

#### **4. TP I e TC I**

# 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<sup>-1G>A</sup>), for which 18 patients were homozygous. Genotype/phenotype correlations were limited; however, E8SJM<sup>-1G>A</sup> 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.

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## 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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## Doença de depósitos de ésteres de colesterol: Revisão de 135 casos de doentes com uma doença subdiagnosticada

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### Resumo

A doença de depósitos de ésteres de colesterol (DDEC) é causada pela deficiência na atividade da lipase ácida lisossomal (LAL), o que resulta predominantemente na acumulação de ésteres de colesterol (EC), particularmente no fígado, baço e nos macrófagos por todo o corpo. Esta doença caracteriza-se pela esteatose microvesicular que leva à insuficiência hepática, à aterosclerose acelerada e à morte precoce. Embora a DDEC seja considerada rara, é provável que muitos doentes não sejam identificados ou que se encontrem incorretamente diagnosticados. Neste artigo são revistos os casos descritos na literatura referentes a 135 doentes com DDEC. Os diagnósticos tiveram como base biópsias hepáticas, a deficiência da LAL e/ou mutações no gene da LAL (LIPA). A hepatomegalia estava presente em 99,3% dos doentes; 74% também apresentaram esplenomegalia. Quando registado, a maioria dos doentes apresentava níveis elevados de colesterol sérico total, de colesterol LDL, de triglicéridos e de transaminases (AST, ALT ou ambas), ao passo que o colesterol HDL se encontrava baixo. Todos os 112 doentes submetidos à biópsia hepática apresentavam a patologia característica, que é progressiva, e inclui a esteatose microvesicular, que leva à fibrose, à cirrose micronodular e, por fim, à insuficiência hepática. Foram também observados nas células hepáticas cristais birrefringentes de EC patognomónicos ou as suas feições remanescentes. Quanto às manifestações extra-hepáticas estas incluíram a hipertensão portal, a presença de varizes esofágicas e a aterosclerose acelerada. A insuficiência hepática registada em 17 doentes levou ao transplante hepático e/ou à morte.

A genotipagem identificou 31 mutações no gene LIPA em 55 doentes; das quais 61% foram referentes à mutação comum no exão 8 na região de junção intrão-exão (E8SJM<sup>-1G>A</sup>), para as quais 18 doentes eram homocigóticos. As correlações genótipo/fenótipo foram limitadas; Contudo, os homocigóticos para E8SJM<sup>-1G>A</sup> apresentaram geralmente um início precoce da doença com progressão lenta. O tratamento de suporte incluiu colestiramina, estatinas e, em última instância, o transplante hepático. A substituição enzimática com LAL recombinante demonstrou ser eficaz em modelos animais e, recentemente, um ensaio clínico de fase I/II comprovou a sua segurança e mostrou o seu potencial e eficácia metabólica.

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### Pontos-chave

- A doença de depósitos de ésteres de colesterol (DDEC) é uma doença hepato-metabólica, progressiva, autossómica recessiva e subdiagnosticada causada pela atividade deficiente da lipase ácida lisossomal (LAL);
- A deficiência da LAL resulta na acumulação de ésteres de colesterol (EC) e de triglicéridos, sobretudo nos hepatócitos e macrófagos, levando à hepatomegalia, à esteatose microvesicular, à cirrose, à dislipidemia, à aterosclerose acelerada e à morte precoce;
- O início das manifestações clínicas pode surgir tanto a partir do primeiro ano de vida como já na idade adulta;
- Na biópsia hepática, a esteatose microvesicular pode ser incorretamente diagnosticada como sendo EHNA, DHGNA ou doença hepática criptogénica. O diagnóstico histológico da DDEC é facilitado pela imunocoloração da proteína lisossomal, cathepsina D, que é realizada regularmente em muitos laboratórios de patologia;
- O tratamento com estatinas não reverte as manifestações da doença que conduzem à insuficiência hepática. Um ensaio clínico de fase II de terapia de substituição enzimática comprovou a sua segurança e mostrou o potencial e eficácia desta abordagem terapêutica.

**Palavras-chave:** Doença de depósitos de ésteres de colesterol; Deficiência da lipase ácida lisossomal; Esteatose microvesicular; Cirrose micronodular; Doença hepática gordurosa não alcoólica (DHGNA); Esteatohepatite não alcoólica; Dislipidemia tipo IIb; Transaminases séricas elevadas; Hepatomegalia; Doença de depósito lisossomal.

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**Abreviaturas:** DDEC, doença de depósitos de ésteres de colesterol; DW, doença de Wolman; LAL, lipase ácida lisossomal; LIPA, gene da lipase ácida lisossomal; DHGNA, doença hepática gordurosa não alcoólica; EHNA, esteatohepatite não alcoólica; ALT, alanina aminotransferase; AST, aspartato aminotransferase; HMG-CoA, 3-hidroxi-3-metilglutaril coenzima A; ApoB, apolipoproteína B; ABCA1, transportador 1 de cassette de ligação de ATP; E8SJM, mutação no exão 8 na região de junção intrão-exão; TSE, terapêutica de substituição enzimática; LAMP, proteína de membrana associada ao lisossoma; LIMP, membrana integral do lisossoma; CHO, ovário de hamster chinês; rhLAL, LAL humana recombinante; EC, ésteres de colesterol.



## Introduction

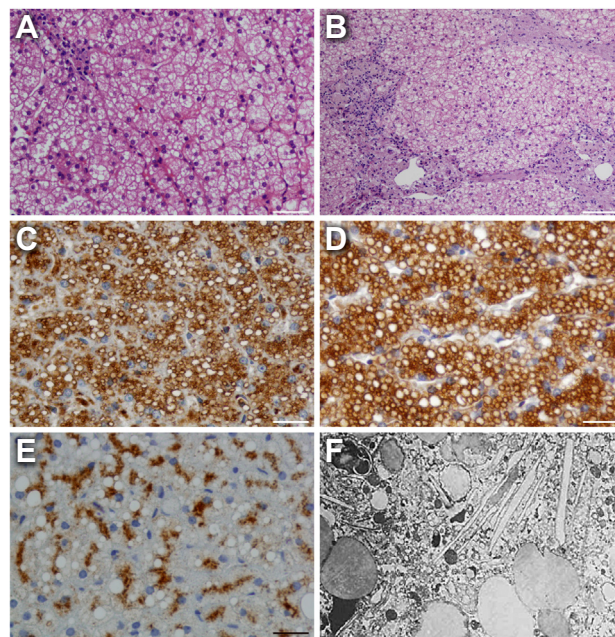
Cholesteryl ester storage disease (CESD; MIM 278000) is an autosomal recessive lysosomal storage disorder caused by mutations in the lysosomal acid lipase gene (*LIPA*) that markedly reduce lysosomal acid lipase activity (LAL; cholesterol ester hydrolase, EC 3.1.13) [1–4]. Deficient LAL activity results 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  $\mu$ m, bar in B 100  $\mu$ m. (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  $\mu$ m. (E) Cathepsin D immunostaining in primarily non-lysosomal liver steatosis ( $\beta$ -oxidation deficiency). The signal for cathepsin D is discrete and restricted to the peribiliary region leaving cytosolic lipid vacuoles free. Bar represents 25  $\mu$ m. (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 $\times$ .

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).

**Introdução**

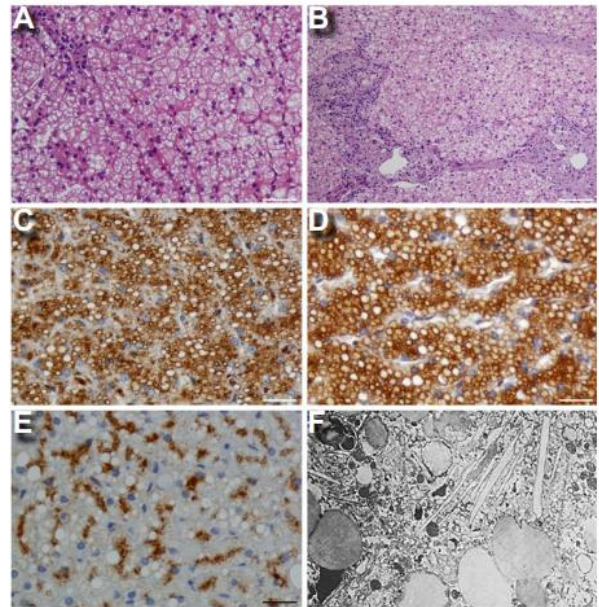
A doença de depósitos de ésteres de colesterol (CESD; MIM 278000) é uma doença lisossomal de sobrecarga autossômica recessiva provocada por mutações no gene da lipase ácida lisossomal (LIPA), que reduzem significativamente a atividade da lipase ácida lisossomal (LAL; hidrólase dos ésteres de colesterol, EC 3.1.13) [1–4]. Esta deficiência na atividade da LAL resulta na acumulação progressiva de ésteres de colesterol (EC), e na acumulação em menor proporção de triglicéridos, predominantemente nos hepatócitos, nas glândulas suprarrenais, nos intestinos e nas células do sistema monocítico-macrofágico por todo o organismo. O envolvimento dos tecidos encontra-se estreitamente correlacionado com a sua participação relativa não só na endocitose mediada por recetores, como também na degradação lisossomal de lipoproteínas [2–6]. Clinicamente, a deficiência da LAL traduz-se em dois fenótipos principais: a doença de Wolman (WD) (MIM 278000), que surge na infância, e a DDEC de início mais tardio, que foram descritas pela primeira vez em 1956 [7,8] e em 1963 [9], respetivamente.

A DW é rara, surge no período neonatal e é considerada um subtipo fulminante onde a atividade normal da LAL se encontra abaixo de 1% ou ausente, o que resulta numa acumulação massiva de EC e de triglicéridos sobretudo no fígado, no baço, nas suprarrenais, na medula óssea, nos gânglios linfáticos e nos macrófagos por todo o organismo, particularmente nas vilosidades intestinais. As crianças afetadas dos dois aos quatro meses apresentam vômitos, diarreia e hepatoesplenomegalia massiva. Destas, cerca de 50% apresentam calcificações suprarrenais. As dificuldades na alimentação e a má absorção conduzem à subnutrição, ao atraso no crescimento e à caquexia que contribuem, conjuntamente com a doença hepática grave, para a morte nos primeiros três a doze meses de vida [2,3,10,11].

Por outro lado, a DDEC não é muitas vezes reconhecida, sendo um subtipo cujo início é mais tardio, podendo apresentar-se também na primeira infância, na infância ou na idade adulta, dependendo da atividade residual *in vitro* da LAL, que varia usualmente entre 1% e ~12 relativamente à atividade normal [2, 3, 12, 13]. A acumulação lisossomal progressiva de EC e de triglicéridos conduz à característica patologia hepática, às transaminases séricas elevadas e ao aumento dos níveis séricos de colesterol LDL e de triglicéridos, acompanhados de níveis normais a baixos de concentrações de colesterol HDL (hiperlipoproteinemia tipo IIb). A morte prematura deve-se à insuficiência hepática e/ou à doença aterosclerótica acelerada, conseqüente à hiperlipidemia crónica [14,15].

