Nomination form requesting assessment of a condition for *addition to* newborn bloodspot screening

Appendix C: Nomination form (addition)

Please submit to the Newborn Bloodspot Screening Program Management Committee via SCoS@health.gov.au

Questions	Response
Name of nominator(s)	**REDACTED**
Organisation(s) (if applicable)	Leukodystrophy Resource & Research Organisation Inc.
Contact details (address, phone, email)	**REDACTED**
Role(s) (for example, clinician, researcher, parent, advocate etc.)	Parent and advocate incorporation for X-linked adrenoleukodystrophy newborn screening. My 9 year old son was finally correctly diagnosed with ALD in 1997 after many misdiagnoses over a period of some 12 months. The lack of knowledge and extended diagnosis time ensured that no intervention then, by way of a Bone Marrow Transplant was possible. He died 20 months later aged 11. Without early diagnosis the male cerebral form is rapid and fatal. Pre-symptomatic detection is critical in saving our boys with a successful HSCT. Upon my son's death I dedicated my life to seeing the introduction of ALD newborn bloodspot screening in Australia so that parents do not have to bury their young sons.
Condition nominated for assessment (specifying form(s), if applicable)	X-linked adrenoleukodystrophy, ALD Childhood cerebral, adrenomyeloneuropathy, Addison's Disease
OMIM* or other names for the condition	OMIM#300100; Addison disease and cerebral sclerosis; Siemerling-Creutzfeldt Disease;

*Online Mendelian Inheritance in Man: http://www.omim.org/

Instructions for completion

- Please complete as many of the 'response' sections within this form as possible, citing relevant references within the text by number, then list and attach all references at section 6.
- It is recommended that a nominee who is not from a newborn bloodspot screening program seeks the advice and guidance of their jurisdiction's newborn bloodspot screening program regarding the required documentation and evidence in order to make a submission for the addition or removal of a condition.
- When the nomination form is complete, it should be submitted to the Newborn Bloodspot Screening Program Management Committee.

1. The condition X-linked adrenoleukodystrophy, X-ALD

The condition should be a serious health problem that leads to significant morbidity or mortality. There should be a benefit to conducting screening in the newborn period; and the natural history of the condition, including development from latent to declared disease, should be adequately understood.

Guiding questions	Response
What is the incidence of the condition in Australia? Is this determined clinically or through screening studies in other countries?	The incidence of X-ALD is determined by neonatal screening to be 1/14,700 male and female births in New York State (1). The incidence of X-ALD was also determined by family screening to be about 1/15,000 in France, the USA, and Canada (2).
What is the burden of disease associated with the condition, including morbidity and mortality? What is the spectrum of disease—in particular, are there mild or late-onset forms?	X-linked adrenoleukodystrophy (X-ALD) is the most common peroxisomal disorder and affects the adrenal cortex and the central nervous system (brain inflammation and spinal cord/peripheral neuropathy). The two most common forms affecting males are the childhood cerebral X-ALD (CCALD) (35%) and adult spinal cord disease, adrenomyeloneuropathy (AMN) (60%). About 90% of males also develop adrenal insufficiency The initial symptoms of CCALD include emotional lability, hyperactive behaviour, school failure and visuospatial impairment followed by worsening cognitive and neurologic disability. As demyelination progresses, boys with X-ALD deteriorate to a vegetative state within two to five years of symptomatic onset and to death thereafter. In contrast, AMN is an adult-onset disorder, with progressive spastic paraplegia, sensory ataxia, and other peripheral nerve and spinal nerve involvement. Although the treatment of adrenal insufficiency is very effective, the identification of adrenal insufficiency is often delayed and may lead to significant morbidity or even death. 65% of heterozygous women also present with the neurological symptoms of AMN, with a range of symptoms from mild to severe disability. Adrenal insufficiency is rare (<1%) in females.

