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Bone Density in Children With Chronic Liver Disease Correlates With Growth and Cholestasis

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Osteopenia and bone fractures are significant causes of morbidity in children with cholestatic liver disease. Dual-energy X-ray absorptiometry (DXA) analysis was performed in children with intrahepatic cholestatic diseases who were enrolled in the Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis in the Childhood Liver Disease Research Network. DXA was performed on participants aged >5 years (with native liver) diagnosed with bile acid synthetic disorder (BASD), alpha-1 antitrypsin deficiency (A1AT), chronic intrahepatic cholestasis (CIC), and Alagille syndrome (ALGS). Weight, height, and body mass index Z scores were lowest in CIC and ALGS. Total bilirubin (TB) and serum bile acids (SBA) were highest in ALGS. Bone mineral density (BMD) and bone mineral content (BMC) Z scores were significantly lower in CIC and ALGS than in BASD and A1AT ($P < 0.001$). After anthropometric adjustment, bone deficits persisted in CIC but were no longer noted in ALGS. In ALGS, height-adjusted and weight-adjusted subtotal BMD and BMC Z scores were negatively correlated with TB ($P < 0.001$) and SBA ($P = 0.02$). Mean height-adjusted and weight-adjusted subtotal BMC Z scores were lower in ALGS participants with a history of bone fractures. DXA measures did not correlate significantly with biliary diversion status. *Conclusion:* CIC patients had significant bone deficits that persisted after adjustment for height and weight and generally did not correlate with degree of cholestasis. In ALGS, low BMD and BMC reference Z scores were explained by poor growth. Anthropometrically adjusted DXA measures in ALGS correlate with markers of cholestasis and bone fracture history. Reduced bone density in this population is multifactorial and related to growth, degree of cholestasis, fracture vulnerability, and contribution of underlying genetic etiology. (Hepatology 2019;69:245-257).

Reduced bone density is a common complication of chronic liver disease in both adults and children. Factors contributing to bone mineral deficits are dependent on the physiology and severity of the underlying liver disease and may include chronic nutrient and calcium malabsorption leading to malnutrition and growth failure, deficiencies of fat-soluble vitamins, level of physical activity, and circulating

Abbreviations: A1AT, alpha-1 antitrypsin deficiency; ALGS, Alagille syndrome; ANOVA, analysis of variance; BASD, bile acid synthetic disorder; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; ChiLDReN, Childhood Liver Disease Research Network; CIC, chronic intrahepatic cholestasis; DXA, dual-energy X-ray absorptiometry; INR, international normalized ratio; JAG1, JAGGED1; LOGIC, Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis; OH, 25-hydroxyvitamin; PFIC, progressive familial intrahepatic cholestasis; SBA, serum bile acid; TB, total bilirubin.

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inflammatory cytokines. High rates of osteoporosis and bone fracture have been observed in adults with chronic cholestatic liver diseases, such as primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC), and cirrhosis of any cause.(1-3) Metabolic bone disease is also common in children with chronic cholestasis or end-stage liver disease, but fewer studies have been conducted to characterize the bone mineral deficits in this vulnerable population.

Osteopenia and pathologic fractures have been reported in children with chronic cholestatic liver disease, including Alagille syndrome (ALGS), progressive familial intrahepatic cholestasis (PFIC), and biliary atresia.(4-7) In some cases, bone disease can be severe enough to be an indication for liver transplantation. In contrast to what is seen in adults, children have remarkable improvement in bone mineral density (BMD) by 1 year after liver transplantation, often accompanied by significant catch-up growth.(8,9) Despite the impact of metabolic bone disease on morbidity and quality of life in pediatric patients with chronic liver disease, few detailed studies have been done to quantify bone deficits and identify risk factors for osteopenia and fracture. In one published study, ALGS patients (n = 31) and 80 healthy control participants underwent evaluation by dual-energy

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X-ray absorptiometry (DXA). The ALGS children were small for age and had reduced bone mineral content (BMC) for age and BMC for height, with bone mineralization positively related to coefficient of fat absorption but not to dietary intake.(10) In another study of 16 nonjaundiced children with biliary atresia, whole-body and lumbar spine BMC values were found to decline with age, indicating that children surviving with native liver are at risk of compromised bone health, even in the absence of elevated bilirubin levels.(11) To date, there have been no published studies of DXA analysis in children with other inherited chronic liver diseases, such as alpha-1 antitrypsin deficiency (A1AT) or PFIC.

The Childhood Liver Disease Research Network (ChiLDReN) is a National Institutes of Health–funded consortium of pediatric centers in North America focused on the study of rare pediatric liver diseases. The Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis (LOGIC; NCT00571272) enrolled children with one of four diagnoses: ALGS, PFIC, A1AT, or bile acid synthetic disorder (BASD). As part of the LOGIC study protocol, participants aged >5 years underwent DXA scanning, the results of which are reported here. The objectives of this study were to address an important knowledge gap by investigating the prevalence of bone mineral deficits in this cohort of children with chronic liver disease and to determine factors associated with lower bone density, including growth parameters, laboratory values, and clinical events such as fracture and biliary diversion.

Participants and Methods

STUDY DESIGN AND PARTICIPANTS

Participants in this study were enrolled in the LOGIC study (NCT00571272) through the National Institute of Diabetes and Digestive and Kidney Diseases–funded multicenter ChiLDReN consortium. The study protocol was approved by the institutional review boards at each participating center and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from parents or guardians or participants 18 years or older, and assent was obtained from participants >7 years of age, per local guidelines.

