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Clinical Features of Lysosomal Acid Lipase Deficiency

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ABSTRACT

Objective: The aim of this study was to characterize key clinical manifestations of lysosomal acid lipase deficiency (LAL D) in children and adults.
Methods: Investigators reviewed medical records of LAL D patients ages ≥5 years, extracted historical data, and obtained prospective laboratory and imaging data on living patients to develop a longitudinal dataset.
Results: A total of 49 patients were enrolled; 48 had confirmed LAL D. Mean age at first disease-related abnormality was 9.0 years (range 0–42); mean age at diagnosis was 15.2 years (range 1–46). Twenty-nine (60%) were male patients, and 27 (56%) were <20 years of age at the time of consent/assent. Serum transaminases were elevated in most patients with 458 of 499 (92%) of alanine aminotransferase values and 265 of 448 (59%) of aspartate aminotransferase values above the upper limit of normal. Most patients had elevated low-density lipoprotein (64% patients) and total cholesterol (63%) at baseline despite most being on lipid-lowering therapies, and 44% had high-density lipoprotein levels below the lower limit of normal. More than half of the patients with liver biopsies (n = 31,

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What Is Known

- Lysosomal acid lipase deficiency presents along an age continuum (infants to adults).
- Presenting features commonly include elevated serum transaminase levels, progressive liver fibrosis, and cirrhosis.
- Nonspecific clinical manifestations can lead to a delay in diagnosis in children and adults.

What Is New

- Current data support previous findings that lysosomal acid lipase deficiency is characterized by progressive hepatic dysfunction, often leading to liver transplantation.
- Hepatic histology with steatosis, often accompanied by fibrosis and cirrhosis, is common.
- Elevated levels of low-density lipoprotein-cholesterol or low levels of high-density lipoprotein-cholesterol along with elevated transaminases represent a potential means for identifying lysosomal acid lipase deficiency earlier in the diagnostic workup.

mean age 13 years) had documented evidence of steatosis (87%) and/or fibrosis (52%). Imaging assessments revealed that the median liver volume was ~1.15 multiples of normal (MN) and median spleen volume was ~2.2 MN. Six (13%) patients had undergone a liver transplant (ages 9–43.5 years). **Conclusion:** This study provides the largest longitudinal case review of patients with LAL D and confirms that LAL D is predominantly a pediatric disease causing early and progressive hepatic dysfunction associated with dyslipidemia that often leads to liver failure and transplantation.

Key Words: cholesteryl ester storage disease, LIPA deficiency, Wolman disease

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Lysosomal acid lipase deficiency (LAL D) (OMIMD 278000) is an autosomal recessive disorder because of mutations in the *LIPA* gene resulting in enzyme deficiency and the accumulation of lysosomal cholesteryl esters (CEs) and triglycerides (TGs) (1). The disease presents with a number of common features including hepatomegaly, elevated serum transaminases, progressive liver fibrosis, and cirrhosis (2–4). The disturbance of hepatic lipid metabolism is accompanied by LAL D–related dyslipidemia, which includes elevated low-density lipoprotein-cholesterol (LDL-C) and low high-density lipoprotein-cholesterol (HDL-C) levels. The disease is most rapidly progressive in infants (historically known as Wolman disease) with death typically before 6 months of age (1,5,6). In children and adults, abnormalities may be overlooked leading to delayed or misdiagnosis.

Current disease understanding is based on published case reports including a recent review of 135 cases, which highlighted the frequent occurrence of complications of chronic liver disease and marked dyslipidemia with early-onset atherosclerotic cardiovascular disease (ASCVD) (2,7,8).

This observational study in children and adults with LAL D was performed to provide more insights into the disease manifestations of LAL D, identify biochemical abnormalities over time, and assess the impact of current disease management strategies by reviewing medical records. In addition, a substudy was initiated to measure liver and spleen volumes and fat content using standardized magnetic resonance–based methods and to determine the short-term variability in key disease-related biochemical abnormalities.

