Journal of Pediatric Gastroenterology and Nutrition, Publish Ahead of Print

DOI : 10.1097/MPG.0000000000003337

Neurodevelopmental Outcomes in Children with Inherited Liver Disease and Native Liver

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ABSTRACT

Objective: To evaluate neurodevelopmental status among children with inherited cholestatic liver diseases with native liver and variables predictive of impairment.

Methods: Participants with Alagille syndrome (ALGS), progressive familial intrahepatic cholestasis (PFIC), and alpha 1 antitrypsin deficiency (A1AT) enrolled in a longitudinal, multicenter study and completed the Wechsler Preschool and Primary Scale of Intelligence-III or Intelligence Scale for Children-IV. Full Scale IQ (FSIQ) was analyzed continuously and categorically (≥100, 85-99, 70-84, <70). Univariate linear regression was performed to study association between FSIQ and risk factors, stratified by disease.

Results: 215 completed testing (ALGS n=70, PFIC n=43, A1AT n=102); median age was 7.6 years (3.0-16.9). Mean FSIQ in ALGS was lower than A1AT (94 vs. 101, p=0.01). Frequency of FSIQ<85 (>1 SD below average) was highest in ALGS (29%) versus 18.6% in

PFIC and 12.8% in A1AT, and was greater than expected in ALGS based on normal distribution (29% vs. 15.9%, p=0.003). ALGS scored significantly lower than test norms in almost all Wechsler composites; A1AT scored lower on Working Memory and Processing Speed; PFIC was not different from test norms. Total bilirubin, alkaline phosphatase, albumin, hemoglobin, and parental education were significantly associated with FSIQ.

Conclusions: Patients with ALGS are at increased risk of lower FSIQ, whereas our data suggest A1AT and PFIC are not. A1AT and ALGS appear vulnerable to working memory and processing speed deficits suggestive of attention/executive function impairment. Malnutrition, liver disease severity, and sociodemographic factors appear related to FSIQ deficits, potentially identifying targets for early interventions.

An infographic is available for this article at: http://links.lww.com/MPG/C554

Keywords: Alagille syndrome (ALGS); progressive familial intrahepatic cholestasis (PFIC); alpha-1 antitrypsin deficiency (A1AT); neurocognitive; intelligence quotient (IQ)

WHAT IS KNOWN

- Alagille syndrome, progressive familial intrahepatic cholestasis, and alpha-1 antitrypsin deficiency represent the most common inherited cholestatic liver disorders in children. Whether specific inherited liver disorders are associated with more neurodevelopmental impairment than others is unknown.

WHAT IS NEW

- In a multi-center observational study, participants with Alagille syndrome were at increased risk of lower Full Scale IQ (FSIQ); malnutrition and liver disease severity were significantly associated with FSIQ and represent potential targets for early intervention.

Trial ID #: Clinicaltrials.gov: NCT00571272

Sources of Funding: This work was supported by funding from the Alpha-1 Foundation (University of Colorado Denver and Saint Louis University School of Medicine) and by U01 grants from the National Institute of Diabetes, Digestive and Kidney Diseases and UL1 grants from the National Center for Advancing Translational Sciences (NCATS): DK062445 [Mt. Sinai School of Medicine], DK062497 and UL1 TR000077 [Cincinnati Children's Hospital Medical Center, University of Cincinnati], DK103149 [Texas Children's Hospital], DK062470 [Children's Healthcare of Atlanta, Emory University], DK062481 and UL1 TR000003 [The Children's Hospital of Philadelphia, University of Pennsylvania], DK062456 [University of Michigan], DK084536 and UL1 TR000006 [Riley Hospital for Children, Indiana University], DK084575 and UL1 TR000423 [Seattle Children's Hospital, University of Washington], DK062500 and UL1 TR000004 [UCSF Children's Hospital, University of California San Francisco], DK062503 and UL1 TR000424 [Johns Hopkins School of

Medicine], DK062466 and UL1 TR000005 [Children's Hospital of Pittsburgh, University of Pittsburgh], DK062453 and UL1 TR002535 [University of Colorado Denver, Children's Hospital Colorado], DK062452 and UL1 TR000448 [Washington University School of Medicine, St. Louis, St. Louis Children's Hospital], DK084538 and UL1 TR000130 [Children's Hospital Los Angeles, University of Southern California], DK062436 and UL1 TR000150 [Ann and Robert H. Lurie Children's Hospital of Chicago, Northwestern University], DK103140 [University of Utah], DK103135 [Hospital for Sick Children (Toronto)].

Conflicts of Interest: ***DHL*** reports grant/research support from Abbvie, Gilead, and CF Foundation; he also serves on advisory panels for Merck and Gilead. ***BMK*** is a consultant for Mirum, Albireo and Audentes; she has unrestricted educational grants from Mirum and Albireo. ***VLN*** is a consultant for Albireo. ***PR*** reports grants from Gilead, Merck, BMS, AbbVie, Travere Therapeutics, Albireo, and Mirum, Arrowhead; and serving as a consultant for Gilead, Mirum, Albireo, Audentes, and Vertex. ***RJS*** reports consulting for Albireo, Mirum/Shire, and Retrophin (all <$10,000 per year). ***KML*** reports consulting for Albireo, Mirum and Retrophin (now known as Travere Therapeutics). The remaining authors declare no conflicts of interest. The study sponsors did not have a role in study design; collection, analysis, and interpretation of data; writing of the report; or the decision to submit the paper for publication.

