Genetic background of intrauterine growth retardation, interactions with other etiological factors

Ph.D. theses

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INTRODUCTION

Intrauterine fetal development is a complex, multifactorial process. Fetal growth is determined by maternal, fetal and environmental factors, and defects in any of these may result in slowing down the growth rate or the eventual development of intrauterine growth restriction (IUGR). One needs to fully appreciate the dynamics of physiological, normal fetal growth before the pathology of IUGR can be fully understood. Although IUGR may be regarded as a fetal adaptive response, it comes at a price as it leads to increased fetal morbidity and mortality.

The pathological mechanisms in the background of IUGR may involve multiple factors including maternal, fetal, placental and environmental. Maternal factors may result from medical illness, nutritional deficiency or a range of socioeconomical problems. Among fetal factors, chromosomal abnormalities, other developmental diseases or intrauterine infections are most prominent. Environmental factors may include alcohol, nicotine or recreational substance abuse, or, less commonly, exposure to radiation or chemicals. Nevertheless, the most common etiology of IUGR is abnormal placental function. Abnormal placental function may result from defective development of the placenta, placental inflammation, partial placental abruption or, less commonly, umbilical cord defects.

Normal physiological fetal growth is regulated through a complex interplay of genetic and endocrine mechanisms. Functional balance of genes involved in the regulation of intrauterine fetal development and the maintenance of the physiological hormonal milieu are both crucial in determining physiological fetal growth.

The placenta plays a fundamental role in guaranteeing adequate oxygen and nutritional supply to the fetus during intrauterine development. This is the primary area where functionality of growth regulating genes becomes significant. While these genes regulate a wide range of physiological processes, it is primarily the balance of these various processes which determines the normality of intrauterine growth; if this balance is disrupted, abnormal growth will ensue.

In the present paper, the expression pattern of several genes involved in the regulation of intrauterine fetal growth is described. In addition, as the changes in expression of the regulating genes were interpreted in face of associated clinical pathology, our findings may provide important new data for the future of clinical practice in this area.

STUDY AIMS

In this paper we describe differences in IGF gene expression patterns, in carbohydrate and insulin metabolism, apoptotic gene activity and in gene activity of the 11β-hydroxysteriod-dehydrogenase 2 gene (thought to be prominent in the regulation glucocorticoid metabolism) between placental samples from IUGR pregnancies versus normal pregnancy. The results of the genetic studies were correlated with clinical findings so that the role of changing patterns of gene expression in the development of clinical pathology could be identified. Associated changes in several clinical parameters were identified that facilitated the clinical interpretation of altered gene expression patterns. These clinical parameters included fetal gender, gestational weight gain, gestational increase in body mass index (BMI), maternal birthweight, type of delivery and presence or absence of impending fetal asphyxia.

Before the study the following set of research questions were asked:

1. *Maternal factors:* Is there a significant difference in median maternal age between the IUGR and normal pregnancy groups? Is there a difference in maternal age distribution? What is the median gestational age at the time of delivery of IUGR fetuses?

2. *Gestational factors:* Was there impending intrauterine fetal asphyxia present during gestation? What was the frequency of cesarian section in IUGR pregnancies?

3. *Nutritional factors:* Is there a difference between gestational weight gain and increase in maternal BMI during gestation?

4. *Birthweight:* What is the distribution between more severe (0-5 percentile birthweight) vs. less severe (5-10 percentile birthweight) cases? Is there a relationship between percentile birthweight and gestational weight gain? Is there a relationship between percentile birthweight and maternal birthweight? What is the distribution of fetal gender in the IUGR group?

5. *Umbilical cord blood testing:* Is there a difference in insulin or glucose levels in umbilical cord samples from IUGR or normal pregnancies?

6. *Gene expression:* Is there a difference in gene expression of the IGF-I, IGF-II and IGFBP-3 genes between the IUGR and normal pregnancy groups? What is the

relationship between expression of these genes and carbohydrate metabolism in the IUGR and normal pregnancy groups?

7. *Gene expression vs. fetal parameters:* Is there a correlation between placental activity of the IGF-I, IGF-II and IGFBP-3 genes and the degree of growth restriction? Is there a correlation between placental activity of the IGF-I, IGF-II and IGFBP-3 genes and fetal gender?

