Cholesteryl ester storage disease of clinical and genetic characterisation: A case report and review of literature

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Abstract

BACKGROUND

Cholesteryl ester storage disease (CESD) is a rare genetic disease. Its symptoms and severity are highly variable. CESD is a systemic disease that can lead to the accumulation of fat and inflammation in the liver, as well as gastrointestinal and cardiovascular disease. The majority of patients require liver transplantation due to decompensated cirrhosis. Enzyme replacement therapy has been approved based on a randomized trial. Our study aims to clinically and genetically evaluate two siblings with CESD who underwent liver transplantation, as well as their first-degree family members.

CASE SUMMARY

The siblings were compound heterozygous for the missense variant in LIPA exon 8, c.894G>A, (p.Gln298Gln) and a single base pair deletion, c.482del (p.Asn161Ilefs*19). Analyses of single nucleotide polymorphisms showed variants with an increased risk of fatty liver disease and fibrosis for both patients. Clinically, both patients show signs of recurrence of CESD in the liver after transplantation and additional gastrointestinal and cardiovascular signs of CESD.
INTRODUCTION

Cholesteryl ester storage disease (CESD) is a type of lysosomal acid lipase (LAL) deficiency. LAL deficiency (LAL-D) is caused by pathogenic variants in the LIPA, which encodes the enzyme LAL[1-3]. LAL-D is inherited in an autosomal recessive manner. Affected individuals are homozygous or compound heterozygous for pathogenic variants in LIPA. The LAL enzyme is required for intracellular hydrolysis of cholesteryl esters and triglycerides within low-density lipoprotein (LDL) particles into free cholesterol and free fatty acids[4]. Deficiency of the enzyme leads to accumulation of mucolipids and mucopolysaccharides. The most common pathogenic variant associated with CESD is variant c.894G>A, p.(Gln298Gln), also named E8SJM, in exon 8 in LIPA[5-8]. In Western countries, the allele frequency in the general population is about 0.0025, corresponding to a carrier frequency of about one in 200. The variant results in a transcript with an in-frame deletion of exon 8 and can result in some residual LAL activity when only small amounts of mRNA are spliced correctly[9]. About 50%-70% of patients with CESD have the c.894G>A, p.(Gln298Gln) variant, which corresponds to a prevalence of the disease ranging from 1 in 40000 to 1 in 300000[10,11]. The variant associated with CESD is variant c.894G>A, p.(Gln298Gln), also named E8SJM, in exon 8 in LIPA[5-8]. In Western countries, the allele frequency in the general population is about 0.0025, corresponding to a carrier frequency of about one in 200. The variant results in a transcript with an in-frame deletion of exon 8 and can result in some residual LAL activity when only small amounts of mRNA are spliced correctly[9]. About 50%-70% of patients with CESD have the c.894G>A, p.(Gln298Gln) variant, which corresponds to a prevalence of the disease ranging from 1 in 40000 to 1 in 300000[10,11].

Three family members who were LIPA heterozygous had a lysosomal acid lipase activity below the reference value. One of these carriers, a seven-year-old boy, was found to have severe dyslipidemia and was subsequently treated with statins.

CONCLUSION

Our study underlines that CESD is a multi-organ disease, the progression of which may occur post-liver transplantation. Our findings underline the need for monitoring of complications and assessment of possible further treatment.

Key words: Lysosomal acid lipase deficiency; Lysosomal storage disease; Non-alcoholic fatty liver disease; Liver transplantation; Sebelipase alfa; Case report

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Core tip: Cholesteryl ester storage disease is a multisystemic disease affecting several organs. Accordingly, the disease will progress after liver transplantation and may recur in the transplanted liver. Abnormal lipid accumulation in the vascular endothelium causes cardiovascular diseases. The occurrence of single nucleotide polymorphisms associated with fatty liver disease may increase the risk of cirrhosis and of recurrence of fatty liver disease after transplantation. Our case presents findings that underline the importance of monitoring and treatment.

