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REVIEW



# Lysosomal acid lipase deficiency: a form of non-obese fatty liver disease (NOFLD)

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**ABSTRACT**

**Introduction:** With the growing obesity epidemic, nonalcoholic fatty liver disease (NAFLD) is rapidly becoming one of the leading causes of liver disease worldwide. Although obesity is a main risk factor for the development of NAFLD, it can also develop in lean subjects and can be encountered in different clinical setting and in association with an array of genetic, metabolic, nutritional, infectious and drug-induced disorders.

**Areas covered:** This article discusses causes of fatty liver in non-obese subjects focusing on Lysosomal acid lipase deficiency (LAL-D), a commonly overlooked disorder reviewing its prevalence, genetics, pathogenesis, clinical features, diagnosis and treatment. It will also review other causes of non-alcoholic fatty liver disease, which can be encountered in the absence of obesity and metabolic syndrome.

**Expert commentary:** Although the prevalence of LAL-D has been estimated in the range of 1 in 40,000 and 1 in 300,000, this estimate is much more than the identified cases reported in the literature, which suggests that that the disease may be considerably under-diagnosed. There is a pressing need to educate clinicians about the disease, especially with the development of new promising therapeutic modalities.

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## 1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the leading diseases affecting the nation and world. In the United States, NAFLD is one of the most common indications for liver transplantation as it represents over 75% of the chronic liver disease [1,2]. NAFLD is a disease of all ages and has been reported in children as young as 2 years of age [3].

NAFLD has always been considered a manifestation of metabolic syndrome in obese individuals. However, NAFLD can be seen in association with an array of disorders, which may result in late or wrong diagnosis. This article aims to review some of these disorders focusing on lysosomal acid lipase deficiency (LAL-D), an under-recognized disorder commonly overlooked or affected individuals misdiagnosed to have more common diseases such as NAFLD, nonalcoholic steatohepatitis (NASH), cryptogenic cirrhosis, heterozygous familial hypercholesterolemia, or familial combined hyperlipidemia.

LAL-D is a rare autosomal recessive lysosomal storage disease that is the result of a mutation in the lysosomal acid lipase (*LIPA*) gene. The mutation creates a reduction in the lysosomal acid lipase activity, resulting in accumulation of cholesteryl esters and, to a lesser degree, triglycerides in multiple organs, including the liver, spleen, adrenal glands, lymph nodes, intestinal mucosa, vascular endothelium, and skeletal muscle [4,5]. Fifty percent of infants with LAL-D have adrenal calcifications [6,7]. There is a correlation between the involvement of tissues and their relative involvement in receptor-mediated endocytosis and lysosomal degradation of lipoproteins [8–12].

Lysosomal acid lipase is the enzyme that catalyzes the hydrolysis of cholesteryl esters and triglycerides that have been internalized via receptor-mediated endocytosis of low-density lipoprotein (LDL) particles, eventually producing free cholesterol and fatty acids [13,14]. The variability in clinical presentations observed in patients with LAL-D may be related to the capricious nature of the mutations in the *LIPA* gene and the consequential level of residual enzyme activity. Other contributing factors, including environmental influences, may also play a role in the disease's progression.

Shteyer et al. have reported that low LAL activity correlates with hepatic steatosis and dysfunction (impairment or abnormality of function) in patients with microvesicular steatosis, cryptogenic cirrhosis, and NAFLD [15]. In another study by Selvakumar et al., which included 168 children, patients with significant fibrosis (stage 2–3) had a significantly lower LAL activity compared to those with mild fibrosis. However, there was no significant difference in LAL activity between children with NASH compared to those without NASH. It is unlikely that these patients with low LAL activity had genetic defects since no patient exhibited LAL activity compatible with genetic LAL-D. These findings suggest a possible role for LAL in the pathogenesis of liver and further studies are needed in order to define the direct role of LAL in liver disease severity and possibly identify patients who may benefit from enzyme replacement therapy [16].

Clinically, the disease can present in two major phenotypes: infantile-onset Wolman disease (WD) and later-onset cholesterol ester storage disease (CESD). WD was first described in

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1956 [17]. A few years later, Fredrickson reported a 12-year-old boy with marked hepatomegaly, hypercholesterolemia, and cholesteryl ester accumulation in the liver; the disorder was named cholesteryl ester storage disease [18]. It was later recognized that both WD and the later-onset CESD share the same molecular defect that results from mutations in the *LIPA* gene [14].

## 2. Genetics and prevalence

LAL-D is an autosomal recessive disease; it results from mutations in the *LIPA* gene, which maps to chromosome 10q. The LIPA gene is approximately 45 kb in length and has 10 exons. Over 50 loss-of-function LIPA mutations have been identified in patients with LAL-D. Thirty-seven percent of the 19 known mutations causing WD are small deletions or insertions, with 26% nonsense, 21% consensus splice-site mutations, 10% missense lesions, and 5% a large deletion. On the other hand, 50% of the 32 known CESD mutations are missense, with 25% small deletions or insertions, 16% nonsense, 6% consensus splice-site mutations, and 3% a large deletion [3,19]. Although occult mutations have been described, affected patients usually have either homozygous or compound heterozygous *LIPA* mutations [5]. Severe mutations associated with markedly reduced or no LAL activity were detected in patients with WD while LIPA mutations that encoded mutant enzymes with residual activity, such as nonsense mutations, frameshift defects, and point mutations resulting in stop codons, were reported in patients with CESD. This may explain the aggressive presentation in affected infants compared to children and older subjects [20].

More than half of patients with LAL-D have an exon 8 splice site mutation or E8SJM (c.894G > A) [21]. The mutation causes a deletion of exon 8 in mRNA by presenting an alternative acceptor splice site. The presence of residual LAL activity can be explained by the fact that some mRNA is spliced properly.

The prevalence of LAL-D has not been precisely determined. It has been estimated that LAL-D prevalence is in the range of 1 in 40,000 and 1 in 300,000. This estimate assumes that 50–70% of affected individuals with LAL-D have the E8SJM mutation [14]. The mutation's prevalence varies widely depending on ethnicity and geographical location [20–23]. This estimate is much more than the identified cases reported in the literature, which indicates that the disease may be considerably underdiagnosed, mostly likely because of a lack of familiarity with it. The disease is common in Jewish infants of Iraqi or Iranian descent, with an estimated incidence of 1 in 4200 in the Los Angeles community [24].