Existe um espectro clínico para a DDEC com alguns doentes a serem diagnosticados na infância, ao passo que outros continuam sem diagnóstico até à idade adulta. Os doentes gravemente afetados podem apresentar na primeira infância manifestações da doença de Wolman tais como a diarreia, a má evolução ponderal, a êmese, a distensão abdominal e até calcificações suprarrenais, mas sobrevivem até à infância ou até à idade adulta. Os doentes apresentam-se usualmente com hepatomegalia, disfunção hepática ou com hiperlipoproteinemia tipo IIb. A hepatomegalia conduz usualmente a uma biópsia hepática que se apresenta visivelmente num amarelo-alaranjado vivo, e é histologicamente marcada por hepatócitos aumentados carregados de lípidos e de células de Kupffer, sendo assim caracterizada pela esteatose microvesicular (Fig. 1A e B) [4,16–18]. O resultado da biópsia hepática pode ser equivocadamente classificado como doença hepática gordurosa não alcoólica (DHGNA), como esteatohepatite não alcoólica (EHNA), ou como doença hepática criptogénica. O depósito progressivo de lípidos leva à fibrose, à cirrose micronodular e, por fim, à insuficiência hepática [4]. A elevação das transaminases séricas, alanina aminotransferase (ALT) e/ou aspartato aminotransferase (AST) juntamente com a hepatomegalia constituem indicadores precoces do compromisso hepático.

O defeito enzimático da LAL traduz-se na redução da hidrólise dos ésteres de colesterol e triglicéridos e na sua retenção massiva, particularmente nos lisossomas das células de Kupffer e hepatócitos, bem como noutras células do sistema monocítico-macrofágico.



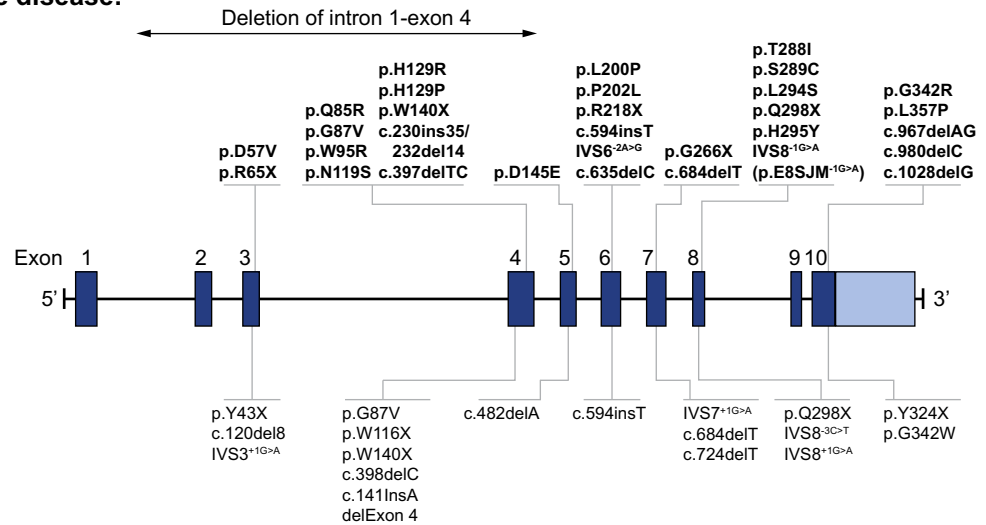
**Fig. 1. Histopatologia hepática e observações ultraestruturais na DDEC.** (A e B) Coloração de secções de parafina com HE que mostram esteatose microvesicular uniforme, tanto numa fase inicial (A), como numa fase mais avançada (B) da DDEC. Note o número de macrófagos espumosos a infiltrar os septos fibrosos dividindo o parênquima hepático em B. A bar em A corresponde a 50 µm e em B corresponde a 100 µm. (C e D) A imunocoloração em secções de parafina tanto para os marcadores lisossomais membranosos (LAMP2) como para os do lúmen (catepsina D), confirma a natureza lisossomal dos vacúolos lipídicos nos hepatócitos na DDEC. (C) Indicador para LAMP2 mostra de forma uniforme a expansão e a ativação do sistema lisossomal nos hepatócitos e nos macrófagos. A LAMP 2 encontra-se em contato próximo com gotículas lipídicas circundando claramente os vacúolos maiores. (D) Foram obtidos resultados comparáveis com o anticorpo contra a catepsina D. A bar corresponde a 25 µm. (E) Imunocoloração da catepsina D na esteatose hepática primária não lisossomal (deficiência da β-oxidação). O indicador para a catepsina D é discreto e restrito à região perilípica, deixando livres os vacúolos lipídicos citosólicos. A bar corresponde a 25 µm. (F) Eletromicrografia que mostra os vacúolos lipídicos ligados à membrana e os cristais de EC em forma de agulha no citoplasma dos hepatócitos de uma criança de 9 anos do sexo feminino com DDEC. Ampliação de 10 000x.

A diminuição do colesterol livre devida à retenção lisossomal dos ésteres de colesterol leva à redução da inibição por feedback da 3-hidroxi-3 metilglutaril coenzima A redutase (HMG-CoA redutase) e ao aumento da síntese do colesterol, bem como ao aumento da síntese da apolipoproteína B (ApoB) e dos recetores LDL nas membranas celulares [19–23].

A expressão desregulada do gene ABCA 1 (*ATP binding cassette transporter 1*) dependente do colesterol LDL, contribui para a redução do colesterol HDL à semelhança do que ocorre na doença de Niemann-Pick tipo C1 [24]. Estas alterações metabólicas conduzem ao aumento dos níveis séricos totais de colesterol LDL e de triglicéridos e à redução dos níveis séricos de colesterol HDL e levam ao diagnóstico da dislipidemia tipo IIb [25]. O aumento das concentrações do colesterol LDL provocam aterosclerose acelerada, sendo que existem registos de doentes com DDEC com aterosclerose precoce, isquemia, AVCs e alvo de cirurgia de bypass coronário [13,15,26–29]. Foram isolados e caracterizados o cDNA e a sequência genómica do gene LIPA [30–33]. O gene tem uma dimensão aproximada de ~36 kb, contém 10 exões, está localizado no cromossoma 10q23.31 e codifica um mRNA de ~2.6 kb [31,33,34]. A enzima lisossomal madura possui 399 aminoácidos. Apesar da enzima humana não ter sido cristalizada a sua estrutura tridimensional foi prevista com base na homologia com a lipase gástrica humana [35]. Até à data foram identificadas mais de 40 mutações no gene LIPA que causam a DDEC e a DW [36] (Fig. 2).

## Review

### Cholesteryl ester storage disease:



### Wolman disease:

**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<sup>-1G>A</sup>; hereafter designated E8SJM<sup>-1G>A</sup>; rs 116928232) has been identified in patients of European ancestry [2,37,38] (Fig. 2). Population screening for E8SJM<sup>-1G>A</sup> among healthy West German individuals indicated a heterozygote frequency of 1 in ~200 individuals. Since E8SJM<sup>-1G>A</sup> 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.

### Literature search and patient demographics

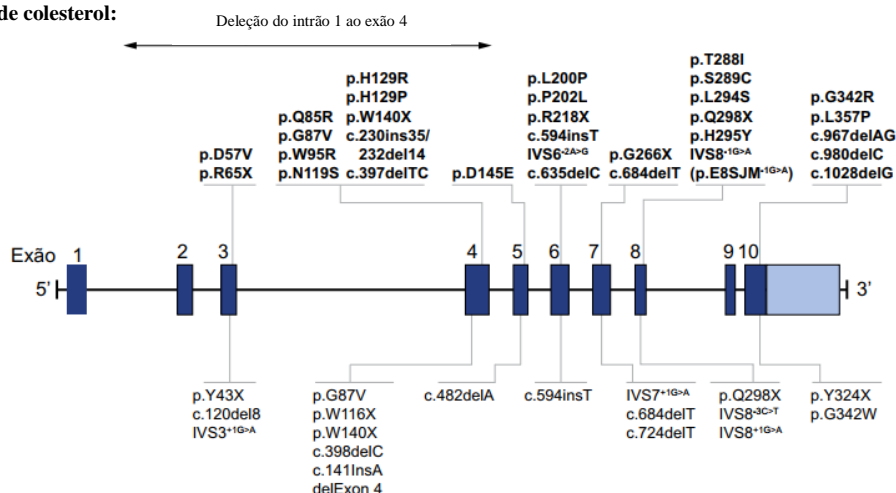
#### Literature search and data collection

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.

**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)

**Doença de depósitos de ésteres de colesterol:**



**Doença de Wolman:**

**Fig.2. Mutações no gene LIPA em doentes com DDEC ou com DW.** Observe que toda a nomenclatura referente às mutações é baseada no cDNA considerando o A da sequência de iniciação ATG como nucleótido 1. Consequentemente, muitas das mutações descritas anteriormente foram redesignadas adicionando os 21 aminoácidos na sequência líder (por exemplo, H108P para H129P). As mutações da DDEC a negrito são as observadas nos doentes aqui descritos.

A DDEC é pan-étnica; contudo a incidência da doença é desconhecida. A estimativa de prevalência nos europeus foi baseada no facto de que a mutação recorrente no exão 8 na região de junção intrão-exão, geralmente designada como E8SJM (c.894 G>A; p.delS 275\_Q298; IVS 8<sup>1G>A</sup>), doravante designada como E8SJM<sup>1G>A</sup>; rs 116928232) ter sido identificada em doentes com ascendência europeia [2,37,38] (Fig. 2). O rastreio populacional da E8SJM<sup>1G>A</sup> entre indivíduos saudáveis da Alemanha ocidental indicou uma frequência em heterozigotia de 1 em ~200 indivíduos. Assim, dado que a E8SJM<sup>1G>A</sup> é responsável por cerca de 50-60% de todas as mutações da DDEC, a frequência em heterozigotia prevista para todas as mutações no gene LIPA foi estimada em 1-100, indicando uma incidência da doença de 1 em ~40 000 [39,40]. Ainda assim a DDEC pode encontrar-se altamente subdiagnosticada particularmente em doentes cuja ascendência seja europeia. Os esforços terapêuticos têm incluído a colestiramina e as estatinas para a redução do colesterol e da síntese da ApoB e o transplante hepático para a insuficiência hepática. Estudos pré-clínicos de substituição enzimática com LAL recombinante em células de fibroblastos humanos e em modelos murinos demonstraram a "prova de conceito" para a terapia de substituição enzimática (TSE) [41- 44]. Recentemente, um ensaio clínico de fase I/II da TSE para a DDEC demonstrou a sua segurança e forneceu evidências que apoiam os seus efeitos metabólicos [45], estimulando desta forma o interesse por esta doença sub-reconhecida. Foram descritos até à data 135 doentes com DDEC na literatura, maioritariamente em estudos de casos individuais ou de pequenas séries de doentes diagnosticados bioquimicamente, histologicamente e/ou geneticamente. Destas séries as maiores incluem apenas sete e treze doentes [46,47]. Neste sentido foram revistos os resultados clínicos, patológicos e genético-moleculares, bem como as observações referentes à história natural e às correlações genótipo-fenótipo presentes em 97 estudos de casos publicados, com o objetivo de alertar hepatologistas, patologistas e lipidologistas sobre esta doença, o seu diagnóstico e o seu tratamento atual e futuro.

