Placenta 76 (2019) 6–9

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Decreased placental glypican expression is associated with human fetal growth restriction

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ARTICLE INFO

Keywords:
Proteoglycans
Heparan sulphate
Glypican
Fetal growth
Restriction

ABSTRACT

Placental mediated fetal growth restriction (FGR) is a leading cause of perinatal morbidity and mortality. Heparan sulphate proteoglycans (HSPG) are highly expressed in placentae and regulate haemostasis. We hypothesise that altered expression of HSPGs, glypicans (GPC) may contribute to the development of FGR and small-for-gestational-age (SGA). GPC expression was determined in first-trimester chorionic villous samples collected from women with later SGA pregnancies and in placentae from third-trimester FGR and gestation-matched uncomplicated pregnancies. The expression of both GPC1 and GPC3 were significantly reduced in first-trimester SGA as well as in the third-trimester FGR placentae compared to controls. This is the first study to report a relationship between altered placental GPC expression and subsequent development of SGA/FGR.

1. Introduction

Fetal growth restriction (FGR) is clinically defined as a birth weight below the 10th percentile for gestational age [1]. The aetiology of approximately 70% of FGR cases remains uncertain and are termed "idiopathic FGR" [2,3] and they are largely associated with placental insufficiency [4]. The exact mechanisms underlying these disturbances are largely unknown.

Proteoglycans (PGs) are macromolecules comprised of a core protein with glycosaminoglycan (GAG) side chains attached. The human placenta contains two major types of PGs; heparan sulphate (HS) and chondroitin sulphate (CS) or dermatan sulphate (DS) PGs [5]. Heparan sulphate proteoglycans (HSPGs) in the placenta include syndecans, glypicans (GPC) and perlecan while CS and DS containing PGs in the placenta include decorin and biglycan.

The GPC family is comprised of 6 GPCs with only GPC1 and GPC3 expressed in placenta. GPCs are attached to the cell membrane via a glycophosphatidylinositol anchor and have important roles during development as their HS side chains are in close proximity to cell surface molecules and growth factors [6,7]. Previous studies have demonstrated reduced GPC1 and GPC3 expression in placentae from pregnancies complicated by preeclampsia compared to gestation-matched controls [8], however, their expression in pregnancies complicated by FGR is unknown.

The overall aim of this study was to determine the expression of placental GPCs in a clinically well-defined cohort of third-trimester pregnancies complicated by FGR compared to gestation-matched controls. In this study, it was hypothesised that reduced GPC expression early in first-trimester contributes to the aetiology of FGR. Small for gestational age (SGA) is often considered as a surrogate for FGR and is

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https://doi.org/10.1016/j.placenta.2018.12.007
Received 21 December 2016; Received in revised form 5 December 2018; Accepted 17 December 2018

Table 1a
The clinical characteristics of the control and CVS samples.

Characteristics	Control (n = 50)	SGA (n = 15)	Significance
Maternal age (years)	38.64 ± 2.45	39.24 ± 2.04	P = 0.08
Gestation age (weeks)	39.64 ± 1.23	39.06 ± 1.67	P = 0.10
Newborn weight (grams)	**3461 ± 225**	**2551 ± 506**	**P < 0.05**

defined as a birth weight below the 10th percentile of a birth weight curve. Therefore, we investigated the relationship between decreased placental GPC expression and subsequent development of SGA/FGR.

2. Methods

2.1. First-trimester chorionic villous sampling (CVS) tissue sample collection and processing

CVS samples were collected between 10 and 12 weeks' gestation at the University Medical Centre of Groningen, The Netherlands [9]. Surplus CVS tissue was collected with informed consent and in accordance with the guidelines of the Federation of Dutch Medical Scientific Societies regarding surplus material not needed for diagnostics. RNA quality determined the selection of n = 15 SGA and n = 50 control pregnancies (see Table 1a for clinical characteristics). Real-time PCR was carried out as previously described [9].

2.2. Third-trimester placental sample collection and processing

Placentae complicated by idiopathic FGR (n = 24) and gestation-matched controls (n = 24) were collected with informed patient consent and approval from the Human Research and Ethics Committees of The Royal Women's Hospital, Melbourne, Australia. The inclusion and exclusion criteria have been described previously [10–13] (see Table 1b for clinical characteristics).

The clinical characteristics of these samples are described in Table 1b. RNA extraction, cDNA preparation, real-time PCR and western immunoblotting were all carried out as previously described [12].

