Newborn Screening for X-Linked Adrenoleukodystrophy (X-ALD):
A Systematic Review of Evidence

Final Report (v5.01)

10/14/2015

**Updated September 2017*

Prepared for:
MATERNAL AND CHILD HEALTH BUREAU

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**September 2017 Update: p. 67 correction.*

Executive Summary

Overview

This report summarizes the evidence regarding benefits and harms of newborn screening for X-linked Adrenoleukodystrophy (X-ALD) and the capability of state newborn screening programs to offer comprehensive testing and follow-up for the condition.

This executive summary highlights key findings from the preliminary version of the complete report developed for the United States Secretary of Health and Human Services' Advisory Committee on Heritable Disorders in Newborns and Children regarding newborn screening for X-ALD. This summary is not intended to replace the complete report, which describes the methods for evidence identification and synthesis and a full discussion of findings. This summary instead provides a high-level review of findings from the complete report.

Adrenoleukodystrophy: Epidemiology and Clinical Course

In the United States, the overall incidence of having at least one mutation associated with X-ALD, regardless of sex, is estimated to be about 6 per 100,000 births. Of these, however, 40% are expected to be hemizygous males, the target of newborn screening. Although females can be homozygous for X-ALD (i.e., a mutation in each gene), the occurrence is extremely rare. The majority of females are heterozygous for the X-ALD mutation.

X-ALD is caused by mutations in the ABCD1 gene located on the X chromosome, leading to defects in the transfer of very long-chain fatty acids into peroxisomes. The clinical phenotype is broad, with severe forms affecting hemizygous males much more often than heterozygous females. Predominant symptoms of X-ALD include adrenocortical insufficiency ("Addison's-only" if the only symptom), cerebral demyelination (child, adolescent, and adult cerebral X-ALD), and progressive paralysis of the lower extremities (adrenomyeloneuropathy, or AMN). The signs and symptoms associated with X-ALD can change and progress over time. Most individuals with X-ALD will experience at least one of the predominant symptoms, with many patients presenting with more than one of the major symptoms in their lifetimes.

In childhood, X-ALD symptoms that appear most frequently include adrenal insufficiency and cerebral demyelination (*Childhood Cerebral X-ALD*). Childhood cerebral X-ALD (C-CALD) is the most serious form of X-ALD. Epidemiologic studies suggest that among males with X-ALD, between 33% and 57% will have cerebral involvement. C-CALD typically presents between 2.5 and 10 years of age and is associated with rapid neurologic decline and death or disability an average 3 years after onset. Adrenal insufficiency has been identified in up to 86% of asymptomatic males prior to any other signs of neurologic involvement, with onset occuring as early as the first year of life through adolescence. Adrenal insufficiency has been reported to co-occur with about 90% of patients with neurologic involvement. In adulthood, neurpoathy and spinal cord involvement frequently appear (adrenomyeloneuropathy [AMN]), though with relatively slower disease progression than the cerebral demyelination.

Screening

Available Screening Methods

New York is the only state in the US that currently screens newborns for X-ALD. The New York NBS program uses a two-tiered approach to screening for X-ALD, plus additional follow-up mutation testing. The first-tier screening is based on measurement of C26:0, a very long chain

fatty acid (VLCFA) in dried blood spots using tandem mass spectrometry (MS/MS) with flow-injection analysis (FIA). The second-tier method uses a combination of high performance liquid chromatography and MS/MS (HPLC-MS/MS) to measure the VLCFA, C26:0 LPC (LC-MS/MS). In the current algorithm used by NY, the first-tier FIA-MS/MS screen yields many initial positive screens, and the second-tier test reduces false positives from the first-tier. Positive screens from the two-tier screening procedure are referred for confirmatory testing. The New York NBS screening program algorithm also conducts mutation analysis of dried blood spots that screen positive for VLCFA (C:26:0-LPC). Mutation analysis, which is perfomed in-house, is conducted primarily to assist referral centers. Mutation analysis is not a necessary component of the newborn screening process.

Outcomes of Prospective Newborn Screening for X-ALD Disease

Newborn Screening Programs in the United States

The New York State NBS Program began screening for X-ALD in December 2013. At the time of this report (through July 2015), 363,755 newborns were screened (51.9% male), of whom 33 screened positive and were referred for confirmatory follow-up testing. Males with X-ALD are most likely to develop significant disease in childhood or adolescence and are the primary targets of newborn screening. In the time period evaluated in this report, of the 33 newborn who screened positive, the New York NBS program has identified 13 males with ABCD1 mutations. Seven of these 13 males have been confirmed to have X-ALD based on short-term follow-up VLCFA testing. The New York NBS program has not yet received the confirmatory results for the remaining 6 males at the time of this report. In addition, 14 females with ABCD1 mutations have been identified. Secondary conditions identified by the follow up diagnostic center in New York include 3 infants with Zellweger's syndrome, and 1 infant with Aicardi-Goutieres syndrome, which is not a peroxisomal disorder.

*Positive Predictive Value*s. The number of cases detected does suggest that the screening algorithm has high sensitivity. However, the positive predictive value is a function of the case definition. If positive cases counted include only males confirmed with X-ALD, the positive predictive value is currently 21% (7/33). This definition is the lower-bound estimate, as it assumes that the 6 males who screened positive but whose confirmatory testing results are false-positive screens, which is unlikely given that they are known to have mutations in the ABCD1 gene. If positive cases counted include all male newborns who screened positive for VLCFA in dried-blood spot testing, the positive predictive value is currently 39% (13/33). If positive cases counted included females with ABCD1 mutations, or any diagnosable condition - peroxisomal or other disorders - the positive predictive value currently approaches 100%.

Published Reports

Among the included published reports in the literature search, one study was identified that included results from a small prospective research study of population-based screening.[1] Investigators used second punch dried blood spot samples from 5,000 prospective newborn screening specimens (52% males) collected from 2008 to 2010 in Maryland. Parental permission was obtained for follow up tracking of any test results from the study that were positive for elevated VLCFA levels. Of 4869 evaluable samples, none were identified as having elevated VLCFA levels. Researchers reported 0 false-positives, and test specificity of 100%. No further follow up was reported for possible false-negatives.

Anticipated Harms of Screening

Predicting timing and severity of disease onset among newborns affected by X-ALD is not possible. All male newborns with X-ALD are expected to be asymptomatic at birth, requiring periodic follow-up and monitoring for symptom onset of adrenal dysfunction and neurologic involvement. Rates of false-negative screening results have not been reported, and, as with newborn screening for most conditions, would be difficult to assess. It is estimated that about 20% of heterozygote females have VLCFA plasma levels within normal limits. However, because females with X-ALD do not typically experience symptoms until adulthood, they are not a target of newborn screening. Genetic counseling and testing is recommended for family members of both males and females affected by X-ALD, which may facilitate identification of X-ALD in otherwise undiagnosed individuals. These otherwise undiagnosed individuals may either be asymptomatic, or may be experiencing symptoms at early or more advanced stages of disease. This information may facilitate clinical care and monitoring, however, the inability to predict timing or severity of onset in newborn screening and family risk identification may also create ethical challenges.

Early Detection and Treatment for X-ALD

Newborn males with X-ALD newborns are asymptomatic at birth. Short-term and long-term follow-up clinical protocols have been established to monitor routinely for primary symptoms of adrenal insufficiency and neurlogical involvement. Adrenal function is tested periodically to guide initiation of adrenal replacement therapy. Monitoring of neurological involvement with magnetic resonance imaging (MRI) ratings of severity and neurological function scores (NFS) appear to predict severity of disease progression and treatment outcomes. Hematopoietic stem cell transplantation (HSCT) is recommended for early stage C-CALD after brain involvement is demonstrated. HSCT can be effective at arresting or slowing progression of cerebral demyelination. HSCT does not appear to impact other major symptoms of X-ALD symptoms (e.g., adrenal insufficiency, peripheral neuropathy). Individuals with advanced cerebral disease are not candidates for HSCT due to poor expected outcome following HSCT. Published evidence consistently demonstrates differences in outcome following HSCT for mildly affected vs. severely affected individuals with C-CALD, as determined by an established MRI rating scale. However, no published study directly compares outcomes for individuals identified presymptomatically who are monitored for disease onset to allow for earlier HSCT versus outcomes for individuals identified through symptomatically through clinical detection.

Unpublished analysis of two datasets (one single medical center and one multi-center study) obtained by the CRW suggests that detection through extended family testing compared with clinical detection is associated with improved survival and less neurologic involvment post-HSCT based on MRI ratings for individuals with C-CALD. Analyses of these two datasets of children with C-CALD also showed that on average, individuals identified through family testing had significantly less neurological involvement pre-HSCT. However, the datasets did no allow direct comparison of long-term outcomes from identification through newborn screening versus usual case detection, and findings may not be generalizable to all individuals who will develop C-CALD. Although adrenal insufficiency is common in boys with C-CALD, no direct evidence was found regarding differences in health outcomes related to adrenal insufficiency comparing presymptomatic detection to usual clinical case detection.

Impact on Public Health of the Population

Based on the published and the limited unpublished data regarding cases of C-CALD detected through family testing versus clinical case detection, newborn screening for X-ALD would lead to 18 deaths averted (range: 7-14), or 37 (range: 17-64) patients who would avert both death and disability (i.e., becoming non-ambulatory and non-communicative) compared with current practices of clinical detection in an annual U.S. birth cohort of 4 million. This estimate reflects the benefit for newborns with C-CALD over the first 15 years of life.

Impact on Public Health Systems

One state (New York) currently offers newborn screening for X-ALD and another four states have mandates to offer screening. Two out of 3 states with mandates that were interviewed reported that it would take between 1 and 3 years to implement screening for X-ALD after approval and allocation of funds. The costs associated with screening and competing public health interests continue to be a challenge.

Clinical referral networks will need to be identified or developed to follow individuals identified with presymptomatic X-ALD. In addition, plans will need to be developed to be able to offer family testing for hemizygous boys and heterozygote females.[2] The availability of experts to provide comprehensive care and the related costs for providing such care are not known.

LIST OF ABBREVIATIONS

Abbreviation	Definition
Advisory Committee, ACHDNC	Secretary's Advisory Committee on Heritable Disorders in Newborns and Children
AO	Addison's Only
AMN	Adrenomyeloneuropathy
CALD	Cerebral Adrenoleukodystrophy
C-CALD	Child/Adolescent Cerebral Adrenoleukodystrophy
CRW	Condition Review Workgroup
FDA	United States Food and Drug Administration
FIA-MS/MS	Flow-injection mass spectrometry
MS/MS	Tandem mass spectrometry
NANC	Non-ambulatory and non-communicative
NBS	Newborn Screening
PD	Peroxisomal Disorder
RUSP	Recommended Uniform Screening Panel
X-ALD	X-linked Adrenoleukodystrophy
VLCFA	Very long chain fatty acids

1. Scope and Methods of the Review

Scope of Review

This report was developed to support the Secretary of Health and Human Services' (HHS) Advisory Committee on Heritable Disorders in Newborns and Children ("Advisory Committee") in making recommendations to the Secretary, HHS, about whether newborn screening for X-ALD disease should be added to the Recommended Uniform Screening Panel (RUSP).

Nomination and Request for Review
X-ALD disease was initially nominated to the Advisory Committee for inclusion in the RUSP in September 2012. At that time, the Committee did not request a systematic review of the potential benefits and harms of screening for X-ALD disease "*based primarily on the determination that sufficient prospective data is not yet available from the large pilot study presently underway at the Mayo Biochemical Genetics Laboratory (MBGL).*" A follow-up nomination was considered by the Advisory Committee at the September 2014 meeting, at which time a formal review of the scientific evidence for newborn screening for X-ALD was requested from the external Condition Review Workgroup. Although the data are not still not available from the MBGL, sufficient other data are avaible to assess newborn screening for X-ALD.

Case Definition

All untreated individuals with X-ALD will have elevated plasma levels of the VLCTA C:26-lysophosphatidylcholine and at least one mutation in the ABCD1 gene. As described previously, the focus of this report is C-CALD.

X-ALD with Childhood/Adolescent Onset. As an X-linked condition, X-ALD primarily affects males during childhood and adolescence with symptoms of cerebral involvement (onset 3-10 years) and adrenal insufficiency (onset 4 – 13 years, though biochemical signs may appear <1 year) the most frequently occuring before adulthood. Current estimates of childhood CALD range between 35%-57%. When accounting for adrenocortical dysfunction with or without CALD, estimates increase to over 80%. The majority of females heterozygous for X-ALD (80%) experience symptom onset in late adulthood, after 60 years of age. Cerebral involvment is not common in females. Therefore, this report will focus on outcomes for boys who may be diagnosed with X-ALD presymptomatically through early detection. Because earlier HSCT may lead to improved outcome for boys with C-CALD, this subgroup will be the focus. However, we will also evaluate the degree to which early identification of adrenal insufficiency leads to improved health outcomes.

Methods – Systematic Evidence Review

The methods guiding this systematic evidence review followed approaches outlined in the Condition Review Workgroup – Manual of Procedures (2012, 2014). These procedures are based on the AHRQ SER Methods Guide,[3,4] the United States Preventive Services Task Force (USPSTF) Procedures Manual,[5] and other established evidence review standards, with adaptations to address the nature of research on rare disorders (e.g., few large RCTs) and the

established review and comment timeline of the ACHDNC. This section describes specific procedures that guided this Condition Review of newborn screening for X-ALD Disease.

Literature Search

Published Literature Search

An experienced medical librarian conducted the initial literature search which included publications through September 2014, and an updated search through July 2015. We identified published literature from MEDLINE, EMBASE, and CINAHL from 1966 (the start of MEDLINE) using the following MeSH terms and associated key words used for each database. Articles were limited to full-text available in English, human subjects only (animal research excluded).

- Publication Dates: Database inception to September 2014, updated through July 2015
- Databases: MEDLINE, EMBASE, & CINAHL
- Keywords and Search Terms: *("Adrenoleukodystrophy"[Mesh]) OR ("Adrenoleukodystrophy"[tiab]) OR ("Adrenoleukodystrophy/therapy"[Mesh]) OR ("X-ALD"[tiab]) OR ("very long-chain fatty acids"[All Fields]) OR ("VLCFA"[tiab]) OR ("Lorenzo's oil"[Supplementary Concept]) OR ("Lorenzo's oil"[tiab]) AND ("humans"[Mesh] NOT "animals"[mesh]) AND Limits: English.*

Literature Screening: Inclusion and Exclusion Criteria

Preliminary Screening

Inclusion Criteria. Articles that reported on studies with human subjects and published in English were included. All study designs were considered, including case reports, case series, observational, studies, uncontrolled, and controlled intervention trials.
Exclusion Criteria. Non-human studies, studies with no English language abstracts, and articles with no new data were excluded.

Literature Review Eligibility Criteria

Following the initial Title and Abstract screen, additional inclusion and exclusion criteria were added to refine the search. minimum sample size requirements and outcomes reported (e.g., if a subset of a larger sample, outcomes must be reported specific to the ALD patient subgroup).

Additional eligibility criteria regarding included Populations, Interventions, Comparators Outcomes, Timing, and Settings for each key topic area (KTA) and question (KTQ) are outlined below. Further details of the article screening procedures and flow diagram can be found in Appendix A.

Full-text review exclusion criteria followed standard rules, with sample size requirements determined after the initial scan of available literature, and are as follows:

- Not Full-text article
- No original data or analyses
- No KTA/KTQ addressed
- No human subjects with ALD

- Other (includes sample size requirements not met)

Published Literature Search Results

Total numbers of articles identified in each of these databases was 2,273 (Pubmed), 730 (Embase), and 154 (CINAHL). After the initial screen for duplicates, the numbers of articles systematically screened and reviewed was 969, 635, and 32, respectively. With database articles combined, an additional 332 reports were screened and removed, for a total of *1,314* articles entered into the Distiller SR program for systematic review. Initial title and abstract screening was conducted by two independent reviewers for relevance and general exclusion and inclusion. An inclusions from at least one reviewer retained an article for further full-text review. After title and abstract screening, 632 articles were excluded, and *682* were advanced for full-text review. Two independent reviewers reviewed the title, abstract, and full-text if needed for exclusion, inclusion. For included articles, key topic area(s) and questions.were identified. At this full-text review stage, disagreements between reviwers were reconciled through discussion or by a third independent reviewer as needed. After the full-text review, 495 articles were excluded, leaving *172* for review, abstraction, and summary review. Screening and Treatment related articles were fully abstracted for content and reviewed by two reviewers using data abstraction forms tailored for this review and incorporporated into Distiller SR. Other Key Topic articles (e.g., Incidence and Epidemiology, Natural History and Clinical Course with Clinical Detection) were summarized in each results section as context. Further details regarding the flow of articles screened, exclusion criteria, and review stages are outlined in Appendix A (PRISMA Diagram).

Key Questions for Evidence Review: X-ALD

The key topic areas and questions for the systematic evidence review were developed from the general analytic framework used by the Condition Review Workgroup (Manual of Procedures-Rev v2.0, 2012, 2014) and the specific needs of the Advisory Committee. The technical expert panel on X-ALD guided refinement of the specific key questions to ensure relevance to the target condition. The Key Questions guiding the review of evidence for newborn screening for a new condition can be organized into four main topic areas, I. Natural History and Clinical Detection, II. Screening and Short-Term Follow Up, III. Treatment and Long-Term Follow Up, and IV. Public Health Impact. The final Key Questions are outlined below, with the refined inclusion and exclusion criteria listed within the Population, Interventions, Comparators, Outcomes, Timing, and Setting (PICOTS) parameters consistent with standard evidence review methods.