8. *Apoptosis:* Is there a difference between gene expression of the proapoptotic Bax gene or the antiapoptotic Bcl-2 gene in the IUGR vs. normal pregnancy groups? What is the role of apoptosis in the development of IUGR?

9. *Apoptosis vs. fetal gender:* Is there a relationship between the Bax and Bcl-2 gene activity with fetal gender?

10. *11β-hydroxysteriod-dehydrogenase 2 gene:* Is there a difference between 11β-hydroxysteriod-dehydrogenase 2 (11β-HSD2) gene activity between the IUGR and normal pregnancy groups? What is the role of abnormal fetomaternal glucocorticoid metabolism in the development of IUGR?

11. *11β-HSD2 and clinical correlations:* Is there a relationship between 11β-HSD2 gene activity and the degree of growth restriction? Is there a relationship between 11β-HSD2 and fetal gender? Is there a relationship between 11β-HSD2 and gestational age? How is 11β-HSD2 placental gene activity affected by impending intrauterine fetal asphyxia?

MATERIALS AND METHODS

During the study period between January 1, 2010 and January 1, 2011 we compared gene expression patterns from 101 IUGR placental samples vs. 140 normal pregnancy placenta samples in the Second Department of Gynecology and Obstetrics, Semmelweis University, Budapest, Hungary. A total of 37 umbilical cord samples were taken for analysis of serum insulin and glucose levels immediately following delivery. Clinical parameters analyzed

included maternal age, gestational age at delivery, distribution of fetal gender, gestational weight gain, and gestational increase in BMI, maternal birthweight, type of delivery and presence or absence of impending intrauterine fetal asphyxia.

Diagnosis of IUGR was established on the basis of percentile birthweight adjusted for fetal gender and gestational age with a cutoff value of 10 percentile. The IUGR group was divided into 2 subgroups on the basis of degree of growth restriction: the more severe growth restriction (0-5 percentile) vs. less severe growth restriction (5-10 percentile). In the majority of cases, the assumed etiology for IUGR were intrauterine infections, chromosomal abnormalities, miscellaneous developmental disorders in the fetus, maternal malnourishment, multiple pregnancy and abnormal placental function diagnosed after ruling out structural placental disorders.

Impending intrauterine fetal asphyxia was diagnosed through cardiotocography and/or Doppler flow study, or presence of meconium.

Placental samples of 2x2x2 cm (8 cm^3) were obtained after delivery. Demographic and clinical data extracted included maternal age, paternal age, obstetric history, history of genetic disorders, general medical history, maternal birthweight, gestational age at delivery, fetal gender, gestational weight gain, gestational increase in BMI, gestational abnormalities of carbohydrate metabolism, miscellaneous medical illnesses during gestation, birthweight and apgar score.

Whole RNA was extracted from placental samples and subsequently total quantity of RNA measured. Reverse transcription was performed. PCR was performed with MX3000 Real-time PCR (Stratagen) equipment.

Changes in gene expression were analyzed through a 2 sample t test with a confidence interval of 95%. Degree of freedom was determined through the *Welch-Satterthwaite correction.* Results of gene expression studies were categorized as (1) overexpression if t $Ln^{2\alpha}>1$, $p<0.05$; (2) underexpression if $Ln^{2\alpha} <-1$, $p<0.05$; (3) no change if $Ln^{2\alpha} <1,>-1$, $p<0.05$.

Models using SPSS software were created for analysis of demographic and clinical variables. ANOVA and linear regression were used for analysis of associations between multiple variables; where dichotomous outcomes were present logistical regression was performed. A cutoff value of $p<0.05$ was chosen for statistical significance.

RESULTS

Median maternal age in the IUGR group was 30.82±4.34 years, not substantially different from median age in the normal pregnancy group (31.45±3.12; $p>0.05$). Median gestational age at delivery was also similar in the two groups, 36±3.02 weeks in the IUGR group and 38±1.76 weeks in the control group ($p>0.05$).

