Severe Neonatal Manifestations of Infantile Liver Failure Syndrome Type 1 Caused by Cytosolic Leucine-tRNA Synthetase Deficiency

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Abstract *Background:* Deleterious mutations in cytosolic leucine-tRNA synthetase (LARS) cause infantile liver failure syndrome, type 1 (ILFS1), a recently recognized, rare autosomal recessive disorder (OMIM151350). Only six families with ILFS1 have been reported in the literature. Patients with ILFS1 are typically diagnosed between 5 and 24 months of age with failure to thrive, developmental delays, encephalopathy, microcytic anemia, and chronic liver dysfunction with recurrent exacerbations following childhood illnesses. Neonatal manifestations of this disorder have not been well documented.

Case Report: We report a premature female newborn with intrauterine growth restriction, failure to thrive, congenital anemia, anasarca, and fulminant liver failure leading to lethal multiple organ failure. Liver failure in this infant was characterized by a disproportionate impairment of liver synthetic function, including severe coagulopathy and hypoalbuminemia without significant defects in liver detoxification or evidence of hepatocellular injury during early phase of the disease. Whole-exome sequencing of child-parent trio identified two inherited missense mutations in *LARS* in this patient. One, c.1292T>A;

p.Val431Asp, has been reported in patients with ILFS1, while the other, c.725C>T; p.Pro242Leu, is novel. Both mutations involve amino acid residues in the highly conserved editing domain of LARS, are predicted to be functionally deleterious, and presumably contribute to the clinical manifestations in this patient.

Conclusion: This is the first case documenting neonatal manifestation of ILFS1, highlighting early, severe, and disproportionate defects in liver synthetic function. Timely diagnosis of ILFS1 is crucial to guide critical clinical management and improve outcomes of this rare and potentially life-threatening disorder.

Introduction

Neonatal acute liver failure (NALF) is a rare and poorly understood disease entity (Taylor and Whittington 2016; Bitar et al. 2017). Determining the etiology of NALF presents a unique and urgent clinical challenge given extensive differential diagnoses and often rapid and fulminant disease progression (Sundaram et al. 2011). Establishing a timely diagnosis is critical to guiding clinical management, as many NALF disorders are medically and/or surgically treatable with favorable outcomes (Bitar et al. 2017; Sundaram et al. 2011; Devictor et al. 2011).

LARS encodes cytosolic leucine-tRNA synthetase, an enzyme critical for incorporating leucine (the most abundant amino acid) during protein synthesis. Deleterious mutations in *LARS* cause infantile liver failure syndrome, type 1 (ILFS1), a recently recognized, rare autosomal recessive disorder (OMIM151350). Only six families have

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been reported in the literature, with the majority of affected individuals from the Irish Traveller population (Casey et al. 2012, 2015; El-Gharbawy et al. 2015; Lin et al. 2017). Reported patients have been diagnosed between 5 and 24 months of age, with variable clinical phenotypes, including liver dysfunction with recurrent exacerbations, microcytic anemia, failure to thrive, encephalopathy, developmental delays, and intellectual disability. Neonatal manifestations of this disorder have not been well documented.

We report a case of a premature female neonate who presented with intrauterine growth restriction (IUGR), congenital anemia, anasarca, and fulminant liver failure who was found to be a compound heterozygote for two deleterious mutations in *LARS*. This is the first report documenting severe neonatal manifestations of *ILFS1* and highlights defects in liver synthetic function as an initial presentation of this rare and potentially lethal genetic disorder.

Case Report

This female infant was born at 29–6/7 weeks of gestation by Cesarean section in the setting of intrauterine growth restriction and maternal preeclampsia. Maternal history was significant for two prior first-trimester pregnancy losses of unknown etiology and a threatened first-trimester loss in this pregnancy. APGAR scores were 7 and 9 at 1 and 5 min of life, respectively. Physical examination at birth was remarkable for a small for gestational age female (weight 760 g, 5th percentile; length 32 cm, 1st percentile; head circumference 25.5 cm, 18th percentile) with a two-vessel cord. Immediate neonatal course was notable for mild respiratory distress, congenital anemia (hemoglobin 9.1 g/dL; MCV 101.1 fL), transient hyponatremia, and apnea of prematurity. Breast milk feedings were initiated but stopped several times due to abdominal distention. At 25 days of age, the infant developed increased work of breathing, abdominal distention, diffuse edema, hematemesis, and hematochezia. Her laboratory studies showed coagulopathy and hypoalbuminemia; her abdominal imaging demonstrated bowel wall thickening and moderate ascites. She was stabilized on mechanical ventilation and treated with empiric antibiotics.