I. Natural History and Epidemiology with Usual Clinical Detection

Key (Context) Question 1: What is the natural history and epidemiology of X-ALD? Specifically, what are the estimated incidence rates for associated X-ALD phenotypes, and the typical course of disease (i.e., ages of reported clinical onset and symptoms, diagnosis, treatment initiation, and death)? What are the phenotypes particularly affecting newborns and children (onset <21 years of age, n>5)? What factors predict morbidity or mortality?

II. Screening, Short-Term Follow-Up, and Diagnostic Confirmation

Key Question 2: What is the direct *and indirect* evidence that newborn screening for ALD disease leads to improved health outcomes compared to usual clinical care?

- Population: n>5, Newborns with no known risk for X-ALD and detected early, or newborns with increased family risk for X-ALD who were identified presymptomatically.
- Interventions: Any care received subsequent to the screening test
- Comparators: Contemporaneous or historical controls affected by X-ALD
- Outcomes: Overall Survival; Survival with major morbidity
- Timing: Any duration of follow-up
- Settings: All settings

Key Question 3: Screening and Short-term follow up/diagnostic confirmation methods

A. What is the analytic validity or clinical validity of the *newborn* screening approaches used to detect X-ALD and associated phenotypes (under high-throughput/population-based conditions)? (a) Cerebral ALD (child and adult forms), (b) Adrenal insufficiency/Addison's Disease (with and without cerebral involvement), (c) female X-ALD carriers, and (d) other phenotypes.
B. What diagnostic testing methods are available to confirm or identify these phenotypes?
C. What screening or diagnostic methods, if any, are available to predict or inform age of onset or disease severity during newborn screening?

There are two standard measures of analytic validity, sensitivity and specificity. To estimate these requires validated proficiency testing samples. Few such data exist. Consequently, one must use screening studies, which represent the combination of analytic and clinical validity.

- Population: n>5, Newborns without known diagnosis of, or risk factor for X-ALD; de-identified dried-blood spots
- Interventions: Any screening methods for X-ALD conducted in the first month of life. For analytic validity, studies should also report proficiency
- Comparators: Diagnosis by genotype and follow-up evaluation or genotype alone
- Outcomes: Sensitivity, specificity, positive predictive value, negative predictive value, reliability, and yield (i.e., prevalence)
- Timing: Any duration of follow-up
- Settings: All settings

Key Question 4: What are the harms associated with newborn screening for X-ALD to the individual or the family?

- Population: n>5, Newborns screened for X-ALD and their families
- Interventions: Any newborn screening for X-ALD
- Comparators: Any population or none
- Outcomes: Systematic assessment of harms, including harm related to false-positive screening results, false-negative screening results, early identification of adult and later-onset disease, or perceived harms or acceptability of screening for X-ALD.
- Timing: Any duration of follow-up
- Settings: All settings

III. Treatment and Long-term Follow Up

Key Question 5: What are the standard treatments for X-ALD and evidence for their effectiveness? Do follow-up protocols exist for the management of X-ALD that do not require immediate initiation of treatment? What is known about the effectiveness of follow-up protocols in modifying intermediate health outcomes?

Does early initiation of treatment improve primary health outcomes (overall survival, other important health outcomes) when the condition is caught early or through newborn screening compared with usual clinical care? How does this vary by phenotype?

- Population: n>3, Newborns and others diagnosed with X-ALD through newborn screening or other methods of presymptomatic detection and diagnosis in childhood
- Interventions: HSCT, adrenal hormone therapy, or other approved therapies
- Comparators: Contemporaneous or historical controls with X-ALD disease or no comparison
- Outcomes: Survival and key health status measures specific to X-ALD
- Timing: Any duration of follow-up
- Settings: All settings

In assessing the impact of early intervention, it is important to distinguish whether cases were identified early through newborn screening or risk (e.g., family history of X-ALD) versus identification of symptoms under usual care (i.e., clinical detection). Those children detected based on symptom onset may have more severe disease, and thus could have worse outcomes. Therefore, it is important to characterize methods of subject X-ALD diagnosis.

Key Question 6: *Does initiation of treatment modify the intermediate health outcomes when X-ALD is detected through newborn screening or other methods of presymptomatic detection and diagnosis in childhood compared with usual clinical care? How does this vary by phenotype? How strong is the association between changes in intermediate outcomes of (e.g., biomarkers) of X-ALD and changes in health outcomes?*

- Population: n>3, Newborns and others diagnosed with X-ALD through newborn screening or other methods of presymptomatic detection and diagnosis in childhood
- Interventions: HSCT, adrenal hormone therapy, or other approved therapies.
- Comparators: Contemporaneous or historical controls with X-ALD disease or no comparator
- Outcomes: Changes in intermediate outcomes, such as improvements in biomarkers or physiologic changes which are related to other health outcomes.
- Timing: Any duration of follow-up
- Settings: All settings

Key Question 7: *What are the effects of treatment on secondary health outcomes?*

- Population: n>3, Newborns and others diagnosed with X-ALD through newborn screening or other methods of presymptomatic detection and diagnosis in childhood

- Interventions: HSCT, adrenal hormone therapy, or other approved therapies.
- Comparators: Contemporaneous or historical controls with X-ALD disease or no comparator
- Outcomes: Other important health outcomes, physical or psychosocial, for the patient or family members
- Timing: Any duration of follow-up
- Settings: All settings

Key Question 8: *What are the harms associated with treatments for X-ALD in early childhood, for symptomatic and presymptomatic patients? How does this vary by phenotype (e.g., child-, adolescent-, and adult cerebral ALD?*

- Population: Any child (or caregiver of child) identified with X-ALD receiving a current treatment
- Interventions: Any systematic assessment of harm
- Comparators: Any population or none
- Outcomes: Any description of harm
- Timing: Any duration of follow-up
- Settings: All settings

Key Question 9: *What is the impact of newborn screening on the Public Health of the population on projected numbers affected? On primary, intermediate, and secondary health outcomes?*

Key Question 10*: What is the impact of implementing newborn screening of X-ALD on the Public Health System? What is the feasibility of population-based screening for X-ALD within the United States? What is the readiness of state newborn screening programs to expand screening panels to include X-ALD?*

Technical Expert Panel

A panel of Technical Experts was identified to advise this review throughout its development; members are listed in Table 1. We first met with technical experts to review our scope of review and methods, identify current issues in research and practice, and to describe the typical care standards for newborn screening and treatment procedures to ensure relevance and applicability of the review. Technical Expert Panel members also met to provide input and feedback throughout development of the decision analysis model to estimate the impact of newborn screening on the population. During the review, additional experts were identified and interviewed to further inform unpublished newborn screening implementation and laboratory practices. Further information about the methods to develop the decision model and the role of the Technical Expert Panel members in the process is detailed in Section 4 – Applying Decision Modeling to Project Population Benefit.

Table 1. Technical Expert Panel Members

TEP Members	Affiliation
Michele Caggana, Sc.D., FAC	New York State Department of Health Director, Newborn Screening Program
Ann Moser, BA	Kennedy Krieger Institute Associate Director – Moser Lab
Joanne Kurtzberg, MD	Duke University School of Medicine – Div of Pediatrics Director, Carolinas Cord Blood Bank
Paul Orchard, MD	University of Minnesota Medical Center – Pediatric Blood and Marrow Transplantation
Gerald Raymond, MD	University of Minnesota Medical Center Department of Neurology
Florian Eichler, MD	Massachusetts General Hospital for Children Director of Leukodystrophy Center

2. Evidence Review Results: Newborn Screening for Adrenoleukodystrohpy

Key Questions for Evidence Review for X-ALD NBS

The key topic areas and questions for the systematic evidence review were developed from the general analytic framework used by the Condition Review Workgroup and the specific needs of the Advisory Committee. The technical expert panel on X-ALD will help to refine the specific key questions. The Key Questions guiding the evidence review fall into 4 main topic areas: 1) Natural history and epidemiology with clinical detection, 2) Screening and Short-term follow up, 3) Treatment and long-term care and management, and 4) Public Health Impact – Population-Level Benefit and Public Health System Impact.

Epidemiology and Natural History of X-ALD with Usual Clinical Detection

Key (Context) Question 1: *What is the epidemiology and natural history of X-ALD? Specifically, what are the estimated incidence rates for X-ALD and the typical course of disease (i.e., ages of reported clinical onset and symptoms, diagnosis, treatment initiation, and death)? What are the phenotypes particularly affecting newborns and children (onset <21 years of age)? What factors predict phenotype or severity?*

Prevalence and Incidence Estimates with Clinical Detection

X-linked Adrenoleukodystrophy (X-ALD) is a peroxisomal disorder affecting the adrenal cortex and the central nervous system (CNS) with cerebral demyelination, chronic myelopathy, or peripheral neuropathogy. Individuals affected by X-ALD are asymptomatic at birth. X-ALD has a highly variable phenotype even within families that ranges in age of onset, presenting symptoms, and severity from childhood through adulthood. X-ALD can affect males throughout the lifespan, while most heterozygote females with X-ALD might experience clinical onset only after the age of 60 years. Based on U.S. cases, female heterozygote X-ALD cases are approximately 1.5 times more prevalent than male X-ALD (see Table 2).

Table 2. Estimated U.S. Prevalence of X-ALD[6]

	Estimated Prevalence in the Total Population
All X-ALD cases *(hemizygote males & heterozygote females)*	1: 16,900
Male X-ALD hemizygotes	1: 42,000
Female X-ALD heterozygotes	1: 28,000

Nine published reports, including the U.S. incidence report described above, were identified that present data on the observed frequency or prevalence of X-ALD to estimate population incidence for regions around the world. These studies are summarized in Table 3. Each of the studies

derives estimated incidence rates for the population from retrospective review of clinical records, family or provider surveys, or targeted and extended family screening, and population birth rates relevant to the base cases reported. Prevalence estimates are calculated or reflect data-based observations.

Studies vary in focus on diagnosed X-ALD males with hemizygote ABCD1 mutations, females with X-ALD with heterozygote ABCD1 mutations, or both males and females. Incidence estimates presented are for the total population of males and females with X-ALD in the total population of the region, or for male-only X-ALD patients in the population, as reported in the individual studies.

Table 3. Estimated Prevalence of X-ALD in Published Reports

[RefID]	Study 1st Author, Year	Country/ Region	Incidence Base Years	Total (M&F) X-ALD per 100,000	Male X-ALD per 100,000
3026	Sereni, 1993	France	1956 - 1986		1
4389	Horn, 2013	Norway	1956 – 1995	0.8	
2692	Heim, 1997	Germany	1978 - 1984	0.8	
843	Kirk, 1998	Australia/NZ	1981 - 1996	1.6	
819	Di Biase, 1998	Italy	1990 – 1995		3.6
314	Takemoto, 2002	Japan	1990 – 1999		2 - 3.3
368	Stradomska, 2009	Poland	1994 - 2004		2.9
696	Bezman, 2001	U.S.	1996 - 1998	5.9	2.38
306	Jardim, 2010	Brazil	2002 – 2006		2.8

A 2001 report[6] estimating minimum prevalence of X-ALD in the population from the United States based on laboratory testing results for male hemizygotes identified as at increased risk through extended family screening or clinical findings. Laboratory results positive for VLCFA levels reported from two primary reference laboratories during 1996 – 1998 (Kennedy Krieger Institute and the Mayo Clinic Rochester) were divided by the total number of live births in the U.S., and multiplied by 2.5 to include their estimate of relative frequency of female heterozygotes (regardless of VLCFA level).

Researchers estimated the minimum prevalence of X-ALD to be 1 in 16,900 total X-ALD cases (male and females) in the total population, or 5.9 cases per 100,000 in the population. Male-only X-ALD prevalence based on observed data and population totals was estimated at 1 in 42,000 (2.38 per 100,000 in the population). As Table 3 shows, the estimated prevalence of X-ALD in the U.S. is generally consistent with those reported from other countries, which range from 1 to 3.6 X-ALD males per 100,000.[7-11] Studies with incidence base years prior to and including the 1980s cover periods before the development and established use of VLCFA screening and diagnostic testing, and may reflect lower-bound estimates.

X-ALD –Phenotypic Spectrum

The heterogenity in presentation of X-ALD has led to many different phenotypic descriptions: adreneoleukodystrophy, childhood-, adolescent-, and adult-cerebral ALD, Addison's-only (for adrenal insufficiency only), adrenomyeloneuropathy (AMN) – with or without cerebral demeylenation, and symptomatic and asymptomatic carriers. The differences in symptom onset across the full lifespan, clinical area affected (e.g., adrenal cortex, cerebral or peripheral nervous

system), and severity of symptoms in the affected area (e.g., from borderline to mild to crisis levels) have complicated and delayed diagnosis of X-ALD by a mean of 9.9 years (range 1–33 years).[12] Recent reviews and clinical guidelines have attempted to simplify characterization of disease presentation and progression and to facilitate diagnosis and management of X-ALD.[13-16] The following sections describe the traditional phenotypic classifications and reported prevalence. However, it is important to note that these descriptive classifications, based primarily on predominant symptoms, do not fully capture the changing nature of disease progression and symptom overlap over time.

Clinical Spectrum of X-ALD

Adrenocortical Insufficiency. ("Addison-only"). Adrenal insufficiency is a major clinical symptom of X-ALD-affected males across the age spectrum. Initially, adrenocortical insufficiency affects glucocorticoid function, though ultimately, mineralocorticoid function becomes deficient in about half of X-ALD patients.[17] In approximately 10% of X-ALD males, adrenal insufficiency can be the primary and only symptom of X-ALD (Addison-only). In most cases, however, it can appear years or decades before neurological symptoms (80-92%).[18,19] If neurological symptoms appear, the primary phenotype classification becomes cerebral ALD or adrenomyelonopathy (AMN), depending on the specific neurological symptom features.

Cerebral ALD (CALD). Cerebral demyelination in the cerebral hemispheres is the most rapidly and progressive symptom in X-ALD. This broad phenotype of CALD can affect children, adolescents, or adults, though the majority of CALD occurs in childhood (Childhood cerebral ALD, C-CALD), with onset between 2.5 and 10 years.[15,16] First symptoms typically appear as cognitive dysfunction or declining school performance and behavioral problems. The majority of C-CALD patients experience rapid neurologic decline. For many childhood CALD patients (63%), the interval from first onset of neurological symptoms to a non-ambulatory and non-communicative state or death is 3 years (standard deviation [sd] 2 years).[20] However, approximately 10% of children with CALD develop "chronic or arrested cerebral X-ALD." In this presentation of CALD, the demelinating process spontaneously arrests, the patient stabilizes for a period of 10-15 years, followed by a sudden onset of rapid neurologic deterioration which progresses to the full neuroinflammatory stage of disease.[13] HSCT in this later stage can be effective.

Adrenomyeloneuropathy (AMN). Most individuals with X-ALD who reach adulthood develop myelopathy and neuropathy, usually in their 30s and 40s. Presenting symptoms include gradually progressive spastic paraperesis, sensory ataxia, and other peripheral nerve involvement and spinal cord symptoms. Among AMN (adult) patients, over time about 20% appear to develop cerebral demelination, similar to childhood CALD.[21] Adrenal insufficiency also reportedly co-occurs in about 70% of adult male AMN patients.[22] In a large natural history report of individuals with ALD in the U.S., the majority of AMN patients experienced mild to severe involvement but were not totally disabled, while after a mean of 13 years from onset, 12% had died or were fully disabled.[20]

Asymptomatic X-ALD. While all X-ALD patients are initially asymptomatic in infancy, it appears that some, especially heterozygote females, will remain asymptomatic through at least later adulthood (>60 years).

Frequency of Male X-ALD Phenotypes and First Symptom Presentation

Available published studies report the most frequently occurring phenotype in males as childhood/adolescent cerebral adrenoleukodystraphy (C-CALD), ranging from 33% to 57%, though in the Netherlands, AMN is the most prevalent (46%) reported form of the condition.[13] Adrenal insufficiency, occuring without other symptoms of cerebral demyelination or neuropathy, is estimated at 10% (referred to as "Addison's-Only (AO))". The olivo-ponto-cerebellar (OPC) form, described as affecting the cerebellar and brain stem involvement in 1-2% of adolescents or adults,[23] has a higher reported prevalence in Japan.[24] This X-ALD phenotype has not been described in other epidemiological reported identified. Table 4 summarizes reported phenotype frequencies in identified studies for the U.S. and other world regions.

Note that these classifications do not fully reflect the evolving and overlapping nature of symptoms that present in X-ALD. In the typically reported phenotype classifications, adrenocortical dysfunction is listed only when occurring in the absence of neurological involvement (i.e., as "Addison's-only"). However, a study evaluating 49 boys (mean age 4.5 years, sd 3.8) diagnosed with X-ALD through family risk who were asymptomatic found 80% of subjects to show abnormal or borderline adrenal function at baseline.[18] assessed by ACTH levels and ACTH stimulation. The mean age of onset of adrenal insufficiency was 4.8 years (sd 3.7 years, range 5 months to 13 years). Researchers report that "70% of patients studied by 2 years of age already showed increased serum ACTH levels." (p. 531). Another study of 55 child and adult X-ALD patients (median age 11 years, range 2-59 years) reported endocrinological symptoms (adrenal impairment) in at least 60% of subjects, of whom at least 61% (n=21) presented adrenal impairment in combination with neurological symptoms[19]. In a review of presentation of first symptoms among 485 case records of boys with X-ALD, 45 (9.3%) presented acutely at a mean age 5.5 years. Of these 45 acute cases, 44% presented with acute adrenal crisis, and 44% with seizures, and 11% with encephalopathy or coma[25].

