UK National Screening Committee

Newborn screening for adrenoleukodystrophy: pilot assessment of topic submission

Version: Draft 3

Solutions for Public Health

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

Abbreviations List

X-ALD	Adrenoleukodystrophy
ACALD	Adult cerebral adrenoleukodystrophy
AdolCALD	Adolescent cerebral adrenoleukodystrophy
AMN	Adrenomyeloneuropathy
CCALD	Childhood cerebral adrenoleukodystrophy
CI	Confidence Interval
HPLC	High performance liquid chromatography
HSCT	Hematopoietic stem cell therapy
ICER	Incremental Cost-Effectiveness Ratio
MRI	Magnetic resonance imaging
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
QALY	Quality Adjusted Life Years
RCT	Randomised Controlled Trial
ScHARR	School of Health and Related Research (Sheffield)
TMS	Tandem Mass Spectrometry
UK	United Kingdom
US	United States
UK NSC	UK National Screening Committee
VLCFA	Very long chain fatty acid

Competing Interest

All SPH authors have completed the ICMJE uniform disclosure form (www.icmje.org/coi_disclosure.pdf) and declare: grants from Public Health England to SPH to undertake the submitted work, no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work

Contents

Introduction

The UK National Screening Committee (UK NSC) is introducing an annual call for topics. This is to ensure that stakeholders have a regular opportunity to suggest new topics which might be evaluated against the UK NSC criteria.

The first call for topics was advertised in September 2016. The closing date for submissions is January 2017.

Topics suggested through this route will be processed in several steps. First, the relevance to the UK NSC remit will be considered. Where relevant, the next step will be to produce an external triage assessment of the suggested topic. Triage assessments of submissions are not intended to provide a definitive statement on the case for screening. The function of the assessment is to distinguish topics which are likely to benefit from further investigation from those which would not. This should be done through a discussion of the papers highlighted as part of the submission. If, at this stage, the suggested topic appears to be well supported by published research evidence, an evidence map may be produced to gauge the volume and type of evidence over and above the submitted papers.

Key issues relating to topics which are successful at the triage stage will be explored through an evidence summary using a rapid review methodology and assessed using the UK NSC reporting checklist for evidence summaries.

This report details an assessment of a topic submitted to UK NSC by the charity ALD Life supporting newborn screening for X-linked adrenoleukodystrophy. The triage assessment is based on a combination of scoping review and evidence map methodology and the resulting document is a vignette of the topic.

Summary of condition and rationale for topic suggestion

The condition

Adrenoleukodystrophy (X-ALD) is a metabolic disorder affecting the adrenal glands, neurological system and testes. It is caused by a defect in the ABCD1 gene either passed on by the mother (X-linked) to her offspring or arising *de novo*.

There are a range of different phenotypic forms of the condition; the four main types are described below based on Engelen et al[4]. Only one phenotype (Childhood cerebral X-ALD) in affected males is the target group for detection with a newborn screening programme.

a) Cerebral X-ALD(CALD) in affected males

CALD is a rapidly progressive demyelination of the brain occurring in affected males. The most common form of CALD occurs during childhood (CCALD) with symptoms typically developing in boys between the ages of 2 to 10 years. Early signs of CCALD include attention deficit and hyperactivity, visual and hearing

difficulties, and coordination problems. It is estimated that 31-57% of males with the ABCD1 mutation will go on to develop CCALD. Without treatment, death or severe disability occurs within about 3 years.

Rarely cerebral demyelination in affected males may occur in adolescence (AdolCALD) and adulthood (ACALD) with similar presenting symptoms but the disease progression is slower.

b) Adrenomyeloneuropathy (AMN) in affected males

AMN is characterised by neurodegeneration of the spinal cord and peripheral nerves in adults. Most patients will develop primary adrenal insufficiency at some stage. The typical presentation is a man in his twenties or middle age who develops progressive stiffness and weakness in the legs, abnormalities of sphincter control, and sexual dysfunction. A small proportion of men with AMN symptoms (~20%) may develop cerebral demyelination. Symptoms can be managed but there are no treatments that have altered disease progression.

c) Addison disease in affected males

Males develop signs of adrenal insufficiency between age two years and adulthood, most commonly by age 7.5 years. When Addison's disease only has developed the signs include unexplained vomiting, weakness, dehydration, dizziness and sweating which can lead to coma. Corticosteroids are the primary treatment for the lack of cortisol and aldosterone.

d) AMN in Female carriers

An increasing number of symptomatic heterozygous women are identified as the first member of the family to be affected by X-ALD and it is estimated that around 80% of carriers of ABCD1 develop symptoms of AMN by the age of 60. Onset of symptoms is usually in mid-life and very similar to those observed in adult males with AMN. Sensory ataxia, fecal incontinence and pain in the legs are often more prominent in women with AMN. Adrenal function is usually normal. Neurologic evaluation for the appearance of symptoms and their management is important.

Incidence of X-ALD

A systematic review[1] summarising the papers determining incidence of X-ALD showed a variation in estimates between 1: 100,000 and 4.7:100,000 (or 1:21,000) male births. This variation is ascribed to differences in data collection methods and populations sampled. The New York State screening programme has released data of the first 18 months of newborn screening for X-ALD and reported that 13 males and 13 heterozygous females out of 365,000 live births with ABCD1 genetic mutation were identified. No studies have determined the incidence in UK populations although a life time risk of 6.6/100,000 has been estimated from British Paediatric Surveillance Unit data[5].

In males childhood cerebral X-ALD (CCALD) and the adult onset adrenomyeloneuropathy (AMN) can occur within the same family. There is no known correlation between genotypes and the presentation or age of onset of the disease so for any individual it is not currently possible to predict what clinical features will develop and when they will occur.

Adrenoleukodystrophy has been under consideration as a disorder amenable to newborn screening in the US for several years. The morbidity and mortality associated with undiagnosed adrenal disease and the emergence of hematopoietic stem cell therapy (HSCT) as a standard therapy for CCALD are reasons to consider screening for X-ALD. The logic of newborn screening is to facilitate early diagnosis of CCALD prior to the onset of adrenal and neurological symptoms to improve outcomes from HSCT.

The focus of the proposed newborn screening programme for X-ALD is to identify all males with gene mutations of ABCD1 and monitor them with a combination of magnetic resonance imaging (MRI) and adrenal function testing in order to identify the point at which they may start developing CCALD with a view to early HSCT. There are currently no interventions for AMN.

The test

There are a number of sequential elements required to determine the group of males with CCALD who may benefit from treatment with HCST. The following sequence of tests is used by the New York State newborn screening programme[6,10].

i) Blood spots are tested using flow injection analysis tandem mass spectrometry(TMS) to detect the very long chain fatty acid (VLCFA) marker C26:0 lysophosphatidylcholine (C26:0LPC). Around 1.8% newborn blood spots will have a positive result.

ii) All blood spots positive for elevated levels of VLCFA markers C26:0LPC are further tested with TMS coupled with High Performance Liquid Chromatography (HPLC) to quantify C26:0LPC. Those blood spots of newborns with peroxisomal disorders have VLCFA markers at >5 fold levels than those from normal subjects.

iii) Of those blood spots with elevated VLCFA's values, Sangar DNA sequencing is undertaken. This confirms which newborns have X-ALD and those that have other peroxisomal disorders such as Zellweger spectrum disorder.

iv) Males with ABCD1 mutation are kept under surveillance and monitored with brain MRI to check for early detection of demyelinating lesions. Brain MRI shows abnormal signal intensities in the corpus callosum, parieto-occipital, or frontal white matter or pyramidal tracts within the brainstem, pons and internal capsules.

The intervention

Males who have been identified as having an ABCD1 mutation and following monitoring have developed CCALD (31-57%) can receive treatment in the form of haematopoietic stem cell transplantation (HCST) administered at an early stage of progressive brain involvement. Most males will not develop CCALD and there are risks associated with HCST so only those who present with early symptoms will be treated. HSCT is not recommended for males with more advanced stages of CCALD as the complications of the procedure outweigh the benefits in respect to survival. Studies of survival show 5 year survival probability for untreated CCALD vs HSCT as 54% vs 95% (X^2=7.47, p=0.006)[8]. No study has specifically evaluated the harms of HSCT for X-ALD related CCALD detected via newborn screening. The impact of carrier testing on families is not available.

Supporting evidence: Description

The table below summarises the supporting evidence included by ALD Life as part of their submission.

Table 3: Description of the evidence submitted

Reference	NSC criteria covered by study: The condition Test Treatment Screening programme	Study type	Included in evidence map	Key points
(1) **The genetic landscape of X-linked adrenoleukodystrophy: inheritance, mutations, modifier genes, and diagnosis** Wiesinger C, Eichler FS, Berger J. Applied Clinical Genetics 2015 8: 109-121[2]	The condition Test Treatment Screening programme	Review article	Yes	Summary of 9 studies reporting worldwide incidence rates for x-linked X-ALD. Estimates incidence rates in males to be between 1:20,000 and 1:30,000 males (states that only newborn screening will be able to accurately update the incidence rates) including de novo mutations (estimated between 5% and 19%). No phenotype-genotype correlation. Raised plasma VLCFA also found in other conditions and affected by dietary intake. ABCD1 mutation necessary but not sufficient to determine if for CCALD will develop. Notes that newborn screening for X-ALD in males has been in place in New York State since 2014.
(2) **General Aspects and Neuropathology of X-Linked Adrenoleukodystrophy** Ferrer I, Aubourg P, Pujol A.	The condition	Review article	No	Describes detailed neuropathology of X-linked X-ALD; several phenotypes including cerebral X-ALD in male children, adolescents and adults, as well as AMN, Addison's Disease and female carriers/presentation.

Brain Pathology 2010 20: 817–830[3]				Notes the lack of phenotype-genotype correlation, as the same ABCD1 gene mutation is associated with several different phenotypes. Suggests that genetic, epigenetic, environmental and stochastic factors contribute to the disease pathway and development of the disease. Highlights the use of raised VLCFAs as a biomarker for X-linked X-ALD.
(3) **X-linked adrenoleukodystrophy (X-adrenoleukodystrophy): clinical presentation and guidelines for diagnosis, follow-up and management** Engelen M, Kemp S, de Visser M et al. The Orphanet Journal of Rare Diseases 2012 7:51[4]	The condition Treatment	Review article/ clinical guideline	No	Describes the following elements of X-ALD: • range of phenotypes and associated frequency • presentation - wide clinical spectrum in males with X-ALD ranging from isolated adrenocortical insufficiency and slowly progressing myelopathy to rapid and severe cerebral de-myelination • differential diagnosis • unpredictable disease progression • clinical management recommended for screen detected cases includes annual MRI before age 3 then every 6 months from age 3- 12 for early signs of CCALD. • allogeneic HSCT may be suitable for screen detected children with very early MRI detected cerebral X-ALD • Screen detected X-ALD asymptomatic male adults are advised to have annual evaluation by neurologist and offer symptomatic treatment when AMN symptoms develop. • There is no curative or preventive treatment

				for majority of patients
(4) **Leukodystrophies and Genetic Leukoencephalopathies in Childhood: A National Epidemiological Study** Stellitano LA, Winstone AM, Van Der Knaap MS, Verity CM. Developmental Medicine & Child Neurology, 7th November 2015[5]	The condition	Prospective national epidemiological study (using British Paediatric Surveillance Unit data)	No	Prospective UK epidemiological study of children registered between May 1997 and Nov 2014, with leukodystrophies defined as progressive intellectual and neurological deterioration (PIND), specifically 'Any child under 16 yrs who has progressive deterioration for 3 months with loss of already attained intellectual or developmental abilities and development of abnormal neurological signs.' 349 children were identified with diagnosed leukodystrophies, 74 with X-linked X-ALD (55 with symptoms). The study includes demographic features (sex, ethnicity, consanguinity and family history) and early symptom and signs of disease. The study estimates the lifetime X-ALD risk 6.6/1,000,000 live births but not incidence. Authors' note that some children with X-ALD will not meet PIND criteria and would not be reported leading to under ascertainment of the condition.
(5) **Newborn screening for X-linked adrenoleukodystrophy (X-ALD): validation of a combined liquid chromatography-tandem mass spectrometric (LC-MS/MS) method.** Hubbard WC, Moser AB, Liu AC, et al. Mol Genet Metab 2009:97:212-20[6]	Test	Diagnostic, two-gated case control study n=1194 (17 with known X-linked X-ALD)	Yes	Use of LC-MS/MS method to test for 26:0-LPC. Levels of 26:0-lyso-PC in blood spots of X-linked X-ALD affected individuals are >5-fold the levels from normal subjects, with no overlap in the levels of 26:0-LPC between affected and normal subjects. All samples were run masked and all individuals with X-ALD were identified with one exception. When the DNA of the

