UK National Screening Committee

Screening for Tyrosinaemia I

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

Version: 3

Bazian Ltd

October 2014

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

Template v1.2, June 2010

Key points

Tyrosinaemia type I is a rare inborn metabolic condition.

The technique used for screening for tyrosinaemia type I is currently used to screen for other conditions.

The sensitivity and specificity of screening for tyrosinaemia type I using succinylacetone as a marker is reportedly approximately 100%. The positive predictive value ranges between 40% and 100%.

Tyrosinaemia type I can be treated with NTBC. There is evidence that treatment with NTBC in combination with a tyrosine and phenylalanine restricted diet can improve survival, improve liver function, reduce the risk or delay the development of hepatocellular carcinoma (liver cancer), correct renal tubular dysfunction, and improve rickets and growth.

There is evidence that early treatment with NTBC leads to better outcomes.

Neurocognitive defects have been observed in patients with tyrosinaemia type I treated with NTBC. The cause of these defects is still unclear and warrants further investigation.

A Canadian study calculated the incremental cost per additional life year saved to be $309,209 Canadian dollars. The economic evaluation in the 2004 HTA report estimated that screening for tyrosinaemia by MS/MS to cost £13,168 per life year gained. No more recent UK studies of cost-effectiveness were identified.

Newborn screening for tyrosinaemia type I could be considered. However, further study into the epidemiology of this condition in Europe; studies determining the feasibility of screening for tyrosinaemia type I in the UK; and investigation of the neurocognitive defects observed in some patients treated with NTBC is required.

Summary

Tyrosinaemia type I is a rare disease of amino acid metabolism caused by mutations in both copies of the *FAH* gene, which codes for the enzyme fumarylacetoacetase. Fumarylacetoacetase is one of the enzymes required for the breakdown of tyrosine, an amino acid.

Without fumarylacetoacetase, tyrosine and intermediate breakdown products accumulate, which can damage the liver, kidneys, nervous system and other organs and tissues.

Tyrosinaemia type I can be divided into two forms: an early onset form and a late onset form. The early onset form classically presents as severe liver disease in young infants. The late onset form is characterised by the development of symptoms at more than six months of age including liver dysfunction, renal (kidney) disease, rickets and/or neurological crises. Tyrosinaemia type I is generally fatal before ten years of age in untreated children.

It is not known how many people in the UK have tyrosinaemia. In studies from European countries, the US and Australia incidence varied from less than one case per 944,000 births to more than one case per 31,000 births.

Tyrosinaemia type I can be detected through newborn screening by measuring the levels of tyrosine or succinylacetone (one of the breakdown products of tyrosine that accumulates in tyrosinaemia type I) in the dried blood spot using a technique called tandem mass spectrometry (MS/MS), which is already used to screen for other conditions in the UK. Succinylacetone is a more sensitive and specific marker of tyrosinaemia type I as tyrosine levels can be elevated due

to other conditions (transient tyrosinaemia, liver disease, or tyrosinaemia type II or type III), and because some infants with tyrosinaemia type I may have normal blood concentrations of tyrosine when the screening sample is taken. Recently, methods to extract succinylacetone from dried blood spots in a manner compatible with newborn screening have been developed.

Screening programmes using succinylacetone as a marker have reported approximately 100% sensitivity (the percentage of newborns with tyrosinaemia type I given a positive screening result) and approximately 100% specificity (the percentage of healthy newborns that are given a negative screening result). However, the positive predictive value (the proportion of positive screening results that are due to newborns with tyrosinaemia) varied between 40% and 100%. Differences between screening programmes may be due to, among others, differences in normal values in the population, the timing of the test, the method used to extract succinylacetone and the cut-offs used for classifying a case as screening positive.

One screening programme reported two cases which were symptomatic at the time of diagnosis. This programme used levels of tyrosine to screen for tyrosinaemia type I, and the age of the infants at diagnosis was unclear.

Tyrosinaemia type I can be treated with 2-(2-nitro-4-trifluoromethylbenzoyl)-1, 3 cyclohexanedione (NTBC). There is evidence that treatment with NTBC in combination with a tyrosine and phenylalanine restricted diet can improve survival, improve liver function, reduce the risk or delay the development of hepatocellular carcinoma (liver cancer), correct renal tubular dysfunction, and improve rickets and growth. There is evidence that early treatment with NTBC leads to better outcomes.

However, neurocognitive defects have been observed in patients with tyrosinaemia type I treated with NTBC. Whether this is due to the drug, raised tyrosine levels, depressed phenylalanine levels, or another factor is not clear. Additional follow up and investigation of this is warranted.

No randomised controlled trials of screening for tyrosinaemia type I were identified.

One cost-effectiveness study for screening for tyrosinaemia published in 2007 was identified. The incremental cost per additional life year was $309,209 Canadian dollars (excluding start up, as tandem MS is already used to screen for other conditions in the UK). However, this study was performed before succinylacetone was routinely used to screen for tyrosinaemia, and was performed from a Canadian perspective. It is unclear how applicable this study would be to the UK. The economic evaluation in the 2004 HTA report estimated that screening for tyrosinaemia by MS/MS to cost £13,168 per life year gained.

Newborn screening for tyrosinaemia type I could be considered. However, further study into the epidemiology of this condition in Europe; studies determining the feasibility of screening for tyrosinaemia type I in the UK; and investigation of the neurocognitive defects observed in some patients treated with NTBC is required.

Introduction

This review will assess screening for tyrosinaemia type I, a disorder of amino acid metabolism.

Tyrosinaemia type I is an autosomal recessive disorder resulting from mutations in the gene encoding fumarylacetoacetase (fumarylacetoacetate hydrolase). Deficiency in this enzyme results in the accumulation of fumarylacetoacetate, succinylacetoacetate and succinylacetone, which are thought to be responsible for the clinical symptoms seen in tyrosinaemia type I: liver dysfunction in 'early onset' and liver dysfunction associated with growth failure and rickets or neurological crises at an older age in patients with 'late-onset' tyrosinaemia type I. The distinction between the two forms of tyrosinaemia type I is based on age at which symptoms develop. If left untreated tyrosinaemia type I usually results in death before ten years of age from liver failure, neurological crisis or hepatocellular carcinoma.

Tyrosinaemia type I can be detected before the onset of symptoms through MS/MS analysis of newborn blood spots, and treatment with 2-(2-nitro-4-trifluoromethylbenzoyl)-1, 3 cyclohexanedione (NTBC) can prevent or delay primary manifestations of the condition. However, treatment with NTBC has been associated with neurocognitive deficits, although whether this is due to NTBC treatment is unclear.

Current policy

Tyrosinaemia type I is not currently screened for in the UK. Currently, screening is offered to all babies in the UK for phenylketonuria, congenital hypothyroidism, sickle cell disease and medium-chain acyl-coenzyme A dehydrogenase deficiency. The UK NSC also recommends that screening should be offered for maple syrup urine disease, homocysteinuria, isovaleric acidaemia and glutaric aciduria type I.

The Newborn Screening Programme Centre recommends that both phenylalanine and tyrosine levels are measured in babies who screen positive for phenylketonuria (phenylalanine levels ≥200 µmol/L). This may lead to the identification of newborns with tyrosinaemia type I.

This report

The clinical and cost-effectiveness of screening for tyrosinaemia type I was last reviewed in 2004 by the Health Technology Assessment (HTA) NHS R&D HTA Programme. The HTA study did not recommend screening for tyrosinaemia type I as there was limited evidence regarding the epidemiology, the reliability of the test and the long term outcomes of treatment.

In 2006, on behalf of the US Health Resources and Services Administration, the American College of Medical Genetics (ACMG) published an analysis of the scientific literature and gathered expert opinion on the effectiveness of newborn screening and on newborn screening programme optimisation to produce:

- a uniform condition panel (including implementation methodology);
- model policies and procedures for State newborn screening programmes (with consideration of a national model);

- model minimum standards for State newborn screening programmes;
- a model decision matrix for consideration of State newborn screening programme expansion;
- and consideration of the value of a national process for quality assurance and oversight.

Twenty-nine conditions were assigned to the core panel. Tyrosinaemia type I was included in the core panel.

A 2012 report on the practices of newborn screening for rare disorders implemented in member states of the European Union, candidate, potential candidate and European Free Trade Association countries reported that screening for tyrosinaemia type I is undertaken in ten countries (Austria, French community of Belgium, Denmark, Hungary, some regions of Italy, the Netherlands, Portugal, Spain and Iceland).[1]

Newborn screening for tyrosinaemia type I was re-assessed against the UK National Screening Committee (NSC) criteria for appraising the viability, effectiveness and appropriateness of a screening programme (National Screening Committee 2003).

Particular areas of interest identified by the National Screening Committee included:

- the condition, including the timing of presentation
- the test, including its timing, benign/uncertain/additional cases detected, performance in premature infants
- treatment, including information on outcome, benefits and harms

For this review an updated systematic search has been performed for relevant publications from 2004 to August 2012. Overall, 457 citations were judged to be relevant (see Methodology section for study breakdown). The full texts of selected papers were retrieved after a first pass appraisal at abstract level. Non-systematic reviews, editorials, other opinion pieces, reports of case series of fewer than four patients, and those with nonhuman data were excluded, as were conference abstracts. Priority was given to studies from Europe, North America and Australia. Additional relevant references identified during the preparation of the report were also included. An overview of the most informative and relevant references regarding the individual screening criteria is given below.

Appraisal against UK NSC Criteria

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

1. The condition should be an important health problem

Tyrosinaemia type I can be divided into several forms.[2] Distinction between the forms is based on the age of onset of symptoms. Tyrosinaemia type I can be divided into a form with symptoms before six months of age and a form which presents later in life. The later onset form is usually milder. Patients with the early onset form have severe liver involvement. Patients with the late onset form present with liver dysfunction associated with growth failure and rickets or neurological crises. If left untreated tyrosinaemia type I usually results in death before ten years of age from liver failure, neurological crisis or hepatocellular carcinoma.[2]

Tyrosinaemia type I is an important health problem and is screened for in the US and other European countries

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A 2012 report on the practices of newborn screening for rare disorders implemented in member states of the European Union, candidate, potential candidate and European Free Trade Association countries reported that screening for tyrosinaemia type I is undertaken in ten countries (Austria, French community of Belgium, Denmark, Hungary, some regions of Italy, the Netherlands, Portugal, Spain and Iceland).[1]

Summary: Criterion 1 met. Tyrosinaemia type I is rare but if left untreated the acute early onset form can be fatal. The late onset form is milder but can lead to liver failure, neurological crisis and hepatocellular carcinoma. Tyrosinaemia type I was included in the US core panel of disorders by the ACMG and is screened for by a number of European countries.

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

Natural history

Tyrosinaemia type I is an autosomal recessive disorder resulting from mutations in the gene encoding fumarylacetoacetase (fumarylacetoacetate hydrolase).[2] This enzyme catalyses the final step in the tyrosine catabolism pathway, the conversion of fumarylacetoacetate (fumarylacetoacetic acid) into fumarate and acetoacetate. Deficiency in this enzyme leads to the accumulation of fumarylacetoacetate. Fumarylacetoacetate is converted into succinylacetoacetate and succinylacetone. The accumulation of fumarylacetoacetate, succinylacetoacetate and succinylacetone is thought to be responsible for the clinical symptoms seen in tyrosinaemia type I.

As mentioned in Criterion 1, tyrosinaemia type I can be divided into several forms depending on the age of onset of symptoms. Patients with the early onset form have severe liver involvement.[2] The later onset form is usually milder, and patients with the late onset form present with liver dysfunction associated with growth failure and rickets or neurological crises. If left untreated tyrosinaemia type I usually results in death before ten years of age from liver failure, neurological crisis or hepatocellular carcinoma.[2]

It is known that mutations in the *FAH* gene encoding fumarylacetoacetase are responsible for tyrosinaemia type I, but so far there is no correlation between clinical presentation and genotype.[2]

The 2004 HTA report stated that:[3]

"Tyrosinaemia type I results from a deficiency of fumarylacetoacetase with resulting accumulation of fumarylacetoacetate and its metabolic precursor maleyl-acetoacetate and inhibition of a variety of other enzyme systems. The characteristics of this disorder are thought

to relate to an accumulation of these toxic metabolites. Fumarylacetoacetase occurs mainly in the liver and kidneys; therefore, patients with tyrosinaemia type I present with liver failure in infancy (acute form) or show a more protracted course resulting in hepatocellular carcinoma (sub-acute and chronic forms).

The majority of children with tyrosinaemia type I present with the acute form. Symptoms such as vomiting, diarrhoea, lethargy and failure to thrive may appear in the first months of life. There are also signs of liver disease, with hypoproteinaemia, hyperbilirubinaemia, defective coagulation capacity and hypoglycaemia. Serum tyrosine is elevated and large amounts of tyrosine metabolites are excreted in the urine. Neurological crises similar to those seen in acute porphyria can occur and may be the presenting feature. The condition is progressive and death from liver failure will occur in the first year of life in untreated patients.

The chronic form is characterised by liver disease, renal tubular dysfunction and hypophosphataemia with vitamin D-resistant rickets. Children characteristically present with rickets and/or hepatomegaly during infancy or at school age. Hepatic cirrhosis, hepatoma and renal failure may develop chronically and patients usually die within the first decade of life if left untreated."

The natural history of tyrosinaemia type I is changed by treatment with 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3 cyclohexanedione (NTBC) in combination with a low tyrosine diet, with treatment improving survival, improving growth, improving liver function, preventing cirrhosis, and correcting renal tubular acidosis and secondary rickets (see Criterion 10).

