

SUPPLEMENTARY MATERIALS – Loomes et al

Supplementary Table S1: Enrollment Criteria for Participants in the LOGIC Protocol

Alagille syndrome	<p>(Participants must meet both 1 and 2)</p> <p>1. Clinical diagnosis of Alagille syndrome as defined by:</p> <ul style="list-style-type: none"> -- Documented mutation in <i>JAG1</i> or <i>NOTCH2</i> -- Family history of ALGS -- Bile duct paucity -- Other clinical criteria <ul style="list-style-type: none"> • Evidence of cholestasis • Cardiac involvement • Ocular findings • Butterfly vertebrae • Characteristic facies • Functional or structural renal anomalies <p>Number of additional clinical criteria required for diagnosis varies depending on presence of documented mutation, family history or bile duct paucity.</p>
Progressive familial intrahepatic cholestasis / benign recurrent intrahepatic cholestasis	<p>2. Clinical, biochemical or histologic evidence of liver disease</p> <p>(Defined by either 1 or 2)</p> <p>1. Definite PFIC or BRIC</p> <p>Genetic testing showing two mutant alleles in <i>ATP8B1</i>, <i>ABCB11</i>, <i>ABCB4</i>, or other genes shown to be confirmed causes of PFIC</p> <hr/> <p>2. Presumed PFIC or BRIC (<i>must meet both A and B</i>)</p> <p>A. History or presence of chronic liver disease (<i>one or more of the following</i>):</p> <ul style="list-style-type: none"> • Duration of biochemical or clinical abnormalities of > 6 months • Clinical/pathologic stigmata of chronic liver disease • Sibling of individual known to be affected by PFIC or BRIC • Recurrent and episodic cholestatic disease occurring on more than two occasions, with episodes separated by at least 3 months and without other known cause <p>B. History or presence of cholestasis (<i>one or more of the following</i>):</p> <ul style="list-style-type: none"> • Fasting total serum bile acid > 3x ULN for age • Direct bilirubin > 2 mg/dL • Fat-soluble vitamin deficiency otherwise unexplainable • GGT > 3x ULN for age • Intractable pruritus explainable only by liver

	disease
	<u>Exclusion criteria:</u> Confirmed diagnosis of another chronic cholestatic liver disease (such as ALGS, biliary atresia, etc.); chronic known infectious hepatitis; short bowel syndrome/TPN cholestasis; chronic known or suspected drug hepatotoxicity; acquired immune deficiency syndrome; acute liver failure; extrahepatic portal vein obstruction.
Alpha-1 antitrypsin deficiency	(Participants must meet both 1 and 2) 1. Low serum alpha-1 antitrypsin concentrations and PIZZ or PISZ phenotype or genotype. For participants after liver transplant, this requirement may be fulfilled by one of the above plus clear histologic evidence of alpha-one antitrypsin deficiency liver disease in the liver explant. 2. Evidence of liver disease associated with alpha-1 antitrypsin deficiency (<i>one or more of the following</i>): <ul style="list-style-type: none"> • History of neonatal cholestasis • Chronic elevation of ALT, AST or GGT > 1.25 times upper limit of normal • Chronic hepatomegaly • Findings or complications of portal hypertension or cirrhosis • Impaired liver synthetic function • Histologic evidence • History of liver transplantation for A1AT deficiency
Bile acid synthetic disorder	(Defined by either 1 or 2) 1. Biochemical evidence of a bile acid synthesis defect documented by Fast Atom Bombardment Mass Spectrometry (FAB-MS) or GC-MS analysis of urine or serum. 2. Two genetic mutations in one of the enzymes in the bile acid synthesis pathway <u>Exclusion criteria:</u> Peroxisomal defect such as Zellweger syndrome, Refsum's syndrome, etc.

Abbreviations: ABCB11, ATP-binding cassette subfamily B member 11; ABCB4, ATP binding cassette subfamily B member 4; ATP8B1, ATPase phospholipid transporting 8B1; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transpeptidase; PFIC, progressive familial intrahepatic cholestasis; ULN, upper limit of normal.

