. If one of more markers were out of range a full metabolic work up was recommended. If initial specimen had very increased marker then initiated straight away. Reference standard: according to standard metabolic criteria
Asia								

Study	Country, time period	Study design	Source and type of material	Age at specimen collection	Method of extraction and derivatisation	Type of MS/MS conditions	Analytes and cut-off	Re-testing of positive samples and reference standard
Mak 2018 [21]	Hong Kong	Pilot screening study 2440 neonates	Heel prick test in compliance with Clinical and Laboratory Standard Institute Guidance	24h-28 days *(24 to 48 hours (n=2064, 84.6%), 3 to 5 days (n=331, 13.6%), 5 to 7 days (n=9, 0.4%), and 7 to 28 days (n=36, 1.5%).*	in compliance with Clinical and Laboratory Standard Institute Guidance	Two commercial DBS assay kits: (1) MassChrom Amino Acids and Acylcarnitines from Dried Blood/ Non-derivatised (Chromsystems Instruments & Chemicals GmBH, Gräfelfing, Germany); and (2) NeoBase Non-derivatized MSMS kit (with succinylacetone assay; PerkinElmer, Waltham manual puncher and an autopuncher for DBS preparation, a fully automated online extraction system (DBS-MS 500; CAMAG, Muttenz, Switzerland)	Not specified which markers were used for LCHADD/MTPD	Positive samples reanalysed. Reference standard: measurements of functional metabolites (mainly plasma amino acid levels, plasma acylcarnitine levels, and urine organic acid levels) and genetic diagnosis by DNA sequencing wherever appropriate.

Study	Country, time period	Study design	Source and type of material	Age at specimen collection	Method of extraction and derivatisation	Type of MS/MS conditions	Analytes and cut-off	Re-testing of positive samples and reference standard
Yang 2018	Jining City, China 2014-2015	Prospective screening study	3.2mm disk US S&S 903	3-37 days (average 5.2±3.8)	Disk dropped into 96-well U bottomed plate. 100uL of extraction solution added (containing a mixture of the respective stable-isotope-labeled internal standard).. Incubated for 45 min at 45 degrees on a shaker set at 650 rpm then incubated at room temperature for 2 hours.	LC-MS/MS kit (NeoBase Non-derivatized MS/MS kit, Perkin Elmer(Triple quadrupole TMS equipped with an ACQUITY TQ detector (Wasters, MA, USA) coupled to a Waters 1525 binary HPLC pump.	LCHADD C16OH cut off >0.04 µmol/L MTPD C18OH >0.03 µmol/L; C18:1OH >0.05 µmol/L	1. MS/MS test repeated and compared to first results. 2. Urinary organic acids determined by GC-MS, or high precision DNA mass spectrometry for 39 genetic mutations

NR: not reported

Table 20. Characteristics of outcome studies: Question 4

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Swedish cohort study											
Fahnehjelm 2008 [49]	Cohort study Average follow-up time: median 7.5 years (2.3-14.8years) Time period/study duration: Not reported Study setting: Karolinska University Hospital and Uppsala University Hospital, Sweden Number of centres: 2	n = 10 LCHADD =10. Asymptomatic screened (AS) n=1 (cascade testing). Age of diagnosis/ treatment: First days of life Clinical presentation of symptoms: n=9 Clinical symptoms (S) but no acute illness: n=4 Severe symptoms (SS) (elevated liver enzymes and cardiomyopathy and/or seizures): n=5 Age of diagnosis/treatment: 0-1m (n=1), 1-6m (n=2), >6m (n=6)	All patients received a dietary treatment of low fat intake and essential fatty acid supplementation. All children also received DHA. Compliance of treatment is not reported	NR	NR	**ERG findings** Asymptomatic screened: Abnormal: 0/1 Pathological: 0/1 S clinical Abnormal: 2/4 (50%) Pathological: 2/4 (50%) SS clinical: Abnormal: 1/5 (20%)[a] Pathological: 4/5 (80%) **Photophobia** Asymptomatic screened: 1/1 (100%) S clinical: 3/4 (75%) SS clinical: 4/4 (100%)[b] **Nyctalopia** Asymptomatic screened: 0/1 S clinical: 0/4 SS clinical: 2/4 (50%)[c]	**Neonatal hypoglycaemia** Asymptomatic screened: 0/1 S clinical: 3/4 (75%) SS clinical: 4/5: (80%)	**Psychomotor development** Asymptomatic screened: DD: 0/1 Severe DD: 0/1 S clinical: DD: 1/4 (25%) Severe DD: 0/4 SS clinical: DD: 3/5 (60%) Severe DD: 1/5 (20%)	**Number of decompensations** (unspecified): Asymptomatic screened: 0-4: 0 5-10: 1/1 (100%) >10: 0 S clinical: 0-4: 1/4 (25%) 5-10: 1/4 (25%) >10: 2/4 (50%) SS clinical: 0-4: 2/5 (40%) 5-10: 2/5 (40%) >10: 1/5 (20%)	Weak Areas of weakness: confounders and blinding

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Fahnehjelm 2016 [50]	Cohort study. Prospective and retrospective data collection Average follow up time: median 15 years (3-26 years). Time period/study duration: Not reported Patients diagnosed between 1990 to using the same treatment guidelines Study setting: Karolinska University Hospital and Uppsala University Hospital, Sweden Number of centres: 2	N=12 LCHADD = 12 Asymptomatic screened n=3 (2 by NBS, 1 unspecified). Age of diagnosis/treatment: First days of life Clinical presentation of symptoms: n=9 Clinical symptoms (S) but no acute illness: n=4 Severe symptoms (SS) (elevated liver enzymes and cardiomyopathy and/or seizures): n=5 Age of diagnosis/treatment: 0-1m (n=1), 1-6m (n=2), >6m (n=6)	All patients received a dietary treatment of low fat intake and essential fatty acid supplementation. 11/12 had DHA. 8/12 continuous night feeds Dietary compliance: Asymptomatic screened: all acceptable S clinical: 1/4 (25%) poor 3/4 (75%) acceptable SS clinical: 3/5 (60%) poor 2/5 (40%) acceptable	NR	NR	NR	**ERG findings** AS: Subnormal: 1/2[d] (50%) Pathological: 0/2 S clinical: Subnormal: 2/4 (50%) Pathological: 1/4 (25%) SS clinical: Subnormal: 1/5 (20%) Pathological: 4/5 (80%) **Best corrected visual acuity** AS: no/mild visual impairment: 2/2 (100%) Missing data 1/3 (33.3%) S clinical: Moderate impairment: 1/4 (25%) No/mild impairment: 3/4 (75%) SS clinical: Blindness: 1/4 (25%) No/mild impairment: 2/4 (50%) Missing data: 1/5 (20%) **Ocular fundi** AS:Normal: 1/3 (33.3%) Subnormal: 2/3 (66.7%) S clinical: Pathological: 4/4 (100%) SS clinical: Pathological 4/5 (80%) Severely pathological 1/5 (20%)	**Epilepsia** Asymptomatic screened: 0/3 S clinical: 1/4 (25%) SS clinical: 2/5 (40%) **Neonatal hypoglycaemia** Asymptomatic screened: 1/3 (33.3%) S Clinical: 3/4 (75%) SS clinical: 4/5 (80%)	NR	NR	Weak Areas of weakness: confounders and blinding

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Haglind 2013 [51]	Cohort study, retrospective data collection of medical reviews Time period/study duration: Not reported Patients' aged up to 20 years Study setting: Karolinska University Hospital and Uppsala University Hospital, Sweden Number of centres: 2	n = 10 LCHADD =10. Asymptomatic screened n=1 (cascade testing). Age of diagnosis/treatment: 2 days Clinical presentation of symptoms: n=9 Clinical symptoms (S) but no acute illness: n=4 Severe symptoms (SS) (elevated liver enzymes and cardiomyopathy and/or seizures): n=5 Age of diagnosis/treatment: mean 6.1 months (up to 13m)	8/10 received DHA. 9/10 had a PEG with continuous night feeds. MCT fat, vitamins, minerals, and trace elements 9/10. Fasting limited to 3-4 hours. 2/10 uncooked corn starch. 1 had carnitine deficiency so given carnitine supplements of 25-50mg/kg/day. Did not record compliance	NR	NR	NR	NR	**Neonatal hypoglycaemia** Asymptomatic screened: 0/1 S clinical: 3/4 (75%) SS clinical: 4/5: (80%)	NR	**No. episodes extra carbohydrate intake:** Asymptomatic screened: >5: 1/1 (100%) S Clinical: <5: 1/4 (25%) >5: 1/4 (25%) >10: 2/4 (50%) SS clinical: <5: 2/5 (40%) >5: 1/5 (20%) >10: 2/5 (40%) **Overweight before 6 years** Asymptomatic screened: 1/1 (100%) S clinical: 4/4 (100%) SS clinical: 3/5 (60%) **ISO BMI** >30kg/m² for 1-5years: Asymptomatic: 1/1 (100%) S clinical: 3/4 (75%) SS clinical: 2/5 (40%) ≥ 25kg/m² at last assessment: AS: 1/1 (100%) S Clinical: 4/4 (100%) SS clinical: 2/5 (40%)	**Moderate** **Areas of weakness: confounders**