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misdiagnosed as non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, or cryptogenic cirrhosis\(^\text{[19]}\). In addition, patients with CESD may experience gastrointestinal symptoms including diarrhoea, abdominal pain, and malabsorption\(^\text{[8]}\). Individuals with CESD often have elevated transaminases and hypercholesterolemia, with elevated serum low-density and very LDL-cholesterol and normal or elevated triglycerides\(^\text{[19]}\). High-density lipoprotein-cholesterol is typically low. Hepatomegaly and hyperlipidaemia may be the only abnormalities prompting the diagnosis, which could explain why LAL-D is underdiagnosed.

The initial test recommended to establish a diagnosis of LAL-D is measurement of the enzymatic activity of LAL in leukocytes in a blood sample or on a dried blood spot\(^\text{[20,21]}\), followed by targeted DNA sequencing of \textit{LIPA} to identify the pathogenic variant(s).

Dietary interventions may help alleviate gastrointestinal symptoms associated with LAL-D\(^\text{[22]}\). In addition, treatment with statins is needed to reduce the risk of cardiovascular disease\(^\text{[23]}\). Unfortunately, the therapeutic response is often insufficient despite high-dose treatment, and upregulation of LDL receptors in hepatocytes may occur. Liver transplantation may be necessary, although longitudinal data about subsequent transplant rejection are scarce\(^\text{[24]}\). Sebelipase alfa is a recombinant human LAL that is produced in egg white from transgenic hens\(^\text{[25]}\). The attached mannose-6-phosphate residue facilitates the lysosomal uptake of the recombinant enzyme, leading to hydrolysis of cholesteryl esters and triglycerides. The drug was approved for the treatment of LAL-D in 2015.

We undertook a cross-sectional study evaluating two siblings with CESD and their first-line family members. The protocol was approved by the Danish Regional Ethics Committee (H-18020170). All participants gave their informed consent. All participants underwent abdominal ultrasound and fibroscan with controlled attenuation parameter (CAP) and routine blood test. LAL activity was determined in dried blood test (DBS) samples. Further, genetic tests were carried out with targeted DNA sequencing of \textit{LIPA} to identify the variant(s) causing LAL-D in the siblings, and potential variants in the family members, was undertaken with whole genome sequencing\(^\text{[12]}\). In addition, genetic variants affecting the interaction between pathways that could increase the risk of developing fibrosis and cirrhosis in CESD were assessed based on analyses for single nucleotide polymorphisms (SNPs)\(^\text{[26-29]}\).

CESD patients underwent extended diagnostic imaging with myocardial perfusion scintigraphy, combined with a cardiac single photon emission computed tomography (SPECT), with stress test as indicated for the diagnosis of coronary artery diseases and to evaluate myocardial blood flow, as well as cardiac magnetic resonance imaging (MRI) and gastroduodenoscopy.

**CASE PRESENTATION**

**Chief complaints**

We assessed two siblings with confirmed CESD, a 59-year-old female and a 61-year-old male, of Scandinavian heritage, in order to systematically evaluate CESD in these patients, and evaluate possible \textit{LIPA} carriers.

**History of present illness**

The patients have experienced persisting gastrointestinal symptoms ever since the first diagnosis.

**History of past illness**

The female patient with CESD was diagnosed as a child after developing symptoms at the age of eight. Symptoms included hepatosplenomegaly, malabsorption and diarrhoea, combined with elevated transaminases and dyslipidemia. As an adult, the female patient underwent surgery for an arterial aneurism in her leg and adrenal insufficiency. At the age of 45, the patient developed progressive liver failure. A liver biopsy revealed microvesicular and micronodular cirrhosis, lobular inflammation and hepatocyte ballooning, and numerous macrophages with Periodic Acid-Schiff positive and diastase-resistant material. The patient underwent liver transplantation. Afterwards, hepatosplenomegaly recurred and a post-transplant biopsy in 2018 showed 60% macrovesicular and microvesicular steatosis, a mild degree of ballooning and lobular inflammation, as well as perisinusoidal fibrosis. The findings were consistent with LAL-D liver pathology.

The male patient with CESD was diagnosed as an adolescent, following his sister’s diagnosis. The patient had elevated transaminases and dyslipidaemia at the time of the diagnosis, but no clinical symptoms. As an adult, the patient developed...
progressive liver failure and a liver biopsy found severe microvesicular and micronodular cirrhosis and inflammation consistent with CESD. The patient underwent liver transplantation at the age of 53. A post-transplant liver biopsy showed mild-to-moderate inflammation and F1 fibrosis. After the liver transplant, the patient also developed cardiovascular disease with an abdominal aortic aneurysm and underwent an endovascular abdominal aortic aneurysm repair.

