Lysosomal Acid Lipase Deficiency (LAL-D)

Presentation Objectives

- Overview of LAL-D
- LAL-D Manifestations of the Liver
- LAL-D Manifestations of the Cardiovascular System
- LAL-D Manifestations of the Spleen and Gastrointestinal Tract
- Diagnostic Testing for LAL-D
- Conclusion
- Appendix

LAL-D, Lysosomal acid lipase deficiency.

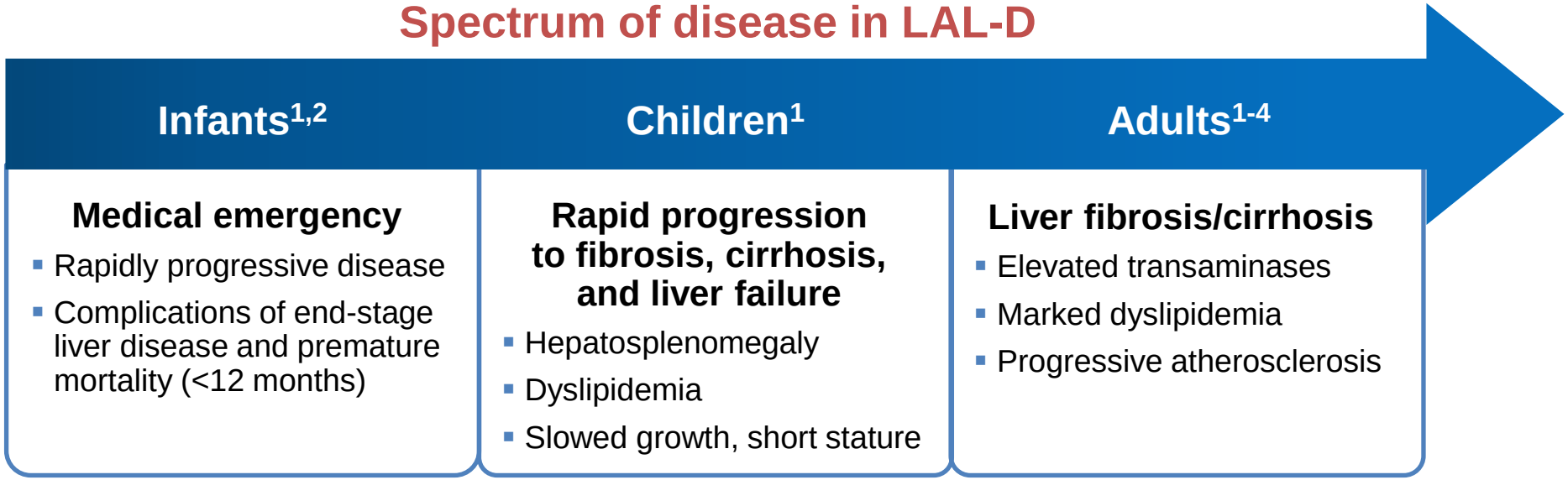
Overview of LAL-D

- Genetics
- Pathophysiology
- Systemic Manifestations
- Diagnosis of LAL-D

Clinical Presentation of LAL-D[1]

- LAL-D is an inherited disease which presents on a spectrum, with varying age of onset and severity[1]
- Regardless of age of onset, manifestations of LAL-D can lead to atherosclerotic progression, complications of end-stage liver disease, and premature death[1,2]

Spectrum of disease in LAL-D



1. Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **2.** Bernstein DL, et al. *J Hepatol.* 2013;58(6):1230-1243. **3**. Elleder M, et al. *J Hepatol*. 2000;32(3):528-534. **4**. vom Dahl S, et al. *J Hepatol.* 1999;31(4):741-746.

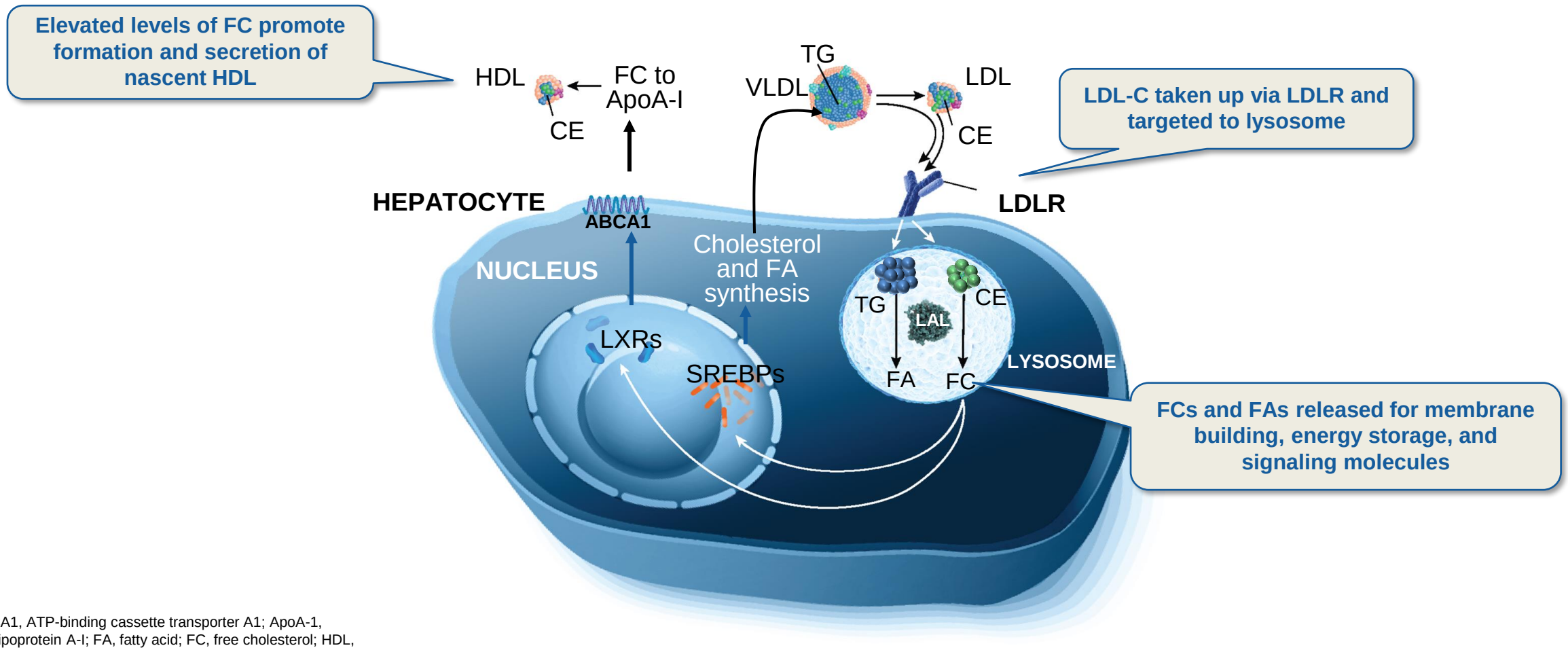
LAL-D Is an Inherited Disease[1]

- LAL-D is an autosomal recessive inherited disease caused by mutations in the *LIPA* gene, resulting in deficient lysosomal acid lipase (LAL) activity[1,2]
  - 214 mutations in the *LIPA* gene have been reported in the ClinVar database as of January 2020[3]
- LOF mutations may result in[4]
  - Reduced expression or complete lack of expression of the gene
  - Production of protein that has reduced, little, or no functional enzyme activity
- 50% to 60% of patients have the c.894G>A mutation, also known as E8SJM, though there is some variability by ethnicity[1,5]

***LIPA* gene structure (chromosome 10)[5]**

1. Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **2.** Valayannopoulos V, et al. *Mol Genet Metab.* 2017;120(1-2):62-66. **3.** ClinVar. https://www.ncbi.nlm.nih.gov/clinvar/?term=LIPA%5Bgene%5D. Accessed January 17, 2020. **4.** Loss-of-function mutation. The Free Dictionary website. https://medical-dictionary.thefreedictionary.com/loss-of-function+mutation. Accessed December 21, 2018. **5.** Bernstein DL, et al. *J Hepatol.* 2013;58(6):1230-1243.

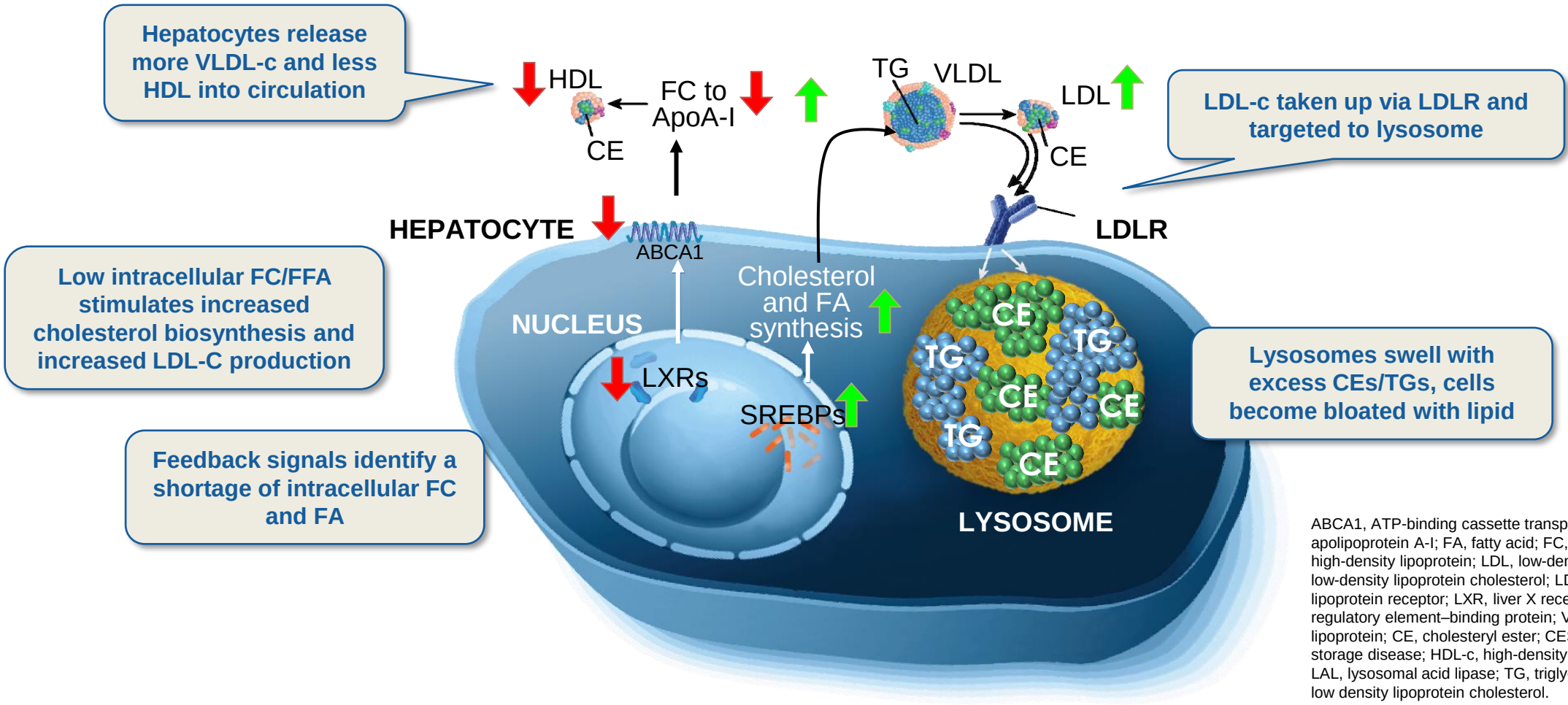
LAL Hydrolyzes Cholesteryl Esters and Triglycerides[1-3]



ABCA1, ATP-binding cassette transporter A1; ApoA-1, apolipoprotein A-I; FA, fatty acid; FC, free cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDL-c, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; LXR, liver X receptor; SREBP, sterol regulatory element–binding protein; VLDL, very low-density lipoprotein.

1. Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **2.** Rader DJ. *N Engl J Med*. 2015;373(11):1071-1073. **3.** Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243.

MUTATIONS IN *LIPA* CAUSE DEFICIENT LAL ENZYME IN PATIENTS WITH LAL-D[1-3]



ABCA1, ATP-binding cassette transporter A1; ApoA-1, apolipoprotein A-I; FA, fatty acid; FC, free cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDL-c, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; LXR, liver X receptor; SREBP, sterol regulatory element–binding protein; VLDL, very low-density lipoprotein; CE, cholesteryl ester; CESD, cholesterol ester storage disease; HDL-c, high-density lipoprotein-cholesterol; LAL, lysosomal acid lipase; TG, triglyceride; VLDL-c, very low density lipoprotein cholesterol.

1. Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30.
2. Rader DJ. *N Engl J Med*. 2015;373(11):1071-1073. **3.** Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243.

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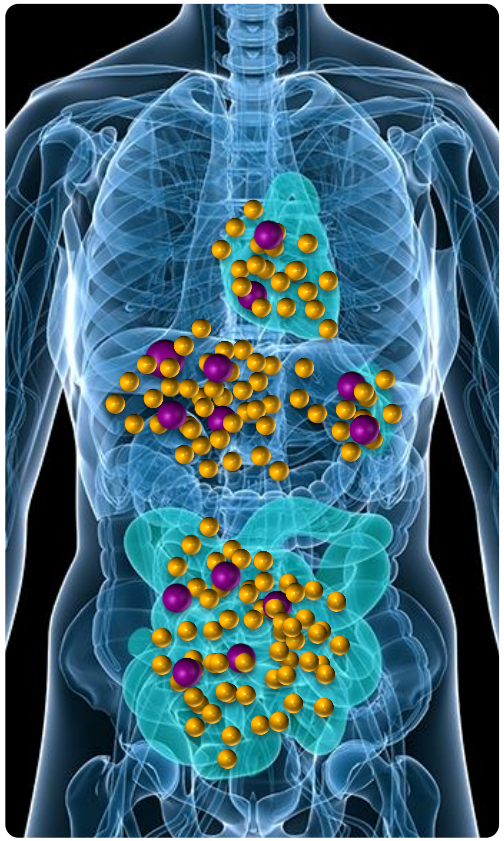
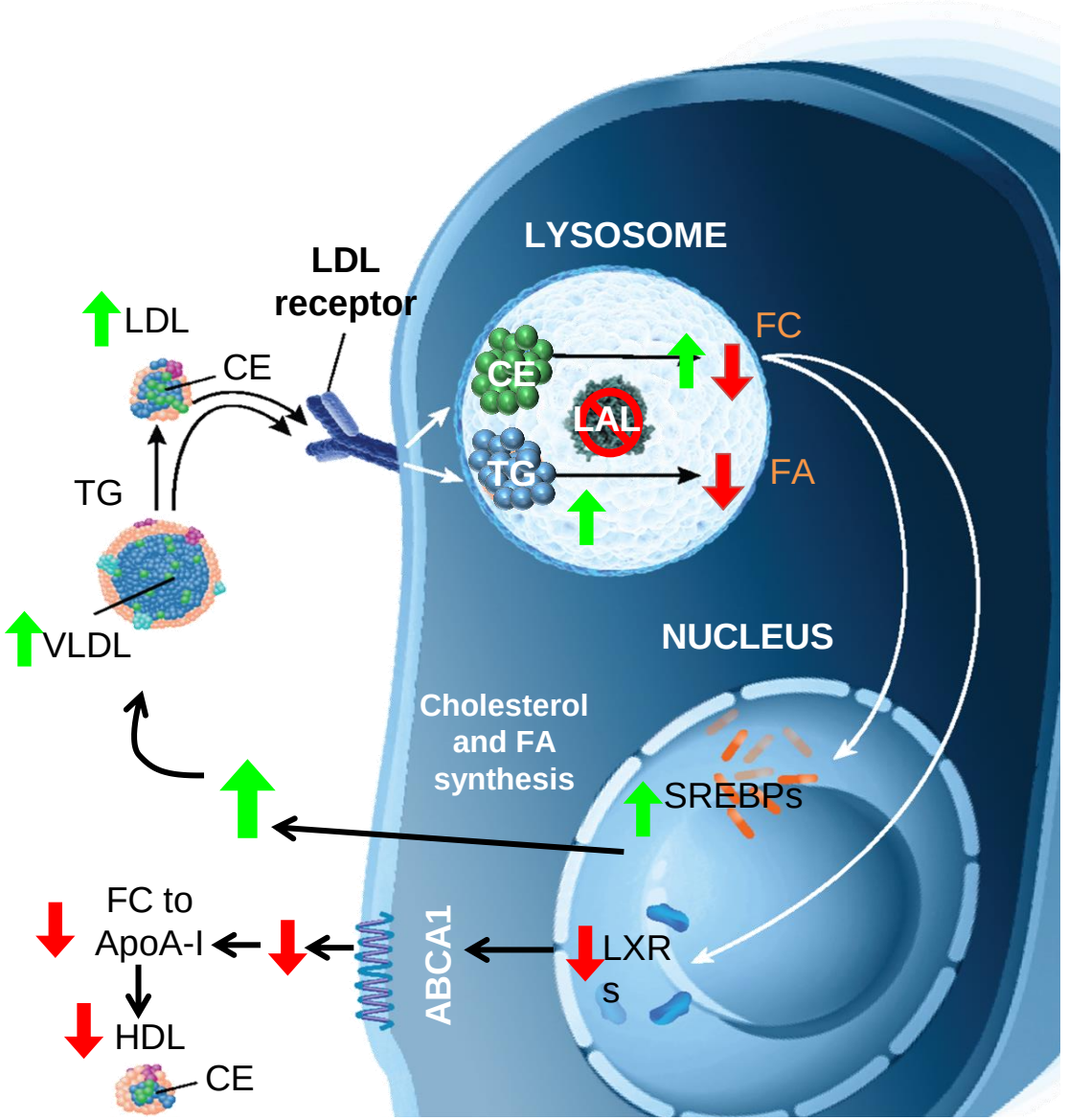
CE and TG Accumulation Is Exacerbated by Increased LDL-C Uptake and Production[1,2]



Imagery supplied by iStock.com.

Hepatocytes upregulate synthesis and packaging of cholesterol, releasing **more** cholesterol that is taken up by LDL receptor-containing cells, such as hepatocytes, macrophages, and vascular endothelial cells[1,3]

- LDL-C
- VLDL-C



HEPATOCYTE

ABCA1, ATP-binding cassette transporter A1; ApoA-1, apolipoprotein A-I; CE, cholesteryl esters; FA, fatty acid; FC, free cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; LXR, liver X receptor; SREBP, sterol regulatory element–binding protein; TG, triglycerides; VLDL-C, very low-density lipoprotein cholesterol.

1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30.
3. Rader DJ. *N Engl J Med*. 2015;373(11):1071-1073.

Systemic Manifestations of LAL-D

CARDIOVASCULAR MANIFESTATIONS: 87%[1,a]

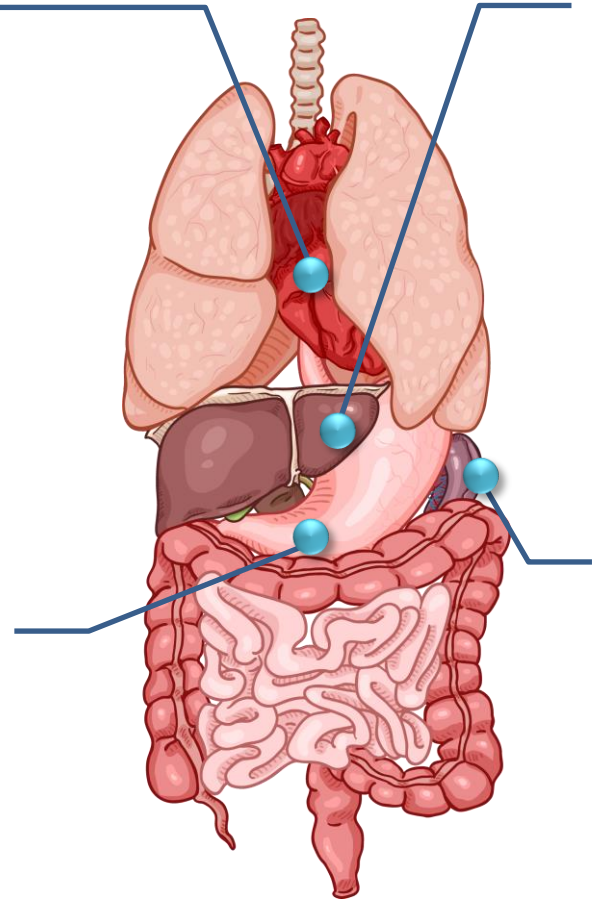
- High LDL-C
- Low HDL-C
- Accelerated atherosclerosis
- Coronary artery disease
- Stroke or suspected stroke
- Myocardial infarction

OTHER MANIFESTATIONS[1,2]

- Growth failure/failure to thrive
- Cachexia
- Adrenal calcification
- Short stature

GASTROINTESTINAL (GI) MANIFESTATIONS: 22%[1,a]

- Abdominal and epigastric pain
- Malabsorption
- Emesis
- Gallbladder dysfunction
- Diarrhea



LIVER MANIFESTATIONS: ~100%[1-3]

- Elevated ALT
- Hepatic steatosis (microvesicular or mixed)
- Hepatomegaly
- Liver dysfunction or failure
- Fibrosis and/or cirrhosis
- Esophageal varices
- Portal hypertension
- Ascites
- Hepatocellular carcinoma
- Cholangiocarcinoma

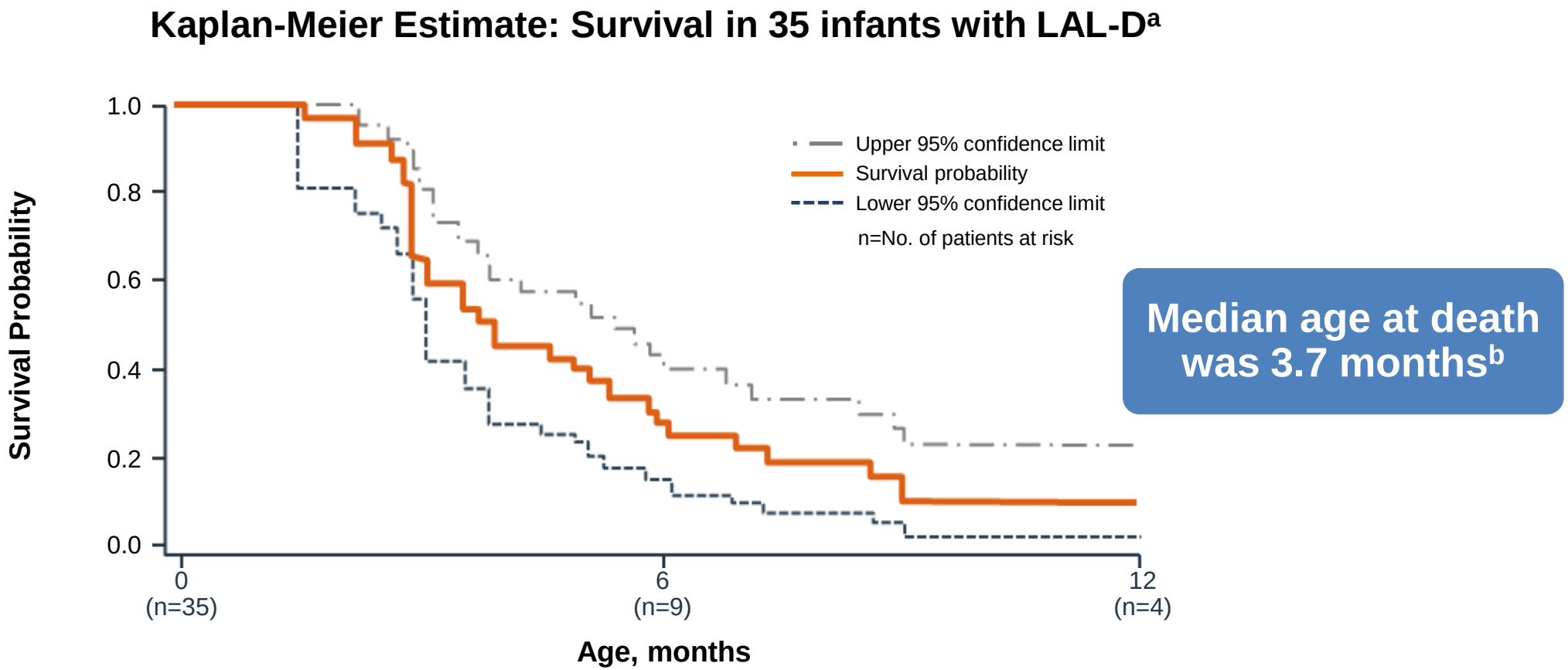
SPLEEN MANIFESTATIONS: 74%[1]

- Splenomegaly

[a]Based on an analysis of 55 genotyped patients with LAL-D in a cohort of 135 cases.

