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Newborn screening for X-linked adrenoleukodystrophy in New York State: Diagnostic protocol, surveillance protocol and treatment guidelines

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ABSTRACT

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

1. Introduction

X-linked adrenoleukodystrophy (X-ALD) is a metabolic disorder affecting the adrenal glands and central nervous system [1]. It is due to mutations in the *ABCD1* gene, located on Xq28. Over 1000 alterations in this gene have been identified, with pathogenic mutations affecting the metabolism of very long chain fatty acids (VLCFAs). X-ALD is the most common peroxisomal disorder, occurring in 1 in 17,000 births and 1 in 20,000 males [2–6].

Most males with X-ALD develop adrenocortical insufficiency (Addison disease), which may present early in life. Two 6-month-old boys with biochemical evidence of adrenal insufficiency were identified at 6 months of age by means of VLCFA screening [4]. Although the treatment of adrenal insufficiency is very effective, the identification of adrenal insufficiency is often delayed and may lead to significant morbidity or even death [3,7].

There are two main neurologic phenotypes: childhood cerebral X-ALD and the adult onset adrenomyeloneuropathy (AMN) [8]. These different neurologic presentations can occur within the same family

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and there is no correlation between genotypes, phenotypes, or age of onset of the disease [1,2,8,9]. A boy with an *ABCD1* gene mutation has a 35–40% risk of developing cerebral X-ALD manifestations between the ages of five and twelve years [1,3,8,9].

Although the earliest reported case presented at 21 months of age, neurological symptoms rarely appear before the age of four years [2,3,10]. Initial symptoms include emotional lability, hyperactive behavior, school failure and visuo-spatial impairment followed by worsening cognitive and neurologic disability [8]. As demyelination progresses, boys with X-ALD deteriorate to a vegetative state within two to five years of symptomatic onset and to death thereafter [8].

In contrast, AMN is an adult onset disorder, with progressive spastic paraplegia occurring at an average age of 28 years [1,3,8]. Adrenal dysfunction occurs in about two-thirds of males with AMN [8]. One in five males with AMN develops cerebral white matter disease between 20 and 35 years of age [8].

Symptoms similar to AMN develop in about 50–65% of women with a heterozygous *ABCD1* gene mutation, but are less severe with onset in the 4th or 5th decades [8]. Addison disease and cerebral disease are rare (less than 1%) in these heterozygous carriers and likely due to skewed X-inactivation.

The only effective treatment for cerebral X-ALD is hematopoietic cell therapy (HCT). Due to the risk of mortality related to the procedure, HCT is recommended only for males with evidence of brain involvement [1,3]. Among inborn errors of metabolism, X-ALD is a frequent indication for HCT, which is now considered standard therapy for boys with cerebral involvement [5,11]. For boys with early evidence of cerebral involvement, HCT may prevent the progression of demyelination [6].

Since there is an available early intervention for X-ALD, there has been a strong interest in improving pre-symptomatic detection. X-ALD has been under consideration by the Discretionary Advisory Committee on Heritable Disorders in Newborns and Children for possible inclusion in the newborn screening panel [12]. Newborn screening would allow for diagnosis of X-ALD prior to the onset of adrenal and neurological symptoms. When a symptomatic proband is identified, family studies often yield biochemical evidence of adrenal insufficiency in an asymptomatic younger brother [4]. Evidence of a "window of opportunity" for treatment of cerebral X-ALD was provided by a study of the cognitive profiles of 52 neurologically asymptomatic boys (mean age 6.7 years ± 3.6 years) with X-ALD and normal brain MRIs in which 48 boys had normal cognitive function [13]. At present, however, half of boys with cerebral X-ALD are already at an advanced stage of the disease when ascertained clinically [2].

Of concern in such a screening program is the current lack of treatment options for newborns with other peroxisomal disorders, who may be identified by X-ALD newborn screening. Other such disorders include Zellweger spectrum disorder (ZSD), acyl-CoA oxidase deficiency and D-bifunctional protein deficiency. Clinical presentation of these disorders is variable and includes vision abnormalities, hearing loss, poor feeding, developmental delay, bony abnormalities, abnormal liver function testing, cysts on renal ultrasound and hypotonia [14]. Treatment for these disorders is only supportive, although, early detection and elimination of the 'diagnostic odyssey' are potential benefits of their identification as secondary targets of X-ALD NBS [15].