METHODS

Study Design and Data Collection

All procedures performed 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. Study-related documents including protocols and informed consent forms were reviewed by an institutional review board or independent ethics committee. Although both living and deceased patients were eligible for inclusion, sites were required to be actively managing ≥1 patient with LAL D. Patients were eligible if they had a documented diagnosis based on LAL enzyme activity testing or *LIPA* gene mutational analysis, were ≥5 years of age, and had the minimum required data in their records. Data collected included the method of diagnosis, demographics, clinical and family history, physical examination, clinical chemistry results, liver biopsy data, details of interventions with diet modification and lipid-lowering medications, and date and cause of death. Patients in the substudy were ≥8 years of age and able to undergo abdominal magnetic resonance imaging (MRI) and magnetic resonance spectroscopy procedures. These patients underwent the following assessments. Biochemical assessment on a minimum of 1 and up to 3 time points including alanine aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, γ-glutamyl transferase (GGT), bilirubin (direct and total), total cholesterol, TGs, HDL-C, and LDL-C. Imaging studies consisting of abdominal MRI (for quantification of liver and spleen volumes), multiecho gradient-echo (MEGE) MRI, and ^{1}H-magnetic resonance spectroscopy images (for quantification of liver and spleen fat content). All imaging scans were acquired using standardized imaging protocols and were read centrally (Biomedical Systems, St Louis, MO; San Diego, CA; Newcastle, UK).

Statistical Analysis

Enrollment of ~30 evaluable patients with LAL D was planned. All analyses were performed using SAS® version 9.2 (SAS Institute, Cary, NC). Three analysis data sets were used for all data summaries. The full analysis set (FAS) included all of the patients with a confirmed diagnosis of LAL D. The dietary intervention (DI; DIs were considered specialized low-fat or low-cholesterol diets; high-protein or low-sodium diets were not considered DIs for the purposes of this study) set and lipid-lowering medication (LLM) set included patients in the FAS who had ≥1 DI or LLM, respectively, with ≥1 preintervention and 1 postintervention value for a serum transaminase or serum lipid parameter.

Longitudinal analyses characterized changes over time in laboratory values. Change from baseline (baseline defined as first recorded value) was summarized for serum transaminase and serum lipid parameters.

Summaries included observed values, changes, and percentage changes from baseline and Wilcoxon sign rank analysis–based *P* values for changes and percentage changes from baseline for each interval. Percentages of patients whose last value in an interval fell above, below, or within normal limits were also provided. Standardized, normal ranges were based on a central lab (if appropriate reference ranges were stratified by age and/or sex). Upper limit of normal values for ALT and AST were selected based on age and sex, set by the central laboratory (based on sex and age) used in a phase 3 study of an investigational enzyme replacement for LAL D (*clinicaltrials.gov* NCT No. 01757184).

In the substudy, the MN for each patient's liver and spleen was calculated by dividing organ volume (in liters) by expected normal value, defined as 2.5% of the patient's body weight (kilograms) for the liver, and 0.2% of the patient's body weight (kilograms) for the spleen.

RESULTS

Enrollment and Demographics

Between June 17, 2011 and January 9, 2013, a total of 49 patients were enrolled. Forty of the 49 patients were identified from a questionnaire-based survey, and 9 were previously undiagnosed patients. Forty-eight patients had a confirmed diagnosis and were included in the FAS; 52% and 48% of patients were included in the DI set and LLM set, respectively. The 1 deceased patient at the time of the study underwent an allogeneic blood stem cell transplant followed by a liver transplant in her 16th year of life. She had ''steroid-induced diabetes'' posttransplant with a ''cushingoid'' appearance and died at 17.6 years of age due to H1N1 infection. Twenty-seven (56%) patients were <20 years old; 8 (17%) were <10 years old. Longitudinal patient data were collected during a median of 12.3 years (range 1.8–38.8).

