Received: 25 September 2020 | Revised: 13 November 2020 | Accepted: 27 November 2020

DOI: 10.1002/ajmg.a.62012

CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A WILEY

Severe course with lethal hepatocellular injury and skeletal muscular dysgenesis in a neonate with infantile liver failure syndrome type 1 caused by novel *LARS1* mutations

Katsuya Hirata[1] | Nobuhiko Okamoto[2] | Chihiro Ichikawa[3] | Shouta Inoue[1] | Masatoshi Nozaki[1] | Kimihiko Banno[4] | Toshiki Takenouchi[5,6] | Hisato Suzuki[6] | Kenjiro Kosaki[6]

[1]Department of Neonatal Medicine, Osaka Women's and Children's Hospital, Izumi, Japan

[2]Department of Medical Genetics, Osaka Women's and Children's Hospital, Izumi, Japan

[3]Department of Laboratory Medicine, Osaka Women's and Children's Hospital, Izumi, Japan

[4]Department of Physiology II, Nara Medical University, Nara, Japan

[5]Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan

[6]Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan

Correspondence
Katsuya Hirata, Department of Neonatal Medicine, Osaka Women's and Children's Hospital, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan.
Email: khirata0513@gmail.com

Funding information
Baby and Infant in Research of Health and Development to Adolescent and Young adult (BIRTHDAY), Grant/Award Number: JP 20gk0110038h

Abstract

Infantile liver failure syndrome type 1 (ILFS1) is a recently recognized autosomal recessive disorder caused by deleterious mutations in the leucyl-tRNA synthetase 1 gene (*LARS1*). The LARS1 enzyme is responsible for incorporation of the amino acid leucine during protein polypeptide synthesis. Individuals with *LARS1* mutations typically show liver failure from infancy to early childhood during periods of illness or other physiological stress. While 25 patients from 15 families with ILFS1 have been reported in the literature, histological reports from autopsy findings are limited. We report here a premature male neonate who presented with severe intrauterine growth retardation, microcytic anemia, and fulminant liver failure, and who was a compound heterozygote for two novel deleterious mutations in *LARS1*. An autopsy showed fulminant hepatitis-like hepatocellular injury and fibrogenesis in the liver and a lack of uniformity in skeletal muscle, accompanied by the disruption of striated muscle fibers. Striking dysgenesis in skeletal muscle detected in the present case indicates the effect of *LARS1* functional deficiency on the musculature. Whole-exome sequencing may be useful for neonates with unexplained early liver failure if extensive genetic and metabolic testing is inconclusive.

KEYWORDS

extremely low birth weight infant, intrauterine growth retardation, *LARS*, neonatal acute liver failure, tRNA synthetase deficiency

1 | INTRODUCTION

The gene leucyl-tRNA synthetase 1 (*LARS1*) encodes cytosolic leucine-tRNA synthetase, which is an enzyme critical for incorporating leucine during protein synthesis. A *LARS1* mutation was first reported in Irish travelers by Casey et al. (2012). *LARS1* mutations cause infantile liver failure syndrome type 1 (ILFS1), which is a rare autosomal recessive disorder (MIM 615438). To date, across 15 families, 25 patients with ILFS1 have been reported in the literature (Areeg El-Gharbawy et al., 2015; Casey et al., 2012; Casey et al., 2015; Fuchs et al., 2019; Lenz et al., 2020; Lin, Zheng, Guo, Cheng, & Song, 2017; Peroutka et al., 2019). These individuals typically show liver failure from infancy to early childhood during periods of illness or other physiological stress. Other reported major symptoms of ILFS1 include abnormalities regarding growth (intrauterine growth retardation [IUGR], small for gestational age [SGA], and preterm birth), the nervous system (neurodevelopmental delay and seizures), blood (microcytic anemia), musculature (muscular hypotonia), and the immune system (frequent infections) (Table S1, Supporting Information) (Lenz et al., 2020).

