Ultrasound Obstet Gynecol 2015; **45**: 190–198
Published online 29 December 2014 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.14685

Longitudinal changes in maternal corin and mid-regional proatrial natriuretic peptide in women at risk of pre-eclampsia

A. KHALIL*, N. MAIZ†, R. GARCIA-MANDUJANO‡, M. ELKHOULI‡ and K. H. NICOLAIDES‡

**Department of Fetal Medicine, St George's University of London, London, UK; †Fetal Medicine Unit, Obstetrics and Gynecology Service, BioCruces Health Research Institute, Hospital Universitario Cruces, University of the Basque Country (UPV/EHU), Barakaldo, Spain; ‡Department of Fetal Medicine, King's College Hospital, London, UK*

KEYWORDS: atrial natriuretic peptide; blood pressure; corin; gestational hypertension; longitudinal; pre-eclampsia; pregnancy screening

ABSTRACT

Objective *Corin, an atrial natriuretic peptide-converting enzyme, has been found to promote trophoblast invasion and spiral artery remodeling. Yet, elevated maternal plasma atrial natriuretic peptide (ANP) and corin levels have been reported in pregnancies complicated by pre-eclampsia (PE). The aim of this study was to investigate longitudinal changes in maternal plasma levels of corin and mid-regional proatrial natriuretic peptide (MR-PANP) in pregnancies that develop PE and gestational hypertension (GH).*

Methods *This was a nested case–control study drawn from a larger prospective longitudinal study in singleton pregnancies identified as being at high risk for PE by screening at 11 + 0 to 13 + 6 weeks' gestation. Blood samples were taken every 4 weeks until delivery. Values were compared in pregnancies that developed preterm PE (requiring delivery before 37 weeks' gestation), term PE, GH and those that remained normotensive.*

Results *A total of 471 samples were analyzed from 122 women, including 85 that remained normotensive, 12 that developed GH, 13 term PE and 12 preterm PE. In the normotensive group, log_{10}corin levels were associated with gestational age (*$P < 0.01$*), whereas log_{10}MR-PANP levels were not. In the preterm-PE group, compared with the normotensive group, corin was significantly lower until 20 weeks' gestation (*$P = 0.001$*). In the GH and term-PE groups, corin did not differ significantly from the normotensive group (*$P = 0.637$ *and* $P = 0.161$*, respectively). Compared with the normotensive group, MR-PANP levels were significantly higher in the pregnancies that developed preterm PE and GH (*$P = 0.046$ *and* $P = 0.019$*, respectively), but not term PE (*$P = 0.467$*).*

Conclusion *Maternal-plasma corin and MR-PANP could potentially be useful biomarkers for the prediction of preterm PE.* *Copyright © 2014 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Pre-eclampsia (PE) is a leading cause of maternal and perinatal mortality and morbidity in both developed and developing countries[1–3]. Despite extensive research, it remains an enigmatic condition. Robust evidence suggests that PE is characterized by impairment of the physiological process of trophoblast invasion of the maternal spiral arteries[4–6]. This leads to placental hypoxia and the release of inflammatory factors that cause endothelial cell activation and the clinical manifestation of PE[4–6]. However, the underlying mechanisms that impair trophoblastic invasion are not fully elucidated. Women who develop PE are at increased risk of cardiovascular disease and stroke in the subsequent decades[7–10]. Furthermore, studies have suggested that prepregnancy cardiovascular risk factors are predictors of PE[11,12].

Mid-regional proatrial natriuretic peptide (MR-PANP) is a precursor of the atrial natriuretic peptide (ANP), whilst corin is a transmembrane serine protease that converts pro-ANP to active ANP[13–16]. Both ANP and corin are considered as biomarkers for cardiovascular disease[17–20]. Studies have reported elevated levels of ANP in pregnancies complicated by PE[21–28]. Studies in pre-eclamptic rats have demonstrated increased expression of MR-PANP in maternal and fetal hearts[21].

Correspondence to: Dr A. Khalil, Fetal Medicine Unit, St George's University of London, Cranmer Terrace, London SW17 0RE, UK (e-mail: asmakhalil79@googlemail.com)

Accepted: 25 September 2014

Furthermore, natriuretic peptide levels correlated significantly with systolic blood pressure, cardiac index and systemic vascular resistance index in pregnancies complicated by PE[28]. In a landmark study, corin, which is also known as ANP-converting enzyme, was found to promote trophoblast invasion and spiral artery remodeling[29]. Pregnant corin-deficient and ANP-deficient mice have demonstrated markedly impaired trophoblast invasion and spiral artery remodeling and developed PE[29]. Furthermore, when compared with normal pregnancies, uterine corin messenger RNA (mRNA) levels were reduced in pregnancies complicated by PE[29]. However, their study also showed that plasma corin was increased in cases of PE.

The aim of this study was to investigate the longitudinal changes in maternal plasma concentrations of corin and MR-PANP, from the first trimester onwards, in women identified as being at high risk of PE following first-trimester screening and who subsequently developed PE, gestational hypertension (GH) or remained normotensive.