Table 4. Reported Phenotype Distribution among X-ALD Males (%), by World Region

	U.S.	SPAIN	NETHERLANDS	AUSTRALIA/ NZ	JAPAN
Study Authors	Moser et al.[20]	Ruiz et al.[26]	van Geel et al.[27]	Kirk et al.[10]	Takemoto et al.[24]
Pub Year	1992	1998	1994	1998	2002
N	n=1,475	n=60	n=77	n=96	n=154
Phenotypes					
Addison's Disease Only	8	12	14	16	0
CCALD/ AdolCALD	57	33	31	52	40 *(29.9, 9.1)*
ACALD	3	16	1	2	21.4
AMN	28	27	46	25	25.3
Asymptomatic	4	12	8	5	4.5
OPC (olivo-ponto-cerebellar)					8.4%

Screening, Short-Term Follow-Up, and Diagnostic Confirmation

Key Question 3: *Methods. What are the screening and short-term follow up/diagnostic confirmation methods available and what is the evidence regarding effectiveness?*

Key Question 2*: Newborn Screening Outcomes. What is the direct and indirect evidence that newborn screening for ALD disease leads to improved health outcomes compared to usual clinical care?*

Key Question 4*: Harms of Screening. What are the harms associated with newborn screening for X-ALD to the individual or the family?*

Screening and Diagnostic Strategies

Screening Methods

Measurement of very long chain fatty acids (VLCFA) as a means to identify individuals at increased risk for or affected by X-ALD was first reported in 1981[28] and laboratory methods refined across two decades to validate detection of X-ALD and related perxisomal disorders.[29] Development of this laboratory test progressed to high-throughput screening methods for X-ALD using assays of very long chain fatty acids (VLCFA), specifically the 26:0-lysophosphatidylcholine (C26:0 LPC).[1,29-32] measured in dried-blood spots (DBSs). Currently, the only method actively used in population-based screening is a two-tier, tandem mass spectrometry (MS/MS) approach implemented by the NY State Health Department Newborn Screening Program.[33] This program uses flow-injection (FIA-MS/MS) for Tier 1 screening of VLCFA levels (specifically C26:0-LPC), followed by a liquid chromatography-tandem mass spectrometry procedures (LC-MS/MS) for Tier 2 retest of VLCFA indicators. The NY newborn screening laboratory procedures currently multiplex X-ALD screening with screening for Krabbe and Pompe disease. Screening was developed to target males, who are more severly affected. Because heterozygote females may have intermediate levels of VLCFA, they may be missed by screening. It has been reported that 15-20% of heterozygote females have "normal" VLCFA plasma levels.[1,16] Screening can also identify secondary peroxysomal conditions, including Zellweger syndrome, which are more rare than X-ALD, associated with death in early infancy, and for which no specific treatment exists.

Researchers at the Mayo Clinic in Rochester, Minnesota[34] are conducting a comparative trial of three different methods for multiplex screening of 13 lysosomal storage disorders (LSDs), Friedrich's ataxia, Wilson's disease, and X-linked adrenoleukodystrophy. The report indicates that X-ALD screening for VLCFA levels may be multiplexed with at least 3 other secondary target disorders (Zellweger syndrome, Acyl-CoA oxidase deficiency, and Bifunctional protein deficiency). Researchers reportedly are testing a more efficient screening method using FIA-MS/MS to multiplex screening for these same 4 conditions, as well as at least 5 other LSDs (Fabry disease, Gaucher disease, Krabbe disease, MPS-I, Pompe disease, and Niemann-Pick A/B). This study is analyzing prospectively 100,000 anonymous dried-blood spots provided by the California newborn screening program, with the aim to identify an effective and efficient testing approach. The study reportedly will include a comprehensive comparative cost analysis of resources needed for each approach (e.g., equipment, space, consumables, hardware, software, personnel effort, repeat rate). In addition, the researchers plan to develop a web site resource for

data, analytical protocols, reference and disease ranges, and interpretive- guides. Results of this study are not yet reported. Of note, approximately 15-20% of females with X-ALD have VLCFA levels within normal limits as measured in plasma,[1,16] suggesting the increased likelihood of missing female carriers. Since female carriers are not a target of newborn screening, for this report, we did not consider this to represent false negatives.

Technical Validity

The systematic evidence review considers and includes prospective studies of screening with diagnostic confirmation. These criteria allow reporting of potentially robust evidence about the expected clinical utility of newborn screening for X-ALD. In development of screening methods, identification of studies which report data on preliminary analytic validation using other non-prospective study designs provide evidence that may contribute to demonstration of availability of technically feasibile high-throughput screening methods.

We identified at least four studies which evaluated MS/MS assays to identify C:26 VLCFA levels in dried-blood spots from subjects with X-ALD disease and normal controls by comparing detected C:26 activity levels in anonymous dried-blood spots or dried blood spots.[1,30-32] In each study, accuracy was high, with the ability to distinguish study cases from normal and affected case controls.

Prospective Pilot Screening

One of these case control studies[1] also included a small propsective pilot research study to test ALD screening methods on additional punch samples (n=4689, 2608 males and 2392 females) retrieved from 5,000 consecutively collected prospective newborn screening samples (2008 to 2010). Informed consent was obtained for research use and follow up contact of any positive screening results. No positive screens were identified to require follow up or as false-positives, suggesting a specificity of 1.0. No further follow up was conducted to validate these results, with males or females.

Current Population-based Screening Programs

The literature search did not identify any published reports on outcomes from propsective, population-based screening for X-ALD.

X-ALD in Newborn Screening Programs in the United States

In the United States, to date, four states, California, Connecticut, New Jersey, and New York, have passed legislation since 2013 mandating newborn screening for X-ALD. The New York newborn screening program is the only state that is currently implementing population-wide screening and reporting results to the state public health newborn screening program.[33] The New York NBS program began full population-based live screening in December 2013, and have screened over 363,755 newborns (50.9% males, n=185,097) through July 2015. Table 5 summarizes screening results from NY State NBS for X-ALD through July 2015.

Table 5. Summary of NY State Newborn Screening for X-ALD (Dec 2013- July 2015)

Total 363,755 newborns screened (Dec 30 2013 – July 2015)
- TIER 1: Re-test rate (same specimen)= 6,679 of 363,755 *infants* = 1.84%
- TIER 2: Repeat rate (independent specimen)= 43 borderline retest results, repeats requested of 363,755 *infants* tested = 0.012%

[405,415 total specimens received, 1,407 ambiguous/unknown/blank specimens]

- **33 referrals** (Referral rate= 33 of 363,755 infants = 0.0091%)

RESULTS

Of 33 Referrals:
- 13 male ABCD1 mutation ……7 confirmed X-ALD with Repeat VLCFA level (Results Pending on 6)
- 14 female heterozygous ABCD1 mutation
- 4 ZSD/PBD…… 3 confirmed ZSD
- 1 Other (Aicardi-Goutieres syndrome) ……..1 confirmed

Diagnosis

Diagnosis of X-ALD is confirmed based on the presence of elevated serum VLCFA, as determined by C:26-LPC. Genotyping of the ABCD1 gene is supportive of the diagnosis, but the lack of genotype-phenotype correlation makes this test less helpful in predicting later phenotype. As described above, the New York state NBS program conducts preliminary in-house genotyping for ABCD1 mutations for positive Tier 2 screens at the time of referrals for confirmatory diagnosis of X-ALD. Further follow up and ongoing clinical assessment of adrenocortical function, MRI, and neurological exam confirm specific phenotype and symptom onset, providing information for indicated treatment(s).

Genetics of X-ALD

X-ALD is caused by mutations of the ABCD1 gene located at Xq28. This is the single known cause of X-ALD. The ABCD1 gene encodes adrenoleukodystrophy protein (ALDP), which supports transport of very long-chain fatty acids (VLCFA) into peroxisomes. ABCD1 abnormalities result in ALDP deficiency, impairing VLCFA beta-oxidations and leading to elongation of VLCFA.

No genotype-phenotype correlation. More than 600 mutations of the gene have been described[35] (see: **http://www.x-ald.nl**). Most mutations are unique and there is no recognized genotype-phenotype correlation within or across families, even with identifical gene mutations.[36,37] A description of the phenotypes appears in the next section.

De novo mutations. Targeted mutation analysis within 489 XALD families identified 20 cases of *de novo* mutations which arose in the mother or maternal grandmother of the index case. This finding supported a new mutation rate of 4.1% in a U.S. sample.[38] Studies across Europe and Asia have reported de novo mutation rates ranging from about 5% to 19%,[2,39,40] with one report[2]

noting that about 93% of index cases in the ALD database inherited mutations from one parent, and suggesting consistency with reported rates of new mutations.

Potential Harms of Newborn Screening for X-ALD

Screening results provided by the NY NBS program indicate that false-positive screens were low to zero after Tier 2 screening was complete. All newborns with a positive screen for VLCFAs were confirmed to have an ABCD1 mutation for X-ALD or other related disorders. Screening is expected to miss up to 20%[1] of females heterozygotes. However, heterozygotes are not the target of screening. To date, no confirmed false-negative screening results have been reported among males with X-ALD.

Attitudes about Presymptomatic Detection Among Families Affected by X-ALD

Two older studies and one case report were identified that describe interview and survey data from family members and patients affected by X-ALD regarding attitudes about presymptomatic detection of X-ALD.[41-43] The case report[43] of an interview with a mother of a son with X-ALD described her experience with genetic counseling. The subject identified the main focus on genetic counseling as providing information, with disregard for counseling or emotional support needs. The subject further identified three main needs during genetic counseling, defined by her multiple roles in the process as a) mother of 2 sons diagnosed with X-ALD, b) family messenger of at-risk status to other family members, and c) newly diagnosed female with X-ALD, with likely later-onset of symptoms. These roles required health information for herself and for other family members, as well as emotional support, not fully provided by the genetic counseling. Two studies[41,42] sent out surveys to family members affected by X-ALD to assess whether they would support presymptomatic screening for X-ALD. Results of the 2 studies were concordant, with the large majority of family members who completed surveys indicating that they would participate in presymptomatic screening for sons (88.7% - 93%) and daughters (89% - 95.4%). The more recent study (2007)[42] further reported that 89.3% of families and patients preferred screening males at birth or prenatally (89.3%), while 8.2% preferred screening before age 4 (but not as a newborn), and 2.5% preferred screening of males after age 4. Attitudes about ages at which to screen females showed that 51.2% preferred screening at birth or prenatally, 31.7% supported screening females before age 18 years (but not as newborns), and 14.6% preferred screening females for X-ALD after age 18 years. Reasons for screening included ability to address health implications, early treatment options, financial planning, and need to inform other family members.

No study directly evaluated the potential harm to individuals associated with family testing resulting from case identification, either clinically or through screening. No study directly evaluated potential harms related to short-term follow-up following the identification of asymptomatic indivuals with X-ALD.

Summary – Screening and Short Term Follow Up

- Measurement of VLCFA levels effectively screens males for X-ALD. This approach misses approximately 15-20% of females for X-ALD, who are not a target of newborn screening.

- Confirmation of elevated VLCFA levels in plasma samples is a standard diagnostic procedure in males. Mutation analysis of the ABCD1 gene can diagnose both males and females.
- A three-tier screening approach for X-ALD appears to be effective for identifying cases of X-ALD and can be used in a high-throughput setting. Data from the New York NBS program demonstrated that X-ALD is able to be multiplexed, or screened in tandem with other lysosomal storage disorders, including Krabbe and Pompe.
- Of 363,755 newborns screened to date in NY State, 13 males and 14 females with ABCD1 mutations have been identified. Of these, 7 males have confirmed diagnoses of X-ALD by repeat VLFCA level measured in a diagnostic laboratory.
- Little is known about the harm related to family testing or the process of follow-up for individuals with asymptomatic X-ALD.

Treatment and Long-term Follow Up

Key Question 5: *What are the standard treatments for X-ALD and evidence for their effectiveness? Do follow-up protocols exist for the management of X-ALD that do not require immediate initiation of treatment? What is known about the effectiveness of follow-up protocols in modifying intermediate health outcomes?*

Does early initiation of treatment improve primary health outcomes (overall survival, other important health outcomes) when the condition is caught early or through newborn screening compared with usual clinical care? How does this vary by phenotype?

Key Question 6*: Compared with usual clinical care, does initiation of treatment when X-ALD is detected through newborn screening or other methods of pre-symptomatic identification modify intermediate health outcomes of X-ALD? How does this vary by phenotype? How strong is the association between changes in intermediate outcomes of (e.g., biomarkers) of X-ALD and changes in health outcomes?*

Key Question 7*: Does initiation of treatment when X-ALD is detected through newborn screening or other methods of pre-symptomatic identification modify secondary health outcomes of X-ALD?*

Key Question 8*: What are the harms associated with treatments, interventions, or follow-up care for X-ALD in early childhood, for symptomatic and presymptomatic patients? How does this vary by phenotypes with childhood onset? (e.g., child-, adolescent cerebral ALD, adrenal insufficiency/Addison's only)?*

Standard Treatment(s) for X-ALD

The two X-ALD phenotypes for which there are primary treatments are in boys with :

1. adrenocortical insufficiency, where steroid replacement therapy may be used, and
2. the childhood/adolescent form of CALD, where HSCT can stop the progression of cerebral white matter deterioration (demyelination), especially when provided at early stages of cerebral involvement.

Monitoring of MRI severity ratings and neurologic function have been found to predict disease progression and HSCT outcomes. Patients in early stage cerebral ALD have been shown to benefit from treatment compared to those with more advanced cerebral involvement. Patients who remain asymptomatic, and patients with higher MRI severity and neurologic impairment are not recommended for transplant, and may experience poorer outcomes from this treatment.

Effectiveness of Early Intervention

Disease Status Measures

Across the studies, common outcome measures include mortality, the presence of adrenal insufficiency, and, for C-CALD, neurocognitive development and neuropsychological status based on a variety of scales, and the degree of cerebral involvement on MRI-based Loes score. Depending on the particular study, these disease status measures can be outcome or predictor measures.

Adrenal Impairment and Insufficiency: ACTH Levels
Measurement and monitoring of adrenal impairment among asympomatic boys with X-ALD is recommended through assessment of serum ACTH levels and ACTH stimulation tests[13,18]. Evidence shows a significant positive association between increasing adrenal dysfunction and age. Although clinical signs of adrenal dysfunction may not be apparent, ACTH levels and stimulation tests have indicated abnormal and borderline function before age 1.[18]

Cerebral Invovlement: Correlated with MRI Severity (Loes Score)
The Loes score was developed as a standardized approach to measure brain involvement in the MRIs of X-ALD patients. As first reported in 1994, the Loes score indicates severity on a scale from 0 to 34 (i.e., higher score indicating greater severity of involvement)[44]. Points are assigned based on location and extent of involvement and the presence of focal and/or global atrophy. Studies confirm the association of the Loes score with functional status. Among a sample of 7 subjects with CCALD (mean age 8 years, none treated with HSCT at the time of the reported assessments, mean Loes score 12.73, performance-intelligence quotient (IQ) mean z-score -2.89) and 8 asymptomatic boys with X-ALD (mean age 8 years, mean Loes score 2.25, performance-IQ mean z-score 0.04), the correlation (*r*) between the Loes score and performance-IQ was -0.764 ($P < 0.0001$).

Without HSCT, observational data suggest that the Loes score among individuals with CCALD worsens over time. One study evaluated the MRI patterns of 140 individuals with X-ALD who did not receive HSCT during the follow-up period of the study.[45] Each MRI was reviewed by two physicians (inter-rater reliability based on correlation: 0.98). Among the subjects, 80 were <10 years and most (80%) had a specific pattern on initial MRI (primary involvement of white matter in the parieto-occipital lobe or splenium of corpus callosum). The mean age of all subjects with this pattern on initial MRI was 10 years. Based on a subset of 34 patients who had contrast MRIs, a prediction formula was developed for Loes score after 1 year: 2.28 * enhancement – 0.07*initial age + 1.05 initial MRI score + 0.87 (enhancement = 1 if perilesional gadolinium enhancement was present; 0 otherwise). These findings support the hypothesis that Loes scores worsen after the initial MRI for boys with this pattern of findings. There are several important limitations of the work: although the individual covariates in this model were reported

to explain 96% of the variability, the model was not validated against another independent dataset; the model only predicts a 1-year change in Loes score from the initial MRI findings; and the applicability of this model to newborns detected through newborn screening is unclear.

Two more recent studies from 2013[46] and 2015,[47] also examine pretransplant and posttransplant Loes scores and other clinical and neurologic assessments with samples of boys with CCALD. The first study includes 8 boys with ALD (mean age 7.9 years, sd 1.55 years) in the U.S. who met clear eligibility criteria, received at least 5 repeated MRI and clinical measures under a standard protocol pre-HSCT <45 days pre-HSCT, and at least 4 repeated measures post-HSCT (T1: 30-60 days, T2: 90-120 days, T3: 180 days, and T4: 1 year after HSCT.[46] A comparison group of age- and sex-matched healthy subjects with normal MR images and no neurologic diagnosis were included for one-time assessment of diffusion tensor imaging (DTI) measure of interest in the study. Pre-HSCT Loes scores among patients with ALD were mean score 8.7 (sd 4.99), and median 11 (range 2-13). At 1-year post-HSCT follow up, 6 of the 8 patients had stablized, while 2 experienced declines in visual, auditory, or motor skills. Overall, post- Loes score were significantly correlated with other clinical scores (DTI measures, neurologic function scores, and severe neuropsychologic scores) at 1 year post-HSCT ($p<0.05$).