Guiding questions	Response
At what age would the condition usually be detected clinically?	The earliest X-ALD male with adrenal insufficiency was 3 months old (7). Median time of onset of adrenal insufficiency is about 14years with 46.8% of affected males presenting between 0-10yrs; 28.6% at 11-40 years and only 5.65% >40years (8). The age of onset of CCALD is usually between ages 2.3 and 10 years, with the peak age of onset at 7 years (3). The earliest reported case of CCALD presented at 21 months of age (5). Onset of AMN in adult males occurs in the 20's and 30's with an average age of 28 years (4). Onset of AMN in heterozygous females is in the 4th or 5th decades of life (4).
What are the benefits of early diagnosis and intervention/treatment? (Consider such benefits as early intervention, prevention of symptoms, reduction of disease severity, provision of a definitive diagnosis, emotional and social benefits and provision of information that would assist families with reproductive decision making.)	Boys with X-ALD who have been identified at birth will be screened for adrenal function and, if found to be deficient, will receive life- saving adrenal hormone replacement therapy and stress doses during illness or trauma (7). Adrenal testing to detect adrenal insufficiency will be recommended every 4-6 months _10 years, annual testing 11-40 years, and solely on demand testing >40 years (8). Boys with X-ALD who have been identified at birth will be referred to a metabolic physician or paediatric neurologist for monitoring of early brain demyelination with an annual MRI once a year starting at 1 year of age until the age of 3 when MRI is recommended every 6 months through the age of 10. After the age of 10, MRI surveillance is once a year. At the first sign of a progressive brain lesion, the X-ALD boy is referred for hematopoietic stem cell transplant (HSCT) if there is an HLA matched donor. Allogeneic HSCT is the most established therapy for CCALD. This procedure has been shown to arrest disease progression only if done at the first sign of abnormal brain MRI prior to the onset of any neurological symptoms. The outcomes are further improved by using a matched related rather than unrelated bone marrow or cord blood donor (9, 10, 11). As HSCT is associated with high morbidity and some mortality, the procedure is not recommended unless there are MRI changes (9).

Guiding questions	Response
	If there is no suitable transplant match, then alternative therapies such as autologous gene therapy should be considered (9, 12,13). Currently in Australia, affected males in families with no known history of X-ALD, usually present too late for HSCT to be a therapeutic option. (Personal note: With early detection not only would my son be alive but so would all the others I have known and watched suffer and die over the past 20 years.) All babies, male and female, with a positive newborn screening test will be referred for genetic counselling. Mothers will be tested to determine if they are carriers and, if so, offered prenatal testing for future pregnancies (5). As the inheritance is X-linked recessive, all extended family members at risk of X-ALD will be offered testing: males by plasma very long chain fatty acids and female relatives by C26:0-lysophosphatidylcholine (which detects all heterozygote females (14)) or by targeted *ABCD1* analysis of the mutation found for the X-ALD baby. Male siblings of the proband have a risk as high as 50% for X-linked adrenoleukodystrophy and could be identified at a critical time for early treatment of adrenal and cerebral disease (5). The X-ALD families will be referred to X-ALD support groups in Australia, the Leukodystrophy Resource & Research Organisation Inc or Leukodystrophy Australia.
What are the possible harms of screening and/or early diagnosis?	The major potential harm is psychological distress regarding the uncertainty of the clinical outcomes for individuals detected by newborn screening. Neither the biochemistry (very long chain fatty acids, C26:0-lysophosphatidylcholine) or mutations of the *ABCD1* gene can predict the clinical course, even in the same family. Individuals and families can become anxious because of the inability to predict phenotypic expression of the condition, which for some males might not be for decades (15). HSCT carries a risk of morbidity and mortality. The mortality is much lower, about 5%, in asymptomatic males with early MRI changes (11). There is a small risk that some males may have their MRI scans misinterpreted and receive HSCT that was not necessary (15). This risk should be eliminated if

Guiding questions	Response
	transplants are only performed in major tertiary centres (as is the case for paediatrics in Australia). The risks of HSCT need to be balanced against the 100% mortality rate within a few years of clinical onset of untreated CCALD (15).

2. The test

There should be a suitable test protocol to identify the presence of the condition, and the test protocol should be socially and ethically acceptable to health professionals and the public.

