Lysosomal Acid Lipase Deficiency Unmasked in Two Children With Nonalcoholic Fatty Liver Disease

Ryan W. Himes, MD,* Sarah E. Barlow, MD, MPH,* Kevin Bove, MD,‡ Norma M. Quintanilla, MD,§ Rachel Sheridan, MD,‡ Rohit Kohli, MBBS, MS¶

*Lysosomal Acid Lipase Deficiency (LAL-D) is a classic lysosomal storage disorder characterized by accumulation of cholesteryl ester and triglyceride. Although it is associated with progressive liver injury, fibrosis, and end-stage liver disease in children and adolescents, LAL-D frequently presents with nonspecific signs that overlap substantially with other, more common, chronic conditions like nonalcoholic fatty liver disease (NAFLD), metabolic syndrome, and certain inherited dyslipidemias. We present 2 children with NAFLD who achieved clinically significant weight reduction through healthy eating and exercise, but who failed to have the anticipated improvements in aminotransferases and γ-glutamyl transferase. Liver biopsies performed for these "treatment failures" demonstrated significant microvesicular steatosis, prompting consideration of coexisting metabolic diseases. In both patients, lysosomal acid lipase activity was low and LIPA gene testing confirmed LAL-D. We propose that LAL-D should be considered in the differential diagnosis when liver indices in patients with NAFLD fail to improve in the face of appropriate body weight reduction.

Lysosomal acid lipase deficiency (LAL-D) is a classic lysosomal storage disorder characterized by accumulation of cholesteryl ester and triglyceride. Although it is associated with progressive liver injury, fibrosis, and end-stage liver disease in children and adolescents, LAL-D frequently presents with nonspecific signs that overlap substantially with other, more common, chronic conditions like nonalcoholic fatty liver disease (NAFLD), metabolic syndrome, and certain inherited dyslipidemias. We present 2 children with NAFLD who achieved clinically significant weight reduction through healthy eating and exercise, but who failed to have the anticipated improvements in aminotransferases and γ-glutamyl transferase. Liver biopsies performed for these “treatment failures” demonstrated significant microvesicular steatosis, prompting consideration of coexisting metabolic diseases. In both patients, lysosomal acid lipase activity was low and LIPA gene testing confirmed LAL-D. We propose that LAL-D should be considered in the differential diagnosis when liver indices in patients with NAFLD fail to improve in the face of appropriate body weight reduction.

Lysosomal acid lipase deficiency (LAL-D) is an autosomal recessive, single gene disorder, caused by mutations in LIPA (OMIM #278000) resulting in lysosomal accumulation of cholesteryl ester and triglyceride in hepatocytes, endothelium, and myeloid-derived cells. Depending on ethnicity and race, prevalence estimates range from 1:40,000 to 1:300,000.1,2 LAL-D may present in early infancy with malabsorption and catastrophic liver failure culminating in death before 6 months of age, a clinical phenotype formerly called Wolman disease. On the other hand, LAL-D may present insidiously in children and adolescents with variable and nonspecific findings like hepatosplenomegaly, hepatic steatosis, and dyslipidemia, elevations in aminotransferases, or a lipid profile characterized by elevated low-density lipoprotein cholesterol (LDL-c) and depressed high-density lipoprotein cholesterol levels. In spite of the later presentation in these patients, there is evidence of clinically significant liver disease and liver-related mortality among children and adolescents with LAL-D, underscoring the importance of identifying affected patients.3

The diagnosis of LAL-D, particularly in children and adolescents, is hampered by the lack of specific clinical findings, the broad spectrum of disease presentation, and significant overlap with more common diseases. In primary care practices, as well as specialty clinics, features of LAL-D, like hepatic steatosis, elevated aminotransferases, and dyslipidemia, are more often seen coexisting with conditions like nonalcoholic fatty liver disease (NAFLD), metabolic syndrome, and dyslipidemias.
and certain inherited dyslipidemias. NAFLD, linked tightly to overweight and obesity, is estimated to affect 33% of adults and 9.6% of children in the general population, making it the most common chronic liver disease in the United States. Unfortunately, there are no laboratory-based diagnostic tests for NAFLD, and although routine liver imaging such as ultrasound may be suggestive of hepatic steatosis, its sensitivity is poor until steatosis exceeds 30%. Thus, only liver biopsy can discriminate between simple hepatic steatosis, a nonprogressive or slowly progressive form of NAFLD, and the more aggressive nonalcoholic steatohepatitis (NASH), which is defined by inflammation and/or fibrosis. Given the invasiveness, cost, and potential risks of applying diagnostic liver biopsy to a condition whose prevalence is nearly 10% of all children, NAFLD is often diagnosed on clinical grounds in overweight or obese individuals after ruling out other conditions through standard investigations.