Supplementary Table S2: Demographic and Clinical Characteristics* of Non-DXA Population by Disease Group

Liver Disease	Alagille Syndrome (n = 72 ^a)	Chronic Intrahepatic Cholestasis (n = 39 ^b)	Alpha 1 Antitrypsin Deficiency (n = 79 ^c)	Bile Acid Synthetic Disorder (n = 7 ^d)	p-value [^]
	Number (%) of participants				
Male gender	45 (63%)	15 (39%)	39 (49%)	1 (14%)	0.02
Race					<0.001
White	51 (71%)	27 (69%)	73 (92%)	1 (14%)	
Black	4 (6%)	6 (15%)	0 (0%)	2 (29%)	
Other	17 (24%)	6 (15%)	6 (8%)	4 (57%)	
Ethnicity					<0.001
Hispanic	20 (28%)	9 (23%)	5 (6%)	5 (71%)	
Non-Hispanic	50 (69%)	29 (74%)	71 (90%)	2 (29%)	
Not reported	2 (3%)	1 (3%)	3 (4%)	0 (0%)	
Any bone fractures**					0.02
No	48 (67%)	34 (87%)	67 (85%)	6 (86%)	
Yes	24 (33%)	5 (13%)	12 (15%)	1 (14%)	
Multiple bone fractures					0.07
0 or 1	62 (86%)	38 (97%)	76 (96%)	7 (100%)	
2 or more	10 (14%)	1 (3%)	3 (4%)	0 (0%)	
Spleen size >2cm and platelet count < 150x10 ³ /μL***	6 (13%)	8 (33%)	9 (17%)	0 (0%)	0.20
BMI Z-score < -2***	7 (11%)	0 (0%)	1 (1%)	0 (0%)	0.06
	Mean (SD)				
	Median (Q1, Q3)				
Age (years)	8.5 (4.5) 6.6 (5.0, 10.7)	9.1 (4.6) 7.7 (5.0, 12.1)	9.1 (4.7) 7.2 (5.0, 11.7)	9.7 (4.5) 12.3 (5.0, 13.3)	0.60
Weight Z-score	-1.6 (1.1) -1.5 (-2.3, -0.8)	-0.2 (1.1) -0.3 (-1.0, 0.1)	0.6 (1.2) 0.7 (-0.3, 1.4)	0.1 (1.0) 0.2 (-1.0, 1.1)	<0.001
Height Z-score	-1.7 (1.2) -1.7 (-2.2, -1.0)	-0.8 (1.2) -0.7 (-1.7, 0.2)	0.5 (1.3) 0.5 (-0.4, 1.2)	-0.4 (1.5) -0.9 (-1.5, 0.9)	<0.001
BMI Z-score	-0.6 (1.1) -0.4 (-1.4, 0.2)	0.4 (1.0) 0.4 (0.0, 1.0)	0.5 (1.1) 0.5 (-0.2, 1.3)	0.6 (1.1) 0.3 (-0.2, 1.2)	<0.001
	Mean (SD)				
Lean body mass (%)*	not done	not done	not done	not done	
Total bilirubin, mg/dL	5.84 (7.40)	2.28 (3.66)	0.59 (0.61)	0.52 (0.36)	<0.001
Direct bilirubin, mg/dL	3.08 (3.95)	1.10 (2.05)	0.21 (0.27)	0.50 (0.57)	0.002
GGT, U/L	427.1 (423.1)	177.9 (428.7)	76.2 (117.8)	22.7 (13.1)	<0.001
Alkaline phosphatase, U/L	586.1 (319.3)	479.2 (302.9)	269.7 (222.9)	316.0 (152.8)	<0.001
ALT, U/L	168.7 (122.4)	92.0 (72.4)	66.4 (45.9)	26.6 (9.8)	<0.001
AST, U/L	167.0 (125.3)	90.6 (67.7)	74.3 (55.6)	49.0 (23.8)	<0.001
Serum bile acids (μmol/L)	not done	not done	not done	not done	
25-OH vitamin D level (ng/mL)	25.3 (14.6)	21.9 (15.4)	34.1 (11.0)	28.6 (14.0)	0.06
INR	1.07 (0.22)	1.16 (0.29)	1.09 (0.17)	1.10 (0.08)	0.29
Albumin, g/dL	3.99 (0.69)	4.10 (0.67)	4.35 (0.58)	4.50 (0.43)	0.004
WBC, x 10 ³ /μL	7.34 (2.66)	8.30 (8.77)	6.77 (3.09)	6.08 (2.04)	0.43
Hemoglobin, g/dL	12.48 (1.42)	12.31 (1.60)	13.25 (1.02)	12.97 (0.75)	0.001

Platelet count, x 10 ³ /μL	264.0 (104.1)	243.6 (159.3)	251.3 (103.6)	242.5 (90.2)	0.86
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^aFor Alagille syndrome, the number of non-missing values for continuous variables ranged from 49 to 72, except for direct bilirubin (n=33).

