Enzyme Inhibitory Assay Using Monoclonal Antibody Against Acid α-D-Glucosidase in Prenatal Diagnosis to Identify Homozygotes of Pompe’s Disease

CHI-YING WEI, GUANG-PENG YEH,1 HSU-FENG CHEN,2 LING-YUE WANG3 and CHING-YUANG LIN3

From January 1985 to January 1990, measurements of acid α-D-glucosidase activity in amniocytes or chorionic villus samplings were done for 24 pregnant mothers who were carriers of Pompe’s disease. 6 women had two subsequent pregnancies. Amniotic fluid was obtained by transabdominal amniocentesis performed on 10 of them, while chorionic villus samplings were obtained in the other 20. The results showed that 7 (23.3%) cases were homozygotes, 16 (53.4%) cases were heterozygotes, and 7 (23.3%) cases were normal. Pregnancies were terminated in the homozygotic group. Final diagnosis was confirmed by either skin fibroblast culture or clinical course. However, we found that there was overlap in the acid α-D-glucosidase activity of amniocytes between homozygotes and heterozygotes due to residual activity of neutral α-D-glucosidase. In an attempt to identify heterozygotes for Pompe’s disease, we established an enzyme inhibitory assay using monoclonal antibody (mAb) against acid α-D-glucosidase. Comparing the differences in α-D-glucosidase activity before and after mAb treatment homozygotes were significantly lower than heterozygotes (P<0.001). There was no more overlap in the difference of acid α-D-glucosidase activity before and after mAb treatment between heterozygotes and homozygotes in amniocytes. This modified enzyme inhibitory assay should facilitate homozygote detection. Comparing acid α-D-glucosidase activity between CVS and amniocytes, the enzyme activity in CVS is about 5 times higher than in...
amniocytes. There was no overlap in the acid α-D-glucosidase activity between homozygotes and heterozygotes. Therefore, CVS is better than amniocentesis in the prenatal diagnosis of Pompe’s disease (Acta Paed Sin 1992; 33:104-11).

Key words: Pompe’s disease, acid α-D-glucosidase, amniocentesis, chorionic villi sampling

INTRODUCTION

Glycogen storage disease type II, Pompe’s disease (McKusick 23230) is an autosomal recessive disorder characterized by impaired glycogen degradation due to a deficiency of lysosomal acid 1,4 alpha-D-glucosidase.1 This lysosomal enzyme has a pH optimum around 4. Several clinical forms of Pompe’s disease different in age of onset, organ involvement, and progression of the disease have been found. In Taiwan, Pompe’s disease is the most common type of glycogen storage disease. Most of the cases are the infantile form (generalized glycogenosis II), nearly all symptoms becoming apparent shortly after birth. Glycogen storage is found in virtually all organs, with muscular weakness, cardiomegaly, hepatomegaly, and macroglossia usually present. Cardiac failure or respiratory tract complications caused by the extensive accumulation of glycogen results in early death in the first year of life. Till now, no practical treatment is available. However, prenatal diagnosis is possible. Therefore, how to make early and accurate prenatal diagnosis of Pompe’s disease is important.

α-D-glucosidase has two major forms. One is acid α-D-glucosidase, the other neutral α-D-glucosidase. The optimal pH value of neutral α-D-glucosidase is pH 6.

The method used for the prenatal diagnosis of Pompe’s disease is the measurement of acid α-D-glucosidase activity either in cultured amniocytes6 or in chorionic villus samples.7,8 However, it is sometimes difficult to differentiate heterozygotes from homozygotes due to their overlapping values of enzyme activity even under the condition of pH 4. There is still residual activity of neutral α-D-glucosidase which comprises up to 15% of the total activity.7 In an attempt to determine a more accurate method for the recognition of homozygotes, we previously established a monoclonal antibody (mAb) against acid α-D-glucosidase which was able to inhibit acid α-D-glucosidase activity. We found that enzyme inhibitory assay could help us to make an accurate prenatal diagnosis. We thought it worthwhile to

MATERIALS

Patient Selection

From 1987 to 1990, there were carriers of Pompe’s disease identified by transabdominal chorionic villus sampling.

Amniocytes

Amniocytes were obtained between 16 and 24 weeks of gestation. They were cultured in Eagle’s minimum essential medium (MEM) containing 15% fetal calf serum (FCS) and antibiotics. Seven days later, the cells were harvested, washed with ice-cold MEM containing 15% FCS, and used for subsequent assays.