Muntoni et al. investigated the impact of heterozygosity in E8SJM carriers on a serum lipid profile. The authors collected E8SJM carriers both from a genetic study-population analysis and from outpatient lipid clinics and measured their serum lipid profiles. Thirteen individuals, mostly Germans, were found to be heterozygote for E8SJM. The 13 subjects had a significant increase in total cholesterol levels in both sexes. These results indicated that the individuals' heterozygotes for E8SJ may have a significant alteration in lipid profile with a polygenic hypercholesterolemia phenotype, leading to an increase in cardiovascular risk [25]. On the other hand, Stitziel et al. genotyped the E8SJM variant in 13,194 individuals of European ancestry and reported E8SJM allele frequency of 0.16%. However, there was no association between the mutations observed in these patients and alterations in plasma lipid levels or an increased risk of myocardial infarction [23].

First-degree relatives of LAL-D who were obligate heterozygotes and heterozygotes detected by genetic screening were reported to have significantly elevated serum cholesterol levels [26–32].

## 3. Pathogenesis

LAL plays a fundamental role in lipid metabolism through the hydrolysis of cholesteryl esters and triglycerides [33] (Figure 1). Free cholesterol and fatty acids and their metabolites stimulate transcription factors (sterol regulatory element-binding proteins [SREBPs]) that regulate the expression of genes controlled in the synthesis and the uptake of cholesterol [34]. The copiousness of free cholesterol results in decreased entry of cholesterol into the cell as SREBP-2 downregulates LDL

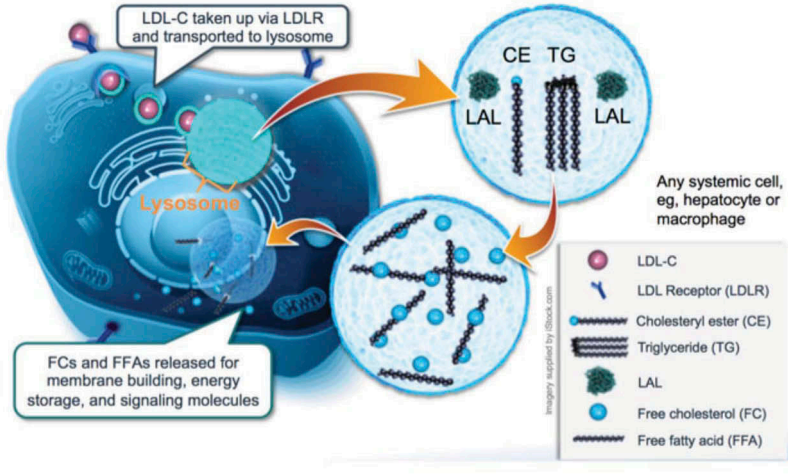


Figure 1. LAL processes CEs and TGs in the cells of healthy individuals. Published with permission of Alexion Pharmaceuticals.

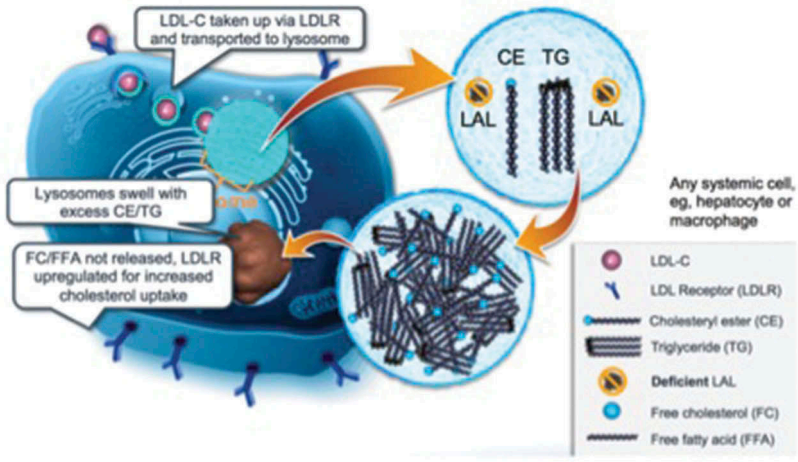


Figure 2. Mutations in *LIPA* cause deficient LAL enzyme in patients with LAL-D. Published with permission of Alexion Pharmaceuticals.

receptors. Cholesterol synthesis is decreased as a result of feedback inhibition of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. At the same time, stimulation of acyl-cholesterol acyltransferase results in enhanced cholesterol esterification. Similarly, an SREBP-1c-mediated downregulation of phospholipid and triglyceride production synthesis results from an abundance of intracellular fatty acids [35].

Reduced levels or the absence of LAL activity is associated with cholesteryl esters and triglyceride accumulation within lysosomes (Figure 2). In addition, the scarcity of intracellular free cholesterol leads to SREBP-mediated upregulation of endogenous cholesterol production by HMG-CoA reductase, enhanced endocytosis via LDL receptors, increased synthesis of apolipoprotein B (ApoB), and very low-density lipoprotein cholesterol (VLDL-C) [36].

Abnormal lipid profile (dyslipidemia) in patients with LAL-D is characterized by elevated serum total cholesterol, high low-density lipoprotein cholesterol (LDL-C), low high-density lipoprotein cholesterol (HDL-C), and elevated triglycerides [37]. The decrease of HDL-C can be explained by a reduction in the formation of mature high-density lipoprotein (HDL) (α-HDL particles). Lipidation of apolipoprotein A-I (apoA-I) and other HDL apolipoproteins by the membrane lipid transporter ATP-binding cassette transporter A1 (ABCA1) is the rate-limiting step in HDL particle formation [38]. Expression of ABCA1 is induced by increased cell cholesterol content, through oxysterol-dependent activation of the nuclear liver X receptors (LXRs). Under normal circumstances, oxysterol (oxidized derivatives of cholesterol)-dependent activation of the nuclear liver X-receptor (LXR), acting on the promoter of the *ABCA1* gene, leads to an expression of the *ABCA1* gene [39,40]. In LAL-D, the trap of cholesteryl esters in the lysosomes leads to reduction of free intracellular cholesterol and thus decreased oxysterol formation and the resultant reduced activation of *ABCA1* expression as well as decrease of free cholesterol in the subcellular compartments and in the plasma membrane that is available for HDL particle formation. Bowden et al. showed that treatment with conditioned medium-containing LAL from normal fibroblasts or with recombinant human LAL rescued ABCA1 expression, apoA-I-mediated cholesterol efflux, HDL particle formation, and the production of 27-hydroxycholesterol (a primary metabolite of cholesterol and an endoplasmic reticulum and LXRs ligand) by CESD human skin fibroblasts. These observations may explain the reduced HDL-C in patients with LAL-D and support the evidence that the rate of release of cholesterol from late endosomes or lysosomes is a major regulator of ABCA1 expression and activity [38].

