



## Pitfalls in the prenatal diagnosis of mucopolidosis II alpha/beta: A case report



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### ARTICLE INFO

#### Article history:

Received 19 November 2013

Revised 28 February 2014

Accepted 5 March 2014

Available online 2 June 2014

#### Keywords:

Mucopolidosis

Prenatal diagnosis

GNPTAB

GlcNAc-1-phosphotransferase

Heterozygote

### ABSTRACT

Mucopolidosis II alpha/beta is an autosomal recessive disorder caused by deficient activity of GlcNAc-1-phosphotransferase. We report the prenatal diagnosis of a fetus who was found to exhibit normal levels of lysosomal enzymes in the amniotic fluid but low levels in amniocytes, and who was found to be heterozygous for the most common GNPTAB mutation. As in some carriers of Mucopolidosis II biochemical abnormalities may hinder prenatal diagnosis, we suggest DNA analysis should be performed whenever possible.

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## Introduction

Mucopolipidosis type II alpha/beta (ML II alpha/beta; OMIM# 252500) is a rare autosomal recessive inborn error of metabolism caused by reduced activity of *N*-acetylglucosamine-1-phosphotransferase (GlcNAc-1-phosphotransferase; EC 2.7.8.17) due to mutations in the *GNPTAB* gene (NG021243; GeneID 79158). GlcNAc-1-phosphotransferase catalyzes the first step in the generation of mannose 6-phosphate recognition marker required for efficient targeting of soluble lysosomal enzymes to lysosomes. Defects in GlcNAc-1-phosphotransferase lead to missorting of several lysosomal enzymes and the lysosomal accumulation of non-degraded macromolecules (Reitman and Kornfeld, 1981). The microscopic pathological findings in these defective cells are cytoplasmic inclusions formed by accumulation of several macromolecules, which culminate in a typical phenotype characterized by coarse facial features, gingival hypertrophy, *dysostosis multiplex*, joint contractures, and death at an early age (Kornfeld and Sly, 2001). There is no specific treatment available for ML II alpha/beta yet, although hematopoietic-cell transplantation can be tried in very early diagnosed cases (Leroy et al., 2008). As expected for autosomal recessive disorders, individuals heterozygous for ML II alpha/beta are not clinically affected, but can exhibit intermediate biochemical abnormalities (Kornfeld and Sly, 2001; Varki et al., 1982; Cathey et al., 2008; Leroy and DeMars, 1967).

Clinical suspicion is the first step in the diagnosis of ML II alpha/beta. Definitive diagnosis depends on demonstration of low GlcNAc-1-phosphotransferase activity using the radioactive [<sup>32</sup>P]UDP-GlcNAc substrate (Reitman and Kornfeld, 1981). However, as this assay is available in only very few laboratories, an indirect diagnosis is usually established by measurement of lysosomal hydrolases both in mesenchymal cells, where their levels should be low, and their surrounding extracellular environment, where their levels should be high (Kornfeld and Sly, 2001). Although no further steps are usually required and *GNPTAB* gene analysis is generally not necessary to confirm the diagnosis, DNA tests are useful when biochemical testing is inconclusive or to diagnose carriers of the condition (Leroy et al., 2008).

Prenatal diagnosis of ML II alpha/beta can be established by the same biochemical approach, assessing fetal material obtained from chorionic villus biopsy, amniocentesis, or cordocentesis (Kornfeld and Sly, 2001). Likewise, DNA can be extracted and isolated from these fetal materials and analyzed as in the postnatal period, although this prenatal DNA-based approach requires additional information about the parental genotype or the flow/nature of disease-causing alleles in the proband's pedigree (Leroy et al., 2008). There is no report of prenatal diagnosis of ML II using cell free fetal DNA in maternal blood.

Although available in clinical and research grounds (Leroy et al., 2008), fetal DNA analysis of *GNPTAB* has not been described in the literature yet. This is the first reported prenatal identification of a fetus heterozygous for ML II alpha/beta.

## Methods

This study, named “Comprehensive study on the Mucopolipidosis II and III in Brazil: an opportunity for understanding the genetic processes that control intracellular trafficking of proteins” was approved by the local Ethics Committee (code GPPG 07.244) and the parents signed an informed consent form.

The biochemical and DNA analysis reported herein were performed at the laboratory of the Medical Genetics Service-Hospital de Clínicas de Porto Alegre, Brazil (LRIEM-HCPA), which is an international reference center for diagnosis of lysosomal disorders. The panel of lysosomal enzymes evaluated in the pre and postnatal periods were chosen according to the experience and protocols available at LRIEM-HCPA at the time of the investigation, as well as to the cells/tissue availability for investigation (Burin et al., 2004). Due to the above reasons, the enzymes evaluated in both periods were not the same (Table 1).

The DNA analysis was performed using genomic DNA extracted from leukocytes (first child) and cultured amniocytes (second child). *GNPTAB* analysis in the first child was performed according to Cury et al. (2013), and included the sequencing of all *GNPTAB* exons and exon–intron boundaries. In the second child, only the whole exon 19 was sequenced. Placental tissue was collected at birth of the second child, in order to have the lysosomal enzymes assayed and the glycosaminoglycans (GAGs) measured. It was not possible to assay the enzymes due to the storage conditions, but placental GAGs were extracted and measured using the Dymethyl Blue technique.

**Table 1**

Mucopolipidosis II alpha/beta: biochemical panel of selected lysosomal hydrolases assessed in both siblings.