Herein, we report 2 children with LAL-D, who were initially presumed to have NAFLD alone. We highlight the diagnostic challenge of identifying a rare, but important disease, which may coexist with, or be misdiagnosed as, the much more common, NAFLD.

**PATIENT 1**

An 8-year-old Hispanic girl presented to her pediatrician after nuchal acanthosis nigricans was identified during a school-wide screening program. Her weight was 38.1 kg (89th percentile), height 134.7 cm (53rd percentile), and BMI 21.1 (94th percentile). Based on her BMI and the presence of acanthosis nigricans, screening laboratory tests were performed that revealed the following: aspartate aminotransferase (AST), 207 U/L (normal, 15–40 U/L); alanine aminotransferase (ALT), 401 U/L (normal, 7–35 U/L); total cholesterol, 235 mg/dL (normal, <170 mg/dL); LDL-c, 167 mg/dL (normal, <110 mg/dL), prompting referral to a pediatric gastroenterologist and recommendations about weight reduction. Her examination at the gastroenterology clinic was remarkable only for the presence of nuchal acanthosis nigricans; she did not have hepatosplenomegaly. A diagnostic evaluation included normal results for α-1 antitrypsin PI-type, antiactin antibody, antinuclear antibody, liver-kidney microsomal antibody, total immunoglobulin (Ig) G level, ceruloplasmin, infectious hepatitis (A/B/C), and tissue transglutaminase IgA. An abdominal ultrasound revealed normal hepatic echogenicity and no organomegaly.

Over the next 3 years, the patient participated in structured programs for weight management and ultimately reduced her BMI to the 85th percentile; follow-up laboratory testing revealed the following: AST, 61 U/L; ALT, 83 U/L; total cholesterol, 200 mg/dL; and LDL-c, 136 mg/dL. Given the persistent aminotransferase elevations in spite of significant BMI improvement, a follow-up liver biopsy was obtained. Light and electron microscopy (EM) revealed widespread microvesicular steatosis and lipid-filled cytoplasmic vesicles in hepatocytes and Kupffer cells (Fig 1C). Minimal lobular inflammation and stage 1 lobular and portal fibrosis were unchanged. Based on the predominantly microvesicular pattern of steatosis, the differential diagnosis was broadened to include metabolic diseases, including LAL-D. Her lysosomal acid lipase activity...
was low, 0.011 nmol/punch/hour, and confirmatory Sanger sequencing of \textit{LIPA} revealed a homozygous mutation, c.894G>A, which alters a splice site and leads to skipping exon 8, resulting in \( \sim 97\% \) reduction in enzyme activity.\(^7\)

**PATIENT 2**

A 16-year-old white girl presented to the emergency department with fever and symptoms suggestive of a urinary tract infection. She had severe obesity, her weight was 137 kg (>99th percentile), height 160.5 cm (35th percentile), with a resultant BMI of 53.18 (>99th percentile).

She underwent a renal ultrasound that confirmed pyelonephritis but also incidentally revealed splenomegaly. Her blood counts suggested hypersplenism with low platelet (78000/μL) and leukocyte (3100/μL) counts. Based on these initial observations, the treating inpatient service requested laboratory tests and consulted gastroenterology. Relevant initial test results included the following: AST, 57 U/L (normal, 15–45 U/L); ALT, 87 U/L (normal, 7–35 U/L); \( \gamma \)-glutamyl transferase (GGT), 108 U/L (normal, 7–32 U/L); LDL-c, 102 mg/dL (normal, <200 mg/dL); high-density lipoprotein cholesterol, 43 mg/dL (normal, >45 mg/dL); and triglycerides, 83 mg/dL (normal, <126 mg/dL).

The gastroenterology team continued the diagnostic evaluation of elevated aminotransferases and GGT in the setting of morbid obesity. This included normal results for \( \alpha \)-1 antitrypsin PI-type, antismooth muscle antibody, antinuclear antibody, liver–kidney microsomal antibody, total IgG level, ceruloplasmin, infective hepatitis (A/B/C/cytomegalovirus/Epstein-Barr virus), iron, ferritin, and tissue transglutaminase IgA. An abdominal ultrasound revealed normal hepatic echogenicity and no organomegaly. An abdominal MRI with liver elastography demonstrated splenomegaly, but also reported a nodular liver with an increased liver stiffness value of 7 kPa; a liver stiffness value of more than 2.7 is consistent with the presence of significant liver fibrosis.\(^8\) The child was discharged with advice to improve lifestyle and diet such as to attempt to lose weight.

