Gastro Hep Advances 2024;3:842–850

ORIGINAL RESEARCH—CLINICAL

Biomarkers Associated With Future Severe Liver Disease in Children With Alpha-1-Antitrypsin Deficiency

Jeffrey H. Teckman,[1] Paula Buchanan,[2] Keith Steven Blomenkamp,[1] Nina Heyer-Chauhan,[3] Keith Burling,[4] and David A. Lomas[3]

[1]Department of Pediatrics and Biochemistry and Molecular Biology, St. Louis University School of Medicine, Cardinal Glennon Children's Hospital, St. Louis, Missouri; [2]Department of Health and Clinical Outcomes Research, St. Louis University School of Medicine, St. Louis, Missouri; [3]Division of Medicine, UCL Respiratory, University College London, London, UK; and [4]Core Biochemical Assay Laboratory, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

BACKGROUND AND AIMS: Children with alpha-1-antitrypsin deficiency (AATD) exhibit a wide range of liver disease outcomes from portal hypertension and transplant to asymptomatic without fibrosis. Individual outcomes cannot be predicted. Liver injury in AATD is caused by the accumulation in hepatocytes of the mutant Z alpha-1-antitrypsin (AAT) protein, especially the toxic, intracellular polymerized conformation. AATD patients have trace Z polymer detectable in serum with unknown significance. **METHODS:** The Childhood Liver Disease Research Network is an NIH consortium for the study of pediatric liver diseases, including AATD. We obtained data and samples with the aim of identifying biomarkers predictive of severe AATD liver disease. **RESULTS:** We analyzed prospective AATD Childhood Liver Disease Research Network data and serum samples in 251 subjects from 2007 to 2015 for outcomes and Z polymer levels. Fifty-eight of 251 had clinically evident portal hypertension (CEPH) at enrollment, and 10 developed CEPH during follow-up. Higher Z AAT polymer levels were associated with existing CEPH ($P = .01$). In infants without CEPH, higher polymer levels were associated with future CEPH later in childhood, but total AAT was not predictive. Higher gamma-glutamyl transferase (GGT) in the first few months of life was also significantly associated with future CEPH, and risk-threshold GGT levels can be identified. A model was constructed to identify subjects at high risk of future CEPH by combining clinical GGT and polymer levels (area under the curve of 0.83; 95% confidence interval: 0.656–1.00, $P = .019$). **CONCLUSION:** High circulating Z polymer levels and high GGT early in life are associated with future CEPH in AATD, and the use of predictive cutoffs may assist in future clinical trial design.

Keywords: Alpha-1-Antitrypsin; Portal Hypertension; Polymer; Gamma Glutamyl Transferase; Cholestasis

Wild-type M alpha-1-antitrypsin (AAT) is synthesized in large quantities in the liver and secreted into the serum.[1] Individuals homozygous for the mutant Z allele of AAT (homozygous ZZ or "PIZZ") have the classic form of AAT deficiency, a genetic disease found in 1 in 2500 to 3500 individuals in North American and European populations. The disease-associated Z allele (Glu342Lys) encodes the production of mutant Z AAT protein, which folds improperly during biogenesis and accumulates in the liver rather than being secreted. Molecules of Z AAT can attain an unusual conformation as chains of polymers within hepatocytes that are hepatotoxic and result in liver injury.[2,3]

ZZ AAT deficiency has a wide range of potential health outcomes in both adults and children. Some individuals are asymptomatic, go undiagnosed, and live a normal life span. About 20% of ZZ newborns develop cholestatic hepatitis. Most children do well, but there is at least a 3% to 5% risk of life-threatening liver disease in childhood, resulting in cirrhosis, portal hypertension, liver transplant, or death.[4–7] There is currently no specific treatment for AAT liver disease other than liver transplant, and there is no way to predict which patients will do well and which will progress to liver failure, portal hypertension, transplant, or death. Our previous studies revealed that the pathological Z AAT polymers can be detected in small amounts (<1% of total serum AAT) in the circulation of homozygous ZZ individuals.[8] Moreover, recent work has suggested that these polymers are secreted from hepatocytes and so may be a biomarker of intrahepatic polymerization and liver cell injury.[9] However, this has not been evaluated in longitudinal samples of children with ZZ AAT deficiency. There is also evidence from various studies indicating a dose-response relationship between mutant Z polymers and liver cell injury; we therefore hypothesized that circulating Z polymer levels would be a biomarker for severe liver injury.[8,10–12]

The Childhood Liver Disease Research Network (ChiLDReN) is a National Institutes of Health (NIH)-supported consortium of pediatric tertiary care centers in North America, 17 of which collected data during this study period

Abbreviations used in this paper: **AAT, alpha-1-antirypsin; ALT, alanine amino transferase; CEPH, clinically evident portal hypertension; ChiLDReN, Childhood Liver Disease Research Network; GGT, gamma-glutamyl transferase; LOGIC, Longitudinal Observational Study of Genetic Causes of Intrahepatic Cholestasis; ROC, receiver operating characteristic.**

Most current article

2772-5723
https://doi.org/10.1016/j.gastha.2024.04.010

and focused on the study of pediatric liver diseases.[6,13] The Longitudinal Observational Study of Genetic Causes of Intrahepatic Cholestasis (LOGIC, NCT00571272) is a study conducted by this network with the goal of describing the natural history and genetic and environmental modifiers of a group of metabolic-cholestatic liver diseases, including AAT deficiency. We have measured Z AAT serum polymer levels from prospectively collected samples from participants in LOGIC and compared their outcomes in the longitudinal database.

