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CASE REPORT

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Mucolipidosis type II with evidence of a novel storage site

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Abstract In a case of infantile mucolipidosis type II (I-cell disease), storage was identified at autopsy in serous-type secretory cells in exocrine pancreas, in the tracheal and sublingual salivary glands and in the chief (zymogenic) cells of the gastric oxyntic glands, suggesting a systemic involvement of this type of secretory cells. The content of specific secretory granules was inversely proportional to the intensity of the storage process. The mucus-producing cells were not affected. The serous glandular system is a novel storage site in I-cell disease. Review of archival material in three further cases confirmed the findings.

Key words Mucolipidosis II · I-cell disease · Lysosomal storage · Serous secretory cells

Introduction

Mucolipidosis type II (ML II) and MLIII are allelic disorders caused by a deficient trans-Golgi enzyme system responsible for creating the M6P label on the mannose residue of lysosomal enzyme glycoproteins. The system encompasses the initial transfer of *N*-acetylglucosaminyl phosphate on the mannose residue of a lysosomal enzyme glycoprotein catalysed by UDP-*N*-acetylglucosamine: lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase (EC 2.7.8.17), found to be deficient in the disorder. In the second step the *N*-acetylglucosamine is removed by a specific glycosidase. At the tissue level the deficiency is reflected in a complex lysosomal storage process involving a variety of still vaguely defined bioconjugates of lipid and/or glcoprotein nature, also encompassing biochemically indefinite residual lysosomal inclusion bodies induced in cultured mutant cells (for review see [5]).

The spectrum of tissues described as affected by storage is not large. Biopsy studies have shown constant involvement of skin fibroblasts, endothelial cells, and Schwann cells of unmyelinated nerve fibres [6] and prominent vacuolation of peripheral lymphocytes [10]. Studies of post-mortem tissues revealed significant involvement of mesenchymal cells, generally contrasting with discrete involvement of epithelial cells and neurons. An exception to this is the extensive involvement of anterior horn cells and the renal glomerular podocytes [3, 7–9]. Screening of post-mortem tissues of our fist case of ML II disclosed changes suggestive of pronounced involvement of exocrine cells of the serous type, a finding confirmed by ultrastructural examination. Results of a review of archival post-mortem specimens are also reported.

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Materials and methods

The skin biopsy sample was double fixed with 10% paraformaldehyde and phosphate-buffered 1% OsO4, dehydrated and embedded in an Epon-Araldite mixture. The thin sections were double contrasted and examined with a JEM 100B electron microscope. The post-mortem samples fixed with 10% formaldehyde were either paraffin embedded and used for routine and special staining or osmicated and examined by EM. Paraffin sections were stained with haematoxylin and eosin, trichrome, PAS, aldehyde fuchsin (after acidified permanganate preoxidation), and Gomori's hot methenamine. Frozen sections were examined for birefringence and autofluorescence, and for apolar lipids using Oil Red O. The peripheral blood smears were examined cytologically using Giemsa-May-Grünwald staining.

Case report

The proband (male) displayed facial dysmorphia soon after birth, with deformed chest, calcaneovalgus deformity of both legs, dysplasia of hip bones, glandular hypospadia and slight cutaneous

syndactyly between the second and third toes. From the second month of life onward slight hepatomegaly, muscular hypertonia, psychomotor retardation and failure to thrive were noted. There were signs of dysostosis multiplex on X-ray, sinistroscoliosis of the thoracic spine, fish-like deformity of vertebral bodies and metaphyseal broadening of long bones. The corneas were clear, and blood chemistry gave normal results except for slightly pathologic liver function tests (AST 72 i.u./l, ALT 168 i.u./l, GMT 108 i.u./l). There was pronounced vacuolation of lymphocytes with clear and semidense vacuoles, slight generalized aminoaciduria, mild nonspecific oligosaccharidurie and mild elevation of heparan and dermatansulphates in the urine. The skin biopsy showed lysosomal storage in fibroblasts and Schwann cells of nonmyelinated nerves and in the vascular endothelium. The lysosomal content was pleiomorphic with the typical ring-like membranous profiles [6]. Eccrine glands were intact. A set of lysosomal enzymes displayed massive increases in activity in the blood serum, most markedly expressed in the case of alpha mannosidase (225×). The infant suffered from repeated respiratory infections and an episode of EB virosis with increased liver function tests. He died of bronchopneumonia at the age of 16 months.