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
European single country studies											
Immonen 2016 [31]	Prospective cohort (followed prospectively but using diagnosis data from retrospectively collected hospital records). Comparison with historical cohort (24/28 diagnosed post mortem) Follow-up time (age of patients at the end of the study): 1-11 years Time period: 1997-2010 Study setting: Hospitals in Finland Number of centres: NR	N = 16 N=11 for some outcomes where cases did not survive All LCHADD cases Asymptomatic screened n=1 (cascade testing) Age at treatment: Birth Symptomatic clinical n=15 Age at presentation: Birth to 0.42 years (~5 months). Mean 0.27 years. Age at diagnosis: Up to 6 months	Age of treatment: 1-30 days of diagnosis All patients in both groups received a low-fat diet, MCT, essential fatty acids and DHA (this was 10 clinical patients as the remainder were not alive). Fasting of more than 3 or 4 hours avoided in infancy and childhood. Good compliance of diet in all bar 1 patient in the 10 patients which survived plus 1 which did then die.	AS screened: 0/1 Symptomatic clinical: 6/15 (40%) Age of death for 5/6 mean 8.5m. Median 5m (3m-2y)	**Cardiomyopathy at diagnosis** Asymptomatic screened: 0/1 S Clinical 6/10 (60%) **Cardiomyopathy at the end of study period**: Asymptomatic screened: 0/1 S Clinical 4/10 (40%)	NR	**Retinopathy** Asymptomatic screened: 0/1 S Clinical: Mild: 7/10 (70%) Moderate: 1/10 (10%) Yes: 1/10 (10%) No: 1/10 (10%)	**Neuropathy** Asymptomatic screened: 0/1 (not detected) S Clinical: Mild: 2/9 (22.2%) Moderate 1/9 (11.1%) None: 2/9 (22.2%) Not detected: 4/9 (44.4%)	NR	**IQ normal** Asymptomatic screened: 1/1 (100%) S Clinical: 8/9 (88.9%) – serious brain damage in one case following metabolic crisis **Obesity** No obesity in either groups	Weak Areas of weakness: confounders and data collection methods

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Sperk, 2010	Case series (6 cases) – clinical histories obtained from referring physicians Maximum follow up until age 5 years Study duration: 3 years Study setting: University Childrens hospital, Dusseldorf, Germany Number of centres: 1	N=6 LCHADD n = 3 MTPD n= 3 Asymptomatic screened: n= 3 (1 LCHADD, 2 MPTD) Symptomatic screened n=3 (2 LCHADD, 1 MTPD) All diagnosed and began treatment 4-5 days	Type of treatment not reported Dietary adherence not reported	Asymptomatic screened: 1/3 (33.3%) LCHADD patient Age at death: 3 months Symptomatic screened: 0/3	**Cardiomyopathy** Asymptomatic screened: 0/3 Symptomatic screened: 2/3 (66.7%) both cases MTP	NR	NR	**Hypoglycaemia** Asymptomatic screened: 1/3 (33.3%) LCHADD case Symptomatic screened: 3/3 (100%)	**Myopathy** Asymptomatic screened: 0/3 Symptomatic screened: 1/3 (33.3%) MTP case	NR	Weak Areas of weakness: confounders and data collection methods
European collaboration studies											

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Karall, 2015 [52]	Retrospective cohort (review of medical records) Study duration: Birth – October 2013 Follow-up time: 0.9-15.4 years (median 7.8 years, mean 6.9 years) Study setting: Metabolic Centres in Austria Graz, Innsbruck, Salzburg, Vienna) and Germany (Munich) Number of centres: 5	N = 14 All LCHADD cases Asymptomatic screened n= 6 Age at diagnosis median (range) : 1.5d (1-10 days) Symptomatic screened n=3 Age at diagnosis median (range) : 15 days Pre NBS clinical:n= 3 Age at diagnosis median (range) : 5m (3-20m) False negative (FN) screen clinical: n=2 Age at diagnosis median (range) : 4.5m (4-5m)	All cases received low-fat diet and MTC. Triheptanoin was used in 2/3 symptomatic screened, 1/3 pre NBS and 1/2 FN NBS Essential fatty acids (walnut oil) were given to all bar 1 pre NBS clinical case DHA given to all asymptomatic screened group, 1/3 in symptomatic screened group, 2/3 in pre NBS and 1/2 in FN NBS PEG used in 1/6 asymptomatic screened, 1/3 in pre NBS and ½ FN NBS Dietary compliance not reported	NR	**Cardiomyopathy** Asymptomatic screened: 1/6 (16.7%) Symptomatic screened: 1/3 (33%) Pre NBS clinical: 3/3 (100%) FN NBS clinical: 2/2 (100%) **Median age of cardiomyopathy** Asymptomatic screened: 4m Symptomatic screened: 9m Pre NBS clinical: 23m (3-156m) FN NBS clinical: 4.5m (4-5m)	**Hepatopathy** Asymptomatic screened: 1/6 (16.7%) Symptomatic screened: 0/3 Pre NBS clinical: 2/3 (66.7%) FN NBS clinical: 2/2 (100%) **Median age of Hepatopathy** Asymptomatic screened: Neonatally Pre NBS clinical: 13m (3-23m) FN NBS clinical: 4.5m (4-5m)	**Retinopathy** Asymptomatic screened: 2/6 (33.3%) Symptomatic screened: 1/3 (33.3%) Pre NBS clinical: 3/3 (100%) FN NBS clinical: 2/2 (100%) **Median age of retinopathy** Asymptomatic screened:53m (50-56m) Symptomatic screened: 39m Pre NBS clinical: 24m (23-108m) FN NBS clinical: 40m (38-42m)	NR	Psychomotor developmental normal in all patients	**Organ failure at diagnosis** Asymptomatic screened: 0/6 Symptomatic screened: 3/3 (100%) Pre NBS clinical: 3/3 (100%) FN NBS clinical: 2/2 (100%) **No. of hospitalisations Mean, median (range)** Asymptomatic screened: 8.2, 6 (2-23) Symptomatic screened: 19, 17 (6-34) Pre NBS clinical: 11, 8 (7-18) FN NBS clinical: 19.9, 19.5 (17-22) Growth normal in all patients	Moderate Areas of weakness: confounders

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Spiekerkoetter, 2009 [41]	Retrospective cohort (questionnaire study) Follow-up time: NR Study setting: Metabolic Centres, Germany/ Switzerland/ Austria/the Netherlands Number of centres: 18	N = 75 Relevant to this review = 27 20 LCHADD, 7 MTPD Screened: n=10 7/10 symptomatic at NBS LCHADD = 7 MTPD = 3 Age at diagnosis: Newborn Clinically diagnosed N=17 LCHADD=13 MTPD = 4 Age at diagnosis LCHADD 5m (3d-11years) MTPD 1 year (1d-4.5y)	Data available on LCT and MCT in 14/27 and 17/27 of LCHADD patients, LCT intake restricted in 13/14 17/17 supplemented with MCT (11/14 received additional carbohydrates 2/14 on continuous overnight nasogastric tube feeding. 1 supplemented with DHA 1 receiving Triheptanoin Dietary compliance not reported	Screened: 2/10 (20%) both MTPD Age of death median (range): 5.5d (3-8d) Clinical: 6/17 (35.3%) 3 LCHADD 3 MTPD Age of death median (range): ~2m (2d-4y)	**Cardiomyopathy** Screened: 4/10 (40%) 1 LCHADD, 3 MTPD Clinical: 8/17 (47%) 7 LCHADD, 1 MTPD **Arrhythmias** Not reported in screened group. Clinical: 1/17 (5.9%)	**Reye syndrome** Screened: 3/10 (30%) 1 LCHADD, 2 MTPD Clinical: 6/17 (35.3%) All LCHADD	**Retinopathy** Screened: NR Clinical: 6/17 (35.3%) 2 LCHADD, 1 MTPD	**Neuropathy** Screened: NR Clinical: 3/17 (17.7%) 2 LCHADD, 1 MTPD **Hypoglycaemia** Screened: 4/10 (40%) 3 LCHADD, 1 MTPD Clinical: 15/17 (83.2%) 13 LCHADD, 2 MTPD	**Hypotonia/ Myopathy** Screened: 4/10 (40%) 2 LCHADD, 2 MTPD Clinical: 14/17 (82.4%) 12 LCHADD, 2 MTPD	NR	Weak Areas of weakness: confounders and data collection methods