Authorship: Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work = DHL, LGS, WY, KH, BMK, KML, EMA, RJS, JCM

Drafting the work or revising it critically for important intellectual content = DHL, LGS, WY, KH, BMK, VLN, KML, EMF, EMA, JEH, SPH, SJK, JPM, PR, RJS, RHS, KSW, JCM

Final approval of the version to be published = DHL, LGS, WY, KH, BMK, VLN, KML, EMF, EMA, JEH, SPH, SJK, JPM, PR, RJS, RHS, KSW, JCM

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved = DHL, LGS, WY, KH, BMK, VLN, KML, EMF, EMA, JEH, SPH, SJK, JPM, PR, RJS, RHS, KSW, JCM

INTRODUCTION

Children with inherited pediatric liver diseases, such as Alagille syndrome (ALGS), progressive familial intrahepatic cholestasis (PFIC), and alpha-1 antitrypsin deficiency (A1AT), are unique and often present with cholestasis or significant fibrosis at an early age.[1-3] ALGS is an autosomal dominant syndrome with cholestasis and extrahepatic involvement, including cardiac, vascular, and renal abnormalities.[4] PFIC is a family of autosomal recessive monogenic liver diseases with altered phospholipid or bile salt transport.[5] A1AT is inherited in an autosomal co-dominant fashion with misfolding and defective secretion of A1AT and can result in cirrhosis and lung disease.[6] These represent the most common inherited cholestatic liver disorders in children, but each has a different etiology and clinical presentation. Whether specific inherited liver disorders have more neurodevelopmental impairment than others is unknown. Neurodevelopmental outcomes in this distinctive cohort of inherited pediatric liver diseases have been limited to small, post-transplant or single-center studies.[7-9] Early predictors of abnormal neurodevelopment in these liver disorders could be used to identify high-risk individuals, potentially leading to interventions and improvement in long-term neurocognitive outcomes. Within the National Institute of Diabetes and Digestive and Kidney Diseases/National Institutes of Health (NIDDK/NIH)-supported Childhood Liver Disease Research Network (ChiLDReN), intelligence testing was completed in a cohort of children with these disorders surviving with their native livers. We hypothesized that children with ALGS, A1AT, and PFIC would demonstrate lower mean intelligence quotient (IQ) relative to test norms, and that specific demographic and clinical features would predict increased risk. Further, we hypothesized that patients with ALGS may have greater neurodevelopmental deficits due to extrahepatic involvement.

METHODS

Study Population

The Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis (LOGIC; Clinicaltrials.gov:NCT00571272) is a prospective study enrolling participants with ALGS, A1AT, or PFIC at 14 clinical sites from birth to age 25 years with annual follow-up. Detailed inclusion and exclusion criteria for LOGIC are listed in the Appendix, http://links.lww.com/MPG/C555. The Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III)[10] and Wechsler Intelligence Scale for Children-IV (WISC-IV)[11] were administered once to English-speaking participants ages 3-5 years and 6-16 years respectively, following enrollment. Informed consent was obtained from parents or guardians at all sites; the protocol was implemented under institutional review board approval.

Intelligence Testing – Measures of IQ

Composite scores for WPPSI-III and WISC-IV include Full Scale IQ (FSIQ), Verbal IQ (VIQ)/Verbal Comprehension Index (VCI), and Performance IQ (PIQ)/Perceptual Reasoning Index (PRI). Processing Speed Index (PSI) and Working Memory Index (WMI) were only available in participants >6 years old (WISC-IV only).[10,11] Although newer versions of both measures became available during study enrollment (WPPSI-IV in 2012; WISC-V in 2014),

we continued using prior versions to preserve data integrity and avoid challenges in interpreting combined data.

Risk Factors

Potential predictors of neurodevelopmental outcome included disease type, demographics, medical history, current pruritus status, history of xanthomas, renal involvement, cardiac disease severity, laboratory results, aspartate aminotransferase (AST) to platelet ratio index (APRI),[12] Fibrosis-4 (FIB-4),[13] and growth parameters[14] obtained ±6 months of intelligence testing. Race and ethnicity were parent-reported. Clinically-evident portal hypertension (CEPH), as defined by ChiLDReN,[15] was analyzed in subgroups of definite (dCEPH), possible (pCEPH), and absent CEPH (aCEPH). Minimal hepatic encephalopathy (MHE) was not captured in the LOGIC study.

Renal involvement and severe cardiac disease were defined *a priori* (Supplemental Digital Content [SDC] Figure 1, http://links.lww.com/MPG/C556). As an exploratory analysis, we examined the effect of *JAG1*, *ATP8B1*, *ABCB11, and ABCB4* genetic mutation class and PI ZZ or PI SZ serum protein phenotype on IQ score in ALGS, PFIC, and A1AT patients, respectively.