Results

Autopsy was performed 2 days after death. Macroscopy was dominated by bilateral pneumonia and moderate hepatomegaly with steatosis.

Despite the 2-day interval between death and autopsy, the most critical sites of autolysis, the gastric mucosa and exocrine pancreas, had perfectly conserved structure. The cytoplasmic appearance of the storage cells ranged from purely vacuolar to purely granular, with many intermediate steps. The granular-type storage process in pure form was difficult to recognize in routinely stained histological sections, especially in cells that display granular cytoplasmic structures normally, such as zymogenic secretory granules. There was the typical pronounced foamy transformation of glomerular podocytes by lucent vacuoles and a granular-type storage in cardiomyocytes resulting from deposition of nonfluorescent, ultrastructurally pleiomorphous material with rare membranous lipid bodies. Hepatocyts displayed apolar nonspecific steatosis. In samples of the brain cortex no pathology was found.

There was extensive uniform storage of the vacuolar type in the acinar exocrine pancreatic cells, with a variable admixture of granular material in the vacuoles. The cytoplasm of the cells in the serous parts of the tracheal and bronchial mucosal glands and of the sublingual gland was distended and predominantly finely granular. Storage with almost lucent vacuoles was seen in the chief (oxyntic) cells of the gastric fundal glands (Figs. 1–3). EM Showed lysosomes with mostly discontinuous pleiomorphic content encompassing membranous structures of vesicular, multilamellar and annular types with a tendency to fragmentation (Fig. 4). The limiting membrane was partly preserved. The size of the expanded lysosomes significantly exceeded that of the persisting serous zymogenic granules of normal appearance, which were generally seriously depleted and only exceptionally present (Fig. 5).

The granular-type stored material tested (cardiocytes, pancreas, salivary gland) was nonfluorescent, isotropic, slightly PAS+, slightly argyrophilic, negative either with Oil Red O or with aldehyde fuchsin after permanganate preoxidation.

