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BRIEF REPORT

Vitamin D status and latitude predict brain lesions in adrenoleukodystrophy

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Abstract

Objectives: Approximately 40% of boys with X-linked adrenoleukodystrophy (ALD) develop inflammatory demyelinating brain lesions (cerebral ALD, cALD) and are at risk for death or severe disability. Risk factors for cALD are poorly understood. Our objective was to evaluate whether vitamin D status, which influences immune function, is associated with risk for cALD. **Methods:** We used two independent cohorts to assess whether low vitamin D status is correlated with cALD. We used complementary proxies for vitamin D status: plasma 25-hydroxyvitamin D levels and latitude. In our first cohort, we measured 25-hydroxyvitamin D in biobanked plasma samples from ALD boys with initially normal brain MRIs followed at two expert centers. In a second cohort, we measured latitude (using home ZIP code) among ALD boys identified in a national administrative database (PHIS) covering 51 US pediatric hospitals. We used logistic regression models to estimate the odds of developing cALD in each cohort. **Results:** In the first cohort, we identified 20 ALD boys with a total of 53 plasma sample timepoints who met inclusion criteria; 50% (n = 10) subsequently developed cALD. Average 25-hydroxyvitamin D levels were lower among boys who developed cALD than those who did not (median 28.9 vs 36.6 ng/ml); p = 0.019. For each 10 ng/mL decrease in 25-hydroxyvitamin D, the odds ratio for developing cALD was 6.94; p = 0.044. In the second cohort, we identified 230 ALD boys across 28 states; 57% of boys (n = 132) developed cALD. Each 2° increase in latitude conferred an odds ratio of 1.17 (95% confidence interval, 1.01, 1.35); p = 0.036 for developing cALD. **Conclusions:** Using independent cohorts, we found that ALD boys with lower pre-morbid plasma levels of 25-hydroxyvitamin D, or more northerly latitude of residence, were more likely to develop cALD. These findings offer complementary lines of evidence that vitamin D and/or ultraviolet light exposure influence cALD risk.

Introduction

X-linked adrenoleukodystrophy (ALD) results from a mutation of the peroxisomal fatty acid transporter gene *ABCD1* leading to accumulation of very long-chain fatty acids in adrenal cortex, blood, and brain tissue.[1] Thirty to forty percent of boys with ALD will develop a disease state termed cerebral ALD (cALD); incidence is concentrated in the first decade.[2,3] cALD is marked by progressive inflammatory demyelinating brain lesions. Left untreated cALD culminates in a vegetative state and death within a few years of onset.[4] Small, early-stage brain lesions can be halted with hematopoietic stem cell transplant.[4,5] Although the presence of a pathogenic *ABCD1* variant confirms an ALD diagnosis, the risk for cALD is discordant in siblings or even identical twins.[1] This suggests a role for environmental or epigenetic risk factors. Understanding etiology would be valuable for surveillance, treatment, and prevention.

Notably, ALD brain lesions share key similarities with multiple sclerosis lesions, including a predilection for the corpus callosum and an inflammatory demyelinating histology characterized by a leading edge of activated microglia, lipid-laden macrophages, and trailing lymphocytes.[6] We reasoned that shared histology could suggest shared risk factors. Lower levels of vitamin D exposure have been robustly linked to higher risk of multiple sclerosis diagnosis, subsequent brain lesions, and improved prognosis.[7–11] Trials of vitamin D supplementation in multiple sclerosis have demonstrated modest benefits, including a reduction in the appearance of new brain lesions.[12,13] Building on this premise, we investigated whether two established proxies for vitamin D exposure, plasma 25-hydroxyvitamin D status and north–south latitude, were correlated with the risk of developing cALD brain lesions.

Methods

The Institutional Review Boards (IRB) at Kennedy Krieger Institute and the Institut National de la Santé et de la Recherche Médicale (INSERM) approved the longitudinal collection of blood for research, which was obtained following written, informed consent. The IRB at Stanford University exempted the subsequent analysis of the previously collected and deidentified blood samples as non-human research. The IRB at the University of Utah exempted the study of latitude and cerebral within the deidentified PHIS database as nonhuman research.