Statistical Methods

Descriptive statistics of patient characteristics were calculated for the total study population and each disease type separately. Categorical and continuous variables were compared across disease groups using chi-square tests and Wilcoxon or Kruskal-Wallis tests. Pairwise comparisons between disease groups were conducted when an overall significant difference was found. Composite IQ scores are standardized, with a mean of 100 and standard deviation (SD) of 15. We compared distribution of IQ scores by disease group to normal distribution using Kolmogorov-Smirnov tests. IQ scores were categorized into bins by z-score: FSIQ $\geq$100 ($z \geq 0$), 85-99 ($-1 \leq z < 0$), 70-84 ($-2 \leq z < -1$), and <70 ($z < -2$) and compared with expected percentages within each bin in the normal distribution using chi-square tests.[10,11] Linear regression was performed to study the association between continuous FSIQ and each risk factor, adjusting for parental education, stratified by disease group. Skewed laboratory variables were modeled on the log base 2 scale to improve model fit. Both original p-values and adjusted p-values using the adaptive Hochberg correction for family-wise error rate are reported.[16]

RESULTS

Study Population

Between November 2007 and April 2018, 647 of 809 LOGIC participants with native liver were eligible for IQ testing. Two-hundred-fifteen children (33.2% of eligible patients) completed testing (ALGS n=70, PFIC n=43, A1AT n=102) at a mean age of 8.2 years (SD 3.8) (Table 1). Reasons for exclusion are detailed in SDC Figure 2, http://links.lww.com/MPG/C556. WPPSI-III and WISC-IV testing was completed in 78 and 137 participants, respectively. Compared with eligible but not tested LOGIC participants (SDC Table 1, http://links.lww.com/MPG/C556), A1AT participants who completed testing

were more likely to be male; PFIC participants were older (both $p<0.05$). Detailed reasons for non-participation by eligible patients were not available.

Demographic and Clinical Variables

Disease groups were similar in age at testing and frequency of IQ test version administered but differed demographically (Table 1). A1AT participants were more white (92.2% vs. 74.4% PFIC and 77.6% ALGS, $p<0.05$), less Hispanic (5.0% vs. 17.1% ALGS, $p<0.05$), and had higher parental education (88.2% with some college or more vs. 69.2% PFIC, $p<0.05$).

Patients with ALGS had significantly lower mean z-scores for height (-1.51 [SD 1.18]), weight (-1.34 [SD 1.25]), and BMI (-0.55 [SD 1.29]) than PFIC and A1AT (all pairwise $p<0.05$). Median APRI was 1.39 in ALGS, significantly higher than PFIC (0.52; $p<0.05$) and A1AT (0.64; $p<0.05$). Median FIB-4 in ALGS was 0.41, significantly higher than PFIC (0.22; $p<0.05$) and A1AT (0.24; $p<0.05$). Total bilirubin, gamma glutamyl transferase (GGT), aspartate (AST), and alanine aminotransferases (ALT) were significantly higher in patients with ALGS versus PFIC and A1AT; alkaline phosphatase (ALP) was the lowest in A1AT versus PFIC and ALGS (Table 1). Among PFIC participants, 41% (n=17/41 with data) had undergone partial external biliary diversion surgery prior to IQ testing. Platelet count was not significantly different among groups. Active or bleeding pruritus was prevalent in ALGS (38.2%) and PFIC (25.6%), but almost completely absent in A1AT (1.0%). Except for 1 PFIC participant, only ALGS participants had xanthomas. Differences among groups in international normalized ratio (INR), albumin, hemoglobin, and creatinine were statistically but not clinically significant; mean values were within normal range.

IQ Scores

The ALGS group scored significantly lower than test norms on all Wechsler composite scores (all p-values ≤ 0.02) except PIQ/PRI (Table 2). The A1AT group scored lower than test norms on WMI and PSI, but higher on VIQ/VCI and PIQ/PRI ($p \leq 0.03$). The PFIC group mean composite scores did not differ from population norms. The ALGS group had lower mean FSIQ, VIQ/VCI, and PIQ/PRI compared with A1AT, ($p<0.05$). These differences persisted after adjustment for parental education using linear regression models. PFIC and A1AT groups did not differ significantly on any composite scores.

Distribution of scores was significantly shifted downward from expected for FSIQ and WMI in ALGS ($p<0.01$), and for WMI and PSI in A1AT ($p<0.05$, Figure 1, SDC Table 2, http://links.lww.com/MPG/C556). In contrast, distribution of VIQ/VCI and PIQ/PRI in A1AT ($p<0.01$) was shifted higher than expected, with a larger proportion of patients scoring at or above average than population norms would suggest. The only observed between-group differences were in VIQ/VCI and PIQ/PRI (SDC Table 2, http://links.lww.com/MPG/C556); ALGS score distribution had a larger proportion in the below-average categories and smaller proportions in the average and above categories compared with A1AT ($p<0.05$).

