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Review

Lysosomal acid lipase deficiency – An under-recognized cause of dyslipidaemia and liver dysfunction

Željko Reiner [a,*], Ornella Guardamagna [b], Devaki Nair [c], Handrean Soran [d], Kees Hovingh [e], Stefano Bertolini [f], Simon Jones [g], Marijana Ćorić [a], Sebastiano Calandra [h], John Hamilton [i], Terence Eagleton [j], Emilio Ros [k,l,**]

[a] University Hospital Center, School of Medicine, University of Zagreb, Kispaticeva 12, 10000 Zagreb, Croatia
[b] Department of Public and Health Sciences, Medical School, University of Turin, Piazza Polonia 94, I-10126 Turin, Italy
[c] Department of Clinical Biochemistry, Royal Free Hospital NHS Foundation Trust, Pond Street, London NW3 2QG, UK
[d] Cardiovascular Trials Unit, Central Manchester University Hospitals NHS Foundation Trust, Oxford Road, Manchester M13 9WL, UK
[e] Department of Vascular Medicine, Academic Medical Center, Meibergdreef 9, Amsterdam 1105 AZ, Netherlands
[f] Department of Internal Medicine, University of Genoa, Viale Benedetto XV n. 6, 16132 Genoa, Italy
[g] Willink Biochemical Genetics Unit, Manchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, St. Mary's Hospital, Oxford Road, Manchester M13 9WL, UK
[h] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Via Campi 287, I-41125 Modena, Italy
[i] Biochemistry Department, Yorkhill Hospital, Glasgow, G3 8SJ, UK
[j] Synageva BioPharma Corp., 33 Hayden Ave., Lexington, MA 02421, USA
[k] Lipid Clinic, Endocrinology & Nutrition Service, Institut d'Investigations Biomèdiques August Pi Sunyer, Hospital Clínic, C. Villarroel, 170, 08036 Barcelona, Spain
[l] CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carloss III (ISCIII), Spain

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ABSTRACT

Lysosomal acid lipase deficiency (LAL-D) is a rare autosomal recessive lysosomal storage disease caused by deleterious mutations in the *LIPA* gene. The age at onset and rate of progression vary greatly and this may relate to the nature of the underlying mutations. Patients presenting in infancy have the most rapidly progressive disease, developing signs and symptoms in the first weeks of life and rarely surviving beyond 6 months of age. Children and adults typically present with some combination of dyslipidaemia, hepatomegaly, elevated transaminases, and microvesicular hepatosteatosis on biopsy. Liver damage with progression to fibrosis, cirrhosis and liver failure occurs in a large proportion of patients. Elevated low-density lipoprotein cholesterol levels and decreased high-density lipoprotein cholesterol levels are common features, and cardiovascular disease may manifest as early as childhood. Given that these clinical manifestations are shared with other cardiovascular, liver and metabolic diseases, it is not surprising that LAL-D is under-recognized in clinical practice. This article provides practical guidance to lipidologists, endocrinologists, cardiologists and hepatologists on how to recognize individuals with this life-limiting disease. A diagnostic algorithm is proposed with a view to achieving definitive diagnosis using a recently developed blood test for lysosomal acid lipase. Finally, current management options are reviewed in light of the ongoing development of enzyme replacement therapy with sebelipase alfa (Synageva BioPharma Corp., Lexington, MA, USA), a recombinant human lysosomal acid lipase enzyme.

* Corresponding author. Tel.: +385 1 2376041; fax: +385 1 2388623.
** Corresponding author. Lipid Clinic, Endocrinology & Nutrition Service, Institut d'Investigations Biomèdiques August Pi Sunyer, Hospital Clínic, C. Villarroel, 170, 08036 Barcelona, Spain. Tel.: +34 93 2279383; fax: +34 934537829.
E-mail addresses: zreiner@kbc-zagreb.hr (Ž. Reiner), eros@clinic.ub.es (E. Ros).

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1. Introduction

Lysosomal acid lipase deficiency (LAL-D) is a rare autosomal recessive lysosomal storage disease characterized by progressive accumulation of cholesteryl esters and triglycerides in the liver, spleen and other organs [1]. Dyslipidaemia is a common finding in patients with LAL-D that has been associated with accelerated development of atherosclerosis, cardiovascular disease and premature mortality [1–3]. Progressive liver disease is another characteristic feature of LAL-D, and patients typically present with hepatomegaly, elevated transaminase levels and/or microvesicular steatosis [1].

LAL-D is an under-recognized condition, with many affected individuals receiving no diagnosis or incorrect diagnoses of heterozygous familial hypercholesterolaemia (HeFH), familial combined hyperlipidaemia (FCH), non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD) or cryptogenic cirrhosis [4–6]. This review paper aims to provide recommendations to guide the timely diagnosis of LAL-D.

2. Current understanding of lysosomal acid lipase deficiency

LAL-D is a heterogeneous disease that presents along a clinical continuum, with signs and symptoms and rate of progression varying between affected individuals [1]. The most rapidly progressive presentation occurs in infants, was first described in 1956 and is referred to as Wolman disease [7]. A few years later, Fredrickson reported the case of a 12-year-old boy with marked hypercholesterolaemia, hepatomegaly and cholesteryl ester accumulation on liver biopsy [8]. This later-onset condition was named cholesteryl ester storage disease (CESD). Since their initial characterization, it has been discovered that Wolman disease and CESD share the same underlying molecular pathology, resulting from mutations in the *LIPA* gene, which encodes lysosomal acid lipase (LAL), the enzyme responsible for hydrolysing the cholesteryl esters and triglycerides within low density lipoprotein (LDL) particles into free cholesterol and free fatty acids [9–12]. The variable rates of progression observed between patients with LAL-D are believed to be related to the nature of the disease-causing mutations and the resulting degree of residual enzyme activity [10,11]. However, there may be other contributing factors (e.g. environmental influences) affecting disease progression.