**Physical examination upon admission**

Both patients presented with steatorrhea and upper abdominal discomfort. The female patient suffered from abdominal pain.

**Laboratory examinations**

The LAL activity was < 0.01 nmol/(punch∙h) for both siblings [normal range: 0.37-2.30 nmol/(punch∙h)]. Routine laboratory tests showed that the female patient had mildly elevated ALT, VLDL, and triglycerides, as well as low platelets (Table 1). The male patient had normal blood tests.

LIPA gene sequencing revealed two previously reported variants, including the well-known missense variant in exon 8, c.894G>A, (p.Gln298Gln), and a single base pair deletion, c.482del (p.Asn161Ilefs*19). Accordingly, the siblings were compound heterozygote for these variants. Additional analyses showed that the female patient had two SNP polymorphisms in the TM6SF2 [Rs58542926(C/T) type CT] and in MBOAT7 [Rs641738(C/T) type CT], suggesting an increased risk of fatty liver disease. The male patient had Rs738409 (C/G) type CG, suggesting an increased risk of fibrosis [28,30].

**Imaging examinations**

The abdominal ultrasound and Fibroscan confirmed that the patients had steatosis with Cap values of 281 and 342 dB/m for the female and male patient, respectively. The male patient also had evidence of mild fibrosis, with a median score of 9.2 kPa in the Fibroscan.

Both patients underwent additional tests to assess evidence of cardiovascular disease. The female patient had a moderate stenosis of up to 70% on ultrasound imaging of the carotid arteries. Transthoracic echocardiography identified a sclerotic aorta valve, mitral annular calcification, and mild left ventricular hypertrophy. Myocardial perfusion scintigraphy combined with SPECT showed a small, reversible perfusion defect near the septal basis and a moderately elevated coronary artery calcium score. The findings were confirmed with cardiac MRI, which found the same reversible perfusion defect near the septal basis and mild left ventricular hypertrophy (Space between the CESD patients).

The male patient had no evidence of stenosis in a carotid artery ultrasound. Transthoracic echocardiography found severe aortic valve stenosis with calcified cusps and mild left ventricular hypertrophy. Myocardial scintigraphy with SPECT showed a small, reversible perfusion defect in the inferior lateral left ventricle and a moderately elevated calcium score. Cardiac MRI showed aortic valves with restrictive movement due to calcified cusps, resulting in severe aorta stenosis and left ventricular hypertrophy. Gastroduodenoscopy was normal. Duodenal biopsies showed no inflammation of foamy cells.

**Family members**

None of the six family members showed evidence of LAL-D in dried blood test (Table 2). Two adult family members and one child were heterozygotes and had LAL activity below the reference value of 0.37 (Table 2, Table 3). The child, a seven-year-old boy, also had elevated LDL cholesterol (166.3 mg/dL; reference value < 116). Both 34-year-old males had increased controlled attenuation parameter values of 330 and 400, respectively they had LAL activity of 0.37 and 0.51 nmol/(punch∙h) (Table 2). The latter was also morbidly obese with a body mass index of 58 kg/m² and had an increased median value of 24.2 Kpa on the Fibroscan. A percutaneous liver biopsy showed steatosis and inflammation, but no fibrosis. Lifestyle interventions were initiated, and the patient was referred for assessment for possible bariatric surgery.

**FINAL DIAGNOSIS**

The final diagnosis of this presented case is LAL-D and its attenuated phenotype CESD disease. SNPs variants associated with development of fatty liver disease were also found in all the participants.
### Table 1  Routine blood tests of patients with cholesteryl ester storage disease and their adult family members