Ten out of the 214 (4.7%) in our overall study population had FSIQ score <70 (at risk of Intellectual Disability) (vs. 2.3% in the population norms), with 8.7% in ALGS, 0% in PFIC, and 3.9% in A1AT.

Risk Factor Analysis

Figure 2 shows risk factors significantly associated (prior to adjustment for multiple comparisons) with FSIQ in at least one disease group identified using univariate analysis adjusting for parental education (SDC Table 3, http://links.lww.com/MPG/C556). Having a parent with some college or more is associated with 11.9 and 16.6 points higher FSIQ compared with less education in A1AT and PFIC ($p<0.05$), respectively. In PFIC, a doubling of total bilirubin and a 1 g/dl decrease in either albumin or hemoglobin were significantly associated with 3.2 (95% CI: 0.5, 5.9), 12.7 (95% CI: 4.5, 20.9), and 3.7 (95% CI: 0.3, 7.2) lower FSIQ, respectively. In ALGS, a doubling of alkaline phosphatase was significantly associated with 6.8 points lower FSIQ (95% CI: 0.3, 13.4), and a 1 g/dl decrease in albumin was significantly associated with 10.9 points lower FSIQ (95% CI: 1.8, 20.1). In A1AT participants, a one-unit increase in BMI-for-age z-score was significantly associated with 3.1 points lower FSIQ (95% CI: 0, 6.1). The only variable that remained significant after adjustment for multiple testing was parental education in the A1AT group (SDC Table 3, http://links.lww.com/MPG/C556). Variables studied that were not significantly associated with FSIQ included CEPH status, pruritus, and age at testing.

In an exploratory sub-analysis among ALGS, cardiac disease severity (n=16 severe vs. n=53 mild or none) was not significantly associated with FSIQ (SDC Figure 1, http://links.lww.com/MPG/C556). Renal involvement was reported in 21.4% of ALGS patients (SDC Table 4, http://links.lww.com/MPG/C556) and was not significantly associated with FSIQ.

The majority of ALGS patients (94%) had *JAG1* mutation testing performed. Among the 60 confirmed *JAG1* genotypes (SDC Table 4, http://links.lww.com/MPG/C556), splicing mutations were most common (n=27, 45%) followed by protein truncation (n=16), missense (n=13), and total gene deletion (n=4). The mean FSIQ of three participants with total gene deletion and an FSIQ score available was the lowest among all JAG1 mutation types (SDC Figure 3a, http://links.lww.com/MPG/C556). Mean FSIQ did not differ among the three PFIC genotypes (*ATP8B1*, *ABCB11*, and *ABCB4*) and variant class types. All A1AT patients with FSIQ<85 (12.8%) had ZZ phenotype (SDC Figure 3b, http://links.lww.com/MPG/C556), except for one with SZ genotype.

DISCUSSION

In this prospective multi-center study, IQ deficits in children ages 3-16 years with inherited cholestatic liver disorders and native liver were greatest among participants with ALGS. Participants with ALGS scored significantly lower than test norms on the WPPSI-III/WISC-IV in FSIQ, VIQ/VCI, WMI, and PSI, but not PIQ/PRI. In contrast, PFIC and A1AT showed fewer IQ deficits. PFIC composite scores were similar to population norms; A1AT scores were lower than test norms only for WMI and PSI. Importantly, among ALGS participants, the frequency of FSIQ<85 was nearly double the expected and the frequency of FSIQ<70 (at risk for Intellectual Disability) was greater than twice expected. In contrast, distribution of FSIQ scores among the A1AT and PFIC groups was no different than population norms. FSIQ, VIQ/VCI, and PIQ/PRI were the lowest among children with ALGS and significantly lower when compared with A1AT.

In addition, we identified socioeconomic and medical risk factors for lower FSIQ among these cholestatic diseases, including lower parental education and measures of nutrition, although specific predictors varied by disease. Surrogates of liver disease progression, namely APRI, FIB-4, and portal hypertension status, were different in ALGS but were not significant predictors of FSIQ. The association of lower albumin with lower FSIQ in ALGS and PFIC may reflect differences in degree of cirrhosis or portal hypertension and nutritional status, as both had negative weight and height z-scores, consistent with suboptimal growth. Similarly, in a biliary atresia (BA) study, low length z-scores at time of testing, a marker of growth stunting, was an independent risk factor for combined BSID-II/Bayley-III variables of physical/motor impairment and low weight z-score for mental/cognitive/language impairment at age 1 year,[17] highlighting nutritional supplementation as a potential intervention.

