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CASE REPORT

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Testis – a novel storage site in human cholesteryl ester storage disease

Autopsy report of an adult case with a long-standing subclinical course complicated by accelerated atherosclerosis and liver carcinoma

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Abstract A case of long-standing subclinical cholesteryl ester storage disease (CESD) manifesting as hyperlipoproteinaemia type IIb without any hepatomegaly is described. The patient underwent surgical vascular interventions because of accelerated atherosclerosis, which dominated his middle age. CESD was an incidental finding when a liver biopsy specimen was taken because liver malignancy was suspected; the patient's condition proved to be due to a cholangiocarcinoma, which led to his death at the of age 52. The autopsy showed moderate-intensity storage in the set of cells characterized by constitutional high-level receptor-mediated LDL endocytosis (hepatocytes, adrenal cortical cells) and also revealed storage in the Leydig cells. The severity with which histiocytes were affected varied regionally, ranging from minimal detectable storage or none at all (gut, lymph nodes, spleen) to extreme lysosomal expansion by cholesteryl ester liquid crystals (bone marrow) or by ceroid (lung, testicular stroma), or by both (liver). The density of the histiocytic population did not correlate with the degree to which parenchymal cells were affected except in the testicular stroma, where it was prominent. The patient was a mixed heterozygote for the G934A and $\Delta C_{673\text{-}5}$ mutations.

Key words CESD · Lysosomal acid lipase deficiency · Storage distribution · Testis · Leydig cells

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Introduction

Lysosomal acid lipase/cholesteryl ester hydrolase (LAL) deficiency is a rare autosomal recessive storage disease that exists in two phenotypic variants [3]. Complete enzyme deficiency, known as Wolman disease [1, 36], leads to death in early infancy as a consequence of massive storage of cholesteryl esters and triglycerides in a variety of cells, the most severely affected being hepatocytes, adrenal cortical spongiocytes, and histiocytes. The second disorder, called cholesteryl ester storage disease (CESD) [12, 29], is associated with some residual LAL activity and takes a much more protracted course with less expression of storage, which is confined mostly to hepatocytes, adrenal cortex and histiocytes, hepatomegaly being the principal and often only clinical sign. CESD poses threats to the patient's health in the form of progressive liver cirrhosis and/or early vascular disease. The latter is due to hypercholesterolaemia, which has been shown to be caused by up-regulation of hepatic apolipoprotein B-100 synthesis [8]. Autopsy reports are rare [4, 6, 9, 13] as the life span is not seriously shortened in CESD. This communication presents an autopsy finding with evidence of a novel storage site – the Leydig cells – , which fits well into the family of cells with constitutional receptor-mediated LDL endocytosis, which are predisposed to storage even in mild variants of LAL deficiency.

Case report

The patient was born in 1944. When he was of 33 hyperlipoproteinaemia was incidentally found and was classified as type IIb. Liver function tests were within normal limits, as were liver and spleen size, for the following asymptomatic 18-year period. At the age of 51 he developed serious atherosclerosis with complications requiring surgical intervention (details of the clinical history are presented elsewhere). Preoperative sonography revealed an increased liver size and discrete hyperechogenic, tumour-like zones in the left liver lobe. The spleen and both adrenals were slightly enlarged. Laparoscopic liver biopsy disclosed a cholangiocarcinoma, micronodular cirrhosis, and a moderate lysosomal storage process with features of LAL deficiency, which was confirmed in the peripheral leucocytes (6% of normal). The patient was found

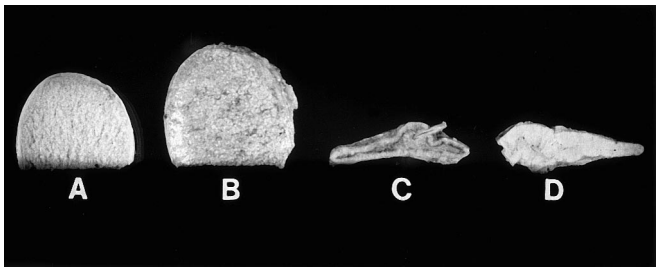

Fig. 1 Testis from the control (**A**) and the CESD patient (**B**). Control adrenal (**C**) and CESD adrenal (**D**). Note the very slight enlargement of both CESD samples and absence of pigmentation of the reticular zone of the adrenal cortex

