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Long-term effects of cholestatic liver disease in childhood on neuropsychological outcomes and neurochemistry.

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Disclosures

JT: Participated in research design, collection of primary data, data analysis and manuscript preparation. SB: Participated in research design, submitted proposal for ethical approval; collection of primary data, data analysis and manuscript preparation. GG: Participated in research design and manuscript preparation. TP: Participated in research design, collected primary data and carried out data analysis and manuscript preparation. DK: Participated in research design, data analysis and manuscript preparation

ABSTRACT

OBJECTIVES. Children with liver disease have increased risk of long-term cognitive deficits. We differentiated between the effects of chronic liver disease from that associated with transplantation by recruiting children with cholestatic liver disease with and without transplantation.

METHODS. Psychometric measures and magnetic resonance spectroscopy were obtained for 3 groups of children: stable liver disease (SLD) without transplantation; cholestatic liver disease (CLD) from birth with transplantation; and individuals healthy to 18 months of age, prior to transplantation for acute liver failure (ALF).

RESULTS. Cognitive outcomes between children with different disease histories were significantly associated with the duration of liver disease but not the effects of transplantation, including that of immune suppression. Lower intellectual ability was most frequently observed in the CLD group, whereas all of the ALF group scored within the normal range. Myo-inositol and glutamate/glutamine concentrations in cortex were significantly associated with disease duration across the cohort. Neuro-metabolite profiles in SLD were consistent with subclinical encephalopathy. Impaired growth in early childhood was associated with later cognitive performance.

CONCLUSION. Children with prolonged liver disease had the poorest cognitive outcomes despite successful transplantation, suggesting that prolonged cholestasis before transplantation adversely impacts neurodevelopment, and reinforces the need for timely interventions.

Key words: Transplantation, cognitive outcomes, pediatric neurodevelopment, magnetic resonance spectroscopy.

What is known.

- Children with liver disease (LD) are at risk of long-term cognitive deficits
- LD has long-term adverse impacts on growth and cognitive outcome

What is new.

- Asymptomatic LD in children is associated with altered brain biochemistry and cognition
- Cognitive ability in later life is associated with disease duration pre-transplant.
- Acute LD associated with higher cognitive outcomes compared to chronic disease
- No relationship between the severity of acute LD on cognitive outcomes.
- No differences in cognitive outcomes between groups of children stratified by transplant history

INTRODUCTION

Chronic liver disease is associated with an array of negative symptoms[1,2]. Improvements in nutritional support, treatment delivery and access to liver transplantation (LT) has resulted in children with liver disease (LD) surviving illnesses that were normally fatal in infancy[2,3]. Children with a history of chronic LD may experience increased risk of long-term deficits in cognitive skills and decreased quality of life, even following LT[4-6]. In contrast, acute liver failure (ALF) is a shorter-term and immediately life threatening condition characterised by hepatic encephalopathy (HE) and cerebral edema, where reports on long-term outcomes are comparatively sparse[5,7].

The aim of this study was to distinguish between the effects of LT, including exposure to immune suppression, from that of LD chronicity, toward evaluating the extent to which these variables influence longer-term cognitive ability. We recruited patients with different disease histories, comparing children with chronic but stable liver disease from birth (SLD) and without LT with two groups of children with LD severe enough to warrant LT, those with cholestatic liver disease (CLD) from birth and a group who were transplanted for ALF after the age of 18 months and who had been healthy previously.

Cognitive ability was assessed using standardised assessments, alongside proton magnetic resonance spectroscopy (^{1}H-MRS). MRS was used to provide proxy measures of the physiological status of neural tissue in cortex, which in both patient and non-patient populations are moderately strong statistical predictors of cognitive ability[8-14]. In cases of neurological dysfunction related to impaired liver function, both hepatic and sub-hepatic encephalopathy (S/HE) have been associated with reductions in MRS-derived myo-inositol (mI) and choline (Cho) concentrations, coupled with an increase in glutamate/glutamine (Glx) signalling[15-17].