LOGIC is a longitudinal study designed to investigate the natural history of several genetic causes of cholestasis, including ALGS, A1AT, BASD, and PFIC. Children and young adults with a confirmed diagnosis of ALGS, A1AT, BASD, or PFIC were eligible for enrollment in LOGIC from birth through 25 years of age. Detailed enrollment criteria for each disease group are presented in Supporting Table S1. In the PFIC group, 20 of 41 participants had a confirmed genetic diagnosis: 10 with mutations in adenosine triphosphatase phospholipid transporting 8B1 (*ATP8B1*), 4 with adenosine triphosphate–binding cassette subfamily B member 11 (*ABCB11*), and 6 with *ABCB4*. Because not all of the participants in the PFIC group have a documented genetic diagnosis, for the purpose of this study, we categorized them as chronic intrahepatic cholestasis (CIC).

In the LOGIC protocol, defined data elements, including clinical events, laboratory values, growth parameters, physical examination findings, and history and location of bone fractures, were collected at enrollment and at yearly visits for 10 years or until liver transplantation or death. LOGIC participants aged >5 years with their native livers were eligible to undergo DXA once during the course of the study. DXA scans were performed at 12 ChiLDReN centers from 2008 through 2013 (2008-2013 for ALGS and CIC participants, 2008-2011 for A1AT, and 2010 for BASD). Interim analysis in 2011 revealed minimal DXA abnormalities in A1AT and BASD participants, so DXA scanning was discontinued in those cohorts at that time. DXA results reported here include those for participants with ALGS (n = 49), A1AT (n = 44), BASD (n = 14), or CIC (n = 41). Supporting Fig. S1 shows the derivation of the sample population.

DXA MEASUREMENTS

DXA scanning of the whole body, spine, and femur was performed using Hologic (Newark, DE) or Lunar (GE Health Sciences, Pittsburgh, PA) equipment. DXA equipment for each brand was standardized using BMIL phantoms provided by the DXA Coordinating Center.(12) The same phantom was used for longitudinal drift correction where necessary. Adjustment between the two brands of scanners was performed with patient-based correction formulas.(13) Standard Z scores were calculated based on age, sex, and race reference data.(14) In addition to these analyses, adjusted Z scores were calculated based on age, sex, race, height, and weight.(15) For a special, supplementary analysis, adjusted Z scores with all of the above listed parameters and percent body fat were also calculated.(15) In most cases, anthropometric measurements were obtained on the same day as the DXA scan; otherwise, the measurements closest to the date of scan were used.

STATISTICAL METHODS

The preplanned analyses of the DXA data included all LOGIC participants with their native liver for whom a DXA was performed. Descriptive statistics are displayed as means and standard deviations or median with first and third quartiles for continuous variables and as frequencies and percentages for categorical variables. Winsorizing refers to a statistical technique in which extreme values are limited in order to reduce the effect of possibly spurious outliers.(16) To reduce the influence of outliers, all DXA *Z* scores were Winsorized using a lower limit of –3 and an upper limit of 3. All DXA *Z* scores presented are adjusted for age, sex, race (black versus nonblack), weight, and height, unless otherwise indicated.

We characterized DXA *Z* scores in two ways—as a continuous variable and as a dichotomous variable based on the proportion of participants <–1.5 versus >–1.5. Comparisons of DXA *Z* scores and other continuous variables among diagnosis groups were tested using analysis of variance (ANOVA) (or by nonparametric Kruskal-Wallis tests if the distributional assumptions of ANOVA were not met), and comparisons of dichotomous DXA *Z* scores and other categorical variables, by diagnosis group, were tested with chi-squared tests. Within diagnosis groups, differences in laboratory values between participants with DXA *Z* scores <–1.5 versus >–1.5 and differences in DXA *Z* scores for participants with and without bone fractures and with and without biliary diversion were compared using two-sample *t* tests.

Pearson correlation coefficients and their corresponding *P* values were used to examine associations between participant anthropometric parameters (height, weight, and body mass index [BMI] *Z* scores) and reference DXA *Z* scores (defined as the standard method to adjust for age, sex, and race) and between DXA *Z* score and laboratory values. Laboratory values were obtained within 1 year of DXA scan. Height, weight, and BMI *Z* scores were calculated based on Centers for Disease Control and Prevention growth charts (reference year 2000; https://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm). Statistical analyses were carried out using SAS version 9.4 (SAS Institute, Cary, NC). Due to the large number of comparisons being made, we considered *P* values <0.01 to be statistically significant.

Results

CHARACTERISTICS OF STUDY COHORT

Our study cohort consisted of a total of 148 participants enrolled in the LOGIC study as part of the ChiLDReN consortium. Supporting Fig. S1 shows the derivation of the study population. Participants had ALGS (n = 49), CIC (n = 41), A1AT (n = 44), or BASD (n = 14). Table 1 displays characteristics of participants in the study by disease group. The A1AT and BASD groups had higher percentages of male participants than the ALGS and CIC groups: 70% and 79% versus 57% and 44%, respectively. Participants were similar across disease groups for race, ethnicity, and age. The rate of bone fracture occurrence was not statistically different among disease groups. Anthropomorphic measurements were significantly different across disease groups (*P* < 0.001), with ALGS and CIC participants having lower mean height, weight, and BMI *Z* scores than participants with A1AT or BASD. Nonparametric Kruskal-Wallis tests confirmed results from ANOVA (data not shown). Across all disease groups, few participants had a BMI *Z* score <–2. The mean percentages of lean body mass were similar among disease groups, ranging from 74% to 76% (Table 1).