Onset of Disease-Related Abnormalities

The mean and median ages at the first report of disease-related abnormalities were 9.0 and 5.8 years (Table 1); 75% of the patients had abnormalities first reported by 10 years of age. The time from the first reported abnormalities to diagnosis was ≤5 years for 77% of patients and >10 years for 15% of patients, with a maximum time of 40 years. Hepatomegaly was the most common initial abnormality (54% of patients). Elevated serum transaminases, dyslipidemia, and splenomegaly were the initial abnormality in 44%, 33%, and 23% of patients, respectively. Other important disease-related abnormalities included a history of cholecystectomy, gallbladder disease, or gallstones (10%) and significant cardiovascular findings in 2 patients including subvalvular aortic stenosis, aortic insufficiency, and infrarenal aortic stenosis (patient 21-002) and tricuspid valve insufficiency and aortic stenosis (patient 21-001).

Family History and Genetic Testing

A total of 38% of the patients had a sibling with LAL D. Two sets of siblings were included in the study. One set were nonidentical twins, one of whom had received a liver transplant at age 9.6 years. The second set included 3 siblings (28.5-year-old twins and a 32.8-year-old sibling). *LIPA* mutation data were available in 19 patients (supplemental Table 1, *http://links.lww.com/MPG/A534*). Eighty-four percent of patients had ≥1 copy of the c.894G>A mutations (also known as Exon 8 splice junction mutation), 4 (21%) were homozygous for c.894G>A (3 were siblings), and 12 (63%) were heterozygous.

Biochemical Abnormalities

The median age of first recorded elevated transaminase was 8.2 years (supplemental Table 2, *http://links.lww.com/MPG/A535*). Mean ALT, AST, and GGT levels were elevated at the time of the first documented measurement (92.4, 87.8, and 52.2 U/L, respectively) and were abnormal in 91.7%, 76.2%, and 20.0% of patients, respectively. ALT and AST elevations (defined as >43 U/L and >59 U/L, respectively) were frequent (92% of available measurements for ALT and 59% for AST) and typically persistent with no evidence of any substantial and/or sustained change following the introduction of dietary modifications and/or LLM (Fig. 1A; supplemental Fig. 1, *http://links.lww.com/MPG/A536* [values, by patient and LLM use, for {A}AST, {B} TG, and {C} HDL-C]). Elevated GGT levels were seen less commonly, with 20% of patients having values >40 U/L at baseline (supplemental Table 2, *http://links.lww.com/MPG/A535*). Among the 15 substudy patients with data available, mean direct bilirubin was 0.29 mg/dL, with a range

TABLE 1. LAL D history and associated medical conditions (frequency and age at onset)

Characteristic	Frequency, n (%)	Age, y	
		Mean (SD)	Median (range)
LAL D history			
First observation*	48 (100)	8.9 (10.4)	5.0 (0.0–39.3)
Onset of disease-related abnormality	48 (100)	9.0 (9.9)	5.8 (0.0–42.0)
Diagnosis	48 (100)	15.2 (14.2)	9.5 (1.2–46.1)
First recorded elevated transaminase	48 (100)	14.5 (14.5)	8.2 (0.0–46.1)
First recorded dyslipidemia	46 (96)	14.5 (14.2)	8.4 (0.9–46.1)
First recorded DI	32 (67)	8.7 (9.8)	5.5 (0.2–43.6)
First recorded LLM	31 (65)	15.8 (12.9)	11.3 (2.0–44.0)
First transplant	6 (13)	23.2 (16.0)	17.0 (9.1–43.5)
LAL D–associated abnormalities			
Hepatomegaly	42 (87.5)	9.38 (10.56)	5.75 (0.00–42.15)
Splenomegaly	38 (79.2)	13.01 (11.34)	9.67 (1.23–42.28)
Persistent abdominal pain	9 (18.8)	15.22 (14.54)	10.67 (2.00–42.87)
History of admissions related to fever or infection	7 (14.6)	11.29 (16.08)	5.37 (0.60–45.57)
Anemia	6 (12.5)	6.16 (5.57)	6.17 (0.36–12.37)
Diarrhea and chronic diarrhea	6 (12.5)	5.09 (5.12)	3.84 (0.13–12.37)
Cholecystectomy, gallbladder disease or gallstones	5 (10.4)	24.68 (14.04)	17.63 (10.11–42.20)
Recurrent infections	4 (8.3)	2.9 (2.62)	2.73 (0.60–5.54)
Splenectomy	3 (6.3)	15.72 (2.98)	15.03 (13.15–18.99)

DI = dietary intervention; LAL D = lysosomal acid lipase deficiency; LLM = lipid-lowering medication; SD = standard deviation.
* First observation is the first time point at which a clinical assessment was documented.