1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30. **3.** Elleder M, et al. *J Hepatol*. 2000;32(3):528-534.

LAL-D Is Rapidly Fatal in Infants Within 12 Months



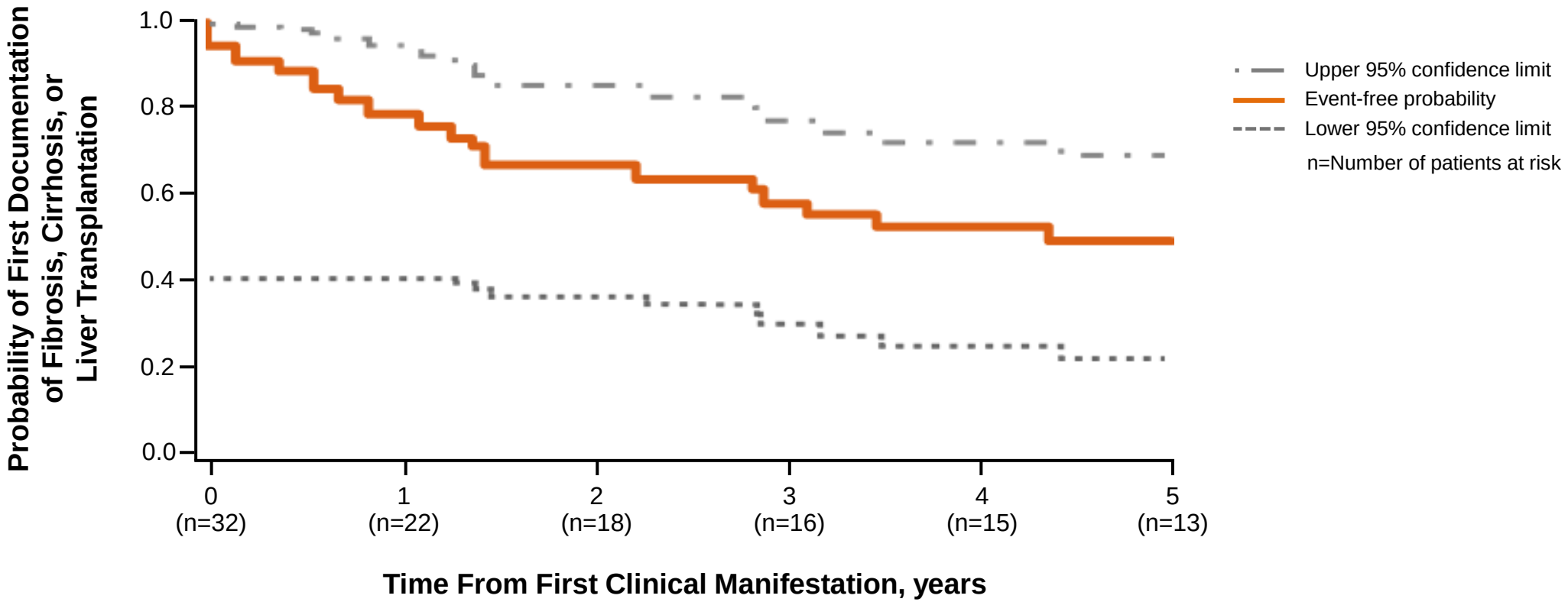
Median age at death was 3.7 months[b]

Adapted by permission from Macmillan Publishers Ltd: *Genet Med,* Jones SA, et al. doi: 10.1038/gim.2015.108.

[a]A retrospective chart review and data extraction of 35 patients diagnosed with LAL-D before age 2 (26 with growth failure before 6 months of age; 9 without). Early growth failure was defined as fulfillment of 1 or more of the following criteria before 6 months of age: (1) decreased body weight across ≥2 of the 11 major percentiles on a standard World Health Organization (WHO) weight-for-age chart; (2) body weight below the tenth percentile on a standard WHO weight-for-age chart and no weight gain within the previous 2 weeks; (3) loss of ≥5% of birth weight in children >2 weeks of age. [b]Range: 1.4 to 46.3 months.
Jones S, et al. *Genet Med*. 2016;18(3):452-458.

Nearly 50% of Patients Progressed to Fibrosis, Cirrhosis, or Liver Transplant Within 3 Years of First Clinical Manifestation

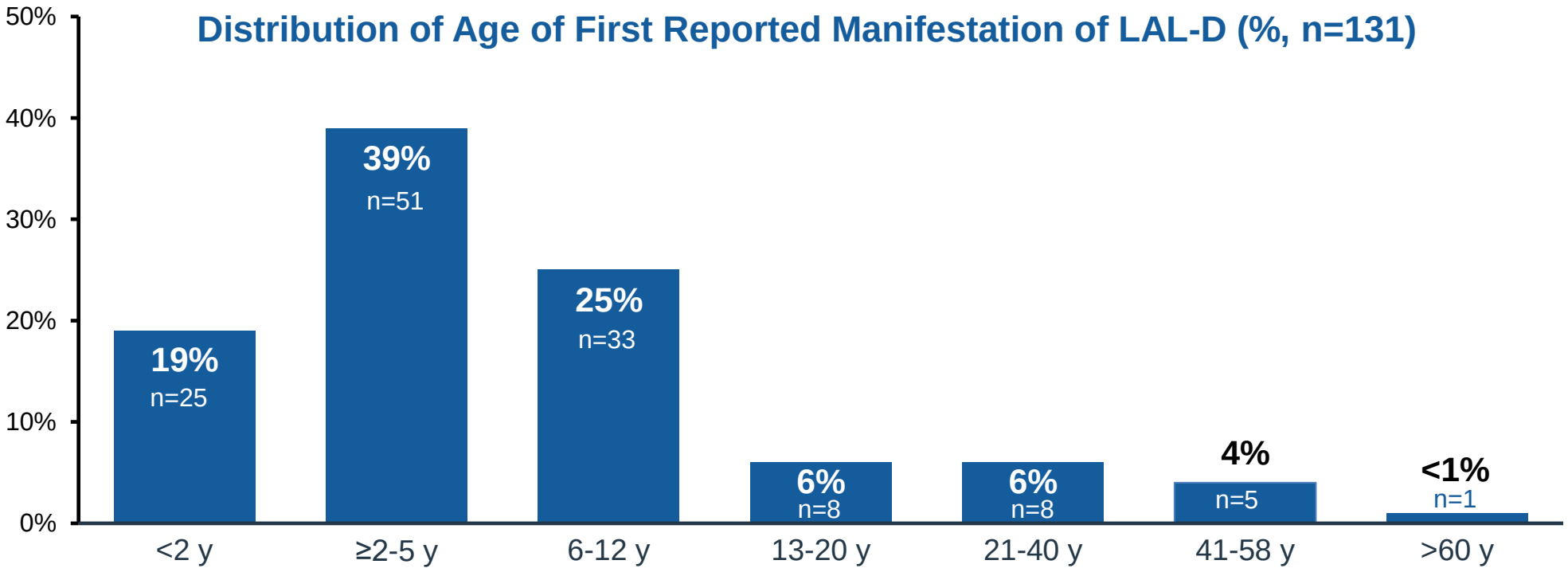
Kaplan-Meier Estimate: Risk from first LAL-D clinical manifestation to first documentation of hepatic fibrosis, cirrhosis, or liver transplant in 32 pediatric and adult patients with LAL-D[a]



[a]Post hoc analysis of an observational study in 31 patients with LAL-D with liver biopsy data and 1 patient with no biopsy who received liver transplantation.
Burton BK, et al. *Curr Med Res Opin*. 2017;33(7):1211-1214.

Patients With LAL-D May Present at Any Age, but Primarily by Young Adulthood

The lower percentage of adults living with LAL-D may be due to underdiagnosis and premature mortality in this group



Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243.

LAL-D Testing Should Be Part of Routine Liver and Lipid Workups[1]

Select Liver diagnostic considerations[1-9]

- ❑ α_1-Antitrypsin disease
- ❑ Drug-induced liver disease
- ❑ Fatty acid oxidation defects
- ❑ Gaucher disease
- ❑ Glycogen storage disorders
- ❑ Hemochromatosis
- ❑ Hepatocellular cancer
- ❑ Hepatitis A/B/C/D/E/Autoimmune
- ❑ **LAL-D**
- ❑ Niemann-Pick disease
- ❑ NAFLD
- ❑ Primary biliary cholangitis
- ❑ Primary sclerosing cholangitis
- ❑ Wilson disease

Select Lipid diagnostic considerations[1-3,7,10]

- ❑ Alcohol
- ❑ Diabetes (types 1 and 2)
- ❑ Drug therapy[a]
- ❑ FCH
- ❑ Familial defective apoB100
- ❑ Familial dysbetalipoproteinemia
- ❑ Heterozygous and homozygous FH
- ❑ Hypopituitarism
- ❑ Hypothyroidism
- ❑ **LAL-D**
- ❑ Nephrotic syndrome

[a]Drug therapies such as corticosteroids, isotretinoin, beta-blockers, oral contraceptives, chemotherapeutic agents, and antiretroviral agents.
FCH, familial combined hyperlipidemia; NAFLD, nonalcoholic fatty liver disease.

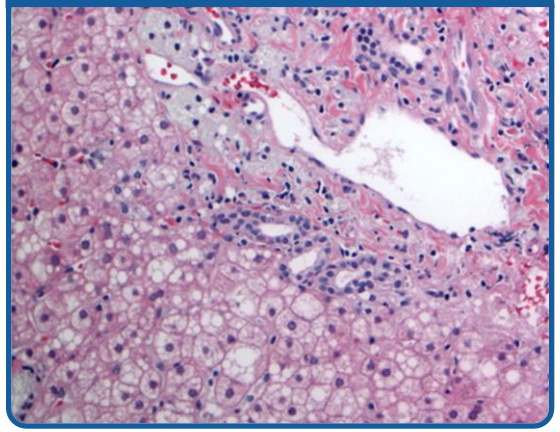
1. Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **2.** Data on File, Alexion Pharmaceuticals. **3.** Longo D. *Harrison's Principles of Internal Medicine.* New York, NY: McGraw-Hill Medical; 2012. Chapter 356:3145-3161. **4.** Longo D. *Harrison's Principles of Internal Medicine.* New York, NY: McGraw-Hill Medical; 2012. Chapter 308:2592-2602. **5.** Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **6.** Burton BK, et al. *N Engl J Med.* 2015;373(11):1010-1020. **7.** Hamilton J, et al. *Clin Chim Acta*. 2012;413(15-16):1207-1210. **8.** Chalasani N, et al. *Gastroenterology.* 2012;142(7):1592-1609. **9.** Vajro P, et al. *J Pediatr Gastroenterol Nutr*. 2012;54(5):700-713.
10. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute. *Pediatrics*. 2011;128(suppl 5):S213-S256.