Mean laboratory measurements of bilirubin, gamma-glutamyl transpeptidase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and serum bile acids (SBAs) were highest in participants with ALGS, intermediate for those with CIC, and lowest in participants with A1AT and BASD. Mean albumin and hemoglobin were lower in ALGS and CIC compared with A1AT and BASD. There were no statistical differences in international normalized ratio (INR), white blood cell count, and platelet count between groups. Few participants met criteria

TABLE 1. Demographic and Clinical Characteristics* of Participant Population by Disease Group

Liver Disease	ALGS (n = 49†)	CIC (n = 41‡)	A1AT Deficiency (n = 44§)	BASD (n = 14$^{\|\|}$)	$P^{\P}$
Number (%) of participants					
Male sex	28 (57%)	18 (44%)	31 (70%)	11 (79%)	0.04
Race					0.16
White	38 (78%)	34 (83%)	43 (98%)	11 (79%)	
Black	5 (10%)	2 (5%)	0 (0%)	1 (7%)	
Other	6 (12%)	5 (12%)	1 (2%)	2 (14%)	
Ethnicity					0.62
Hispanic	4 (8%)	7 (17%)	3 (7%)	3 (21%)	
Non-Hispanic	44 (90%)	33 (80%)	40 (91%)	11 (79%)	
Not reported	1 (2%)	1 (2%)	1 (2%)	0 (0%)	
Any bone fractures#					0.20
No	30 (61%)	31 (76%)	35 (80%)	11 (79%)	
Yes	19 (39%)	10 (24%)	9 (20%)	3 (21%)	
Frequency of bone fractures					0.06
0 or 1	40 (82%)	38 (93%)	43 (98%)	13 (93%)	
2 or more	9 (18%)	3 (7%)	1 (2%)	1 (7%)	
Spleen size >2 cm and platelet count <150 × 10^3/μL**	6 (16%)	4 (13%)	5 (12%)	0 (0%)	0.59
BMI *Z* score <–2**	3 (6%)	2 (5%)	1 (2%)	1 (7%)	0.73
Mean (SD) Median (Q1, Q3)					
Age (years)	10.8 (4.6)	11.0 (5.0)	11.0 (5.3)	12.0 (6.7)	0.90
	9.8 (7.7, 13.1)	10.9 (6.6, 15.0)	8.7 (6.5, 14.5)	8.4 (6.8, 20.0)	
Weight *Z* score	–1.3 (1.3)	–1.1 (1.5)	0.6 (0.9)	0.9 (1.4)	<0.001
	–1.1 (–2.3, –0.4)	–1.1 (–2.0, 0.0)	0.7 (0.1, 1.2)	1.1 (–0.2, 1.7)	
Height *Z* score	–1.5 (1.1)	–1.3 (1.8)	0.5 (1.2)	–0.1 (0.7)	<0.001
	–1.6 (–2.1, –0.7)	–1.2 (–2.3, –0.3)	0.5 (–0.1, 1.1)	0.1 (–0.5, 0.2)	
BMI *Z* score	–0.6 (1.2)	–0.2 (1.0)	0.4 (1.0)	1.0 (1.4)	<0.001
	–0.3 (–1.3, 0.3)	–0.1 (–0.8, 0.4)	0.6 (–0.2, 0.9)	1.3 (0.4, 1.9)	

Continued

TABLE 1. (Continued)

Liver Disease	ALGS (n = 49[†])	CIC (n = 41[‡])	A1AT Deficiency (n = 44[§])	BASD (n = 14[‖])	P[¶]
Mean (SD)					
Lean body mass (%)[††]	73.8 (5.6)	76.4 (7.4)	75.8 (7.7)	74.6 (8.5)	0.31
TB, mg/dL	3.7 (5.1)	1.6 (1.9)	0.8 (1.2)	0.3 (0.3)	<0.001
Direct bilirubin, mg/dL	2.4 (3.2)	0.5 (0.9)	0.1 (0.1)	—	<0.001
GGT, U/L	353.6 (309.4)	97.9 (172.5)	57.0 (66.9)	23.6 (4.9)	<0.001
Alkaline phosphatase, U/L	501.9 (345.1)	457.0 (229.8)	234.9 (114.4)	215.6 (122.7)	<0.001
ALT, U/L	164.9 (120.4)	76.2 (53.8)	59.0 (39.2)	26.1 (22.4)	<0.001
AST, U/L	151.8 (110.8)	78.5 (51.5)	61.1 (48.6)	51.8 (17.0)	<0.001
Serum bile acids (μmol/L)	117.3 (106.3)	69.8 (102.2)	16.6 (19.0)	27.5 (43.4)	<0.001
OH vitamin D level (ng/mL)	34.1 (19.0)	37.8 (16.4)	37.8 (10.6)	25.9 (9.9)	0.12
INR	1.08 (0.16)	1.07 (0.17)	1.06 (0.10)	1.03 (0.10)	0.67
Albumin, g/dL	4.2 (0.6)	4.2 (0.5)	4.5 (0.4)	4.6 (0.4)	0.005
WBC, $\times 10^3$/μL	6.5 (2.8)	5.9 (2.2)	5.9 (2.5)	6.8 (2.2)	0.44
Hemoglobin, g/dL	12.9 (1.7)	13.0 (1.4)	13.8 (1.1)	14.4 (1.3)	<0.001
Platelet count, $\times 10^3$/μL	232.5 (105.0)	272.7 (142.2)	232.1 (114.8)	249.1 (109.4)	0.39

Note: Nonparametric Kruskal-Wallis tests confirmed results from ANOVA.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase; WBC, white blood cell.