^bFor chronic intrahepatic cholestasis, the number of non-missing values for continuous variables ranged from 26 to 39, except for direct bilirubin (n=21), and vitamin D (n=20).

^cFor alpha 1 antitrypsin deficiency, the number of non-missing values for continuous variables ranged from 48 to 74, except for direct bilirubin (n=23), and vitamin D (n=18).

^dFor bile acid synthetic disorder, the number of non-missing values for continuous variables ranged from 5 to 7, except for direct bilirubin (n=2).

^ep-value based on ANOVA reported for mean rows and from Chi-square or Fisher's Exact test for discrete outcomes.

*Values closest to the time when participants would have been eligible for a DXA scan were used in the analysis. Measurements were required to be within 1 year of that date.

**Bone fractures could have occurred before or after the time of DXA eligibility.

***Percentages are among participants with non-missing data.

****Lean body mass was measured by DXA, so it was not available for this sample.

Note: non-parametric Kruskal-Wallis tests confirmed results from ANOVA.

Supplementary Table S3: DXA Measurement Z-Scores by Disease Group

	<i>Mean (SD)</i>	Alagille Syndrome (n = 46-49)	Chronic Intrahepatic Cholestasis (n = 39-41)	Alpha-1 Antitrypsin Deficiency (n = 43-44)	Bile Acid Synthetic Disorder (n = 14)
Whole body DXA Z-scores					
BMD	Reference	-0.82 (1.78)	-1.39 (1.32)	-0.51 (1.49)	-0.67 (1.06)
	Weight- and height-adjusted	0.14 (1.78)	-0.46 (1.54)	-0.38 (1.38)	-0.36 (1.00)
	Weight-, height- and % fat-adjusted	0.47 (1.89)	-0.45 (1.61)	-0.24 (1.34)	-0.43 (1.07)
BMC	Reference	-1.27 (1.46)	-1.83 (1.36)	0.13 (1.24)	-0.22 (1.11)
	Weight- and height-adjusted	0.02 (1.47)	-1.05 (1.17)	-0.29 (1.59)	-0.29 (1.22)
	Weight-, height- and % fat-adjusted	0.41 (1.73)	-1.05 (1.34)	-0.09 (1.56)	-0.41 (1.20)
Whole body minus head DXA Z-scores					
BMD	Reference	-1.10 (1.57)	-1.58 (1.11)	-0.74 (1.43)	-0.70 (1.10)
	Weight- and height-adjusted	0.23 (1.65)	-0.37 (1.45)	-0.55 (1.34)	-0.16 (1.01)
	Weight-, height- and % fat-adjusted	0.60 (1.69)	-0.33 (1.45)	-0.43 (1.20)	-0.31 (1.03)
BMC	Reference	-1.23 (1.45)	-1.80 (1.33)	0.18 (1.26)	-0.08 (1.00)
	Weight- and height-adjusted	0.36 (1.34)	-0.76 (1.16)	-0.22 (1.60)	-0.13 (1.38)
	Weight-, height- and % fat-adjusted	0.76 (1.54)	-0.72 (1.35)	-0.00 (1.58)	-0.27 (1.26)
Spine DXA Z-scores					
BMD	Reference	-0.08 (1.66)	-1.01 (1.36)	0.10 (1.24)	0.14 (1.24)
	Weight- and height-adjusted	0.13 (1.65)	-1.15 (0.98)	-0.67 (1.22)	-0.03 (1.17)
	Weight-, height- and % fat-adjusted	0.45 (1.76)	-1.15 (1.02)	-0.58 (1.08)	-0.13 (1.27)
BMC	Weight- and height-adjusted	-0.27 (1.35)	-1.21 (1.29)	-0.78 (1.46)	-0.29 (1.19)
	Weight-, height- and % fat-adjusted	0.17 (1.59)	-1.24 (1.24)	-0.65 (1.42)	-0.39 (1.27)
Hip neck DXA Z-scores					
BMD	Reference	-1.24 (1.44)	-1.72 (1.11)	-0.26 (1.45)	0.33 (0.79)
	Weight- and height-adjusted	-0.96 (1.41)	-1.54 (1.16)	-0.68 (1.45)	-0.06 (1.11)
	Weight-, height- and % fat-adjusted	-0.64 (1.53)	-1.56 (1.11)	-0.65 (1.35)	-0.14 (1.12)
BMC	Weight- and height-adjusted	-0.70 (1.23)	-1.63 (1.06)	-0.82 (1.54)	-0.09 (1.12)