Guiding questions	Nominator's response
Describe a detailed methodology for the test (for example, tandem mass spectrometry, immunoassay, molecular), including any second-tier testing required. Provide reference to a published methodology and describe any modifications required.	There are several methods that are established for the measurement of C26:0-lysophosphatidylcholine (C26:0-LPC). Depending on the number of newborn samples screened, and what tests are multiplexed with X-ALD screening, the screening laboratories in the USA have chosen either three or two-tier testing program. The larger states such as New York and California have a three-tier X-ALD newborn screen. The first-tier is high throughput flow-injection analysis tandem mass spectrometry (FIAMS/MS) combined with testing for Krabbe disease and/or other lysosomal disorders. The first-tier has a higher number of positives due to the presence of isobaric contaminants to C26:0- LPC. All the positives (about 1.8% of samples (15)) from the first-tier are reanalysed by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), which separates the C26:0-LPC from the isobaric contaminants. (5, 15,16,17,18) The X-ALD positive samples from the second-tier are referred for sequencing of the *ABCD1* gene as there are other disorders such as the Zellweger spectrum disorders (ZSD) and the single enzyme defects of peroxisomal fatty acid oxidation, (acyl-CoA oxidase, D-bifunctional enzyme), *ABCD5* and the contiguous *ABCD1 DXS* gene deficiencies that also would have an elevation of C26:0-LPC in the newborn blood spot. The Zellweger spectrum and other disorders have a collective birth incidence of about 1/50,000. There is no effective treatment for these disorders; however, neonatal detection can shorten the diagnostic odyssey and provide appropriate genetic counselling to their families (19,20,21,22).

Guiding questions	Nominator's response
	States in the USA with a lower birth rate than New York or California are using a two-tier testing program for XALD newborn screening. The first-tier is tandem mass spectrometry for C26:0-LPC in either positive or negative mode. The second-tier is sequence analysis of the *ABCD1* gene or, in some states, follow-up testing only includes biochemical studies such as plasma very long chain fatty acid (VLCFA) analysis and referral of the baby to a geneticist.
Can the test be performed on the same dried bloodspot specimen that is used currently? If not, what additional sample would be required?	Yes, both C26:0-LPC and sequencing of the *ABCD1* gene can be done on the same bloodspot that is used currently. In some cases where the C26:0-LPC value is at the cut off value for X-ALD newborn screening, a second dried bloodspot is used for confirmation before sequencing the *ABCD1* gene.
For the proposed testing protocol, comment on the:	
clinical and analytic validity	The elevation of C26:0-LPC in the newborn blood spot has been shown to be a valid analytical marker for ALD and other disorders that have defects in peroxisomal fatty acid oxidation (14,16,17,18,23). The clinical validity has not been determined for all X-ALD newborns; however there are now reports of X-ALD boys identified through newborn screening where the X-ALD male infants have been identified with adrenal dysfunction and several XALD boys, ages 3 and 4 years, under MRI surveillance that have developed progressive brain lesions and have been referred for hematopoietic cell transplant.
sensitivity	The MS/MS analysis of C26:0-LPC is a sensitive biomarker for X-ALD newborn screening. A recent study showed that C26:0-LPC detected all newborns with XALD as well as all women with X-ALD, including those with normal plasma C26:0 levels. To date, no false negative cases are known from newborn screening so sensitivity is 100% (15). Newborn screening for X-ALD has occurred for <5 years so it will be a long time before the true false negative rate is known. NB: There is no data on NBS programs which use VLCFA as their final tier rather than *ABCD1* sequencing.
specificity	Based on 365,000 newborn screens in New York over 18 months to July 2015, from the first 2 tiers (FIA-MS/MS C26:0-LPC and HPLC-MS/MS C26:0-LPC) the specificity is 99.99%. After the third tier, *ABCD1*

Guiding questions	Nominator's response
	sequencing, the specificity is 100% (15) NB: There is no data on NBS programs which use VLCFA as their final tier rather than *ABCD1* sequencing.
false positive rate	Using LC- MS/MS analysis of C26:0-LPC there is a very low false positive rate. Of the 365,000 babies screened in New York for 18 months to July 2015, there were 7 false positives from the first 2 tiers of screening (false positive rate of 0.001%). After the third tier, *ABCD1* screening, the false positive rate was 0% (15). In New York where *ABCD1* screening occurs before notification, there have been no false positives in nearly 1 million babies screened for X-ALD since screening started in December of 2013. NB: There is no data on NBS programs which use VLCFA as their final tier rather than *ABCD1* sequencing.
false negative rate	To date there are no known false negative cases. Newborn screening for X-ALD has occurred for <5 years so it will be a long time before the true false negative rate is known (15). NB: There is no data on NBS programs which use VLCFA as their final tier rather than *ABCD1* sequencing.
positive predictive value	Based on the 365,000 babies screened to July 2015, after the first 2 tiers the positive predictive value was 78.79%. After the third tier of *ABCD1* screening the positive predictive value was 100% (15) NB: There is no data on NBS programs which use VLCFA as their final tier rather than *ABCD1* sequencing.
negative predictive value	To date there are no known false negative cases so the negative predictive value is 100%. Newborn screening for X-ALD has occurred for <5 years so it will be a long time before the true false negative rate is known. NB: There is no data on NBS programs which use VLCFA as their final tier rather than *ABCD1* sequencing.
Can the test be multiplexed?	The C26:0-lysophophatidylcholine biomarker for X-ALD can be multiplexed with galactose-1-phosphate uridyltransferase (galactosaemia), biotinidase, Pompe Disease, mucopolysaccharidosis 1H (Hurler syndrome), Krabbe disease and other lysosomal storage disorders using liquid chromatography-tandem mass spectrometry. For the lysosomal storage disorders, both enzymatic activities and biomarkers can be incorporated (5,24).
What other conditions may be detected (clinical or of unknown significance)?	The Zellweger spectrum disorders and the single enzyme defects of peroxisomal fatty acid oxidation (acyl-CoA oxidase, D-bifunctional enzyme), *ABDC5* and