Infants with LAL-D typically present in the first weeks of life and die within 6–12 months due to multi-organ failure [10]. Clinical signs may even arise during pregnancy, with reports of foetal ascites and polyhydramnios detected by prenatal ultrasonography [13]. The hallmarks of the disease in infants consist of prominent hepatosplenomegaly, diarrhoea and vomiting, resulting in malabsorption, growth failure and liver failure. These infants quickly develop liver fibrosis and cirrhosis due to the massive accumulation of cholesteryl esters and triglycerides in the liver [14]. Abnormal lipid accumulation has also been described in the spleen, adrenal glands, lymph nodes, intestinal mucosa, vascular endothelium and skeletal muscle [14,15]. Approximately 50% of infants with LAL-D have adrenal calcifications [1,16].

In children and adults, LAL-D has a more variable clinical course than in infants. Mean age at symptom onset has been reported to be 5 years in both male and female patients, although clinical presentation has been documented as late as 44 years old in men and 68 years old in women [1]. Lipid abnormalities may be present at all ages, with a lipid profile that is indistinguishable from that of more common genetic hypercholesterolaemias, such as HeFH [17]. Liver dysfunction is common, with hepatomegaly being an almost universal finding at diagnosis [1,18]. These phenotypic features of LAL-D are non-specific and overlap with other diseases, which may explain the common under-diagnosis of this condition.

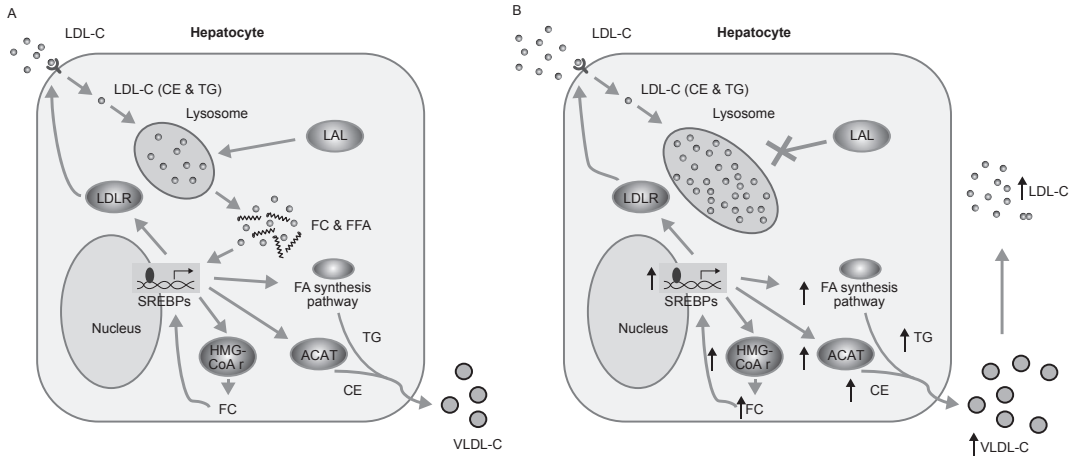

Fig. 1. Schematic view of cellular cholesterol homoeostasis in (A) healthy individuals and (B) patients with LAL-D. ACAT, acyl-cholesterol acyltransferase; CE, cholesteryl esters; FA, fatty acid; FC, free cholesterol; FFA, free fatty acid; HMG-CoA r, hydroxymethylglutaryl-coenzyme A reductase; LAL, lysosomal acid lipase; LAL-D, LAL deficiency; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; SREBPs, sterol regulatory element binding proteins; TG, triglyceride; VLDL-C, very-low-density lipoprotein cholesterol.

2.1. Inheritance and genetics

LAL-D arises from mutations in the *LIPA* gene, which maps to chromosome 10q23.2, has 10 exons and is approximately 45 kb in length [15]. More than 40 loss-of-function mutations have been identified to date (Supplemental Tables S1 and S2) [1,19]. LAL-D is an autosomal recessive disease and affected individuals are typically either homozygous or compound heterozygous for *LIPA* mutations, although some patients may have occult mutations. The most severe alterations, such as nonsense mutations, frameshift defects and point mutations resulting in stop codons, are generally detected in affected infants (Supplemental Fig. S1A). Less severe mutations are thought to occur in children and adults (Supplemental Fig. S1B) [20].

The most commonly inherited defect is the exon 8 splice site mutation, E8SJM (c.894G > A), which is found in more than half of all children and adults with LAL-D [21,22]. The mutation introduces an alternative acceptor splice site, resulting in the deletion of exon 8 in mRNA. A small amount of the mRNA is spliced correctly, which may result in the expression of some residual LAL activity. Studies in the general population showed that the frequency of the E8SJM allele is as follows: 0.0013 in Caucasians (USA, 0.0017; Germany, 0.0025; EU, 0.0012), 0.0017 in US Hispanics, 0.0010 in US Ashkenazi Jews, 0.0005 in Asians and 0.0000 in African–Americans [23,24]. Based on the assumption that 50–70% of children and adults with LAL-D have the E8SJM mutation [21,22], it has been estimated that the overall disease prevalence may be between 1 in 40,000 and 1 in 300,000 depending on ethnicity and geographical location [1,15,21,23]. This estimate conflicts with the small number of cases of LAL-D reported in the literature, indicating that the disease may be substantially underdiagnosed, especially in patients of European ancestry [1]. No formal studies of the incidence of LAL-D in Europe have been performed. A frequency of less than 1 in 700,000 has been reported in Australia [25]. Jewish infants of Iraqi or Iranian origin appear to be most at risk of LAL-D, with an estimated incidence of 1 in 4200 in the Los Angeles community [26].