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
International studies											
Boese 2016 [23]	Retrospective case series (cohort) Time period: 20/9/1994 – 18/8/2015 Follow up period: Median 5.6 years (0.3-20.2y) Study setting: Oregon health and science University (OHSU) Casey Eye Institute, USA Number of centres: 1	N= 21 LCHADD n=18 MTPD n=3 Screened: 7 LCHADD: 6 MTPD:1 Age at diagnosis: newborn 1 LCHADD case symptomatic at screening Clinical: 14 LCHADD: 12 MTPD: 2 Age at diagnosis: 4.5m (1 day – 3y)	A diet low in long-chain fatty acids and were supplemented with MCT. All subjects and/or guardians were counselled to avoid fasting. Some subjects were prescribed oral carnitine supplements. Dietary intake assessed by 24 hour recall	NR	NR	NR	**Best corrected visual acuity visit 1** Screened: 4/7 (57.1%) CSM (1 symptomatic case was CMS; 3 LCHADD) Clinical 4/14 (28.6%) CSM **Vision visit 1 (calculated by reviewers)[e]** Screened: 7/7 (100%) normal Clinical: 14/14 (100%) normal **Best corrected visual acuity visit 2** Screened: 0/7 CMS Clinical: 2/14 CSM (11.8%) (1 LCHADD, 1 MTPD) **Vision visit 2 (calculated by reviewers)[e]** Screened: 7/7 (100%) normal Clinical: 2/14 impaired (14.3%)	All LCHADD clinical cases presented with hypoketotic hypoglycaemia	**>1 episode of rhabdomyolysis** Screened: 6/7 (85.7%) Clinical: 13/13 (100%)	NR	Moderate Areas of weakness: confounders

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
De Biase 2017 [26]	Retrospective cohort (chart review) Average follow-up time: nearly 10 years (9.2± 5.9 years) Study setting: Metabolic Clinic University of Utah, USA Number of centres: 1	N=5 LCHADD =4 MTPD=1 Asymptomatic screened: 1 LCHADD Age at diagnosis: birth (NBS) Symptomatic screened: 2 1 LCHADD, 1 MTPD Age at diagnosis: birth (NBS) Symptomatic clinical: 2 LCHADD Age at diagnosis: 5m (4-6m)	All patients received low-fat diet, MCT, essential fatty acids and carnitine. All patients bar symptomatic clinical treated received cornstarch. Both screened symptomatic cases and 1/2 clinical symptomatic patients received DHA One late treated patient is noted to have followed dietary therapy with variable compliance.	NR	**Arrhythmias** Asymptomatic Screened: 0/1 Symptomatic screened: 0/2 Symptomatic clinical: 1/2 (50%) LCHADD	NR	**Retinopathy** Asymptomatic screened: 1/1 (100%) LCHADD Symptomatic screened: 1/2 (50%) LCHADD Symptomatic clinical: 2/2 (100%) LCHADD	**Neurological symptoms** Asymptomatic screened: 0/1 Symptomatic screened: 0/2 Symptomatic clinical: 1/2 (50%) LCHADD **Hypoglycaemia at diagnosis** Asymptomatic screened: 0/1 Symptomatic screened 2/2 (100%) 1 LCHADD, 1 MTPD Symptomatic clinical: 1/2 (50%) LCHADD	**Myoglobinuria** Asymptomatic screened: 0/1 Symptomatic screened: 0/2 Symptomatic clinical: 1/2 (50%) LCHADD	**Failure to thrive** Asymptomatic screened: 0/1 Symptomatic screened 2/2 (100%) 1 LCHADD, 1 MTPD Symptomatic clinical: 0/2	Weak Areas of weakness: selection bias, confounders and data collection methods

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Gillingham 2017 [28]	Randomised double blind parallel RCT (retrospective data collected on time of diagnosis) Follow-up time NR Study setting: Oregon Health and Science University and University of Pittsburgh, USA Number of centres: 2	N = 24 Included for this review n=12 LCHADD = 8 MTPD = 4 Asymptomatic screened n= 7 5 LCHADD 2 MTPD Age at diagnosis/treatment (range): newborn (0-2m). Symptomatic clinical n= 5 3 LCHADD 2 MTPD Age at diagnosis/treatment (range): Infancy (2m-2y) or childhood (2y-10y).	All patients received a low-fat diet and MCT. 3/7 (42.86%) from the asymptomatic group received Triheptanoin (2 LCHADD one MTP) 4/7 (57.14%) from the asymptomatic group received Trictanoin (3/4 LCHADD, one MTP) 4/5 (80%) from the symptomatic group received Triheptanoin (2 LCHADD, 2 MTP) 1/5 (20%) from the symptomatic group received Trictanoin (LCHADD) Dietary compliance not reported	NR	**Cardiac complications** Asymptomatic screened: 0/7 Symptomatic clinical: 3/5 (60%) All 3 LCHADD	NR	NR	NR	NR	NR	Weak Areas of weaknes s: selection methods, confounders and data collection methods

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Kang, 2018 [33]	Retrospective cohort Follow-up time: ~10 years Time period: May 2002 – February 2016 Study setting: Department of Medical Genetics, Asan Medical Center Children's Hospital, Seoul Korea Number of centres: NR	N=22 Included for this review n=7 LCHADD/MTPD not differentiated but genotypes are suggestive of MTPD Asymptomatic Screened: 1 Symptomatic clinical: 6	Screened patient educated to avoid prolonged fasting, MCT diet with long chain fat restriction Dietary compliance not reported	Asymptomatic screened: 1/1 (100%) Mean age of death (range): 49d Symptomatic clinical: 2/6 (33.3%) Mean age of death (range): 6.5d (4-9d)	NR	NR	NR	NR	NR	NR	Moderate Areas of weakness: confounders
International collaboration studies											

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Lund, 2012 [53]	Case-control study Follow-up time: 2-109 months Time period: Feb 1st 2002 – Mar 31st 2011 (trial 2002 – 2009) Study setting: Statens Serum Institut, Copenhagen. Cases from Denmark, Faroe Islands and Greenland Number of centres: 1	N = 5 Don't differentiate between LCHAD and MTP deficiency Asymptomatic Screened n=3 Age at diagnosis/treatment median (range): 6 days (1d-5d) Symptomatic clinical n=2 Age at diagnosis/treatment median (range): 4.5 months	Type of dietary management not reported Dietary compliance not reported	Asymptomatic: 0/3 Symptomatic clinical: 1/2 (50%) Age of death: 4m	**Cardiomyopathy** Asymptomatic 0/3 Symptomatic clinical: 2/2 (100%)	**Hepatopathy** Asymptomatic: 0/3 Symptomatic clinical: 1/2 (50%)	NR	NR	NR	**Acute metabolic encephalopathy** Asymptomatic 0/3 Symptomatic clinical: 1/2 (50%) **Developmental delay** Asymptomatic: 0/3 Symptomatic clinical: 1/2 (50%)	Moderate Weak rating for confounders

Study	Study design	Participants	Treatment	Main findings relating to the patient							
				Death	Heart related problems	Liver related problems	Visual problems	Neural problem or hypoglycaemia	Motor/ muscular problems	Other	Global quality rating
Sykut-Cegielska, 2011 [6]	Retrospective cohort Follow-up time: Up to 17 years Time period: 1992-2009 Study setting: Children's memorial health institute (CMHI) and Institute of Mother and Child (IMC) Warsaw, Poland Number of centres: 2	N = 59 All LCHADD Asymptomatic screened n=15 Age at diagnosis/treatment: 14 days (4d-8w) Detected by cascase testing, TMS pilot screening or by chance from PKU screening Symptomatic n=44 Age at diagnosis/treatment median (range): 6m (1m-18y1m) Group includes all those tested due to suspicions of metabolic disorders post mortems and diagnoses established abroad	Type of dietary management not reported Dietary compliance not reported	Asymptomatic screened: 1/15 (6.7%) Age at death: 7d Symptomatic 19/44 (43%) Age at death mean, median (range): 23.95m; 6m (4d-10y1m)	NR	NR	NR	NR	NR	NR	Weak Areas of weakness: confounders and data collection methods

CSM, Steady central and maintained; FN, false negative; NR, not reported; IQR, interquartile range; NR, not reported; SD, standard deviation; DD. Developmental delay; S, symptomatic; SS, severe symptomatic

a Progressive subnormal

b No information on one person

c Further visual measures are provided in the paper

d 1 no ERG

e The World Health Organization established criteria for low vision using the LogMAR scale. **Low vision** is defined as a best-corrected visual acuity worse than 0.5 LogMAR but equal or better than 1.3 LogMAR in the better eye. **Blindness** is defined as a best-corrected visual acuity worse than 1.3 LogMAR in the better eye. Normal defined as above 0.5

Appendix 4 - Quality Appraisal

Appraisal for quality and risk of bias

Quality assessments of included studies are reported below.