Experimental Procedures

This report includes participants with AAT deficiency with their native livers enrolled in LOGIC from November 30, 2007, through 2015. Institutional review board approvals and written informed consents were obtained at each institution consistent with the ethical guidelines of the 1975 Declaration of Helsinki.[6,13] Database access and sharing were approved under NIH guidelines. Eligibility for enrollment of AAT participants in the LOGIC study includes homozygous ZZ or compound double mutant SZ (PIZZ or PISZ) serum protein phenotype or genotype (although only ZZ were included in this analysis), with a corresponding low serum level of AAT, age birth to 25 years, and evidence of liver disease as defined by documentation of one of the following: neonatal cholestasis (conjugated hyperbilirubinemia and jaundice within the first 3 months of life); $\geq$1.25 $\times$ the upper limit of normal alanine aminotransferase (ALT), aspartate aminotransferase, or gamma-glutamyl transferase (GGT); chronic hepatomegaly; clinical findings or complications of portal hypertension or cirrhosis; impaired liver synthetic function; or abnormal liver biopsy histology, other than globular inclusions of AAT, showing liver injury such as inflammation, fibrosis, or necrosis. At enrollment, medical history and physical exam were obtained, including review of available medical records and standard of care labs. Updates in medical history and physical exams were documented at annual follow-up visits, and other data were collected as previously described. Participants with clinically evident portal hypertension (CEPH) were identified during clinical care by systematically documenting the criteria of splenomegaly (palpable spleen $>$ 2 cm below rib margin) and thrombocytopenia (platelet count $<$150,000) with validation and supplemental analysis as previously published.[6]

Serum samples were obtained from the specimen repository from enrollment and from each annual visit and matched to clinical data collected. Serum Z protein AAT polymer levels were determined by investigators blind to the participants' clinical characteristics using Meso Scale Discovery (MSD) assay, using monoclonal antibodies that are specific for different AAT forms described previously.[8] MSD normal-bind immunoassay plates were coated overnight at 4 °C with 30 μL/well of purified 3C11 or 2C1 mAb at 3 μg/mL in phosphate buffered saline. The next day, the plates were washed and incubated with 150 μL/well of blocking solution for 1 hour at room temperature. Standards and samples were prediluted in phosphate buffered saline containing 1% bovine serum albumin; 10 μL of each was added to the plate along with 30 μL/well 30 μl DELFIA Diluent II (Perkin Elmer) and incubated for 2 hours at room temperature. Bound total or polymeric AAT was detected with 25 μl of 1 ng/ml rabbit anti-human alpha-1-antitrypsin polyclonal antibody (Dako) in MSD Diluent 100 solution and incubated for 2 hours. The plate was then washed before adding 25 μl of 500 ng/ml of goat anti-rabbit Sulpho-TAG antibody (MSD) in MSD Diluent 100 and incubated at room temperature for 30 minutes. After a further wash step, 150 μl of $\times$1 MSD read buffer was added to the wells. The plate was then immediately read on the MSD s600 plate reader. AAT (μg/mL) concentrations were determined by interpolation of absorbance values on the standard curve using MSD workbench software. Oligomer analysis was performed on non-denatured gels with standard techniques.[9]

For all continuous variables, median, first, and third quartiles were reported for the whole sample and also for the 2 subgroups: no CEPH at baseline and CEPH at baseline. Mann-Whitney U-tests were performed to make comparisons between these 2 subgroups. Receiver operating characteristic (ROC) curves were used to illustrate the predictive ability of Z AAT polymer and GGT on the development of portal hypertension. Logistic regression was used to obtain the predictive probability of developing CEPH given the lab values. We then ran a ROC analysis on the predictive probability to determine the optimal sensitivity and specificity and a combination of sensitivity and specificity. Following this, we reverse-calculated using the logistic regression equation to determine the lab values that gave us the predictive value associated with sensitivity, specificity, and the combination values.

Results

Cohort Characteristics

We examined data and stored serum samples from sequential homozygous ZZ AAT deficient participants in the LOGIC study, which began enrolling in November of 2007 through our data lock of 2015. Two hundred fifty-one subjects had adequate samples and data for analysis, including at least one data collection after baseline resulted in a total of 686 patient-years of follow-up. The median age at enrollment was 65 months (range 3–269 months) (Table 1). Fifty-eight of the 251 (23%) subjects had clinically evident portal hypertension (CEPH) at enrollment, and 10 (4%) of the remaining subjects developed CEPH after enrollment during follow-up observation. For these 10, the median and interquartile range time to CEPH from enrollment was 616 days (186–763).

Correlation of Total AAT and CEPH Status

First, we examined the data for a relationship between total AAT serum level and CEPH, which had not been previously reported. It has been hypothesized that if more of the hepatotoxic Z AAT is secreted, then less would remain in the liver to cause injury. However, several published studies have shown that the efficiency of intracellular degradation of the Z AAT in hepatocytes, not secretion, is associated with severe liver injury in children.[14–16] The results showed that the total serum AAT level at diagnosis of AAT deficiency was not related to future CEPH. However, lower total AAT levels at enrollment in LOGIC were associated with existing CEPH (Figure 1A–B, and Figure A1). We were unable to identify any clinical utility of the total AAT determination with regard to liver disease beyond the routine measurement typically determined on a single

Table 1. Patient and Clinical Characteristics by CEPH Status at Enrollment Into LOGIC and at Diagnosis of AAT Deficiency

	Overall (N = 251)	No PHT at baseline[a] (N = 193)	PHT baseline (N = 58)	
Criteria	Median (IQR)	Median (IQR)	Median (IQR)	*P*-value[b]
Age at baseline (mo)	65 (21–115)	57.8 (19.0–107.8)	78.8 (40.3–163.0)	.03
Follow-up time (y)[c]	1.18 (0–4.87)	2.05 (0–5.07)	0.54 (0–4.40)	.36
Follow-up time for those with follow-up, N = 135 (y)[c]	4.45 (2.97–7.26)	4.61 (3.07–7.29)	4.39 (2.18–7.15)	.34
AAT Z-polymer at baseline (μg/mL)	11.1 (7.2–18.7)	10.3 (6.7–17.3)	14.3 (8.6–23.9)	.01
Total AAT at baseline (mg/dL)	13.8 (9.5–18.4)	14.7 (9.9–18.5)	11.5 (7.8–17.7)	.03
Total AAT at diagnosis (mg/dL)	32.0 (29.0–39.0)	32.0 (29.3–38.0)	33.5 (27.0–40.0)	.93
GGT at baseline (IU/L)	n = 117 39 (20.5–112.0)	n = 91 29.0 (18–61)	n = 26 153 (62.5–287)	<.001
GGT when no PHT (IU/L) Max value Min value Avg value	n = 96 34.0 (21.3–98.3) 20 (12.3–35.8) 26.4 (16.8–66.8)	n = 96 34.0 (21.3–98.3) 20 (12.3–35.8) 26.4 (16.8–66.8)		
GGT when no PHT (IU/L) Max value Min value Avg value	N = 105 35 (23.5–112) 20 (13–45) 28.7 (18.2–72.7)	N = 105 35 (23.5–112) 20 (13–45) 28.7 (18.2–72.7)		
GGT at time of PHT developed, IU/L	n = 31 161 (62–308)	n = 8 63.0 (39.5–302.8)	n = 23 188.0 (65.0–299.0)	.28
GGT post-PHT development (n = 49), IU/L Max value Min value Avg value	n = 37 174 (85–405.5) 99 (44–211) 138 (74.7–335.0)	n = 9 130 (57–319.5) 45 (16.5–157.5) 127.0 (43.7–223.4)	n = 28 178.5 (95.3–454.3) 104.5 (59.5–231.8) 149.0 (79.9–362.4)	 .34 .09 .29

