Biallelic Mutations in the *LSR* Gene Cause a Novel Type of Infantile Intrahepatic Cholestasis

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We identified biallelic pathogenic mutations in the *Lipolysis-stimulated lipoprotein receptor* (*LSR*) gene in a patient with infantile intrahepatic cholestasis. We established that mutations in the *LSR* gene, which encodes a protein which is critical for the formation of tricellular tight junctions in the liver, are a novel cause of pediatric cholestasis. *(J Pediatr 2020; ■:1-4).*

Progressive familial intrahepatic cholestasis (PFIC) refers to a group of genetic disorders that are characterized by defects in the transport or excretion of bile salts. The phenotypes of patients with PFIC include cholestasis, often with onset during early infancy, pruritus, and growth retardation. Mutations of the *ATP8B1*,[1] *ABCB11*,[2] *ABCB4*,[3] *TJP2*,[4] and *NR1H4*[5] genes have been implicated in the etiology of PFIC, in which the cholestasis often progresses to liver cirrhosis.

The lipolysis-stimulated lipoprotein receptor (LSR) is expressed in the liver and other organs and represents a component protein of tricellular tight junctions, where 3 epithelial cells meet.[6] Complete loss of function of the LSR protein has been shown to result in liver hypoplasia and fetal death in mice.[7] The roles of LSR in human health and disease have not yet been established. One patient with intrahepatic cholestasis with a rare variant of *LSR* has been reported.[8] The patient was identified through exome analysis of patients with abnormal bile acid metabolism and cholestatic disease following negative clinical sequencing of known cholestasis-related genes. The patient was born to consanguineous parents and had the homozygous nonsynonymous variant p.Glu235Gly in *LSR*.[8] It remains unclear whether the homozygosity for the *LSR* p.Glu235Gly variant contributed to the intrahepatic cholestasis phenotype or whether the association occurred by chance. Unequivocal demonstration of biallelic pathogenic variants of *LSR* in another patient with the same or overlapping phenotype would confirm the causality.[9] Here we report a patient who fulfilled this criterion, establishing LSR deficiency as a novel cause of human liver disease.

gGT	Gamma-glutamyltransferase
H&E	Hematoxylin and eosin
LSR	Lipolysis-stimulated lipoprotein receptor
PFIC	Progressive familial intrahepatic cholestasis

Clinical Report

The proband was a 5-year-old female patient who was the first child of healthy, nonconsanguineous Japanese parents. She was born at 39 weeks of gestation with a birth weight of 3102 g (+0.7 SD) and a birth length of 51.0 cm (+1.2 SD). Jaundice was not unduly prolonged during the neonatal period and was never apparent during infancy. At age 5 months, she developed severe and uncontrollable itching. She could not sleep well at night, and her skin became thickened because of the severe itching. The results of laboratory studies at age 9 months were serum total bilirubin, 0.9 mg/dL (normal range, <1.2 mg/dL); serum aspartate aminotransferase, 95 U/L (normal range, <30 U/L); serum alanine aminotransferase, 111 U/L (normal range, <30 U/L); serum total bile acids, 71 μmol/L (normal range, <14.4 μmol/L); serum gamma-glutamyltransferase (gGT), 48 U/L (normal range, <73 U/L); and serum alkaline phosphatase, 3629 U/L (normal range, <338 U/L).

At age 15 months, the child was started on treatment with ursodeoxycholic acid (which represses CYP7A, an important enzyme for bile acid synthesis), alfacalcidol, and tocopherol acetate. Despite this treatment, her serum bile acid levels remained high, and severe itching persisted. The ursodeoxycholic acid treatment was discontinued at age 23 months. Treatment of the uncontrollable severe itching with nalfurafine hydrochloride was attempted between age 3 and 5 years but proved ineffective. Her verbal milestones were slightly delayed; she could not speak any