Table 21. Study quality of included studies (key question 1) using JBI tool for prevalence studies

Study	Risk of Bias								
	Q1 - representative sample	Q2 - appropriate recruitment	Q3 - sample size	Q4 - setting/subjects described	Q5 - coverage	Q6 - objective criteria	Q7 - condition reliably measured	Q8 - statistics	Q9 - Response rate
Al -Jasmi 2015[13]	Yes	Yes	No	Yes	Yes	Unclear	Unclear	Yes	Yes
Chien 2013[14]	Yes	Unclear	No	Yes	Yes	Yes	Yes	Yes	Yes
Hassan 2016[20]	Unclear	Unclear	No	No	Unclear	Yes	Yes	Yes	Yes
Lim 2014[15]	Yes	Unclear	No	No	No	Unclear	Unclear	Yes	Yes
Mak 2018 [21]	Unclear	Unclear	No	No	No	Unclear	Unclear	No	Yes
Rocha 2014[10]	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes
Shibata 2018[11]	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes
Smon 2018[16]	Yes	Yes	Yes	No	NA	Unclear	Unclear	Yes	Yes
Therell 2014[17]	Yes	Yes	Yes	No	Unclear	Unclear	Unclear	Yes	Yes
Yang 2018[18]	Yes	Yes	No	No	Unclear	Unclear	Unclear	Yes	Yes
Yunas[19]	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes

Table 22. Study quality of included studies (key question 2) using JBI tool for analytical cross sectional studies

Study	Risk of Bias							
	Q1 Inclusion defined	Q2. Study subjects	Q3. Exposure	Q4. Measurement of condition	Q5. Confounders	Q6. Strategies for confounders	Q7. Outcome measurement	Q8. Statistics
Bo 2017[22]	Yes	Yes	Yes	Yes	No	Unclear	Unclear	NA
Boese 2016[23]	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Boutron 2011[24]	Yes	No	Yes	Yes	No	No	Unclear	NA
Choi 2007[25]	No	Yes	Yes	Yes	NA	NA	Yes	Yes
De Biase 2017[26]	No	Yes	Yes	Yes	No	No	Yes	No
Diekman 2013[27]	No	Yes	Yes	Yes	NA	NA	Yes	NA
Gillingham 2017[28]	Yes	No	Yes	No	No	No	Yes	NA
Hayes 2007[29]	No	No	Yes	Yes	NA	No	Unclear	NA
Hintz 2002[30]	No	Yes	Yes	Yes	No	NA	Unclear	NA
Immonen 2016[31]	Yes	Yes	Yes	Yes	NA	NA	Yes	Yes
Joost 2011[32]	No	No	Yes	Yes	NA	NA	Unclear	NA
Kang 2018[33]	No	Yes	Yes	Yes	NA	NA	Unclear	NA
Lundy 2013[34]	No	Yes	Yes	Yes	NA	NA	Yes	NA
Purevsuren 2009[35]	No	No	Yes	No	NA	NA	Unclear	NA
Sander 2005[36]	Yes	Yes	Yes	Yes	No	NA	Unclear	No
Schwab 2003[37]	No	Yes	Yes	Yes	NA	NA	Yes	NA
Sperk 2010[38]	Yes	No	Yes	Yes	No	No	Unclear	NA
Spiekerkoetter 2002[40]	No	No	Yes	Yes	NA	NA	Yes	NA
Spiekerkoetter 2003[42]	No	No	Yes	Unclear	No	No	Unclear	NA

Study	Risk of Bias							
	Q1 Inclusion defined	Q2. Study subjects	Q3. Exposure	Q4. Measurement of condition	Q5. Confounders	Q6. Strategies for confounders	Q7. Outcome measurement	Q8. Statistics
Spiekerkoetter 2009[58]	No	No	Yes	Yes	Yes	NA	Unclear	NA
Spiekerkoetter 2004[39]	No	No	Yes	Yes	No	No	Unclear	NA
Strandqvist 2015[43]	Yes	No	Yes	Yes	No	No	Unclear	NA
Sykut-Cegielska 2011[43]	Yes	No	Yes	Yes	No	No	Unclear	NA
Tuuli 2016[44]	No	No	Unclear	Unclear	NA	NA	Unclear	NA
Vockley 2016[45]	Yes	No	Unclear	Unclear	No	No	Yes	Yes
Waisbren 2013[46]	No	No	Unclear	Unclear	NA	NA	Unclear	NA
Yang 2002[47]	Yes	Yes	Yes	Yes	No	No	Yes	Yes

Table 23. Study quality of included studies (key question 3) according to tailored QUADAS-2 (100% checked)

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference test	Flow and timing	Patient selection	Index test	Reference test
Bonham[9]	Low	High	Unclear	High	Low	Low	Low
Couce[59]	Low	Unclear	High	High	High	High	Low
Frazier[60]	Unclear	High	Unclear	High	High	Low	Unclear
Lindner[67]	Unclear	Low	High	High	High	Unclear	Low
Lund[5]	Low	Low	High	High	Unclear	Low	Low
Mak [21]	Unclear	Unclear	High	High	High	Unclear	Unclear
Sander[36]	Unclear	Low	Unclear	High	High	High	Unclear
Smon[16]	Unclear	High	Unclear	High	High	Low	Unclear
Yang[18]	Unclear	High	Unclear	High	Unclear	Low	Unclear
Zytkovicz[48]	Unclear	High	Unclear	High	High	Low	Unclear

Table 24. Study quality of included studies (key question 4) according to EPHPP quality assessment tool [65]

Study	Global rating from sections A-F						Global rating for this study
	A) Selection bias	B) Study design	C) Confounders	D) Blinding	E) Data collection methods	F) Withdrawals and drop-outs	
Swedish study							
Fahnehjelm 2008 [49]	Moderate	Moderate	Weak	Moderate	Moderate	Strong	Weak
Haglind 2013 [51]	Moderate	Moderate	Weak	Moderate	Moderate	Strong	Moderate
Fahnehjelm 2016 [50]	Moderate	Moderate	Weak	Moderate	Moderate	Strong	Weak
European single centre studies							
Immonen 2015 [31]	Strong	Moderate	Weak	Moderate	Weak	Strong	Weak
Sperk 2010 [38]	Strong	Moderate	Weak	Moderate	Weak	Strong	Weak
Gillingham 2017 [28]	Weak	Strong	Weak	Strong	weak	Strong	Weak
European collaboration studies							
Karall 2015 [52]	Moderate	Moderate	Weak	Moderate	Moderate	Strong	Moderate
Spiekerkoetter 2009 [41]	weak	Moderate	Weak	Moderate	Weak	moderate	Weak
International single studies							
Boese 2016 [23]	Strong	Moderate	Weak	Moderate	Moderate	Strong	Moderate
De Biase 2017 [26]	Weak	Moderate	Weak	Moderate	Weak	Strong	Weak
Kang 2018 [33]	strong	Moderate	Weak	Moderate	Moderate	Strong	Moderate
Internation collaboration studies							
Lund 2012 [5]	Strong	Moderate	weak	Moderate	Moderate	Strong	Moderate
Sykut-Cegielska 2011 [6]	Strong	Moderate	Weak	Moderate	Weak	Strong	Weak

Appendix 5 - Analyses

Table 25. Genotype and phenotype data – question 2

Phenotypes		
Genotype Group	**Death**	**Age at death**
LCHADD homozygous 1528G>C /E474Q	Hintz 2002 0/1 Joost 2011 1/3 (33.3%) Lundy 2003 1/3 (33.3%) Tuuli 2016 1/12 (8.3%) Vockley 2016 0/2 Yang 2002 1/6 (16.7%) Boutron 2011 11/19 (58%) Immonen 2016 6/16 (37.5%) Sander 2005 0/5 Sperk 2010 1/3 (33.3%) Spiekerkoetter 2009 3/20 (15%) Sykut-Cegielska 2011 13/45 (28.9%)	Joost 2011 6m Lundy 2003 31d Immonen 2016 5m (3m-2y)* missing data for one patient Spiekerkoetter 2009 4m (3d-4y) Sykut-Cegielska 2011 5m (3m-9y7m)
LCHADD heterozygous 1528G>C/E474Q	Joost 2011 2/2 (100%) Yang 2002 1/6 (16.7%) Boutron 2011 4/15 (27%) Sykut-Cegielska 2011 3/7 (42.9%)	Joost 2011 16.75m (3.5m-30m) Sykut-Cegielska 2011 18m (7d-2y5m)
LCHADD unclear (2nd mutation unspecified)	Sander 2005 0/2 Sykut-Cegielska 2011 4/7 (57.1%)	Sykut-Cegielska 2011 3y4.5m (4d-10y1m)
MTP Beta subunit mutations	Bo 2017 3/10 (30%) Diekman 2012 2/2 (100%) Purevsuren 2009 2/5 (40%) Schwab 2003 2/2 (100%) Spiekerkoetter 2003 4/15 (26.7%) Boutron 2011 6/7 (85.7%) Kang 2018 1/5 (20%) Sperk 2010 0/2	Bo 2017 ~1 month (8d-3m) Diekman 2012 20.5 days (10-31d) Purevsuren 2009 1.5m (8d-3m) Schwab 2003 2m (12h-4m) Spiekerkoetter 2003 10.5d (8d-1 Kang 2018 9 days
MTP Alpha subunit mutations	Bo 2017 3/3 100% Hintz 2002 1/1 (100%) Spiekerkoetter 2002 2/2 (100%) Boutron 2011 5/11 (45.5%) Choi 2007 2/2 (100%) Kang 2018 2/2 (100%) Sperk 2010 0/1	Bo 2017 21 days (6-44 days) Hintz 2002 ~day 2 Spiekerkoetter 2002 infancy and neonatal period Choi 2007 37d (26-48d) Kang 2018 26.5d (4-49d)
MTP subunit unspecified	Lundy 2003 0/1 Vockley 2016 1/2 (50%) Sander 2005 2/2 (100%) Spiekerkoetter 2009 5/7 (71.4%)	Vockley 2016 ~3m Sander 2005 6.5m (8d-13m) Spiekerkoetter 2009 78d (2d-5m)