Cardiomyopathy has also been observed in patients with tyrosinaemia type I. Arora et al. (2006) described 20 children with tyrosinaemia type I who were referred to the Children's Hospital NHS Trust, Birmingham, between 1986 and 2002.[4] All were initially treated with dietary therapy, and since 1992 with NTBC. Six (30%) had cardiomyopathy at initial assessment, although no child had symptoms relating to the cardiovascular system. After a median follow-up of 3.6 years, five of the six had complete resolution of cardiomyopathy and the other patient showed significant improvement. No child with a normal initial echocardiograph subsequently developed cardiomyopathy.[4]

Incidence

The HTA concluded that "*the expected incidence of tyrosinaemia type I is 1.8 cases per 100,000 births and may be appreciably higher in ethnic groups such as the Pakistani population than in northwest European populations*" based on one study which reported on the incidence of tyrosinaemia type I in one region of the UK between 1981 and 1991 (Neonatal Screening Programme, West Midlands, UK).[3]

Sanderson et al. (2006) reported the incidence (assumed to be equivalent to birth prevalence) of amino acid disorders in the West Midlands, UK between 1999 and 2003, although incidences for individual disorders was not given.[5] Based on definitive diagnosis resulting from the investigation of a clinically-presenting patient the birth prevalence of amino acid disorders excluding PKU was 1 in 5,354.[5]

No studies investigating the incidence of tyrosinaemia type I in the UK were identified. Studies which have assessed epidemiology, screening or treatment can offer some estimate of the incidence of the condition. The incidences calculated from these studies in European, North American and Australian populations are shown in Table 1.

Incidences of tyrosinaemia type I range from 1:30,672 (Germany, during a pilot screening trial using succinylacetone as a marker)[6] and 1:944,078 (North Carolina, US, one missed case of tyrosinaemia type I from a cohort of 944,078 screened infants).[7] It was not possible to calculate incidences in studies that did not identify any cases.

Two studies present results which allow the impact of screening programmes on incidence to be assessed.

In Australia, the incidence of tyrosinaemia type I was 1:517,067 in a cohort of unscreened infants born between 1994 and 2002 who were either born before the introduction of screening or were born in a region that had not yet introduced screening.[8] In the screened cohort (born between 1998 and 2002), two cases of tyrosinaemia type I were identified. The incidence of tyrosinaemia type I was higher, 1:230,750. However, both of these cases were missed by screening (tyrosine used as the marker analyte).

Lund et al. (2012) compared the incidence of tyrosinaemia in Denmark, the Faroe Islands and Greenland in the decade before newborn screening and the decade after the introduction of newborn screening.[9] The incidence remained similar after the introduction of screening. In the decade before the screening, the incidence was 1:112,459. After the introduction of screening the incidence was 1:140,565.

In conclusion, the incidence of tyrosinaemia type I varied widely between reports from different countries, from more than one case per 31,000 births to less than one case per 944,000 births. Where a screened cohort was compared to a contemporaneous or historical unscreened cohort in the same country, one study found that the incidence of tyrosinaemia type I was similar. Another study found the incidence increased, despite the fact that the cases were missed by screening. It should be noted that individual cases of the disease have a big impact on calculated incidences as tyrosinaemia type I is a rare disease.

Table 1: Estimates of the incidence of tyrosinaemia type I. NR, not reported; NA, not applicable.

Study	Country and time period	Screening programme in operation?	Number of identified cases	Incidence	Notes
Bliksrud et al. (2012)[10]	Norway 1991 to end of 2010	No	14	1:74,800	Incidence adjusted for undiagnosed cases based on the age of patients at the time of diagnosis
Lund et al. (2012)[9]	Demark, the Faroe Islands and Greenland January 1992 to December 2001	No	NR	1:112,459	
Lund et al. (2012)[9]	Demark, the Faroe Islands and Greenland 2009 to 2011	Yes	1	1:140,565	
Couce et al. (2011)[11]	Galicia, Spain 2000 to 2010	Yes	3 (2 identified by screening)	1:105,082 1:70,055 if case missed by screening included	1 case of tyrosinaemia type I given a false negative result.
Lindner et al. (2011)[12]	South-West Germany January 1999 to April 2005	Yes	2	1:291,777	An additional suspected case died before tyrosinaemia could be confirmed
Morrissey et al. (2011)[13]	New York, US December 2007-December 2009	Yes	2	1:250,000	
Kasper et al. (2010)[14]	Austria 2002 to 2010	Yes	5	1:124,498	

Study	Country and time period	Screening programme in operation?	Number of identified cases	Incidence	Notes
Wilcken et al. (2009)[8]	Australia Born between 1994 and 2002	No	3	1:517,067	
Wilcken et al. (2009)[8]	Australia Born between 1998 and 2002	Yes	2 (0 cases identified by screening)	1:230,750 if cases missed by screening included	2 cases were missed by screening using tyrosine only as the marker analyte
la Marca et al. (2008) [15]	Tuscany, Italy January 2002 to end of 2007	Yes	1 (0 cases identified by screening)	1:160,000 if case missed by screening included	160,000 infants screened 1 case missed by screening
Masurel-Paulet et al. (2008)[16]	France 1990-NR	No	74	<1:200,000	
Feuchtbaum et al. (2006)[17]	California, US January 2002 to June 2003	Yes	0	NA	353,894 infants screened
Frazier et al. (2006)[7]	North Carolina, US July 1997 to July 2005	Yes	1 (0 cases identified by screening)	**1:944,078** if case missed by screening included	944,078 infants screened 1 case missed by screening
Sander et al. (2006)[6]	Germany 16 weeks	Yes	2	**1:30,672**	Pilot screening trial using succinylacetone as a marker for tyrosinaemia type I
Comeau et al. (2004)[18]	New England Screening Program January 1999 to February 2003	Yes	0	NA	318,535 infants were screened

Detectable risk factor or disease marker

In tyrosinaemia type I, deficiency in fumarylacetoacetase (fumarylacetoacetate hydrolase) blocks the tyrosine catabolic pathway.[2] This leads to accumulation of the substrate of fumarylacetoacetase, fumarylacetoacetate. Fumarylacetoacetate is converted into succinylacetoacetate and succinylacetone. Succinylacetone interferes with the activity of parahydroxyphenylpyruvic acid dioxygenase, resulting in elevation in plasma tyrosine concentration, and PBG synthase (ALA dehydratase), which has a number of knock-on effects.[2]

The increase in plasma tyrosine levels, the levels of succinylacetone in blood and urine and the activity of PBG synthase have all been used as markers of tyrosinaemia type I (see Criterion 5). Tyrosine and succinylacetone concentrations are the common markers analysed from dried blood spots by tandem mass spectrometry (MS/MS) in newborn screening programmes. Succinylacetone is a more sensitive and specific marker of tyrosinaemia type I as tyrosine levels can be elevated due to transient tyrosinaemia, liver disease, or tyrosinaemia type II or type III, and because some infants with tyrosinaemia type I may have normal blood concentrations of tyrosine when the screening sample is taken.

Latent period or early symptomatic stage

The length of time before the onset of symptoms of tyrosinaemia type I varies, and no studies were found which identified a marker that could discriminate between the early and late onset forms. Several studies were identified which reported whether identified infants were symptomatic before the results of newborn screening were available. Lindner et al. (2011) reported on the efficacy and outcome of a pilot expanded newborn screening programme for metabolic diseases in South-West Germany (1999-2005).[12] Two cases of tyrosinaemia type I were identified, both before the onset of symptoms. However, a suspected case of tyrosinaemia type I died before diagnosis could be confirmed. The case of tyrosinaemia type I identified in Denmark, the Faroe Islands and Greenland between 2002 and 2011 was not symptomatic at the time of diagnosis.[9] However, Couce et al. (2011), reported that the two cases of tyrosinaemia type I identified between 2000 and 2010 in Galicia, Spain, were symptomatic when the condition was diagnosed.[11] In Galicia, samples of blood and urine were collected for screening between the 5th and 8th day of life between 2000 and 2002. In 2003, this changed to the 3rd day of life.

Summary: Criterion 2 partly met. No studies were identified that reported the incidence or prevalence of tyrosinaemia type I in the UK. The natural history of the condition is well established. Tyrosinaemia type I can be detected through MS/MS analysis of newborn blood spots. The concentrations of tyrosine and succinylacetone can be used to screen for tyrosinaemia type I. Succinylacetone is a more sensitive and specific marker of tyrosinaemia type I as tyrosine levels can be elevated due to transient tyrosinaemia, liver disease, or tyrosinaemia type II or type III, and because some infants with tyrosinaemia type I may have normal blood concentrations of tyrosine when the screening sample is taken. The duration of the latent asymptomatic period varies, and no marker has been identified that can discriminate between the early and late onset forms. It has been reported that infants with tyrosinaemia type I can develop symptoms before the results of a newborn dried blood spot screen are available.

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

Criterion 3 not applicable. Tyrosinaemia type I is a genetic diseases.

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

This report will focus on the use of tandem mass spectrometry, or MS/MS to quantify levels of markers in punches from dried blood spots.

The screening test identifies individuals with abnormal levels of markers in the blood, rather than screening for the presence of a mutation. No reports of the identification of individuals heterozygous for mutations (carriers) through MS/MS screening were identified.

If a child is diagnosed with tyrosinaemia type I, it would mean that the parents are obligate carriers. This would also be the case if infants with this condition are identified due to presentation with symptoms, although more infants with this condition may be diagnosed if newborn screening is implemented (i.e. fewer infants may remain undiagnosed).

Criterion 4 not applicable. The screening test does not identify carriers of a mutation.

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

This report will focus on the use of tandem mass spectrometry, or MS/MS to quantify levels of markers in punches from dried blood spots. MS/MS was the only technology considered in the 2004 HTA of the clinical and cost-effectiveness of neonatal screening for inborn errors of metabolism, and has been introduced by several newborn-screening programmes around the world for the detection of amino acid disorders.[3]

Since 2009 all UK laboratories have used tandem mass spectrometry as the screening technology for screening for phenylketonuria.[19] Multiple analytes can be simultaneous assayed, allowing the detection of a range of metabolic disorders using the same dried blood-spot sample collected as part of the current screening programme.

The 2004 HTA report concluded that the “evidence regarding the sensitivity and specificity of neonatal screening for tyrosinaemia type I using tandem MS is limited”.[3]

Since the publication of the HTA report, the results of several expanded screening programmes which have used MS/MS on a dried blood spot to screen for tyrosinaemia type I have been published.

Screening for tyrosinaemia type I

Tyrosinaemia type I was initially screened for using elevated tyrosine levels as a marker. However, hypertyrosinaemia also occurs in other physiological and pathological conditions, and

tyrosine levels are not consistently elevated early in tyrosinaemia type I. Therefore, screening with tyrosine as a marker was associated with the identification of both false positives and false negatives. Succinylacetone is a specific marker of tyrosinaemia type I, but is not extracted from dried blood spots using the conventional methanol technique. Several studies were identified in the update search that had developed protocols for extracting succinylacetone from dried blood spots using methods suitable for newborn screening.

Allard et al. (2004) described a method to extract succinylacetone from residual dried blood spots which had already undergone normal methanol extraction for routine MS/MS analysis.[20] Succinylacetone was extracted by adding an acetonitrile and water solution (80:20 by volume) containing 0.1% formic acid and 15mmol/L hydrazine hydrate (0.1% by volume) to residual dried blood spots. Succinylacetone concentrations were then analysed in a separate MS/MS run.

Sander et al. (2006) used the method described in Allard et al. (2004)[20] in a 16-week study in Germany.[6] During the study 61,344 dried blood spots were prospectively analysed. Two affected children were identified, no false positives and no false negatives at the time of publication. In all cases identified, the authors report that they would not have been identified by tyrosine screening alone. The sensitivity and specificity of the test were 100%.

Morrissey et al. (2011) reported results for 24 months from December 2007 for screening for tyrosinaemia type I in New York using succinylacetone as a marker, extracted using a similar technique to that described in Allard et al. (2004)[20] and Sander et al. (2006)[6].[13] Succinylacetone analysis was performed one day after amino acid/acylcarnitine analysis. In the New York screening programme, any sample with an initial succinylacetone value of 3μmol/L or more is flagged and retested in duplicate. For any specimen with an average succinylacetone value (initial and retest results) of between 3μmol/L and 5μmol/L, a repeat blood specimen is requested. For any sample with an average succinylacetone value of 5μmol/L or more an immediate referral is made to the appropriate speciality care centre. Between 2008 and 2009, approximately 500,000 samples were screened. There were five screen positives: two were in the range 3-5μmol/L and required repeat samples, which were negative. Three samples had levels requiring immediate referral to a metabolic centre. Two patients were diagnosed with tyrosinaemia type I, the other patient was a false positive. In the patients with tyrosinaemia type I, the initial tyrosine values were well below the cut-off used for the tyrosine analysis. The sensitivity of the test was 100% and the specificity was 99.9994%. The positive predictive value was 40%.

The Mayo Clinic developed a two tier assay, in which succinylacetone was measured if tyrosine levels were elevated.[21,22] However, as this approach still relies on elevated tyrosine levels as a first screen the risk of false negatives remains. This method will not be discussed further.

Turgeon et al. (2008) reported a technique in which amino acids and acylcarnitines and then succinylacetone were extracted sequentially, but the extracts were then pooled so that only a single MS/MS run is required.[23] In this technique, amino acids and acylcarnitines are extracted using methanol, and then succinylacetone is extracted in an acetonitrile:water solution containing hydrazine. However, there were concerns that this protocol could result in the presence of underivatized acylcarnitines in the pooled extract, which could complicate the analysis of the mass spectra. An improved method, including extra wash steps of the residual dried blood spot to reduce the underivatized acylcarnitines was published by Chase et al. (2009).[24]

La Marca et al. (2008) developed a technique in which succinylacetone could be co-extracted with acylcarnitines and amino acids by using a mixture of hydrazine hydrate (1mmol/L) in water and methanol.[25] This technique has been used in Tuscany, Italy. A report in 2009 stated that since 2007, 87,000 newborns from Tuscany have been screened using this extraction technique, and that during this period one infant with tyrosinaemia type I has been identified through newborn screening.[26] Dhillion et al. (2011) also developed a single extraction method for succinylacetone, amino acids and acylcarnitines, using an acetonitrile-water-formic acid mixture containing hydrazine.[27] The extract is then derivatized with n-butanolic-HCl and analysed by MS/MS.