To assess correlation of premorbid plasma 25-hydroxyvitamin D and subsequent development of cALD, we analyzed previously biobanked plasma samples collected as part of longitudinal clinical cohort studies of ALD boys at two centers (Kennedy Krieger Institute, Baltimore, Maryland, USA & INSERM, Paris, France) between May 1, 1999 and November 8, 2012. All individuals were followed with serial magnetic resonance imaging (MRIs) every 6–12 months to screen for brain lesions according to standard care guidelines.[1] ALD brain lesions were defined as any gadolinium-enhancing white matter lesion on brain MRI. Plasma samples were collected longitudinally and stored at −80°C. Inclusion criteria for plasma analysis were: (i) molecular/biochemical diagnosis of ALD, (ii) male sex, (iii) age <11 years at sample collection (corresponding with period of maximal risk for cALD[2,3]), (iv) normal brain MRI prior to plasma collection, and (v) availability of plasma samples from at least two timepoints prior to study endpoint. Study endpoints were (i) identification of cALD brain lesion on MRI or (ii) no ALD brain lesion identified at time of last follow-up. We excluded samples collected after a brain lesion was identified. We measured 25-hydroxyvitamin D levels (Heartland Assays Inc.) and C26:0 lipid levels (Kennedy Krieger Institute) using tandem mass spectrometry. Lipid levels were measured at the time of plasma collection. We used a two-tailed Mann–Whitney *U* test to assess differences between groups (Prism v8.4, GraphPad Software, San Diego, CA). We used a logistic regression analysis to estimate the odds of developing brain lesion based on each patient's average 25-hydroxyvitamin D level (Stata Stastical Software v11, StataCorp, College Station, TX).

To assess correlation of latitude and cALD, we determined the total number of ALD boys in the Pediatric Health Information System (PHIS) database. Patients had to be male, age <19 years, with a valid US ZIP code, and presenting between October 1, 2015 and June 30, 2019; with an ICD10 code for ALD, including E71520—Childhood cerebral X-linked adrenoleukodystrophy; E71521—Adolescent X-linked adrenoleukodystrophy; E71522—Adrenomyeloneuropathy; E71528—X-linked adrenoleukodystrophy NEC; and E71529—X-linked adrenoleukodystrophy NOS. We assessed for the primary clinical outcome, the development of brain lesions, by the presence of an ICD9 or ICD10 code for at least one of the following categories indicating CNS involvement: bone marrow transplant/hematopoietic stem cell transplant; cerebral degeneration or neurological dysfunction; or cortical visual loss/blindness (full list in Supporting Information Table S1). We used ZIP codes to determine latitude; we collapsed latitude into 2° ranges to allow a minimum of two ALD cases in each category. We excluded latitude ranges that had no ALD cases (19–24° north and >49° north). For each latitude range, we calculated the unadjusted probability of cALD among total ALD cases. We used a logistic regression model to

calculate the probability of cALD among ALD patients, adjusting for the size of PHIS population. Finally, we modeled the relationship between latitude ranges and probability of cALD compared to ALD using logistic regression, adjusting for total PHIS population size in that latitude (SAS v9.4, SAS Institute, Cary, NC).

Results

We identified 20 ALD boys with plasma samples collected at 53 timepoints over an average of 3.8 ± 3.1 years between first plasma collection and clinical endpoint. All 20 boys had normal MRIs at the start of surveillance and at the time of plasma collection. Ten boys developed brain lesions during surveillance with a median Loes score of 1 at the time of detection. All but one of the lesions originated in the splenium of the corpus callosum. All lesions demonstrated gadolinium enhancement; no gadolinium-negative lesions were observed. Although plasma samples were collected at similar ages in both groups, patients who never developed cALD were followed longer (Table 1). Average 25-hydroxyvitamin D levels were significantly lower among boys who developed cALD than those who did not (median 28.9 vs. 36.6 ng/ml); $p = 0.019$ (Table 1). For each 10 ng/ml decrease in 25-hydroxyvitamin D level, the odds ratio (OR) for developing cALD was 6.94 (95% confidence interval [CI], 1.05, 45.8); $p = 0.044$. Fatty acid biomarkers associated with ALD were available for 16 patients; they were similar across groups (Table 1).

