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Insights From Minnesota on Newborn Screening for Adrenoleukodystrophy: A 5-Year Update

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ABSTRACT

Our objectives are to report on the outcomes of adrenal insufficiency (AI) and cerebral ALD (cALD) in children diagnosed with X-linked adrenoleukodystrophy (ALD) identified by newborn screening (NBS) in Minnesota in the first 5 years following initiation of NBS in 02/2017. A retrospective chart review was conducted for children diagnosed with ALD via Minnesota NBS from 02/06/2017 through 02/06/2022. Data reviewed included newborn screening data, diagnostic very long chain fatty acid levels, *ABCD1* molecular testing results, serial measurements of ACTH and cortisol, and serial brain MRI results. Thirty-two boys and 11 girls were molecularly and/or biochemically confirmed to have ALD. Of these 32 boys, six (2–7 years; median age:18 months) developed AI. Two boys developed cALD and underwent stem cell transplantation, one of whom also has been diagnosed with AI. All the pathogenic/likely pathogenic variants detected during the first 5 years had initial C26:0 lysophosphatidylcholine (C26:0 lysoPC) values over 0.3 μmol/L at the time of newborn screening. The addition of ALD to NBS in Minnesota has allowed for early detection of asymptomatic AI in six young patients and asymptomatic cALD in two patients. Data from our study shows a positive correlation between high newborn screening LysoPC levels and variant pathogenicity.

1 | Background

Adrenoleukodystrophy (ALD; MIM # 300100) is a peroxisomal disorder characterized by the accumulation of very long chain fatty acids (VLCFA) in the nervous system, adrenal glands, and testes. With an estimated birth prevalence of 1 in 17,000, ALD is an X-linked disorder with variability in clinical presentations and incomplete penetrance (Bezman et al. 2001; Moser et al. 2016). Historically, nearly one-third of children with ALD develop progressive cerebral disease, while others develop adolescent or adult-onset cerebral ALD, adrenomyeloneuropathy (AMN), or adrenal insufficiency (AI) after a presymptomatic period (Ruiz et al. 1998). AI is the most common manifestation of ALD, and in one study, 80% of boys diagnosed with ALD developed AI, and the majority developed it prior to adulthood, with a typical onset between 3 and 10 years of life (Dubey et al. 2005). Development of AI may start even earlier than what has been previously reported, with biochemical evidence reported in two patients who were 5 weeks and 4.5 months old (Regelmann et al. 2018). Primary

AI, if not treated, can progress to a life-threatening adrenal crisis characterized by severe dehydration, hypotension, hypoglycemia, and altered mental status (Raymond, Jones, and Moser 2007). No clear genotype–phenotype associations have been established in ALD to date. Early detection and treatment can prevent the progression of the cerebral disease, though it has not been shown to change the course of AI or AMN (Petryk et al. 2012).

Given the significant morbidity/mortality of cerebral disease and AI, ALD was added to the Recommended Uniform Screening Panel (RUSP) in 2015. Newborn screening (NBS) for ALD was first introduced in New York in 2013 and in Minnesota on February 6, 2017. The objective of this project was to describe the prevalence and clinical outcomes and explore potential biomarkers for phenotype prediction in children diagnosed with ALD by newborn screening in Minnesota during the initial 5 years after the initiation of NBS.

2 | Methods

The NBS process for ALD in the state of Minnesota has been described previously by Wiens et al. in 2019 (Wiens et al. 2019). Briefly, C26:0 Lysophosphatidylcholine (C26LPC) levels are assayed by negative ion-mode liquid chromatography-tandem mass spectrometry (LC–MS/MS). Any sample with a C26:0-LPC value $\geq 0.30\,\mu mol/L$ is considered screen positive and C26:0-LPC values between 0.16 and $0.29\,\mu mol/L$ are considered borderline and are requested to have a repeat newborn screen. With two borderline screens or with a screen positive, these newborns are referred to specialists for further evaluation and diagnosis. Although results are returned to primary care providers, close coordination and follow-up between the Minnesota Department of Health and specialists at designated referral centers ensures that positive screens are followed up at least to the initial evaluation. A retrospective chart review was conducted for children screened for ALD via Minnesota NBS from February 6, 2017, to February 5, 2022. The inclusion criteria were the following: (i) Abnormal NBS for ALD in a child born in the state of Minnesota (ii) Medical records available for review. These children are followed at the University of Minnesota/M Health Fairview Masonic Children's Hospital (UMN), Mayo Clinic, or Children's Minnesota. The majority of patients ($n = 39$) are currently being followed within the Comprehensive ALD Clinic at UMN, where boys with confirmed ALD are monitored for complications of ALD including AI and cerebral ALD (cALD). Following the identification of an abnormal newborn screen, neonates are seen in the clinic for a comprehensive evaluation including physical examination to assess for other possible peroxisomal disorders. Following confirmation with VLCFA and molecular genetic analysis, children undergo recommended adrenal surveillance, as recommended by the Pediatric Endocrine Society Drug and Therapeutics/Rare Diseases Committee in 2018. This includes monitoring of adrenal function every 3–4 months until 2 years of age and every 6 months thereafter until 18 years (Regelmann et al. 2018). High-dose ACTH stimulation testing is performed if ACTH > 100 pg/mL and cortisol < 10 mcg/dL are documented on serial monitoring. Children are diagnosed with partial adrenal insufficiency if ACTH is elevated to > 100 pg/mL and peak cortisol on high-dose ACTH stimulation testing is sub-optimal, usually between 10 and 18 mcg/dL. Children are diagnosed with complete adrenal insufficiency when ACTH is elevated (usually > 300 pg/mL) and peak cortisol on ACTH stimulation testing is significantly less than 18 mcg/dL (usually less than 10 mcg/dL).