				one missed sample was sequenced no X-ALD was found.
(6) **Cerebral X-linked adrenoleukodystrophy: the international hematopoietic cell transplantation experience from 1982 to 1999** Peters C, Charnas LR, Tan Y et al. Blood. 2004 Aug 1;104(3):881-8. Epub 2004 Apr 8[7]	Treatment	Retrospective uncontrolled cohort study	No	N=126 boys with cerebral X-ALD who received HSCT between 1982 and 1999 43 international centres Results: Dataset available for analysis: n=92/126 from 32 treatment centres Survival at 5 years=92% Survival at 8 years = 56% Overall 5 year survival higher in early stage disease (0-1 neurological deficit, MRI severity score <9 at baseline) vs others (overall 5 year survival 45%). P<0.01
(7) **Survival analysis of haematopoietic cell transplantation for childhood cerebral X-linked adrenoleukodystrophy: a comparison study** Mahmood A1, Raymond GV, Dubey P, Peters C, Moser HW Lancet Neurol. 2007 Aug;6(8):687-92[8]	Treatment	Retrospective survival analysis	No	Retrospective survival analysis of 283 CCALD non-transplanted patients plus comparison of survival outcomes of 19 HSCT patients with 30 matched non transplanted patients with CCALD (from Peters et al 2004 – see reference 6 above) Key finding for 5 year survival probability for untreated vs HSCT: 54% vs 95% (X^2=7.47, p=0.006)
(8) **Hematopoietic Stem Cell Transplantation and Hematopoietic Stem Cell Gene Therapy in X-Linked Adrenoleukodystrophy** Cartier N, Aubourg P Brain Pathology, 2010 20(4): 857–862[9]	Treatment	Review article	No	Narrative review of the effectiveness of allogeneic HSCT for early stage cerebral demyelination in X-linked X-ALD in boys. Includes references to primary research including Baumann et al 2003 (n=12 case reports) and Mahmood et al 2007, a retrospective comparative survival analysis of HSCT vs non-transplant group (see reference 7 above).

				Narrative considers limitations of HSCT in adults with CCALD, limitations of HSCT (morbidity and mortality risks), patient selection and the emergence of HSC gene therapy as a possible alternative to HSCT.

(9) **Personal Communication from Joseph Orsini New York State Screening Laboratory**
Orsini J, Caggana M, Bradley S and Saavedra-Matiz C, New York State Department of Health, Albany, NY
http://www.aphl.org/conferences/Documents/Session-8.pdf[10]
	4: Screening programme	Conference abstract	No	Description of the New York State 3 tiered screening tests and results from the first 4 months of screening:
				• 75,885 newborns screened
				  • 8 referred for diagnostic evaluation/DNA testing
				  ○ 3 boys confirmed X-ALD (with 2 pending)
				  ○ 1 female carrier and
				  ○ 2 newborns confirmed with no ABCD1 mutation despite raised C26:0-lyso-PC)
				• By Sept 2014, 165,142 newborns screened
				  • 3,609 raised C26:0-lyso-PC
				  • 14 referred for diagnostic evaluation and DNA test
				  ○ 10 newborns confirmed with ABCD1 mutation
				  ○ 2 confirmed with Zellweger spectrum disorder
				  ○ 2 other disorders

(10) **Cost effectiveness of including X-ALD in the NHS Newborn Screening Programme – Report for consultation.** Bessey A, Leaviss J, Chilcott J, Sutton A School of Health and Related Research	The condition Test Treatment Screening programme	Cost effectiveness model using decision tree	No	Cost effectiveness study based on assumptions from systematic literature reviews of evidence published before data published by new York State X-ALD screening programme.

(ScHARR), University of Sheffield, 2015[11]				The model suggests that newborn screening for X-ALD is cost effective at £14,249 per QALY due to both increased QALYs gained and reduction in social and education costs of caring for affected individuals. (See Supporting evidence: Summary below for more in depth critique)
(11) **Newborn screening for X-linked adrenoleukodystrophy: evidence summary and advisory committee recommendation** Kemper AR, Brosco J, Comeau AM et al. Genet Med. 2016 Jun 23[1]	The condition Test Treatment Screening programme	Summary of systematic evidence review	Yes	Summary of systematic evidence review by the Condition Review workgroup and Summary of Advisory Committee recommendation. Full review not accessible at online HRSA.gov (as at 7thDec2016) as some data from a range of centres informing the review is embargoed until centres have published. (See Supporting evidence: Summary below for more in depth critique)

Supporting evidence: summary

The supporting evidence submitted covers the latest findings about the condition of X-ALD, testing, treatment and screening for the condition. Two of the papers, based on systematic reviews with a focus on newborn screening, give a comprehensive summary of the evidence. These are the evidence summary and recommendation to the advisory committee in the US[1] and the UK cost effectiveness study by ScHARR[11]. These are considered in more detail below as the first includes full information about newborn screening for X-ALD and the second has applied latest evidence to model newborn screening in a UK context. It should be noted that the full systematic review by Kemper et al[1] could not be accessed and the cost effectiveness report has not been published and forms part of the grey literature.

Newborn screening for X-linked adrenoleukodystrophy: evidence summary and advisory committee recommendation (Kemper et al 2016)[1]

The New York State X-ALD newborn screening programme has recently published the data emerging from the first 18 months following implementation. This was in-conjunction with a systematic review of the literature which together formed the basis of the recommendation that newborn screening for X-ALD should be added to the list of screening programmes nationally recommended by the US Department of Health and Human Services.

In the 18 months to July 2015 approximately 365,000 newborns (male and female) were screened for X-ALD. Of those 6,570 had an elevated C26:0LPC level and had their DNA sequenced. 33 individuals had a positive DNA test of which 13 were male X-ALD who would be monitored for CCALD. A further 13 females also carried the X-ALD mutation and 6 cases of other peroxisomal conditions. One other incidental diagnosis was also identified.

Table 4: New York State X-ALD newborn screening programme: number of newborns screened and outcome

Newborns tested in 18 months to July 2015	Newborns with elevated C26:0LPC	Males and females with ABCD1 following DNA sequencing	Other peroxisomal conditions identified	Other incidental diagnoses
365,000	6,570(1.8%)	13(0.004%) males and 13 (0.004%)females	6(0.002%)	1(0.0003%)

Of the 13 males monitored with X-ALD between 4 and 8 (31-57%) are expected to develop CCALD before age 18.

Table 5: New York State X-ALD newborn screening programme: expected interventions for those newborns detected with DNA sequencing

	Newborns detected with a condition	Males with X-ALD	Female carriers X-ALD	Other peroxisomal conditions	Other finding
	33	13	13	6	1
Intervention	DNA sequencing	Under surveillance 31-57% expected to develop CCALD and require HCST Remainder highly likely to develop AMN in early midlife and require symptom management Case finding of other family members	Information given about the condition and development of symptoms in later midlife. Case finding of other family members	Managed as clinically indicated	Managed as clinically indicated

The proportion of the target group of 13 males with the ABCD1 mutation under surveillance and expected to develop CCALD is between 31 to 57% or 4 to 8 children. Theoretically HSCT treatment would be triggered in children with the earliest detected neurological deficit (0 or 1 neurological deficit and an MRI severity score of less than 9). The MRI scoring system used was developed by Loes et al 1994[12] and 2003[13] describes 5 patterns of progressive cerebral demyelination typical in X-ALD patients. Patterns 1 and 5 were typically seen in childhood (CCALD) 2 and 3 in adolescence (AdolCALD) and 3 in adults (ACALD)

Kemper et al[1] has reported that there are no recent data regarding the mortality risk associated with HCST in the literature but data from between 1982-1999 indicated that 5 year survival varied with number of neurological deficits, disability rating and MRI score. It was estimated that children treated with the earliest detected neurological deficit and MRI severity scoring would achieve survival 92% at 5 years[7]. It isn't clear what proportion of the survivors will experience other complications of HSCT (eg: graft vs host disease).

Kemper et al[1] reports that the knowledge about management of X-ALD detected pre-symptomatically is still developing and there is a risk that an individual could be treated with HCST sooner than is clinically indicated. There were no studies identified that evaluated these potential harms for X-ALD newborn screening.

Of the 33 positive DNA test results between 25.6 (78%) and 29(88%) newborns will not receive treatment but will need supportive care and life long management of symptoms. These include:

- between 5.6 and 9 X-ALD males monitored who do not develop CCALD but are highly likely to subsequently develop AMN
- 13 females of which approximately 10 (80%) will develop AMN in mid to later life
- 7 newborns who have incidental diagnoses mostly of other disabling or fatal peroxisomal conditions with no current treatment

Apart from the detection rate of peroxisomal disease types from DNA sequencing all other outcomes of the screening programme between January 2014 and July 2015 are estimates as the newborns diagnosed are still under surveillance at the time of writing. CCALD would be expected to develop in 4-8 of the 13 males from age 3-10 yrs (January 2017-July 2025) The actual outcome of this cohort of newborns will not be fully known until the 18 year surveillance period is completed (July 2033).

It is expected that case finding within families of newborns identified with X-ALD will also be undertaken. Kemper et al[1] did not identify any data regarding the impact of carrier testing on families or the uptake or outcomes of extended family testing for X-ALD.

Challenges for the screening programme include developing an infrastructure for both screenng and follow up care, overdiagnosis arising from identification of infants with ABCD1 mutation who do not develop CCALD and maximising the effectiveness of HSCT whilst minimising the morbidity and mortality risks of the treatment.

Cost effectiveness of including X-X-ALD in the NHS Newborn Screening Programme – Report for consultation (Bessey et al 2015[11])

A cost effectiveness study of adding X-ALD newborn screening to the NHS newborn blood spot screening programme was commissioned from the School of Health and Related Research (ScHARR) at Sheffield University by ALD Life[11]. The evidence used to inform the model parameters was based on the available published evidence prior to the publication of the Kemper et al[1] evidence summary, so did not include data from the New York State screening programme. The analysis recognises the limitations and lack of robustness around the evidence base used to populate the parameters. These include:

- no published Quality Adjusted Life Years (QALY) estimates for X-ALD or comparable disorder were found
- uncertainty about incidence and phenotype distributions world wide and no UK based studies
- uncertainty about the benefits and dis-benefits for the 78-88% of newborns and their families identified via DNA sequencing who will not receive HCST

Assumptions were made around these uncertainties and the results of the modelling suggest that screening all newborns for X-ALD is cost–effective with an increase in Quality Adjusted Life Years and an overall reduction in total cost. The incremental cost-effectiveness ratio (ICER) was calculated as £14,326 and achieved a level below £20,000 for 73% of the 10,000 runs of the model. Social care costs were reduced significantly in the screening scenario compared to no screening, however health care and screening programme costs would increase from £785,000 to £1,424,000 per annum.

Supporting evidence; evaluation

This section focusses on the 3 questions in step 3 of the scoping review process and includes a critique of the two most recent relevant publications about newborn screening for X-ALD.

a. Does the supporting evidence indicate a good understanding of the natural history of the condition?

The supporting evidence indicates that there is some understanding about the natural history of the target condition (CCALD) but less understanding about the natural history of AMN and other conditions identified via the screening programme such as Zellweger syndrome. More understanding about what might trigger the development of CCALD which phenotypically can't be correlated to genotype, even within families, would be useful.

b. Can a gold standard diagnostic test be identified from the supporting evidence?

For this screening programme a sequence of tests are required in order to detect the population that will require treatment with HSCT.

i) TMS and HPLC test for quantification of VLCFA's

The results of Hubbard's[6] two gate cohort study has reported a specificity of 1 as all anonymised X-ALD samples (n=17) were detected amongst a larger control sample of 1177 with a 5 fold difference between 26:0lyso-PC picomol levels in control blood spots vs X-ALD bloodspots. Studies using a two gate design have been criticized for over inflating diagnostic accuracy especially if the sample size of either the control or those with the disorder is small as the range of values detected in the study may not reflect those in the population. The authors recognize this and point to further studies with larger study populations to further confirm their findings.

The sensitivity of the initial TMS test will not be known as those who may have a false negative result are not followed up. If they were followed up it would take 18 years from newborn bloodspot test to determine if any males with X-ALD who developed CCALD had been missed.

ii) DNA sequencing of those positive for VLCFA's to detect the ABCD1 mutation

Confirmatory testing using Sangar sequencing to detect an X-linked mutation of ABCD1 is considered to have high validity and will identify all newborns with X-ALD within a set of samples.

iii) Brain MRI scan for males identified as having X-ALD to detect those that develop CCALD.