All the techniques for extracting succinylacetone described so far involve hydrazine. Metz et al. (2012) evaluated a commercially available mass spectrometry kit which does not contain hydrazine (MassChrom® Amino Acids and Acylcarnitines form Dried Blood; Chromsystems, Munich, Germany).[28]

Reports of screening programmes for multiple disorders

The results of several expanded newborn screening programmes using MS/MS have been published since 2004. Unfortunately, most of the reports do not present enough detail for the sensitivity and the specificity of MS/MS screening for individual disorders to be calculated. In addition, many screening programmes have used the same analyte as a marker for multiple disorders.

The results of the screening studies identified are summarised below and in Table 3. Where possible, if insufficient data was presented to calculate the sensitivity and specificity of screening for tyrosinaemia type I, the sensitivity and specificity of the screening programme as a whole has been calculated.

Lund et al. (2012) described the results of expanded newborn screening in Denmark, the Faroe Islands and Greenland (2002 to March 31st 2011).[9] Flagged samples were re-analysed in duplicate. If abnormal profiles were reproduced, The Centre for Inherited Metabolic Disorders, Copenhagen University Hospital, was immediately contacted. A specialist in metabolic disorders subsequently contacted the child's local paediatric department, which then contacted the families and initiated confirmatory testing of the child. Screening for tyrosinaemia type I, using succinylacetone as a marker, started in 2009, with the introduction of the PerkinElmer NeoBase non-derivatized MS/MS kit to extract analytes. The results of screening are shown in Table 2. One true positive case of tyrosinaemia type I was identified, and no false positives or false negatives.

Table 2: Screening results, and sensitivity and specificity of MS/MS screening for tyrosinaemia type I using results from Lund et al. (2012)[9]

Disorder	Number screened	True positives	False Positives	False Negatives	True negatives	Sensitivity	Specificity
Tyrosinaemia type I	140,565	1	0	0	140,564	100%	100%

Couce et al. (2011) reported on newborn screening in Galicia, Spain between 2000 and 2010.[11] During this period, 210,165 infants were screened and 137 cases of inborn errors of metabolism

were identified. There were 43 false positives and four false negative results. Therefore the screening programme as a whole had a sensitivity of 97.16%, a specificity of 99.98% and a positive predictive value 76.11%. Enough detail is provided to calculate the sensitivity and specificity of screening for tyrosinaemia type I. Screening for tyrosinaemia type I was performed by analysing tyrosine levels. Two true positives, no false positives and one false negative were identified, corresponding to a sensitivity of 66.67%, a specificity of 100% and a positive predictive value of 100%.

Lindner et al (2011)[12] reported on expanded newborn screening for metabolic diseases in South-West Germany. Tyrosinaemia type I was initially included in the screening panel, but was not part of the legal screening panel in Germany implemented from April 2005 onwards. The authors report that for the programme overall (until June 2009, 1,084,195 children screened) confirmatory testing was recommended for 377 cases and in 373 a metabolic disorder was confirmed. In addition they report that there have been no false-negative cases of any disorder reported. If it is assumed that confirmatory testing was recommended in all screen positives, we can calculate that the programme overall had a sensitivity of 100% and a specificity of 99.9996%.

Kasper et al. (2010) described the MS/MS screening programme in Austria between April 2002 and December 2009.[14] During this period, 622,489 infants were screened. In the Austrian programme, if a dried blood spot screened positive, another disk from the same dried blood spot was punched. If the result indicated that an infant was at risk of acute metabolic decompensation the infant was immediately recalled for confirmatory/diagnostic testing, otherwise a repeat dried blood spot was obtained prior to confirmatory/diagnostic testing. The results for the screening programme of a whole are reported. 1,728 newborns had positive result on initial screening, and 218 were diagnosed with an inborn error of metabolism. A total of four infants with false negative results were reported (two cases of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and two cases of methylmalonic academia). The overall sensitivity was 98.20% and specificity was 99.76%. The positive predictive value was 12.62%.

Wilcken et al. (2009) described the results of screening in Australia between 1998 and 2002, during which period 461,000 infants were screened.[8] The programme had a false positive rate of 0.18%, and seven cases were missed by screening (false-negatives). The number of false positives per condition was not reported.

la Marca et al. (2008) described the six year experience of screening (January 2002 to October 2004 pilot, all infants since November 2004) for more than 40 inborn errors of metabolism in Tuscany.[15] Infants who screen positive for disorders with possible acute metabolic decompensation are immediately recalled and clinical examinations and confirmatory tests are performed. Infants who screen positive for other disorders provide a second bloodspot. If this also screens positive clinical examinations and confirmatory tests are performed. Not enough details were provided to calculate the sensitivity or specificity of the test.

Feuchtbaum et al. (2006) described a pilot MS/MS screening programme in California between January 2002 and June 2003.[17] During the pilot, 353,894 infants were screened. No cases of tyrosinaemia type I were identified. For the whole programme, 701 results were flagged, and 461 were classified as screen positive and were referred. Of these, 51 were diagnosed with a disorder. Three cases of MS/MS detectable diseases were missed. For the screening programme as a whole, the sensitivity was 94.4% and the specificity was 99.9%.

Frazier et al. (2006) described the MS/MS screening programme in North Carolina between 1997 and 2005.[7] For most analytes, both a 'border line' and 'diagnostic' cut-off were established. If the screening result was above the borderline cut-off, another disc was punched from the same sample card and the analysis was repeated before a report was generated. If the screening result was above the diagnostic cut-off, a metabolic specialist was contacted who immediately contacted the infant's primary care provider with results and recommendations. Results shown in Table 3 are from 2003 to 2004 only, the year that the cut-offs were implemented. During this period 239,415 newborns were screened. For the programme as a whole, 27 infants had repeat samples above the borderline cut-off and were screened positive, and 82 infants had levels above the diagnostic cut-off and were screened positive. There were a total of 58 confirmed diagnoses. The positive predictive value for all disorders screened for was 53%.

Comeau et al. (2004) reported results from the New England Screening Program between January 1999 and February 2003.[18] Data was presented for 19 metabolic disorders together, including tyrosinaemia type I. Considering the panel of 19 metabolic conditions as a whole, 425 infants screened positive; 121 were referred to a specialist, and 28 infants were diagnosed with a condition (318,535 infants screened). Therefore there were 15 screen positives per case and four specialist referrals per case. Tyrosinaemia was the disorder accounting for the most false positive screens (76 of the 425 screen positives and 4 of 121 specialist referrals, but no confirmed cases).

The timing of the test

The timing of blood spot sampling is a consideration. The levels of markers may vary physiologically, and the optimal time period for sampling may vary between conditions. Where reported, the age at which blood spots were taken was reported in Table 3. An additional consideration is the need for screening results before the onset of symptoms. As discussed in Criterion 2, there have been reported cases of patients who became symptomatic before screening results were available.

In the UK, the blood spot sample is taken on day five of life (in exceptional circumstances between day five and day eight) for all babies regardless of medical condition, milk feeding and prematurity. Premature infants are retested at 28 days of age.[29] The time of blood spot collection may need to be earlier to screen for tyrosinaemia type I.

The sensitivity and specificity of the test in premature infants

No studies were identified that analysed the sensitivity and specificity of the test in premature infants. Several screening programmes reported that additional samples were taken from premature infants later in life (see

Table 3). In addition, we identified one study that looked at the normal ranges of analytes in premature and acutely ill infants (Oladipo et al. [2011][30]). This study found that tyrosine levels were significantly elevated in premature infants but not elevated in acutely ill infants. Variation in succinylacetone was not explored, but levels of this metabolite should be low in all normal infants (see Criterion 6).

Summary: Criterion 5 met. Screening for tyrosinaemia had been improved by the development of methods to extract succinylacetone from dried blood spots in a manner compatible with newborn screening. Succinylacetone levels should be low in all infants without tyrosinaemia, including premature infants. Screening programmes using succinylacetone as a marker have reported 100% sensitivity and 100% specificity. However, other studies have reported the identification of false positives. Differences between screening programmes may be due to, among others, differences in normal values in the population, the timing of the test, the method used to extract succinylacetone and the cut-offs used for classifying a case as screening positive.

Table 3: Screening for tyrosinaemia type I. NR, not reported.

Study	Country, period	Age at which sample collected	Analyte and cut-off	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
Lund et al. (2012)[9]	Denmark, the Faroe Islands and Greenland February 2009 to March 31st 2011	2.5 days (median, February 2009 to March 2011)	Succinylacetone Cut-off: >2.1U	PerkinElmer NeoBase non-derivatized MS/MS kit	140,565	1	0	0	All preterm newborns had their expanded biochemical screening test repeated at gestational age 32 weeks or when oral feeding had been established.
Couce et al. (2011)[11]	Galicia, Spain 2000 to 2010	Between the 5 and 8 days of life (between 2000 and 2002) 3 days of life (2003 onwards)	Tyrosine Cut-off: NR	NR	210,165	2	0	1	In Galicia, blood and urine samples are collected and tested for all newborns
Lindner et al. (2011)[12]	South-West Germany	Between 3 and 5 days of life (before	Details given in Schulze et al. (2003)[31]:	Details given in Schulze et al. (2003)[31]:	583,553	2	NR	0	A suspected case of tyrosinaemia type I died

Study	Country, period	Age at which sample collected	Analyte and cut-off	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
	January 1999 to April 2005	2002) and between 36 and 72 hours of life thereafter	Tyrosine Cut-off: >200μmol/L plus positive succinylacetone test	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)					before tyrosinaemia type I could be confirmed.
Morrissey et al. (2011)[13]	New York, US 2008 to 2009	NR	Succinylacetone Cut-off: 3μmol/L (retest) 5μmol/L (immediate referral)	Succinylacetone extracted from residual blood spots using an acetonitrile: water solution containing hydrazine	500,000	2	3	0	Total number screened approximate
Kasper et al. (2010)[14]	Austria April 2002 to December 2009	Between 36 and 72 hours of life.	Two stage screen: Elevated tyrosine; 5-aminolevulinic acid dehydratase activity Cut-off: NR	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	622,489	5	NR	0	A second screening sample was obtained and assayed after 14 days of life from all infants born prior to 32 weeks gestational age

Study	Country, period	Age at which sample collected	Analyte and cut-off	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
Wilcken et al. (2009)[8]	Australia 1998 and 2002	Between 48 to 72 hours of age	NR	NR	461,500	0	NR	2	
la Marca et al. (2008)[15]	Tuscany, Italy 2002 to 2008	Between 48 and 72 hours of life	Elevated tyrosine Cut-off: 200μmol/L Succinylacetone from January 2007 Cut-off: >2μmol/L (Tyrosine a secondary marker, with a cut-off >250μmol/L)	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters. From 2007, samples extracted with hydrazine hydrate in water and methanol and then converted to their butyl esters (derivatized)	160,000	0	NR	1 (during period when tyrosine was the marker for tyrosinaemia)	For premature infants, a sample is collected between 48 hours and 72 hours of life, then 2 additional samples at 15 and 30 days. For babies on parenteral nutrition, including premature babies, a second sample at 48 hours after the ending of parenteral nutrition is collected. In

Study	Country, period	Age at which sample collected	Analyte and cut-off	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
									all transfused newborns, a new sample is collected seven days after the end of transfusion
Feuchtbaum et al. (2006)[17]	California, US January 2002 to June 2003	NR	NR	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	353,894	0	NR	0	
Frazier et al. (2006)[7]	North Carolina, US 2003 to 2004	39 hours	Elevated tyrosine Cut-off: Borderline: >500μmol/L; Diagnostic: >900μmol/L	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	239,415	0. However elevated tyrosine was used to screen for other disorders, and one true positive for another disorder was	27 patients had repeated levels above the borderline cut-off or a screen result above the diagnostic cut-off, and no cases of tyrosinaemia type I were	Uncertain. One case of tyrosinaemia was missed during the screening programme, but it is unclear when this case was screened.	Cut-offs used from January 2003

Study	Country, period	Age at which sample collected	Analyte and cut-off	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
						identified.	identified		
Sander et al. (2006)[6]	Germany 16 weeks	36 to 72 hours of life	Succinylacetone Cut-off: >10μmol/L	Succinylacetone extracted from residual blood spots using an acetonitrile: water solution containing hydrazine	61,344	2	0	0	Unclear whether the cut-off was selected *a priori*.
Comeau et al. (2004)[18]	New England, US January 1999 to February 2003	NR	NR	NR	318,535	0	76	NR	

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

A US Region 4 project, a Regional Genetics and Newborn Screening Collaborative, aimed to achieve uniformity of testing panels by MS/MS, improve overall analytical performance and set and sustain the lowest achievable rates of false positive and false negative results. It initially included seven US states, but has expanded into an international collaboration, including 80 programmes in 45 countries, in addition to 47 US states and Puerto Rico. The Region 4 Stork has collected data on:

- Five selected percentiles of individual markers and ratios in the normal population,
- Cut-off values used in routine screening practice
- The complete set of all available amino acid and acylcarnitine results in true positive cases (defined as cases meeting the case definition established by local protocols and/or professional guidelines)
- Performance metrics (detection rate, false positive rate, and positive predictive values)
- Other information relating to newborn screening, including source of reagents, use of derivatization, date of collection and punch size.