We report a premature male neonate with severe IUGR, SGA, microcytic anemia, and fulminant liver failure who was a compound heterozygote for two novel deleterious mutations in *LARS1*. The autopsy findings showed fulminant liver cirrhosis and skeletal muscular dysgenesis.

2 | METHODS

2.1 | Editorial policies and ethical considerations

This study was approved by the Institutional Review Board of Osaka Women's and Children's Hospital and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from the parents of the patient.

2.2 | Molecular analysis

DNA was isolated from peripheral blood from the patient and both parents, and from the umbilical cord of the patient's older sibling for whole-exome sequencing (WES) and direct sequencing as described previously (Uehara et al., 2020). The NEBNext Ultra II DNA Library Prep Kit for Illumina (Biolabs, Ipswich, MA) was used for preparation of the library. Sequencing was performed on the Illumina HiSeq X platform (Illumina, San Diego, CA).

3 | CLINICAL REPORT

A Japanese male infant was born to a 33-year-old multiparous woman. There was a family history for infantile death due to liver failure of his older sister, who was born at 29 weeks of gestation with a birth weight of 594 g (−3.9 *SD*). Pregnancy was achieved spontaneously but complicated by severe IUGR from 18 weeks of gestation. Hypertensive disorders of pregnancy were not observed. Cesarean delivery was performed at 29 weeks and 6/7 days of gestation for non-reassuring fetal heart tracing. He was delivered with a birth weight of 606 g (−4.3 *SD*), length of 32 cm (−3.1 *SD*), head circumference of 23.2 cm (−2.1 *SD*), and Apgar scores of 3 and 7 at 1 and 5 min, respectively. He was intubated immediately, provided with surfactant, and admitted to the neonatal intensive care unit. A physical examination at birth was remarkable for prominent hepatomegaly (Figure S1a). Breast feeding was initiated and gradually increased, reaching 100 ml kg^{-1} day^{-1} on Day 11 of life.

During his hospital course, persistent anemia (nadir hemoglobin level, 6.9 g/dl; normal, 12–16 g/dl), which required repeated red cell transfusions, and hypoalbuminemia (nadir albumin level, 2.0 g/dl; normal, 3.9–4.9 g/dl) refractory to repeated albumin transfusions were observed. Cholestasis with progressive elevation of serum direct bilirubin levels was observed from Day 5 of life without any triggers such as infections or surgery (peak direct bilirubin level, 21.6 mg/dl; normal, 0–0.2 mg/dl) (Figure 1). Evaluations for causes of infectious hepatitis, including cytomegalovirus, and hepatitis B and C, were negative. A bright liver was first detected on Day 6 by ultrasonography, and this progressed to liver cirrhosis-like findings on Day 28 (Figure S1b,c). Markers of liver fibrosis were already elevated on Day 14 as follows: type III procollagen peptide (19 U/ml; normal, <1 U/ml), type IV collagen (1,695 ng/ml; normal, <137 ng/ml), and hyaluronic acid (544 ng/ml; normal, <50 ng/ml). He had severe coagulopathy (prothrombin time international normalized ratio, 1.55–3.51 [normal, 0.9–1.1]), activated partial thromboplastin time, 78–>200 s [normal, 25–45 s]), and low fibrinogen levels (<50–206 mg/dl; normal, 180–306 mg/dl). With progression of his disease, plasma ammonia

