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

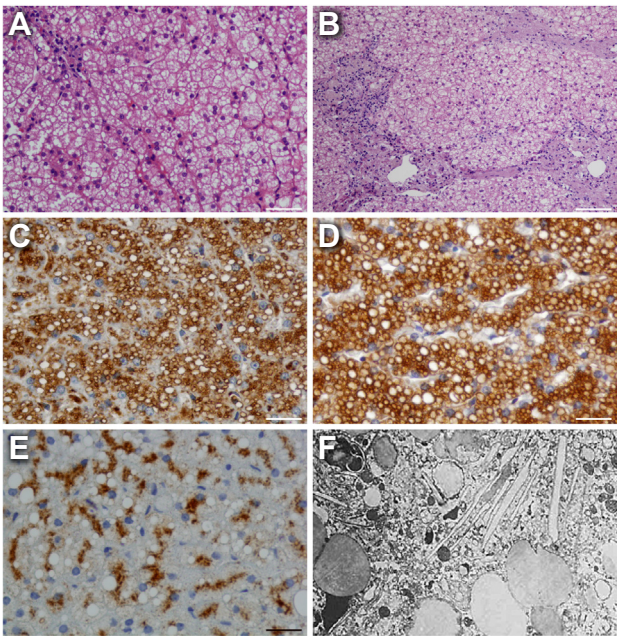

Fig. 1. Liver histopathology and ultrastructural findings in CESD. (A and B) Paraffin sections stained with H&E showing uniform microvesicular steatosis in both (A) early and (B) later stages of CESD. Note the number of foam macrophages infiltrating fibrous septa dividing the liver parenchyma in B. Bar 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).

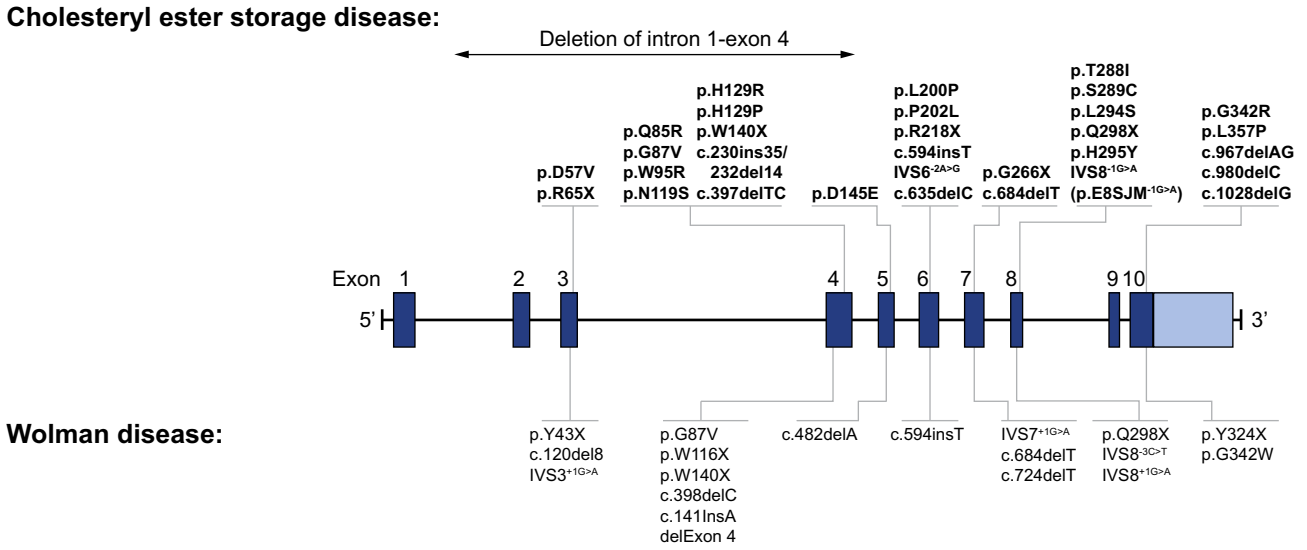

Fig. 2. *LIPA* gene mutations in patients with CESD and WD. Note that all mutation nomenclature is based on the cDNA with the A of the initiation sequence ATG as nucleotide 1. Thus, many of the previously reported mutations have been redesignated by adding the 21 bases in the leader sequence (e.g., H108P to H129P). Note, CESD mutations in bold are those in the reported patients described here.

CESD is pan-ethnic; however, the disease incidence is unknown. Prevalence estimates in Europeans have been based on the fact that a recurrent exon 8 splice-junction mutation, commonly referred to as E8SJM (c.894G>A; p.delS275_Q298; IVS8$^{-1G>A}$; hereafter designated E8SJM$^{-1G>A}$; rs 116928232) has been identified in patients of European ancestry [2,37,38] (Fig. 2). Population screening for E8SJM$^{-1G>A}$ among healthy West German individuals indicated a heterozygote frequency of 1 in ~200 individuals. Since E8SJM$^{-1G>A}$ accounts for about 50–60% of all CESD mutations, the predicted CESD heterozygote frequency for all *LIPA* mutations was estimated to be 1 in ~100, indicating a disease incidence of 1 in ~40,000 [39,40]. Thus, CESD may be grossly underdiagnosed, especially in patients of European ancestry.

Therapeutic efforts have included cholestyramine and statins to decrease cholesterol and ApoB synthesis, and liver transplantation for liver failure. Preclinical studies of LAL replacement in human fibroblast cells and murine models demonstrated "proof-of-concept" for enzyme replacement therapy (ERT) [41–44]. Recently, a phase I/II clinical trial of ERT for CESD indicated its safety and provided evidence supporting its metabolic effects [45], stimulating interest in this under-recognized disease.

To date, 135 CESD patients have been described in the literature, mostly in single case reports or small series of biochemically, histologically, and/or gene-diagnosed patients. The two largest series had only seven and 13 patients [46,47]. Therefore, the clinical, pathologic, biochemical, and molecular genetic findings, as well as the natural history and genotype-phenotype correlations in the 97 published reports were reviewed, with the objective of alerting hepatologists, pathologists, and lipidologists to this disease, its diagnosis, and current and future treatment.

Table 1. Characteristics of reported CESD patients.

