Review

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Cholesteryl ester storage disease: Review of the findings in 135 reported patients with an underdiagnosed disease

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Summary

Cholesteryl ester storage disease (CESD) is caused by deficient lysosomal acid lipase (LAL) activity, predominantly resulting in cholesteryl ester (CE) accumulation, particularly in the liver, spleen, and macrophages throughout the body. The disease is characterized by microvesicular steatosis leading to liver failure, accelerated atherosclerosis and premature demise. Although CESD is rare, it is likely that many patients are unrecognized or misdiagnosed. Here, the findings in 135 CESD patients described in the literature are reviewed. Diagnoses were based on liver biopsies, LAL deficiency and/or LAL gene (*LIPA*) mutations. Hepatomegaly was present in 99.3% of patients; 74% also had splenomegaly. When reported, most patients had elevated serum total cholesterol, LDL-cholesterol, triglycerides, and transaminases (AST, ALT, or both), while HDL-cholesterol was decreased. All 112 liver biopsied patients had the characteristic pathology, which is progressive, and includes microvesicular steatosis, which leads to fibrosis, micronodular cirrhosis, and ultimately to liver failure. Pathognomonic birefringent CE crystals or their remnant clefts were observed in hepatic cells. Extrahepatic manifestations included portal hypertension, esophageal varices, and accelerated atherosclerosis. Liver failure in 17 reported patients resulted in liver transplantation and/or death. Genotyping identified 31 *LIPA* mutations in 55 patients; 61% of mutations were the common exon 8 splice-junction mutation (E8SJM$^{-1G>A}$), for which 18 patients were homozygous. Genotype/phenotype correlations were limited; however, E8SJM$^{-1G>A}$ homozygotes typically had early-onset, slowly progressive disease. Supportive treatment included cholestyramine, statins, and, ultimately, liver transplantation. Recombinant LAL replacement was shown to be effective in animal models, and recently, a phase I/II clinical trial demonstrated its safety and indicated its potential metabolic efficacy.

Key Points

- Cholesteryl ester storage disease (CESD) is an underdiagnosed, autosomal recessive, progressive, metabolic liver disease due to the deficient activity of lysosomal acid lipase (LAL)
- LAL deficiency results in cholesteryl ester (CE) and triglyceride storage, primarily in hepatocytes and macrophages, leading to hepatomegaly, microvesicular steatosis, cirrhosis, dyslipidemia, accelerated atherosclerosis, and early demise
- Onset of the clinical manifestations can present from the first year of life and into adulthood
- On liver biopsy, the microvesicular steatosis may be misdiagnosed as NASH, NAFLD, or cryptogenic liver disease. The histologic diagnosis of CESD is facilitated by immunostaining for the lysosomal protein, cathepsin D, which is routinely performed in many pathology laboratories
- Treatment with statins does not reverse the disease manifestations, which lead to liver failure. A phase II clinical trial of enzyme replacement therapy indicated the potential safety and effectiveness of this therapeutic approach

Key words: Cholesteryl ester storage disease; Lysosomal acid lipase deficiency; Microvesicular steatosis; Micronodular cirrhosis; Non-alcoholic fatty liver disease (NAFLD); Non-alcoholic steatohepatitis; Type 2b dyslipidemia; Elevated serum transaminases; Hepatomegaly; Lysosomal storage disease.
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Abbreviations: CESD, cholesteryl ester storage disease; WD, Wolman disease; LAL, lysosomal acid lipase; *LIPA*, lysosomal acid lipase gene; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ApoB, apolipoprotein B; *ABCA1*, ATP binding cassette transporter 1; E8SJM, exon 8 splice-junction mutation; ERT, enzyme replacement therapy; LAMP, lysosomal associated membrane protein; LIMP, lysosomal integral membrane; CHO, Chinese hamster ovary; rhLAL, recombinant human LAL; CE, cholesteryl ester.

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Introduction

Cholesteryl ester storage disease (CESD; MIM 278000) is an autosomal recessive lysosomal storage disorder caused by mutations in the lysosomal acid lipase gene (*LIPA*) that markedly reduce lysosomal acid lipase activity (LAL; cholesterol ester hydrolase, EC 3.1.13) [1–4]. Deficient LAL activity results in progressive lysosomal accumulation of cholesteryl esters (CE), and to a lesser extent, triglycerides, predominantly in hepatocytes, adrenal glands, intestines, and cells of the macrophage-monocyte system throughout the body. The involvement of tissues closely correlates with their relative participation in receptor-mediated endocytosis and lysosomal degradation of lipoproteins [2–6]. Clinically, LAL deficiency results in two major phenotypes: infantile-onset Wolman disease (WD) (MIM 278000) and later-onset CESD, which were first described in 1956 [7,8] and in 1963 [9], respectively.

WD is a rare, neonatal-onset, fulminant subtype with absent or less than 1% of normal LAL activity, resulting in massive lysosomal accumulation of CEs and triglycerides, predominantly in the liver, spleen, adrenals, bone marrow, lymph nodes, and in macrophages throughout the body, particularly in the intestinal villi. Affected infants present by two to four months of age with vomiting and diarrhea, and massive hepatosplenomegaly. About 50% have adrenal calcifications. Feeding difficulties and malabsorption lead to malnutrition, growth retardation, cachexia, which together with the severe liver disease, contribute to demise in the first three to 12 months of life [2,3,10,11].

