Mid trimester maternal ADAM12 levels differ according to fetal gender, in pregnancies complicated by preeclampsia.

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Abstract

An over representation of adverse pregnancy outcomes has been observed in pregnancies associated with a male fetus. We investigated the association between fetal gender and candidate biomarkers for preeclampsia. Proteins were quantified in samples taken at 20 weeks from women recruited to the SCOPE study (preeclampsia n=150; no preeclampsia n=450). In contrast to PlGF, sEng and IGFALs, levels of ADAM12 at 20 weeks were dependent on fetal gender in pregnancies complicated by preeclampsia, male (n=73) 1.3 multiples of the median (MoM) [IQR 1.1-1.5] vs female (n=75) 1.1 [1.0-1.3]; p<0.01. Prediction of preeclampsia using ADAM12 levels was improved for pregnancies associated with a male rather than a female fetus, Area under Receiver Operator Curve (AUC) 0.73 (95% CI 0.67-0.80) vs 0.62 (0.55-0.70), (p=0.028). The data presented here fit a contemporary hypothesis that there is a difference between the genders in the response to an adverse maternal environment and suggest that an alteration in ADAM12 may reflect an altered placental response in pregnancies subsequently complicated by preeclampsia.

Introduction

Many investigators have observed disparities between male and female fetuses in relation to a number of adverse pregnancy outcomes. The ratio of male fetuses to female fetuses has been shown to be increased in fetal loss due to miscarriage[1] and stillbirths[2]. Disorders of pregnancy associated with poor placentation such as abruption[2], preeclampsia[3] and fetal growth restriction (FGR) associated with placental insufficiency[4, 5] have also been shown to occur more frequently when the fetus is male. Furthermore, sex specific alterations in placental genes (e.g. JAK1, IL2RB, Clusterin, LTBP, CXCL1 and IL1RL1 and TNF receptor)[6] have been observed to be upregulated in female placentas suggesting that aberrations in placental function can occur in a sex specific manner[7].

Placental complications such as preeclampsia and fetal growth restriction represent highly significant, but potentially modifiable health and economic burdens throughout the world. Preeclampsia continues to be a major cause of maternal mortality[8], and there are strong associations between both conditions and stillbirth[9, 10]. Effects of preeclampsia and FGR are not limited to very early life; surviving babies are at increased risk of cardiovascular disease, chronic hypertension, type 2 diabetes and schizophrenia in adulthood[11]. To prevent the complications of preeclampsia and FGR, women at high risk of the condition need to be identified early in pregnancy. Consequently there is much interest in predictive tests using combinations of clinical risk factors, biophysical measurements and biochemical tests[12-16]. Although there have been significant advances in the reported performance of these combinations, to date no screening test has achieved the requisite sensitivity and specificity to be useful and cost effective in a clinical setting.

Much of the data on early pregnancy biomarkers is conflicting[17-20], with several studies reporting positive and negative findings for the same markers. We have previously identified the biomarkers placental growth factor (PlGF), soluble endoglin (sEng), metallopeptidase Domain 12 (ADAM-12) and insulin-like growth factor acid labile subunit (IGFALS)) as being relevant to the prediction of preeclampsia and FGR[21]. In this study, we aimed to investigate whether there was a significant association between fetal gender and the levels of these protein biomarkers. In addition, the influence of fetal gender on the predictive performance of potential biomarkers was assessed.

Methods

Local ethical committee approval was granted and written informed consent was obtained from all participants. Women recruited into the SCOPE study, a prospective screening study of low risk nulliparous women recruited in Australia, New Zealand, United Kingdom and Ireland between November 2004 and February 2011 (ACTRN12607000551493) participated in this study[12]. A research midwife interviewed participants at 14-16 weeks' and 19-21 weeks' gestation and pregnancy outcomes were prospectively tracked. At the time of interview, blood pressure, height and weight were measured and data were entered on an internet accessed central database (MedSciNet). Two consecutive manual blood pressure measurements were recorded. Blood samples were collected on EDTA at 14-16 and 19-21 weeks' gestation and plasma was stored at -80°C within four hours of collection.

