Growth Hormone & IGF Research 21 (2011) 31–36

Contents lists available at ScienceDirect

Growth Hormone & IGF Research

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

Decreased placental expression of *hPGH*, *IGF-I* and *IGFBP-1* in pregnancies complicated by fetal growth restriction

Maria Koutsaki [a], Stavros Sifakis [b], Apostolos Zaravinos [a], Dimitrios Koutroulakis [b], Ourania Koukoura [b], Demetrios A. Spandidos [a,*]

[a] Laboratory of Clinical Virology, School of Medicine, University of Crete, Heraklion, Crete, Greece
[b] Department of Obstetrics & Gynecology, University Hospital of Crete, Heraklion, Crete, Greece

ARTICLE INFO

Article history:
Received 5 May 2010
received in revised form 14 November 2010
accepted 5 December 2010
Available online 5 January 2011

Keywords:
Fetal growth
Fetal growth restriction
Human Placental Growth Hormone
Insulin-like Growth Factor-I
IGF-binding Protein 1
IGF-binding Protein 3

ABSTRACT

Objective: The human Placental Growth Hormone (*hPGH*) and the Insulin-like Growth Factor (IGF) system are implicated in fetal development. This study aimed to evaluate the expression of *hPGH*, *IGF-I*, *IGFBP-1* and *IGFBP-3* genes in placentas from pregnancies complicated by fetal growth restriction (FGR).
Design: The study group was comprised of term placentas from 47 FGR-complicated pregnancies of no recognizable cause. Thirty-seven placentas from normal pregnancies with appropriate for gestational age birth weight were used as controls. The expression status of the genes was evaluated by quantitative real-time PCR.
Results: *hPGH*, *IGF-I* and *IGFBP-1* exhibited significantly lower expression compared to the controls ($p = 0.003$, $p = 0.049$ and $p = 0.001$, respectively). Numerically, lower *IGFBP-3* expression was also demonstrated in the FGR-affected group, without however reaching statistical significance ($p = 0.129$). Significant co-expression patterns were detected among the study genes in both the FGR and normal pregnancies.
Conclusion: Decreased placental expression levels of *hPGH*, *IGF-I* and *IGFBP-1* were demonstrated in pregnancies with FGR. Whether these alterations are a causative factor of FGR or accompany other pathogenetic mechanisms requires further investigation.

1. Introduction

Fetal growth restriction (FGR) is an important obstetric complication affecting 5% of pregnancies [1] This condition represents an in utero shift from the expected pattern of fetal growth potential into reduced birth weight leading to increased risk for intrauterine compromise, stillbirth, iatrogenic preterm birth and adverse perinatal and long-term outcomes [2]. The pathophysiological mechanisms leading to FGR remain unknown in particular in the absence of any antenatally recognizable cause — a condition called idiopathic FGR [1].

Human placental growth hormone (*hPGH*) and the insulin-like growth factor (IGF) system are implicated in normal fetal development [3–6]. *hPGH* is the product of the GH-V gene [7] specifically expressed in the syncytiotrophoblast layer of the human placenta [8] and gradually replaces pituitary GH in the maternal circulation. *hPGH* is also detectable in the fetal compartment according to recent studies in cord blood and amniotic fluid [9–11]. *hPGH* has important implications for physiological adjustment to gestation by increasing nutrient availability for the fetoplacental unit [4,5]. This action is exercised indirectly, probably by regulating the maternal levels of the insulin-like growth factor I (*IGF-I*) [4,5,12]. Furthermore, the presence of *hPGH* receptors in extravillous trophoblast suggests that its physiological role also includes a direct influence upon placental development and function via an autocrine and/or paracrine mechanism [13].

IGF-I is a strong mitogen that promotes cell proliferation and differentiation, possessing a critical role in mediating fetal and postnatal growth [6,14,15]. The actions of IGF-I in the circulation and the extracellular matrix are modulated by the presence of the insulin-like growth factor-binding proteins, mainly 1 and 3 (*IGFBP-1* and *IGFBP-3*) [14,15]. The human *IGFBP-1* and *IGFBP-3* genes are contiguous and located on chromosome 7 [16]. *IGFBP-3* is the most abundant IGFBP in the circulation and prolongs IGF half-life in plasma providing an IGF reservoir for target tissues. *IGFBP-1* and *IGFBP-3* also show IGF-independent actions, such as inhibition of growth and stimulation of apoptosis [14,15].

Several studies have demonstrated altered concentrations of *hPGH*, *IGF-I* and IGFBPs in the maternal circulation and fetal compartment in pregnancies with FGR [9,10,12,17–27]. Little is known, however, about the actual expression profile of these molecules in the human placenta of FGR-affected pregnancies. Our study aimed to evaluate the expression status of the *hPGH*, *IGF-I*, *IGFBP-1* and *IGFBP-3* genes in placentas from human FGR pregnancies of no apparent etiology.

* Corresponding author. Laboratory of Clinical Virology, Medical School, University of Crete, P.O. Box 2208, Heraklion 71003, Crete, Greece. Tel.: +30 210 722 7809; fax: +30 210 725 2922.
E-mail address: spandidos@spandidos.gr (D.A. Spandidos).

