Fabry disease: studies on diagnosis, screening and patients' perspectives
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ANALYSIS OF PLACENTAL TISSUE IN FABRY DISEASE WITH AND WITHOUT ENZYME REPLACEMENT THERAPY

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ABSTRACT

There are only a few reports on the histology of placental tissue of pregnancies from mothers with Fabry disease. Fabry disease is a lysosomal disorder caused by α-galactosidase A deficiency. Extensive glycosphingolipid (GSL) accumulation in fetal and maternal placenta tissue obtained from a Fabry mother and her affected male newborn has previously been reported. Here we report the evaluation of placenta tissue of two pregnancies in Fabry mothers, one of an unaffected male newborn (placenta A) and one of an affected female newborn (placenta B). The mother of the female affected offspring was treated with recombinant α-galactosidase A (enzyme replacement therapy, ERT) during the pregnancy (placenta B). Storage material was only detected in smooth muscle cells of the umbilical cord of placenta B. No accumulation was seen in both placentae. Combining these results with the outcome in two earlier described placentae, a heterogeneous picture emerges. This may be due to differences in disease severity in the mothers or severity of disease in their offspring. In addition, a possible effect of ERT on placental GSL accumulation could also explain lack of GSL storage in placenta B.
INTRODUCTION

Deficiency of the lysosomal enzyme α-galactosidase A (α -Gal A) leads to the X-linked lysosomal storage disorder Fabry disease (OMIM #301500). This disorder is characterized by accumulation of glycosphingolipids (GSL) in different cell types. Classically, the disease manifests during childhood with acroparesthesia, angiokeratoma and hypohidrosis, and progresses to renal, cardiac and neurological morbidity 1.

Storage of GSL already occurs in utero 2-4. In a fetus with a gestational age of 19 weeks, storage was already seen in kidney and myenteric plexuses 3. Vedder et al. described extensive accumulation of GSL in placental and umbilical cord tissue of an affected newborn born to a female Fabry disease patient 2. Here we report on two additional pregnancies in Fabry patients in whom we evaluated placental tissue and umbilical cord. One was treated with recombinant α-galactosidase A (rh- α-Gal A) during pregnancy.

CASES AND PLACENTAL ANALYSIS

Placenta A was from a 39-year-old woman diagnosed with Fabry disease at the age of 24, following a positive family history. She had no complaints consistent with Fabry disease and no abnormalities were detected on cerebral magnetic resonance imaging (MRI), echocardiography, audiography and studies of renal function. No enzyme therapy had been initiated. α-Galactosidase A (α -Gal A) activity in leucocytes was decreased (13.5 nmol/h/mg protein; reference 32–60). Analysis of the GLA gene revealed a base substitution (c.1232T > C, F18S). During her uneventful pregnancy, prenatal evaluation showed that her male foetus was not affected with Fabry disease.

Placenta B was from a 24 years old female, who suffered from acroparesthesia since childhood. Leukocyte α -Gal A activity was decreased (15.7 nmol/h/mg protein). GLA gene analysis revealed a base substitution (c.901C > T, R310X). Her renal function was normal (103 mL/min/1.73 m2), but she was proteinuric (0.7 g/24 h). There was no left ventricular hypertrophy on echocardiogram. Brain MRI showed multiple white matter abnormalities. She started at the age of 21 with enzyme supplementation therapy (agalsidase beta 1.0 mg/kg/14 days), which was continued when she became pregnant. After an uneventful pregnancy she gave birth to a girl in whom the same mutation was detected.

Placenta C and D were previously described 2. In short, placenta C was derived from an affected mother with severe acroparesthesia giving birth to an affected boy. Placenta D was from a non-affected mother who gave birth to an affected girl. None of these mothers received enzyme replacement therapy before or during pregnancy.
Both patients gave informed consent for histological studies of placental tissue. The placentae were examined, weighed and five biopsies of 1 cm³ were taken from the umbilical cord (1), the maternal (2) and fetal side of the placenta (2). Samples were fixed in Karnovsky’s fixative for electron microscopy (EM). The details of EM analysis have been described elsewhere\(^2\). In brief, after fixation, the material was post fixed in 1% osmium tetroxide, block-stained with 1% uranyl acetate, one-step dehydration in dimethoxypropane and embedded in epoxyresin LX-112. Light microscopy sections were stained with toluidine blue. EM sections were stained with tannic acid, uranyl acetate and lead citrate, followed by examination in a Philips CM10 (FEI). Photographs were taken with a Morada digital camera (S.I.S.).