The second recently published study[47] from Poland included 7 boys diagnosed with CCALD who underwent HSCT, and had conventional pre- and post-HSCT assessments with MRIs, scored with Loes severity rating scale, DTI, and VLCFA levels. Mean age at HSCT was 8.14 years (range 5-10 years). Two of the subjects were diagnosed pre-symptomatically (ages 5 and 9 years, MRI scores 7 and 6, respectively). The other 5 subjects were 7 to 10 years of age, with mean MRI score of 10.9, range 8 to 16). After transplantation (conventional follow up at 11 weeks) the two boys with MRI severity scores <8 showed no clinical or MRI progression (no change in Loes scores). The 5 boys with higher pre-HSCT MRI severity scores (mean 10.9) all experienced clinical progression following transplantation, with a mean post-HSCT MRI score of 20.9 (range 16.5 to 25). One subject died (age 7 years, MRI severity score 17).

Effect of Early Intervention Based on Adrenal Insufficiency

Although experts suggest that one of the benefits of early identification of X-ALD is the treatment of adrenal insufficiency before cases would otherwise come to clinical diagnosis, no report that met inclusion for this report was identified that describes outcomes related to the treatment of adrenal insufficiency before clinical case detection. At least two reports.[8,18] identified X-ALD patients with adrenal insufficiency who were noted to receive adrenal hormone/steroid replacement therapy. One study did not follow up or report outcomes of the 80% - 86% of X-ALD boys with signs of impaired adrenal function.[18] Another study noted that 7 of 11 male X-ALD patients received steroid replacement therapy, and described present dosages, suggesting survival and favorable outcomes of the therapy.[8] This study, as well as guidelines, state that steroid replacement therapy is “effective” and “straightforward”[48] for adrenal insufficiency, though no empirical evidence is reported or has been identified within this X-ALD review. We were not able to identify reports describing morbidity or mortality related to untreated adrenal insufficiency in patients with C-CALD. HSCT does not resolve the need to treat adrenal insufficiency.[49] It is unknown whether early HSCT could prevent the development of adrenal insufficiency.

One case series[50] provides evidence that delayed recognition of adrenal insufficiency as a sign of X-ALD leads to worse outcomes. This study compared the outcomes for 7 cases of C-CALD with a delay of diagnosis of more than 12 months after identification of adrenal insufficiency (median age when adrenal insufficiency diagnosed: 4.9 years) to 10 cases who were diagnosed within 12 months of developing adrenal insufficiency (median age when adrenal insufficiency diagnosed: 6.3 years) from a database of 90 subjects with X-ALD. The study reports that adrenal insufficiency was the presenting sign in 40% of the cases of C-CALD. The causes of delay were not described. Two in the late diagnosis group and one in the early diagnosis had not received HSCT. Among those treated, the median Loes score was worse pre- and post-transplant for those with a "late" (i.e., >12 months after adrenal insufficiency, median Loes score ≥12) relative to those with an "early" (i.e., ≤12 months after adrenal insufficiency, median Loes score ≤10). This study does not provide direct evidence about identification in infancy because of the ages of the children.

Effect of Early Intervention Based on Neurologic Impairment in C-CALD

Case studies and case series demonstrate that HSCT leads to improved survival and neurodevelopmental and neuropsychological outcome for individuals with C-CALD.[49-60] For example, one comparative case series described 19 cases of C-CALD treated with HSCT compared to 30 cases that did not receive HSCT, matched on the degree of neurological impairment and Loes score.[54] The cases were selected from a database that included 283 subjects. The average age of onset for the group that did not receive HSCT was 6.9 years and was 7.8 years for those who did receive HSCT. The average Loes score at baseline was 4 for the untreated group and 3.5 for the group that received HSCT. The probably of survival at 5 years was 95% among those who received HSCT, and 54% among those who were not transplanted.

The present report focuses on the benefit of HSCT to improve health outcomes in early-detected cases of C-CALD. As a proxy for addressing this issue, studies have instead focused on the degree to which HSCT with less disease involvement leads to better outcomes (e.g., lower Loes score).

One report[58] described a study to determine prognostic factors based on 12 subjects with HSCT for C-CALD between 1995 and 2000. There were two other subjects that were excluded from this analysis because they died from HSCT-related complications. The subjects were divided into two groups of 6 subjects: one that deteriorated (including 2 deaths) and one that had stable neurological or neuropsychological outcomes over the follow-up period of 1.9 to 5.5 years. Across all subjects, the age of onset of symptoms ranged from 5.1 years to 12.3 years and the age of HSCT ranged from 5.9 years to 12.9 years. One subject, who became symptomatic at 5.9 years of age, had a 4-year period before HSCT and then died 6 months later. The other subject that died developed symptoms at 6.1 years, received HSCT at 7.5 years, and died 6 months later. In this case, the time from onset to HSCT was similar for those who survived. These two subjects along with a third had "rapidly progressive" neurologic involvement after HSCT. These three subjects also had the highest degree brain involvement by MRI prior to HSCT. The study was underpowered to develop a prediction rule for HSCT. However, the authors proposed an algorithm that recommends HSCT for individuals with the development of signs or symptoms of progressive disease but against HSCT for those with a Loes score >10, marked cognitive

impairment (performance IQ < 75), or severe neurological symptoms. Limitations of this work include an insufficient sample size to develop the algorithm and lack of information about how these subjects were identified and referred for treatment. There is variation in timing of the outcome assessments.

Similar findings were reported from two other case series. One included 126 subjects treated with HSCT from 1992 to 1999, of which complete data were available and analyzed for the 94 subjects with CCALD (median age 9.1 years), most (71%) identified after development of symptoms.[57] The eight-year survival across the group was 56% (95% CI: 44%-68%). The likelihood of survival 5 years after HSCT was significantly greater (p<0.01) for those subjects with at most one neurological deficit and Loes score < 9 (92%, 95% CI: 81%-100%) compared with patients with ≥2 neurologic deficits or Loes score ≥9 (45%, 95% CI: 23%-67%).

The other case series evaluated 60 cases of C-CALD within a single center.[52] The median age at HSCT was 8.7 years and the overall survival rate was 78% at 3.7 years. As illustrated below, the likelihood of survival at 5 years was significantly greater for those with a Loes score <10 at the time of HSCT compared with patients with a baseline Loes score ≥10 (89% vs. 60%, respectively, p=0.03).

Another case series suggests that better neurologic outcome is also associated with HSCT with a lower Loes score.[55] This report describes 12 subjects with median age 7 years (range: birth to 9.75 years) who received HSCT at a median age of 7.1 years (range: 2.4 years to 11.7 years). One child died after initiation of chemotherapy thought to be related to an adrenal crisis and two others died due to complications of HSCT. Another subject required a second HSCT. Patients with a Loes score ≤10 showed improved cognitive and gross motor outcomes. Patients with Loes scores >10 experienced cognitive and motor function below average. The case series did not evaluate the relationship between how the cases were identified and these outcomes.

We identified one case report[53] that described the outcomes of a case identified during prenatal testing because of the death of a sibling with C-CALD. At 16 months of age, the child had a Loes score of 2.5 and spasticity in one foot. The child received HSCT based on worsening MRI findings. Twenty-two months after transplant, the subject was neurologically normal with a Loes score of 1.5. This case is unusual because of the young age at the time of HSCT.

A second report[61] described outcomes of 4 patients with ALD, 3 of whom are brothers. Patient 1 presented with attention deficits and clumsiness at age 9.5 years, was diagnosed with ALD at 10 years, with overall pre-transplant IQ of 82-88, and transplanted at 10.5 years of age. At 3.5 year follow up, his neurological symptoms have continued to progress, with clinical signs of dementia. Patients 2 and 3 each presented with adrenal insufficiency at 6 and 4.5 years and transplant at 8 and 6.5 years, respectively. Patient 2 had variable cognitive skills ranging from normal to -3 Z-scores below normal. At 1.5 years post HSCT, his disease had progressed and the boy died. Patient 3 (Patient 2's younger brother), who was identified early, remains health at 3.5 years post-transplant, with no decline in overall IQ (remaining in the average to high average range across cognitive functions). Patient 4, also a younger brother of Patient 2 who was

identified early and with no presenting symptoms. He is followed closely, continues to have normal MRI, though has shown deficits in attention and sensorimotor functions on neuropsychological testing. A search for a matching HLA-identifical donor is underway in case a future transplant is needed.

The treatment studies demonstrate that outcome is significantly worse with a Loes score above 9. The Technical Expert Panel described current clinical care as recommending HSCT as early as possible upon the confirmation of CCALD, including any radiographic evidence of progressive neurologic involvement (e.g., Loes score of 0.5 increasing to 1.5). The rationale is that CCALD is a progressive disease and that treatment does not restore lost function, neurologic status can rapidly worsen, and that poor outcomes are expected with Loes score of 9 or more.

Effectiveness of Presymptomatic Detection – Unpublished Evidence

No study included in this review specifically evaluated outcomes for a series of individuals identified presymptomatically compared to usual clinical case detection apart from small case series typically focusing on individual families. Newborn screening is too recent to provide information regarding long-term outcomes of early detection. Researchers in the field have focused on outcomes based on the degree of involvement at the time of identification or at the time of HSCT. To address this gap, two data sources were identified related to C-CALD. Findings are described in the next two sections. The subjects described below do not overlap.

Unpublished Data – C-CALD Patients at a Single Center
To address this gap, data were obtained regarding patients with CCALD evaluated at a major U.S. medical center in the northeast from 2006-2015 and who had a pre-treatment Loes score on record[1]. For each patient, the baseline Loes score for this analysis was taken from the one on record, even if obtained from another institution. . However, this does not necessarily reflect the first Loes score measured on each patient.

Sample characteristics. Overall, 30 patients had baseline Loes score on file, of which 17 were detected through family testing (F) and 13 were detected by symptoms (S). Primary health outcomes data were available for 19 subjects (7 F, 12 S) regarding survival (alive or dead) and alive with major disability (non-communicative and non ambulatory). Outcomes information for the remaining 11 subjects was not available due to participation in ongoing treatment trials. These patients were excluded from the present analysis.

HSCT status Of the 19 included in the present analyses, 3 of 7 in the family-testing group (43%) and 7 of 12 (58%) in the symptom-detected group received BMT (p=0.65). For the family-testing group the age of HSCT was 7 years (IQR 4-9) compared to 8 years (IQR: 6-9) in the symptom-detected group (p >0.99). The reason for no HSCT in the family-testing group was that 1 subject was undergoing pre-transplant evaluation and 3 subjects had arrested cerebral ALD (e.g., no progression of the cerebral lesions, often preceded by the disappearance of contrast enhancement). In contrast, among the symptom-detected group, 4 had advanced disease and 1 had self-halted ALD (p=0.05).

[1]*F. Eichler, MD, and colleagues, Personal communication. Confidential, unpublished data from F. Eichler, MD for the express purpose of this review only. Not for distribution without permission from Dr. Eichler.*

MRI Severity: Loes Scores
The median age of first Loes score for those detected by family testing was 5 years (interquartile range [IQR]: 4-6) and 7 years (IQR: 6-9) for those detected by symptoms (p=0.19).
For the subjects detected by family testing, the first Loes score was 0 (IQR:0-1) compared to 12 (IQR:11-20) for those detected by symptoms (p<0.001).

The most recent Loes scores among the family-detected group, which occurred at a median age of 10 years (range 8-15) was 3 (IQR:2-4). The most recent Loes scores among the survivors in the symptom group, which occurred at a median age of 11 years (8, 11, and 19), was 11, 12, and 20, respectively.

Primary Health Outcomes
Primary health outcomes (survival [Live], non-ambulatory and non-communicative [NANC], and Dead) are presented in Table 6 for the overall group and by detection method, regardless of treatment (HSCT) status.

Table 6. Primary Health Outcomes Overall and by Detection Group

	status			
detection	Live	NANC	Dead	Total
F	7	0	0	7
	100.00	0.00	0.00	100.00
S	3	7	2	12
	25.00	58.33	16.67	100.00
Total	10	7	2	19
	52.63	36.84	10.53	100.00

Fisher's exact = 0.004

The median age for NANC boys was 7 years (IQR:6-9) and the two deaths were at age 7 and 12 years. The known age of survival (i.e., the last known follow-up age) for those detected by family testing was 10 years (IQR:8-15) and was 8, 12, and 19 years for those detected by symptoms.

HSCT vs. no HSCT
Primary health outcomes by detection group for subjects treated with BMT compared with those who did not receive a BMT are presented in the table below.

Table 7. Primary Health Outcomes by Detection Group for Patients with HSCT v. no HSCT

detection	status: Live	NANC	Dead	Total
F	3	0	0	3
	100.00	0.00	0.00	100.00
S	3	3	1	7
	42.86	42.86	14.29	100.00
Total	6	3	1	10
	60.00	30.00	10.00	100.00

Fisher's exact = 0.625

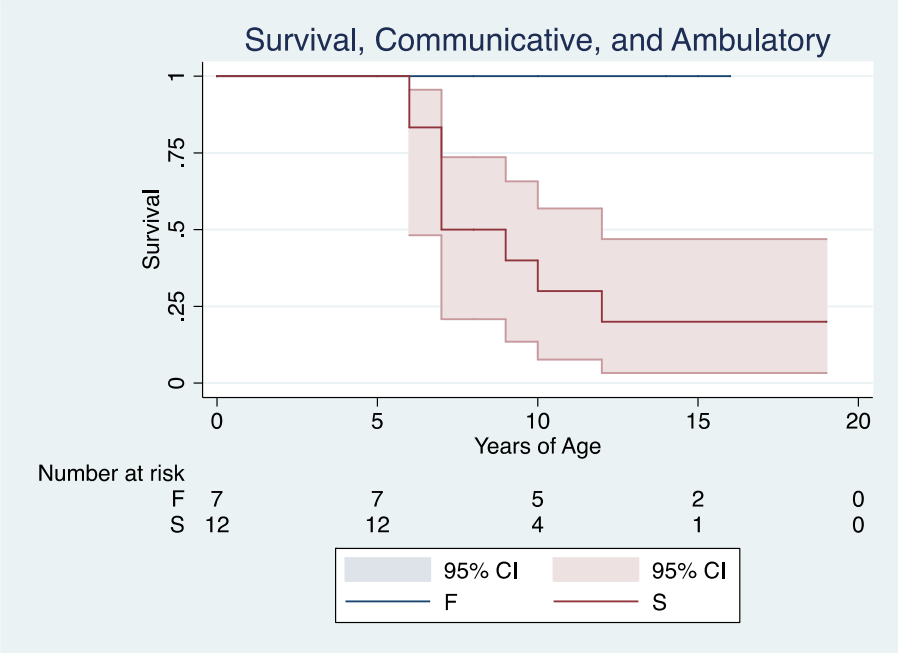
The following table summarizes key elements from the above description. Because of the small sample size, p-values are only provided for the baseline data.

Table 8. Primary Outcomes and Ages by Detection Group –U.S. Single Center (n=19)

Primary Outcomes	**Detection Group**		Statistical Sig of Differences *(p<0.05)*
	Family-risk (F) (n=7)	**Symptom (S)** (n=12)	
First Available Loes Score (median)	0 (IQR 0-1)	12 (IQR 11-12)	$p<0.001$
Median Age at First Available Loes	5 years (IQR 4-6)	7 years (IQR 6-9)	$p<0.19$
Most Recent LOES Score (median)	3 (IQR 2-4)	12 (IQR 11-20)	
Median Age at Most Recent Loes Score	10 years (Range 8-15)	11 years (IQR 8-19)	
Received HSCT	3 (43%)	7 (58%)	
Median Age at HSCT	7 years (Range 4-7)	8 years (IQR 6-9)	
Survival with mobility and communication	7 (100%)	3 (25%)	
Median Age at last known follow up of survivors	*10 years*	*12 years*	

The following figure compares outcomes for individuals with CCALD, regardless of treatment. This Kaplan-Meier curve illustrates the combined outcome of survival and communicative and ambulatory. Too few deaths occurred within the series to separately evaluate these outcomes.

Figure 1. Kaplan-Meier Combined Survival Curve (Live, Communicative, and Ambulatory) for C-CALD Patients



Unpublished Data: C-CALD Patients Treated at Multiple Sites
Sample Characteristics. We obtained data from an international, multicenter, retrospective chart review study of patients with C-CALD treated with HSCT treated in 2001 or later.[2] The present data included 59 patients, 25 who were detected through extended family testing and 34 patients who were detected after the development of symptoms. These data did not overlap with the data obtained from the unpublished single-center previously described.

HSCT Status
In the present data, all subjects included were treated with BMT. The median age at BMT was similar for family-detected and symptom-detected groups (8 [IQR:5-9) years vs. 8 [IQR:7-10] years, *p*=0.44).

Primary Outcomes
MRI Severity: Loes Scores. Among those detected by family testing, the median age at the first recorded Loes score was 8 years (IQR:5-8), similar to symptom-detected cases (median age 8, IQR: 6-9 years, *p*=0.27). However, the Loes score was lower for family-detected vs. symptom-detected cases (4 [IQR:2-5] vs. 7.5 [IQR: 3-11], *p*=0.02).

Mortality. There was 1 subject in the family testing group that died (4%; age=18 years) and 8 (24%) in the symptom-detected group that died (*p*=0.07; age 13.5 years [IQR: 8-14]). Among

[2] *Asif Paker, MD, MPH; BluebirdBio; Personal communications. Confidential, unpublished data from A. Paker, MD, MPH for this review, manuscript in preparation. Not for distribution without permission from Dr. Paker and BluebirdBio.*

the survivors, the most recent available Loes scores, at age 10.5 years (IQR:9-14.5) for those detected by family testing and at age 10 years (IRQ: 8-13) for the screening detected subjects (p=0.68) was 5.75 (IQR: 2-11.5) and 13 (IQR: 6.5-18) respectively (p=0.02).