Based on recent evidence/data on cortisol values derived from Elecsys II immunoassay, we as a group lowered our cortisol cut-off to $15\,\mu g/dL$ on the ACTH stimulation test within the past few months (Javorsky et al. 2021). When the patients for this report were being evaluated, a cut-off of $18\,\mu g/dL$ was being used, which could lead to increased false positives when the diagnosis is based on cortisol solely. However, given that we are screening using random ACTH and cortisol levels and only proceeding to a high-dose ACTH stimulation test if ACTH is rising, the likelihood of false positives is low.

The recommended surveillance for cerebral ALD includes: brain MRI at 18 and 36 months of age followed by brain MRI every 6 months until they reach 12 years of age, after which it is repeated annually (Mallack et al. 2021). Brain MRIs are scored for indication of leukodystrophy as previously described by Loes et al., with scores of 1 or more indicating cerebral demyelinating disease onset (Loes et al. 1994). In addition to being followed at the Comprehensive ALD Clinic, 25/32 (78%) participants in this publication were also enrolled in the National ALD Registry housed at UMN (https://aldnr.umn.edu).

During the retrospective chart review, the following data were collected from the medical records: gestational age, C26:0-lysophosphatidylcholine (C26:0-LPC), age, sex, VLCFA, genotype, parental inheritance, adrenal function tests, brain MRI, Loes score, and clinical phenotype.

ABCD1 variants were reported from the clinical testing labs classified as Pathogenic (P), Likely Pathogenic (LP), or Variant of Uncertain Significance (VUS). *ABCD1* variants in this cohort were re-evaluated in March 2024. The re-interpretation of variants considered classifications in ClinVar (www.ncbi.nlm.nih.gov/clinvar) and the ALDinfo *ABCD1* variant database (Mallack et al. 2022). ACMG-AMP criteria were evaluated using Franklin (franklin.genoox.com) to assist with the aggregation of publicly available evidence including population data and computational predictors. Published reports associated with variants were reviewed, locating these via the resources above. Variant curation was done independently of biochemical data in this report. Some variants were noted as having conflicting interpretations from uncertain to pathogenic (U/P conflict). Additional details of re-classification criteria are included in the supplemental methods.

Statistical comparisons were performed using GraphPad Prism 9. With data in some groups not normally distributed and with small samples in some groups, nonparametric statistics were used for comparisons (Mann Whitney tests for comparisons with only two groups and Kruskal Wallis ANOVA tests with Dunn's multiple comparisons test for > 2 groups).

This study was approved by the Institutional Review Board of the University of Minnesota (STUDY00017318).

3 | Results

During the study period, 323,314 newborns were screened by the Minnesota Department of Health. Among these, 52 newborns (34 boys and 18 girls) screened positive for ALD (Figure 1). Further diagnostic evaluation led to the confirmation of a diagnosis or carrier state of ALD in 43 children (32 boys and 11 girls; Table 2). Among the total screen positives, there were three (6%) false positives (2 boys and 1 girl). One of the boys concluded to be a false positive and underwent extensive testing including a negative Zellweger panel, Aicardi-Goutieres panel, chromosomal microarray, and exome sequencing, while the other case had normal *ABCD1* sequencing/deletion/duplication analysis and a negative Zellweger panel. The girl who was concluded to be a false positive had normal VLCFAs. Three girls with elevated C26:0 lysoPC were diagnostically confirmed to have other peroxisomal disorders such as the Zellweger spectrum or D-bifunctional protein deficiency. All three children with other peroxisomal disorders did not survive beyond the perinatal period. To date, no cases of false negative NBS for X-ALD have been reported in Minnesota. The estimated birth prevalence of ALD in the state of Minnesota is 1 in 7519 newborns (1:5000 males, 1:14,000 females) when including all *ABCD1* variants (Pathogenic (P), Likely Pathogenic (LP) and Variants of Uncertain Significance (VUS)). However, the prevalence is 1:15,396 (1:19,488 females and 1:12,589 males) when including only children with P or LP variants.

We are aware of 10 additional children with a single borderline screen who had diagnostic testing initiated in lieu of a repeat newborn screen. These included four males and three females who had normal subsequent diagnostic testing and three females who were diagnosed with ALD after these diagnostic studies. These cases cannot be counted as positive newborn screens as it is unclear if their second screening result would have been sufficient to merit additional testing and, hence, are not included in the table.

3.1 | Cerebral ALD

Two children (UMN10 and M01) diagnosed with abnormal NBS (C26LPC:1.12 and1.06) developed cerebral ALD at the age of 5 and 3.6 years, respectively. UMN10 was found to have T2 hyperintensity along the bilateral aspects of the splenium of the corpus callosum at 5 years of age during routine imaging surveillance with a Loes score of 1 consistent with cALD. He underwent an 8/8 human leukocyte antigen (HLA) matched unrelated donor (MUD) transplant at 5.5 years of age and continues to be 100% engrafted in the myeloid and 93% engrafted in the lymphoid compartment at day +180 with stable imaging findings. M01 developed abnormal imaging findings including T2 hyperintensities in the splenium of the corpus callosum at the age of 3.6 years, leading to the diagnosis of cALD and had a Loes score of 1. He underwent a 10/10 HLA-matched MUD transplant at 4 years of age. The post-transplantation course was complicated by secondary graft failure, likely due to adenoviremia. He underwent a second HCT using haploidentical peripheral blood stem cells from his father at 4.3 years. This transplant course was complicated by transplant-associated thrombotic microangiopathy and stage 3 skin graft versus host disease that was effectively treated. He continues to be 100% engrafted in myeloid and lymphoid compartments at Day 200 after the second transplant and has stable imaging findings to date.