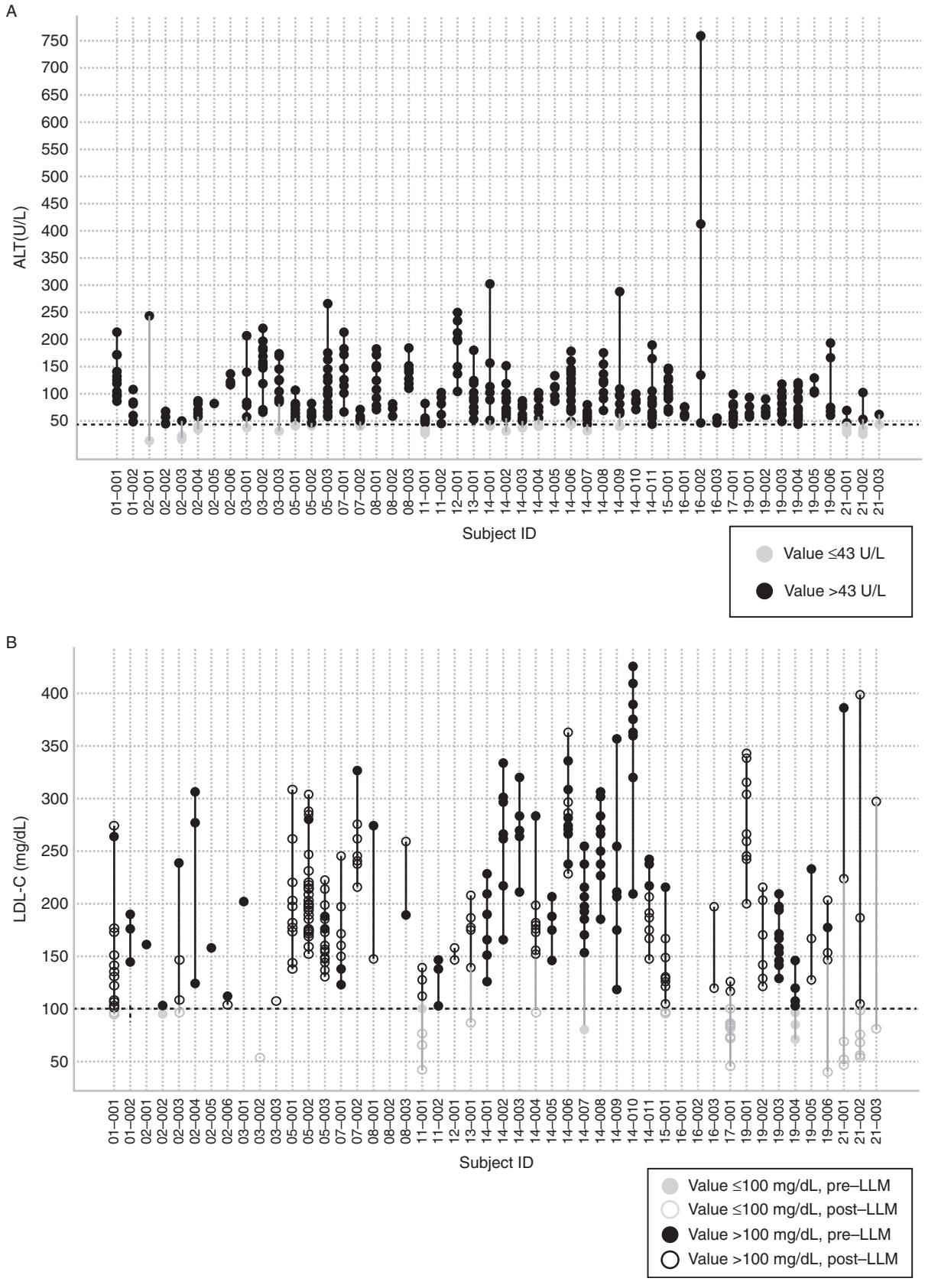

FIGURE 1. Values, by patient, for (A) ALT, and (B) LDL-C. ALT = alanine aminotransferase; LDL-C = low-density lipoprotein-cholesterol.

