Clinical and genetic characterisation of Infantile Liver Failure Syndrome Type 1, due to recessive mutations in LARS

Jillian P. Casey[1,2], Suzanne Slattery[3], Melanie Cotter[4], AA Monavari[3], Ina Knerr[3], Joanne Hughes[3], Eileen P Treacy[3], Deirdre Devaney[5], Michael McDermott[6], Eoghan Laffan[7], Derek Wong[8], Sally Ann Lynch[1,2,9], Billy Bourke[10], Ellen Crushell[2,3].

(1) Genetics Department, Temple Street Children's University Hospital, Dublin 1, Ireland
(2) UCD Academic Centre on Rare Diseases, School of Medicine and Medical Science, University College Dublin, Belfield, Dublin 4, Ireland
(3) National Centre for Inherited Metabolic Diseases, Temple Street Children's University Hospital, Dublin 1, Ireland
(4) Department of Haematology, Temple Street Children's University Hospital, Dublin 1, Ireland
(5) Histopathology Department, Temple Street Children's University Hospital, Dublin 1, Ireland
(6) Pathology Department, Our Lady's Children's Hospital, Crumlin, Dublin 12, Ireland
(7) Department of Radiology, Temple Street Children's University Hospital, Dublin 1, Ireland.
(8) Department of Pediatrics, David Geffen School of Medicine, University of California, Los Angeles, CA 90095
(9) National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12, Ireland
(10) Our Lady's Children's Hospital, Crumlin, Dublin 12, Ireland.

Correspondence
Dr Ellen Crushell, National Centre for Inherited Metabolic Diseases, Children's University Hospital, Temple Street, Dublin 1, Ireland.
Fax:+353 1 874 7439 T: +353 1 878 4317 E: ellen.crushell@cuh.ie

Summary

Background

Recessive *LARS* mutations were recently reported to cause a novel syndrome, Infantile Liver Failure Syndrome Type 1 (ILFS1), in six Irish Travellers. We have since identified four additional patients, including one of Ashkenazi origin, representing the largest ILFS1 cohort to date. Our study aims to define the ILFS1 clinical phenotype to help guide diagnosis and patient management.

Methods

We clinically evaluated and reviewed the medical records of 10 ILFS1 patients. Clinical features, histopathology and natural histories were compared and patient management strategies reviewed.

Results

Early failure to thrive, recurrent liver dysfunction, anemia, hypoalbuminemia and seizures were present in all patients. Most patients (90%) had developmental delay. Encephalopathic episodes triggered by febrile illness have occurred in 80% and were fatal in two children. Two patients are currently >28 years old and clinically well. Leucine supplementation had no appreciable impact on patient well-being. However, we suggest that the traditional management of reducing/stopping protein intake in patients with metabolic hepatopathies may not be appropriate for ILFS1. We currently recommend ensuring sufficient natural protein intake when unwell.

Conclusions

We report the first non-Irish ILFS1 patient, suggesting ILFS1 may be more extensive than anticipated. Low birth weight, early failure to thrive, anemia and hypoalbuminemia are amongst the first presenting features, with liver dysfunction before age 1. Episodic hepatic dysfunction is typically triggered by febrile illness, and becomes less severe with increasing age. While difficult to anticipate, two patients are currently >28 years old, suggesting that survival beyond childhood may be associated with a favourable long-term prognosis.

Synopsis

ILFS1 is clinically defined by recurrent liver dysfunction and microcytic anemia and the traditional management of reducing/stopping protein intake in patients with metabolic hepatic disease may not be appropriate in this cohort

Compliance with Ethics Guidelines

Conflict of Interest

Jillian P. Casey, Suzanne Slattery, Melanie Cotter, AA Monavari, Ina Knerr, Joanne Hughes, Eileen P Treacy, Deirdre Devaney, Michael McDermott, Eoghan Laffan, Derek Wong, Sally Ann Lynch, Billy Bourke and Ellen Crushell declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for which identifying information is included in this article.

Author contributions

EC, JPC and SAL were responsible for study concept and design and obtained funding. SS, AM, IK, JH, ET, BB, DW, SAL and EC acquired, reviewed and interpreted the clinical data. MC, MM and EL were responsible for haematological, histological and radiological investigations and interpretation respectively. JPC was responsible for analysis and interpretation of the genetics data. JPC and EC drafted the manuscript. All authors were involved in critical revision of the manuscript.

Introduction

We recently identified recessive mutations in the cytoplasmic leucyl-tRNA synthetase gene (*LARS*) as the cause of a multisystem disorder involving infantile liver dysfunction and acute liver failure, anemia, decompensation with minor illness, developmental delay and, in some cases, seizures and encephalopathy (Casey et al 2012). This disorder is now referred to as Infantile Liver Failure Syndrome type 1 (ILFS1 MIM #615438). We have identified nine patients, seven male and two female, from two extended Irish Traveller families with a genetic diagnosis of ILFS1 (Fig. 1A-B). Six of the nine Irish patients were reported in our original study (Casey et al 2012). Three additional Irish patients have been identified; two relatives of previously reported patients and a child from an unrelated Irish Traveller family. A tenth patient, a female of non-consanguineous Ashkenazi Jewish background, has also recently been diagnosed with ILFS1 (Fig. 1C).

Mutations in eight cytoplasmic and ten mitochondrial aminoacyl tRNA synthetase (ARS) genes have been associated with a number of human disorders, mainly those with metabolic, neurological, mitochondrial and muscular involvement (Table S1). There is one previous report of infantile liver failure syndrome due to mutations in methionyl-tRNA synthetase (*MARS*), now classified as ILFS2. ILFS1 and ILFS2 are similar except that interstitial lung disease is additionally present in ILFS2. *LARS* and *MARS* are the first two cytoplasmic amino-acyl tRNA synthetase genes to be associated with a hepatic disorder which may suggest an overlapping disease mechanism (Casey et al 2012, van Meel et al 2013).

The pathophysiology of ILFS1 is currently unknown. However, the LARS enzyme is responsible for incorporation of the amino acid leucine during protein polypeptide synthesis. It is therefore plausible that proteins with the highest percentage content of leucine, which are

largely immune-related proteins, will be most severely affected if LARS is not functioning efficiently (Casey et al 2012). One possible theory is that when children with *LARS* mutations are ill, the requirement for leucine increases due to the induction of immune-related pathways that involve proteins with high leucine content and the mutant LARS may not cope with this spike in demand. This effect would be further exacerbated by low protein intake, and hence low leucine intake, when the children are unwell. Alternatively, the disease mechanism responsible for recurrent liver crises may relate to the fever associated with infection. It is possible that the mutant LARS enzyme is thermolabile; it may retain reduced functionality at body temperature but is destroyed at higher temperatures associated with fever. Further cellular studies are required to investigate these hypotheses as possible mechanisms underlying the recurrent hepatic and cerebral decompensations in these patients.