The collaboration recently published a paper which aimed to clinically validate cut-off values for newborn screening using tandem mass spectrometry.[32]

From the data submitted, tyrosine and succinylacetone cumulative percentiles in normal neonatal dried blood spots were calculated. These are shown in Table 4. The authors note that similar values for normal ranges between sites were seen for most markers, with the exception of argininosuccinic acid and succinylacetone. Succinylacetone levels varied between sites, and this may be due to pre-analytical variables. However, the authors state that succinylacetone is essential for the reliable detection of tyrosinaemia type I. As noted previously, elevated tyrosine concentration can be a marker for several conditions.

Table 4: Succinylacetone and tyrosine cumulative percentiles (markers of interest for the screening of tyrosinaemia type I) in the normal population, as submitted by participants of the Region 4 Stork Collaborative project (as of December 1, 2010). CV coefficient of variation (calculated from values within the interquartile range: median ± [(75 percentile – 25 percentile) x 1.5]), n= number of participants that submitted percentile values. Modified from McHugh et al. (2011)[32]

			Normal population (μmol/L)					
			1 percentile		50 percentile		99 percentile	
Marker	**Condition(s) marker screens for**	**N**	**Value**	**CV**	**Value**	**CV**	**Value**	**CV**
Succinylacetone	Tyrosinaemia type I	22	0.21	85%	0.66	54%	1.4	84%
Tyrosine	Tyrosinaemia type I Tyrosinaemia type II Tyrosinaemia type III Transient tyrosinaemia	97	34	19%	80	16%	207	17%

Succinylacetone and tyrosine ranges in true positive cases identified by the participants of the Region 4 Stork Project were also reported (Table 5).

Table 5: Marker ranges in neonatal dried blood spots analysed by tandem mass spectrometry in affected newborn cases, from participants of the Region 4 Stork Collaborative Project (as of December 1, 2010). Modified from McHugh et al. (2011)[32]

		Percentiles of disorder ranges (μmol/L)								
Condition	**Marker**	**N**	**1%**	**5%**	**10%**	**25%**	**50%**	**75%**	**90%**	**99%**
Tyrosinaemia type I	Succinylacetone	59	3.8	8.8	13	20	35	47	66	148
Tyrosinaemia type I	Tyrosine	86	66	89	111	141	201	302	489	834

Using this data, the authors calculated clinically relevant cut-off ranges. For succinylacetone and tyrosine, screens above the cut-off would be considered positive (Table 6). The cut-off ranges were defined as the interval between the cumulative 99th percentile of the normal population and the lowest 5th percentile of all disorder ranges of the same marker (if the analyte is informative for multiple conditions). One or both limits of the target range were adjusted depending on the degree of overlap between the normal population and the disorder range, to balance sensitivity and specificity.

Table 6: Cut-offs for succinylacetone and tyrosine suggested by the Region 4 Stork Collaborative Project.[32] The cut-off ranges were defined as the interval between the cumulative 99th percentile of the normal population (lower end of range, 'low' in the table) and the lowest 5th percentile of all disorder ranges of the same marker (if the analyte is informative for multiple conditions, 'high' in the table). One or both limits of the target range were adjusted depending on the degree of overlap between the normal population and the disorder range, to balance sensitivity and specificity.

			Cut-off range (μmol/L)	
Marker	**Number of cases**	**Number of conditions**	**Low**	**High**
Succinylacetone	60	1 (Tyrosinaemia type I)	1.4	7.5
Tyrosine	204	4 (Tyrosinaemia type I, tyrosinaemia type II, tyrosinaemia type III, transient tyrosinaemia)	207	226

Recently, this collaboration has reported the development of multivariate pattern-recognition software designed to convert metabolic profiles into a composite score driven by the degree of overlap between the normal population and the disease range.[33] The software has been developed using the MS/MS profiles of 12,077 patients affected with 60 metabolic disorders and 644 heterozygotes carriers for 12 conditions. The authors report that, as of 15 December 2011, a total of 90 active tools were available, 37 of which were applicable to the differential diagnosis of two or more conditions. The authors report that an "all conditions" tool, designed to evaluate a full amino acid and acylcarnitine profile to suggest any possible diagnosis is soon to be released. The tools are intended to generate a score that drives the interpretation and resolution of cases with potentially abnormal MS/MS results.[33]

Cut offs from individual screening studies

Individual screening studies have also reported cut-offs (see Table 7). As can be seen, the marker analysed and the cut-offs used vary. This may be linked to the age at which specimens are collected and the protocol used to extract markers from the dried blood spot. The differences in cut-offs and markers used may explain some of the variation in the number of false-positives and false-negatives identified by different screening programmes (see Criterion 5).

Table 7: Marker and cut-offs used to screen for tyrosinaemia type I

Study	Primary marker	Cut-off	Secondary marker	Cut-off	Notes
Lund et al. (2012)[9]	Succinylacetone	>2.1U			Cut-offs at end of reported period (March 31st, 2011) (underivatized)
Morrissey et al. (2011)[13]	Succinylacetone	3μmol/L (retest) 5μmol/L (immediate referral)			
la Marca et al. (2008)[15]	Succinylacetone	>2μmol/L	Tyrosine	>250μmol/L	
Sander et al. (2006)[6]	Succinylacetone	>10μmol/L			Unclear whether this cut-off was selected *a priori;* reported to produce no false positive results
Frazier et al. (2006)[7]	Tyrosine	Borderline: >500μmol/L; Diagnostic: >900μmol/L			Cut-offs used from January 2003

Cut-offs in premature infants

As mentioned in Criterion 5, Oladipo et al. (2011) tried to define the range of amino acid concentrations encountered in normal, premature and acutely ill infants in the US by measuring the concentrations of 25 amino acids in residual plasma samples by MS/MS.[30] Although this study was done on plasma samples, the authors say that tyrosine is equally distributed between erythrocyte and plasma water, so that the results should hold for newborn screening. Samples were from full term infants (>38 weeks) with uncomplicated nursery stays and discharge in less than 72 hours (n=206); premature babies born between 32 and 37 weeks gestation that remained in nursery for fewer than 10 days and were discharged home without major health concerns (n=50); and acutely ill infants in neonatal intensive care (regardless of gestational age, n=98), diagnoses included sepsis, respiratory distress, cardiac malformation/malfunction, and gastrointestinal disorders. The distributions of 13 amino acids were significantly different in premature infants when compared to healthy, full-term infants. The distribution of 16 amino acids was significantly different in the acutely ill population when compared to healthy, full-term infants. The median concentration and 2.5 to 97.5 percentile distributions of tyrosine in healthy, premature and acutely ill infants are shown in Table 8. Tyrosine levels were significantly elevated in premature infants but not elevated in acutely ill infants.[30]

Table 8: Median concentrations and 2.5 to 97.5 percentile distributions of tyrosine in plasma from healthy, premature and acutely ill infants less than 10 days of age.[30]

Amino acid (μmol/L) Median (2.5 to 97.5 percentiles)	Healthy (n=206)	Premature (n=50)	Acutely ill (n=98)
Tyrosine	88 (38 to 258)	115 (20 to 420)	84 (23 to 680)

Summary: Criterion 6 uncertain. Although an international collaboration has suggested cut-offs for both tyrosine and succinylacetone, individual studies have used different markers and cut-offs to screen for tyrosinaemia type I. Some of these differences in cut-offs used may be linked to the age at specimens collection and the protocol used to extract markers from the dried blood spot.

An international collaboration has published levels of tyrosine and succinylacetone in the normal population and in cases of tyrosinaemia type I. However, it stated that succinylacetone levels varied between sites, possibly due to pre-analytic variables.

The distribution of markers in a UK population after specimen collection at day five of life is unknown.

7. The test should be acceptable to the population

MS/MS is performed on amino acids and succinylacetone extracted from dried blood spots on Guthrie cards, which are already collected as part of the newborn screening programme.

Summary: Criterion 7 met. MS/MS is performed on extracts from the dried blood spot already collected as part of the newborn screening programme.

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

The US National Academy of Clinical Biochemistry (NACB) has produced laboratory medicine practice guidelines for the follow-up testing for metabolic diseases identified by the expanded newborn screening programme, grading the strength of recommendations using criteria adopted from the US Preventative Services Task Force.[34] The NACB recommendations for confirmatory testing for tyrosinaemia type I are shown in Table 9.

Table 9: NACB recommendations.[34] Evidence: A-I, the highest level of evidence, the NACB strongly recommends adoption, there is good evidence that it improves important health outcomes and the NACB concludes that benefits substantially outweigh harms. The evidence includes consistent results from well-designed, well-conducted studies in representative populations.

	Screening marker	Follow-up analyses	Follow-up markers	Additional testing	Evidence

Tyrosinaemia Type I	Tyrosine	Urine organic acids	Succinylacetone	No additional testing	A-I

The New York Mid-Atlantic Consortium for Genetic and Newborn Screening Services (NYMAC, region 2), one of the seven US regional collaboratives, has developed guidelines for the confirmation of diagnoses of conditions in the newborn screening panel- the NYMAC Newborn Screening Diagnostic Guidelines.[35,36] Extracts from the relevant guideline (last updated November 30, 2010) are displayed in Table 10.

Table 10: Diagnostic guideline for tyrosinaemia type I. Extracted from the NYMAC Diagnostic Guidelines for Confirmation of Screen-Positive Newborn Screening Results.[36]

Tyrosinaemia type I	
Abnormal newborn screening metabolite(s):	Elevated tyrosine Elevated succinylacetone
Initial diagnostics at referral centre	Plasma amino acids (PAA) Urine organic acids (UOA) including succinylacetone Liver function tests
Recommended additional testing to consider at time of initial consultation	Alpha fetoprotein
Abnormal Metabolites Expected	Elevated tyrosine, methionine (PAA) Elevated succinylacetone and succinylacetoacetate (UOA) Liver function tests may be abnormal in sick children Elevated alpha fetoprotein
If initial testing is negative has the disorder been ruled out?	Yes
Diagnostic Confirmation	Presence of succinylacetone is diagnostic
Differential Diagnosis	Tyrosinaemia type II Tyrosinaemia type III Total parenteral nutrition Transient tyrosinaemia of the newborn

The American College of Medical Genetics have also produced ACTion sheets and algorithms.[37]

In addition, some screening reports have given the criteria they have used to make a diagnosis. Kasper et al. (2010) report that for amino acidopathies, confirmatory testing included a repeated newborn screen and plasma amino acid analysis in Austria.[12] Additional testing is performed for some disorders, for example analysis of urine organic acids for tyrosine metabolism disorders.[12] Frazier et al. (2006) similarly report that a repeat newborn screen and plasma amino acid analysis is performed for suspected amino acidopathies, and urine organic acids for tyrosine metabolism disorders in North Carolina.[7] Diagnostic criteria used in South West Germany

(Lindner et al. [2011][12]) and the confirmatory tests employed in Denmark, the Faroe Islands and Greenland (Lund et al. [2012][9]) were also described (see Table 11 and Table 12).

Table 11: Minimal criteria to accept diagnosis as confirmed. South West Germany. Lindner et al. (2011)[12]

Disorder	Minimal criteria to accept diagnosis as confirmed
Tyrosinaemia Type I	Elevated succinylacetone in urine

Table 12: Confirmatory tests employed in Denmark, the Faroe Islands and Greenland. Lund et al. (2012)[9]

Disorder	Confirmatory tests
Tyrosinaemia type I	Urine Organic Acids Plasma Amino Acids Mutation analysis

Summary: Criterion 8 met. Example guidance on the diagnostic investigations that that should be performed if tyrosinaemia type I is suspected is available from the US, and this guidance tallies with the confirmatory tests reported in studies of newborn screening.

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

Criterion 9 not applicable.

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

The aim of treatment is to prevent manifestations of the disease. For tyrosinaemia type I these include liver dysfunction, renal tubular dysfunction, growth failure, rickets, neurological crises and hepatocellular carcinoma.

The 2004 HTA report concluded that "*effective treatments for tyrosinaemia type I included orthotopic liver transplantations which improved clinical, and biochemical outcomes and enhanced the quality of life in contrast to NTBC therapy*" based on the one study of patients presenting with tyrosinaemia type I between 1989 and 1997 who were treated by orthotopic liver transplantation.[3] However, the Pollitt report (a previous HTA report) concluded that "*early diagnosis through neonatal screening followed by treatment with NTBC will preserve hepatic and renal function and prevent porphyria-like attacks. However, it is unclear whether NTBC*

treatment prevents or merely delays the onset of malignancy and whether there are any long-term adverse effects of the drug."

Treatment options for tyrosinaemia type I

Options include:[2]

- NTBC treatment in combination with a low tyrosine diet
- Liver transplantation.

NTBC prevents the accumulation of fumarylacetoacetate and its conversion to succinylacetone. Treatment with NTBC should begin as soon as the diagnosis of tyrosinaemia type I is confirmed, in combination with a low tyrosine and phenylalanine diet. Liver transplantation is reserved for children who have severe liver failure at presentation, fail to respond to NTBC therapy, or have documented evidence of malignant changes in hepatic tissue.