The role of heterozygosity in LAL-D has not yet been studied in depth. Evaluation of 13 individuals heterozygous for the E8SJM mutation showed an altered lipid profile similar to that seen in polygenic hypercholesterolaemia, conferring an increased cardiovascular risk [27]. To investigate this further, another study group evaluated the frequency and impact of heterozygosity for the E8SJM mutation on lipid levels in 13,194 individuals of European ancestry [24]. In addition, to assess the impact of partial loss of *LIPA* function on risk for myocardial infarction (MI) or coronary artery disease (CAD), the frequency and impact of the E8SJM variant was evaluated in 12,747 patients with MI/CAD and 14,725 controls free of MI and CAD [24]. In both studies, no association between heterozygosity and plasma lipid levels or risk for MI/CAD was observed [24]. It should be emphasized, however, that the number of heterozygotes was rather small; thus additional studies are warranted to substantiate whether heterozygotes develop some clinical features of LAL-D and may be predisposed to sequelae such as atherosclerosis and liver dysfunction.

2.2. Pathogenesis

LAL plays a key role in lipid metabolism through the hydrolysis of cholesteryl esters and triglycerides in lysosomes (Fig. 1A). As LDL-derived neutral lipids (cholesteryl esters and, to a lesser extent, triglycerides) are degraded by LAL, the resulting free cholesterol and fatty acids act as critical mediators in cellular cholesterol homoeostasis [28]. These lipids or their oxidized derivatives interact with transcription factors (sterol regulatory element-binding proteins [SREBPs]) that directly modulate the expression of genes involved in the synthesis and uptake of cholesterol and lipogenesis [29]. Normally, an intracellular abundance of free cholesterol leads to SREBP-2-mediated down-regulation of LDL receptors (resulting in reduced entry of cholesterol into the cell), feedback inhibition of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase (resulting in reduced cholesterol synthesis), and stimulation of acyl-cholesterol acyltransferase (resulting in enhanced cholesterol esterification). At the same time, intracellular fatty acid enrichment leads to inhibition of phospholipid and triglyceride production via SREBP-1c-mediated down-regulation of fatty acid synthesis [30].

When LAL activity is absent or reduced, cholesteryl esters and triglycerides are not degraded and accumulate within lysosomes (Fig. 1B). The consequent scarcity of intracellular free cholesterol causes the SREBP-mediated up-regulation of endogenous cholesterol production by HMG-CoA reductase and of endocytosis via LDL receptors, as well as increased synthesis of apolipoprotein B (ApoB) and markedly increased production of very-low-density

lipoprotein cholesterol (VLDL-C) [31]. Enhanced expression of HMG-CoA reductase is the primary outcome of SREBP-2 sensing intracellular cholesterol depletion, leading to an increase in free cholesterol levels.

The impact of any increase in free cholesterol levels resulting from the HMG-CoA reductase up-regulation observed in LAL-D is not entirely understood, but it may cause feedback inhibition of LDL receptor activity and reduced clearance of LDL-C from the circulation. However, cholesterol trafficking in LAL-D does not seem to follow this scheme. For example, the cellular uptake of LDL-C was increased in LAL-deficient fibroblasts and uptake of ApoB was normal in a patient with LAL-D [28,32]. In LAL-deficient hepatocytes, increases in cholesterol synthesis lead to great increases in VLDL–C production and secretion, the natural way of exporting cholesterol from the liver; this in turn leads to enhanced LDL–C production and thus may be an important contributor to hypercholesterolaemia in LAL-D [32].

In this context, the use of statins in patients with LAL-D raises some concerns. Firstly it is unlikely that statins would reduce the liver damage associated with accumulation of cholesterol esters in the liver and there is evidence of persistence of elevation of serum transaminases and continued progression of liver fibrosis to cirrhosis in affected patients on statins [1,33]. Secondly, statins are expected to reduce cholesterol synthesis, thus reducing the secretion of ApoB containing lipoproteins and increasing the expression of LDL receptors. This would translate into a reduction of plasma LDL-C, but, in view of the higher receptor-mediated uptake of plasma LDL-C, it would also be expected to accelerate lysosomal accumulation of cholesteryl esters, with potentially deleterious effects on liver function.

Interestingly, in sharp contrast with LAL-D, in another lysosomal sterol storage disease, Niemann–Pick disease type C1 (NPC1), circulating levels of total and LDL-C are reduced, not increased [34]. In patients with NPC1, free cholesterol accumulates in lysosomes due to defects in NPC proteins involved in transporting the sterol to the cytosolic compartment. However, some of the high levels of intralysosomal free cholesterol may still be able to diffuse through the organelle membrane to the exit site, resulting in a lesser compensatory cholesterol synthesis (and presumably less VLDL-C–ApoB secretion) than in LAL-D, as shown in experimental studies with *lal*$^{-/-}$, *npc1*$^{-/-}$, and *npc2*$^{-/-}$ mice [35].

Patients with LAL-D typically present with dyslipidaemia with elevated serum total cholesterol, high LDL-C, low high-density lipoprotein cholesterol (HDL-C) and may also have elevated triglycerides [17]. The increase in total cholesterol and triglycerides is due to accumulation in plasma of ApoB-containing lipoproteins such as VLDL-C and LDL-C [36]. The reduction in HDL-C levels has been linked, at least *in vitro*, to reduced formation of mature HDL (α-HDL particles), secondary to decreased expression of the adenosine triphosphate-binding cassette transporter A1 (ABCA1). Expression of the *ABCA1* gene is induced by increased cell cholesterol content, predominantly through an oxysterol-dependent activation of the nuclear liver X-receptor (LXR), acting on the promoter of the *ABCA1* gene [37,38]. In LAL-D, the entrapment of cholesteryl esters in lysosomes, and thus the reduction of the intracellular free cholesterol pool, translates into: i) reduced oxysterol formation and the consequent decreased activation of *ABCA1* expression; and ii) a reduced amount of cholesterol in the subcellular compartments and in the plasma membrane that is available for the ABCA1-mediated transfer to the extracellular lipid-poor apolipoprotein A1, the key event in the formation of α-HDL particles. These concepts are supported by the observation that the addition of recombinant LAL to LAL-deficient cells rescues the ABCA1-mediated cholesterol efflux to ApoA-I and HDL particle formation [39].