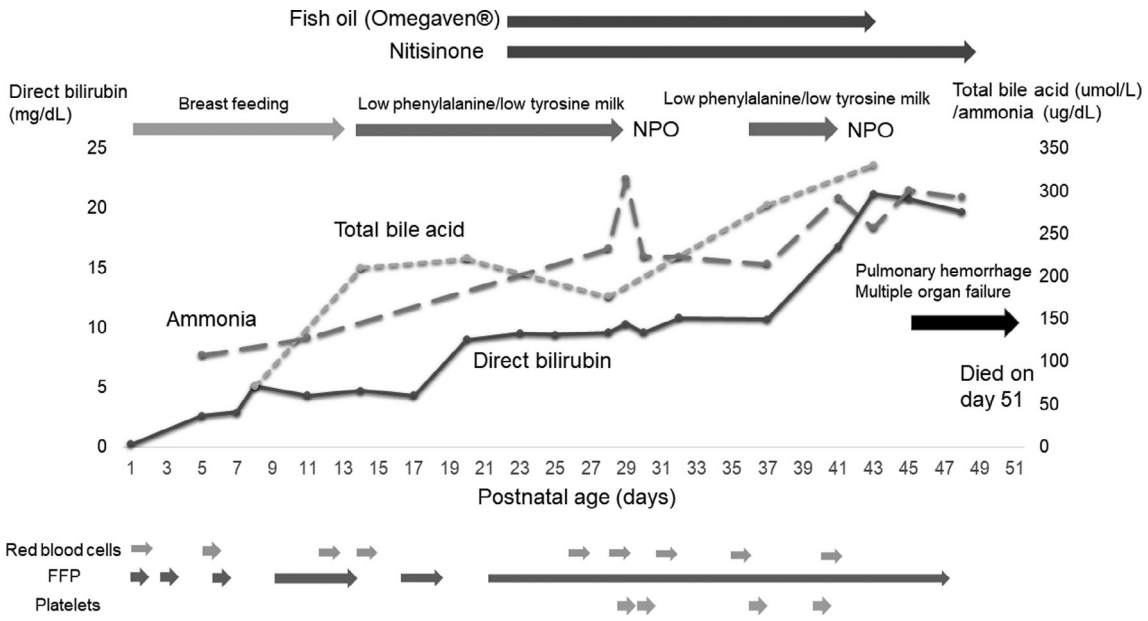

FIGURE 1 The patient's clinical course from birth to death. FFP, fresh frozen plasma; NPO, nil per os

levels (108–315 μg/dl; normal, 30–86 μg/dl) and total bile acid levels (70.7–330.6 μmol/L; normal, 0–10 μmol/L) increased (Figure 1). Serum transaminases were normal to slightly elevated throughout the clinical course (aspartate aminotransferase levels, 14–77 IU/L [normal, 8–38 IU/L]; alanine aminotransferase levels, 8–135 IU/L [normal, 4–43 IU/L]). Serum ferritin levels were slightly elevated (330 ng/ml; normal, 25–280 ng/ml), but creatine kinase levels were not (22–105 U/L; normal, 32–187 U/L). Plasma amino acids showed elevated tyrosine levels (282–2,593 nmol/ml; normal, 38.4–-89.4 nmol/ml). Urine organic acids showed increased excretion of p-hydroxyphenylpyruvate, p-hydroxyphenylacetate, and N-acetyltyrosine, which suggested tyrosinemia type 1. Therefore, we started low-dose tyrosine and phenylalanine milk administration from Day 14 and nitisinone from Day 22. However, tyrosinemia type 1 was ruled out because elevated urine succinylacetone was not detected (0.01 nmol/ml; normal, <1.0 nmol/ml), despite measurements being