**Pesquisa bibliográfica e dados demográficos dos doentes**

Foi realizada uma pesquisa bibliográfica no site do PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>) de modo a identificar todas as publicações que descrevessem os doentes com DDEC desde a descrição inicial [9]. Os doentes diagnosticados com DW que faleceram no primeiro ano de vida foram excluídos.

**Tabela 1. Características observadas nos doentes com DDEC.**

Todos os doentes relatados, n (%):	135 (100)	
Sexo masculino	56 (45)	
Etnia/origem, n (%)		
Caucasiana/Europa	85 (65)	
Caucasiana/ América do Norte	23 (17)	
Caucasiana/ América Latina	14 (10)	
Caucasiana/ Médio Oriente	4 (3)	
Asiática	4 (3)	
Africana	1 (0.3)	
Idade no início e último relatório, anos:	Início	Último relatório
Sexo masculino (n = 56)		
Média	6	17.7
Mediana	5	13
Intervalo	<1-44	1-52
Sexo feminino (n = 68)		
Média	4	18.49
Mediana	5	13
Intervalo	<1-68	2.2-82
Sexo desconhecido (n = 11)		
Média	5.5	12.95
Mediana	5.6	12.2
Intervalo	0.1-13	3.3-20.8
Distribuição, n (%)	131 (97)	130 (96)
<2 anos	25 (19)	2 (<1)
≥2-5 anos	51 (39)	23 (18)
6-12 anos	33 (25)	32 (25)
13-20 anos	8 (6)	32 (25)
21-40 anos	8 (6)	28 (22)
41-58 anos	5 (4)	12 (9)
>60 anos	1 (<1)	1 (<1)

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].

#### 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].

**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)
<i>LIPA</i> mutation detection:	
Patients <i>LIPA</i> genotyped, n (%)	55 (41)
Total mutant alleles detected	106
Number different mutations	31
E8SJM <sup>-1G&gt;A</sup> alleles, n (%)	65 (61)
E8SJM <sup>-1G&gt;A</sup> 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.

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<sup>-1G>A</sup> 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,



Foram analisados oitenta e dois artigos revistos por pares em inglês e 15 artigos em língua estrangeira.

Dos 135 doentes, encontravam-se disponíveis os dados longitudinais para 99, os quais foram acompanhados durante dois a >30 anos desde o seu diagnóstico inicial ou desde o início dos sintomas, incluindo-se aqui também 21 doentes descritos em estudos posteriores, sendo assim fornecidas informações relativas à história natural que pudessem ser correlacionadas, quando disponíveis, com os genótipos do LIPA dos doentes. Foram registadas, quando relatadas, as seguintes informações para cada doente: manifestações clínicas, idade de início da doença e última avaliação, presença e grau da hepatomegalia e/ou da esplenomegalia, envolvimento de outros sistemas de órgãos, patologia hepática, atividade da LAL, mutações no gene LIPA, colesterol sérico total, colesterol LDL e HDL, níveis dos triglicéridos, atividade da AST e ALT séricas e tratamentos que incluam a colestiramina/sequestrantes dos ácidos biliares, as estatinas e o transplante hepático acompanhadas da idade e causa da morte.

*Dados demográficos dos doentes*

A tabela 1 fornece uma síntese da caracterização demográfica e inclui o sexo, a etnia, o local de origem e as idades no início da doença e no último relatório. Os doentes eram europeus (65%), norte-americanos (17%), latino-americanos (10%), asiáticos provenientes da Índia ou da Tailândia (3%) e médio-orientais (3%). Surgiram estudos de casos individuais de um doente australiano e de um afro-americano [48,49].

**Manifestações clínicas**

*Idade no início e apresentação clínica*

Foram registadas informações clínicas sobre todos os doentes, embora com graus de detalhe variáveis. A idade referente ao início dos sintomas e/ou diagnóstico foi registada para 131 (97%) doentes (Tabela 1). A idade mediana de início da doença para 56 (45%) homens e para 68 (55%) mulheres foi aos cinco anos de idade para ambos os sexos, variando do nascimento aos 44 anos e de um mês aos 68 anos, respetivamente. Onze estudos de casos não especificaram o sexo. Destes 131 doentes, a idade de início para 35 (27%) das crianças gravemente afetadas situou-se entre o nascimento e os dois anos, ao passo que para 81 (62%) dos doentes esta situou-se entre os três anos de idade e os 12, sendo que para 15 (11%) dos doentes a doença surgiu ou diagnosticou-se durante a adolescência ou já na idade adulta. O diagnóstico foi realizado em cinco doentes na autópsia [5,50–52]. Clinicamente, a hepatomegalia esteve presente em 134 (99,3%) dos doentes e 74% também apresentaram esplenomegalia. Um homem de 27 anos ligeiramente afetado não apresentou hepatomegalia, apesar de possuir patologia hepática e transaminases elevadas [53]. Os doentes apresentaram geralmente hepatomegalia ou hepatoesplenomegalia nos exames físicos ou de imagem quando avaliados inicialmente devido à atividade das transaminases séricas elevadas ou febre, a infeções respiratórias e/ou devido a outros sintomas [54, 55]. A tabela 2 fornece uma síntese dos métodos de diagnóstico que incluem a biópsia hepática, a atividade da LAL e as mutações no gene LIPA em doentes genotipados, bem como inclui a atividade das transaminases séricas e os níveis lipídicos, quando registados. Atividades elevadas da AST ou ALT estavam presentes em todos os casos em que o doseamento das atividades das transaminases séricas foi reportado com flutuações significativas em diferentes pontos temporais (Tabela 2). Os perfis lipídicos não foram usualmente registados para muitos dos doentes pediátricos, ao passo que os adultos apresentavam frequentemente hiperlipidemia, que apresentava respostas variáveis aos tratamentos com estatinas ou com colestiramina. Os irmãos de indivíduos afetados foram diagnosticados quando inicialmente sintomáticos ou ainda pré-sintomáticos mediante ensaios à atividade da LAL ou por análise das mutações no gene LIPA [26,47,56–58].

**Tabela 2. Método de confirmação do diagnóstico, atividades das transaminases séricas e níveis lipídicos nos doentes com DDEC.**

Confirmação diagnóstica, n (%):	
Biópsia hepática	112 (83)
Cristais patognomônicos/fendas	21 (16)
Atividade patológica da LAL	114 (84)
Deteção da mutação no gene LIPA:	
Doentes genotipados, n (%)	55 (41)
Total de alelos mutantes detetados	106
Número de mutações diferentes	31
E8SJIM <sup>-1G&gt;A</sup> alelos, n (%)	65 (61)
E8SJIM <sup>-1G&gt;A</sup> homozigóticos	17
Atividades das transaminases séricas elevadas:	
AST, n	78
n em UI/L (intervalo)	54 (9-5240)
ALT, n	73
n em UI/L (intervalo)	52 (15-2340)
Lípidos séricos em mg/dl, n (intervalo):	
Colesterol total	110 (104-620)
Colesterol LDL	43 (119-360)
Colesterol HDL	65 (8-50)
Triglicéridos	96 (69-425)

\*Dados retirados da literatura sobre doentes em que foram registados os métodos de diagnóstico e os níveis das transaminases séricas e de lípidos.

Regra geral as crianças mais gravemente afetadas foram também as mais facilmente diagnosticadas comparativamente com os doentes com DDEC, que apresentavam uma progressão lenta da doença hepática e diferentes níveis de lípidos séricos [13,53,59]. Dado que se trata de um diagnóstico exigente é provável que muitos adultos com DDEC permaneçam sem diagnóstico ou se encontrem incorretamente diagnosticados como tendo DHGNA, EHNA ou doença hepática criptogénica [4,26,60].

*Colesterol sérico e níveis dos triglicéridos (tabela 2)*

O colesterol total encontrava-se elevado em todos os 110 doentes para os quais havia registo, embora 26% estivessem a ser tratados com inibidores da HMG-CoA redutase. Dos 43 doentes para os quais haviam registos do colesterol LDL sérico, 79% apresentam níveis elevados (>200 mg/L) e mais de 95% apresentam níveis acima do normal, incluindo 49% que estavam a ser tratados com inibidores da HMG-CoA redutase. Já no caso dos 65 doentes para os quais existiam registos dos níveis de colesterol HDL, estes oscilavam entre os 8 a 50 mg/dl, sendo que 71% apresentavam níveis de colesterol HDL entre os 20 e os 40 mg/dl e 18% possuíam níveis abaixo dos 20 mg/dl, ainda que 11% tivessem níveis de >40 mg/dl. Curiosamente, foram descritas pelo menos 10 famílias em que parentes em primeiro grau dos probandos (heterozigóticos obrigatórios), assim como os heterozigóticos detetados através do *screening* da mutação E8SJIM<sup>-1G>A</sup>, possuíam níveis elevados de colesterol total sérico [12,13,49,61–71,131]. Surgiram vários estudos que documentaram a presença de doença arterial coronária ou de aterosclerose nos pais dos doentes, contudo, foram poucos os heterozigóticos que conheceram a doença hepática concomitante [49,67].

*Outras manifestações*

As observações extra-hepáticas mais comuns incluíram a diarreia frequente, a dor abdominal e epigástrica, a êmese, a anemia, a má absorção, a colestase, a esteatorreia, o crescimento deficiente, a disfunção da vesícula biliar e a doença cardiovascular [72–76,125].

## Review

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.

## Revisão

Os doentes com cirrose micronodular apresentam hipertensão portal, ascite, caquexia, varizes esofágicas, hemorragia gastrointestinal e outras complicações secundárias ligadas à insuficiência hepática que resultaram em coma, morte ou em transplante hepático [51, 77]. As varizes esofágicas foram observadas em 12 doentes, incluindo nove doentes, dos cinco aos 20 anos [26, 46, 50, 78–84]. Existiram dois casos de carcinoma hepatocelular que foram observados aos 11 e aos 52 anos [28, 85] sendo que também foram observadas calcificações suprarrenais que ocorrem em ~50% dos doentes com DW e que se encontravam presentes em nove doentes com DDEC com idades compreendidas dos <1 aos 10 anos. Como tal, a calcificação das suprarrenais pode ocorrer em qualquer um dos fenótipos [52, 64, 78, 84, 86–92]. A acumulação gastrointestinal de lípidos e de EC foi uma observação comum nas vilosidades da lâmina própria incluindo nas células endoteliais dos vasos lacteais, no músculo liso, nos pericitos vasculares e no duodeno e na mucosa intestinal onde macrófagos espumosos estavam presentes (e.g., [51, 93–96]). As manifestações cardiovasculares envolveram predominantemente a doença arterial coronária, o aneurisma e o AVC [6, 13, 22, 26, 29].