avg, average; IQR, interquartile range; Max, maximum; min, minimum; PHT, portal hypertension.
Enrollment: December 11, 2004–December 2, 2014.
[a]Ten patients developed PHT after baseline.
[b]*P*-value compares between those with no PHT at baseline and those with PHT at baseline using the Mann-Whitney U-test for continuous variables and chi-square test for categorical variables.
[c]Total follow-up time: 685.8 years, mean (SD): 2.73 (3.24) years, range: 0–12.85 years, for only those with follow-up (N = 135); mean (SD) time was 5.08 (2.75) years.

occasion at the time of diagnosis for comparison to the genotype or phenotype.

AAT Polymer Level and GGT Associated With CEPH

Next, we examined the relationship of serum Z AAT polymer and the clinical lab determination of GGT to CEPH. We hypothesized that since many studies show a dose-response relationship between intracellular Z AAT polymer levels and cell and liver injury, this would be a potentially valuable marker if the polymer in the circulation was proportional to intrahepatic polymer. GGT has also been previously known to be elevated in many diseases causing CEPH in children, including AAT deficiency.[4,10] Our previously published work on this database indicated a lack of utility of measures of aspartate aminotransferase, ALT, albumin, platelets, and many other common clinical chemistries to build forward looking predictive models, which is why we are now focused on GGT and Z polymers.[6,13]

First, we plotted AAT polymer level at enrollment vs age and noted that some participants had much higher than typical levels in the first few months of life. Previously, we found that only a small percentage of the serum AAT in a ZZ individual is in the polymerized conformation, but it was not previously known that early in life some patients would have much higher levels (Figure 2).[8] We then found that subjects with CEPH at enrollment had significantly higher AAT polymer levels compared to those without CEPH at enrollment (Figure 2B; $P < .01$). When subjects who did not have CEPH at enrollment but who developed CEPH during follow-up were examined, it was found that they had higher, but not significantly, serum polymer than those who did not develop CEPH ($P = .13$). However, it was significantly higher ($P = .003$) in those with CEPH compared to those who did not develop CEPH during follow-up (Figure 2C and D). Next, we made a model of the Z polymer level over time by CEPH status (Figure A2), which showed a significantly higher Z polymer level at enrollment when CEPH was present compared to absent, but then, over several years, a decline. This decline was in the same time frame as a total AAT drop and paralleled a likely drop in liver synthetic function as liver failure progressed. Similarly, serum GGT at enrollment was higher in those with CEPH at enrollment compared to