Determining the earliest point at which cerebral demyelination has developed is a clinical judgement using the Loes scoring system. There is currently a risk of overdiagnosis arising from identification of infants with ABCD1 mutation who do not develop CCALD. The sensitivity and specificity of this scoring system was not included in any of the supporting evidence.

c. Is there an intervention with some evidence that it reduces mortality/morbidity?

The use of HSCT has shown reduced mortality compared to no treatment of children in the early stages of developing CCALD.

The supporting evidence from the ALD Life submission includes:

- a systematic review and data from a recently implemented X-ALD screening programme in the US
- published results of a diagnostic accuracy study using a platform (Tandem Mass Spectrometry) that is also used in the NHS newborn screening programme.
- some evidence of greater survival of people with X-ALD treated with HSCT compared to no treatment
- a cost-effectiveness analysis model suggesting a screening for X-ALD has a potentially acceptable cost benefit.

Based on the evaluation of the supporting evidence it is considered helpful to identify further recent literature with an evidence map of X-ALD. This was produced by UK NSC.

Evidence map: Identify research question

Below are the triage questions based on UK NSC criteria that inform the evidence map search strategy undertaken by UK NSC.

Question 1: Test and screening	Has the clinical value of newborn screening tests for X-ALD been established in prospective studies of large, unselected or representative, populations?
Population	Newborn
Intervention	Newborn screening using C26:0-lysophosphatidylcholine (C26:0 LPC) analysis by LC-MS/MS in newborn bloodspots
Comparator	Other tests No screening
Outcomes	Diagnostic accuracy of screening test
Study design	HTA, SR, Meta analysis, RCT, non randomised T, cohort study

Question2: Treatment following screening detection	What is the evidence base (in terms of study type and volume) relating to the improvement in treatment outcomes that will be achieved by screening for X-ALD?
Population	Newborn
Intervention	Haematopoietic stem-cell transplant
Comparator	No treatment Other treatment modality
Outcomes	Delayed or reduced impact of disease
Study design	HTA, SR, Meta analysis, RCT, non randomised T, cohort study.

Evidence map: Search strategy

This evidence map has been produced as part of the triage process to assess whether a more in depth UK NSC evidence review should be carried out for newborn screening for adrenoleukodystrophy. The search focuses on the test, screening and treatment.

A systematic review (Musolino et al, 2014[14]) on the treatment for adrenoleukodystrophy was identified during the scoping work. The search for this systematic review included references up until 2012. Therefore, 2012 was taken as the start date for the treatment search. This systematic review was retrieved by the search and is included in the results.

SOURCES SEARCHED: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present, Embase 1996 to 2016 Week 33, and the Cochrane Library.

DATES OF SEARCH: 2004-2016 (the test and screening), 2012-2016 (the treatment)

After automatic and manual de-duplication, 835 unique references were sifted for relevance to the review.

Inclusions and exclusions:

- case reports, conference abstracts and publications not in English were excluded
- studies were included if the number of patients was 10 or more

47 references were deemed to be relevant and are included in the evidence map.

Evidence map: Results

The table below summarises the information available from the abstracts identified from the evidence map search.

Table 5: Summary of abstracts identified from the evidence map for newborn screening for X-ALD (published between 2004 and 2016 for the test and screening programme and 2012 to 2016 for the intervention

Study type	Comments	Test	Intervention	Screening programme	Total
Systematic reviews (CEBM Evidence level 1)*	One systematic review covered studies of treatment using HSCT in X-ALD and the other focussed on the X-ALD screening programmes in the US		1	1	2
Cohort studies (CEBM Evidence Level 2)*	Both studies focussed on HSCT in X-ALD		2		2
Retrospective cohort study (CEBM Evidence level 2)*	One study focussed on HSCT in X-ALD and the other on treatment in leukodystrophies		2		2
Validation studies	These mostly focus on the refinement of a standard method of analysis of C26:0-LPC in dried blood spots that is simple fast with high through-put for newborn screening purposes	9			9
Drug evaluation	The paper describes the development of a potential new methodology using stem cell lines that would enable greater understanding of pathophysiology of X-ALD and support the development of new drugs		1		1
Reviews (not systematic)	These reviews summarise the current understanding about diagnosing, treating and screening for X-ALD	3	18	8	29
Survey	Survey of X-ALD affected families and their attitudes towards genetic testing in different settings (newborn, preconception, prenatal etc)			1	1
Guideline protocol	X-ALD Newborn screening guideline protocol. Programme follow up protocol needs development as symptoms may not emerge for many years			1	1

*OCEBM Levels of Evidence Working Group. "The Oxford 2011 Levels of Evidence". Oxford Centre for Evidence-Based Medicine. http://www.cebm.net/index.aspx?o=5653

Summary of evidence about X-ALD from evidence map

Of the 47 unique references identified by the evidence map 3 had been included (out of 11) in the X-ALD submission of supporting evidence by ALD Life. The 8 references not identified by the evidence map would not have been detected by the literature search as they were unpublished; conference abstracts or published before the search date. Of the references identified 29/47 (62%) were non systematic review articles. Test validation studies made up 20% of the references whilst intervention studies took the form of one systematic review and 4 cohort studies.

The systematic review by Musolino et al (2014)[14] identified the worldwide literature on numbers of leukodystrophy patients undergoing HSCT as well as the safety and efficacy of the procedure in the patient population. There is limited experience on the overall long-term neurological development and outcome of children after bone marrow transplantation in the leukodystrophies. A total of 152 studies were included which combined reported on a total of 689 patients. Study quality ranged from poor to good and many had small sample sizes. A meta-analysis of larger studies indicated that transplantation at earlier stages of disease was more successful than at later stages. Beyond survival, few functional or neurological outcomes were reported. Of 88 ALD patients undergoing HSCT, the complications reported included 50% had graft vs host infections, 26% had other infections, 16% had graft failure and 15% had other complications. The review concluded that further studies are needed to determine neurological outcome following HSCT.

Discussion

This scoping review has identified the main evidence base and themes concerning newborn screening for X-ALD.

There is one newborn screening programme for X-ALD which has an agreed test using a newborn blood spot that is analysed for elevated levels of C26:0LCP. Screen-positive samples are further analysed with DNA sequencing. Approximately 12-22% of newborns receiving a positive DNA test result will be males with X- ALD who will be monitored throughout childhood for signs of CCALD. The sensitivity and specificity of the overall testing process is unclear.

It is uncertain what the impact will be on families of X-ALD affected males who are monitored for 18 years but who do not develop CCALD.

Seventy eight to 88% of those with a positive DNA test result will be incidental findings of newborns who are carriers of X-ALD, who may or may not develop symptoms of AMN in later life and those diagnosed with other untreatable peroxisomal conditions. This level and severity of incidental findings would raise difficult ethical issues that the UK NSC would have to consider.

Experience of HSCT for patients with CCALD is limited because the condition is rare and this has inevitably led to a low volume of high quality evidence about the effectiveness, safety and efficacy of the procedure in the patient population. It is clear that leaving CCALD untreated leads to severe disability and death and that treating with HSCT at an early stage of brain involvement will reduce morbidity and mortality. However the management of X-ALD detected pre-

symptomatically is still developing and more needs to be known about long term outcomes from this approach. This also suggests that the clinical indications prompting timely treatment are not well described and there is a risk that an individual could be treated unnecessarily or at a less than optimal point.

ScHARR has developed and populated a model that shows that X-ALD newborn screening would be cost effective if implemented in the UK with an ICER of £14,326. Due to a paucity of consistent evidence the model has had to make broad assumptions about incidence, the distribution of X-ALD phenotypes, the treatment outcomes and the benefits and dis-benefits for the 78-88% of newborns identified with a condition but who will not receive HCST. The health decrement effect on the cost effectiveness estimate is more pronounced if girls are included presumably because they don't suffer from CCALD and the long term impact of identifying adult onset AMN may be serious. There is also uncertainty about the screening strategy that would be used. Overall there is not enough certain evidence informing the assumptions of the model to feel confident in its predictions.

The table below shows the incidence of X-ALD within the New York State newborn screening programme. These rates have been applied to the UK newborn population (2015) as a very rough estimate of the numbers a UK newborn screening programme may generate.

Table 4: Estimate of potential cases of X-ALD that could be identified via a UK newborn screening programme based on 18 months data from the New York State X-ALD newborn screening programme

	New York state programme (18 months)	Rates per 100,000 population screened newborns	X-ALD in the UK population (per year) when NYS rates applied
Number of newborn screened	365,000 (18 months)		697,852 (2015 live births)
Number of cases with elevated C26:LPC (1.8% of those screened)	6,570	1,800	12,561
Number of cases confirmed by DNA sequencing (0.5% of those with elevated C26:0LPC)	33	9	62
Number of cases of male X-ALD (who will be monitored)	13	3.5	24.4
Number of cases of female X-ALD	13	3.5	24.4
Number of cases of other peroxisomal conditions	6	1.6	11.3
Other	1	0.27	1.86
Males expected to develop CCALD and require HCST (31-57% males with ABCD1 mutation) during childhood (to age 18)	4-7.4	1.1-2	7.5 - 14
Males expected to survive HCST (5 year=92%)	3.68-6.8	1-1.8	7-12.8

Recommendation

From studies identified in this scoping review significant gaps in the evidence base around newborn screening for X-ALD are clear. As the New York State newborn screening programme continues and more data emerges there may be more clarity about the management of screen detected X-ALD patients and screen detected patients with incidental conditions. More evidence about the safety and efficacy and timing of HSCT for these patients may also be published.

It is recommended that due to the current gaps in evidence of many elements of the screening pathway for X-ALD a full UK NSC evidence review is not undertaken at this time.

Appendix 1: Evidence map newborn screening and treatment for adrenoleukodystrophy

BACKGROUND: this evidence map has been produced as part of the triage process to assess whether adrenoleukodystrophy should be considered as a newborn screening programme. The search focuses on the test, screening and treatment.

A systematic review (Musolino et al.) on the treatment for adrenoleukodystrophy was identified during the scoping work. The search for this systematic review included references up until 2012. Therefore, 2012 was taken as the start date for the treatment search. This systematic review was retrieved by the search and is included in the results.

2004 was chosen as the start date for the test and screening as this was when the US abandoned the idea of newborn screening for the condition.

SOURCES SEARCHED: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and

Ovid MEDLINE(R) 1946 to Present, Embase 1996 to 2016 Week 33, and the Cochrane Library.

DATES OF SEARCH: 2004-August 2016 (the test and screening), 2012-August 2016 (the treatment)

SEARCH STRATEGIES:

Medline:
1. Adrenoleukodystrophy/ (1575)
2. adrenoleukodystrophy.tw. (1795)
3. leukodystroph$.ti. (1479)
4. "x linked X-ALD".tw. (40)
5. 1 or 2 or 3 or 4 (3545)
6. Neonatal Screening/ (8308)
7. (screen$3 or detect$3).tw. (2210779)
8. ((advance or early or earlier) adj3 (identif$ or recogni$)).tw. (41301)
9. Genetic Testing/ (30616)
10. (genetic$ adj3 (identif$ or test or tests or testing)).tw. (44054)
11. (test or tests or testing).tw. (1812082)
12. 6 or 7 or 8 or 9 or 10 or 11 (3724212)
13. Hematopoietic Stem Cell Transplantation/ (33028)
14. ((hematopoietic or haematopoietic) adj2 stem cell transplant$).tw. (15859)
15. ((hematopoietic or haematopoietic) adj cell transplant$).tw. (4192)
16. (therap$ or treat$).ti. (1792384)
17. cord blood.tw. (23782)
18. bone marrow.tw. (185313)
19. 13 or 14 or 15 or 16 or 17 or 18 (2003184)
20. 5 and 12 (725)
21. limit 20 to yr="2004 -Current" (325)
22. 5 and 19 (481)
23. limit 22 to yr="2012 -Current" (110)
24. 21 or 23 (415)