### *Insuficiência hepática e causas de morte*

A disfunção hepática e/ou insuficiência verificou-se em todos os 135 doentes. Dos 11 óbitos relatados a maioria (73%) foi devida à insuficiência hepática, sendo que a progressão para as varizes esofágicas foi relatada em 12 casos. Dos 112 (83%) doentes submetidos à biópsia hepática, todos apresentaram descobertas importantes que se encontram descritas abaixo. A morte devida à progressão da doença hepática ocorreu dos sete aos 56 anos e 50% dos óbitos são referentes a doentes com menos de 21 anos [5,50,52,78]. Existe apenas um estudo de uma doente que sobreviveu para lá dos 58 anos [13], contudo informações relativas ao acompanhamento a longo prazo não se encontravam disponíveis para a maioria dos doentes. Adicionalmente registaram-se mais quatro óbitos por insuficiência hepática após os respetivos estudos de casos [26,57]; (comunicação pessoal Dr. C. Gashe, Viena, Áustria; Dra. V. McIn, Genebra, Suíça; (Tabela 3: doentes 35, 36, 50 e 54).

### **Patologia hepática**

A acumulação massiva de EC e triglicéridos conduz à esteatose microvesicular difusa que envolve os hepatócitos, as células de Kupffer e os macrófagos (Fig. 1 A e B), progredindo para a fibrose e derradeiramente para a cirrose micronodular [4,58,91,97]. Os resultados das biópsias hepáticas foram constantes entre os doentes independentemente da idade, do genótipo e de outros fatores. No exame macroscópico os espécimes de fígado mostram uma acentuada aparência amarelo-alaranjada. Já na microscopia de luz (Fig. 1), a observação geral foi a esteatose microvesicular difusa e uniforme com diferenças mínimas nas zonas do lóbulo hepático [4]. Encontravam-se já presentes macrófagos espumosos contendo lípidos e cerídeos nos sinusóides e nos tratos portais de doentes jovens. Tendo sido observado em fases mais avançadas um aumento do número de macrófagos de armazenamento e uma progressão para a fibrose. Contrariamente aos macrófagos, a acumulação de cerídeos não acompanhou a acumulação lisossomal de lípidos nos hepatócitos [4]. A esteatose microvesicular não é comum entre as outras causas metabólicas de doença hepática, apesar de terem sido relatados casos raros induzidos pela hepatotoxicidade mediada pela inibição grave da β-oxidação mitocondrial, por exemplo, quando associada ao uso de ácido valproico ou à síndrome de Reye. A síndrome de Reye apresenta um aumento mitocondrial característico que pode ser visualizado ultraestruturalmente [98–104].

Recentemente, Hulkova e Elleder reavaliaram uma série de 19 biópsias hepáticas de doentes com DDEC de modo a serem identificados critérios histopatológicos fiáveis que distinguissem a DDEC de outras formas de esteatose microvesicular, particularmente, da DHGNA e de outras doenças hepáticas gordurosas não lisossomais em crianças [4]. Os mesmos constataram que com a imunocoloração para a LAMP1, LAMP2 e LIMP2 ou com uma proteína do lúmen lisossomal (catepsina D), que foi fácil identificar a acumulação lipídica como sendo lisossomal, facilitando assim o diagnóstico da DDEC, anulando-se a necessidade de uma demonstração ultraestrutural da acumulação lisossomal de lípidos (Fig. 1C–E). Os patologistas devem assim ter em conta os seus métodos específicos, os seus fornecedores comerciais de anticorpos e as características histopatológicas únicas da DDEC [4]. Posto isto, a coloração adicional de uma secção de parafina identificará doentes para procederem à confirmação mediante a análise da atividade enzimática da LAL e/ou sequenciação genética do LIPA.

Os cristais birrefringentes de EC patognomónicos foram observados nos hepatócitos e/ou nas células de Kupffer em tecidos frescos e congelados sob luz polarizada. Foram observadas nas secções fixas embebidas em parafina, as fendas remanescentes onde o lípido foi extraído durante procedimentos de desidratação. Estes cristais ou as suas fendas remanescentes quando observados pelo microscópio eletrónico encontravam-se delimitadas por uma única membrana lisossomal ou livres no citoplasma. Embora apenas 65 das 112 biópsias tenham descrito especificamente os cristais birrefringentes de EC em forma de agulha, 26 (23%) dos casos adicionais relataram a acumulação de EC nos hepatócitos e dois relataram a presença de fendas remanescentes de EC, sendo assim contabilizados 93 casos dos 112 (ou 83%), não obstante de que presumivelmente estes cristais de EC estariam presentes em todos os casos se os espécimes estivessem congelados e fossem vistos através da microscopia polarizada ou processados para a microscopia eletrónica.

Dos 112 doentes submetidos à biópsia, 72 (64%) apresentam fibrose e/ou cirrose, incluindo-se a fibrose sinusoidal, portal/periportal ou septal apresentada em 56 doentes (50%), já a cirrose esteve presente em 33 doentes (29%). Adicionalmente, foram observados 17 doentes (15%) que apresentam fibrose e cirrose em biópsias iniciais ou posteriores [105–107]. Alguns estudos de casos não fizeram a distinção entre a fibrose e a cirrose [58,79,84]. A necrose dos hepatócitos foi observada em oito (7%) dos doentes.

Igualmente de interesse, a esteatose microvesicular e a acumulação de EC encontrava-se já presente num feto diagnosticado no período pré-natal às 17 semanas de gestação [89]. O feto afetado foi detetado numa família cujo filho anterior havia sido diagnosticado aos dois anos. O fígado fetal apresentava uma acumulação significativa de EC ligados à membrana nos hepatócitos e nos sincitiotrofoblastos das vilosidades coriônicas, bem como infiltração de colesterol e necrose das suprarrenais aumentadas. A fibrose portal e periportal foi observada na biópsia hepática do irmão afetado de dois anos, mas não no feto, o que sugere a natureza progressiva da DDEC quando associada à doença hepática [89].

### **Diagnóstico**

Os diagnósticos de todos os 135 doentes relatados foram baseados na deficiência da LAL e/ou nas mutações do gene LIPA, ou mediante os resultados patológicos da biópsia hepática. A notória deficiência na atividade da LAL foi verificada em 114 (84%) dos doentes dos quais 55 (42%) também foram submetidos a análises de mutações no gene LIPA (Fig. 2). Os restantes 21 (16%) dos doentes foram diagnosticados pela presença de cristais de EC patognomónicos, ou pelas fendas remanescentes presentes nas suas biópsias hepáticas. Um feto afetado com DDEC foi diagnosticado no período pré-natal ao demonstrar deficiência da LAL em células cultivadas de líquido amniótico [89]. O diagnóstico pré-natal também pode ser realizado fiavelmente em vilosidades coriônicas cultivadas através da análise da atividade enzimática da LAL ou pela análise de mutações.

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

Patient	LIPA genotype HGMD	LIPA genotype (as reported <sup>1</sup> )	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]

Tabella 3. Dados clínicos, patológicos e laboratoriais referentes aos doentes com DDEC genotipados.

Doente	LIPA genótipo HGMD	Genótipo LIPA (como relatado <sup>1</sup> )	Sexo	Idade aparecimento	Idade último relatório/morte (causa)	Manifestações clínicas	Patologia fígado/vicido	Resultados laboratoriais	[Ref.]
1	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	M	<5	22	I	MVS, BRC	Ilb	[131]
2	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	M	13	23	SM, GF	PBF, BRC, HV	Ilb, ET	[38, 116]
3	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	n.r.	3	3	D (fam hx MI 30s + 40s)	MVS, BMV	Ilb, ET	[90]
4	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	F	8	13	HT	NB, HS on U/S	ET, HC	[55, 113]
5	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	F	<5	41	D, caquexia, má absorção	MVS, F, BRC, FC, HL, BP	ET	[75]
6	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	F	0.16	5	FTT, D, AD, SM,	PI, HV, KV, F	ET, HC, HTG	[122]
7	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	M	6	9	SM	PI, HV, KV, F	ET, HC	[122]
8	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup> (p.S275_Q298del) + c.894G>A	F	4	10	SM	Esteatose, PI, F, FC	Ilb, ET	[113]
9	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup> (p.S275_Q298del) + c.894G>A	M	n.r.	22	SM	F, FC	Ilb, ET	[113]
10	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup> (p.S275_Q298del) + c.894G>A	F	3	3	n.r.	NB	Ilb, ET	[113]
11	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	M	0.8	15	SM	NB	Ilb, ET	[117]
12	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	M	2	18	SM	NB	Ilb, ET	[117]
13	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	F	2	36	SM, ML, I, MLD, ATH, AFIB, AVC, GU, ECC, X, CA, FC	HS em U/S, FC, BMV	Ilb, A, ALF	[29]
14	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup> c.934G>A/ c.934G>A	F	6	24	SM	DH	Ilb	[47]
15	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup> c.934G>A/ c.934G>A	F	6	24	HTN	DH	Ilb	[47]
16	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup> c.934G>A/ c.934G>A	F	4	22	HTN	DH	Ilb	[47]
17	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup> c.934G>A/ c.934G>A	F	5	16	SM, Iarngite	DH	Ilb	[47]
18	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup>	E8SJIM <sup>102</sup> /E8SJIM <sup>102</sup> c.934G>A/ c.934G>A	F	53	m. 58 (MI)	AP, MI	DH, BMV	HC	[47]
Inferido de genótipos infantis									
19	E8SJIM <sup>102</sup> /c.594insT	E8SJIM <sup>102</sup> /fsA176X190	M	3	18	GI	HV, G, MBL, F,	Ilb	[119]
20	E8SJIM/c.980delC	E8SJIM <sup>102</sup> /fs→330X	M	28	28	SM	NB	n.r.	[90]
21	E8SJIM/c.397-398delTC	E8SJIM <sup>102</sup> /fs→137X	M	11	22	SM	NB	n.r.	[90]
22	E8SJIM <sup>102</sup> /c.230ins35/ c.245del114	E8SJIM <sup>102</sup> /c.G77fsX82	F	68	m. 82	HF, CA, SM	NB	n.r.	[13]
23	E8SJIM <sup>102</sup> /c.635delIC	E8SJIM <sup>102</sup> /fs→195X (934G>A)/DC673-5	M	33	m. 52 (insuficiência hepática) GA	CA, EA, ATH, aneurismas, GA	BRC, C	Ilb	[28, 47]
24	E8SJIM <sup>102</sup> /R65X	E8SJIM <sup>102</sup> /c.C233T R44→fsX (Exão3)	F	11	34	SM, MH	DH	Ilb	[47]
25	E8SJIM <sup>102</sup> /R65X	E8SJIM <sup>102</sup> /c.C233T R44→fsX (Exão3)	M	37	49	AP	BRC, F, FC, HV, DU	Ilb, ET	[95, 96]