METHODS

Participants

Participants were recruited by a nurse specialist who screened families to establish their willingness to participate. Informed consent was obtained subsequently from all participants and their guardians, under a protocol consistent with the tenets of the Declaration of Helsinki and with approval from the Black Country Research Ethics Committee (08/H1202/38) and Aston University's Human Subjects Ethics Committee (REG/00/175).

Participants were patients with a prior history of liver disease, either from birth or as a result of ALF, who attended long-term follow-up and lived near enough to the hospital to accommodate a full day of testing. Cases were excluded from consideration for testing if they had co-morbidities associated with adverse cognitive development (for e.g., microcephaly, autistic spectrum disorder, multi-organ transplant). Following screening, 56 individuals met the inclusionary criteria: 25 had CLD from birth requiring transplant, 10 had LT for ALF at 18 months of age or older, and 21 had a history of SLD. Children younger than 8 years of age were not recruited because of the increased likelihood that they would be unable to tolerate the research protocol.

Of the eligible patients, 32 consented and were recruited for the study (see Figure 1). The 24 patients who declined to participate did so because the time commitment was considered too great, the study dates were inconvenient, or for no other specific reason. The range of diagnoses of the patients who declined to take part in the study was similar to that of the participants. Four children were recruited into the study but were subsequently unable to complete the testing (non-attendance $n = 2$; extreme fatigue $n = 2$). Diagnostic information for the 28 participants are shown in Table 1a.

Eight of the study group had not received LT and were stable with well-compensated liver disease from infancy (SLD group) and were receiving medical treatment (e.g., fat soluble vitamins). None were receiving antibiotics or immune suppression medication. The other 20 children had experienced LT. Twelve had required transplant between 5.7 months and 12.8 years of age for CLD present from birth. Another 8 had received emergency LT following ALF at which they were aged between 18.7 months and 9.3 years.

Disease severity was defined by the need for transplantation, based on holistic clinical assessment, incorporating five laboratory measures and the pediatric end-stage liver disease (PELD) equation, which was used to assign priority for organ transplantation[18]. At the time of transplant, encephalopathy was present in all of the ALF patients (severity grade 2; $n = 2$; grade 3, $n = 6$), and in (3/12) of the CLD patients (severity grade 1, $n = 1$; grade 2, $n = 2$), using the West Haven criteria[19]. None of the SLD patients were symptomatic of encephalopathy at the time of testing or at any point earlier in their clinical history. Symptoms of chronic liver disease, including varices (6/12) and pruritus (5/12) were present in members of the CLD group before LT, but not in the ALF group. The SLD group did not have pruritus at the time of testing, but 25% (2/8) had asymptomatic varices, detected during surveillance oeso-gastroduoendoscopy. The duration of deteriorating liver disease prior to day of testing was identified from retrospective review of clinical records from birth (CLD group) or from time of clinical presentation (SLD and ALF groups). The CLD children received calcineurin-inhibitor (CNI) based immune suppression (Cyclosporin, $n = 8$ or Tacrolimus, $n = 4$) but by the time of testing only 2 were still receiving Cyclosporin and 6 were receiving Tacrolimus (See Table, Supplementary Digital Content 1, http://links.lww.com/MPG/B647, which describes immune suppression regimens for patient participants). The ALF group received long term prednisolone in addition to CNIs (Cyclosporin, $n = 4$ or Tacrolimus, $n = 4$) but by the time of testing only 2 were still receiving

Cyclosporin; 4 remained on Tacrolimus. Patients in the SLD group were not exposed to immune suppression.

Procedure

Children in the three patient groups were assessed as day cases during planned out-patient review, as summarised in Tables 1b and 2. Baseline clinical data shown in Table 1a were obtained at a point on average 10 years prior to study participation, corresponding to the time of transplant for the CLD and ALF groups, and at a comparable timepoint prior to study participation for the SLD group.