Embase:
1. adrenoleukodystrophy/ (2058)
2. adrenoleukodystrophy.tw. (1495)
3. leukodystroph$.ti. (943)
4. "x linked X-ALD".tw. (33)
5. 1 or 2 or 3 or 4 (3064)
6. newborn screening/ (12903)
7. (screen$3 or detect$3).tw. (2200201)
8. ((advance or early or earlier) adj3 (identif$ or recogni$)).tw. (48350)
9. genetic screening/ (55414)
10. (genetic$ adj3 (identif$ or test or tests or testing)).tw. (55483)
11. (test or tests or testing).tw. (1889301)
12. 6 or 7 or 8 or 9 or 10 or 11 (3745661)
13. exp hematopoietic stem cell transplantation/ (44641)
14. ((hematopoietic or haematopoietic) adj2 stem cell transplant$).tw. (27082)
15. ((hematopoietic or haematopoietic) adj cell transplant$).tw. (6749)
16. (therap$ or treat$).ti. (1419929)
17. cord blood.tw. (26932)
18. bone marrow.tw. (183686)
19. 13 or 14 or 15 or 16 or 17 or 18 (1635090)
20. 5 and 12 (802)
21. limit 20 to yr="2004 -Current" (628)
22. 5 and 19 (730)
23. limit 22 to yr="2012 -Current" (289)
24. 21 or 23 (845)

Cochrane
#1 MeSH descriptor: [Adrenoleukodystrophu] this term only (12)
#2 adrenoleukodystrophy:ti,ab,kw (14)
#3 leukodsystroph*:ti (4)
#3 #1 or #2 or #3 (18)

All searches carried out on 17th August 2016

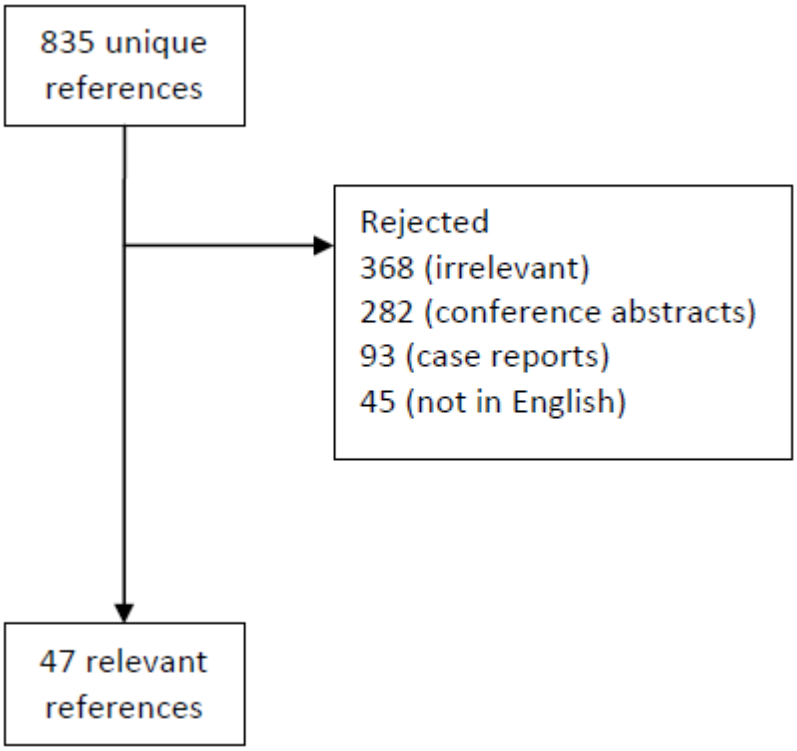
	The test/ screening	**Treatment**	**Combined**
Medline	325	110	425
Embase	628	289	845
Cochrane Library	---	---	9
Total			1,269

After automatic and manual de-duplication, 835 unique references were sifted for relevance to the review.

Inclusions and exclusions:

- Case reports, conference abstracts and publications not in English were excluded.
- Studies were included if the number of patients was 10 or more.

47 references were deemed to be relevant



These references are categorised as follows:

The test Validation studies (9) Non-systematic reviews (3)	12
The treatment **a. Hematopoietic stem cell transplant in adrenoleukodystrophy** Systematic review (1) Cohort studies (2) Retrospective cohort (1) Non-systematic review (1) • **In leukodystrophies** Retrospective cohort (1) Non-systematic reviews (7) • **Potential/emerging** Drug evaluation (1) Non-systematic reviews (10)	24
Screening • **For adrenoleukodystrophy** Systematic review (1) Guideline protocol (1)	11

Survey (1) Non-systematic reviews (2) • **Newborn screening** Non-systematic reviews (6)	
Total	**47**

The abstracts for the studies identified are listed below

The test (12)

Validation studies (9)

1. Tortorelli S, Turgeon CT, Gavrilov DK, Oglesbee D, Raymond KM, RinX-ALDo P, et al. Simultaneous Testing for 6 Lysosomal Storage Disorders and X-Adrenoleukodystrophy in Dried Blood Spots by Tandem Mass Spectrometry. Clinical Chemistry. 2016;20:20

BACKGROUND: Newborn screening for lysosomal storage disorders (LSD) has revealed that late-onset variants of these conditions are unexpectedly frequent and therefore may evade diagnosis. We developed an efficient and cost-effective multiplex assay to diagnose six LSDs and several peroxisomal disorders in patients presenting with diverse phenotypes at any age.

METHODS: Three 3-mm dried blood spot (DBS) punches were placed into individual microtiter plates. One disc was treated with a cocktail containing acid sphingomyelinase-specific substrate and internal standard (IS). To the second DBS we added a cocktail containing substrate and IS for beta-glucosidase, acid alpha-glucosidase, alpha-galactosidase A, galactocerebrosidase, and alpha-L-iduronidase. The third DBS was extracted with methanol containing d4-C26 lysophosphatidylcholine as IS and stored until the enzyme plates were combined and purified by liquid-liquid and solid-phase extraction. The extracts were evaporated, reconstituted with the extract from the lysophosphatidylcholine plate, and analyzed by flow injection tandem mass spectrometry.

RESULTS: Reference intervals were determined by analysis of 550 samples from healthy controls. DBS from 3 confirmed patients with 1 of the 6 LSDs (n = 33), X-adrenoleukodystrophy (n = 9), or a peroxisomal biogenesis disorder (n = 5), as well as carriers for Fabry disease (n = 17) and X-adrenoleukodystrophy (n = 5), were analyzed for assay validation. Prospective clinical testing of 578 samples revealed 25 patients affected with 1 of the detectable conditions.

CONCLUSIONS: Our flow injection tandem mass spectrometry approach is amenable to high-throughput population screening for Hurler disease, Gaucher disease, Niemann-Pick A/B disease, Pompe disease, Krabbe disease, Fabry disease, X-adrenoleukodystrophy, and peroxisomal biogenesis disorder in DBS.

2. Mashima R, Tanaka M, Sakai E, Nakajima H, Kumagai T, Kosuga M, et al. A selective detection of lysophosphatidylcholine in dried blood spots for diagnosis of adrenoleukodystrophy by LC-MS/MS. Molecular Genetics and Metabolism Reports. 2016;7:16-9

X-linked adrenoleukodystrophy (X-X-ALD) is a rare inherited metabolic disorder characterized by an impaired beta-oxidation of very long chain fatty acids in the peroxisomes. Recent studies have suggested that 1-hexacosanoyl-2-hydroxy-sn-glycero-3-phosphocholine (Lyso-PC 26:0) can be a sensitive biomarker for X-X-ALD. Although approximately 10-fold increase in the

concentration of Lyso-PC 26:0 in DBSs from X-X-ALD-affected individuals were reported, whether the carriers might be distinguished from the healthy controls remained unclear. To address this question, we have validated previously developed LC-MS/MS-based analytical procedures using QC DBS. We found that the recovery of Lyso-PC 26:0 from the QC DBSs was 73.6 +/- 0.3% when 2 muM of Lyso-PC 26:0 was spiked into the blood. Based on this result, the amounts of Lyso-PC 26:0 in the controls and X-ALD-affected individuals were 0.090 +/- 0.004 (n = 11) and 1.078 +/- 0.217 (n = 4) pmol/DBS, respectively. Interestingly, the concentration of Lyso-PC 26:0 in the carriers were 0.548 +/- 0.095 pmol/DBS (n = 3), indicating that the carriers and the healthy controls can be distinguished. These results suggest that LC-MS/MS-based technique can be used for the detection of asymptomatic carriers and X-X-ALD-affected subjects in the newborn screening.

3. Haynes CA, De Jesus VR. Simultaneous quantitation of hexacosanoyl lysophosphatidylcholine, amino acids, acylcarnitines, and succinylacetone during FIA-ESI-MS/MS analysis of dried blood spot extracts for newborn screening. Clinical Biochemistry. 2016;49(1):161-5

Objectives: The goal of this study was to include the quantitation of hexacosanoyl lysophosphatidylcholine, a biomarker for X-linked adrenoleukodystrophy and other peroxisomal disorders, in the routine extraction and analysis procedure used to quantitate amino acids, acylcarnitines, and succinylacetone during newborn screening. Criteria for the method included use of a single punch from a dried blood spot, one simple extraction of the punch, no high-performance liquid chromatography, and utilizing tandem mass spectrometry to quantitate the analytes. Design and methods: Dried blood spot punches were extracted with a methanolic solution of stable-isotope labeled internal standards, formic acid, and hydrazine, followed by flow injection analysis-electrospray ionization-tandem mass spectrometry. Results: Quantitation of amino acids, acylcarnitines, and hexacosanoyl lysophosphatidylcholine using this combined method was similar to results obtained using two separate methods. Conclusions: A single dried blood spot punch extracted by a rapid (45. min), simple procedure can be analyzed with high throughput (2. min per sample) to quantitate amino acids, acylcarnitines, succinylacetone, and hexacosanoyl lysophosphatidylcholine.

4. Turgeon CT, Moser AB, Morkrid L, Magera MJ, Gavrilov DK, Oglesbee D, et al. Streamlined determination of lysophosphatidylcholines in dried blood spots for newborn screening of X-linked adrenoleukodystrophy. Molecular Genetics & Metabolism. 2015;114(1):46-50

BACKGROUND: Pre-symptomatic hematopoietic stem cell transplantation is essential to achieve best possible outcomes for patients with the childhood cerebral form of X-linked adrenoleukodystrophy (X-X-ALD). We describe a high-throughput method for measurement of C20-C26 lysophosphatidylcholines (LPCs) and biochemical diagnosis of X-X-ALD using the same dried blood spots (DBS) routinely used for newborn screening.

METHODS: LPCs are extracted from 3-mm DBS punch with methanol containing an isotopically labeled LPC as internal standard. This extract is transferred to a 96-well plate, evaporated and then reconstituted in mobile phase for flow injection analysis tandem mass spectrometry (FIA-MS/MS) in selected reaction monitoring mode for measurement of four different LPCs (C20, C22, C24, C26) and the internal standard (d4-C26-LPC). Analysis time is 1.5min per sample. 4

RESULTS: The mean CVs from the intra- and inter-assay experiments for LPCs were 6.3-15.1% for C20-LPC, 4.4-18.6% for C22-LPC and 4.5-14.3% for C24-LPC. Limits of detection were determined for C20-LPC (LOD=0.03mug/mL), C22-LPC (0.03mug/mL), C24-LPC (0.03mug/mL) and C26-LPC (0.01mug/mL). Reference ranges were established from DBS of 130 newborns and 20 adults.

Samples of patients with X-X-ALD (n=16), peroxisomal biogenesis disorders (n=8), and X-X-ALD carriers (n=12) were analyzed blindly and all were correctly identified.

CONCLUSION: Analysis of LPC species by FIA-MS/MS is a fast, simple and reliable method to screen for X-X-ALD and other peroxisomal disorders in DBS. To maximize specificity, abnormal results can be verified by a 2nd tier assay using LC-MS/MS.

5. Theda C, Gibbons K, DeFor TE, Donohue PK, Golden WC, Kline AD, et al. Newborn screening for X-linked adrenoleukodystrophy: Further evidence high throughput screening is feasible. Molecular Genetics & Metabolism. 2014;111:55-7

X-linked adrenoleukodystrophy (X-ALD) is characterized by adrenal insufficiency and neurologic involvement with onset at variable ages. Plasma very long chain fatty acids are elevated in X-ALD; even in asymptomatic patients. We demonstrated previously that liquid chromatography tandem mass spectrometry measuring C26:0 lysophosphatidylcholine reliably identifies affected males. We prospectively applied this method to 4689 newborn blood spot samples; no false positives were observed. We show that high throughput neonatal screening for X-ALD is methodologically feasible.

