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The association between angiogenic markers and fetal sex: Implications for preeclampsia research

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ABSTRACT

Objective: Current research suggests sexual dimorphism between the male and female fetoplacental units, but with unknown relevance for preeclampsia. We investigated the association between fetal sex and concentrations of the angiogenic markers soluble Fms-like kinase 1 (sFlt-1), placental growth factor (PlGF), and sFlt-1/PlGF ratio in first and second-third trimester in women with/without preeclampsia, and the impact of fetal sex on the prognostic value of angiogenic markers for preeclampsia.
Study design: Observational study in a prospective, population-based cohort of 2110 singleton pregnancies with 150 preeclampsia cases.
Results: Higher sFlt-1 concentrations were observed for women carrying female fetuses in first trimester (all, 1107.65 vs. 992.27 pg/ml; preeclampsia cases, 1118.79 vs. 934.49 pg/ml, $p < 0.05$) and in second-third trimester (all, 1130.03 vs. 1043.15 pg/ml; preeclampsia, 1480.30 vs. 1152.86 pg/ml, $p < 0.05$), with similar findings for the sFlt-1/PlGF ratio concentrations in first (29.67 vs. 27.39 $p < 0.05$) and second-third trimester (3.56 vs. 3.22, $p < 0.05$). In first trimester, log transformed concentrations of PlGF, sFlt-1 and sFlt-1/PlGF (all participants) and sFlt-1 (preeclampsia cases) associated with fetal sex in adjusted analyses ($p < 0.05$). In second-third trimester, only log(sFlt-1) associated with fetal sex (all, $p = 0.028$; preeclampsia, $p = 0.067$) In receiver operating curve analysis, prediction of early-onset preeclampsia by sFlt-1/PlGF tended to be superior in pregnancies with female vs. male fetuses ($p = 0.06$).
Conclusion: Sexual dimorphism was observed for concentrations of angiogenic markers. Female fetal sex was associated to higher sFlt-1 and sFlt-1/PlGF ratio concentrations in both healthy pregnancies and women developing preeclampsia. Fetal sex should be considered in research and clinical use of angiogenic markers.

1. Introduction

Preeclampsia remains a leading cause of maternal and perinatal morbidity and mortality worldwide, with a global incidence of 3–5% of all pregnancies (Sibai et al., 2005). In spite of decades of research, the exact mechanisms behind the disease remain unclear. Sex-specific differences in fetal development and prognosis of the newborn are well established. Male fetuses are on average larger than female fetuses, and more male infants are born relative to female infants, however, the male sex has an increased risk of neonatal mortality (Vatten and Skjaerven, 2004). Very preterm births are substantially more common in pregnancies carrying a male fetus, whereas preterm preeclampsia is more common in pregnancies with female fetuses (Vatten and Skjaerven, 2004).

In addition, several *in vitro* studies have demonstrated that the human placenta displays sexual dimorphism with different patterns of gene and protein expression depending on fetal sex

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(Sood et al., 2006; Clifton 2010; Osei-Kumah et al., 2011). Fetal sex-specific hormones may play a role, also in placenta. In an experimental setting, stimulation of endometrial cells with estrogen regulated their production of angiogenic proteins VEGF-A and angiopoietin (Tsuzuki et al., 2013). Furthermore, Brown and colleagues demonstrated that the angiogenic factors and biomarkers for preeclampsia development, soluble Fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF), demonstrated sex specific changes already in first trimester of healthy pregnancies (Brown et al., 2014).

PlGF and sFlt-1 are both released from the placenta and the maternal endothelium (Levine et al., 2004; Powe et al., 2011). PlGF is a member of the VEGF family, potentiating the effects of VEGFA (Park et al., 1994). The levels of PlGF are decreased early in pregnancies later complicated by preeclampsia (Thadhani et al., 2004). sFlt-1 is a scavenger receptor, preventing the binding of VEGF-A and PlGF to their other receptors (Breier et al., 1995). A high serum concentration of sFlt-1 is thought to reduce the effect of VEGF-A and PlGF in the maternal circulation, thus exerting an anti-angiogenic function.

Angiogenic factor concentrations can correctly classify a large proportion of women with preeclampsia at time of disease onset (Verlohren et al., 2010; Benton et al., 2011; Alvarez-Fernandez et al., 2014; Andersen et al., 2015), and predict preeclampsia development, 1–4 weeks prior to clinical diagnosis (Zeisler et al., 2016).

The aim of this study was to investigate the association between fetal sex in singleton pregnancies and angiogenic marker concentrations in first and second-third trimester. The specific objectives were (1) to determine whether angiogenic factor concentrations differ by fetal sex, (2) to determine such differences in women with and without preeclampsia, and (3) to evaluate the impact of fetal sex on the prognostic value of angiogenic markers for preeclampsia prediction.