Chorionic Villus Sampling

CVS was performed under the preliminary guidance of ultrasonography and located transabdominally by ultrasound techniques. The chorion from a section of chorionic villus fragments was cut, placed in maternal dextran-coated flask, and observed under microscope, Research, USA. After washing with Eagle’s MEM containing 15% FCS, the supernatant was used for enzyme assay.

α-D-Glucosidase

The α-D-glucosidase activity was determined in amniocytes and chorionic villi samples using a specific colorimetric method.
report our experience.

MATERIALS AND METHODS

Patient Selection

From January 1985 to January 1990, thirty pregnant mothers who were carriers of Pompe's disease, were included. Amniotic fluid was obtained by transabdominal amniocentesis performed on ten of them while chorionic villus samples were obtained in the other twenty.

Amniocytes Culture

Amniotic fluid obtained by transabdominal amniocentesis was obtained between the 14th and 18th gestational weeks under sonographic guidance. Cells were collected by centrifugation and cultures were maintained in 37°C, 5% CO₂ incubator with RPMI 1640 supplemented with 15% fetal calf serum. Subcultured amniocytes were used in the measurement of acid α-D-glucosidase activity.

Chorionic Villus Sampling (CVS)

CVS was performed as an outpatients procedure and the chorionic tissue was obtained between the 8th and the 12th week of gestation. A preliminary sonographic examination determined embryonic age and vitality and located the trophoblast. All CVS procedures were performed using a transcervical aspiration technique. Villus tissue was obtained under ultrasound guidance by passing a Portex catheter transcervically to the chorion frondosum. Suction was applied using a 20 ml syringe to aspirate villus fragments as the catheter was withdrawn. Chorionic villi were separated from maternal desidual tissue using fine forceps under a dissecting stereo microscope, then stored at 4°C and transported to the Department of Medical Research, Veterans General Hospital. For enzyme assay, chorionic villi were washed with normal saline 3 times then pelleted in pH 4 citrate phosphate buffer and put into an eppendorf tube. The cell membranes were disrupted by ultrasonic sonificator then centrifuged at 1,300 rpm for 15 min. The supernatants were used in the measurement of acid α-D-glucosidase.

α-D-Glucosidase Assay

The activity of acid α-D-glucosidase was assayed with 4-
methylumbelliferyl-\(\alpha\)-D-glucopyranoside as substrate with the following modifications.\(^4\) Twenty-microliters cell suspensions or homogenated muscle tissues were incubated with 50 \(\mu\)l of 1mM 4-methylumbelliferyl-\(\alpha\)-D-glucopyranoside in citrate phosphate buffer pH 4 and 6 for 2 hours adding 1 ml of 400 mM glycine-NaOH buffer, pH 10.5. Fluorescence was determined in a Hitachi or an Aminco-Bowmen spectrofluorometer with an excitation wavelength of 360 nm and an emission wavelength of 450 nm. The standard contained 0.05 mM (8.81 mg/liter) of 4-methylumbelliferone in 0.05 M glycine carbonate buffer. Protein was determined by the method of Lowry et al.\(^10\)

**Enzyme Inhibitory Assay of \(\alpha\)-D-Glucosiaase**

mAb (8-23) against acid \(\alpha\)-D-glucosidase were prepared in our laboratory previously.\(^11\) This mAb can recognize 70 KD mature form acid \(\alpha\)-D-glucosidase by immunoprecipitation. The isotype determinant of this mAb was IgG1 in immunoglobulin subclass. Ascites of hybridoma of this mAb were obtained by an intraperitoneal injection of 1x10⁷ hybrid cells into BALB/c mice. The mice were sacrificed after 7 to 10 days, and the ascites were collected, centrifuged, passed through a Sephadex G-25 column and purified by the protein A method. Briefly, enzyme inhibitory assay of fifty microliters of tissue extract either from amniocytes or chorionic villi were incubated with the same amount of mAb in pH 4 citrate phosphate buffer then centrifuged shortly. The supernatant was used for the determination of the acid \(\alpha\)-D-glucosidase activity.

**Confirmation Test**

The final diagnosis was confirmed by either skin fibroblast cultrue of the aborted fetus or enzyme assay of mononuclear cells obtained postnatally as shown in Table 2 and 4.

