Placenta 31 (2010) 178–185

Contents lists available at ScienceDirect

Placenta

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

Differences in gene expression dependent on sampling site in placental tissue of fetuses with intrauterine growth restriction

A.A. Tzschoppe [a], E. Struwe [a], H.G. Dörr [a], T.W. Goecke [b], M.W. Beckmann [b], R.L. Schild [c], J. Dötsch [a,*]

[a] Department of Pediatrics and Adolescent Medicine, University of Erlangen-Nuremberg, Loschgestr. 15, D-91054 Erlangen, Germany
[b] Department of Obstetrics & Gynaecology, University of Erlangen-Nuremberg, Germany
[c] Department of Obstetrics & Gynaecology, Diakonische Dienste Hannover, Hannover, Germany

ARTICLE INFO

Article history:
Accepted 3 December 2009

Keywords:
Corticotropin releasing hormone
Insulin-like growth factor-binding protein-1
Intrauterine
Growth restriction
Leptin
Prolactin

ABSTRACT

Objective: The human placenta as part of the feto-placental unit may influence fetal endocrine systems and may therefore represent a very important link between intrauterine growth restriction (IUGR) and metabolic disorders in later life. We aimed to analyze the effect of sample origin on gene expression of placental factors potentially involved in fetal programming in IUGR versus appropriate for gestational age growth (AGA) to standardize sample collection procedure for a multicenter approach.
Design: Placental gene expression of insulin-like growth factor-binding protein (IGFBP)-1, prolactin, corticotropin releasing hormone (CRH) and leptin was measured and compared between proximal, intermediate and peripheral region of the placenta in 22 IUGR (proven by anomalous placental Doppler velocimetry) and 19 AGA neonates.
Results: Whereas no difference in gene expression was seen in the proximal portion, in the intermediate placental region mRNA expression of IGFBP-1 ($p = 0.01$), prolactin ($p = 0.04$), CRH ($p = 0.01$) and leptin ($p = 0.04$) was increased in IUGR samples compared to controls. At the placental periphery, gene expression of these placental transcripts showed a higher expression level in IUGR placentas without statistical significance, except for leptin ($p = 0.03$).
Conclusion: Placental sampling site seems to be relevant for detecting differences in gene expression between IUGR and AGA neonates.

1. Introduction

Intrauterine growth restriction (IUGR) is not only associated with an increased risk for perinatal morbidity and mortality, but can also lead to the development of the metabolic syndrome later in life including obesity, diabetes type 2, hypertension, and coronary artery disease [1,2]. Among the various causes of IUGR, placental dysfunction associated with poor placental perfusion and hypoxia is one factor for 'idiopathic' IUGR [3]. Metabolic alterations in the fetal milieu change the early programming process [4], which may influence the regulation of endocrine function later in life. In this respect the central role of the placenta is well recognized and may therefore represent a very important link between IUGR and metabolic disorders in later life.

Elevated placental gene expression and cord blood concentrations associated with IUGR have been described for different placental endocrine regulators, e.g. insulin-like growth factor-binding protein-1 (IGFBP-1) [5–7], corticotropin releasing hormone (CRH) [8–10], leptin [11,12] and prolactin [13].

When comparing gene expression of specific placental transcripts, it is important to consider the sampling site and control for placental inhomogeneity, as placental architecture and blood flow are not uniform across the chorioallantoic human placental disk [14,15]. It has been shown that the expression of hypoxia-related transcripts is dependent on the sampling site and reflects the pattern of maternal arterial blood flow in the human placenta [16]. Therefore, differences in the sampling site may contribute to variability in gene expression across the placental disk. IUGR placentas represent diseased tissue and show characteristic hypoxic/ischemic changes, including increased syncytial knots, infarction, or hypercapillarization [17,18], which might result in more dramatic changes in gene expression levels.

We established a prospective multicenter study (FIPS-study) to identify placental genes with predictive value for the development of obesity and metabolic disorders after intrauterine growth restriction based on the hypothesis of an alteration in fetal programming. As we define IUGR by anomalous placental Doppler velocimetry, all of the IUGR pregnancies in this study experience

* Corresponding author. Tel.: +49 9131 85 33117; fax: +49 9131 85 33706.
E-mail address: joerg.doetsch@uk-erlangen.de (J. Dötsch).

0143-4004/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.placenta.2009.12.002

placental insufficiency. In annual follow-up examinations, clinical and biochemical characteristics of the enrolled IUGR and AGA infants especially with regard to childhood obesity, growth failure and hypertension are monitored until the age of 6 years. The ultimate goal is to prevent childhood morbidity in neonates after IUGR.

In view of this multicenter approach, we tried to establish a feasible concept of sample collection for all participants. To provide a basis for reliable investigations, we compared placental gene expression of factors potentially involved in fetal programming between IUGR and AGA newborns at different sampling sites.

2. Methods

2.1. Patients

Placental tissue was collected from 22 mothers with IUGR neonates (10 males, 12 females) and 19 healthy women with AGA newborns (10 males, 9 females) born prematurely or at term. Inclusion and exclusion criteria are presented in Table 1. All children participated in the FIPS-study and had regular follow-up examinations after birth, therefore strict exclusion criteria comprising any condition affecting postnatal growth were applied according to the study protocol. The clinical characteristics of the 2 groups at birth are shown in Table 2. For the purpose of the study, percentiles of Voigt et al. for German newborns were used [19]. Birth weight Z-score was calculated using the following formula: birth weight *Z*-score = (neonates' birth weight – mean birth weight)/standard deviation. Gestational age was determined from the date of the last menstrual period of the mother and was confirmed by ultrasound measurements before the 14th week of gestation. Prematurity was defined as a gestational age <37 weeks.