[*] Values closest to the time of the DXA scan for each participant were used in the analysis. Measurements were required to be within 1 year of the scan.

[†] For ALGS, the number of nonmissing values for continuous variables ranged from 38 to 49, except for direct bilirubin (n = 22).

[‡] For CIC, the number of nonmissing values for continuous variables ranged from 35 to 41, except for direct bilirubin (n = 22) and vitamin D (n = 33).

[§] For A1AT deficiency, the number of nonmissing values for continuous variables ranged from 38 to 44, except for direct bilirubin (n = 24) and vitamin D (n = 13).

[¶] For BASD, the number of nonmissing values for continuous variables ranged from 10 to 14, except for direct bilirubin (n = 0).

[**] *P* value based on ANOVA reported for mean rows and from chi-squared or Fisher's exact test for discrete outcomes.

[††] Bone fractures could have occurred before or after the DXA scan was performed.

[‡‡] Percentages are among participants with nonmissing data.

[§§] Lean body mass was measured by DXA.

TABLE 2. DXA Reference and Height-Adjusted and Weight-Adjusted Z Scores by Disease Group

Mean (SD) % with $Z < -1.5$	ALGS (n = 46-49)	CIC (n = 39-41)	P*	Winsorized[†] Values, n (%)
BMD measures				
Total body minus head				
Reference Z score	–1.10 (1.57) 46.9%	–1.58 (1.11) 56.1%	0.10	13 (14%)
Adjusted Z score	0.23 (1.65) 20.8%	–0.37 (1.45) 20.0%	0.07	9 (10%)
Spine				
Reference Z score	–0.08 (1.66) 20.4%	–1.01 (1.36) 43.9%	0.005	9 (10%)
Adjusted Z score	0.13 (1.65) 20.8%	–1.15 (0.98) 40.0%	<0.001	4 (5%)
Total hip				
Reference Z score	–0.78 (1.38) 23.4%	–1.17 (1.23) 47.5%	0.16	10 (11%)
Adjusted Z score	–0.46 (1.41) 21.7%	–1.17 (1.30) 43.6%	0.02	7 (8%)
BMC measures				
Total body minus head				
Reference Z score	–1.23 (1.45) 44.9%	–1.80 (1.33) 63.4%	0.06	25 (28%)
Adjusted Z score	0.36 (1.34) 10.4%	–0.76 (1.16) 27.5%	<0.001	7 (8%)

Tests for differences DXA Z scores between disease groups were performed using two-sample t tests.
[†] Values <–3 were set to –3, and values >3 were set to 3.

for portal hypertension as defined by spleen size >2 cm below the left costal martin and platelet count <150,000/μL,[(17)] with no significant differences across disease groups (Table 1).

Overall, LOGIC participants who underwent DXA scanning were similar to those who did not, with a few exceptions. Supporting Table S2 shows demographic and clinical characteristics of participants who were eligible but did not undergo DXA scan. In the BASD group, some differences were identified in sex, race, and ethnicity between participants who did (n = 14) and did not (n = 7) undergo DXA. The mean age tended to be younger in the non-DXA participants. In the CIC group, participants who underwent DXA had lower weight and BMI Z scores. In the ALGS and CIC groups, DXA study participants had higher 25-hydroxyvitamin (OH) vitamin D levels than those who did not undergo DXA scanning. Minor differences in hemoglobin were also identified in the BASD and A1AT groups.

DXA SCORES

The A1AT and BASD participants did not have significant bone mineral deficits as measured by DXA (Supporting Table S3); therefore, the results are focused on the ALGS and CIC groups. Table 2 shows summary measures of reference as well as height-adjusted and weight-adjusted BMD and BMC Z scores resulting from DXA scans by disease group for ALGS and CIC participants. The reference scores take into account the participants' age, sex, and race. Mean DXA reference Z scores were similar between ALGS and CIC participants, except for spine BMD, which was lower in the CIC group (Table 2). Because some of the children in the study cohort have significant growth deficits, we examined correlations between DXA reference Z scores and height, weight, and BMI Z scores by disease group (Table 3). All DXA reference Z scores correlated strongly with height Z

TABLE 3. Pearson Correlations Between DXA Reference Z Scores* and Height, Weight, and BMI Z Scores for ALGS and CIC

	ALGS (n = 47-49)			CIC (n = 40-41)		
	Height	Weight	BMI	Height	Weight	BMI
BMD measures						
Total body	0.62[§]	0.53[§]	0.18	0.56[‡]	0.55[‡]	0.23
Total body minus head	0.67[§]	0.59[§]	0.24	0.59[§]	0.55[‡]	0.19
Spine	0.58[§]	0.44[†]	0.09	0.53[‡]	0.54[‡]	0.29
Total hip	0.57[§]	0.48[‡]	0.19	0.33	0.32	0.18
Hip neck	0.56[§]	0.42[†]	0.16	0.33	0.35	0.24
BMC measures						
Total body	0.76[§]	0.69[§]	0.34	0.73[§]	0.68[§]	0.28
Total body minus head	0.81[§]	0.73[§]	0.34	0.74[§]	0.68[§]	0.27

*Reference Z score is adjusted for age, sex, and race (black versus nonblack).
[†] $P < 0.01$.
[‡] $P < 0.001$.
[§] $P < 0.0001$.