TABLE 2. Patients in the FAS with biopsy evidence of steatosis, fibrosis, and/or cirrhosis

Patient number	Age at diagnosis	Biopsy evidence of steatosis (age at first documentation, y)	Biopsy evidence of fibrosis (F) and/or cirrhosis (C) (age at first documentation, y)
01-001	3.2	Yes (3.1)	F (3.1)
02-003	41.4	Yes (41.0)	C (41.0)
02-006	16.8	Yes (16.3)	F (16.3)
05-003	3.0	Yes (7.1)	F (2.7)
07-001	19.4	Yes (19.4)	F (19.4)
08-002	6.0	Yes (5.6)	C (5.6)
11-001	13.1	Yes (17.6)	C (17.6)
11-002	1.8	Yes (16.4)	C (15.0)
12-001	2.3	Yes (1.9)	F (1.9)
14-002	9.0	Yes (5.0)	F (5.0)
14-005	11.1	Yes (10.6)	F (10.6)
14-008	7.9	Yes (4.0)	F (4.0)
14-010	7.8	Yes (6.9)	F (6.9)
14-011	13.5	Yes (5.9)	F (5.9)
15-001	12.6	Yes (17.3)	F (12.5)
17-001	38.7	Yes (37.9)	F (37.9)
19-001	10.4	No	F (9.8)/C (9.8)
19-004	12.6	Yes (12.2)	F (12.2)
19-005	2.0	Yes (1.9)	F (1.9)
19-006	4.2	No	F (3.5)

FAS = full analysis set.

of 0.05 to 0.51 mg/dL. Three (20%) patients had a direct bilirubin value above the upper limit of normal. The mean total bilirubin was 1.46 mg/dL (range 0.298–6.64 mg/dL). Mean LDL-C total cholesterol, and TG levels were elevated at the time of the first documented measurement (202.9, 269.5, and 184.4 mg/dL, respectively) and were abnormal in 64.4%, 62.5%, and 27.1% of patients, respectively. Mean HDL-C at the first measurement was 37.5 mg/day (girls 39.0 mg/dL, boys 36.4 mg/dL) and was abnormal in 43.5% of patients. Most of the available LDL-C values (88%; 270/306) were >100 mg/dL, with many values in a range indicative of substantial dyslipidemia in the study population (Fig. 1B). Overall, although reductions in LDL-C with LLM were seen, these reductions were typically small with substantial overlap in the range of elevated LDL-C levels seen in patients not receiving LLM.

Imaging and Liver Biopsy

Abnormal imaging findings included hepatomegaly (n = 36; 77%), splenomegaly (n = 30; 64%), and fatty liver/steatosis (including findings of increased liver echogenicity) (n = 24; 51%). Adrenal calcification was seen on imaging in 3 patients (finding first seen at ages 8.8, 13.1, 14.8, and 8.8 years). In the substudy, median liver volume was 1.15 MN (range 0.74–2.20). The median hepatic fat fraction (FF) percent was 8.0% (range 1.17%–14.83%). The majority of patients with liver MEGE performed had a liver FF >5%. Median splenic volume was 2.20 MN (range 0.85–4.97). Histology data were available from 45 liver biopsies from 31 (65%) patients. The mean age at the time of first biopsy was 13 years, with 25 of the 31 patients ages <18 years. Steatosis was reported in 87% (27/31) of patients, and evidence of fibrosis and/or cirrhosis was reported in 64.5% of the biopsied patients. Evidence of cirrhosis and fibrosis was seen on the first biopsy in 5 and 16 patients, respectively (Table 2). A total of 85% (17/20) of the patients with biopsy evidence of fibrosis and/or cirrhosis were <18 years.