All reported patients, n (%):	135 (100)	
Male	56 (45)	
Ethnicity/country of origin, n (%):		
Caucasian/Europe	85 (65)	
Caucasian/North American	23 (17)	
Caucasian/Latin American	14 (10)	
Caucasian/Middle Eastern	4 (3)	
Asian	4 (3)	
African	1 (0.3)	
Age at onset and last report, yr:	Onset	Last report
Males (n = 56)		
Mean	6	17.7
Median	5	13
Range	<1-44	1-52
Females (n = 68)		
Mean	4	18.49
Median	5	13
Range	<1-68	2.2-82
Sex unknown (n = 11)		
Mean	5.5	12.95
Median	5.6	12.2
Range	0.1-13	3.3-20.8
Distribution, n (%)	131 (97)	130 (96)
<2 yr	25 (19)	2 (<1)
≥2-5 yr	51 (39)	23 (18)
6-12 yr	33 (25)	32 (25)
13-20 yr	8 (6)	32 (25)
21-40 yr	8 (6)	28 (22)
41-58 yr	5 (4)	12 (9)
>60 yr	1 (<1)	1 (<1)

Literature search and patient demographics

Literature search and data collection

A literature search was undertaken to identify all publications describing CESD patients in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) since the original description [9]. Patients diagnosed with WD who died in the first year of life were excluded.

Eighty-two peer-reviewed articles in English and 15 foreign language articles were reviewed.

Of the 135 patients, longitudinal data were available for 99 who were followed for two years to >30 years from their initial diagnoses or symptom onset, including 21 patients who were described in subsequent reports, providing natural history information that could be correlated with their *LIPA* genotypes when available. For each patient, the following information was recorded, if reported: clinical manifestations, age at onset and last examination, presence and degree of hepatomegaly, and/or splenomegaly, other organ system involvement, liver pathology, LAL activity, *LIPA* mutations, serum total cholesterol, LDL- and HDL-cholesterol, and triglyceride levels, serum AST and ALT activities, and treatment including cholestyramine/bile acid sequestrants, statins, liver transplantation, and age and cause of death.

Patient demographics

Table 1 summarizes the demographic characteristics including gender, ethnicity, and country of origin, and ages at onset and last report. Patients were European (65%), North American (17%), Latin American (10%), Asian from India or Thailand (3%) and Middle Eastern (3%). There were single case reports of an Australian and African-American patient [48,49].

Table 2. Method of diagnostic confirmation, serum transaminase activities, and lipid levels in CESD patients*.

Diagnostic confirmation, n (%):	
Pathologic liver biopsy	112 (83)
Pathognomonic crystals/clefts	21 (16)
Pathologic LAL activity	114 (84)
LIPA mutation detection:	
Patients *LIPA* genotyped, n (%)	55 (41)
Total mutant alleles detected	106
Number different mutations	31
E8SJM$^{-1G>A}$ alleles, n (%)	65 (61)
E8SJM$^{-1G>A}$ homozygotes	17
Elevated serum transaminase activities:	
AST, n	78
n in IU/L (range)	54 (9-5240)
ALT, n	73
n in IU/L (range)	52 (15-2340)
Serum lipids in mg/dl, n (range):	
Total cholesterol	110 (104-620)
LDL-cholesterol	43 (119-360)
HDL-cholesterol	65 (8-50)
Triglycerides	96 (69-425)

*Data abstracted from the literature for patients in which the diagnostic methods and serum transaminases and lipids were reported.

Clinical manifestations

Age of onset and clinical presentation

Clinical information was reported on all patients, although with varying detail. The age at earliest symptom onset and/or diagnosis was recorded for 131 (97%) patients (Table 1). Median age of onset for the 56 (45%) males and 68 (55%) females was five years for both genders, ranging from birth – 44 years and 1 month-68 years, respectively. Eleven case reports did not specify gender. Of these 131 patients, age at onset for 35 (27%) severely affected children was between birth and two years, 81 (62%) presented between age 3 and 12 years, and 15 (11%) had onset or diagnosis during adolescence or as adults. There were five patients whose diagnoses were made at autopsy [5,50–52].

Clinically, hepatomegaly presented in 134 (99.3%) patients, and 74% also had splenomegaly. One mildly affected 27-year old male had no hepatomegaly, although he had liver pathology and elevated transaminases [53]. Typically, the patients had hepatomegaly or hepatosplenomegaly on physical exam or imaging studies when initially evaluated for elevated serum transaminase activities or for fever, respiratory infections, and/or other symptoms [54,55].

Table 2 summarizes the method of diagnosis, including liver biopsy, LAL activity, and *LIPA* mutations of genotyped patients, as well as the serum transaminase activities and lipid levels, when reported. Elevated AST and/or ALT activities were present in all cases reporting serum transaminase activities, with significant fluctuations at different time points (Table 2). The lipid profiles were not commonly reported for many pediatric patients, while adult patients often presented with hyperlipidemia that had variable responses to treatment with statins or cholestyramine. Siblings of affected individuals were diagnosed when initially symptomatic or pre-symptomatically by LAL assays or LIPA mutation analyses [26,47,56–58].

In general, the more severely affected children were more readily diagnosed than the CESD patients who had slower progression of their liver disease, and varying levels of serum lipids [13,53,59]. Because the diagnosis is challenging, it is likely that many adult CESD patients are being misclassified as having NAFLD, NASH, or cryptogenic liver disease, or remain undiagnosed [4,26,60].

Serum cholesterol and triglyceride levels (Table 2)

Total cholesterol was elevated in all 110 patients for whom it was reported, even though 26% were being treated with HMG-CoA reductase inhibitors. Of the 43 patients in whom serum LDL-cholesterol was reported, 79% had elevated levels (>200 mg/L), and over 95% had levels above the normal range, including 49% who were treated with HMG-CoA reductase inhibitors. There were 65 patients for whom HDL-cholesterol levels were reported, ranging from 8 to 50 mg/dl; 71% had HDL-cholesterol levels between 20 and 40 mg/dl, and 18% had levels below 20 mg/dl, while 11% had levels >40 mg/dl. Interestingly, at least 10 kindred were reported in which first-degree relatives of the probands who were obligate heterozygotes, as well as heterozygotes detected by E8SJM$^{-1G>A}$ screening, had significantly elevated serum total cholesterol levels [12,13,49,61–71,131]. Several reports documented the presence of coronary artery disease or atherosclerosis in the parents of affected patients, though few heterozygotes had known concomitant liver disease [49,67].