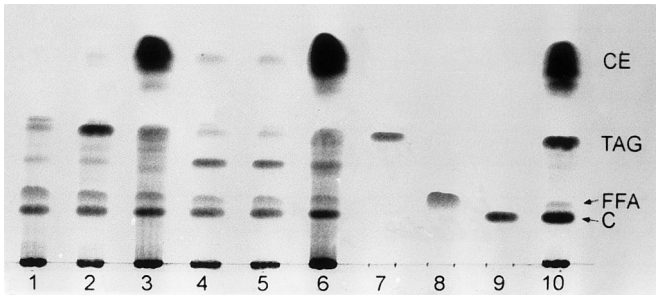

Fig. 2 Chromatography performed on HPTLC plates (Kieselgel 60; Merck, Germany) of apolar lipids extracted from liver and testis. Stepwise solvent system hexan–diethylether (1:1; v/v) for the first half of the chromatogram and hexan-diethylether (97:3; v/v) to the top of the chromatogram. Detection: cupric sulphate. The samples were loaded as follows: *lanes 1* and *2* control liver, *lane 3* CESD liver, *lanes 4* and *5* control testis, *lane 6* CESD testis, *lane 7* tripalmitin standard, *lane 8* oleic acid standard, *lane 9* cholesterol standard; *lane 10* serum lipid mixture. *C* cholesterol, *CE* cholesteryl ester, *FFA* free fatty acids, *TAG* triacylglycerol

to be a compound heterozygote with the prevalent G934A (exon 8 splice site) mutation and deletion of $C_{673\text{-}5}$ in exon 6 of the LAL gene (Dr. P. Lohse, Munich, Germany). The final stage of the disease was characterized by ascites, icterus, and cachexia. The patient died at the age of 52.

Autopsy findings

Autopsy findings were dominated by extensive ulcerothrombotic atherosclerosis of the aorta, with thrombosed saccular aneurysm of its abdominal part, thrombosed saccular aneurysms of both common iliac arteries, and severe atherosclerotic stenoses of the femoral arteries. The enlarged liver (3850 g) displayed micronodular cirrhosis and a multitude of tumour nodules in both lobes, without tumour progression into the hepatic or portal veins. Ascites (1200 ml) and mild splenomegaly (180 g) were observed. Tumour metastases were confined to the subhepatic lymph nodes. The adrenal glands were moderately increased in size, without calcifications and lacking pigmentation of the reticular zone. The testes were slightly enlarged, and their surface was stippled with discrete yellowish granules (Fig. 1). The heart was dilated, with moderate hypertrophy of the left ventricle (18 mm thickness) and dispersed myofibrosis.

Materials and methods

Histology and electron microscopy were performed as described elsewhere [10]. Lipopigment was detected by UV autofluorescence in paraffin-embedded and fresh-frozen sections mounted in Immu-mount (Shandon). Cholesteryl ester (CE) accumulation was assessed using birefringence and oil red O staining. Macrophages were identified immunohistochemically with monoclonal CD68