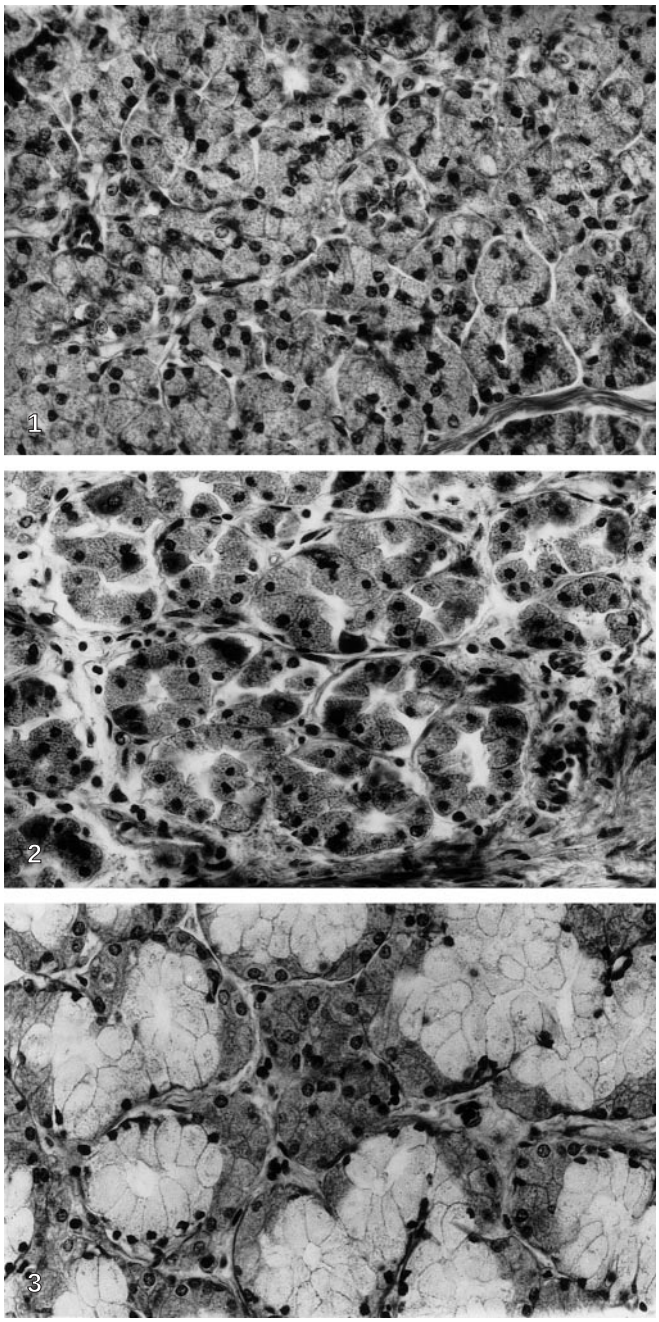

Fig. 1 Pancreatic acini. Fine uniform vacuolation of the acinar cells with a very discrete eosinophilic content. Note intactness of the structure. Hematoxylin-eosin, ×750

Fig. 2 Base of gastric fundal gland. Absence of autolysis. The oxyntic cells are systematically finely vacuolated. The parietal cells dominate as darkly stained disperse elements. Trichrome, ×750

Fig. 3 Sublingual salivary gland. Intact mucous secretory cells and slightly distended finely granular zymogenic cells. Hematoxylin-eosin stain, ×750

Pancreatic islet cell were unaffected histologically: as the aldehyde fuchsin stain showed relatively compact granular staining. Other cell types in the glands exam-

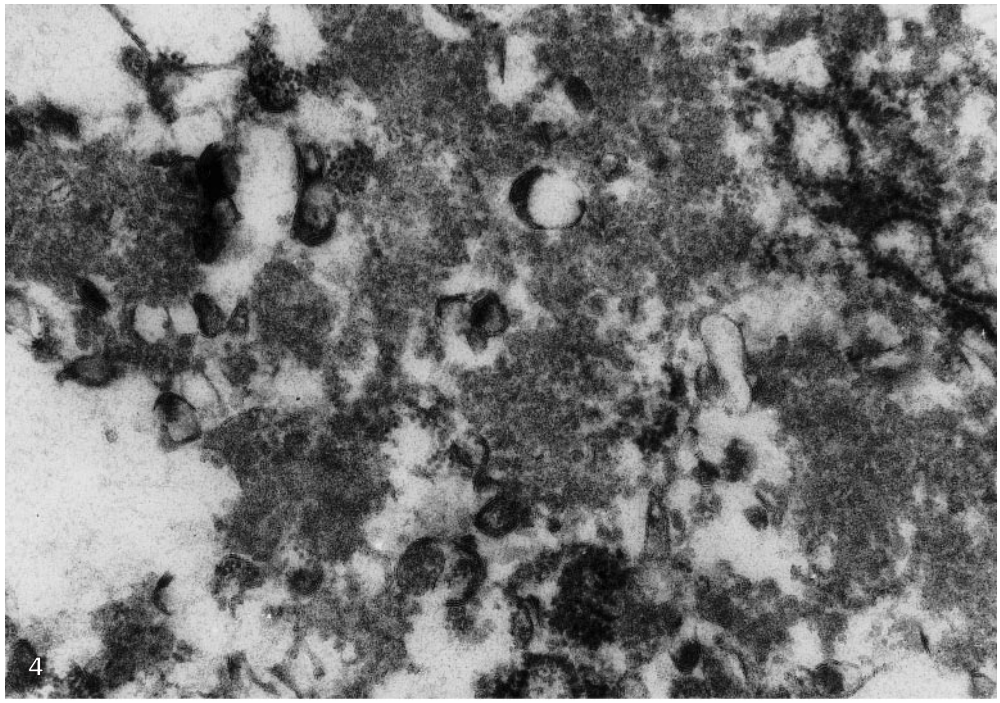

Fig. 4 Electronogram. Higher magnification of the residual storage material in the pancreatic lysosomes. Note the discrete membranous substructure with tendency to annular configuration. ×44,000