## 4. Clinical features

The severity of a clinical presentation of LAL-D can vary widely depending on the residual activity of the LAL enzyme. During infancy, the disease's course can be severe and associated with high mortality rates because of complete absence or substantially reduced enzyme activity [11,41–44]. On the other hand, older children and adults with LIPA mutations and higher levels of LAL enzyme residual activity can have a milder course. Therefore, the disease in older patients can be easily overlooked or misdiagnosed because the manifestations can be very similar to more common disorders such as NAFLD, metabolic syndrome, and inherited dyslipidemias. Himes et al. [45] reported two children with NAFLD who managed to reduce their weight by adopting healthy diet and exercising. However, the improvement in body mass index (BMI) was not associated with an improvement in their hepatic biochemical parameters. A liver biopsy showed intense microvesicular steatosis, and the diagnosis of LAL-D was confirmed by measuring LAL activity and LIPA gene testing. These cases denotes the importance of increased awareness of LAL-D among health-care providers and signifies the need to consider LAL-D in the differential diagnosis of common hepatic disorders, such as NAFLD, especially when liver parameters in patients with NAFLD do not improve despite appropriate drop in the patient's body weight.

LAL-D usually presents in infants with an acute-severe course. Gastrointestinal manifestations are usually the initial symptoms, including vomiting, diarrhea, steatorrhea, and poor weight gain. Abdominal distension is remarkable because of hepatosplenomegaly. Adrenal calcification that is detected radiologically can be seen in 50% of patients [7,46,47]. Gall bladder dysfunction and stroke have also been reported [48]. As the disease progresses, manifestations of anemia, malnutrition, and

Table 1. Symptoms, signs, and biochemical features of LAL-D.

| | |
|---|---|
| Symptoms and signs | Abdominal pain |
| | Vomiting |
| | Diarrhea |
| | Malabsorption |
| | Steatorrhea |
| | Anemia |
| | Failure to thrive |
| | Cachexia |
| | Gall bladder dysfunction |
| | Coronary artery disease |
| | Aneurysm |
| | Stroke |
| | Adrenal calcification |
| | Jaundice |
| | Abdominal distension |
| | Esophageal varices |
| | GI bleeding |
| Biochemical markers | Elevated serum transaminases |
| | Elevated total cholesterol |
| | Elevated triglycerides |
| | Elevated plasma LDL-C |
| | Elevated plasma ApoB |
| | Decreased HDL-C |

multiorgan failure may emerge, such as jaundice, ascites, gastrointestinal bleeding, and coma (Table 1).

In a review by Bernstein et al. [41], the median age at the earliest onset of symptoms, diagnosis, or both in 131 patients with LAL-D was 5 years for both males (range birth–44 years) and females (range 1 month–68 years). Sixty-two percent of the patients presented between age 3 and 12, and 11% had onset or diagnosis during adolescence or as adults. There were five patients whose diagnoses were made at autopsy. Patients with severe disease were more readily diagnosed than those with a mild disorder. In 35 patients with a severe case of the disease, the median age of onset was between birth and 2 years old [11,13,49,50]. There is a report of a female diagnosed at the age of 80 years, probably with a mild phenotype [51].

In the same review by Bernstein et al. [41], hepatomegaly was identified clinically or by imaging modalities in 134 out of 135 subjects [52]. The only patient with a mild version of the disease who did not have hepatomegaly had elevated transaminases and pathological changes documented by liver biopsy. Splenomegaly was detected in 74% of the patients.

Dyslipidemia is one of the hallmarks of LAL-D. Children and adults with LAL-D usually have elevated total cholesterol, LDL-C, ApoB, and decreased HDL-C levels [52–62]. Dyslipidemia is a known risk factor for atherosclerosis and premature cardiovascular disease [46]. Beaudet et al. reported three siblings diagnosed with CESD; two died at ages 7 and 9 years, respectively, with hepatic scarring and portal hypertension. Aortic plaques were detected in the older child [47].

In three large case-controlled, genome-wide association studies, increased expression of *LIPA* in blood monocytes has been shown to be a risk factor for coronary artery disease (CAD). Increased intrinsic *LIPA* expression might augment intracellular release of fatty acids and cholesterol from the lysosomes, possibly explaining the association of the risk allele with impaired endothelial function, a precursor of atherosclerosis [63–65]. Interestingly, despite evidence that the risk allele was associated with higher *LIPA* gene expression (suggesting that both under- and overactivity of LIPA increase CAD risk), it was not significantly associated with abnormal lipid profiles. This observation may indicate that the increased risk of CAD may be because of a different mechanism that is independent of the altered lipid levels.

In the same review by Bernstein et al. [41], total cholesterol was elevated in all 110 patients for whom serum cholesterol was reported (26% were undergoing treatment with HMG-CoA reductase inhibitors). Thirty-four out of 43 patients with elevated serum LDL-C had elevated levels above 200 mg/L, and 41 had levels above the normal range, including 20 patients who were on treatment with HMG-CoA reductase inhibitors. In the same study, 65 patients were reported as having low HDL-cholesterol (ranges from 8 to 50 mg/dl). Seventy-one percent of the 65 patients had HDL-cholesterol levels between 20 and 40 mg/dl, 18% had levels below 20 mg/dl, and 11% had levels above 40 mg/dl.

## 5. Morbidity and mortality

There is a lack of longitudinal survival data for LAL-D. However, LAL-D is characterized by accelerated progression to fibrosis and cirrhosis [66]. In an acid lipase replacement investigating safety and efficacy (ARISE) trial, 36, or 78%, of the liver biopsies showed bridging fibrosis, cirrhosis, or both. An interesting observation was that 8 out of the 10 patients with cirrhosis did not have clinical or biochemical evidence of cirrhosis at the time of the biopsy. In another study that included 31 patients with LAL-D under the age of 18, 85% of liver biopsies showed evidence of fibrosis, cirrhosis, or both [67].