METHODS

At University College London Hospitals, the risk for development of PE was routinely assessed at 11 + 0 to 13 + 6 weeks of gestation, using a combination of maternal history, uterine artery Doppler mean pulsatility index, mean arterial pressure and serum pregnancy-associated plasma protein A[30]. Those considered to be at high risk for early-onset PE were followed up in a specialist hypertension clinic, in which blood samples were collected every 4 weeks until delivery. The study took place between December 2009 and May 2012. Written informed consent was obtained from all women participating in the study, which was approved by the London-Surrey Borders Research Ethics Committee. None of these pregnancies was complicated by aneuploidy or major structural abnormalities.

Maternal-plasma corin and MR-PANP levels were measured in 122 women: 12 later developed PE and required delivery before 37 weeks' gestation (preterm PE); 13 developed PE requiring delivery at or after 37 weeks' gestation (term PE); 12 developed GH; and 85 were unaffected controls. The controls had one or more samples within the preselected gestational age windows of 11–14, 15–19, 20–24, 25–29, 30–34 and 35–38 weeks' gestation, delivered phenotypically normal babies of appropriate weight for gestational age at term and did not develop any hypertensive disorder of pregnancy. None of the samples in this nested case–control study was previously thawed and refrozen.

Patient characteristics recorded included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian or mixed), method of conception (spontaneous or assisted requiring the use of ovulation-inducing drugs), cigarette smoking during pregnancy, history of chronic hypertension or pre-existing diabetes mellitus, history of PE in the mother of the patient and obstetric history including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks' gestation) and previous pregnancy with PE. Maternal weight and height were also measured, and body mass index (BMI) was calculated.

The scientist performing the assays was not aware of the clinical data of the patients and was blinded to the pregnancy outcome. Plasma corin was measured using an enzyme-linked immunosorbent assay (ELISA) (Quantikine corin ELISA kit; R&D Systems Europe Ltd, Abingdon, UK) and the lower limit of detection of the assays was 5.64 ng/L[31]. Plasma MR-PANP was measured using the B.R.A.H.M.S assay based on TRACE technology, which measures the signal that is emitted from an immunocomplex with time delay (B.R.A.H.M.S Biomarkers, Clinical Diagnostics Division, Thermo Fisher Scientific, Hennigsdorf, Germany)[32]. The lower limit of detection of the assays was 2.1 pmol/L. The samples were analyzed in duplicate and the tests were performed according to the manufacturer's recommendations.

Data on pregnancy outcomes were collected from the hospital maternity records of the women. The obstetric records of women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was chronic hypertension, PE or GH. The diagnosis of PE or GH was made according to the guidelines of the International Society for the Study of Hypertension in Pregnancy[33]. GH is defined by systolic blood pressure of 140 mmHg or more and/or diastolic blood pressure of 90 mmHg or more on at least two occasions, 4 h apart, developing after 20 weeks of gestation in a previously normotensive woman in the absence of significant proteinuria. PE is defined by GH with proteinuria of 300 mg or more in 24 h, or two readings of at least ++ on dipstick analysis of a midstream or catheter urine specimen, if no 24-h collection is available. PE superimposed on chronic hypertension is defined as significant proteinuria (as defined above) developing after 20 weeks of gestation in a woman with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks of gestation in the absence of trophoblastic disease).

Statistical analysis

Maternal baseline characteristics were compared using the chi-square test or Fisher's exact test for categorical variables and the Kruskal–Wallis test for continuous variables, and comparisons between different outcome groups were made using the Mann–Whitney *U*-test with *post-hoc* Bonferroni correction for multiple comparisons. Data are presented as median (interquartile range) for continuous data and as *n* (%) for categorical variables.

The distribution of maternal plasma corin and MR-PANP were made Gaussian after $\log_{10}$ transformation and was tested using the Kolmogorov–Smirnov test. Analysis of repeated measures with a multilevel mixed-effects linear model (fixed effects and random effects) was performed. The fixed-effect component included up to third-order polynomial terms of gestational

age, hypertensive disorders (PE or GH) and first-order interaction between gestational age and each hypertensive disorder. The random-effect component included the intercept and linear effects of gestational age. Repeated measurements at different weeks of gestation in the same woman constituted Level 1 and each individual constituted Level 2. The multilevel model was compared with the one-level model using the likelihood radio (LR) test. Before performing the regression analysis, continuous variables were centered by subtracting the mean from each measured value (70 from maternal weight in kg, 164 from maternal height in cm and 32 from maternal age in years).

The software programs MLwiN 2.30 (Centre for Multilevel Modelling, University of Bristol, Bristol, UK) and IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA) were used for the statistical analysis[34].

RESULTS

The maternal characteristics of each outcome group are summarized in Table 1. The median maternal weight was higher in women who developed term PE ($P = 0.036$). There were no significant differences between the different outcome groups in maternal age ($P = 0.164$), height ($P = 0.095$), racial origin ($P = 0.079$), parity ($P = 0.970$), smoking status ($P = 0.638$), mode of conception ($P = 0.117$) or history of chronic hypertension ($P = 0.067$).