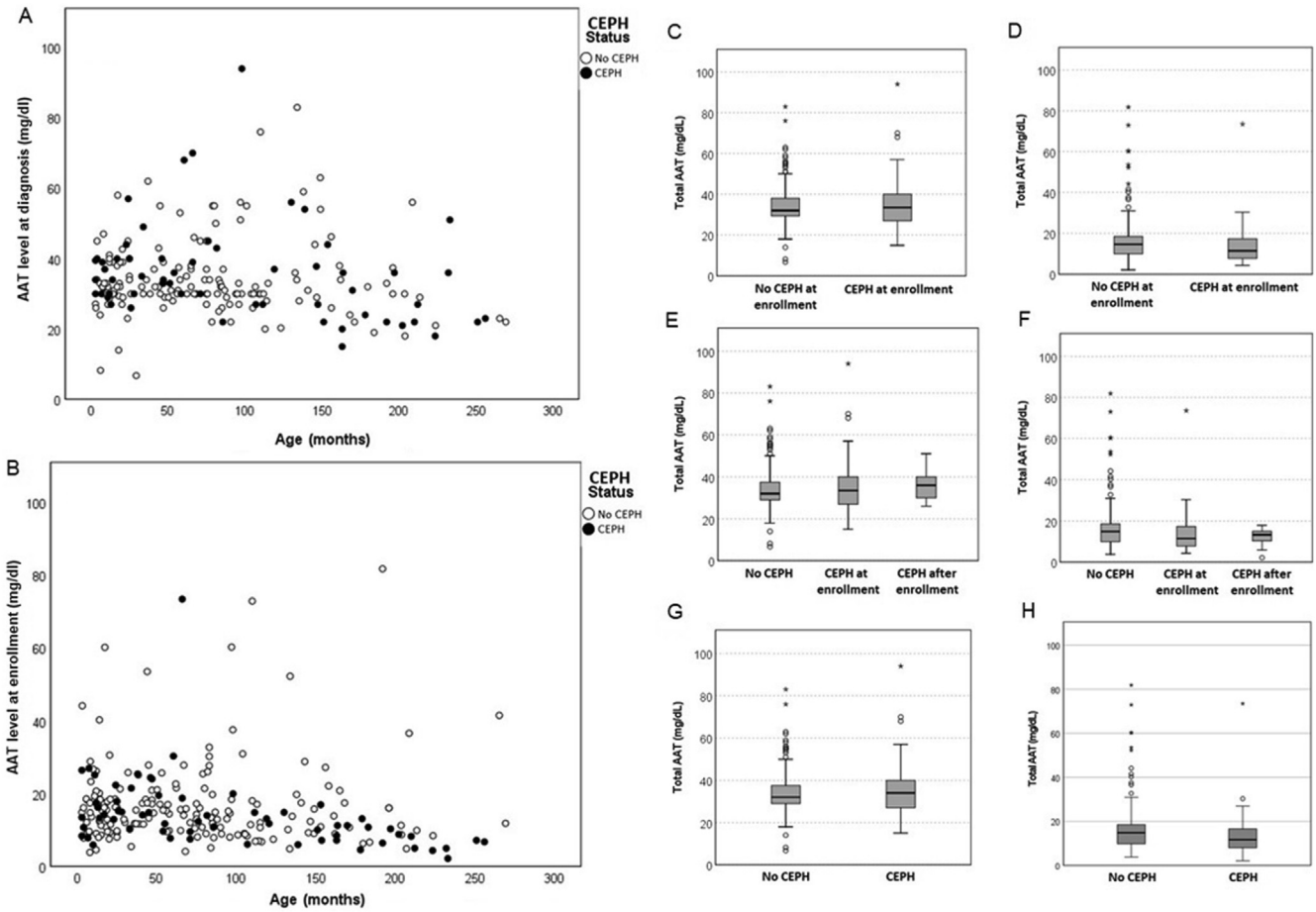

Figure 1. (A–H) Total serum AAT (mg/dL) at (A) diagnosis and at (B) enrollment vs age by CEPH. Status and total AAT (mg/dL) at baseline enrollment and diagnosis by CEPH status at enrollment. (A) depicts the serum total AAT at diagnosis of AAT deficiency vs age at enrollment in months by CEPH status; (B) depicts the total AAT at enrollment vs age at enrollment in months by CEPH status. Box and whiskers plot depicting (C) total AAT levels at **diagnosis** vs presence of CEPH at enrollment, median (IQR) no CEPH at baseline: 32.0 (29.3–38.0), CEPH at enrollment: 33.5 (27.0–40.0), Mann-Whitney U test $P = .93$. (D) Total AAT levels at enrollment vs presence of CEPH at enrollment, median (IQR) no CEPH at enrollment: 14.66 (9.88–18.46), CEPH at enrollment: 11.46 (7.80–17.67), Mann-Whitney U test $P = .02$. (E) Total AAT levels at **diagnosis** vs CEPH status, median (IQR) no CEPH ever: 32.0 (29.0–37.8), CEPH at enrollment: 33.5 (27.0–40.0), CEPH after enrollment: 36.0 (30.0–40.0), Kruskal-Wallis $P = .57$. (F) Total AAT levels at enrollment vs CEPH status, median (IQR) no CEPH ever: 14.86 (9.87–18.94), CEPH at enrollment: 11.46 (7.80–17.67), CEPH after baseline: 13.28 (9.2–15.06), Kruskal-Wallis $P = .03$. Post hoc tests: no CEPH ever vs CEPH at enrollment, $P = .02$; no CEPH ever vs CEPH after baseline, $P = .16$; CEPH at enrollment vs CEPH after enrollment, $P = .80$. (G) Total AAT levels at **diagnosis** vs presence of CEPH ever, median (IQR) no CEPH: 32.0 (29.0–38.0), CEPH: 34.0 (27.0–40.0), Mann-Whitney U test $P = .57$. (H) Total AAT levels at enrollment vs presence of CEPH ever, median (IQR) no CEPH: 14.86 (9.87–18.94), CEPH: 11.74 (7.93–16.77), Mann-Whitney U test $P = .01$. IQR, interquartile range.

those without (mean 153 IU/L vs 29 IU/L, $P < .001$), and higher GGT was found in participants who developed CEPH during follow-up (Figure 3). We used these data to plot the 80% probability and 20% probability of CEPH by chronological age based on these data (Figure 3D). Overall, the association of GGT with future CEPH was stronger than Z polymer, but the difference was not statistically significant.

Next, we hypothesized that a useful algorithm to predict CEPH could be developed from these data. We noted an empiric peak cutoff of serum AAT polymer of 50 ug/ml if reached before 50 months of age, which identified a group highly associated with future CEPH. We generated ROC curves with significant association to future CEPH in young subjects, which could then be combined with GGT values from the clinical lab to form a table predictive of risk (Table 2, Figure 4, and Figure A3). We note that the GGT, which is commonly measured as part of clinical care, might have significant utility, depending on the cutoff chosen and the age of the patient. Previous attempts to develop predictive models from clinical ChiLDReN data such as ALT and albumin have not been successful.[6,13] The AAT-Z polymer also suggested utility at the empiric cutoff we identified. A model that combined GGT and Z polymer is possibly the most predictive and useful for subjects under 50 months of