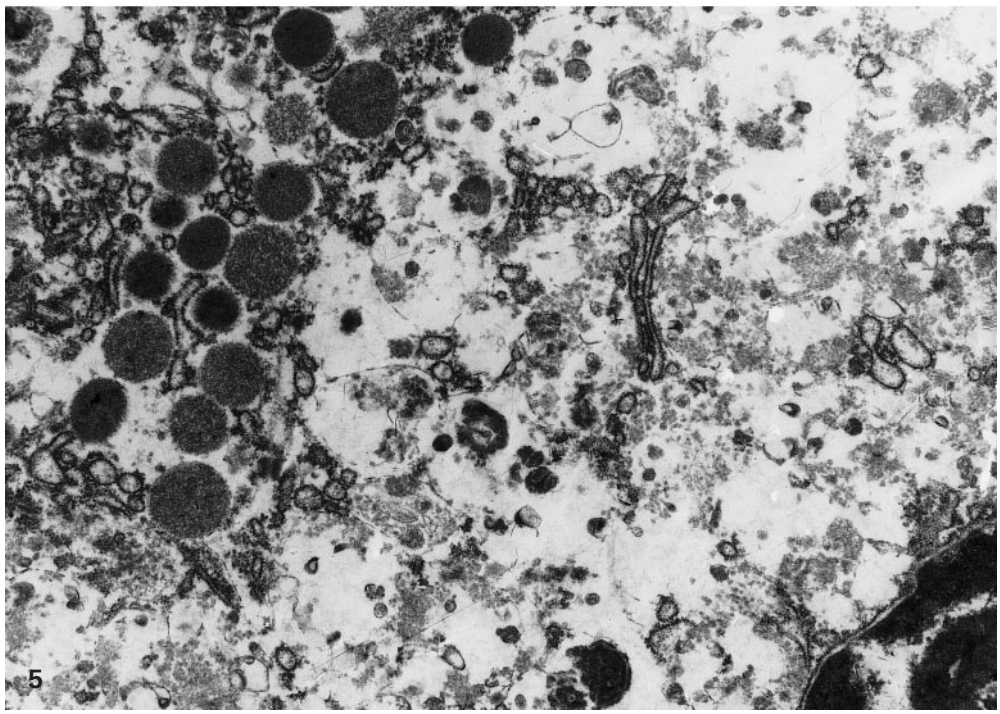

Fig. 5 Electronogram. Pancreatic acinar cell with signs of lysosomal storage. The lysosomes are of almost lucent type. Some exceptionally persisting secretory granules are also seen. ×10,000

ined were either unaffected (mucous cells) or harboured a few storage lysosomes detectable in EM (parietal cells). The colonic epithelium was normal.

The pancreatic cells in the previously published cases of MLII [7, 8] also displayed a predominantly vacuolar type of storage. The ventricular oxyntic cells were uniformly finely vacuolated. The Paneth cells harboured dense eosinophilic granules of typical appearance.

Discussion

In ML II there is marked involvement of the system of serous exocrine glands by lysosomal storage, leading to depletion of the secretory granule pool. The reported morphological spectrum of the expanding lysosomal content ranges from the lucent type, generally supposed to be caused by partly degraded low molecular weight glycoconjugates [5] or membranous fragments distinct from the lipid membranous bodies and consisting probably of proteins. The histochemical properties of deposits

of this type in our case (absence of both sudanophilia and autofluorescence) were not compatible with those established for lipopigments [2].

Comparison with data from the literature is difficult, as the serous glands have never been included in the list of tissues examined. Revision of the archival paraffin samples from previously published cases [7, 8] showed the same mode and degree of involvement of the exocrine pancreas and the oxyntic cells of gastric fundal glands, indicating a regular incidence of the lysosomal storage in ML II in the serous type of exocrine glands. Storage has also been identified in exocrine pancreas in a revised case of ML II (B.D. Lake, personal communication).

Involvement of serous glands by lysosomal enzymopathies is a very common phenomenon. It has been monitored systematically in the pancreas, which seems to be affected particularly often [4]. This suggests a high lysosomal turnover, indicating an important role of lysosomal enzymes in these secretory cells. Gastric glands have not been examined systematically in storage diseases. Discrete involvement has been demonstrated in sulphatidosis [1] (unpublished observations), in Niemann-Pick disease type C, and in late infantile neuronal ceroid lipofuscinosis (M. Elleder, unpublished observation).

Clinical consequences of serous gland involvement are likely to be mainly in the form of malabsorption, which was not tested for in our patient, whose symptomatology was governed by failure to thrive. Gastrointestinal symptoms are briefly mentioned only by Martin et al. [7]. It is suggested that pancreatic and gastric secretions should be included in the list of clinical examinations in patients with ML II disease. It is tempting to assume that the cause of the minimal autolysis may have been a serious deficiency of proteolytic enzymes of the respective serous granules.

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