1096-6374/$ – see front matter
doi:10.1016/j.ghir.2010.12.002

2. Materials and methods

2.1. Participants

Our study group was comprised of term placentas from 47 pregnancies complicated by FGR of no apparent etiology. A panel of 37 placentas from pregnancies that resulted in the birth of healthy neonates with birth weight appropriate for gestational age and gender was used as the control group. The gestational age, for both FGR and control pregnancies, was calculated by last menstrual period dates and sonographic assessment at 11–13 weeks of pregnancy. Patients in the FGR group did not display clinical, sonographic or laboratory evidence of preeclampsia or other antenatally recognizable causes of FGR, such as congenital abnormalities or chromosomal aberrations of the fetus, and intrauterine infections by the STORCH group. They were recruited among pregnant women who were followed up for FGR at the Division of Maternal Fetal Medicine. The Research and Ethics Committee of the University Hospital of Heraklion, Crete, Greece, approved the study, and written informed consent was obtained from all participants.

Birth weight was expressed as a percentile corrected for gestation at delivery and included variables such as sex, maternal racial origin, weight, height and parity. The newborn was considered to be intra utero growth restricted if the birth weight percentile was less than 5% [28]. Demographic data of the mothers were recorded prior to and after birth (age, parity, previous medical and gynaecological history, potential pregnancy-associated complications, weight and maximal weight gained at pregnancy, height, body mass index (BMI), duration of gestation, mode of delivery, smoking and ethnicity of both parents). None of the pregnant women included in the study were diabetic, and the glucose tolerance test during pregnancy was normal in all the participants. At birth, clinical data concerning the newborns and the perinatal outcome were recorded.

2.2. Tissue collection and RNA extraction

Placental samples were collected at delivery from both groups. The biopsy specimens were taken immediately after the extraction of the placenta from the uterus. Six basal plate biopsy specimens of the maternal–fetal interface, approximately 5 mm^3 in size, were randomly excised from each placenta in a way that each sample contained the deciduas basalis and villous placenta. Areas involving calcification or infarcts were avoided. The tissue specimens were immediately frozen at −80 °C and stored until processed. Total RNA was extracted from each specimen using a power homogenizer and the TRIzol® reagent (Invitrogen, Carlsband, CA) according to the manufacturer's instructions. RNA concentration and purity were calculated after measuring its absorbance (260 nm) and absorbance ratio (260/280 nm), respectively, on a UV spectrophotometer.

2.3. Reverse transcription and real-time PCR

Reverse transcription was performed using the RETROscript first strand synthesis kit (AMBION, Austin, TX), from 0.5 μg of total RNA. Random hexamers were used as amplification primers. Real-time PCR was applied to quantify the mRNA expression of all four genes (*PGH*, *IGF-I*, *IGFBP-1* and *IGFBP-3*). The reactions were carried out using the Mx3000P real-time PCR system (Stratagene, La Jolla, CA) with the application of the SYBR® Green I Master Mix (Stratagene, La Jolla, CA), according to the manufacturer's instructions. Data were collected and analyzed using Mx3000P real-time PCR software version 2.00, Build 215 Schema 60 (Stratagene La Jolla, CA). Primer pair sequences and PCR product sizes are listed in Table 1. GAPDH and RPL13A genes were used as internal controls. However, normalization of the gene expression levels was determined with GAPDH, the most stable housekeeping gene. The primer pairs were designed to span at least one intron in order to avoid amplification of the contaminating genomic DNA along with cDNA.

Table 1
Primer sequences used for quantitative real-time RT-PCR.

Gene	Primer pair sequence (5′-3′)	Product size (bp)
IGF-I	CCTCCTCGCATCTCTTCTACCTG CTGCTGGAGCCATACCCTGTG	166
IGFBP-1	TAACTGAGGAGGAGCTCCTGGATA TTTGGAAATTTCTTCTCCTGATGTC	215
IGFBP-3	AAGACAGCCAGCGCTACAAAG TACGGCAGGGACCATATTCTG	102
hPGH	AGAACCCCCAGACCTCCCT TGCGGAGCAGCTCTAGGTTAG	96
GAPDH	GGAAGGTGAAGGTCGGAGTCA GTCATTGATGGCAACAATATCCACT	101
RPL13A	CCTGGAGGAGAAGAGGAAA TGAGGACCTCTGTCTATTTGTC	125

One μL cDNA from normal or FGR-complicated placental samples, respectively, was amplified in a PCR reaction with 2× Brilliant SYBR® Green QPCR Master Mix (containing 2.5 mM MgCl2), 300 nM of each primer and 30 μM Rox passive reference dye in a final volume of 20 μl. After initial denaturation at 95 °C for 10 min, samples were subjected to 40 amplification cycles comprising denaturation at 95 °C for 30 s, annealing at a specific temperature for each gene for 30 s, and elongation at 72 °C for 30 s. Annealing temperatures for each gene, were as follows: 60 °C for *IGF-I*, *hPGH*, *IGFBP-3* and *GAPDH*; 53 °C for *RPL13A* and 55 °C for *IGFBP-1*. Amplification and elongation steps were followed by a melt curve analysis in which the temperature was increased from 55 °C to 95 °C at a linear rate of 0.2 °C/s. Data collection was performed during both annealing and extension, with two measurements at each step and at all times during melt curve analysis. To verify the results of the melt curve analysis, PCR products were analyzed by electrophoresis on 2% agarose gel, stained with ethidium bromide and photographed on a UV light transilluminator. Two negative controls were included in each PCR reaction, one with no cDNA template and one with no reverse transcription treatment. Gene transcription levels were calculated as previously reported [29].