Four children (UMN04, UMN11, UMN13, and UMN30) had nonspecific, non-progressive white matter T2 hyperintensities detected on MRI not suggestive of cALD. Similarly, UMN19 with a diagnosis of ALD and Down syndrome had evidence of

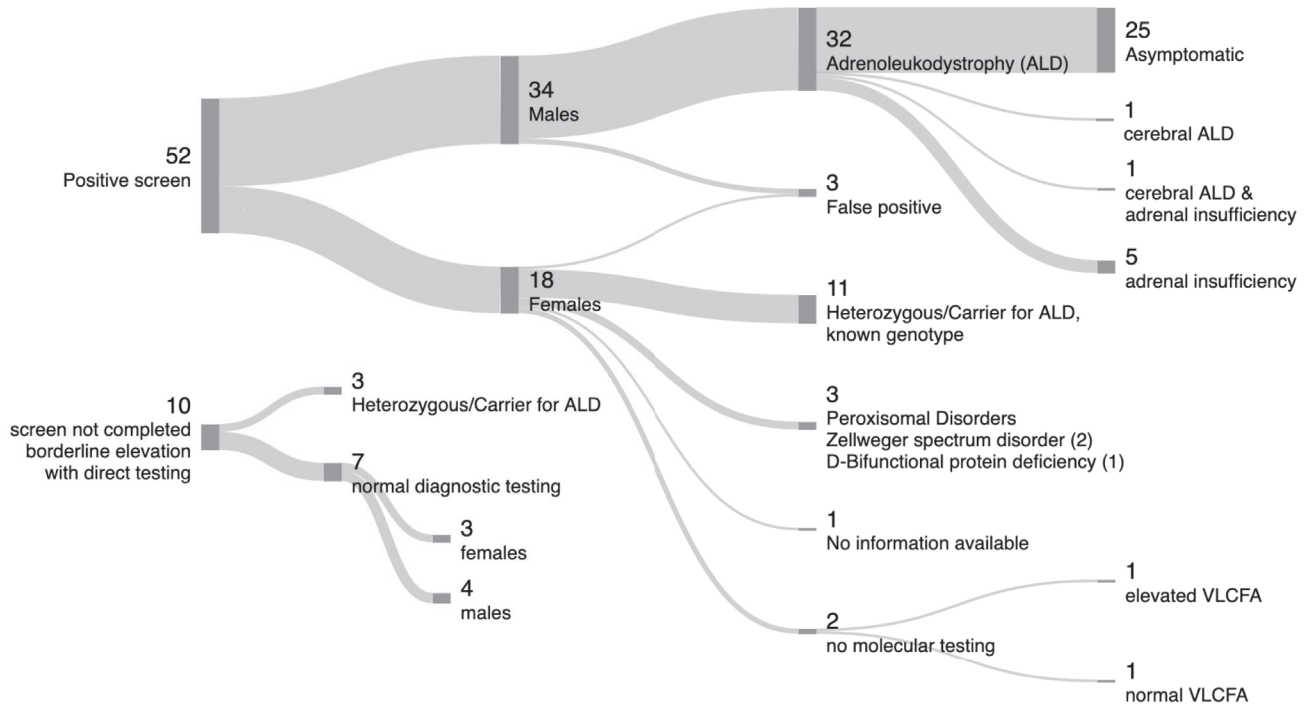

FIGURE 1 | Patient cohort described in the study. Figure prepared using SankeyMATIC.com.

chronic micro hemorrhage with mild bilateral hippocampal volume loss on brain MRI.

3.2 | Adrenal Insufficiency

Six of the 32 boys were diagnosed with abnormal NBS (Table 1). One child (UMN 27) developed complete AI at 8 months of age. He had higher than normal ACTH levels from time of first laboratory check. A high-dose ACTH stimulation test was performed at 5 months of age, which resulted in an optimal peak cortisol level of 18.1 μg/dL despite a baseline ACTH level of 279 pg/mL. Stimulation testing was repeated 3 months later and was notable for a very elevated ACTH level of 1031 pg/mL with a low peak cortisol of 11.6 μg/dL. He was started on maintenance and stress dose hydrocortisone.

The remaining five were initially diagnosed with partial AI between the ages of 9 and 30 months (median age 25 months). In these five children, screening morning/random ACTH levels were greater than 100 pg/mL, and cortisol levels were less than 10 μg/dL. On subsequent high-dose ACTH stimulation testing, ACTH levels and peak cortisol levels ranged from 113 to 202 pg/mL and 11 to 17.2 mg/dL, respectively, consistent with a diagnosis of partial AI. They were assessed as requiring stress dose hydrocortisone only as appropriate, without scheduled maintenance replacement hydrocortisone. These five boys were largely asymptomatic at the time of diagnosis with partial AI.

Three of the five proceeded to develop complete AI requiring maintenance hydrocortisone over a span of 2–4 years (between the ages of 3 and 5 years 8 months). On high-dose ACTH stimulation testing, ACTH levels ranged from 342 to 1031 pg/mL and peak cortisol levels ranged from 10 to 12.9 mg/dL.

3.3 | Genotype

Among confirmed cases of ALD or carrier state, 14 (31%) and 19 (42%) had pathogenic (P) or likely pathogenic (LP) *ABCD1* variants, respectively, as assessed by diagnostic laboratories (Table 2). Nine children (20%) with positive screen and biochemical confirmation by elevated C26-VLCFA had VUS. Biochemically diagnosed cases with VUS are frequently seen with *ABCD1*, since rare or novel missense variants without prior association with a *clinical* phenotype (cALD, AMN, AI) may be reported as VUS, that is if only associated with a biochemical phenotype. One child (UMN03) was not found to have any detectable *ABCD1* variant but was considered confirmed due to mildly elevated VLCFA levels. Functional studies confirmed his diagnosis (on a research basis, immunoblotting for ALDP showed reactivity of 5% of control, and VLCFA beta-oxidation and VLCFA synthesis were in the ALD range; Stephan Kemp, private communication). Two females, one with elevated VLCFA and one with normal VLCFA, did not undergo molecular testing since the families declined. All children in our cohort with identifiable *ABCD1* variants had a variant that was maternally inherited except UMN08 and UMN38, which had variants that were found to be *de novo* in origin.

