Placenta 35 (2014) 359–364

Contents lists available at ScienceDirect

Placenta

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

Fetal sex specific differences in human placentation: A prospective cohort study

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ARTICLE INFO

Article history:
Accepted 20 March 2014

Keywords:
Fetal sex
placental biomarkers

ABSTRACT

Introduction: Our objective was to assess fetal sex specific differences in first trimester placental biomarkers of both physiological and pathological pregnancies and their interaction with environmental influences. This study is embedded in the Generation R Study, a prospective cohort study.
Methods: Only live singleton births were included. Linear regression was performed to assess the effect of sex on first trimester placental biomarkers. Interaction analyses were performed to assess interaction of fetal sex with environmental influences. First trimester soluble fms-like tyrosine kinase (s-Flt1), placental growth factor (PLGF), plasminogen activator inhibitor (PAI-2) and homocysteine levels were assessed.
Results: Significant fetal sex specific differences in placental biomarkers were observed. S-Flt1, PAI-2 and PLGF log transformated concentrations were 0.08 ng/mL (95% CI 0.05; 0.11), 0.07 ng/mL (95% CI 0.06; 0.09) and 0.04 pg/mL (95% CI 0.01; 0.06) higher in case of female as compared to male placentas. In pregnancies complicated by pre-eclampsia (PE), preterm birth (PTB) or a newborn being small for gestational age (SGA) no fetal sex specific differences were observed. Interaction analyses suggest that concentrations of s-Flt1, PLGF and PAI-2 decrease in male placentas in the case of hyperhomocysteinemia but remain equal in female placentas.
Discussion: Fetal sex affects early placentation processes with discrepancies regarding pregnancies complicated by PE, PTB or a newborn being SGA. This suggests that other mechanisms causing these complications may dominate the fetal sex effect. The differences concerning homocysteine suggest that fetal sex dependent placental gene–environment interactions exist.
Conclusion: Fetal sex specific differences in placental biomarkers exist.

1. Introduction

Pregnancy induces several placental-mediated hemodynamic adaptations of maternal physiology to deal with the increased demands of the developing fetal-placental unit. Placental gene expression is known to be fetal sex specific [1,2]. Furthermore, earlier studies showed that the fetoplacental unit influences maternal physiology in a sex specific manner with stronger expressions of placental cytokine mRNA among female placentas resulting in altered maternal asthma symptomatology between women carrying a female and women carrying a male fetus [3–5]. Likewise, a different ratio of male to female fetus concerning miscarriages and pre-eclamptic pregnancies has led to the hypotheses that sex specific associations with respect to placentation exist [6]. Given the knowledge that the placenta is affected by environmental influences that may modify epigenetic marks and gene expression and subsequently placental development and function [1,7], we hypothesize that fetal sex specific alterations in early placentation exist, and that these alterations differ according to environmental influences. We examined sex specific differences among soluble fms-like tyrosine kinase 1 (s-Flt1), placental growth factor (PLGF) and plasminogen activator inhibitor-2 (PAI-2) representing

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http://dx.doi.org/10.1016/j.placenta.2014.03.014
0143-4004/

important angiogenic and fibrinolytic factors implicated in placental development and function [8–10]. Concerning environmental influences we assessed smoking, age, parity, folic acid use and educational level as a proxy of behavioural status. Furthermore, we assessed homocysteine concentrations as a proxy for nutritional status. Homocysteine is an important substance regarding DNA methylation and crucial for placental development and functioning [11,12].

2. Materials and methods

The study was embedded in The Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, The Netherlands [13]. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, The Netherlands. Written informed consent was obtained from all the participants. Women were enrolled prenatally between 2001 and 2005 ($n = 8880$). For the present study, women with a live singleton birth were considered eligible ($n = 8631$). Only women of which at least one placental biomarker was assessed in the first trimester were included ($n = 6040$). We firstly aimed to assess the fetal sex specific differences in the total study population. Secondly, we aimed to assess these differences in subgroups of our study population, i.e. pregnancies complicated by the placental syndrome encompassing pre-eclampsia (PE, $n = 125$), spontaneous preterm birth (PTB, $n = 221$) and a newborn being small for gestational age (SGA, $n = 609$). PE was defined after the completion of the pregnancy according to the International Society for the Study of Hypertension in Pregnancy criteria [14]. PE was defined as the novo hypertension (an absolute blood pressure 140/90 mmHg or greater) after the 20th gestational week with concurrent proteinuria (0.3 g or greater in a 24-h urine specimen or 2+ or greater [1 g/L] on a voided specimen, or 1+ or greater [0.3 g/L] on a catheterized specimen). The occurrence of hypertension and hypertension related complications were cross-validated using hospital registries [15]. PTB was defined as a spontaneous onset of birth <37 weeks of gestation. SGA was defined as a birth weight under the 10th percentile based on our own cohort [16]. Regarding markers of placentation, maternal non-fasting venous blood samples were drawn in the first trimester (median 13.2 weeks of gestational age, 90% range 10.5–17.2 weeks). Details of processing procedures have been described previously [17,18]. Plasma s-Flt1 and PLGF concentrations were analyzed using an immunoelectrochemoluminence assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, The Netherlands) and plasma PAI-2 concentrations were determined by enzyme-linked immunosorbent assay. Serum homocysteine concentrations were analyzed using a microparticle-enhanced immunoassay on the AxSYM and Architect system. Data on maternal age, educational level, folic acid use smoking habits in early pregnancy and parity were obtained through self-administered questionnaire at enrollment (response rate 93% [13]). Information on maternal outcomes, including PE, PTB and SGA was obtained from medical records, completed by community midwives and obstetricians.