A 2-fold increased or decreased expression was considered to be significant (over-expression or down-regulation, respectively). All samples were treated in duplicate.

2.4. Statistical analysis

Numerical values were expressed as the median (range). Differences between FGR and control samples were calculated using Mann–Whitney U test. Correlation analysis between the mRNA levels of the genes was performed using Pearson's bivariate-correlation coefficient test. *P*-values <0.05 were considered as statistically significant. Analyses were performed using SPSS v.15 (SPSS Inc., Chicago, IL).

3. Results

3.1. Demographic characteristics

Table 2 summarizes the main clinical data of both mothers and neonates. No statistically significant difference was detected between the study and control groups, except for cigarette smoking that was more frequent in the FGR group. With regard to pregnancy outcome, the FGR-affected pregnancies exhibited shorter gestational age at delivery compared to the controls, and higher caesarean section rate, as anticipated ($p<0.001$).

3.2. Expression in the placenta

The Real-Time PCR results are shown in Fig. 1. The median (range) mRNA expression for *hPGH* was 0.06 (0.0004–0.95) in the control

Table 2
Main characteristics of the women enrolled in the study and the outcome of normal and FGR complicated pregnancies.

Maternal characteristics	FGR pregnancies	Normal pregnancies	*p* value
	N = 47	*N* = 37	
Age in years: mean (range)	28.34 (19–43)	28.62 (18–44)	0.831[a]
Primiparity: n (%)	25 (53.1)	20 (54.0)	0.662[b]
Cigarette smoker: n (%)	14 (29.7)	10 (27.02)	0.004[b]
BMI in kg/m^2: mean (±SD)	23.74 (±4.78)	22.15 (±2.85)	0.107[a]
Pregnancy outcome			
Fetal gender male: n (%)	18 (38.2)	22 (61.1)	0.109[b]
Gestational age at delivery in days: mean (±SD)	251.54 (±27.37)	271.13 (±9.91)	<0.001[a]
Caesarean section: n (%)	34 (72.3)	10 (27)	<0.001[b]

BMI: Body Mass Index; SD: Standard Deviation.
[a] *t*-test for two independent samples.
[b] Chi-square test.

group and 0.03 (0.0002–0.15) in the FGR group, with a statistically significant difference between the two groups ($p = 0.003$). The median (range) mRNA expression for *IGF-I* and *IGFBP-1* in the control group was 0.002 (7×10^{-6}–0.026) and 0.01 (0.0001–1.03) respectively. The corresponding values for the FGR-affected group were 0.002 (1.1×10^{-5}–0.008) and 0.01 (0.0001–0.18), respectively, with a statistically significant difference between the two groups, $p = 0.049$ for *IGF-I* and $p = 0.001$ for *IGFBP-1*. In the case of *IGFBP-3*, although the median expression was lower in the FGR group 0.12 (0.0002–1) compared with the controls 0.5 (0.0003–2.73), the difference was not statistically significant ($p = 0.129$). The differences in the expression of the examined genes in the groups of comparison remained statistically significant after adjustment for age and smoking. Hence, we found that in 3 out of 4 of the genes studied, the expression in FGR placentas was statistically significantly lower compared with control placentas obtained from normal pregnancies.

3.3. Correlation analysis

Correlations between the mRNA expression levels of each gene and clinical information, including maternal age, parity, smoking, BMI, gestational age at delivery, fetal gender and birth weight percentile were examined. No significant correlation was produced. In the FGR group, significant co-expression patterns were detected among all of the genes studied pairwise (Table 3). In addition, a strong positive correlation was revealed between the genes of *hPGH* and *IGFBP-1* ($p < 0.001$), *hPGH* and *IGFBP-3* ($p < 0.001$), *IGFBP-1* and *IGFBP-3* ($p < 0.001$) and *IGF-I* and *IGFBP-3* ($p < 0.001$). In the control group, *hPGH* and *IGFBP-3* was the unique couple of genes that correlated positively ($p = 0.012$). Adjustment for age and smoking did not change the co-expression patterns.

4. Discussion

As *hPGH* and the IGF axis have a critical role in fetal and postnatal growth [5,15] we focused concomitantly on the expression profile of 4 genes *hPGH*, *IGF-I*, *IGFBP-1* and *IGFBP-3*. We found decreased placental expression of *hPGH*, *IGF-I* and *IGFBP-1*, along with positive gene co-expression patterns, in pregnancies demonstrating idiopathic FGR. Table 4 displays the results of similar studies by the use of qRT-PCR, which included a relatively limited number of examined cases.