NTBC treatment

Since the last report, NTBC (Orfadin) was authorised for the treatment of tyrosinaemia type I in combination with dietary restriction of tyrosine and phenylalanine in Europe (in 2005).[38] The drug was originally authorised under 'Exceptional Circumstances' due to the limited information available at approval due to the rarity of the disease. The 'Exceptional Circumstances' ended in 2009. Evidence of clinical efficacy was based mainly on the compassionate use of NTBC in 207 patients enrolled in the NTBC study, a large uncontrolled study (between February 1991 and August 1997).[39] NTBC was found to improve survival relative to an historical cohort of patients treated with dietary measures alone. Patients who had had onset of symptoms at less than two months of age had two and four year survival probabilities of 29% when treated with dietary measures alone in the historical cohort. In contrast, children who started NTBC therapy at less than two months of age had two year and four year survival probabilities of 88%. Patients who had onset of symptoms between two and six months of age had survival probabilities of 74% and 65%, respectively, when treated with dietary therapy compared with 95% for those who started treatment with NTBC. Two and four year survival probabilities were similar for dietary restriction and NTBC treatment when symptoms/treatment started at greater than 6 months of age. Therefore NTBC seems particularly effective in preventing mortality in the early onset "acute" forms of tyrosinaemia type I.

In addition, early NTBC treatment seems to reduce the incidence of hepatocellular carcinoma. Data for a total of 566 patients with tyrosinaemia type I treated for varying periods of time between 1991 and the end of 2003 were presented.[39] During this period, 44 children had died, 70 had undergone liver transplantation, five had withdrawn and six had been lost to follow-up. Amongst all 566 patients that had been treated with NTBC, a total of 24 cases of hepatocellular carcinoma were identified. It was found that age at which NTBC therapy was started correlated with the development of hepatocellular carcinoma despite the fact that the dose and time on NTBC treatment did not differ significantly between those who developed and those who did not develop cancer. The majority of patients who developed hepatocellular carcinoma initiated NTBC therapy after 12 months of age. Initiating NTBC after 12 months of age was associated with a relative risk of 13.53 (95% CI 4.07 to 44.89) of developing hepatocellular carcinoma compared to starting NTBC treatment before 12 months of age. This suggests that either children with the early onset form are less susceptible to hepatocellular carcinoma or that early treatment is beneficial.

Further studies have demonstrated the benefit of NTBC treatment.

Masurel-Paulet et al. (2008) described the long-term outcomes of 46 French patients with tyrosinaemia type I who had been treated with NTBC.[16] The mean administered dose of NTBC was 0.95mg/kg per day (given as two doses) and all patients were given a low-protein diet with supplementation of a special amino acids mixture without phenylalanine and tyrosine in order to try and maintain plasma tyrosine levels below 500μmol/L. The overall survival in this cohort, after a mean duration of NTBC treatment of 4 years and 9 months, was 97.5%. With treatment growth was normal, rickets were cured in all patients presenting with this symptom, and renal tubulopathy improved in 23 of 36 patients with this symptom, although it persisted in 13 patients. No patient suffered a porphyric crisis.

Thirty three of the cases were diagnosed when children were less than six months old, either because they presented symptomatically with the so-called 'acute' form of the disease or because they were identified by screening due to familial history or after screening for hyperphenylalaninaemia. In the majority of these cases, treatment with NTBC also started before 6 months of age, although for two patients treatment didn't start until the patients were more than 24 months old (188.9 months old and 35.9 months old, respectively). No patient who had been diagnosed before 6 months of age died. Liver transplant was required in two patients: one who had been diagnosed and treated before 6 months of age but who did not respond to NTBC therapy, and one patient who had been initially treated with dietary therapy due to NTBC not being available. This patient had received NTBC therapy before liver transplantation for hepatocellular carcinoma.

Ten patients were diagnosed between 6 months and 24 months of age ('sub-acute' form) and treatment started during the same period. In this subgroup there were no deaths, no cases of hepatocellular carcinoma and no patient received a liver transplant.

Three patients were diagnosed when they were more than 24 months old. There was one death and two liver transplants. One patient developed hepatocellular carcinoma, underwent liver transplantation and died from complications of the transplant. One patient had a liver transplant due to cirrhosis and repeat episodes of liver failure.[16]

Couce et al. (2011) reported the long term outcomes of 34 Spanish patients with tyrosinaemia type I treated with NTBC.[40] All patients were treated according to standard protocol (NTBC at 1mg/kg per day divided into 2 doses). Dosage was adjusted thereafter according to NTBC plasma levels. The mean dose was 0.87mg/kg per day. A tyrosine and phenylalanine restricted diet was also prescribed to maintain plasma tyrosine levels below 400μmol/L and phenylalanine levels between 35 and 120μmol/L. Patients were followed up for between 3.3 and 9.2 years. In this cohort the survival rate was 100%.

Patients were diagnosed with tyrosinaemia type I due to early onset of symptoms (at <2 months of age) in 6 cases, early onset in 17 cases, and late presentation in five cases. Six cases were detected by newborn screening. The average time in treatment was 6.7 years. Only one patient required liver transplantation. This patient had been diagnosed at 5 months of age and had received only dietary treatment until 14 years of age, when NTBC was begun. Liver transplantation was required after one year of treatment because of the development of cirrhosis and oesophageal varices. All other patients had good outcome to date, and no patient developed hepatocellular carcinoma.[40]

Koelink et al. (2006) reviewed the cases of 11 Dutch patients with tyrosinaemia type I (diagnosed due to elevated succinylacetone levels in combination with confirmed diagnosis in a sibling or decreased fumarylacetoacetase activity and/or DNA mutations) who had data on NTBC treatment (see Table 13).[41] Prior to 1992 patients received dietary treatment. After 1992, NTBC treatment was started as soon as possible. Four patients developed liver cancer and underwent liver transplantation. Of the patients who developed liver cancer, two started NTBC treatment before 24 months of age and two started treatment later. Of the patients who have not developed liver cancer, five started NTBC treatment before 24 months of age and two started treatment after 24 months of age. All patients were alive at follow-up.

Table 13: 11 cases of tyrosinaemia type I. From Koelink et al. (2006)[41]

Patient	Age at time of diagnosis (months)	Age at start of NTBC treatment (months)	Age at liver cancer diagnosis (months)
1	9	77	116
2	26.5	27	100
3	10	14	145
4	0.5	0.5	6
5	15	15	NA
6	8	9	NA
7	5	5	NA
8	9	33	NA
9	0.5	0.5	NA
10	1.5	1.5	NA
11	3	68	NA

NTBC treatment has also been found to improve renal tubular dysfunction. In addition to the findings of Masurel-Paulet et al. (2008)[16], another study, Santra et al. (2008), looked specifically at renal tubular dysfunction in 21 patients with tyrosinaemia type I who had been treated with NTBC for at least 12 months.[42] All 21 patients had biochemical evidence of renal tubular dysfunction at presentation. After NTBC and dietary treatment were started, plasma phosphate, urinary protein/creatinine ratio and tubular reabsorption of phosphate (markers of tubular function) normalised within 1 year. NTBC also improved rickets in patients that had them at presentation. The only child with nephrocalcinosis (calcium deposited in the liver) presented clinically after 1 year of age, but none of the children diagnosed in infancy had, or developed, nephrocalcinosis.

Early versus late treatment with NTBC

Larochelle et al. (2012) reported the long term outcomes of 78 patients born between February 1984 and February 2004 who were diagnosed with tyrosinaemia type I in Québec.[43] During this period newborn screening for tyrosinaemia type I was performed, and diet therapy and liver

transplantation were available. However, NTBC treatment only became available in 1994. Doses of NTBC were initially fixed at 0.6 or 1.0mg/kg daily in two daily oral doses. After 1999, NTBC dose was titrated in order to minimise urine levels of succinylacetone. Dietary restriction was also prescribed, aiming to maintain plasma tyrosine at 200 to 400μmol/L. Patients were followed up until hepatic transplantation, death or August 1st 2009.

Patients were divided into three groups: those who never received NTBC (28 patients), those who were first treated after 1 month of age (late-treatment, 26 patients) and those treated before 1 month of age (early treatment, 24 patients).

Survival and need for liver transplant are shown in Table 14. Eight patients who did not receive NTBC died before liver transplantation, at a mean age of 16 months. No patients receiving NTBC died before transplantation ($p<0.01$ vs. non-NTBC treated). Liver transplantation was performed in 20 non-NTBC treated patients (71%), at a median of 26 months of age. The indications for transplant were cirrhosis or cancer (13 patients), acute hepatic failure (2 patients) and previous neurological crises (5 patients). Seven late treated patients (26%, $p<0.001$ vs. non-NTBC treated), and no early treated patient ($p<0.001$) required transplantation. All seven late-treated patients had chronic liver abnormalities prior to receiving NTBC and were transplanted because abdominal imagining suggested macronodular cirrhosis. Cirrhosis was confirmed, and hepatocellular carcinoma was present in two livers and the other five had dysplastic foci. Following transplant there were two deaths in both the never treated and late-treated group (not statistically different). No early-treated patient had developed detectable liver disease after more than five years of NTBC therapy.

Of note, six cases of tyrosinaemia I were missed by screening, either due to birth outside Quebec or 'screening failure'. Screening failure was not defined in the paper, but was taken to mean missed by screening (i.e. patients were given a false negative result). Five of these patients were treated late, and one patient was never treated. Four patients required transplantation: one never treated with NTBC and three of the late treated patients. These patients are included in the analysis above.

Table 14: Effect of NTBC Treatment on liver transplantation and death due to tyrosinaemia type I in Quebec. From Larochelle et al. (2012)[43]

	No treatment with NTBC	**Treated with NTBC**		
	Never treated	**Treated at any time (late treated + early treated**	**Late-treated**	**Early- treated**
Number of patients	28	50	26	24
Transplantation	20	7	7	0
Death	10	2	2	0
Death before transplantation	8	0	0	0
Death after transplantation	2	2	2	0

The effect of NTBC treatment on hospitalisations due to acute complications of tyrosinaemia type I were also monitored (Table 15). No hospitalisations for acute complications of tyrosinaemia type I occurred during 5,731 months of NTBC treatment (early treated patients and late treated patients once NTBC therapy had started combined), versus 184 during 1,312 months without treatment (p<0.001).

Table 15: Effect of NTBC treatment on hospitalisations for acute complications of tyrosinaemia type I. From Larochelle et al. (2012)[43]

	Not treated		Treated			
	Total non-NTBC	Never treated	Late treated		Early treated	Total NTBC
	[Never treated plus late treated pre NTBC]		Pre NTBC	NTBC		[Early treated plus late treated with NTBC]
Follow-up (patient months)	1312	777	535	3138	2593	5731
Months with tyrosinaemia-related hospitalisations (including neurological crises)	184	141	43	0	0	0
Months with neurological crises	88	71	17	0	0	0

Liver transplantation

Since the introduction of NTBC, a UK study has reported that liver transplantation is only indicated if a patient does not respond to NTBC or where the development of hepatocellular carcinoma is suspected.[4]

Arnon et al. (2011) reviewed cases of liver transplantation in patients with tyrosinaemia type I which had taken place between October 1987 and May 2008 in the US United Network for Organ Sharing (UNOS) database.[44] It found that outcomes after liver transplant were good, with one and 5 year survival over 90%.

During the period of the study, 125 liver transplants for hereditary tyrosinaemia type I occurred. The number of patients receiving transplants reduced over time. Forty patients received transplants between 1987 and 1991 (NTBC became available for clinical trials in 1991); 37 patients received transplants between 1992 and 1996; 30 patients received transplants between

1997 and 2001; and 18 patients received transplants between 2002 and 2008 (NTBC approved by the FDA in 2002).

The mean age at liver transplant was 2.46 ± 3.58 years. The mean age during the first 10 years of the study was significantly lower than during second 10 years of the study (1.82 years versus 3.70 years, p=0.01).

One year survival after transplantation was 90.4% and 5 year survival was 90.4%. There was no improvement over time in 1 year and 5 year survival. The primary cause of death was graft failure.

Unfortunately, the UNOS database does not contain information on all variables, including medical therapy before transplant (i.e. use of NTBC) or the presence of hepatocellular carcinoma in patients. The authors speculate that the decrease in rate of liver transplantation and the increase in age at transplant are most probably due to early diagnosis via newborn screening and treatment with NTBC.

Pierik et al. (2005) reported on long-term renal follow-up (mean follow up 11 years) after liver transplantation of nine patients with tyrosinaemia type I treated in one centre in the Netherlands.[45] All patients were alive at the end of the study. After transplantation, succinylacetone continued to be excreted in urine. There was no change in glomerular filtration rate; however, tubulopathy persisted in some patients- only two patients had no abnormal findings on tubular function tests at their most recent evaluation after transplantation.

Summary: Criterion 10 met. NTBC is an effective treatment for tyrosinaemia type I, with evidence that treatment with NTBC in combination with a tyrosine and phenylalanine restricted diet can improve survival, improve liver function, reduce the risk or delay the development of hepatocellular carcinoma, correct renal tubular dysfunction, and improve rickets and growth. There is evidence that early treatment with NTBC leads to better outcomes. If liver transplantation is required due to non-responsiveness to NTBC or the development of hepatocellular carcinoma, evidence suggests that one and five year survival is over 90%.

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

Criterion 11 not assessed.

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

Criterion 12 not assessed. However no studies were found which explored the proportion of tyrosinaemia cases identified through current practice of measuring tyrosine levels in babies testing positive for phenylketonuria (when phenylalanine is ≥200µmol/L).

13. There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person

being screened to make an "informed choice" (eg. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened

The 2004 HTA report stated that "RCTs of screening for rare disorders are difficult because of the enormous numbers that would be needed for adequate power."[3]

No RCTs assessing whether screening for tyrosinaemia type I is effective in reducing mortality or morbidity were identified in the update search. However a study comparing outcomes between screened and unscreened cohorts was identified, as well as several studies that have described the outcomes of cases identified by screening.