2.1. Data analyses

To test the differences in baseline characteristics between males and females Student *t*, Mann–Whitney U and Chi-square tests were firstly performed. Second to assess fetal sex specific differences in s-Flt1, PLGF and PAI-2 concentrations Mann–Whitney *U* test was used as not all markers of placentation were normally distributed. Next, log transformation of s-Flt1, PLGF and PAI-2 concentrations were performed with subsequent linear regression analyses to relate the placental biomarker concentrations to fetal sex, adjusted for the gestational age at sampling and placental weight. We tested fetal sex specific differences in markers of placentation in response to different environmental influences known to affect placentation. Effect modification was tested by multiplying fetal sex with the covariables maternal smoking, age, parity, educational level as a proxy of behavioural status and serum homocysteine concentrations as a proxy for nutritional status. If $p < 0.10$ was fulfilled linear regression analyses were performed in strata of that specific determinant for both the sexes. All statistical analyses were performed using SPSS version 20.0 for Windows (IBM SPSS INC, Chicago, IL, USA).

3. Results

No differences between women carrying a male fetus and women carrying a female fetus concerning maternal age, ethnicity, education level, BMI at intake, i.e. enrollment in the study, folic acid use, gravidity, parity, gestational age at birth and placental weight were observed (Table 1).

The non-adjusted plasma concentrations of s-Flt1, PLGF and PAI-2 are presented in Table 2 in the first column of each biomarker. Associations between fetal sex and the plasma concentrations of the placental biomarkers in the first trimester of pregnancy are presented in the same table in the second column. In the total group consisting of 6040 participants, all placental biomarkers were higher in women carrying a female fetus compared with women carrying a male fetus. Subsequently, we stratified the total group into 4 groups: PE ($n = 125$), SGA ($n = 609$), PTB ($n = 221$) and women without the previous pregnancy complications (i.e. uncomplicated pregnancies, $n = 5137$). There were 52 women with more than one complication, these women contribute to both complication groups. In women with PE, SGA or PTB all fetal sex specific differences disappeared. In uncomplicated pregnancies, all results were comparable with the total group, i.e. all placental biomarkers were higher in women carrying a female fetus compared with women carrying a male fetus.

We did not find a fetal sex specific interaction concerning maternal smoking, folic acid use, and maternal age at intake, educational level and parity. However, in the case of high homocysteine concentrations (>97.7th percentile) interactions were found as demonstrated in Fig. 1. In women carrying a male fetus plasma levels of PLGF and PAI-2 decreased in the case of hyperhomocysteinemia. However, in women carrying a female fetus these effects were no longer present. Concerning plasma levels of s-Flt1 no effect modification was found.

4. Discussion

4.1. Main findings

We demonstrate fetal sex related differences in biomarkers of early placentation among physiologic pregnancies as well as among pregnancies with complications belonging to the placental syndrome [19]. We further show evidence of fetal sex specific placental gene–environmental interactions.

4.2. Interpretation

Normal human pregnancy involves important changes to maternal vascular function that allow for the large increase in blood flow to the fetoplacental unit [20]. Abnormal vascular adaptations are associated with pregnancy complications including PE and SGA. The angiogenic and fibrinolytic growth factors PLGF, s-Flt1 and PAI-2 are well known for their associations with placental development and function and consequently placenta-mediated vascular adaptation to pregnancy [18]. Previously, it was demonstrated that maternal vascular function can be affected by the sex of the fetus and that this process varies in the presence of PE [1,21]. In normal physiologic pregnancies, maternal microvascular vasodilatation induced by placentally derived corticotrophin releasing hormone is enhanced in women with a male fetus compared to women with a female fetus [21]. However, in pre-eclamptic pregnancies this process seems to be different with reduced microvascular vasodilatation in women pregnant with a male fetus compared to normotensive women pregnant with a male fetus. Importantly, in women pregnant with a female fetus, no differences are observed [1]. As changes in maternal vascular function are associated with changes in placental metabolic function, these results are in line with our data showing not only that the placental release of circulating angiogenic and fibrinolytic factors is influenced by fetal sex, but also that these fetal sex associated differences in angiogenic and fibrinolytic factors alter in pregnancies complicated by the placental syndrome, including PE, SGA and PTB. Secondly, concerning the fact that we observed different associations in complicated versus uncomplicated pregnancies we know that placentation in the case of PE, SGA or PTB in the first trimester is already altered compared with an uncomplicated pregnancy [20]. Impaired trophoblast invasion of myometrial arteries and poor