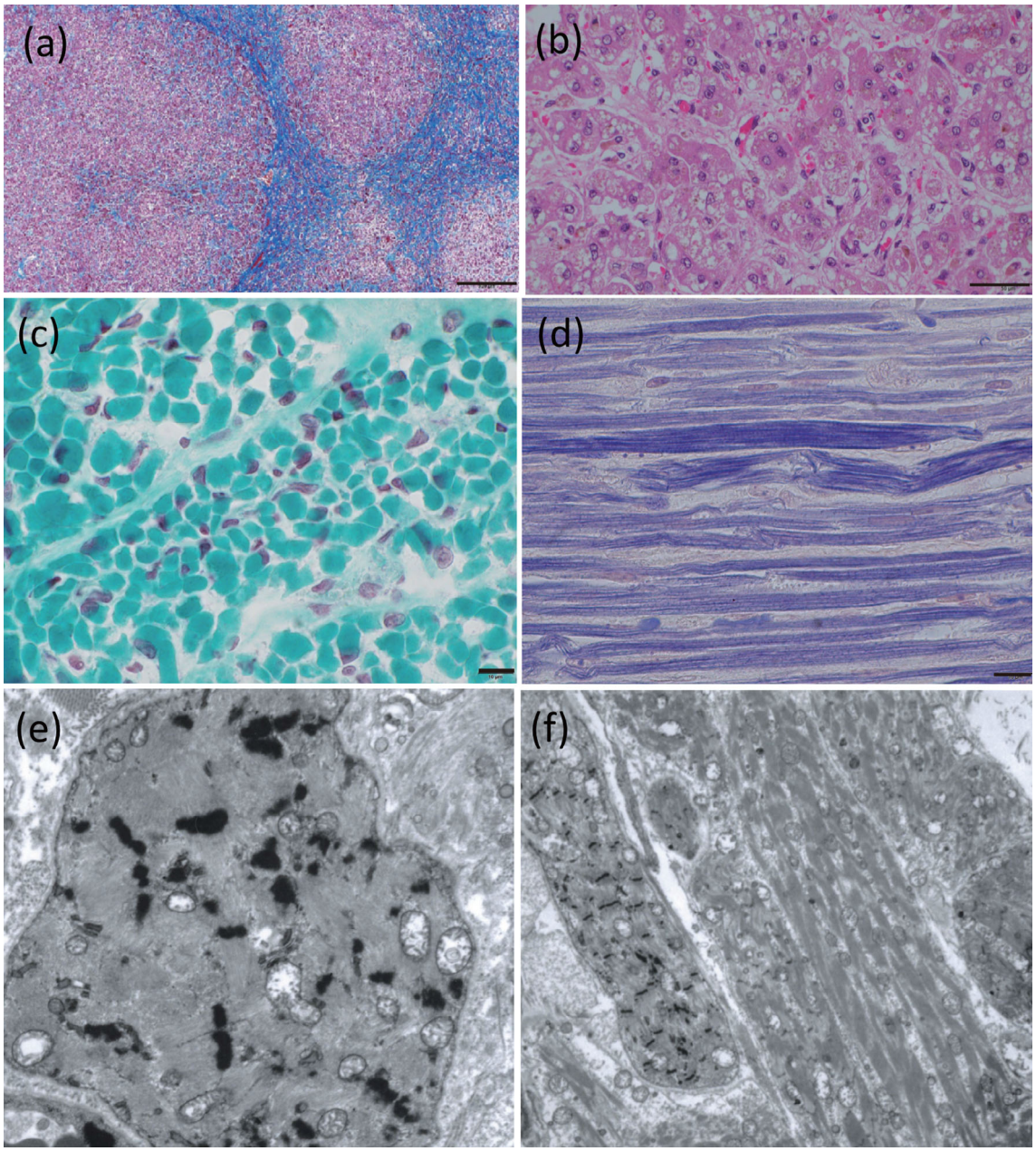

FIGURE 2 Autopsy findings of the patient. (a) Masson trichrome staining of the liver showing varying sizes of foci of regenerative nodules. Scale bar = 500 μm. (b) Hematoxylin and eosin staining of the liver showing ballooning and cellular cholestasis, indicating marked cellular injury. Scale bar = 50 μm. (c) Gomori–trichrome staining of skeletal muscle showing that muscle fibers are thin and variable in size. No signs of inflammation, necrotic muscle fibers, or ragged red fibers were observed. Scale bar = 10 μm. (d) Phosphotungstic acid hematoxylin staining of skeletal muscle showing that cross-striations were not observed in some muscle fibers. Scale bar = 10 μm. (e) (f) Electron microscopy of skeletal muscle showing that the sarcomere structure was not observed or it was disordered. (e) (×3,000) and (f) (×8,000)

repeated. We started fish oil from Day 22, but liver failure progressed. Enteral feeding was stopped on Day 28 because of notable elevations of ammonia (Figure 1). He required mechanical ventilation throughout the clinical course. He suffered from pulmonary hemorrhage and severe intraventricular hemorrhage and died of multiple organ failure caused by liver dysfunction at 51 days. No apparent seizures or hypotonia were observed during the clinical course.