Given wide variability in interpretations of *ABCD1* gene variants across clinical laboratories, including patients in our cohort with differing pathogenicity classifications for the same variant (Table 3), we re-evaluated pathogenicity classifications of *ABCD1* variants based on the public databases and resources and literature evidence. Curated variant assessments were mostly consistent with classification on the diagnostic laboratory reports (Table 3). Twenty-five of 28 variants from cases identified by NBS were missense variants with two frameshift variants and one canonical splice variant. Five variants designated as VUS by diagnostic laboratories (p.Ala170Thr, p.Ala247Thr, p.Arg280His, p.Thr483Met, and p.Ala634thr) and seven variants called as likely pathogenic (p. Tyr27Ser, p.Ala170Thr, p.Asp194Glu, p.Leu229Val, p.Arg275Trp, p.Lys533Gln, and p.Val583Met) were noted in our analysis as having conflicting evidence of pathogenicity and were put in an intermediate category with conflicting interpretations from uncertain to pathogenic (U/P conflict). In all of these U/P conflict cases, while the variants have been reported in association with biochemical findings, we were unable to identify associated cases with clinical disease features. One variant,

TABLE 1 | Clinical outcomes in children diagnosed with ALD by NBS in Minnesota in the first 5 years.

	Partial adrenal insufficiency			Complete adrenal insufficiency			cALD
	Age	ACTH (pg/mL)	Cortisol (mg/dL)	Age	ACTH (pg/mL)	Cortisol (mg/dL)	Age
1	30 months	113	17.2[a]	5 years 8 months	342	12.9	N/A
2	11 months	230	12.6[a]	3 years	532	10	N/A
3	N/A	N/A	N/A	8 months	1031	11.6[a]	N/A
4	28 months	N/A	11[a]	N/A	N/A	N/A	N/A
5	25 months	117	12.5[a]	N/A	N/A	N/A	N/A
6[b]	9 months	202 pg/mL	8.8 mg/dL	4 years	On physiologic dosing since transplant[b]		4 years
7	N/A	N/A	N/A	N/A	N/A		5 years

Note: Six children diagnosed with adrenal insufficiency and two children diagnosed with cALD.
[a]Peak cortisol on high-dose ACTH stimulation test.
[b]Evaluation at OSH.

TABLE 2 | Clinical and biochemical features of children diagnosed with ALD by abnormal NBS.

Subjects	Current age[a] (age at recent MRI if different from the current age)	Gestational age in weeks	Sex	Genotype/Classification/Inheritance[b]	C26:0 LPC μmol/L (first/second screen)[c]	C26:0 μg/ml	C24/C22	C26/C22	Partial AI (yes/no)	Complete AI (yes/no)	MRI brain/Loes score	Clinical phenotype
UMN01	7	39	M	c.823C>T (p.Arg275Trp)/LP/mat	0.35	1.26	1.807	0.093	No	No	Normal/0	Asymptomatic
UMN02	6	39	M	c. 823C>T (p.Arg275Trp)/LP/mat	0.47	NA	NA	NA	No	No	Normal/0	Asymptomatic
UMN03	6	NA	M	None detected/mat	0.26	1.325	1.58	0.059	No	No	Normal/0	Asymptomatic
UMN04	7	37	M	c.1597A>C (p.Lys533Gln)/LP/mat	0.4	1.202	1.66	0.055	No	No	Faint T2 hyperintensity/0	Asymptomatic
UMN05	7	39	M	c.80A>C (p.Tyr27Ser)/LP/mat	0.2/0.16	0.607	1.46	0.026	No	No	Normal/0	Asymptomatic
UMN06	7	37	M	c.487C>T (p.Arg163Cys)/LP/mat	0.76	2.737	1.76	0.12	No	No	Normal/0	Asymptomatic
UMN07	7	NA	F	c.1635-16_1645delinsCACAGACATGTAGGGC/LP/mat	0.54	0.83	1.465	0.054	N/A	N/A	NA	Discharged from clinic
UMN08	6	41	F	c.1978C>T (p.Arg660Trp)/P/dn	0.57	0.85	1.412	0.047	N/A	N/A	NA	Discharged from clinic
UMN09	6	36	F	c.1553G>A (p.Arg518Gln)/P/mat	0.64	1.05	1.37	0.063	N/A	N/A	NA	Discharged from clinic
UMN10	6	39	M	c.1973C>T (p.Thr658Ile)/LP/unk	1.12	3.2	2.006	0.164	No	No	T2 hyperintensity within the splenium of the corpus calloum (Loes score of 1) and Chiari type 1/0	cALD s/p HCT at 5.5 years
UMN11	6	37	M	c. 593C>T (p.Thr198Met)/LP/mat	0.43	0.762	1.45	0.046	No	No	T2 white matter hyperintensity in the central left parietal white matter/0	Asymptomatic
UMN12	5	Term	M	c. 593C>T (p.Thr198Met)/LP/mat	0.31	0.94	1.342	0.055	No	No	Normal/0	Asymptomatic

(Continues)

TABLE 2 | (Continued)