3. Signs and symptoms of lysosomal acid lipase deficiency

Presenting signs and symptoms of LAL-D may vary considerably between patients. Many of the most common clinical manifestations, namely dyslipidaemia, hepatomegaly and liver cell damage (as evidenced by increased serum transaminases with progression to fibrosis and cirrhosis), are shared with other cardiovascular, liver and metabolic diseases that are more prevalent than LAL-D (Table 1).

Infants with LAL-D typically have a more acute clinical course compared with children and adults. Gastrointestinal symptoms (vomiting, diarrhoea with steatorrhoea and abdominal distension) and growth failure are often the first manifestations to be observed [15]. Abdominal distension is a striking feature, occurring primarily due to massive hepatosplenomegaly. Characteristic punctate calcifications of the adrenal glands may be observed on radiological images in around 50% all infants and may also be seen in children [1,15,16,40]. Anaemia may also be observed. Later manifestations are related to multi-organ failure, particularly to liver cirrhosis/failure, and include jaundice and cachexia [15]. Effects on the central nervous system are uncommon and those that have been reported appear to be related to malnutrition and/or specific nutritional deficiencies, or to complications of bone marrow transplantation, rather than being related to LAL-D directly [15,41].

The natural history of LAL-D in children and adults is less well defined than in infants and detection of the disease is often incidental [18]. For instance, a 36-year-old woman was admitted to hospital because of hepatosplenomegaly and anaemia, and was later diagnosed with LAL-D; however, a review of her medical records showed that at the age of 2 years she had developed jaundice, hepatomegaly and slightly raised brownish skin spots, which would have provided an early indication of the disease [42]. Approximately one-third of children experience severe gastrointestinal symptoms, including frequent diarrhoea, vomiting, abdominal pain, malabsorption and steatorrhoea [1,18,43]. Cholestasis, poor growth, adrenal calcification, stroke and gallbladder dysfunction have also been reported [1,40,44].

Table 1
Summary illustrating range of clinical features, serum markers and liver biopsy findings in children and adults with LAL-D.

Clinical signs and symptoms	Hepatomegaly/hepatosplenomegaly
	Diarrhoea
	Abdominal and epigastric pain
	Vomiting
	Anaemia
	Malabsorption
	Cholestasis
	Steatorrhoea
	Poor growth
	Gallbladder dysfunction
	Coronary artery disease
	Aneurysm
	Stroke
	Adrenal calcification (not required for diagnosis)
	Oesophageal varices
Serum markers	Elevated total cholesterol
	Elevated low-density lipoprotein cholesterol
	Decreased high-density lipoprotein cholesterol
	Elevated serum transaminases
Liver biopsy findings	Bright yellow–orange in colour
	Enlarged lipid-laden hepatocytes and Kupffer cells
	Microvesicular steatosis (may be mixed with macrovesicular steatosis)
	Fibrosis
	Micronodular cirrhosis

LAL-D, lysosomal acid lipase deficiency.

3.1. Dyslipidaemia

Total plasma triglyceride and cholesterol levels are often normal in infants with LAL-D, but elevated triglycerides and VLDL-C levels have been reported in addition to low plasma HDL-C levels [45–48].

Children and adults with LAL-D often have type IIa or type IIb hyperlipidaemia [17,18,49–55], exemplified by elevated total cholesterol, elevated LDL-C, elevated ApoB and decreased HDL-C levels [1,56]. Dyslipidaemia has been associated with atherosclerosis and premature cardiovascular disease [1–3,5,42,57], as demonstrated by the detection of an aortic plaque in a child with LAL-D who died at the age of 9 years [40]. Intriguingly, three large case-controlled genome-wide association studies showed that increased expression of *LIPA* in blood monocytes is a risk factor for coronary artery disease. [58–60] *LIPA* expression is markedly upregulated during differentiation to macrophages and it is unclear what relevance the small observed differences in monocytes have to final expression levels in tissue macrophages. In practice, however, morbidities related to liver disease are recognized more frequently than vascular events in patients with LAL-D.

3.2. Effects on the liver and spleen

Infants with LAL-D often present with failure to thrive, hepatomegaly, with rapid progression to hepatocellular failure along with liver fibrosis and cirrhosis [14,15]. The spleen may also be enlarged and can reach over 20 times normal size by 2–3 months of age [61]. Hepatomegaly and splenomegaly are observed in approximately 99% and 74%, respectively, of children and adults with LAL-D [1,62]. Elevated levels of serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) are early indicators of liver damage, although values may only be slightly raised and can vary widely between patients. Clinical manifestations range from severe liver disease (i.e. oesophageal varices, liver failure) that may be present in early childhood, to asymptomatic low grade liver injury (i.e. elevated transaminases), with silent progression to cirrhosis in late adulthood.