Infants and toddlers with cholestatic liver disease are at high risk for neurodevelopmental deficits.[9,18] This was confirmed in a recent multi-center study showing increased risk for delays at ages 12 and 24 months in children with BA with native livers, despite successful hepatoportoenterostomy (HPE).[17] Furthermore, those with unsuccessful HPE (persistent cholestasis or more severe disease) were >4 times more likely to have neurodevelopmental impairment compared with those with successful HPE.[17] In contrast to BA, however, neither GGT nor total bilirubin was a predictor of poor neurodevelopmental outcome in ALGS (or any subgroup) despite being 4-fold and 10-fold higher respectively, than the A1AT group. However, ALGS participants had the highest mean APRI, FIB-4, AST, and ALT, all markers of progressive liver disease. In a parallel study to this one, health-related quality of life was impaired in children with ALGS compared with healthy children and A1AT-affected children and positively correlated with better growth and inversely with total bilirubin.[19] The association of higher alkaline phosphatase levels with lower FSIQ in ALGS is noteworthy. It is possible that elevations in alkaline phosphatase may be a surrogate marker of more severe cholestasis. High alkaline phosphatase can also be related to rapid bone growth or bone disorders (e.g., osteopenia or hepatic osteodystrophy). Bone mineral deficits are prevalent in children with ALGS, correlating in severity with degree of cholestasis and fracture history.[20] There is also evidence for intrinsic trabecular and cortical bone abnormalities in ALGS children,[21] further supporting a role for *JAG1* and the Notch pathway in skeletal bone development.[22]

Wechsler composite score profiles provide more detailed neurodevelopmental outcomes. The ALGS group had FSIQ, VIQ/VCI, and PIQ/PRI scores consistently 6-7.5 points lower than A1AT, with a significantly higher proportion of below average scores than test norms and A1AT. Higher VIQ/VCI and PIQ/PRI scores in A1AT likely reflect the specific demographic characteristics (>88% some college or more) and cannot be generalized to the A1AT population at-large. Nevertheless, there appear to be disease-specific neurodevelopmental profiles. WMI and PSI (low in ALGS and A1AT) are more vulnerable to the influence of attention, ability to engage, illness, fatigue, and central nervous system (CNS) injury.[23-25] Processing speed and working memory, defined as "the ability to actively maintain information in conscious awareness, perform some operation or manipulation with it, and produce a result", are critical components in fluid reasoning.[11] These findings suggest that ALGS patients may be more globally impacted by their disease, whereas A1AT patients may

have specific weaknesses in attention and executive functioning. Patients with ALGS have additional comorbidities, including physical disabilities[26] and severe pruritus, which may disrupt sleep, school attendance, mood, and concentration.[27]

Patients with ALGS may be at greater risk for neurodevelopmental deficits due to a combination of factors shared with other cholestatic liver diseases (stunted growth/nutritional deficits, severity of liver disease and associated complications) in addition to extra-hepatic factors such as intracranial vascular anomalies. While our sub-analyses did not demonstrate a significant association between cardiac disease severity or *JAG1* mutation class with neurodevelopmental delay, median FSIQ among ALGS were lower in those with gene deletions and more severe cardiac lesions. Deletion of *JAG1* located in 20p12.2 is known to occur in ALGS and is associated with developmental delay, speech delay, intellectual disability, or poor coordination.[28,29] It is also well-known that children with congenital heart disease demonstrate challenges in IQ, language, motor skills, attention, and executive function.[30-33]

There were no significant differences in mean FSIQ among PFIC genotypes. Interestingly, while mean FSIQ among A1AT ZZ participants were similar to SZ, all but one participant with FSIQ≤85 had the ZZ variant. The small sample size of these comparison groups should be noted; thus, our findings are not conclusive, but may be potentially clinically relevant.

There were several potential limitations. Completion of IQ testing was lower than planned for unknown reasons; time required for testing, scheduling challenges, and illness severity likely contributed. Our analysis is limited by small disease subgroups, which differed demographically and clinically. Our tested sample under-represents females (35%) compared with the eligible but untested group (49%). However, sex was not a significant univariate predictor of low FSIQ. High socioeconomic status (SES) across disease groups, reflected in parent education (69%-88% with some college/trade school or more) is notable compared with the normative sample (approximately 55%-60% for WPPSI-III and WISC-IV).[10,11] Additionally, because only patients with native liver were included, the study cohort may have skewed towards healthier participants, which suggests that our findings likely under-estimate concerns in this population. MHE status was not collected within the parent LOGIC study; hence, no correlation could be made with FSIQ. Although parental education was the only variable that remained significant after adjustment in the A1AT group, this should be interpreted with caution. Because the adaptive method assumes independence between all p-values, it very likely leads to larger type 2 error, given that many of our risk factors were correlated. IQ testing represents only one instrument to assess neurocognitive ability, but is ubiquitously accepted, robust and reliable, provides some level of detail with composite scores, and represents a first step in characterizing these diseases on a large scale that has not been adequately captured previously. The WPPSI-III/WISC-IV tests were one version behind, which could have artificially inflated scores to some extent via the Flynn effect.[34] Note that the WPPSI-III and WISC-IV are not equivalent measures, although they are correlated almost at the same level as WISC-IV test-retest (r=0.89 vs r=0.93).[11]

In conclusion, this is the first and largest prospective multi-center analysis of neurodevelopmental status in children with inherited cholestatic liver disorders who have not

undergone liver transplantation. Our data show that children with ALGS are at increased risk of lower FSIQ but A1AT and PFIC are not. Both A1AT and ALGS appear to be more vulnerable to WMI and PSI deficits suggestive of attention and executive function impairment. Malnutrition, liver disease severity, and sociodemographic factors appear related to these FSIQ deficits. Lower albumin and higher alkaline phosphatase are associated with lower FSIQ in ALGS, potentially identifying nutrition as an important and early intervention. Early screening for and identification of neurodevelopmental deficits in children with ALGS should prompt therapies that can maximize developmental outcomes. Further, attention and executive functioning should be closely monitored across disease groups since these domains can significantly impact learning and everyday functioning.