Decreased maternal serum levels of *hPGH* and *IGF-I* have been observed in pregnancies with impaired fetal growth attributed to placental insufficiency [12,18,24,25]. Regarding the placental expression of these molecules, we found decreased expression of *IGF-I*, in

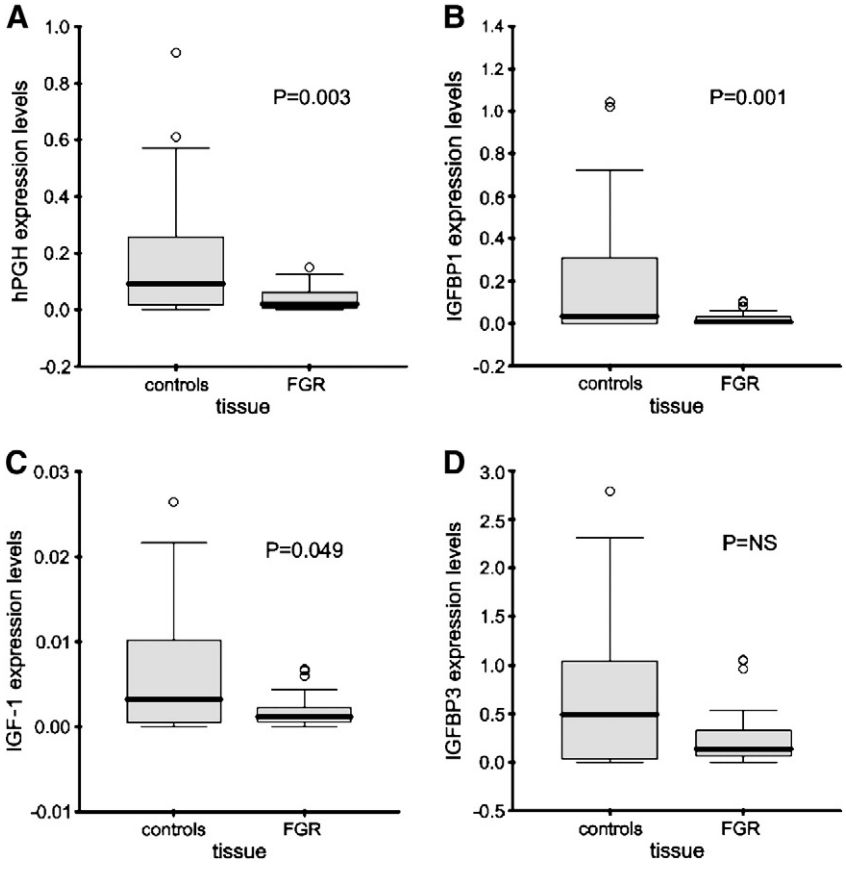

Fig. 1. Placental hPGH, *IGF-I*, *IGFBP-1* and *IGFBP-3* gene expression levels in pregnancies demonstrating fetal growth restriction and control samples. Boxplots show the 25th, 50th (median), and 75th percentile values. Whiskers show the minimum and maximum values.

Table 3
Correlations of the placental expression of the *IGF-I*, *IGFBP-1*, *IGFBP-3* and *hPGH* in FGR and control pregnancies.

FGR pregnancies		*IGF-I*	*IGFBP-1*	*IGFBP-3*	*hPGH*
IGF-I	CC				
	P				
IGFBP-1	CC	0.506			
	P	**0.002**[a]			
IGFBP-3	CC	0.667	0.827		
	P	<**0.001**[a]	<**0.001**[a,b]		
hPGH	CC	0.506	0.677	0.773	
	P	**0.002**[a]	<**0.001**[a,b]	<**0.001**[a,b]	
Control pregnancies		*IGF-I*	*IGFBP-1*	*IGFBP-3*	*hPGH*
IGF-I	CC				
	P				
IGFBP-1	CC	0.153			
	P	0.352			
IGFBP-3	CC	0.026	0.017		
	P	0.877	0.915		
hPGH	CC	0.021	0.066	0.376	
	P	0.899	0.673	**0.012**[b]	

Bold writing implies statistically significant correlations; CC: Correlation Coefficient.
[a] Correlation is significant at the level of $P<0.01$ (2-tailed).
[b] Correlation is significant at the level of $P<0.05$ (2-tailed).

agreement with a previous report by Calvo et al. (2004) [31] in a comparable small cohort of idiopathic FGR pregnancies (Table 4). We also found decreased *hPGH* expression, contrary to other reports where no differences were reported between FGR-affected and normal pregnancies ([33,34], Table 4). Barrio et al. (2009) showed that placental mRNA levels of *hPGH* exhibited a tendency towards a down-regulation [34]. In a number of studies using in situ hybridization or immunohistochemistry conflicting results showed that the transcription of *hPGH* and *IGF-I* was either increased [37–39] or decreased [40]. Our results contradict the increased *IGFBP-1* placental expression shown by others ([32,35], Table 4). Furthermore, *IGFBP-1* levels in both fetal and maternal circulations are inversely correlated with birth weight [20,24,25,41] Our study is the first one assessing the placental expression of *IGFBP-3* in FGR pregnancies and found no differences between affected and normal pregnancies, while increased circulating levels have been reported in pregnancies demonstrating FGR [24,25]. Further investigation is required for the evaluation of both the transcription and the circulating levels of *IGFBP-1* and *IGFBP-3* in pregnancies complicated with impaired fetal growth.