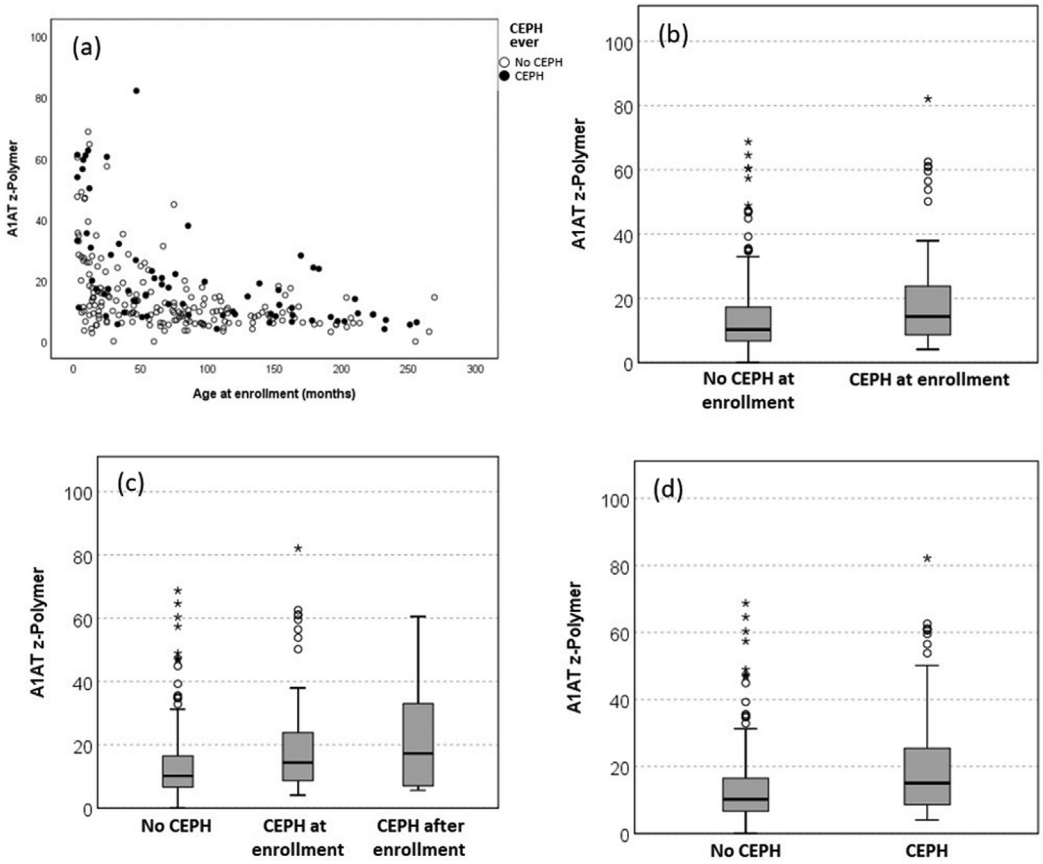

Figure 2. (A–D) Z AAT polymer level (μg/mL) at enrollment vs age in months at enrollment by CEPH status and Z AAT polymer level (μg/mL) at enrollment by CEPH status. (A) Depicts Z AAT polymer level (μg/mL) at enrollment vs age at enrollment in months by CEPH status. Box and whiskers plot depicting Z AAT polymer level (μg/mL), median (IQR), (B) no CEPH: 10.27 (6.69–17.26) vs CEPH at enrollment: 14.34 (8.63–23.91), Mann-Whitney U test $P = .01$. (C) No CEPH: 10.17 (6.60–16.56) vs CEPH at enrollment: 14.34 (8.63–23.91) vs CEPH after enrollment: 17.26 (6.96–33.62), Kruskal-Wallis $P = .01$, pair-wise post-hoc test shows a difference in no CEPH vs CEPH at enrollment, $P = .01$, but no differences in no CEPH vs CEPH after enrollment, $P = .13$ or CEPH at enrollment vs CEPH after enrollment, $P = .82$. (D) No presence of CEPH ever: 10.17 (6.60–16.56), vs CEPH: 15.04 (8.60–26.05), Mann-Whitney U test $P = .003$. IQR, interquartile range.

age (area under the curve 0.83; 95% confidence interval: 0.66–1.00, $P = .017$).

Persistence of Circulating Oligomers Early in Life Associated With CEPH

Finally, we questioned if polymer length was related to CEPH. Within hepatocytes, most of the polymers are very long and insoluble, but polymers in the serum appear to be shorter length chains that are soluble. Our serum assay is based on immunological recognition but did not specifically examine polymer length. Therefore, we used nondenatured gels to examine polymer length from samples collected at annual visits from each subject who developed CEPH during follow-up matched to a subject of the same age and sex who did not develop CEPH (n = 7 pairs with adequate samples and data before 7 years of age). We used this matched design since we had identified the above changes in Z polymer with age early in life. The results showed in the first few years of life that all subjects had easily detected 3 and 4-mer AAT polymers in serum. These forms usually disappear by 5 years of age. However, in 6 of the 7 (86%) of those who developed CEPH, these forms remained detectable in serum. (Figure 5). Densitometric quantification of the combined laddered band densities of the 2-, 3-, and 4-mer forms for samples from each annual visit after 5 years of age was significantly greater in those with CEPH compared to those without ($P = .04$).

Discussion

Homozygous ZZ AAT deficiency is a common genetic disease identified in infants with liver disease but is widely underdiagnosed and misdiagnosed in adults. This is partly the result of the very wide range of potential presentations throughout all ages of life. Although equally as frequent as cystic fibrosis and many other genetic diseases, AAT deficiency is not on the newborn screen in any country or US state. The most common presentation in childhood is neonatal cholestatic hepatitis, but it has been impossible to predict which of the infants who are diagnosed after presenting with this syndrome will do well and which will go