For latitude analysis, we identified 230 ALD boys across 28 states and 21° of latitude. The racial breakdown was as follows: 108 (47%) White, non-Hispanic, 28 (12%) Black, 32 (14%) Hispanic, 32 (14%) mixed-race, 20 (9%) Asian/American Indian/other, and 10 (4%) unknown. One hundred and thirty-two boys (57%) developed cALD. Each 2° increase in latitude conferred an OR of 1.17 (95% CI, 1.01, 1.35); $p = 0.036$ for developing cALD (Table 2 and Figure 1).

Discussion

Using independent cohorts, we found that ALD boys with lower pre-morbid plasma levels of 25-hydroxyvitamin D, or more northerly latitude of residence, were more likely to develop inflammatory brain lesions (cALD). These findings offer complementary lines of evidence implicating vitamin D and/or ultraviolet light exposure as a regulator of cALD risk.

We derived the concept of vitamin D as a risk factor for cALD based on histologic similarities between ALD and multiple sclerosis brain lesions.[6,14] In multiple sclerosis, vitamin D's therapeutic mechanism is attributed to its role in immune homeostasis.[12,14] Similar immunologic mechanisms are plausible in reducing risk of developing the inflammatory demyelination that similarly characterizes cALD. However, because the ALD genotype disrupts peroxisomal fatty acid metabolism and causes elevated very long-chain fatty acid levels, our findings may implicate

Table 1. Demographics and plasma levels from 20 ALD boys followed prospectively at two academic medical centers

	Subsequent cALD ($n = 10$)	No cALD ($n = 10$)	p
Age at time of plasma sample, years, median (IQR)	5.3 (4.2–7.0)	6.6 (4.2–7.8)	0.36
Age at endpoint, years	6.7 (5.5–7.7)	8.4 (7.8–10.4)	0.02
Duration of observation from first plasma sample to endpoint, years	2.0 (1.2–2.3)	5.2 (3.4–5.9)	0.007
MRI lesion score (Loes score) at time of last MRI (scale 0–34)	1 (1–1.8)	0	n/a
Race/ethnicity			
White, non-Hispanic	8	6	n/a
Black	1	2	n/a
Hispanic	1	0	n/a
Other or unknown	0	2	n/a
Unique time points with plasma samples available prior to clinical endpoint (minimum 2 per patient), total	21	32	n/a
Plasma 25-hydroxyvitamin D, all timepoints ($n = 53$), ng/ml, median (IQR)	27.5 (23.4–31.9)	33.8 (29.3–42.8)	0.007
Plasma 25-hydroxyvitamin D, each patient's timepoints averaged to a single value ($n = 20$), ng/ml, median (IQR)	28.9 (25.9–32.6)	36.6 (33.1–42.0)	0.019
C26:0, ($n = 8$ patients with 18 plasma samples), µg/ml, median (IQR)	1.0 (0.5–1.1)	0.6 (0.4–1.0)	0.30
C26:0-lysophosphatidylcholine ($n = 8$ patients with 18 plasma samples), ug/ml, median (IQR)	0.3 (0.2–0.4)	0.3 (0.2–0.3)	0.48

All boys had normal brain MRIs at the start of observation and were followed with serial MRIs to detect early-stage cALD. Clinical endpoints assigned according to whether patient developed cALD during period of observation.
cALD, cerebral X-linked adrenoleukodystrophy; IQR, interquartile range; MRI, magnetic resonance imaging.