On biopsy, the liver appears bright yellow–orange in colour, and histological analysis shows varying degrees of portal and perilobular fibrosis and intense microvesicular steatosis due to accumulation of cholesteryl esters and triglycerides in the lysosomes of hepatocytes [4]. A characteristic feature is the presence of markedly hypertrophic Kupffer cells and portal macrophages, with a foamy, tan-coloured cytoplasm that stains strongly with periodic acid–Schiff (PAS). The membranes of such vacuoles are well stained by PAS diastase (Fig. 2). In unfixed biopsy samples, the presence of birefringent-stored liquid crystals of cholesteryl esters is an additional diagnostic clue in LAL-D. Immunohistochemical detection of luminal cathepsin D and lysosomal markers (lysosomal-associated membrane protein [LAMP1, LAMP2] and lysosomal integral membrane protein 2) around the lipid droplets is also a very useful indication of LAL-D [4]. Cholesteryl esters and triglyceride droplets of varying size can be noted in both parenchymal and Kupffer cells, mostly surrounded by a single membrane. Some of the lipid vacuoles may contain star-like crystalline imprints of cholesteryl esters that dissolved during sample preparation, giving the stored material a moth-eaten appearance [14].

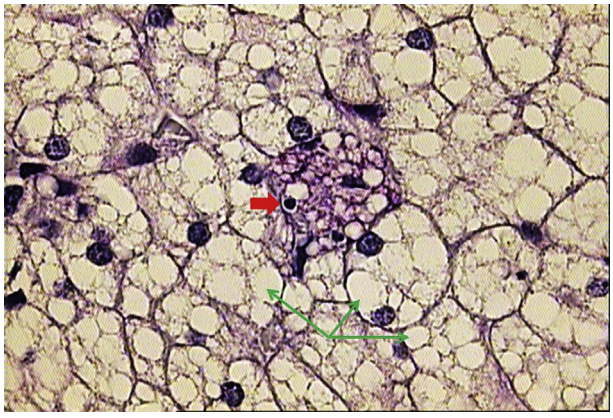

Fig. 2. . Hepatocytes show microvesicular steatosis (green arrows). The foamy Kupffer cell (red block arrow) in the perivenular zone is PAS positive (PAS after diastase digestion, original magnification × 630). PAS, periodic acid–Schiff. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Progressive lipid deposition leads to fibrosis (present in two-thirds of individuals affected during childhood or adulthood) and, eventually, micronodular cirrhosis [4,18]. Complications associated with cirrhosis include portal hypertension, ascites, oesophageal varices, gastrointestinal bleeding, cachexia and coma, which often progress to liver failure and death [1].

4. Differential diagnoses

Owing to its similarity with other cardiovascular, liver and metabolic diseases, the differential diagnosis of LAL-D can be challenging. Without appropriate investigation, these similarities can lead to misdiagnosis and a delay in appropriate management [1,17].

Serum lipid levels in LAL-D may vary, but most children and adults present with elevated total cholesterol and LDL-C and reduced HDL-C levels [1]. Common misdiagnoses include HeFH, familial defective ApoB, FCH and polygenic hypercholesterolaemia [17,18,51–55]. Nevertheless, it is possible to distinguish LAL-D from these conditions based on a few simple principles. First, a detailed family history/pedigree analysis can delineate autosomal dominant disorders (e.g. HeFH, FCH and polygenic hypercholesterolaemia) from autosomal recessive disorders (e.g. LAL-D and sitosterolaemia). Secondly, total cholesterol and LDL-C levels in LAL-D may not be as high as in HeFH. Additionally, HDL-C levels are usually lower in LAL-D than in HeFH, but may overlap with levels seen in patients with HeFH [63]. The potential for similar lipid profiles suggest that physicians should beware of the possibility of LAL-D when considering HeFH as a diagnosis.

Although liver abnormalities observed in LAL-D are well characterized, the rate of progression and therefore presentation of symptoms of liver disease are not always consistent [2]. For example, hepatomegaly is observed in most, but not all [24,64], patients, and some may have only slightly elevated or, rarely, normal levels of serum transaminases. In those with hepatomegaly and persistently elevated serum transaminases, incorrect diagnoses may include NAFLD, NASH or cryptogenic liver disease, or no diagnosis at all [1]. A full viral/immunological profile should be carried out to exclude more common disorders such as viral hepatitis and autoimmune liver disease [50]. Unlike individuals with metabolic syndrome, which is also associated with dyslipidaemia, fatty liver and elevated liver transaminases, patients with LAL-D may not be obese. Therefore, non-obese patients with these liver manifestations may help in differentiating LAL-D from other conditions like metabolic syndrome.

5. Screening for lysosomal acid lipase deficiency

To aid the timely diagnosis of LAL-D, a diagnostic algorithm is proposed based on the clinical experience of the authors (Fig. 3). In light of the heterogeneous phenotype of LAL-D, it is not necessary for a patient to fulfil all of the criteria listed before LAL-D is