2.3. Statistical analysis

All data are reported as Mean ± SEM (standard error of mean) and were assessed using One- Mann-Whitney *U* Test, and GraphPad Prism 6 (San Diego, CA, USA). The data were tested for normality and were not normally distributed. A probability value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

Table 1a depicts the demographic data collected at delivery for both SGA and control pregnancies for samples obtained via CVS. A significant decrease in birth weight was observed in the SGA group compared with controls. The clinical characteristics of the third-trimester FGR and control placental samples are demonstrated in Table 1b. As expected, a significant reduction in newborn and placental weight is observed in the FGR samples compared to their respective controls.

Placental *GPC1-6* mRNA relative to *18S* rRNA determined by real-time PCR demonstrated no significant differences in GPC 2, 4, 5 and 6 between first-trimester SGA and controls and in third-trimester FGR and control pregnancies (provided as a supplementary data). However, a significant decrease in placental *GPC1* (Fig. 1) and *GPC3* (Fig. 2) mRNA was observed in both first-trimester SGA (Figure A) and in third-trimester FGR (Figure B) compared to respective control pregnancies. The decrease in placental GPC1 and GPC3 protein was confirmed in third-trimester FGR and gestation matched control pregnancies using immunoblotting. As shown, a 62 kDa immunoreactive GPC1 protein (Fig. 1C) and a 55 kDa immunoreactive GPC3 protein (Fig. 2C) was detected in all placental samples tested. GAPDH (40 kDa) protein was used as a total placental protein loading control. Immunoblotting for GPC1 and GPC3 in CVS samples was not performed due to the small sample volume and low protein yield.

Table 1b
The clinical characteristics of control and third-trimester FGR samples.

Characteristics	Control (n = 24)	FGR (n = 24)	Significance
Maternal age (years)	31.9 ± 6.5	33.2 ± 5.7	P = 0.4
Gestation age (weeks)	35.8 ± 6.6	34.4 ± 6.5	P = 0.25
Newborn weight (grams)	**2603.8 ± 857.0**	**2051.4 ± 637.0**	**P < 0.05**
Placental weight (grams)	**525.0 ± 148.2**	**409.3 ± 110.3**	**P < 0.005**

Consistent with this study, we have previously demonstrated significant reductions of other PGs including, decorin, biglycan and syndecan in third-trimester idiopathic FGR-affected pregnancies compared to gestation-matched controls [10,12,13]. Furthermore, Chen et al., have reported significant increases in PG expression in other pregnancy complications such as gestational diabetes, which is usually associated with fetal overgrowth [14]. Thus, it is likely that PG homeostasis is critical in the development of these pregnancy complications.

GPC's are important modulators of growth factor and cytokine signalling as demonstrated by studies on human inherited disorders and genetic experiments in fruit flies [15], and mice [16]. Whether GPCs have a direct regulatory role on fetal growth is yet to be determined.

The molecular processes that lead to FGR may occur very early in placental development [17]. GPCs may also have a direct gestation specific regulation on feto-placental growth. PGs including GPC are expressed in the first-trimester placenta and control trophoblast differentiation [18–24]. For example, in early gestation, GPC may contribute to an increase in placental growth to establish feto-maternal circulation, by regulating trophoblast function and by modulating signalling pathways that are important for biological processes such as cell structure and motility. However, later in gestation, GPCs may modulate branching angiogenesis in the villous vasculature by increasing endothelial cell proliferation and by regulating the expression of angiogenic molecules. Therefore, the direct or indirect mechanisms by which GPC1 and GPC3 may regulate feto-placental growth warrants further investigation.

Aberrant PG expression may initiate the processes that lead to FGR. The results from this study support a significant relationship between reduced GPC1 and GPC3 expression in both first-trimester SGA and in third-trimester FGR pregnancies, consistent with a causal role for placental GPC1 and GPC3 in the success of pregnancy outcome. This study selected first-trimester samples from SGA cases using a definition of birth weight below the 10th percentile. It is important to note that SGA and FGR are not necessarily interchangeable as some SGA babies will not have FGR and *vice-versa* [25]. Additional clinical diagnoses including Doppler studies and fetal growth trajectories, were unavailable, thus a tighter definition of FGR would be helpful for future studies, including longitudinal parameters of fetal growth and placental function [25] to ensure all cases are FGR and not just SGA.

One of the major limitations of our study is that GPC expression was not performed in the placental tissues from the same cohort of pregnant women, as such source of third trimester placental tissues were not readily available. In summary, this study was able to demonstrate that GPC1 and GPC3 expression levels are altered in both the first trimester and third trimester in pregnancies associated with reduced fetal growth. It is therefore plausible that FGR may be occurring as a result of reduced GPC expression, although it is more likely to be a collective reduction in PG expression as opposed to reduced expression of a single PG.