Other manifestations

The most common extrahepatic findings were frequent diarrhea, abdominal and epigastric pain, emesis, anemia, malabsorption, cholestasis, steatorrhea, poor growth, gallbladder dysfunction,

and cardiovascular disease [72–76,125]. Patients with micronodular cirrhosis had portal hypertension, ascites, cachexia, esophageal varices, gastrointestinal bleeding, and other secondary complications of liver failure resulting in coma, death or liver transplantation [51,77]. Esophageal varices were reported in 12 patients, including nine from 5 to 20 years of age [26,46,50,78–84]. Two cases of hepatocellular carcinoma were reported by the age of 11 and 52 years [28,85], and adrenal calcifications, which occur in ~50% of patients with WD, were present in nine CESD patients who ranged in age from <1 to 10 years. Thus, adrenal calcification may occur in either phenotype [52,64,78,84,86–92]. Gastrointestinal lipid and CE accumulation were a common finding, including in the core villi of the lamina propria, lacteal endothelium, smooth muscle, vascular pericytes, and in the duodenum and bowel mucosa where foamy macrophages were present (e.g., [51,93–96]). Cardiovascular manifestations predominantly involved coronary artery disease, aneurysm and stroke [6,13,22,26,29].

Liver failure and causes of death

Liver dysfunction and/or failure occurred in all 135 patients. Of the 11 reported deaths, the majority (73%) were due to liver failure, and progression to esophageal varices was reported in 12 cases. Of the 112 (83%) patients who had liver biopsies, all had significant findings as described below. Death due to liver disease progression occurred at 7 to 56 years of age, and 50% of deaths were in patients under 21 years of age [5,50,52,78]. Only one reported patient survived beyond 58 years [13], but long-term follow-up information was not available for most patients. Four, known additional deaths from liver failure occurred after their case reports [26,57]; (personal communication Dr. C. Gasche, Vienna, Austria; Dr. V. McLin, Geneva, Switzerland); (Table 3: patients 35, 36, 50, and 54).

Liver pathology

The massive lysosomal accumulation of CE and triglycerides leads to a diffuse microvesicular steatosis involving hepatocytes, Kupffer cells, and macrophages (Fig. 1A and B), which progresses to fibrosis, and ultimately, to micronodular cirrhosis [4,58,91,97]. Liver biopsy findings were consistent among patients, and appeared independent of age, genotype, or other factors. On gross examination, the liver specimens appeared a striking orange-yellow in color. On light microscopy (Fig. 1), the universal finding was the diffuse, uniform microvesicular steatosis with minimal zonal differences within the hepatic lobule [4]. Foamy macrophages containing lipid and ceroid were already present in the sinusoids and portal tracts of young patients. Increased numbers of storage macrophages and progression to fibrosis were observed at later stages. In contrast to macrophages, ceroid accumulation did not accompany lysosomal lipid accumulation in hepatocytes [4].

Microvesicular steatosis is uncommon in other metabolic causes of liver disease, though rare cases induced by severe mitochondrial beta-oxidation-mediated hepatotoxicity, for example associated with valproic acid use or Reye's syndrome, have been reported. Reye's syndrome has distinctive, mitochondrial enlargement that can be seen ultrastructurally [98–104].

Recently, Hulkova and Elleder re-evaluated a series of 19 CESD liver biopsies to identify reliable histopathologic criteria that would distinguish CESD from other forms of microvesicular steatosis, and in particular from NAFLD and other non-lysosomal fatty liver diseases in children [4]. They found that immunostaining for LAMP1, LAMP2, LIMP2, or with a lysosomal luminal protein (cathepsin D), readily identified the lipid accumulation as lysosomal, facilitating the diagnosis of CESD, abrogating the need for the ultrastructural demonstration of lysosomal lipid deposition (Fig. 1C–E). Pathologists should note their specific methods, commercial antibody sources, and additional unique histopathologic features of the CESD pathology [4]. Thus, one additional stain of a paraffin section would identify patients for confirmatory LAL and/or *LIPA* gene sequencing.

Pathognomonic birefringent CE crystals were observed in hepatocytes and/or Kupffer cells in fresh-frozen tissues under polarized light. In fixed paraffin-embedded sections, remnant clefts were observed where the lipid was extracted during dehydrating procedures. These crystals, or their remnant clefts, observed by electron microscopy were limited by a single lysosomal membrane or were free in the cytoplasm. Although only 65 of the 112 biopsies (58%) specifically described birefringent, needle-shaped CE crystals, 26 (23%) additional cases reported CE hepatocyte accumulation, and two reported CE remnant clefts, thereby accounting for 93 of the 112 cases (or 83%), although CE crystals presumably would be present in all cases if specimens were frozen and viewed by polarization microscopy or processed for electron microscopy.

Of the 112 biopsied patients, 72 (64%) had fibrosis and/or cirrhosis, including sinusoidal, portal/periportal or septal fibrosis in 56 (50%), whereas cirrhosis was present in 33 (29%). There were 17 patients (15%) who had both fibrosis and cirrhosis in initial or subsequent biopsies [105–107]. Some reports did not distinguish between fibrosis and cirrhosis [58,79,84]. Hepatocyte necrosis was reported in eight (7%) patients.

Of interest, the microvesicular steatosis and CE accumulation were already present in a prenatally diagnosed fetus at 17 weeks of gestation [89]. The affected fetus was detected in a family who had a previous child diagnosed at 2 years of age. The fetal liver had marked membrane-bound CE accumulation in the hepatocytes and syncytiotrophoblasts of the chorionic villi, as well as cholesterol infiltration and necrosis of enlarged adrenal glands. Portal and periportal fibrosis were observed in the affected two-year old sibling's liver biopsy, but not in the fetus, suggesting the progressive nature of CESD-associated liver disease [89].

Diagnosis

The diagnoses of all 135 reported patients were based on deficient LAL activity and/or *LIPA* gene mutations, or the pathologic liver biopsy findings. Markedly deficient LAL activity was demonstrated in 114 (84%) patients of whom 55 (42%) also had *LIPA* gene mutation analyses (Fig. 2). The remaining 21 (16%) patients were diagnosed by the presence of the pathognomonic CE crystals, or remnant clefts, in their liver biopsies. An affected CESD fetus was diagnosed prenatally by demonstrating LAL deficiency in cultured amniotic fluid cells [89]. Prenatal diagnosis can also be reliably made in cultured chorionic villi by LAL assay and/or by mutation analysis.

Table 3. Clinical, pathologic, and laboratory data for genotyped CESD patients.