Guiding questions	Nominator's response
	the contiguous *ABCD1 DXS* gene deficiencies also have an elevation of C26:0- LPC in the neonatal bloodspot. The Zellweger spectrum and other disorders have a collective birth incidence of about 1/50,000. (19,20,21,22) There was one case of Aicardi-Goutiéres Syndrome detected in the NY state newborn screening for X-ALD; however, clinically these are conditions that usually are symptomatic at birth (25). The false positive cases have mainly been genetic disorders so, although there are no specific treatments, the early diagnosis shortens the diagnostic journey for these families and gives them the opportunity for prenatal testing in subsequent pregnancies. There are some X-ALD babies who have *ABCD1* variants of unknown significance, VUS, (mutations in the gene with unknown significance). These X-ALD babies will receive the same referral for follow-up as those who have known disease causing *ABCD1* mutations. Diagnosis can be confirmed by VLCFA and family studies and prenatal diagnosis would need to be performed with VLCFA and C26:0-LPC until the VUS can be confirmed pathogenic or non-pathogenic.
What would be the cost of the test?	In the USA there is a kit that has been approved by the FDA for X-ALD newborn screening and is available for purchase from Perkin Elmer Genetics at the cost of $5 USD per patient. For those states using FIA-MS/MS, the cost of X-ALD newborn screening using available published procedures, and multiplexing with other lysosomal disorders, followed by the second-tier LCMS/MS is approximately $2 USD per sample. *ABCD1* sequencing cost about $1,000 AUD but may be cheaper if it is done in-house by the newborn screening laboratory. The C26:0-LPC, other fatty acid LPCs, and the internal standard for the measurement of C26:0-LPC, a 4-deuterium labelled C26:0-LPC, is available from Avanti Polar Lipids (Alabaster, Alabama)
If DNA analysis is required, would testing include common mutations, a panel or full sequencing?	*ABCD1* analysis is done by full sequencing. There are also peroxisomal testing panels available in some referral testing laboratories in the USA.
What are the potential harms associated with the test protocol?	The test protocol uses the same newborn screening card as existing newborn screening tests so there are no additional collection risks. There is a risk of false positive results. To date, these have been mainly other genetic disorders with one critically ill infant. The early diagnosis reduces the

Guiding questions	Nominator's response
	diagnostic journey for the family and allows them the option of prenatal testing with future pregnancies. There is also a risk of false negative results. Although these have not yet been reported, newborn screening for X-ALD has only been occurring since December 2013 so there has been insufficient time for missed cases to present clinically. A false negative affected male may miss the opportunity for HSCT or may die or suffer damage from collapse due to unrecognised adrenal insufficiency in an illness. These risks currently occur as we have no newborn screening; but there is some risk that doctors may become deskilled in recognising adrenal insufficiency (as has happened with recognising cystic fibrosis since newborn screening started). This is particularly as newborn screening for congenital adrenal hyperplasia is also occurring. Affected patients may be lost to follow up during the surveillance program which may go on for decades. This puts them at risk of unrecognised adrenal failure. There is a very small risk of unnecessary transplants because of misdiagnosis of an MRI. This is unlikely in Australia as all paediatric transplant units are in tertiary paediatric hospitals. The risk could be reduced further by having a single centre in Australia as the reference centre for Loes scores (the scoring system to quantitate the MRI changes in X-ALD) (10), similar to what currently happens with Gaucher disease radiology. Parental and patient anxiety about the uncertainty of the clinical course because of the prolonged surveillance program and the inability to predict the clinical course of the disease is a definite risk.