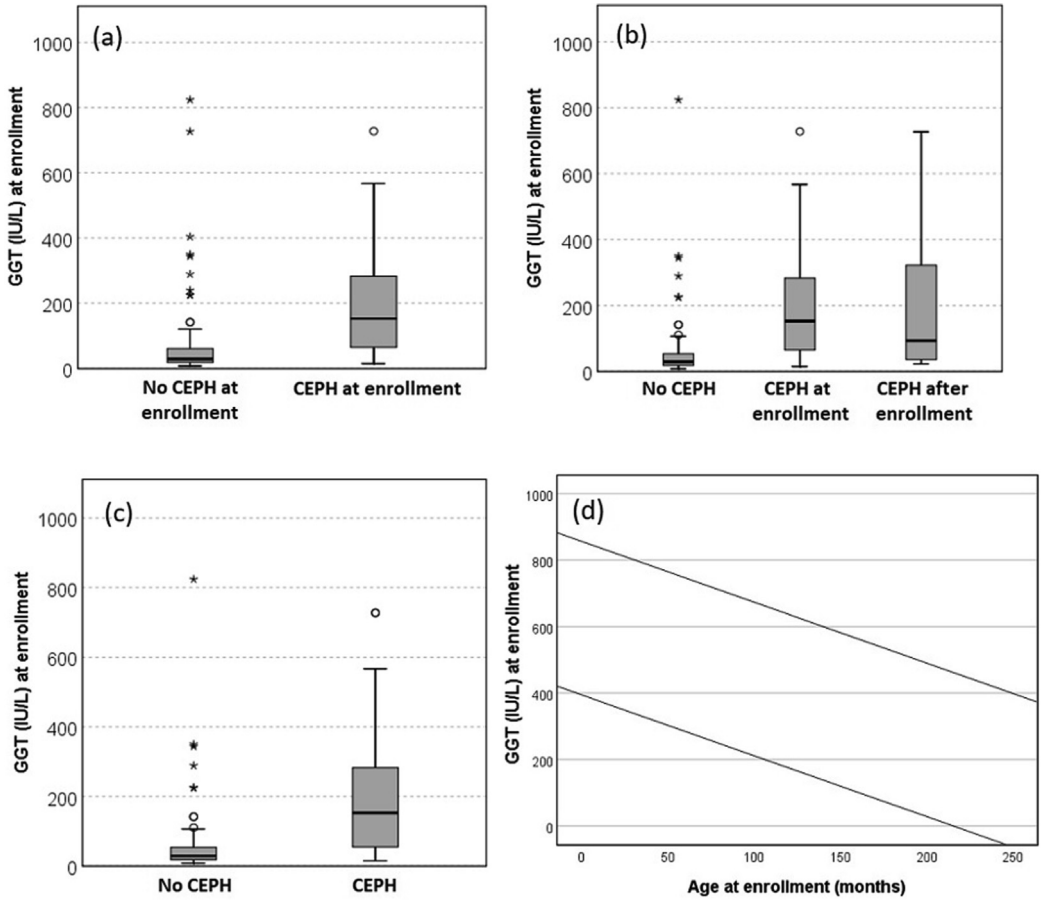

Figure 3. (A–D) GGT (IU/L) at enrollment by CEPH status. Box and whiskers plot depicting GGT (IU/L), median (IQR), at enrollment for (A) no CEPH: 29.0 (18.0–65.0) vs CEPH at enrollment: 153.0 (62.5–287.0), Mann-Whitney U test $P < .001$. (B) No CEPH: 29.0 (18.0–55.0) vs CEPH at baseline: 153.0 (62.5–287.0) vs CEPH after enrollment: 180.5 (41.25–399.5), Kruskal-Wallis $P < .001$, pair-wise post-hoc test shows a difference in no CEPH vs CEPH at enrollment, $P < .001$, no CEPH vs CEPH after enrollment, $P = .005$, but no differences in CEPH at enrollment vs CEPH after, $P = .67$. (C) No presence of CEPH ever: 29.0 (18.0–55.0), vs CEPH: 153.0 (62.5–315.25), Mann-Whitney U test $P < .001$. (D) GGT vs chronological age with an 80% probability (top line) and a 20% probability (bottom line) of developing future CEPH. IQR, interquartile range.

Table 2. Optimal Cutoffs of GGT and Z A1AT Polymers for Predicting PHT, (N = 251)

	AUC (95% CI)	*P*-value	Optimal cutoff[a]	Sensitivity	Specificity
GGT					
Ever	0.82 (0.74–0.91)	<.001	65	0.765	0.795
At baseline	0.81 (0.71–0.90)	<.001	51	0.846	0.703
After baseline	0.79 (0.64–0.94	.008	36	0.875	0.614
After baseline for those <50 mo	0.84 (0.68–1.00)	.02	0.005	1.000	0.575
A1AT					
Ever	0.62 (0.55–0.70)	.003	16.44	0.471	0.754
At baseline	0.61 (0.53–0.70)	.009	17.67	0.414	0.777
After baseline	0.64 (0.45–0.84)	.13	17.15	0.600	0.765
After baseline for those <50 mo	0.68 (0.46–0.90)	.12	16.90	0.857	0.593
Combined GGT and A1AT					
Ever	0.83 (0.75–0.91)	<.001			
At baseline	0.82 (0.74–0.90)	<.001			
After baseline	0.75 (0.55–0.95)	.02			
After baseline for those <50 mo	0.83 (0.66–1.00)	.02			

AUC, area under the curve as determined via ROC analysis; CI, confidence interval; PHT, portal hypertension.
[a]The optimal cutoff was defined as the value that maximized sensitivity + specificity.