Wilcken et al. (2009) and Norman et al. (2009a) compared the outcomes for patients with amino acid, organic acid and fatty acid metabolism (excluding PKU) disorders identified in a screened cohort and those identified in contemporaneous and historical cohorts of unscreened patients in Australia.[8,46] Before 1998, no MS/MS screening was performed. It was introduced to three of the five Australian laboratories in 1998, 1999 and 2002. Cases identified during the period (1998 to 2002) and in the areas that screening was available were compared to a contemporaneous cohort in an unscreened region, and to a historical unscreened cohort (1994 to 1998). In Wilcken et al. data for specific conditions was not presented.[8] However, the study found that overall, MS/MS newborn screening is associated with a better outcome for patients at 6 years of age, with fewer deaths and fewer clinically significant disabilities.[8] In Norman et al. disorders were divided. Although data was not presented separately for tyrosinaemia type I, data was presented for deaths in patients with aminoacidurias (including tyrosinaemia type I) (see Table 16).[46]

Table 16: Identification of aminoacidurias and mortality from these diseases. From Norman et al. (2009a)[46] *Aminoacidurias included five cases of tyrosinaemia type I. One case was diagnosed through newborn screening, but due to method used at the time, it would be expected that cases would be missed. Both deaths (in the clinical diagnosis control groups) were due to tyrosinaemia type I. ‡Number of patients screened taken from Wilcken et al. (2009)[8]

	Newborn Screening Diagnosis 1998-2002			Clinical Diagnosis Contemporaneous Control Group, 1998-2002			Clinical Diagnosis Historical control Group, 1994-1998		
Number of patients screened‡	**461,500**			**533,400**			**1,017,800**		
Disorders	**Total cases**	**Death by 7 days**	**Death later**	**Total cases**	**Death by 7 days**	**Death later**	**Total cases**	**Death by 7 days**	**Death later**
Aminoacidurias*	10	0	0	4	0	1	6	0	1

If deaths before the age of 7 days are assumed to occur too early for screening to have an impact, and only deaths after the age of 7 days are considered, it can be seen that there were no deaths from aminoacidurias during the period of screening and two deaths, both due to

tyrosinaemia type I, in the pooled cohort that presented clinically (1:775,600 unscreened infants).

Couce et al. (2011) reported on the results of newborn screening in Galicia (Spain) between 2000 and 2010.[11] Both of the two patients with tyrosinaemia type I identified by newborn screening were symptomatic at diagnosis and therefore would have presented clinically even without screening. Long term follow up for the patients identified by screening is shown in Table 17. No comparison with an unscreened cohort was performed.

Table 17: Characteristics of cases of tyrosinaemia type I identified by newborn screening in Galicia, Spain. From Couce et al. (2011)[11]. PDI/IQ Psychomotor Development Index/Intellectual Quotient

Diagnosis	Number of subjects	Clinical symptoms at diagnosis	Mean follow-up (months)	PDI/IQ (mean)	Present status
Tyrosinaemia type I	2	Yes	99	89	Free of symptoms

Summary: Criterion 13 not met. No randomised controlled trials of screening were identified. An observational study comparing outcomes for patients with aminoacidurias (including tyrosinaemia type I) identified by screening with patients in unscreened cohorts suggests that screening is associated with better outcomes. This finding was independent of the detection rate, suggesting that the identification of mild variants was not responsible for the improvements seen.

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

Criterion 14 not assessed.

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

No formal assessments of the balance of benefits and harms of screening were identified.

Harms of screening

As with all screening tests there is likely to be harm from false-positive results and results with unknown clinical significance.

Carriers are not identified by the screening test. The presence of a family member with the disease could lead to the genetic testing of other members of the family and the identification of individuals carrying the mutation. This is likely to be the case whether screening is implemented or whether infants are identified due to presentation with symptoms, although more infants may receive a diagnosis if newborn screening is implemented (i.e. fewer cases may remain undiagnosed).

Harms of treatment

The European Public Assessment Report (EPAR) for the public lists the following as common side effects of NTBC (seen in between one and ten patients in 100):[38]

- thrombocytopenia (low blood platelet counts)
- leucopenia (low white blood cell counts)
- granulocytopenia (low levels of a type of blood cell called granulocytes)
- conjunctivitis (inflammation of the membrane that lines the eyelid)
- corneal opacity (clouding of the transparent layer [cornea] in the front of the pupil)
- keratitis (inflammation of the cornea)
- photophobia (increased sensitivity of the eyes to light)
- eye pain

Ocular effects

Couce et al. (2011) which reported the long term outcomes of 34 Spanish patients with tyrosinaemia type I treated with NTBC reported that four patients developed mild transient ocular side effects: itching and red eyes, without evidence of corneal crystals at slit lamp examination; one of them associated with albinism. These patients had high levels of plasma tyrosine (range 405 to 728mmol/L).[40]

Neurological effects

However, recently there has been concern about the neuropsychological effects of NTBC treatment. This is a concern because neuropsychological defects have not been described as part of the natural history of tyrosinaemia type I.

Masurel-Paulet et al. (2008) reported that schooling difficulties affected eight of the 23 school-age patients (35%) and major cognitive disturbances were noted in six (memory and concentration difficulties, and slowness).[16]

De Laet et al. (2011) performed a retrospective analysis of the neuropsychological outcomes of NTBC-treated patients living in Belgium in 2004.[47] Three of the ten patients had an IQ below 85. Two of these patients had had a severe clinical presentation at diagnosis. IQ was between 85 and 100 in six patients, and over 100 in one patient. It has been suggested that elevated tyrosine levels could be responsible for neurodevelopmental delay, and therefore IQ in relation to tyrosine and phenylalanine concentrations was looked at. During treatment the aim was to keep plasma tyrosine levels below 500μmol/L, although this limit was often exceeded. The number of patients treated with NTBC and the neuropsychological tests administered to the patients differed, meaning that no statistical tests could be applied. However, the authors report that mean tyrosine concentrations were in the same range in patients with normal and lower IQs, although patients with the lowest IQ had mean phenylalanine concentrations below 40μmol/L for the first two years of treatment.

Thimm et al. (2012) described the neurocognitive development of nine NTBC treated tyrosinaemia type I patients in Germany.[48] Neurocognitive development was assessed using standardized psychometric test batteries with respect to cognition, motor abilities and speech.

This study concluded that there is abnormal intellectual development in a high percentage of patients with tyrosinaemia type I under long term treatment with NTBC.

NTBC treatment was started on the first day in life in one patient (diagnosed prenatally due to affected sibling), in the neonatal period for four patients (9 days to 4 weeks, diagnosed by screening), at 2 to 3 months of age in three patients and 12 months of age in one patient (all four presented clinically).[48] The mean duration of NTBC treatment was 4 years 8 months. Development in patients under the age of 3 years of age was assessed using the Bayley Scales of Infant Development. Patients between 3 and 5 years of age were assessed with the Snijder Oomen test (SON-R). Psychological evaluation in patients above 5 years of age was performed with the Kaufmann-Assessment Battery for Children (K-ABC). Motor abilities in children over 3 years of age were assessed with the Movement Assessment Battery for Children. Outcomes are given in Table 18.

Table 18: Neurocognitive outcomes of patients with tyrosinaemia type I treated with NTBC. From Thimm et al. (2012).[48] *Patients with significant differences in scores on the different tests had inhomogeneous test profiles, demonstrating deficiencies in different facets of neurocognitive function.

Patient	Age	Test Battery	Standard value	Significant difference between test subscales ($p<0.05$)*	Evaluation M-ABC
1	8y8m	K-ABC	77	+	No pathological findings
2	8y6m	K-ABC	83	+	Critical
3	7y	K-ABC	92	+	No pathological findings
4	5y7m	K-ABC	110	+	No pathological findings
5	3y10m	SON-R	83		Therapy needed
6	3y9m	SON-R	50		Therapy needed
7	3y3m	SON-R	73	+	Therapy needed
8	2y3m	Bayley		+	
9	1y	Bayley			

The two youngest patients were tested with the Bayley Scales of Infant Development. Both patients achieved age appropriate performance in cognitive and motor tasks. However, performance of the language subtest was significantly worse than performance on other subtests in one patient.

Among the seven children aged 3 years old or older:

- Five had standard test values below normal total IQ score
- Five presented with an inhomogeneous test score (significant difference in score on different subscales)
- Four patients had motor difficulties

Additionally, five patients underwent logopaedic evaluation (analysis of language development, the other four patients were either too young or did not speak enough German to be tested). Only one patient demonstrated language understanding, production and memory appropriate

to age. However, it should be noted that German was not the native language of eight of the nine children.

Summary:

Criterion 15 uncertain. No formal assessments of the balance of benefits and harms of screening were identified. Screening for tyrosinaemia type I can be done on a dried blood spot. There is likely to be some harm caused by the identification of false positives and false negatives, although if succinylacetone levels are used for the identification of patients with tyrosinaemia type I few false positives and false negatives should be identified (see Criterion 5). NTBC is an effective treatment for tyrosinaemia type I, with evidence that treatment with NTBC in combination with a tyrosine and phenylalanine restricted diet can improve survival, improve liver function, reduce the risk or delay the development of hepatocellular carcinoma, correct renal tubular dysfunction, and improve rickets and growth. However, neurocognitive defects have been observed in patients with tyrosinaemia type I treated with NTBC. Whether this is due to the drug, raised tyrosine levels, depressed phenylalanine levels, or another factor is not clear. Additional follow up and investigation of this is warranted.

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

The economic evaluation in the 2004 HTA report estimated that screening for tyrosinaemia by MS/MS to cost £13,168 per life year gained and screening for urea cycle disorders to cost £2,965 per life-year gained.[3]

No UK based cost-effectiveness analyses were identified.

From the update search, only one study was identified that considered the cost-effectiveness of screening for tyrosinaemia type I individually by MS/MS. Cipriano et al. (2007) performed a cost effectiveness analysis from a societal perspective of replacing screening for phenylketonuria using the Guthrie bacterial inhibition assay with expanded screening for up to 21 inherited metabolic disorders using MS/MS in Ontario, Canada.[49] Two programme strategies were assessed: each disease was assessed independently, including the entire capital cost of investing in the MS/MS technology, the cost of screening and the cost of programme maintenance; and diseases were assessed in bundles, as MS/MS allows several diseases to be screened for at the same time. Using a decision analytic model, with life years saved as the outcome, the analysis considered:

- the incidence and the severity of the conditions
- the sensitivity, specificity and positive predictive rate of the test
- the health benefits
- the start-up costs of MS/MS screening
- the cost of confirmatory testing

- the cost of treatment, hospitalisation, social services and education

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

Table 19: Incremental cost effectiveness of each disease evaluated independently and a breakdown of incremental costs, savings and life years gained per patient screened. All costs given in 2004 Canadian dollars. From Cipriano et al. 2007[49]

Disease	Incremental cost ($) (including start-up*)	Incremental cost ($) (excluding start-up)	Incremental life years gained ($) ($x10^{-5}$)	ICER† ($) (including start-up)	ICER ($) (excluding start up)	Order of cost-effectiveness‡
Tyrosinaemia type I	32.51	14.14	4.57	711,379	309,209	16

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

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

‡Position relative to the 21 inherited metabolic disorders considered

Tyrosinaemia type I was amongst the least cost-effective disorders to screen for. However, it should be noted that the cost-effectiveness was calculated before succinylacetone was routinely used to screen for tyrosinaemia type I, and the authors report that they considered the positive predictive rate of screening for tyrosinaemia type I to be 2%.

Several other studies were identified that considered the cost-effectiveness of screening.

Norman et al. (2009b) performed a systematic review of studies examining the cost-effectiveness of screening for rare metabolic conditions using tandem mass spectrometry published between January 1997 and March 2008.[50] The systematic review found that despite the substantial differences in the methods employed, the consensus is positive in favour of MS/MS screening.

Norman et al. (2009a) analysed the cost-effectiveness of screening for a panel of disorders including tyrosinaemia type I in Australia, using the phased introduction of screening using MS/MS in Australia.[46] The cost effectiveness per life years gained and deaths averted are shown in Table 20.

Table 20: Cost-effectiveness outcomes. Costs given in Australian dollars.

Outcome measure	Outcome, number per 100,000 screens	Cost per 100,000 screens. A$	Cost effectiveness A$ per outcome
Life years gained	32.378	349,010 (cost of screening [218,000] plus additional cost of treatment [131,010])	10,779
Deaths averted	0.738		472,913

Feuchtbaum and Cunningham (2006) performed cost-effectiveness and cost-utility analyses from a payer perspective (costs to the public, i.e. excluding additional costs borne by families) of MS/MS screening for all MS/MS detectable disorders in California, US.[51] Costs and benefits were derived from the results of the California MS/MS screening pilot.[17]

The model found that incremental cost of screening was approximately $5.7 million per 540,000 births. Per 540,000 births screened, 83 affected newborns would be identified, and in the base case analysis eight deaths would be prevented. The incremental cost of screening per life saved was $708,063. An estimated 949 QALYs would be saved, and the saving per QALY would be $1,628. These calculations and other calculations relating to medical costs saved are shown in Table 21.

Table 21: Summary of economic impact of MS/MS screening in California, in the base case scenario and estimating total lifetime medical care costs at $1,000,000 for a severely mentally retarded person. Results were calculated per 83 diagnosed cases.