Only speculations can be given for the discrepancies in the assessment of *hPGH* and IGF-axis genes in the placenta and the maternal circulation of FGR-affected pregnancies. Firstly, the differences in the examined population due to the large heterogeneity and the diversity of the underlying causes of FGR [2] as well as the variety in the gestational age of maternal sampling or placental biopsies. In particular, Tzschoppe et al. (2010) showed that differences in the sampling site may contribute to variability in gene expression across the placental disk in placentas from FGR-affected pregnancies. Since these placentas represent diseased tissue and show characteristic hypoxic/ischemic changes, it may result in more dramatic changes in gene expression levels [42]. Secondly, posttranscriptional and/or posttranslational modifications may affect the measured levels of the hormones/proteins in the circulation, not always corresponding to the placental expression [37,43,44]. These modifications are associated with protein stability, alterations in the release mechanism of the protein, impaired protein transport across the placenta or modified serum stability of the hormones [44]. Furthermore, a change in the circulating levels of placental origin hormones/proteins, such as *hPGH* and PAPP-A, has been shown between the preclinical stage and the clinical manifestation of conditions associated with retarded fetal growth and placental insufficiency [9,10,12,18,25,45]. Lastly, the multiple roles of the *IGFBP-1* and *IGFBP-3* in placental development and fetal growth that includes both *IGF-I*-dependent and independent actions [14,15] may have an impact on the results obtained in the particular assessment.

The above observations and speculations might partly explain the absence of correlation between mRNA expression levels and the birth weight percentiles in the FGR pregnancies. However, limitations of this study such as the lack of measurements of maternal serum concentrations of the proteins, the placental weights, and the protein expression in the placenta do not allow further elucidation at this point. Despite the differences in mRNA placental expression between the growth restricted and the control group it remains to be elucidated whether the downregulation of *hPGH*, *IGF-I*, *IGFBP-1* genes comprises part of the underlying pathogenetic mechanisms or is an unrelated observation.

We revealed significant positive co-expression patterns between all of the genes studied in the FGR group. It is considered that *IGF-I* is probably regulated by *hPGH* [3,4] independent of the gestational age [12,18]. Furthermore, several studies have found a positive correlation between serum *hPGH* and *IGF-I* values in cross-sectional and longitudinal studies both in normal and FGR pregnancies [3,5,12,25]. The co-expression pattern of the placental expression of *hPGH* and *IGF-I* in our study supports these findings. We observed a strong correlation between *IGFBP-3* and *IGF-I* in the FGR group, which has also been shown in a study assessing cord blood in FGR pregnancies [27]. *IGFBP-3* is the primary binding protein that extends the half-life of *IGF-I* in the circulation [6] and could be considered as an index of

Table 4
Placental expression of *hPGH*, *IGF-I*, *IGFBP-1*, and *IGFBP-3* in human pregnancies complicated by FGR, using qRT-PCR. With the exception of the study by Trollmann et al., all studies include FGR-affected pregnancies unrelated to any known pathology.

Author, year (Ref. #)	Samples (n)		*hPGH*	*IGF-1*	*IGFBP-1*	*IGFBP-3*
	FGR	Controls				
Abu-Amero et al., 1998 [30]	9	9		NS		
Calvo et al., 2004 [31]	31	42		↓$P=0.008$		
Street et al., 2006 [26]	16	20		NS	NS	
Okamoto et al., 2006 [32]	4	4			↑	
Trollmann et al., 2007 [33]	6/22*	28	NS	NS		
Barrio et al., 2009 [34]	50	50	NS			
Struwe et al., 2010 [35]	27	35			↑	
Tschoppe et al., 2009 [36]	14	15			NS	
present study	47	37	↓$P=0.003$	↓$P=0.049$	↓$P=0.001$	NS

↓: Statistically significant down-regulation in the expression of the particular gene;
↑: Statistically significant up-regulation in the expression of the particular gene;
NS: Non- significant variation in the expression;
*: 22 pregnancies complicated by chronic placental hypoxia including idiopathic FGR (6 cases), preeclampsia (8 cases), birth weight <10th centile (5 cases), gestational diabetes (2 cases), and nicotine abuse (1 case).

the functionality of the growth hormone-*IGF-I* axis in the nonpregnant state [46]. We also found a strong correlation between *IGFBP-3* and *hPGH* in the FGR group, which is in concordance with other investigators [25]. Interestingly, this co-expression pattern was the only one maintained in the control group.