Here, we sought to clinically define ILFS1 based on comprehensive review of the clinical symptoms, natural history and current management strategies in ten ILFS1 patients (six previously reported and four unreported).

Methods

Patient recruitment

We identified ten patients (six previously reported) from three families diagnosed with ILFS1 based on clinical and genetic findings. The two Irish families were identified by authors between 2012-2014 after referral for early failure to thrive, anemia and recurrent liver dysfunction. The proband, of Ashkenazi origin from the USA, was referred to pediatric genetics for a suspected metabolic disorder. Clinical exome sequencing led to a diagnosis of ILFS1. This study presents the clinical evaluations of the ten ILFS1 patients and the results of medical record review.

Clinical evaluations

All patients were physically examined by at least one of the authors. Clinical evaluation included biochemical testing (10 patients), molecular testing (10 patients), assessment of development (10 patients), analysis of liver biopsies (4 patients), analysis of blood films and bone marrow aspirates (4 patients), Magnetic Resonance Imaging (MRI) of brain (7 patients) and Magnetic Resonance Spectroscopy (MRS) of the brain (voxel on left basal ganglia) (3 patients). Development was assessed clinically by a general and/or developmental pediatrician.

Known metabolic and genetic causes of liver failure including disorders of intermediary metabolism, storage disorders, peroxisomal disorders, glycosylation disorders and mitochondrial disorders were excluded through clinical, biochemical and genetic analyses (Casey et al 2012).

Clinical definitions

While a spectrum of liver injury was observed in this study, we sought to distinguish the severity of episodes by the following terms:

We defined the term "acute liver failure (ALF)" as the rapid development of severe impairment of hepatic synthetic function including the development of coagulopathy. In contrast, "liver dysfunction" was defined as periods of abnormal biochemical markers of liver function (in particular the transaminases – alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) but without significant impairment of synthetic function (i.e. without coagulopathy). As hypoalbuminemia was a consistent finding amongst the cohort, it was not included in defining the severity of episodes of liver dysfunction.

Coagulopathy was determined by a prolonged blood prothrombin time (PT) and/or a prolonged blood activated partial thromboplastin time (aPTT).

Encephalopathy was defined as a depressed level of consciousness with accompanying electroencephalographic (EEG) evidence of encephalopathy.

Mutation detection

The nine Irish Traveller patients were tested for the *LARS* c.245A>G and c.1118A>G variants by Sanger sequence analysis, as described (Casey et al 2012). Patient CII:1 underwent clinical exome trio sequencing at the Orphan Disease Testing Center, UCLA Medical Center, USA. Exome enrichment was performed using an Agilent SureSelect Human All Exon 50 Mb kit (Agilent Technologies, Santa Clara) and the enriched libraries were sequenced on an Illumina HiSeq 2500 (Illumina, California).

Review of medical records

We reviewed medical records of the ten patients with a diagnosis of ILFS1 due to recessive *LARS* mutations to document and define the ILFS1 phenotype. A detailed description of the natural history in each patient is provided in Supplementary Table S2.

Results

Molecular diagnosis

All nine Irish Traveller patients are homozygous for both *LARS* c.245A>G (p.Lys82Arg; rs112954500; minor allele frequency 0.003) and c.1118A>G (p.Tyr373Cys; rs201861847;

minor allele frequency unknown). It is not known if (i) both variants contribute to the phenotype or (ii) only one variant is causal and the other variant is in linkage. Segregation analysis was not informative when trying to infer pathogenicity as none of the twelve healthy relatives tested are homozygous for either mutant allele. However, of the two variants, c.1118A>G is more likely to be damaging based on *in silico* predictions and its location within the critical editing domain of the enzyme.

Patient CII:1 is the first child with ILFS1 to be reported outside of the Irish Traveller population. Trio-based clinical exome sequencing identified compound heterozygous variants in *LARS* as the most likely cause of the patient's multisystem disorder; a paternally inherited missense variant in exon 16 (c.1511C>T; p.Ala504Val) and a maternally inherited missense variant in exon 19 (c.1842C>G; p.Asn614Lys). These variants are not present in dbSNP135, 1000 Genomes database or the NHLBI ESP database supporting the likelihood that they are rare pathogenic variants. Of note, the exon 11 p.Tyr373Cys variant in the Irish Travellers and the exon 16 p.Ala504Val variant in patient CII:1 are both located within the LARS editing domain. Further studies are required to determine if these variants have a deleterious effect on editing which would support mistranslation as a possible contributing factor to ILFS1.

Clinical features: current status

The patients currently range from 16 months to 35 years of age. Two children have died, aged 4 and 8 years, during lower respiratory tract infections; H1N1 influenza in patient AIV:1 and bronchopneumonia (presumed viral in origin as no organism was identified) in patient AIII:6. Both children developed status epilepticus and encephalopathy following the onset of symptoms of respiratory tract infection and died despite Intensive Care management.

Clinical features: **neonatal period**

Six patients were delivered before 37/40 weeks gestation and all patients had low birth weight (<2.7kg). All unaffected siblings of the cohort weighed >3kg at birth. Poor feeding and early failure to thrive (FTT) were noted in all patients. Notable physical features include cherubic faces with full cheeks despite low body weight (Fig. 1D).

Hepatic presentation

All patients had evidence of abnormal liver function within the first year of life. A prominent finding was hypoalbuminemia, even prior to obvious liver dysfunction. Fecal alpha-1-antitrypsin levels in three patients and small bowel biopsies in two patients were normal. Six patients developed acute liver failure aged 2-8 months, with recurrence in four patients to date (Table 1). One episode (patient AIV:2) of ALF was associated with rapid rise of AST to a maximum of 8578 U/l (NR 8-45) and ALT to 3429 U/l (NR 10-45) two days after the onset of a febrile illness (respiratory syncytial virus and parainfluenza viruses isolated in sputum). Total bilirubin was 114 mmol/l and conjugated bilirubin was 100 mmol/l (NR 5-17). PT was 48 seconds (NR 12-14.5) and aPTT was 52 seconds (NR 28-36). Lactate was 1.5 mmol/l (NR 1-2.2), albumin 27 mmol/l (NR 36-50) and ammonia 60 mmol/l (NR<55). He was treated in the Intensive Care unit with supportive management and total parenteral nutrition (containing 2.5g protein/kg/day) and all parameters slowly recovered over the following three weeks.