In the infant, trio-based WES after his death showed compound heterozygosity for two potentially pathogenic variants in *LARS1* [NM_020117.10]. One missense variant, c.1351A>T; p.Ile451Phe in exon 14, was paternally inherited. This amino acid substitution is located in the *LARS1* editing domain, which is crucial for protein function. In silico analysis showed that the amino acid substitution of isoleucine by phenylalanine resulted in the alignment of sequencing reads, and variant calling was performed by DRAGEN Bio-IT Platform v3.2 (Illumina). The combined annotation-dependent depletion score, which reflects the relative pathogenicity of human variants (Kircher et al., 2014), was 24.5 for p.Ile451Phe. Variant effect prediction tools showed that p.Ile451Phe was probably damaging (PolyPhen2; http://genetics.bwh.harvard.edu/pph2/) and deleterious (SIFT; http://sift.bii.a-star.edu.sg/). The other variant, which was maternally inherited, was a splice donor site variant (c.213+1G>A) in intron 3. This upstream-located mutation in the splice donor site inhibits normal splicing, which leads to deleterious changes in protein function by skipping exon 3 or activating cryptic splice sites. These two variants have not been reported previously in ILFS1. The same two variants in *LARS1* were detected in subsequent analysis of the preserved umbilical cord of the patient's older sister who died with a similar course.

An autopsy showed fulminant hepatitis-like hepatocellular injury, and fibrogenesis accompanied by proliferation in the bile duct were detected in the liver (Figure 2a,b). Electron microscopy showed normal mitochondria. Macrovesicular and microvesicular steatosis was not detected in the present case. In the skeletal muscle, muscle fibers lacked uniformity, with diameters of 1.5–4.5 μm (normal, 7 μm), accompanied by the disruption of striated muscle fibers (Figure 2c,d). Mitochondria were normal and the sarcomere structure was not observed or it was abnormal in electron microscopy of skeletal muscle (Figure 2e,f). Pulmonary edema, pulmonary hemorrhage, and hyperinflation of the lungs were detected compatible with bronchopulmonary dysplasia. Acute tubular necrosis was found in the kidney. There were no major findings in the heart, pancreas, esophagus, stomach, small intestine, colon, adrenal gland, or bone marrow. A brain examination was not performed.

4 | DISCUSSION

ILFS1 is a newly recognized, rare autosomal recessive disorder caused by deleterious mutations in *LARS1* (Casey et al., 2012). The LARS1 enzyme is responsible for incorporation of the amino acid leucine during protein polypeptide synthesis. As well as the 17 mutations in *LARS1* reported previously (Lenz et al., 2020), the present case identified two novel mutations (Table S2). Clinical presentation in the present case is consistent with ILFS1 (Table S1). Among the 25 previously reported patients with ILFS1, only one had severe manifestations in the neonatal period, which is similar to our case (Peroutka et al., 2019). The presence of an upstream-located splice donor mutation (c.213+1G>A) in intron 3 may explain the severity of our case, combined with another deleterious missense variant (c.1351A>T) in exon 14. Either skipping exon 3 (88 bp deletion) or activating another possible cryptic splice site in intron 3 (resulting in the extension of exon 3 by more than 37 bp at the 3′ end) results in a shift of the open reading frame, creating a premature stop codon which will shorten a transcribed protein to remove most of the editing domain. This transcript also leads to mRNA degradation through nonsense-mediated decay. Healthcare providers should be prepared to assess the mutational sites in *LARS1*, which may affect the severity as well as onset of this disease.