A drawback of this study may be the lack of gestational age-matched controls that would offer more appropriate groups for comparison. There are no studies to show changes in the placental expression of *hPGH,* and *IGFBP-3* throughout the last weeks of normal pregnancy. In a study by Street et al. (2006) the expression of *IGF-I* and *IGFBP-1* gene in the placenta decreases from 35–37 weeks to 38–40 weeks [47]. In pregnancies complicated with preterm delivery decreased levels of *IGFBP-1* in placental extracts [48] increased levels of *IGFBP-1* in cervical secretion, and the amniotic fluid [49–51] and decreased *IGFBP-3* concentrations in the maternal circulation [52] have been found. This may reflect changes in the placental expression of these genes, which, however, has not yet been investigated. As preterm delivery may be the consequence of pathological conditions of the pregnancy with unknown effects on the gene expression in the placenta, we opted to include in the normal group placentas from uncomplicated pregnancies ending in term delivery. Despite the difference in the gestational age of sampling (35.9 ± 3.9 vs. 38.7 ± 1.4 weeks, Table 2), we consider that the results of our study retain their value in the evaluation of the expression of *hPGH*, *IGF-I*, *IGFBP-1* and *IGFBP-3* genes in the third trimester placentas of FGR-affected pregnancies. Furthermore, the difference in the mode of delivery between the studied groups probably had no effect on the obtained results. No differences have previously been found in the concentration of *hPGH* in the maternal blood and the amniotic fluid in respect to caesarean section or vaginal delivery [53,54]. Despite the lack of data regarding the particular genes of this study, it has been shown that gene expression in a near term placenta is not significantly altered by labor, suggesting that the mode of delivery has no major implication in the interpretation of results of genomic studies on placenta [55].

Additional studies are required to elucidate whether the decreased placental expression of *hPGH, IGF-I* and *IGFBP-1* in pregnancies with idiopathic FGR is etiologically related to this condition or accompanies other pathogenetic mechanisms.