Table 1
Inclusion and exclusion criteria for the study.

Inclusion criteria for IUGR
- Gestational age ≥30 + 0 and <41 + 0 weeks
- Birth weight <10th percentile [19]
- Ultrasound examination within the first trimester of gestation to confirm gestational age and to evaluate fetal morphology
- Proven placental insufficiency [31]:

elevated pulsatility index (PI) in at least one uterine artery (>1,2) with or without early diastolic notches
and elevated pulsatility index (PI) in the umbilical arteries (>2 SD) or absent/reverse diastolic flow
- Informed consent of parent

Inclusion criteria for AGA
- Gestational age ≥30 + 0 and <41 + 0 weeks
- Birth weight >25th and <75th percentile [19]
- All pregnancies were dated correctly by ultrasound during the first trimester of gestation
- Normal uterine and umbilical artery Doppler velocimetry during pregnancy [32]
- Informed consent of parent

Exclusion criteria
- Multiple (twins/triplets) pregnancies
- Chromosomal aberration/syndrome
- Congenital infection/TORCH
- Primary sepsis evidenced by clinical symptoms or biochemical signs (immature-to-total neutrophil ratio (IT ratio) > 0,25, CRP > 10 mg/l or positive blood cultures)
- Preeclampsia
- Fetal alcohol syndrome or maternal drug ingestion
- Other congenital or acquired disease leading to postnatal growth restriction (severe cardiac malformation, bronchopulmonary dysplasia, intracranial hemorrhage >II°, hydrocephaly, short bowel syndrome or other severe malformation)
- Large for gestational age (LGA)-newborns > 75. percentile [19]

Table 2
Birth characteristics of IUGR ($n = 22$) and AGA ($n = 19$) neonates.

	IUGR	AGA
Maternal age (years)	28.1 ± 1.3(16–38)	31.0 ± 1.3(20–38), NS
Gestational age (weeks)	34.5 ± 0.6(30–39)	36.0 ± 0.6(30–39), NS
Birth weight	1556 ± 118 (770–2700)	2722 ± 138 (1420–3570)†
Birth weight Z-score	−2.3 ± 0.2(−3.9–1.0)	−0.4 ± 0.1(−1.1–0.2)†
Male/female	10/12	10/9, NS
Spontaneous delivery/Section‡	1/21	9/10*
Maternal smoking‡	2	2, NS
Placental weight (g)	367 ± 28(190–660)	470 ± 32(320–780)*

Values are shown as mean ± SEM (range). Significant differences between the IUGR and AGA group are marked (unpaired *t*-test, ‡Fishers's exact test).
NS, not significant.
*$p < 0.05$.
†$p < 0.0001$.

As a subgroup, we furthermore analyzed placental tissue of 10 SGA neonates (5 males, 5 females). SGA was defined by a birth weight *Z*-score <10th percentile and normal uterine and umbilical artery Doppler velocimetry during pregnancy. Exclusion criteria were the same as for IUGR and AGA newborns (Table 1). Birth weight *Z*-score ranged from a minimum of −2.5 to a maximum of −1.4 with a mean of −2.0. Mean gestational age was 39.0 weeks (range 36–41). Caesarean section was performed in 4 cases, 6 neonates were delivered spontaneously. Mean placental weight was 442 g (range 315–690 g).

The study was reviewed and approved by the ethics committee of the University of Erlangen–Nuremberg. It was explained to each parent who signed a written consent.

2.2. Placental tissue acquisition

Fresh samples of human placentas derived from 22 IUGR to 19 AGA infants were obtained within 30 min after placental delivery in collaboration with the Department of Obstetrics and Gynecology at the University of Erlangen-Nuremberg. Using a sterile scalpel, we excised three quadrangular segments (approximately 2 × 2 cm) along the placental thickness from basal towards chorionic surface, which were localized at specific portions of the placenta. To establish a feasible standardized concept of sample collection even for cases with lateral insertion of the cord or very irregularly shaped placenta, we defined sample location by the percentage of distance between umbilical cord insertion site (0%) and placental margin (100%). Thus, probes taken from an area of up to 10% were called proximal, the sample location at 30–50% was designated as intermediate and at 60–80% as peripheral.

After a rinse of the samples with normal saline, the amniotic membranes and the maternal decidua were removed, then the samples were snap frozen in liquid nitrogen and stored at −80 °C until further processing.

2.3. RNA extraction and reverse transcription

Total cellular RNA was extracted from the placental tissue by TRIzol® reagent (TRIzol®, Invitrogen GmbH, Karlsruhe, Germany). RNA concentrations were determined spectrophotometrically. One μg of RNA was reversely transcribed in a volume of 20 μl at 37 °C for 60 min to synthesize cDNA (chemicals from Boehringer Mannheim, Germany).

2.4. TaqMan real-time PCR

TaqMan real-time PCR (Perkin–Elmer, Foster City, CA) was used to quantify the expression of genes in placenta of IUGR and AGA