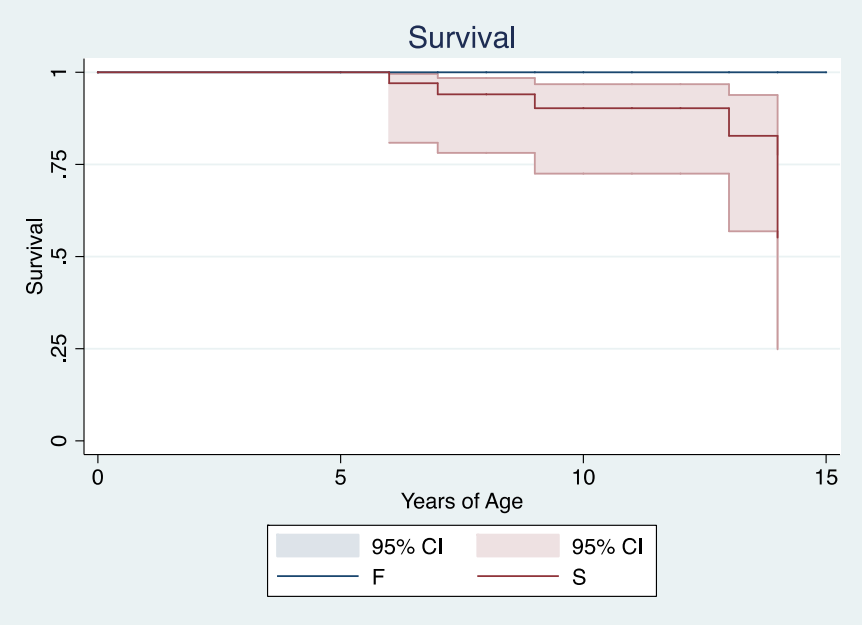
The following table summarizes the primary outcomes and ages, by detection group, described above.

Table 9. Primary Outcomes and Ages by Detection Group – Multicenter (n=59)

Primary Outcomes	**Detection Group**		Statistical Sig of Differences *(*p<0.05)*
	Family-risk (F) (n=25)	**Symptom (S)** (n=34)	
Received HSCT	25 (100%)	34 (100%)	
Median Age at HSCT	8 years (Range 5-9)	8 years (IQR 7-10)	p=0.44
First Available Loes Score (median)*	4 (IQR 2-5)	7.5 (IQR 3-11)	*p<0.02
Median Age at First Available Loes	8 years (IQR 5-8)	8 years (IQR 6-9)	p<0.27
Most Recent LOES Score (median)*	5.75 (IQR 2-11.5)	13 (IQR 6.5-18)	*p<0.02
Median Age at Most Recent Loes Score	10.5 years (IQR 9-14.5)	10 years (IQR 8-13)	p=0.68
Deaths	1 (4%)	8 (24%)	p=0.07
Median Age at death	18 years --	13.5 years (IQR 6.5-18)	*p=0.02

The following figure illustrates survival through age 15, the last age at which at least 5 subjects in each group were followed up. Seven family-detected and 5 symptom-detected subjects were followed through age 15. Attrition was similar in the two groups, with 18 of 25 (72%) family-detected subjects and 22 of 34 (65%) symptom-detected subjects lost to follow-up prior to age 15. There were no deaths prior to age 15 among family-detected subjects who were followed, compared with 7 deaths among symptom-detected subjects, at ages 6, 7, 9, 13, and 14 years (3 subjects). The difference in survival curves is statistically significant (p=0.03).

Figure 2. Kaplan-Meier Combined Survival Curve for C-CALD Patients



Of 6 subjects in the family-detected subjects followed past age 15 years, one died at 18 years of age and the others were lost to follow-up at ages 16 (2 subjects), 17, and 19 (2 subjects). Of 5 symptom detected subjects tracked past age 15, one died at 18 years of age and the others were lost to follow-up at ages 17, 18 (2 subjects), and 19 years.

Other Unpublished Data

A recent conference abstract[62] reported a dataset of 91 patients undergoing cord blood transpalant for X-ALD. Five-year survival was reported to be 58%, but the findings were not stratified by factors related to the timing of diangosis and the degree of disease involvement. In the future, this might be a helpful resource for evalating the benefit of early identification.

Summary – Evidence for Treatment Outcomes from Early Detection

- The Loes score is commonly used to measure the degree of brain involvement and is associated with functional status in more advanced cases. One study suggests that the Loes score in individuals with CCALD can change by 2 points in one year and developed a predictive 1-year predictive model. However, the prediction is based on a small number of subjects older than the individuals who would be identified by newborn screening.
- HSCT outcomes (morbidity, neurological) are better for patients with a pre-HSCT Loes score < 9 compared to those with a higher Loes score. Experts recommend treating individuals with a much lower Loes score. Although there is no clear ideal threshold, experts suggest that any patient with progressive changes on MRI should receive HSCT.
- Although adrenal insufficiency is common in CCALD, no data were found regarding the impact of early intervention specifically for adrenal insufficiency on subsequent morbidity or mortality. HSCT does not resolve adrenal insufficiency. Clinicians should consider the possibility of X-ALD when adrenal insufficiency is diagnosed.

- Although published treatment studies included cases identified presymptomatically through family history, these cases were not separately evaluated. One case report of a child identified prenatally based on family history reported favorable neurological outcomes through 3 years of age after HSCT in the second year of life.
- Unpublished data suggest a benefit to detection through family testing compared to symptom detection for individuals with CCALD. The small sample size of patients, all from specialty treatment centers may affect generalizability of the findings. Insufficient data are available to assess the specific ages that subjects first came to attention and the factors leading to detection.

3. Public Health Impact – Population Outcomes

Key Question 9: *What is the impact of newborn screening on the Public Health of the population in terms of projected numbers affected by screening and projected health outcomes?*

OVERVIEW OF PROCESS

Evidence Evaluation and Methods Workgroup
In May 2012, an Evidence Evaluation and Methods Workgroup was convened to consider methods and approaches utilized by the External Condition Review Workgroup (CRW) for the SACHDNC. One of the recommendations from this Workgroup was to incorporate the application of decision analysis into the evidence review process. Since this recommendation, decision analytic modeling has been used as part of the evidence review process for hyperbilirubinemia, Pompe disease, and mucopolysaccharidosis type I disease (MPS I). X-linked adrenoleukodystrophy (X-ALD) is the fourth condition to incorporate decision analytic modeling into the evidence review and synthesis process.

Objectives of Decision Analysis
Decision analysis is a systematic approach to decision making under conditions of uncertainty that has been applied to clinical and public health problems.[1] Decision analytic models can be used to simulate randomized clinical trials for new health interventions, to project beyond the clinical trial time frame, or to compare treatment protocols not directly compared in head-to-head trials. The decision analytic approach allows the decision maker to identify which alternative is expected to yield the most health benefit. It can also allow researchers to characterize the uncertainty associated with projections of clinical and economic outcomes over the long-term[2], which is important given the lack of long-term outcomes data for most conditions considered for newborn screening. A decision analytic model (or decision tree) defines the set of alternatives and short-and long-term outcomes associated with each alternative. In the application to screening for X-ALD disease, this approach was anticipated to aid in the estimation of the range of health outcomes that could be expected for universal newborn screening of X-ALD disease compared with clinical identification.

Applying Decision Analysis to Screening for X-ALD Disease
Published literature for rare phenomena including X-ALD disease is usually very limited with respect to data for prevalence, natural history, or response to treatment. For this review, we are able to utilize preliminary data from the newly-implemented screening program in New York state, in combination with additional published and unpublished data. By utilizing modeling, we can supplement the evidence base identified through the systematic review by providing projections of key health outcomes at the population level for newborn screening compared with clinical identification. This process also serves to highlight evidence gaps and areas with the most uncertainty, thereby enhancing the overall decision making process.

Expert Panel Meeting Process
Clinical and scientific experts in the screening and treatment of X-ALD disease were identified and invited to serve on an Expert Panel (see Table 1 for list of expert panelists). Expert panel members were asked to provide input on the design and assumptions of the decision analysis model, including the identification of key health outcomes to be included in the analysis. A

series of three technical expert panel meetings (4/14/15; 6/11/15; 7/28/15) were conducted to identify sources for input probabilities for each outcome in the model; to provide feedback on the structure of the initial and revised decision analytic models, including the relevant timeframe for key health outcomes; and to develop assumptions where little or no data were available. All meetings were conducted via webinar. Expert panel participants received a discussion guide that included background information, a schematic of the model structure, proposed data inputs, and proposed modeling inputs for discussion by the group. The identification of data sources and the development of a decision analytic model is typically an iterative process.

Table 10. Timeline - Application of Decision Analytic Model for X-ALD Disease Screening

Date	Decision Analysis Milestones
September 2014	X-ALD disease nominated for addition to uniform newborn screening panel; referred to external condition review group
March 2015	Initial development of decision analytic model to evaluate newborn screening for X-ALD Disease
April 2015	Technical Expert Panel 1 – Review Model Structure
June 2015	Technical Expert Panel 2 – Review Revised Model Structure and Preliminary Evidence Review Summary
July 2015	Technical Expert Panel 3 – Review Revised Input Assumptions and Preliminary Results
August 2015	Final X-ALD evidence review report and decision analysis findings presented to Advisory Committee

METHODS

An initial decision analysis model was developed concurrently with the evidence review process. The initial model was reviewed with the expert panel in April 2015. A schematic of the final X-ALD newborn screening decision model is shown in Figure 3 and Figure 4.

The **key features** of the decision analytic model are as follows:

- Target population: Annual newborn cohort for the US, excluding newborns at higher risk for X-ALD disease (i.e., with a family history of ALD), of 4 million newborns.
- Interventions: A strategy of universal newborn screening (NBS) is compared with diagnosis through clinical identification (CI). The analysis assumes that identified cases of severe X-ALD disease meeting treatment criteria will be treated with HSCT whether they are diagnosed through newborn screening or through clinical identification. In other words, the key difference in determining outcomes between the two modeled cohorts – newborn screened or clinically-identified – indicates the benefits of earlier diagnosis and treatment.
- Key health endpoints: Mortality, survival with limitations (non-ambulatory, non-communicative (NANC) at 10 years

Two additional expert panel meetings were held in June 2015 and July 2015 to review the decision tree, proposed set of parameter inputs for the decision model, and preliminary results. Parameter inputs were based on published and unpublished data. The model structure and parameter estimates were revised following each expert panel based on additional data sources identified during the expert panel and supplemented by expert opinion in cases where no data were available. The sources of published and unpublished data are listed in Table 11. The final set of parameter inputs and associated ranges for the analysis are shown in the Tables 12 and 13 below.

Table 11. Sources of Data for Decision Model Inputs

Bezman, L., et al., Adrenoleukodystrophy: incidence, new mutation rate, and results of extended family screening. Ann Neurol, 2001. 49: 512-7.
Caggana, M. & Orsini, J. *Personal communications,* NY State NBS X-ALD Screening, Dec 2013 – July 2015.
Eichler, F., et al., *Personal communications,* CCALD patients evaluated at Massachusetts General Hospital Leukodystrophy clinic from 2006-2015. Unpublished raw data. 2015.
Jardim, L.B., et al., X-linked adrenoleukodystrophy: clinical course and minimal incidence in South Brazil. Brain Dev, 2010. 32(3): p. 180-90.
Kirk, EP, et al., X-linked adrenoleukodystrophy: the Australasian experience. Am J Med Genet, 1998. 76: 420-3.
Stradomska, TJ & Tylki-Szymanska, A. Serum very-long-chain fatty acids levels determined by gas chromatography in the diagnosis of peroxisomal disorders in Poland. Folia Neuropathol, 2009. 47: 306-13.
Takemoto, Y., et al., Epidemiology of X-linked adrenoleukodystrophy in Japan. J Hum Genet, 2002. 47: 590-3.

Table 12. Key probability inputs, X-ALD disease and phenotype[1]

	Universal newborn screening (NBS)				Clinical Identification (CI)			
X-ALD	**Most Likely**	**Min**	**Max**	**Sources**	**Most Likely**	**Min**	**Max**	**Sources**
Confirmed X-ALD disease (all types)	3.6 per 100,000	1.6 per 100,000	5.3 per 100,000	New York Screening Program	2.3 per 100,000	1.6 per 100,000	3.3 per 100,000	Bezman et al, 2001; Kirk et al, 1998; Jardim et al, 2010; Takemoto et al, 2002; Stradomska et al, 2009
Distribution of X-ALD Phenotypes for Confirmed Diagnoses in Males								
Childhood/ Adolescent Cerebral ALD	0.32	0.32	0.50	Evidence Review/ Assumption	0.50	--	--	Evidence Review
Adrenal Insufficiency Only	0.08	0.08	0.13		0.13	--	--	
Adult Cases (CALD & AMN)	0.59	0.38	0.59		0.37	--	--	

[1] 95% confidence interval derived using a binomial distribution

Identification, Diagnosis, and Treatment of Severe Cases of X-ALD

- The base case estimates assume that the same number of cases of childhood/adolescent CALD and adrenal insufficiency are identified under newborn screening and clinical identification. This is a conservative assumption because it is possible that there would be dfferences in the number of cases identified under newborn screening if some cases were

missed for patients who died prior to diagnosis under clinical identification or if some patients were diagnosed after age 18 and would not have been counted as childhood/adolescent CALD under clinical identification (i.e., identified as adult cases under clinical identification). The ranges allow for the possibility that there are differences in the incidence of childhood/adolescent CALD and adrenal insufficiency under newborn screening compared with clinical identification. In other words, the number of cases of childhood/adolescent CALD identified could be higher under newborn screening.

- We assume that patients identified with X-ALD will receive HSCT if they meet certain clinical criteria – in other words, the same treatment criteria will apply if identified under newborn screening or clinical identification.
- Data on potential health benefits of newborn screening are derived from 2 data sources that evaluate outcomes for (1) data for a series of patients identified through family history or clinical identification both treated and untreated (Eichler et al, 2015), and (2) data on treated patients identified through family history or clinical identification (multisite study described above in the evidence review).

Results

Projected Newborn Screening Outcomes

Table 13. Projected newborn screening outcomes for X-ALD

	Per 100,000		Per 4 million	
	Newborn Screening (n)	**Range**	**Newborn Screening (n)**	**Range**
Total confirmed positive screens (all types)	9	6-13	363	240-520
Repeat Screens*	12	9-16	473	342-637
Second-tier screens**	1,836	1,793-1,880	73,445	71,710-75,211

*Independent samples
**Repeated on same sample

Projected Cases of X-ALD disease and Long-term Outcomes

We projected the annual number of X-ALD cases and associated phenotypes that would be identified with newborn screening compared with clinical identification, presented in the following table:

Table 14. Projected cases for newborn screening for X-ALD disease compared with clinical identification for a cohort of 4 million children (US population)

	Newborn Screening	Clinical Detection
Total X-ALD (ABCD1 Mutation)	**143** (64-211)	**92** (64-132)
Childhood/Adolescent Cerebral ALD*	**46** (32-68)	**46** (32-66)

Adrenal Insufficiency Only	**12** (8-18)	**12** (8-17)
Adult Cases (CALD and AMN)	**85** (24-125)	**34** (24-49)
Heterozygote ABCD1 – Female Carriers	**154** (103-221)	**143**
Peroxisomal/Other Disorders	**66** (45-95)	--

*Includes cases with adrenal insufficiency

Table 15. Long-term Health Outcomes[1]

Projected survival without NANC (unpublished data from Eichler et al, 2015), most likely values (ranges)

	Survival without NANC, at 15 years of age	Deaths or cases of NANC, at 15 years of age
Screened/Treated if Indicated		
Most Likely	46	0
(Min, Max)	(5, 68)	(0, 57)
Clinically Diagnosed/Treated if Indicated		
Most Likely	9	37
(Min, Max)	(1, 31)	(17, 64)

Projected survival, treated patients only (unpublished data from multisite study), most likely values (ranges)

	Survival, at 15 years of age	Deaths, at 15 years of age
Screened/Received Transplant		
Most Likely	46	0
(Min, Max)	(22, 68)	(0,21)
Clinically Diagnosed/Received Transplant		
Most Likely	28	18
(Min, Max)	(11, 52)	(7, 44)

[1]Minimum and maximum values derived from 95% CIs assuming a binomial distribution.