In the remaining four patients who had intermittent liver dysfunction but who did not develop acute liver failure, hepatic transaminases (ALT, AST) were intermittently elevated up to three to four times the upper limit of the normal range, albumin was decreased but coagulation screens and ammonia levels were normal. Typical presenting features in this group included intermittent liver dysfunction, hepatomegaly, infantile FTT and anemia. In all patients,

hepatic dysfunction occurred during febrile illnesses and became less prominent with increasing age.

Six liver biopsies were performed on four patients. The most prominent and consistent feature was marked macro- and micro-vesicular steatosis (Fig. 2A). Electron microscopy was performed on one sample from patient CII:1 and showed normal mitochondria and needle shaped crystals of unknown etiology. Because of ultrasonographic evidence of evolving hepatic nodularity in one child (AIII:8) at age 4 years, a follow-up liver biopsy was undertaken which showed steatosis, fibrosis, nodular regeneration and early cirrhotic change (Fig. 2B). Similar findings were seen in a post-mortem sample of another child (AIV:1) (Fig. 2C-F). Neither of the two adult patients have had liver imaging. No patient has splenomegaly or thrombocytopenia or other clinical stigmata of chronic liver disease.

Development

Nine of ten patients (AII:9, AII:13, AIII:4, AIII:6, AIII:8, AIII:10, AIV:2, BII:3 and CII:1) had developmental delay (DD) with eight children having mild-moderate DD with predominately early motor delay. One child had severe global DD (AIII:6); he did not walk until age 5 years, had no speech by age 8 years and had sensorineural deafness. Extensive investigations did not identify a second disorder in this child and the reason for his severe phenotype is not known. All other children attended mainstream school, with most requiring learning support. Patient CII:1 had excellent cognitive development (reading at age 3) but delayed motor development. One patient (deceased AIV:1) had normal development. Despite infantile FTT, growth improved over time. The two adults (28-35 years) and two of the older children (6-12 years) now have normal stature (Table 1).

Brain Imaging

MRI of brain was performed in seven children. In four (AIII:4, AIII:6, AIII:10 and AIV:1), the scan showed nonspecific mild cerebral atrophy. One MRI was normal (AIII:8). In one child (BII:3) Leigh-like changes were noted in the thalami and basal ganglia during a presentation with status epilepticus (Fig. 3A). No common Leigh disease mutations were found in this child and the appearances on follow up had resolved (Fig. 3B). Another patient (CII:1) had evidence of progressive infarcts on sequential scanning but Magnetic Resonance Angiography of brain was normal. MRS in three children (AIII:10, AIV:1 and BII:3) was normal. Computed Tomograghy of brain performed during an encephalopathic episode in one patient (AIV:2) was normal while, on another (AIV:1), CT of brain following prolonged status epilepticus showed cerebral oedema with evidence of unilateral cerebral infarction (this episode was fatal).

Seizures and encephalopathy

All ten patients experienced seizures associated with intercurrent illnesses. The age at first presentation with seizures varied from 3 months to 6 years. Some of these episodes presented as prolonged status epilepticus and encephalopathy while, in others, the presentation was of isolated self-resolving generalised tonic clonic seizures. Encephalopathic episodes were seen in eight patients. Encephalopathy was not associated with hyperammonemia. Two patients died during encephalopathic episodes, both of which were triggered by viral respiratory infections. For one of these patients (AIV:1), it was his first encephalopathic presentation while the other patient (AIII:6) had already had a prior encephalopathic episode from which he recovered. Both children developed seizures and coma requiring ventilatory support. Both deteriorated systemically clinically with cardiovascular instability. We believe that the encephalopathy is not primarily hepatic but is due to a local brain effect as some episodes

occur in association with minimal liver dysfunction and, conversely, ALF has not always been associated with encephalopathy.

A typical episode of encephalopathy, as experienced by patient AIII:10 during a Noroviral gastroenteritis at age 21 months, started with four short generalised seizures over the course of a day. This was followed by respiratory depression, requiring intubation and ventilation, and a comatose state persisting after anticonvulsant and sedative drugs had worn off. EEG on day 3 of this episode (while still comatose) showed a slow background with 0.5Hz delta rhythm with no active seizure activity. She regained consciousness over the following 2-3 days yet her speech and other developmental skills were slow to return to baseline prompting a further EEG 11 days later. This showed ongoing encephalopathic features with a persistently slow background. Her recovery continued and all skills returned to baseline approximately three weeks after the episode. A similar pattern has also been observed in other children.

Anemia

All patients have had persistent microcytic hypochromic anemia, with three patients requiring repeated red cell transfusions for a period of time in infancy (AIII:4, AIV:1 and AIV:2). Iron studies have been normal and no response to iron treatment was noted in any patient. Reticulocyte counts were appropriate. Peripheral blood films showed microcytosis with hypochromasia and target cells (Fig. 4A). Bone marrow aspirate from one patient (AIII:4) showed evidence of dyserythropoiesis and abnormal iron distribution with ringed sideroblasts (Fig. 4B). Bone marrow aspirate from two other patients (AIII:8 and AIV:2) also showed evidence of dyserythropoiesis with prominent siderotic granules in erythroblasts. The anemia typically resolves in childhood however abnormal red cell morphology is still evident in the

blood films of the two adult patients. It is unclear at this point what the underlying mechanism of the anemia is but the findings are consistent. It is interesting to note that the patient reported by Cole and colleagues with mutations in *MARS* also had a period of transfusion-dependent anemia in infancy (van Meel et al 2013).

During a critical illness with viral infection, two patients developed thrombocytopenia along with elevation of bilirubin and lactate dehydrogenase and a blood film suggestive of microangiopathic hemolytic anemia (MAHA). The findings resolved with supportive measures. Mutation screening of *ADAMTS13* in one patient did not identify any pathogenic variants. No evidence of MAHA was seen in the other patients, even during critical illnesses.

Renal dysfunction

To date, two patients have had self-resolving acute renal failure. In one patient (AIII:6) this was during a critical illness where he also had acute liver failure. In the other patient (AIII:4), it appeared to be isolated during a minor illness and was associated with proteinuria, hypertension and elevated urea and creatinine. No cause was identified and spontaneous resolution occurred.