Patient	*LIPA* genotype HGMD	*LIPA* genotype (as reported[1])	Sex	Age at onset, yr	Age at last report/death, yr (cause)	Clinical manifestations	Liver/tissue pathology	Lab findings	[Ref.]
1	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	M	<5	22	I	MVS, BRC	IIb	[131]
2	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	M	13	23	SM, GF	PBF, BRC, HV	IIb, ET	[38, 116]
3	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	n.r.	3	3	D (fam hx MI 30s + 40s)	MVS, BMV	IIb, ET	[90]
4	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	F	8	13	HT	NB, HS on U/S	ET, HC	[55, 113]
5	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	F	<5	41	D, cachexia, malabsorption	MVS, F, BRC, FC, HL, BP	ET	[75]
6	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	F	0.16	5	FTT, D, AD, SM,	PI, HV, KV, F	ET, HC, HTG	[122]
7	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	M	6	9	SM	PI, HV, KV, F	ET, HC	[122]
8	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$ (p.S275_Q298del) + c.894G>A	F	4	10	SM	Steatosis, PI, F, FC	IIb, ET	[113]
9	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$ (p.S275_Q298del) + c.894G>A	M	n.r.	22	SM	F, FC	IIb, ET	[113]
10	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$ (p.S275_Q298del) + c.894G>A	F	3	3	n.r.	NB	IIb, ET	[113]
11	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	M	0.8	15	SM	NB	IIb, ET	[117]
12	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	M	2	18	SM	NB	IIb, ET	[117]
13	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	F	2	36	SM, ML, I, MLD, ATH, AFIB, stroke, GU, ECC, X, CA, FC	HS on U/S, FC, BMV	IIb, A, ALF	[29]
14	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$ c.934G>A/c.934G>A	F	6	24	SM	DH	IIb	[47]
15	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$ c.934G>A/c.934G>A	F	6	24	HTN	DH	IIb	[47]
16	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$ c.934G>A/c.934G>A	F	4	22	HTN	DH	IIb	[47]
17	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$ c.934G>A/c.934G>A	F	5	16	SM, laryngitis	DH	IIb	[47]
18	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$	E8SJM$^{-1G>A}$/E8SJM$^{-1G>A}$c.934G>A/c.934G>A Inferred from children's genotypes	F	53	d. 58 (MI)	AP, MI	DH, BMV	HC	[47]
19	E8SJM$^{-1G>A}$/c.594insT	E8SJM$^{-1G>A}$/fsA178X190	M	3	18	GI	HV, G, MBL, F,	IIb	[119]
20	E8SJM/c.980delC	E8SJM$^{-1G>A}$/fs→ 330X	M	28	28	SM	NB	n.r.	[90]
21	E8SJM/c.397-398delTC	E8SJM$^{-1G>A}$/fs→137X	M	11	22	SM	NB	n.r.	[90]
22	E8SJM$^{-1G>A}$/c.230ins35/c.245del14	E8SJM$^{-1G>A}$/G77fsX82	F	68	d. 82	HF, CA, SM	NB	n.r.	[13]
23	E8SJM$^{-1G>A}$/c.635delC	E8SJM$^{-1G>A}$/fs→195X (934G>A)/DC673-5	M	33	d. 52 (liver failure)	CA, EA, ATH, aneurysms, GA	BRC, C	IIb	[28, 47]
24	E8SJM$^{-1G>A}$/R65X	E8SJM$^{-1G>A}$/c.C233T R44→fsX (Exon3)	F	11	34	SM, MH	DH	IIb	[47]
25	E8SJM$^{-1G>A}$/R65X	E8SJM$^{-1G>A}$/c.C233T R44→fsX (Exon3)	M	37	49	AP	BRC, F, FC, HV, DU	IIb, ET	[95, 96]

Table 3. (*continued*)

Patient	*LIPA* genotype HGMD	*LIPA* genotype (as reported[1])	Sex	Age at onset, yr	Age at last report/death, yr (cause)	Clinical manifestations	Liver/tissue pathology	Lab findings	[Ref.]
26	E8SJM$^{-1G>A}$/c.967delAG	E8SJM$^{-1G>A}$/S323L>fs>X366 (c.967delAG)	M	0.8	38	AP, SM	HV	ET	[38]
27	E8SJM$^{-1G>A}$/W95R	E8SJM$^{-1G>A}$/W74R (c.323T>A)	M	2	30	CF	DH	IIb	[11, 47]
28	E8SJM$^{-1G>A}$/D145E	E8SJM$^{-1G>A}$/475T>A	M	3	28	n.r.	DH	IIb	[47]
29	E8SJM$^{-1G>A}$/D145E	E8SJM$^{-1G>A}$/475T>A	M	14	36	n.r.	DH	IIb	[47]
30	E8SJM$^{-1G>A}$/H295Y	E8SJM$^{-1G>A}$/H295Y (p.S275_Q298del) + [c.883C>T]	F	19	19	n.r.	HS on U/S	IIb, ET	[113]
31	E8SJM$^{-1G>A}$/H295Y	E8SJM$^{-1G>A}$/H295Y (p.S275_Q298del) + [c.883C>T (p.H295Y)]	M	4	n.r.	n.r.	HS on U/S	IIb	[113]
32	E8SJM$^{-1G>A}$/L200P	E8SJM$^{-1G>A}$/L179P	F	6	n.r.	n.r.	NB	IIb	[132]
33	E8SJM$^{-1G>A}$/L200P	E8SJM$^{-1G>A}$/L179P	M	8	n.r.	n.r.	NB	IIb	[132]
34	E8SJM$^{-1G>A}$/L200P; G266X	E8SJM$^{-1G>A}$/L179P;G245FsX	M	5	12	SM	MVS, BRC	IIb	[8, 37]
35	E8SJM$^{-1G>A}$/H129P	E8SJM$^{-1G>A}$/H108P	F	<52	58 (d. age unk, pers. commun.)	ATH, CAD	MVS, FC, BRC, C	n.r.	[26, 57]
36	E8SJM$^{-1G>A}$/H129P	E8SJM$^{-1G>A}$/H108P	M	44	46 (liver transplant, age unk, pers. commun.)	Lipomas, ET, HC	F, FC, MVS	n.r.	[26, 57]
37	E8SJM$^{-1G>A}$/H129P	E8SJM$^{-1G>A}$/H108P	F	32	d. 56	EV	FC, MVS, C		[26, 57]
38	E8SJM$^{-1G>A}$/L357P	E8SJM$^{-1G>A}$/L336P	F	<14	19	SM	NB	IIb	[12]
39	E8SJM$^{-1G>A}$/D57V	E8SJM (934G>A)/210A>T	M	11	24	n.r.	DH	IIb, ET	[47]
40	E8SJM$^{-1G>A}$/G342R	E8SJM$^{-1G>A}$/G342R	M	9	9	HS on U/S	NB	ALF, IIb	[113]
41	E8SJM$^{-1G>A}$/N119S	E8SJM$^{-1G>A}$/N98S	F	26	26	AP	MVS, FC, PI	ALF, IIb	[49]
42	E8SJM$^{-1G>A}$/W140X	E8SJM$^{-1G>A}$/W140X c.894G>A (p.S275_Q298del) + [c.419G>A (p.W140*)]	M	7	7	n.r.	MVS	IIb	[113]
43	E8SJM$^{-1G>A}$/c.1028delG	E8SJM$^{-1G>A}$/c.DG1064-8	M	0.1	24	AD (birth)	DH,	ALF, IIb	[47]
44	E8SJM$^{-1G>A}$/G87V	E8SJM$^{-1G>A}$/G66V	M	6	21	SM	HV	IIb, HTG	[124]
45	E8SJM$^{-1G>A}$/Q85R	E8SJM$^{-1G>A}$/Q64R	F	6	11	n.r.	F, FC	ET	[88]
46	E8SJM$^{-1G>A}$/R218X	R218X/del S275_Q298.c.652 C>T/c.894 G>A, del c.823_894	F	9	18	n.r.	NB	IIb, ET	[13]
47	E8SJM$^{-1G>A}$/R218X	R218X/del S275_Q298.c.652 C>T/c.894 G>A, del c.823_894	F	9	18	n.r.	F, MVS, BRC, FC, HL	IIb, ET	[13]
48	E8SJM$^{-1G>A}$/UNK	E8SJM$^{-1G>A}$/UNK	F	51	51	2 sibs with CESD	NB	IIb	[90]
49	E8SJM$^{-1G>A}$/UNK	E8SJM$^{-1G>A}$/UNK	F	30	d. 47 (stroke)	X, stroke	DH, BMV	HC, HTG	[47]
50	H129R/large deletion (intron 1- exon 4)	H108R/large gene deletion (intron 1- exon 4)	M	1.5	3 (liver transplant, age unk, pers. commun.)	SM	DH	n.r.	[57]
51	T288I/T288I	T267I/T267I	F	0.67	8 (liver transplant, 7)	D, FTT, AC (8 mo)	HV, BRC (2 yr), C (6 yr)	n.r.	[88]
52	H295Y/H295Y	H274Y/H274Y	F	1.5	14 (liver transplant, 11)	n.r.	HV, BRC	n.r.	[114]
53	P202L/IVS6-2A>G	181L/E7SJM (ISVA>G D205-253)	M	5	13 (liver transplant, 11)	n.r.	BRC, C, HV	HC, HTG	[124]
54	G342R/S289C	G342R/S289C	F	1.8	3.2 (liver transplant, 18) d. 18 (pers. commun.)	HT, AC	BRC, F, TAO, BMV, FC	HC, ET	[90]
55	L294S/UNK	L273S/UNK	n.r.	5	>15	SM	NB	n.r.	[124]