**Controls**

Either amniocytes or CVS were used as controls each time. Acid \(\alpha\)-D-glucosidase measurements were obtained from 30 healthy normal pregnant women. The indications for either amniocentesis or CVS sampling in these controls were being a carrier of hereditary disease other than Pompe's disease, chromosome anomaly and age over 35. They also proved to have normal acid \(\alpha\)-D-glucosidase activity.

**RESULTS**

**Prenatally Determined**

A total of 20 women were enrolled in the study. All women had normal pregnancies required only monitoring. No abnormalities were found either in the amniocytes or CVS.

**Controls**

The final diagnosis was confirmed by either skin fibroblast cultrue of the aborted fetus or enzyme assay of mononuclear cells obtained postnatally as shown in Table 2 and 4.

**Statistical Analysis**

The results are expressed as mean ± standard error. The statistical analysis was performed using Student's t-test, or student's t'-test, according to the data being compared across groups being analyzed.

**Enzyme Inhibitory Assay**

For enzyme inhibitory assay, acid \(\alpha\)-D-glucosidase activity using tissue extract from both homozygotes and heterozygotes was incubated for 2 hours with mAb, and then centrifuged. The supernatant was used for the determination of the acid \(\alpha\)-D-glucosidase activity.
Statistical Analysis

The differences between groups were assessed by the approximate t test, or student's t test, depending on the equality of the variances of the two groups being compared.

RESULTS

Prenatally Diagnosed Abnormalities

A total of 24 women who were carriers of Pompe's disease, were enrolled in this study, including 10 amniocentesis and 20 CVS subjects. 6 women had two subsequent pregnancies. All the cases undergoing CVS required only one catheter insertion. There were no unsuccessful samplings either in the amniocentesis or CVS group.

Results of the genetic counselling in the amniocentesis and CVS groups showed 3 cases of homozygotes, 5 cases of heterozygotes and 2 cases normal in the amniocentesis group and 4 cases of homozygotes, 11 cases of heterozygotes and 5 cases normal in the CVS group (Table 1). The total homozygotes were 23.3%, heterozygotes 53.4% and normal 23.3%. Comparing acid α-D-glucosidase activity between amniocytes and CVS, CVS had about 5 times higher enzyme activity then amniocytes (Table 2). There was no overlap in the acid α-D-glucosidase activity of CVS between homozygotes and heterozygotes. However, we found that there was overlap in the acid α-D-glucosidase activity of amniocytes among homozygotes, heterozygotes and normal fetus as shown in Table 2.

Enzyme Inhibition Assay with the Addition of mAb

For the differentiation among homozygotes, heterozygotes and normal fetuses in amniocytes, we added mAb to inhibit acid α-D-glucosidase activity using mAb enzyme inhibitory assay. The groups of homozygotes and heterozygotes could be distinguished clearly as shown in Table 3. After incubation with mAb, there was a statistical difference of reduced enzyme activity between homozygotes and heterozygotes.
Table 1. Results in Prenatal Diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Homozygote</th>
<th>Heterozygote</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniocentesis (n=10)</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>CVS (n=20)</td>
<td>4</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Percentage</td>
<td>23.3% (7/30)</td>
<td>53.4% (16/30)</td>
<td>23.3% (7/30)</td>
</tr>
</tbody>
</table>

Table 2. Activity of the Acid α-D-Glucosidase (pH 4) in Cultured Amniocytes, CVS and/or Lymphocytes After Birth [Mean ± SD (Range)]

<table>
<thead>
<tr>
<th></th>
<th>Culture Amniocytes</th>
<th>CVS</th>
<th>Lymphocytes (After Birth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygotes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.72±0.23</td>
<td>8.32±3.16</td>
<td>N.D.</td>
</tr>
<tr>
<td>(0.22-9.32) n=3</td>
<td>(1.62-18.68) n=4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygotes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.62±5.34</td>
<td>60.42±8.76</td>
<td>10.12±3.82</td>
</tr>
<tr>
<td>(8.96-29.42) n=5</td>
<td>(31.91-75.52) n=11</td>
<td></td>
<td>(7.12-14.32)</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.61±58.34</td>
<td>174.21±21.42</td>
<td>24.12±8.14</td>
</tr>
<tr>
<td>(27.61-58.34) n=2</td>
<td>(78.35-479.21) n=11</td>
<td></td>
<td>(19.14-32.62)</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.32±8.16</td>
<td>165.72±23.42</td>
<td>24.62±10.32</td>
</tr>
<tr>
<td>(27.45-63.21) n=15</td>
<td>(76.21-434.16) n=5*</td>
<td></td>
<td>(18.32-37.14)</td>
</tr>
</tbody>
</table>