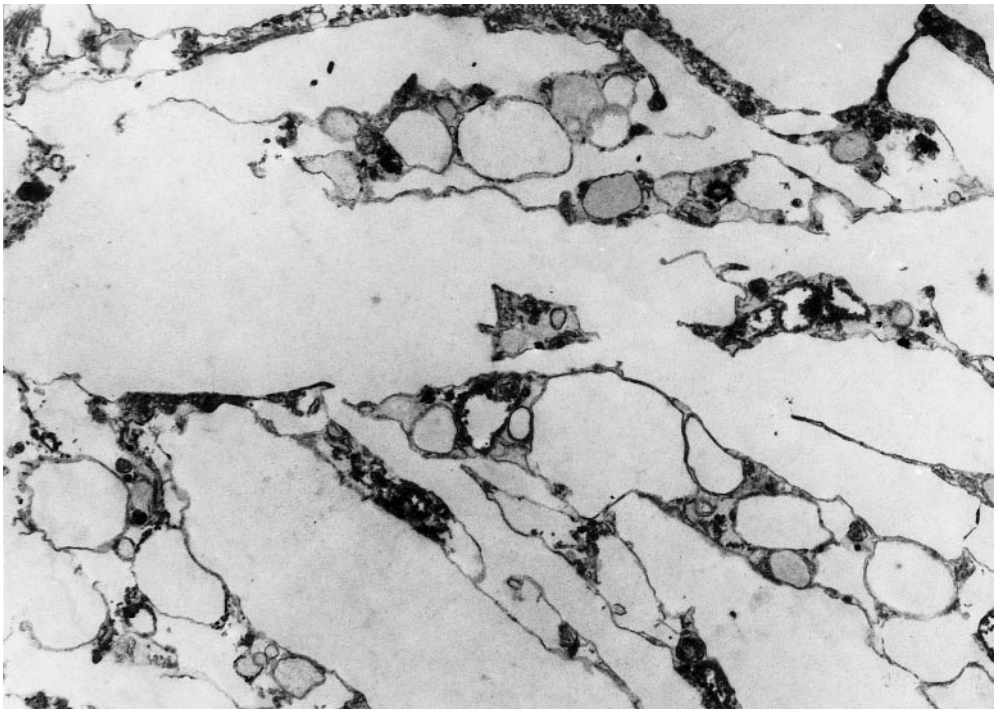

Fig. 3 Ultrastructure of the storing Leydig cell with cytoplasm occupied by giant storage lysosomes with crystalline shape. ×10,000

antibodies PG-M1 and KP1 (Dako, Glostrup, Denmark), followed by incubation with secondary and tertiary Px-coupled antibodies ($SWAM_{Px}$, $RASW_{Px}$; Sevac, Praha, CZ) as already published [11]. Lipids were analysed by HPTLC (see Fig. 2 for details) following extraction by the method of Schnaar and Needham [31]. LAL activity was measured using MU palmitate [18].

Results

The storage process displayed the typical features: presence of both liquid and solid birefringent CE within the lysosomes of several cell types. Lipid chromatography showed an excess of CE in liver and testis (Fig. 2), while TG levels were normal. In histiocytes, the lipid storage was accompanied by various degrees of ceroid accumulation, which sometimes dominated, whereas in hepatocytes and cortical suprarenal spongiocytes the lipid accumulation interfered with age pigment deposition so that the latter was present in rudimentary form. Electron microscopy demonstrated a single limiting membrane around lysosomes with any kind of storage material (Fig. 3). The ceroid pigment was ultrastructurally heterogeneous with an amorphous background varying in electron density and pleiomorphic membranous and vesicular profiles (Fig. 5). Immunohistochemistry with macrophage-specific CD68 markers demonstrated a decrease in staining intensity, which was proportional to the degree of storage.

Histology and storage distribution.

There was micronodular florid cirrhosis of the liver and moderate hepatocytic CE storage in the relatively narrow peribiliary zone. Numerous lipid- and ceroid-storing histiocytes were found around the tumour, especially in the areas of tumour regression, while they were scarce in the liver lobules. The carcinoma itself was free of CE storage. The adrenal gland displayed a steadily increasing gradient of vacuolization of the cortical epithelium, reaching its maximum in the reticular zone, where the cells were almost devoid of lipofuscin. The rare sinusoidal histiocytes were small and displayed discrete lipid and ceroid deposition. There were also patchy cortical necroses without calcification. The medulla was histologically normal. The lung was infiltrated by histiocytes loaded with ceroid granules, but with very few CE droplets. The ventricle and the gut were free of histochemically detectable CE accumulation. The smooth muscle cells contained only a small amount of lipofuscin. There was discrete deposition of ceroid in scarce spleen and lymph node histiocytes, while CE storage was undetectable. Sinus histiocytes in lymph nodes were cytologically normal. Foamy histiocytes were scarce in the haematopoietically active vertebral bone marrow but numerous in the inactive adipose femoral compartment, and lipid storage dominated over ceroid accumulation. Brain (including the choroid plexus), eccrine glands of the skin, exocrine and endocrine pancreas, and heart and