Log_{10}corin was significantly higher in women of Afro-Caribbean racial origin and increased significantly with maternal age (Table 2), but was not significantly affected by maternal weight ($P = 0.403$), height ($P = 0.289$), smoking status ($P = 0.798$), mode of conception ($P = 0.097$), history of PE ($P = 0.427$) or chronic hypertension ($P = 0.505$). A random slope model provided a significantly better fit to the data than did a single-level model (LR = 298, degrees of freedom = 3, $P < 0.001$) or a random intercept model (LR = 8.36, degrees of freedom = 2, $P < 0.05$).

In the normotensive group, there was a fourth-order (quadratic) polynomial association between log_{10}corin and gestational age (Table 2, Figure 1). In the preterm-PE group, compared with the normotensive group, the concentration of corin was significantly lower until 20 weeks' gestation, but not after 20 weeks' gestation and the corin concentration increased more rapidly (Table 2, Figure 2). In the GH and term-PE groups, the corin concentration did not differ significantly from that in the normotensive group ($P = 0.637$ and $P = 0.161$, respectively).

Log_{10}MR-PANP increased significantly with maternal height, decreased significantly with maternal weight and was significantly higher in pregnancies achieved by *in-vitro* fertilization (Table 2), but was not significantly affected by maternal age ($P = 0.313$), history of PE ($P = 0.224$) or chronic hypertension ($P = 0.168$), smoking status ($P = 0.964$), racial origin ($P = 0.077$) or gestational age ($P = 0.338$). A random slope model provided a significantly better fit to the data than did a single-level model (LR = 147.4, degrees of freedom = 3, $P < 0.001$) or a random intercept model (LR = 26.1, degrees of freedom = 2, $P < 0.001$).

There was no significant association between log_{10}MR-PANP and gestational age in any of the outcome groups (Table 2, Figures 3 and 4). In the preterm-PE and GH groups, compared with the normotensive group, MR-PANP was significantly higher (Table 2, Figure 4). In term PE, MR-PANP was not significantly different from the normotensive group ($P = 0.467$).

Table 1 Maternal characteristics, according to outcome group, of 122 pregnant women assessed for development of pre-eclampsia (PE) and gestational hypertension (GH)

Characteristic	*Controls* (n = *85*)	*GH* (n = *12*)	*Term PE* (n = *13*)	*Preterm PE* (n = *12*)
Maternal age (years)	32.0 (28.5–35.5)	34 (30.3–38.8)	28.0 (25.5–33.5)	28.0 (26.3–36.5)
Weight (kg)	65.0 (56.5–75.0)	72.5 (65.0–83.9)	78.0 (64.5–93.3)*	62.5 (56.8–65.3)
Height (cm)	164.0 (160.3–167.8)	165.0 (162.7–169.0)	162.0 (157.0–167.6)	157.0 (152.3–167.3)
Racial origin				
Caucasian	66 (77.6)	8 (66.7)	5 (38.5)	6 (50.0)
Afro-Caribbean	8 (9.4)	3 (25.0)	3 (23.1)	3 (25.0)
South Asian	7 (8.2)	1 (8.3)	3 (23.1)	3 (25.0)
East Asian	2 (2.4)	—	2 (15.4)	—
Mixed	2 (2.4)	—	—	—
Parous	24 (28.2)	3 (25.0)	3 (23.0)	4 (33.3)
Cigarette smoker	4 (4.7)	1 (8.3)	—	—
Mode of conception				
Spontaneous	80 (94.1)	10 (83.3)	12 (92.3)	12 (100)
Ovulation induction	3 (3.5)	2 (16.7)	1 (7.7)	—
In-vitro fertilization	2 (2.4)	—	—	—
Chronic hypertension	1 (1.2)	—	2 (15.4)	1 (8.3)
Gestational age at delivery (weeks)	39.7 (38.9–40.6)	40.2 (38.0–41.4)	39.3 (39.4–40.0)	33.0 (29.3–36.5)**

Data are given as median (interquartile range) or *n* (%). Comparisons between outcome groups by chi-square test for categorical variables and Kruskal–Wallis for continuous variables. Comparison of each outcome group with normal outcome by Mann–Whitney *U*-test with *post-hoc* Bonferroni correction. *$P < 0.005$. **$P < 0.001$.

Table 2 Application of multilevel linear mixed-effects model on log_{10}corin and mid-regional proatrial natriuretic peptide (MR-PANP) in 122 pregnant women assessed for development of pre-eclampsia (PE) and gestational hypertension (GH)