Phenotypes					
	Severity				
Genotype Group	**Asymptomatic at diagnosis**	**Asymptomatic at study end**	**Neonatal severe**	**Infant hepatic**	**Late onset neuromyopathic**
LCHADD homozygous 1528G>C /E474Q	Strandqvist 2015 1/5 (20%) Yang 2002 2/6 (33.3%) Sperk 2010 1/3 (33.3%)	Spiekerkoetter 2009 3/20 (15%)	Sperk 2010 1/3 (33.3%)	Yang 2002 2/6 (33.3%) Boutron 2011 19/19 (100%) Sperk 2010 1/3 (33.3%)	NR
LCHADD heterozygous 1528G>C/E474Q	Strandqvist 2015 0/3 Yang 2002 0/6		Boutron 2011 1/15 (6.7%)	Yang 2002 3/6 (50%) Boutron 2011 13/15 (86.7%)	Boutron 2011 1/15 (6.7%)
LCHADD unclear (2nd mutation unspecified)	NR	NR	NR	NR	NR
MTP Beta subunit mutations	NR	NR	Bo 2017 4/10 (40%) Diekman 2012 2/2 (100%) Purevsuren 2009 2/5 (40%) Spiekerkoetter 2003 4/15 (26.7%) Boutron 2011 6/7 (85.7%) Sperk 2010 2/2 (100%)	Bo 2017 2/10 (20%) Purevsuren 2009 2/5 (40%) Spiekerkoetter 2003 2/15 (13.3%) Boutron 2011 1/7 (14.3%)	Bo 2017 4/10 (40%) Purevsuren 2009 1/5 (20%) Spiekerkoetter 2003 9/15 (60%) Spiekerkoetter 2004 11/11 (100%) Boutron 2011 0/7
MTP Alpha subunit mutations	Sperk 2010 1/1 (100%)	NR	Bo 2017 3/3 (100%) Spiekerkoetter 2002 2/2 (100%) Boutron 2011 5/11 (45.5%)	Boutron 2011 2/11 (18.2%)	Boutron 2011 4/11 (36.4%)
MTP subunit unspecified	NR	Spiekerkoetter 2009 0/7	NR	NR	Bo 2017 1/1 (100%)

Phenotypes						
	Heart/breathing issues					
Genotype Group	**Cardiac failure/ Biventricular dysfunction**	**Cardiomyopathy**	**Cardiac complications/ cardiac arrest/ Cardiopulmonary arrest**	**Arrhythmias/ tachypnoea**	**Respiratory failure**	**Other**
LCHADD homozygous 1528G>C /E474Q	Lundy 2003 1/3 (33.3%) Vockley 2016 1/2 (50%) Heart failure; 0/2 biventricular dysfunction initial presentation	Hayes 2007 1/1 (100%) Joost 2011 0/2 Strandqvist 2015 1/5 (20%) at diagnosis Vockley 2016 1/2 (50%) initial presentation 2/2 (100%). Age 53m (10m-8y) Immonen 2016 6/11 (54.5%) at start of study 4/11 (36.4%) end of study Sander 2005 2/5 (40%) Sperk 2010 0/3 Spiekerkoetter 2009 8/20 (40%)	Boese 2016 1/9 (11.1%) Gillingham 2017 1/6 (16.7%)	Spiekerkoetter 2009 1/20 (5%) Tacypnoe Lundy 2003 0/3		Apnea Strandqvist 2015 1/5 (20%) at diagnosis ARDS Lundy 2003 3/3 (100%)
LCHADD heterozygous 1528G>C /E474Q	NR	Hayes 2007 1/1 (100%) Joost 2011 1/2 (100%) Strandqvist 2015 0/3 at diagnosis	Boese 2016 1/7 (14.3%) Gillingham 2017 2/4 (50%)	NR	NR	Apnea Strandqvist 2015 0/3 at diagnosis
LCHADD unclear (2nd mutation unspecified)	NR	Sander 2005 0/2	Boese 2016 0/2	NR	NR	NR
MTP Beta subunit mutations	NR	Bo 2017 2/10 (20%) Symptom at onset Diekman 2012 2/2 (100%) Hayes 2007 0/1 Purevsuren 2009 2/5 (40%) Spiekerkoetter 2003 5/15 (33.3%) Sperk 2010 2/2 (100%)	Boese 2016 0/3 Cardiopulmonary arrest Bo 2017 1/10 (10%	NR	Bo 2017 2/10 (20%) Symptom at onset Diekman 2012 1/2 (50%) Spiekerkoetter 2004 mean age 10y (1y-21y) 5/11 (45.5%) Spiekerkoetter 2004 first symptom 1/11 (9.1%) Spiekerkoetter 2004 5/11 (45.5%)	Pericardial effusion Choi 2007 1/1 (100%) Artificial ventilation Bo 2017 1/10 (10%) currently

Phenotype					
	Liver issues				
Genotype Group	**Hepatomegaly/hepatic failure**	**Renal failure**	**Liver steatosis**	**Elevated liver enzymes/liver enlargement**	**Liver dysfunction**
LCHADD homozygous 1528G>C/E474Q	Hayes 2007 1/1 (100%) Joost 2011 2/3 (66.7%) Lundy 2003 3/3 (100%)	Lundy 2003 1/3 (33.3%)	Joost 2011 0/3	Strandqvist 2015 2/5 (40%) at diagnosis	
LCHADD heterozygous 1528G>C/E474Q	Hayes 2007 1/1 (100%) Joost 2011 0/2	NR	Joost 2011 1/2 (50%)	Strandqvist 2015 2/3 (66.7%) at diagnosis	NR
LCHADD unclear	NR	NR	NR	NR	NR
MTP Beta subunit mutations	Hayes 2007 0/1	Choi 2007 1/1 (100%)	NR	NR	NR
MTP Alpha subunit mutations	NR	Hintz 2007 1/1 (100%)	NR	NR	Purevsuren 2009 3/5 (60%) Choi 2007 1/2 (50%)
MTP subunit unspecified	Lundy 2003 1/1 (100%)	Lundy 2003 0/1	NR	Choi 2007 1/1 (100%)	NR

Phenotype					
	Motor issues				
Genotype Group	**Motor delay**	**Motor retardation**	**Foot deformities**	**Skeletal myopathy**	**Polyneuropathy**
LCHADD homozygous 1528G>C/E474Q	NR	Sander 2005 0/5	NR	NR	Tuuli 2016 8/12 (66.7%)
LCHADD heterozygous 1528G>C/E474Q	NR	NR	NR	NR	NR
LCHADD unclear (2nd mutation unspecified)	NR	Sander 2005 1/2 (50%)	NR	NR	NR
MTP Beta subunit mutations	Spiekerkoetter 2004 first symptom 3/11 (27.3%)	NR	Spiekerkoetter 2004 4/11 (36.4%)	Spiekerkoetter 2003 8/15 (53.3%)	NR
MTP Alpha subunit mutations	NR	NR	NR	NR	NR
MTP subunit unspecified	NR	Sander 2005 1/2(50%)	NR	NR	NR