Lives saved (*a*)	8
Incremental programme costs (*b*)	$5,664,500
Incremental costs per live saved (*b/a*)	**$708,063**
Incremental costs per case detected (*b/83*)	$68,247
Lifetime medical costs with screening (*c*)	$5,321,052
Total costs with screening (*d=b+c*)	$10,985,552
Total costs per case detected (*d/83*)	$132,356
Total costs without screening (*e*)	$12,530,204
Total costs saved with screening (*f=e-d*)	$1,544,652
QALY saved (*g*)	969
Saving per QALY (*h=f/g*)	**$1,628**
Values of lives saved (*i=a x $5,700,000*)	$45,600,000
Net incremental benefit (*j=f+i*)	$47,144,652

Summary: Criterion 16 not met. The economic evaluation in the 2004 HTA report estimated that screening for tyrosinaemia type I by MS/MS to cost £13,168 per life year gained. No UK based studies of cost effectiveness were identified. Analyses that have considered screening for a panel of MS/MS detectable disorders have found screening to be cost effective. However, in the one study that considered screening for disorders individually, screening for tyrosinaemia type I was amongst the least cost-effective disorders to screen for. This analysis was published in 2007 and performed from a Canadian perspective and it is unclear how applicable this study would be to the UK.

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

Criterion 17 not assessed. Other potential options for improving outcomes for tyrosinaemia type I include interventions to improve awareness of this condition so that this disease is suspected and diagnosed in a timely manner.

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

The European Research Network for the evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism (ERNDIM) aims to reach a consensus between European Biochemical Genetics Centres on reliable and standardised procedures for diagnosis, treatment and monitoring of inherited metabolic diseases.[52] This is achieved through provisions of quality control schemes on a European wide scale. Quantitative schemes, for example for amino acids, are planned and managed by members of the ERNDIM Scientific Advisory Board and organised in partnership with SKML (the Dutch Foundation for Quality Assessment in Medical Laboratories), a Quality Assurance (QA) provider based in the Netherlands. SKML dispatches QA samples to scheme participants and provides a website for on-line submission of results and access to scheme reports by participants. In 2011, the scheme consisted of 8 lyophilised samples.[53]

The US Centres for Disease Control and Prevention (CDC) provides QA for dried blood spot screening tests. All laboratories in the US that test dried blood spots participate voluntarily in the Newborn Screening Quality Assurance Program (NSQAP).[54] The CDC accepts international participants into the QA programme. The CDC provides Quality Control materials, proficiency testing services and technical support. The proficiency testing programme provides laboratories with quarterly panels of blind-coded dried blood spot specimens and gives the laboratory an internal assessment of performance.

Summary: Criterion 18 not directly assessed. US and European quality assurance systems are in place, which could provide examples for UK practice.

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

Criterion 19 not assessed.

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

Criterion 20 not assessed.

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

Children Living with Inherited Metabolic Diseases (Climb), the National Information Centre for Metabolic Diseases, reports on their website that they have been working alongside medical professionals and families to add Inherited Metabolic Diseases to the Newborn Screening Programme.[55]

Other stakeholders with an interest in screening for these conditions include:

- Genetic Alliance UK
- Institute of Child Health
- Royal College of General Practitioners
- Royal College of Midwives
- Royal College of Paediatrics and Child Health

Summary: Criterion 21 uncertain. Climb report that they have been working alongside medical professionals and families to add Inherited Metabolic Diseases to the Newborn Screening Programme, but specific disorders are not mentioned.

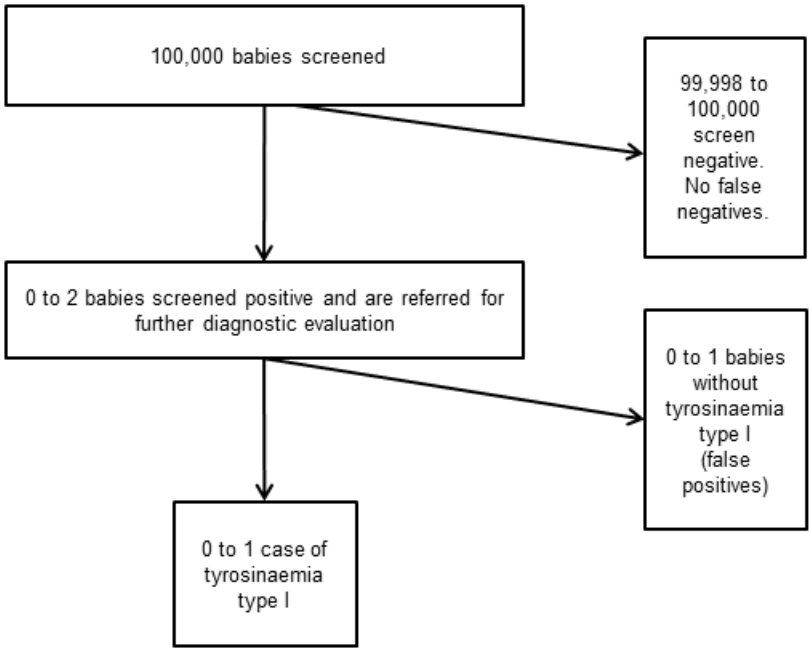
22. If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members

Criterion 22: Not applicable.

Screening flow chart

A flow chart showing what happens to 100,000 babies screened for tyrosinaemia type I by analysing levels of succinylacetone, based on the results of screening programmes that gave values for the number of true positives, the number of false positives and the number of false negatives identified, is shown in Figure 1.

Figure 1: Screening flow chart for tyrosinaemia type I. Based on the results of screening programmes that used succinylacetone as a marker for tyrosinaemia type I and that reported the number of true positive, false positives and false negatives identified (Sander et al.[2006][6], Morrissey et al.[2011][13] and Lund et al. [2012][9])



Conclusions

The condition

Tyrosinaemia type I is an autosomal recessive disorder resulting from mutations in the gene encoding fumarylacetoacetase (fumarylacetoacetate hydrolase). Deficiency in this enzyme results in the accumulation of fumarylacetoacetate, succinylacetoacetate and succinylacetone, which are thought to be responsible for the clinical symptoms seen in tyrosinaemia type I: liver dysfunction in 'early onset' and liver dysfunction associated with growth failure and rickets or neurological crises at an older age in patients with 'late-onset' tyrosinaemia. The distinction between the two forms of tyrosinaemia type I is based on age at which symptoms develop. If left untreated tyrosinaemia usually results in death before ten years of age from liver failure, neurological crisis or hepatocellular carcinoma.

Tyrosinaemia type I can be detected before the onset of symptoms through MS/MS analysis of newborn dried blood spots. The concentrations of tyrosine and succinylacetone can be used to screen for tyrosinaemia type I. Succinylacetone is a more sensitive and specific marker of tyrosinaemia type I as tyrosine levels can be elevated due to transient tyrosinaemia, liver disease, or tyrosinaemia type II or type III, and because some infants with tyrosinaemia type I may have normal blood concentrations of tyrosine when the screening sample is taken. However, the duration of the latent asymptomatic period varies. Reports of cases of

tyrosinaemia that were symptomatic before the results of a newborn blood spot screen were available were identified.

The incidence of tyrosinaemia type I in the UK is uncertain. The incidence of the disease can be estimated from studies performed in other European countries, the US and Australia. Incidence varied from less than one case per 944,000 births to more than one case per 31,000 births. The introduction of screening in Australia caused the incidence rate to approximately double, although all the affected cases were missed by screening, whereas it had little effect in Denmark, the Faroe Islands and Greenland. It should be noted that the detection of individual cases of the disease in small populations contribute to the wide variations in calculated incidences, as tyrosinaemia type I is a rare condition.

The screening test

Screening for tyrosinaemia type I can be performed using MS/MS on dried blot spots. Dried blood spots are already collected as part of the newborn screening programme, and MS/MS technology is used to screen for phenylketonuria in the UK.

Screening for tyrosinaemia type I has been improved by the development of methods to extract succinylacetone from dried blood spots in a manner compatible with newborn screening. Screening programmes using succinylacetone as a marker have reported up to 100% sensitivity and 100% specificity. However, other studies have reported the identification of false positives. Differences between screening programmes may be due to, among others, differences in normal values in the population, the timing of the test, the method used to extract succinylacetone and the cut-offs used for classifying a case as screening positive.

An international collaboration has attempted to define the normal range of markers of tyrosinaemia type I and to establish cut-offs. **However, it stated that succinylacetone levels varied between sites, possibly due to pre-analytic variables.**

Treatment

NTBC is an effective treatment for tyrosinaemia type I, with evidence that treatment with NTBC in combination with a tyrosine and phenylalanine restricted diet can improve survival, improve liver function, reduce the risk or delay the development of hepatocellular carcinoma, correct renal tubular dysfunction, and improve rickets and growth. There is evidence that early treatment with NTBC leads to better outcomes. However, neurocognitive defects have been observed in patients with tyrosinaemia type I treated with NTBC. Whether this is due to the drug, raised tyrosine levels, depressed phenylalanine levels, or another factor is not clear. Additional follow up and investigation of this is warranted. If liver transplantation is required due to non-responsiveness to NTBC or the development of hepatocellular carcinoma, evidence suggests that one and five year survival is over 90%.

The screening program

No randomised controlled trials of screening were identified. An observational study comparing outcomes for patients with aminoacidurias (including tyrosinaemia type I) identified by screening with patients in unscreened cohorts suggests that screening is associated with better

outcomes. This finding was independent of the detection rate, suggesting that the identification of mild variants was not responsible for the improvements seen. However, details for tyrosinaemia type I were not presented separately.

The economic evaluation in the 2004 HTA report estimated that screening for tyrosinaemia by MS/MS to cost £13,168 per life year gained. No additional UK based studies of cost effectiveness were identified. Analyses that have considered screening for a panel of MS/MS detectable disorders have found screening to be cost effective. However, in the one study that considered screening for disorders individually, tyrosinaemia type I was amongst the least cost-effective disorders to screen for. This analysis was published in 2007 and performed from a Canadian perspective and it is unclear how applicable this study would be to the UK.

US and European quality assurance systems are in place. No UK based reports were identified as screening for tyrosinaemia type I is not currently provided in the UK. However, if screening was to be implemented in the UK, plans for managing and monitoring the screening programme and quality assurance standards could be formulated based on the systems used in Europe or the US, or the UK could join one of these systems.

Implications for research

The following areas could provide useful avenues for further research:

- Large European based epidemiological studies, as uncertainty remains over the epidemiology of tyrosinaemia type I.
- Studies to determine the feasibility of screening for tyrosinaemia type I in the UK, including:
    - Studies to determine whether the UK bloodspot screening process can detect early-onset cases before they are symptomatic
    - Studies to determine the distribution of markers of tyrosinaemia type I in a UK population on day five of life
    - Studies to determine the predictive value of the screening test in a UK population
    - Studies to determine the number of cases detected through current UK practice
- Further studies into the long term outcomes of treatment with NTBC for tyrosinaemia type I, to determine the cause of the neurological effects reported in some studies, and whether these can be avoided.

Methodology

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

Search strategy

BACKGROUND: A systematic review on this topic was published in 2004: Pandor A et al, Clinical effectiveness and cost effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry: a systematic review, March 2004

SOURCES SEARCHED: Medline, Embase, Cochrane Library.

DATES OF SEARCH: Medline 2004- July Week 4 2012; Embase 2004-2012 Week 31, Cochrane Library 2012 Issues 7 and 3.

SEARCH STRATEGY:

Medline (OVID interface)

1 Neonatal Screening/
2 ((neonat* or newborn*) adj2 screen*).tw.
3 Mass Screening/
4 exp Infant, Newborn/
5 1 or 2 or (3 and 4)
6 Tandem Mass Spectrometry/
7 exp spectrum analysis, mass/
8 (tandem adj2 mass).tw.
9 or/6-8
10 5 and 9
11 Tyrosinemias/
12 tyrosin?emi*.tw.
13 (((fumarylacetoacetate adj hydrolase) or fumarylacetoacetase or fah) adj2 deficien*).tw.
14 or/11-13
15 Citrullinemia/
16 citrullin?emi*.tw.
17 citrullinuri*.tw.
18 (argininosuccinate adj2 (synthase or synthetase) adj2 deficien$).tw.
19 ass deficien*.tw.
20 or/15-19
21 Argininosuccinic Aciduria/
22 ((Argininosuccinic adj Aciduria) or Argininosuccinicaciduria).tw.
23 ((Argininosuccinate or Argininosuccinase or asl or asal) adj deficien*).tw.
24 or/21-23
25 amino acid metabolism, inborn errors/
26 14 or 20 or 24 or 25
27 prevalence/
28 incidence/
29 (prevalen* or inciden*).tw.
30 exp epidemiological studies/
31 "predictive value of tests"/

32 "sensitivity and specificity"/
33 ((positive or negative) adj predictive value*).tw.
34 (false adj (positive* or negative*)).tw.
35 (sensitiv* or specific*).tw.
36 early diagnosis/
37 Delayed Diagnosis/
38 disease progression/
39 prognosis/
40 "quality of life"/
41 exp treatment outcome/
42 morbidity/
43 mortality/
44 Tyrosinemias/di, dh, dt, ep, mo, su, th [Diagnosis, Diet Therapy, Drug Therapy, Epidemiology, Mortality, Surgery, Therapy]
45 Citrullinemia/di, dh, dt, ep, mo, su, th
46 Argininosuccinic Aciduria/di, dh, dt, ep, mo, su, th
47 exp Diet Therapy/
48 Liver Transplantation/
49 nitisinone.mp.
50 Phenylbutyrates/
51 exp Renal Dialysis/
52 Phenylacetates/
53 5 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 47 or 48 or 49 or 50 or 51 or 52
54 26 and 53
55 44 or 45 or 46 or 54
56 10 or 55
57 amino acid metabolism, inborn errors/di, dh, dt, ep, mo, su, th
58 56 or 57
59 limit 58 to yr="2004 -Current"

A similar search strategy was used in Embase, and a simplified strategy in the Cochrane Library.

RESULTS

All results were downloaded into an Excel spreadsheet, and 491 duplicates removed.
A total of 1916 citations remained.