Projected Health Outcomes for Males with X-ALD with Childhood/Adolescent Onset

It is anticipated the earlier identification, diagnosis, and treatment of males with X-ALD could result in additional cases of childhood and adolescent X-ALD and improved long-term outcomes:

- The projected number of cases of childhood/adolescent CALD diagnosed in the US each year ranges from 46 (32-68) for newborn screening and 46 (32-66) for clinical identification. The additional number of childhood/adolescent CALD cases identified through NBS compared with CI ranges from 0 to 19.
- Projected benefits at 15 years of age for the two sources of long-term data show:
    - The averted number of cases of death/survival with NANC ranged from 17 to 64
    - The averted number of deaths ranged from 7 to 44 for treated patients

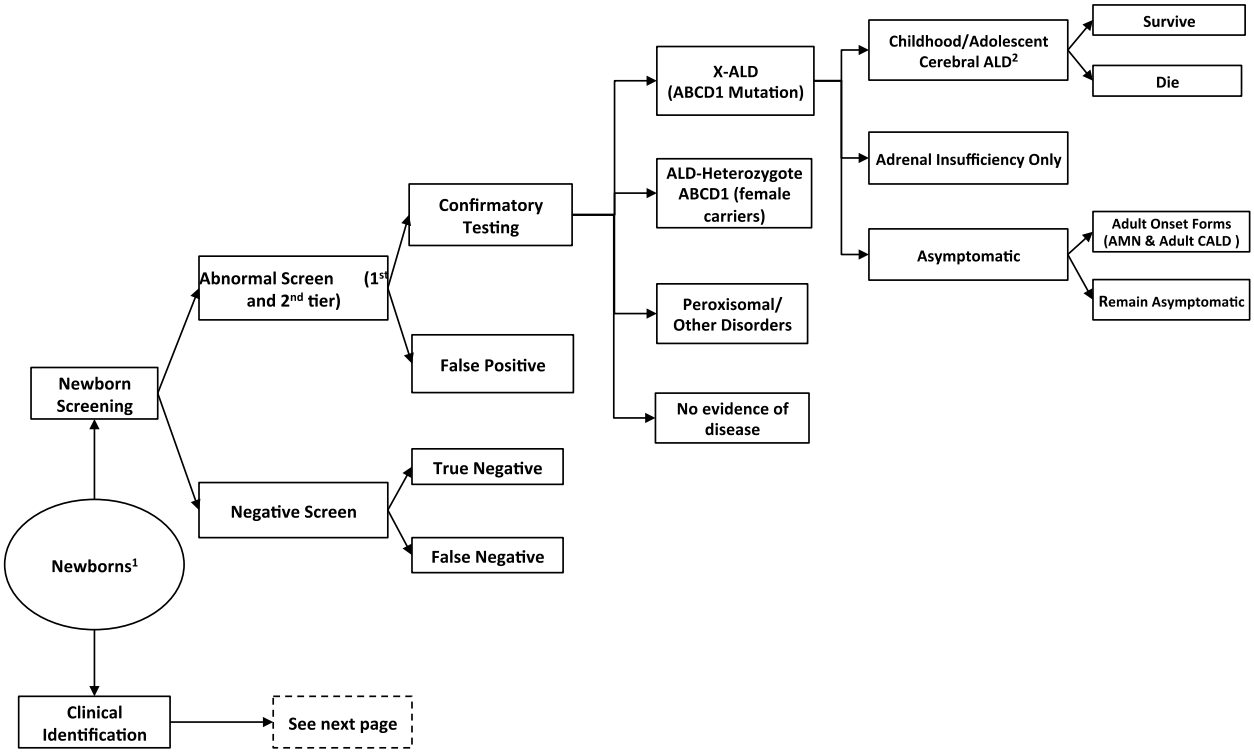
Limitations

Limited data were available for a number of parameter inputs. In particular, very little data were available for the distribution of phenotypes following confirmatory testing.

Summary of Population Health Benefits

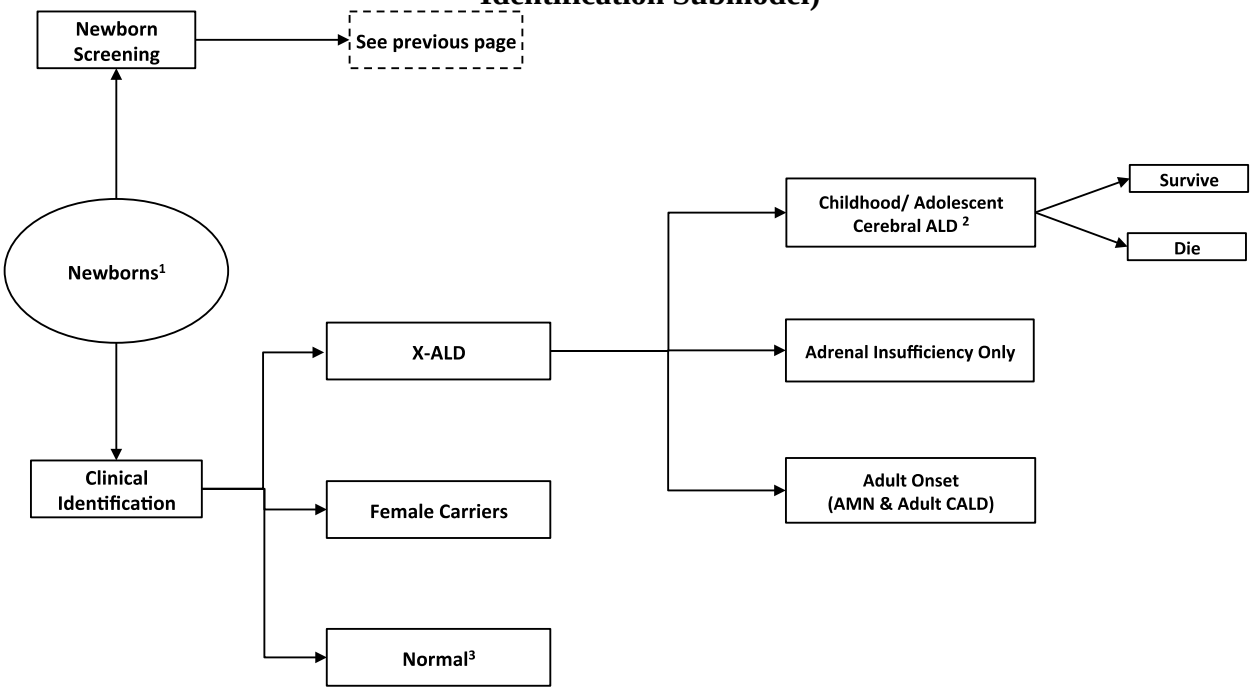
- Newborn screening may result in a higher incidence of X-ALD. This may reflect a higher incidence attributable to missed cases, but may also be attributable to spectum bias.
- Projected health benefits in terms of survival or survival without NANC project 18 deaths averted (range:7-44) or 37 (range:17-64) cases of death or NANC averted annually for newborn screening compared with clinical identification by 15 years of age for the US population.
- Under certain scenarios, the additional number of adult cases of AMN identified is projected to be as high as 76 cases annually.

Figure 3. Schematic for X-ALD Newborn Screening Decision Model (Screening Submodel)



[1]Not at high risk
[2]With or without adrenal insufficiency

Figure 4. Schematic for X-ALD Newborn Screening Decision Model (Clinical Identification Submodel)



[1]Not at high risk
[2]With or without adrenal insufficiency
[3]Can also include peroxisomal/other disorders

Table 16. Technical Data: Decision Model Inputs

A. Universal Newborn Screening (NBS)	Most Likely	Low	High	Source(s)
Probability of abnormal screen (1st and 2nd tier – independent sample)	0.0185	0.0180	0.0189	New York Screening Program
Conditional probability of true positive given an abnormal screen	0.0049	0.0034	0.0069	
Conditional probability of transient positive screen given an abnormal screen	0.9951	0.9931	0.9966	
Conditional probability of true negative given an abnormal screen	1.0000	0.99998	1.0000	
Conditional probability of false negative given an abnormal screen	0.0000	0.0000	0.00002	
Conditional probability of X-ALD (all types) given a positive confirmatory screen	0.3939	0.2624	0.4050	
Conditional probability of childhood/adolescent CALD with and without adrenal insufficiency given X-ALD diagnosis	0.3217	0.3217	0.5000	Evidence Review
Conditional probability of adrenal insufficiency only given X-ALD diagnosis reen	0.0839	0.0839	0.1250	
Conditional probability of adult –onset CALD/AMN/Asymptomatic given X-ALD diagnosis	0.5944	0.3750	0.5944	
Conditional probability of ALD-heterozygote ABCD1 (carriers) given a positive confirmatory screen	0.4242	0.4165	0.5163	New York Screening Program
Conditional probability of peroxisomal/other disorders	0.1818	0.1785	0.2213	
Conditional probability of survival beyond 15 years – treated	1.0000	0.6915	1.0000	Unpublished data from multisite study
Conditional probability of survival beyond 15 years (without NANC) – treated and untreated	1.0000	0.1581	1.0000	Unpublished data from Eichler et al, 2015
B. Clinical Identification (CI)	**Most Likely**	**Low**	**High**	**Source(s)**
Probability of X-ALD (all types)	0.000023	0.000016	0.000033	Bezman et al, 2001; Kirk et al, 1998; Jardim et al, 2010; Takemoto et al, 2002; Stradomska et al, 2009
Conditional probability of childhood/adolescent CALD with and without adrenal insufficiency given a diagnosis of X-ALD	0.5000	--	--	Evidence Review
Conditional probability of Addison's Only	0.1316	--	--	
Conditional probability of Adult CALD/AMN	0.3182	--	--	
Probability of ALD-Heterozygote ABCD1 (Carriers)	0.0000357	--	--	Bezman et al, 2001
Conditional probability of survival beyond 15 years (treated)	0.5984	0.3398	0.7833	Unpublished data from multisite study
Conditional probability of survival beyond 15 years (without NANC) – treated and untreated	0.2000	0.0327	0.4690	Unpublished data from Eichler et al, 2015

4. Public Health System Impact Assessment for X-ALD

Key Question 10: *What is the impact of implementing newborn screening of X-ALD on the Public Health System? What is the feasibility of population-based screening methods for X-ALD? What is the state of Readiness of State Newborn Screening Programs to Screen for X-ALD?*

As part of the evidence review procedures, a Public Health System Impact (PHSI) assessment of expanding newborn screening for X-linked adrenoleukodystrophy (X-ALD) was conducted by the Association of Public Health Laboratories (APHL). From March 2015 to June 15, APHL evaluated individual state NBS programs' capability to implement screening for X-ALD. Minor revisions were made to the existing survey and interview instruments for ease of use and relevance to A-ALD as needed. The process and results from the X-ALD assessment are described in this report.

Methods

Feasibility and Readiness

Feasibility is based on the degree to which the following exist:

- An established and available screening test
- A clear approach to diagnostic confirmation
- Acceptable treatment plan, and
- Established approach to long-term follow-up plans

Some of the key issues related to feasibility extend beyond the public health system and into personal medical care services.

Readiness refers to the overall national ability to adopt a condition into state NBS panels and is classified as:

- Ready: most NBS programs could implement within 1 year
- Developmental Readiness: most NBS programs could implement within 1–3 years
- Unprepared: most NBS programs would take more than 3 years to implement

Fact Sheet

The fact sheet, which was created in collaboration with APHL, members from the CRW and individuals from state NBS programs, provided background information pertaining to X-ALD to assist individuals with completing the survey (Appendix B). The fact sheet was sent to NBS program directors along with an X-ALD survey. The X-ALD fact sheet included information such as incidence of the disorder, screening methods, resources/materials, workstation resources and capacity, personnel requirements, quality control and reported screening results, estimated costs, short-term follow up, and treatments.

Limitations of the fact sheet were that cost data were based on projections, and screening performance and health outcomes were based on data from the only state NBS program conducting screening.

Survey
APHL developed a web-based survey instrument intended to evaluate states' readiness to implement comprehensive screening for X-ALD. The survey was pilot-tested with five beta testers and feedback was incorporated into the final survey instrument (Appendix B). NBS programs that contract services did not receive questions pertaining to the screening test itself or to laboratory capabilities. The survey instrument included questions related to implementation challenges, resources/factors that can hinder or aid in implementation and timeframe to complete implementation activities.

The survey link was sent to one state designee (e.g., program director) in 53 U.S. states and territories via email. The survey email emphasized that the individual completing the survey should collaborate with necessary stakeholders (e.g., laboratory experts, follow-up staff, medical specialists, Title V directors, advocates, public health commissioners) prior to completing the survey link. The timeframe to complete the survey was from May 12, 2015 to June 19, 2015. All survey data was submitted electronically to APHL.

Interviews
NBS programs that had a requirement or other mandate to screen for X-ALD, either as part of a pilot program or across the entire population were excluded from the web-based survey; instead, representatives from such NBS programs were interviewed by telephone. These respondents were provided the interview questions in advance and were asked to consult with stakeholders in their public health system. Stakeholders were encouraged to be on the call. APHL designed a combination of open- and close-ended interview questions (Appendix C) meant to assess challenges and successes. The interview tool included questions related to progress with regards to implementation, factors that will aid and hinder implementation, and timeframe for implementation activities.

Webinar and Outreach
APHL conducted a webinar on May 14, 2015 to discuss the purpose of the public health system impact assessment, benefits of completing the survey, and the X-ALD factsheet.

APHL discussed the public health system impact assessment and survey at several meetings and conference calls. Additionally, emails were distributed to the Principal Investigators of the seven Genetics and Newborn Screening Regional Collaborative groups. The email discussed the importance of their input to ensure that the point of contact for each state in their region would follow through on completing the survey.

Throughout May and June 2015, APHL conducted active follow-up with survey non-responders through phone calls and emails to improve participation.

Data Analysis
Data were kept secure and reviewed for accuracy. Quantitative and qualitative data from the surveys were aggregated for analysis using Qualtrics and Excel. Interview data were de-identified for anonymity.

Results

Interview Analysis

The following state NBS programs were excluded from the web-based survey and completed an in-depth interview.

Figure 5. NBS Programs Interviewed

	Legislative Mandate	Statewide Screening
New York		X
California	X	
Connecticut	X	
New Jersey	X	

The four state NBS programs that were interviewed are either conducting statewide screening or have a mandate for X-ALD. Illinois NBS program (not listed here) has a mandate to screen but it has not been signed into law; thus they were not interviewed. Each program varies with regard to its progress made towards considering implementation.

State Conducting X-ALD Screening

The NBS program that has begun screening for X-ALD, uses a three-tier approach for screening: 1) MS/MS for C26:0 2) HPLC and MS/MS for C26:0 LPC and 3) mutation analysis of the ABCD1 gene. Sequencing of the ABCD1 gene is not a necessary component of the screening test, however it is required for diagnostic testing. Many NBS programs in the U.S. do not have onsite molecular capabilities and this activity would typically take place at a diagnostic center. The second-tier, which is a requirement for X-ALD screening, reduces many of the false positives that are identified by the first-tier screening approach.

X-ALD is capable of being multiplexed, or screened in tandem, with lysosomal storage disorders (LSDs). A benefit to screening in this manner is that some of the instrumentation (tandem mass spectrometers) may not be required if a NBS program is already screening for LSDs and can handle the additional workload. According to the director for the state conducting X-ALD screening, it would be reasonable to expect that a program would need two or three tandem mass spectrometers (one for back-up) to process 100,000 specimens annually. In addition to this instrumentation, a program would need a high performance liquid chromatography (HPLC) column and possibly a liquid handler, depending on the number of specimens needing to be processed. The director of the program screening for X-ALD indicated that 1.5 FTE would be required to process 100,000 specimens annually using the basic two-tier approach used in New York.

Challenges that were mentioned by this program during the interview include: validating the X-ALD assay; determining how to multiplex the assay with LSDs to get consistent results; adjusting the screening cutoff to the appropriate level in order to capture as many cases as possible; and resolving follow up issues that come as a result of identifying asymptomatic males and secondary targets (e.g., female carriers and females with peroxisomal disorders). Although LSD and ALD extracts can be combined to run together on a mass spectrometer, setting up the multiplex method is not trivial for laboratories, particularly those conducting high volume testing. Some of the issues include:

- The source conditions to quantify the LSD internal standard and enzymatic products for the LSDs are quite different than the source conditions required to run the ALD markers (C20-C26-LPCs [lysophosphatidylcholines]).

- In the first-tier ALD test, unknown interferents account for the majority of marker signal (C26-LPC) measured in ALD-negative samples. Since the interferents are unknown, the accuracy is unknown as there is not a matched internal standard. Hence, it is common to see variable background levels of this interferent in negative samples across mass spectrometers. This can be adjusted for using varied relative response factors. These factors are much more variable across the instruments than would be expected.

- With high volume screening, evaporative loss can become a problem because it can take a long time to get to the final plates in a run. A heat sealing aluminum foil may be used to eliminate evaporation, resulting in more stable results across a run.

- C26:0 LPC sticks to the glass container when in cold storage, causing accuracy issues. To address this issue, a laboratory should store the internal standard solution in a refrigerator and on day of use place in incubator for an hour and then let come to room temperature to ensure it is in solution.

Although, the validation process has its challenges, a two-tier screening approach allows for a more robust test. During the interview, several factors aiding implementation of X-ALD were discussed. Some of these factors include consistent communication/developing relationships with specialty centers, health care providers, and diagnostic centers; not needing to procure new equipment; and having existing resources for screening.

As reported by the NY NBS program, from January 2013 to July 2015, 363,755 newborns were screened (405,415 specimens), of whom 185,097 were males (50.9%). Of the newborns screened, 13 males were identified with ABCD1 mutations. In addition, 14 females were identified with ABCDI mutations who were referred for genetic counseling. Other secondary disorders identified included Zellweger spectrum disorder/peroxisome biogenesis disorders (4). The NY NBS program reports 0 false positives identified through screening (for *any* detectable condition, X-ALD or other). Other interesting findings from this NBS program include:

- Four referrals (1 confirmed case, 1 female carrier and two open cases) of *de novo* mutations in which mutation is not detected in the mother.
- Three brothers identified through referrals of new brothers. One case was brother and grandfather with same mutation.
- One brother and one father identified through referrals of females. One brother had been diagnosed with ALD previously. One of the carrier's father also had X-ALD.
- One case in which an uncle was identified and diagnosed. His niece had a daughter who was found to be a carrier. The uncle had mobility problems beginning at age 20. Identification of his great niece lead to his diagnosis.
- A male with Klinefelter syndrome (heterozygous for a mutation) was identified.
- A male identified had alterations in the splenium of his corpus callosum and was transplanted at 10 months.

- A male was identified and diagnosed with adrenal symptoms at 6 months.
- A diagnosed male already had a strong family history.

Details regarding the X-ALD screening algorithm, requirements for screening and outcomes from the state NBS program conducting screening can be found in the fact sheet (Appendix B).