Regarding type of delivery, vaginal delivery occurred in 39 out of 101 cases (38.6%) in the IUGR group, while 61.4% delivered via cesarean section. The respective numbers in the normal pregnancy group were 89 out of 140 cases (61.3%) for vaginal delivery and 38.7% for cesarean section. Impending intrauterine fetal asphyxia was a more common clinical indication for cesarean section in the IUGR group (31 cases; 30.7%) compared to the normal pregnancy group (33cases; 23.6%).

Mean gestational weight gain was 10.9 kg in the IUGR group vs. 14.8 kg in the normal pregnancy group. Corresponding changes in BMI were an increase of 4.1 in the IUGR group and 5.3 in the normal pregnancy group.

Within the IUGR group severe growth restriction (birthweight 0-5 percentile) was seen in 31 cases (30.7%) while the less severe growth restriction (birthweight 5-10 percentile) occurred in 80 cases (69.3%). The more severe form of growth restriction was also associated with gestational weight gain: it was most commonly observed with a weight gain of 3-9 kg or 14-17 kg compared to those pregnancies where gestational weight gain fell between 10-13 kg. The more severe growth restriction also correlated with maternal birthweight: those women who gave birth to infants with more severe growth restriction had had a median birthweight of 2830 gram, which was significantly different from the median birthweight of women (3120 gram) who gave birth to infants with less severe growth restriction ($p<0.05$). Fetal gender distribution was 37 males and 64 females (M:F 0.58) in the IUGR group vs. 73 males and 67 females (M:F 1.09) in the normal pregnancy group.

In umbilical cord samples, both insulin and glucose levels were found to be significantly higher in the normal pregnancy group than in the IUGR group.

In the gene expression studies it was found that both the IGF-II gene and the IGFBP-3 gene were overexpressed when compared to the normal pregnancy group (1.67-fold overexpression for IGFII, $p<0.04$; 1.55-fold overexpression for IGFBP-3, $p<0.03$). In contrast, there was no difference in IGF-I gene activity between the two groups.

There was no correlation between fetal gender and placental expression of the IGF-I and IGFBP-3 genes. However, in placental samples taken from pregnancies with male fetal gender, a 1.02-fold overexpression ($p<0.03$) of the IGF-II gene was detected. There was no association between fetal gender and the degree of growth restriction within the IUGR group.

There was no difference between gene activity of the proapoptotic Bax gene between the two groups. In contrast, the antiapoptotic Bcl-2 gene was significantly underexpressed in the IUGR group as compared to the normal pregnancy group (-2.17-fold decrease in gene activity; $p<0.04$).

There was no association between fetal gender and Bax or Bcl-2 gene expression within the IUGR group. Similarly, no correlation could be observed between Bax and Bcl-2 genes and the degree of growth restriction within the IUGR group.

The 11β-HSD2 gene was also underexpressed in the IUGR group with a -1.28-fold decrease in gene activity ($p<0.05$) vs. the normal pregnancy group. On the other hand, no significant association was observed between 11β-HSD2 gene expression and the degree of growth restriction in IUGR ($Ln2^{\alpha}$: 0.22; $p<0.03$). Similarly, no association could be detected between 11β-HSD2 gene expression and fetal gender in the IUGR group ($Ln2^{\alpha}$: 0.09; $p<0.06$).

No change in gene expression of the 11β-HSD2 gene could be detected when the subgroup of IUGR pregnancies where IUGR was diagnosed before the 33rd gestational week was compared to the normal pregnancy group ($p<0.02$). At the same time, in the subgroups where IUGR was detected either between gestational weeks 33-37 or gestational week >37 there was a significant and roughly similar decrease in 11-βHSD2 gene expression with a -1.19-fold and -1.31-fold decrease in gene activity, respectively ($p<0.04$). In the IUGR pregnancies where impending intrauterine fetal asphyxia was diagnosed, 11β-HSD2 gene expression decreased both compared to normal pregnancies with impending intrauterine fetal asphyxia present (-1.24-fold decrease in gene activity; $p<0.04$) and to IUGR pregnancies without impending intrauterine fetal asphyxia (-1.41-fold decrease in gene activity; $p<0.03$).

CONCLUSIONS

Intrauterine growth restriction was found to be significantly more common in maternal age groups 17-24 years and 35-44 years compared to age groups 25-31 years or 32-34 years.