	Corin			*MR-PANP*		
Parameter	*Estimate*	*SE*	P	*Estimate*	*SE*	P
Fixed part						
Intercept	−0.092539	0.838820	0.912	1.296470	0.017241	< 0.001
GH	0.030929	0.065384	0.637	0.114157	0.048199	0.019
Term PE	0.092735	0.065657	0.161	−0.034879	0.047773	0.467
Preterm PE	−0.263254	0.069792	< 0.001	0.103143	0.051167	0.046
GA (weeks)	0.517377	0.152870	0.001			
GA (weeks)^2	−0.032282	0.009960	0.001			
GA (weeks)^3	0.000856	0.000277	0.002			
GA (weeks)^4	−0.000008	0.000003	0.004			
Interaction:						
GH with GA (weeks)	0.001009	0.002004	0.616			
Term PE with GA (weeks)	−0.001468	0.002029	0.471			
Preterm PE with GA (weeks)	0.008385	0.002448	0.001			
Weight (−70)*				−0.002053	0.000879	0.021
Height (−164)†				0.007417	0.002328	0.002
Racial origin						
Caucasian (reference)						
Afro-Caribbean	0.142854	0.043865	0.001			
South Asian	0.013396	0.047527	0.779			
East Asian	0.107239	0.086807	0.219			
Mixed	0.132665	0.114652	0.250			
Maternal age (−32)‡	0.008275	0.002839	0.004			
Mode of conception						
Spontaneous (reference)						
Ovulation induction				−0.063707	0.065448	0.332
In-vitro fertilization				0.290695	0.109411	0.009
Random part						
Level 2						
Variance (constant)	0.018933	0.006950		0.079991	0.016544	
Variance (GA)	0.000005	0.000007		0.000063	0.000018	
Covariance (constant, GA)	0.000040	0.000196		−0.001988	0.000521	
Level 1						
Residual	0.009203	0.000907		0.016115	0.001528	

*Subtracted from maternal weight in kg. †Subtracted from maternal height in cm. ‡Subtracted from maternal age in years. GA, gestational age; SE, standard error.

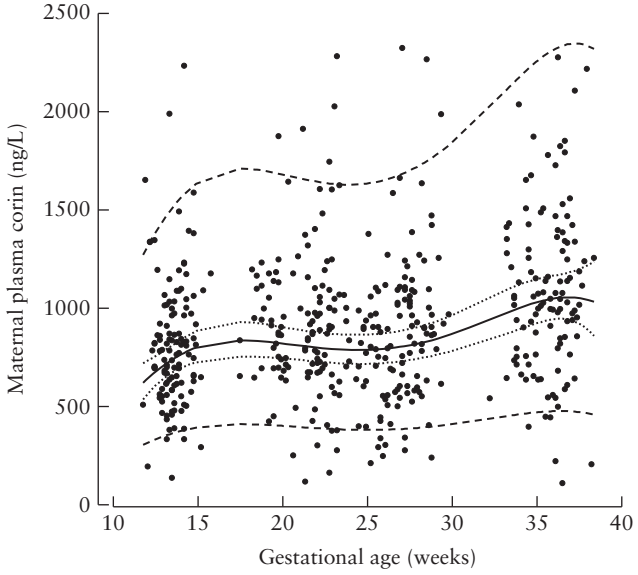

Figure 1 Maternal plasma corin levels in 85 pregnancies with normal outcome. ——, mean; - - - -, 5th and 95th centiles; ....., 95% CI of mean.

DISCUSSION

This study has demonstrated that in normotensive pregnancies there is a quadratic increase in plasma corin level with gestational age, whereas plasma MR-PANP levels do not change significantly with gestation. In preterm PE, compared with normotensive controls, plasma corin is significantly lower from an early stage in pregnancy until 20 weeks of gestation. Maternal plasma MR-PANP levels are significantly higher in pregnancies complicated by preterm PE and GH than in those that remain normotensive. In term PE, neither corin nor MR-PANP levels are significantly different from those of the normotensive group. Maternal plasma corin levels are similar in pregnancies complicated by GH and in normotensive pregnancies.

The major strengths of this study are its prospective longitudinal design and the use of a well-defined methodology (e.g. the assay used to measure MR-PANP, which is produced in amounts equimolar to the mature hormone, has been shown to detect more stable parts of the precursors of ANP[32]). Other strengths include the