States with X-ALD Mandate

NBS program directors from the three states with a mandate to screen but have not yet started indicated that they had funds to begin some of the implementation activities for X-ALD screening, but not sufficient funding for sustained screening. In order to continue screening, the laboratory directors explained that they would need an appropriation of funds and/or would need to increase their NBS fee. One program director mentioned that the NBS program would contract testing services, at least initially, if X-ALD were added to the RUSP. Two states contain language in their bills that require several conditions to be met before screening for X-ALD can begin. One state contains language in its bill requiring screening to begin immediately upon addition to the RUSP.

Figure 6. State Legislation and Requirements for X-ALD Screening

State	Year Mandate Received	Screening REQUIRED immediately once added to RUSP	Conditions to be met before screening begins	Timeframe to fulfill conditions
CA	2014	Yes	None	Not specified
CT	2013	No	• Development and validation of method or FDA approved kit • Availability of necessary reagents for tests	Not specified
NJ	2013	No	• Development of a reliable test • Availability of quality assurance materials • Inclusion on the RUSP • Review by the Department of Health • Acquisition of equipment	Six months after condition is added to RUSP

Figure 7. Time Frame and Factors for Implementation

State	Time frame to complete implementation activities	What has your program done to prepare for implementation *Examples were mentioned during interview and may not be comprehensive	What does your program need to do to prepare for implementation *Examples were mentioned during interview and may not be comprehensive
CA	1 to 2 years	• Attended conference/information sharing • Formed internal workgroup	• Determine laboratory procedures/method development and validation • Develop education materials and/or follow up protocols • Form relationships with/communicate with follow-up centers, health professionals, and providers
CT	2 to 3 years	• Attended conference/information sharing	• Determine laboratory procedures/method development and validation • Develop education materials and/or follow up protocols • Form relationships with/communicate with follow-up centers, health professionals, and providers • Commissioner's approval
NJ	2 to 3 years	• Attended conference/information sharing • Hired personnel • Acquired equipment	• Determine laboratory procedures/method development and validation • Develop education and/or follow up protocols • Form relationships with/communicate with follow-up centers, health professionals, and providers • Additional hiring • Resolve follow-up issues

NBS program directors identified the following challenges with regards to X-ALD implementation: having a realistic time frame to accomplish implementation activities; working with neurologists for the first time in NBS; deciding on a referral process; determining how long to track patients; choosing a medical home for cases; dealing with follow-up issues that result from identifying asymptomatic males and secondary targets (e.g., female heterozygotes and peroxisomal disorders); ensuring availability of specimens for laboratory validation; and hiring challenges. Follow-up issues were the most commonly reported challenges by the NBS programs interviewed.

The NBS program directors identified factors that will aid X-ALD implementation. These factors include the following: communicating and sharing information with other NBS programs; attending national trainings and conferences; having adequate clinical and follow-up data; the addition of other disorders on the RUSP propelling X-ALD; observing experiences from states that are screening; and having adequate time to complete implementation activities. Communicating and sharing information with other NBS programs was the most commonly reported facilitator. Qualitative data from interviews in combination with data from surveys were useful in assessing readiness and feasibility.

Survey Analysis

A total of 37 completed surveys were received from 53 U.S. states and territories, for a response rate of 70%. Four state NBS programs were excluded from the analysis because they participated in the interview. Of the 33 responses included in the analysis, 27 came from state NBS programs that have laboratory and follow-up components and six came from programs that contract NBS laboratory services regionally or commercially. Results from the survey can be found in the figures below.

Figure 8. Duration for X-ALD Authorization

Full Question Text: If ALD was added to the Recommended Uniform Screening Panel (RUSP) tomorrow, how long would it take to get authorization to screen for ALD in your state?

Answer	Response	%
Less than 1 year	5	15.2%
1 to 3 years	20	60.6%
More than 3 years	8	24.2%
Never	0	0.0%
Total	33	100.0%

Figure 9. Duration for X-ALD Funds

Full Question Text: Once you received authorization to screen, how long would it take to have funds allocated for ALD?

Answer	Response	%
Less than 1 year	5	15.2%
1 to 3 years	19	57.6%
More than 3 years	7	21.2%
Never	2	6.1%
Total	33	100.0%

Figure 10. Figure 6: X-ALD Implementation Challenges

Full Question Text: Please select the top 3 challenges related to ALD implementation.

Answer	Response	%
Provide screening test	20	60.6%
Short-term follow-up of abnormals	20	60.6%
Increase of NBS fee	16	48.5%
Long-term follow up for carriers and individuals with peroxisomal disorders	15	45.5%
Support to ALD specialists	12	36.4%
Treatment support for ALD	8	24.2%
Other-please specify	3	9.1%

Figure 11. X-ALD Implementation Resources

Full Question Text: Please indicate your NBS program's readiness to implement screening for ALD by evaluating the following resources.

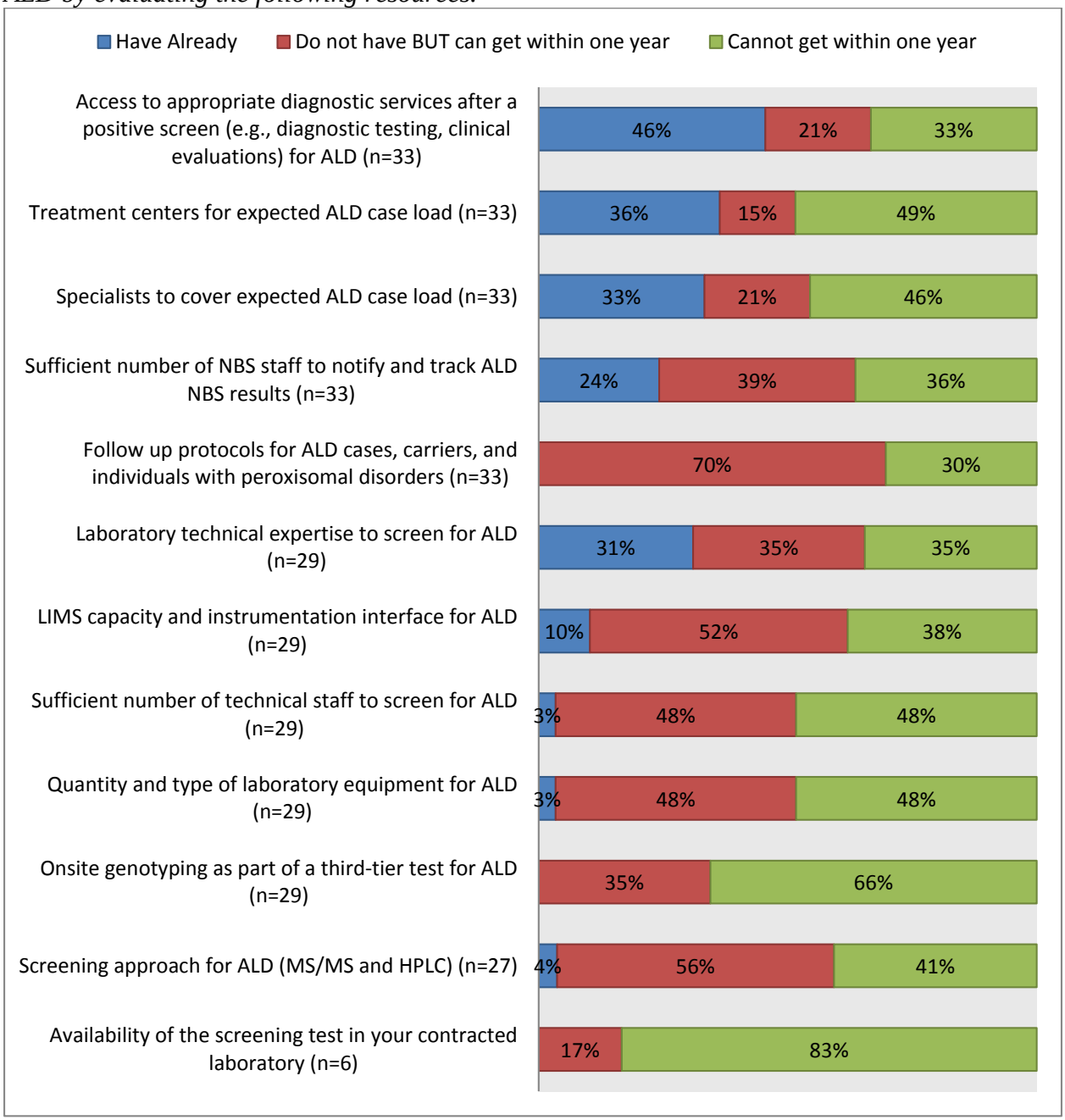


Figure 12. X-ALD Implementation Factors

Full Question Text: To what extent do the factors below impede or facilitate the adoption of screening for ALD in your NBS program?

	Major Barrier		Minor Barrier		No Impact		Minor Facilitator		Major Facilitator	
	n	%	n	%	n	%	n	%	n	%
Cost per specimen to conduct ALD screening (personnel, equipment, reagents) (n=33)	19	58%	13	39%	1	3%	0	0%	0	0%
Other ongoing NBS program activities (e.g., addition of other conditions, other quality improvements) (n=33)	16	49%	17	52%	0	0%	0	0%	0	0%
Cost of treatment for newborns diagnosed with ALD (n=33)	9	27%	11	33%	13	39%	0	0%	0	0%
Other non-NBS public health priorities within your state (n=33)	9	27%	12	36%	12	36%	0	0%	0	0%
Expected clinical outcomes of newborns identified with ALD from screening (n=33)	8	24%	4	12%	8	24%	7	21%	6	18%
Expected cost-benefit of screening for ALD in your state (n=33)	6	18%	6	18%	11	33%	7	21%	3	9%
Advocacy for screening for ALD (n=33)	2	6%	2	6%	11	33%	14	42%	4	12%
Extent to which the screening test for ALD can be multiplexed with other disorders (n=29)*	6	21%	9	31%	3	10%	4	14%	7	24%
Predicted run time to screen for ALD as it relates to other workload (n=29)*	4	14%	14	48%	9	31%	2	7%	0	0%

*Question only asked to labs with a state NBS program or commercial contract

Figure 13. Duration for Implementation Activities

Full Question Text: How long would it take your NBS program to complete the following activities?

Q8. What is the most significant barrier to implementing screening for X-ALD in your program?

Respondents identified multiple barriers to screening. Fifteen NBS programs cited funding/costs as the most significant barrier for implementing screening for ALD. Eight programs noted the most significant barrier was staffing/hiring. Six NBS programs noted that the most significant barrier was getting legislative approval or not having the condition on the RUSP. Other barriers included waiting for contract laboratory to get the screening test, competing public health priorities, not having an FDA approved kit, development of follow-up protocols, and identification of clinics and specialists.

Q9. What is the most significant facilitator to implementing screening for X-ALD in your program?

Respondents identified multiple facilitators to screening. Eight NBS programs cited the potential of multiplexing with other disorders as being the most significant facilitator for X-ALD screening. Six NBS programs noted the most significant facilitator as addition to the RUSP. Three NBS programs noted the benefits of early detection as being the most important facilitator to implementing screening for X-ALD. Other responses included readiness of contract laboratory/other program that can perform testing, advocacy, a reliable test with low false positive rate, funding/legislation in place, existing infrastructure/equipment, and laboratory and follow-up expertise.

Discussion

The PHSI attempted to assess NBS programs' readiness and feasibility to implement new disorders to the RUSP. Although APHL was not able to evaluate opinions and experiences from every state NBS program, the survey response rate of 70% was a strength. An additional strength of the PHSI was that it was able to assess both real experiences through interviews as well as perceptions about implementing X-ALD via a survey based on NBS programs' experiences with implementing other disorders.

Feasibility

When assessing feasibility to screen, it is important to consider the following components of the definition separately.

Does an established and available screening test exist?

MS/MS for C26:0 LPC followed by HPLC and MS/MS for C26:0 LPC is a reliable method for X-ALD. Additionally, the Centers for Disease Control are in the process of making quality assurance/quality control and proficiency testing materials available for X-ALD. Although there have been challenges, X-ALD is capable of being multiplexed with other LSDs, allowing for quicker, more efficient testing. The positive predictive value for the X-ALD screening test is extremely good (~96%) if it includes identification of secondary targets, but drops dramatically (~35%) if it includes identification of X-linked ALD only.

Is there a clear approach to diagnostic confirmation?
X-ALD in males and females can be confirmed by the presence of an ABCD1 mutation, confirmatory VLCFA analysis, or plasmalogen evaluation. The X-ALD dried blood spot test for VLCFA levels also detects positive screens for secondary targets and other disorders, including peroxisomal and other related disorders.

Is there an acceptable treatment plan?
Newborns identified with X-ALD are asymptomatic at birth. Clinical management plans established for X-ALD males involve long-term follow up with several specialists including endocrinologists and neurologists. Follow up care typically includes periodic evaluations, usually every six months, though this may vary for individual patients. Males identified with an X-ALD receive serial evaluations to monitor adrenal function and MRI severity ratings to detect early stages of disease. Patients with early signs of disease are referred for treatment (i.e., steroid replacement therapy or HSCT) and follow-up care, which may continue at a specialty care center.

Is there a long-term follow up plan?
Short-term follow-up ends with a diagnosis of X-ALD (males and heterozygote females), other specific peroxisomal disorder, other condition, or disconfirmed positive screen. Among NBS programs, there generally appears to be confusion regarding a long-term follow-up plan for X-ALD. At the time of diagnosis, there can be much uncertainty regarding age of onset, whether some males with the mutation will remain asymptomatic, and which form of the disorder will develop. Traditionally, NBS identifies disorders that manifest during the newborn period; however, with X-ALD, the disorder often does not manifest until childhood (> 4 years of age). Additionally, NBS program directors interviewed indicated that they were unclear how the referral process should be made and how long to track patients over time. Guidance in this area is needed.

Twenty out of the 33 (61%) NBS programs surveyed responded that it would take between 1 to 3 years to get authorization to screen for X-ALD in their state. Additionally, 19 NBS programs surveyed (66%) noted that it would take 1 to 3 years to implement screening for X-ALD after the allocation of funds. Although APHL did not get a response from every state in the U.S., it is reasonable to conclude that NBS programs across the U.S. are, at best, developmentally ready to implement X-ALD screening. The time it takes for the addition of the condition to the RUSP, obtaining legislative approval, and funding for screening may significantly slow down the process.

Although NBS programs, as a whole, are developmentally ready to implement X-ALD screening there is quite a bit of variation from program to program in terms of readiness. For example, forty-six percent of survey respondents (n=33) reported already having access to appropriate diagnostic services after getting a positive screen; 21% reported not having, but being able to get within one year; and 33% reported not being able to get within one year, respectively. Other factors that varied greatly included treatment centers for expected X-ALD caseload and access to specialists to cover expected caseloads. Of concern, is that eleven of 27 (41%) of NBS programs reported that they could not get the X-ALD screening test within a year. Sixty-one percent (61%) of NBS programs considered providing the screening test as a challenge.

Results from the PHSI survey emphasized the importance of being able to multiplex LSDs with X-ALD for efficiency and cost effectiveness purposes. Among NBS programs, there was debate regarding X-ALD being multiplexed with other disorders. Approximately 52% of the survey respondents reported that the extent to which the screening test for X-ALD can be multiplexed with other disorders was a major or minor barrier to implementation; 10% reported it had no impact; and 38% reported it was a minor or major facilitator. More research in this area may be warranted.

Without a federal decision regarding MPS-1, the evidence to screen for other disorders, such as X-ALD becomes less compelling from the NBS program perspective. NBS programs that have a mandate to screen or are screening for LSDs will likely be further ahead in their decision making or capacity to implement screening for X-ALD. Although we did not conduct a full evaluation, interview data showed these programs as being more likely to have researched testing methodology, formed workgroups, begun discussions with providers, acquired necessary equipment, hired personnel, made changes to their computer systems, and created follow-up protocols. The addition of a condition onto the RUSP was a driving consideration, and often a requirement for many programs in order to implement.

The addition of conditions on the RUSP will likely propel implementation of disorders on a national level. The program directors with mandates indicated that they would begin screening immediately or shortly after X-ALD was added to the RUSP. Some programs had specific conditions that needed to be met before screening could begin. If there is a quick push to implement many disorders at once, it is quite possible that NBS programs that do not have testing resources/capabilities may contract these services to other NBS programs/commercial laboratories. Although laboratories that contract services were underrepresented in our analysis (6 out of 14), five of them noted that they would not be able to get the screening test in their contracted laboratory. The availability of a screening test by the contract laboratory was a commonly noted concern.

There were several limitations with the PHSI assessment. In many of the survey questions, respondents were asked to assume approval had occurred and funds allocated. This was not meant to underestimate the importance and time commitment involved with these steps, but rather to have responders consider specific implementation activities outside of funding and legislation. It is plausible to assume that getting approval and acquiring funds could add years to the timeframe for implementation. Additionally, although NBS program directors likely relied on experiences implementing other conditions, the questions in the survey were hypothetical and responses were subjective. Interviews assisted in gathering additional information pertaining to real world barriers and facilitators as well as screening outcomes.

Summary of PHSI Assessment

Most (61%) of the state NBS programs that were surveyed and 2 out of 3 states with mandates reported that it would take between 1 and 3 years to implement screening for X-ALD after approval and allocation of funds. Follow-up challenges were commonly reported in this assessment. The costs associated with screening and competing public health interests continue to be an issue hindering implementation of conditions. The state NBS program that has begun to offer screening for X-ALD has identified 13 X-ALD males since 2013 and provides important

lessons about resources and challenges related to implementation. One of the most important factors in aiding implementation is consistent communication/developing relationships with necessary stakeholders.