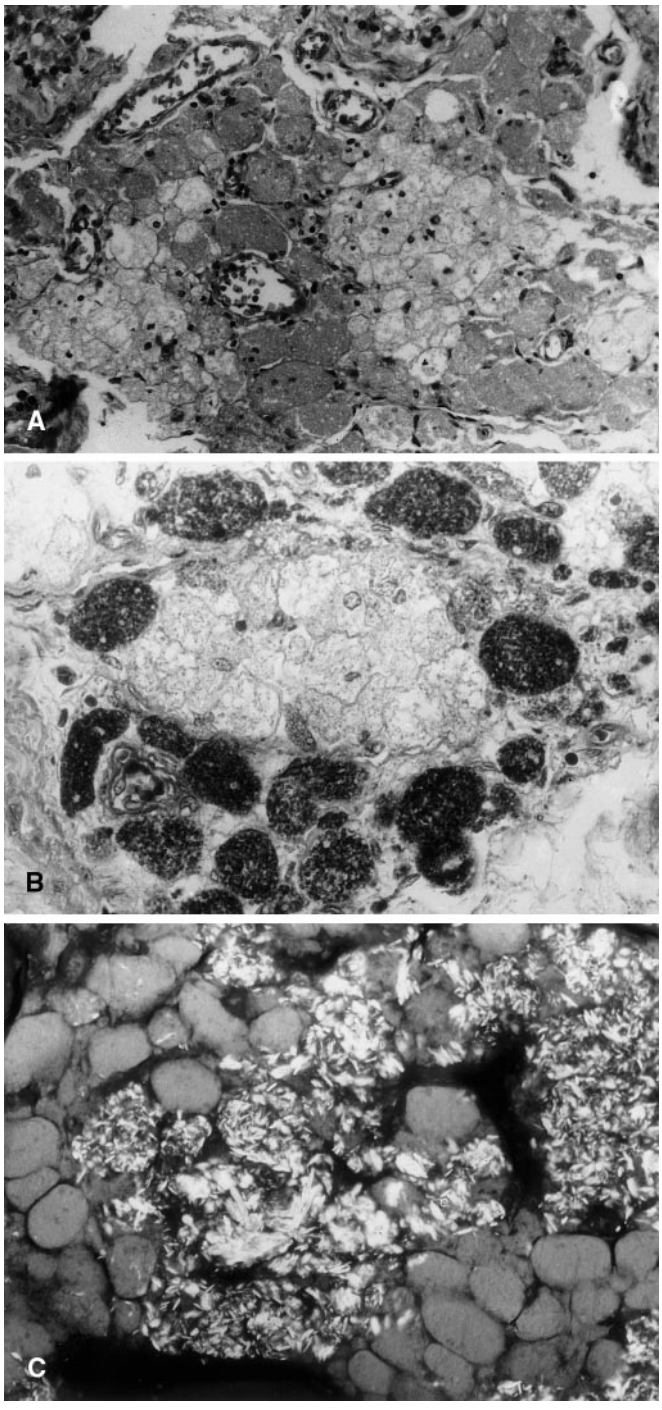

Fig. 4 A, B Paraffin-embedded and **C** frozen sections of the CESD testis. Haematoxylin–eosin **A** Massive infiltration of the testicular stroma with light foamy, cohesive Leydig cells which are segregated from the less cohesive, granular histiocytic storage cells. ×160 **B** Strong staining of the ceroid-loaded histiocytic storage cells with Sudan Black B, while Leydig cells remain unstained. ×330 **C** Combined examination with polarized light and autofluorescence shows solid birefingent crystal CE deposits within Leydig cell nests and autofluorescence of the ceroid-bearing histiocytes. ×330