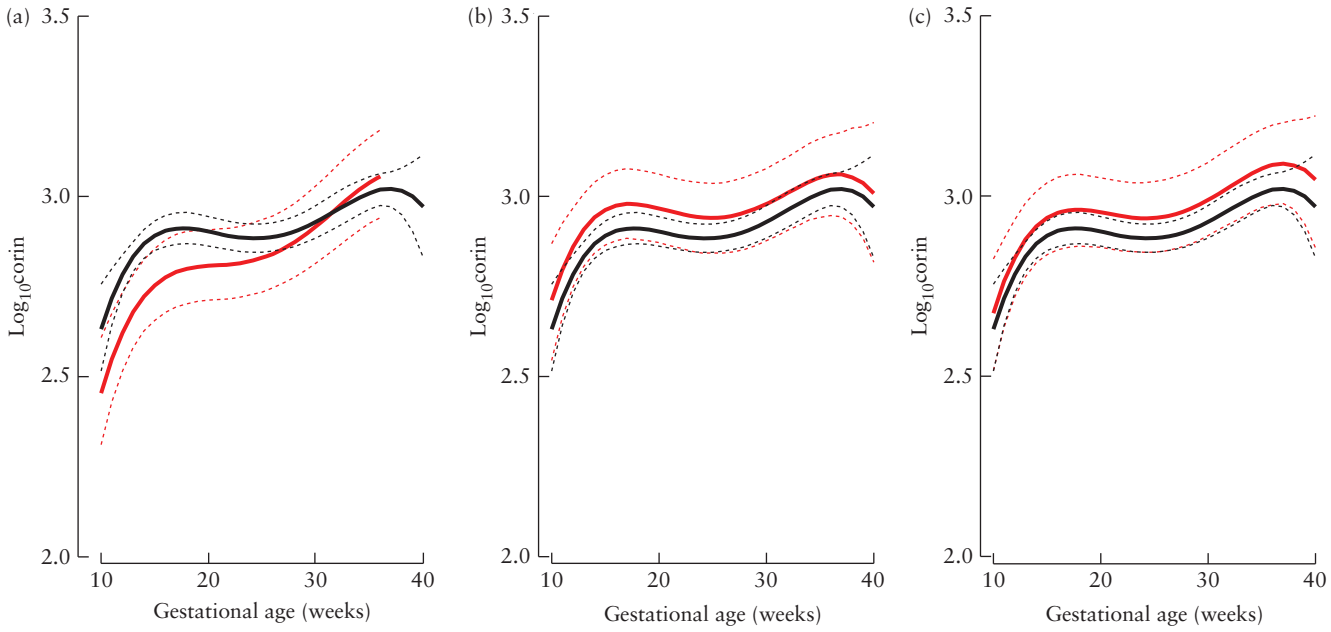

Figure 2 Mean predicted maternal plasma corin levels in pregnancies that remained normotensive (——) and in those complicated (——) by preterm pre-eclampsia (PE) (a), term PE (b) or gestational hypertension (c), for a 32-year-old Caucasian woman. Mean values with 95% CIs are shown.

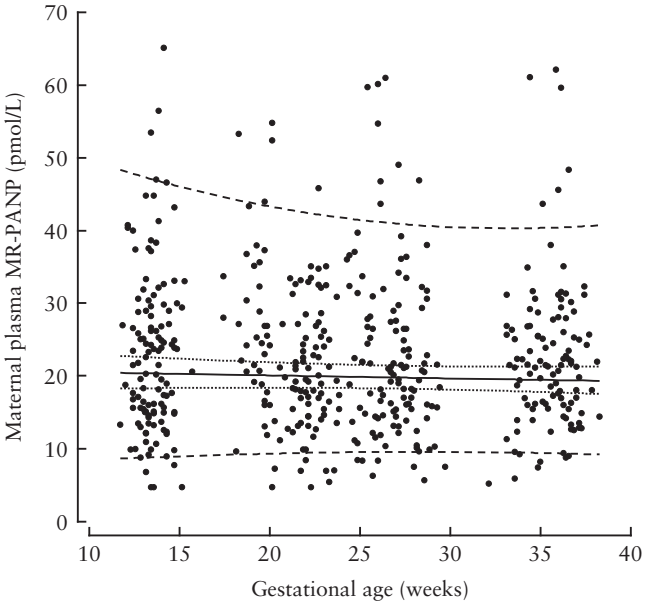

Figure 3 Maternal plasma mid-regional proatrial natriuretic peptide (MR-PANP) levels in 85 pregnancies with normal outcome. ——, mean; - - - -, 5th and 95th centiles; ....., 95% CI of mean.

application of a robust statistical approach that takes into account not only the difference in marker levels in the outcome groups but also the change with gestational age. This approach is different from the calculation of trends with gestation based on large numbers of cross-sectional and unrelated measurements. A limitation of the study is the relatively small number of cases.

ANP is a cardiac hormone that plays a key role in blood pressure regulation through its diuretic and vasodilator effects[26]. Both corin knockout and ANP knockout mice exhibit a similar hypertensive phenotype[35,36]. Plasma corin levels are lower in patients with heart failure than in healthy individuals[31,37]. Corin is expressed in several tissues, but mainly in cardiac myocytes[38–43]. The fact that corin expression has been detected in the pregnant but not in the non-pregnant uterus suggests that it might be upregulated in the decidua as an adaptive mechanism to regulate blood pressure in pregnancy[29,38,44,45]. ANP receptors are also expressed in the pregnant uterus[46–50]. Studies have reported hypertension, proteinuria, glomerular damage, placental cell necrosis and calcium deposits in pregnant corin knockout mice[29,51]. Furthermore, uterine corin mRNA and protein levels are significantly lower in women with PE than in normotensive pregnant women[29]. However, maternal plasma corin levels have been found to be higher in PE compared with normotensive pregnancies[29,52]. In our cohort, maternal plasma corin levels were reduced early in pregnancy in those destined to develop PE, followed by an increase from mid-pregnancy onwards. These paradoxical findings could suggest an extrauterine source of the elevated plasma corin levels reported in PE. This could represent an adaptive response to the impaired trophoblast invasion-related production of corin, reflected in the low levels we have demonstrated in maternal blood early in pregnancy. This would result in low levels of corin in the uterus and hence in the plasma early in pregnancy, followed by an adaptive response and release of corin into the maternal circulation, resulting in a gradual increase in the plasma corin level as the pregnancy advances, and reaching significantly higher levels at the clinical onset of PE.