LAL-D Manifestations of the Liver

LAL-D Causes Progressive Liver Damage[1]

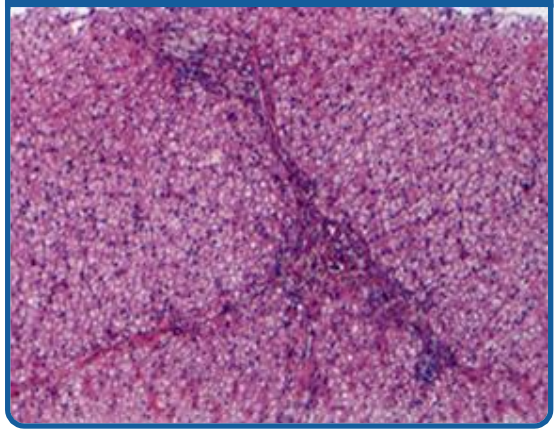
- ~100% of patients with LAL-D experience hepatic manifestations[1,a]
- LAL-D is characterized by progressive liver injury accompanied by elevated transaminases, often at an early age[1,2]
  - Marked lysosomal accumulation of CE and TG leads to diffuse microvesicular steatosis[2]
  - Microvesicular steatosis progresses to fibrosis and micronodular cirrhosis[2]

Steatosis[3]



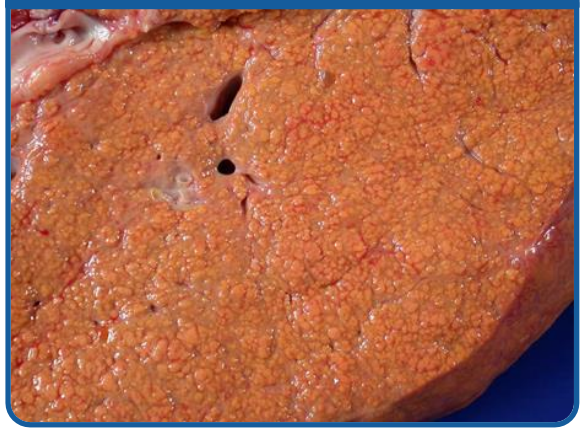
Accumulation of cholesteryl esters and triglycerides[1]

Fibrosis[3]



Presence of scar tissue, which distorts internal structure and can interfere with blood flow[4]

Micronodular Cirrhosis[3]



Cirrhosis is late-stage fibrosis resulting in distortion of normal hepatic architecture; characterized by regenerative nodules surrounded by dense fibrotic tissue[5]

Images courtesy of Dhanpat Jain, MD, Yale University School of Medicine, New Haven, CT.

Image provided by Geneva University Hospitals.

[a]Longitudinal data for 99 patients who were followed for two years to >30 years from their initial diagnoses or symptom onset, including 21 patients who were described in subsequent reports, providing natural history information that could be correlated with their *LIPA* genotypes when available.

1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **3.** Data on file. Alexion Pharmaceuticals. **4.** Civan JM. Fibrosis of the liver. Merck Manual Consumer Version website. https://www.merckmanuals.com/home/liver-and-gallbladder-disorders/fibrosis-and-cirrhosis-of-the-liver/fibrosis-of-the-liver. Updated December 2019. Accessed January 16, 2020. **5** Civan JM. Cirrhosis. Merck Manual Professional Version website. https://www.merckmanuals.com/professional/hepatic-and-biliary-disorders/fibrosis-and-cirrhosis/cirrhosis. Updated December 2019**.** Accessed January 16, 2020.

LAL-D Causes Progressive Liver Damage[1]

Figure 1: Birefringent cholesteryl ester (CE) crystals are present in Kupffer cells in the liver of patients with LAL-D[2,a]

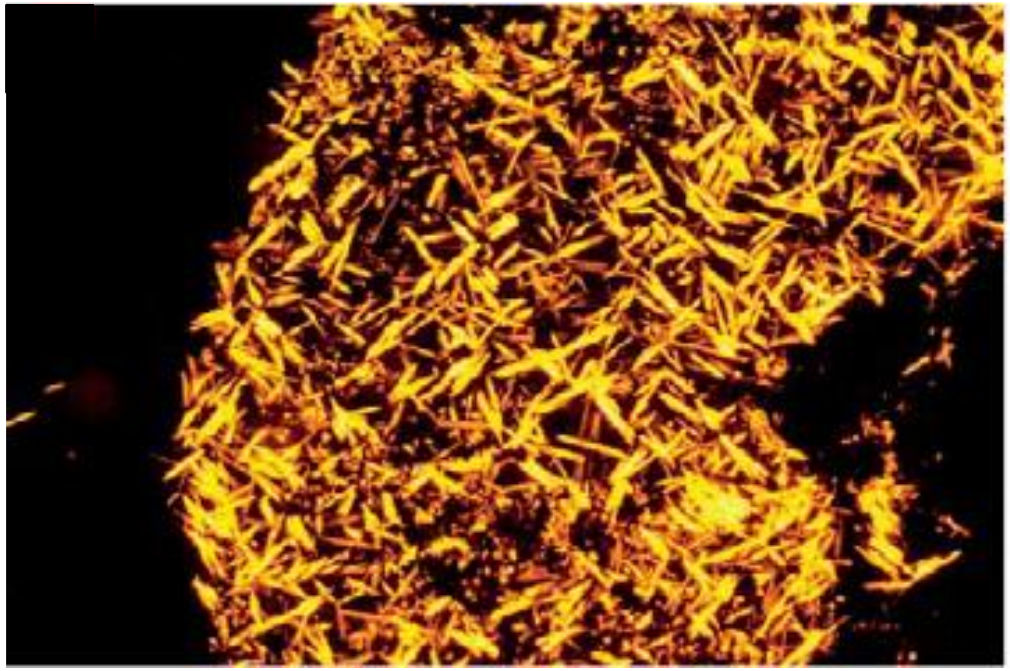
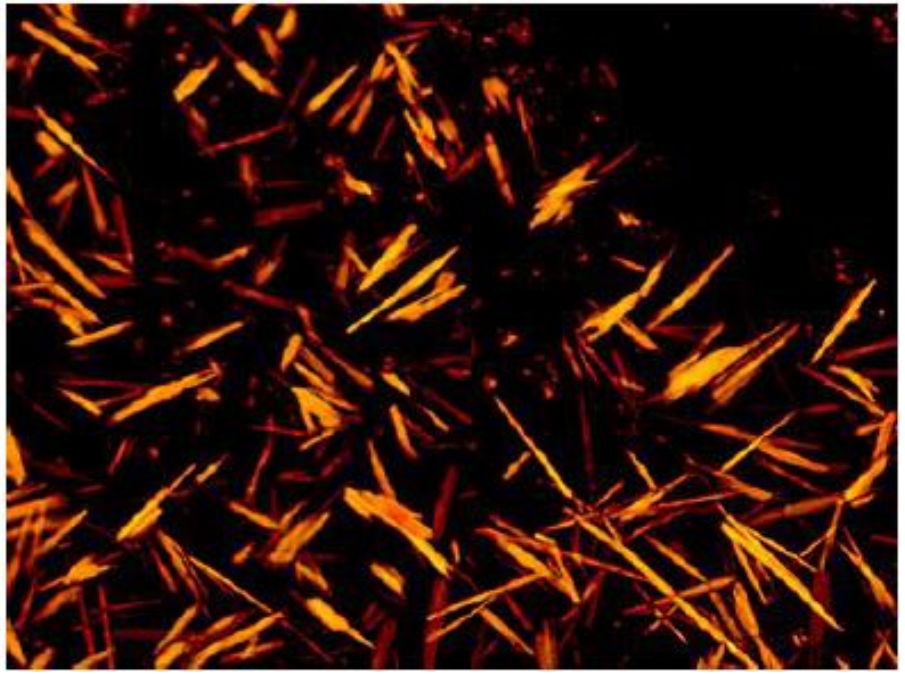


Image from Ivashkin V, Zharkova M. *N Engl J Med*. 2017;376(9):e14. doi: 10.1056/NEJMicm1610060.

Figure 2: Frozen liver biopsy sample reveal accumulation of CE crystals in Kupffer cells under polarized light[3,b]



Zharkova M, et al. *Case Rep Gastroenterol*. 2019;13(3):498-507.

[a]An 18-year old female who presented with elevated transaminases after varicella infection. [b] An 17-year old female who presented with elevated transaminases (3 times normal) and with varicella infection.

1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Ivashkin V, Zharkova M. *N Engl J Med.* 2017;376(9):e14. doi: 10.1056/NEJMicm1610060. **3.** Zharkova M, et al. *Case Rep Gastroenterol.* 2019;13(3):498-507.

Complications of Progressive Liver Damage

- Progressive liver injury stimulates hepatocellular hyperplasia and angiogenesis, potentially leading to fibrosis and cirrhosis
- Progressive complications due to loss of hepatic function may lead to:
  - Coagulopathy
  - Hepatorenal syndrome
  - Hepatic encephalopathy
- Portal hypertension is the most common serious complication of cirrhosis, and can cause complications such as esophageal varices
- Signs that cirrhosis has progressed to a decompensated stage may include:
  - Edema
  - Ascites
  - Jaundice

Civan JM. Cirrhosis. Merck Manual Professional Version website. https://www.merckmanuals.com/professional/hepatic-and-biliary-disorders/fibrosis-and-cirrhosis/cirrhosis. Updated December 2019. Accessed January 20, 2020.

Liver Damage Progresses Rapidly in LAL-D When Compared With Hepatitis C and NAFLD[1]

In 3 separate studies:

LAL-D

- A literature search found that 64% (72/112) of patients with LAL-D had fibrosis and/or cirrhosis (cirrhosis was present in 29% [33/112] of patients)[1]

Hepatitis C

- In a study of pediatric patients, cirrhosis was only seen in 2% (2/121) of patients with evidence of persistent infection[2,3]

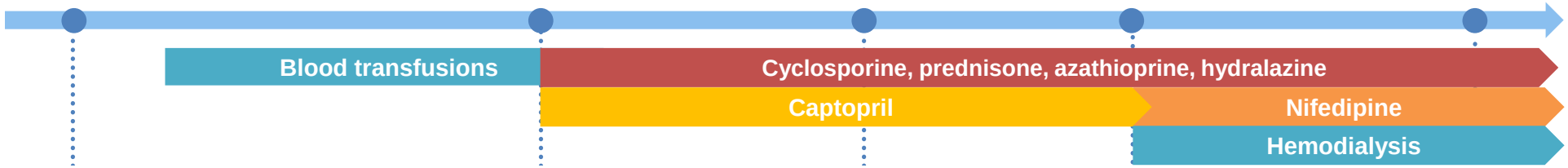
NAFLD

- Cross-sectional studies of 67 pediatric patients found clinically significant fibrosis in 14.7% and no cirrhosis[3]

1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Goodman ZD, et al. *Hepatology.* 2008;47(3):836-843. **3.** Burton BK, et al. *J Pediatr Gastroenterol Nutr*. 2015;61(6):619-625.

Progressive Liver Damage in LAL-D Can Have Severe Consequences

Case: Female patient with LAL-D[1,2]



Hepatosplenomegaly	
Liver cholestasis and fibrosis	
Total-c	238 mg/dL
HDL-C	46 mg/dL
TG	178 mg/dL
AST	240 U/L

LAL protein 1.37 nmol/min/mg

Pre transplant: Slowed growth rate, Ascites Portal hypertension	
AST	81 U/L
ALT	26 U/L
Gastritis, upper gastrointestinal bleeding post transplant	
Biliary obstruction and infection post transplant	

Liver transplant

Splenomegaly	
Hypertension	
Total-c	142 mg/dL
HDL-C	33 mg/dL
LDL-C	54 mg/dL
TG	274 mg/dL
AST	Normal

5-7 years post liver transplant:

- Abnormal kidney function
  - Atherosclerosis, lipid deposits, atrophy, and fibrosis shown on renal biopsy
- Worsening hypertension
- Required emergency hemodialysis to relieve pulmonary edema

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; LAL, lysosomal acid lipase; total-c, total cholesterol; TG, triglycerides.