ACKNOWLEDGEMENTS

Heather Van Doren, Senior Medical Editor with Arbor Research Collaborative for Health, provided editorial assistance on this manuscript. The authors thank the children and their families whose participation made this study possible.

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FIGURE LEGENDS

Figure 1. Distribution of IQ Scores by Disease Group. Distribution of Full Scale and subdomain IQ scores are binned by z-score and compared to expected percentages from the normal distribution using chi-square tests.

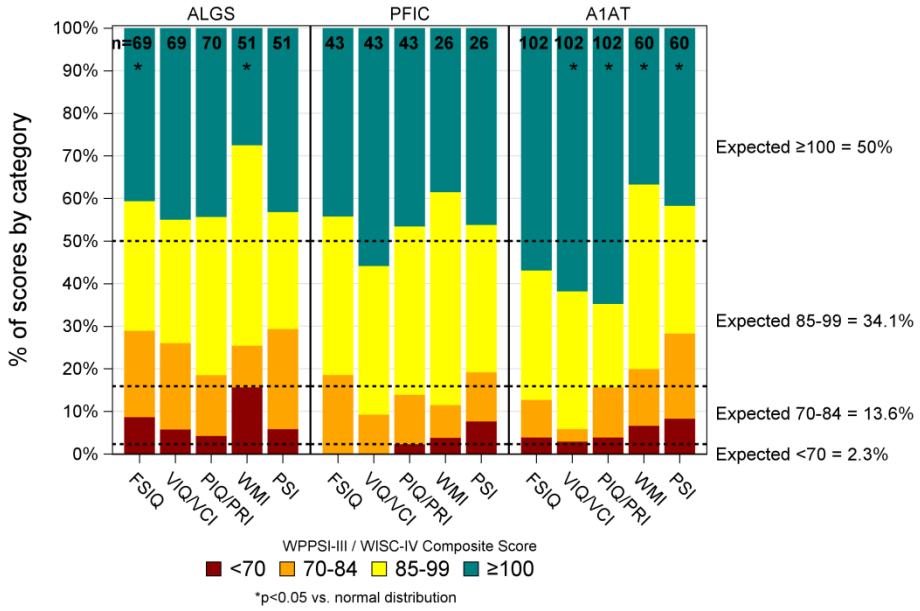

Figure 2. Univariate Predictors of Continuous FSIQ. Linear regression adjusted for parental education was performed, stratified by disease. Variables associated with FSIQ at p<0.05 (unadjusted) in at least one disease group are shown. P-values adjusted for multiple comparisons are also included for reference.

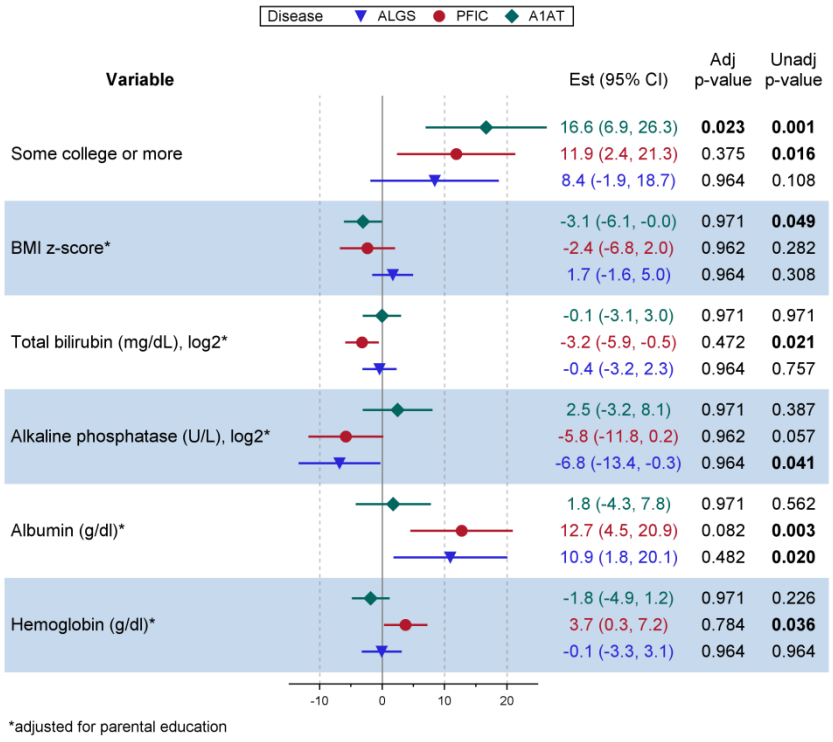

Table 1. Demographics and Clinical Characteristics of Children with Inherited Cholestatic Liver Disease

