ABCD1 Molecular Testing For Second-Tier Confirmatory Newborn Screening

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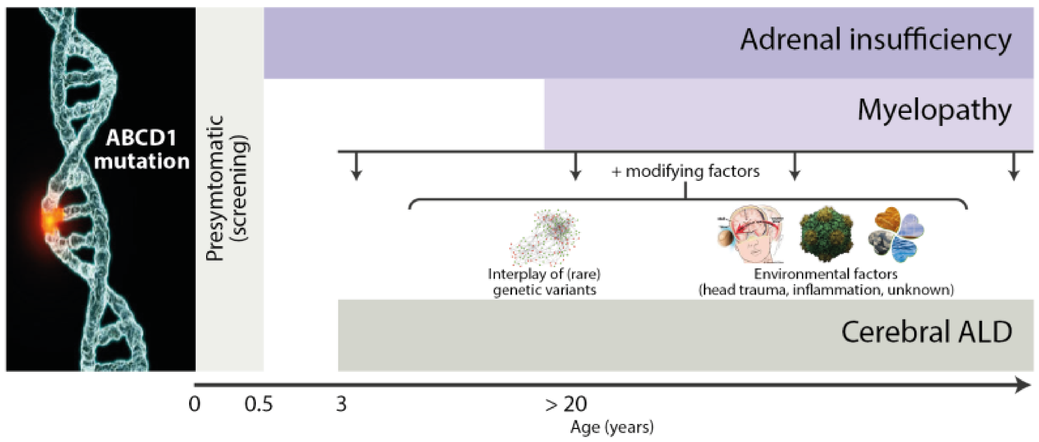
INTRODUCTION

- X-linked adrenoleukodystrophy (X-ALD) is a metabolic disorder with an estimated incidence of 1:17,000.
- It is characterized by the accumulation of very long chain fatty acids (VLCFA) in the nervous system, including the brain, spinal cord, and peripheral nerves, and in the adrenal glands.
- Phenotypic features range from adrenocortical insufficiency (Addison disease), to a progressive adult on-set paraparesis with adrenal gland dysfunction (adrenomyeloneuropathy, AMN), to the most severe and fatal cerebral form of X-ALD with damage to the brain and nervous system occurring in males later in childhood or in the early 20's.
- *ABCD1,* encoding the adrenoleukodystrophy protein (ALDP), is the only gene known to cause X-ALD.
- X-ALD is inherited in an X-linked manner with males more severely affected than females. About 80% of female carriers will develop neurological symptoms, usually in the fifth decade.



- Newborn screening (NBS) is a public health program that aims to identify medically manageable conditions in presymptomatic newborns to prevent mortality, morbidity and disabilities. NBS is critical for detection of asymptomatic individuals in the newborn period and therefore has the potential of improving therapy.
- With improved treatment options and development of high-throughput screening tests, additional conditions, especially those with severe neuropathy, have been included into NBS programs.
- Although adrenal steroid supplement is an effective approach for individuals with adrenocortical dysfunction, hematopoietic stem cell transplantation (HSCT) prior to the onset of neurological manifestations determined by MRI is proving to be a promising treatment for X-ALD.
- California (CA) added X-ALD to its NBS program in mid-2016.
- With 20 years of NBS experience using state-of-the-art molecular technology to test over 6.5 million babies, PerkinElmer Genomics (PKIG) started offering *ABCD1* full gene Sanger sequence analysis for newborns with a positive NBS in 2017 as a second tier confirmatory test performed on the original NBS blood spot.

CONCLUSION

- Second tier confirmatory NBS testing of *ABCD1* has allowed ascertaining affected individuals before symptoms progress, which will promote therapy and survival.
- Future areas for study can include using this molecular data to aid in adjusting the NBS cut-off values for calling positive X-ALD samples and correlating biochemical with molecular data for more accurate interpretation of variants.

RESULTS

- PKIG received and tested 94 dried blood spot (DBS) samples from CA, including 45 males and 49 females, from February 2017 through March 2018.
- A pathogenic/likely pathogenic (P/LP) variant or a variant of unknown significance (VUS) was detected in approximately 63.8% (60 of 94) of samples while approximately 36.3% (34 of 94) of cases yielded a negative result (Fig. 1).
- The number of cases with a P/LP variant or a VUS was 23 (38.3%) and 37 (61.7%), respectively. P/LP variants were distributed throughout the entire *ABCD1* gene (Fig. 2).
- Among the 23 cases with P/LP *ABCD1* variants, nine were hemizygous males, one was a mosaic male (Fig. 3), and thirteen were heterozygous females.
- Parental targeted testing was performed for five female and seven male probands. All seven males inherited the *ABCD1* P/LP variant or VUS from their mothers. Of the five females, two inherited the pathogenic variants from their mothers, two inherited VUSs from their fathers, and one carried a *de novo* VUS variant.

