PRENATAL EXCLUSION OF POMPE DISEASE BY ELECTRON MICROSCOPY

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Abstract. Pompe disease is a lysosomal storage disorder caused by α-glucosidase deficiency. The disease is characterized by accumulation of glycogen in the lysosomes. The accumulation has unique ultrastructural features which enable a prenatal diagnosis by electron microscopy. We describe prenatal electron microscopic testing in a fetus of a mother whose previous child died of Pompe disease. The disease in the affected child was diagnosed by a decrease in α-glucosidase activity of his skin fibroblasts. Electron microscopy of the chorionic villus sample and amniocytes revealed normal findings, thus predicting an unaffected fetus. The study was confirmed by the birth of a normal neonate who was still healthy at the age of 12 months. Electron microscopy is useful in the first and second trimesters to exclude Pompe disease prenatally. This test can be use prenatally and provides families with reassurance.

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 (Reuser and Hirschhorn, 2001).

The estimated incidence of Pompe disease is 1 in 40,000 births (Martiniuk *et al*, 1998; Ausems *et al*, 1999). A prenatal diagnosis can be made by several methods. Herein, we report the use of prenatal electron microscopic testing in a fetus whose mother previously had a child affected by Pompe disease.

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MATERIALS AND METHODS

Case evaluation

A 31-year-old woman had a previous child with Pompe disease.

The baby boy was subsequently delivered by uncomplicated spontaneous vaginal delivery. He developed hypotonia and symptoms of congestive heart failure at the age of 4 months. He later developed macroglossia and suffered from recurrent pneumonia. ECG showed a short PR interval and giant QRS waves. The diagnosis of Pompe disease was made by electron microscopy, which demonstrated vacuoles filled with glycogen from his skin biopsy (Fig 1), and confirmed by decreased enzymatic activity of fibroblast acid α-glucosidase (0.09 nmol/mg protein/hour; normal range >60.00). The patient died of cardiac failure at the age of 11 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, chorionic villus sampling was performed at 11 weeks gestation without complications. The material obtained was sent for electron microscopy and chromosome studies. No abnormalities were detected on electron microscopy. Chromosome studies could not be done due to no growth of cells from the chorionic villus sampling specimen. The parents opted to perform an amniocentesis for chromosome studies. The amniocentesis was performed at 16 weeks gestation without complications. One part of the amniotic fluid was sent for chromosome studies. The other part was sent for electron microscopy. The results revealed a normal karyotype (46,XX) and normal electron microscopy. The pregnancy was continued without complications.

At 38 weeks gestation, the mother delivered a normal appearing female infant weighing 3,050 g with apgar scores of 9 and 10 at 1 and 5 minutes by spontaneous vaginal delivery. The baby was still normal at 12-months follow-up.

RESULTS

Electron microscopy and results

Five milligrams of tissue was 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. There was no vacuoles filled with glycogen identified in the fibrocytes.

Ten milliliters of amniotic fluid was centrifuged and 5 mg of amniocytes were proceed for electron microscopy, the same as the fibrocytes from the CVS. There were no vacuoles filled with glycogen identified in the amniocytes either.

Fig 1–Electron microscopic findings of the skin biopsy from the affected child. Skin biopsy demonstrated vacuoles filled with glycogen (arrows).

DISCUSSION

Pompe disease can present as infantile, juvenile, or adult onset forms (Reuser and Hirschhorn, 2001). The heterogeneous presentation of Pompe disease is from the different mutations in the lysosomal acid α-glucosidase gene (Umapathysivam *et al*, 2000). 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 patient's first child. 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 (Reuser and Hirschhorn, 2001; Shotelersuk *et al*, 2002).

Enzyme-replacement therapy has recently become available. The cost is extremely high and the results need further confirmation. Hence, early and rapid prenatal diagnosis of this disease is very important.

Our affected baby had a severe form of infantile onset Pompe disease. He died at the age of 11 months. The disease is an autosomal recessive disorder of glycogen metabolism. The severity of the first child was the main factor influencing the parents' decision in the next pregnancy. Due to a 25% recurrence risk, they decided to undergo a prenatal diagnostic testing for their second child.

Many methods have been reported to diagnose Pompe disease in a fetus. Prenatal diagnosis can be made by the determination of the acid α-glucosidase activity in cultured amniotic cells and/or in chorionic villus biopsies (Park *et al*, 1992; Kleijer *et al*, 1995) and also by mutation analysis (Kleijer *et al*, 1995). Recently, electron microscopy of uncultured amniotic cells or chorionic villus biopsies has been proposed to enhance rapid prenatal diagnosis (Hug *et al*, 1984, 1991; Phupong *et al*, 2005). Many procedures, including chorionic villus sampling (CVS), amniocentesis and cordocentesis, can obtain embryonic or fetal cells for such studies. With the risks of complications with CVS, early amniocentesis, second trimester amniocentesis and cordocentesis of 3.7, 2.5, 0.5 and 2.7%, respectively (Cunningham *et al*, 2004), the couple chose to undergo CVS in the first trimester. Chorionic villus tissue was collected for electron microscopy. The result in this second pregnancy was negative for Pompe disease. The prenatal electron microscopy was confirmed by normal physical examination at 12-months of age.

The downside of CVS in this case was the inability to perform chromosome studies. This may have been due to an inadequate sample, which is found in 0.7-1.9% (Cunningham *et al*, 2004). This leads couples to decide for an amniocentesis, another prenatal diagnosis test.

In conclusion, we report the usefulness of electron microscopy in the first and second trimester to exclude Pompe disease prenatally. The results of prenatal testing allow counseling and reassurance of families.

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