Table 2. Incidence risk of cALD and latitude in a cohort of 230 boys with ALD from the PHIS database

Degrees latitude[a]	Total PHIS population	Unadjusted probability of cALD (% with cALD)	Adjusted[b] probability of cALD—latitude excluded from model (% with cALD)	Adjusted[b] probability of cALD—latitude included in model (% with cALD)
25°–26°	101,841	50.0%	54.7%	36.7%
27°–28°	106,949	25.0%	54.8%	40.2%
29°–30°	323,873	30.0%	55.4%	42.8%
31°–32°	438,066	50.0%	55.5%	46.4%
33°–34°	881,280	40.0%	56.6%	48.2%
35°–36°	472,796	43.8%	55.6%	53.9%
37°–38°	437,657	75.8%	55.5%	58.0%
39°–40°	1,060,064	56.0%	57.2%	58.5%
41°–42°	698,861	72.1%	56.1%	64.2%
43°–44°	156,815	50.0%	54.8%	69.9%
45°–46°	51,321	0.0%	54.6%	73.4%
47°–48°	72,553	60.0%	54.6%	76.3%

cALD rates at each latitude range are shown before and after adjustments for Pediatric Healthy Information System (PHIS) population. The inclusion of latitude in the regression model significantly affected cALD rates. To protect patient confidentiality, we have not provided numerators (cALD patients) and denominators (all ALD patients) by latitude category since almost half the latitude ranges have either (i) fewer than 10 patients or (ii) fewer than three hospitals.

[a]Collapsed to get at least two cases of ALD in each category. [b]Adjusted for PHIS population.

cALD, cerebral X-linked adrenoleukodystrophy.

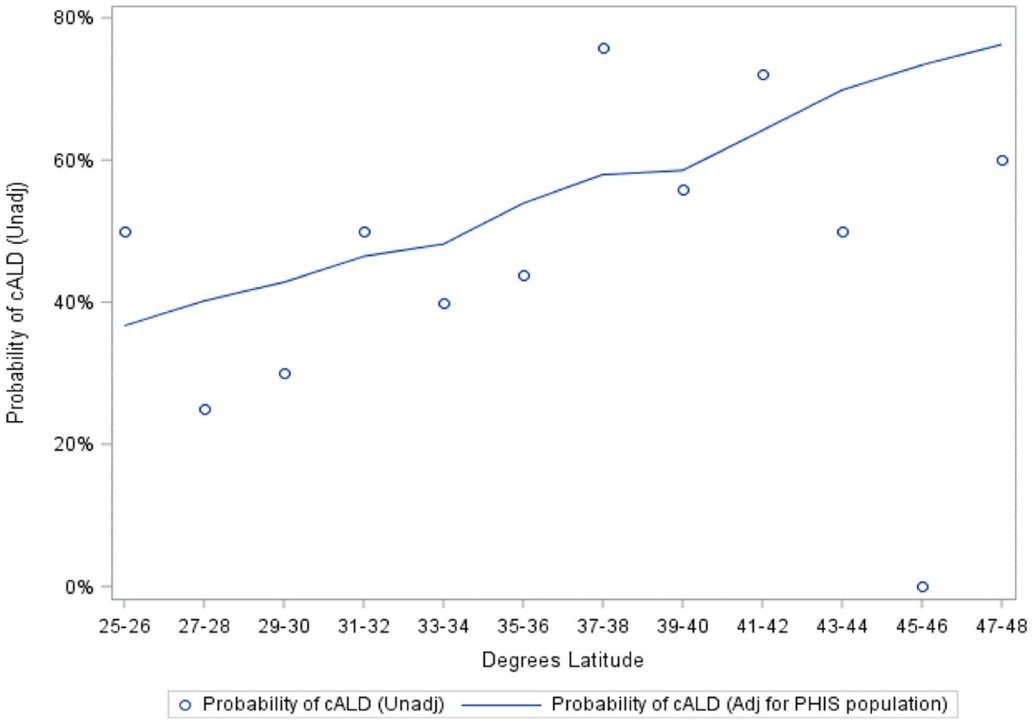

Figure 1. The incidence risk of an ALD brain lesion compared to latitude. Each 2° increase in latitude conferred an odds ratio of 1.17 (95% confidence interval 1.01, 1.35); $p = 0.036$ for developing cALD. cALD, cerebral X-linked adrenoleukodystrophy.

vitamin D in mediating fatty acid metabolism and/or its role in immunologic homeostasis.[15–17] Moreover, a growing body of evidence, including randomized controlled trials, also suggest vitamin D may favorably influence several of the proposed pathogenic cALD mechanisms, including oxidative stress, immune homeostasis, and cerebral blood flow.[15–23]