Cognitive measures

Cognitive assessments included age-standardised measures of intellectual ability. The Wechsler Intelligence Scale for Children (WISC-IV) was used for children aged between 6 and 16 years and the Wechsler Abbreviated Scale of Intelligence (WASI) for two participants aged 16 years and older[20,21]. Scores on the component subscales were used to derive indices for verbal IQ (VIQ), non-verbal/performance IQ (PIQ), full-scale IQ (FSIQ) and information processing speed (PSI).

Magnetic Resonance Spectroscopy

^{1}H-MRS data, obtained *in vivo* without sedation, were acquired using standard acquisition software and a quadrature head coil on a Siemens 3-Tesla Trio scanner. Single-voxel acquisitions were obtained following anatomical localisation of an 8cm^{3} voxel of interest using a 5-plane localiser (TR: 20ms, TE: 5ms, 10 slices at 5mm thickness), followed by a stimulated echo acquisition mode (STEAM) pulse sequence (TR: 2000ms, TE: 30ms, 96 averages), including water suppression, for voxels identified within posterior occipito-parietal cortex. Metabolite values were obtained post-acquisition using LCModel[22]. All analyses

have adopted the convention of reporting metabolite concentrations as a ratio to that obtained for Creatine (Cr). Data from two successive scans of the same voxel were averaged prior to analysis.

RESULTS

Statistical analyses

Between-groups contrasts assessed the effects of disease history, including disease duration, and the presence or absence of LT. Analyses employed conservative, distribution free, nonparametric tests for independent samples to assess group differences on cognitive and neurochemistry variables, evaluated against an alpha level of .05 (two-tailed). Where appropriate, significant, group-wise statistical tests were followed by planned, post-hoc comparisons. Bivariate relationships between continuous measures were evaluated using non-parametric measures of statistical association.

On the day of testing all three patient groups were statistically comparable in their age, height, weight, and for biomarkers of liver function (see Table 1b). CNI regimes were not markedly different between transplanted groups on the day of testing (See Table, Supplementary Digital Content, http://links.lww.com/MPG/B647).

Cognitive measures

Results from the cognitive measures of the three patient groups and that for the controls are shown in Table 2, along with MR spectroscopy data. There were no statistically significant associations ($p > .05$) between the ranks on cognitive variables and either rank age at LT or time since transplant, across the entire cohort who received LT. The ALF group scored within the normal range on all IQ measures, with one participant scoring more than 1 *SD* below the population mean on one measure (PSI). Members of the CLD group had the

lowest cognitive abilities, with 41.6% (5/12) of the group scoring 1 *SD* or below the mean on each of the individual cognitive indices. The SLD group had ability levels ranging from the lower tail of the normal population distribution to values near the population mean. Kruskal-Wallis tests for multiple independent samples showed that among the IQ indices, only PSI did not significantly differ across the 3 patient groups.

To evaluate the effects of the presence of chronic liver disease on cognitive variables, we pooled the data from the CLD and SLD groups (i.e., the *chronic* LD groups) and compared them with the ALF group as a non-chronic LD control. Mann-Whitney tests for independent groups revealed that this chronic LD group obtained lower mean ranks than the ALF group on FSIQ (p =.008), VIQ (p =.013) and PIQ (p =.016), but not on PSI (p =.070). An important control analysis in this context is the impact of LT on cognitive ability, irrespective of the presence of LD. This was evaluated by comparing data from the two transplant groups (CLD & ALF) to the SLD group as a non-transplant control. There were no significant differences (p >.05) in the mean ranks of cognitive variables between the groups stratified by LT history. However, lower ranked IQ scores were systematically associated with longer pre-intervention illness durations across the entire patient cohort (FSIQ, r_s = -.72, p<.001; PIQ, r_s = -.68, p<.001; VIQ, r_s= -.67, *p<.001;* PSI*, r_s = -.51, p=.005*). A representative scatterplot of these strong associations is shown in Figure 2a.