Figure 1. *ABCD1* variants in NBS positive for VLCFA accumulation

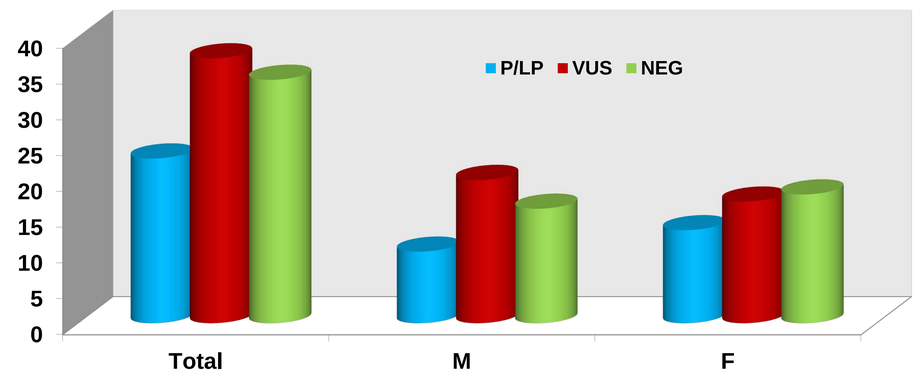


Figure 2. Distribution of *ABCD1* pathogenic variants

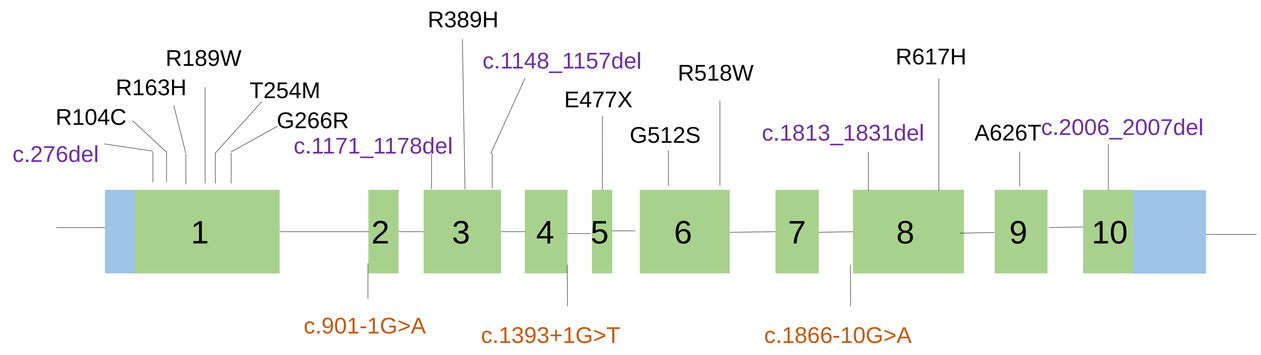
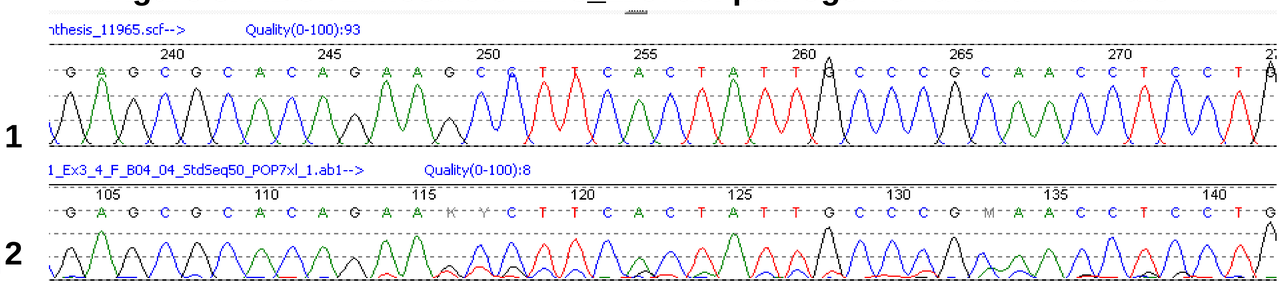


Figure 3. *ABCD1* mosaic c.1148_1157del pathogenic variant in a male



1: reference sequence; 2: patient sequence

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