	<i>Mean (SD)</i>	Alagille Syndrome (n = 46-49)	Chronic Intrahepatic Cholestasis (n = 39-41)	Alpha-1 Antitrypsin Deficiency (n = 43-44)	Bile Acid Synthetic Disorder (n = 14)
	Weight-, height- and % fat-adjusted	-0.32 (1.46)	-1.72 (1.06)	-0.74 (1.45)	-0.24 (1.14)
Total hip DXA Z-scores					
	Reference	-0.78 (1.38)	-1.17 (1.23)	-0.18 (1.33)	0.39 (0.69)
BMD	Weight- and height-adjusted	-0.46 (1.41)	-1.17 (1.30)	-0.60 (1.40)	0.04 (0.99)
	Weight-, height- and % fat-adjusted	-0.07 (1.60)	-1.20 (1.28)	-0.50 (1.32)	-0.14 (0.96)
BMC	Weight- and height-adjusted	-0.38 (1.18)	-1.18 (1.22)	-0.83 (1.41)	0.16 (0.99)
	Weight-, height- and % fat-adjusted	0.06 (1.46)	-1.30 (1.19)	-0.81 (1.36)	-0.13 (1.12)

Supplementary Table S4: Pearson Correlations between DXA Reference Z-scores^a and Height, Weight and BMI Z-Scores for A1AT and BASD.

	Alpha 1 Antitrypsin Deficiency (n = 44)			Bile Acid Synthetic Disorder (n = 14)		
	Height	Weight	BMI	Height	Weight	BMI
BMD Measures						
Total Body	0.16	0.28	0.11	0.60	0.58	0.43
Total Body – Head	0.18	0.38	0.19	0.62	0.59	0.42
Total spine	0.19	0.42*	0.30	0.30	0.39	0.40
Total hip	0.05	0.26	0.16	0.51	0.66*	0.56
Hip neck	0.12	0.29	0.13	0.35	0.58	0.47
BMC Measures						
Total Body	0.45*	0.44*	0.13	0.63	0.65	0.52
Total Body – Head	0.48*	0.54**	0.21	0.63	0.70*	0.56

^aReference Z-score is adjusted for age, gender, and race (Black vs non-Black)

*p<0.01

**p<0.001

***p<0.0001

Supplementary Table S5: Differences in Laboratory Measurements by DXA Height- and Weight-Adjusted Z-Score Groups

Alagille Syndrome (n=49)

	Total Bilirubin (mg/dL)		Serum Bile Acids (μ mol/L)		Vitamin D (ng/dL)		INR		Albumin (g/dL)	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
BMD measures										
Total body minus head										
Z<-1.5	9	9.8 (7.49)	10	151.8 (95.4)	10	27.5 (25.17)	10	1.2* (0.16)	10	3.7** (0.50)
Z \geq -1.5	33	2.0 (2.47)	28	107.9 (110.0)	28	36.5 (16.16)	35	1.0* (0.15)	37	4.3** (0.49)
Spine										
Z<-1.5	9	9.4 (7.74)	10	156.0 (120.0)	9	30.2 (26.19)	10	1.1 (0.14)	10	3.8* (0.68)
Z \geq -1.5	33	2.1 (2.64)	28	106.4 (100.9)	29	35.3 (16.57)	35	1.1 (0.17)	37	4.3* (0.48)
Total hip										
Z<-1.5	10	9.4* (7.40)	9	170.9 (117.8)	8	28.4 (26.81)	10	1.2*** (0.16)	10	3.6*** (0.50)
Z \geq -1.5	30	1.6* (1.60)	28	106.1 (100.9)	29	36.4 (16.33)	33	1.0*** (0.13)	35	4.4*** (0.47)
BMC measures										
Total body minus head										
Z<-1.5	5	11.1 (9.33)	5	147.6 (132.8)	4	13.0 (8.54)	5	1.1 (0.13)	5	3.5* (0.55)
Z \geq -1.5	37	2.6 (3.42)	33	115.2 (104.2)	34	36.6 (18.37)	40	1.1 (0.17)	42	4.3* (0.51)

Tests for differences between groups based on DXA Z-scores (Z<-1.5 vs. Z \geq -1.5) were performed using two-sample t-tests.