Discussion

We undertook a comprehensive review of all known ILFS1 patients (six previously reported and four unreported), in order to clinically define ILFS1 and help guide diagnosis and patient management. Nine of the ten patients are from the Irish Traveller population, an endogamous nomadic group of ~30,000-40,000 in the Republic of Ireland (All Ireland Traveller Health Study Team 2011). Patient CII:1 is the first reported patient with ILFS1 outside of the Irish Traveller population, suggesting that ILFS1 may be more extensive than originally

anticipated. Her phenotype is in-keeping with the Irish cohort (Table 1) with the exception of cerebrovascular strokes which we have not observed in Irish patients. However, patient CII:1 is known to have other vascular risk factors; she is heterozygous for the prothrombin G20210A mutation and has the Plasminogen Activator Inhibitor-1 4G/5G genotype.

We compared the frequency of clinical features in the patient cohort (n=10); recurrent liver dysfunction (10/10; 100%), acute liver failure in infancy (6/10; 60%), recurrent liver failure (4/10; 40%), early failure to thrive (10/10; 100%), microcytic anemia (10/10; 100%), seizures (10/10; 100%), encephalopathy (8/10; 80%), developmental delay (9/10; 90%), acute kidney injury (2/10; 20%) and sensorineural deafness (1/10; 10%). Recurrent liver dysfunction and microcytic anemia appear to be the defining clinical features of ILFS1. The liver crises are most severe in infancy and become less severe with age, which may be related to an increased ability to cope with infection or fever in later years, or an increased ability to synthesise enough protein with increasing liver size. It is difficult to predict the long-term prognosis – while two patients are 28 and 35 years and have no biochemical or clinical evidence of chronic liver disease, two of the children have developed liver nodularity with well-compensated cirrhosis. While this remains clinically stable over a number of years, there is concern about possible progression and the development of complications of cirrhosis including portal hypertension, hepatic synthetic dysfunction and hepatocellular carcinoma.

No patient has had a liver transplant although this treatment was considered acutely on three separate occasions for three different patients who had ALF. In all cases, the liver improved with supportive measures and transplantation was deferred. In the two children who died, their final illness was primarily an encephalopathic presentation triggered by respiratory infection and therefore liver transplantation was not considered.

Hypoalbuminemia, at times severe, was a prominent and consistent finding in infancy even when protein intake was adequate and liver function appeared to be otherwise normal. Where studied, there was no evidence of enteropathy or excess protein loss in the stools or urine We speculate that endogenous albumin synthesis may be compromised in these patients due to reduced LARS activity. Albumin is very sensitive to amino acid depletion, particularly in the case of leucine, isoleucine and tryptophan (Hutson et al 1987). Leucine is one of the most abundant amino acids in albumin (Table S3). While these children do not show any measurable leucine deficiency, it is possible that homeostasis is maintained but is not adequate to support synthesis of an abundant leucine-rich protein such as albumin, resulting in low albumin. Furthermore, given that the patient's mutation is located in the LARS editing domain, there may be incorporation of incorrect amino acids during protein synthesis, leading to accumulation of misfolded proteins. In turn, this may lead to endoplasmic reticulum (ER) stress, up-regulation of the unfolded protein response and, at maximum ER stress levels, activation of apoptosis which is a known contributor to human liver disease (Jiang et al 2014). This would be particularly true for proteins with a high percentage content of leucine. In the case of albumin, a misfolded version of the protein may be insoluble and hence will not be secreted. Despite a good protein intake (3.6g/kg/day), one infant (AIV:2) was given a 1 month trial of leucine supplements (100 mg TDS). However, no effect on his low albumin levels (which were at times <20 mmol/l) was seen. Hypoalbuminemia improved in all after the first two years of age.

Identification of the genetic basis for the liver failure in these patients has resulted in a new management protocol within our unit. Patients are admitted promptly to hospital even with minor viral infections to help pre-empt the hepatic and encephalopathic episodes. We believe

that the traditional management of reducing/stopping protein intake in patients with metabolic hepatic disease is not appropriate and may even be detrimental in this cohort. Currently, we now ensure that the patient receives a minimum 2.5g/kg of whole protein while unwell either enterally or parenterally. It has been observed that the patients' liver function and overall condition begins to improve once this intake has been established. However, it is too soon to comment as to whether this approach will prevent ALF or encephalopathy. Blood leucine levels in the patient cohort are normal. Nevertheless, a short trial (1 month) of leucine supplements (L-leucine 100mg TDS) was given to one child (AIV:2) and no appreciable difference in the child's clinical status or biochemical parameters was seen. However, no side effects were observed.

The molecular mechanism underlying ILFS1 is currently unknown. However, LARS has recently been shown to be an activator of mTORC1, the latter of which inhibits autophagy (Han et al 2012). Autophagy is an important quality-control mechanism in the human body that serves to degrade long-lived and damaged cytoplasmic organelles to generate new substrates for energy production. This is particularly important during periods of stress, starvation and infection and this homeostatic function protects against a wide variety of disorders, including liver disease (Levine and Yuan 2006, Murrow and Debnath 2013). The autophagy process must be tightly regulated; too much or too little can be deleterious (Chen and Klionsky 2011). Han and colleagues showed that deficiency in LARS prevents activation of mtorc1 (autophagy inhibitor) resulting in increased levels of autophagy (Han et al 2012). Furthermore, reduced hepatic mTORC1 activity has been shown to cause liver cell damage (Umemura et al 2014). Could mutations in *LARS* result in a multisystem disorder through reduced mTORC1 activity and abnormal autophagy? Further work examining the role of

autophagy in ILFS1 is required as autophagy is a pathway amenable to drug treatment and this may lead to new therapeutic options.

In conclusion, while the ILFS1 phenotype is variable, all children suffer from early failure to thrive, infantile liver failure or intermittently abnormal liver function, hypoalbuminemia in infancy, microcytic anemia and seizures with or without encephalopathy. Most children also have at least mild developmental delay. We suspect that there are many more cases of ILFS1 yet to be identified and recommend sequencing of the *LARS* gene in patients presenting with unexplained infantile liver failure, recurrent liver dysfunction, failure to thrive and persistent anemia. Importantly, ILFS1 patients are at high risk of episodic life-threatening encephalopathic and/or hepatic episodes triggered by viral infections. We suggest that aggressive intervention by early hospital admission, antipyretic treatment and maintenance of dietary protein intake may help reduce the likelihood of liver failure, encephalopathy or death during inter-current infections.

References

All Ireland Traveller Health Study Team, School of Public Health, Physiotherapy and Population Science, University College Dublin, Ireland. All Ireland Traveller Healthy Study. Our Geels, 2011.