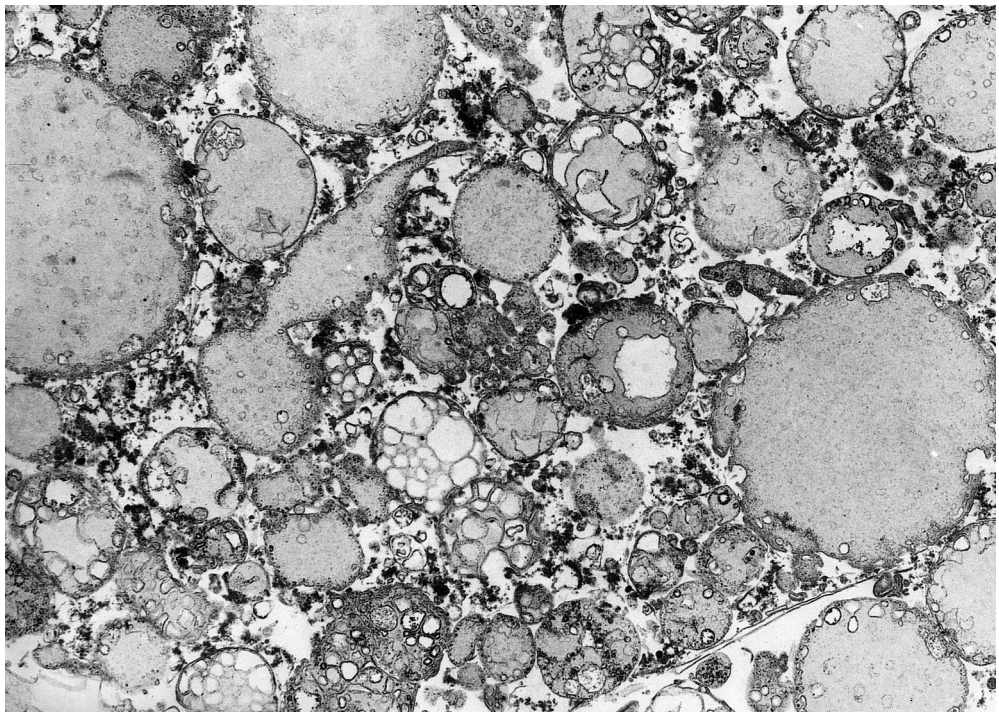

Fig. 5 Ultrastructure of the ceroid loaded macrophage in the testicular stroma. Rounded lysosomes with hemidense amorphous content, with many vesicular membranous profiles of various sizes. ×10,000

skeletal muscle were free of CE storage. Arteries displayed fibroatheromatous changes with scarce foam cells.

The stroma of the testes was extensively infiltrated by two types of storage cells: (1) foamy, CE-loaded, highly cohesive cells, mostly around 30–50 μm in diameter, arranged in clusters, in which electron microscopy showed lucent lysosomes mostly of crystalline outline (Fig. 3); (2) granular, ceroid-loaded, less cohesive, larger (approximately 60–80 μm in diameter) and sometimes multinuclear cells, arranged in a network-like fashion ensheathing the clusters of CE-storing cells (Fig. 4). The ceroid-bearing cells were the only cell type that was immunostainable with CD68 marker KP1. Their ultrastructure was rich in vesicular and loose membranous profiles (Fig. 5). Normal Leydig cells, in contrast, were absent, and ultrastructural examination also failed to disclose Leydig cell features, i.e. abundant smooth endoplasmic reticulum, tubular transformation of mitochondrial cristae, or Reinecke crystals [5], in any type of stromal storing cells. The seminiferous tubules were atrophic to varying degrees, with thickened basement membranes and without signs of storage. The epithelium of the epididymis contained a relatively large amount of lipofuscin with discrete vacuolization, suggesting a mild degree of CE storage.