Characteristic autopsy findings in the present case were fulminant hepatitis-like hepatocellular injury and fibrogenesis in the liver and a lack of uniformity in skeletal muscle, accompanied by the disruption of striated muscle fibers. Marked macrovesicular and microvesicular steatosis in the liver was reported as a prominent and consistent feature in six biopsies from four patients with ILFS1 (Casey et al., 2015). In the present case, vesicular steatosis was not detected, but further severe findings were seen that were compatible with the severe clinical course. Although muscular hypotonia has frequently been reported in ILFS1 cases, histological findings of the musculature are limited (Lenz et al., 2020). Striking dysgenesis in skeletal muscle detected in the present case indicates the effect of the deficiency in *LARS1* function on the musculature. Further investigations with molecular approaches are required to determine the pathophysiology of ILFS1.

Extremely low birth weight infants who are severely SGA are risk of parenteral nutrition-associated cholestasis (Baserga & Sola, 2004) (Robinson & Ehrenkranz, 2008). However, similar to our case, some reports showed early liver fibrosis in extremely low birth weight infants who were SGA, despite minimal parenteral nutrition (Arai et al., 2010). The possibility of a genetic disorder, such as ILFS1, should be taken into account in severely SGA premature infants who develop early liver failure, despite minimum parenteral nutrition or adequate enteral breast feeding, and in those with exclusion of other causes of neonatal liver failure (e.g., infectious hepatitis, metabolic disorders, and alloimmune liver disease). WES may be considered for neonates with unexplained early liver failure if extensive genetic and metabolic testing is inconclusive (Lunke et al., 2020).

ACKNOWLEDGMENTS

This work was supported by the project for Baby and Infant in Research of Health and Development to Adolescent and Young adult (BIRTHDAY) (Grant No. JP 20gk0110038h) from the Japan Agency for Medical Research and Development. We also thank Drs Norio Sakai, Tomiko Kuhara, Hirokazu Arai, Satoshi Ohnishi, Maiko Wagata, Jun Sasahara, Makoto Takeuchi, and Yuri Etani for supporting this study.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Katsuya Hirata: Study design; interpretation of the data; manuscript writing. **Nobuhiko Okamoto:** Supervision. **Chihiro Ichikawa:** Collection and interpretation of the clinical data. **Shouta Inoue:** Collection and interpretation of the clinical data. **Masatoshi Nozaki:** Collection and interpretation of the clinical data. **Kimihiko Banno:** Analysis and interpretation of the genetic data. **Toshiki Takenouchi:** Analysis and interpretation of the genetic data. **Hisato Suzuki:** Analysis and interpretation of the genetic data. **Kenjiro Kosaki:** Supervision. All authors were involved in revising the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Katsuya Hirata https://orcid.org/0000-0003-3148-9892
Toshiki Takenouchi https://orcid.org/0000-0002-7311-4135
Hisato Suzuki https://orcid.org/0000-0002-8122-7180

REFERENCES

Arai, H., Noguchi, A., Goto, R., Matsuda, T., Nakajima, H., & Takahashi, T. (2010). Liver fibrosis in an extremely small infant for gestational age. *Tohoku Journal of Experimental Medicine*, *221*(3), 181–185. https://doi.org/10.1620/tjem.221.181

Areeg El-Gharbawy, J. S., Ghaloul-Gonzalez, L., Mroczkowski, J., Goldstein, A., Venkat, V., Dobrowolski, S., … Vockley, J. (2015). LARS mutations in non-Irish travelers: An under-recognized multi-system disorder characterized by infantile hepatopathy during physiological stress. *Mitochondrion*, *24*, S40–S41.

