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Elevation of glycosaminoglycans in the amniotic fluid of a fetus with mucopolysaccharidosis VII:

Prenatal diagnosis of an MPS VII fetus

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Abstract

Objective—The aim of this study was to quantify GAGs in amniotic fluid (AF) from an MPS VII fetus compared with age-matched fetuses obtained from normal pregnancies.

Method—Disaccharides were measured by liquid chromatography tandem mass spectrometry (LC/MS/MS), compared to age-matched controls. Enzyme assay was performed in AF supernatant or cultured amniocytes. *GUSB* was analyzed by next generation sequencing using Ion Torrent Personal Genome Machine with a customized panel.

Results—No activity of β-glucuronidase was detected in fetal cells. The pregnancy was spontaneously terminated in the third trimester. Genetic studies identified a homozygous mutation of p.N379D (c.1135A>G) in the *GUSB* gene. LC/MS/MS showed that chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate levels were markedly increased in the MPS VII AF, compared to those in age-matched control AF (DS, HS, and C6S more than 10 × than age-matched controls; C4S and KS more than 3 times higher).

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Conflict of Interest: Francyne Kubaski, Ana Carolina Brusius-Facchin, Robert W. Mason, Pravin Patel, Maira G. Burin, Kristiane Michelin-Tirelli, Rejane G. Kessler, Fernanda Bender, Sandra Leistner-Segal, Carolina Moreno, Denise P. Cavalcanti, Roberto Giugliani, and Shunji Tomatsu declare that they have no conflict of interests.

Conclusion—This is the first report of specific GAG analysis in AF from an MPS VII fetus, indicating that GAG elevation in AF occurs by 21 weeks of gestation and could be an additional tool for prenatal diagnosis of MPS VII and potentially other MPS types.

Keywords

Mucopolysaccharidosis VII; Sly syndrome; prenatal diagnosis; glycosaminoglycans; β-glucuronidase; tandem mass spectrometry

INTRODUCTION

Mucopolysaccharidosis VII (MPS VII; Sly syndrome) (OMIM#253220) is an autosomal recessive lysosomal storage disorder (LSD) caused by a deficiency of β-glucuronidase (GUSB). This enzyme deficiency leads to accumulation of chondroitin sulfate (CS), dermatan sulfate (DS), and heparan sulfate (HS).[1] The first MPS VII case was described in 1973 by Dr. William S. Sly.[2]

Patients with MPS VII have a broad range of clinical signs and symptoms including; coarse facies, skeletal dysplasia, short stature, hernias, hepatosplenomegaly, neurological impairment, and corneal clouding. The clinical spectrum ranges from a severe form with lethal hydrops fetalis to attenuated forms with survival into adulthood despite physical and cognitive impairment.[3–12]

GUSB is localized to chromosome 7q21.11, and the 21kb gene contains 12 exons.[13–16] Several groups have independently reported many mutations within *GUSB* that result in different MPS VII phenotypes.[10, 17–32] Sixty-three mutations have been described according to the Human Gene Mutation database (HGMD) as of November, 2016.

The incidence of MPS VII is not well documented, and many cases are not diagnosed due to spontaneous abortion.[33–42] Clinical manifestations of MPS usually do not appear at birth; however, accumulation of GAGs has been reported histopathologically in the human fetus (MPS I, II, III, IVA) and placenta (MPS II, VI),[43–45] indicating that the disease process starts and can be detected prior to appearance of clinical signs and symptoms.

Prenatal studies for MPS VII have been performed primarily to measure GUSB deficiency and for histophatologic analysis.[46–54] Until now, no study has reported on the quantification of GAGs in fetal specimens with MPS VII. The aim of this study was to quantify GAGs in amniotic fluid (AF) from an MPS VII fetus and to compare with age-matched AF obtained from normal pregnancies.

METHODS

Tandem mass spectrometry

Chondroitin-4-sulfate (C4S), chondroitin-6-sulfate (C6S), dermatan sulfate (DS), heparan sulfate (HS-0S, NS and diS_1) and keratan sulfate (mono-KS and di-KS) were measured in AF by liquid chromatography tandem mass spectrometry (LC/MS/MS) and compared with AF of five age-matched controls corrected by protein.