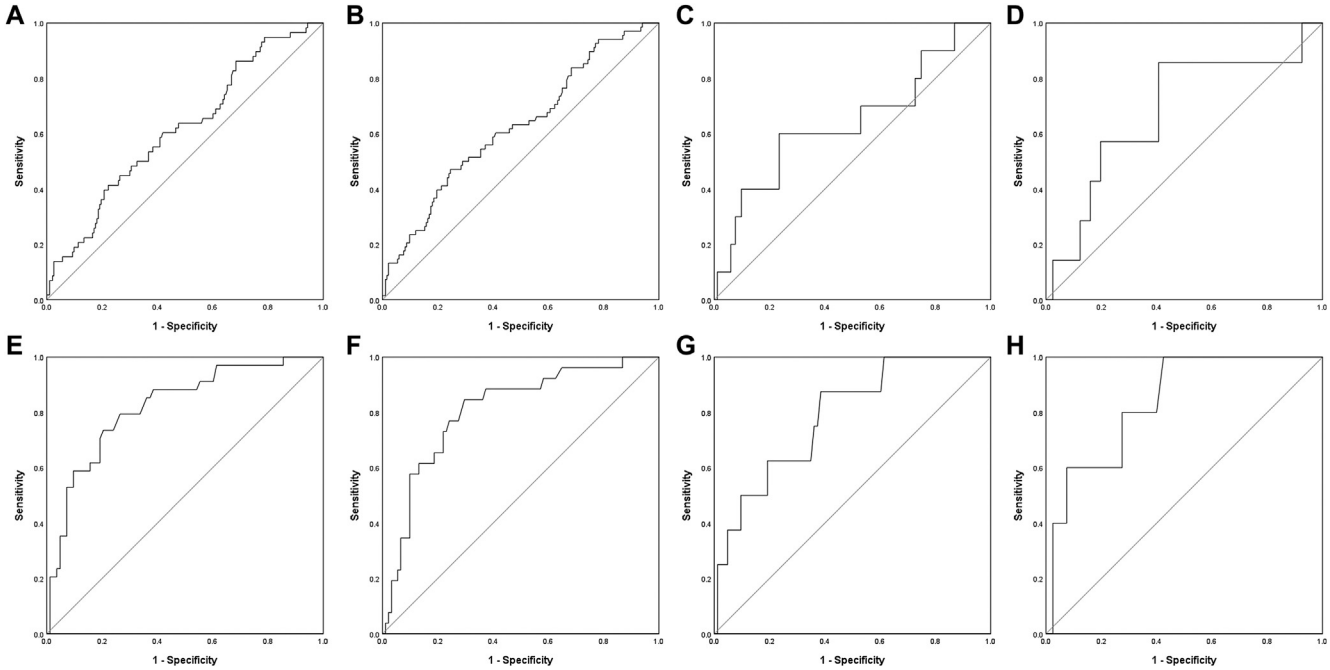

Figure 4. (A–H) ROC analysis of Z A1AT polymer or GGT in predicting CEPH. (A) Indicates that baseline enrollment Z A1AT polymer is better than chance alone at predicting CEPH status at baseline, AUC (95% CI): 0.61 (0.53–0.70), $P = .01$. (B) Indicates that A1AT polymer is better than chance alone at predicting if the patient ever developed CEPH, AUC (95% CI): 0.62 (0.55–0.70), $P = .003$. (C) Indicates that enrollment in Z A1AT polymer is no better than chance alone at predicting if the patient developed CEPH given that they did not have CEPH at enrollment, AUC (95% CI): 0.64 (0.45–0.84), $P = .13$. (D) Indicates that enrollment in Z A1AT polymer is no better than chance alone at predicting if the patient developed CEPH given that they did not have CEPH at enrollment for those <50 months of age at enrollment, AUC (95% CI): 0.68 (0.46–0.90), $P = .12$. (E) Indicates that enrollment in GGT is better than chance alone at predicting CEPH status at enrollment, AUC (95% CI): 0.81 (0.71–0.90), $P < .001$. (F) Indicates that enrollment in GGT is better than chance alone at predicting if the patient ever developed CEPH, AUC (95% CI): 0.82 (0.74–0.91), $P < .001$. (G) Indicates that enrollment in GGT is better than chance alone at predicting if the patient developed CEPH given that they did not have CEPH at enrollment, AUC (95% CI): 0.79 (0.64–0.94), $P = .008$. (H) Indicates that enrollment in GGT is better than chance alone at predicting if the patient developed CEPH given that they did not have CEPH at enrollment for those <50 months of age at enrollment, AUC (95% CI): 0.84 (0.68–1.00), $P = .02$. AUC, area under the curve; CI, confidence interval.

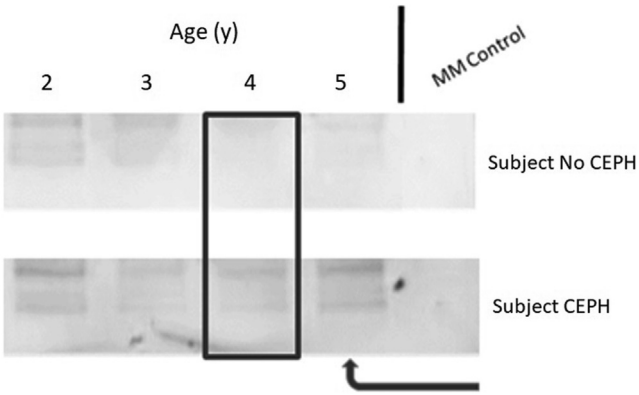

Figure 5. Changes in polymers with the shortest lengths with CEPH. Subject serum from annual visits at ages shown on nondenatured gel and AAT anti-polymer antibody immunoblot, with wild-type AAT MM serum from a 3-year-old that does not contain polymers as a control. When examining the pattern of polymer length, the results of the Western blot showed that bands representing 2, 3, and 4-mer oligomers of Z protein are present in infants and then disappear over a period of several years in childhood. However, in 86% of subjects, these bands reappear, or are never reduced, when CEPH develops.

on to CEPH, liver transplant, or death.[6,13] It is also known that there is a risk of CEPH in childhood, even without preceding neonatal cholestasis, but this also cannot be predicted. These data provide a biomarker and algorithm useful in identifying a group of ZZ children at high risk of severe liver disease. We not only show how the clinically obtained GGT level might be used but also show the potential utility of the circulating polymer measurement. Further study, as more participants are enrolled and followed in this cohort will allow further refinement. The majority of these participants were treated with ursodeoxycholic acid at some point in their care, as part of local standard of care, but not in a controlled way. Currently, we are not able to assess the impact of any specific standard of care intervention on serum polymer.

Our findings are critically important and timely. In recent years, there has been a burst of activity in the development of new therapies for the liver disease of AAT deficiency, including siRNA strategies to knock down expression of the toxic protein and studies of the potential role of small molecules to improve folding, reduce accumulation, and increase secretion.[17] While none are yet approved, many clinical trials are newly opened

and enrolling liver-affected ZZ individuals. At present, these newly enrolling studies are limited to enrolling adults and studying adult outcomes. Older ZZ adults have a greater risk of cirrhosis, perhaps as high as 40% lifetime risk, than children, which may have a 3% to 5% overall cirrhosis risk in childhood, and 15% of those with neonatal cholestasis. These data will allow identification of a group of infants at high risk of severe disease soon after diagnosis. Enrollment of children into trials can then be focused on this high-risk group, sparing many participants from needless trials and speeding the effective treatments to proof of efficacy and approval. Studies are also now underway comparing the liver burden of Z polymer to the serum level and to clinical outcomes. This will be another important question to examine.

While exciting and, for this disease, likely a critical finding, this report leaves a major question unanswered. What is the role of Z protein polymer in the circulation, and what can that teach us about disease pathophysiology or normal physiology? Our previous work has shown that there is secretion of these small quantities of Z polymer from hepatocytes. However, could small amounts of Z polymer also be leaking out of dying hepatocytes nonspecifically, and if so, how is this important[2,9]? It is well documented that Z polymer can be found in the lungs and other tissues of ZZ individuals. Previous studies demonstrate that extracellular polymer has pro-inflammatory properties, such as stimulating neutrophil chemotaxis, and polymers have also been implicated as part of the pathophysiology of lung injury in this disease.[18,19] These data are further limited by the lack of a currently available, Clinical Laboratory Improvement Amendment approved assay for serum polymer. It is currently a test only available for research.

The other major limitation of this work is the fairly low number of "events" (development of CEPH) during the observation period. While we have a large data set of existing CEPH compared to no CEPH, it is the prospective data collection before the CEPH develops that is most useful in developing predictive associations from baseline data. We will continue to gather data, and we intend to repeat this analysis over time, but at current rates, this is likely to take several years. However, with the power of these data, this report will be important in stimulating additional study of these findings.

AAT liver disease has no treatment and no cure at the present time. These newly identified markers associated with a high-risk group of infants will stimulate and accelerate human trials and likely bring nearer the day when there are no more liver transplants required for AAT deficiency.

Supplementary Materials

Material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.gastha.2024.04.010.