Age-standardised measures of height obtained early in the clinical treatment pathway (see Table 1a) were associated with individual differences in cognitive ability measured at long-term follow-up (FSIQ, r_s =.51, p =.006; VIQ, r_s, =.60, p =.001; PIQ, r_s =.44, p =.018). The impact of LT on physical growth is illustrated by comparing z-scores for height and weight on the day of testing with pre-transplant growth data. The CLD group were consistently below average at the time of transplant compared with the ALF group. However, when they

were re-evaluated in our study, their *z*-scores had improved so that group differences in ranks for height were no longer significant statistically (Table 1b).

Magnetic Resonance Spectroscopy

Kruskall-Wallis tests for multiple independent samples revealed significant differences for Glx (p =.041) across the three patient groups (see Table 2). The group effects were strongly associated with disease duration, but not transplant-history. Namely, no significant differences across mean ranks on spectroscopy variables were found between groups differing in transplant history, but significant effects for Glx (p =.007) were present when groups were collapsed across disease history. Differences in neurochemistry in SLD were related to comparatively increased Glx concentrations, consistent with the presence of S/HE in at least some members of this group. The duration of symptomatic LD was significantly and inversely correlated with rank mI concentration (r_s = -.38, p =.043) (Figure 2b), and in the expected opposite direction to that with Glx (r_s = .44, p=.018) (Figure 2c). Associations with Cho were not significant. At the date of testing, age was not significantly associated with group differences in neurometabolite concentrations in this sample.

DISCUSSION

The success of LT for previously fatal health conditions has led to an increased focus on the outcomes associated with longer-term quality of life in these patient groups[3 -7]. A recent systematic review has highlighted both the high frequency of neuro-developmental deficits in children undergoing LT for liver diseases and the comparative paucity of similar outcome data for children who experience disease but retain their native livers[5]. In this context, this study compared neurodevelopmental outcomes of children with SLD maintained on medical treatment, but without LT, with two groups of children who received LT in contrasting clinical contexts. The stratification of the sample across both disease and transplant history

enabled comparisons between the effects of transplantation, including exposure to immune suppression, with that of LD chronicity. We found that individual differences in cognitive ability were strongly and significantly associated with two main clinical factors: the presence of cholestatic LD from birth, and the duration of deteriorating liver function prior to intervention. In contrast to these effects, no significant relationships were identified between either the severity of LD or the experience of LT on cognitive ability assessed as an outcome variable. Together, these analyses suggest that adverse cognitive outcomes associated with LD are more strongly related to the duration of symptomatic disease than with the potential iatrogenic effects on health and neurodevelopment associated with LT.

In this context, we acknowledge the limitations of our study with respect to issues of external validity. Our sample was based on selection of participants with LD with clearly contrasting disease histories and without comorbid conditions already known to adversely affect cognitive development. We included patients with autoimmune liver disease within the ALF group only, excluding them from the SLD and CLD groups, because the design of our study focussed on patients with liver disease present from birth. We were also unable to recruit patients with Alagille syndrome because of its common presentation as a multi-system (co-morbid) disease. The design of the study also did not enable assessment of the impact of chronic graft injury in the CLD and ALF groups, although at the time of testing the liver function tests in all 3 groups fell within the normal range (see Table 1b).

Previous studies of the effects on cognition associated with transplant and associated immune suppression have yielded mixed evidence. Gilmour *et al*.[23] reported that up to 23% of intra-individual variance in patients' verbal IQs could be explained by immune suppression exposure, including CNIs. A cross-sectional study of patients with a mean age at transplant of 35 months, however, did not identify a similarly strong statistical relationship between CNI exposure and IQ variables[24]. In contrast to the null effects associated with post-

transplant exposure to immune suppression in our study, we identified the strong relationship between the comparative duration of symptoms referable to deterioration of liver and developmental delay early in the course of the patient's disease history and individual differences in psychometric IQ variables measured at post-operative follow-up in the long term. Similar findings regarding the covariance between age at LT, maturational variables and cognitive ability measured at post-operative follow-up were reported by Kaller *et al*.[24]. This general pattern of result suggests that systemic insult, particularly at the early stages of pediatric maturation, can manifest as sustained impairments of cognitive ability.