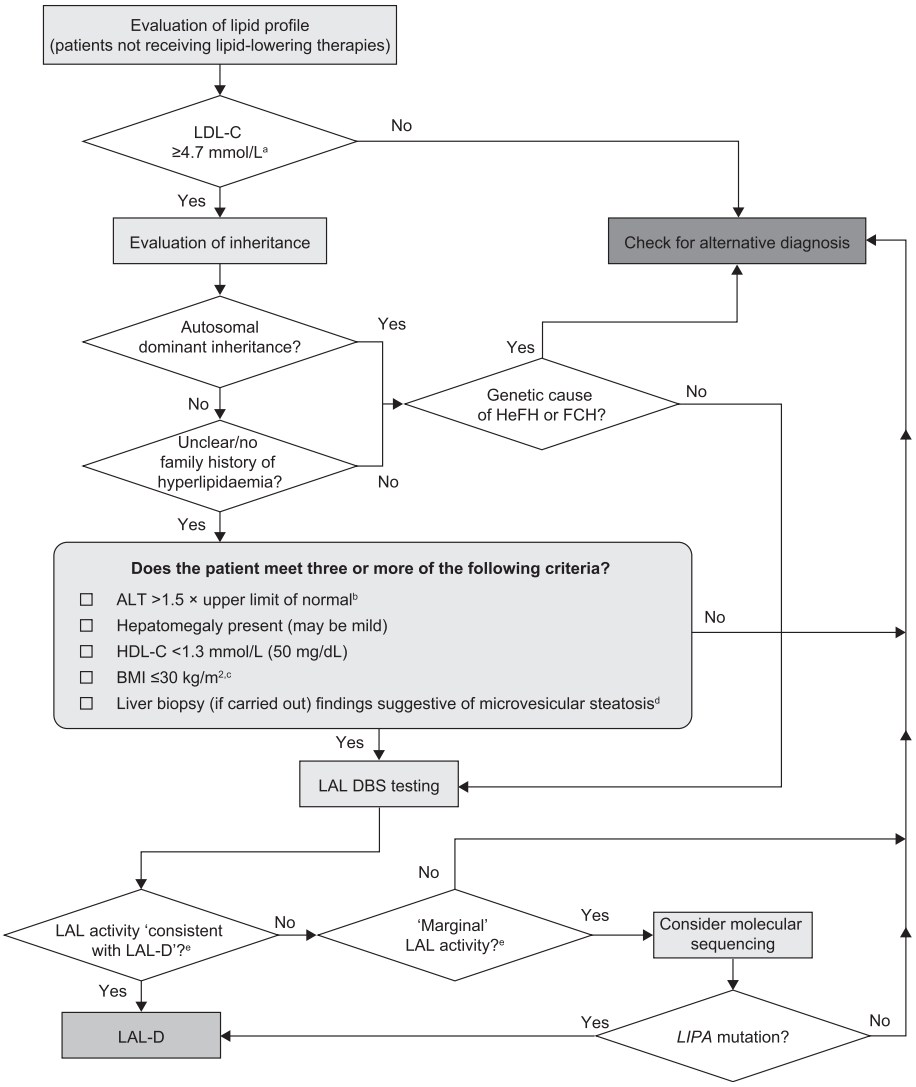

Fig. 3. Recommended screening criteria for LAL-D in patients at baseline assessment or not receiving lipid-lowering therapies. ALT, alanine aminotransferase; BMI, body mass index; DBS, dried blood spot; FCH, familial combined hyperlipidaemia; HeFH, heterozygous familial hypercholesterolaemia; HDL-C, high-density lipoprotein cholesterol; LAL, lysosomal acid lipase; LAL-D, lysosomal acid lipase deficiency; LDL-C, low-density lipoprotein cholesterol; LAL gene. [a]Or below the 95th percentile for age and sex in children and adults [65]. It should be noted that LDL-C might be lower in some patients with LAL-D, especially in those receiving statins. [b]Upper limit of normal for age and sex in healthy individuals, with no clear explanation (e.g. viral hepatitis, excessive alcohol consumption). It should be noted that, owing to periodic fluctuations in ALT levels, this sign may not always be detected. [c]Or above the 95th percentile for age and sex in children and adults [65]. It should be noted, however, that BMI may be above 30 in some patients with LAL-D, depending on diet. [d]Please note that liver biopsy is not recommended as a diagnostic method for LAL-D. [e]When measured by DBS testing, mean LAL activity is approximately 1.00 nmol/punch/hour in healthy individuals [66]. LAL activity less than or equal to 0.03 nmol/punch/hour (3% mean normal) is considered consistent with LAL-D. Marginal LAL activity is defined as a measurement between 0.03 nmol/punch/hour and 0.15 nmol/punch/hour (i.e. 3–15% mean normal). Patients with LAL activity in this range should be referred for molecular sequencing of the *LIPA* gene. Readers should note that reference ranges will vary between laboratories.

considered in the differential diagnosis. Rather, the criteria have been designed to be sufficiently broad so that a patient meeting any three or more of the listed criteria should be tested for LAL-D.

The absence of family history of hyperlipidaemia may indicate autosomal recessive inheritance, as would the absence of a genetic mutation indicative of HeFH. Although liver biopsy is not recommended as a diagnostic procedure, the presence of microvesicular steatosis on biopsy may be suggestive of LAL-D.

It should be noted that the reference ranges for some parameters are age-dependent, and this must be taken into account. For instance, reference ranges for LDL-C levels will typically be lower in children and adolescents than in adults [65]; however, experience is limited, especially in very young children [18]. To avoid a missed diagnosis, clinical suspicion should be raised for any child with a lipid profile consistent with HeFH or FCH (i.e. above the 95th percentile for age and sex) [65].

6. Investigations to diagnose LAL-D

A diagnosis of LAL-D can be obtained by demonstration of deficient LAL activity or mutations in the *LIPA* gene [1]. Biopsy findings and radiological findings are not considered diagnostic, but help raise the suspicion of LAL-D.

6.1. Measurement of LAL activity

LAL-D can be confirmed biochemically by measuring enzyme activity in cultured fibroblasts, peripheral leukocytes or liver tissue.

However, substrates in these assays (e.g. 4-nitrophenyl palmitate) may not be specific for LAL, so it is theoretically possible for a false-negative result to be generated. A new method for determining LAL activity in dried blood has been developed and has been found to detect affected patients [66]. LAL activity is measured using the fluorimetric substrate 4-methylumbelliferyl palmitate. Other lipases in whole blood may interfere with the measurement of LAL in dried blood spots (DBS) [67], so a LAL inhibitor is used. Lalistat 2 (Chemical Tools, South Bend, IN, USA) is a highly specific inhibitor of LAL that has been developed previously as a potential therapeutic target for NPC1 [68]. LAL activity is determined by comparing total lipase activity to lipase activity in the presence of Lalistat 2; the difference between the two results can be attributed to LAL enzyme. The method demonstrates excellent differentiation between healthy and affected individuals, with carriers showing intermediate LAL activity. A feature of the assay is that significantly reduced activity (<3% of normal) falls below the limit of detection, meaning that it is not possible to distinguish between LAL-D presenting in infants versus presentation in older patients.