Over the next year, the child reduced her BMI from a peak of 56 to 46, but her liver indices, though improved, remained elevated despite significant weight loss: AST, 33 U/L; ALT, 58 U/L; and GGT, 105 U/L. A repeat MRI revealed an improved, though still elevated, liver stiffness (3.83 kPa) value. Given these continued abnormalities, a liver biopsy was obtained that revealed panlobular microvesicular steatosis, minimal lobular inflammation, and stage 3 to 4 fibrosis (Fig 2 A, B, and C). EM revealed prevalent membrane-bound lipid droplets, as well as diffuse mitochondrial abnormalities typical of metabolic stress in obese patients with metabolic syndrome (Fig 2D). Based on the microvesicular pattern of steatosis and the EM findings, the differential diagnosis was broadened to include metabolic diseases, particularly LAL-D. Her lysosomal acid lipase activity was 0.0 nmol/punch/hour and sequencing of \textit{LIPA} revealed 2 variants: c.920C>A (p.A307D) and c.1055_1057delACG (p.D352del). Each has a population frequency of <0.0001 in the Exome Aggregation Consortium database, with the former predicted by in silico modeling to be pathogenic and the latter disrupting an aspartate residue that is conserved in all mammalian species.
DISCUSSION

We report 2 children with LAL-D, who were initially diagnosed and treated for NAFLD. Their diagnoses were established only after liver biopsies, performed 1 or more years into follow-up, demonstrated findings atypical for NAFLD. Both were consistent with predominantly microvesicular steatosis, suggesting the possibility of a competing or coexisting diagnosis. Indeed, we believe that these patients had both NAFLD and LAL-D, the latter coming to attention only after additional studies were performed, when clinically meaningful weight reduction failed to achieve expected improvements of laboratory parameters. In case 2, the severity of hepatic fibrosis at such an early age would not be expected in NASH alone. Moreover, diffuse mitochondrial stress changes are not a component of LAL-D, but are frequently observed in NASH. These phenotypically similar diseases are not necessarily mutually exclusive.

In a practical sense, the diagnostic evaluation of suspected NAFLD should, at a minimum, address conditions that are potentially life-threatening or those that are treatable; Wilson disease and autoimmune hepatitis are examples of conditions routinely tested for in this context. LAL-D, which can be ruled out with a blood-based enzyme analysis, has not, up to this point, been part of this diagnostic evaluation in most centers. This is in spite of clinical guidelines from Europe⁶ and the United States¹⁰ that endorse consideration of monogenic causes of steatosis and utilization of noninvasive testing initially in children, before they undergo liver biopsy.

It is now recognized that LAL-D may be both underdiagnosed² and associated with significant morbidity and mortality, even among pediatric and adolescent patients. In a literature review, Bernstein et al³ identified 135 patients with LAL-D presenting after infancy; 4 of 8 liver-related deaths occurred in patients under 21 years of age and children aged 5 to 14 years old accounted for all 9 liver transplants reported. Burton et al¹¹ presented a similar rate of pediatric liver transplantation, 8.1%, among a group of 49 patients with LAL-D. On this background, there appears to be value in identifying individuals with LAL-D, whether for closer medical supervision or for consideration of treatment. Encouraging results from clinical trials¹²–¹⁴ of recombinant lysosomal acid lipase, now approved for use in the United States, may portend a viable therapy for this group of patients. We conclude, given the accessibility of clinical laboratory-based testing for LAL-D, availability of potential curative treatments, and the data from our report, there is a supportive rationale for testing for LAL-D among patients with suspected NAFLD who do not respond to routine lifestyle interventions.

ABBREVIATIONS

ALT: alanine aminotransferase  
AST: aspartate aminotransferase  
EM: electron microscopy  
GGT: γ-glutamyl transferase  
Ig: immunoglobulin  
LAL-D: lysosomal acid lipase deficiency  
LDL-c: low-density lipoprotein cholesterol  
NAFLD: nonalcoholic fatty liver disease  
NASH: nonalcoholic steatohepatitis

POTENTIAL CONFLICT OF INTEREST: Dr Himes has served on an advisory board, speakers’ bureau, and been a site investigator for an epidemiology study on lysosomal acid lipase deficiency; for Alexion Pharmaceuticals; Dr Barlow has served as site investigator for an observational database on lysosomal acid lipase deficiency, for Alexion Pharmaceuticals; Dr Kohli has served on an advisory board and speakers’ bureau for Alexion Pharmaceuticals; and Drs Bove, Quintanilla, and Sheridan have indicated they have no potential conflicts of interest to disclose.

REFERENCES


Lysosomal Acid Lipase Deficiency Unmasked in Two Children With Nonalcoholic Fatty Liver Disease
Ryan W. Himes, Sarah E. Barlow, Kevin Bove, Norma M. Quintanilla, Rachel Sheridan and Rohit Kohli
Pediatrics originally published online September 13, 2016;
Lysosomal Acid Lipase Deficiency Unmasked in Two Children With Nonalcoholic Fatty Liver Disease

Ryan W. Himes, Sarah E. Barlow, Kevin Bove, Norma M. Quintanilla, Rachel Sheridan and Rohit Kohli

*Pediatrics* originally published online September 13, 2016;

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://pediatrics.aappublications.org/content/early/2016/09/11/peds.2016-0214