Subjects	Current age[a] (age at recent MRI if different from the current age)	Gestational age in weeks	Sex	Genotype/Classification/Inheritance[b]	C26:0 LPC μmol/L (first/second screen)[c]	C26:0 μg/ml	C24/C22	C26/C22	Partial AI (yes/no)	Complete AI (yes/no)	MRI brain/Loes score	Clinical phenotype
UMN13	6	32	M	c.293C>T (p.Ser98Leu)/P/mat	1.06	4.205	1.79	0.205	N/A	Yes	T2 hyperintensities in the paraventricular area/0	ADHD/hyperactivity, Adrenal insufficiency
UMN14	3	34	F	c.293C>T (p.Ser98Leu)/P/mat	0.44	NA	NA	NA	N/A	N/A	NA	Discharged from clinic
UMN15	5	Term	M	c.1747G>A (p.Val583Met)/LP/mat	0.49	1.48	1.791	0.092	No	No	Normal/0	Asymptomatic
UMN16	5	33	F	Undetermined[e]	0.2	0.29	1.011	0.018	N/A	N/A	NA	NA
UMN17	5	37	M	c.1028G>T (p.Gly343Val)/P/mat	0.74	2.56	1.921	0.157	N/A	Yes	Normal/0	Adrenal insufficiency
UMN18	5	39	F	c.1817C>T (p.Ser606Leu)/P/unk	0.45	1.6	1.559	0.081	N/A	N/A	NA	Discharged from clinic
UMN19	4	39	M	c.625G>A (p.Ala209Thr)/VUS/mat	0.31/0.28	0.71	1.238	0.052	No	No	Chronic microhemorrhages and Questionable bilateral mild hippocampal volume loss/0	Down syndrome
UMN20	4	38	M	c.685C>G (p.Leu229Val)/LP/mat	0.33	0.73	1.474	0.043	No	No	Normal/0	Asymptomatic
UMN21	4	38	M	c.685C>G (p.Leu229Val)/LP/mat	0.2	0.663	1.31	0.041	No	No	Normal/0	NA
UMN22	4	39	M	c.685C>G (p.Leu229Val)/LP/mat	0.26	0.928	1.54	0.043	N/A (No f/up)	N/A (No f/up)	NA? Lost to follow up	NA
UMN23	4	37	M	c.1781G>T (p.Gly594Val)/LP/mat	0.44	0.92	1.899	0.086	No	No	Normal/0	Asymptomatic
UMN24	4	38	F	c.565C>T (p.Arg189Trp)/LP/pat	0.31	0.63	1.358	0.044	N/A	N/A	NA	Discharged from clinic
UMN25	3	41	M	c.593C>T (p.Thr198Met)/P/unk	0.33	0.48	1.511	0.051	No	No	Normal/0	Asymptomatic
UMN26	3	39	M	c.508G>A (p.Ala170Thr)/LP/mat	0.23	NA[d]	NA[d]	NA[d]	No	No	Normal/0	Asymptomatic

(Continues)

TABLE 2 | (Continued)

Subjects	Current age[a] (age at recent MRI if different from the current age)	Gestational age in weeks	Sex	Genotype/Classification/Inheritance[b]	C26:0 LPC μmol/L (first/second screen)[c]	C26:0 μg/ml	C24/C22	C26/C22	Partial AI (yes/no)	Complete AI (yes/no)	MRI brain/Loes score	Clinical phenotype
UMN27	3	39	M	c.1876G>A (p.Ala626Thr)/P/mat	0.96	1.69	1.66	0.116	N/A	Yes	Normal/0	Adrenal insufficiency
UMN28	3	40	M	c.1876G>A (p.Ala626Thr)/P/mat	0.39	1.13	1.729	0.059	No	No	Normal/0	Asymptomatic
UMN29	2 (1.5)	39	M	c.1448C>T (p.Thr483Met)/VUS/mat	0.34	0.85	1.794	0.067	No	No	Normal/0	Asymptomatic
UMN30	4	39	M	c.582C>G (p.Asp194Glu)/LP/unk	0.27	0.45	1.383	0.032	No	No	Non-specific punctate foci/0	Asymptomatic
UMN31	3	39	M	c.839G>A (p.Arg280His)/VUS/mat	0.28	1.06	1.758	0.072	No	No	Stable T2 hyperintensity within white matter and corpus callosum without contrast enhancement or diffusion restriction/0	Asymptomatic
UMN32	4	39	M	c.739G>A (p.Ala247Thr)/VUS/mat	0.28/0.18	0.916	1.38	0.036	No	No	Normal/0	Asymptomatic
UMN33	3	39	M	c.739G>A (p.Ala247Thr)/VUS/mat	0.35	0.86	1.585	0.041	No	No	Normal/0	Asymptomatic
UMN34	3	38	F	c.1553G>A (p.Arg518Gln)/P/nonmat	0.34	1.11	1.353	0.056	N/A	N/A	NA	Discharged from clinic
UMN35	4	37	M	c.1900G>A (p.Ala634Thr)/VUS/mat	0.27	0.9	1.519	0.049	No	No	Normal/0	Asymptomatic
UMN36	3	39	M	c.1415_1416delAG (p.Gln472Argfs*83)/P/mat	0.92	1.638	1.5	0.099	Yes	No	Normal/0	Adrenal insufficiency
UMN37	2 (1.5)	40	M	c.1876G>A (p.Ala626Thr)/P/mat	1.03	1.45	1.75	0.1	Yes	No	Stable nonspecific T2 hyperintensities in the periatrial white matter/0	Adrenal insufficiency
UMN38	2 (1.5)	40	F	c.1814T>C (p.Leu605Pro)/LP/dn	0.36	0.83	1.208	0.037	N/A	N/A	NA	Discharged from clinic
UMN39	2 (1.5)	39	M	c.1747G>A (p.Val583Met)/LP/mat	0.3	1.71	1.86	0.07	No	No	Normal/0	Asymptomatic

(Continues)

TABLE 2 | (Continued)