Premature atherosclerosis and cardiovascular disease have been reported in association with LAL-D; however, there is a lack of conclusive studies that show increased cardiovascular mortality in LAL-D [40,68,69].

It is unusual for infants with LAL-D to survive beyond a year, except for those who receive liver transplants or hematopoietic stem cell transplants. In a study by Jones et al., the median age at death was 3.7 months, and the estimated probability of survival past age 12 months was 0.114. Among patients with early growth failure, the median age at death was 3.5 months and the estimated probability of survival past age 12 months was 0.038. Patients who underwent hematopoietic stem cell transplants and liver transplants survived longer than untreated patients (median survival 8.6 months) [70].

Liver dysfunction is universal in patients with LAL-D and was reported in all the 135 patients described by Bernstein et al. [34]. In the same report, 8 out of the 11 deaths were because of liver failure and occurred at 7–56 years of age (4 patients were under 21 years of age [46]. Four additional deaths from liver failure occurred after the case reports were published [34].

## 6. Diagnosis

LAL enzyme activity can be measured in cultured liver tissue, fibroblasts, or peripheral leukocytes. However, this technique may not be very accurate because the substrates used in these assays (e.g. 4-nitrophenyl palmitate) are not very specific to LAL. Dried blood spot (DBS) is a new diagnostic tool that can measure LAL activity in peripheral leukocytes. DBS has been found to identify patients who have LAL-D [71]. LAL activity is measured using the fluorimetric substrate 4-methylumbelliferyl palmitate.

Because other lipases in the blood may affect the measurement of LAL activity in dried blood, lalistat 2 (specific inhibitor of LAL) is used to determine LAL activity [72,73]. LAL activity is measured in the presence and absence of lalistat 2 by comparing total lipase activity in both situations. LAL activity as measured by DBS reflects the total activity in blood including peripheral leukocytes. DBS has several advantages, including long-term stability and the fact that it only requires a small blood sample. Therefore, DBS is a promising diagnostic modality that can be used in the future for newborn screening [70].

LAL-D can be diagnosed by comprehensive sequencing of the coding regions of *LIPA* [5]. Screening for E8SJM (the most common mutation) may fail to detect many affected individuals because it is detected in only 50–70% of mutant alleles in patients with LAL-D. Moreover, E8SJM has a low prevalence in some populations, such as Asians and African Americans [22]. Genetic testing has another caveat: some patients may have an intronic mutation (nucleotide sequence within a gene that is removed by RNA splicing during maturation of the final RNA product). This type of mutation may not be detected with regular genetic screening. The absence of enzyme activity in patients with WD who usually have the null allelic variants results in a severe course and rapid progression. On the other hand, residual enzyme activity can be detected in patients with CESD.

Determining the genotype can help in distinguishing WD from CESD but does not predict residual enzyme activity since varying levels of LAL enzyme activity have been reported in patients with the same genotype. Similarly measuring residual LAL enzyme activity is not useful in anticipating the course of the disease as the severity of the disease vary significantly among patients with similar enzyme level [34]. Unfortunately, for the time being we lack any parameters that can predict the prognosis of the disease since patients with similar genetic mutations and similar residual activity can have different clinical courses.

Intense microvesicular steatosis is usually seen in a liver biopsy in patients with LAL-D (Figures 3 and 4). However, microvesicular steatosis is not specific for LAL-D because it can be associated with multiple disorders. Other specific features include hypertrophic Kupffer cells and portal macrophages with a periodic acid-Schiff-positive foamy tan-colored cytoplasm. The presence of luminal and membrane lysosomal markers around lipid vacuoles in fixed paraffin-embedded material, as well as cholesteryl ester crystals in unfixed samples, is pathognomonic for LAL-D [10].

Liver biopsy is commonly performed during the diagnostic process in patients who might have LAL-D. However, liver biopsy carries some morbidity and a very rare mortality risk [74]. Thelwall et al. demonstrated that magnetic resonance spectroscopy can identify and quantify the hepatic lipid distinctive features associated with LAL-D. Following treatment with sebelipase alfa (LAL replacement therapy), a substantial reduction in hepatic CE was observed in LAL-deficient rats. This method may be a less-invasive approach and an alternative to repeated liver biopsies for the diagnoses and monitoring of patients with LAL-D [75].

## 7. Treatment

### 7.1. Lipid-lowering agents

Dyslipidemia is a characteristic feature in patients with LAL-D. Therefore, it is rational to try lipid-lowering therapies in these patients. Statins (HMG-CoA-reductase inhibitors) are LDL-C-lowering agents commonly used to decrease the risk of cardiovascular disease in patients with dyslipidemia [76]. Statins were used as monotherapy or in combination with other lipid-lowering agents in patients with LAL-D, and mixed results were found. Although a decrease in LDL was described in some studies, no change or an actual increase of LDL were described in the reports [77–81]. Although improvement of hepatomegaly was reported in patients with LAL-D, statins do not seem to improve liver histology or halt the progression of liver fibrosis [82].

Ezetimibe (a cholesterol-absorption inhibitor) was reported to normalize liver transaminases and reduce total cholesterol and LDL-C levels (30% and 25%, respectively) in a 15-year-old boy with LAL-D after 6 months of treatment. Serum levels of cytokines and oxidative stress parameters, which were elevated at baseline, were also found to normalize after 1 year of ezetimibe therapy [83].

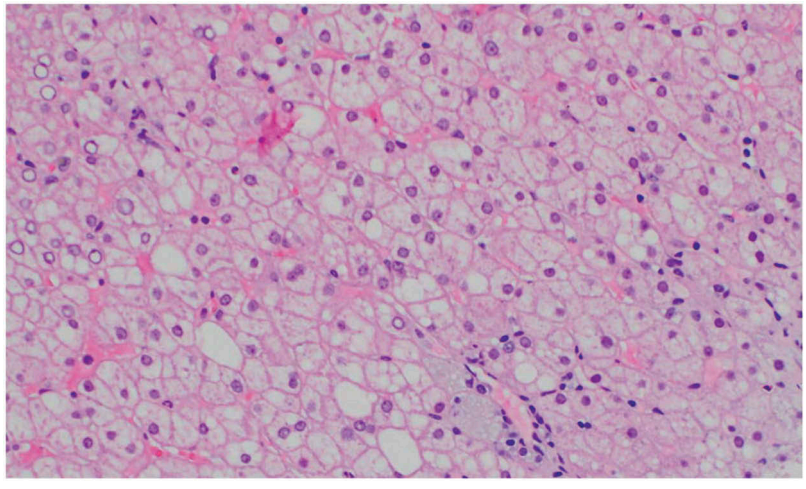

Figure 3. Liver biopsy in a patient with LAL-D showing microvesicular steatosis, intranuclear glyglycogen and storage material. H&E – 20x.