3. The intervention

There should be an accepted intervention for patients with recognised disease, and facilities for diagnosis and management should be available so that these services can be offered if there is a positive screening result.

Guiding questions	Nominator's response
What diagnostic testing is necessary? Is it available and reliable? What is its associated cost?	*ABCD1* sequencing if not performed by the neonatal screening laboratory and plasma very long chain fatty acids (VLCFA) are suggested as confirmatory tests to elevated C26:0-LPC level in the neonatal screening. These tests are the "gold standard" diagnostic tests for

Guiding questions	Nominator's response
	X-ALD. The plasma VLCFA measurement costs $AUD 80. The *ABCD1* sequence analysis costs around $1000 AUD. Note that no symptoms are expected before 6 months of age so there is time for the gene sequencing to occur.
What is the established intervention/treatment for this condition?	For X-ALD male infants, testing for adrenal dysfunction should be done within the first 6 months of age and regularly after that. If adrenal insufficiency is found, steroid replacement therapy should commence. MRI surveillance for a progressive brain lesion should begin at 1 year and repeat every year until the age of 3 years after which MRI should be performed every 6 months until the age of 10 years, and annually thereafter (5,15). Males showing MRI evidence of disease progression should be referred for HSCT. There is no treatment for AMN apart from steroid replacement therapy if needed.
Do all patients require an intervention or treatment upon diagnosis? If not, can those who require treatment be distinguished from those who do not?	No infants with X-ALD will need treatment at diagnosis. There is currently no biochemical or genetic test that can predict either which boys will develop adrenal insufficiency or cerebral disease or when disease onset will occur. 90% of males with X-ALD will develop adrenal insufficiency at some stage and all diagnosed males will be monitored for that with treatment started once it is detected. About 35% of males will develop CCALD, the cerebral form of X-ALD, and male infants should be monitored for early brain white matter changes on MRI from 1 year of age. The most common age of onset is 3-10yrs of age and MRI monitoring will occur 6 monthly during this period. If MRI changes develop, then that boy is referred for HSCT. Families of all X-ALD male and female babies who screen positive for X-ALD, require genetic follow-up and their families should receive genetic counselling and atrisk family members should be screened for X-ALD. The counselling should occur soon after the diagnosis is confirmed. There is no treatment for AMN in either males or females but the identification and treatment of adrenal insufficiency in affected males can be life-saving (15).

Guiding questions	Nominator's response
How effective is the intervention/treatment? (Does it alleviate symptoms, slow/halt progression?)	The treatment of adrenal insufficiency is extremely effective. Higher doses are required during periods of stress such as illness and/or trauma. Treatment with corticosteroid replacement is lifelong. Hematopoietic stem cell transplantation (HSCT), whether through an allogeneic donor or autologous ex vivo gene correction, remains the only therapeutic intervention for CCALD. HSCT can halt the progression of CCALD provided the procedure is performed at an early stage of the disease. Unfortunately, this therapeutic window is narrow and usually missed in cases diagnosed clinically. This is why early diagnosis by newborn screening is essential for effective treatment. HSCT stabilises cognitive function if performed when MRI changes are early (26). HSCT does not modify adrenal insufficiency or other types of myelopathy or neuropathy (15).
What are the impacts on quality of life?	Adherence to the surveillance protocols may cause worry and stress to the X-ALD families and most likely to the male child with X-ALD as well. This stress will be maximal in the first 10-12 years of life as that is when CCALD is most likely to occur. If HSCT is performed, there is a prolonged period of hospitalization followed by possible graft vs. host disease and other possible long-term complications of HSCT; however, there is a close to 90-95% survival of males with X-ALD who are identified early either through neonatal or family screening and who thus receive HSCT at the first MRI signs of progressive brain disease (10,11). These early treated males have a 27% chance of graft versus host disease of grade 2 or higher (11). It is important to remember that without the HSCT these boys would die within a few years of clinical onset (3,15). There is good cognitive outcome for boys transplanted with early MRI changes of X-ALD (26) and beyond the transplant period, quality of life is good. The first patient to receive HSCT for X-ALD in Australia has been cognitively stable for over 25 years.
How urgent is the intervention/treatment? Must it be initiated before symptoms present?	There is no immediate urgency for treatment. Adrenal insufficiency can occur from a few months of age and CCALD from a few years of age. There is therefore time to complete *ABCD1* sequencing before notifying the family as this gives the best specificity and positive predictive value for the test.