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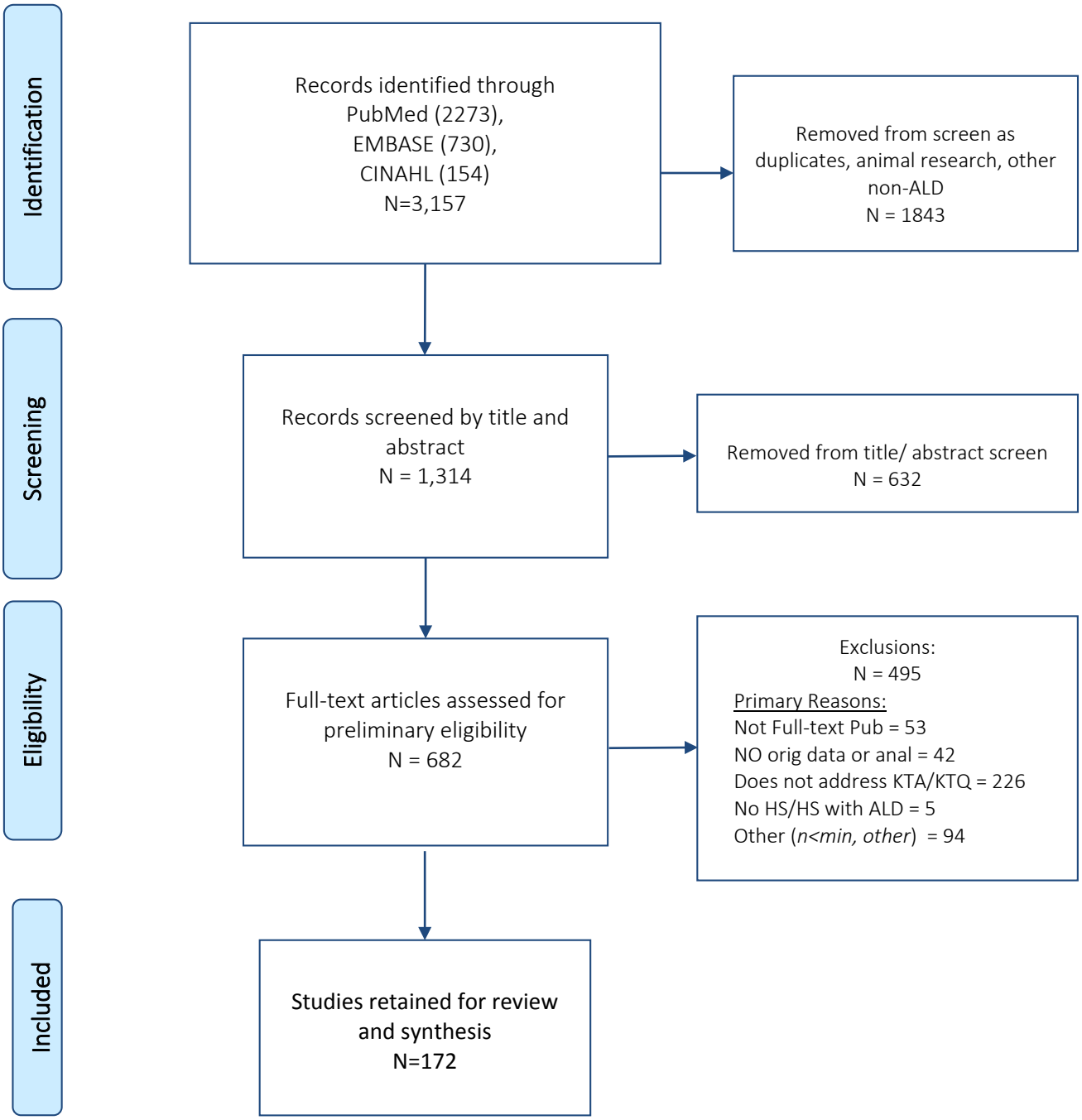
Appendix A. Systematic Evidence Review Technical Methods

PRISMA[63] Flow Diagram of Literature Search for Newborn Screening for X-ALD



Appendix B. PHSI Assessment: Fact Sheet for X-ALD Screening

Condition	ALD
Description	Metabolic disorder affecting the adrenal glands and central nervous system. It is due to mutations in the ABCD1 gene and affects the metabolism of very long chain fatty acids (VLCFA). X-ALD presents as a spectrum of disease, typically with progressive neurological decline (Cerebral ALD) and/or adrenal insufficiency ("Addison's Disease") presenting across the lifespan. Most boys (~90%) with childhood cerebral ALD (CALD) also experience adrenal insufficiency. Neurological involvement and/or adrenal insufficiency may also occur later in adolescence or adulthood (adult-onset adrenomyeloneuropathy [AMN]), or as Addison's Disease, respectively. Females may be identified with a heterozygote ABCD1 mutation, and usually present with neurological symptoms in later adulthood.
Expected Incidence	Clinical detection= ~1 in 20,000 male births[3] Detection by laboratory screening= 1 in 14,238 male infants screened; NYS NBS Program with data collected from 12/30/2013 to 7/27/2015; 363,755 infants screened including 185,097 males Clinically 35-40% of patients have childhood onset of cerebral ALD[1]

Screening Methods	
Measurement Method	First tier- MS/MS (required for referral) Second tier- HPLC MS/MS (required for referral) ***Sequencing of ABCD1 gene is a next step toward diagnosis (optional as third tier for NBS program)***
Data Source(s)	NY NBS Program uses a three-tiered screening approach and screened over 316,000 infants
Screening Marker	C26:0 lysophosphatidylcholine (C26:0 LPC)
Screening Strategy	Measurement of analyte

Resources and Materials	
Minimum Instrumentation, Equipment and Requirements Necessary to Process 100,000 Specimens Annually (Includes	• At least two MS/MS with one for back-up • One liquid handler is helpful (can be done without liquid handler in smaller volume laboratory)

[3] Vogel BH et al., 2015. Newborn Screening for X-linked Adrenoleukodystrophy in New York State: Diagnostic protocol, surveillance protocol and treatment guidelines. *Molecular Genetics and Metabolism,* 114 (4), 599-603.

Conventional Redundancies)	
Equipment Suppliers and Availability of Kits, Reagents and Consumables	Tips and plates; HPLC column

Workstation Resources and Capacity	
Tech Time to Prepare Specimens	3 hours for 10-24 plates (870-2,088 specimens)
Instrument Time	1.5 min. per run per specimen 2.5 hrs. per plate
Maximum Number of Specimens to Be Analyzed at One Workstation During An 8 Hour Shift	Up to 24 plates (2,088 specimens) per lab staff person
Minimum Space Requirements (Supporting Equipment Not Included)	Cu ft. for two MS/MS and liquid handlers and hood space for solvent and extraction (dependent on instrumentation)

Personnel Requirements	
FTE Needed to Process 100,000 Specimens Annually	1.5 FTE
Other Considerations	
LIMs Adjustments	Variable (dependent on vendor)
Training	MS/MS and chromatography

QC and Reported Screening Results	
Availability of Quality-Control Specimens	Yes
Reported Rate of Second-Tier Test	6,679 samples of 363,755 samples received = 1.8%
Reported Rate of Repeat Requests (Independent Specimen)	43 borderlines requiring second specimen out of 363,755 samples tested = 0.012%
Rate of Referrals	33 of 363,755 specimens = 0.0091%

Reported Outcomes	**# by type(s):** (n=363,755 specimens; 185,097 male infants) Confirmed ALD = 13 boys with ABCD1 mutations Carriers = 14 females heterozygous for an ABCD1 mutation Zellweger spectrum disorder/PBD = 4 Infant expired = 1 (likely PBD; unconfirmed) Other = 1 Aicardi-Goutieres syndrome determined using whole exome sequencing (elevated VLCFA and normal plasmalogen); pending deletion/duplication analysis False positives = 0 Lost to follow-up = 0

Estimated $$ Costs	
Equipment Cost (Overhead)	Two MS/MS- $500,000-$600,000 DNA Sequencer- $160,000 if purchasing (not required) Liquid Handler- $100,000-$250,000 if purchasing (not required; used in NY because of LSD assay; varies by capacity; can use multi-channel pipettors)
Estimated Cost to Laboratory of Reagents or FDA-Approved Kit	$35,000 annually [solvent, tips, plate, columns]
Estimated Reagent Rental Cost	N/A
Estimated Personnel Cost To Screen 50,000 to 100,000 Specimens Annually (Follow-Up Not Included)	$109,000-$135,000 (per person salary, fringe, and indirect)
Estimated Diagnostic Assay Cost	$160-$320 depending on laboratory (VLCFA only)
Estimated Diagnostic Molecular Testing Costs	$500 per sample (approximate actual reagent and personnel cost; not laboratory charge)

Short-Term Follow-Up	
Description	Confirmation of diagnosis of X-ALD and female ABCD1 carriers by determination of VLCFA levels; assessment of endocrine status, genetic analysis, and MRI/neurological exam.
Case Definition (manifests in childhood)	X-ALD is a rare demyelinating disease of the central nervous system that is inherited as an X-linked recessive trait primarily affecting males in childhood; characterized by progressive neurological decline, blindness, deafness, tonic spasms, and mental deterioration.
Diagnostic Method & Criteria	• ABCD1 mutation (this is not necessary if screening program offers Tier 3 testing) • Confirmatory VLCFA analysis

	• Plasmalogen evaluation is performed if no mutation or an unknown variant is detected on DNA sequencing ABCD1 mutation and elevated VLCFA in males suggests ALD; ABCD1 mutation in females and normal VLCFA and plasmalogen suggests carrier; clinical symptoms and low plasmalogen in females suggests peroxisomal disorder. Multiple phenotypes of X-ALD can be seen in families.
Availability of Diagnostic Testing Laboratories	The diagnostic studies recommended at this phase of testing (VLCFA, plasmalogen, ABCD1) can be performed in a number of laboratories.

Current Treatment(s)	
Description and Current Treatment Guidelines with Clinical Identification	Hematopoietic stem cell therapy (HSCT) is recommended for males with cerebral X-ALD. This is generally NOT done in infants, rather, identified boys are followed closely in infancy and early childhood with serial MRI's to optimize time of HSCT. HSCT can prevent progressive cerebral demyelination. Gene therapy research is currently experimental and not yet approved. Corticosteroid replacement therapy is used for adrenal insufficiency.
Specialty Providers or Centers	Screen positive infants are referred to inherited metabolic disease specialists in NYS for evaluation and genetic counseling. Short term follow-up ends with a diagnosis of X-ALD, ABCD1 carrier, specific peroxisomal disorder or other condition. See "Reported Outcomes" above. Once a diagnosis of X-ALD is made, the following specialists are involved: • Endocrinologists- to conduct serial evaluations and treatment for adrenal insufficiency (usually at the specialty center). • Neurologists- to conduct evaluations, arrange for se MRI's beginning at 6 months of life, and refer for HSCT if appropriate. • HSCT centers- there are very few centers specializing in pediatric HSCT for metabolic disorders; X-ALD patients may need to go out of state for treatment. Follow-up care may continue at a specialty care center.

Appendix C. X-ALD Public Health System Impact Assessment Survey

The purpose of this survey is to inform the Secretary of Health and Human Services Advisory Committee on Heritable Disorders in Newborns and Children about the ability to add newborn screening (NBS) for Adrenoleukodystrophy (ALD) using information gathered from most of the Newborn Screening (NBS) programs in the U.S.
Please refer to the ALD screening factsheet to answer the following questions about the ability to add NBS for ALD in your NBS program. Please also consult with others in your NBS program, including laboratory and follow-up staff, medical professionals and specialists, prior to completing the survey. When unsure about a response, **please provide your best estimate**.

1. Within the last 3 years, has your NBS program [Check all that apply]
    - o Included ALD as part of the routine NBS panel (*end survey*)
    - o Included ALD as any type of pilot evaluation (*end survey*)
    - o Received a mandate to screen for ALD (*end survey*)
    - o None of the above (*go to question 2*)

2. If ALD was added to the Recommended Uniform Screening Panel (RUSP) tomorrow, how long would it take to get authorization to screen for ALD in your state?

    - o Less than 1 year
    - o 1 to 3 years
    - o More than 3 years
    - o Never (go to question 4)

3. Once you received authorization to screen, how long would it take to have funds allocated for ALD?

    - o Less than 1 year
    - o 1 to 3 years
    - o More than 3 years
    - o Never

4. Please select the top 3 challenges related to ALD implementation.

    - o Provide screening test
    - o Increase of NBS fee
    - o Short-term follow-up of abnormals
    - o Support to ALD specialists
    - o Treatment support for ALD
    - o Long-term follow up for carriers and individuals with peroxisomal disorders
    - o Other – please specify

FOR QUESTIONS 5-8, PLEASE ASSUME THAT ALD HAS BEEN AUTHORIZED FOR ADDITION TO YOUR STATE'S PANEL AND THAT FUNDS FOR LABORATORY TESTING AND FOLLOW UP HAVE BEEN MADE AVAILABLE.

5. Please indicate your NBS program's readiness to implement screening for ALD by evaluating the following resources.

Resource	Have Already	Do not have BUT can get within 1 year	Cannot get within 1 year
Screening approach for ALD (MS/MS and HPLC) Shown to state NBS programs only			
Quantity and type of laboratory equipment for ALD Shown to all except regional contract			
Laboratory technical expertise to screen for ALD Shown to all except regional contract			
Sufficient number of technical staff to screen for ALD Shown to all except regional contract			
Availability of the screening test in your contracted laboratory Shown to regional contract and commercial contract			
Onsite genotyping as part of a third-tier test for ALD Shown to all except regional contract			
LIMS capacity and instrumentation interface for ALD Shown to all except regional contract			
Sufficient number of NBS staff to notify and track ALD NBS results			
Access to appropriate diagnostic services after a positive screen (e.g., diagnostic testing, clinical evaluations) for ALD			
Specialists to cover expected ALD case load			
Treatment centers for expected ALD case load			
Follow up protocols for ALD cases, carriers, and individuals with peroxisomal disorders			

**This question only applies if you reported using a contracted laboratory at question 2.*

6. To what extent do the factors below impede or facilitate the adoption of screening for ALD in your NBS program? Please see the definitions below.*

Factor	Major Barrier	Minor Barrier	No Impact	Minor Facilitator	Major Facilitator
Predicted run time to screen for ALD as it relates to other workload Shown to all except regional contract					
Extent to which the screening test for ALD can be multiplexed with other disorders Shown to all except regional contract					
Advocacy for screening for ALD					
Other ongoing NBS program activities (e.g., addition of other conditions, other quality improvements)					
Cost per specimen to conduct ALD screening (personnel, equipment, reagents)					
Cost of treatment for newborns diagnosed with ALD					
Expected clinical outcomes of newborns identified with ALD from screening					
Expected cost-benefit of screening for ALD in your state					
Other non-NBS public health priorities within your state					

**Major barrier- Will prevent testing from being done effectively and/or timely.*
Minor barrier- May compromise testing so it is not performed effectively and/or timely.
Minor facilitator- May allow testing to be done effectively and/or timely.
Major facilitator- Will allow testing to be done effectively and/or timely.

7. How long would it take to complete the following activities assuming your current NBS program and laboratory infrastructure?

	Activity already completed	< 1 year	1 to 3 years	> 3 years	Activity not required
Obtain and procure equipment for ALD screening Shown to all except regional contract					
Select, develop, and validate the ALD screening test within your laboratory assuming you are multiplexing with other disorders Shown to all except regional contract					
Select, develop, and validate the ALD screening test within your laboratory assuming you are NOT multiplexing with other disorders Shown to all except regional contract					
Hire necessary laboratory and follow-up staff for ALD					
Consult with medical staff and specialists to add test for ALD					
Develop follow-up protocols for ALD					
Add the ALD screening test to the existing outside laboratory contract Shown to regional contract and commercial contract					
Pilot test the ALD screening process within your state, after validation has taken place Shown to all except regional contract					
Entire process from obtaining equipment to full reporting and implementing statewide ALD screening (assuming that some activities may occur simultaneously) Shown to all except regional contract					

**This question only applies if you reported using a contracted laboratory at question 2.*

8. What is the most significant barrier to implementing screening for ALD in your program?

9. What is the most significant facilitator to implementing screening for ALD in your program?

10. Please share any additional information regarding implementation of screening for ALD.

Appendix D. X-ALD Interview Questions for State NBS Programs

1) When did your state receive a mandate to screen for X-ALD? How was the decision made?

2) Please describe the process for adding the condition to your state's NBS panel.

3) When do you plan to begin screening?

4) Since the decision was made to screen, what if anything has your state NBS program done to prepare for implementation?

5) In an attempt to better understand timeframe for a variety of implementation activities we would like to know how long it will likely take/has taken you to complete the following from the beginning (answer options < 1yr., 1-2 yrs., 2-3 yrs. >3 years):

- Obtain and procure equipment for screening
- Hire necessary laboratory and follow-up
- Consult with medical staff and specialists
- Select, develop, and validate the screening test within your
- Develop follow-up protocols
- Pilot test the screening process within your state, after validation has taken place
- Implement statewide screening for all newborns, including full reporting and follow-up of abnormal screens after validation and pilot
- Entire process from obtaining equipment to implementing statewide screening (assuming that some activities occurred simultaneously)

6) What major steps need to be made before you can begin statewide screening?

7) What challenges do you envision for this screening?

8) What do you think will most aid in implementation?

9) Is there anything else you would like us to know?

10) Who assisted you in preparing for the interview today?

Appendix E. Evidence Tables – X-ALD Systematic Evidence Review

- **Incidence and Prevalence**
- **Treatment for X-ALD**