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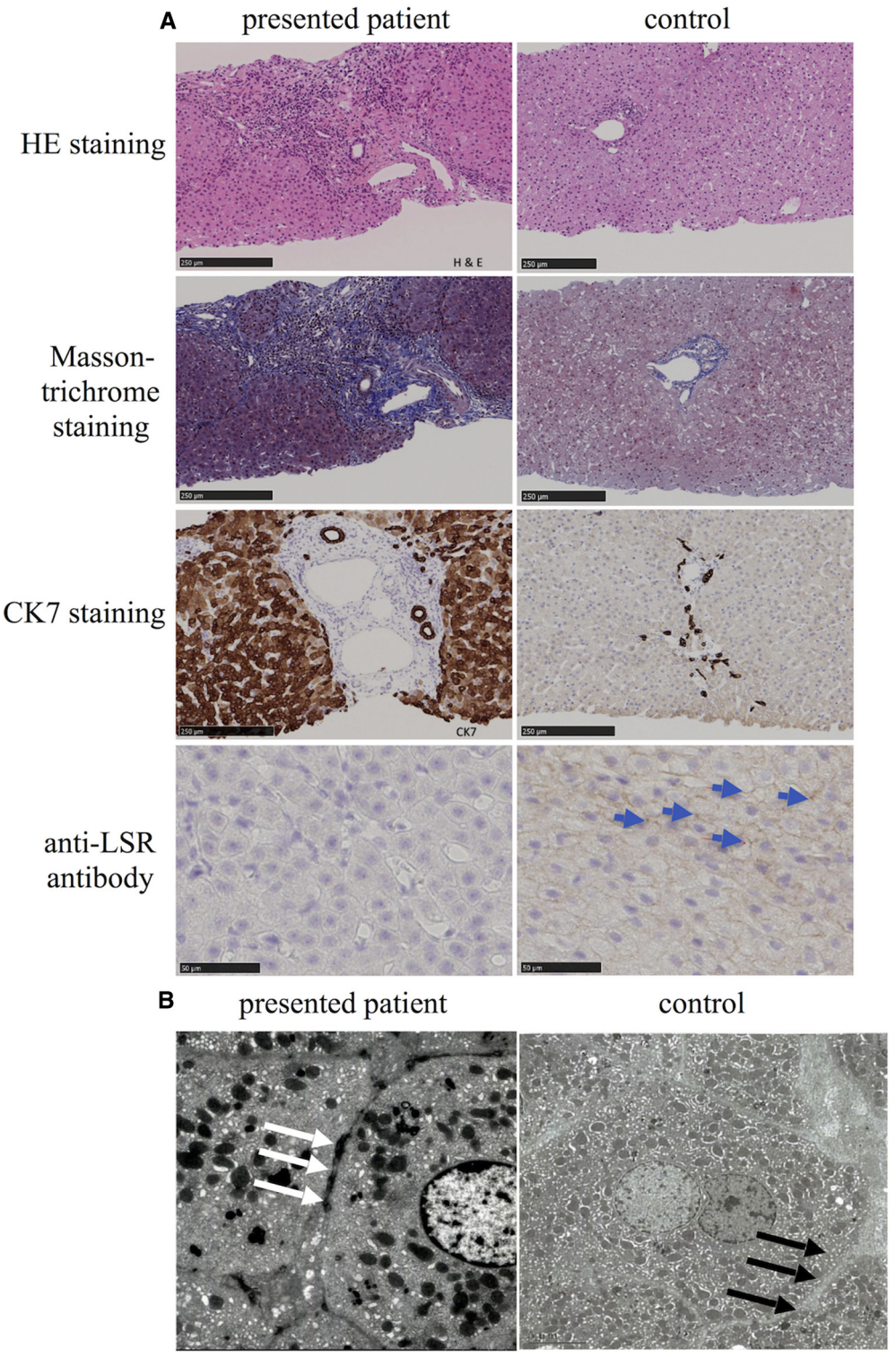

Figure. (Left) Liver biopsy samples obtained from the patient with infantile intrahepatic cholestasis and biallelic *LSR* mutations reported herein. (Right) Liver biopsy samples obtained from a 1-year, 7-month-old girl with borderline liver enlargement and normal liver function, which served as the control samples in the immunohistochemical and electron microscopy studies. **A,** Histological findings of the liver biopsy samples assessed by H&E staining, Masson trichrome staining, and immunostaining with anti-cytokeratin 7 antibodies and anti-LSR antibodies. Note the dystrophic regression with fibrosis (Masson trichrome staining), oxyphilic degeneration, ductular reaction (immunostaining with anti-cytokeratin 7 antibody), and absence of LSR expression (immunostaining with anti-LSR antibody). Arrows indicate the expression of LSR at the tight junctions in the liver samples from the control patient. **B,** Electron micrographs of liver biopsy samples obtained from the patient reported here (left) and the control patient (right). Note the congestion of the liver with uniformly dense bile (*white arrows*) in the patient with biallelic *LSR* mutations compared with the control patient.