Guiding questions	Nominator's response
	The X-ALD baby's family should be referred to genetic counselling as soon as the diagnosis is confirmed. This is to allow identification of female carriers and undiagnosed, pre-symptomatic males in the extended family pedigree. Early referral to an endocrinologist is essential to formalise the adrenal surveillance protocol and to alert the family to signs of adrenal insufficiency. Treatment should be started as soon as reduced adrenal function is identified. There is a critical window of opportunity for HSCT. Early referral to a metabolic physician or neurologist will ensure that the MRI surveillance protocol is explained and implemented. MRI changes are scored via the Loes scale of 0-34 (0 is unaffected) (27) and the best outcomes occur with Loes scores of 1-3. HSCT is generally not offered with Loes scores of 10 or above (26). HSCT needs to occur as soon as possible after MRI changes are noted.
What are the potential harms of the intervention/treatment?	The MRI tests are done under anaesthesia until the boy is old enough to hold still for the procedure. General anaesthetics have a small risk of complications.The bone marrow transplant procedure has a risk of mortality and associated graft vs. host disease. The most recent data indicated that the mortality from HSCT in patients with Loes Scores of 1-3 is 5% (1 of 22 patients with median post-transplant period of 17 months) and morbidity from graft versus host disease of grade 2 or higher being 27% (11). Without HSCT these boys will die within a few years of clinical onset. The potential risks from steroid treatment are due to overtreatment which is prevented by regular monitoring of the dose by the endocrine team. There is also a risk of collapse if insufficient steroid is not given during a period of stress such as an illness. This can be prevented by the endocrine team educating the patient and family and by the family and patient complying with treatment recommendations, particularly the unwell steroid regime. Untreated patients can die during a period of stress.
What is the cost of the intervention/treatment?	All costs are covered by the public health system in Australia. Each MRI test costs about $555AUD with a general anaesthetic and $405AUD without anaesthetic. The

Guiding questions	Nominator's response
	HSCT procedure costs about $150,000AUD for a related donor and $250,000AUD for an unrelated donor. Steroid treatment is available on the PBS and is required whether or not newborn screening occurs so is not costed.
What facilities are required to deliver the intervention/treatment? Do current health care facilities in each state and territory have capacity, and are they of sufficient quality, to support the intervention/treatment? Is there equitable access to the intervention/treatment?	Each state in Australia has a HSCT transplant unit for children. Access is equitable but patients who live outside of capital cities may need to be temporarily accommodated in the capital city during the transplant procedure. The transplant units arrange this and accommodation is subsidised. Screening will only increase the workload of each unit by a small number of patients per year. Each tertiary paediatric hospital in Australian states have metabolic, endocrine and neurology units who provide telehealth services to the regional centres in the state. Regional paediatricians are able to provide all necessary follow up care after the HSCT as well as ongoing monitoring of steroid replacement therapy. Adrenal monitoring can be arranged at any health centre. Although regional centres could do the MRIs, these are best arranged at the tertiary hospital to develop the expertise of 1 or 2 radiologists per centre in interpreting X-ALD MRIs. ACT has 2 paediatric endocrinologists at the Canberra Hospital, but transplants would need to be done in Sydney, as would neurology follow up. Northern Territory patients would need to go to either Brisbane or Adelaide for HSCT.

4. Cost-effectiveness

Guiding questions	Nominator's response
Provide any available evidence for the cost-effectiveness of screening for this condition, either from Australia or internationally.	A recent article from the UK looked at the economic impact for newborn screening of X-ALD and concluded that it will save a lot of money. In one year the cost of screening is estimated to be £402,000. Estimated lifetime health, social care and education costs leading to a discounted cost saving of £3,040,000 per year. Patients with CCALD were estimated to gain 8.5 quality adjusted life years (QALYs) each giving an overall program benefit of 82 QALYs (28).

Guiding questions	Nominator's response
	The authors did not take into consideration the negative impact of screened positive males who did not have CCALD and may have needed screening for decades, but felt that any disbenefit was unlikely to negate the benefit of identifying CCALD boys and treating them early.