*p<0.01

**p<0.001

***p<0.0001

Chronic intrahepatic cholestasis (n=41)

	Total Bilirubin (mg/dL)		Serum Bile Acids ($\mu\text{mol/L}$)		Vitamin D (ng/dL)		INR		Albumin (g/dL)	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
BMD measures										
Total body minus head										
Z<-1.5	6	1.1 (0.9)	7	109.7 (159.3)	5	30.4 (6.3)	6	1.1 (0.1)	8	4.2 (0.8)
Z \geq -1.5	31	1.6 (2.0)	26	60.9 (84.1)	28	39.1 (17.4)	28	1.1 (0.2)	32	4.2 (0.4)
Spine										
Z<-1.5	14	2.2 (2.6)	15	100.2 (122.9)	11	38.3 (21.7)	14	1.1 (0.1)	16	4.0 (0.6)
Z \geq -1.5	23	1.1 (1.1)	18	47.1 (79.6)	22	37.5 (13.7)	20	1.1 (0.2)	24	4.3 (0.4)
Total hip										
Z<-1.5	15	2.0 (2.6)	16	78.7 (114.4)	13	39.4 (19.8)	15	1.1 (0.1)	17	4.1 (0.6)
Z \geq -1.5	21	1.1 (1.1)	16	66.8 (97.4)	20	36.7 (14.4)	19	1.1 (0.2)	22	4.3 (0.4)
BMC measures										
Total body minus head										
Z<-1.5	9	2.7 (3.2)	11	76.6 (130.4)	8	38.4 (22.3)	9	1.1 (0.1)	11	4.0 (0.7)
Z \geq -1.5	28	1.1 (1.0)	22	68.6 (90.4)	25	37.6 (14.7)	25	1.1 (0.2)	29	4.3 (0.4)

No significant differences between groups based on DXA Z-scores (Z<-1.5 vs. Z \geq -1.5) were found using two-sample t-tests.

Abbreviations: BMC, bone mineral content; BMD, bone mineral density; DXA, dual energy x-ray absorptiometry; INR, international normalized ratio; SD, standard deviation.

Supplementary Figure Legends

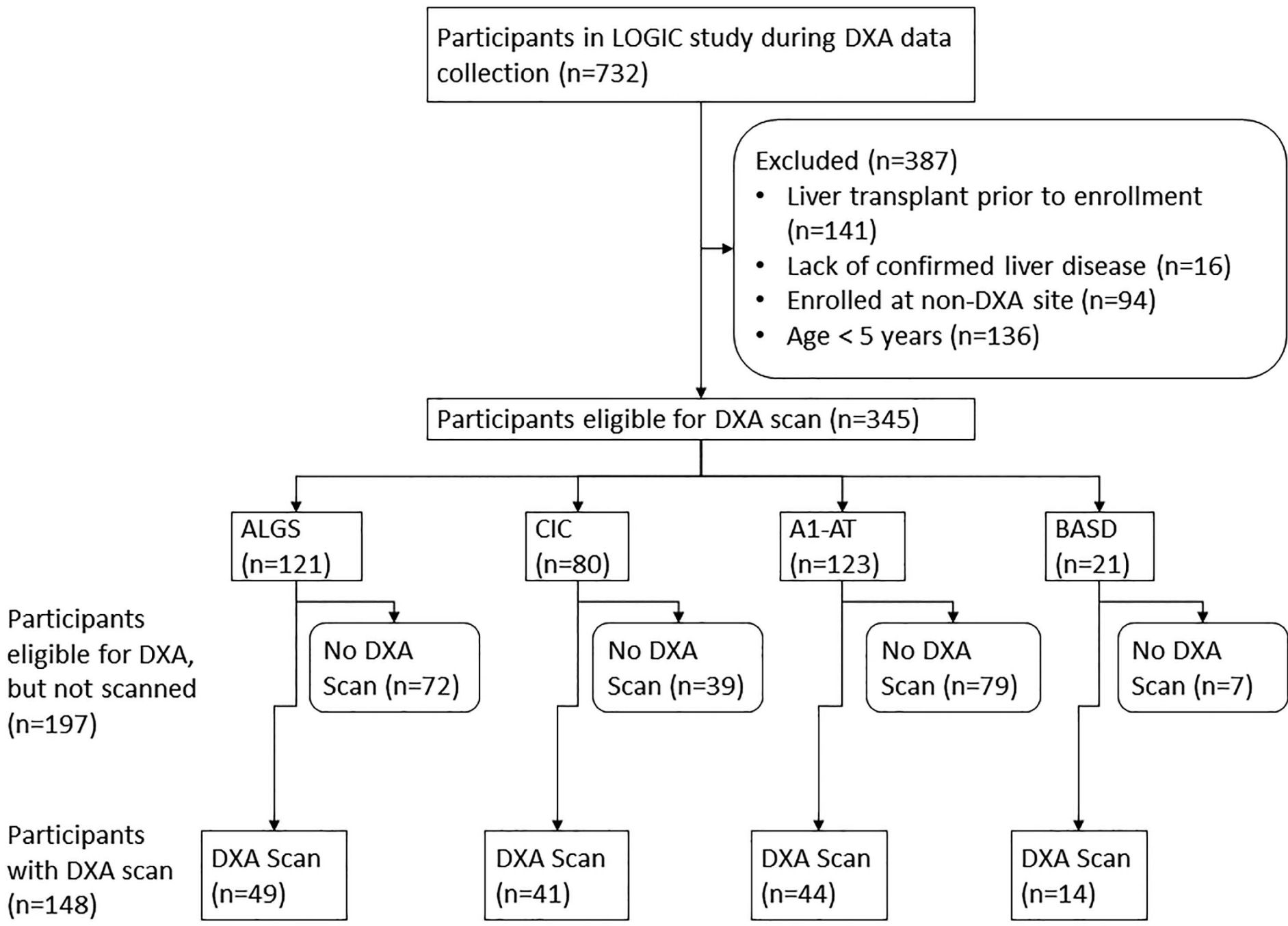
Supplementary Figure S1: Derivation of sample population.