Both placentae had a centrally inserted umbilical cord and showed no abnormalities on macroscopic evaluation. The placentae weighed 590 and 565 g respectively. In placenta A and in the umbilical cord, no GSL storage (absence of zebra bodies), was found. In placenta B no GSL accumulation was found either, but smooth muscle cells of the umbilical cord showed inclusion bodies typically seen in Fabry disease (Fig. 1). No abnormalities were found in the placentae.

**DISCUSSION**

Combining these findings with a previously reported study on placental tissue in FD \(^2\), a heterogeneous picture emerges (Table 1). This heterogeneity may be caused by both maternal and foetal factors. An additional explanation could be the effect of enzyme replacement therapy with recombinant \(\alpha\)-galactosidase A.

A potential maternal factor influencing the degree of placental GSL accumulation might be the severity of Fabry disease in the mother. Placenta A and D were from a respectively mildly affected FD mother and a mother who

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**Figure 1A.** Vascular smooth muscle cell with zebra bodies (arrows) in vein of umbilical cord.  
**Figure 1B.** Higher magnification of endothelial cell with zebra bodies (arrow), periodicity 4.8 nm (arrow). N = nucleus.
had no FD. In both these placentae (A and D) no GSL accumulation could be detected. In the two mothers who did have symptoms of Fabry disease, placental GSL accumulation was found in one placenta (placenta C), and in the umbilical cord of both placentae (placenta B and C).

The observed variability in placental or umbilical GSL accumulation may also be influenced by fetal factors, of which most prominent would be disease status and gender. Complete absence of GSL storage was seen in placenta A from an unaffected male, whereas only mild storage was seen in the umbilical cord of placenta B from an affected female newborn. Complete absence of storage was found in placenta D of an affected female newborn. This minimal or absent storage is in contrast to the extensive number of zebra bodies found in placenta C of an affected male. These differences could possibly be explained by the amount of enzyme in the newborn. The absent activity in the male newborn might have resulted in the GSL accumulation in placenta C, whereas the minimal or absent storage in placenta B and D could have been the result of significant residual α-Gal A activity.

Passive transport of the rh-αGal A that was given to mother B, thus resulting in reduced GSL accumulation, is unlikely because of the molecular weight of the enzyme of ~100 kD. However, an influence of rh-α-Gal A treatment by either clearing the maternal part of the placenta, or active vesicular transport of the recombinant enzyme to the newborn cannot be excluded. Additional observations are needed in placentae of treated patients to substantiate a possible benefit with regard to placental GSL clearance.

Table 1. Characteristics of FD mothers and histological evaluation of placentae.

<table>
<thead>
<tr>
<th>Characteristics of mother</th>
<th>Placenta A</th>
<th>Placenta B</th>
<th>Placenta C</th>
<th>Placenta D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabry disease</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Age</td>
<td>39</td>
<td>24</td>
<td>23</td>
<td>NA</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td>13.3</td>
<td>15.7</td>
<td>26.2</td>
<td>NA</td>
</tr>
<tr>
<td>Mutation in GLA gene</td>
<td>F18S</td>
<td>R301X</td>
<td>Tyr134MetfsX31</td>
<td>NA</td>
</tr>
<tr>
<td>Clinical evaluation</td>
<td>No abnormalities</td>
<td>Proteinuria, WMLs</td>
<td>Acroparesthesia</td>
<td>NA</td>
</tr>
</tbody>
</table>

Histological evaluation (EM)

| Foetal placenta           | -         | -         | +         | -         |
| Maternal placenta         | -         | -         | +         | -         |
| Umbilical cord            | -         | +         | +         | -         |

WML: white matter lesions; NA: not available; + / -: GBS accumulation present/ absent; ¹ As described previously (Vedder et al 2006); ² α-Galactosidase activity in leukocytes (normal value 32-60 nmol/mg.hr)
We conclude that there is a wide variability in GSL accumulation in placental tissue of pregnant Fabry disease patients. This could be caused by maternal or fetal factors, but the effect of treatment with rh-α-Gal A cannot be ruled out.

REFERENCES