Latitude mechanistically limits potential vitamin D production, such that at 51° latitude and higher, cutaneous vitamin D production is not physiologically possible during

portions of the winter months.[24] Higher latitudes also confer colder climates, which prompt behavioral changes that limit UV exposure including more time spent indoors and less exposed skin when outdoors. Consequently, people living closer to the poles must fortify their vitamin D levels.[25] However, latitude is not the sole physical geographical factor influencing vitamin D production. Atmospheric contents such as clouds, aerosols, and smog can reduce the duration of vitamin D synthesis considerably at any latitude.[24] Moreover, components of sunlight influence biologic functions beyond vitamin D production, including the production of nitric oxide, serotonin, and melatonin, all of which could alter neuroimmune function.[26]

Further limitations of our study include the retrospective nature of our analyses and modest sample sizes, the latter of which limits our ability to assess for putative confounders such as race, socioeconomic status, and similar indicators. Plans for a larger, prospective study of these phenomena are currently underway with the intent to control for these variables. Notably, our cohorts have an over-representation of cALD cases (50% in the vitamin D cohort and 57% in the latitude cohort) compared to historical natural history rates of 40%. This could be the result of small numbers in the case of the vitamin D cohort where all participants' follow-up began prior to onset of cALD. In the latitude cohort, which was drawn from a pediatric hospital consortium database, this is more likely a result of systemic bias toward diagnosis in symptomatic cases. Nonetheless, neither situation would be expected to systematically bias toward high or low vitamin D status or latitude. Although these analyses represent the first reported assessments of vitamin D and latitude as a risk factor for cALD, prospective studies with or without intervention would further validate and refine the magnitude of risk associated with low vitamin D status in boys with an ALD genotype.

The advent of universal newborn screening for ALD has increased the number of ALD boys who can benefit from prospective MRI surveillance. Because ALD brain lesions rarely manifest before 2 years of age, widespread newborn screening could facilitate a trial of early life vitamin D supplementation to test its potential as a preventive therapy against ALD brain lesions.

In summary, we describe two complementary lines of evidence implicating low vitamin D status as a risk factor for the development of brain lesions among ALD boys. Future studies should expand on these observations via larger, prospective studies that control for a larger number of environmental and demographic variables.

Author Contributions

Keith P. van Haren: Conceptualization; Data curation; Funding acquisition; Investigation; Project administration; Writing – original draft; Writing – review and editing. **Jacob Wilkes**: Data curation; Formal analysis; Writing – review and editing. **Ann B. Moser**: Data curation; Investigation; Resources; Writing – review and editing. **Gerald V. Raymond**: Conceptualization; Data curation; Investigation; Methodology; Resources; Writing – review and editing. **Troy Richardson**: Formal analysis; Investigation; Methodology; Software; Visualization; Writing – review and editing. **Patrick Aubourg**: Data curation; Investigation; Resources; Writing – review and editing. **Timothy W. Collins**: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Visualization; Writing – review and editing. **Ellen M. Mowry**: Formal analysis; Methodology; Writing – review and editing. **Joshua L. Bonkowsky**: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Visualization; Writing – review and editing.

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Conflicts of Interest

All authors have completed the ICMJE uniform disclosure form and declare that the study described in the submitted work was supported, in part, by the Child Neurology Foundation Scientific Award and NIH/NINDS K23NS087151; KV has received research grants from Bluebird bio and Minoryx for clinical trials in ALD participants, separate from the submitted work; consulting fees from bluebird bio, Minoryx, Viking Therapeutics, Poxel, and Orpheris for ALD therapy development separate from the submitted work. He participates in advisory boards for Poxel (paid), Viking (paid), ALD Connect (unpaid), and the United Leukodystrophy Foundation (unpaid). GVR has received consulting fees from bluebird bio, from Viking Therapeutics, and for therapy development outside the submitted work. JLB has received research support from Sanofi and Autobahn as well as consulting fees from Neurogene, Passage Bio, Takeda, and Autobahn all for work outside the submitted work. He is an unpaid board member at ALD Connect and wFluidx. No other

relationships or activities that could appear to have influenced the submitted work.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