Samples:

150 women who developed preeclampsia and 450 controls were randomly selected from the 5605 women recruited in Australia, New Zealand, London, Manchester, Leeds, UK and Cork, Ireland. Controls were selected from those who did not have preeclampsia at the same center and included women with uncomplicated pregnancies and those with complications such as small for gestational age, preterm birth, gestational hypertension and gestational diabetes[21]. Preeclampsia was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or both, on at least two occasions four hours apart after 20 weeks' gestation but before the onset of labour, or postpartum, with either proteinuria (24 hour urinary protein ≥300 mg or spot urine protein:creatinine ratio ≥ 30 mg/mmol creatinine or urine dipstick protein ≥++) or any multisystem complication of preeclampsia[12]. Preterm preeclampsia was defined as women who required delivery prior to 37 weeks' gestation. Small for gestational age (SGA) was used as a proxy for FGR and was defined as infants weighing <10th customized centile[12].

Measurement of proteins

Proteins were quantified as part of a biomarker validation study using targeted mass spectrometry assays based on an SRM peptide quantification method, using custom-built assays as previously reported[21]. In brief, plasma samples were depleted of albumin and IgG, denatured and spiked with mixture of isotopically-labelled peptides serving as internal reference. Following tryptic digestion, and peptide separations, quantitative data was obtained using a triple quadrupole MS instrument. The readout of an assay in each sample was the ratio of the analyte signal area (endogenous peptide) over the common internal standard signal area. Comparison of ratios between different samples

represents the relative quantification of the protein. The details of the peptide transitions used are described in the supplementary information of our previous study[21].

The sample order was randomised prior to every analytical step, and laboratory personnel were blinded to the pregnancy outcome related to each sample. Technical variation was estimated by preparing and measuring 10% of the samples, in duplicate, in a randomized order. Coefficient of variation were 11%, 12% and 10% for sEng, ADAM12 and IGFALS, respectively, with <5% missing data for each biomarker[21].

PlGF was measured in all samples using DELFIA time resolve fluorescence technology (PerkinElmer, Turku, Finland). Interassay coefficients of variation for the PlGF assay were 3% at 16.8 pg/mL and 8% at 852 pg/mL.

Data analysis

The sample size was determined by the previous study which aimed to identify a panel of predictive markers for preeclampsia[21]. R and bioconductor were used to perform all statistical analyses[22]. The characteristics of the preeclampsia group and controls were compared using student *t* test, Wilcoxon rank sum test and χ^2 test. Amongst the controls, there was a significant relationship between ADAM12 concentration and gestational age at sampling and BMI and therefore ADAM12 was converted to multiples of the median (MoM). sEng concentration was also expressed as MoM to correct for BMI. Median differences in biomarker levels were assessed using Mann Whitney-U and differences between areas under receiver operator characteristic curves (AUC) were assessed using DeLong's test. For the multiple of the mean (MoM) the regressed medians were computed using a log-linear regression of the first degree on the control population.

Results

Baseline characteristics and pregnancy outcome for the patient cohort investigated are shown in Table 1. As expected, significant differences between groups were observed for the parameters known to be associated with preeclampsia, such as early pregnancy BMI and blood pressure. There were significantly more preterm births in the preeclampsia groups, in addition to a higher proportion of small for gestational age infants compared with the group that did not develop preeclampsia (control group).