α-D-glucosidase was measured in n mole/mg protein/hr
* 10 of CVS control group woman received amniocentesis between the 14th and 18th gestational weeks
N.D.: not done

Follow-up

Seven cases of homozygote which were diagnosed either by amniocytes or CVS, received termination of the pregnancies. The enzyme activity in muscle and cultured fibrobiasts are shown in Table 4. The 16 heterozygotes and 7 normal fetuses were fullterm delivery. All of them were regularly followed up in the outpatient clinic for more than one and half years. Till now, there has been no development of Pompe's disease. There was also no complication after CVS and amniocentesis. The acid α-D-glucosidase measurement from Patient's peripheral blood lymphocytes confirmed the results of prenatal diagnosis (Table 2).

DISCUSS

β-glucosidase of Chinese a

Table 4. Activity of the Acid α-D-Glucosidase (pH 4) in Cultured Amniocytes, CVS and/or Lymphocytes After Birth [Mean ± SD (Range)]

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<td>8.32±3.16</td>
<td>N.D.</td>
</tr>
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<td>(1.62-18.68) n=4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygotes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
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</tr>
<tr>
<td>(27.45-63.21) n=15</td>
<td>(76.21-434.16) n=5*</td>
<td></td>
<td>(18.32-37.14)</td>
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</tbody>
</table>

α-D-glucosidase was measured in n mole/mg protein/hr
* 10 of CVS control group woman received amniocentesis between the 14th and 18th gestational weeks
N.D.: not done

Follow-up

Seven cases of homozygote which were diagnosed either by amniocytes or CVS, received termination of the pregnancies. The enzyme activity in muscle and cultured fibrobiasts are shown in Table 4. The 16 heterozygotes and 7 normal fetuses were fullterm delivery. All of them were regularly followed up in the outpatient clinic for more than one and half years. Till now, there has been no development of Pompe's disease. There was also no complication after CVS and amniocentesis. The acid α-D-glucosidase measurement from Patient's peripheral blood lymphocytes confirmed the results of prenatal diagnosis (Table 2).
Table 3. Decreased α-D-Glucosidase Activity (pH 4) Before and After Incubation with mAb (8-23) in Culture & Amniocytes [Mean ± SD (Range)]

<table>
<thead>
<tr>
<th></th>
<th>Before Incubation with mAb (8-23)</th>
<th>After Incubation with mAb (8-23)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygotes</td>
<td>4.72±0.23* (0.22-9.32)</td>
<td>0.12±0.06 (0.03-1.40)</td>
<td>0.58±0.14** (0.19-7.92)</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>14.62±5.34 (8.96-29.42)</td>
<td>3.12±0.48 (0.04-4.42)</td>
<td>17.12±3.45** (8.90-25.00)</td>
</tr>
<tr>
<td>Controls</td>
<td>35.32±8.16 (27.45-63.21)</td>
<td>5.32±1.28 (4.12-9.52)</td>
<td>37.12±8.46* (23.42-52.71)</td>
</tr>
</tbody>
</table>

*: mean ± SD; α-D-glucosidase was measured in n mole/mg protein/hr difference = (before) - (after)

**.: P<0.001

Table 4. Activities of Acid α-D-Glucosidase Either in Chorionic or Cultured Amniocytes, Fibroblasts and Muscle Cells [Mean ± SD (Range)] in Aborted Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Chorionic villi</th>
<th>Amniocytes</th>
<th>Fibroblast</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.65</td>
<td>0.70</td>
<td>2.66</td>
<td>0.432</td>
</tr>
<tr>
<td>2</td>
<td>4.62</td>
<td>—</td>
<td>2.14</td>
<td>0.26</td>
</tr>
<tr>
<td>3</td>
<td>9.72</td>
<td>—</td>
<td>3.18</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>89.59</td>
<td>—</td>
<td>3.09</td>
<td>0.28</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>0.22</td>
<td>0.82</td>
<td>0.12</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>2.86</td>
<td>1.12</td>
<td>0.20</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>9.32</td>
<td>2.86</td>
<td>0.30</td>
</tr>
<tr>
<td>Normal</td>
<td>165.72±23.42</td>
<td>35.32±8.16</td>
<td>58.42±16.74</td>
<td>14.12±5.32</td>
</tr>
<tr>
<td></td>
<td>(n=25)</td>
<td>(n=15)</td>
<td>(n=15)</td>
<td>(n=10)</td>
</tr>
<tr>
<td></td>
<td>(76.21±434.16)</td>
<td>(27.45±63.21)</td>
<td>(35.21±84.36)</td>
<td>(10.32±21.64)</td>
</tr>
</tbody>
</table>

