Arch Gynecol Obstet (2005) 271: 259–261
DOI 10.1007/s00404-004-0620-3

CASE REPORT

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Prenatal diagnosis of Pompe disease by electron microscopy

Received: 14 October 2003 / Accepted: 11 March 2004 / Published online: 14 May 2004

Abstract *Introduction*: Pompe disease is one of the lysosomal storage disorders caused by α-glucosidase deficiency. The disease is characterized by accumulation of glycogen in the lysosome. The accumulation has unique ultrastructural features, which enable a prenatal diagnosis possible by electron microscopy. *Materials and methods*: A prenatal diagnosis of Pompe disease by electron microscopic study of chorionic villus biopsies is described in a fetus of a mother whose previous child had died of the disease. *Results*: Electron microscopy revealed fibrocytes with typical vacuoles filled with glycogen. A prenatal diagnosis of Pompe disease was made and subsequently confirmed by the autopsy study of the abortus. *Conclusion*: We report the usefulness of electron microscopy for prenatal diagnosis in the first trimester of Pompe disease.

Keywords Prenatal diagnosis · Pompe disease · Electron microscopy · Chorionic villus sampling

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Introduction

Pompe disease (OMIM 232300) or glycogen storage disease type II is one of more than 40 distinct genetic diseases, referred to as lysosomal storage disorders. It is an autosomal recessive disorder of glycogen metabolism, caused by deficiency of lysosomal acid α-glucosidase. Patients with Pompe disease are unable to degrade glycogen stored in the lysosome, leading to the accumulation of this substrate in lysosomal storage vacuoles. As a result, there is an increase in the size and number of lysosomes in the cell [8].

The estimated incidence of Pompe disease is 1 in 40,000 births [1, 6]. A prenatal diagnosis can be made by several methods. Herein, we report the use of electron microscopy in a prenatal search for Pompe disease in a fetus whose mother previously had a child affected by the disease.

Case report

Case evaluation

A 24-year-old woman had her first child with Pompe disease (case 1 in [10]).The baby boy was subsequently delivered by uncomplicated spontaneous vaginal delivery. At the first admission at the age of 7 months, the child had pneumonia, hypotonia, cardiomegaly with congestive heart failure. The diagnosis was made by electron microscopy, which demonstrated vacuoles filled with glycogen from his skin biopsy. He died of cardiopulmonary failure with septicemia at the age of 22 months.

One year later, the mother became pregnant. After extensive counseling about the risk of Pompe disease in her second pregnancy, she and her husband chose to undergo a prenatal diagnosis. After informed consent was obtained, the chorionic villus sampling was per-

formed at 11 weeks' gestation without complications. The material obtained was sent for electron microscopic study.

Electron microscopic study and results

Five milligrams of the tissue were fixed immediately in 3% glutaraldehyde with 0.1 M phosphate (pH 7.2). The fixed cells were routinely processed and embedded in plastic. Thin sections were stained with lead citrate and examined under electron microscopy. Vacuoles filled with glycogen were identified in the fibrocytes (Fig. 1).

After knowing the result, the parents opted to confirm the study by amniocentesis. Due to inadequate amniotic cells sampled, the result of the amniocentesis performed at 16 weeks' gestation was unsuccessful. However, based on the result of the electron microscopic study, the parents finally decided to terminate the pregnancy. The induced abortion with vaginal misoprostal 200 μg every 8 h was initiated. A male abortus was aborted 24 h later without maternal complication.

At the postabortion examination, the body weighted 500 g. No gross abnormalities were detected. However, distinctive changes on routine hematoxylin and eosin stain similar to those of the previous child were observed; a few distended neurons were detected in the deep gray structures of the brain. In addition, Periodic acid Schiff (PAS) staining method highlighted increased PAS-positive diastase-sensitive intracytoplasmic glycogen granules in the striated and cardiac muscles, the hepatocytes, as well as the abnormal neurons (Fig. 2). The distribution of glycogen accumulation is consistent with that of Pompe disease.

Discussion

Pompe disease can present as infantile, juvenile, or adult onset forms [8]. The heterogeneous presentation of Pompe disease is from the different mutations in the lysosomal acid α-glucosidase gene [11]. The infantile onset form is characterized by massive cardiomegaly, macroglossia, progressive muscle weakness (including respiratory muscles), and marked hypotonia, with death occurring within the first 2 years of life as found in our case. The juvenile and adult onset forms manifest as slower progressive muscular disorders that are limited to skeletal muscles, and death usually occurs from respiratory failure [8].