Subjects	Current age[a] (age at recent MRI if different from the current age)	Gestational age in weeks	Sex	Genotype/Classification/Inheritance[b]	C26:0 LPC μmol/L (first/second screen)[c]	C26:0 μg/ml	C24/C22	C26/C22	Partial AI (yes/no)	Complete AI (yes/no)	MRI brain/Loes score	Clinical phenotype
M01	5	39	M	c.900+1G>A (p.Val301fs*?)/P/mat	1.06	1.82	1.41	0.074	Yes	No	T2 hyperintensity within the splenium of the corpus callosum (Loes score of 1)	cALD s/p HCT at 4 yr., Adrenal insufficiency
M02	2 (1.5)	39	M	c.508G>A (p.Ala170Thr)/VUS/mat	0.18	0.782	1.18	0.024	No	No	Normal	Asymptomatic
CH01	No data available	NA	F	c.508G>A (p.Ala170Thr)/VUS/mat	0.17	0.849	NA	NA	NA	NA	NA	NA
CH02	5	39	F	Molecular testing not done	0.3	0.801	1.19	0.028	NA	NA	NA	Asymptomatic
CH03	5	37	F	False Positive	0.24	NA	NA	NA	NA	NA	NA	NA
CH04	4	37	F	c.401T>G (p.Leu134Arg)/VUS/mat	0.38	0.988	1.13	0.052	NA	NA	NA	NA
CH05	3	40	F	c.1876G>A (p.Ala626Thr)/P/mat	0.46	0.920	1.19	0.037	NA	NA	NA	NA
CH06	5	39	M	False positive	0.23	0.571	0.72	0.021	NA	NA	NA	Discharged from clinic
CH07	4	NA	M	False positive	0.26	0.242	0.93	0.011	NA	NA	NA	Discharged from clinic
CH08	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Note: Variant classifications from clinical report: VUS—variant of uncertain significance, LP—likely pathogenic, P—pathogenic. Inheritance: Inheritance was described as maternal "mat" (if the proband's mother had positive genetic testing or if multiple sons or maternal relatives were confirmed to have X-ALD), paternal "pat" (if the proband's father had positive genetic testing or VLCFA studies), de novo "dn" (if the mother of a male proband or both parents of a female proband had negative testing), not maternal "nonmat" (if paternal testing for a female proband was not completed but maternal testing was negative), or unknown "unk" (if no parental testing was completed).
Abbreviations: AI, adrenal insufficiency; NA, not available for review.
[a]Age at the time of the most recent brain MRI and adrenal insufficiency labs for asymptomatic children.
[b]Genotypes reported with reference to transcript NM_000033.4.
[c]Where only one C26:0-LPC value since first screen was above borderline range only one value to report in table.
[d]Confirmatory test sent included only C26:0 Lyso PC: 258.5 pmol/mL (X-ALD range).
[e]Undetermined: Molecular testing not done.

TABLE 3 | *ABCD1* variants observed in newborn screening.

ABCD1 (NM_000033.4) genotype	Subject (s)	Clinical results report variant interpretation(s) in this cohort	ALD database classification[a]	ClinVar classification (s)	ClinVar variation ID	Franklin automated caller classification[a]	Variant Re-classification from this study[a]
c.80A>C [p.Tyr27Ser]	UMN05	LP	VUS	LP	623,112	LP	U/P conflict
c.293C>T [p.Ser98Leu]	UMN13, UMN14	P, P	P	P	458,641	P	P
c.401 T>G [p.Leu134Arg]	CH04	VUS	LP	N/A	N/A	LP	VUS
c.487C>T [p.Arg163Cys]	UMN06	LP	LP	P/LP	623,113	P	LP
c.508G>A [p.Ala170Thr]	UMN26, M02, CH01	LP, VUS, VUS	LB	VUS/LP	644,432	LP	U/P conflict
c.565C>T [p.Arg189Trp]	UMN24	LP	P	LP/P	430,349	P	P
c.582C>G [p.Asp194Glu]	UMN30	LP	VUS	VUS/LP	570,073	LP	U/P conflict
c.593C>T [p.Thr198Met]	UMN11, UMN12, UMN25	LP, LP, P	P	LP/P	623,114	P	LP
c.625G>A [p.Ala209Thr]	UMN19	VUS	VUS	N/A	N/A	LP	VUS
c.685C>G [p.Leu229Val]	UMN20, UMN21, UMN22	LP, LP, LP	LP	VUS/LP	995,582	VUS	U/P conflict
c.739G>A [p.Ala247Thr]	UMN32, UMN33	VUS	VUS	VUS/LP	528,342	LP	U/P conflict
c. 823C>T [p.Arg275Trp]	UMN01, UMN02	LP	VUS	VUS/LP	384,589	P	U/P conflict
c.839G>A [p.Arg280His]	UMN31	VUS	VUS	VUS/LP/P	953,948	P	U/P conflict
c.900+1G>A	M01	P	P	N/A	N/A	LP	P
c.1028G>T [p.Gly343Val]	UMN17	P	P	P	1,800,630	P	P
c.1415_1416delAG	UMN36	P	P	P	11,303	P	P
c.1448C>T [p.Thr483Met]	UMN29	VUS	VUS	VUS/LP	585,354	P	U/P conflict
c.1553G>A [p.Arg518Gln]	UMN09, UMN34	P, P	P	P	92,317	P	P
c.1597A>C [p.Lys533Gln]	UMN04	LP	VUS	LP/P	623,117	P	U/P conflict
c.1635-16_1645delins CACAGACATGTAGGGC	UMN07	LP	P	N/A	N/A	LP	LP
c.1747G>A [p.Val583Met]	UMN15, UMN39	LP, LP	VUS	VUS/LP/P	566,124	P	U/P conflict
c.1781G>T [p.Gly594Val]	UMN23	LP	VUS	N/A	N/A	LP	VUS

(Continues)

TABLE 3 | (Continued)