| Blood tests                      | Patients                  | F  | M  | M, 5 yr | F, 9 yr | M, 31 yr | M, 34 yr | M, 34 yr |
|----------------------------------|---------------------------|----|----|---------|---------|----------|----------|----------|
| Alanine amino transferase (U/L) |                           | 86 | 38 | 19      | 19      | 66       | 28       | 18       |
| Alkaline phosphatase (U/L)       |                           | 49 | 96 | 269     | 234     | 61       | 79       | 52       |
| Total bilirubin (mg/dL)          |                           | 0.94 | 0.35 | 0.58 | 0.29 | 0.41 | 0.94 | 0.41 |
| International normalised ratio (INR) |                    | 0.9 | 1.0 | 1.1 | 1.1 | 0.9 | 1.0 | 0.9 |
| Haemoglobin (g/L)                |                           | 132.1 | 137 | 120.8 | 127.3 | 133.7 | 148.2 | 161.1 |
| Platelets (10⁹/L)                |                           | 121 | 168 | 230 | 329 | 234 | 192 | 229 |

F: Female; M: Male. Blood tests for 7 years child unavailable.

### TREATMENT

Treatment has been limited to statins. Both patients receive ongoing treatment with statin. However, the female patient has dyslipidemia and does not respond to statin medication. The male patient shows effect of the latter and has normal blood concentration of lipids. Previously, the female patient had received treatment with enzyme replacement therapy, Sebelipase alfa. Unfortunately, the treatment was assessed as having no clear effect and treatment was stopped. The price and availability of the medicine have blocked new attempts to retry the enzyme replacement therapy.

### OUTCOME AND FOLLOW-UP

Our clinical work-up showed evidence of recurrence of LAL-D pathology in liver and abnormal lipid accumulation in the vascular endothelium leading to cardiovascular diseases in both patients. Consequently, these findings have led to multidisciplinary follow up control of the patients by the department of hepatology and cardiology. They will be assessed once a year to monitor the disease progression. None of the family members showed any symptoms and they all had normal LAL activity. One of the family members, the seven-year-old boy was referred for treatment with statins.

### DISCUSSION

This is a clinical and genetic study of two siblings with CESD and their first-degree family members. The siblings were compound heterozygotes for the missense variant in \( LIPA \), c.894G>A, (p.Gln298Gln) and a single base pair deletion, c.482del (p.Asn161Ilefs*19). Analyses of SNPs showed an increased risk of fatty liver disease and fibrosis. One patient was treated with Sebelipase alfa and was classified as non-responsive. It is possible that genetic factors influence the response to therapy. Both patients underwent liver transplantation, and both had symptoms in the gastrointestinal tract, as well as cardiovascular disease, underlining that CESD is a systemic disease. None of the family members had CESD, but one child had dyslipidemia and required treatment.

Our clinical examinations showed evidence of abnormal lipid accumulation in the transplanted liver and vascular endothelium in both patients. Our findings included reversible perfusion defects in coronary arteries and severe aorta valve stenosis, with calcified cusps and mild left ventricular hypertrophy in the male patient. Both patients underwent surgery for arterial aneurysms. Additionally, our findings confirm previous evidence that liver transplantation does not prevent the multi-systemic progression of CESD\(^{16}\). Patients have increased mortality and morbidity post-transplantation, which underlines the importance of adequate monitoring and treatment of complications.

Recurrence of CESD in a transplanted liver may occur when deficient bone marrow-derived monocyte macrophages migrate to the liver and mature to Kupffer cells, thereby repeating the pre-liver transplantation pathophysiology\(^{31,32}\). In our patients, the histological assessment found mild-to-moderate inflammation, fibrosis, and microvesicular steatosis, which is likely an outcome of the combination of their medication and, for one patient, her obesity, but is also consistent with recurrent...
Table 2  Lysosomal acid lipase activity and LIPA variants in family members of two patients with cholesteryl ester storage disease

| Age | Gender | DBS | LIPA variant                  | Genotype          |
|-----|--------|-----|--------------------------------|--------------------|
| 5   | Male   | 1.03| c.482del, p.Asn161Ilefs*19    | Heterozygote       |
| 7   | Male   | 0.34| c.894G>A, p.Gln298Gln         | Heterozygote       |
| 9   | Female | 1.13| WT                            | Normal             |
| 31  | Male   | 0.22| c.894G>A, p.Gln298Gln         | Heterozygote       |
| 34  | Male   | 0.37| c.482del, p.Asn161Ilefs*19    | Heterozygote       |
| 34  | Female | 0.51| c.482del, p.Asn161Ilefs*19    | Heterozygote       |

DBS: Dried blood spot nmol/punch per hour; WT: Wildtype.