[1]Both the HGMD nomenclature and the mutations as described in the original publications are listed. Previously, amino acid substitutions were described as 21 bases shorter when numbering was based on the peptide post-translational modification.

Abbreviations: Clinical manifestations: AC, adrenal calcifications or enlargement; AD, abdominal distension; AP, abdominal pain; ATH, atherosclerosis; CA, cancer; CAD, coronary artery disease; CF, chronic fatigue; D, diarrhea; ECC, ecchymoses; EV, esophageal varices; FTT, failure to thrive; GA, gonadal atrophy; GF, Growth failure (Ht/Wt <5th centile); GI, gastrointestinal manifestations; GU, perforated gastric ulcer; HF, heart failure; HT, hypertension; I, icterus; SM, splenomegaly; X, xanthelomatous skin lesions.

Liver and other tissue pathologies: BP, bowel pathology (bowel bx: mucosal edema, foamy macrophages); FC, foam cells; BMV, bone marrow vacuolization; BRC, birefringent crystals/clefts (massive); C, cirrhosis, (micronodular cirrhosis); DH, diagnostic hepatopathology; DU, duodenal bx lipid, foam cells; F, fibrosis; G, intracytoplasmic glycogen; kV, Kupffer cell vacuolization; HL, hepatocyte lipid deposition; HS, hepatic steatosis on ultrasound; HV, hepatocyte vacuolization; KL, Kupffer cell lipid deposition; MBL, membrane bound lipid; ML, mesenteric lipodystrophy; MLD, metabolic liver disease; MVS, microvesicular steatosis; NB, no biopsy (or not reported); PBF, portal-to-portal bridging fibrosis; PI, portal inflammation; TAO, trabecular architecture obliteration; VL, vacuolated lymphocytes, histiocytes.

Lab findings: IIb, type IIb hyperlipoproteinemia; A, anemia; ALF, abnormal liver function; HC, high cholesterol; HTG, hypertriglyceridemia; ET, elevated transaminases. n.r., not reported.

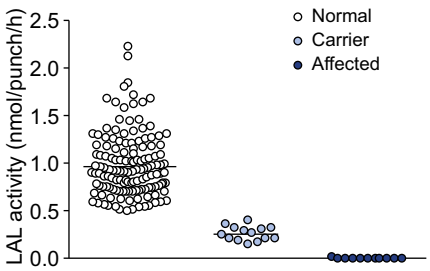

Fig. 3. Dried blood spot LAL activity in affected homozygotes, heterozygotes, and normal individuals. This assay is LAL specific, as it incorporates a specific inhibitor, Lalistat 2, which reduces the activity of the other lipases. LAL activity in 140 normal controls, 11 LAL deficient patients and 15 carriers. Limit of detection = 0.02 nmol/punch/h. From reference [109], with permission.

Deficient LAL activity

LAL activity was determined in cultured fibroblasts, peripheral leukocytes and liver tissue using various lipase substrates that were not specific for LAL, thereby precluding direct comparisons of the residual LAL activities among patients. The enzyme activities reported in the 114 patients ranged from 'undetectable' to 16% of normal mean values, but were typically between <1% and 10% of normal mean values for peripheral leukocytes or cultured fibroblasts [13,29,53,62–64,108]. Due to assay variability, the reported residual enzyme activity did not necessarily predict disease severity. In contrast, the LAL activities in cultured fibroblasts or leukocytes from patients with the more severe LAL deficient subtype, WD, had little, if any, detectable activity [3]. Recently, a LAL-specific assay was reported which determined the LAL activity in dried blood spots (DBS) using 4-methylumbelliferyl-palmitate as the enzyme substrate, and the LAL-specific inhibitor, lalistat 2. This assay resulted in good separation of the activities for normal controls and CESD homozygotes and heterozgyotes [109] (Fig. 3).