significant words at age 2 years, spoke her first word at age 3 years, and was able to verbally communicate by age 5 years. Her fine motor milestones were not delayed; she could draw circles at age 3 years, triangles at 4 years, and squares at 5 years. Her height at age 5 years, 6 months was 109.0 cm (−0.1 SD), and her body weight at 5 years, 9 months was 21.05 kg (+1.0 SD).

Tissue sections obtained by needle biopsy of the liver, performed at age 5 years, were examined after hematoxylin and eosin (H&E) staining, Masson trichrome staining, and immunostaining with anti-cytokeratin 7 antibodies and anti-LSR antibodies. The H&E-stained sections showed mild inflammatory cell infiltration in the portal tracts (**Figure**, A). Masson trichrome staining of the sections showed diffuse dystrophic regression of the hepatocytes, with the cells replaced by fibrosis (**Figure**, A). Immunostaining with antibody for cytokeratin 7, which is expressed exclusively in the bile duct epithelial cells in the normal liver, revealed strongly aberrant expression in the hepatocytes (**Figure**, A). This ectopic expression suggests activation of hepatic progenitor cells or a regenerative phenomenon of the hepatocytes (so-called "ductular reaction"). No bile plugs, giant cell formation, or feathery degeneration were observed (**Figure**, A).

Serum, urine, and stool samples were also obtained from the patient at age 5 years (**Table**). Bile acid levels were analyzed using high-performance liquid chromatography electrospray ionization/multiple reaction monitoring mass spectrometry with negative ion detection. Samples obtained from 3 age-matched children and one patient with PFIC with *ABCB11* mutations were also analyzed as negative and positive controls, respectively. The results showed elevated bile acid levels in the serum and urine and decreased bile acid levels in the stool (**Table**). No unusual bile acids were detected.

Electron microscopy examination revealed a marked increase of uniformly dense bile in the tight junction complexes (**Figure**, B, left, white arrows) in the patient compared with controls (**Figure**, B, right, black arrows), which was thought to be related to tight junction dysfunction (4000×). Cholestasis with bile thrombi was also found.

On approval from the local Institutional Review Board and informed consent from the parents of the child, molecular studies were performed. Genomic DNA extracted from the peripheral blood of both the patient and her parents were analyzed. Whole-exome analysis of the samples from the patient and her parents was performed using the HiSeq platform (Illumina, San Diego, California) and SureSelectXT Human All Exon V6 (Agilent Technologies, Santa Clara, California). The quality scores were >Q30 for more than 93.7% of the nucleotides. The variants in the patient and her parents were filtered for candidate de novo mutations using DeNovoCheck. The nonsynonymous variant of the *LSR* gene (ENST00000361790), chr19(GRCh37): g.35749851C>T (c.602C>T, p.Ala201Val) was detected in exon 3. The c.602C>T variant was absent in the database of 3552 normal Japanese individuals (Japanese Multi Omics Reference Panel; https://jmorp.megabank.tohoku.ac.jp/201911/),[10] and also absent in the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/). Furthermore, the heterozygous frameshift variant chr19(GRCh37):g.35758119dup, (c.1396dup, p.Arg466ProfsTer51) was detected in exon 8 and was derived from the mother, and the minor allele frequency was 0.0014 in the jMorp database. Both variants were confirmed by Sanger sequencing.

