Mosaicism, *De Novo* Occurrence and Subclinical Parents: Lessons Learned From Two-Year *ABCD1* Second-Tier Confirmatory Testing

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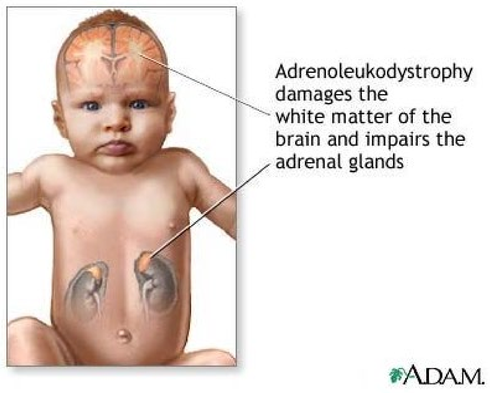
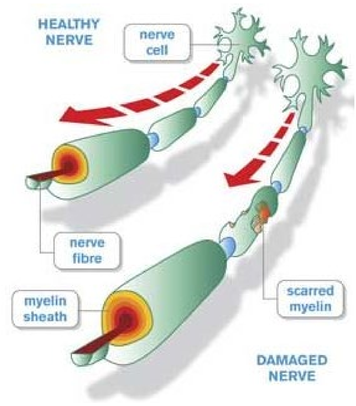
INTRODUCTION

- X-linked adrenoleukodystrophy (X-ALD) is a metabolic disorder with an estimated incidence of 1:17,000.
- It is characterized by progressive deterioration of the adrenal glands, spinal cord, white matter of the nervous system.
- 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.
- *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 service designed to identify individuals who may be at an increased risk of a variety of severe conditions at birth.
- It allows doctors to start treatment before some of the harmful effects happen.
- NBS is not a diagnostic test. It identifies individuals who may have the condition so that confirmatory follow-up testing can be offered to determine if the condition is truly present.

RESULTS

- PKIG received and tested 206 dried blood spot (DBS) samples, including 94 males and 112 females, from February 2017 through February 2018.
- A pathogenic/likely pathogenic (P/LP) variant or a variant of uncertain significance (VOUS) was detected in approximately 66.5% (64M+73F) of samples while approximately 33.5% (30M+39F) of cases yielded a negative result (Fig. 1).
- The number of cases with a P/LP variant or a VUS was 56 (27.2%) and 81 (39.3%), respectively.
- Among 19 males with parental testing, one pathogenic and one VOUS *ABCD1* variant occurred *de novo*; and three pathogenic variants were maternally inherited.
- In 21 female newborns with parental testing, three pathogenic variants were *de novo*, two were inherited maternally and one was inherited from a reportedly unaffected father (Table 1).
- Three *ABCD1* VOUS carried by females were inherited from their reportedly asymptomatic fathers while four females inherited *ABCD1* VOUS from their mothers (Table 1).
- Two males with a *ABCD1* pathogenic variants were mosaic (Fig. 2) and one with potential deletion of exons 8-10 (Fig. 3).

CONCLUSION

- Second-tier confirmatory NBS testing of *ABCD1* is essential to identify and ascertain affected individuals with positive VLCFA accumulation.
- The molecular confirmatory data will be helpful in assessing the NBS biochemical cut-off values for calling positive result.
- Future in depth study of biochemical and molecular data will also benefit accurate interpretation of *ABCD1* variants as well as genotype-phenotype correlation.

REFERENCES

1. GeneReviews: X-Linked Adrenoleukodystrophy (www.ncbi.nlm.nih.gov/books/NBK1315/)
2. Adrenoleukodystrophy Database (www.x-ald.nl)