2. Study population and participants

We studied participants in the population-based Odense Child Cohort (OCC) in Denmark, a cohort including newly pregnant women between January 1st 2010 and December 31st 2012. The cohort has earlier been described in details (Kyhl et al., 2015). The women were predominantly Danish, with only 3.5% originating from non-Western countries. All participants gave informed consent.

From a population base of 7032 pregnant women, 6707 were offered inclusion and a total of 2874 pregnant women (42.9%) enrolled in OCC up to December 31st, 2012. Twin pregnancies and early pregnancy fetal loss (miscarriage or abortion on request) were excluded from the present study. Of the remaining participants, 1016 donated a blood sample at gestational age 8 < 14 weeks, and 1595 donated a blood sample at gestational age 20 < 34 weeks, Fig. 1. A total of 2110 blood samples were available for analysis of sFlt-1 and PlGF in these two time intervals together, as some women only contributed a blood sample in one of the gestational age intervals.

Diagnosis of hypertensive disease of pregnancy was validated retrospectively as previously described (Luef et al., in review). Briefly, preeclampsia was defined if a participant had two or more episodes of *de novo* hypertension defined as >140/90 mmHg with at least 4 h between, or previous existing hypertension, with *de novo* onset of proteinuria (>0.3 g/24 h) defined as at least +1 on sterile urine dipstick, after gestational week 20 + 0. Early-onset preeclampsia was defined as preeclampsia with debut before gestational week 34 + 0.

Information on due date, pre-gestational body mass index (BMI), maternal age, smoking habits and parity were extracted from self-reported data at the first antenatal visit. Maternal parental country of origin was extracted from municipality records, and stratified by Danish, Western and non-Western countries. Smoking was defined as smoking during early pregnancy in any quantity. Maternal age at the time of delivery was recorded. Mode of delivery, date of birth, birth weight, placental weight and other birth data were extracted from hospital files.

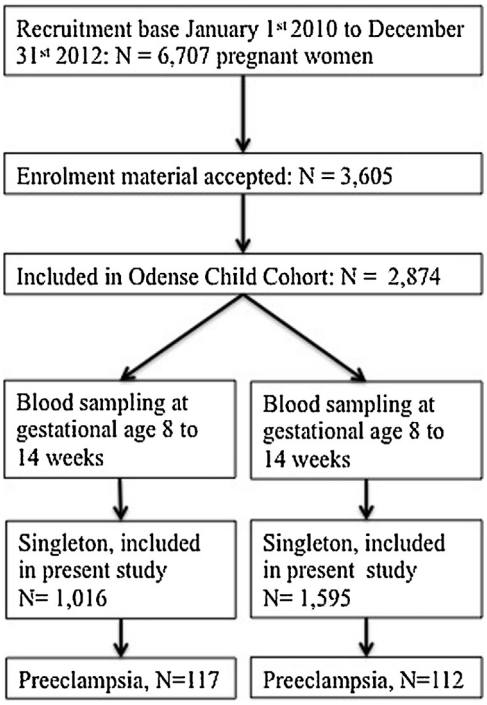

Fig. 1. Inclusion flowchart.

3. Description of assay methods

Measurements for BRAHMS sFlt-1 and PlGF KRYPTOR assays were performed on the fully automated KRYPTOR compact Plus system (KRYPTOR PlGF and KRYPTOR sFlt-1; Thermo Fisher Scientific) according to the manufacturer's instructions as described in detail previously (Andersen et al., 2015). The staff carrying out assay measurements was blinded to individual participant's status in regard to preeclampsia and maternal covariates. According to the manufacturer's instructions for use, the KRYPTOR sFlt-1 assay covered a measuring range of 22–90,000 pg/ml. The limit of detection is 22 pg/ml and the limit of quantization (functional sensitivity) is 29 pg/ml. The KRYPTOR PlGF assay covers a measuring range of 3.6–7.000 pg/ml. The limit of detection is 3.6 pg/ml and the limit of quantitation is 6.9 pg/ml.

4. Statistical analyses

Pregnancy and maternal variables were reported as mean and standard deviation (SD), or 95% confidence intervals. Crude comparisons of continuous variables between groups were performed with Student's *t*-test. Comparisons between proportions of groups were calculated using Fischer's exact test. As concentrations of angiogenic markers demonstrated very skewed distributions, data was log-transformed with the natural logarithm as base, prior to further analyses. Means and 95% confidence intervals were back-transformed to the geometric equivalents using the exponential function, prior to presentation in text and Table 2 as suggested by Bland and Altman (Bland and Altman, 1996). Multiple linear