Discussion

This patient had one of the slowly progressing CESD variants that have a long-standing silent course [9, 10, 15, 23] and become manifest clinically through the cumulative effect of permanent hypercholesterolaemia and LAL deficiency, both widely recognized factors in acceleration of the atherosclerotic process (for a review see [3]). Other known risk factors, hyperhomocysteinaemia and LpA, were within normal limits (data not shown). A low level of storage was associated with the mixed heterozygous state, the prevalent G934A CESD mutation with incomplete splicing defect [2] compensating for the Wolman-type mutation on the second allele and its serious disruption of LAL activity (for details see [26]).

The storage distribution pattern corresponds essentially to that described in the very rare autopsy reports relating to CESD, that is to say hepatocytes were affected, which is a constant feature of LAL deficiency of any type [3]. Storage in the adrenal cortex is one of the regular findings, except in the case described by Cagle et al. [6], in which both the macroscopical findings and the histology were reported as normal. However, reports on storage in other steroidogenic cells in LAL deficiency are extremely scarce and incomplete. Foam cell infiltration of the testicular stroma and of the ovaries has been observed in Wolman disease [7, 17, 27, 35], but the cell type affected was not specified. Dincsoy et al. [9] described massive involvement of both ovaries in a 57-year-old patient with a subclinical course of CESD, again without further specifying the origin of the closely packed foam cells. On the other hand, in the ovaries of an 18-year-old woman only “numerous foamy histiocytes in the hilum” were found [6], suggesting that the time factor should be considered.

This report is the first one describing the storage in the testis. Thanks to the advanced stage of storage modulating the cell’s morphological phenotype the situation

requires a brief comment. The foamy, CE-bearing cells are considered to be Leydig cells with secondary hyperplasia, for the following reasons: (1) on average, they are smaller and more cohesive than histiocytes; (2) they are free of lipofuscin, a normal constituent of Leydig cells in adults, which is in agreement with the observation of an inhibitory effect of CE storage on lipofuscin deposition seen in hepatocytes and adrenal cortical cells of CESD patients [11] (unpublished observations); (3) they are also free of ceroid, which is strikingly associated with the macrophages and can be seen as their marker in CESD (M. Elleder, unpublished results); (4) involvement of Leydig cells in the storage appears to be logical, as the sterol cell system utilizes lipoprotein-bound CE in large part as the substrate for hormone synthesis [14, 16, 22, 28, 34].

However, storage in the sterol-producing cells does not appear to have an adverse effect on hormone production generally. There are no clinical indications of either masculine or feminine hypogonadism or of symptomatic adrenal cortical insufficiency even in Wolman disease [3] Fertility also does not seem to be affected. Two of our female patients have had normal pregnancies [10, 25]. The expansive stromal storage in the testis may cause secondary atrophy and might lead to sterility in a very advanced stage of the disease. The steroidogenic cells have several possible ways of maintaining adequate hormone synthesis: increasing de novo cholesterol synthesis, utilizing the HDL-mediated cholesterol/CE uptake pathway [14, 19–21, 24, 28, 30, 32, 34] to maintain a constant level of cytoplasmic free cholesterol, and reactive hyperplasia. The last may be a common reaction to a lysosomal storage process in mitotically active tissues.

The histiocytic expression of storage was lower in this case than in other reports so far published, and it varied noticeably. While in some regions there was no detectable storage in the bulk of the histiocytes (lymph nodes, spleen), their pulmonary population was greatly expanded and loaded with ceroid. The liver histiocytic population was small on average, with ceroid prevailing over CE, while that in the bone marrow was inversely proportional to the haematopoiesis, with storage dominated by CE. In contrast to earlier reports [29] (see [3] for a review), the gut was completely free of storage histiocytes.

The massive accumulation of CE-storing macrophages in the testes contrasted with the situation in the tissues mentioned above. This suggests that histiocytes may play a significant part in normal testicular lipoprotein turnover and supports the observation of a functional relationship between them and Leydig cells [33].

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