CESD in the transplanted liver[16]. A previous study assessing 18 patients with LAL-D who underwent liver transplantation found severe disease progression post-transplant[32]; six of these patients died.

The siblings described in our paper were carriers of the most commonly reported variant in the literature, c.894G>A, (p.Gln298Gln) in a compound heterozygous state[17,33]. The other variant was a single base pair deletion, c.482del (p.Asn161Ilefs*19), which has an allele frequency rate of 0.0032%[7,34], compared with 0.0090% for c.894G>A, (p.Gln298Gln). This corresponds with the critically low LAL activity of < 0.01 nmol/(punch∙h). In addition, our female patient had two SNP polymorphisms in TM6SF2 [Rs58542926 (C/T) type CT] and MBOAT7 [Rs641738 (C/T) type CT], suggesting a significant predisposition to fatty liver disease development, in addition to the impact of the pathogenic LIPA variants[33,38]. The male patient had CG genotype in Rs738409 (C/G), suggesting an increased risk of fibrosis in alcoholic liver disease[27,30]. It is possible that the additional genetic assessment of patients with LAL-D, including SNPs associated with liver disease, may provide important prognostic information and be an indicator of treatment requirements.

The role of LAL-D heterozygosity in presenting LAL-D phenomena has not yet been defined, and there are few reports assessing LAL-D carriers. However, we found a correlation between heterozygosity and LAL activity. Three heterozygous family members presented a LAL activity value below the reference value of 0.37 nmol/(punch-h) (Figure 1). Two of the adult heterozygous family members had moderate steatosis, one of them verified with a liver biopsy showing severe steatosis and inflammation. It is possible that LAL activity can be used as a biomarker in heterozygotes[6,36-38]. In addition, one of the family members in the study was a seven-year-old boy with LAL activity of 0.34 nmol/(punch-h). The child also had severe dyslipidemia, indicating that (as previously suggested[19]) reduced LAL activity may contribute to dyslipidemia, as well as to non-specific symptoms[8,12,16,39]. The choice was therefore made to monitor the child closely.

Treatment for LAL-D has been limited to lipid-lowering medications, statins, or liver transplantation. The patients in our study have been treated with statins for more than 15 years. Our results indicate that this treatment does not prevent the progression of CESD. Even with statins, the female patient continued to have elevated VLDL and triglycerides. These findings accord with the conclusions of previous studies that treatment with statins is not beneficial to the progression of liver diseases associated with LAL-D due to enhanced acceleration of intracellular accumulation of cholesterol ester[35,36-39]. The current treatment of choice is Sebelipase alfa, based on a randomized controlled trial showing beneficial effects on several clinical outcomes[40]. Our assessment of the male patient suggests that Sebelipase alfa might have beneficial effects on cardiovascular outcomes. However, based on its price and availability in Denmark, treatment is not readily available without prior approval.

CONCLUSION

Our study underlines that CESD is a multi-organ disease, the progression of which may occur post-transplantation. The findings reiterate the need for monitoring of complications and assessment of possible additional treatment. The findings also suggest that despite the high carrier frequency for CESD, one must consider testing family members for possible dyslipidemia, as well as LAL-D, as even heterozygote patients might present with dyslipidemia.
Table 3  Analysis of single nucleotide polymorphisms in family members of patients with cholesteryl ester storage disease

| Age  | Gender | MBOAT7          | PNPLA3          |
|------|--------|-----------------|-----------------|
| 5    | Male   | Rs641738 [C/T]; CT |                |
| 7    | Male   | Rs641738 [C/T]; CT |                |
| 9    | Female | Rs641738 [C/T]; TT |                |
| 31   | Male   | Rs641738 [C/T]; CT | Rs641738 [C/T]; CT |
| 34   | Male   | Rs641738 [C/T]; CT | Rs738409 [C/G]; CG |

1The patient also had TM6SF2 Rs58542926 [C/T]; CT.

Figure 1  Family pedigree with lysosomal acid lipase activity and single nucleotide polymorphisms. CESD: Cholesteryl ester storage disease; LAL: Lysosomal acid lipase.

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