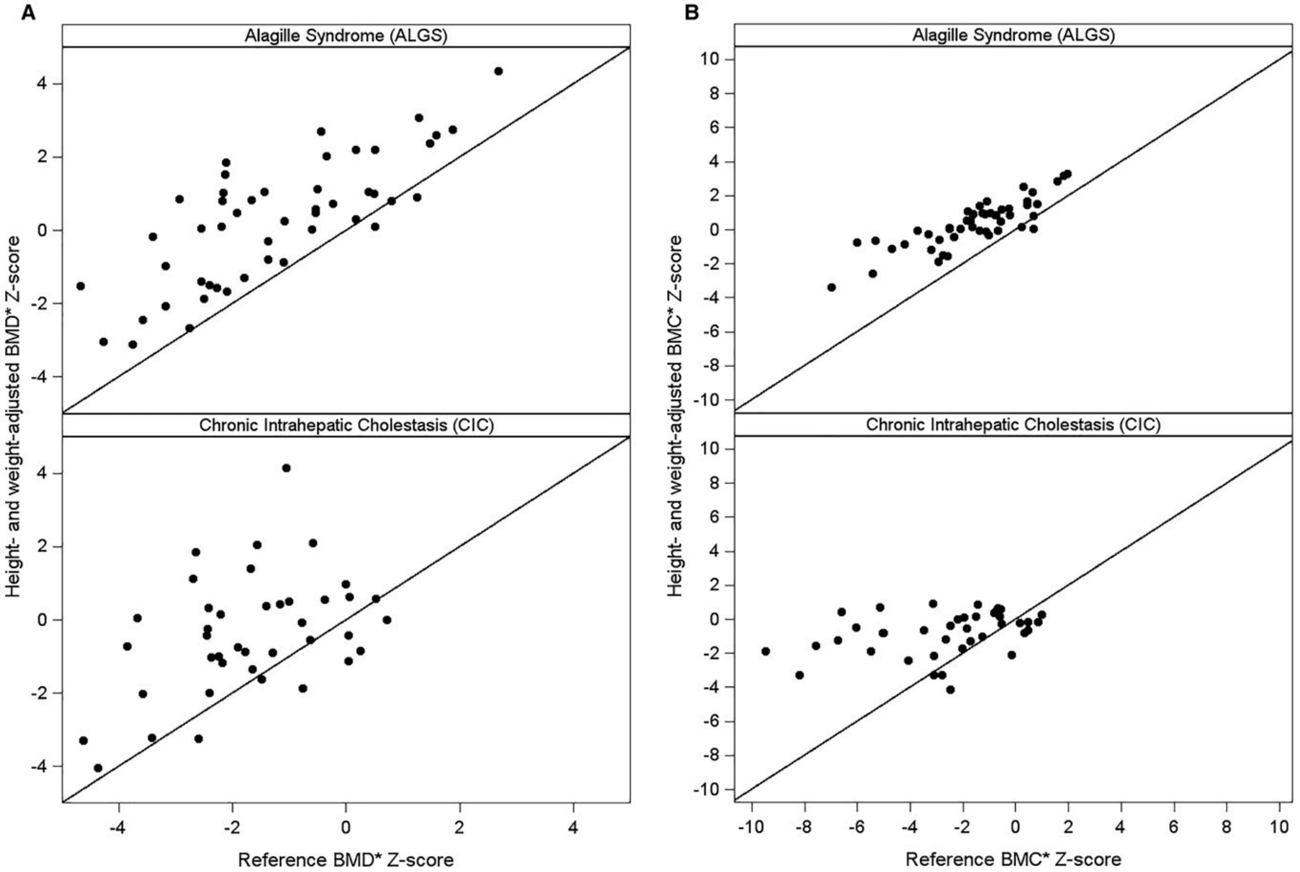

FIG. 1. Comparison between DXA reference and height-adjusted and weight-adjusted *Z* scores for total body minus head measurements (top, BMD; bottom, BMC) for the ALGS and CIC disease groups. The reference line indicates equality between the reference and adjusted *Z* scores. Points above the line indicate observations where the *Z* score adjusted for height and weight is higher than the reference *Z* score. Points below the line indicate observations where the adjusted *Z* score is lower than the reference *Z* score. *Non-Winsorized BMD values presented.

scores in the ALGS participants ($P < 0.0001$). In the ALGS group, weight *Z* scores also correlated strongly with total body and total body minus head BMD and BMC DXA reference *Z* scores ($P < 0.0001$) and, to a lesser extent with spine, total hip, and hip neck DXA reference *Z* scores (Table 3). Height and weight *Z* scores for the CIC participants correlated with total body and total body minus head BMD and BMC DXA reference *Z* scores. Fewer correlations were identified in A1AT and BASD (Supporting Table S4), but these participants also did not have significant growth deficits at the time of DXA (Table 1). No correlations were found between DXA reference *Z* scores and BMI *Z* scores for any disease group. Because DXA reference *Z* scores were so tightly correlated with growth parameters, we elected to adjust all DXA *Z* scores for weight and height according to the method of Short et al.(15)

After adjustment for height and weight, mean total body minus head BMD and BMC *Z* scores were higher (than when calculated with the reference methods) in both ALGS and CIC groups, indicating that these bone deficits are at least in part due to small size (Table 2). Interestingly, the CIC group had deficits in total spine and total hip BMD reference *Z* scores that persisted after adjustment for height and weight, with total spine and total hip BMD *Z* scores in the CIC group of –1.15 and –1.17, respectively. Over 40% of the CIC cohort had a *Z* score <–1.5, in contrast to the expected distribution of 7% for the reference population.