When the Recommended Uniform Screening Panel (RUSP) was developed, X-ALD was considered but not recommended because of the lack of a validated screening test [11,16]. There were also concerns about the available treatment options and about the manner of selecting patients for the different therapeutic interventions [11].

Since the RUSP was initially selected, there have been many advances in validating a suitable assay. As discovered by Moser et al. in 1981, plasma very long-chain fatty acids (VLCFAs) are elevated in males with ALD [2,8] and are elevated in up to 80–85% of heterozygotes [2,3,8]. This biochemical abnormality is present at birth. In 2006, Hubbard et al. developed a combined LC–MS/MS method for the analysis of VLCFA in dried blood spots [17]. Refinements in the method have followed [18]. In 2013, 4689 newborn blood spot samples were tested with no false positives to show that high throughput screening is feasible [19].

These technological advances removed a major barrier to the implementation of newborn screening for ALD (ALD NBS). On April 29, 2012, New York resident Aidan Seeger died from X-ALD and his mother, Elisa Seeger, began advocating for ALD NBS. The combination of an improved DBS assay and advocacy, allowed for consideration of ALD NBS by NYS. On March 31, 2013, Aidan's Law went into effect mandating the New York State Newborn Screening Program to begin screening for the disorder. On December 30, 2013, newborn screening for X-ALD commenced in NYS.

2. Methods

Since ALD is a rare disorder there are presently no guidelines by any professional bodies for prospective monitoring or treatment. Prior to the institution of screening, the New York State Newborn Screening Program developed a workgroup to create diagnostic guidelines, surveillance protocols, treatment initiation recommendations, parental educational materials and long-term follow-up data elements for ALD NBS. The group consisted of the director from each metabolic center in NYS, personnel from the NYS Newborn Screening Program, and others. Over the months preceding the roll out, a series of conference calls took place to cover diagnosis, surveillance, therapy, and education. Since the diagnosis of ALD has a significant impact beyond just the identified newborn, a session was held with genetic counselors from each specialty care center to discuss counseling considerations of ALD NBS.

ALD NBS in NYS is accomplished using a three-tier algorithm. The first tier is MS/MS of C26:0 and the second tier is the measurement of C26:0 LPC using HPLC–MS/MS [8,20]. All newborns are screened with the first tier and newborns with an out-of-range result are screened with the more specific second tier. If the C26:0 LPC remains elevated on the second tier, then third tier testing is done, sequencing of the *ABCD1* gene. Each of the 10 exons of *ABCD1* is sequenced [8].

3. Results and discussion

3.1. Diagnostic protocol

The diagnostic protocol differs based on the outcome of the third tier of ALD NBS and the newborn's gender. Three categories were created: a male with an *ABCD1* mutation, a female with an *ABCD1* mutation, or no mutation identified (Fig. 1).

Confirmatory VLCFA analysis to an independent laboratory and a repeat specimen to NYS NBS is done for every newborn male with an *ABCD1* gene mutation (Fig. 1A). If VLCFAs are elevated at an independent laboratory, it is consistent with a diagnosis of X-linked ALD. Each mother of a male is offered carrier testing at the NBS Program.

A female with an *ABCD1* mutation is likely only a carrier of X-linked ALD, but could have another peroxisomal disorder (Fig. 1B). If there are clinical symptoms of a peroxisomal disorder, then the diagnostic evaluation continues. Other peroxisomal disorders are often symptomatic in the newborn period, but may be further discerned by appropriate biochemical testing (e.g. plasmalogens) and genetic testing. If there is no clinical evidence of peroxisomal disease, then the newborn is a carrier of ALD and genetic counseling is recommended. Both parents are offered gene mutation testing by the NYS NBS Program.

If an *ABCD1* mutation is not identified, then VLCFA and plasmalogen are ordered [15].

- If VLCFAs are elevated and plasmalogens are low, additional studies should be done to evaluate for clinical evidence of a Zellweger spectrum disorder (ZSD) (Fig. 1C).
- If VLCFAs are elevated, but plasmalogens are normal, then further evaluation for clinical evidence of a peroxisomal disorder