6. Sandlers Y, Moser AB, Hubbard WC, Kratz LE, Jones RO, Raymond GV. Combined extraction of acyl carnitines and 26:0 lysophosphatidylcholine from dried blood spots: Prospective newborn screening for X-linked adrenoleukodystrophy. Molecular Genetics & Metabolism. 2012;105(3):416-20

X-linked adrenoleukodystrophy (X-X-ALD) is a severe genetic disorder that affects the nervous system, and the adrenal cortex. Newborn screening for X-X-ALD has been proposed to allow improved diagnosis along with prospective monitoring and treatment for this severe disorder. Newborn dried whole blood spot (DBS) 26:0 lysophosphatidyl choline was validated as a diagnostic marker for X-X-ALD and other peroxisomal disorders of peroxisomal beta-oxidation.In this study, we developed a new one step extraction procedure that simultaneously extracts acyl carnitines and the lysophosphatidyl cholines from DBS. Further analysis of these metabolites has been performed by two different high throughput LC-MS/MS methods. The 26:0 lysophosphatidyl choline levels in this study were consistent with previously published values and discriminate between healthy and abnormal profiles. There is a very minor modification to the original acyl carnitine extraction procedure and our data indicates that there is no significant effect on acyl carnitine levels in DBS.Our new method potentially can be complementary to the current newborn screening panel. It successfully combines the existing method for acyl carnitine analysis and 26:0 lysophosphatidyl choline that can be applied for prospective X-X-ALD newborn screening.

7. Haynes CA, De Jesus VR. Improved analysis of C26:0-lysophosphatidylcholine in dried-blood spots via negative ion mode HPLC-ESI-MS/MS for X-linked adrenoleukodystrophy newborn screening. Clinica Chimica Acta. 2012;413(15-16):1217-21

Background: X-linked adrenoleukodystrophy (X-X-ALD) is the most common human peroxisomal disorder, and is caused by mutations in the peroxisomal transmembrane X-ALD protein (X-ALDP, ABCD1). The biochemical defect associated with X-X-ALD is an accumulation of very long-chain fatty acids (VLCFA, e.g. C24:0 and C26:0), which has been shown to result in the accumulation of C26:0-lysophosphatidylcholine (C26:0-LPC). Methods: We describe the analysis of C26:0-LPC in dried-blood spots (DBS) using a rapid (30. min) and simple extraction procedure, isocratic HPLC

resolution of LPC, and structure-specific analysis via negative ion mode tandem mass spectrometry. Results: In putative normal DBS specimens from newborns (N = 223) C26:0-LPC was 0.09 +/- 0.03mumol/l whole blood, while in peroxisomal biogenesis disorder (including X-X-ALD) patients (N = 28) C26:0-LPC was 1.13 +/- 0.67mumol/l whole blood. Both multiple reaction monitoring and a neutral loss scan (225.1. Da) analysis of DBS were used to analyze LPC. Conclusions: Compared to a previous report of C26:0-LPC analysis in DBS, the method described here is simpler, faster, and more structure-specific for LPC with C26:0 acyl chains. © 2012.

8. Hubbard WC, Moser AB, Liu AC, Jones RO, Steinberg SJ, Lorey F, et al. Newborn screening for X-linked adrenoleukodystrophy (X-X-ALD): validation of a combined liquid chromatography-tandem mass spectrometric (LC-MS/MS) method. Molecular Genetics & Metabolism. 2009;97(3):212-20

Newborn screening for X-linked adrenoleukodystrophy (X-X-ALD) has until now been limited in implementation because of the lack of an accepted standard methodology. We have previously reported a technique using LC-MS/MS analysis that could provide the basis for screening of newborns for X-X-ALD. The target analyte diagnostic for X-X-ALD and other peroxisomal disorders of peroxisomal beta-oxidation is 1-hexacosanoyl-2-lyso-sn-3-glycero-phosphorylcholine (26:0-lyso-PC). We report here the validation of the analytical method using an authentic standard of the target compound. The method possesses sensitivity of <1.0fmole injected on column with a correlation coefficient (R(2)) of 0.9987. A tetradeuterated analog of 26:0-lyso-PC served as the internal standard. The sensitivity of this clinical method was confirmed using 17 newborn samples of individuals with peroxisomal disorders retrieved from state newborn screening programs. These samples were run masked with over 1000 newborn samples. All affected individuals were identified with one exception. One sample which was retrieved as an affected did not have the biochemical or genetic abnormality of X-X-ALD and thus is considered an error in sample identity. These studies clearly show that the method is highly sensitive and accurate in identifying individuals with a defect in peroxisomal beta-oxidation such as X-X-ALD.

9. Hubbard WC, Moser AB, Tortorelli S, Liu A, Jones D, Moser H. Combined liquid chromatography-Tandem mass spectrometry as an analytical method for high throughput screening for X-linked adrenoleukodystrophy and other peroxisomal disorders: Preliminary findings. Molecular Genetics & Metabolism. 2006;89(1-2):185-7

Utilizing combined liquid chromatography-tandem mass spectrometry (LC-MS/MS) as the analytical method, we have demonstrated a ten to sixtyfold excess of lysophosphatidyl choline containing hexacosanoic acid (26:0) in dried blood spots on a filter paper matrix from 25 male patients with X-linked adrenoleukodystrophy and nine patients with peroxisome biogenesis disorders compared to 19 controls. There was no overlap between normal subjects versus affected subjects.

Non-systematic reviews (3)

1. Klouwer FCC, Huffnagel IC, Ferdinandusse S, Waterham HR, Wanders RJA, Engelen M, et al. Clinical and Biochemical Pitfalls in the Diagnosis of Peroxisomal Disorders. Neuropediatrics. 2016;47(4):205-20

Peroxisomal disorders are a heterogeneous group of genetic metabolic disorders, caused by a defect in peroxisome biogenesis or a deficiency of a single peroxisomal enzyme. The peroxisomal disorders include the Zellweger spectrum disorders, the rhizomelic

chondrodysplasia punctata spectrum disorders, X-linked adrenoleukodystrophy, and multiple single enzyme deficiencies. There are several core phenotypes caused by peroxisomal dysfunction that clinicians can recognize. The diagnosis is suggested by biochemical testing in blood and urine and confirmed by functional assays in cultured skin fibroblasts, followed by mutation analysis. This review describes the phenotype of the main peroxisomal disorders and possible pitfalls in (laboratory) diagnosis to aid clinicians in the recognition of this group of diseases.

2. Parikh S, Bernard G, Leventer RJ, van der Knaap MS, van Hove J, Pizzino A, et al. A clinical approach to the diagnosis of patients with leukodystrophies and genetic leukoencephelopathies. Molecular Genetics & Metabolism. 2015;114(4):501-15

Leukodystrophies (LD) and genetic leukoencephalopathies (gLE) are disorders that result in white matter abnormalities in the central nervous system (CNS). Magnetic resonance (MR) imaging (MRI) has dramatically improved and systematized the diagnosis of LDs and gLEs, and in combination with specific clinical features, such as Addison's disease in Adrenoleukodystrophy or hypodontia in Pol-III related or 4H leukodystrophy, can often resolve a case with a minimum of testing. The diagnostic odyssey for the majority LD and gLE patients, however, remains extensive--many patients will wait nearly a decade for a definitive diagnosis and at least half will remain unresolved. The combination of MRI, careful clinical evaluation and next generation genetic sequencing holds promise for both expediting the diagnostic process and dramatically reducing the number of unresolved cases. Here we present a workflow detailing the Global Leukodystrophy Initiative (GLIA) consensus recommendations for an approach to clinical diagnosis, including salient clinical features suggesting a specific diagnosis, neuroimaging features and molecular genetic testing. We also discuss recommendations on the use of broad-spectrum next-generation sequencing in instances of ambiguous MRI or clinical findings. We conclude with a proposal for systematic trials of genome-wide agnostic testing as a first line diagnostic in LDs and gLEs given the increasing number of genes associated with these disorders.Copyright Published by Elsevier Inc.

3. Moser HW, Mahmood A, Raymond GV. X-linked adrenoleukodystrophy. Nature Clinical Practice Neurology. 2007;3(3):140-51

X-linked adrenoleukodystrophy (X-X-ALD) is caused by a defect in the gene ABCD1, which maps to Xq28 and codes for a peroxisomal membrane protein that is a member of the ATP-binding cassette transporter superfamily. X-X-ALD is panethnic and affects approximately 1:20,000 males. Phenotypes include the rapidly progressive childhood, adolescent, and adult cerebral forms; adrenomyeloneuropathy, which presents as slowly progressive paraparesis in adults; and Addison disease without neurologic manifestations. These phenotypes are frequently misdiagnosed, respectively, as attention-deficit hyperactivity disorder (ADHD), multiple sclerosis, or idiopathic Addison disease. Approximately 50% of female carriers develop a spastic paraparesis secondary to myelopathic changes similar to adrenomyeloneuropathy. Assays of very long chain fatty acids in plasma, cultured chorion villus cells and amniocytes, and mutation analysis permit presymptomatic and prenatal diagnosis, as well as carrier identification. The timely use of these assays is essential for genetic counseling and therapy. Early diagnosis and treatment can prevent overt Addison disease, and significantly reduce the frequency of the severe childhood cerebral phenotype. A promising new method for mass newborn screening has been developed, the implementation of which will have a profound effect on the diagnosis and therapy of X-X-ALD.

The treatment (24)

• Hematopoietic stem cell transplant in adrenoleukodystrophy

Systematic review (1)

1. Musolino PL, Lund TC, Pan J, Escolar ML, Paker AM, Duncan CN, et al. Hematopoietic stem cell transplantation in the leukodystrophies: A systematic review of the literature. Neuropediatrics. 2014;45(3):169-74

Objective The objective of this study is to systematically review the literature on worldwide numbers of leukodystrophy patients undergoing hematopoietic stem cell transplantation (HSCT) as well as the safety and efficacy of the procedure in this patient population. Materials and Methods A PubMed and EMBASE search up to June 2012 was conducted with a manual search of references from relevant articles. Selected studies were evaluated using internationally accepted criteria. The effect estimates of HSCT upon survival in early-stage disease versus late-stage disease were compared. Results One hundred and fifty-two studies qualified for inclusion and reported on a total of 689 patients. Study quality ranged from poor to good; no study was rated excellent. Small sample sizes limited most studies. Meta-analysis in a subset of larger studies indicates that transplantation in earlier stages of disease fairs better than in the late stages. Beyond survival, little longitudinal data on functional outcome is reported and neurological outcome is sparse. Conclusion Further studies are needed to determine the neurological outcome following HSCT in the leukodystrophies. HSCT in the early stages of cerebral disease is still recommended for select leukodystrophies. © 2014 Georg Thieme Verlag KG Stuttgart New York.

Cohort studies (2)

1. Miller WP, Mantovani LF, Muzic J, Rykken JB, Gawande RS, Lund TC, et al. Intensity of MRI gadolinium enhancement in cerebral adrenoleukodystrophy: A biomarker for inflammation and predictor of outcome following transplantation in higher risk patients. AJNR American Journal of Neuroradiology. 2016;37(2):367-72

Background and Purpose: Outcomes following hematopoietic stem cell transplantation for higher risk childhood-onset cerebral adrenoleukodystrophy are variable. We explored whether a brain MR imaging gadolinium intensity scoring system improves prediction of neurologic outcome. Materials and Methods: We developed a 4-point scale of gadolinium intensity relative to the choroid plexus: 0=no enhancement; 1 = hypointense; 2 = isointense; 3 = hyperintense. The interobserver concordance of the scale was assessed on 30 randomly chosen studies. Scores were generated for 64 evaluable patients and compared with CSF chitotriosidase levels, a known inflammatory marker correlating with outcomes following transplantation. 7

For 25 evaluable higher risk patients (Loes =10), the gadolinium intensity score was compared with longer term posttransplantation clinical change. RESULTS: The gadolinium intensity scoring system showed good interobserver reproducibility (kappa=0.72). Of 64 evaluable boys, the score positively correlated with average concomitant CSF chitotriosidase activity in nanograms/milliliter/hour: 0: 2717, n = 5; 1: 3218, n = 13; 2: 6497, n = 23; and 3: 12,030, n = 23 (P kappa.01). For 25 evaluable higher risk patients, more intense pretransplantation brain MR imaging gadolinium enhancement predicted greater average loss on the adrenoleukodystrophy neurologic function scale following transplantation: 0/1: adrenoleukodystrophy neurologic function scale score difference = 4.3, n = 7; 2/3: adrenoleukodystrophy neurologic function scale score difference = 10.4, n = 18 (P =.05). Conclusions: Gadolinium enhancement intensity on

brain MR imaging can be scored simply and reproducibly for cerebral adrenoleukodystrophy. The enhancement score significantly correlates with chitotriosidase. In boys with higher risk cerebral disease (Loes =10), the enhancement score itself predicts neurologic outcome following treatment. Such data may help guide treatment decisions for clinicians and families.