Head ultrasound showed mild asymmetric prominence of the left lateral ventricle without hemorrhage. Abdominal ultrasound showed increasing echogenicity of the right kidney, intermittent reversal of blood flow within the main portal vein, reversal of blood flow within the left portal vein, and moderate ascites, but without significant hepatosplenomegaly. Echocardiography showed pulmonary hypertension, a small muscular ventricular septum defect, and normal biventricular function. Exploratory laparotomy (performed due to a concern for necrotizing enterocolitis)

demonstrated approximately 30 mL of clear ascites but no evidence of bowel necrosis or malrotation. A catheterized urine specimen showed moderate leukocyte esterase and grew *Candida lusitanae* at 3 days; the patient was treated with 14 days of intravenous fluconazole. Evaluations for causes of infectious hepatitis, including herpes simplex virus, cytomegalovirus, enterovirus, parvovirus, and hepatitis B and C, were negative.

During her hospital course, laboratory studies showed persistent anemia (nadir 7.9 g/dL; normal 14.1–20.1 g/dL) despite several packed red cell transfusions and hypoalbuminemia (1.5–2.8 g/dL; normal, 3.5–5.3 g/dL) refractory to multiple albumin transfusions and protein intake of up to 3.5 g/kg/day from total parenteral nutrition. She had severe coagulopathy (PT 16.5–44.9 s [normal 10.0–13.6 s]; PTT 34.2–166.4 s [normal 26.9–62.5 s]; INR 1.6–4.6 [normal 0.86–1.22]). Plasma ammonia levels were normal (55–129 µmol/L; normal <150 µmol/L). Serum bilirubin levels were mildly elevated (peak total bilirubin 6.3 mg/dL; peak direct bilirubin 4.6 mg/dL) at 29 days of life, and serum transaminases were normal to borderline elevated: aspartate aminotransferase (26–99 IU/L; normal <31 IU/L), alanine aminotransferase (7–68 IU/L; normal <31 IU/L), and γ-glutamyltransferase (23–27 U/L; normal 12–123 U/L). As her illness progressed, she developed thrombocytopenia (nadir 23 K/mL; normal 180–327 K/mL), low factor VII activity (5%; normal >25%), low fibrinogen level (47 mg/dL; normal 82–383 mg/dL), and elevated D-dimer (6.42–7.55 mg/L FEU; normal 0.11–0.42 mg/L FEU), which did not correct after multiple fresh frozen plasma and cryoprecipitate transfusions. Despite maximal ventilation support, aggressive treatment of infection, and optimized nutrition, she developed severe respiratory failure, vasopressor-refractory hypotension, renal failure, and profound anasarca. She died due to multi-organ failure at 53 days of age.

The infant had an elevated immunoreactive trypsinogen (IRT level >468 ng/mL; normal <100 ng/mL) on her initial state newborn screen. However, molecular testing for 23 common mutations for cystic fibrosis (core pathogenic mutations recommended by American College of Medical Genetics) was negative, and a repeat IRT also was normal. Her serum lactate levels were intermittently elevated (4–6.9 mmol/L; normal, 0.5–2.2 mmol/L). Total and fractionated carnitine levels and acylcarnitine profile did not suggest a known inborn error of metabolism. Plasma amino acids showed nonspecific moderate elevation of several amino acids including tyrosine (361–748 µmol/L; normal 20–108 µmol/L), methionine (96–148 µmol/L; normal 7–43 µmol/L), and glycine (745–927 µmol/L; normal 87–323 µmol/L). Urine organic acids showed increased excretion of 4-hydroxyphenyllactate and 4-hydroxyphenylpyruvate, suggesting either liver

immaturity or dysfunction. Quantitative blood succinylacetone was normal (1.9 nmol/mL; normal <5 nmol/mL). Carbohydrate-deficient transferrin electrophoresis showed a normal profile. Alpha-fetoprotein levels were age-appropriate (6,964–11,601 ng/mL; normal <22,062 ng/mL). Serum ferritin levels were normal to borderline elevated (314–952 ng/mL; normal 200–600 ng/mL).