The blood test for LAL has been a powerful tool in allowing screening programmes and large population-based surveys for LAL-D to be undertaken and it may be adapted for newborn screening. The DBS technique has many advantages, including a small sample volume (50 μL whole blood) and transport to specialist laboratories at ambient temperature. Sample stability shows a 15% fall in LAL activity after 7 days at room temperature. With prompt transport of samples, however, LAL activity in DBS is adequate for diagnostic purposes. Long-term stability at −20 °C is good, with 87% activity remaining at 100 days [66]. Supplemental Table S3 lists laboratories capable of performing the analysis of DBS samples.

6.2. Genetic testing

Complete sequencing of the coding regions of *LIPA* enables characterization of the genetic status of individuals with suspected LAL-D [15]. Although the most common mutation, E8SJM, is present in 50–70% of mutant alleles in children and adults with LAL-D, the low frequency of the E8SJM mutation in some populations (e.g. those of African–American and Asian descent) means that screening for the common mutation via the polymerase chain reaction assay may not be adequate in these populations [23].

Although most affected patients are homozygous or compound heterozygous for *LIPA* mutations, some patients may have intronic mutations that go undetected in routine genetic screening. In light of this, the utilization of sequencing to diagnose LAL-D might be replaced with the easily accessible, accurate and low-cost DBS assay.

6.3. Liver biopsy

Liver biopsy is generally regarded as the most reliable method for evaluating liver abnormalities. However, the risk of biopsy-related morbidity and mortality and the costs associated with this procedure limit its widespread application [69]. Furthermore, sampling error can make diagnosis by this method difficult. Current guidelines suggest that liver biopsy should only be used to obtain a diagnosis if a conclusion cannot be reached by other non-invasive means, such as a blood test [69,70].

The presence of microvesicular steatosis on liver biopsy is not unique to LAL-D, so other histological signs are needed to confirm a diagnosis. Hypertrophic Kupffer cells and portal macrophages with a foamy, tan-coloured cytoplasm are a characteristic feature of LAL-D in children and adults. The presence of luminal and membrane lysosomal markers around lipid vacuoles is indicative of LAL-D in fixed paraffin-embedded material, as are pathognomonic cholesteryl ester crystals in unfixed samples [4].

6.4. Radiological techniques

Hepatic magnetic resonance spectroscopy using a 3T magnetic resonance imaging (MRI) scanner has recently been described as a useful non-invasive method to identify and to quantify the hepatic lipid signature associated with LAL-D [71]. This approach may provide a more favourable alternative to repeated biopsy sampling for diagnosis and disease monitoring.

7. Management and therapies in development

No disease-specific treatments are currently available for patients with LAL-D. Existing approaches focus on supportive therapies to reduce the burden of disease complications. Clinical trials are ongoing to investigate the safety and efficacy of sebelipase alfa (Synageva BioPharma Corp., Lexington, MA, USA), a recombinant human lysosomal acid lipase that addresses the underlying defect in LAL-D patients.

7.1. Lipid-lowering therapies

Statins (HMG-CoA-reductase inhibitors) are well-tolerated LDL-C-lowering agents known to reduce the risk of cardiovascular disease [72]. In children and adults with LAL-D, statins as monotherapy or in combination with other lipid-lowering drugs were found to reduce LDL-C in many cases reported in the literature, but elevations were still reported in some patients [5,17,32,36,64,73–77]. A recent observational study also noted that dyslipidaemia persists despite treatment with lipid-lowering therapies in many patients with LAL-D [33]. In some patients with LAL-D, statin therapy was associated with a significant decrease in endogenous cholesterol synthesis in fibroblasts [32,36]. In another study, the authors concluded that a decrease in circulating LDL-C was due to reduced hepatic production of ApoB-containing lipoproteins [36]. Although statin therapy can lower plasma LDL-C levels and reduce cardiovascular risk in many patient populations, there is evidence that hepatic damage progresses in patients with LAL-D in spite of treatment [1,36]. A reduction in liver size has been reported in some patients with LAL-D treated with statins [36,73]; however, the liver fibrosis continued to progress in all individuals followed long term [1,15,77]. Similarly, a recent review revealed that in 12 patients treated with statins, liver histology did not improve [1]. In subsequent biopsies, all 12 patients had progressive liver disease that was more advanced than before, demonstrating the progressive nature of liver disease in LAL-D. In fact, six patients treated with statins required transplantation or died from liver failure [1]. Further research in this field is needed.

Ezetimibe (a cholesterol absorption inhibitor) has been 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 [78]. Serum levels of cytokines and oxidative stress parameters, which were elevated at baseline, were also found to normalize after 1 year of ezetimibe therapy. [78]

7.2. Vitamin E

In an *in vitro* study, tocopherol, a compound with vitamin E activity, was found to promote lysosomal exocytosis and thus reduce lipid accumulation in fibroblasts harvested from patients with NPC1 and infants with LAL-D [79]. Unfortunately, translating these cell-based results into *in vivo* studies in animals and

patients remains a challenge owing to the unfavourable pharmacokinetics of tocopherol. To achieve a therapeutic effect *in vitro*, a supraphysiological concentration of tocopherol was deployed; even with dietary supplementation, it is unlikely that high concentrations will be achievable *in vivo* owing to rapid oxidation of vitamin E derivatives by the cytochrome P450 enzyme CYP4F2.

7.3. Haematopoietic stem cell and liver transplantation

Haematopoietic stem cell transplantation has been performed in a few infants with LAL-D [13,80]. However, this approach has had limited success in addressing the multi-system nature of the disease and has been associated with high toxicity and challenges with sustained engraftment in target tissues [80–82]. Follow-up data show survival in some patients with LAL-D up to 5 years after liver transplantation, but for many there is limited information on long-term outcomes and other co-morbidities [1,83,84].