1. Ferry GD, et al. *J Pediatr Gastroenterol Nutr*. 1991;12(3):376-378. **2.** Kale AS, et al. *J Pediatr Gastroenterol Nutr.* 1995;20(1):95-97.

Guidelines Recommend Ruling Out LAL-D When Evaluating Children and Adults for NAFLD[1,2]

2017 NASPGHAN Guidelines: Differential Diagnosis for Pediatric Patients with Hepatic Steatosis[1]

Genetic/metabolic disorders	Medications	Dietary causes	Infections
Nonalcoholic fatty liver disease	Amiodarone	Protein-energy malnutrition (Kwashiorkor)	Hepatitis C (genotype 3)
Fatty acid oxidation and mitochondrial disorders			
Citrin deficiency	Corticosteroids	Alcohol abuse	
Wilson disease	Methotrexate	Rapid surgical weight loss	
LAL-D	Certain antipsychotics	Parenteral nutrition	
Uncontrolled diabetes	Valproic acid		
Lipodystrophies	Certain antidepressants		
FCH	HAART		
Abeta/hypobeta-lipoproteinemia			

Used with permission from Vos MB, et al. *J Pediatr Gastroenterol Nutr.* 2017;64(2):319-333.

2017 AASLD Guidelines: Common Causes of Secondary Hepatic Steatosis in Adults[2]

Macrovesicular Steatosis	Microvesicular Steatosis
Excessive alcohol consumption	Reye's syndrome
Hepatitis C (genotype 3)	Medications (valproate, anti retroviral medicines)
Wilson disease	Acute fatty liver of pregnancy
Lipodystrophy	HELLP syndrome
Starvation	Inborn errors of metabolism (eg, LCAT deficiency, LAL-D)
Parenteral nutrition	Medications (eg, amiodarone, methotrexate, tamoxifen, corticosteroids)
Abetalipoproteinemia	

Adapted from Chalasani N, et al. *Hepatology.* 2018;67(1):328-357.

AASLD, American Association for the Study of Liver Diseases; FCH, familial combined hyperlipidemia; HAART, highly active antiretroviral therapy; HELLP, hemolysis, elevated liver enzymes, low platelet count; LCAT, lecithin-cholesterol acyltransferase; LAL-D, lysosomal acid lipase deficiency; NAFLD, non-alcoholic fatty liver disease; NASPGHAN, North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition. [See https://celiac.org/event/north-american-society-for-pediatric-gastroenterology-hepatology-and-nutrition-naspghan-annual-meeting/]

1. Vos MB, et al. *J Pediatr Gastroenterol Nutr.* 2017;64(2):319-334. **2.** Chalasani N, et al. *Hepatology.* 2018;67(1):328-357.

Test for LAL-D in Patients With Elevated ALT and LDL-C[1]

Test for LAL-D when evaluating patients for fatty liver disease:

1. If LDL-C ≥130 mg/dL in children or ≥160 mg/dL in adults[2,3,a]
2. Or, if cholesterol levels are not available, consider testing for LAL-D when testing for Wilson disease[4,5]

[a]Based on baseline LDL-C levels in 66 patients with LAL-D from a multicenter study.

ALT, alanine aminotransferase; LAL-D, lysosomal acid lipase; LDL-C, low density lipoprotein cholesterol.

1. Maciejko JJ. *Am J Cardiovasc Drugs.* 2017;17(3):217-231. **2.** Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **3**. Burton BK, et al. *N Engl J Med*. 2015;373(11):1010-1020.
4. Manolaki N, et al. *J Pediatr Gastroenterol Nutr.* 2009;48(1):72-77. **5**. Roberts EA, Schilsky ML. *Hepatology.* 2008;47(6):2089-2111.

Elevated LDL-C Is More Prevalent in LAL-D Than NAFLD[1-4]

Patients with elevated LDL-C in 3 separate studies of NAFLD/NASH and LAL-D

Patient population	LDL-C ULN	Patients with elevated LDL-C (%)
Pediatric NAFLD (n=173)[1,a]	≥130 mg/dL	**22%**
Adult NAFLD/NASH (n=247)[2,b]	≥160 mg/dL	**16%**
Patients with LAL-D (n=66)[3,4,c]	≥130 mg/dL	**94%**

[a]All Individuals are from the Treatment of NAFLD in Children (TONIC) trial were included (N =173). In the TONIC trial, children with NAFLD were randomized to vitamin E, metformin, or placebo for 96 weeks. [b]Individuals in the Pioglitazone vs. Vitamin E vs. Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial with paired liver biopsies and fasting lipid levels were included (N = 222). In the PIVENS trial individuals were randomised to pioglitazone 30 mg, vitamin E 800 IU or placebo for 96 weeks. [c]A total of 66 subjects were enrolled in multicentre, randomised, placebo-controlled study was designed to evaluate the safety and efficacy of sebelipase alfa in patients with LAL Deficiency of whom 36 were randomly assigned to sebelipase alfa and 30 were randomly assigned to placebo. Median LDL-C was 204 mg/dL with a range between 70 and 378 mg/dL. A multicenter, randomized, double-blind, placebo-controlled study involving 66 patients who were evaluated for the safety and effectiveness of enzyme-replacement therapy with sebelipase alfa (administered intravenously at a dose of 1 mg per kilogram of body weight every other week); the placebo-controlled phase of the study was 20 weeks long and was followed by open-label treatment for all patients
NOTE: These studies represent individual publications using different patient populations. Data are not directly comparable.
NASH, nonalcoholic steatohepatitis; ULN, upper limit of normal.

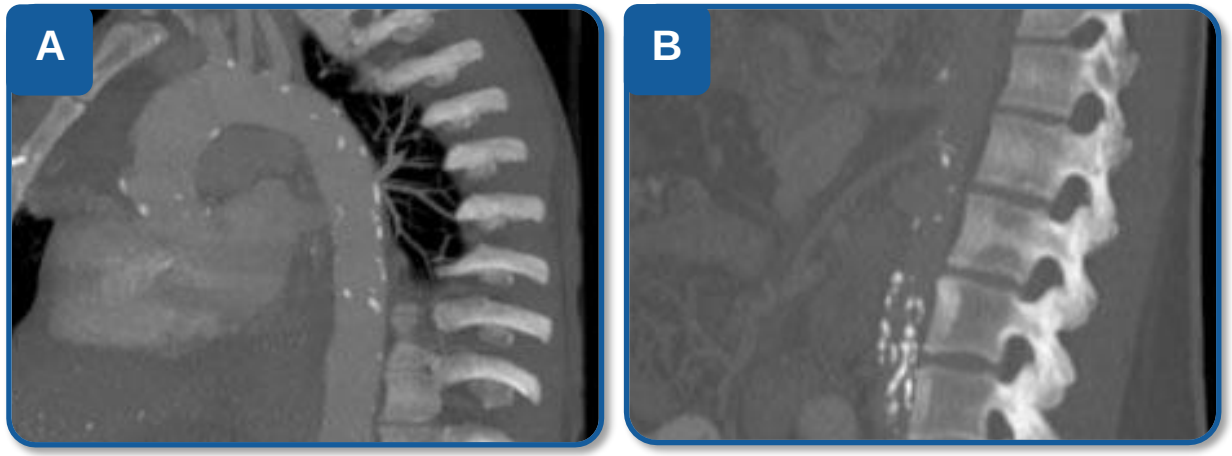
1. Corey KE, et al. *J Pediatr Gastroenterol Nutr*. 2015;60(3):360-367. **2.** Corey KE, et al. *Aliment Pharmacol Ther*. 2015;41(3):301-309. **3.** Burton BK, et al. *N Engl J Med*. 2015;373(11):1010-1020. **4.** Data on file, Alexion Pharmaceuticals. **2.**

LAL-D Manifestations of the Cardiovascular System

Cardiovascular Consequences in LAL-D

87% of patients with LAL-D have CV consequences[1,a]

- Dyslipidemia
- Accelerated atherosclerosis
- Coronary artery disease
- Aneurysm or stroke
- Myocardial infarction



Sagittal CT images of the major arteries showing extensive calcification in the (A) thoracic aorta and (B) abdominal aorta; female, age 42 years, with LAL-D[2]

Springer Science+Business Media: JIMD Reports, Orthotopic liver transplantation in an adult with cholesteryl ester storage disease, 8, 2013, 41-46, Ambler GK, et al with permission of Springer.

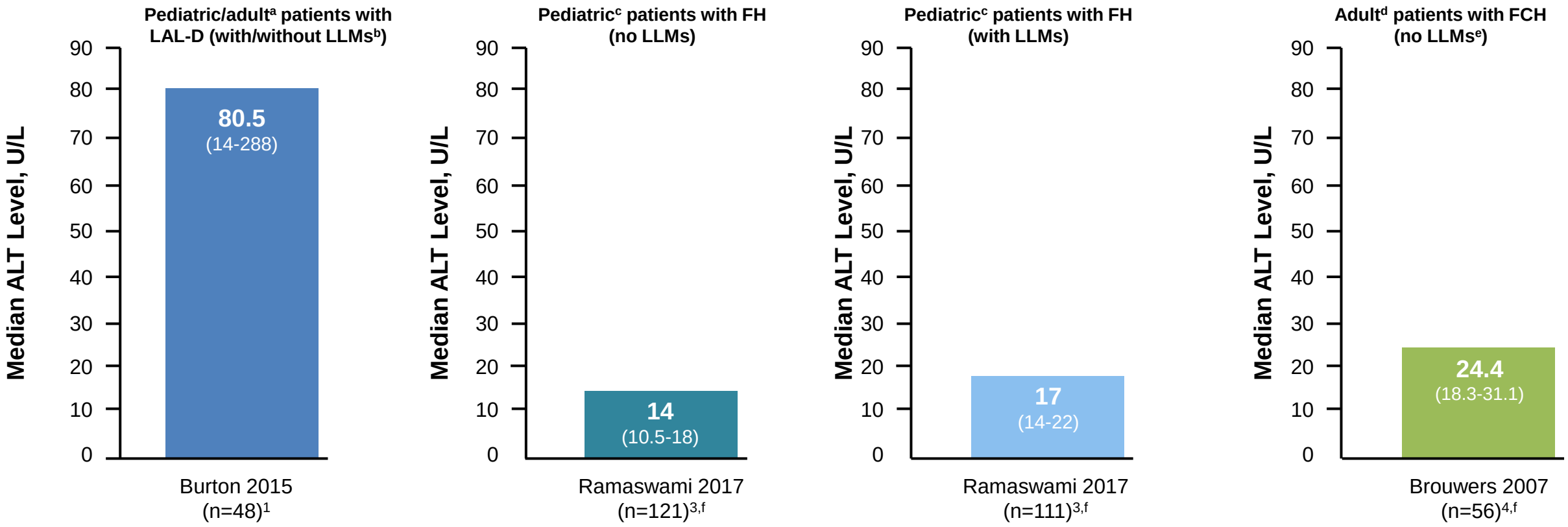
[a] Based on an analysis of 55 genotyped LAL-D patients in a cohort of 135 cases.[1]

CT, computed tomography.

1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Ambler GK, et al. *JIMD Rep.* 2013;8:41-46. doi: 10.1007/8904_2012_155.

Elevated ALT May Help Differentiate LAL-D From Hereditary Dyslipidemias[1,2]

Median (range) ALT levels in LAL-D, FH, and FCH studies



NOTE: These studies represent individual publications using different patient populations. Data are not directly comparable.

LAL-D, lysosomal acid lipase; FH, familial hypercholesterolemia; FCH, familial combined hyperlipidemia; LLM, lipid-lowering medication; NICE, National Institute for Health and Care Excellence.

[a]In the observational study of patients with LAL-D, the mean ± SD age at first recorded elevated transaminase was 14.5 ± 14.5 years. [b]81% of patients reported use of LLMs at some point during disease course; ALT elevations typically persisted with no evidence of substantial and/or sustained change following diet modifications or LLMs. [c]In the FH study, NICE CG71 recommends testing of children at risk of HeFH by the age of 10 years; therefore, subjects are split into 2 categories: group ≤10 years and group ≥10 years old. The mean ± SD age of the ≤10 years old group is 7.3 ± 2.2 years old; mean ± SD age of the ≥10 years old group is 12.6 ± 1.7 years old. [d]In the FCH study, mean ± SD age of subjects with FCH was 50 ± 13 years old. [e]LLMs were withdrawn 2 weeks prior to the study. [f]Interquartile range.