Phenotype						
	Muscle issues					
Genotype Group	**Hypotonia**	**Myoglobinuria Myalgia**	**Myopathy**	**Rhabdomyolysis**	**Weakness/lethargy/ fatigue**	**Muscle pain/weakness**
LCHADD homozygous C1528G>C/ E474Q	Hayes 2007[29] 1/1 (100%) Joost 2012[32] 1/3 (33.3%) Lundy 2003[34] 0/3 Strandqvist 2015 [43]2/5 (40%) at diagnosis Spiekerkoetter 2009[58] 14/20 (70%)	NR	Sperk 2010[38] 0/3	Hayes 2007[29] 0/1 Vockley 2016[45] 1/2 (50%) initial presentation Boese 2016 [23] 0/9 Immonen 2016 [31] 4/10 (40%) Sander 2005[36] 1/5 (20%)	Hayes 2007[29] 0/1 Strandqvist 2016[43] at diagnosis 2/5 (40%)	De Biase 2017[26] 4/4 (100%)
LCHADD heterozygous 1528G>C /E474Q	Hayes 2007[29] 0/1 Joost 2012[32] 1/2 (50%) Strandqvist 2016[43] 1/3 (33.3%) at diagnosis	NR	NR	Hayes 2007 [29]1/1 (100%) Boese 2016 [23]0/7	Hayes 2007 [29]0/1 Strandqvist 2016[43] at diagnosis 0/3	Waisbren 2013 [46]1/1 (100%)
LCHADD unclear	NR	NR	NR	Boese 2016 [23] 0/2 Sander 2005 [36] 1/2 (50%)	NR	NR
MTP Beta subunit mutations	Bo 2017[22] 1/10 (10%) currently Spiekerkoetter 2004 [39] first symptom 2/11 (18.2%)	Choi 2007[25] 1/1 (100%) Bo 2017[22] 1/10 (10%) symptom at onset	Sperk 2010[38] 1/2 (50%)	Bo 2017[22] 2/10 (20%) symptom at onset 4/10 (40%) currently Hayes 2007[29] 1/1 (100%) Choi 2007[25] 1/1 (100%) Boese 2016 [23] 1/3 (33.3%) Kang 2018[33] 4/4 (100%)* in surviving patients	Bo 2017[22] 2/10 (10%) Symptom at onset Hayes 2007 [29]1/1 (100%) Spiekerkoetter 2004 [39]first symptom 1/11 (9.1%)	Purevsuren 2009[35] 1/3 (33.3%) Spiekerkoetter 2004 [39]first symptom 4/11 (36.4%) Progressive and severe episodic weakness 9/11 (81.8%)
MTP Alpha subunit mutations	Bo 2017[22] 1/3 (33.3%) Symptom at onset Hayes 2007 0/1	NR	Sperk 2010[38] 0/1	NR	NR	NR
MTP subunit unspecified	Lundy 2003[34] 1/1 (100%) Spiekerkoetter 2009[41] 4/7 (57.1%)	Bo 2017 1/1 [22](100%) symptom at onset	NR	Bo 2017[22] 1/1 (100%) Symptom at onset 1/1 (100%) currently Vockley 2016[45] 0/2 heart failure; 1/2 biventricular dysfunction at initial presentation Sander 2005[36] 1/2 (50%)	Choi 2007 [25]1/1 (100%)	De Biase 2016[26] 0/1

Phenotypes					
	Visual issues				
Genotype Group	**Vision V1**	**Vision visit 2**	**Retinopathy**	**Retinis pigmentosa**	**Reye syndrome**
LCHADD homozygous 1528G>C/E474Q	Boese 2016[23] 0/9 impaired	Boese 2016[23] 1/9 (11.1%) impaired	Hayes 2007[29] 0/1 Waisbren 2013 [46]mild retinal function deficits 1/1 (100%) Immonen 2016 [31] 9/11 (81.8%) Sperk 2010[38] 0/3 Spiekerkoetter 2009[41] 5/20 (25%)	De Biase 2016[26] 4/4 (100%)	Spiekerkoetter 2009[58] 7/20 (35%)
LCHADD heterozygous 1528G>C/E474Q	Boese 2016[23] 0/7 impaired	Boese 2016[23] 1/7 (14.3%) impaired	Hayes 2007 [29] 1/1 (100%) Waisbren 2013 [46]mild retinal function deficits 1/1 (100%)	NR	NR
LCHADD unclear (2nd mutation unspecified)	Boese 2016[23] 0/2 imparied	Boese 2016[23] 0./2 impaired	NR	NR	NR
MTP Beta subunit mutations	NR	NR	Hayes 2007 [29] 1/1 (100%) Sperk 2010 0/2[38]	NR	NR
MTP Alpha subunit mutations	NR	NR	Sperk 2010[38] 0/1	NR	NR
MTP subunit unspecified	Boese 2016[23] 0/3 impaired	Boese 2016[23] 0/3 impaired	Spiekerkoetter 2009 [58] 1/7 (14.3%)	De Biase 2016 0/1	Spiekerkoetter 2009 2/7 (28.6%)

Phenotypes							
	IQ and development						
Genotype Group	**Brain damage**	**Learning difficulties**	**Mental retardation**	**Developmental delay**	**Developmentally normal**	**Speech delay**	**Failure to thrive**
LCHADD homozygous 1528G>C /E474Q	Immonen 2016[31] 1/10 (10%)	Hayes 2007[29] 0/1	NR	Waisbren 2013[46] 1/1 (100%)	Hintz 2002 [30] 1/1 (100%)	NR	Strandqvist 2015 [43]0/5 at diagnosis Yang 2002[47] 0/6 De Biase 2016 [26] 1/4 (25%)
LCHADD heterozygous 1528G>C /E474Q	NR	Hayes 2007[29] 1/1 (100%)	NR	NR	NR	Waisbren 2013 [46]1/1 (100%)	Strandqvist 2016[43] 1/3 (33.3%) at diagnosis Yang 2002[47] 1/6 (16.7%)
LCHADD unclear (2nd mutation unspecified)	NR	NR	NR	NR	NR	NR	NR
MTP Beta subunit mutations	NR	Hayes 2007 [29] 0/1	Bo 2017[22] 1/10 (10%)	Bo 2017[22] 1/10 (10%) Purevsuren 2009[35] 3/3 (2 died)	NR	NR	NR
MTP Alpha subunit mutations	NR	NR	NR	NR	NR	NR	NR

MTP subunit unspecified	NR	NR	NR	NR	NR	NR	De Biase 2016[26] 1/1 (100%)

Phenotype		
	Neuropathy	
Genotype Group	**Peripheral neuropathy**	**Neuropathy**
LCHADD homozygous 1528G>C/E474Q	Hayes 2007[29] 0/1	Immonen 2016 [31] 3/10 (30%) 5/10 not detected Sperk 2010[38] 0/3 Spiekerkoetter 2009[58] 2/20 (10%)
LCHADD heterozygous 1528G>C/E474Q	Hayes 2007 [29] 1/1 (100%)	NR
LCHADD unclear (2nd mutation unspecified)	NR	NR
MTP Beta subunit mutations	Bo 2017 [22] 4/10 (40%) Currently Hayes 2007[29] 1/1 (100%) Spiekerkoetter 2003[42] 7/15 (46.7%) Spiekerkoetter 2004 [39]8/11 (72.7%) Kang 2018[33] 4/4 (100%) * in surviving patients	Spiekerkoetter 2003 [42]7/15 (46.7%) Sperk 2010 [38] 0/2

Phenotype						
	Chemical/hormonal imbalances					
Genotype Group	**Metabolic acidosis/ lactic acidosis/ lactic acidemia**	**Hyperammonemia**	**Hypoglycaemia**	**Hypoparathyroidism**	**Periventricular leukomalacia**	**hypocalcaemia**
LCHADD homozygous 1528G>C /E474Q	Strandqvist 2016[43] 1/5 (20%) at diagnosis	NR	Hayes 2007 [29] 1/1 (100%) Hintz 2002 [30]1/1 (100%) Joost 2011 [32]2/3 (66.7%) Lundy 2003 [34]2/3 (66.7%) Strandqvist 2015[43] 2/5 (40%) at diagnosis Vockley 2016[45] 0/2 initial presentation Boese 2016[23] 6/9 (66.7%) De Biase 2017 [26] 2/4 (50%) at diagnosis Sperk 2010 [38] 2/3 (66.7%) Spiekerkoetter 2009 16/20 (80%) Sander 2005 [36] 2/5 (40%)	NR	Strandqvist 2016[43]0/5 at diagnosis	Hintz 2002[30] 1/1 (100%)
LCHADD heterozygous 1528G>C/ E474Q	Strandqvist 2015[43] 0/3 at diagnosis	NR	Hayes 2007[29] 1/1 (100%) Joost [32]1/2 (50%) Strandqvist 2015 [43] 2/3 (66.7%) at diagnosis Boese 2016 [23]5/7 (71.4%)	NR	Strandqvist 2015[43] 1/3 (33.3%) at diagnosis	NR
LCHADD unclear (2nd mutation unspecified)	NR	NR	Boese 2016[23] 2/2 (100%) Sander 2005 [36]0/2	NR	NR	NR
MTP Beta subunit mutations	Bo 2017[22] 1/10 (10%) Symptom at onset Purevsuren 2009 [35] 1/5 (20%)	Purevsuren 2009 2/5 (40%)	Hayes 2007 [29] 0/1 Purevsuren 2009 [35] 2/5 (40%) Spiekerkoetter 2003 [42] 7/15 (46.7%) Spiekerkoetter 2004 [39]first symptom 2/11 (18.2%) Boese 2016 [23]1/3 (33.3%) Sperk 2010 [38]2/2 (100%)	Bo [22] 4/10 (40%) currently	NR	NR
MTP Alpha subunit mutations	Spiekerkoetter 2002 [40] 1/2 (50%) Choi 2007 1/2 (50%)	NR	Spiekerkoetter 2002 [40]1/2 (50%) Choi 2007 [25]1/2 (50%) Sperk 2010 [38](0/1)	NR	NR	NR

MTP subunit unspecified	NR	NR	Lundy 2003[34] 0/1 Vockley 2016[45] 2/2 (100%) initial presentation De Biase 2016 [26]1/1 (100%) Spiekerkoetter 2009 [58]3/7 (42.9%) Sander 2005 [36]1/2 (50%)	NR	NR	NR