Figure 1 shows the levels of ADAM12, s-ENG and IGFALS and PlGF at 20 weeks in women with preeclampsia compared to controls. There was no effect of gender on sEng, PlGF or IGFALS levels in the control group or in women with pregnancies complicated by preeclampsia or SGA ($p>0.05$). In contrast, levels of ADAM12 at 20 weeks were dependent on fetal gender in women with preeclampsia, with significantly higher ADAM12 levels observed in women carrying male fetuses (n=73) compared to women carrying female fetuses (n=75) (1.3 MoM [IQR 1.1-1.5] vs 1.1 [1.0-1.3]; $p<0.01$; Mann-Whitney U test). There was no effect of fetal gender in the control group on ADAM12 levels. ADAM12 levels were associated with significantly higher AUCs (prediction of preeclampsia) for pregnancies associated with a male rather than a female fetus, 0.73 (95% CI 0.67-0.80) vs 0.62 (0.55-0.70), respectively ($p=0.028$; DeLong's test; Figure 2). The MoM levels of ADAM12 were also dependent on fetal gender in pregnancies complicated by SGA (male n=39, female n=49; $p<0.01$; Figure 3). The prediction of SGA using ADAM12 was poor and the AUC was not significantly altered by fetal gender (AUC females 0.55 [95% CI 0.45-0.64] vs males 0.60 [0.51-0.69]; $p>0.05$; DeLong's test). No significant relationship between ADAM12 and the other markers IGFALS (rho: 0.25), PlGF (rho: 0.16) or s-ENG (rho: 0.42) was observed.

Discussion

Previous work has suggested that there are altered responses to pregnancy stressors according to fetal gender. In this study we aimed to determine the potential impact of fetal gender on levels of a number of pregnancy biomarkers. Of the proteins quantified in this cohort, there was a strong association of ADAM12 with fetal gender in pregnancies subsequently complicated by preeclampsia and/or SGA. ADAM12 was upregulated in pregnancies complicated by preeclampsia but discrimination between cases and controls was more distinct for pregnancies with a male rather than a female fetus. This gender effect was not observed for the other proteins measured.

Both naturally occurring splice variants, ADAM12-L and ADAM12-S, are highly expressed in the human placenta[23], and ADAM12-S is found in maternal serum from the early first trimester[24]. At the beginning of pregnancy (up to week 8) ADAM12 is closely correlated to the size of the placenta[25]. In normal pregnancy, the ADAM12-S levels associate with gestational age and a 60-fold increase between 8 weeks and full term pregnancy (>38 weeks)[24].

It has been suggested that ADAM12 is involved in the regulation of fetal growth through proteolysis of insulin-like growth factor binding proteins (IGFBPs). Proteolysis of IGFBPs results in the release of insulin-like growth factors (IGFs) which play an important role in the development of the placenta and fetus [26]. The cleavage of IGFBP3 (the most abundant IGFBP in serum) and IGFBP5 by ADAM12-S is thought to be one of the factors which regulates the bioavailability of IGF-I and IGF-II and reduced 1st trimester ADAM-12 levels have been reported in SGA pregnancies[27]. Several other publications have reported the potential of ADAM12 to be used as an early pregnancy marker for both preeclampsia and fetal growth restriction (FGR), although the results are inconclusive[17, 28]. In these studies, 1st trimester maternal serum ADAM12 concentrations were influenced by fetal gender, gestational age (increasing), ethnicity (higher in African American), maternal weight (decreasing), smoking (lower in smokers) and maternal age (lower in women ≥40 years). The authors, however, commented that smoking and maternal age accounted for the largest intergroup differences in ADAM12, whereas gender-related differences were negligible[28].

The data regarding changes in ADAM12 in early pregnancy are conflicting[17, 19, 20, 29, 30] with previous studies reporting a down regulation of ADAM12 in pregnancies subsequently complicated by preeclampsia and more recent reports suggesting an upregulation. Some of these disparities may relate to different sampling methods, particularly different gestational ages. In the current study, significant differences in ADAM12 were observed at 20 weeks, a later gestation than many of the previous reports.