References

1. Patel D, McAllister SL, Teckman JH. Alpha-1 antitrypsin deficiency liver disease. Transl Gastroenterol Hepatol 2021;6:23.
2. Strnad P, McElvaney NG, Lomas DA. Alpha(1)-Antitrypsin deficiency. N Engl J Med 2020; 382(15):1443–1455.
3. Lomas DA, Evans DL, Finch JT, et al. The mechanism of Z alpha 1-antitrypsin accumulation in the liver. Nature 1992;357(6379):605–607.
4. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med 1976;294(24):1316–1321.
5. Suri A, Patel D, Teckman JH. Alpha-1 antitrypsin deficiency liver disease. Clin Liver Dis 2022;26(3): 391–402.
6. Teckman J, Rosenthal P, Hawthorne K, et al. Longitudinal outcomes in young patients with alpha-1-antitrypsin deficiency with native liver reveal that neonatal cholestasis is a poor predictor of future portal hypertension. J Pediatr 2020;227:81–86.e4.
7. Joly P, Lachaux A, Ruiz M, et al. SERPINA1 and MAN1B1 polymorphisms are not linked to severe liver disease in a French cohort of alpha-1 antitrypsin deficiency children. Liver Int 2017;37(11):1608–1611.
8. Tan L, Dickens JA, Demeo DL, et al. Circulating polymers in alpha1-antitrypsin deficiency. Eur Respir J 2014; 43(5):1501–1504.
9. Fra A, Cosmi F, Ordoñez A, et al. Polymers of Z alpha1-antitrypsin are secreted in cell models of disease. Eur Respir J 2016;47(3):1005–1009.
10. Clark VC, Marek G, Liu C, et al. Clinical and histologic features of adults with alpha-1 antitrypsin deficiency in a non-cirrhotic cohort. J Hepatol 2018;69(6):1357–1364.
11. Lindblad D, Blomenkamp K, Teckman J. Alpha-1-antitrypsin mutant Z protein content in individual hepatocytes correlates with cell death in a mouse model. Hepatology 2007;46(4):1228–1235.
12. Greene CM, Marciniak SJ, Teckman J, et al. Alpha1-antitrypsin deficiency. Nat Rev Dis Prim 2016;2:16051.
13. Teckman JH, Rosenthal P, Abel R, et al. Baseline analysis of a young alpha-1-antitrypsin deficiency liver disease cohort reveals frequent portal hypertension. J Pediatr Gastroenterol Nutr 2015;61(1):94–101.
14. Qu D, Teckman JH, Omura S, et al. Degradation of a mutant secretory protein, alpha1-antitrypsin Z, in the endoplasmic reticulum requires proteasome activity. J Biol Chem 1996;271(37):22791–22795.
15. Wu Y, Whitman I, Molmenti E, et al. A lag in intracellular degradation of mutant alpha 1-antitrypsin correlates with the liver disease phenotype in homozygous PiZZ alpha 1-antitrypsin deficiency. Proc Natl Acad Sci U S A 1994; 91(19):9014–9018.
16. Teckman JH, Perlmutter DH. The endoplasmic reticulum degradation pathway for mutant secretory proteins alpha1-antitrypsin Z and S is distinct from that for an unassembled membrane protein. J Biol Chem 1996; 271(22):13215–13220.
17. Strnad P, Mandorfer M, Choudhury G, et al. Fazirsiran for liver disease associated with alpha(1)-antitrypsin deficiency. N Engl J Med 2022;387(6):514–524.

18. Jagger AM, Waudby CA, Irving JA, et al. High-resolution ex vivo NMR spectroscopy of human Z alpha(1)-antitrypsin. Nat Commun 2020;11(1):6371.
19. Parmar JS, Mahadeva R, Reed BJ, et al. Polymers of alpha(1)-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. Am J Respir Cell Mol Biol 2002;26(6):723–730.

Received February 16, 2024. Accepted April 19, 2024.

Correspondence:
Address correspondence to: Jeffrey H. Teckman, MD, Department of Pediatrics, Saint Louis University School of Medicine, Cardinal Glennon Children's Hospital, 1465 South Grand Blvd, St. Louis, Missouri 63104. e-mail: jeff.teckman@health.slu.edu.

Authors' Contributions:
Jeffrey H. Teckman designed the query, led the analysis, and was the primary author. Paula Buchanan led statistics. Keith Burling, Nina Heyer-Chauhan, Keith Steven Blomenkamp, and David A. Lomas supervised various aspects of the analysis. All authors edited the final manuscript.

Conflicts of Interest:
These authors disclose the following: Jeffrey H. Teckman has no relationships that directly impact this publication, but he is a consultant and accepts grant funding from the following: NIH, Alpha-1 Foundation, Arrowhead Inc, BioMarin, KorroBio, Beam Therapeutics, Intellia, Takeda Inc, Vertex. David A. Lomas has no relationships that directly impact this publication, but he is an inventor on patent PCT/GB2019/051,761 that describes the development of small molecules to block the polymerization of Z α1-antitrypsin. The intellectual property has been licensed to BioMarin by UCL Business. Keith Steven Blomenkamp has no relationships that directly impact this publication, but he is an independent consultant for the following: AIRNA and BridgeBio. The remaining authors disclose no conflicts.

Funding:
Jeffrey H. Teckman is supported by the Childhood Liver Disease Research Network (ChiLDReN): NIH: DK 62453 and UL1 TR002535 (University of Colorado Denver, Children's Hospital Colorado, subcontracts), Alpha-1 Foundation, Saint Louis University Liver Center, Cardinal Glennon Children's Hospital Foundation, and University of Michigan Sample and Data Coordinating Center; NIH-DK062456 (collection and distribution of biosamples and data). David A. Lomas is supported by the Medical Research Council (UK) (MR/NO24842/1 and MR/V034243/1), the Alpha-1 Foundation (USA), Alpha-1 Awareness, and the NIHR UCLH Biomedical Research Centre. He is an NIHR Senior Investigator.

Ethical Statement:
Participants were consented at each site under local and central IRB as directed by the network.

Data Transparency Statement:
All Childhood Liver Disease Research Network data is available as directed under NIH guidelines. Questions regarding other data outputs and analysis will be supplied by the authors upon request.

Reporting Guidelines:
STROBE.