Revisão

**Table 3. (continued)**

Patient	LIPA genotype HGMD	LIPA genotype (as reported <sup>1</sup> )	Sex	Age at onset, yr	Age at last report/death, yr (cause)	Clinical manifestations	Liver/tissue pathology	Lab findings	[Ref.]
26	E8SJIM <sup>-1(G&gt;A)</sup> /c.967delAG	E8SJIM <sup>-1(G&gt;A)</sup> /S323L>fs>X366 (c.967delAG)	M	0.8	38	AP, SM	HV	ET	[38]
27	E8SJIM <sup>-1(G&gt;A)</sup> /W95R	E8SJIM <sup>-1(G&gt;A)</sup> /W74R (c.323T>A)	M	2	30	CF	DH	Ilb	[11, 47]
28	E8SJIM <sup>-1(G&gt;A)</sup> /D145E	E8SJIM <sup>-1(G&gt;A)</sup> /475T>A	M	3	28	n.r.	DH	Ilb	[47]
29	E8SJIM <sup>-1(G&gt;A)</sup> /D145E	E8SJIM <sup>-1(G&gt;A)</sup> /475T>A	M	14	36	n.r.	DH	Ilb	[47]
30	E8SJIM <sup>-1(G&gt;A)</sup> /H295Y	E8SJIM <sup>-1(G&gt;A)</sup> /H295Y (p.S275_Q298del) + [c.883C>T]	F	19	19	n.r.	HS on U/S	Ilb, ET	[113]
31	E8SJIM <sup>-1(G&gt;A)</sup> /H295Y	E8SJIM <sup>-1(G&gt;A)</sup> /H295Y (p.S275_Q298del) + [c.883C>T (p.H295Y)]	M	4	n.r.	n.r.	HS on U/S	Ilb	[113]
32	E8SJIM <sup>-1(G&gt;A)</sup> /L200P	E8SJIM <sup>-1(G&gt;A)</sup> /L179P	F	6	n.r.	n.r.	NB	Ilb	[132]
33	E8SJIM <sup>-1(G&gt;A)</sup> /L200P	E8SJIM <sup>-1(G&gt;A)</sup> /L179P	M	8	n.r.	n.r.	NB	Ilb	[132]
34	E8SJIM <sup>-1(G&gt;A)</sup> /L200P; G266X	E8SJIM <sup>-1(G&gt;A)</sup> /L179P;G245FsX	M	5	12	SM	MVS, BRC	Ilb	[8, 37]
35	E8SJIM <sup>-1(G&gt;A)</sup> /H129P	E8SJIM <sup>-1(G&gt;A)</sup> /H108P	F	<52	58 (d. age unk, pers. commun.)	ATH, CAD	MVS, FC, BRC, C	n.r.	[26, 57]
36	E8SJIM <sup>-1(G&gt;A)</sup> /H129P	E8SJIM <sup>-1(G&gt;A)</sup> /H108P	M	44	46 (liver transplant, age unk, pers. commun.)	Lipomas, ET, HC	F, FC, MVS	n.r.	[26, 57]
37	E8SJIM <sup>-1(G&gt;A)</sup> /H129P	E8SJIM <sup>-1(G&gt;A)</sup> /H108P	F	32	d. 56	EV	FC, MVS, C	Ilb	[26, 57]
38	E8SJIM <sup>-1(G&gt;A)</sup> /L357P	E8SJIM <sup>-1(G&gt;A)</sup> /L336P	F	<14	19	SM	NB	Ilb, ET	[12]
39	E8SJIM <sup>-1(G&gt;A)</sup> /D57V	E8SJIM (934G>A)/210A>T	M	11	24	n.r.	DH	Ilb, ET	[47]
40	E8SJIM <sup>-1(G&gt;A)</sup> /G342R	E8SJIM <sup>-1(G&gt;A)</sup> /G342R	M	9	9	HS on U/S	NB	ALF, Ilb	[113]
41	E8SJIM <sup>-1(G&gt;A)</sup> /N119S	E8SJIM <sup>-1(G&gt;A)</sup> /N98S	F	26	26	AP	MVS, FC, PI	ALF, Ilb	[49]
42	E8SJIM <sup>-1(G&gt;A)</sup> /W140X	E8SJIM <sup>-1(G&gt;A)</sup> /W140X c.894G>A (p.S275_Q298del) + [c.419G>A (p.W140*)]	M	7	7	n.r.	MVS	Ilb	[113]
43	E8SJIM <sup>-1(G&gt;A)</sup> /c.1028delG	E8SJIM <sup>-1(G&gt;A)</sup> /c.DG1064-8	M	0.1	24	AD (birth)	DH,	ALF, Ilb	[47]
44	E8SJIM <sup>-1(G&gt;A)</sup> /G87V	E8SJIM <sup>-1(G&gt;A)</sup> /G66V	M	6	21	SM	HV	Ilb, HTG	[124]
45	E8SJIM <sup>-1(G&gt;A)</sup> /Q85R	E8SJIM <sup>-1(G&gt;A)</sup> /Q64R	F	6	11	n.r.	F, FC	ET	[88]
46	E8SJIM <sup>-1(G&gt;A)</sup> /R218X	R218X/del S275_Q298.c.652 C>T/c.894 G>A, del c.823_894	F	9	18	n.r.	NB	Ilb, ET	[13]
47	E8SJIM <sup>-1(G&gt;A)</sup> /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	Ilb, ET	[13]
48	E8SJIM <sup>-1(G&gt;A)</sup> /UNK	E8SJIM <sup>-1(G&gt;A)</sup> /UNK	F	51	51	2 sibs with CESD	NB	Ilb	[90]
49	E8SJIM <sup>-1(G&gt;A)</sup> /UNK	E8SJIM <sup>-1(G&gt;A)</sup> /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]

Tabela 3. (continuação)

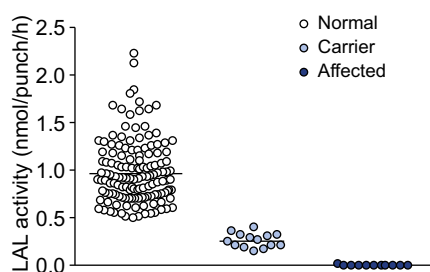
Doente	LIP A genótipo HGMD	Genótipo LIP A (como relatado <sup>1</sup> )	Sexo	Idade aparecimento	Idade último relatório/morte (causa)	Manifestações clínicas	Patologia fígado/veículo	Resultados laboratoriais	[Ref.]
26	E8SJM <sup>+</sup> o <sup>+</sup> /c.967delAG	E8SJM <sup>+</sup> o <sup>+</sup> /S323L>f>X366 (c.967delAG)	M	0.8	38	AP, SM	HV	ET	[38]
27	E8SJM <sup>+</sup> o <sup>+</sup> /W95R	E8SJM <sup>+</sup> o <sup>+</sup> /W74R (c.323T>A)	M	2	30	CF	DH	lb	[11, 47]
28	E8SJM <sup>+</sup> o <sup>+</sup> /D145E	E8SJM <sup>+</sup> o <sup>+</sup> /475T>A	M	3	28	n.r.	DH	lb	[47]
29	E8SJM <sup>+</sup> o <sup>+</sup> /D145E	E8SJM <sup>+</sup> o <sup>+</sup> /475T>A	M	14	36	n.r.	DH	lb	[47]
30	E8SJM <sup>+</sup> o <sup>+</sup> /H295Y	E8SJM <sup>+</sup> o <sup>+</sup> /H295Y (p.S275_Q298del) + [c.883C>T]	F	19	19	n.r.	HS em U/S	lb, ET	[113]
31	E8SJM <sup>+</sup> o <sup>+</sup> /H295Y	E8SJM <sup>+</sup> o <sup>+</sup> /H295Y (p.S275_Q298del) + [c.883C>T (p.H295Y)]	M	4	n.r.	n.r.	HS em U/S	lb	[113]
32	E8SJM <sup>+</sup> o <sup>+</sup> /L200P	E8SJM <sup>+</sup> o <sup>+</sup> /L179P	F	6	n.r.	n.r.	NB	lb	[132]
33	E8SJM <sup>+</sup> o <sup>+</sup> /L200P	E8SJM <sup>+</sup> o <sup>+</sup> /L179P	M	8	n.r.	n.r.	NB	lb	[132]
34	E8SJM <sup>+</sup> o <sup>+</sup> /L200P; G266X	E8SJM <sup>+</sup> o <sup>+</sup> /L179P;G245F>X	M	5	12	SM	MVS, BRC	lb	[β, 37]
35	E8SJM <sup>+</sup> o <sup>+</sup> /H129P	E8SJM <sup>+</sup> o <sup>+</sup> /H108P	F	<52	58 (m. id. desc., comu. pess.)	ATH, CAD	MVS, FC, BRC, C	n.r.	[26, 57]
36	E8SJM <sup>+</sup> o <sup>+</sup> /H129P	E8SJM <sup>+</sup> o <sup>+</sup> /H108P	M	44	46 (transplante hepático, id. desc., comu. pess.)	Lipomas, ET, HC	F, FC, MVS	n.r.	[26, 57]
37	E8SJM <sup>+</sup> o <sup>+</sup> /H129P	E8SJM <sup>+</sup> o <sup>+</sup> /H108P	F	32	m. 56	EV	FC, MVS, C	lb	[26, 57]
38	E8SJM <sup>+</sup> o <sup>+</sup> /L357P	E8SJM <sup>+</sup> o <sup>+</sup> /L336P	F	<14	19	SM	NB	lb	[12]
39	E8SJM <sup>+</sup> o <sup>+</sup> /D57V	E8SJM (934G>A)210A>T	M	11	24	n.r.	DH	lb, ET	[47]
40	E8SJM <sup>+</sup> o <sup>+</sup> /G342R	E8SJM <sup>+</sup> o <sup>+</sup> /G342R	M	9	9	HS em U/S	NB	ALF, lb	[113]
41	E8SJM <sup>+</sup> o <sup>+</sup> /N119S	E8SJM <sup>+</sup> o <sup>+</sup> /N98S	F	26	26	AP	MVS, FC, PI	ALF, lb	[49]
42	E8SJM <sup>+</sup> o <sup>+</sup> /W140X	E8SJM <sup>+</sup> o <sup>+</sup> /W140X;c.894G>A (p.S275_Q298del) + [c.419G>A (p.W140Y)]	M	7	7	n.r.	MVS	lb	[113]
43	E8SJM <sup>+</sup> o <sup>+</sup> /c.1028delG	E8SJM <sup>+</sup> o <sup>+</sup> /c.DG1064-8	M	0.1	24	AD (nascimento)	DH	ALF, lb	[47]
44	E8SJM <sup>+</sup> o <sup>+</sup> /G87V	E8SJM <sup>+</sup> o <sup>+</sup> /G66V	M	6	21	SM	HV	lb, HTG	[124]
45	E8SJM <sup>+</sup> o <sup>+</sup> /Q85R	E8SJM <sup>+</sup> o <sup>+</sup> /Q64R	F	6	11	n.r.	F, FC	ET	[88]
46	E8SJM <sup>+</sup> o <sup>+</sup> /R218X	R218X/del S275_Q298;c.652 C>T;c.894 G>A, del.c.823_894	F	9	18	n.r.	NB	lb, ET	[13]
47	E8SJM <sup>+</sup> o <sup>+</sup> /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	lb, ET	[13]
48	E8SJM <sup>+</sup> o <sup>+</sup> /UNK	E8SJM <sup>+</sup> o <sup>+</sup> /UNK	F	51	51	2 irmãos com DDEC	NB	lb	[90]
49	E8SJM <sup>+</sup> o <sup>+</sup> /UNK	E8SJM <sup>+</sup> o <sup>+</sup> /UNK	F	30	m. 47 (AVC)	X, AVC	DH, BMV	HC, HTG	[47]
50	H129R/grande deleção (infrão 1- exão 4)	H108R/grande deleção no gene (infrão 1- exão 4)	M	1.5	3 (transplante hepático, id. desc., comu. pess.)	SM	DH	n.r.	[57]
51	T288I/T288I	T267I/T267I	F	0.67	8 (transplante hepático, 7)	D, FTT, AC (8 meses)	HV, BRC (2 anos), C (6 anos)	n.r.	[88]
52	H295Y/H295Y	H274Y/H274Y	F	1.5	14 (transplante hepático, 1)	n.r.	HV, BRC	n.r.	[114]
53	P202L/VS6-2A>G	181L/E7SJM (ISVA>G D205-253)	M	5	13 (transplante hepático, 1)	n.r.	BRC, C, HV	HC, HTG	[124]
54	G342R/S289C	G342R/S289C	F	1.8	3.2 (transplante hepático, 18) m. 18 (comu. pess.)	HT, AC	BRC, F, TAO, BMV, FC	HC, ET	[90]
55	L294S/UNK	L273S/UNK	n.r.	5	>15	SM	NB	n.r.	[124]