1. Burton BK, et al. *J Pediatr Gastroenterol Nutr.* 2015;61(6):619-625. **2.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30. **3.** Ramaswami U, et al. *Arch Dis Child.* 2017;102(3):255-260. **4.** Brouwers MC, et al. *Clin Sci.* 2007;113(9):375-381.

Patient Profile to Test

LAL-D should be considered in high-risk patient groups

- In any patient with elevated LDL-C accompanied by elevated (even mildly) ALT[1]
- In patients with a clinical diagnosis of FH without genetic confirmation[2,3]
- In patients with suspected FCH with an absence of family history[4,5]
- In patients with suspected metabolic syndrome with elevated LDL-C, elevated ALT, and/or suboptimal response to LLMs[1,4,5]

Test for LAL-D with an enzymatic blood test[6]

ALT, alanine aminotransferase; FCH, familial combined hyperlipidemia; FH, familial hypercholesterolemia; LAL-D, lysosomal acid lipase deficiency; LDL-C, low-density lipoprotein cholesterol;; LLM, lipid-lowering medication.

1. Bernstein DL, et al. *J Hepatol.* 2013;58(6):1230-1243. **2**. Chora JR, et al. *J Clin Lipidol*. 2017;11(2):477-484.e2. **3.** Sturm AC, et al. *J Am Coll Cardiol.* 2018;72(6):662-680. **4.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30. **5.** Maciejko JJ. *Am J Cardiovasc Drugs*. 2017;17(3):217-231. **6**. Lukacs Z, et al. *Clin Chim Acta*. 2017;471:201-205.

LAL-D Should Be Considered in Individuals With a Negative FH Genetic Test

FH Genetic Testing Recommendations Based on Patient Category

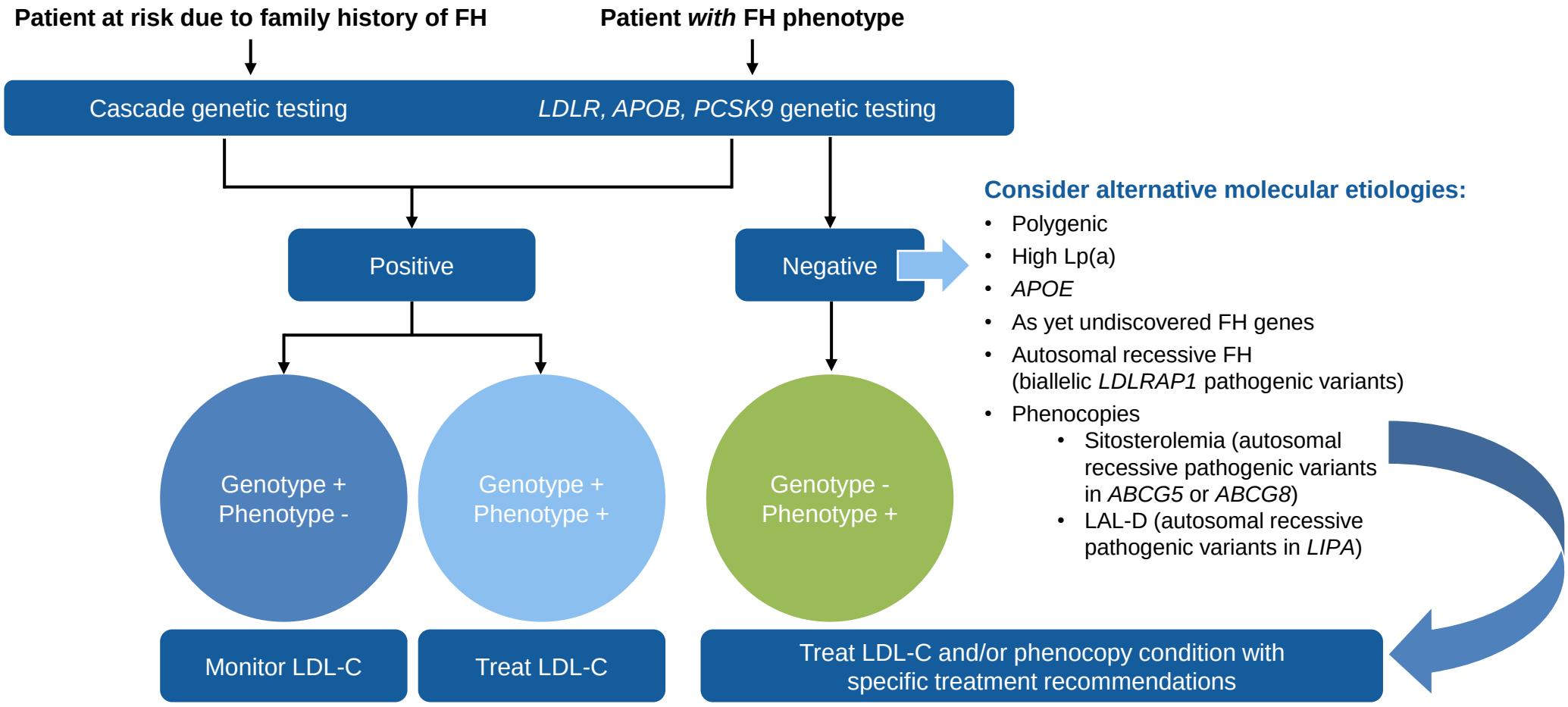


Image from Sturm AC, et al.. *J Am Coll Cardiol*. 2018;72(6):662-680.

APOB, apolipoprotein B; APOE, apolipoprotein E; FH, familial hypercholesterolemia; LAL-D, lysosomal acid lipase deficiency; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; LDLRAP1, LDLR adaptor protein 1; Lp(a), lipoprotein; PCSK9, proprotein convertase subtilisin/kexin 9.

Sturm AC, et al.. *J Am Coll Cardiol.* 2018;72(6):662-680.

LAL-D Manifestations of the Spleen and Gastrointestinal Tract

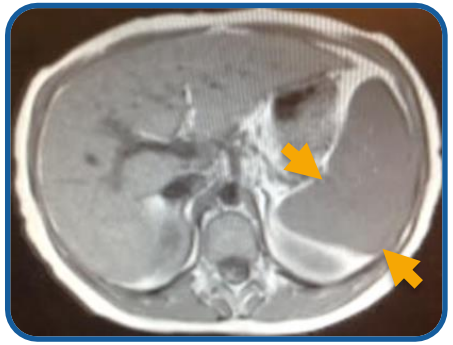
LAL-D Can Cause Splenomegaly and Other Spleen-Related Manifestations

74% of patients with LAL-D have splenomegaly. Other spleen manifestations include:[1]

- Thrombocytopenia[2]
- Anemia[1]

Consequences of splenomegaly[3]

- Risk of traumatic splenic rupture
- Risks associated with splenectomy



9 yo female with LAL-D: Spleen enlarged 12 cm[4]

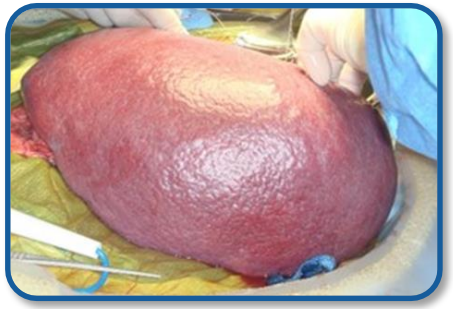
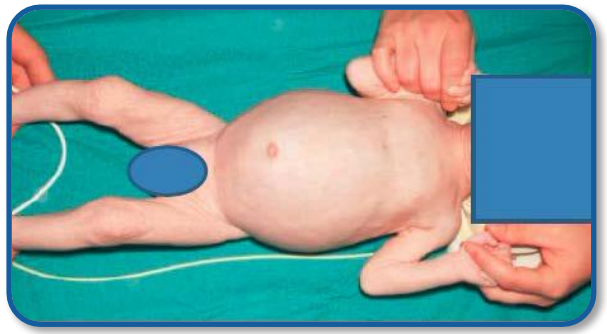


Image courtesy of Nadia Ovchnisky, MD, Montefiore Hospital, New York.

Splenectomy from child with LAL-D and persistent debilitating abdominal pain[4]

Infant with LAL-D: Rapid progression of abdominal distension



24 days

Alexion image permissions on file.

1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Taurisano R, et al. *Eur J Pediatr*. 2014;173(10):1391-1394. **3.** Radhakrishnan N, Sacher RA. In: Splenomegaly. Medscape. http://misc.medscape.com/pi/android/medscapeapp/html/A206208-business.html. Accessed December 14, 2017. **4.** Data on file. Alexion Pharmaceuticals.

LAL-D Can Cause Progressive Gastrointestinal Damage[1,2]

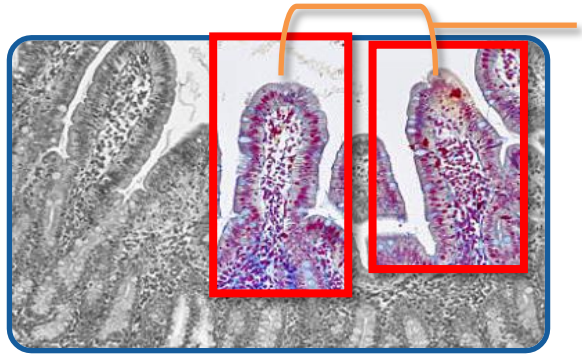
22% of LAL-D patients have gastrointestinal manifestations[1,a]

- Abdominal and epigastric pain[1,2]
- Emesis[1,2]
- Diarrhea[1,2]
- Chronic malabsorption[1,2]

Consequences may include[2]

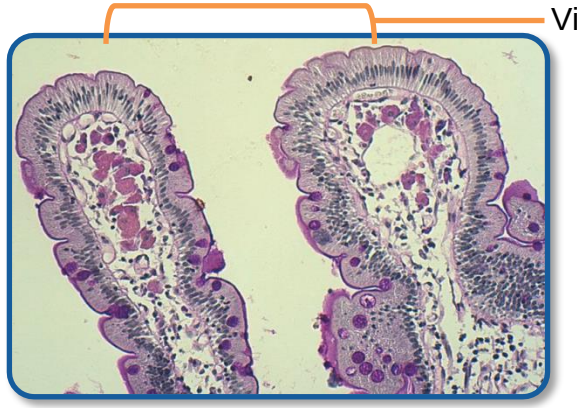
- Failure to thrive/growth failure
- Cachexia
- Short stature

Healthy



Biophoto Associates / Science Source.

LAL-D[3]



Santillan-Hernandez Y, et al. *World J Gastroenterol.* 2015;21(3):1001-1008.
© The Authors, 2015.