There was a significantly higher occurrence of cesarean section in the IUGR group compared to the normal pregnancy group. Impending intrauterine fetal asphyxia was also significantly more common in the IUGR group than in the normal pregnancy group. Our findings confirm that emergent delivery occurs more commonly in IUGR compared to normal pregnancy and the most common clinical indication for this remains impending intrauterine fetal asphyxia.

Gestational weight gain and BMI were also found to be different between the groups. Specifically, IUGR was more commonly seen with either very low (3-9 kg) or higher than average (14-17 kg) weight gain compared to average weight gain (10-13kg); increase in BMI was also significantly less in IUGR compared to the normal pregnancy group. A correlation with a similar pattern was detected between gestational weight gain and the degree of growth restriction within the IUGR group: a more severe growth restriction with 0-5 percentile birthweight was more commonly seen with either very low (3-9 kg) or higher than average (14-17 kg) gestational weight gain compared to average weight gain (10-13kg).

The more severe form of growth restriction was also associated with a lower birth weight in the mother compared with maternal birthweight in the less severe form of growth restriction. This raises the possibility that genetic factors may play an important role in the background of intrauterine growth restriction.

In IUGR pregnancies post-delivery umbilical cord glucose and insulin levels are significantly lower than in normal pregnancy. Placental gene expression of IGF-I was not different in the IUGR and normal pregnancy groups; at the same time both IGF-II and IGFBP-3 were overexpressed. We speculate that this increased activity of the IGF-II gene may reflect an important role of IGF-II in a prioritized organ distribution of energy in situations where fetal energy supply is reduced.

Severity of growth restriction in IUGR may not be primarily determined by the activity of the IGF system; other factors possibly including genetic influences may play a more prominent role. There was a significant gender-associated difference in gene activity of the IGF-2 gene in IUGR with an overexpression of IGF-2 in male fetuses.

As we had a relatively large patient population, our findings strongly support the idea that IUGR is associated with a decreased activity of the Bcl-2 gene resulting in decreased inhibition of apoptosis in IUGR. Thus, these findings partly fulfill prior expectations regarding a change in gene expression of apoptosis-regulating genes. However, this change in gene expression is confined to a diminished activity of antiapoptotic regulatory genes; an increase in proapoptotic gene activity was not seen. There was no change in Bax or Bcl-2

gene expression in association with either the degree of restriction or fetal gender distribution in the IUGR group.

In our relatively large patient population we could also confirm that the gene coding for the type 2 isoenzyme of 11-beta-hydroxysteriod-dehydrogenase is underexpressed in IUGR as compared to normal pregnancy. We found no correlation between placental expression of the 11-βHSD2 gene and postnatal growth rate following a normal pregnancy. We propose that abnormalities in the fetomaternal glucocorticoid metabolism play an important role in the development of fetal programming and that a change in 11β-HSD2 gene activity is an integral part of this mechanism. A resultant increase in glucocorticoid exposure during gestation may predict an increased propensity for cardiovascular, metabolic and neuroendocrine disorders later in adult life.

It seems that there is no direct correlation between 11β-HSD2 gene expression and the degree of growth restriction in IUGR. Similarly, 11β-HSD2 gene expression does not seem to depend on fetal gender. On the other hand, the change in expression pattern of the 11β-HSD2 gene is dependent on the time point in gestation at which IUGR first appears: when IUGR is diagnosed before gestational week 33, gene expression of the 11β-HSD2 gene shows no change compared to normal pregnancy cases. However, when IUGR is detected at a later gestational age, a decrease in 11β-HSD2 gene activity can be seen. We conclude that the starting point for the decline in 11β-HSD2 gene activity must be around gestational week 33. This raises the possibility that it is the last 7 to 8 weeks of the gestational period that plays the key role in the development of fetal programming.

Gene activity of 11β-HSD2 is further lowered in IUGR cases where impending intrauterine fetal asphyxia is also present. A reduced fetal oxygen supply seems to be an important factor in the decline of 11β-HSD2 gene activity; this decline in 11β-HSD2 gene activity may be in turn an important mediator of the deleterious effects by the low oxygen environment.

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