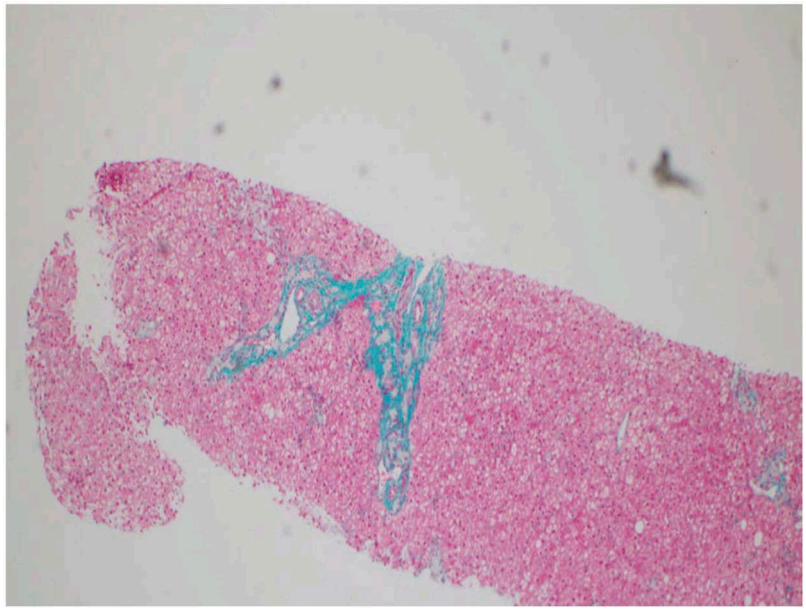

Figure 4. Liver biopsy in a patient with LAL-D. Trichrome stain showing microvesicular steatosis and stage 2 fibrosis, 10x.

### 7.2. Vitamin E

In an *in vitro* study, Xu et al. reported that δ-tocopherol efficiently reduced lysosomal cholesterol accumulation, decreased lysosomal volume, increased cholesterol efflux, and alleviated pathological phenotypes in the fibroblasts of patients with Niemann–Pick type C1 and WD. The authors speculated that the reduction of these abnormalities may be facilitated by a δ-tocopherol-induced intracellular $Ca^{2+}$ response and the consequent augmentation of lysosomal exocytosis. The mechanism of action appeared to be independent of either the distorted enzyme or the storage substance because the authors reported that δ-tocopherol reduced the pathological phenotypes in the fibroblasts of patients who had other lysosomal storage diseases, including Niemann–Pick type C2, Batten (ceroid lipofuscinosis, neuronal 2, CLN2), Fabry, Farber, Niemann–Pick disease type A, Sanfilippo type B (mucopolysaccharidosis type IIIB, MPSIIIB), and Tay–Sachs [84].

Unfortunately, the application of these findings in clinical practice may be challenging because of unfavorable pharmacokinetics. Achieving adequate serum and brain levels of δ-tocopherol is difficult because of the rapid degradation of Vitamin E metabolites. In the future, molecular configuration modifications to improve Vitamin E pharmacokinetic properties may help to increase plasma and tissue concentrations. However, these data suggest that regulating exocytosis may represent a possible novel therapeutic target for drug development that can treat lysosomal storage disease, including patients with LAL-D.

### 7.3. Enzyme replacement therapy (ERT)

ERT can be used as well; it is carried out by targeting the enzyme to the lysosomes that allows correction of metabolic derangements associated with LAL-D and ameliorates the pathological features of the disorder. An infusion of recombinant human LAL in the mouse model of LAL-D resulted in histological improvement in several tissues, including Kupffer cells [85]. Other modalities for targeting the recombinant enzyme have been reported, including adenovirus-mediated gene transfer in mice and the production of human LAL in Chinese hamster ovary cells [86,87].

ERT has been shown to be an effective therapeutic approach in patients with lysosomal storage diseases [88]. Sebelipase alfa (Kanuma) is a recombinant human LAL enzyme that has been approved recently by the FDA for the treatment of patients with LAL-D. The recommended dose for infants under 6 months is 1 mg/kg every week; however, in adults and older children with later-onset LAL-D (CESD), the recommended dosage is 1 mg/kg every 2 weeks.

Sebelipase alfa is a recombinant human LAL synthesized in genetically modified hens, with the protein secreted into the egg white [89–91]. It is a glycoprotein with a molecular weight of 55 kDa, including mannose-terminated *N*-linked glycan structures. The glycan element enables targeted delivery of LAL to the lysosomal compartment through mannose 6-phosphate receptors and the mannose receptor on reticuloendothelial cells. Data from an ongoing global study assessing the safety and efficacy of sebelipase alfa have shown that this treatment may provide a significant survival benefit and improve most of the signs and symptoms of LAL-D [92].

Recently, new long-term data from an ongoing, open-label extension of the pivotal Phase 3 ARISE trial of Kanuma in children and adults with LAL-D were presented at the 2016 annual meeting of the American Association for the Study of Liver Diseases (AASLD) in Boston [93]. In a double-blind study, 65 subjects were treated with either Kanuma (35 patients) or a placebo (30 patients) for up to 130 weeks. Paired liver biopsies were taken in 20 patients (ages 5–59 years, where 12 patients received Kanuma and 8 patients received the placebo) at baseline and week 52 of the study. Two-thirds of the patients treated with Kanuma for 52 weeks had a reduction in liver fibrosis from the baseline, as measured by Ishak score. In

addition, half of the patients achieved at least a two-stage reduction, including five patients with fibrosis and one with cirrhosis at the baseline. Improvement of liver fibrosis was accompanied by sustained improvements in alanine aminotransferase (ALT), LDL-C, and hepatic steatosis through the 52 weeks of Kanuma therapy.

