Brief Communication

Placenta analysis of prenatally diagnosed patients reveals early GAG storage in mucopolysaccharidoses II and VI

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**Abbreviations:** MPS II, mucopolysaccharidoses type II; MPS VI, mucopolysaccharidoses type VI; GAGs, glycosaminoglycans; LSD, lysosomal storage disorder; EM, electron microscopy.

1. Introduction

Some lysosomal storage disorders (LSDs) may present prenatal storage of large molecules, observed as membrane bound vacuoles in endothelial cells, trophoblasts and fibroblasts [1]. Among these LSDs there have been reports of vacuolated cells in some mucopolysaccharidoses (MPS), including MPS I and MPS VII [2]. We report — for the first time, to the best of our knowledge — analyses of placental tissue in fetuses with MPS II (iduronate sulphatase deficiency) and MPS VI (arylsulfatase B deficiency). The cases were diagnosed prenatally and in both situations the families decided to continue the pregnancy (abortion is, in most cases, illegal in Brazil), and placentas were collected at birth for analysis.

2. Clinical and laboratory report

In the first case, the mother (who had a brother with the severe form of MPS II) had 2 previous pregnancies (1 severe MPS II child, 1 spontaneous abortion). Prenatal diagnosis in amniocytes was performed (enzyme activity measurement and DNA analysis for the family mutation p.R88H) and confirmed MPS II. The parents decided to continue the pregnancy and planned to attempt all possible therapeutic alternatives. The baby was born at term (37 weeks 2 days), birth weight of 3320 g, height of 51 cm and OFC of 34 cm. Clinical examination at birth revealed subtle lumbar gibbus, with corresponding radiological abnormality (L3–L5); neurological examination disclosed hyper-reactivity, compatible with a more premature baby. Abdominal and cerebral ultrasound, echocardiogram, ophthalmologic and auditory evaluations and pulmonary function tests (oxymetry, pulmonary expansion and pulmonary resistance) were normal. Activity of iduronate sulphatase on the newborn was low both in plasma (1.2 nmol/4 h/ml — normal range: 122–463) and leukocytes (4.3 nmol/4 h/ml — normal range: 31–110). Placenta was collected and processed for electron microscopy (EM) analysis and glycosaminoglycan (GAG) measurement. Analysis of GAG content was performed using the dimethyl blue assay after GAG extraction [3]. Levels were about 4-fold higher than normal range (1265 μg GAG/g...
tissue, normal range 286–318 μg/g). Extensive EM examination disclosed discrete storage in lysosomes with ultrastructure compatible with the group of MPS disorders (Fig. 1). They were localized in occasional endothelial cells and in pericytes. Fibroblasts were free of storage. Hofbauer cells contained normal lysosomal apparatus which has been described to be physiologically distended [4]. There were no signs of storage in the trophoblast layer.

In the second case, there was a recurrence risk of MPS VI, because the couple already had two children affected with the severe form of MPS VI (confirmed by biochemical diagnosis). The previous sibs had the rapid progressing form of the disease (early diagnosis, hepatosplenomegaly, dysostosis multiplex, severe joint restrictions, significant heart disease and pulmonary disease). Despite these occurrences, the family looked for medical advice regarding genetic risks only on the third trimester of the new pregnancy. Cordocentesis was performed at 34 weeks, being the measurement of arylsulphatase B activity performed in dried fetal blood spots [5]. No clinical abnormalities were observed at birth: clinical examination, skeletal radiographs and echocardiogram were normal. Placenta was collected at birth, placed in buffered formalin, embedded in paraffin and analyzed using both H–E and Alcian Blue staining. A discrete storage in pericytes was observed, with no other convincing sign of storage at cytological level. As in the MPS II case, GAG levels were increased (490 μg/g tissue). Unfortunately we were not able to perform EM studies on this sample.

3. Conclusion

Our results suggest that some abnormalities related to MPS storage, although not pronounced, may be already observed in placental tissue of patients affected by MPS II and MPS VI. These results are especially important as they reinforce the prenatal onset of storage. Since treatment with enzyme replacement therapy for these two conditions is available, the presence of early storage may indicate that earlier introduction of therapy could probably lead to better results, as highlighted by the case described by McGill et al. [6]. Long-term results on a reasonable number of patients should be obtained to verify the efficacy of this approach, but the present study highlights the evidence for an early GAG accumulation and provides a rationale for a prenatal and/or newborn diagnosis (potentially with newborn screening) to enable earlier introduction of therapy, which could contribute to improve clinical outcomes on these conditions.

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References