<sup>1</sup>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 heterozygotes [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<sup>-1G>A</sup>, has been found only in CESD patients and the two exon 8 splice-junction variants, E8SJM<sup>+1G>A</sup> and E8SJM<sup>+3C>T</sup>, occurred only in WD patients [3,36]. The common donor-splice-site mutation, E8SJM<sup>-1G>A</sup> 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]. Non-sense, 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<sup>-1G>A</sup> 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.

Review

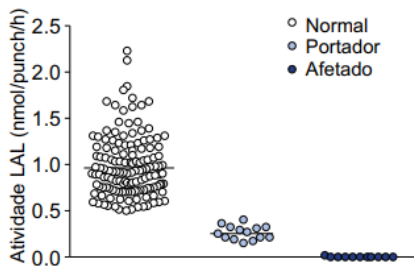


<sup>1</sup> As mutações são apresentadas tanto na nomenclatura HGMD (base de dados de mutações em genes humanos), como na nomenclatura utilizada nas publicações originais. Anteriormente as substituições de aminoácidos foram descritas como tendo 21 aminoácidos a menos quando a numeração foi baseada na modificação pós-traducional do péptido.

**Abreviaturas: Manifestações clínicas:** AC, calcificação das suprarrenais ou aumento; AD, distensão abdominal; AFIB, fibrilação auricular; AP, dor abdominal; ATH, aterosclerose; CA, cancro; CAD, doença arterial coronária; CF, fadiga crónica; D, diarreia; ECC, equimoses; EV, varizes esofágicas; FTT, má evolução ponderal; GA, atrofia gonadal; GF, deficiências de crescimento (altura/peso < percentil 5); GI, manifestações gastrointestinais; GU, úlcera gástrica perfurada; HF, insuficiência cardíaca; HTN, hipertensão; I, icterícia; SM, esplenomegalia; X, lesões cutâneas xantomatosas.

**Fígado e outras patologias dos tecidos:** BP, patologia dos intestinos (bx intestinal: edema da mucosa, macrófagos espumosos); FC, células espumosas; BMV, vacuolização da medula óssea; BRC, cristais birrefringentes/fendas (massivos); C, cirrose, (cirrose micronodular); DH, diagnóstico de hepatopatologia; DU, bx duodenal lípido, células espumosas; F, fibrose; G, glicogénio intracitoplasmático; kV, vacuolização das células de Kupffer; HL, deposição de lípidos nos hepatócitos; HS, esteatose hepática em ultrassonografia; HV, vacuolização dos hepatócitos; KL, deposição de lípidos nas células de Kupffer; MBL, lípido ligado à membrana; ML, lipodistrofia mesentérica; MLD, doença hepática metabólica; MVS, esteatose microvesicular; NB, sem biópsia (ou não relatada); PBF, fibrose porto-portal; PI, inflamação portal; TAO, obliteração da arquitetura trabecular; VL, linfócitos vacuolados, histiócitos.

**Resultados laboratoriais:** IIb, hiperlipoproteinemia tipo IIb; A, anemia; ALF, função hepática anormal; HC, colesterol alto; HTG, hipertrigliceridemia; ET, transaminases elevadas. n.r., não relatado.



**Fig. 3.** Teste da gota seca (*dried blood spot testing*) da atividade da LAL em homocigóticos, heterocigóticos e em indivíduos normais. Esta análise é específica da LAL, pois incorpora um inibidor específico, o lalistat 2, que reduz a atividade de outras lipases. Atividade da LAL em 140 controles normais, em 11 doentes com deficiência da LAL e 15 portadores. Limite de deteção = 0.02 nmol/punch/h. Da referência [109], com permissão.

**Atividade deficiente da LAL**

A atividade da LAL foi determinada em fibroblastos cultivados, em leucócitos periféricos e no tecido hepático utilizando vários substratos de lipase não específicos para a LAL, excluindo-se assim comparações diretas das atividades residuais da LAL entre os doentes. As atividades enzimáticas encontradas em 114 doentes variaram de “indetectável” a 16% da média dos valores dos controlos, encontrando-se usualmente entre os <1% e os 10 % da média dos valores dos controlos para leucócitos periféricos ou fibroblastos cultivados [13,29,53,62–64,108]. Devido à variabilidade dos ensaios, a atividade enzimática residual não se correlaciona necessariamente com a gravidade da doença. Por outro lado, as atividades da LAL em fibroblastos cultivados ou em leucócitos de doentes com um subtipo de deficiência da LAL mais grave, a DW, apresentaram pouca, se alguma, atividade detetável [3]. Foi relatada recentemente uma análise específica para a LAL que determinou a sua atividade em amostras de sangue seco (DBS) utilizando 4-metilumbeliferona palmitato como substrato enzimático e o inibidor específico para a LAL, lalistat 2. Esta análise resultou numa boa separação das atividades para controlos normais e para homocigóticos e heterocigóticos da DDEC [109] (Fig. 3).

**Mutações no gene LIPA**

Até à data foram identificadas mais de 40 mutações com perda de função no gene LIPA em doentes com DDEC e com DW (<http://www.hgmd.cf.ac.uk/ac/index.php>) (Fig. 2). Das 19 mutações conhecidas por causarem a DW a maioria (37%) são pequenas deleções/inserções, 26% são do tipo *non-sense*, 21% são mutações em sequências consenso *splicing*, 10% são lesões do tipo *missense* e 5% são causadas por uma grande deleção. Já no que concerne as 32 mutações conhecidas da DDEC a maioria (50%) são do tipo *missense*, 25% são

deleções/inserções, 16% são do tipo *non-sense*, 6% são mutações em sítio de junção e 3% são causadas por uma grande deleção.

Quanto à mutação mais comum, E8SJM<sup>-1G>A</sup>, esta encontra-se apenas em doentes com DDEC e as duas variantes no exão 8 na junção intrão-exão, E8SJM<sup>+1G>A</sup> e a E8SJM<sup>-3C>T</sup>, surgiram apenas em doentes com DW [3,36]. A mutação comum em sítio dador E8SJM<sup>-1G>A</sup> causa *splicing* alternativo conduzindo à deleção do exão 8, que codifica uma enzima LAL mutante sem atividade bem como 2% a 4% do mRNA *wild-type* normalmente processado, que codifica ~3–8% da atividade normal da LAL [8,24,37,48]. Foram encontradas mutações dos tipos *non-sense*, pequenas deleções/inserções, *splicing* e *missense* em doentes com DDEC e com DW. Contudo, as alterações mais graves que resultaram na redução acentuada ou na ausência da atividade da LAL foram detetadas em doentes com DW, ao passo que as mutações no gene LIPA que codificam enzimas mutantes com atividade residual foram encontradas em doentes com DDEC [110]. Sendo importante salientar que a G87V (também descrita como G66V) é a mutação fundadora da DW entre indivíduos com ascendência judaico-persa e Bukharin-judaica, com uma frequência alélica de ~1 em 32 [88,111].

Estudos de expressão do cDNA LIPA in vitro avaliaram a atividade residual da LAL causada por várias mutações *missense* [110]. Estes estudos demonstraram que as mutações do tipo *missense* quando associadas com pouca ou nenhuma atividade, particularmente quando em homocigotia causam DW. Contrariamente, mutações do tipo *missense* que codificam uma enzima com atividade enzimática residual (1–5%) in vitro, foram encontradas em doentes com DDEC [36, 110].

Como mencionado anteriormente os métodos de ensaio da LAL usam substratos e procedimentos diferentes, que nem sempre são específicos para a LAL, e que, por conseguinte, não são comparáveis. Por exemplo, o doente com DDEC com a maior atividade enzimática da LAL relatada (16 % da atividade normal média em fibroblastos) apresentava uma forma grave da doença com início aos seis meses de vida, com cirrose hepática aos três anos de idade, com hipertensão portal e varizes esofágicas aos sete e foi alvo de transplante hepático aos 13 anos [80,81,112].

**Correlações genótipo-fenótipo**

Geralmente o subtipo da doença (DW ou DDEC) e a sua gravidade são baseados principalmente na ausência ou nos níveis de atividade residual da LAL determinada por dois alelos mutantes no gene LIPA. As mutações no gene LIPA em doentes com DW resultam principalmente em pouca ou nenhuma atividade da LAL, enquanto que os doentes com DDEC apresentam pelo menos uma mutação que resulta em alguma atividade enzimática residual. Caso um doente possua o alelo comum E8SJM<sup>-1G>A</sup>, ou uma mutação do tipo *missense* que codifique atividade enzimática residual significativa, este encontra-se protegido da DW e possuirá DDEC independentemente da gravidade da mutação presente no outro alelo.