Phenotype										
	Other									
Genotype Group	**Intraventricular/ intraparenchymal haemorrhage grades III-IV**	**Necrotizing enterocolitis**	**Organ failure**	**Epilepsy**	**Neonatal hypothermia**	**Seizure**	**Encephalopathy**	**Bilateral tensions pneumothoraces**	**Symptoms induced by illness**	**Symptoms induced by exercise**
LCHADD homozygous 1528G>C/E474Q	Strandqvist 2015[43] 0/5 at diagnosis	NR	Strandqvist 2015[43] 1/5 (20%) at diagnosis (renal liver and heart)	Strandqvist 2015 [43]1/5 (20%)	Strandqvist 2015 [43]0/5	Hintz 2002[30] 1/1 (100%)	Lundy 2003[34] 2/3 (66.7%)	Lundy 2003[34] 1/3 (33.3%)	NR	NR
LCHADD heterozygous	Strandqvist 2015[43] 1/3 (33.3%) at diagnosis	NR	Strandqvist 2015[43] 0/3 at diagnosis	Strandqvist 2015 [43]1/3 (33.3%)	Strandqvist 2015 [43]1/3 (33.3%)	NR	NR	NR	NR	NR
MTP Beta subunit	NR	Diekman 2012[27] 1/2 (50%)	Diekman 2012[27] 1/2 (50%)	NR	NR	Bo 2017[22] 1/10 (10%) symptom at onset	NR	NR	Spiekerkoetter 2004[39] 9/11 (81.8%)	Spiekerkoetter 2004[39] 7/11 (63.6%)
MTP Alpha subunit mutations	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
MTP subunit unspecified	NR	NR	NR	NR	NR	NR	Lundy 2003[34] 0/1	Lundy 2003[34] 0/1	NR	NR

Table 26. Follow up analyses of early vs late groups and LCHAD deficiency vs MTP deficiency

Analyses	Mortality	Heart problems	Liver problems	Visual problems	Neurological problems	Motor/muscular	Other
Asymptomatic vs symptomatic	Immonen 2016[31] 0/1 vs 6/15 (p=1) Sperk 2010[38] 1/3 vs 0/3 (p=1) Kang 2018[33] 1/1 vs 2/6 (p=1) Lund 2012[5] 0/3 vs 1/2 (p=0.4) Skyut-Cegielska 2011[6] *1/15 vs 13/37* (**p<0.05**)	**Cardiomyopathy** Immonen 2016 [31] Start 0/1 vs 6/10 (p=0.46) End 0/1 vs 4/10 (p=0.64) Sperk 2010[38] 0/3 vs 2/3 (p=0.4) Karall 2015[52] 1/6 vs 6/8 (p=1) Lund 2012[5] 0/3 vs 2/2 (p=1) **Arrhythmias** De Biase [26]2016	**Hepatopathy** Karall 2015[52] 1/6 vs 4/8 (p=0.4) Lund 2012[34] 0/3 vs 1/2 (p=0.4)	**ERG findings** Swedish cohort Fahnehjelm 2016[50] Normal vs subnormal/pathological 1/2 vs 8/9 (p=0.35) **Ocular Fundii** Normal vs subnormal/pathological/ severely pathological 2/3 vs 9/9 (p=0.25) **Photophobia** Swedish cohort Fahnehjelm 2008[49] 1/1 vs 7/8 (p=1) **Nyctalopia** Swedish cohort Fahnehjelm 2008[49] 0/1 vs 2/8	**Epilepsia** Swedish Cohort Fahnehjelm 2008[49] 0/3 vs 3/8 (p=0.51) **Neurological symptoms** De Biase 2016[26] 0/1 vs 1/4 (p=1)	**Psychomotor development** Swedish Cohort Fahnehjelm 2008[49] 0/1 vs 5/9 (p=1) Karall 2015[52] 0/6 vs 0/8 NA **Myopathy** Sperk 2010[38] 0/3 vs 1/3 (p=1) De Biase [26](2016) 0/1 vs 1/4 (p=1)	**Hypoglycaemia** Swedish cohort study Fahnehjelm 2016 [50] 1/3 vs 7/9 (p=0.27) Sperk 2010[38] 1/3 vs 3/3 (p=0.4) **BMI and obesity** Swedish cohort Haglind 2013[51] *Overweight before 6* 1/1 vs 7/9 (p=1) *ISO BMI* >30 kg/m² 1-5 years 1/1 vs 5/9 (p=1) >25 kg/m² last assessment 1/1 vs 6/9 (p=1) Normal weight last assessment 0/1 vs 3/9 (p=1) *Obesity* Immonen 2016

Analyses	Mortality	Heart problems	Liver problems	Visual problems	Neurological problems	Motor/muscular	Other
	Spiekerkoetter 2009[58] 0/7 vs 8/20 (p=0.17)	0/1 vs 1/4 (p=1) **Cardiac complications** Gillingham 2017[28] 0/7 vs 3/5 **(p<0.05)**		(p=1) **Retinopathy** Immonen 2016[31] 0/1 vs 9/10 (p=0.22) Karall 2015[52] 2/6 vs 6/8 (p=0.28) De Biase 2016[26] 1/1 vs 4/5 (p=1)		**>1 episode of rhabdomyolysis** Boese 2016[23] 5/6 vs 14/14 (p=0.3)	0/1 vs 0/10 **IQ normal** Immonen 20161/1 vs 8/9 (p=1) **Failure to thrive** De Biase 2016[26] 0/2 vs 2/4 (p=0.47) **Acute metabolic encephalopathy** Lund 2012[5] 0/3 vs 1/2 (p=0.4) **Developmental delay** Lund 2012[5] 0/3 vs 1/2 (p=0.4)
Screened vs unscreened	Lund 2012 0/3 vs 1/2 (p=0.4) Immonen 2016	**Cardiomyopathy** Karall 2015 2/9 vs 5/5 **P=0.02**	**Hepatopathy** Karall 2015 1/9 vs 4/5 **P=0.02**	**ERG findings** Swedish cohort Fahnehjelm 2016 Normal vs subnormal/pathological	**Epilepsia** Swedish Cohort Fahnehjelm 2008 0/3 vs 3/8	**Psychomotor development** Swedish Cohort	**Hypoglycaemia** Swedish cohort study Fahnehjelm 2016 1/3 vs 7/9 (p=0.27)

Analyses	Mortality	Heart problems	Liver problems	Visual problems	Neurological problems	Motor/muscular	Other
	0/1 vs 6/15 (p=1) Kang 2018 1/1 vs 2/6 (p=1) Spiekerkoetter 2009 2/10 vs 6/17 P=0.67 Skyut-Cegielska 2011 *1/15 vs 13/37* (**p<0.05**)	Lund 2012 0/3 vs 2/2 (p=1) Immonen 2016 Start 0/1 vs 6/10 (p=0.46) End 0/1 vs 4/10 (p=0.64) Spiekerkoetter 2009 4/10 vs 8/17 P=1 **Arrhythmias** De Biase 2016 0/3 vs 1/2 (p=0.4) **Cardiac complications** Gillingham 2017 0/7 vs 3/5 **(p<0.05)**	Lund 2012 0/3 vs 1/2 (p=0.4) **Reye syndrome** Spiekerkoetter 2009 3/10 vs 6/17 P=0.69	1/2 vs 8/9 (p=0.35) **Ocular Fundi** Normal vs subnormal/pathological/ severely pathological 2/3 vs 9/9 (p=0.25) **Photophobia** Swedish cohort Fahnehjelm 2008 1/1 vs 7/8 (p=1) **Nyctalopia** Swedish cohort Fahnehjelm 2008 0/1 vs 2/8 (p=1) **Retinopathy** Karall 2015 3/9 vs 5/5 **P=0.03** De Biase 2016 2/3 vs 2/2 P=1	(p=0.51) **Neurological symptoms** De Biase 2016 0/3 vs 1/2 (p=0.4)	Fahnehjelm 2008 0/1 vs 5/9 (p=1) **Myoglobinuria** De Biase (2016) 0/3 vs 1/2 (p=0.4) **Myopathy/hypotonia** Spiekerkoetter 2009 4/10 vs 14/17 **P=0.03** **>1 episode of rhabdomyolysis** Boese 2016 5/6 vs 14/14 (p=0.3)	Spiekerkoetter 2009 4/10 vs 15/17 **P=0.02** **BMI and obesity** Swedish cohort Haglind 2013 *Overweight before 6* 1/1 vs 7/9 (p=1) *ISO BMI* >30 kg/m² 1-5 years 1/1 vs 5/9 (p=1) >25 kg/m² last assessment 1/1 vs 6/9 (p=1) Normal weight last assessment 0/1 vs 3/9 (p=1) *Obesity* Immonen 2016 0/1 vs 0/10 **Developmental delay** Lund 2012 0/3 vs 1/2 (p=0.4)