2. McKinney AM, Benson J, Nascene DR, Eisengart J, Salmela MB, Loes DJ, et al. Childhood Cerebral Adrenoleukodystrophy: MR Perfusion Measurements and Their Use in Predicting Clinical Outcome after Hematopoietic Stem Cell Transplantation. AJNR American Journal of Neuroradiology. 2016;14:14

BACKGROUND AND PURPOSE: MR perfusion has shown abnormalities of affectedWMin cerebral X-linked adrenoleukodystrophy, but serial data is needed to explore the import of such findings after hematopoietic stem cell transplantation. Our aim was to prospectively measure MR perfusion parameters in patients with cerebral adrenoleukodystrophy pre- and post-hematopoietic stem cell transplantation, and to correlate those measurements with clinical outcome.

MATERIALS AND METHODS: Ten patients with cerebral adrenoleukodystrophy prospectively underwent DSC-MR perfusion imaging at <45 days pre- (baseline), 30-60 days post-, and 1 year post-hematopoietic stem cell transplantation. MR perfusion measurements in the 10 patients and 8 controls were obtained from the parieto-occipital WM, splenium of the corpus callosum, leading enhancing edge, and normal-appearing frontal white matter. MR imaging severity scores and clinical neurologic function and neurocognitive scores were also obtained. MR perfusion values were analyzed in the patients with cerebral adrenoleukodystrophy at each time point and compared with those in controls. Correlations were calculated between the pre-hematopoietic stem cell transplantation MR perfusion values and 1-year clinical scores, with P value adjustment for multiple comparisons.

RESULTS: At baseline in patients with cerebral adrenoleukodystrophy, both relative CBV and relative CBF within the splenium of the corpus callosum and parieto-occipital WM significantly differed from those in controls (P = .005-.031) and remained so 1 year post-hematopoietic stem cell transplantation (P = .003-.005). Meanwhile, no MR perfusion parameter within the leading enhancing edge differed significantly from that in controls at baseline or at 1 year (P = .074-.999) or significantly changed by 1 year post-hematopoietic stem cell transplantation (P = .142-.887). Baseline Loes scores correlated with 1-year clinical neurologic function (r =0.813, P < .0001), while splenium of the corpus callosum relative CBV also significantly correlated with 1-year neurologic function scale and the neurocognitive full-scale intelligence quotient and performance intelligence quotient scores (r = -0.730-0.815, P = .007-.038).

CONCLUSIONS: Leading enhancing edge measurements likely remain normal post-hematopoietic stem cell transplantation in cerebral adrenoleukodystrophy, suggesting local disease stabilization. Meanwhile, parieto-occipital WM and splenium of the corpus callosum relative CBV and relative CBF values worsened; this change signified irreversible injury. Baseline splenium of the corpus callosum relative CBV may predict clinical outcomes following hematopoietic stem cell transplantation.

Retrospective cohort (1)

1. Petryk A, Polgreen LE, Chahla S, Miller W, Orchard PJ. No evidence for the reversal of adrenal failure after hematopoietic cell transplantation in X-linked adrenoleukodystrophy. Bone Marrow Transplantation. 2012;47(10):1377-8

Full text: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4547590/

Non-systematic review (1)

1. Engelen M, Kemp S, Poll-The BT. X-linked adrenoleukodystrophy: Pathogenesis and treatment. Current Neurology and Neuroscience Reports. 2014;14(10):1-8

X-linked adrenoleukodystrophy (X-ALD) is a puzzling inborn error of metabolism with a strikingly heterogeneous clinical spectrum. All patients have mutations in the ABCD1 gene and accumulate very long chain fatty acids in all tissues. Virtually all male X-ALD patients develop adrenocortical insufficiency in childhood and progressive myelopathy and peripheral neuropathy in adulthood. A subset of male patients, however, develops a fatal cerebral demyelinating disease, cerebral adrenoleukodystrophy. Female patients also develop progressive myelopathy and peripheral neuropathy, but generally at a later age than males. They only very rarely develop adrenocortical insufficiency or cerebral adrenoleukodystrophy. This review proposes to simplify the classification of the clinical spectrum of X-ALD and reviews the largely unresolved pathophysiological mechanisms and the current treatment options.

- **In leukodystrophies**

Retrospective cohort (1)

1. Mitchell R, Nivison-Smith I, Anazodo A, Tiedemann K, Shaw PJ, Teague L, et al. Outcomes of haematopoietic stem cell transplantation for inherited metabolic disorders: a report from the Australian and New Zealand Children's Haematology Oncology Group and the Australasian Bone Marrow Transplant Recipient Registry. Pediatric Transplantation. 2013;17(6):582-8

We report a retrospective analysis of 53 haematopoietic stem cell transplants for inherited metabolic disorders performed at ANZCHOG transplant centres between 1992 and 2008. Indications for transplant included Hurler syndrome, X-ALD, and MLD. The majority of transplants utilized unrelated donor stem cells (66%) with 65% of those being unrelated cord blood. Conditioning therapy was largely myeloablative, with Bu plus another cytotoxic agent used in 89% of recipients. Primary graft failure was rare, occurring in three patients, all of whom remain long-term survivors following the second transplant. The CI of grade II-IV and grade III-IV acute GVHD at day +100 was 39% and 14%, respectively. Chronic GVHD occurred in 17% of recipients. TRM was 12% at day +100 and 19% at one yr post-transplant. OS at five yr was 78% for the cohort, 73% for patients with X-ALD and 83% for patients with Hurler syndrome. There was no statistically significant difference in overall survival between unrelated marrow and unrelated cord blood donor groups. The development of interstitial pneumonitis was an independent variable shown to significantly impact on TRM and OS. In summary, we report a large cohort of patients with inherited metabolic disorders with excellent survival post-allogeneic transplant. Copyright © 2013 John Wiley & Sons A/S.

Non-systematic reviews (7)

1. Guilcher GMT. Hematopoietic stem cell transplantation in children and adolescents. Pediatrics in Review. 2016;37(4):135-44

Hematopoietic stem cell transplantation (HSCT) refers to the infusion of either allogeneic or autologous hematopoietic stem cells. * Newer techniques to reduce the risk of complications are expanding the applicability of HSCT. * Nonmalignant disease indications for HSCT are increasing. * Observational and cohort studies (level C evidence) indicate that acute and long-term toxicities remain an important consideration for patients, families, and clinicians in making a recommendation for HSCT and warrant lifelong surveillance. (11)(12)(13)(21) * Based on overwhelming evidence from observational studies (level B evidence), graft-versus-host disease can be a significant cause of morbidity and mortality in allogeneic HSCT. (22)(24) * General pediatricians and subspecialists should be aware of evolving and newly established nonmalignant indications for HSCT to make appropriate referrals (level D evidence). (28)(29)(30).

2. Helman G, Van Haren K, Bonkowsky JL, Bernard G, Pizzino A, Braverman N, et al. Disease specific therapies in leukodystrophies and leukoencephalopathies. Molecular Genetics and Metabolism. 2015;114(4):527-36

Leukodystrophies are a heterogeneous, often progressive group of disorders manifesting a wide range of symptoms and complications. Most of these disorders have historically had no etiologic or disease specific therapeutic approaches. Recently, a greater understanding of the pathologic mechanisms associated with leukodystrophies has allowed clinicians and researchers to prioritize treatment strategies and advance research in therapies for specific disorders, some of which are on the verge of pilot or Phase I/II clinical trials. This shifts the care of leukodystrophy patients from the management of the complex array of symptoms and sequelae alone to targeted therapeutics. The unmet needs of leukodystrophy patients still remain an 9

overwhelming burden. While the overwhelming consensus is that these disorders collectively are symptomatically treatable, leukodystrophy patients are in need of advanced therapies and if possible, a cure.

3. Boelens JJ, Orchard PJ, Wynn RF. Transplantation in inborn errors of metabolism: Current considerations and future perspectives. British Journal of Haematology. 2014;167(3):293-303

Inborn errors of metabolism (IEM) comprise an assorted group of inherited diseases, some of which are due to disordered lysosomal or peroxisomal function and some of which might be improved following haemopoietic cell transplantation (HCT). In these disorders, the onset in infancy or early childhood is typically accompanied by rapid deterioration, resulting in early death in the more severe phenotypes. Timely diagnosis and immediate referral to an IEM specialist are essential steps in optimal management. Treatment recommendations are based on the diagnosis, its phenotype, rate of progression, prior extent of disease, family values and expectations and the risks and benefits associated with available therapies, including HCT. International collaborative efforts are of utmost importance in determining outcomes of therapy for these rare diseases, and have improved those outcomes significantly over recent decades. This discussion focusses on HCT in IEM, providing an international perspective on progress, limitations, and future directions.

4. Orchard P, Boelens JJ, Raymond G. Multi-Institutional Assessments of Transplantation for Metabolic Disorders. Biology of Blood and Marrow Transplantation. 2013;19(1 SUPPL.):S58-S63

5. Miranda CO, Brites P, Sousa MM, Teixeira CA. Advances and pitfalls of cell therapy in metabolic leukodystrophies. Cell Transplantation. 2013;22(2):189-204

Leukodystrophies are a group of disorders characterized by myelin dysfunction, either at the level of myelin formation or maintenance, that affect the central nervous system (CNS) and also in some cases, to a lesser extent, the peripheral nervous system (PNS). Although these genetic-based disorders are generally rare, all together they have a significant impact in the society, with an estimated overall incidence of 1 in 7,663 live births. Currently, there is no cure for leukodystrophies, and the development of effective treatments remains challenging. Not only leukodystrophies generally progress very fast, but also most are multifocal needing the simultaneous targeting at multiple sites. Moreover, as the CNS is affected, the blood-brain barrier (BBB) limits the efficacy of treatment. Recently, interest on cell therapy has increased, and the leukodystrophies for which metabolic correction is needed have become first-choice candidates for cell-based clinical trials. In this review, we present and discuss the available cell transplantation therapies in metabolic leukodystrophies including fucosidosis, X-linked adrenoleukodystrophy, metachromatic leukodystrophy, Canavan disease, and Krabbe's disease. We will discuss the latest advances of cell therapy and its pitfalls in this group of disorders, taking into account, among others, the limitations imposed by reduced cell migration in multifocal conditions, the need to achieve corrective enzyme threshold levels, and the growing awareness that not only myelin but also the associated axonopathy needs to be targeted in some leukodystrophies. © 2013 Cognizant Comm. Corp.

6. Weber SL, Segal S, Packman W. Inborn errors of metabolism: Psychosocial challenges and proposed family systems model of intervention. Molecular Genetics and Metabolism. 2012;105(4):537-41

Inborn errors of metabolism result in psychosocial crises that challenge individual and familial modes of functioning across the life cycle. Increased stress, mood disorders, interpersonal challenges, decreased quality of life, and grief reactions are all common for patients and their families. To effectively care for these patients, a holistic approach to their care, which incorporates their social context, is essential. Patients and their families need support as they focus on immediate practical demands, grieve over illness-related losses, and reorient future expectations. A family systems based model provides a flexible and individualized approach to care that allows for optimal psychosocial adjustment throughout the disease process. © 2012.

7. Perlman SJ, Mar S. Leukodystrophies. Neurodegenerative Diseases. 2012;Advances in Experimental Medicine and Biology. 724:154-71

Leukodystrophies comprise a broad group of progressive, inherited disorders affecting mainly myelin. They often present after a variable period of normalcy with a variety of neurologic problems. Though the ultimate diagnosis is not found in many patients with leukodystrophies, distinctive features unique to them aid in diagnosis, treatment and prognostication. The clinical characteristics, etiologies, diagnostic testing and treatment options are reviewed in detail for some of the major leukodystrophies: X-linked adrenoleukodystrophy, Krabbe disease, metachromatic leukodystrophy, Pelizaeus-Merzbacher disease, Alexander disease, Canavan disease, megalencephalic leukoencephalopathy with subcortical cysts and vanishing white matter disease. © 2012 Landes Bioscience and Springer Science+Business Media.