Most of the adverse effects observed during the study were mild to moderate in severity and included nasopharyngitis, headache, fever, and cough. No patient discontinued the open-label study because of adverse effects. Infusion-associated reaction was reported in 20% of the patients. One patient had a serious reaction, and the treatment was stopped; however, the patient was able to resume therapy later.

During the same meeting (AASLD), researchers presented new long-term data from the ARISE study, showing that 76 weeks of Kanuma treatment was associated with rapid and sustained improvement in liver enzymes and lipid profiles in children and adults with LAL-D. Ninety-eight percent of the patients had a persistent 56% reduction in ALT levels, with a mean reduction from baseline of 56% [94].

These encouraging data may indicate that Kanuma therapy can halt or even reverse the advancement of liver damage. It is interesting that the greatest effect was observed in those patients with substantial liver fibrosis at baseline. This emphasizes the importance of early recognition, as well as prompt and long-term treatment with Kanuma. Currently, there is no recommendation regarding the use of sebelipase alfa during pregnancy. Although animal studies did not indicate any detrimental effects with respect to the reproductive system, it is advisable to avoid use of sebelipase alfa in pregnant women.

Kanuma is a foreign protein; therefore, it was not surprising that development of antibodies against the drug during therapy has been reported. These antibodies may affect cellular uptake and enzyme efficacy. Antibody development associated with enzyme replacement in other lysosomal disorders, such as mucopolysaccharidosis disorders and Pompe disease, have been described [95–97]. No antidrug antibodies (ADA) have been reported in adults receiving Kanuma; however, in seven infants tested for antibody formation, four patients had detectable ADA, two of which developed neutralizing antibodies. However, all the patients could continue therapy. Three out of the four patients who developed ADA were receiving a dosage of 1 mg/kg once a week, and the fourth patient was receiving 3 mg/kg once a week. The ADA titers were detected within the first 2 months of exposure. One of the four ADA-positive patients had persistent ADA titers; while in three patients, ADA titers decreased to undetectable levels, and the patients continued therapy at a dosage of 3 mg/kg once a week [98].

Despite these encouraging results, the outcomes of long-term therapy still need to be determined. Although sebelipase alfa trials have shown significant improvements in the lipid profiles of the included subjects, the question remains if long-term therapy will prevent vascular disease and decrease cardiovascular disease morbidity and mortality. Another question is what will be the effect of therapy on patients with advanced liver disease, decompensated cirrhosis, and liver comorbidities because these patients were excluded from the study [91].

### 7.4. Stem cell or bone marrow transplantation

Stem cell or bone marrow transplantation for patients with LAL-D is hypothetically curative because normal LAL activity will be derived from donor cells [99]. Tolar et al. reported their experience with hematopoietic stem cell transplantation (HSCT) in four WD patients, two of whom, are long-term survivors (4 and 11 years old). Following HSCT, diarrhea resolved within weeks, liver functions normalized, and hepatosplenomegaly improved. In one child, adrenal function was restored back to normal. Both survivors had normal adaptive functions. However, one patient developed mild to moderate neurocognitive deficits [100]. In another study, Stein et al. reported the success of HSCT in an infant with WD who received unrelated HLA-mismatched umbilical cord blood-derived stem cells. Following a transplant, LAL levels were restored back to normal, and the child was reported to be surviving at the age of 4 [101].

A case of two brothers with WD who were treated with HSCT was published. Unfortunately, both died from end-stage liver disease complications [102]. Another unsuccessful outcome of HSCT was reported in a child who died following the transplant because of hepatic complications [103].

### 7.5. Liver transplantation

A liver transplant is a viable therapeutic option in patients with LAL-D in the context of liver failure. Liver transplantation was reported in nine pediatric patients who were 5 to 14 years old at the time of transplantation [34]. Ferry et al. [104] reported a child with LAL-D who underwent a liver transplant for liver cirrhosis, portal hypertension, ascites, and gastrointestinal bleeding. The child continued to do well for 4.5 years following the transplant, except for mild hypersplenism and elevated creatinine and blood urea nitrogen. There was no evidence of progressive renal, vascular, or pulmonary disease. Although serum cholesterol normalized, triglycerides remained elevated. Seven years after the transplant, the patient developed end-stage renal disease secondary to glomerular sclerosis and atherosclerosis, with extensive vascular lipid accumulation and tubular atrophy, as well as interstitial fibrosis. The patient required chronic hemodialysis by 21 years of age [105]. Although multiple organ dysfunctions, including kidney disease, has been reported in association with CESD [50,106], this was the first reported case of a patient progressing to end-stage renal disease. It is possible that cyclosporine, which is a known nephrotoxic drug and was used in the patient for immune suppression, compromised kidney function and contributed to the rapid decline of renal function. In 1991, Arterburn et al. reported a successful liver transplant in a 13-year-old child [107].

In 1995, Leone et al. described a 5-year-old boy with CESD who had severe hepatosplenomegaly, adrenal gland calcifications, hypercholesterolemia, hypertriglyceridemia, increased ALT values, and 16% residual activity of lysosomal acid lipase. The patient was treated with a low-fat diet, cholestyramine and simvastatin. Despite an improvement in lipid profile and a decrease in hepatomegaly, the patient's liver enzymes did not change. After 4 years of medical therapy, he underwent a

successful liver transplant and was reported to do well 10 months after the transplant, showing normal liver enzymes and lipid profile [108].

Hepatic malignancy (cholangiocarcinoma) has been reported in an adult patient with CESD [109]. Riva et al. described an 11-year-old patient with LAL-D who developed micronodular cirrhosis and received an extended right lobe graft (segments I + IV + VIII) obtained by *in situ* split-liver procurement from a 13-year-old donor. During surgery, a 1.4-cm nonencapsulated nodule of trabecular hepatocellular carcinoma (G1/G2) was identified in segment VII. No extrahepatic malignancy was discovered, and the child was reported to do well for up to 30 months posttransplant [110].

Ambler et al. reported the first case of orthotopic liver transplantation for CESD in an adult. A 42-year-old woman diagnosed with CESD in childhood ran an indolent course presented with cirrhotic hydrothorax and underwent a liver transplant. The patient later required retransplantation for hepatic artery thrombosis. The patient was reported to do well, with excellent graft function 2 years after the second transplant and remained free of vascular events, with excellent liver function and a normal lipid profile. However, the patient's peripheral LAL levels remained low. Atherosclerosis was evident at the initial assessment and may have contributed to hepatic artery thrombosis [111].