10 μl of AF was added to a 96 well Omega 10K filter plate (Pall Co, MI) with 90 μl of 50 mM Tris HCL (pH 7.0) with a 96-well receiver plate at the bottom. Samples were incubated with a mixture of internal standard (chondrosine), chondroitinase (B, ABC and ACII), heparitinase and keratanase II (Seikagaku Co, Japan) according to the same method described by Kubaski et al.[55]

DS and C4S were measured as 2-acetamido-2-deoxy 3-O- (β-D-gluco-4-enepyranosyluronic acid)- 4- O- D- sulfo-galactose digested by chondroitinase B or ACII to generate DS or C4S, respectively. C6S was measured as 2-acetamido-2-deoxy-3-O- (β-D-gluco-4-enepyranosyluronic acid)- 6-O-D-sulfo-galactose digested by chondroitinase ABC. HS was measured by 2-acetamido-2-deoxy-4-O-(4-deoxy-α-L-threo-hex-enopyranosyluronic acid)-D-glucose (HS-0S), 2-deoxy-2-sulfamino 4-O-(4-deoxy-α-L-threo-hex-4-enopyranosyluronic acid)-D-glucose (HS-NS) and 2-deoxy-2-sulfamino-4-(4-deoxy-α-L-threo-hex-4-enopyranosyluronic acid)-6-O-sulfo-D-glucose (HS-diS_1) digested by heparitinase. KS was measured by Gal(6S)β1 → 4GlcNAc(6S) (di-KS) and Galβ1 → 4GlcNAc(6S) (mono-KS) digested by keratanase II.

Specific precursor ion and its product, *m/z* and liquid chromatography were used to quantify each disaccharide, respectively (354.3, 193.1 for IS; 378.3, 175.1 for HS-0S and DS; 458.4, 300.2 for C4S; 458.4, 282.1 for C6S; 416, 138.1 for HS-NS; 496.3, 416.3 for HS-diS_1; 542, 461.9 for di-KS; 462, 97 for mono-KS).

Molecular analysis

Molecular analyses were conducted by next generation sequencing using Ion Torrent Personal Genome Machine (Thermo Scientific™) with a customized panel (Ion AmpliSeq™ Thermo Scientific™) including the *GUSB* gene. Data was analyzed on Ion Torrent suite and Ion reporter (Thermo Scientific™) version 5.0.

This study was approved by the ethics committee of Hospital de Clínicas de Porto Alegre (project # 03066) and informed consent was obtained from the parents for prenatal diagnosis and molecular analysis.

RESULTS

The proband was a female fetus from non-consanguineous parents that presented with hydrops fetalis at 19 weeks of pregnancy and spontaneously died at 25 weeks of pregnancy. The mother, 31 year-old, had three previous pregnancies (one stillbirth and two children that died in the first year of life). No investigation was performed on these previous siblings.

Since the most common causes of non-immune hydrops fetalis, as congenital infection, malformations and chromosomal abnormalities, were ruled out, a lysosomal storage disorder was suspected. Thus, the levels of GAGs in AF at 21 weeks of pregnancy were measured using tandem mass spectrometry.

We observed that the concentration of GAGs found within the patient's AF was greatly elevated (≥10 SD) when compared to age-matched controls (Table 1).

Levels of DS, HS, and C6S were at least 10 fold higher in MPS VII than those in the age-matched controls, and C4S and KS were over 3 fold higher. C4S, C6S, HS-0S, and HS-NS were elevated as primary storage materials. Levels of mono-sulfated and di-sulfated KS were also elevated, secondarily. HS-diS1 level was not elevated (Table 1).

Due to the elevated levels of DS, HS, and C6S, a diagnosis of MPS VII was suspected. Biochemical analysis showed the very low enzymatic activity of GUSB in cultured amniocytes and AF, confirming the initial suspicion. The moderately low activity of β-galactosidase and a slight reduction in the activity of neuraminidase were also observed, but these alterations were not considered clinically relevant (Table 2). α-Mannosidase and total hexosaminidase were evaluated in AF supernatant to exclude mucolipidosis II/III.[56]

Molecular analysis

Molecular analyses were conducted by next generation sequencing with a customized panel including the *GUSB* gene. 36,000 reads were obtained with 1,500 reads per amplicon. The Asn379Asp (p.N379D) (c.1135A>G) alteration was found in a homozygous state for the affected fetus and in a heterozygous state in both parents. This is the first report of p.N379D substitution in GUSB.