In a study conducted before the widespread availability of LT intervention, Stewart *et al*.[1] reported that children with chronic LD scored lower than typically developing children of the same age on both IQ scores and physical variables - particularly height. Similarly, Ng et al[3] have reported poorer cognitive outcomes in children with biliary atresia, including an association between height measured at 18 months and a set of broad-based neurocognitive variables assessed longitudinally at 24 months. These data are consistent with our finding of the relationship between pre-transplant percentiles for height, and IQ measured at long-term follow-up.

The occurrence of HE related to underlying liver disease (e.g., cirrhotic and non-cirrhotic portal hypertension) is well-established. Increased Glx and mI concentrations in neural tissue are associated with both hepatic and sub-hepatic encephalopathy (S/HE)[16,17,19]. MRS-derived neurometabolic profiles associated with ALF may normalise within a time span as little as 22 weeks[27], a result that is in keeping with reports of the beneficial effects on cognitive outcomes associated with shorter illness durations[28] . Children in our asymptomatic LD group - who had not been transplanted (SLD) - had higher Glx concentrations when compared to the children who had experienced LT (i.e., the CLD and ALF groups). This result illustrates the limitations of the West Haven criteria for diagnosing S/HE, especially in

children[19], but is in keeping with previous findings from adult patients with cirrhosis[15] and those with extra-hepatic portal vein obstruction[29].

A novel element of our study was the inclusion of MR spectroscopy data in patient groups with contrasting disease and transplant histories. The ALF group, who had a shorter experience of illness before LT, had normal mI and Glx concentrations, but across the all 3 patient groups there was an association between reduced mI signal and duration of symptomatic liver disease. A longitudinal MRS study would be needed to directly evaluate the hypothesis that prolonged liver dysfunction is associated with longer term changes in neural health, but the findings of this study are consistent with observations in patients with extra hepatic portal vein obstruction[29], in which reduced concentrations of mI are most prevalent in those with the longest disease durations.

In conclusion, our results augment the literature by reporting the long term effects of liver disease experienced in early childhood on cognitive function and associated neurometabolites[5,6,24,28, 30]. The exclusionary criteria applied in this study enabled the evaluation of the impact of liver disease and transplantation on cognitive outcomes, independently from associated factors that could also explain these effects. Although the study was not designed to interrogate possible causal factors prior to LT; the identification of early onset (prior to 18 months of age) and chronicity of LD as adverse factors on both cognitive and neurodevelopmental markers, combined with evidence of growth restriction before LT, points to the important role of nutritional and associated metabolic factors as mediators of long–term outcomes in these patient groups.

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Figure Legends

Figure 1. Flow diagram showing the pipeline for subject recruitment in the study and the patients' diagnostic status.