Baserga, M. C., & Sola, A. (2004). Intrauterine growth restriction impacts tolerance to total parenteral nutrition in extremely low birth weight infants. *Journal of Perinatology*, *24*(8), 476–481. https://doi.org/10.1038/sj.jp.7211137

Casey, J. P., McGettigan, P., Lynam-Lennon, N., McDermott, M., Regan, R., Conroy, J., … Ennis, S. (2012). Identification of a mutation in LARS as a novel cause of infantile hepatopathy. *Molecular Genetics and Metabolism*, *106*(3), 351–358. https://doi.org/10.1016/j.ymgme.2012.04.017

Casey, J. P., Slattery, S., Cotter, M., Monavari, A. A., Knerr, I., Hughes, J., … Crushell, E. (2015). Clinical and genetic characterisation of infantile liver failure syndrome type 1, due to recessive mutations in LARS. *Journal of Inherited Metabolic Disease*, *38*(6), 1085–1092. https://doi.org/10.1007/s10545-015-9849-1

Fuchs, S. A., Schene, I. F., Kok, G., Jansen, J. M., Nikkels, P. G. J., van Gassen, K. L. I., … van Hasselt, P. M. (2019). Aminoacyl-tRNA synthetase deficiencies in search of common themes. *Genetics in Medicine*, *21*(2), 319–330. https://doi.org/10.1038/s41436-018-0048-y

Kircher, M., Witten, D. M., Jain, P., O'Roak, B. J., Cooper, G. M., & Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nature Genetics*, *46*(3), 310–315. https://doi.org/10.1038/ng.2892

Lenz, D., Smith, D. E. C., Crushell, E., Husain, R. A., Salomons, G. S., Alhaddad, B., … Staufner, C. (2020). Genotypic diversity and phenotypic spectrum of infantile liver failure syndrome type 1 due to variants in LARS1. *Genetics in Medicine*, *22*, 1863–1873. https://doi.org/10.1038/s41436-020-0904-4

Lin, W. X., Zheng, Q. Q., Guo, L., Cheng, Y., & Song, Y. Z. (2017). Clinical feature and molecular diagnostic analysis of the first non-caucasian child with infantile liver failure syndrome type 1. *Zhongguo Dang Dai Er Ke Za Zhi*, *19*(8), 913–920.

Lunke, S., Eggers, S., Wilson, M., Patel, C., Barnett, C. P., Pinner, J., … Stark, Z. (2020). Feasibility of ultra-rapid exome sequencing in critically ill infants and children with suspected monogenic conditions in the Australian public health care system. *JAMA*, *323*(24), 2503–2511. https://doi.org/10.1001/jama.2020.7671

Peroutka, C., Salas, J., Britton, J., Bishop, J., Kratz, L., Gilmore, M. M., … Wang, T. (2019). Severe neonatal manifestations of infantile liver failure syndrome type 1 caused by cytosolic leucine-tRNA synthetase deficiency. *JIMD Reports*, *45*, 71–76. https://doi.org/10.1007/8904_2018_143

Robinson, D. T., & Ehrenkranz, R. A. (2008). Parenteral nutrition-associated cholestasis in small for gestational age infants. *Journal of Pediatrics*, *152*(1), 59–62. https://doi.org/10.1016/j.jpeds.2007.06.002

Uehara, T., Yamada, M., Umetsu, S., Nittono, H., Suzuki, H., Fujisawa, T., … Kosaki, K. (2020). Biallelic mutations in the LSR gene cause a novel type of infantile intrahepatic cholestasis. *Journal of Pediatrics*, *221*, 251–254. https://doi.org/10.1016/j.jpeds.2020.01.064

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Hirata K, Okamoto N, Ichikawa C, et al. Severe course with lethal hepatocellular injury and skeletal muscular dysgenesis in a neonate with infantile liver failure syndrome type 1 caused by novel *LARS1* mutations. *Am J Med Genet Part A*. 2020;1–5. https://doi.org/10.1002/ajmg.a.62012