Fig. 1 displays the reference and adjusted *Z* scores for total body minus head BMD and BMC for the

TABLE 4. Pearson Correlations Between DXA Height-Adjusted and Weight-Adjusted *Z* Scores and Laboratory Measures of Cholestasis and Liver Synthetic Function

	ALGS (n = 37-47[*])					CIC (n = 32-40[*])				
	TB (mg/dL)	SBA (µmol/L)	Vitamin D (ng/mL)	INR	Albumin (g/dL)	TB (mg/dL)	SBA (µmol/L)	Vitamin D (ng/mL)	INR	Albumin (g/dL)
BMD										
Total body minus head	−0.54[‡]	−0.37	0.07	−0.41[†]	0.35	−0.12	−0.29	0.19	0.08	−0.08
Spine	−0.59[§]	−0.31	0.27	−0.33	0.44[†]	−0.36	−0.29	0.09	0.00	0.20
Total hip	−0.66[§]	−0.36	0.14	−0.44[†]	0.53[‡]	−0.24	−0.09	0.03	0.13	0.22
BMC										
Total body minus head	−0.63[§]	−0.39	0.33	−0.31	0.48[‡]	−0.39	−0.23	−0.09	0.01	0.29

[*] Number of participants varies due to missing values for DXA or lab measurements.
[†] $P < 0.01$.
[‡] $P < 0.001$.
[§] $P < 0.0001$.
Abbreviations: INR, international normalized ratio.

ALGS and CIC groups (results for A1AT and BASD disease groups are shown in Supporting Fig. S2). For both the ALGS and CIC disease groups, the data indicate that low weight and height for age contribute to observed bone mineral deficits. Supporting Table S3 shows data for additional DXA measurements (total body and hip neck) and all DXA measurements for the A1AT and BASD disease groups. All DXA measures are also presented with adjustment for weight, height, and percent body fat (Supporting Table S3). Of note, differences in DXA *Z* scores among body areas are not unexpected due to intrinsic differences in cortical and trabecular bone composition.

ASSOCIATIONS BETWEEN DXA AND LABORATORY MEASUREMENTS

We examined potential associations between DXA height-adjusted and weight-adjusted *Z* scores and laboratory measurements using Pearson correlation coefficients. The correlations for ALGS and CIC are shown in Table 4. In ALGS participants, all of the height-adjusted and weight-adjusted BMD and BMC measures were negatively correlated with serum total bilirubin (TB; $P < 0.001$). In the ALGS group, many of the DXA measurements were also correlated with INR and albumin. There were no significant correlations found in the CIC (Table 4), A1AT, or BASD groups (data not shown).

Supporting Table S5 compares mean lab values for ALGS and CIC participants with DXA *Z* scores above and below −1.5. There were no significant differences found in the CIC group. In ALGS, albumin was lower in participants with height-adjusted and weight-adjusted BMD *Z* scores <-1.5 compared with those ≥ -1.5.

DIFFERENCES IN DXA Z SCORES BY BONE FRACTURE AND BILIARY DIVERSION

Table 5 shows differences in DXA height-adjusted and weight-adjusted *Z* scores, TB, and SBAs by whether or not the participant had a bone fracture. There were no significant differences seen for any of the DXA measurements or the lab values in the CIC group. In ALGS, adjusted spine BMD and total body minus head BMC *Z* scores were lower in the fracture group (−0.6 versus 0.6, $P = 0.02$; and −0.2 and 0.7, $P = 0.02$). Serum TB and SBAs were both higher in the fracture group (6.1 versus 2.0, $P = 0.03$; and 157 versus 80, $P = 0.02$) for ALGS participants. However, the *P* value of 0.02 for these comparisons does not meet our cutoff for statistical significance of $P < 0.01$.

We also examined DXA scores by biliary diversion status. There were 9 (18%) ALGS participants with a biliary diversion performed prior to the DXA scan. The median time between diversion and the DXA scan was 7 years. The CIC group had 23 (56%) participants with a biliary diversion performed prior to the DXA scan. The median time between diversion and DXA was 6 years. Table 6

TABLE 5. Differences in DXA Height-Adjusted and Weight-Adjusted *Z* Score Measures and Lab Values by Occurrence of Bone Fracture*

	ALGS (n = 49)			CIC (n = 41)		
	n	Mean (SD)	$P^†$	n	Mean (SD)	$P^†$
BMD						
Total body minus head			0.07			0.33
Bone fracture	19	−0.3 (1.8)		10	−0.8 (1.7)	
No bone fracture	29	0.6 (1.5)		30	−0.2 (1.4)	
Spine			0.02			0.12
Bone fracture	19	−0.6 (1.7)		10	−1.6 (1.0)	
No bone fracture	29	0.6 (1.4)		30	−1.0 (0.9)	
Total hip			0.21			
Bone fracture	18	−0.8 (1.6)		10	−1.5 (1.5)	0.41
No bone fracture	28	−0.2 (1.3)		29	−1.1 (1.2)	
BMC						
Total body minus head			0.02			0.96
Bone fracture	19	−0.2 (1.4)		10	−0.8 (1.2)	
No bone fracture	29	0.7 (1.2)		30	−0.8 (1.2)	
Lab measures						
TB			0.03			0.89
Bone fracture	17	6.1 (6.8)		9	1.5 (1.4)	
No bone fracture	25	2.0 (2.7)		29	1.6 (2.1)	
SBA			0.02			0.58
Bone fracture	19	156.9 (119.2)		8	87.6 (95.7)	
No bone fracture	20	79.7 (77.9)		26	64.3 (105.3)	

*Bone fracture could have occurred before or after the DXA scan.
$^†P$ value based on two-sample *t* test.

shows mean DXA height-adjusted and weight-adjusted *Z* scores for ALGS and CIC by the diversion status. The spine DXA *Z* score was lower in ALGS participants with biliary diversion compared to those without (–1.1 versus 0.4, $P = 0.013$). In participants with CIC, the mean total body minus head BMD *Z* score was higher in the diversion group (0.1 versus –1.0, $P = 0.03$). However, these differences did not reach the threshold of $P < 0.01$ for statistical significance in this study.