• Potential/emerging

Drug evaluation (1)

1. Jang J, Yoo JE, Lee JA, Lee DR, Kim JY, Huh YJ, et al. Disease-specific induced pluripotent stem cells: A platform for human disease modeling and drug discovery. Experimental and Molecular Medicine. 2012;44(3):202-13

The generation of disease-specific induced pluripotent stem cell (iPSC) lines from patients with incurable diseases is a promising approach for studying disease mechanisms and drug screening. Such innovation enables to obtain autologous cell sources in regenerative medicine. Herein, we report the generation and characterization of iPSCs from fibroblasts of patients with sporadic or familial diseases, including Parkinson's disease (PD), Alzheimer's disease (AD), juvenile-onset, type I diabetes mellitus (JDM), and Duchenne type muscular dystrophy (DMD), as well as from normal human fibroblasts (WT). As an example to modeling disease using disease-specific iPSCs, we also discuss the previously established childhood cerebral adrenoleukodystrophy (CCCALD)- and adrenomyeloneuropathy (AMN)-iPSCs by our group. Through DNA fingerprinting analysis, the origins of generated disease-specific iPSC lines were identified. Each iPSC line exhibited an intense alkaline phosphatase activity, expression of pluripotent markers, and the potential to differentiate into all three embryonic germ layers: the ectoderm, endoderm, and mesoderm. Expression of endogenous pluripotent markers and downregulation of retrovirus-delivered transgenes [OCT4 (POU5F1), SOX2, KLF4, and c-MYC] were observed in the generated iPSCs. Collectively, our results demonstrated that disease-specific iPSC lines characteristically resembled hESC lines. Furthermore, we were able to differentiate PD-iPSCs, one of the disease-specific-iPSC lines we generated, into dopaminergic (DA) neurons, the cell type mostly affected by PD. These PD-specific DA neurons along with other examples of cell models derived from disease-specific iPSCs would provide a powerful platform for examining the pathophysiology of relevant diseases at the cellular and molecular levels and for developing new drugs and therapeutic regimens. © 2012 by the Korean Society for Biochemistry and Molecular Biology.

Non-systematic reviews (10)

1. Pujol A. Novel therapeutic targets and drug candidates for modifying disease progression in adrenoleukodystrophy. Endocrine Development. 2016;30:147-60

X-linked adrenoleukodystrophy (X-X-ALD) is the most frequent inherited monogenic demyelinating disease. It is often lethal and currently lacks a satisfactory therapy. The disease is caused by loss of function of the ABCD1 gene, a peroxisomal ATP-binding cassette transporter, resulting in the accumulation of very-long-chain fatty acids (VLCFA) in organs and plasma. Recent findings on pathomechanisms of the peroxisomal neurometabolic disease X-X-ALD have provided important clues on therapeutic targets. Here we describe the impact of chronic redox imbalance caused by the excess VLCFA on mitochondrial biogenesis and respiration, and explore the consequences on the protein quality control systems essential for cell survival, such as the proteasome and autophagic flux. Defective proteostasis, together with mitochondrial malfunction, is a hallmark of the most prevalent neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, and of the aging process. Thus, we discuss molecular targets and emerging treatment options that may be common to both multifactorial neurodegenerative disorders and X-X-ALD. New-generation antioxidants, some of them mitochondrial targeted, mitochondrial biogenesis boosters such as pioglitazone and resveratrol, and the mTOR inhibitor temsirolimus hold promise as disease-modifying therapies.

2. Cefalo MG, Carai A, Miele E, Po A, Ferretti E, Mastronuzzi A, et al. Human iPSC for Therapeutic Approaches to the Nervous System: Present and Future Applications. Stem Cells International. 2016;2016 (no pagination)(4869071)

Many central nervous system (CNS) diseases including stroke, spinal cord injury (SCI), and brain tumors are a significant cause of worldwide morbidity/mortality and yet do not have satisfying treatments. Cell-based therapy to restore lost function or to carry new therapeutic genes is a promising new therapeutic approach, particularly after human iPSCs became available. However, efficient generation of footprint-free and xeno-free human iPSC is a prerequisite for their clinical use. In this paper, we will first summarize the current methodology to obtain footprint- and xeno-free human iPSC. We will then review the current iPSC applications in therapeutic approaches for CNS regeneration and their use as vectors to carry proapoptotic genes for brain tumors and review their applications for modelling of neurological diseases and formulating new therapeutic approaches. Available results will be summarized and compared. Finally, we will discuss current limitations precluding iPSC from being used on large scale for clinical applications and provide an overview of future areas of improvement. In conclusion, significant progress has occurred in deriving iPSC suitable for clinical use in the field of neurological diseases. Current efforts to overcome technical challenges, including reducing labour and cost, will hopefully expedite the integration of this technology in the clinical setting.

3. Aubourg P. Gene therapy for leukodystrophy: Progress, challenges and opportunities. Expert Opinion on Orphan Drugs. 2016;4(4):359-67

Introduction: Leukodystrophies have long been considered untreatable. Recently, autologous transplantation of hematopoietic stem cells genetically corrected with lentiviral vectors showed clear benefit in X-linked adrenoleukodystrophy and metachromatic leukodystrophy, either at a very early stage of disease or in pre-symptomatic patients. These first successes still need, however, to be extended to a larger number of patients.Areas covered: The rationale, preclinical proof-of-concept research, and outcome of hematopoietic stem cell gene therapy trials with lentiviral vector in X-linked adrenoleukodystrophy and metachromatic leukodystrophy are provided. Potential application of this gene therapy strategy to Krabbe disease (globoid cell leukodystrophy) is discussed. Progresses in adeno-associated virus vectorology and vector delivery to target therapeutic gene in oligodendrocytes and astrocytes for many other leukodystrophies are explained.Expert Opinion: Each leukodystrophy will require a specific gene therapy strategy, from vector design to viral vector delivery and window of therapeutic intervention. Yet, the very encouraging results obtained in two leukodystrophies, as well as the successes of gene therapy in various severe inherited diseases of the blood, eye and immune systems open a real hope that, in a relatively near future, several leukodystrophies will become treatable by gene therapy.

4. Aubourg P. Gene therapy for rare central nervous system diseases comes to age. Endocrine Development. 2016;30:141-6

Gene therapy for rare inherited neurologic diseases has entered the clinics. One strategy relies upon the replacement of brain microglia using hematopoietic stem cell gene therapy with lentiviral vectors. Therapeutic success using this approach has been obtained in X-linked adrenoleukodystrophy and metachromatic leukodystrophy. The other strategy relies upon the intracerebral administration of adeno-associated virus vectors encoding lysosomal enzymes. Therapeutic trials are ongoing in Batten's disease, metachromatic leukodystrophy, and Sanfilippo type A and B diseases.

5. Helman G, Van Haren K, Escolar ML, Vanderver A. Emerging Treatments for Pediatric Leukodystrophies. Pediatric Clinics of North America. 2015;62(3):649-66

The leukodystrophies are a heterogeneous group of inherited disorders with broad clinical manifestations and variable pathologic mechanisms. Improved diagnostic methods have allowed identification of the underlying cause of these diseases, facilitating identification of their pathologic mechanisms. Clinicians are now able to prioritize treatment strategies and advance research in therapies for specific disorders. Although only a few of these disorders have well-established treatments or therapies, a number are on the verge of clinical trials. As investigators are able to shift care from symptomatic management of disorders to targeted therapeutics, the unmet therapeutic needs could be reduced for these patients.

6. Phillips MI, Burns AB. The emergence of gene therapy for rare diseases. Expert Opinion on Orphan Drugs. 2014;2(11):1197-209

Introduction: Gene therapy is emerging from a checkered past to success in several diseases, most of them are rare. Currently, the goal of most gene therapy trials is gene replacement, gene correction or knockdown of gene products for monogenetic diseases via viral vectors that cure or slow disease progression. The definition can be broadened to include nonviral vectors, such as antisense oligonucleotides and small interfering RNA, to control too much gene expression. Gene modification of stem cells is also a gene therapy and works by providing cells with corrected genes. Areas covered: Diseases that have been shown to benefit from gene therapy in clinical trials include adenosine deaminase-severe combined immunodeficiency (ADA-SCID), X-linked SCID, homozygous familial hypercholesterolemia, lipoprotein lipase deficiency, Leber's congenital amaurosis and adrenoleukodystrophy. There is reason for the optimism that gene therapies will eventually provide treatments and perhaps cures for disease that currently have no or inadequate therapies. Many rare diseases may benefit. Expert opinion: This resurgence of hope for gene therapy applications is based on several recent positive approvals and ongoing trials for gene therapy with positive results. Here we consider orphan drugs and orphan status/designations that are currently being tested. Keywords: adeno-associated virus, antisense, exon skipping, FDA designations, gene therapy, Lentivirus, rare diseases

7. Bigger BW, Wynn RF. Novel approaches and mechanisms in hematopoietic stem cell gene therapy. Discovery Medicine. 2014;17(94)

Hematopoietic stem cell gene therapy is one of the most exciting clinical tools to emerge from the gene therapy stable. This technology combines the expansion capability of hematopoietic stem cells, capable of replacing the entire blood and immune system of an individual, with the capacity for long-term replacement of one or more gene copies using integrating gene therapy vectors. Hematopoietic stem cell gene therapy benefits significantly from the pre-existing experience of standard blood and marrow transplantation, whilst at the same time having the capacity to deliver a safer and more effective therapy to a wider range of diseases. In this review we summarize the potential of hematopoietic stem cell gene therapy to expand the scope of hematopoietic stem cell transplantation, including the evolution of vector delivery systems and the success and failures of current clinical experience with this treatment. In particular we deal with the incidence of vector mediated transformation in patients and the steps that have been taken to minimize this risk. Finally we discuss the innovations in preclinical development that are likely to drive the future of this field, including the expansion to many more genetic diseases, particularly those affecting the brain. © Discovery Medicine.

8. Yu DX, Marchetto MC, Gage FH. Therapeutic translation of iPSCs for treating neurological disease. Cell Stem Cell. 2013;12(6):678-88

Somatic cellular reprogramming is a fast-paced and evolving field that is changing the way scientists approach neurological diseases. For the first time in the history of neuroscience, it is feasible to study the behavior of live neurons from patients with neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, and neuropsychiatric diseases, such as autism and schizophrenia. In this Perspective, we will discuss reprogramming technology in the context of its potential use for modeling and treating neurological and psychiatric diseases and will highlight areas of caution and opportunities for improvement. © 2013 Elsevier Inc.

9. Wang H, Doering LC. Induced pluripotent stem cells to model and treat neurogenetic disorDers. Neural Plasticity. 2012;2012 (no pagination)(346053)

Remarkable adVances in cellular reprogramming have made it possible to generate pluripotent stem cells from somatic cells, such as fibroblasts obtained from human skin biopsies. As a result, human diseases can now be investigated in releVant cell populations Derived from induced pluripotent stem cells (iPSCs) of patients. The rapid growth of iPSC technology has turned these cells into multipurpose basic and clinical research tools. In this paper, we highlight the roles of iPSC technology that are helping us to unDerstand and potentially treat neurological diseases. Recent studies using iPSCs to model various neurogenetic disorDers are summarized, and we discuss the therapeutic implications of iPSCs, including drug screening and cell therapy for neurogenetic disorDers. Although iPSCs have been used in animal models with promising results to treat neurogenetic disorDers, there are still many issues associated with reprogramming that must be addressed before iPSC technology can be fully exploited with translation to the clinic. © Copyright 2012 Hansen Wang and Laurie C. Doering.

10. Sng J, Lufkin T. Emerging stem cell therapies: Treatment, safety, and biology. Stem Cells International. 2012;(no pagination)(521343)

Stem cells are the fundamental building blocks of life and contribute to the genesis and development of all higher organisms. The discovery of adult stem cells has led to an ongoing revolution of therapeutic and regenerative medicine and the proposal of novel therapies for previously terminal conditions. Hematopoietic stem cell transplantation was the first example of a successful stem cell therapy and is widely utilized for treating various diseases including adult T-cell leukemia-lymphoma and multiple myeloma. The autologous

transplantation of mesenchymal stem cells is increasingly employed to catalyze the repair of mesenchymal tissue and others, including the lung and heart, and utilized in treating various conditions such as stroke, multiple sclerosis, and diabetes. There is also increasing interest in the therapeutic potential of other adult stem cells such as neural, mammary, intestinal, inner ear, and testicular stem cells. The discovery of induced pluripotent stem cells has led to an improved understanding of the underlying epigenetic keys of pluripotency and carcinogenesis. More in-depth studies of these epigenetic differences and the physiological changes that they effect will lead to the design of safer and more targeted therapies. © 2012 Joel Sng and Thomas Lufkin.