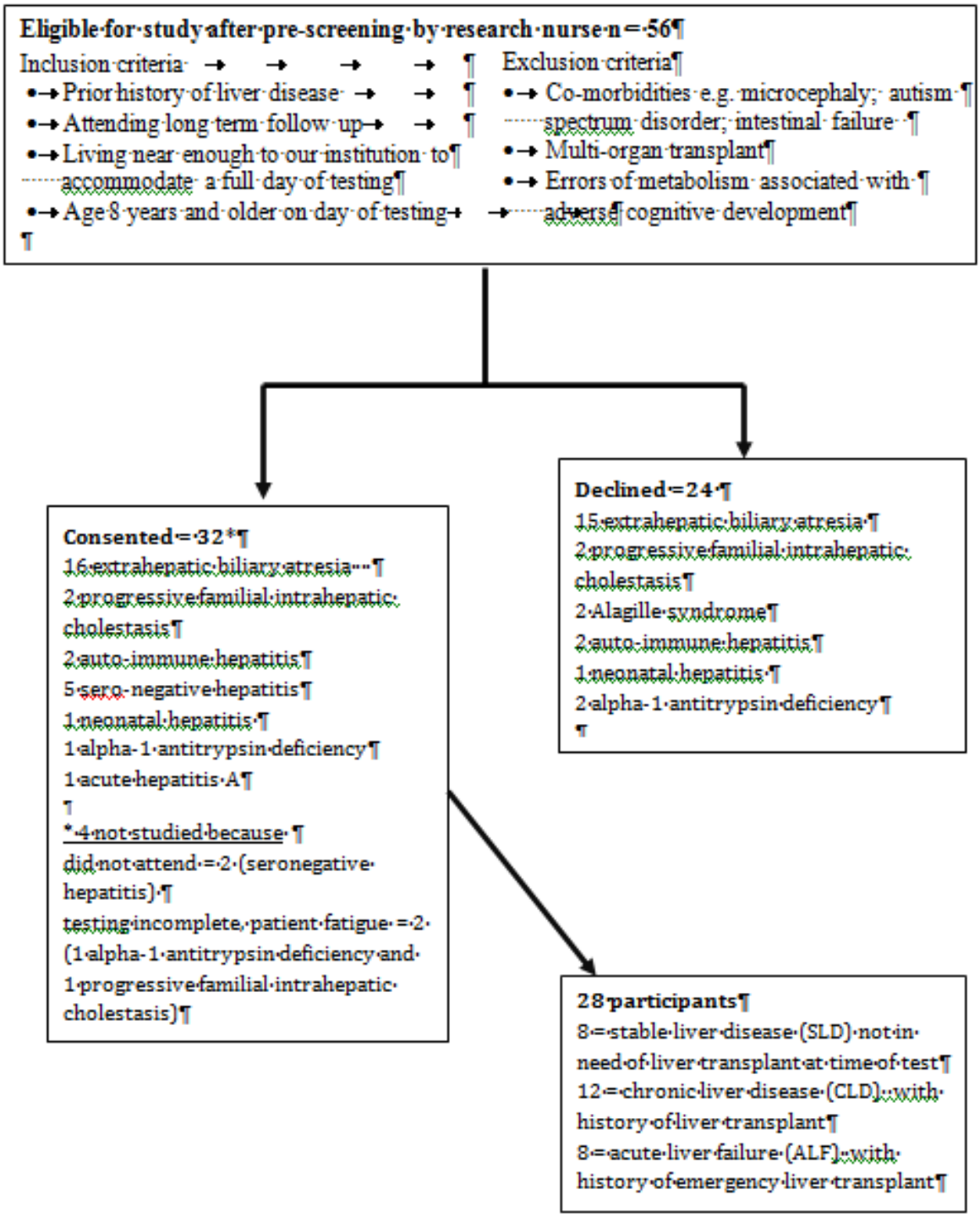

Figure 2. Scatterplots of the association between the duration of uncompensated liver disease in months and, a. Full-scale IQ; b. MRS-derived mI:Cr ratios in brain; c. MRS-derived Glx: Cr ratios across the three patient groups, CLD (circles), SLD (squares), ALF (triangles). The SLD group in panel b and the ALF group in panel c contain one data point for which the symbol represents two overlapping cases.