Enzyme	Enzyme activity (RV)					
	First child (affected)			Second child (heterozygous)		
	Postnatal			Prenatal		Postnatal <sup>a</sup>
	Blood plasma <sup>b</sup>	Leukocytes <sup>c</sup>	Cultured fibroblasts <sup>c</sup>	Amniotic fluid supernatant <sup>b</sup>	Cultured amniocytes <sup>c</sup>	Blood plasma <sup>b</sup>
Total hexosaminidase	27,427 (1000–2875)	NP	696 (3000–13,490)	NP	2318 (2631–2622)	1401 (1000–2857)
β-Glucuronidase	875 (30–300)	196 (23–151)	23 (62–361)	72 (26–86)	97 (40–254)	NP
α-Mannosidase	2640 (17–56)	127 (60–400)	4.6 (93–359)	14 (1.2–21)	NP	59 (17–56)
β-Galactosidase	NP	78 (78–280)	6.5 (394–1440)	NP	314 (521–1783)	NP
α-L-Iduronidase	NP	23 (32–56)	3.4 (74–148)	NP	29 (92–264)	52 (6.8–13.3)

RV, reference values.

<sup>a</sup> Only plasma sample was available.<sup>b</sup> nmol/h/mL.<sup>c</sup> nmol/h/mg of protein.

## Case report

The case reported herein is the second child of a young consanguineous couple. The firstborn male was diagnosed with ML II alpha/beta at age 1 year due to neurologic and psychomotor impairment, typical coarse facial features, gingival hypertrophy, kyphosis, *pectus carinatum*, and joint contractures, and subsequently died at age 25 month (Table 1). DNA analysis revealed the homozygous *GNPTAB* mutation c.3503\_3504delTC at exon 19, which is the most common ML II alpha/beta-causing mutation worldwide. Unfortunately, there was no parental DNA available to confirm the analysis.

The male and female progenitors were 31 and 36 years old, respectively, at the second conception. Prenatal obstetric ultrasound showed normal nuchal translucency, skeletal development and other fetal characteristics. At 16 weeks of pregnancy, amniocentesis was performed for biochemical and DNA testing, aiming at a very early performance of hematopoietic stem-cell transplantation, if the fetus was affected. The biochemical investigation (Table 1) showed inconclusive results, since the levels β-glucuronidase and α-mannosidase were within the normal range in the amniotic fluid (as expected for non-affected fetus), but in amniocytes only the level of β-glucuronidase was normal (as expected for non-affected fetus) while the levels of total hexosaminidase, β-galactosidase and α-iduronidase were below the normal range values (as expected for an affected fetus). DNA analysis showed the fetus was heterozygous for the c.3503\_3504delTC mutation. The child was delivered at 39 weeks of pregnancy, weighing 3670 g, measuring 50 cm and normal physical examination. In the postnatal period, biochemical testing was conducted only in plasma and showed slight increases in α-L-iduronidase and α-mannosidase activities, as well as normal levels of total hexosaminidase. Placental GAG levels were 69 μg GAG/mg of protein, within the normal range (41–78 μg/mg). No further cells were available for additional analysis (Table 1).

## Discussion/conclusions

To the best of our knowledge, this is the first reported prenatal investigation of ML II alpha/beta by a DNA-based approach and the first prenatal statement of ML II alpha/beta heterozygosity described in the literature as well. Reports of prenatal diagnosis of ML II alpha/beta are scarce and usually based on abnormal sonographic findings such as fetal hydrops, bone dysplasia and growth restriction (Heo et al., 2012; Chen et al., 2010; Yuksel et al., 2007; Saul et al., 2005; Carey et al., 1999; Ben-Yoseph et al., 1988). The case reported by Ben-Yoseph et al. (1988) was found to have heterozygote levels of GlcNAc-1-phosphotransferase in chorionic villi, but DNA analysis was not yet available at that time.

This case report corroborates the concept that biochemical diagnosis of ML II in the prenatal period can sometimes be inaccurate, as heterozygotes may exhibit intermediate activity of GlcNAc-1-phosphotransferase and, hence, milder abnormalities in lysosomal enzyme activities (Kornfeld and Sly, 2001; Leroy and DeMars,

1967; Cathey et al., 2010; Varki et al., 1982). However, as expected for “true” autosomal recessive disorders, this intermediate activity is not sufficient to cause storage of the substrates of the lysosomal hydrolases (e.g., GAGs) without clinical compromise.

In the present report, three out of the 4 enzymes evaluated in amniocytes showed low activities, but both the two enzymes evaluated in the amniotic fluid showed normal values, which suggests that intracellular levels of lysosomal enzymes can be more frequently abnormal than extracellular ones in fetuses heterozygous for ML II alpha/beta.

Prenatal diagnosis of autosomal recessive diseases is usually associated with some ethical dilemmas such as the possibility of identification of a heterozygous fetus that will never become symptomatic. However, if the fetus is affected, it could enable not only an early diagnosis but also an early treatment (e.g. hematopoietic stem-cell transplantation). Therefore, since this case demonstrates why biochemical analyses alone cannot be sufficient for prenatal diagnoses, we suggest DNA analysis should be performed whenever possible.

## Acknowledgements

This study was financed by the Brazilian National Council for Scientific and Technological Development (CNPq, nº 4800848/2007-0 and 305147/2007-2) and of the Rio Grande do Sul Research Foundation (FAPERGS); and by the Hospital de Clínicas de Porto Alegre Research and Event Incentive Fund (FIPE). We would like to thank Sandra Leistner-Segal, PhD, for her assistance with DNA analysis.

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