Treatments and Interventions

Lipid-lowering medication use, most commonly statin, and DIs were reported for 81% and 68% of the patients, respectively. The mean ages at the first LLM use and the first DI were 15.8 and 8.7 years, respectively, with 20 patients receiving their first LLM before the age of 18 years. Thirteen percent of patients had undergone liver transplant; 4 underwent transplant during childhood (9–18 years). Only 9 patients ages ≥40 years were identified in this study; of these, 2 underwent liver transplant at ages 43.3 and 43.5 years, respectively. Patients who underwent liver transplantation generally had lower or decreasing ALT levels, higher AST/ALT ratios, lower TG and HDL-C levels, a higher incidence of cytopenia, and lower albumin levels than other LAL D patients in this study.

DISCUSSION

This study aimed to better characterize the clinical features of LAL D, gain insights into the identification and progression of known biochemical abnormalities over time and assess the impact of current disease management approaches. Although most patients were identified as a result of an initial questionnaire, 18.4% of patients were previously undiagnosed patients identified during the course of the study, which is consistent with previous suggestions based on genetic epidemiology that the disease is likely to be underdiagnosed (9,10). For instance, 3 patients in this study were clinically unapparent cases presenting as an autosomal recessive hypercholesterolemia that were diagnosed by exome sequencing of the index case (11). The long duration between initial disease and diagnosis in some patients is consistent with other metabolic diseases (12) and is likely due in large part to poor awareness and knowledge of LAL D leading to patients being misdiagnosed or going undiagnosed. In particular, the noted abnormalities of hepatomegaly, elevated liver enzymes and/or fatty liver and dyslipidemia, may be confused with more common disorders such as nonalcoholic fatty liver disease (NAFLD), nonalcoholic

steatohepatitis (NASH), metabolic syndrome, and/or familial hypercholesterolemia.

This study focused mainly on living patients with the inclusion of deceased patients if the participating site had access to charts from the patients. Although the study did not focus on the investigation of survival and time to liver transplantation, some insights into these events were provided. Only 1 deceased patient was included in the study, with progression to cirrhosis and liver transplantation at the age of 16 and death at 17.6 years of age, which is consistent with disease progression seen in previously described cases (2). The overall frequency of liver transplantation was 13%, which is high given the age structure and duration of follow-up of the study population. In comparison in a 20-year follow-up of a cohort of 66 children with NAFLD with a mean age at presentation of 13.9 ± 3.9 years, only 3% ($n = 2$) of the patients required liver transplantation (13). In this study of LAL D patients, the proportion of older patients identified was low with only 18.7% of affected patients $\geq$40 years, which is markedly different from the expected number in a representative population in which 46.7% of the population is $\geq$40 years of age (14). These findings are consistent with a recent extensive case report review in which $<$10% of the 135 reported patients were $>$40 years of age (2). Unlike publication-based reviews, the enrolled patients in this study are not subject to the same biases related to the selection and the acceptance of case reports for publication. In contrast to other rare diseases characterized by early mortality and morbidity, disease progression in LAL D can occur relatively silently, and therefore one potential explanation for the underrepresentation of older patients, which requires further investigation, is that these patients are dying prematurely and that in some cases death may occur without a definitive diagnosis of LAL D.