ABCD1 (NM_000033.4) genotype	Subject (s)	Clinical results report variant interpretation(s) in this cohort	ALD database classification[a]	ClinVar classification (s)	ClinVar variation ID	Franklin automated caller classification[a]	Variant Re-classification from this study[a]
c.1814T>C [p.Leu605Pro]	UMN38	LP	P	VUS/LP	1,679,949	LP	LP
c.1817C>T [p.Ser606Leu]	UMN18	P	P	P	11,310	P	P
c.1876G>A [p.Ala626Thr]	UMN27, UMN28, UMN37, CH05	P, P, P, P	P	LP/P	576,910	P	P
c.1900G>A [p.Ala634Thr]	UMN35	VUS	VUS	VUS/LP	282,253	LP	U/P conflict
c.1973C>T [p.Thr658Ile]	UMN10	LP	P	LP	623,118	P	LP
c.1978C>T [p.Arg660Trp]	UMN08	P	P	P	585,302	P	P

Note: Acknowledging the limitations of current variant classification criteria to reach consensus across multiple resources particularly for this gene and disorder, variants with conflicting classifications ranging from VUS to likely pathogenic/pathogenic across multiple sources reported only through newborn screening or only associated with biochemical phenotype and without published association with a defined, clinical phenotype were re-classified in a separate category, variants with conflicting interpretations from uncertain to pathogenic (U/P).

[a]Data from various sources were reviewed to inform variant classification. Variant classifications from publicly available databases (ALD database—https://adrenoleukodystrophy.info/mutations-and-variants-in-abcd1, Clinvar—https://www.ncbi.nlm.nih.gov/clinvar/, and Franklin—franklin.genoox.com) were reviewed in March of 2024.

p.Gly594Val, was ultimately judged as of uncertain significance even though initially classified as a likely pathogenic variant by the diagnostic laboratory.

3.4 | Biochemical Characteristics

In this cohort, higher variant pathogenicity at clinical reporting generally correlated with higher C26LPC values. Cases with variants of uncertain significance (VUS) as well as likely pathogenic (LP) variants judged by the clinical labs had generally lower C26LPC values, whereas those with pathogenic (P) alleles had statistically elevated C26LPC values compared to VUS ($p = 0.142$) or LP ($p = 0.0058$; Figure 2a). Following our variant curation process, cases with likely pathogenic and pathogenic variants all had screening C26LPC values higher than 0.3 mM/L and a significantly higher distribution of C26LPC values (0.31–1.12; median: 0.54; SD:0.28) than cases with variants classified as VUS or U/P conflict variants (0.17–1.88; median: 0.28; SD:0.3) ($p < 0.0001$). This effect appears largely due to reclassification of multiple variants clinically reported as likely pathogenic but for which there has not been a clear association with clinical disease into our U/P conflict category (Figure 2b). In contrast, there were no significant differences in the ranges of C26:0 values when considered with respect to variant classification (Figure 2c,d).

4 | Discussion

The addition of adrenoleukodystrophy to newborn screening panels in many states has the potential to further define the natural history of adrenoleukodystrophy and to reduce the associated morbidity and mortality of patients developing more advanced disease. Early identification of cases is essential in ALD since the only therapy able to arrest cerebral demyelinating disease, hematopoietic stem cell transplantation, is only beneficial if performed early in disease progression (Peters et al. 2004; Warren et al. 2007; Cartier and Aubourg 2010). In our cohort, cases were able to be transplanted with only minimal cerebral disease, highlighting the benefits of screening. In addition, early identification of adrenal insufficiency reduces the morbidity and mortality associated with adrenal crises. Newborn screening allows for monitoring and then for treatment of ALD complications before symptoms are apparent because once the disease is clinically evident, it may be too late to treat.

Our data support that early evolving cases of AI can be identified through routine screening of adrenal function of children diagnosed with ALD through newborn screening. In our six patients who were started on hydrocortisone, AI developed prior to any neurological signs/symptoms and any cerebral findings on brain MRI. We suspect, as noted especially in the cases of UMN 13, UMN 17, and M01 that AI largely develops gradually, and the ability to mount an adequate cortisol response during illness is compromised before the development of any daily symptoms. Therefore, in the absence of newborn screening and subsequent routine screening of adrenal function, affected individuals would be at high risk of morbidity/mortality from undiagnosed AI. While our data suggest asymptomatic adrenal insufficiency can start earlier in childhood compared to what