A single nucleotide polymorphism (SNP) microarray identified an 807 kb interstitial duplication at 17p12 of unknown clinical significance which was inherited from her mother who is asymptomatic. Mitochondrial DNA sequencing and deletion/duplication analysis found no pathogenic variants. Whole-exome sequencing utilizing the child-parent trio revealed compound heterozygosity in the infant for two potentially pathogenic variants in *LARS* [NM_020117.9] (Fig. 1). One missense variant, c.1292T>A; p.Val431Asp in exon 14 [NM_020117.9], was maternally inherited, has a cumulative allele frequency of 0.0003612, and has not been observed in a homozygous state in large population cohorts (gnomad.broadinstitute.org). This amino acid substitution is located in the *LARS* editing domain crucial to protein function (Fig. 1) (Han et al. 2012; Huang et al. 2014) and occurs at a position where only amino acids with similar properties to valine (isoleucine and leucine) appear tolerated across species. In silico analysis indicates that amino acid substitution of valine by aspartate results in significant changes in polarity, charge, and size of the residue and predicts a probably damaging effect on protein structure and/or function [PolyPhen-2: 0.994; probably damaging (<http://genetics.bwh.harvard.edu/pph2/>) and CADD-PHRED: 29.5 (<https://cadd.gs.washington.edu/>)]. Importantly, this variant has been previously described in two unrelated, non-Irish Traveller patients with ILFS1 (El-Gharbawy et al. 2015). Five of the eight previously reported mutations in ILFS1 patients are located in this *LARS* editing domain (Fig. 1). The second missense variant, c.725C>T; p.Pro242Leu in

exon 8 [NM_020117.9], was paternally inherited, has a cumulative allele frequency of 0.00001628, and has not been observed in a homozygous state in large population cohorts (gnomad.broadinstitute.org). This amino acid substitution is located in the CH1 hairpin motif of the editing domain of *LARS* at an amino acid position that is highly conserved across species (Fig. 1) (Lin et al. 2017). In silico analysis predicts that it is probably damaging to protein structure and/or function (PolyPhen-2: 0.998; probably damaging; CADD-PHRED: 32). This novel variant has not been reported previously in ILFS1. Subsequent testing confirmed that a healthy 3-year-old sister of this patient is heterozygous for this variant.

Discussion

ILFS1 is a newly recognized, rare autosomal recessive disorder caused by deleterious mutations in *LARS*. Patients typically present from infancy to early childhood during periods of illness or other physiological stress. Noted clinical features include preterm delivery, intrauterine growth restriction, failure to thrive, microcytic anemia, hypoalbuminemia, coagulopathy, recurrent liver dysfunction, liver fibrosis and cirrhosis, renal dysfunction, encephalopathy, developmental delays, and intellectual disability (Table 1). Chronic liver dysfunction with intermittent exacerbations (triggered by fever, infection, and physical and/or metabolic stress conditions) leads to progressive liver failure, fibrosis, and cirrhosis (Table 1). In addition to premature birth, IUGR, and congenital anemia, our patient presented with a fulminant course of liver failure leading to multi-organ failure. This case provides valuable clues for initial recognition and underscores importance of early diagnosis of this potentially lethal genetic disorder. Although available genetic findings and core clinical manifestations of this patient are consistent with ILFS1,

Fig. 1 Cytosolic leucine-tRNA synthetase domains and mutations found in ILFS1 patients. Brackets indicate the editing, catalytic, and c-terminal domains of human cytosolic leucine-tRNA synthetase. The HIGH motif is the ATP binding site; CH1 and CH2 represent CP1 hairpin 1 and 2; KMSKS is the mobile pentapeptide motif regulating conformational changes of tRNA synthetase, with amino acid position

of each motif in parentheses. *c.725C>T*; *p.Pro242Leu* (novel) is the paternal variant for this patient and is *italicized*. **c.1292T>A**; **p.Val431Asp** (previously reported) is the maternal variant for this patient marked in **bold**. All other variants are previously reported variants

Table 1 Clinical features of reported patients with infantile liver failure syndrome type 1 caused by cytosolic leucine-tRNA synthetase deficiency