In contrast, CESD is an often unrecognized, later-onset subtype that may present in infancy, childhood, or adulthood, depending on the residual *in vitro* LAL activity, which typically ranges from 1% to ~12% of normal [2,3,12,13]. The progressive lysosomal CE and triglyceride accumulation leads to the characteristic liver pathology, elevated serum transaminases, and elevated serum LDL-cholesterol and triglycerides, with normal to low HDL-cholesterol concentrations (type IIb hyperlipoproteinemia). Premature demise is due to liver failure and/or accelerated atherosclerotic disease secondary to the chronic hyperlipidemia [14,15].

There is a clinical spectrum for CESD with some patients diagnosed in childhood, while others remain undiagnosed until adulthood. Severely affected patients may present in infancy with Wolman-like manifestations, such as diarrhea, failure to thrive, emesis, abdominal distension and even adrenal calcifications, but survive into childhood or adulthood. Patients typically present with hepatomegaly and liver dysfunction or type IIb dysliproteinemia. Hepatomegaly typically leads to a liver biopsy which grossly appears bright yellow-orange in color, and histologically is characterized by enlarged lipid-laden hepatocytes and Kupffer cells, and is characterized as microvesicular steatosis (Fig. 1A and B) [4,16–18]. The liver biopsy diagnosis may be misclassified as non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), or cryptogenic liver disease. The progressive lipid deposition leads to fibrosis, micronodular cirrhosis, and ultimately to liver failure [4]. Elevation of serum transaminases, alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST), and hepatomegaly are early indications of liver impairment.

The LAL enzyme defect results in the reduced hydrolysis of cholesteryl esters and triglycerides and their massive sequestration, particularly in the lysosomes of Kupffer cells and hepatocytes, as well as other cells of the macrophage/monocyte system. The lack of free cholesterol due to lysosomal trapping

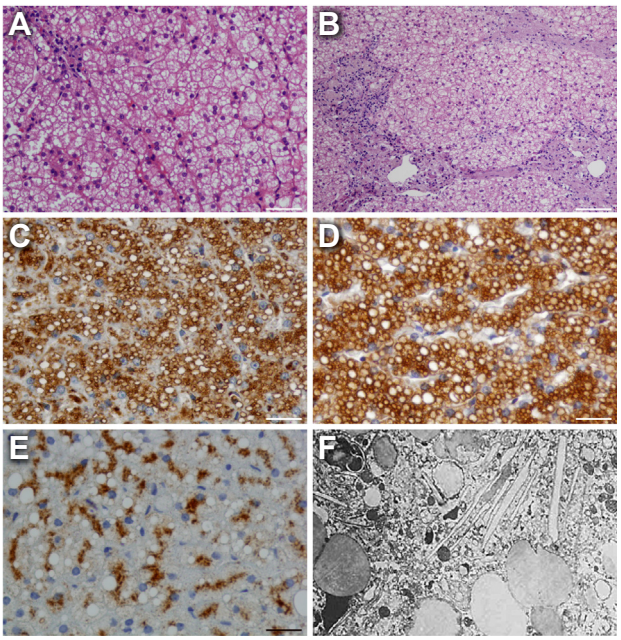

Fig. 1. Liver histopathology and ultrastructural findings in CESD. (A and B) Paraffin sections stained with H&E showing uniform microvesicular steatosis in both (A) early and (B) later stages of CESD. Note the number of foam macrophages infiltrating fibrous septa dividing the liver parenchyma in B. Bar in A represents 50 μm, bar in B 100 μm. (C and D) Immunostaining for both membranous (LAMP2) and luminal (cathepsin D) lysosomal markers in paraffin sections confirms lysosomal nature of lipid vacuoles in hepatocytes in CESD. (C) Signal for LAMP2 showing uniformly expanded and activated lysosomal system in both hepatocytes and macrophages. LAMP2 is in close contact with lipid droplets, clearly surrounding larger vacuoles. (D) Comparable results achieved with antibody against cathepsin D. Bars represent 25 μm. (E) Cathepsin D immunostaining in primarily non-lysosomal liver steatosis (β-oxidation deficiency). The signal for cathepsin D is discrete and restricted to the peribiliary region leaving cytosolic lipid vacuoles free. Bar represents 25 μm. (F) Electron micrograph demonstrating membrane-bound lipid vacuoles and needle-shaped CE crystals in the cytoplasm of hepatocytes from a 9-year old female with CESD. Magnification 10,000×.

of cholesteryl esters leads to reduced feedback inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and increased synthesis of cholesterol, as well as upregulation of apolipoprotein B (ApoB) synthesis and LDL-receptors on cell membranes [19–23]. The dysregulated expression of the LDL-cholesterol-dependent ATP binding cassette transporter 1 (*ABCA1*) gene contributes to HDL-cholesterol reduction in a manner similar to that in Niemann-Pick type C1 disease [24]. These metabolic alterations lead to increased serum total- and LDL-cholesterol and triglycerides, and decreased serum HDL-cholesterol, and the diagnosis of type IIb dyslipidemia [25]. The increased LDL-cholesterol concentrations cause accelerated atherosclerosis, and CESD patients have been reported who had premature atherosclerosis, ischemia, strokes, and coronary bypass surgery [13,15,26–29].

The *LIPA* cDNA and genomic sequence have been isolated and characterized [30–33]. The ~36 kb gene containing 10 exons is located on chromosome 10q23.31 and encodes an ~2.6 kb mRNA [31,33,34]. The mature lysosomal enzyme has 399 amino acids. Although the human enzyme has not been crystallized, its three-dimensional structure has been predicted based on homology with human gastric lipase [35]. To date, over 40 *LIPA* mutations causing CESD and WD have been identified [36] (Fig. 2).