Supplementary Figure S2: Comparison between DXA Reference and Height- and Weight-Adjusted Z-scores for Total Body minus Head Measurements

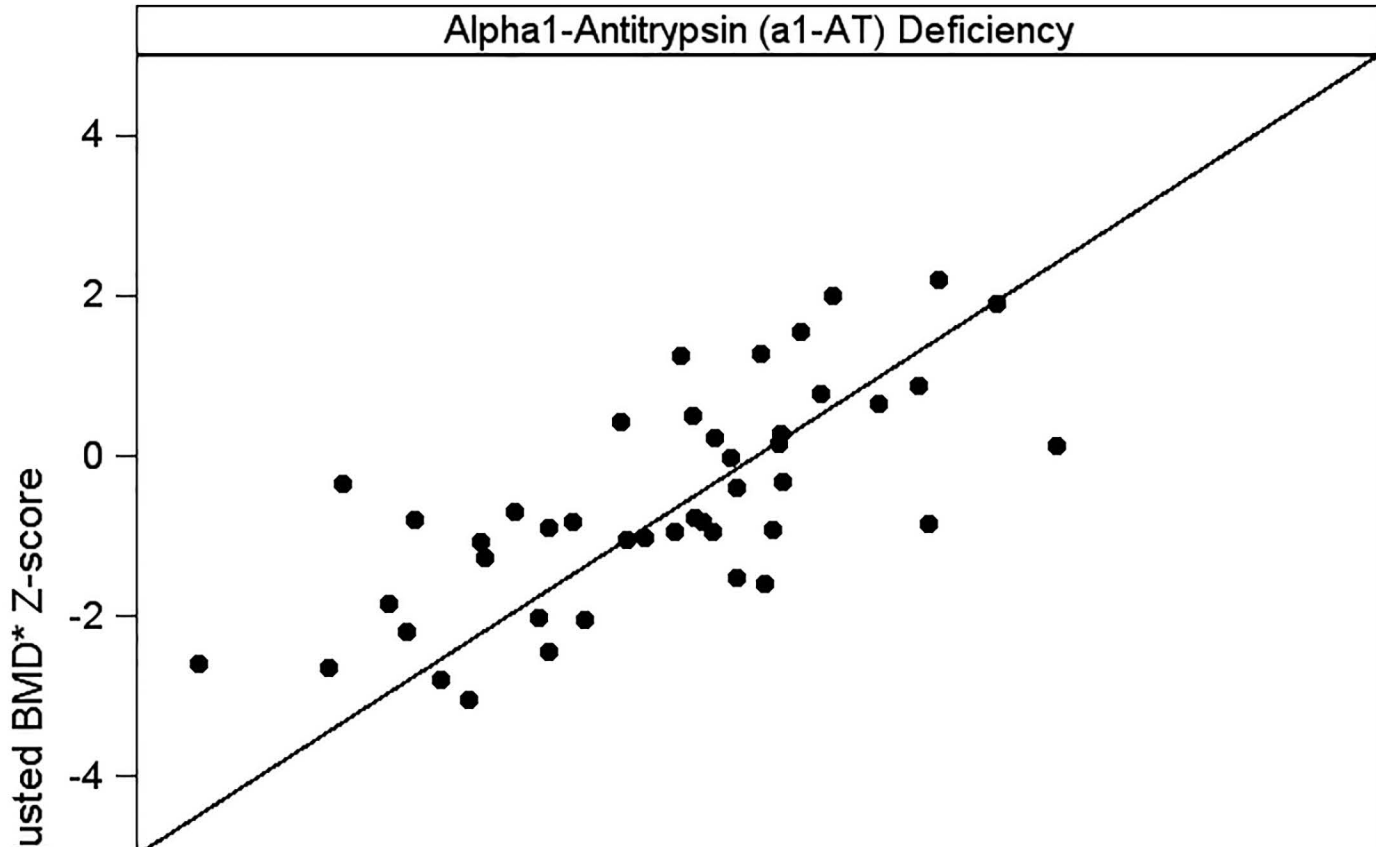
(Supplementary Figure S2.1 BMD; Supplementary Figure S2.2 BMC) for A1-AT and BASD disease groups. The reference line indicates equality between the reference and adjusted Z-scores. Points above the line indicate observations where the Z-score adjusted for height and weight is higher than the reference Z-score. Points below the line indicate observations where the adjusted Z-score is lower than the reference Z-score.