α-D-glucosidase was measured in n mole/mg protein/hr

DISCUSSION

Pompe's disease type II glycogenosis, is one of the most common types of glycogenosis in Taiwan. The disease involves mainly Southern Chinese and is rare in Northern Chinese and Japanese. In our estimation the

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frequency of heterozygotes in Taiwan is about 0.5% to 1%. This is a high frequency hereditary disease. Therefore, genetic counselling is important.

The prenatal diagnosis was performed by measurement of acid $\alpha$-D-glucosidase activity either from cultured amniocytes or CVS.\(^{6-9}\)

In the present study, we demonstrated 7 homozygotes, 16 heterozygotes and 7 normal diagnosed in the 30 pregnant mothers who were carriers of Pompe's disease. Although the assay of acid $\alpha$-D-glucosidase activity in amniocytes is useful in the detection of homozygotes and heterozygotes of this disease, we found, however, that there was overlap in the acid $\alpha$-D-glucosidase activity of amniocytes among homozygotes, heterozygotes and normal fetuses. This is due to residual activity of neutral $\alpha$-D-glucosidase up to 15% of the total activity in the homozygote group.\(^{7,13}\) Therefore, we tried to improve the sensitivity of the method using mAb against acid $\alpha$-D-glucosidase activity for a better differentiation of the homozygotes. If the acid form $\alpha$-D-glucosidase is in fact present, the enzyme activity in the homozygote is unchanged before and after mAb treatment. In the heterozygotes, the decrease of acid $\alpha$-D-glucosidase activity is higher than in the homozygotes after mAb treatment, which means the overlap portion is due to neutral form instead of acid form. This result suggests that mAb inhibitory assay is useful in the distinguishing of homozygotes and heterozygotes.

Prenatal diagnosis of lysosomal disease either using amniocytes or CVS is now a common procedure. The disadvantage of amniocentesis is late pregnancy termination, so psychological and physical complications can occur when a therapeutic abortion for homozygotic fetus is indicated. Therefore, evaluation of possible first-trimester prenatal diagnosis in lysosomal disease by CVS has been developed.\(^{7,9}\) Although some complications or problems of CVS can occur such as amniotic band syndrome, disturbed blood circulation resulting in anomaly, unreliable data due to maternal contamination, nevertheless, when the procedure was carefully and skillfully performed, such complications could be prevented. In the present study, we demonstrate in Table 2 that the acid $\alpha$-D-glucosidase activity in chorionic villi is about 5 times higher than in amniotic fluid cells.\(^{9}\) There is no overlap between homozygotes and heterozygotes. In addition, a further advantage for the first-trimester diagnosis is reducing complications of termination in pregnancy. Therefore, for the prenatal diagnosis of Pompe's disease CVS is better than amniocentesis.

In conclusion, genetic counselling and prenatal diagnosis of Pompe's disease is important.

CVS is better than amniocentesis. mAb inhibitory assay is useful in the distinguishing of homozygotes and heterozygotes.

REFERENCES


6. Fujim: First trimester prenatal diagnosis of glycogen.

7. Grub: First trimester prenatal diagnosis of glycogen.

8. Shin T: First trimester prenatal diagnosis of glycogen.

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6. Fujim: First trimester prenatal diagnosis of glycogen.

7. Grub: First trimester prenatal diagnosis of glycogen.

8. Shin T: First trimester prenatal diagnosis of glycogen.
disease is important in Taiwan due to its high frequency and poor prognosis. CVS is better than amniocentesis in the prenatal diagnosis of Pompe’s disease. mAb inhibitory assay should be used to facilitate homzygote detection.

ACKNOWLEDGMENTS: This study was partially supported by a grant from the National Science Council of the Republic of China (NSC 78-04120-B 075-81).

REFERENCES


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