5. Any other comments

If females develop AMN this does not occur until later adult life. There is no specific treatment for AMN and <1% of females develop adrenal insufficiency. An argument can therefore be made for only screening males. The advantage of screening females is from identifying at risk individuals in their extended family pedigree.

For the statistics, data is given for sensitivity, specificity and PPV etc after the tandem mass spec analyses and then after the third tier of DNA sequencing. There is time to do the ABCD1 sequencing before notifying the results and this would be preferable as it eliminates most of the false positives. This is how New York State manages their program. Other US states are notifying after the tandem mass spec results. We would recommend sequencing the gene before notification.

6. References

1.Moser AB, Jones RO, Hubbard WC, et al. Newborn screening for X-linked adrenoleukodystrophy. *Int J Neonatal Screen*. 2016; 2 (15):1–5.

2.Bezman L, Moser AB, Raymond GV, et al. Adrenoleukodystrophy: incidence, new mutation rate, and results of extended family screening. *Ann Neurol*, 2001; 49:512–517.

3.Raymond GV, Moser AB**,** and Fatemi A. X-linked Adrenoleukodystrophy. 1999 Mar 26 [updated 2018 Feb 15] In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. PMID: 20301491 Available from: https://www.ncbi.nlm.nih.gov/books/NBK1315/

4. Kemp S, Berger J, Aubourg P, X-linked adrenoleukodystrophy: clinical, metabolic, genetic and pathophysiological aspects, Biochim. Biophys. Acta 1822 (9) (2012) 1465–1474.

5. Vogel BH, Bradley SE, Adams DJ, et al. Newborn screening for X-linked adrenoleukodystrophy in New York State: diagnostic protocol, surveillance protocol and treatment guidelines. *Mol Genet Metab*. 2015; 114 (4):599-603.

6. Engelen M, Barbier M, Dijkstra IM, Schür R, de Bie RM, Verhamme C, Dijkgraaf MG, Aubourg PA, Wanders RJ, van Geel BM, de Visser M, Poll-The BT, Kemp S.X-linked adrenoleukodystrophy in women: a cross-sectional cohort study Brain 2014;137(3):693-706

7.Burtman E, Regelmann MO. Endocrine dysfunction in X-linked adrenoleukodystrophy. *Endocrinol Metab Clin N Am*. 2016; 45:295-309. Endo.theclinics.com

8. Huffnagel IC, Laheji FK, Aziz-Bose R, Tritos NA, Marino R, Linthorst GE, Kemp S, Engelen M, Eichler F. The natural history of adrenal insufficiency in X-linked adrenoleukodystrophy: an international collaboration. J Clin Endocrinol Metab. 2018 Sep 24. doi: 10.1210/jc.2018-01307

9. Moser AB, Fatemi A. Newborn screening and emerging therapies for X-linked adrenoleukodystrophy. JAMA Neurology 2018;75(10);1175-1176

10. Miller WP, Rothman SM, Nascene D, et al. Outcomes after allogeneic hematopoietic cell transplantation for childhood cerebral adrenoleukodystrophy: the largest single-institution cohort report. *Blood.* 2011; 118 (7):1971-1978.

11. Chiesa R, Boelens JJ, Duncan C, Chin W, McNeil E, Orchard P. An observational study of patients with cerebral adrenoleukodystrophy (CALD) treated with allogeneic hematopoietic stem cell transplant. J Inherit Met Dis 2018, 41(Suppl 1): S40

12. Eichler F, Duncan C, Musolino PL, et al. Hematopoietic stem-cell gene therapy for cerebral adrenoleukodystrophy. *N Engl J Med*. 2017; 377 (17):1630-1638.

13. Gissen P, Eichler F, Thrasher AJ, Duncan C, Orchard PJ, De Oliveira S, Lund TC, Amartino H, Smith NJC, Chin W, McNeil E, Aubourg P, Williams DA. Lenti-D hematopoetic stem cell gene therapy for cerebral adrenoleukodystrophy: safety and efficacy outcomes from an ongoing Ph 2/3 trial. J Inherit Met Dis 2018, 41(Suppl 1): S40

14. Huffnagel IC, van de Beek MC, Showers AL, Orsini JJ, Klouwer FCC, Dijkstra IME, Schielen PC, van Lenthe H, Wanders RJA, Vaz FM, Morrissey MA, Engelen M, Kemp S. Comparison of C26:0carnitine and C26:0lysophosphatidylcholine as diagnostic markers in dried blood spots from newborns and patients with adrenoleukodystrophy. Mol Genet Metab. 2017 Dec;122(4):209-215