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

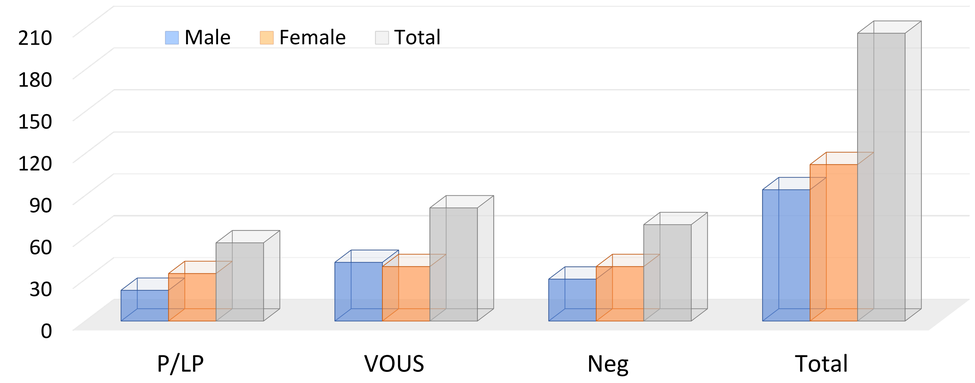


Table 1. *De novo ABCD1* pathogenic variants and Subclinical parents

Gender of Proband	*ABCD1* Variant	Maternal/ Clinical Presentation	Paternal/ Clinical Presentation	Classification
Male	c.1225-1G>A	negative	-	Pathogenic
Male	c.674C>T	negative	negative	VOUS
Male	c.1813_1831del	c.1813_1831del Asymptomatic	-	Pathogenic
Male	c.1661G>A	c.1661G>A Asymptomatic	-	Pathogenic
Male	c.760dupA	c.760dupA Asymptomatic	-	Pathogenic
Female	c.253dup	-	-	Pathogenic
Female	c.1690delG	-	-	Pathogenic
Female	c.1516dupA	-	-	Pathogenic
Female	c.276delG	-	c.276delG Asymptomatic	Pathogenic
Female	c.1393+1G>T	c.1393+1G>T	-	Pathogenic
Female	c.2006_2007delAC	c.2006_2007delAC	-	Pathogenic
Female	c.1900G>A	-	c.1900G>A Asymptomatic	VOUS
Female	c.1192G>A	-	c.1192G>A Asymptomatic	VOUS
Female	c.508G>A	-	c.508G>A Asymptomatic	VOUS

Figure 2. *ABCD1* mosaic c.1148_1157del pathogenic variant in a male

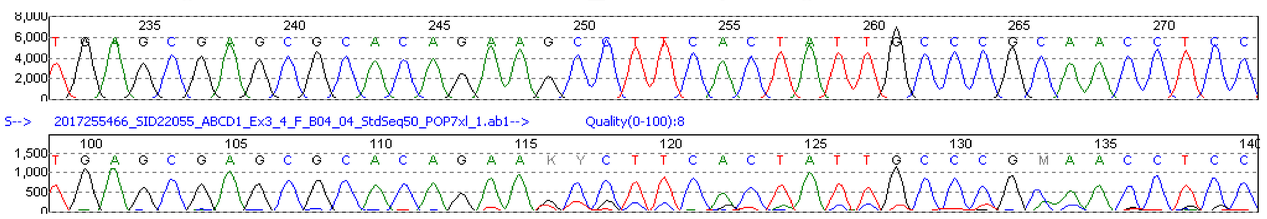


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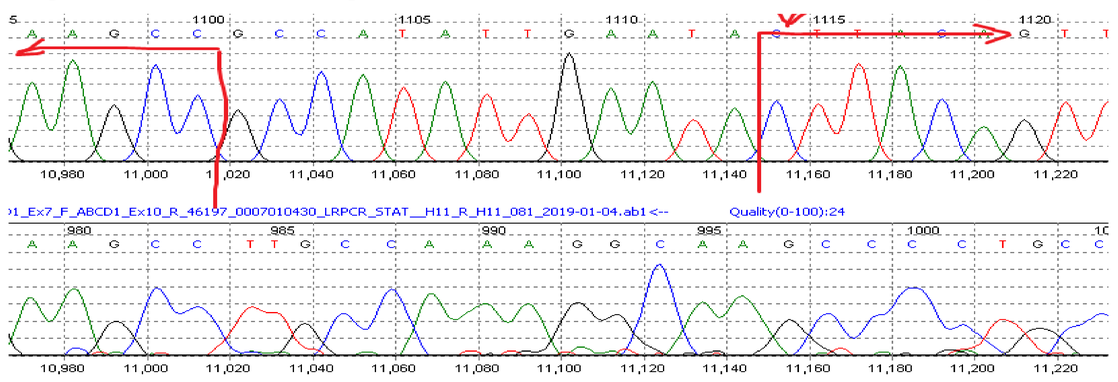
Figure 3. Potential intragenic *ABCD1* deletion of exons 8-10 in a male patient



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