Variable		*ALGS (N=70)*		*PFIC (N=43)*		*A1AT (N=102)*		*Total (N=215)*		*Overall p-value*
Demographics and clinical variables		*n*	*freq (%) or mean (SD)*	*n*	*freq (%) or mean (SD)*	*n*	*freq (%) or mean (SD)*	*n*	*freq (%) or mean (SD)*	
Age at testing (years)		70	8.7 (3.7)	43	8.5 (4.3)	102	7.7 (3.5)	215	8.2 (3.8)	0.207
Female		70	25 (35.7%)	43	22 (51.2%)[c]	102	29 (28.4%)[c]	215	76 (35.3%)	**0.033**
Race	White	67	52 (77.6%)[b]	43	32 (74.4%)[c]	102	94 (92.2%)[b,c]	212	178 (84.0%)	**0.008**
	Black		6 (9.0%)		5 (11.6%)		0 (0.0%)		11 (5.2%)	
	Other*		9 (13.4%)		6 (14.0%)		8 (7.8%)		23 (10.8%)	
Hispanic		70	12 (17.1%)[b]	42	5 (11.9%)	100	5 (5.0%)[b]	212	22 (10.4%)	**0.036**
Parental education	High school equivalent or less	66	14 (21.2%)	39	12 (30.8%)[c]	93	11 (11.8%)[c]	198	37 (18.7%)	**0.032**
	Some college/trade school or more		52 (78.8%)		27 (69.2%)		82 (88.2%)		161 (81.3%)	
Test type	WPPSI-III (age 3 to 5)	70	19 (27.1%)	43	17 (39.5%)	102	42 (41.2%)	215	78 (36.3%)	0.151
	WISC-IV (age 6 to 16)		51 (72.9%)		26 (60.5%)		60 (58.8%)		137 (63.7%)	
Height-for-age z-score		68	-1.51 (1.18)[a,b]	43	-0.90 (1.24)[a,c]	97	0.45 (1.03)[b,c]	208	-0.47 (1.43)	**<.001**
Weight-for-age z-score		66	-1.34 (1.25)[a,b]	43	-0.47 (1.14)[a,c]	99	0.71 (1.05)[b,c]	208	-0.18 (1.45)	**<.001**
BMI z-score		66	-0.55 (1.29)[a,b]	43	0.23 (1.03)[a,c]	96	0.70 (1.04)[b,c]	205	0.20 (1.25)	**<.001**
Pruritus	None/mild	68	42 (61.8%)[b]	43	32 (74.4%)[c]	99	98 (99.0%)[b,c]	210	172 (81.9%)	**<.001**
	Active/bleeding		26 (38.2%)		11 (25.6%)		1 (1.0%)		38 (18.1%)	
History of xanthoma(s)		69	18 (26.1%)[a,b]	43	1 (2.3%)[a]	102	0 (0.0%)[b]	214	19 (8.9%)	**<.001**
CEPH	aCEPH	70	46 (65.7%)[b]	43	34 (79.1%)	102	79 (77.5%)[b]	215	159 (74.0%)	0.100
	pCEPH		14 (20.0%)		5 (11.6%)		7 (6.9%)		26 (12.1%)	
	dCEPH**[1]		10 (14.3%)		4 (9.3%)		16 (15.7%)		30 (14.0%)	
Laboratory variables		*n*	*freq (%) or median (IQR)*	*n*	*freq (%) or median (IQR)*	*n*	*freq (%) or median (IQR)*	*n*	*freq (%) or median (IQR)*	
Total bilirubin (mg/dl)		63	1.6 (0.7, 3.4)[a,b]	43	0.7 (0.4, 1.9)[a,c]	95	0.4 (0.3, 0.7)[b,c]	201	0.6 (0.4, 1.6)	**<.001**
GGTP (U/L)		49	353 (233, 869)[a,b]	35	19 (12, 48)[a,c]	80	31 (19, 91)[b,c]	164	55 (19, 275)	**<.001**
AST (U/L)		64	153 (102, 192)[a,b]	43	60 (36, 113)[a]	97	62 (42, 93)[b]	204	77 (48, 147)	**<.001**
ALT (U/L)		63	179 (119, 242)[a,b]	43	50 (32, 106)[a]	96	68 (43, 110)[b]	202	90 (46, 179)	**<.001**
Alkaline phosphatase (U/L)		61	506 (369, 653)[b]	42	474 (331, 553)[c]	93	255 (211, 337)[b,c]	196	347 (236, 513)	**<.001**
Platelet count (x 10^3/mm^3)		58	244 (171, 338)	36	305 (206, 377)	88	263 (201, 324)	182	266 (189, 333)	0.333
INR		55	1.0 (0.9, 1.1)[b]	33	1.0 (1.0, 1.1)	64	1.1 (1.0, 1.1)[b]	152	1.0 (1.0, 1.1)	**0.016**