There is lack of information regarding the long-term outcome of patients with LAL-D post-liver transplant. The question remains as whether the disease will recur in the graft, considering the fact that the LAL activity may not normalize [111]. Another concern is that renal failure, which can be associated with lipid deposition following transplant, may indicate that liver transplant may only improve hepatic function, not necessarily the systemic lysosomal lipid deposition. An additional child with LAL-D who received a liver transplant at 5 years of age rejected the transplant and developed progressive, congestive heart failure [112].

Prolongation of life expected after transplant warrants close monitoring of the cardiovascular, pulmonary, and renal functions, taking into consideration that immunosuppressive therapy in transplanted patients may have adverse effects on multiple organs.

## 8. Other forms of nonobese fatty liver disease

### 8.1. Fatty liver in lean subjects

Beside LAL-D, a long list of disorders can be overlooked and affected patients can receive late or wrong diagnosis as NAFLD. NAFLD has always been considered a manifestation of metabolic syndrome in obese individuals. Although obesity is a main risk factor for the development of NAFLD, fatty liver disease can also arise in lean subjects at all ages including children [113–117]. Having lower BMI may not be protective in certain populations. Cohort studies have shown that despite having significantly lower BMI than other ethnic groups, Asians have an unexpectedly high prevalence of NAFLD [118].

Bellentani et al. studied the prevalence and risk factors for hepatic steatosis in Northern Italy and reported that the prevalence of NAFLD in the lean, nondrinking population in Italy is approximately 16% [119]. In another study, 22% of nondiabetic NAFLD patients had normal BMI [115]. Marchesini et al. reported that approximately one of the eight NAFLD patients at their tertiary center has normal BMI. These patients had higher levels of ALT/AST compared to the overweight or obese NAFLD patients [113]. Studies of liver histology obtained from liver donors [120], car accidents victims [121], autopsy results [122], and clinical liver biopsies [123] suggest that the prevalence rates of steatosis and steatohepatitis in nonobese individuals are approximately 15% and 3% versus 65% and 20% and 85% and 40% in obese and extremely obese persons, respectively. Wei et al. in a study using proton-magnetic resonance spectroscopy reported that one-fifth of the general nonobese Chinese population has NAFLD. Although nonobese patients with NAFLD did not seem to have a higher risk to advance to steatohepatitis and fibrosis, the authors recommended that patients with metabolic syndrome and PNPLA3 G allele carriage should be screened for severe NAFLD [124]. In another study from an Indian rural area with low prevalence of NAFLD, Das et al. showed that 75% of NAFLD patients had a BMI lower than 25 kg/m$^2$ and 54% were neither obese nor overweight [116].

### 8.2. Bariatric surgery

Steatosis is the most common hepatic side effect of jejunoileal bypass (a procedure commonly performed in patients with morbid obesity). Steatosis usually maximizes during the phase of rapid weight loss. The vast majority of patients develop steatosis by the end of the first year after the procedure [125]. The rapid weight loss seen following the procedure may lead to metabolic stress resulting in hepatocellular injury. Although steatosis improves rapidly after the bypass, inflammation usually worsens [126]. Eventually, the histological changes will improve in association with improvement in insulin sensitivity and the associated metabolic abnormalities. Similarly, changes in serum amino acids have been also reported in patients with Kwashiorkor, a disorder of protein–calorie malnutrition characterized by edema, irritability, ulcerating dermatoses, and fatty liver. Protein deficiency can result in reduced VLDL synthesis and inhibited VLDL transport due to decreased apolipoprotein synthesis [127,128].

### 8.3. Prolonged total parenteral nutrition (TPN)

TPN can lead to depletion of cytoplasmic carnitine, a critical factor for the transport of fatty acids to the mitochondria for beta-oxidation. Cytoplasmic choline, an essential factor for lipoprotein secretion, is also decreased in patients on long-term TPN. Pathological features of TPN-associated liver disease include steatosis, intrahepatic cholestasis, and bile duct proliferation [129].

### 8.4. Celiac disease

Several factors may be involved in the development of hepatic steatosis in patients with celiac disease including diabetes mellitus. Several reports of substantial fatty infiltration of the

liver in patients with celiac disease have been published [130–132].

### 8.5. Cystic fibrosis

Steatosis is one of the most common hepatobiliary manifestations in patients with cystic fibrosis, occurring in 23–75% of cases [133]. The pathogenesis does not seem to be directly related to a defective *CFTR* gene. More likely, steatosis is secondary to malnutrition and deficiencies of essential fatty acids, carnitine or choline [134]. Cystic fibrosis has been also reported to be associated with insulin resistance [135].

### 8.6. Inflammatory bowel disease

Hepatic steatosis is also a common finding in patients with inflammatory bowel disease (IBD). In a study which included 511 patients (311 patients with Crohn's disease (CD) and 200 patients with ulcerative colitis (UC), hepatomegaly with mild-to-moderate and severe liver steatosis were found in 25.7% and 39.5% of CD patients and in 25.5% and 35.5% of UC patients, respectively [136]. The severity of colitis correlated with the fatty liver changes in patients with IBD especially UC in a study of 484 patients with IBD [137].

### 8.7. Drug-induced liver injury (DILI)

The liver is vulnerable to drug injury due to its fundamental role in drug metabolism and clearance. DILI is an important cause of hepatic failure and a common indication for liver transplantation [138]. Although DILI may arise in a dose-dependent mode, the majority of events are idiosyncratic and dose-independent. The precise incidence of DILI is difficult to define; however, it is estimated to be in the range of 1/10,000–1/1,000,000 prescription-years [139].

Steatohepatitis is usually considered a rare form of DILD, with NASH representing fewer than 2% of NASH [140]. However, data from the Drug Induced Liver Injury Network (DILIN) has shown that some form of steatosis was detected in 26% of cases of DILI, with macrovesicular steatosis displayed as a prevailing pattern in more than 70% of the cases [141].

Grieco et al. have divided drugs known to incite steatosis and steatohepatitis into three broad groups: those that cause steatosis and steatohepatitis independently (e.g. amiodarone, perhexiline maleate); drugs which can precipitate latent NASH (e.g. tamoxifen); and drugs which induce sporadic events of steatosis/steatohepatitis (e.g. carbamazepine). The histological features of drug-induced steatosis and steatohepatitis are similar to those observed in alcohol liver disease including Mallory bodies formation, balloon degeneration and intense cellular infiltration [142]. Several drugs have been reported to cause steatotic changes with different features.