The early development of fibrosis and cirrhosis that also occurs in infants within the first 6 months of life suggests that the lysosomal accumulation of CE and TG in the liver is a potent inducer of fibrosis (2–4). In this study, the frequency of fibrosis and/or cirrhosis was high relative to other chronic liver diseases given the age range of the patients, with 64.5% demonstrating histological evidence of fibrosis and/or cirrhosis, and 16.1% evidence of cirrhosis on the first biopsy with a mean age at the time biopsy of only 13 years. In comparison in cross-sectional studies of 67 consecutive pediatric patients with biopsy-proven NAFLD, clinically significant fibrosis was only seen in 14.7% of patients, and cirrhosis was not seen in any cases (15), and in a broader age range of patients with NASH with a median age of 50.5 years, cirrhosis was seen in 17% of patients (16). The frequency of cirrhosis in patients with LAL D also appears substantially higher than that seen with hepatitis C. As an example, in a study of pediatric patients with hepatitis C, cirrhosis was only seen in 2% of patients with evidence of persistent infection (17). In patients with LAL D, in addition to the nature of the fibrosis-inducing insult, the duration of liver injury is also likely to be important. It is well recognized for other chronic diseases, including hepatitis B and C, NAFLD, alcoholic liver disease, and various metabolic diseases, that failure to remove the causative factor producing liver fibrosis is associated with progression to cirrhosis (18). The longitudinal data in this study shows for the first time that liver injury begins early in childhood and is persistent in patients with LAL D. Imaging data from this study shows that the elevations in serum transaminases are accompanied by increases in liver volume and liver FF assessed by MEGE consistent with the presence of accumulated lysosomal lipid in the liver. Although the FF measurements assessed by MEGE are relatively low compared with values in NAFLD patients (19), the recent demonstration of marked reductions in FF assessed using this method with enzyme replacement provides further support for the presence of abnormal amounts of lipid in the liver in patients with LAL D (20). The relatively low apparent hepatic FF compared with patients with NAFLD may relate to differences in the composition (predominance of CE vs TG) and/or cellular location (lysosomal vs cytosolic) of the accumulated lipid (21).

Although less well studied, patients with LAL D also demonstrate complications related to ASCVD, which are likely related to both the dyslipidemia, because of abnormal hepatic lipid metabolism, and the impairment of cholesterol efflux from lipid-containing LAL-deficient macrophages (7,8,22–24). Although the young age of the affected patients in this study limits the insights into the risk of ASCVD because complications associated with cumulative LDL-C exposure may not manifest as cardiovascular events until adulthood (25,26), complications related to ASCVD were seen in 4.2 % of the overall population, and in 12.5% (2/16) of patients in this study over the age of 25 years with both of these patients showing LDL-C levels $>$350 mg/dL. The mean LDL-C value of 202.9 mg/dL for the first recorded LDL-C for the enrolled patients was in the very high range ($>$190 mg/dL). The LDL-C levels are substantially higher than those seen in patients with NAFLD/NASH, which is an important consideration for the differential diagnosis (27,28). Historical LDL-C measurements from case records, in addition to showing for the first time that these abnormalities are present from early in life, also demonstrated that LDL-C elevations are persistent and not substantially impacted by DI or LLMs. These findings are important because insights from other genetic conditions that impact LDL-C levels early in life, highlight the importance of duration of exposure to LDL-C and the absolute level of LDL-C in determining overall ASCVD risk (25). Because LAL D is an autosomal recessive disease, the absence of a family history of cardiovascular disease and/or hypercholesterolemia and the current inconsistencies in pediatric lipid screening may delay early recognition of the lipid abnormalities in LAL D patients, in contrast to the other autosomal dominant inherited conditions such as familial hypercholesterolemia in which family history can assist in early diagnosis.

In addition, the information for this study was obtained from medical records with variability in the depth of their descriptions; thus, definitive conclusions regarding the differences between LAL D and other common diseases, such as NAFLD/NASH, cannot be made.

In summary, this is the first detailed characterization of a large number of children and adults with LAL D to provide important insights into a number of aspects of the disease that are difficult to address from published case reports. The majority of living cases are children, and there is a striking underrepresentation of patients $>$40 years of age. The high frequency of liver transplantation, fibrosis, and cirrhosis at a young age in patients with LAL D, relative to other more common causes of chronic liver diseases is important given the low disease awareness and potential for confusion with other more common liver diseases such as NAFLD/NASH. Moreover, although persistent liver injury is present from early in life, the serum transaminase levels alone may not lead to a specialist referral and/or a detailed liver workup. The high LDL-C or low HDL-C levels seen in patients with LAL D, however, provide an opportunity to potentially distinguish these patients earlier during the diagnostic workup. In totality, these study data indicate that LAL D is predominantly a pediatric disease that primarily targets the liver, causing progressive hepatic dysfunction with fibrosis (and commonly cirrhosis), which, combined with the systemic manifestations of dyslipidemia, put these patients at risk for significant morbidity and early mortality.

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