Casey JP, McGettigan P, Lynam-Lennon N et al (2012) Identification of a mutation in LARS as a novel cause of infantile hepatopathy. *Mol Genet Metab* 106(3): 351-358.

van Meel E, Wegner DJ, Cliften P et al (2013) Rare recessive loss-of-function methionyl-tRNA synthetase mutations presenting as a multi-organ phenotype. *BMC Med Genet* 14: 106.

Han JM, Jeong SJ, Park MC et al (2012) Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. *Cell* 149(2): 410-424.

Hutson SM, Stinson-Fisher C, Shiman R et al (1987) Regulation of albumin synthesis by hormones and amino acids in primary cultures of rat hepatocytes. *Am J Physiol* 252(3 Pt1): E291-E298.

Jiang S, Yan C, Fang Q et al (2014) Fibroblast growth factor 21 is regulated by the IRE1α-XBP1 branch of the unfolded protein response and counteracts endoplasmic reticulum stress-induced hepatic statosis. *J Biol Chem* 298: 29751-29765.

Levine B, Yuan J (2006) Autophagy in cell death: an innocent convict? *J Clin Invest* 115(10): 2679-2688.

Murrow L, Debnath J (2013) Autophagy as a stress-response and quality-control mechanism: implications for cell injury and human disease. *Annu Rev Pathol* 8: 105-137.

Chen Y, Klionsky DJ (2011) The regulation of autophagy – unanswered questions. *J Cell Sci* 124(Pt 2): 161-170.

Umemura A, Joong Park E, Taniguchi K et al (2014) Liver damage, inflammation, and enhanced tumorigenesis after persistent mTORC1 inhibition. *Cell Metab* 20(1): 133-144.

Financial support

The genetic studies were supported by The Children's Fund for Health, Temple Street Children's University Hospital, Dublin, Ireland (PAC 09131). Jillian Casey is supported by a Medical Research Charities Group (MRCG) grant from the Health Research Board (HRB) and the Children's Fund for Health, Temple Street Children's University Hospital (MRCG/2013/02).

Acknowledgements

We sincerely thank the patients and their families who have contributed to this study and helped our understanding of childhood liver disease. We would also like to thank Prof . David Wilson (Professor of Paediatric Gastroenterology and Nutrition at the University of Edinburgh) for his insightful discussions on albuminemia and amino acid homeostasis and Dr Seamus Hussey and Dr Annemarie Broderick for stimulating discussions on mechanisms of liver disease.

Figures

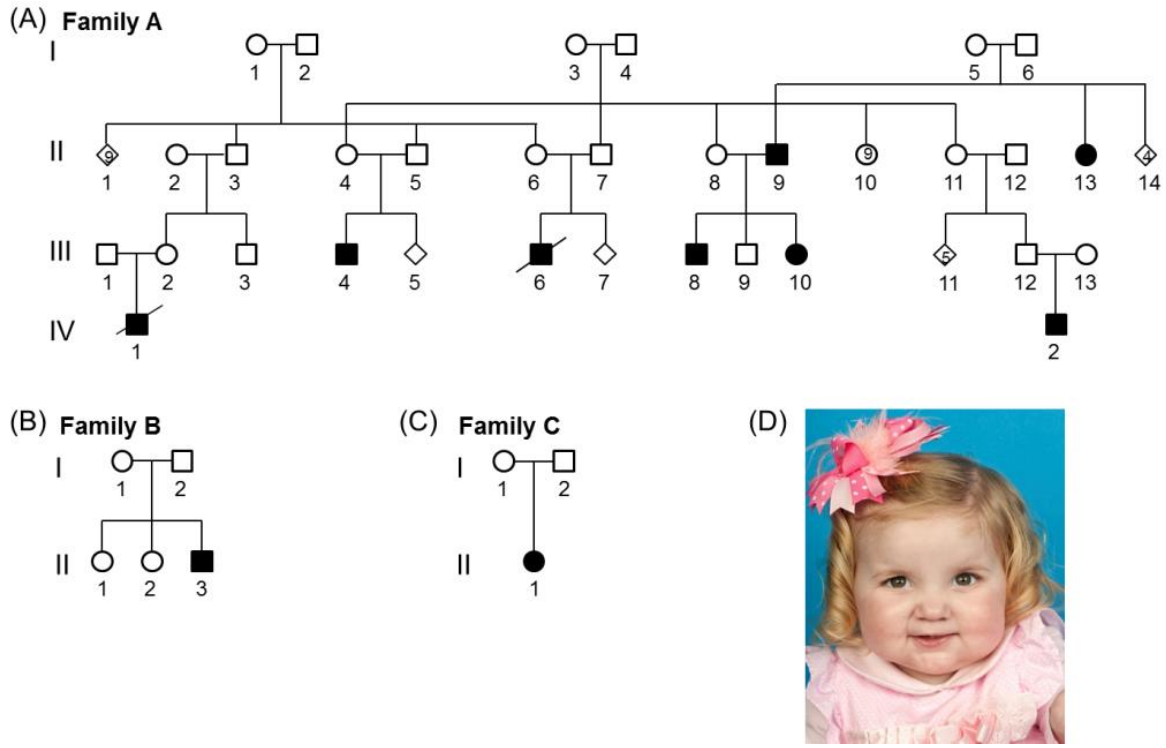

Fig. 1. Pedigrees of three families with ILFS1 due to recessive LARS mutation. (A) Family A is an Irish Traveller family which includes eight individuals (3 months to 35 years of age) from three generations diagnosed with ILFS1. (B) Family B are members of the Irish Traveller population and includes one child with ILFS1. (C) Family C is a non-consanguineous family of Ashkenazi Jewish background from the United States of America. They have one affected children diagnosed with ILFS1. (D) Photograph of one of the ILFS1 patients showing a cherubic face with full cheeks despite normal or low body weight.