References

[1] A. Romo, R. Carceller, J. Tobajas, Intrauterine growth retardation (IUGR): epidemiology and etiology, Pediatr. Endocrinol. Rev. 6 (2009) 332–336.
[2] E.K. Pallotto, H.W. Kilbride, Perinatal outcome and later implications of intrauterine growth restriction, Clin. Obstet. Gynecol. 49 (2006) 257–269.
[3] M. Chellakooty, K. Vangsgaard, T. Larsen, T. Scheike, J. Falck-Larsen, J. Legarth, et al., A longitudinal study of intrauterine growth and the placental growth hormone (GH)-insulin-like growth factor I axis in maternal circulation: association between placental GH and fetal growth, J. Clin. Endocrinol. Metab. 89 (2004) 384–391.
[4] M.C. Lacroix, J. Guibourdenche, J.L. Frendo, F. Muller, D. Evain-Brion, Human placental growth hormone — a review, Placenta 23 (2002) S87–S94.
[5] J. Fuglsang, P. Ovesen, Aspects of placental growth hormone physiology, Growth Horm. IGF Res. 16 (2006) 67–85.
[6] R. Randhawa, P. Cohen, The role of the insulin-like growth factor system in prenatal growth, Mol. Genet. Metab. 86 (2005) 84–90.
[7] F. Frankenne, M.L. Scippo, J. Van Beeumen, A. Igout, G. Hennen, Identification of placental human growth hormone as the growth hormone-V gene expression product, J. Clin. Endocrinol. Metab. 7 (1990) 15–18.
[8] F. Frankenne, F. Rentier-Delrue, M.L. Scippo, J. Martial, G. Hennen, Expression of the growth hormone variant gene in human placenta, J. Clin. Endocrinol. Metab. 64 (1987) 635–637.
[9] E. Papadopoulou, S. Sifakis, E. Giahnakis, et al., Increased human placental growth hormone at midtrimester pregnancies may be an index of intrauterine growth retardation related to preeclampsia, Growth Horm. IGF Res. 16 (2006) 290–296.
[10] P. Mittal, J. Espinoza, S. Hassan, et al., Placental growth hormone is increased in the maternal and fetal serum of patients with preeclampsia, J. Matern. Fetal. Neonatal Med. 20 (2007) 651–659.
[11] S. Sifakis, E. Papadopoulou, A. Konstantinidou, et al., Increased levels of human placental growth hormone in the amniotic fluid of pregnancies affected by Down syndrome, Growth Horm. IGF Res. 19 (2009) 121–125.
[12] A. Caufriez, F. Frankenne, G. Hennen, G. Copinschi, Regulation of maternal IGF-I by placental GH in normal and abnormal human pregnancies, Am. J. Physiol. Endocrinol. Metab. 265 (1993) E572–E577.
[13] F. Frankenne, E. Alsat, M.L. Scippo, A. Igout, G. Hennen, D. Evain-Brion, Evidence for the expression of growth hormone receptors in human placenta, Biochem. Biophys. Res. Commun. 182 (1992) 481–486.
[14] M.M. Rechler, D.R. Clemmons, Regulatory actions of insulin-like growth factor-binding proteins, Trends Endocrinol. Metab. 9 (1998) 176–183.
[15] R. Monzavi, P. Cohen, IGFs and IGFBPs: role in health and disease, Best Pract. Res. Clin. Endocrinol. Metab. 16 (2002) 433–447.
[16] P.D. Lee, L.C. Giudice, C.A. Conover, D.R. Powell, Insulin-like growth factor binding protein-1: recent findings and new directions, Proc. Soc. Exp. Biol. Med. 216 (1997) 319–357.
[17] C. Lassarre, S. Hardouin, F. Daffos, F. Forestier, F. Frankenne, M. Binoux, Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in subjects with intrauterine growth retardation, Pediatr. Res. 29 (1991) 219–225.
[18] V. Mirlesse, F. Frankenne, E. Alsat, M. Pounchelet, G. Hennen, D. Evain-Brion, Placental growth hormone levels in normal pregnancy and in pregnancies with intrauterine growth retardation, Pediatr. Res. 34 (1993) 439–442.
[19] K. Langford, W. Blum, K. Nicolaides, J. Jones, A. McGregor, J. Miell, The pathophysiology of the insulin-like growth factor axis in fetal growth failure: a basis for programming by under nutrition? Eur. J. Clin. Invest. 24 (1994) 851–856.
[20] L.C. Giudice, F. de Zegher, S.E. Gargosky, et al., Insulin-like growth factors and their binding proteins in the term and pre-term human fetus and neonate with normal and extremes of intrauterine growth, J. Clin. Endocrinol. Metab. 80 (1995) 1548–1555.
[21] K.S. Langford, K.H. Nicolaides, J. Jones, A. Abbas, A.M. McGregor, J.P. Miell, Serum insulin-like growth factor-binding protein-3 (IGFBP-3) levels and IGFBP-3 protease activity in normal, abnormal and multiple human pregnancy, J. Clin. Endocrinol. Metab. 80 (1995) 21–27.
[22] J.A. Spencer, T.C. Chang, J. Jones, S.C. Robson, M.A. Preece, Third trimester fetal growth and umbilical venous blood concentrations of IGF-I, IGFBP-1 and growth hormone at term, Arch. Dis. Child. Fetal Neonatal Ed. 73 (1995) F87–F90.
[23] J. Leger, J.F. Oury, M. Noel, et al., Growth factors and intrauterine growth retardation. I. Serum growth hormone, insulin-like growth factor (IGF)-I, IGF-II, and IGF binding protein 3 levels in normally grown and growth-retarded human fetuses during the second half of gestation, Pediatr. Res. 40 (1996) 94–100.
[24] R. Holmes, R. Montemagno, J. Jones, M. Preece, C. Rodeck, P. Soothill, Fetal and maternal plasma insulin-like growth factors and binding proteins in pregnancies with appropriate or retarded fetal growth, Early Hum. Dev. 49 (1997) 7–17.
[25] H.D. McIntyre, R. Serek, D.L. Crane, et al., Placental growth hormone (GH), GH-Binding Protein and insulin-like growth factor axis in normal, growth-retarded, and diabetic pregnancies: correlations with fetal growth, J. Clin. Endocrinol. Metab. 85 (2000) 1143–1150.
[26] M.E. Street, P. Seghini, S. Fieni, et al., Changes in interleukin-6 and IGF system and their relationships in placenta and cord blood in newborns with fetal growth restriction compared with controls, Eur. J. Endocrinol. 155 (2006) 567–574.
[27] R. Verkauskiene, J. Beltrand, O. Claris, et al., Impact of fetal growth restriction on body composition and hormonal status at birth in infants of small and appropriate weight for gestational age, Eur. J. Endocrinol. 157 (2007) 605–612.
[28] J. Gardosi, A. Francis, Software program for the calculation of customized birth weight percentiles, Version 6.2, 2000-2007, , 15 April, 2010, Available at: http://www.gestation.net. Retrieved.
[29] A. Zaravinos, J. Bizakis, D.A. Spandidos, RKIP and BRAF aberrations in human nasal polyps and the adjacent turbinate mucosae, Cancer Lett. 264 (2008) 288–298.
[30] S.N. Abu-Amero, Z. Ali, P. Bennett, J.I. Vaughan, G.E. Moore, Expression of the insulin-like growth factors and their receptors in term placentas: a comparison between normal and IUGR births, Mol. Reprod. Dev. 49 (1998) 229–235.
[31] M.T. Calvo, A. Romo, J.J. Gutiérrez, E. Relaño, E. Barrio, A. Ferrández Longás, Study of genetic expression of intrauterine growth factors IGF-I and EGFR in placental tissue from pregnancies with intrauterine growth retardation, J. Pediatr. Endocrinol. Metab. 17 (2004) 445–450.
[32] A. Okamoto, H. Endo, B. Kalionis, et al., IGFBP1 and Follistatin-like 3 genes are significantly up-regulated in expression profiles of the IUGR placenta, Placenta 27 (2006) 317–321.
[33] R. Trollmann, K. Klingmüller, R.L. Schild, W. Rascher, J. Dötsch, Differential gene expression of somatotrophic and growth factors in response to in vivo hypoxia in human placenta, Am. J. Obstet. Gynecol. 197 (2007) 601.e1–601.e6.
[34] E. Barrio, M.T. Calvo, M.D. Miramar, et al., Study of apoptosis and related proteins, CRH and hpGH in placentas of newborns small for gestational age (SGA), Pediatr. Endocrinol. Rev. 6 (2009) 337–342.
[35] E. Struwe, G. Berzl, R. Schild, et al., Microarray analysis of placental tissue in intrauterine growth restriction, Clin. Endocrinol. (Oxf) 72 (2) (2010) 241–247.
[36] A. Tzschoppe, E. Struwe, H. Blessing, et al., Placental 11beta-HSD2 gene expression at birth is inversely correlated with growth velocity in the first year of life after intrauterine growth restriction, Pediatr. Res. 65 (2009) 647–653.
[37] S. Sheikh, P. Satoskar, D. Bhartiya, Expression of insulin-like growth factor-1 and placental growth hormone mRNA in placentae: a comparison between normal and intrauterine growth retardation pregnancies, Mol. Hum. Reprod. 7 (2001) 287–292.
[38] H. Dalçik, M. Yardimoğlu, B. Vural, et al., Expression of insulin-like growth factor in the placenta of intrauterine growth-retarded human fetuses, Acta Histochem. 103 (2001) 195–207.
[39] S. Ozkan, B. Vural, C. Dalçik, A. Taş, H. Dalçik, Placental expression of insulin-like growth factor-I, fibroblast growth factor-basic and neural cell adhesion molecule in pregnancies with small for gestational age fetuses, J. Perinatol. 28 (2008) 468–474.