Database	No. citations retrieved	Exclusive
Medline	1009	1005
Embase	1333	857
Cochrane Library	65	54
	Total=2407	**Total = 1916**

The title and abstracts of these citations, and where necessary and available the full text, were examined for relevance to newborn screening for amino acid disorders or expanded newborn screening using tandem mass spectrometry. Articles commenting on other papers are listed with the original paper. 457 citations remained, and have been classified as follows:

Category	No. of citations
Systematic reviews	
– general/amino acid disorders	6
– Tyrosinaemia I	1
Guidelines	
– general/amino acid disorders	6
– Tyrosinaemia I	1
Non-systematic reviews	
– general/amino acid disorders	58
– Argininosuccinate lyase deficiency	2
– Citrullinaemia	3
– Tyrosinaemia I	6
Natural history	
– general/amino acid disorders	4
– Argininosuccinate lyase deficiency	6
– Citrullinaemia	45
– Tyrosinaemia I	27
Prevalence of condition	
– general/amino acid disorders	27
Outcomes	
– general/amino acid disorders	6
– Argininosuccinate lyase deficiency	2
– Citrullinaemia	1
– Tyrosinaemia I	5
Delayed diagnosis	
– general/amino acid disorders	1
Screening test	
– general/amino acid disorders	55
– Citrullinaemia	2
– Tyrosinaemia I	23
Treatment	

– general/amino acid disorders	7
– Argininosuccinate lyase deficiency	8
– Citrullinaemia	22
– Tyrosinaemia I	56
Screening programme	
– general/amino acid disorders	69
– Argininosuccinate lyase deficiency	2
– Citrullinaemia	2
– Tyrosinaemia I	4
Total	457

Quality

Non-systematic reviews, editorials, other opinion pieces, reports of case series of fewer than four patients, and those with nonhuman data were excluded. Conference abstracts were also excluded. Additional relevant references identified during the preparation of the report were also included. Priority was given to studies from Europe, North America and Australia.

References

1. Burgard P, Cornel M, Di Filippo F et al. Report on the practices of newborn screening for rare disorders implemented in Member States of the European Union, Candidate, Potential Candidiate and EFTA Countries. 2012. Available from: http://ec.europa.eu/eahc/documents/news/Report_NBS_Current_Practices_20120108_FINAL.pdf.

2. Siderman King L, Trahms C, Scott CR. Tyrosinemia Type I [Internet]. Seattle (WA): GeneReviews; 2006 [updated 25 August 2011; cited 29 January 2013]. [26 screens]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1515/.

3. Pandor A, Eastham J, Beverley C et al. Clinical effectiveness and cost-effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry: a systematic review. Health technology assessment (Winchester, England). 2004;8(12):iii-121.

4. Arora N, Stumper O, Wright J et al. Cardiomyopathy in tyrosinaemia type I is common but usually benign. J Inherit Metab Dis. 2006;29(1):54-7.

5. Sanderson S, Green A, Preece MA et al. The incidence of inherited metabolic disorders in the West Midlands, UK. Arch Dis.Child. 2006;91(11):896-9.

6. Sander J, Janzen N, Peter M et al. Newborn screening for hepatorenal tyrosinemia: Tandem mass spectrometric quantification of succinylacetone. Clinical chemistry. 2006;52(3):482-7.

7. Frazier DM, Millington DS, McCandless SE et al. The tandem mass spectrometry newborn screening experience in North Carolina: 1997-2005. J Inherit Metab Dis. 2006;29(1):76-85.

8. Wilcken B, Haas M, Joy P et al. Expanded newborn screening: outcome in screened and unscreened patients at age 6 years. Pediatrics. 2009;124(2):e241-e248.

9. Lund AM, Hougaard DM, Simonsen H et al. Biochemical screening of 504,049 newborns in Denmark, the Faroe Islands and Greenland--experience and development of a routine program for expanded newborn screening. Mol Genet Metab. 2012;107(3):281-93.

10. Bliksrud YT, Brodtkorb E, Backe PH et al. Hereditary tyrosinaemia type I in Norway: incidence and three novel small deletions in the fumarylacetoacetase gene. Scand J Clin Lab Invest. 2012;72(5):369-73.

11. Couce ML, Castineiras DE, Boveda MD et al. Evaluation and long-term follow-up of infants with inborn errors of metabolism identified in an expanded screening programme. Mol Genet Metab. 2011;104(4):470-5.

12. Lindner M, Gramer G, aege G et al. Efficacy and outcome of expanded newborn screening for metabolic diseases--report of 10 years from South-West Germany. Orphanet journal of rare diseases. 2011;6:44.

13. Morrissey MA, Sunny S, Fahim A et al. Newborn screening for Tyr-I: two years' experience of the New York State program. Mol Genet Metab. 2011;103(2):191-2.

14. Kasper DC, Ratschmann R, Metz TF et al. The national Austrian newborn screening program - eight years experience with mass spectrometry. past, present, and future goals. Wiener klinische Wochenschrift. 2010;122(21-22):607-13.

15. la Marca G, Malvagia S, Casetta B et al. Progress in expanded newborn screening for metabolic conditions by LC-MS/MS in Tuscany: update on methods to reduce false tests. J Inherit Metab Dis. 2008;31 Suppl 2:S395-S404.

16. Masurel-Paulet A, Poggi-Bach J, Rolland MO et al. NTBC treatment in tyrosinaemia type I: long-term outcome in French patients. J Inherit Metab Dis. 2008;31(1):81-7.

17. Feuchtbaum L, Lorey F, Faulkner L et al. California's experience implementing a pilot newborn supplemental screening program using tandem mass spectrometry. Pediatrics. 2006;117(5 Pt 2):S261-S269.

18. Comeau AM, Larson C, Eaton RB. Integration of New Genetic Diseases into Statewide Newborn Screening: New England Experience. American journal of medical genetics.Part C, Seminars in medical genetics. 2004;125 C(1):35-41.

19. UK Newborn Screening Programme Centre. A laboratory guide to newborn screening in the UK for phenylketonuria. London: UK Newborn Screening Programme Centre; 2011. Available from: http://newbornbloodspot.screening.nhs.uk/getdata.php?id=11648.

20. Allard P, Grenier A, Korson MS et al. Newborn screening for hepatorenal tyrosinemia by tandem mass spectrometry: analysis of succinylacetone extracted from dried blood spots. Clin Biochem. 2004;37(11):1010-5.

21. Matern D, Tortorelli S, Oglesbee D et al. Reduction of the false-positive rate in newborn screening by implementation of MS/MS-based second-tier tests: the Mayo Clinic experience (2004-2007). J Inherit Metab Dis. 2007;30(4):585-92.

22. Magera MJ, Gunawardena ND, Hahn SH et al. Quantitative determination of succinylacetone in dried blood spots for newborn screening of tyrosinemia type I. Mol Genet Metab. 2006;88(1):16-21.

23. Turgeon C, Magera MJ, Allard P et al. Combined newborn screening for succinylacetone, amino acids, and acylcarnitines in dried blood spots. Clinical chemistry. 2008;54(4):657-64.

24. Chace DH, Lim T, Hansen CR et al. Improved MS/MS analysis of succinylacetone extracted from dried blood spots when combined with amino acids and acylcarnitine

butyl esters. Clinica chimica acta; international journal of clinical chemistry. 2009;407(1-2):6-9.

25. la Marca G, Malvagia S, Pasquini E et al. The inclusion of succinylacetone as marker for tyrosinemia type I in expanded newborn screening programs. Rapid Commun Mass Spectrom. 2008;22(6):812-8.

26. la Marca G, Malvagia S, Funghini S et al. The successful inclusion of succinylacetone as a marker of tyrosinemia type I in Tuscany newborn screening program. Rapid communications in mass spectrometry : RCM. 2009;23(23):3891-3.

27. Dhillon KS, Bhandal AS, Aznar CP et al. Improved tandem mass spectrometry (MS/MS) derivatized method for the detection of tyrosinemia type I, amino acids and acylcarnitine disorders using a single extraction process. Clinica Chimica Acta. 2011;412(11-12):873-9.

28. Metz TF, Mechtler TP, Merk M et al. Evaluation of a novel, commercially available mass spectrometry kit for newborn screening including succinylacetone without hydrazine. Clinica chimica acta; international journal of clinical chemistry. 2012;413(15-16):1259-64.

29. UK Newborn Screening Programme Centre. Guidelines for Newborn Blood Spot Sampling- February 2012. London: UK Newborn Screening Programme Centre; 2012. Available from: http://newbornbloodspot.screening.nhs.uk/getdata.php?id=11952.

30. Oladipo OO, Weindel AL, Saunders AN et al. Impact of premature birth and critical illness on neonatal range of plasma amino acid concentrations determined by LC-MS/MS. Mol Genet Metab. 2011;104(4):476-9.

31. Schulze A, Lindner M, Kohlmuller D et al. Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. Pediatrics. 2003;111(6 Pt 1):1399-406.

32. McHugh D, Cameron CA, Abdenur JE et al. Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. Genetics in medicine : official journal of the American College of Medical Genetics. 2011;13(3):230-54.

33. Marquardt G, Currier R, McHugh DMS et al. Enhanced interpretation of newborn screening results without analyte cutoff values. Genetics in medicine : official journal of the American College of Medical Genetics. 2012;14(7):648-55.

34. Dietzen DJ, Rinaldo P, Whitley RJ et al. National academy of clinical biochemistry laboratory medicine practice guidelines: follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry; executive summary. Clinical chemistry. 2009;55(9):1615-26.

35. Kronn D, Mofidi S, Braverman N et al. Diagnostic guidelines for newborns who screen positive in newborn screening. Genetics in medicine : official journal of the American College of Medical Genetics. 2010;12(12 Suppl):S251-S255.

36. NYMAC. Diagnostic Guidelines for Confirmation of Screen-Positive Newborn Screening Results. 2010. Available from: http://www.wadsworth.org/newborn/nymac/docs/DX_Guidelines_11-30-2010.doc.

37. American College of Medical Genetics. Newborn Screening ACT Sheets and Confirmatory Algorithms [Internet]. Bethesda (MD): American College of Medical Genetics; 2001 [updated 2001 Jan 01; cited 2012 Jan 31]. [7 screens]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK55827/.

38. EMA. Orfadin (nitisinone) European Public Assessment Report (EPAR) Summary for the public. London: European Medicines Agency; 2009. Available from: http://www.emea.europa.eu/docs/en_GB/document_library/EPAR_-_Summary_for_the_public/human/000555/WC500049190.pdf.

39. EMA. Orfadin: EPAR- Scientific Discussion. London: European Medicines Agency; 2007. Available from: http://www.emea.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000555/WC500049192.pdf.

40. Couce ML, Dalmau J, Del Toro M et al. Tyrosinemia type 1 in Spain: mutational analysis, treatment and long-term outcome. Pediatr Int. 2011;53(6):985-9.

41. Koelink CJL, van Hasselt P, van der Ploeg A et al. Tyrosinemia type I treated by NTBC: how does AFP predict liver cancer? Mol Genet Metab. 2006;89(4):310-5.

42. Santra S, Preece MA, Hulton SA et al. Renal tubular function in children with tyrosinaemia type I treated with nitisinone. J Inherit Metab Dis. 2008;31(3):399-402.

43. Larochelle J, Alvarez F, Bussieres JF et al. Effect of nitisinone (NTBC) treatment on the clinical course of hepatorenal tyrosinemia in Quebec. Mol Genet Metab. 2012;107(1-2):49-54.

44. Arnon R, Annunziato R, Miloh T et al. Liver transplantation for hereditary tyrosinemia type I: analysis of the UNOS database. Pediatr Transplant. 2011;15(4):400-5.

45. Pierik LJWM, Van Spronsen FJ, Bijleveld CMA et al. Renal function in tyrosinaemia type I after liver transplantation: a long-term follow-up. J Inherit Metab Dis. 2005;28(6):871-6.

46. Norman R, Haas M, Chaplin M et al. Economic evaluation of tandem mass spectrometry newborn screening in Australia. Pediatrics. 2009;123(2):451-7.

47. De Laet C, Munoz VT, Jaeken J et al. Neuropsychological outcome of NTBC-treated patients with tyrosinaemia type 1. Dev Med Child Neurol. 2011;53(10):962-4.

48. Thimm E, Richter-Werkle R, Kamp G et al. Neurocognitive outcome in patients with hypertyrosinemia type I after long-term treatment with NTBC. J Inherit Metab Dis. 2012;35(2):263-8.

49. Cipriano LE, Rupar CA, Zaric GS. The cost-effectiveness of expanding newborn screening for up to 21 inherited metabolic disorders using tandem mass spectrometry: results from a decision-analytic model. Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research. 2007;10(2):83-97.

50. Norman R, Haas M, Wilcken B. International perspectives on the cost-effectiveness of tandem mass spectrometry for rare metabolic conditions. Health Policy. 2009;89(3):252-60.

51. Feuchtbaum L, Cunningham G. Economic evaluation of tandem mass spectrometry screening in California. Pediatrics. 2006;117(5 Pt 2):S280-S286.

52. ERNDIM. ERNDIM 2011 [cited 28 January 2013]. Available from: http://www.erndim.org/home/start.asp.

53. ERNDIM. Annual Report ERNDIM-EQAS 2011. 2011. Available from: http://cms.erndimqa.nl/getdoc/79f6c4ae-aced-493e-8279-18c00ee429f9/AnnualReportAA2011.aspx.

54. CDC. CDC Newborn Screening Quality Assurance Program [Internet]. Atlana (GA): 2013 [updated 2013 Jan 22; cited 2013 Jan 28]. [2 screens]. Available from: http://www.cdc.gov/labstandards/nsqap.html.

55. CLIMB. Children Living with Inherited Metabolic Diseases 2012 [updated 30 January 2013; cited 31 January 2013]. Available from: www.climb.org.uk .