Variable		*ALGS (N=70)*		*PFIC (N=43)*		*A1AT (N=102)*		*Total (N=215)*		*Overall p-value*
Albumin (g/dl)		61	4.3 (4.0, 4.5)[b]	42	4.2 (3.9, 4.5)[c]	95	4.4 (4.1, 4.7)[b,c]	198	4.3 (4.0, 4.6)	**0.012**
BUN (mg/dl)		45	16 (14, 19)[a,b]	31	12 (9, 14)[a]	56	12 (11, 15)[b]	132	13 (11, 16)	**<.001**
Creatinine (mg/dl)		49	0.44 (0.37, 0.50)[a]	32	0.35 (0.30, 0.50)[a,c]	60	0.40 (0.33, 0.54)[c]	141	0.40 (0.30, 0.50)	**0.042**
Hemoglobin (g/dl)		57	12.9 (12.0, 13.5)[b]	36	12.6 (11.8, 13.5)[c]	89	13.5 (13.0, 14.0)[b,c]	182	13.1 (12.3, 13.9)	**<.001**
APRI		58	1.39 (0.98, 2.96)[a,b]	36	0.52 (0.33, 0.96)[a]	87	0.64 (0.37, 1.32)[b]	181	0.83 (0.41, 1.83)	**<.001**
	<1		15 (25.9%)[a,b]		28 (77.8%)[a]		63 (72.4%)[b]		106 (58.6%)	
	1-1.5		16 (27.6%)		1 (2.8%)		4 (4.6%)		21 (11.6%)	
	>1.5		27 (46.6%)		7 (19.4%)		20 (23.0%)		54 (29.8%)	
FIB-4		57	0.41 (0.21, 0.89)[a,b]	36	0.22 (0.14, 0.38)[a]	86	0.24 (0.13, 0.36)[b]	179	0.26 (0.14, 0.53)	**0.003**

Abbreviations: A1AT, alpha one antitrypsin deficiency; aCEPH, absent clinically-evident portal hypertension; ALGS, Alagille Syndrome; ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; CEPH, clinically-evident portal hypertension; dCEPH, definite clinically-evident portal hypertension; FIB-4, Fibrosis-4; GGTP, gamma glutamyl transpeptidase; INR, international normalized ratio; pCEPH, possible clinically-evident portal hypertension; PFIC, progressive familial intrahepatic cholestasis.

*Other race includes Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Other, Multiracial, or Unknown.

**Renal involvement was defined as dysplastic kidney, single kidney, renal tubular acidosis, or other renal abnormality reported on initial history.

[1]Bass LM, Shneider BL, Henn L, et al. Clinically evident portal hypertension: An operational research definition for future investigations in the pediatric population. *J Pediatr Gastroenterol Nutr* 2019;68:763-767.

[a]ALGS vs. PFIC, $p<0.05$

[b]ALGS vs. A1AT, $p<0.05$

[c]PFIC vs. A1AT, $p<0.05$

Overall p-value refers to a simultaneous comparison of all three disease groups. $P<0.05$ indicates a significant difference in at least one group compared with the others.

Table 2. WPPSI-III/WISC-IV IQ Scores by Disease Group

		ALGS (N=70)		*PFIC (N=43)*		*A1AT (N=102)*		
WPPSI-III / WISC-IV Composite Score		*n*	*Mean (SD)*	*n*	*Mean (SD)*	*n*	*Mean (SD)*	*Overall p-value*
Full Scale IQ (FSIQ)		69	94.2 (16.7)*	43	98.9 (14.4)	102	100.8 (16.3)*	**0.030**
	p-value vs. norms		0.020		>0.250		>0.250	
Verbal IQ / Verbal Comprehension Index (VIQ/VCI)		69	95.0 (16.1)*	43	100.4 (11.3)	102	102.5 (15.2)*	**0.016**
	p-value vs. norms		0.008		>0.250		0.027	
Performance IQ / Perceptual Reasoning Index (PIQ/PRI)		70	96.9 (16.1)*	43	100.7 (16.3)	102	102.9 (17.3)*	**0.033**
	p-value vs. norms		>0.250		>0.250		0.015	
Working Memory Index (WMI)**		51	92.6 (18.2)	26	97.3 (13.7)	60	94.3 (14.0)	0.601
	p-value vs. norms		0.003		>0.250		0.021	
Processing Speed Index (PSI)**		51	93.1 (15.2)	26	96.2 (14.5)	60	91.8 (16.6)	0.527
	p-value vs. norms		0.017		>0.250		0.002	

Abbreviations: A1AT, alpha one antitrypsin deficiency; ALGS, Alagille Syndrome; IQ, intelligence quotient; PFIC, progressive familial intrahepatic cholestasis; WISC-IV, Wechsler Intelligence Scale for Children-IV; WPPSI-III, Wechsler Preschool and Primary Scale of Intelligence-III.

*ALGS vs. A1AT, $p<0.05$

No significant differences found between ALGS vs. PFIC or PFIC vs. A1AT ($p>0.05$ for all).

**WMI and PSI are only available for WISC-IV.