[Footnote] *Non-Winsorized BMD values presented

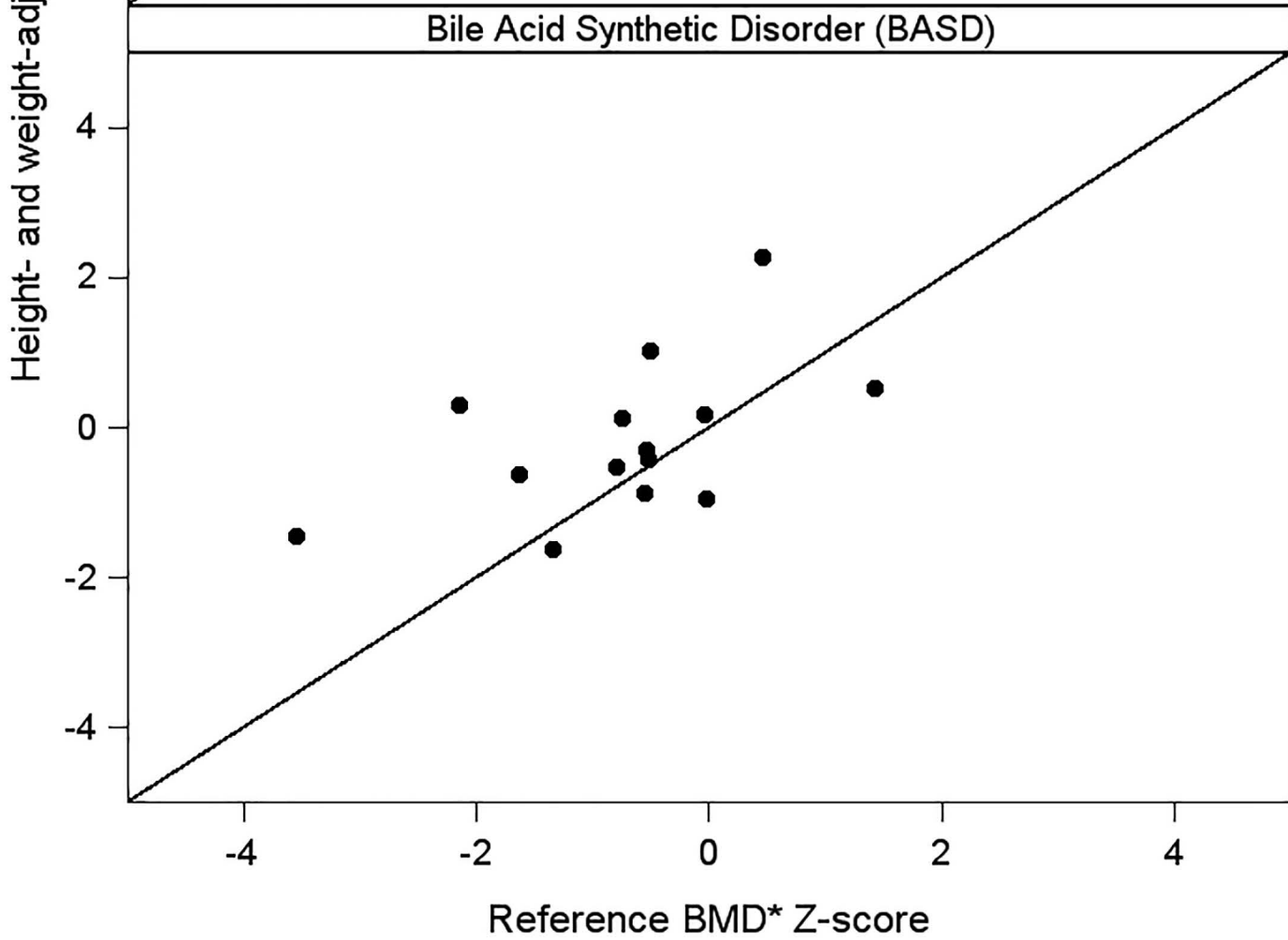
Abbreviations: BMC, bone mineral content; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry.