LIPA gene mutations

To date, over 40 loss-of-function *LIPA* mutations have been identified in patients with CESD and WD (http://www.hgmd.cf.ac.uk/ac/index.php) (Fig. 2). Of the 19 known mutations causing WD, most (37%) are small deletions/insertions, with 26% non-sense, 21% consensus splice-site mutations, 10% missense lesions, and 5% a large deletion. Of the 32 known CESD mutations, most (50%) were missense, with 25% small deletions/insertions, 16% non-sense, 6% consensus splice-site mutations, and 3% a large deletion. The most common mutation, E8SJM$^{-1G>A}$, has been found only in CESD patients and the two exon 8 splice-junction variants, E8SJM$^{+1G>A}$ and E8SJM$^{+3C>T}$, occurred only in WD patients [3,36]. The common donor-splice-site mutation, E8SJM$^{-1G>A}$ causes alternative splicing leading to the deletion of exon 8, which encodes a mutant LAL enzyme with no activity, as well as about 2% to 4% of normally spliced, wild-type mRNA which encodes ~3–8% of normal LAL activity [8,24,37,48]. Nonsense, small deletions/insertions, splicing, and missense mutations were found in patients with both CESD and WD. However, the most severe alterations that resulted in markedly reduced or no LAL activity were detected in patients with WD, while *LIPA* mutations that encoded mutant enzymes with residual activity were found in patients with CESD [110]. Of note, G87V (also described as G66V) is the *LIPA* founder mutation for WD among individuals of Persian Jewish and Bukharin Jewish ancestry, with an allele frequency of ~1 in 32 [88,111].

In vitro LIPA cDNA expression studies have assessed the residual LAL activity caused by various missense mutations [110]. These studies have shown that the missense mutations associated with little or no activity, particularly when homozygous, caused WD. In contrast, missense mutations which encoded residual enzyme activity (1–5%) *in vitro* were found in CESD patients [36,110].

As noted above, most LAL assay methods use different substrates and procedures, are not always LAL-specific, and therefore, are not comparable. For example, the CESD patient with the highest reported LAL activity (16% of normal mean fibroblast activity) had severe disease with onset at 6 months of life, hepatic cirrhosis by 3 years, portal hypertension and esophageal varices by seven years, and was liver transplanted at 13 years of age [80,81,112].

Genotype/phenotype correlations

In general, the disease subtype (WD or CESD) and severity are primarily based on the absence or amount of residual LAL activity determined by the two *LIPA* mutant alleles. *LIPA* mutations in patients with WD primarily result in little or no LAL activity, while patients with CESD have at least one mutation that results in some residual enzyme activity. If a patient has the common E8SJM$^{-1G>A}$ allele, or a missense mutation that encodes significant residual enzyme activity, the patient will be protected from having WD and will have CESD, no matter how severe the other allelic mutation.

Among CESD patients, who have some residual LAL activity, the clinical spectrum varies from early onset, rapidly progressive disease, to a later-diagnosed, more attenuated phenotype. The CESD severity should reflect the *LIPA* genotype, and the total residual activity encoded by both alleles. The residual enzymatic activity will correlate with the disease subtype, age at clinical onset, and rate of disease progression. The common E8SJM$^{-1G>A}$ and missense mutations, which predict residual LAL activity, were the most frequent among the genotyped CESD patients.

Of the 135 reported patients, *LIPA* mutations were identified in 55 patients, including four for whom only one mutation was detected, but whose diagnoses were based on LAL deficiency and/or CE crystals in the liver biopsies. Among these 55 patients, 31 *LIPA* mutations were identified including 17 missense, 3 nonsense, one small insertion, one complex small insertion/deletion, one double mutation (2 mutations on the same allele), five small deletions, one large gene deletion spanning four exons, and two consensus splice site mutations, including an intronic splice-site mutation resulting in a deletion of 48 base pairs (exon 7), as well as the common E8SJM$^{-1G>A}$ mutation (Fig. 2, in bold). Six mutations, H295Y, G342R, L200P, R44X, G87V, and E8SJM$^{-1G>A}$ have been identified in at least two or more unrelated kindred, and the L179P, 195X (c.673_675delC), and G87V mutations have been identified in both CESD and WD patients [56,88,111,132]. Among the 106 *LIPA* mutations detected in these 55 patients, the common E8SJM$^{-1G>A}$ allele was present in 61% of mutant alleles, which included 17 patients who were E8SJM$^{-1G>A}$ homozygotes. One additional patient was inferred to be an E8SJM$^{-1G>A}$ homozygote based on molecular testing of the proband's three children [47].

To date, there has been no genotype/phenotype correlations reported for CESD. Such correlations have been limited by the fact that there are few affected sibs or unrelated affected individuals with the same genotypes for clinical comparison (Table 3). The limitations of genotype/phenotype correlations are further highlighted by the diversity among the18 E8SJM$^{-1G>A}$ homozygotes. This is the largest group of reported CESD patients with the same genotype. Almost all E8SJM$^{-1G>A}$ homozygotes had onset in the first years of life, followed by progression of their liver disease into adult life. Of the 15 E8SJM$^{-1G>A}$ homozygotes whose ages of onset were reported, 13 were diagnosed in the first year of life to 6 years of age, and two cases were diagnosed at ages 8 and 13 years. One additional female inferred to be homozygous, based on her children's genotypes, who was first diagnosed at 53 years of age had a fatal myocardial infarction at 58 years [47]. Liver disease findings among the reported E8SJM$^{-1G>A}$ homozygotes ranged from microvesicular steatosis to fibrosis, and fibrosis with septal bridging, indicative of cirrhosis. Extrahepatic involvement among E8SJM$^{-1G>A}$ homozygotes included gastrointestinal lipid accumulation, severe, acute and chronic diarrhea, malabsorption, abdominal pain, perforated gastric ulcer, weight and height below the 5th percentile, anemia, frequent hospitalizations for respiratory infections, coronary artery disease including atherosclerosis, heart failure, aortic calcifications, myocardial infarction, and stroke. While disease progression in some patients was rapid, in others the progression was slow, although ultimately leading to hepatic fibrosis and complications of atherosclerosis [29,113].