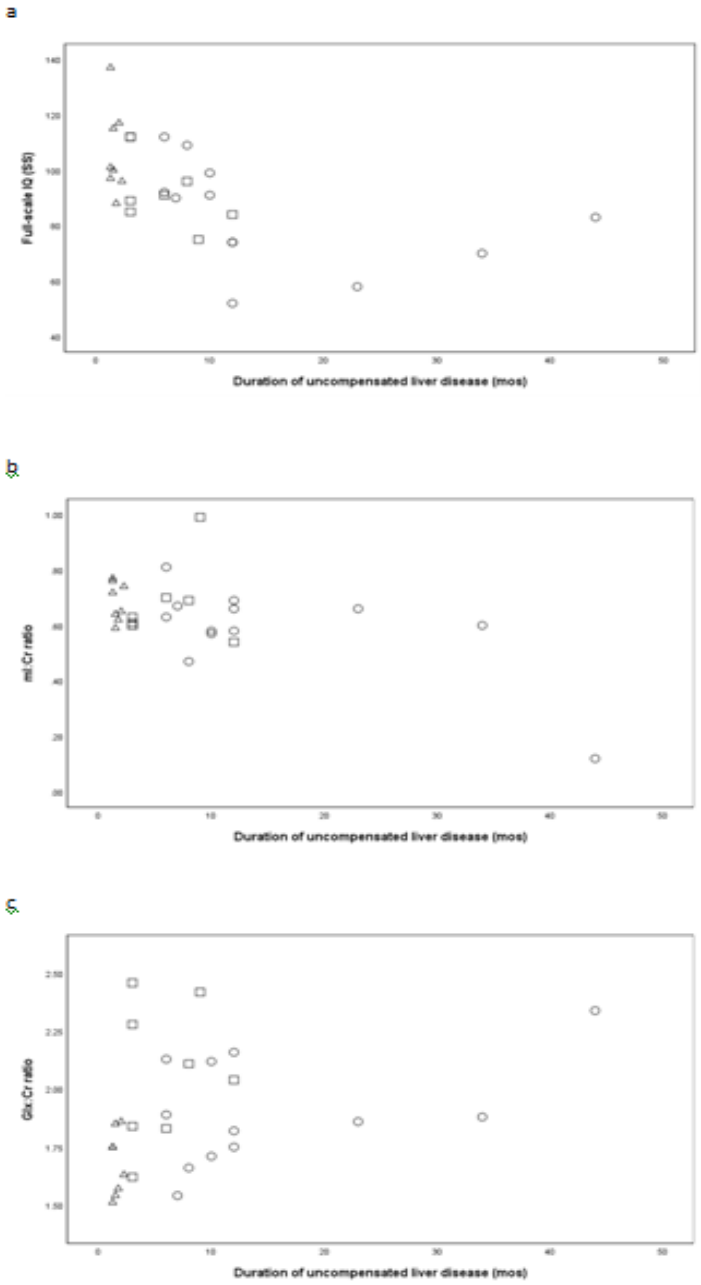

Table 1. Descriptive data for the 3 patient groups obtained: a) at baseline; b) on the day of cognitive testing and neuroimaging.

a.

	SLD[1] (*n* = 8)	CLD[2] (*n* = 12)	ALF[3] (*n* = 8)	
Measure (unit)	*Median (SIQR)*			
Age (mos)	61.5 (13.4)	15.5 (11.2) *	44.0 (34.9)*	**; 1>2,1>3,3>2
Gender (m:f)	4:4	8:4	2:6	nc
Albumin (g/dL)	41 (1.9)	30 (5.2)	28.5 (3.6)	***; 1>2,1>3
Platelet (x 10^9L)	151 (63.6)	127 (64.6)	187.5 (73.9)	
AST (int. units/L)	50 (22.2)	247 (76.4)	895 (316.2)	***; 3>2>1
Prothrombin time (s)	11.5 (5.6)	15 (2.5)	49 (12.0)	***, 3>2>1
Bilirubin (mg/dL)	.5 (.2)	16.9 (10.1)	20.4 (2.3)	***;1<2,1<3
PELD score	0 (0) □	16.0 (7.1)	37.0 (3.4)	***, 1<2<3
Encephalopathy (% cases)	0	16.7	100	nc
Height (z-score)	.4 (1.4)	-1.6 (1.1)	-.2 (.6)	*; 2<3
Weight (z-score)	1.0 (1.5)	-1.0 (1.4)	0 (.1)	