### 8.8. Hepatitis C virus (HCV)

HCV infection is a major cause of chronic liver disease in the United States and worldwide. Hepatic steatosis is a common histological feature of chronic hepatitis C. There are two distinct forms of steatosis detected in patients with HCV infection. Steatosis may develop in patients with risk factors (such as obesity, hyperlipidemia, and insulin resistance) and can also be a result of direct cytopathic effect of the virus [143]. Both types of steatosis seem to accelerate the progression of fibrosis. Hepatic steatosis is more common in patients infected with HCV genotype 3 than other genotypes and eradication of the virus with peginterferon α-2a plus ribavirin has been reported to clear the steatosis [144]. Further studies are warranted to investigate the effect of HCV eradication on hepatic steatosis and progression to fibrosis, cirrhosis, and hepatocellular in the era of direct-acting antivirals.

The mechanism of developing hepatic steatosis in HCV-infected patients may be due to binding of the viral X gene product to LXR-alpha resulting in upregulation of the transcription factor SREBP-1c. Steatosis is usually mild and non-zonal in contrast to steatosis seen in patients with NAFLD [145].

The relationship between steatosis and sustained viral response (SVR) following treatment of HCV infection remains to be determined. In a retrospective study of 174 patients with chronic HCV hepatitis, obesity (and not steatosis) was a negative predictor of SVR [146]. Several reports have suggested that steatosis seems to negatively affect SVR [147,148]. However, these observations seem to be restricted to steatosis unrelated to HCV infection as in patients with genotype 3 the presence of severe steatosis did not influence SVR [149].

### 8.9. Hepatitis B

Although hepatic steatosis has been linked with HCV infection, an association with hepatitis B virus (HBV) infection remains controversial. In a meta-analysis reported by Machado et al., steatosis in HBV patients seemed to be as common as in the general population and lower than in patients HCV. An interesting observation was that there was a strong negative association between viral load and hepatic steatosis, suggesting a possible protective effect of the virus against hepatic steatosis [150].

### 8.10. Wilson disease

Wilson disease is an autosomal dominant disease characterized by excess copper accumulation in tissues. It is caused by mutations in the gene encoding the copper-transporting ATPase ATP7B [151]. This mutation prevents copper transport into the Golgi complex and binding of copper to apoceruloplasmin. The defect of copper transport leads to copper deposition in different tissues including the liver, which results in mitochondrial dysfunction impairing beta-oxidation of fatty acids and steatosis [145].

## 9. Expert commentary

The lack of awareness about LAL-D highlights the great need for educating clinicians about the disease, taking into consideration the fact that promising therapeutic modalities are being developed. Although the prevalence of LAL-D has been estimated in the range of 1 in 40,000 and 1 in 300,000, this estimate is much more than the identified cases reported in the literature, which suggests that the disease may be considerably underdiagnosed and it is very likely that many patients are undiagnosed or misdiagnosed. There is a pressing

need for further research into developing additional diagnostic tools and therapeutic modalities.

Several studies have suggested a possible role for LAL in the pathogenesis of liver disease and reported that low LAL activity correlates with hepatic steatosis and dysfunction in patients with microvesicular steatosis, cryptogenic cirrhosis and NAFLD. These patients do not exhibit LAL activity compatible with genetic LAL-D. As a matter of fact, this is a fascinating observation that underlines the need for further research to better understand the role of LAL in the development and the prognosis of liver disease in the absence of genetic LAL-D. It is possible that in the future modifying LAL level may alter the disease course or provide therapeutic benefit.

Fatty liver disease in both obese and nonobese individuals affects a substantial portion of the population. There is lack of awareness among health-care providers that fatty liver disease is not limited to overweight individuals. Increased awareness is imperative. There is a tenacious need for the development of effective therapeutic options for fatty liver disease in all patients obese or nonobese. However, the lack of complete understanding of the pathogenesis of fatty liver disease is the biggest hurdle to develop novel therapeutic modalities that can target the metabolic derangements implicated in the development of the disorder. The search continues for an effective and safe therapy. Several drugs are under investigation. However, none has been so far established as an effective therapeutic option.

## 10. Five-year view

The search continues for novel therapeutic modalities to treat the LAL-D and fatty liver disease. An active and promising area of research for enzyme replacement is the development of Kanuma. Although current data may indicate that Kanuma therapy can halt or even reverse the progress of liver damage, the sequels of long-term therapy still need to be defined. Hopefully, in the near future other therapeutic options will become available.

## Key issues

- Nonalcoholic fatty liver disease (NAFLD) is one of the most prominent causes of liver disease worldwide.
- Although obesity is a main risk factor for the development of NAFLD, it can also develop in non-obese subjects and can be encountered in different clinical setting Including genetic, metabolic, nutritional, infectious and drug-induced disorders
- LAL-D is an autosomal recessive disease resulting from mutations in the *LIPA* gene.
- LAL-D can present in two major phenotypes: infantile-onset Wolman disease (WD) and later-onset cholesterol ester storage disease (CESD).
- The prevalence of LAL-D has been estimated in the range of 1 in 40,000 and 1 in 300,000.
- The diagnosis of LAL-D can be made by measuring enzyme activity or by comprehensive sequencing of the coding regions of *LIPA*.
- LAL-D should be suspected in patients with unexplained hepatomegaly, microvesicular or mixed steatosis, rapidly progressive neonatal liver disease and cryptogenic cirrhosis.
- LAL-D should be considered in patients suspected to have NAFLD with persistent elevation of liver enzymes despite weight loss.
- The diagnosis of LAL-D should be contemplated in patients with lipid abnormalities (LDL-c: ≥130 mg/dL or i ≥ 130 mg/dL in children and adults respectively) and high liver enzymes or absence of family history of lipid abnormalities [41,67].
- Treatment strategies include lipid-lowering agents, bone marrow transplant, liver transplant and enzyme replacement therapy.
- Increased awareness, early recognition and prompt intervention of LAL-D are crucial.

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## Declaration of interest

The author is a consultant and speaker for Alexion Pharmaceuticals, a company marketing Kanuma, an enzyme replacement therapy approved for the treatment of patients with LAL-D. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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