Source	Casey et al. (2012, 2015)	Casey et al. (2015)	El-Gharbawy et al. (2015)	El-Gharbawy et al. (2015)	Lin et al. (2017)	Current report
Ethnicity	Irish Travellers	Ashkenazi Jewish	Caucasian, non-Irish Traveller	Caucasian, non-Irish Traveller	Chinese	Caucasian, non-Irish Traveller
No. of reported families (no. of patients)	2 (9)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Variant 1	c.1118A>G; p.Tyr373Cys	c.1511C>T; p.Ala504Val	c.242G>T; p.Gly81Val	c.903delG; p.Gln302fs	c.1183G>A; p.Asp395Asn	c.725C>T; p.Pro242Leu
Variant 2	c.1118A>G; p.Tyr373Cys	c.1842C>G; p.Asp614Lys	c.1292T>A; p.Val431Asp	c.1292T>A; p.Val431Asp	c.2133_2135del; p.Leu712del	c.1292T>A; p.Val431Asp
In silico analysis with PolyPhen-2; prediction (score)	p.Tyr373Cys; damaging (0.993)	NR	NR	NR	p.Asp395Asn; damaging (0.999)	p.Pro242Leu; probably damaging (0.998) p.Val431Asp; probably damaging (0.994)
Age at presentation	1 month–2 years	5 months	17 months	13 months	18 months	Neonatal (<1 month)
Sex (no. individuals)	Male (7); female (2)	Female	Female	Male	Male	Female
Pregnancy complications	NR	NR	Recurrent pregnancy loss	Recurrent pregnancy loss; HELLP	NR	Recurrent pregnancy loss; preeclampsia
Premature birth	6/9	NR	Y	Y	N	Y
Low birth weight	9/9	Y	NR	NR	Y	Y
Failure to thrive	9/9	Y	Y	NR	Y	Y
Microcytic anemia	7/9	Y	Y	NR	Y	Y
Acute liver failure (age of first episode)	5/9 (2–8 months)	Y (5 months)	Y (17 months)	NR	NR	Y (<1 month)
Intermittent liver dysfunction (within first year)	4/9	NR	NR	Y	Y	Y
Hypoalbuminemia	9/9	Y	NR	NR	Y	Y
Coagulopathy (elevated PT/PTT/INR)	9/9	Y	Y	Y	Y	Y
Hyperbilirubinemia	9/9	Y	NR	Y	Y	Y
Hepatomegaly	3/9	NR	NR	NR	Y	N
Hepatic steatosis	4/4	NR	Y	Hepatic necrosis	Y	NR
Liver fibrosis, nodules, cirrhosis	2/4	NR	Y	NR	Y	NR
Developmental delays	8/9	Y	NR	Y	NR	NR
Seizure disorders	9/9	Y	NR	NR	NR	N
Renal failure or Fanconi syndrome	2/9	NR	Y	NR	NR	Y
Brain imaging studies	Mild cerebral atrophy 4/7	Stroke	NR	NR	NR	Asymmetric prominence of the left lateral ventricle
Other biochemical genetic testing ^a	Negative	Negative	Negative	Elevation of plasma tyrosine	Negative	Elevation of plasma tyrosine
Additional notes						Ascites; anasarca; DIC

Y yes/present, N no/absent, NR not reported, DIC disseminated intravascular coagulation, HELLP hemolysis, elevated liver enzymes, and low platelets, INR international normalized ratio, PT prothrombin time, PTT partial thromboplastin time

^a Targeted biochemical testing such as plasma amino acids, urine organic acids, succinylacetone, acylcarnitine profile, and fractionated carnitine

functional characterizations of these variants are warranted to establish a definitive causal relationship.

Prematurity, IUGR, and anemia are relatively nonspecific findings commonly seen in neonates admitted to the neonatal intensive care unit (NICU). Impaired liver function (with elevated transaminases and γ -glutamyl transferase, hyperbilirubinemia, hyperammonemia, hypoglycemia, hypoalbuminemia, and coagulopathy in neonates) can be caused by infectious hepatitis, gestational alloimmune liver disease, histiophagocytic lymphohistocytosis, systematic illnesses (such as hypoxia and sepsis), and metabolic disorders (including tyrosinemia type I, galactosemia, alpha-1-antitrypsin deficiency, and mitochondrial disorders). In the majority of these disorders, hepatocellular injury results in elevated transaminases and, ultimately, defects in liver detoxification and liver synthetic function. Although ILFS1 patients develop recurrent hepatic dysfunction leading to progressive liver fibrosis and cirrhosis with disease progression, this case highlights the disproportionately impaired liver synthetic function causing severe and persistent coagulopathy and hypoalbuminemia during early course of the disease. In our patient, liver transaminases and γ -glutamyltransferase were normal or only mildly elevated, suggesting absence of significant hepatocyte and biliary tree injury. Additionally, liver detoxification functions of ammonia, bilirubin, and bile acids were normal or only mildly affected. This liver failure profile, coupled with congenital anemia, prenatal and postnatal growth restriction, and negative evaluations for other common causes of neonatal liver failure, serves as a valuable clue for early diagnosis of ILFS1 during the neonatal period. Additionally, early WES-based diagnosis could potentially establish or eliminate inborn errors of metabolism and other genetic disorders presenting with neonatal liver failure and reduce expansive diagnostic workups. Data from WES analysis also may provide neonatologists and pediatric critical care specialists with prognostic and therapeutic guidance in managing such diseases. In ILFS-1, for example, initiation of early, aggressive nutrition support has been proposed to benefit affected patients (El-Gharbawy et al. 2015).