Revisão

## Review

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<sup>-1G>A</sup> 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<sup>-1G>A</sup> mutation (Fig. 2, in bold). Six mutations, H295Y, G342R, L200P, R44X, G87V, and E8SJM<sup>-1G>A</sup> 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<sup>-1G>A</sup> allele was present in 61% of mutant alleles, which included 17 patients who were E8SJM<sup>-1G>A</sup> homozygotes. One additional patient was inferred to be an E8SJM<sup>-1G>A</sup> 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 the 18 E8SJM<sup>-1G>A</sup> homozygotes. This is the largest group of reported CESD patients with the same genotype. Almost all E8SJM<sup>-1G>A</sup> homozygotes had onset in the first years of life, followed by progression of their liver disease into adult life. Of the 15 E8SJM<sup>-1G>A</sup> 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<sup>-1G>A</sup> homozygotes ranged from microvesicular steatosis to fibrosis, and fibrosis with septal bridging, indicative of cirrhosis. Extrahepatic involvement among E8SJM<sup>-1G>A</sup> 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<sup>-1G>A</sup> allele

(genotype H129P/E8SJM<sup>-1G>A</sup>). Three siblings with the E8SJM<sup>-1G>A</sup>/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<sup>-1G>A</sup>/c.323T>A and E8SJM<sup>-1G>A</sup>/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-

## Revisão

Entre os doentes com DDEC que apresentam alguma atividade residual da LAL, o espectro clínico varia desde o início precoce com progressão rápida da doença, a um diagnóstico mais tardio caracterizado por um fenótipo mais atenuado. A gravidade da DDEC deverá refletir o genótipo do LIPA e a atividade residual total codificada por ambos os alelos. A atividade enzimática residual será correlacionada com o subtipo da doença, idade de início e com o ritmo de progressão da mesma. A mutação comum E8SJM<sup>1G>A</sup> e as mutações do tipo *missense*, cuja presença se prevê originar a atividade residual da LAL foram as mais frequentes entre os doentes genotipados com DDEC. Dos 135 doentes relatados foram identificadas mutações no gene LIPA em 55 doentes, incluindo quatro nos quais foi detetada apenas uma mutação, mas cujos diagnósticos foram baseados na deficiência da LAL e/ou nos cristais de EC presentes nas biópsias hepáticas. Foram identificadas nestes 55 doentes 31 mutações no gene LIPA, incluindo 17 do tipo *missense*, três do tipo *non-sense*, uma pequena inserção, uma pequena inserção/deleção complexa, mas cujas duplas (duas mutações no mesmo alelo), cinco deleções pequenas, uma grande deleção estendendo-se por 4 exões e duas mutações em sequências consenso *splicing*, incluindo uma mutação intrónica num local de *splicing* que resulta na deleção de 48 pares de bases (exão 7) e na mutação comum E8SJM<sup>1G>A</sup> (Fig. 2, a negrito). Seis mutações H295Y, G342R, L200P, R44X, G87V, e a E8SJM<sup>1G>A</sup> foram identificadas em pelo menos duas ou mais famílias não relacionadas, sendo que as mutações L179P, 195X (c.673\_675delC) e a G87V foram identificadas em doentes com DDEC e com DW [56,88,111,132]. Entre as 106 mutações detetadas no gene LIPA nestes 55 doentes, o alelo comum E8SJM<sup>1G>A</sup> encontrava-se presente em 61% dos alelos mutantes, incluindo 17 doentes que eram homocigóticos para a E8SJM<sup>1G>A</sup>. Adicionalmente, deduziu-se que um outro doente fosse homocigótico para a E8SJM<sup>1G>A</sup> ao ter como base a análise molecular dos três filhos do probando [47].

Até à data não foram relatadas correlações genótipo-fenótipo para a DDEC. Sendo estas correlações limitadas pelo facto de que existem poucos irmãos afetados ou indivíduos afetados não relacionados com os mesmos genótipos para ser realizada uma comparação clínica (Tabela 3). Estas limitações referentes às correlações genótipo-fenótipo tornaram-se ainda mais evidentes dada a diversidade encontrada entre os 18 homocigóticos para a E8SJM<sup>1G>A</sup>. Sendo este o maior grupo relatado de doentes com DDEC com o mesmo genótipo. Praticamente em todos os homocigóticos para a E8SJM<sup>1G>A</sup> o início da doença deu-se nos primeiros anos de vida, seguido de progressão da doença hepática até à idade adulta. Dos 15 homocigóticos para a E8SJM<sup>1G>A</sup> cujas idades de início da doença foram relatadas, 13 foram diagnosticados entre o primeiro ano de vida ou até aos seis anos, e em dois casos foram diagnosticados aos oito e aos 13 anos. Adicionalmente deduziu-se que uma mulher fosse homocigótica ao ter como base os genótipos dos seus filhos, tendo sido a mesma primeiramente diagnosticada aos 53 anos e sofrido de um enfarte do miocárdio fatal aos 58 anos [47]. As observações da doença hepática entre os homocigóticos relatados para a E8SJM<sup>1G>A</sup> variaram entre a esteatose microvesicular à fibrose incluindo a fibrose porto-portal indicativa de cirrose. O envolvimento extra-hepático entre os homocigóticos para a E8SJM<sup>1G>A</sup> incluiu a acumulação gastrointestinal de lípidos, a diarreia grave, aguda e crónica, a má absorção, a dor abdominal, a úlcera gástrica perfurada, o peso e altura abaixo do percentil 5, a anemia, internamentos frequentes devidos a infeções respiratórias, a doença arterial coronária incluindo a aterosclerose, a insuficiência cardíaca, calcificações aórticas, o enfarte do miocárdio e o AVC. Ao passo que a progressão da doença foi para alguns doentes rápida, noutros esta foi lenta embora tenha em última instância conduzido à fibrose hepática e a complicações de aterosclerose [29,113].

Muitos dos doentes eram heterocigóticos compostos para a mutação do tipo *missense* (histidina para prolina) H129P (que in vitro apresentou 4,6% da atividade enzimática normal) e para o alelo comum E8SJM<sup>1G>A</sup> (genótipo H129P/E8SJM<sup>1G>A</sup>).

Três irmãos com o genótipo E8SJM<sup>1G>A</sup>/H129P foram diagnosticados já adultos (32, 44 e <52 anos) [26]. Estes três apresentaram doença hepática acentuada incluindo cirrose e varizes esofágicas e um morreu devido a insuficiência hepática aos 56 anos de idade [26]. Desde a publicação um segundo irmão também morreu e o terceiro foi alvo de transplante hepático (comunicação pessoal, Dr. C. Gasche, [26]). Pelo contrário, um doente com heterocigotia composta para a H129R (histidina para arginina) e para uma deleção do gene, tinha 18 meses quando surgiu a doença [57]. Após a publicação este doente também desenvolveu insuficiência hepática e foi alvo de transplante hepático aos nove anos, quatro anos depois de o estudo de caso ter sido publicado (comunicação pessoal Dra. V. McLin). Estes doentes ilustram a importância de ambos os alelos e da alteração conformacional da proteína causada pela substituição de aminoácidos no prognóstico da gravidade da doença num dado doente. Revela-se importante salientar que os doentes com DDEC com a T288I/T288I (a proteína T288I in vivo, apresentava 3,6% da atividade normal) e com os genótipos G342R/S289C apresentaram uma manifestação semelhante à da DW, que incluiu o início na infância, diarreia, calcificações suprarrenais e a má evolução ponderal. Contudo, estes doentes possuíam atividade residual da LAL suficiente para sobreviverem até à segunda ou terceira década de vida após a realização do transplante hepático. O doente homocigótico para a T288I foi alvo de transplante hepático aos sete anos [88,90]. O doente com o genótipo G342R/S289C sobreviveu até aos 18 anos quando a insuficiência hepática levou a um transplante hepático e subsequente morte (Tabela 3: doente 54) [92]. Os pacientes homocigóticos para a H295Y (com 2,9% da atividade normal) também apresentaram início da doença na infância necessitando de um transplante hepático aos 11 anos e dois doentes não relacionados com os genótipos E8SJM<sup>1G>A</sup>/c.323T>A e E8SJM<sup>1G>A</sup>/S344L (c.967delAG >fs→338X) apresentaram também início da doença na infância mas sobreviveram até aos trinta de acordo com o último acompanhamento [38,47,114]. Estes doentes demonstram que o início precoce das manifestações desta doença pode progredir rapidamente na infância ou na adolescência, ou que pode progredir mais lentamente até à idade adulta conduzindo à insuficiência hepática, e, por sua vez, ao transplante ou à morte.

### Tratamento

#### Estratégias de redução de colesterol

Apenas 35 (26%) dos doentes relatados foram tratados com inibidores da HMG-CoA redutase para a hiperlipidemia [13–15, 26, 28, 29, 38, 47, 65–67, 79, 84, 95, 96, 107, 115–121]. O primeiro uso das estatinas para controlo das alterações na síntese do colesterol na DDEC foi relatado numa menina de nove anos com hepatomegalia, com níveis de colesterol sérico total e LDL significativamente elevados e de HDL reduzidos com acumulação massiva de EC, com fibrose, e, com cirrose na biópsia hepática [107]. Após oito meses de tratamento com 5 mg/d de lovastatina a sua dosagem foi aumentada para 20 mg/d tendo reduzido os triglicéridos plasmáticos, o colesterol e o colesterol LDL, embora os níveis de colesterol HDL não tenham aumentado significativamente. Dado que os volumes do fígado e do baço desta doente não sofreram alterações desde a base de referência, os autores colocaram a hipótese de que a produção hepática dos EC possa ter aumentado em resposta ao aumento da expressão do recetor de LDL com fluxo mediado pela ApoB [107]. Sendo esta hipótese apoiada subsequentemente por dois estudos de casos posteriores que documentaram respostas favoráveis à sinvastatina (0,28 mg/kg) e à colestiramina após dois anos de tratamento que resultou em reduções significantes do colesterol LDL sérico e triglicéridos e num aumento das concentrações de colesterol HDL com diminuição do volume do fígado e sem alterações nos níveis das transaminases, da esplenomegalia ou das calcificações das suprarrenais.

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-phosphate 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

Após três anos de tratamento a biópsia hepática revelou aumento da fibrose com subsequente hipertensão portal e após cinco anos de tratamento dada a progressão da doença hepática foi necessária a realização do transplante hepático [84, 115]. Dos 35 doentes a tomar estatinas, em relação a oito não eram relatados resultados da biópsia hepática, 15 apresentavam resultados de biópsia com fibrose, cirrose ou hepatopatologia associada à DDEC, não tendo sido relatado o acompanhamento sequencial ou a longo prazo [18, 29, 47, 53, 60, 65–67, 89–91, 95, 96, 117, 118, 120–122]. Os restantes 12 doentes a inibidores da HMG-CoA redutase tinham realizado múltiplas biópsias hepáticas, providenciando dados longitudinais. Não existiram casos em que a histologia hepática tivesse melhorias e todos os 12 doentes apresentaram doença hepática progressiva mais avançada em biópsias subsequentes, demonstrando-se a natureza progressiva da doença hepática na DDEC. Com efeito, foram seis os doentes tratados com inibidores da HMG-CoA redutase que necessitaram de transplante ou morreram de insuficiência hepática [15, 26, 28, 38, 47, 48, 62, 79, 84, 115, 116, 119, 123] (comunicação pessoal, Dr. C. Gasche, Dra. V. McLin). Estas observações salientaram a falta de eficácia das estatinas na melhoria da doença hepática ou na prevenção da sua progressão.