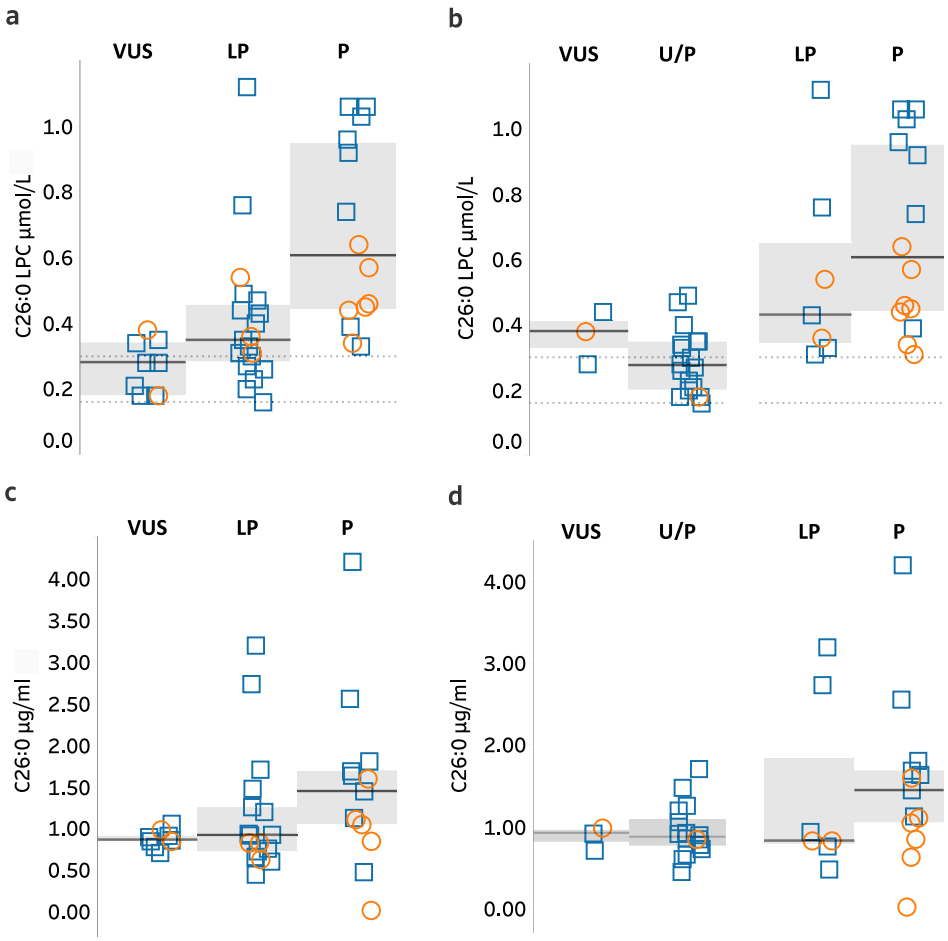

FIGURE 2 | (a) C26:0 Lyso PC (C26LPC) levels in different subsets of variant classification as reported by the diagnostic laboratory. (b) C26LPC in the different subsets as per author's variant reclassification. (c) C26:0 levels in different subsets of variant classification as reported by the diagnostic laboratory. (d) C26LPC levels in different subsets as per author's variant reclassification. Circle represents levels in females and square represents the levels in males. Dashed lines at 0.16 (borderline) and 0.3 (positive/abnormal) indicate cutoffs associated with newborn screening in Minnesota. Solid lines indicate median and the gray bar represent the interquartile range (Q1–Q3). P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance; U/P, interpretations with conflicting criteria between uncertain and pathogenic.

has previously been described, the overall prevalence of adrenal insufficiency and cerebral disease is likely to be higher in the cohort as they age.

Prior to the advent of newborn screening, the prevalence of ALD was thought to be 1:14,000 female births and 1:21,000 male births. Data from New York, the first state to add ALD to the newborn screen, indicate a higher incidence in males compared to the previous literature—1: 15,400 (Regelmann et al. 2018). It is speculated that the prior discrepancy may have been due to the mortality of boys secondary to undetected adrenal insufficiency. However, the birth prevalence in the state of Minnesota reported in 2019 was 1 in 3878 males and 1 in 6586 females, much higher than what is reported in the literature (Wiens et al. 2019). After 5 years of screening, the Minnesota estimated incidence is less frequent (1:5000 males, 1:14,000 females) than the first-year estimate, yet retaining the observed gender difference in identifying twice as many males as females.

It was believed that 95% of the cases are inherited from one of the parents, with 5% being *de novo* (Bezman et al. 2001; Wang et al. 2011; Coll et al. 2005). However, a recent population-based Norwegian study reported a higher rate of *de novo* mutation at 19% (Horn et al. 2013). It is unclear whether this discrepancy in the *de novo* rate is related to the limited sample size. In our cohort presented here, only two children were found to have an apparent *de novo* variant (2.3%), whereas 97.6% inherited the variant from their mother. 15%–31% of ALD carrier females have been reported to have normal C26:0 (Engelen et al. 2014; Moser et al. 1999). As a result, the girls in our cohort (UMN16 and CH06) who had normal VLCFA without any associated molecular testing have been classified as undetermined.

As previously described in the literature, an increased frequency of variants of uncertain significance in *ABCD1* was detected in our newborn screening cohort (Kemp et al. 2023). The application of ACMG criteria for specific *ABCD1* gene variants has been diversely applied across laboratories, necessitating an independent review of variants to apply consistent criteria across cases. After this curation, a pattern emerged biochemically with regard to C26LPC, separating variants with clinically established pathogenicity from novel, missense variants of uncertain significance, even when those variants have been associated with VLCFA elevation.

5 | Conclusion

The addition of adrenoleukodystrophy to the newborn screening conditions in Minnesota has allowed for the early detection of AI in six very young patients and cerebral ALD in two patients, where early diagnosis and management helped reduce morbidity and mortality associated with this disease. This highlights the potential for benefits from initiating screening in jurisdictions that have not yet adopted NBS for ALD. Additionally, our findings suggest that C26:0-lysophosphatidylcholine (C26LPC) may better align with variant pathogenicity than C26:0 VLCFA. Larger studies, potentially using databases such as the National ALD Registry, are warranted to validate these results and to improve our understanding of this disease.

Author Contributions

Arpana Rayannavar: conceptualization, data curation, formal data analysis, writing – original draft, and writing – review and editing. **Charles J. Billington Jr:** conceptualization, formal data analysis, writing – original draft, writing – review and editing. **Rebecca Tryon:** conceptualization, formal data analysis, writing – original draft, writing – review and editing. **Tory Kaye:** data curation, formal data analysis, and writing – review and editing. **Ashish Gupta:** writing – review and editing. **Troy C. Lund:** writing – review and editing. **Aida Lteif:** data curation and writing – review and editing. **Katherine Adriatico:** data curation and writing – review and editing. **Paul J. Orchard:** writing – review and editing. **Bradley S. Miller:** writing – review and editing. **Nishitha R. Pillai:** conceptualization, data curation, formal data analysis, writing – original draft, and writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

IRB

This study was approved by the Institutional Review Board of the University of Minnesota (STUDY00017318).

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