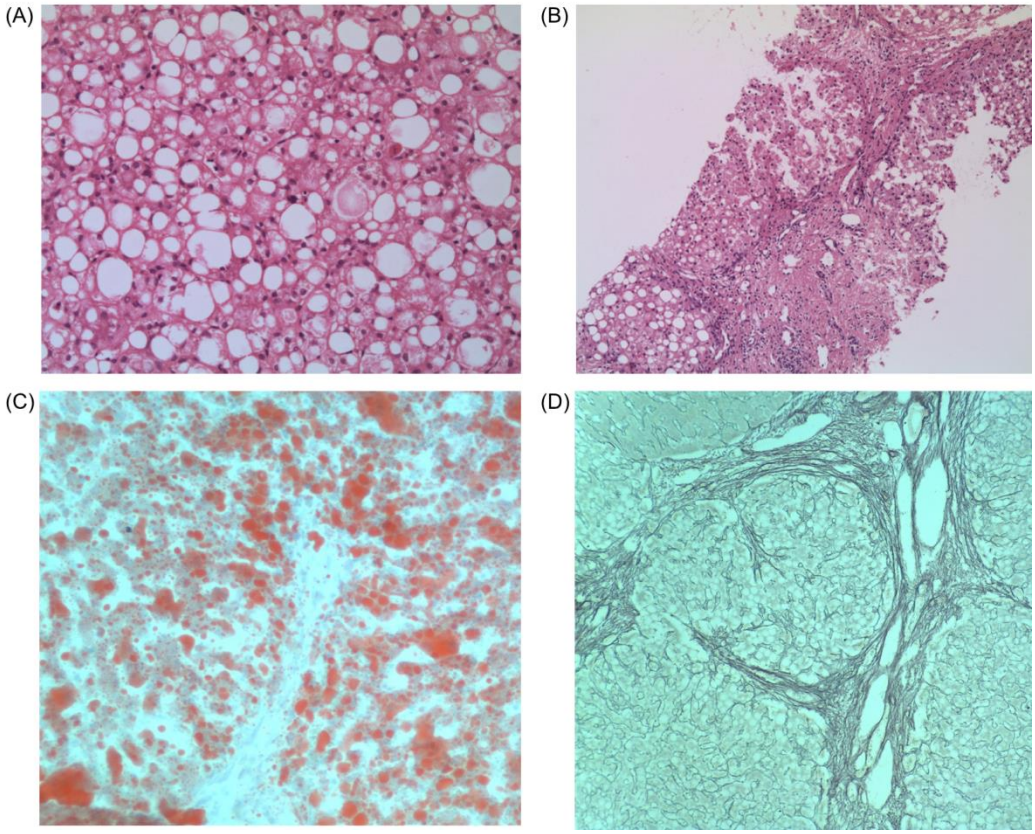

Fig. 2. Liver histology. (A) Photomicrograph of initial liver biopsy in patient AIII:8 showing marked steatosis with rare necrotic hepatocytes. (B) Photomicrograph of follow-up liver biopsy in patient AIII:8 four years later showing marked fibrous expansion of portal tracts with bridging fibrosis and persistent steatosis in residual hepatic lobules. (C-D) Post mortem liver histology images from patient AIV:4 confirms the diffuse macro- and micro-vesicular steatosis throughout the liver (Figure 2C - Oil Red O stain) and also the liver fibrosis with nodule formation and early cirrhosis (Figure 2D - Reticulin stain).

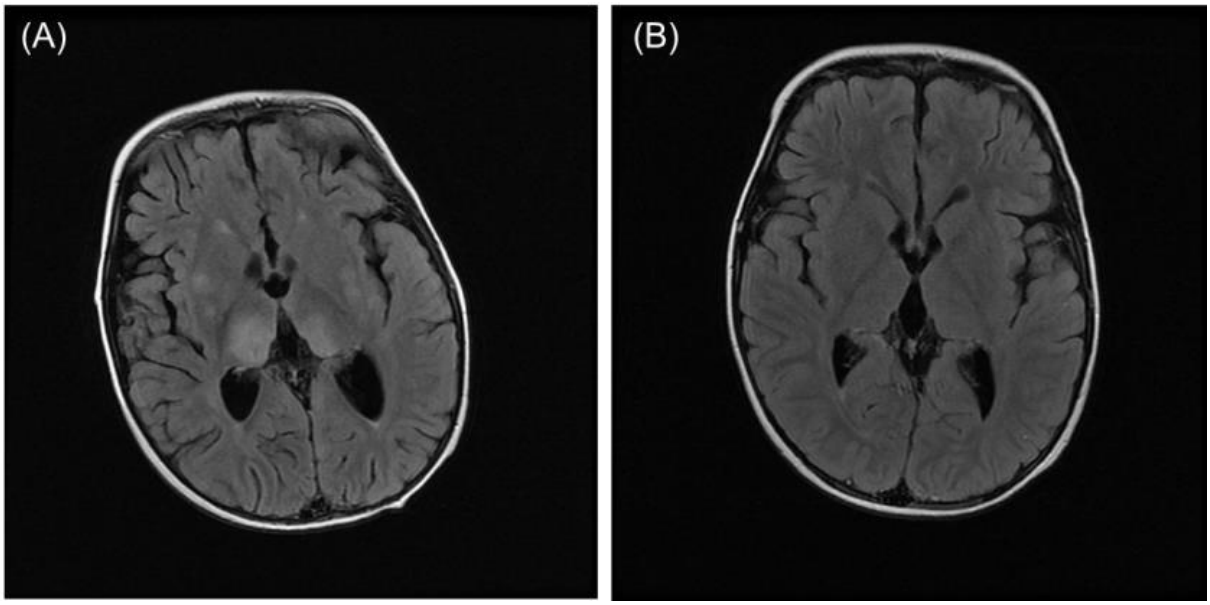

Fig. 3. Brain imaging for patient BII:3. (A) Brain MRI of patient BII:3 aged 2.5 years during his first episode with status epilepticus and liver dysfunction demonstrates quite pronounced T2 hyperintensity of the thalami (R>L) and basal ganglia, suggestive of acute Leigh-like changes. (B) Brain MRI of patient BII:3 was repeated one year later when he presented with a further encephalopathic episode. The Leigh-like changes previously observed had resolved with no recurrence one year later. Mild cerebral atrophy is evident.