Analyses	Mortality	Heart problems	Liver problems	Visual problems	Neurological problems	Motor/muscular	Other
				Immonen 2016 0/1 vs 9/10 (p=0.22) **Vision[a]** Boese 2016[22] Normal visit 1: 7/7 vs 14/14 P is NA Normal Visit 2: 7/7 vs 12/14 P=0.53			**IQ normal** Immonen 1/1 vs 8/9 (p=1) **Acute metabolic encephalopathy** Lund 2012 0/3 vs 1/2 (p=0.4) **Failure to thrive** De Biase 2016 2/3 vs 0/2 (p=0.4)
Asymptomatic screened, symptomatic screened and symptomatic clinically diagnosed	Spiekerkoetter 2009[58] 0/4 vs 2/6 vs 6/17 (p=0.42)	**Cardiomyopathy** Karall 2015[52] 1/6 vs 1/3 vs 5/5 **(p<0.05)** **Arrhythmias** De Biase 2016[26] 0/1 vs 0/2 vs 1/2 (p=1)	**Hepatopathy** Karall 2015[52] 1/6 vs 0/3 vs 4/5 (p=0.06)	**Retinopathy** Karall 2015[52] 1/6 vs 1/3 vs 4/5 **(p=0.05)** De Biase 2016[26] 1/1 vs 1/2 vs 2/2 (p=1) **Best corrected visual acuity** Boese 2016[23] Central steady and maintained Visit 1: 3/6 vs 1/1 vs 4/14 (p=0.33)	**Neurological symptoms** De Biase 2016[26] 0/1 vs 0/2 vs 1/2 (p=1)	**Myoglobinuria** De Biase 2016[26] 0/1 vs 0/2 vs 1/2 (p=1)	**Hypoglycaemia at diagnosis** De Biase 2016[26] 0/1 vs 2/2 vs 1/2 (p=0.6) **Failure to thrive** De Biase 2016[26] 0/1 vs 2/2 vs 0/2 (p=0.2)

Analyses	Mortality	Heart problems	Liver problems	Visual problems	Neurological problems	Motor/muscular	Other
				Visit 2: 0/6 vs 0/1 vs 2/14 (p=1) **Vision[a]** Boese 2016[23] Normal visit 1: 6/6 vs 1/1 vs 14/14 P value NA Normal Visit 2: 6/6 vs 1/1 vs 12/14 (p=1)			
LCHADD vs MTPD	Spiekerkoetter 2009[58] 3/20 vs 5/7 **(p= 0.01)** Sperk 2010[38] 1/3 vs 0/3 (p=1)	**Cardiomyopathy** Spiekerkoetter 2009[58] 8/20 vs 4/7 (p=0.66) Sperk 2010[38] 0/3 vs 2/3 (p=0.4) **Cardiac complications** Gillingham 2017[28]	**Reye syndrome** Spiekerkoetter 2009[58] 7/20 vs 2/7 (p=1)	**Vision at visit 2[a]** Boese 2016 Rates of impaired 2/18 vs 0/3 (p=1) **Retinopathy** De Biase 2016 De Biase, 2017 #5} 4/4 vs 0/1 (p=0.2)	**Neurological symptoms** De Biase 2016 1/4 vs 0/1 (p=1)	**Hypotonia/ Myopathy** Spiekerkoetter 2009[58] 14/20 vs 4/7 (p=0.65) Sperk 2010[38] 0/3 vs 1/3 (p=1) **>1 episode of**	**Hypoglycaemia** Spiekerkoetter 2009[58] 16/20 vs 3/7 (p=0.14) Sperk 2010[38] 2/3 vs 2/3 P value NA **Hypoglycaemia at diagnosis** De Biase 2016 De Biase, 2017 #5}

Analyses	Mortality	Heart problems	Liver problems	Visual problems	Neurological problems	Motor/muscular	Other
		2/8 vs 1/4 (p= 0.75) **Arrhythmias** De Biase[26] 2016 1/4 vs 0/1 (p=1)				**rhabdomyolysis** Boese 2016[23] 16/17[b] vs 3/3 (p=1) **Myoglobinuria** De Biase 2016 De Biase, 2017 #5} 1/4 vs 0/1 (p=1)	2/4 vs 1/1 (p=1) **Failure to thrive** De Biase 2016 De Biase, 2017 #5} 1/4 vs 1/1 (p=0.4)

ERG, electroretinography; LCHADD, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; MTPD, Mitochondrial Trifunctional Protein Disorder
a Vision calculated by reviewers. The World Health Organization established criteria for low vision using the LogMAR scale. **Low vision** is defined as a best-corrected visual acuity worse than 0.5 LogMAR but equal or better than 1.3 LogMAR in the better eye. **Blindness** is defined as a best-corrected visual acuity worse than 1.3 LogMAR in the better eye. Normal defined as above 0.5
b 1 case lost to follow up
Bold figures highlight values which reached significance (p<0.05).

Appendix 6 - UK NSC reporting checklist for evidence summaries

All items on the UK NSC Reporting Checklist for Evidence Summaries have been addressed in this report. A summary of the checklist, along with the page or pages where each item can be found in this report, is presented in Table 27. UK NSC reporting checklist for evidence summaries.

Table 27. UK NSC reporting checklist for evidence summaries

	Section	Item	Page no.
1.	TITLE AND SUMMARIES		
1.1	Title sheet	Identify the review as a UK NSC evidence summary.	1
1.2	Plain English summary	Plain English description of the executive summary.	5
1.3	Executive summary	Structured overview of the whole report. To include: the purpose/aim of the review; background; previous recommendations; findings and gaps in the evidence; recommendations on the screening that can or cannot be made on the basis of the review.	7
2.	INTRODUCTION AND APPROACH		
2.1	Background and objectives	Background – Current policy context and rationale for the current review – for example, reference to details of previous reviews, basis for current recommendation, recommendations made, gaps identified, drivers for new reviews Objectives – What are the questions the current evidence summary intends to answer? – statement of	15

		the key questions for the current evidence summary, criteria they address, and number of studies included per question, description of the overall results of the literature search. Method – briefly outline the rapid review methods used.	
2.2	Eligibility for inclusion in the review	State all criteria for inclusion and exclusion of studies to the review clearly (PICO, dates, language, study type, publication type, publication status etc.) To be decided *a priori*.	22
2.3	Appraisal for quality/risk of bias tool	Details of tool/checklist used to assess quality, e.g. QUADAS 2, CASP, SIGN, AMSTAR.	26
3.	SEARCH STRATEGY AND STUDY SELECTION (FOR EACH KEY QUESTION)		
3.1	Databases/ sources searched	Give details of all databases searched (including platform/interface and coverage dates) and date of final search.	19
3.2	Search strategy and results	Present the full search strategy for at least one database (usually a version of Medline), including limits and search filters if used. Provide details of the total number of (results from each database searched), number of duplicates removed, and the final number of unique records to consider for inclusion.	19
3.3	Study selection	State the process for selecting studies – inclusion and exclusion criteria, number of studies screened by title/abstract and full text, number of reviewers, any cross checking carried out.	19
4.	STUDY LEVEL REPORTING OF RESULTS (FOR EACH KEY QUESTION)		
4.1	Study level reporting, results and	For each study, produce a table that includes the full citation and a summary of the data relevant to the	Question 1: 27

	risk of bias assessment	question (for example, study size, PICO, follow-up period, outcomes reported, statistical analyses etc.). Provide a simple summary of key measures, effect estimates and confidence intervals for each study where available. For each study, present the results of any assessment of quality/risk of bias.	Question 2: 32 Question 3: 51 Question 4: 59
4.2	Additional analyses	Describe additional analyses (for example, sensitivity, specificity, PPV, etc.) carried out by the reviewer.	Question 2: 35 Question 3: 55 Question 4: 65
5.	QUESTION LEVEL SYNTHESIS		
5.1	Description of the evidence	For each question, give numbers of studies screened, assessed for eligibility, and included in the review, with summary reasons for exclusion.	Question 1: 28 Question 2: 33 Question 3: 52 Question 4: 60
5.2	Combining and presenting the findings	Provide a balanced discussion of the body of evidence which avoids over reliance on one study or set of studies. Consideration of four components should inform the reviewer's judgement on whether the criterion is 'met', 'not met' or 'uncertain': quantity; quality; applicability and consistency.	Question 1: 29 Question 2: 35 Question 3: 55 Question 4: 63
5.3	Summary of findings	Provide a description of the evidence reviewed and included for each question, with reference to their eligibility for inclusion. Summarise the main findings including the quality/risk of bias issues for each question. Have the criteria addressed been 'met', 'not met' or 'uncertain'?	Question 1: 49 Question 2: 49 Question 3: 57 Question 4: 80
6.	REVIEW SUMMARY		

6.1	Conclusions and implications for policy	Do findings indicate whether screening should be recommended? Is further work warranted? Are there gaps in the evidence highlighted by the review?	81
6.2	Limitations	Discuss limitations of the available evidence and of the review methodology if relevant.	84

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