[40] J.A. Chowen, D. Evain-Brion, J. Pozo, E. Alsat, L.M. García-Segura, J. Argente, Decreased expression of placental growth hormone in intrauterine growth retardation, Pediatr. Res. 39 (1996) 736–739.
[41] H.S. Wang, J. Lim, J. English, L. Irvine, T. Chard, The concentration of insulin-like growth factor-I and insulin-like growth factor-binding protein-1 in human umbilical cord serum at delivery: relation to fetal weight, J. Endocrinol. 129 (1991) 459–464.
[42] A.A. Tzschoppe, E. Struwe, H.G. Dörr, et al., Differences in gene expression dependent on sampling site in placental tissue of fetuses with intrauterine growth restriction, Placenta 31 (3) (2010) 178–185.
[43] M.L. Brizot, E. Jauniaux, A.T. Mckie, F. Farzaneh, K.H. Nicolaides, Placental expression of alpha and beta subunits of human chorionic gonadotrophin in early pregnancies with Down's syndrome, Hum. Reprod. 10 (1995) 2506–2509.
[44] M.L. Brizot, J.A. Hyett, A.T. Mckie, N.A. Bersinger, F. Farzaneh, K.H. Nicolaides, Gene expression of human pregnancy-associated plasma protein-A in placenta from trisomic pregnancies, Placenta 17 (1996) 33–36.
[45] K. Spencer, C.K. Yu, M. Savvidou, A.T. Papageorghiou, K.H. Nicolaides, Prediction of pre-eclampsia by uterine artery Doppler ultrasonography and maternal serum pregnancy-associated plasma protein-A, free beta-human chorionic gonadotropin, activin A and inhibin A at 22 + 0 to 24 + 6 weeks' gestation, Ultrasound Obstet. Gynecol. 27 (2006) 658–663.
[46] A. Juul, K.W. Kastrup, S.A. Pedersen, N.E. Skakkebaek, Growth hormone (GH) provocative retesting of 108 young adults with childhood-onset GH deficiency and the diagnostic value of insulin-like growth factor I (IGF-I) and IGF-binding protein-3, J. Clin. Endcorinol. Metab. 82 (1997) 1195–1201.
[47] M.E. Street, P. Seghini, M.A. Ziveri, et al., Interleukin-6 and insulin-like growth factor system relationships and differences in the human placenta and fetus from the 35th week of gestation, Growth Horm. IGF Res. 16 (2006) 365–372.
[48] F.A. Hills, L.K. Gunn, P. Hardiman, S. Thamaratnam, T. Chard, IGFBP-1 in the placenta, membranes and fetal circulation: levels at term and preterm delivery, Early Hum. Dev. 44 (1996) 71–76.
[49] M. Kekki, T. Kurki, T. Kärkkäinen, V. Hiilesmaa, J. Paavonen, E.M. Rutanen, Insulin-like growth factor-binding protein-1 in cervical secretion as a predictor of preterm delivery, Acta Obstet. Gynecol. Scand. 80 (2001) 546–551.
[50] H.M. Tanir, T. Sener, Z. Yildiz, Cervical phosphorylated insulin-like growth factor binding protein-1 for the prediction of preterm delivery in symptomatic cases with intact membranes, J. Obstet. Gynaecol. Res. 35 (2009) 66–72.
[51] S.E. Lee, B.D. Han, I.S. Park, R. Romero, B.H. Yoon, Evidence supporting proteolytic cleavage of insulin-like growth factor binding protein-1 (IGFBP-1) protein in amniotic fluid, J. Perinat. Med. 36 (2008) 316–323.
[52] S.M. Cooley, J.C. Donnelly, C. Collins, M.P. Geary, C.H. Rodeck, P.C. Hindmarsh, The relationship between maternal insulin-like growth factors 1 and 2 (IGF-1, IGF-2) and IGFBP-3 to gestational age and preterm delivery, J. Perinat. Med. 38 (2010) 255–259.
[53] J. Fuglsang, P. Sandager, N. Møller, S. Fisker, H. Orskov, P. Ovesen, Kinetics and secretion of placental growth hormone around parturition, Eur. J. Endocrinol. 154 (2006) 449–457.
[54] P. Mittal, S.S. Hassan, J. Espinoza, et al., The effect of gestational age and labor on placental growth hormone in amniotic fluid, Growth Horm. IGF Res. 18 (2008) 174–179.
[55] V. Sitras, R.H. Paulssen, H. Grønaas, A. Vårtun, G. Acharya, Gene expression profile in labouring and non-labouring human placenta near term, Mol. Hum. Reprod. 14 (2008) 61–65.