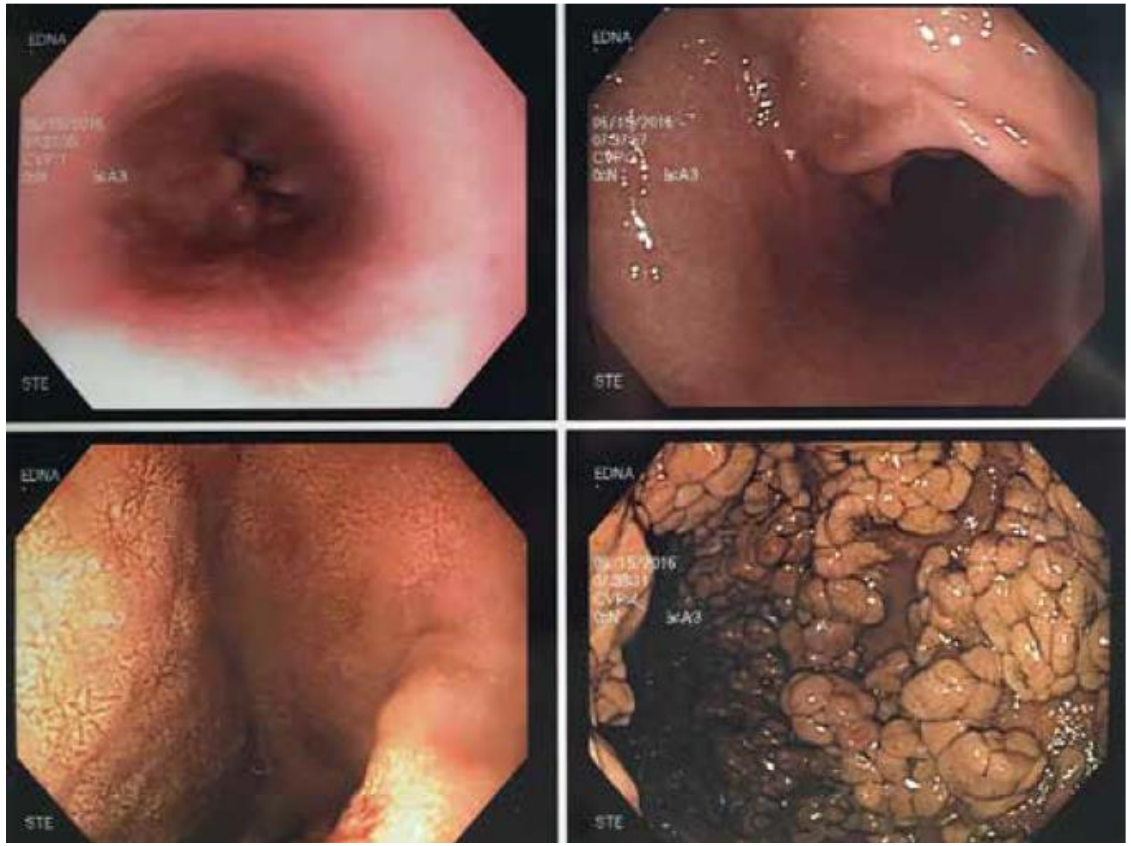
Duodenal biopsy: Histiocytes with ceroid material in lamina propria of villi[4]

[a]Based on an analysis of 55 genotyped LAL-D patients in a cohort of 135 cases.

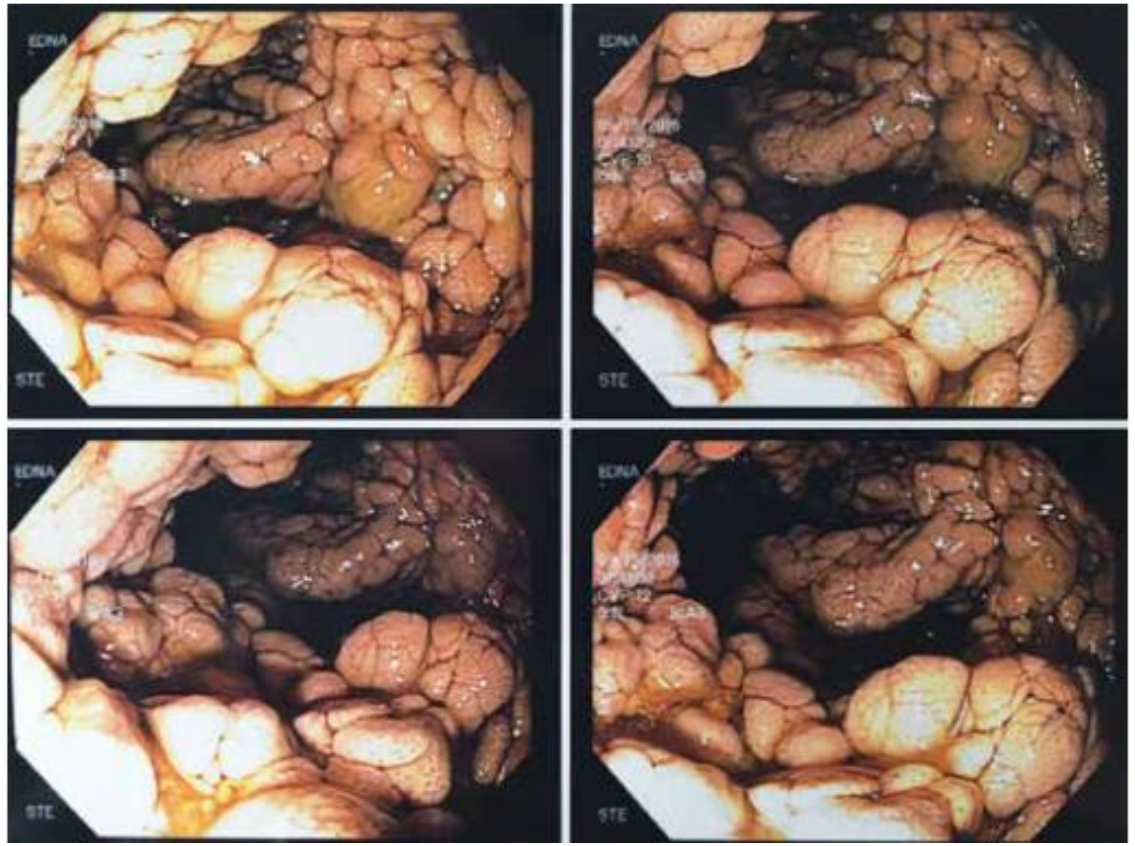
1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30. **3.** Santillán-Hernández Y, et al. *World J Gastroenterol.* 2015; 21(3):1001-1008. **4.** Detlefson S, et al. *Hum Pathol.* 2013;44(5):683-696.

Upper GI Endoscopy of an Adult With LAL-D

Duodenal bulb + Distal duodenum



Distal duodenum



Images courtesy of Ana Maria Martins, MD.

Diagnostic Testing for LAL-D

LAL-D Is Diagnosed with an Enzyme Activity Test[1]

- LAL enzyme activity can be measured in multiple sample types[1]

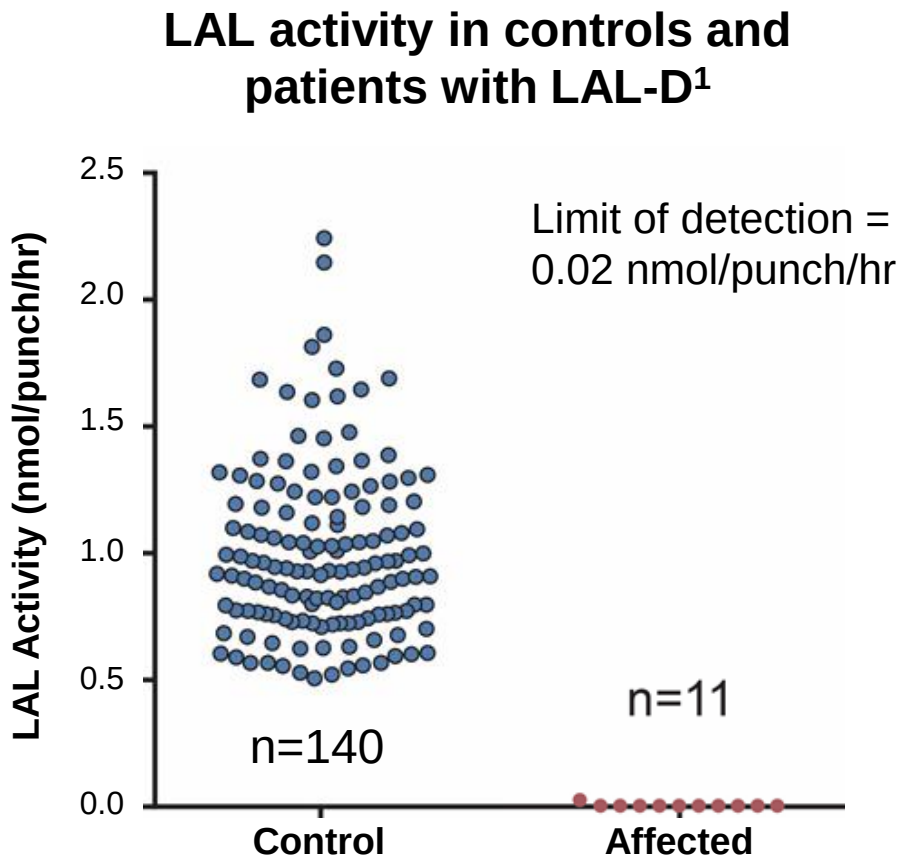
Dried blood spot (DBS) | **Leukocytes from whole blood** | **Fibroblasts**

- While LAL enzyme activity testing is confirmatory for LAL-D, further evaluations may support the diagnosis[2]
  - *LIPA* gene sequencing is not diagnostic but helps support a positive diagnosis and provides information for genetic counseling, family screening, and variant databases[2,3]
    - Possible exception when enzyme activity is inconclusive and 2 known pathogenic *LIPA* mutations are found[4]
  - Liver biopsy may be suggestive of LAL-D with the presence of microvesicular steatosis[2]

LAL, lysosomal acid lipase; LAL-D, lysosomal acid lipase deficiency.

1. Civallero G, et al. *Gene*. 2014;539(1):154-156. **2.** Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **3.** Thorogood A, et al. *Genet Med.* 2017;19(7):838-841. **4.** Lukacs Z, et al. *Clin Chim Acta*. 2017;471:201-205.

Interpreting the Results of Enzyme-Based Biochemical Tests for LAL Activity



Reprinted from Clin Chim Acta, 413(15-16):1207-1210, Hamilton J, et al. A new method for the measurement of lysosomal acid lipase in dried blood spots using the inhibitor Lalistat 2, © 2012, with permission from Elsevier B.V.

- DBS or leukocyte testing is highly accurate and easy to interpret with "Affected" or "Unaffected" results[2]
  - Indeterminate results should be retested per individual lab protocols, as they are likely caused by poor sample quality
- Patients with manifestations consistent with LAL-D, but with marginal LAL enzyme activity, may require additional consultation or genetic analysis[2]

DBS, dried blood spot.

1. Hamilton J, et al. *Clin Chim Acta*. 2012;413(15-16):1207-1210. **2.** Lukacs Z, et al. *Clin Chim Acta*. 2017;471:201-205.

Establishing Interpretation Thresholds

DBS[1]

- In published validations of enzyme activity in patients with LAL-D, LAL activity by DBS was <5% of mean normal, making this a suggested cutoff for the diagnosis of LAL-D
  - Sample quality must be confirmed to report a result of <5% mean normal as positive for LAL-D

Fibroblasts and leukocytes[1,2]

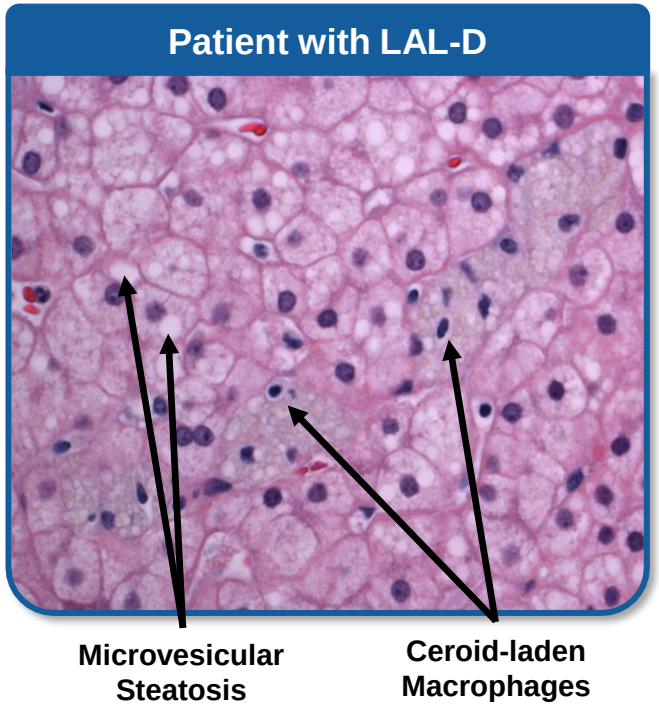
- Residual LAL activity is detectable and quantifiable by leukocyte or fibroblast testing
  - Because of possible residual activity, the threshold for reporting a test as positive for LAL-D may be higher in these sample types compared to DBS
- Laboratories select a cutoff based on local experience with the assay, but it is advised to have the threshold be a percentage of mean normal activity
  - Equipment settings must be optimized for each laboratory

DBS, dried blood spot; LAL, lysosomal acid lipase; LAL-D, lysosomal acid lipase deficiency.