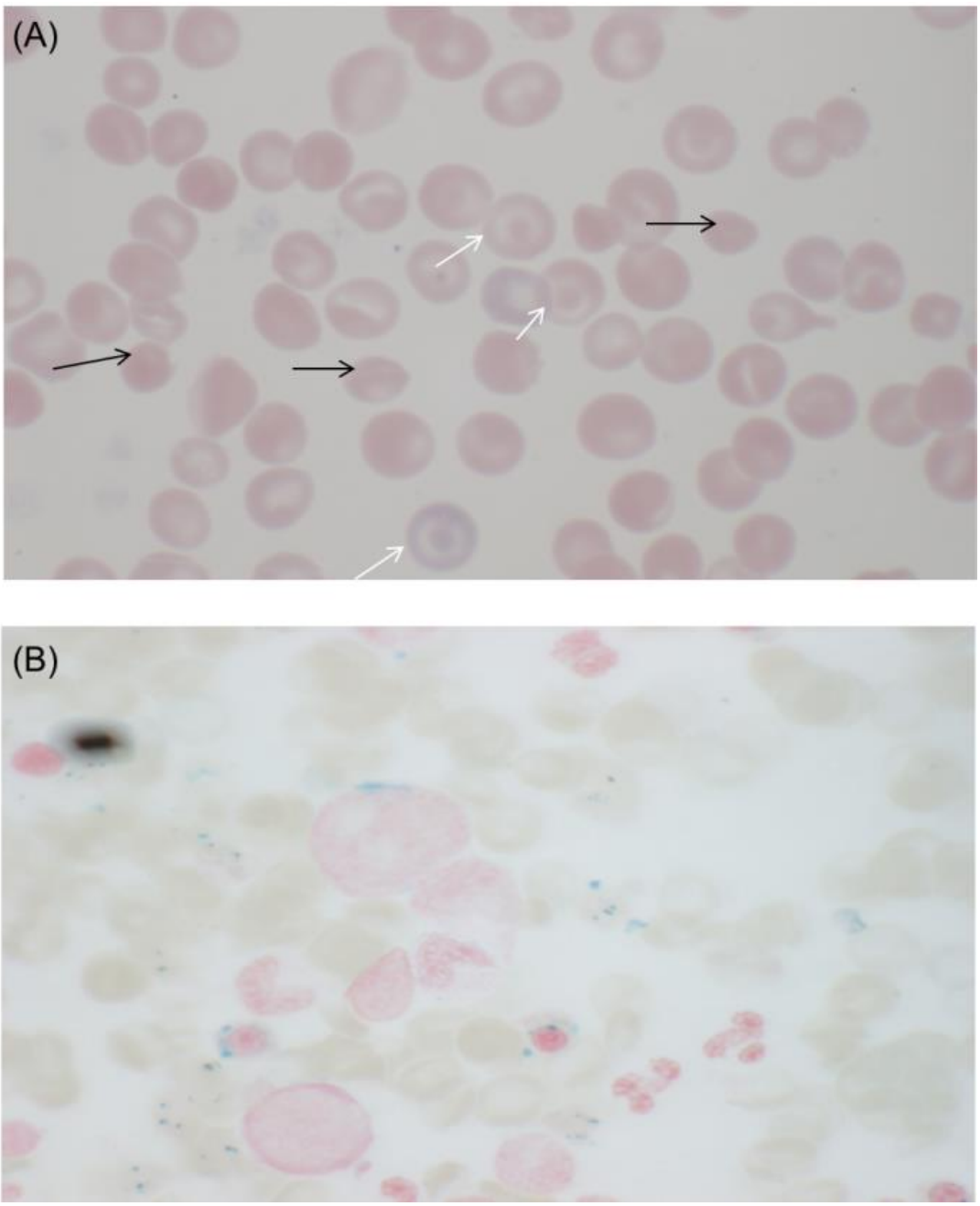

Fig. 4. Anemia phenotype. All patients in our cohort had hypochromic microcytic anemia which was resistant to treatment with iron supplementation. Two patients were transfusion dependent for a short period of time. (A) A peripheral blood film from patient AIII:4 showed target cells (white arrow) and small irregularly contracted red cells (black arrow). (B) A bone marrow aspirate from patient AIII:4 showed evidence of dyserythropoiesis, staining with Prussian blue showed abnormal iron distribution with ringed sideroblasts.

Table 1. Clinical features of patients with ILFS1.

Family ID	**A III:8**	**A III:10**	**A II:9**	**A II:13**	**A IV:1**	**A III:4**	**A III:6**	**A IV:2**	**B II:3**	**C II:1**
Age at presentation	2 mo	2 mo	< 1yr	6 wks	2 mo	1 mo	3 mo	1 mo	2 yrs	5 mo
Presenting features	ALF, MA	ALF, MA	LDF, HM, MA, FTT	LDF, HM, MA, FTT	ALF	LDF, MA, FTT, HG	ALF	FTT, HT, MA, HA, HG	LDF, HM, MA, SE	ALF, MA, FTT
ALF	2 epis 2mo, 17mo	2 epis 2 mo, 22mo	-	-	3 epis 2mo, 4mo, 11mo	-	1 epis 3mo	2 epis 8 mo, 18mo	-	1 epis 5mo
Microcytic Anemia	++	+	+	+	++	++	+	++	+	+
Hypoalbuminemia in infancy	++	++	?	?	+	+	+	++	+	+
Seizures with illness	+	+	+	+	+	+	+	+	+	+
DD	+	+	+	+	-	+	+++	++	+	+
Stature at last review	norm	<2 pc	norm	norm	<2 pc	<2 pc	<2 pc	<2 pc	norm	<2 pc
Other	-	-	-	-	-	CAH, ARF, MAHA	SND, ARF	MAHA	-	stroke
Current age	11y	3y	35y	28y	RIP age 4y	5y	RIP age 8y	16mo	4y	7y

Features that are present, absent or unknown are represented by +, - and ? respectively. Symptom severity: symptom present (+), significant (++), severe (+++). Abbreviations: ALF, acute liver failure; ARF, acute renal failure; CAH, congenital adrenal hyperplasia; DD, developmental delay; FTT, failure to thrive; HG, hypoglycaemia; HA, hypoalbuminemia; HM, hepatomegaly; HT, hypotonia; LDF, liver dysfunction; MA, microcytic anemia; MAHA; microangiopathic haemolytic anemia; mo, months; norm, normal; SE, status epilepticus; SND, sensorineural deafness; y, years; <2 pc, less than the second height percentile for age and sex.

Supplementary Information

Table S1. Amino acyl tRNA synthetase genes associated with human disorders.