Several patients were compound heterozygotes for the H129P (histidine to proline) missense mutation (which *in vitro* had 4.6% of normal enzyme activity) and the common E8SJM$^{-1G>A}$ allele (genotype H129P/E8SJM$^{-1G>A}$). Three siblings with the E8SJM$^{-1G>A}$/H129P genotype were diagnosed as adults (32, 44, and <52 years) [26]. All three had significant liver disease including cirrhosis and esophageal varices, and one died of liver failure at 56 years of age [26]. Since the publication, a second sibling passed away, and the third received a liver transplant (personal communication, Dr. C. Gasche, [26]). In contrast, a patient with compound heterozygosity for H129R (histidine to arginine) and a gene deletion, had onset at 18 months of age [57]. After publication, this patient also developed liver failure and received a liver transplant at 9 years of age, four years after the case report was published (personal communication Dr. V. McLin). These patients illustrate the importance of both alleles and the protein conformational alteration by the amino acid substitution in predicting patient's severity. Of note, the CESD patients with the T288I/T288I (*in vivo* the T288I protein had 3.6% of normal activity), and G342R/S289C genotypes both had a WD-like presentation, including infantile-onset, diarrhea, adrenal calcifications and failure to thrive. However, they had sufficient residual LAL activity to survive into the second or third decades of life, after liver transplantation. The patient homozygous for T288I was liver transplanted at 7 years of age [88,90]. The patient with the G342R/S289C genotype survived to age 18 years, when liver failure led to liver transplantation, and subsequent demise (Table 3: patient 54) [92]. The patients homozygous for H295Y (which had 2.9% of normal activity) also had infantile-onset CESD, requiring liver transplantation at age 11 years, and two unrelated patients with an E8SJM$^{-1G>A}$/c.323T>A and E8SJM$^{-1G>A}$/S344L (c.967delAG >fs→338X) genotype, both had infantile-onset disease, but survived into their 30s at last follow-up [38,47,114]. These patients illustrate the early onset of disease manifestations, which may rapidly progress in childhood or adolescence, or more slowly progress into adulthood when liver failure leads to transplantation or death.

Treatment

Cholesterol reduction strategies

Only 35 (26%) of the reported patients were treated with HMG-CoA reductase inhibitors for hyperlipidemia [13–15,26,28,29,38,47,65–67,79,84,95,96,107,115–121]. The first use of statins to control the cholesterol synthesis abnormalities in CESD was in a 9-year old girl with hepatomegaly, markedly elevated serum total and LDL-cholesterol and decreased HDL-cholesterol levels with massive CE accumulation, fibrosis, and cirrhosis on liver biopsy [107]. After 8 months of treatment with 5 mg/d of lovastatin, her dose was increased to 20 mg/d and the patient's plasma triglycerides, cholesterol and LDL-cholesterol decreased, although the HDL-cholesterol level was not significantly increased. Since her liver and spleen volumes were unchanged from baseline, the authors hypothesized that hepatic CE production was increased in response to ApoB flux-mediated LDL receptor upregulation [107]. This hypothesis was subsequently supported by two later case reports that documented favorable responses to simvastatin (0.28 mg/kg) and cholestyramine after two years of treatment, which resulted in significant reductions in serum LDL-cholesterol, triglycerides and increased HDL-cholesterol concentrations, with decreased liver volume, and no changes in the transaminase levels, splenomegaly or adre-

nal calcifications. After 3 years of treatment, a liver biopsy revealed increased fibrosis with ensuing portal hypertension, and after five years of treatment, the liver disease progression necessitated liver transplantation [84,115].

Of the 35 patients taking statins, eight had no liver biopsy findings reported, 15 had biopsy findings with fibrosis, cirrhosis or CESD-associated hepatopathology, but no long-term or sequential follow-up data reported [18,29,47,53,60,65–67,89–91,95,96,117,118,120–122]. The 12 remaining patients on HMG-CoA reductase inhibitors had multiple liver biopsies, providing longitudinal data. There were no cases whose liver histology improved, and all 12 patients had progressive liver disease that was more advanced in subsequent biopsies, demonstrating the progressive nature of liver disease in CESD. In fact, six patients treated with HMG-CoA reductase inhibitors required transplantation or died from liver failure [15,26,28,38,47,48,62,79,84,115,116,119,123] (personal communication, Dr. C. Gasche, Dr. V. McLin). These findings emphasize the lack of efficacy of statins in ameliorating liver disease or preventing its progression.

Liver transplantation

Liver transplantation was reported in nine patients who were five to 14 years of age at transplantation [81,83–85,88,114,124,125]. At least three additional patients developed liver failure requiring transplantation after the publication of their case reports, two of whom subsequently died (Dr. C. Gasche, personal communication [26]; Table 3: patients 36, 50, and 54). There is limited information on the long-term follow-up for the majority of transplanted patients, with several exceptions [80,81,83,112,126,127]. Six liver-transplanted patients were followed from 10 months to three years, reportedly without complications, despite one case who had hepatocellular carcinoma that was found incidentally in the removed liver. An additional patient who was transplanted at five years of age had hyperbilirubinemia and elevated transaminases suggestive of transplant rejection, and developed progressive, congestive heart failure [126]. Only two transplanted patients had documented follow-up for over five years. One, transplanted at 14 years of age, had a subsequent biliary infection and obstruction requiring surgery. She developed end-stage renal failure seven years post-transplant due to glomerular sclerosis and atherosclerosis with extensive vascular lipid accumulation and tubular atrophy, as well as interstitial fibrosis, and required chronic hemodialysis by 21 years of age. The lipid deposition in the renal vascular system raised concern that the transplant may have ameliorated only the liver disease, but not the systemic lysosomal CE accumulation. The second patient for whom long-term follow-up was reported had no renal involvement six years after transplant [80,81,112].

Enzyme replacement therapy

ERT in CESD was first performed in cultured CESD fibroblasts using a *Pseudomonas*-derived LAL (which was cross-linked to albumin and/or conjugated to insulin or ApoB) [41]. These enzyme preparations were incubated in the media of the cultured CESD fibroblasts, were taken up, and the intracellular enzyme degraded lysosomally-accumulated CEs. Subsequent efforts to develop ERT awaited the generation and/or characterization of murine CESD models and the purification of LAL from various sources.

Preclinical studies of ERT have been conducted in naturally-occurring rats and in knockout mice with LAL deficiency [128,129]. Rats with autosomal recessive LAL deficiency were identified by Yoshida and Kuriyama in 1990 [128]. These rats had ~19% of wild-type hepatic LAL activity. Clinically, the rats have hepatosplenomegaly, enlarged lymph nodes, and thickened, dilated intestines. They have been characterized pathologically and biochemically, and were found to closely mimic the liver disease pathology in humans, having microvesicular steatosis of the hepatocytes and Kupffer cells, which progressed to fibrosis and micronodular cirrhosis. They do not have adrenal calcifications. More recently, a *LIPA* knockout mouse model was generated by recombinant DNA techniques and characterized [122]. The mice had no LAL enzyme activity or protein, and had massive hepatic accumulation of CEs and triglycerides. In addition, the mice developed the typical CESD hepatic, adrenal, and intestinal pathology (i.e., hepatocyte, Kupffer cell, and other macrophage and adrenocortical storage), survived to adulthood, and produced progeny. These mice were used to evaluate ERT [42–44] and adenovirus-mediated gene therapy [130].