1. Lukacs Z, et al. *Clin Chim Acta*. 2017;471:201-205. **2.** Civallero G, et al. *Gene.* 2014;539(1):154-156.

Liver Biopsy Is an Optional Clinical Evaluation, but Not a Diagnostic Test[1]

- Non-invasive means should be utilized in the initial evaluation and diagnosis of LAL-D[1]
  - Enzyme blood test
  - Radiologic imaging
- Biopsy may be used for staging and following disease progression[1]
  - Features may include microvesicular or mixed steatosis and altered macrophages and Kupffer cells
- Importantly, many features observed on liver biopsy are not unique to LAL-D[1,2]



5-year-old male with hepatosplenomegaly and ALT 3x normal

Image courtesy of Dhanpat Jain, MD, Yale University School of Medicine, New Haven, CT.

ALT, alanine aminotransferase.

1. Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **2.** Bernstein DL, et al. *J Hepatol.* 2013:58(6):1230-1243.

Specific Considerations for Diagnosis of Rapidly Progressive Infantile LAL-D

- Rapidly progressive infantile LAL-D is a medical emergency that could result in liver failure and premature mortality (<12 months)[1,2]
- Rapid diagnosis of these infant patients is vital[1,2]
- Clinicians and laboratories play a critical role in expediting diagnosis of affected individuals[3]

  - If rapidly progressive infantile LAL-D is suspected, it is essential to obtain a prompt sample for LAL enzyme activity testing[2]
  - Deterioration is rapid in infants, and turn-around time should be a consideration in all patients, but especially those with infantile-onset LAL-D[2]

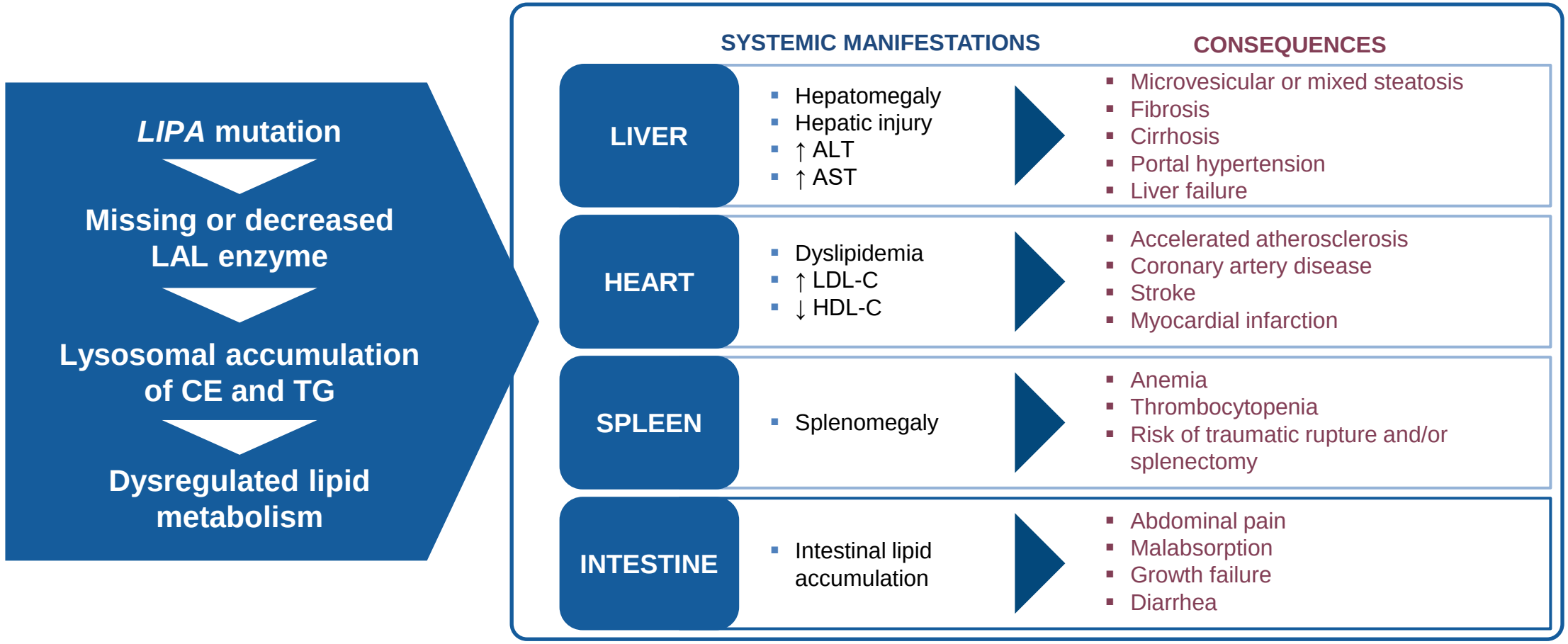
LAL, lysosomal acid lipase; LAL-D, lysosomal acid lipase deficiency.

1. Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **2.** Lukacs Z, et al. *Clin Chim Acta.* 2017;471:201-205. **3.** Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243.

Conclusion

Systemic Manifestations of LAL-D

LAL-D is a life-threatening genetic disease with ongoing, progressive, multiorgan damage[1-3]



ALT, alanine aminotransferase; AST, aspartate aminotransferase; CE, cholesteryl esters; HDL-C, high-density lipoprotein cholesterol; LAL-D, lysosomal acid lipase deficiency; LAL, lysosomal acid lipase; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30. **3.** Radhakrishnan N, Sacher RA. Splenomegaly. Medscape website. http://misc.medscape.com/pi/android/medscapeapp/html/A206208-business.html. Accessed December 21, 2018.

Conclusions

- LAL-D is rapidly progressive and associated with severe morbidities and premature mortality[1]
  - Early recognition and accurate diagnosis of LAL-D are critical[2]
- An enzyme-based biochemical test can be used to identify patients with LAL-D[2]
  - Genetic testing and liver biopsy provide additional supportive information when LAL enzyme activity is low
- Unexplained liver and/or lipid abnormalities should raise suspicion of LAL-D[2]
  - LAL-D should be considered in the differential diagnosis of Wilson disease, NAFLD/NASH, metabolic syndrome, FCH, and HeFH[2-4]

1. Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30. **3.** Vos MB, et al. *J Pediatr Gastroenterol Nutr.* 2017;64(2):319-334. **4.** Himes RW, et al. *Pediatrics*. 2016;138(4).

Appendix

Ordering and Submitting a LAL-D Enzymatic Blood Test

LAL enzyme activity testing is confirmatory for LAL-D

Step 1: Determine if LAL-D is an available test in your EMR

- The test may be listed as LAL-D, Lysosomal Acid Lipase, Acid Lipase, LAL, Wolman, Cholesteryl Ester Storage Disease, or CESD. This may differ by order entry system
- If not listed in the EMR, select “other” or “miscellaneous,” and type “Lysosomal Acid Lipase (LAL) test.” Enter your lab of choice and the appropriate test code

Step 2: Order LAL-D enzyme testing when you encounter patients with elevated ALT and LDL-C

DBS Sample Requirements – All DBS Labs[1,2]	
Specimen	Whole blood (ethylenediamine tetraacetic acid [EDTA])
Anticoagulant	Preferred: Lavender-top (EDTA) tube; Also accepted: Yellow top (acid citrate dextrose [ACD]) or Green top (sodium heparin)
Required sample volume	1-3 mL
Sample storage and shipping	Refrigerated sample must arrive within 72 hours

Leukocyte Requirements – Lysosomal Diseases Testing Laboratory[3]	
Specimen	Whole blood (EDTA)
Anticoagulant	Lavender-top (EDTA) or Green top (sodium heparin) tube
Required sample volume	4-6 mL (minimum is 2 mL)
Sample storage and shipping	Ship sample overnight (Mon-Thurs) at room temperature in well-insulated container. Do not chill or spin

Leukocyte Requirements – Baylor Genetics Laboratory[4]	
Specimen	Whole blood
Anticoagulant	Yellow top (ACD) tube
Required sample volume	7-10 mL adult/child; 3-5 mL (Infant <2 years)
Sample storage and shipping	Ship at room temperature in an insulated container by overnight courier to arrive within 36 hours of collection. Do not heat or freeze

CESD, cholesteryl ester storage disease; EMR, electronic medical record.

1. ARUP Laboratories. Lysosomal acid lipase activity, Dried blood spot. https://ltd.aruplab.com/Tests/Pub/2012266. Accessed May 11,2020. **2.** LabCorp. Lysosomal acid lipase deficiency. https://www.labcorp.com/tests/402300/lysosomal-acid-lipase-lal-deficiency. Accessed May 11, 2020. **3.** Thomas Jefferson University. Sample requirements for leukocyte lysosomal enzyme screen. https://www.jefferson.edu/university/jmc/departments/neurology/programs/neurogenetics/lysosomal_diseases/sample/leukocyte.html. Accessed April 6, 2020. **4.** Baylor Genetics. Medical genetics test details. https://www.bcm.edu/research/medical-genetics-labs/test_detail.cfm?testcode=4504&show=1&CFID=14409331&CFTOKEN=51729810. Accessed April 6, 2020.

Ordering and Submitting a LAL-D Enzymatic Blood Test

Step 3: Create a preference list that includes LAL-D along with other commonly ordered tests related to either fatty liver or lipid abnormalities

Select Liver diagnostic considerations[1-9]

- ❑ α_1-Antitrypsin disease
- ❑ Drug-induced liver disease
- ❑ Fatty acid oxidation defects
- ❑ Gaucher disease
- ❑ Glycogen storage disorders
- ❑ Hemochromatosis
- ❑ Hepatocellular cancer
- ❑ Hepatitis A/B/C/D/E/Autoimmune
- ❑ **LAL-D**
- ❑ Niemann-Pick disease
- ❑ NAFLD
- ❑ Primary biliary cholangitis
- ❑ Primary sclerosing cholangitis
- ❑ Wilson disease

Select Lipid diagnostic considerations[1-3,7,10]

- ❑ Alcohol
- ❑ Diabetes (types 1 and 2)
- ❑ Drug therapy[a]
- ❑ FCH
- ❑ Familial defective apoB100
- ❑ Familial dysbetalipoproteinemia
- ❑ Heterozygous and homozygous FH
- ❑ Hypopituitarism
- ❑ Hypothyroidism
- ❑ **LAL-D**
- ❑ Nephrotic syndrome

[a]Drug therapies such as corticosteroids, isotretinoin, beta-blockers, oral contraceptives, chemotherapeutic agents, and antiretroviral agents.
FCH, familial combined hyperlipidemia; NAFLD, nonalcoholic fatty liver disease.

1. Reiner Ž, et al. *Atherosclerosis.* 2014;235(1):21-30. **2.** Data on File, Alexion Pharmaceuticals. **3.** Longo D. *Harrison's Principles of Internal Medicine.* New York, NY: McGraw-Hill Medical; 2012. Chapter 356:3145-3161. **4.** Longo D. *Harrison's Principles of Internal Medicine.* New York, NY: McGraw-Hill Medical; 2012. Chapter 308:2592-2602. **5.** Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **6.** Burton BK, et al. *N Engl J Med.* 2015;373(11):1010-1020. **7.** Hamilton J, et al. *Clin Chim Acta*. 2012;413(15-16):1207-1210. **8.** Chalasani N, et al. *Gastroenterology.* 2012;142(7):1592-1609. **9.** Vajro P, et al. *J Pediatr Gastroenterol Nutr*. 2012;54(5):700-713.
10. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; National Heart, Lung, and Blood Institute. *Pediatrics*. 2011;128(suppl 5):S213-S256.

Thank You!