The data presented here fit a contemporary hypothesis stating that when exposed to insults during pregnancy female and male offspring "react" differently[7]. Previous studies have demonstrated that cord blood levels of IGF I and IGFBP-3 are different between male and female fetuses[31]. In addition, a prospective study of pregnancies complicated by maternal asthma investigated the relative influences of asthma, corticosteroid use, smoking and components of the IGF axis on birthweight[32]. A gender-specific difference in the relationship between IGF I and IGFBP on birthweight was observed; in pregnancies with a male fetus IGF I levels were not influenced by maternal factors whereas IGF I was significantly decreased in pregnancies complicated by asthma and smoking when the fetus was female. The authors hypothesized that this data was supportive of different response strategies by male and female fetuses to adverse pregnancy conditions[7]. Males are believed to adapt for continued growth in an adverse maternal environment while females reduce growth in an attempt to survive further maternal insults. The data presented here suggest that an alteration in ADAM12 may reflect an altered placental response, perhaps mediated by changes in IGF bioavailability, in pregnancies subsequently complicated by preeclampsia. It is interesting that there was no observed effect of fetal gender on the other biomarkers investigated in this study. This may indicate that regulation of angiogenic factors (sEng and PlGF) is independent of fetal gender, although this would need to be confirmed in independent studies. Whilst IGFALS mRNA is expressed by the placenta[33], it is likely that the majority of circulating IGFALS is maternal in origin[34] and therefore unlikely to be affected by fetal gender.

Limitations of study

Whilst this study has used data from a large prospective cohort, the number of pregnancies studies subsequently affected by preeclampsia is modest. In addition, the analysis described in this study represents a post hoc analysis of data generated as part of a previous study and may therefore be subject to bias.

Significance

Gender differences may account for disparate data relating to ADAM12 as a predictive marker for preeclampsia. Given the heterogeneity of preeclampsia as a diagnosis, the influence of factors such as fetal gender, not traditionally considered in screening algorithms, may be important in the pursuit of more accurate predictive tests for pregnancy complications. Novel technologies which allow us to determine fetal gender from maternal blood are likely to be more affordable in the future; this may allow the inclusion of fetal gender in predictive algorithms. Both prediction studies and ex vivo studies need to consider the effect of fetal gender in the development of pregnancy complications.

Ethical approval

Ethical approval was obtained from local ethics committees [New Zealand AKX/02/00/364, Australia REC 1712/5/2008, London, Leeds and Manchester 06/MRE01/98 and Cork ECM5 (10) 05/02/08]

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Disclosures

GT, RT, YVH, ROD are employed by Pronota which has a commercial interest in the development of predictive tests for preeclampsia. JM, PNB and RAN have received consultancy fees (paid to their institution) from Pronota.

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Figure legends

Figure 1

Levels of ADAM12, PlGF, sEng and IGFALs in samples taken at 20 weeks from women with and without preeclampsia subdivided by fetal sex. The dotted lines represent the median of the control and preeclampsia groups for both sexes combined .

Figure 2

Univariate area under the receiver operator curve (AUC) with 95% CI for the candidate biomarkers, subdivided by fetal sex.

Figure 3

Levels of ADAM12, PlGF, sEng and IGFALs in samples taken at 20 weeks from women with (n=88) and without SGA (n=512) subdivided by fetal sex. The dotted lines represent the medians of the control and SGA groups for both sexes combined. ADAM12 was significantly different between SGA pregnancies associated with male vs female fetus ($p< 0.01$), but not different in women without SGA.

Table 1 Demographic characteristics

Characteristics	Preeclampsia (n=150)	No Preeclampsia (n=450)	P value
Maternal age (years)	28 (23-32)	29 (24-32)	NS
Fetal gender male	75 (50%)	232 (52%)	NS
Caucasian Ethnicity	128 (90%)	402 (90%)	NS
Smoker at 15 wks	12 (8%)	61 (14%)	NS
Body mass index at 15 wks(kg/m^2)	26.6 (23.1-30.5)	24.1 (21.7-27.7)	<0.001
Gestation at sampling (wks)	20.4 (0.7)	20.4 (0.8)	NS
Systolic blood pressure (mmHg)	116 (11)	109 (10)	<0.001
Diastolic blood pressure (mmHg)	69 (9)	65 (7)	<0.001
Gestation age at delivery (weeks)	38 (37-40)	40 (39-41)	<0.001
Preterm birth (<37 wks)	42 (28%)	19 (4%)	<0.001
Small for gestational age	39 (26%)	49 (11%)	<0.001
Gestational hypertension	0 (0%)	39 (9%)	<0.001
Gestational diabetes mellitus	8 (7%)	8 (3%)	NS

Results are expressed as mean (SD), median (interquartile range, IQR) or n (%).