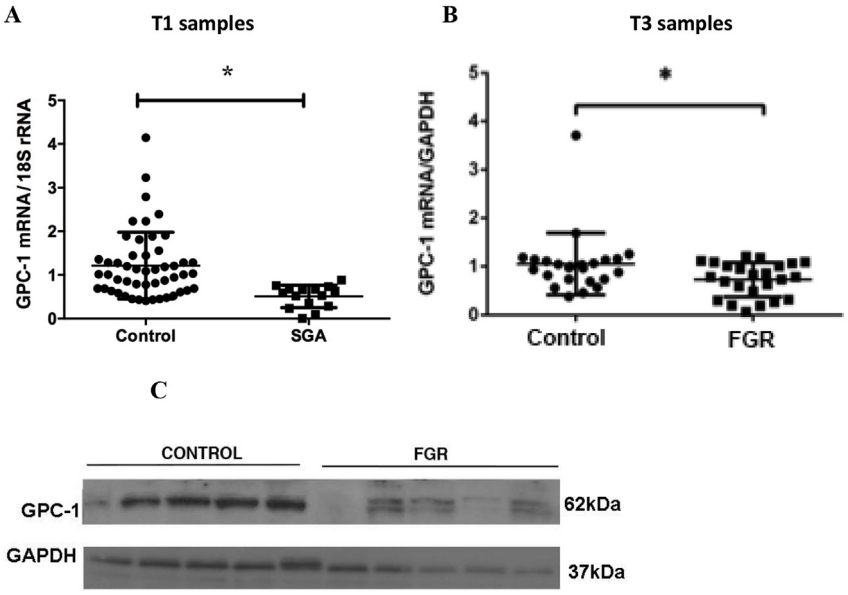

Fig. 1. Placental glypican-1 (GPC1) expression in SGA and FGR-affected pregnancies compared with matched controls.

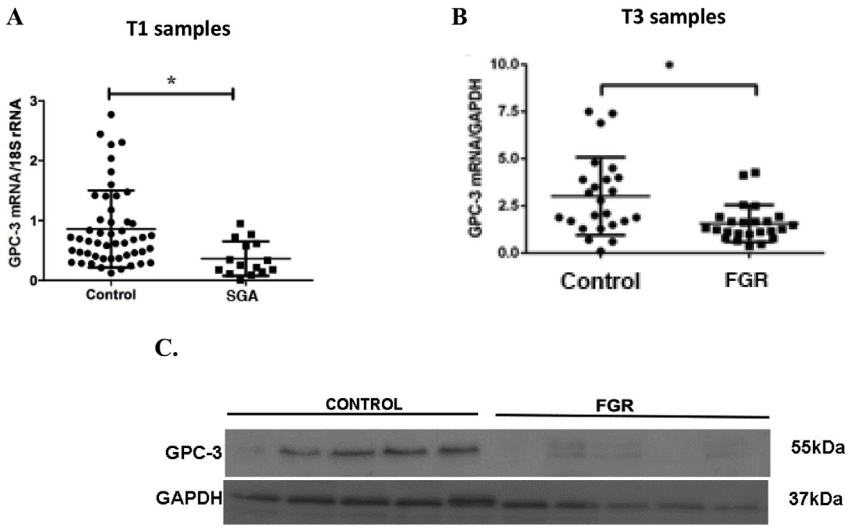

Fig. 2. Placental glypican-3 (GPC3) expression in SGA and FGR-affected pregnancies compared with matched controls. The relative expression of GPC1 (Fig. 1A) and GPC3 (Fig. 2A) in CVS samples obtained from SGA-affected pregnancies (T1, n = 15) were significantly reduced compared to control pregnancies (n = 50). This reduction was also demonstrated in third-trimester FGR placental samples (T3, n = 24) for GPC1 (Fig. 1B) and GPC3 (Fig. 1C) compared to their respective controls (n = 24). Protein expression for GPC1 (Fig. 1C) and GPC3 (Fig. 2C) was also consistently reduced in third-trimester FGR placental samples compared to controls. The presented graphs indicate mean ± SEM.* = P < 0.05, Mann-Whitney *U* test.

Acknowledgements

This work was supported by National Health and Medical Research Council Project Grants, Australia (grant number: 1004952 and 1042239).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2018.12.007.

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