Preclinical trials of ERT were evaluated in the *LIPA* knockout mice [42–44]. These studies evaluated the effectiveness of human recombinant LAL expressed in *Pichia pastoris* [42], Chinese hamster ovary (CHO); [43], or in *Nicotiana benthamiana* (tobacco) cells [44]. Expression in yeast resulted in a recombinant human LAL (rhLAL) with primarily mannose-terminated oligosaccharide chains, while expression in CHO cells produced a human LAL with both mannose- and mannose-6-phosphate-terminated oligosaccharides [42–44]. Ten intraperitoneal doses of each enzyme over 30 days were administered. The mannose-terminated *Pichia pastoris* rhLAL was delivered via the mannose-receptor to macrophages and macrophage-derived cells in various organs. The CHO cell-produced rhLAL had mannose-6-phophate terminated oligosaccharides and cleared both hepatocytes, Kupffer cells, and macrophage-delivered cells. The plant-derived rhLAL had mannose-receptor-dependent uptake and, following 10 intravenous injections (every 3 days), resulted in decreased hepatic CE and triglyceride concentrations, and diminished foamy macrophages in the liver, spleen, and intestinal villi [44]. When each enzyme was administered to double knockout mice for *LIPA* and the macrophage mannose-receptor, only the CHO-derived enzyme cleared both hepatocyte and macrophage cell lipid accumulations [43].

These preclinical studies provided the rationale for clinical trials of ERT in WD and CESD with rhLAL produced in egg whites [45]. A phase 1/2 randomized, double-blind, placebo-controlled, open label, dose-escalation study was performed with rhLAL that had both mannose- and mannose-6-phosphate oligosaccharides. Three doses were evaluated (0.35, 1.0, and 3.0 mg/kg) weekly for four weeks in CESD patients, followed by an extension study with all participants receiving 1 mg/kg every other week. These studies demonstrated the safety of the egg-white-derived rhLAL, reduced the serum transaminase activities, and resulted in serum LDL-cholesterol elevations evidencing the release of free cholesterol from the accumulated CEs in lysosomes [45].

Disease management

Based on the clinical, pathological, and biochemical studies of the patients reported in the literature, the following evaluations are suggested to monitor disease progression. Annual laboratory tests should include liver function tests (AST, ALT, prothrombin

time, bilirubin), complete blood counts, chitotriosidase, (a macrophage inflammatory marker reported elevated in CESD; [29]), and a lipid panel to assess dyslipidemia. Magnetic resonance imaging should be performed periodically to determine liver and spleen volumes and magnetic resonance spectroscopy can be used to measure the hepatic fat fraction. Imaging studies, such as abdominal ultrasound or esophagoduodenoscopy to monitor portal hypertension and variceal bleeding, may be indicated. Cardiovascular evaluations include an annual electrocardiogram and echocardiogram, as well as carotid intima media thickness, and a neurovascular evaluation for stroke risk should be considered.

Discussion

Previously, there have been no prospective or retrospective natural history studies of CESD patients. Of the 135 CESD cases reported in the literature, most were diagnosed incidentally by the characteristic liver pathology, and/or confirmed by deficient LAL enzyme activity or by *LIPA* gene analyses. All reported cases had significant liver disease characterized by microvesicular steatosis that progresses to micronodular cirrhosis and liver failure.

Clinically, CESD has a variable age of onset, and is often unrecognized, particularly in adults, until the unexplained hepatomegaly, with or without splenomegaly, elevated transaminase activities, and/or type IIb hyperlipoproteinemia lead to diagnostic investigation. Typically, a liver biopsy will reveal the striking yellow-orange color, foamy lipid-laden hepatocytes and macrophages (Fig. 1A and B), and the pathognomonic lysosomal CE crystals, best observed by ultrastructural examination (Fig. 1F). However, awareness of this lysosomal storage disease is limited, and many patients were originally missed clinically. The liver biopsy may be misdiagnosed as NASH, NAFLD, or cryptogenic cirrhosis [4]. Diagnostic suspicion can be confirmed by demonstrating the markedly deficient LAL activity (Fig. 2) [109], or by *LIPA* mutation analyses, which accurately detect both affected patients and heterozygotes for this autosomal recessive disease.

Based on this review, affected children tend to have a severe course that leads to early liver failure and transplantation. Although liver transplantation has been effective in preventing death from liver failure, extrahepatic organ involvement, even in transplanted patients, resulted in significant disease burden and in some patients premature demise. Patients diagnosed later in life tend to have a more attenuated course; however, there are no long-term data on the morbidity and mortality of these patients. The later-onset patients may be the most underdiagnosed cohort, since they often appear asymptomatic, other than having type IIb hyperlipoproteinemia, until stroke, aneurysm, aorto-coronary disease or premature sudden death from liver failure lead to the diagnosis.

Given that ERT clinical trials for CESD are now underway, the need for increased disease awareness is paramount. LAL deficiency should be included in the differential diagnosis for all patients with elevated serum total cholesterol and LDL-cholesterol who also may have mildly to moderately decreased HDL-cholesterol, elevated transaminases and hepatomegaly. The availability of non-invasive LAL enzyme and *LIPA* gene molecular diagnoses can abrogate the need for a liver biopsy to confirm the CESD diagnosis. However, CESD should be suspected for any liver biopsy that is orange-yellow in color, with microvesicular steatosis and/or micronodular cirrhosis. The CESD diagnosis can be established by immunostaining for LAMP1, LAMP2, LIMP2 or cathepsin D, or by demonstrating pathognomonic CE crystals or their remnant clefts by electron microscopy. It is anticipated that awareness of CESD by hepatologists, pathologists, cardiologists, and neurologists will lead to greater detection of patients, particularly among adults, leading to improved management and treatment.

Conflict of interest

DLB and RJD are consultants to Synageva BioPharma, the company that is developing enzyme therapy for Cholesteryl Ester Storage Disease – now in Phase 3 trial. Dr. Desnick has stock options to Synageva BioPharma, and serves on their Scientific Advisory Board. HH, and MGB have no conflicts.

Authors' contributions

DLB and RJD designed the study, analyzed the data, and wrote and edited the paper. HH contributed the section on liver pathology and MGB and HH reviewed the manuscript. All authors approved the final manuscript.

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