Cytoplasmic amino acyl tRNA synthetases		**Mitochondrial amino acyl tRNA synthetases**	
Gene	**Human disorder**	**Gene**	**Human disorder**
AARS	Charcot-Marie-Tooth disease, axonal, type 2N	*AARS2*	Infantile cardiomyopathy
CARS	-	*CARS2*	-
DARS	Hypomyelination with brainstem and spinal cord involvement and leg spasticity (HBSL)	*DARS2*	Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL)
EPRS	-	*EARS2*	Leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL)
FARSA *FARSB*	-	*FARS2*	Combined oxidative phosphorylation deficiency 14; infantile onset of a fatal encephalopathy with refractory seizures, lack of psychomotor development, lactic acidosis
GARS	Charcot-Marie-Toothdisease	*N/A*	-
HARS	Usher syndrome type 3B	*HARS2*	Perrault syndrome 2
IARS	-	*IARS2*	-
KARS	Recessive intermediate Charcot-Marie-Tooth neuropathy; Congenital visual impairment with progressive microcephaly	*N/A*	-
LARS	Liver dysfunction, anemia, decompensation, developmental delay and seizures (ILFS1)	*LARS2*	Mitochondrial encephalomyopathy lactic acidosis and stroke-like symptoms (MELAS); Perrault syndrome 4
MARS	Liver dysfunction, anemia, decompensation, developmental delay, seizures and interstitial lung disease (ILFS2)	*MARS2*	Spastic ataxia with leukoencephalopathy
NARS	-	*NARS2*	-
N/A	-	*PARS2*	-
QARS	-	*N/A*	-
RARS	-	*RARS2*	Pontocerebellar hypoplasia, type 6
SARS	-	*SARS2*	Hyperuricemia, pulmonary hypertension, renal failure in infancy and alkalosis
TARS	-	*TARS2*	-
VARS	-	*VARS2*	-
WARS	-	*WARS2*	-
YARS	Charcot-Marie-Tooth disease, dominant intermediate C	*YARS2*	MLSA: Myopathy, lactic acidosis, sideroblastic anemia

Variation in 8 cytoplasmic and 10 mitochondrial aminoacyl tRNA synthetase genes has been associated with human disorders. To date, only two ARS genes (*LARS* and *MARS*) have been implicated in a liver phenotype.

Table S2. Detailed natural history of each of the 10 patients with ILFS1.

Natural History
Patient AIII:8 has had two episodes of ALF with minor illness. His last episode occurred at 17 months of age. At age 12 years he has liver fibrosis with nodularity evident on ultrasound without other clinical or biochemical evidence of chronic liver disease.
Patient AIII:10 has had three episodes of ALF, with the last episode occurring at 22 months of age which was also associated with seizures and encephalopathy. Now aged 3 years she has mild gross motor delay.
Patient AII:9 is the eldest patient (35 years) and is currently well. He has had multiple episodes of liver dysfunction with illness and he had a single encephalopathic episode associated with measles infection at age 4 years. His last episode of liver dysfunction was noted at 26 years of age when he had a viral gastroenteritis. At this time he also had a tonic-clonic seizure. His hepatic transaminases and hepatic synthetic function are normal when well. He has not had a liver biopsy or detailed hepatic assessment.
Patient AII:13, the adult sister of AII:9, now aged 28 years, experienced multiple episodes of liver dysfunction in infancy beginning at 6 weeks with gastroenteritis. She had seizures in the first year of life and these recurred aged 11 years with a febrile illness. She had microcytic anemia requiring transfusions in early childhood. Her last known episode of liver dysfunction was with a viral upper respiratory tract infection at age 16 years. She is now of normal stature with no abnormalities on examination or on routine biochemical assessment. Her blood film shows microcytes with mild anemia.
Patient AIII:4 had persistent severe anemia requiring multiple red cell transfusions with hypoalbuminemia and mild liver dysfunction in infancy. He was very well from 1 year until age 5 years when he presented with anemia, oedema and a single seizure in the context of a febrile illness. He was found to have ARF which resolved and is currently being treated with anti-hypertensive agents.
Patient AIII:6 developed ALF at age 3 months, in the setting of a coryzal illness. During this critical illness he also developed epileptic encephalopathy, microangiopathic haemolytic anemia and acute renal failure, from which he recovered with supportive care. He had profound global developmental delay and sensorineural deafness but subsequently had few hospitalisations until age 8 years when he died during an encephalopathic episode triggered by a respiratory tract infection.

Patient AIV:1 had three episodes of ALF in infancy. At 4 years old he presented with pneumonia (Influenza A H1N1 infection) and had prolonged drug resistant status epilepticus and subsequently died in ICU. CT brain showed cerebral oedema with unilateral infarction. Post mortem examination confirmed hypoxic ischaemic encephalopathy which had progressed to unilateral infarction.
Patient AIV:2 had recurrent severe hypoalbuminaemia and hypoketotic hypoglycaemia in infancy and required gastrostomy feeding. He has had two episodes of ALF triggered by viral infection. During the last episode, at 18 months, (triggered by parainfluenza and RSV infection) he became encephalopathic requiring intubation and ventilation for 4 days. Ammonia level was normal. CT brain during this period was normal with no evidence of cerebral oedema or raised intracranial pressure. Blood film during this episode was consistent with microangiopathic haemolytic anemia. While initial neurological recovery was slow, two months later, he has made a full recovery. He is now 20 months and has mild global developmental delay.
Patient BII:3 has had two episodes of prolonged status epilepticus (at age 2.5 and 3.5 years) with encephalopathy during febrile illnesses. He was noted to have hepatomegaly and had been investigated in infancy for hepatomegaly, liver dysfunction, hypoalbuminemia and microcytic anemia. He has mild global developmental delay.
Patient CII:1 had ALF at 5 months of age. She developed seizures with fever and/or viral illness at age 1.5 years. She has had numerous episodes, some status epilepticus and other were generalised tonic-clonic seizures. MRI brain showed areas of multiple infarcts, old and new, she has thrombotic risk factors. She has had microcytic anemia. She has gross motor developmental delay but excellent cognition.

Abbreviations: ALF: acute liver failure; ARF: acute renal failure; CT: Computed tomography, RSV: MRI: magnetic resonance imaging

Table S3. Amino acid composition of human albumin.

Amino acid symbol	Amino acid	Number of times each amino acid is present in the protein	% of total amino acid content (X/609)
L	Leucine	64	10.51
A	Alanine	63	10.34
E	Glutamic acid	62	10.18
K	Lysine	60	9.85
V	Valine	43	7.06
D	Aspartic acid	36	5.91
F	Phenylalanine	35	5.75
C	Cysteine	35	5.75
T	Threonine	29	4.76
S	Serine	28	4.60
R	Arginine	27	4.43
P	Proline	24	3.94
Q	Glutamine	20	3.28
Y	Tyrosine	19	3.12
N	Asparagine	17	2.80
H	Histidine	16	2.63
G	Glycine	13	2.13
I	Isoleucine	9	1.48
M	Methionine	7	1.15
W	Tryptophan	2	0.33

Breakdown of the amino acid composition of human albumin. Albumin contains 609 amino acid residues. Leucine is one of the most abundant amino acids present in this protein.