The nonsynonymous variant p.Ala201Val located within the Ig domain had a Combined Annotation Dependent Depletion score of 28.8 and was predicted to be deleterious.[11] According to the method Evolutionary Amino acid and Structural Encodings with Multiple Models (EASE-MM) method, the non-synonymous variant would decrease the protein stability.[12] Furthermore, immunohistochemical staining using anti-LSR antibodies showed absent expression of LSR at the tricellular tight junctions in the patient reported herein, whereas well-defined LSR expression was observed at the tricellular junctions in the control case (**Figure**, A, arrows). Overall, both *LSR* variants were considered pathogenic according to the Standards and Guidelines for the Interpretation of Sequence Variants of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[13]

No other candidate genes were identified in the autosomal recessive model. In addition, no mutations were identified in any of the known causative genes for cholestasis, including *JAG1, NOTCH1* (Alagille syndrome), *ATP8B1*, *ABCB11*, *ABCB4*, *TJP2*, *FXR* (PFIC), *VPS33B* (arthrogryposis/renal/cholestasis), *TJP2*, *BAAT*, *EPHX1* (hypercholemia), *DHCR7* (Smith–Lemli–Opitz syndrome), or *MYO5B* (microvillus inclusion disease).

Table. Bile acid analyses of blood, urine, and stool in the present patient, a patient with PFIC2, and 3 healthy children as negative controls

Total bile acid level	Present patient	Patient with PFIC2	Negative control 1	Negative control 2	Negative control 3
Urine, mmol/molCr	8.74	55.39	0.94	1.01	0.38
Blood, μmol/L	75.11	361.29	7.90	1.62	0.49
Stool, μmol/g	2.76	2.58	6.98	7.04	40.57

Discussion

Here we report a female patient with cholestasis and hepatic fibrosis, features consistent with the diagnosis of infantile intrahepatic cholestasis, and biallelic mutations in the *LSR* gene (ENST00000361790): 1 maternally derived frameshift variant in exon 8 and a de novo nonsynonymous variant in exon 3. Immunostaining using anti-LSR antibody showed absent staining for LSR protein at the tricellular tight junctions in the patient's liver (**Figure**, A). Before this case, another similar patient with homozygous nonsynonymous

mutations of the *LSR* gene manifesting the features of infantile intrahepatic cholestasis was reported from Saudi Arabia.[8] Yet, the causal relationship between *LSR* and infantile intrahepatic cholestasis was not established from a standpoint of function with respect to the nonsynonymous changes. Documentation of biallelic pathogenic variants of the *LSR* gene and lack of LSR protein expression in the liver in our patient establishes *LSR* deficiency as a novel cause of infantile intrahepatic cholestasis. The later onset, the rather benign histology with regard to fibrosis, and the lack of progression suggest that this new disease phenotype could be milder than most of the other forms of PFIC described. One could speculate the existence of compensatory mechanisms, such as enhanced clearance of bile acids through the urine rather than through the stool. Both the present patient and the patient from Saudi Arabia reportedly have the homozygous *LSR* nonsynonymous variant manifested mild speech delay and intellectual disability.[8] It is possible that these developmental problems represent extrahepatic features of LSR deficiency, given that LSR is also known to be expressed in the central nervous system.

Striking similarities were observed between the present patient and Saudi Arabian patient; in both patients, the onset of symptoms, consisting mainly of intractable itching, occurred several months after birth. In our patient, the transformation of cytokeratin 7 expression from the bile ducts to the hepatocytes, shown by the strong staining of the hepatocytes for cytokeratin 7, and elevated serum levels of the primary bile acids were the features indicative of intrahepatic cholestasis.[8] The conditions considered to be forms of PFIC are characterized by the serum gGT levels in the normal or low range for the degree of cholestasis (except for the condition associated with MDR3 deficiency). Consistent with this, our patient and the patient with the homozygous *LSR* nonsynonymous variant reported from Saudi Arabia had normal serum gGT levels.

The present patient with *LSR* mutation had high serum bile acid levels in serum and low bile acid levels in stool (**Table**). This combination is indicative of reduced secretion of bile acids into the intestine. In addition, electron microscopy examination showed congestion of the liver with uniformly dense bile. It is likely that the tricellular tight junction protein LSR plays a role in one or more of the following processes of bile acid transport: transport from sinusoids to hepatocytes, transport in hepatocytes, and transport from hepatocytes to the canalicular membrane. Although the histopathology of the lesion observed in our patient was comparable to that in patients with mutations of *TJP2*, which also encodes a component protein of the tight junctions in the liver,[5] the role of the integrity of tricellular tight junctions in the pathogenesis of infantile intrahepatic cholestasis requires further investigation. ■

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