TABLE 6. Differences in DXA Height-Adjusted and Weight-Adjusted *Z* Score Measures by Biliary Diversion*

	ALGS (n = 49)			CIC (n = 41)		
	n	Mean (SD)	$P^†$	n	Mean (SD)	$P^†$
BMD						
Total body minus head			0.34			0.03
Diversion	9	−0.2 (1. 9)		23	0.1 (1.5)	
No diversion	39	0.4 (1.6)		17	−1.0 (1.2)	
Spine			0.013			0.90
Diversion	9	−1.1 (1. 5)		23	−1.1 (1.0)	
No diversion	39	0.4 (1.6)		17	−1.2 (1.0)	
Total hip			0.10			
Diversion	9	−1.1 (1.1)		22	−1.0 (1.4)	0.40
No diversion	37	−0.3 (1.4)		17	−1.4 (1.1)	
BMC						
Total body minus head			0.13			0.65
Diversion	9	−0.2 (1.5)		23	−0.7 (1.2)	
No diversion	39	0.5 (1.3)		17	−0.9 (1.2)	

*All diversions were performed prior to DXA scans. The median (interquartile range) times between diversion and scan for ALGS and CIC were 7 (7-10) and 6 (5-10) years, respectively.
$^†P$ value based on two-sample *t* test.

Discussion

In this multicenter study, we report the results of DXA analysis in children with four types of intrahepatic cholestasis—ALGS, CIC (including genetically determined PFIC), A1AT, and BASD. Importantly, participants with A1AT or BASD did not have meaningful bone mineral deficits detected by DXA, but these participants were also only mildly cholestatic at the time of the DXA scan (Table 1). In contrast, study participants with ALGS and CIC had significant bone mineral deficits that correlated strongly with growth parameters. Adjustment for height and weight normalized DXA BMD and BMC *Z* scores in ALGS but to a lesser extent in CIC. In ALGS, weight-adjusted and height-adjusted DXA *Z* scores correlated with laboratory indicators of cholestasis and hepatic synthetic function. Bone fracture occurrence correlated with DXA parameters and lab values in the ALGS group, who were prone to fractures. In this cohort, biliary diversion did not have a major impact on DXA *Z* scores.

DXA is a two-dimensional projection technique that summarizes total bone mass within the projected bone area. This measurement of areal BMD (grams per square centimeter) is known to systematically underestimate volumetric BMD (grams per cubic centimeter) in children with poor growth,(18) and various methods have been used to adjust for body size.(19) In light of this intrinsic limitation of DXA analysis and the fact that in our study cohort we observed a strong correlation between DXA measurements and anthropometrics (Table 3), we elected to adjust for height and weight using the method of Short et al.(15)

Although the ALGS and CIC participants were similar with respect to weight, height, and BMI *Z* scores, only the ALGS group showed a significant normalization of DXA *Z* scores after anthropometric adjustment. The reasons underlying this divergent phenotype are not entirely clear but are likely to be multifactorial. Individuals with ALGS are at risk of growth delay associated with the multisystem involvement of the disease and the underlying genetic defect in Notch pathway signaling. Both ALGS and CIC patients can have profound cholestasis that would be expected to impact absorption of nutrients to a similar extent. A subset of CIC patients, specifically those with mutations in *ATP8B1*, may have intestinal manifestations that could further contribute to malabsorption. Cytokine and immune-mediated alterations may also contribute to bone mineral deficits in patients with chronic cholestatic liver disease. One study of 16 pediatric patients with biliary atresia, ALGS, and PFIC found elevated circulating levels of interleukin-8, which correlated with severity of hepatic fibrosis.(20) In addition, immune dysregulation has been reported in ALGS as a result of altered cluster of differentiation 46–Notch pathway crosstalk.(21,22) In our study, we did not measure levels of inflammatory cytokines that could have a potential impact on bone health in this patient population. Further studies will be required to determine the etiology of persistent bone mineral deficits in the CIC cohort after adjustment for patient size.

One of the key findings in this study is the strong correlation between weight-adjusted and height-adjusted DXA *Z* scores and laboratory measures of cholestasis and liver synthetic function, specifically in ALGS (Table 4). Similar correlations were not identified in the CIC group. Notably, OH vitamin D levels were not correlated with DXA *Z* scores in this cholestatic population. The effect of cholestasis on bone metabolism has been studied both *in vitro* and *in vivo*. Ruiz-Gaspà et al. treated human primary osteoblasts with bilirubin or serum from jaundiced patients and found decreased osteoblast viability and differentiation, reduced expression of osteogenic transcription factors, and up-regulation of factors inducing osteoclastogenesis.(23) Histomorphometric analysis of 50 patients with PBC and PSC at the time of liver transplantation showed decreased bone formation in both male and female patients and increased bone resorption only in female patients.(24) These studies support the hypothesis that cholestasis has direct effects on bone remodeling. The divergent results for the ALGS and CIC groups with respect to correlations between DXA measures and cholestasis are not well understood but may be explained to some extent by the fact that ALGS participants in this study had higher mean TB and SBA levels compared with the CIC group. However, the majority of the CIC participants (56%) had undergone biliary diversion, indicating a history of long-standing and profound cholestasis at some point in the past, which is unlikely to be reflected by current laboratory values. Varying clinical response to biliary diversion, ranging from complete resolution of cholestasis to ongoing issues with fat-soluble vitamin deficiencies, complicates interpretation of these results.