The action of corin is likely to be mediated through its activation of ANP[15,16]. Studies have shown that

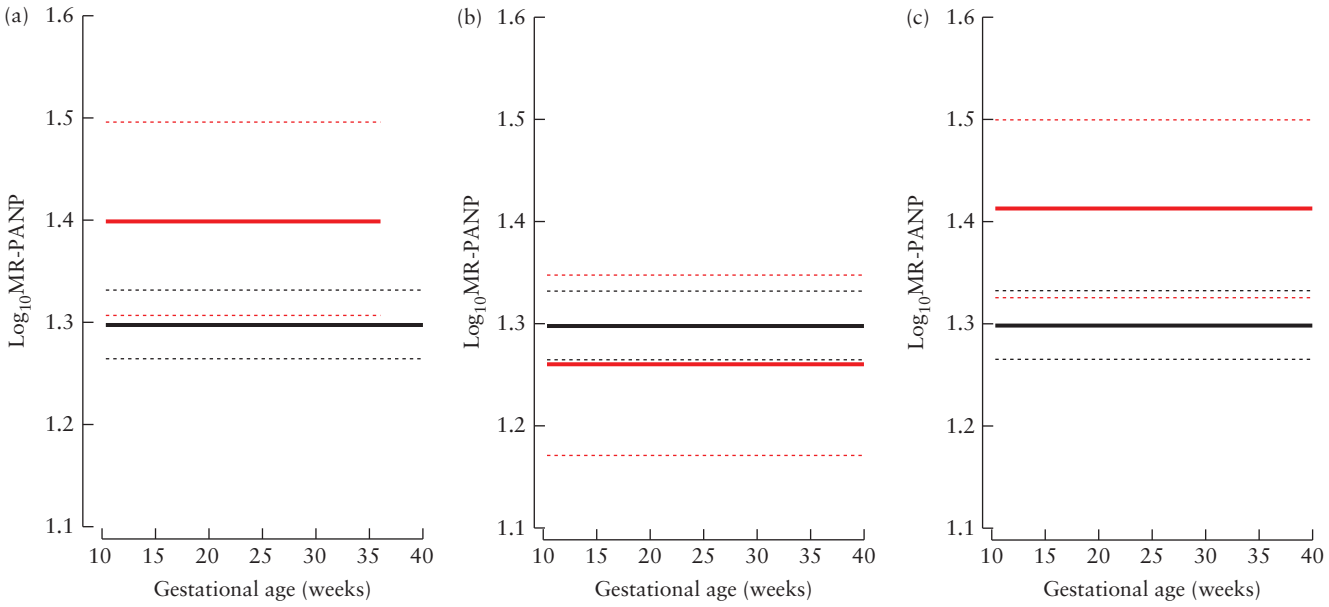

Figure 4 Mean predicted maternal plasma mid-regional proatrial natriuretic peptide (MR-PANP) levels in pregnancies that remained normotensive (——) and in those complicated (——) by preterm pre-eclampsia (PE) (a), term PE (b) or gestational hypertension (c), for a woman weighing 70 kg, 164 cm tall and who had conceived spontaneously. Mean values with 95% CIs are shown.

ANP is a vasodilator, antagonizes the contractile effect of angiotensin II and endothelin-I on uteroplacental vessels[53,54], suppresses vascular smooth muscle cell proliferation[55,56], controls endothelial cell growth and migration[57,58] and stimulates angiogenic endothelial regeneration[59,60]. Therefore, this could be an explanation of the recently described role of corin in promoting trophoblast invasion and spiral artery remodeling[29]. The PE phenotype in pregnant corin knockout and ANP knockout mice is markedly similar, adding further evidence that the corin action is facilitated by its pro-ANP activity[29]. Moreover, two corin mutations, Lys317Glu and Ser472Gly, leading to a marked reduction in pro-ANP activity, have been reported in women who developed PE[29].

Interestingly, we found that corin levels were significantly lower in women of Afro-Caribbean racial origin, who are known to be at increased risk of PE, when compared with Caucasian women[61]. Previous studies have reported corin variants with reduced activity in African-American women[20,62,63].

In our cohort, maternal plasma MR-PANP levels were significantly higher from the first trimester, in pregnancies that developed preterm PE and GH. Studies investigating maternal plasma ANP levels, in pregnancies complicated by PE, have reported conflicting results, with some showing increased levels[21–28] and others demonstrating similar levels to those in normotensive pregnancies[64–66]. These conflicting results could be explained by the underestimation of the quantity of the circulating ANP by the ANP assays used in some of these studies. ANP is an unstable peptide, which is susceptible to early degradation of crucial epitopes at the extreme ends of the precursor molecule[32], whereas MR-PANP is more stable than the N- or C-terminal parts of the precursor and is released in a ratio equimolar to ANP[67]. For this reason, we measured MR-PANP in our study cohort.