15. Kemper AR, Brosco J, Comeau AM, Green NS, Grosse SD, Jones E, Kwon JM, Lam WK, Ojodu J, Prosser LA, Tanksley S. Newborn screening for Xlinked adrenoleukodystrophy: evidence summary and advisory committee recommendation. Genet Med. 2017 Jan;19(1):121-126

16.Turgeon CT, Moser AB, M_rkrid L, et al. Streamlined determination of lysophosphatidylcholines in dried bloodspots for newborn screening of X-linked adrenoleukodystrophy. *Mol Genet Metab*. 2015; 114:46–50.

17.Haynes CA, De Jesús 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; 413:1217–1221.

18.Hubbard WC, Moser AB, Liu A, et al. Newborn Screening for X-Linked Adrenoleukodystrophy (X-ALD): Validation of a Combined Liquid Chromatography-Tandem Mass Spectrometric (LC-MS/MS) Method. *Mol Genet Metab*. 2009; 97:212-220.

19.Braverman NE, Raymond GV, Rizzo WB, Moser AB, Wilkinson ME, Stone EM, Steinberg SJ, Wangler MF, Rush ET, Hacia JG, Bose M. Peroxisome biogenesis disorders in the Zellweger spectrum: An overview of current diagnosis, clinical manifestations, and treatment guidelines. *Mol Genet Metab.* 2016, 117:313.321.

20.Waterham HR, Ferdinandusse S, Wanders RJ. Human disorders of peroxisome metabolism and biogenesis. *Biochim Biophys Acta*. 2016; 1863:922-933.

21. Ferdinandusse S, Falkenberg KD, Koster J, Mooyer PA, Jones R, van Roermund CWT, Pizzino A, Schrader M, Wanders RJA, Vanderver A, Waterham HR. ACBD5 deficiency causes a defect in peroxisomal very longchain fatty acid metabolism. *J Med Genet.* 2017; 54:330-337.

22.Van de Kamp JM, Errami A, Howidi M, Anselm I, Winter S, Phalin-Roque J, Osaka H, van Dooren SJ, Mancini GM, Steinberg SJ, Salomons GS. Genotype-phenotype correlation of contiguous gene deletions of SLC6A8, BCAP31 and ABCD1. *Clin Genet* 2015, 87:141-147

23.Theda C, Gibbons K, and DeFor TE, et al. Newborn screening for X-linked adrenoleukodystrophy: Further evidence high throughput screening is feasible. *Mol Genet Metab.* 2014; 111:55–57.

24. Hong X, Kumar AB, Scott CR, Gelb MH. Multiplex tandem mass spectrometry assay for newborn screening of X-linked adrenoleukodystrophy, biotinidase deficiency, and galactosemia with flexibility to assay other enzyme assays and biomarkers. Molec Genet Metab 2018;124:101-108

25.Armangue T, Orsini JJ, Takanohashi A , Gavazzi F, Conant A, Ulrick N , Morrissey MA, Nahhas N, Helman G, Gordish-Dressman H, Orcesi S, Tonduti D, Stutterd C, van Haren K, Toro C, Iglesias AD, van der Knaap MS, Goldbach Mansky R, Moser AB, Jones RO, Vanderver A. Neonatal detection of Aicardi Goutières Syndrome by

increased C26:0 lysophosphatidylcholine and interferon signature on newborn screening blood spots. *Mol Genet Metab.* 2017; 122:134-139.

26.Pierpont EI, Eisengart JB, Shanley R, Nascene D, Raymond GV, Shapiro EG, Ziegler RS, Orchard PJ, Miller WP. Neurocognitive trajectory of boys who received a hematopoietic stem cell transplant at an early stage of childhood cerebral adrenoleukodystrophy. *Jama Neurol* 2017; 74(6): 710-717 PMID: 28418523

27. Loes DJ, Fatemi A, Melhem ER, Gupte N, Bezman L, Moser HW, Raymond GV. Analysis of MRI patterns aids prediction of progression in X-linked adrenoleukodystrophy. Neurology 2003;61:369-374

28. Bessey A, Chilcott JB, Leaviss J, Sutton A. Economic impact of screening for X-linked Adrenoleukodystrophy within a newborn blood spot screening programme. Orphanet J Rare Dis. 2018 Oct 11;13(1):179