ALGS is an autosomal dominant disorder caused by mutations in the Notch ligand JAGGED1 (*JAG1*) gene in >90% of cases, with mutations in *NOTCH2* found in a small percentage of patients.(25,26) *JAG1* encodes the Jagged1 ligand in the Notch signaling pathway, which is essential for cell fate decisions in multiple tissues and cell types, accounting for the multisystem involvement in ALGS.(27) Skeletal anomalies, primarily facial dysmorphism and butterfly vertebrae, are well-recognized clinical features of ALGS. Interestingly, a genome-wide association study in Chinese women identified a single-nucleotide polymorphism (SNP) in the *JAG1* gene to have a protective effect on BMD and osteoporotic fractures.(28) The risk SNP was found to increase *JAG1* expression in human bone-derived cells. This finding, which has now been replicated in other ethnic cohorts, indicates a role for *JAG1* and Notch signaling in bone homeostasis. Additional studies support an important function for Notch signaling in skeletal development, as well as postnatal bone maintenance and remodeling. Data derived from animal models suggest that the underlying genetic defect in ALGS may lead to structural abnormalities of the cortical and trabecular bone, which may not be adequately characterized by DXA analysis.(29-31)

In this study, weight-adjusted and height-adjusted DXA *Z* scores were lower in ALGS and CIC participants who had fractures compared to those who did not, and TB and SBAs were higher in the ALGS fracture group (Table 5). In ALGS, but not in CIC, differences in DXA measures in participants with a history of fracture reached a marginal statistical significance. It is unclear why DXA measures would be lower in ALGS patients with a fracture history and not in CIC; it is possible that Notch signaling deficits play a prominent role in fracture development separate from the severity of cholestasis in ALGS. In this case, the CIC cohort would be an ideal cholestatic control population for study of this relationship. However, variable timing of bone fracture and biliary diversion complicates interpretation of the DXA data in the CIC group. In healthy children, there is evidence that bone mass is lower in those with a fracture history. BMC and areal BMD measures were lower at all body sites in normal children who had sustained fractures compared with those who had not.(32) Modest decreases in bone mass have been observed in children with a fracture history.(33) Previous studies have shown increased fracture vulnerability in ALGS. In a cohort of ALGS patients, 12 of 42 (28%) reported 27 fractures,(4) which occurred at a young age, predominantly in the lower extremities and with minimal trauma. Femur fractures were 50 times more likely than expected for the general population.(4) The modest differences in DXA *Z* scores identified in our ALGS cohort do not provide an explanation for predisposition to pathologic fracture in ALGS. The genetic underpinnings of ALGS may lead to intrinsic abnormalities of bone strength and composition that are underestimated by DXA imaging techniques.

In this study, we found no statistically significant differences in height-adjusted and weight-adjusted DXA *Z* scores between participants with and without biliary diversion. Variable clinical response to biliary diversion significantly complicates the interpretation of the results of these analyses.(34) Some patients may respond to biliary diversion with normalization of bilirubin and bile acids, but low intestinal luminal bile acid levels may lead to profound fat-soluble vitamin deficiencies. Limited numbers of participants who underwent biliary diversion precluded subanalysis of bile flow after the procedure with regard to clinical response and vitamin D status. After successful drainage procedures, some patients develop bouts of cholestasis that can last months, akin to benign recurrent intrahepatic cholestasis.(35) As such, the timing of DXA scanning relative to intermittent cholestasis events could affect the DXA findings.

Our study has some limitations. Because our study is cross-sectional in design, it was not possible to correlate bone mineral deficits with progression or improvement of underlying liver disease. Given that these inherited pediatric liver disorders are all quite rare, population sizes were limited and did not allow for multiple comparisons. In addition, only 40% of eligible ALGS participants and 51% of eligible CIC participants underwent DXA analysis. Within the CIC cohort, small numbers precluded subanalysis of the specific genetic etiology of liver disease. This was especially true in the analysis of those individuals with biliary diversion. In this group, it would have been ideal to subdivide the patients by level of cholestasis and vitamin D sufficiency, but this was not possible due to limited numbers of participants.

In conclusion, our results represent the largest study, to date, of DXA analysis in a well-characterized cohort of children with intrahepatic cholestatic liver diseases. In particular, children with ALGS and CIC have significant bone mineral deficits that can impact clinical outcomes. There are clearly complex and poorly understood multifactorial influences on bone mineralization in this population, with varying effects on growth, degree of cholestasis, fracture vulnerability, and direct contribution of underlying genetic etiology. Fractures in this population are unlikely to be simple manifestations of vitamin D deficiency but are the result of a much more complex pathophysiology. Further studies will be required to fully characterize bone health in this unique patient population, to develop methods to predict patients at high risk for fracture, and to generate new approaches to prevent those fractures.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.30196/suppinfo.