The MR-PANP values reported in pregnancies complicated by PE are similar to those in acute ischemic stroke[68]. Khaleghi *et al.* has demonstrated, in non-pregnant adults, that plasma MR-PANP levels correlate with the severity of hypertension and proposed that it might be a marker of arterial stiffness[69]. Previous studies have reported higher indices of arterial stiffness, both at the time of, and before, the onset of the clinical diagnosis of PE[70–75]. We have previously reported that central systolic blood pressure was increased in preterm PE as early as the first trimester[76]. In preterm PE, compared with the normotensive group, markers of arterial stiffness, including pulse wave velocity and augmentation index, are significantly higher from 16–17 weeks' gestation and the difference for both increased with gestational age[76].

This study describes the longitudinal changes in maternal plasma corin and MR-PANP levels in high-risk pregnancies that remained normotensive and those that developed GH or PE. Corin and MR-PANP are potentially useful markers in the early prediction of PE.

ACKNOWLEDGMENTS

The study was supported by a grant from The Fetal Medicine Foundation (UK Charity No: 1037116). The biochemical assays were performed by Dr Tracey Drew, Department of Biochemistry, King's College Hospital, London, UK.

REFERENCES

1. Cantwell R, Clutton-Brock T, Cooper G, Dawson A, Drife J, Garrod D, Harper A, Hulbert D, Lucas S, McClure J, Millward-Sadler H, Neilson J, Nelson-Piercy C, Norman J, O'Herlihy C, Oates M, Shakespeare J, de Swiet M, Williamson C, Beale V, Knight M, Lennox C, Miller A, Parmar D, Rogers J, Springett A. Saving Mothers' Lives: Reviewing maternal deaths to make motherhood safer: 2006–2008. The Eighth Report of the Confidential Enquiries into Maternal Deaths in the United Kingdom. *BJOG* 2011; **118** (Suppl 1): 1–203.
2. Duley L. The global impact of pre-eclampsia and eclampsia. *Semin Perinatol* 2009; **33:** 130–137.
3. World Health Organization. *Make Every Mother* and *Child Count. World Health Report*, 2005. *Geneva*, Switzerland: World Health Organization, 2005.
4. Redman CWG. Pre-eclampsia and the placenta. *Placenta* 1991; **12:** 301–308.
5. Roberts JM, Redman CW. Pre-eclampsia: more than pregnancy-induced hypertension. *Lancet* 1993; **341:** 1447–1451.
6. Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. Pathophysiology of hypertension during pre-eclampsia linking placental ischemia with endothelial dysfunction. *Hypertension* 2001; **38:** 718–722.
7. Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. *BMJ* 2001; **323:** 1213–1217.
8. Smith GC, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. *Lancet* 2001; **357:** 2002–2006.
9. Ray JG, Vermeulen MJ, Schull MJ, Redelmeier DA. Cardiovascular health after maternal placental syndromes (CHAMPS): population-based retrospective cohort study. *Lancet* 2005; **366:** 1797–1803.
10. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ* 2007; **335:** 974–986.
11. Hale SA, Badger GJ, McBride C, Magness R, Bernstein IM. Prepregnancy vascular dysfunction in women who subsequently develop hypertension during pregnancy. *Pregnancy Hypertens* 2013; **3:** 140–145.
12. Magnussen EB, Vatten LJ, Nilsen TIL, Salvesen KÅ, Smith GD, Romundstad PR. Prepregnancy cardiovascular risk factors as predictors of pre-eclampsia: population based cohort study. *BMJ* 2007; **335:** 974–983.
13. Anderson JV, Donckier J, McKenna WJ, Bloom SR. The plasma release of atrial natriuretic peptide in man. *Clin Sci (Lond)* 1986; **71:** 151–155.
14. Rodeheffer RJ, Tanaka I, Imada T, Hollister AS, Robertson D, Inagami T. Atrial pressure and secretion of atrial natriuretic factor into the human central circulation. *J Am Coll Cardiol* 1986; **8:** 18–26.
15. Wu Q, Xu-Cai YO, Chen S, Wang W. Corin: new insights into the natriuretic peptide system. *Kidney Int* 2009; **75:** 142–146.
16. Yan W, Wu F, Morser J, Wu Q. Corin, a transmembrane cardiac serine protease, acts as a pro-atrial natriuretic peptide-converting enzyme. *Proc Natl Acad Sci USA* 2000; **97:** 8525–8529.
17. Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, Wolf PA, Vasan RS. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* 2004; **350:** 655–663.
18. Maisel A, Mueller C, Nowak R, Peacock WF, Landsberg JW, Ponikowski P, Mockel M, Hogan C, Wu AH, Richards M, Clopton P, Filippatos GS, Di Somma S, Anand I, Ng L, Daniels LB, Neath SX, Christenson R, Potocki M, McCord J, Terracciano G, Kremastinos D, Hartmann O, von Haehling S, Bergmann A, Morgenthaler NG, Anker SD. Mid-region pro-hormone markers for diagnosis and prognosis in acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial. *J Am Coll Cardiol* 2010; **55:** 2062–2076.
19. Chan JC, Knudson O, Wu F, Morser J, Dole WP, Wu Q. Hypertension in mice lacking the proatrial natriuretic peptide convertase corin. *Proc Natl Acad Sci U S A* 2005; **102:** 785–790.
20. Dries DL, Victor RG, Rame JE, Cooper RS, Wu X, Zhu X, Leonard D, Ho SI, Wu Q, Post W, Drazner MH. Corin gene minor allele defined by 2 missense mutations is common in blacks and associated with high blood pressure and hypertension. *Circulation* 2005; **112:** 2403–2410.
21. Sugulle M, Herse F, Hering L, Mockel M, Dechend R, Staff AC. Cardiovascular biomarker midregional proatrial natriuretic peptide during and after preeclamptic pregnancies. *Hypertension* 2012; **59:** 395–401.
22. Rafik Hamad R, Larsson A, Pernow J, Bremme K, Eriksson MJ. Assessment of left ventricular structure and function in preeclampsia by echocardiography and cardiovascular biomarkers. *J Hypertens* 2009; **27:** 2257–2264.
23. Hatjis CG, Greelish JP, Kofinas AD, Stroud A, Hashimoto K, Rose JC. Atrial natriuretic factor maternal and fetal concentrations in severe pre-eclampsia. *Am J Obstet Gynecol* 1989; **161:** 1015–1019.
24. Pouta AM, Vuolteenaho OJ, Laatikainen TJ. An increase of the plasma N-terminal peptide of proatrial natriuretic peptide in preeclampsia. *Obstet Gynecol* 1997; **89:** 747–753.
25. Thomsen JK, Storm TL, Thamsborg G, de NM, Bodker B, Skouby S. Atrial natriuretic peptide concentrations in pre-eclampsia. *BMJ (Clin Res Ed)* 1987; **294:** 1508–1510.
26. Zunker P, Happe S, Louwen F, Evers S, Ringelstein EB. Peripartal temporal course of endothelin 1, angiotensin II, and atrial natriuretic peptide in pre-eclampsia and normotensive pregnancy. *Fetal Diagn Ther* 1998; **13:** 309–314.
27. Borghi C, Esposti DD, Immordino V, Cassani A, Boschi S, Bovicelli L, Ambrosioni E. Relationship of systemic hemodynamics, left ventricular structure and function, and plasma natriuretic peptide concentrations during pregnancy complicated by preeclampsia. *Am J Obstet Gynecol* 2000; **183:** 140–147.
28. Tihtonen KM, Kööbi T, Vuolteenaho O, Huhtala HS, Uotila JT. Natriuretic peptides and hemodynamics in preeclampsia. *Am J Obstet Gynecol* 2007; **196:** 328.e1–7.
29. Cui Y, Wang W, Dong N, Lou J, Srinivasan DK, Cheng W, Huang X, Liu M, Fang C, Peng J, Chen S, Wu S, Liu Z, Dong L, Zhou Y, Wu Q. Role of corin in trophoblast invasion and uterine spiral artery remodelling in pregnancy. *Nature* 2012; **484:** 246–250.
30. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11–13 weeks. *Prenat Diagn* 2011; **31:** 66–74.
31. Dong N, Chen S, Yang J, He L, Liu P, Zheng D, Li L, Zhou Y, Ruan C, Plow E, Wu Q. Plasma soluble corin in patients with heart failure. *Circ Heart Fail* 2010; **3:** 207–211.
32. Morgenthaler NG, Struck J, Thomas B, Bergmann A. Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. *Clin Chem* 2004; **50:** 234–236.
33. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* 2001; **20:** 9–14.
34. Rasbash J, Charlton C, Browne WJ, Healy M, Cameron B. *MLwiN Version 2.1*. Centre for Multilevel Modelling, University of Bristol: Bristol, 2009.
35. John SW, Krege JH, Oliver PM, Hagaman JR, Hodgin JB, Pang SC, Flynn TG, Smithies O. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science* 1995; **267:** 679–681.