Asn379 is conserved in GUSB in 50 species including humans, mice, and *E. coli*, and is a buried residue[57]. Consequently, alteration of the neutral Asn to acidic Asp is likely to disrupt the structure of the protein. The PredicSNP[2] program indicates that this change is likely deleterious to the protein structure[58]. More molecular studies are needed to confirm the function of Asn379 in the structure or function of GUSB.

DISCUSSION

We have demonstrated that AF surrounding a fetus with MPS VII at 21 weeks of gestational age has a marked elevation of GAGs, including secondary storage of KS, indicating that AF is valuable for measuring GAG level to detect MPS before birth. In 1993, Chabás et al. described the use of chorionic villus (CVS) and amniotic fluid (AF) to measure GUSB activity for prenatal diagnosis.[49] Reduced levels of GUSB activity have subsequently been detected in these tissues for several MPS VII fetuses.[50, 51, 53, 59] Groener et al. used two-dimensional electrophoresis to demonstrate elevated DS and CS in fetal blood in a confirmed MPS VII fetus.[54] However, no prior study has been reported on quantification of the levels of GAGs in CVS or AF. Our study shows that all GAGs and their subclasses, except for HS-diS1, are noticeably elevated in AF of the affected fetus compared with those in the age-matched controls and that the biochemical finding of GAGs is matched with the previous pathological findings of GAG accumulation in the affected fetuses as described above.[60–62] We and others have shown that KS is secondarily elevated in plasma (serum) and urine in several types of MPS and LSDs (in addition to MPS IV in which the deficient enzyme is directly involved in the catabolism of KS).[63–65] We previously proposed that secondary elevation of KS can be caused by several factors including the release of KS from damaged bone and cartilage.[66–68] However, newborn DBS from MPS I, II, or III subjects showed no elevation of KS at birth.[69] The elevated KS in AF surrounding the MPS VII fetus

in this study could suggest a more severe phenotype, including skeletal damage and/or developmental impairment *in utero*.

CONCLUSION

In this first report of quantification of GAGs in AF surrounding an MPS VII fetus using LC/MS/MS, we show that GAG elevation is present at 21 weeks of gestation. Thus, we suggest that GAG measurements in AF may become a valuable additional tool for the diagnosis of MPS VII and potentially other MPS types.

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What is already known about the topic?

- Mucopolysaccharidosis VII (MPS VII; Sly Syndrome) is an autosomal recessive disorder caused by deficiency of β-glucuronidase, leading to accumulation of primary glycosaminoglycans (GAGs);
- MPS VII has a broad clinical spectrum from the most severe lethal hydrops fetalis to attenuated forms with survival into adulthood despite somatic and cognitive impairment;
- GAGs are elevated in a fetus with MPS VII.

What does this study add?

- Not only primary GAGs (HS, DS) but KS are accumulated as early as 21 weeks of gestation;
- This is the first report of p.N379D substitution in GUSB.
- Quantification of GAGs by mass spectrometry applies to prenatal diagnosis, prognosis, and screening for MPS;

Table 1

AF GAG levels from patient and age-matched controls (mean ± SD)

	GAG levels (ng/mg protein)		
GAG	**Age-matched controls (mean ± SD)**	**Patient**	**z-score**
DS	61 ± 44	712	15
C4S	142 ± 56	543	7
C6S	57 ± 19	621	29
HS-0S	59 ± 25	891	33
HS-NS	12 ± 5	142	25
HS-diS_1	4 ± 3	2	−1
mono-KS	131 ± 91	361	2
di-KS	6 ± 2	25	8

DS: dermatan sulfate; HS: heparan sulfate; CS: chondroitin sulfate; KS: keratan sulfate.

Table 2

Biochemical diagnosis of MPS VII by enzyme activity assay in cultured amniocytes or AF supernatant

Enzyme	Enzyme Activity	
	Normal Range	Patient
β-glucuronidase[1]	40–254	0.34
α-iduronidase[1]	92–264	156
α-mannosidase[2]	1.25–21	20
Neuraminidase[1]	30–68	25
β-galactosidase[1]	521–1783	281
β-glucosidase[1]	207–596	218
N-acetylgalactosamine 6 sulfate sulfatase[1]	55–212	71
Hexosaminidase[2]	378–2901	979

[1]Enzyme activity in cultured amniocytes (nmol/h/mg protein)

[2]Enzyme activity in amniotic fluid supernatant (nmol/h/mL)