The pathophysiology of LARS deficiency is incompletely understood. The failure to incorporate leucine efficiently during protein synthesis is particularly detrimental to organs that depend on high-protein synthesis rate (such as the liver and bone marrow) during periods of rapid physical growth in infancy and early childhood. We speculate that this mechanism contributes to the observed intrauterine growth restriction and failure to thrive in ILFS1 patients. This mechanism also may in part explain why clinical symptoms are improved in ILFS1 patients during later adolescence and adulthood when physical growth and

demands for protein synthesis slow down. Consistent with this mechanism, tissues with high demand for protein synthesis, such as liver, hematopoietic cells, and immune systems, are commonly affected in ILFS1 patients (Casey et al. 2012, 2015; El-Gharbawy et al. 2015; Lin et al. 2017). Finally, recent studies showed that LARS functions as a leucine sensor in regulating the mTORC1-signaling pathway, which plays a key role in many cellular processes, including autophagy, and cell growth (Han et al. 2012).

Only six families with ILFS1 were reported in the literature (Table 1). Two families, including the original family with eight patients, are Irish Travellers (Casey et al. 2012, 2015). However, current literature supports an expanding awareness of ILFS1 in populations outside of this group. *LARS* mutations have been found in unrelated ILFS1 patients of Ashkenazi Jewish, Caucasian non-Irish Traveller, and Chinese descent (Casey et al. 2015; El-Gharbawy et al. 2015; Lin et al. 2017) (Table 1). LARS deficiency-related ILFS1 likely is under-recognized for several reasons. Early clinical features such as IUGR, failure to thrive, anemia, and liver dysfunction are relatively nonspecific in premature infants. In older children, ILFS1 exacerbations are often triggered by common childhood illnesses such as fever and systematic infection, and the associated hepatic and red cell abnormalities may be attributed to the illness itself. Thus, high clinical vigilance is needed to recognize the profile of ILFS1 manifestations at different ages leading to early diagnosis and optimized treatment to achieve desirable clinical outcomes and prevent permanent liver damage and other severe complications.

Though specific treatment to ILFS1 is not yet available, patients with ILFS1 should be admitted, even with minor illnesses, for aggressive treatment and supportive care of the underlying causes of physical and/or metabolic stress. High dextrose infusions (to suppress catabolism) and sufficient protein intake (to promote protein synthesis) are indicated during acute exacerbations to minimize liver damage and reduce risks of complications involving multi-organs (Casey et al. 2015; El-Gharbawy et al. 2015).

Details of the Contributions of Individual Authors

Christina M. Peroutka, Jacqueline L. Salas, Juliet Bishop, and Jacquelyn F. Britton contributed to collection, analyses, and interpretation of data and drafting the article. W. Christopher Golden, Maureen M. Gilmore, Lisa Kratz, Jill A. Fahrner, and Tao Wang contributed to conception and design, data analysis and interpretation, and drafting the article or revising it critically for important intellectual content.

All authors read and approved the final manuscript.

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Ethics Statement

This study is in accordance with the ethical standards of the responsible conduct on human subject research (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Parental Consent

The authors obtained parental consent to use protected health information presented in this report.

Synopsis

The triad of congenital anemia, prenatal and postnatal growth restriction, and severely impaired liver synthetic functions are early neonatal manifestation of infantile liver failure syndrome 1 caused by a deficiency of cytosolic leucine-tRNA synthetase.

Compliance with Ethics Guidelines

Christina Peroutka, Jacqueline Salas, Jacquelyn Britton, Juliet Bishop, Lisa Kratz, Maureen M. Gilmore, Jill Fahrner, W. Christopher Golden, and Tao Wang declare that they have no conflict of interest.

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