#### Transplante hepático

A idade de realização do transplante hepático foi relatada em nove doentes dos cinco aos 14 anos [81, 83–85, 88, 114, 124, 125]. Adicionalmente foram pelo menos mais três os doentes que desenvolveram insuficiência hepática após a publicação dos seus estudos de casos, dois dos quais posteriormente morreram (Dr. C. Gasche, comunicação pessoal [26]; Tabela 3: doentes 36, 50, e 54). Havendo uma limitação da informação referente ao acompanhamento a longo prazo dos doentes transplantados, com algumas exceções [80, 81, 83, 112, 126, 127]. Foram seguidos seis doentes alvo de transplante hepático entre 10 meses a três anos, sem complicações registadas, excluindo-se o caso de um doente que apresentou carcinoma hepatocelular encontrado incidentalmente no fígado removido. Um outro doente que fora transplantado aos cinco anos apresentou hiperbilirrubinemia e transaminases elevadas sugestivas da rejeição do transplante, tendo desenvolvido progressivamente insuficiência cardíaca congestiva [126]. Apenas dois doentes transplantados foram acompanhados durante mais de cinco anos. Uma das doentes que fora transplantada aos 14 anos apresentou subsequentemente infeção e obstrução biliar necessitando de cirurgia. A mesma desenvolveu insuficiência renal terminal sete anos após o transplante devido a glomeruloesclerose e aterosclerose com elevada acumulação vascular de lípidos e atrofia tubular, bem como fibrose intersticial necessitando de hemodiálise crónica aos 21 anos de idade. A deposição lipídica no sistema vascular renal suscitou a preocupação de que o transplante tenha apenas melhorado a doença hepática, mas não a acumulação sistémica lisossomal de EC. O segundo doente para o qual o acompanhamento a longo prazo foi relatado não apresentou envolvimento renal seis anos após o transplante [80, 81, 112].

#### Terapia de substituição enzimática

A TSE foi primeiramente realizada em fibroblastos cultivados da DDEC usando LAL derivada de pseudomonas (em ligação cruzada com albumina e/ou conjugada com insulina ou ApoB) [41]. Estas preparações enzimáticas foram incubadas no meio de culturas de fibroblastos da DDEC, foram internalizadas, e a enzima intracelular degradou os EC acumulados nos lisossomas. As tentativas subsequentes para o desenvolvimento da TSE aguardaram pela geração e/ou caracterização dos modelos murinos da DDEC, bem como pela purificação da LAL a partir de várias fontes.

Os estudos pré-clínicos da TSE foram realizados em ratos em que a doença ocorre espontaneamente e em ratos *knockout* com deficiência da LAL [128, 129]. Os ratos com deficiência da LAL autossómica recessiva foram identificados por Yoshida e Kuriyama em 1990 [128]. Estes ratos apresentavam ~19% da atividade da LAL *wild-type* no fígado. Clinicamente, estes ratos manifestaram hepatoesplenomegalia, aumento dos gânglios linfáticos e intestinos mais grossos e dilatados. Estes ratos foram caracterizados a nível patológico e bioquímico constatando-se que reproduzem de forma semelhante a patologia da doença hepática presente nos humanos, manifestando estes esteatose microvesicular dos hepatócitos e células de Kupffer, que progrediu para a fibrose e para a cirrose micronodular. Não apresentaram calcificações suprarrenais. Mais recentemente um modelo murino LIPA *knockout* foi gerado por técnicas recombinantes de DNA e caracterizado [122]. Os ratos não apresentavam atividade enzimática da LAL ou proteína e apresentavam acumulação hepática massiva de EC e de triglicéridos. Adicionalmente os ratos desenvolveram a patologia hepática, suprarrenal e intestinal comum da DDEC (i.e. hepatócitos, células de Kupffer e outros macrófagos e acumulação adrenocortical), sobreviveram até à idade adulta e produziram descendência. Estes ratos foram usados para avaliar a TSE [42–44] e a terapia génica mediada por adenovírus [130].

Os ensaios pré-clínicos da TSE foram avaliados nos ratos *knockout* para o gene LIPA [42–44]. Estes estudos avaliaram a eficácia da LAL humana recombinante em células de *Pichia pastoris* [42], de ovário de hamster chinês (CHO); [43] ou em células de *Nicotiana benthamiana* (tabaco) [44]. A expressão na levedura resultou numa LAL humana recombinante (rhLAL) com cadeias oligossacarídeas terminadas principalmente em manose, ao passo que a expressão nas células CHO produziu uma LAL humana com oligossacarídeos terminados em manose e manose-6-fosfato [42–44]. Foram administradas 10 doses de cada enzima por via intraperitoneal ao longo de 30 dias. A rhLAL da *Pichia pastoris* terminada em manose foi entregue via recetor de manose a macrófagos e a células derivadas de macrófagos em vários órgãos. A rhLAL produzida nas células CHO tinha oligossacarídeos terminados em manose-6-fosfato e desobstruiu os hepatócitos, as células de Kupffer e as células derivadas de macrófagos. Quanto à rhLAL derivada da planta do tabaco esta teve captação dependente do recetor de manose, e, após 10 injeções por via intravenosa (a cada três dias), resultou na diminuição hepática dos EC e das concentrações de triglicéridos, diminuindo também os macrófagos espumosos no fígado, baço e nas vilosidades intestinais [44]. Quando cada enzima foi administrada a ratos duplo *knockout* para o gene LIPA e para o recetor da manose em macrófagos, apenas a enzima derivada do CHO eliminou as acumulações lipídicas nos hepatócitos e nos macrófagos [43].

Estes estudos pré-clínicos providenciaram a fundamentação para os ensaios clínicos da TSE na DW e na DDEC onde a rhLAL foi produzida em claras de ovo [45]. Um estudo de fase I/II, randomizado, duplamente cego, controlado por placebo, aberto, com escalonamento de dose foi realizado com a rhLAL que continha oligossacarídeos de manose e de manose-6-fosfato. Foram avaliadas três dosagens (0,35 1,0 e 3,0 mg/kg) semanalmente durante quatro semanas em doentes com DDEC, seguindo-se um estudo de extensão onde todos os participantes receberam 1 mg/kg em semanas alternadas. Demonstrando estes estudos a eficácia da rhLAL derivada de claras de ovo ao reduzir as atividades das transaminases séricas e ao resultar na elevação sérica do colesterol LDL comprovando-se a libertação do colesterol livre proveniente dos EC acumulados nos lisossomas [45].

#### Gestão da doença

Tendo como base os estudos clínicos, patológicos e bioquímicos dos doentes relatados na literatura, as seguintes avaliações são as propostas para a monitorização da progressão da doença. Os exames laboratoriais anuais devem incluir testes de função hepática (AST, ALT, Tempo de Protrombina, bilirrubina), hemograma completo, quitotriosidase (um marcador inflamatório

## Review

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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## Revisão

de macrófagos descrito como estando elevado na DDEC; [29]) e o perfil lipídico para avaliar a dislipidemia. A ressonância magnética deve ser realizada periodicamente para determinar os volumes do fígado e do baço e a espectroscopia por ressonância magnética pode ser usada para medir a fração de gordura hepática. Podem ser indicados estudos de imagem, como a ultrassonografia abdominal ou a esofagoduodenoscopia para controlar a hipertensão portal e o sangramento das varizes. As avaliações cardiovasculares incluem anualmente um eletrocardiograma e um ecocardiograma, devendo ser também consideradas a progressão na espessura da camada íntima-média da carótida e a avaliação neurovascular de risco de AVC.

### Discussão

Anteriormente, não existiam estudos prospectivos ou retrospectivos sobre a história natural dos doentes com DDEC. Dos 135 doentes relatados na literatura a maioria foi diagnosticada incidentalmente dada a patologia hepática característica e/ou devido ao diagnóstico confirmado a partir da atividade deficiente da LAL ou tendo em conta as análises do gene LIPA. Todos os casos relatados apresentaram doença hepática grave caracterizada pela esteatose microvesicular com progressão para cirrose micronodular e para insuficiência hepática.

Clinicamente, a DDEC apresentou uma idade de início variável sendo frequentemente não diagnosticada até que a hepatomegalia inexplicada com ou sem esplenomegalia, as atividades das transaminases elevadas e/ou a hiperlipoproteinemia tipo IIb levem à investigação do diagnóstico. Geralmente a biópsia hepática revelará uma acentuada aparência amarelo-alaranjada, hepatócitos e macrófagos espumosos carregados de gordura (Fig. 1A e B) e cristais de EC patognomônicos, que são melhor observados no exame ultraestrutural (Fig. 1F). Todavia, o conhecimento desta doença é limitado e inicialmente muitos doentes foram incorretamente diagnosticados. A biópsia hepática pode ser diagnosticada incorretamente como EHNA, DHGNA ou como cirrose criptogénica [4]. A suspeita do diagnóstico pode ser confirmada pela demonstração da deficiência notória na atividade da LAL (Fig. 2) [109], ou mediante análises de mutações no gene LIPA, que detetam com precisão tanto os doentes como os indivíduos heterozigóticos para esta doença autossômica recessiva.

Com base nesta revisão as crianças afetadas tendem a apresentar uma evolução acentuada que conduz à insuficiência hepática precoce e ao transplante. Embora o transplante hepático se tenha revelado eficaz na prevenção da morte devida à insuficiência hepática, o envolvimento extra-hepático mesmo em doentes transplantados resultou numa carga de doença significativa e na morte precoce em alguns doentes. Os doentes diagnosticados numa fase mais tardia na vida tendem a apresentar uma evolução mais atenuada, não existindo dados a longo prazo sobre a morbidade e a mortalidade destes doentes. Os doentes que apresentam um início da doença mais tardio poderão ser a coorte mais subdiagnosticada, uma vez que estes parecem assintomáticos, não apresentando nada para além de hiperlipoproteinemia tipo IIb, sendo apenas diagnosticados quando surge um AVC, um aneurisma, a doença arterial coronária ou até que se dê a morte precoce causada pela insuficiência hepática.

Dado que os ensaios clínicos da TSE se encontram em curso revela-se de extrema importância a sensibilização para esta doença. A deficiência da LAL deveria estar incluída num diagnóstico diferencial para todos os que apresentam colesterol sérico e LDL elevado e que também possam ter níveis ligeiramente a moderadamente reduzidos de colesterol HDL, transaminases elevadas e hepatomegalia. A disponibilidade de diagnósticos moleculares não-invasivos da enzima LAL e do gene LIPA podem anular a necessidade de realização de biópsia hepática na confirmação do diagnóstico da DDEC. Contudo, deverá suspeitar-se de DDEC em qualquer biópsia hepática com aparência amarelo-alaranjada e com esteatose microvesicular e/ou cirrose micronodular. O diagnóstico da DDEC pode ser estabelecido mediante a imunocoloração para a LAMP1, LAMP2 e LIMP2 ou para a catepsina D ou ao ser demonstrada via

microscopia eletrónica a presença de cristais de EC patognomônicos ou as suas fendas remanescentes. Prevê-se que a sensibilização para a DDEC por parte de hepatologistas, patologistas, cardiologistas e neurologistas leve a uma maior identificação destes doentes, particularmente entre os adultos, conduzindo assim a uma melhor gestão e tratamento desta doença.

### Conflito de interesses

Os autores DLB e RJD são consultores na Synageva BioPharma, a empresa que se encontra a desenvolver a terapia enzimática para a doença de depósitos de ésteres de colesterol à data em fase III de ensaio. O Dr. Desnick possui opções sobre ações na Synageva BioPharma e exerce funções no Conselho Consultivo Científico. No que concerne os autores HH e MGB estes não possuem qualquer conflito.

### Contribuições dos autores

O estudo foi concebido por DLB e RJD que analisaram os dados e escreveram e editaram o artigo. Tendo contribuído a autora HH com a secção sobre patologia hepática e tendo sido revisto o manuscrito por MGB e HH. Todos os autores aprovaram o manuscrito final.

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