Legend: SIQR semi-interquartile range; * age at LT; AST aspartate aminotransferase; PHD pediatric hepatology score; PELD pediatric end-stage liver disease score. □ PELD score not validated for the patient group that did not require LT. Diagnoses: [1] SLD = 7 biliary artresia; 1 neonatal hepatitis; [2] CLD = 9 biliary artresia, 1 alpha 1-anti trypsin, 2 progressive familial intrahepatic cholestasis; [3] ALF = 5 sero-negative; 2 auto-immune, 1 Hepatitis A. Encephalopathy % reflects the percentage of patients in each group with grade 2 severity or greater using the West Haven criteria. * p<.05; ** p<.01; p<.001, Kruskal-Wallis Test for multiple groups. Significant post-hoc, pairwise statistical comparisons between patient groups by Mann-Whitney, denoted by group differences at p ≤<05. nc, between groups comparisons for categorical data not computed.

b.

	SLD[1] (n = 8)	CLD[2] (n = 12)	ALF[3] (n = 8)	
Measure (unit)	*Median (IQR)*			
Age (yrs)	14.8 (4.5)	13.4 (1.8)	15.6 (2.7)	
Disease duration (mos)	4.5 (2.9)	11 (6.5)	1.5 (.3)	***,1<2, 1>3,2>3
Albumin (g/dL)	41.5 (2.0)	42.5 (1.8)	43.0 (4.5)	
Platelet (x 10^9/L)	99.5 (65.0)	199.5 (28.1)	168 (47.4)	
AST (int. units/L)	35.5 (9.0)	29 (6.4)	35 (27.9)	
Bilirubin (mg/dL)	.5 (.4)	.6 (.27)	.6 (.9)	
Prothrombin time (s)	11.0 (.5)	11.5 (.5)	11.0 (0)	
Height (z-score)	-.2 (.6)	-.7 (1.2)	-.4 (.9)	
Weight (z-score)	.4 (1.3)	.6 (1.2)	.4 (1.2)	

Legend: As for Table 1a. Disease duration: Duration of deteriorating and decompensated liver function prior to day of testing.

Table 2. Descriptive statistics for the cognitive and spectroscopic measures for the participant groups

	SLD[1] *(n = 8)*	CLD[2] *(n = 12)*	ALF[3] *(n = 8)*	
Measure (unit)	*Median (SIQR) nsub*	*Median (SIQR) nsub*	*Median (SIQR) nsub*	
FSIQ (SS)	90.0 (12.0) 3	86.5 (13.0) 5	100.5 (10.0) 0	*; 3>2
VIQ (SS)	91.0 (12.5) 2	86.0 (14.0) 5	105.0 (13.5) 0	*; 3>2
PIQ (SS)	90.5 (8.0) 1	87.0 (20.0) 5	101.0 (11.5) 0	*; 3>2,1
PSI (SS)	92.0 (5.5) 1	89.5 (12.0) 5	104.0 (9.0) 1	
	Median (SIQR)	*Median (SIQR)*	*Median (SIQR.)*	
	n = 8	*n = 12*	*n = 8*	
Cho/Cr	.23 (.03)	.24 (.01)	.24 (.02)	
NAA/Cr	1.71 (.33)	1.71 (.12)	1.74 (.12)	
mI/Cr	.62 (.05)	.62 (.05)	.68 (.06)	
Glx/Cr	2.08 (.28)	1.87 (.21)	1.69 (.14)	*; 1>3

Legend: Intelligence quotient (IQ) data are standard scores (SS) referenced to age-matched norms (M = 100, s.d. = 15) for full-scale (FS), verbal (V), performance (P) IQ, and for the processing speed index (PSI), nsub: number of subjects per group with scores 1 or more s.d. units below population mean. SIQR, semi-interquartile range. MRS data are shown as ratios relative to Creatine (Cr) for Choline (Cho),, N-acetylaspartate (NAA), myoinositol (mI) and glutamate, glutamine (Glx) concentrations