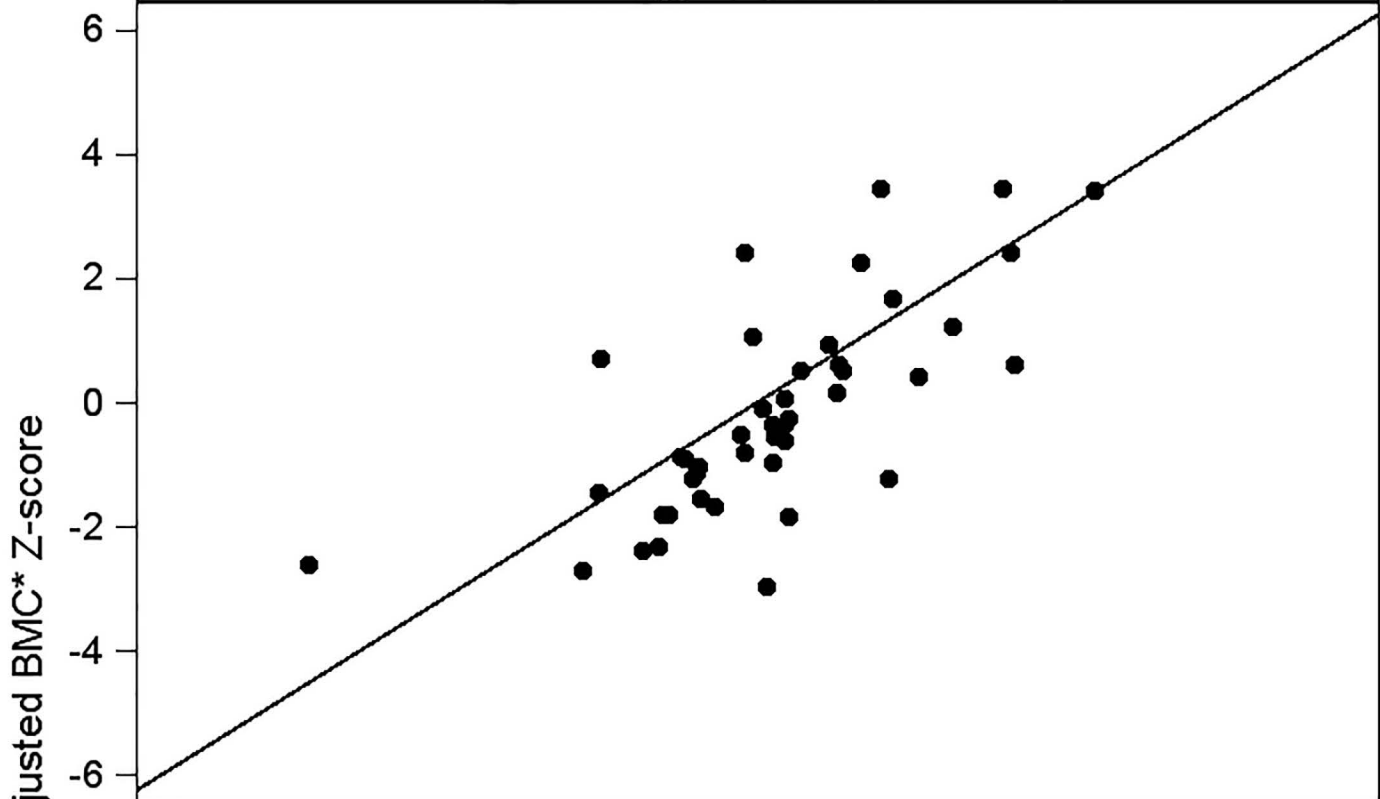
Alpha1-Antitrypsin (a1-AT) Deficiency



Bile Acid Synthetic Disorder (BASD)



Alpha1-Antitrypsin (a1-AT) Deficiency



Bile Acid Synthetic Disorder (BASD)