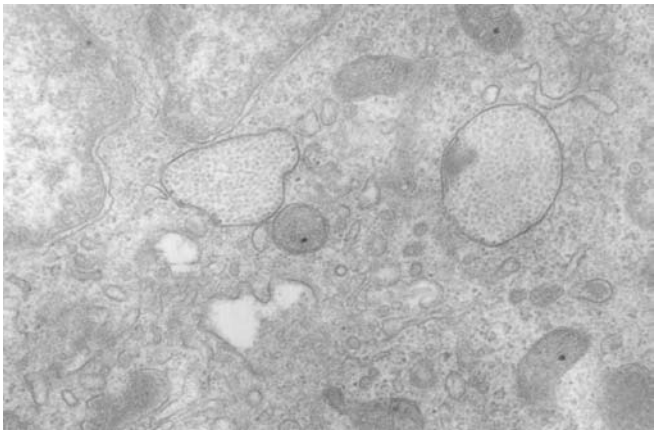

Fig. 1 Electron microscopic finding: vacuoles filled with glycogen in the fibrocytes

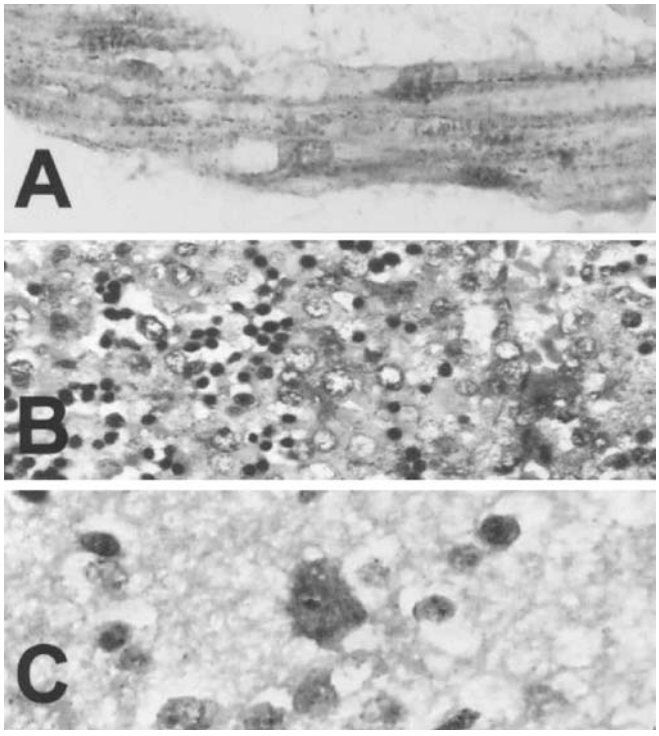

Fig. 2A–C Light microscopic findings of Pompe disease. Periodic acid Schiff (PAS) stained sections demonstrate increased glycogen in **A** striated muscle fibers and **B** hepatocytes. **C** PAS-positive glycogen granules in the cytoplasm are depicted

The definite treatment for Pompe disease is currently unavailable in the world. However, two main treatment strategies are being proposed. Enzyme-replacement therapy, which has shown promising results in animal studies, is currently undergoing Phase II clinical trial in Pompe patients. Gene transfer of the α-glucosidase gene with viral vectors is being pursued, with successful correction of several cell types in vitro being reported [9]. Hence, the early and rapid prenatal diagnosis of this disease is very important.

Our first affected baby had a severe form, infantile onset of Pompe disease. He died at the age of 22 months. The disease is an autosomal recessive disorder of glycogen metabolism. Severity of the first child was a main factor influencing the parents' decision in the next pregnancy. Due to the 25% recurrence risk, they decided to undergo a prenatal diagnostic test for their second child.

Many methods have been reported to be able to diagnose fetus with Pompe disease. Prenatal diagnosis

can be made by the determination of the acid α-glucosidase activity in cultured amniotic cells and/or in chorionic villus biopsies [5, 7] and also by mutation analysis [5]. Recently, the electron microscopy of uncultured amniotic cells or chorionic villus biopsies has been proposed to enhance the rapid prenatal diagnosis [3, 4]. Many procedures, including chorionic villus sampling (CVS), amniocentesis and cordocentesis, can obtain embryonic or fetal cells for such studies. With the risks of complications in CVS, early amniocentesis, second trimester amniocentesis and cordocentesis of 3.7, 2.5, 0.5, and 2.7% [2] respectively; the couple chose to undergo a CVS in the first trimester. Chorionic villus tissue was collected for electron microscopic study. The result in this second pregnancy was positive for Pompe disease. This prenatal electron microscopic study was confirmed by the autopsy of the abortus.

In conclusion, we report the use of first trimester prenatal diagnosis with electron microscopic study in Pompe disease. The result of the prenatal diagnosis allows counseling, reassuring the families and offering the choice of termination of pregnancy.

Acknowledgements The authors wish to thank Ms. Vanee Wattanaseamkit for her preparation of the pathological slides.

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