7.4. Enzyme replacement therapy

Enzyme replacement therapy (ERT) has been used successfully in other lysosomal storage diseases and is a potential future therapy for patients with LAL-D [85]. The goal of ERT for LAL-D is to reinstate near-physiological enzyme levels to prevent the accumulation of cholesteryl esters and triglycerides, and ultimately restore normal organ function.

Sebelipase alfa, a recombinant human LAL enzyme, is currently in phase 3 clinical trials. Data from the first human studies with sebelipase alfa, an open-label phase 2 trial (LAL-CL01) in 9 adults with LAL-D and an ongoing extension study (LAL-CL04) in 7 of these patients, have been reported [86,87]. In the first study, patients received four once-weekly infusions, which were well tolerated and resulted in a rapid decrease of liver transaminases and increases of total cholesterol, LDL-C and triglycerides, suggesting mobilization of accumulated lipid in tissues [86]. After the LAL-CL01 trial was completed and patients stopped sebelipase alfa, both liver enzymes and lipid levels returned to baseline values. Patients who enrolled in the extension study again received four once-weekly infusions of sebelipase alfa before transitioning to long-term every-other week infusions (1 or 3 mg/kg). After 78 weeks of treatment with sebelipase alfa in seven patients, both ALT and AST continued to be reduced compared with baseline, falling into the normal range. In addition, sebelipase alfa treatment for these patients, many of whom were on stable doses of lipid-lowering agents, led to mean 52% and 40% reductions in LDL-C and triglyceride levels, respectively, and a mean 37% improvement in HDL-C [87]. Reductions from baseline were also observed for liver fat fraction (mean, 55%) and liver volume (mean, 12%) at week 52, the latest time point for these assessments [87]. More than 250 infusions have been administered thus far in the trial and no serious safety concerns have emerged with long-term dosing. There were no drug-related serious adverse events. The majority of adverse events were mild and unrelated to sebelipase alfa. Infusion-related reactions were uncommon and most were mild gastrointestinal events (diarrhoea, abdominal cramping).

The efficacy and safety of sebelipase alfa for LAL-D are being assessed in a randomized, double-blind, placebo-controlled, phase 3 trial ("ARISE" trial; ClinicalTrials.gov identifier: NCT01757184). In addition, an open-label, multicentre, phase 2/3 study in infants with LAL-D presenting with growth failure is also ongoing (ClinicalTrials.gov identifier: NCT01371825). Data from these studies are expected to be released in 2014.

8. Disease monitoring

Given the progressive nature of LAL-D, patients should undergo annual evaluations to monitor disease progression. Recommended laboratory procedures include liver function tests, full blood counts, lipid profile and measurement of plasma levels of chitotriosidase (a chitinase that is markedly increased in many lysosomal storage diseases) [1]. Dyslipidaemia and any other cardiovascular risk factors should be managed according to current guidelines for high-risk patients [88]. Imaging studies should be performed periodically to evaluate liver and spleen volumes. Similarly, magnetic resonance spectroscopy or multi-echo gradient-echo MRI can be used to monitor hepatic fat content [71]. Imaging studies to monitor liver fibrosis, portal hypertension and risk of variceal bleeding should be considered according to clinical judgement.

9. Prognosis

Disease progression and the impact of clinical manifestations on daily life vary from patient to patient. Some children with LAL-D suffer early liver failure and require transplantation [1], and cardiovascular complications of LAL-D may include coronary artery disease, aneurysm and stroke [5,42,57,89,90]. Clearly, these severe signs and symptoms will have a negative effect on quality of life; however, no validated patient-reported outcome or quality of life measures have been developed for LAL-D. Individuals with less obvious signs and symptoms may remain undetected or misdiagnosed until a premature cardiovascular event or sudden death from liver failure.

10. Discussion

Dyslipidaemia is a characteristic feature of LAL-D in children and adults, and many patients are likely to be referred to lipid clinics before diagnosis. Given the similarities between the lipid profile observed in patients with LAL-D and that of common genetic hyperlipidaemias such as HeFH and FCH, it is important that lipidologists remain vigilant to avoid misdiagnosis. Similarly, the hepatic manifestations of the disease may be confused with disorders such as NAFLD, NASH or cryptogenic liver disease.

Early recognition and diagnosis of individuals with LAL-D is essential if appropriate care is to be provided. After reviewing the relevant data, we have proposed a diagnostic algorithm that can be used in most lipid clinics. The recent development of a blood test for LAL-D provides physicians with a method for rapid diagnosis of LAL-D.

Current management of LAL-D focuses on lipid control and amelioration of liver complications. The limited success of statins in treating all aspects of LAL-D means that alternative, disease-specific treatments are needed. The ongoing development of sebelipase alfa offers a disease-specific therapeutic approach that may change the natural course of the disease. However, there is a need for enhanced awareness of LAL-D so that early diagnosis can help limit disease-associated morbidity and mortality.

Lipidologists, endocrinologists, cardiologists and hepatologists are likely to be confronted with LAL-D in clinical practice and should be aware of the hallmarks of the disease. We all have a responsibility to raise awareness of LAL-D as a potential diagnosis in patients presenting with signs and symptoms usually associated with more common cardiovascular, liver and metabolic diseases.

Declaration of conflicts of interest

DN has no conflicts of interest to declare.

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SC has no conflicts of interest to declare.

SJ has received lecturer's honoraria and consultancy fees from Synageva BioPharma Corp., and is a clinical trial investigator for sebelipase alfa.

TE was employed at Synageva BioPharma Corp.

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Appendix A. Supplementary data

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