Screening (11)

• For adrenoleukodystrophy

Systematic review (1)

1. Kemper AR, Brosco J, Comeau AM, Green NS, Grosse SD, Jones E, et al. Newborn screening for X-linked adrenoleukodystrophy: evidence summary and advisory committee recommendation. Genetics in Medicine. 2016;23:23

The secretary of the US Department of Health and Human Services in February 2016 recommended that X-linked adrenoleukodystrophy (X-X-ALD) be added to the recommended uniform screening panel for state newborn screening programs. This decision was informed by data presented on the accuracy of screening from New York, the only state that currently offers X-X-ALD newborn screening, and published and unpublished data showing health benefits of earlier treatment (hematopoietic stem cell transplantation and adrenal hormone replacement therapy) for the childhood cerebral form of X-X-ALD. X-X-ALD newborn screening also identifies individuals with later-onset disease, but poor genotype-phenotype correlation makes predicting health outcomes difficult and might increase the risk of unnecessary treatment. Few data are available regarding the harms of screening and presymptomatic identification. Significant challenges exist for implementing comprehensive X-X-ALD newborn screening, including incorporation of the test, coordinating follow-up diagnostic and treatment care, and coordination of extended family testing after case identification.Genet Med advance online publication 23 June 2016Genetics in Medicine (2016); doi:10.1038/gim.2016.68.

Guideline protocol (1)

1. Vogel BH, Bradley SE, Adams DJ, D'Aco K, Erbe RW, Fong C, et al. Newborn screening for X-linked adrenoleukodystrophy in New York State: Diagnostic protocol, surveillance protocol and treatment guidelines. Molecular Genetics and Metabolism. 2015;114(4):599-603

Purpose: To describe a diagnostic protocol, surveillance and treatment guidelines, genetic counseling considerations and long-term follow-up data elements developed in preparation for X-linked adrenoleukodystrophy (X-X-ALD) newborn screening in New York State. Methods: A group including the director from each regional NYS inherited metabolic disorder center, personnel from the NYS Newborn Screening Program, and others prepared a follow-up plan for X-X-ALD NBS. Over the months preceding the start of screening, a series of conference calls took place to develop and refine a complete newborn screening system from initial positive screen results to long-term follow-up. Results: A diagnostic protocol was developed to determine for each newborn with a positive screen whether the final diagnosis is X-X-ALD, carrier of X-X-ALD, Zellweger spectrum disorder, acyl CoA oxidase deficiency or D-bifunctional protein deficiency. For asymptomatic males with X-X-ALD, surveillance protocols were developed for use at the time of diagnosis, during childhood and during adulthood. Considerations for timing of treatment of adrenal and cerebral disease were developed. Conclusion: Because New York was the first newborn screening laboratory to include X-X-ALD on its panel, and symptoms may not develop for years, long-term follow-up is needed to evaluate the presented guidelines.

Survey (1)

1. Schaller J, Moser H, Begleiter ML, Edwards J. Attitudes of families affected by adrenoleukodystrophy toward prenatal diagnosis, presymptomatic and carrier testing, and newborn screening. Genetic Testing. 2007;11(3):296-302

Families affected by adrenoleukodystrophy (X-ALD) and adrenomyeloneuropathy (AMN) were surveyed to elicit attitudes toward prenatal, presymptomatic and carrier testing, and newborn screening in order to determine the level of support that these families have for current and future genetic testing protocols. Identifying attitudes toward genetic testing, including newborn screening, is especially important because of new data regarding therapeutic options and the possible addition of X-ALD to newborn screening regimens. The Kennedy Krieger Institute (KKI) database identified 327 prospective participants. Families that were willing to participate in the study received an anonymous questionnaire for completion. Frequencies were generated using SPSS© software for Windows. Questionnaires were returned from 128 families for a response rate of 39%. Sons who were at risk for inheriting the X-ALD gene would be tested by 93% of respondents, and 89.3% would ideally have this testing performed prenatally or in the newborn period. Eighty-nine percent would test an at-risk daughter and 51.2% would ideally have this testing performed prenatally or shortly after birth. X-ALD newborn screening for males and females was supported by 90% of respondents. If newborn screening for X-ALD/AMN commences, or there is a new diagnosis of X-ALD, genetic professionals need to be prepared to have extensive conversations with families regarding the benefits and limitations of current therapeutic and genetic testing options. © 2007 Mary Ann Liebert, Inc.

Non-systematic reviews (2)

1. Wiesinger C, Eichler FS, Berger J. The genetic landscape of X-linked adrenoleukodystrophy: Inheritance, mutations, modifier genes, and diagnosis. Application of Medical Genetics. 2015;8:109-21

X-linked adrenoleukodystrophy (X-X-ALD) is caused by mutations in the ABCD1 gene encoding a peroxisomal ABC transporter. In this review, we compare estimates of incidence derived from different populations in order to provide an overview of the worldwide incidence of X-X-ALD. X-X-ALD presents with heterogeneous phenotypes ranging from adrenomyeloneuropathy (AMN) to inflammatory demyelinating cerebral X-ALD (CCALD). A large number of different mutations has been described, providing a unique opportunity for analysis of functional domains within ABC transporters. Yet the molecular basis for the heterogeneity of clinical symptoms is still largely unresolved, as no correlation between genotype and phenotype exists in X-X-ALD. Beyond ABCD1, environmental triggers and other genetic factors have been suggested as modifiers of the disease course. Here, we summarize the findings of numerous reports that aimed at identifying modifier genes in X-X-ALD and discuss potential problems and future approaches to address this issue. Different options for prenatal diagnosis are summarized, and potential pitfalls when applying next-generation sequencing approaches are discussed. Recently, the measurement of very long-chain fatty acids in lysophosphatidylcholine for the identification of peroxisomal disorders was included in newborn screening programs.

2. Raymond GV, Jones RO, Moser AB. Newborn screening for adrenoleukodystrophy: Implications for therapy. Molecular Diagnosis and Therapy. 2007;11(6):381-4

X-Linked adrenoleukodystrophy (X-X-ALD) is a progressive metabolic condition affecting the adrenal glands and nervous system of males. Although variable in the age of onset and presentation in families, X-X-ALD does present in characteristic phenotypes including a devastating childhood form that affects 35% of boys with this genetic condition. The majority of males with X-X-ALD will also develop adrenal insufficiency, which may result in crisis. Early detection is desirable in order to prevent morbidity from this condition. We have recently developed a tandem mass spectroscopy method that allows this to be done during newborn

screening for other genetic disorder. In this review, we discuss the rationale for early detection, its effect on treatment, and some of the uncertainties.

• **Newborn screening**

Non-systematic reviews (6)

1. Martinez-Morillo E, Prieto Garcia B, Alvarez Menendez FV. Challenges for Worldwide Harmonization of Newborn Screening Programs. Clinical Chemistry. 2016;62(5):689-98

BACKGROUND: Inherited metabolic disorders (IMDs) are caused by a defect in a metabolic pathway, leading to malfunctioning metabolism and/or the accumulation of toxic intermediate metabolites. To date, hundreds of IMDs have been identified. Many of these diseases are potentially fatal conditions that are not apparent at birth. Newborn screening (NBS) programs involve the clinical and laboratory examination of neonates who exhibit no health problems, with the aim of discovering those infants who are, in fact, suffering from a treatable condition.

CONTENT: In recent years, the introduction of tandem mass spectrometry has allowed the expansion of screening programs. However, this expansion has brought a high degree of heterogeneity in the IMDs tested among different NBS programs. An attempt to harmonize the metabolic conditions recommended to be screened has been carried out. Two uniform screening panels have been proposed in the US and European Union, by knowledgeable organizations. Here, we review current evidence-based processes to assess and expand NBS programs. We also discuss the IMDs that have recently been introduced in some screening programs, such as severe combined immunodeficiencies, lysosomal storage disorders, and adrenoleukodystrophy.

SUMMARY: NBS programs have been an established public health function for more than 50 years to efficiently and cost-effectively identify neonates with severe conditions. However, NBS is not yet optimal. This review is intended to elucidate the current degree of harmonization of NBS programs worldwide as well as to describe the major controversial points and discuss the multiple challenges that must be confronted in expanded NBS strategies.

2. Wilcken B, Wiley V. Fifty years of newborn screening. Journal of Paediatrics and Child Health. 2015;51(1):103-7

Newborn screening has evolved fast following recent advances in diagnosis and treatment of disease, particularly the development of multiplex testing and applications of molecular testing. Formal evidence of benefit from newborn screening has been largely lacking, due to the rarity of individual disorders. There are wide international differences in the choice of disorders screened, and ethical issues in both screening and not screening are apparent. More evidence is needed about benefit and harm of screening for specific disorders and renewed discussion about the basic aims of newborn screening must be undertaken.

3. Therrell BL, Padilla CD, Loeber JG, Kneisser I, Saadallah A, Borrajo GJC, et al. Current status of newborn screening worldwide: 2015. Seminars in Perinatology. 2015;39(3):171-87

Newborn screening describes various tests that can occur during the first few hours or days of a newborn's life and have the potential for preventing severe health problems, including death. Newborn screening has evolved from a simple blood or urine screening test to a more comprehensive and complex screening system capable of detecting over 50 different conditions.

While a number of papers have described various newborn screening activities around the world, including a series of papers in 2007, a comprehensive review of ongoing activities since that time has not been published. In this report, we divide the world into 5 regions (North America, Europe, Middle East and North Africa, Latin America, and Asia Pacific), assessing the current NBS situation in each region and reviewing activities that have taken place in recent years. We have also provided an extensive reference listing and summary of NBS and health data in tabular form.

4. Matern D, Oglesbee D, Tortorelli S. Newborn screening for lysosomal storage disorders and other neuronopathic conditions. Developmental Disabilities Research Reviews. 2013;17(3):247-53

Newborn screening (NBS) is a public health program aimed at identifying treatable conditions in presymptomatic newborns to avoid premature mortality, morbidity, and disabilities. Currently, every newborn in the Unites States is screened for at least 29 conditions where evidence suggests that early detection is possible and beneficial. With new or improved treatment options and development of high-throughput screening tests, additional conditions have been proposed for inclusion into NBS programs. Among those are several conditions with a strong neuronopathic component. Some of these conditions have already been added to a few national and international screening programs, whereas others are undergoing pilot studies to determine the test performance metrics. Here, we review the current state of NBS for 13 lysosomal storage disorders, X-adrenoleukodystrophy, Wilson disease, and Friedreich ataxia. © 2013 Wiley Periodicals, Inc.

5. Mak CM, Lee HCH, Chan AYW, Lam CW. Inborn errors of metabolism and expanded newborn screening: Review and update. Critical Reviews in Clinical Laboratory Sciences. 2013;50(6):142-62

Inborn errors of metabolism (IEM) are a phenotypically and genetically heterogeneous group of disorders caused by a defect in a metabolic pathway, leading to malfunctioning metabolism and/or the accumulation of toxic intermediate metabolites. To date, more than 1000 different IEM have been identified. While individually rare, the cumulative incidence has been shown to be upwards of 1 in 800. Clinical presentations are protean, complicating diagnostic pathways. IEM are present in all ethnic groups and across every age. Some IEM are amenable to treatment, with promising outcomes. However, high clinical suspicion alone is not sufficient to reduce morbidities and mortalities. In the last decade, due to the advent of tandem mass spectrometry, expanded newborn screening (NBS) has become a mandatory public health strategy in most developed and developing countries. The technology allows inexpensive simultaneous detection of more than 30 different metabolic disorders in one single blood spot specimen at a cost of about USD 10 per baby, with commendable analytical accuracy and precision. The sensitivity and specificity of this method can be up to 99% and 99.995%, respectively, for most amino acid disorders, organic acidemias, and fatty acid oxidation defects. Cost-effectiveness studies have confirmed that the savings achieved through the use of expanded NBS programs are significantly greater than the costs of implementation. The adverse effects of false positive results are negligible in view of the economic health benefits generated by expanded NBS and these could be minimized through increased education, better communication, and improved technologies. Local screening agencies should be given the autonomy to develop their screening programs in order to keep pace with international advancements. The development of biochemical genetics is closely linked with expanded NBS. With ongoing advancements in nanotechnology and molecular genomics, the field of biochemical genetics is still expanding rapidly. The potential of

tandem mass spectrometry is extending to cover more disorders. Indeed, the use of genetic markers in T-cell receptor excision circles for severe combined immunodeficiency is one promising example. NBS represents the highest volume of genetic testing. It is more than a test and it warrants systematic healthcare service delivery across the pre-analytical, analytical, and post-analytical phases. There should be a comprehensive reporting system entailing genetic counselling as well as short-term and long-term follow-up. It is essential to integrate existing clinical IEM services with the expanded NBS program to enable close communication between the laboratory, clinicians, and allied health parties. In this review, we will discuss the history of IEM, its clinical presentations in children and adult patients, and its incidence among different ethnicities; the history and recent expansion of NBS, its cost-effectiveness, associated pros and cons, and the ethical issues that can arise; the analytical aspects of tandem mass spectrometry and post-analytical perspectives regarding result interpretation. © 2013 Informa Healthcare USA, Inc.

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