36. Melo LG, Veress AT, Chong CK, Pang SC, Flynn TG, Sonnenberg H. Salt-sensitive hypertension in ANP knockout mice: potential role of abnormal plasma renin activity. *Am J Physiol* 1998; **274:** R255–R261.
37. Ibebuogu UN, Gladysheva IP, Houng AK, Reed GL. Decompensated heart failure is associated with reduced corin levels and decreased cleavage of pro-atrial natriuretic Peptide. *Circ Heart Fail* 2011; **4:** 114–120.
38. Yan W, Sheng N, Seto M, Morser J, Wu Q. Corin, a mosaic transmembrane serine protease encoded by a novel cDNA from human heart. *J Biol Chem* 1999; **274:** 14926–14935.
39. Hooper JD, Scarman AL, Clarke BE, Normyle JF, Antalis TM. Localization of the mosaic transmembrane serine protease corin to heart myocytes. *Eur J Biochem* 2000; **267:** 6931–6937.
40. Polzin D, Kaminski HJ, Kastner C, Wang W, Krämer S, Gambaryan S, Russwurm M, Peters H, Wu Q, Vandewalle A, Bachmann S, Theilig F. Decreased renal corin expression contributes to sodium retention in proteinuric kidney diseases. *Kidney Int* 2010; **78:** 650–659.
41. Ichiki T, Huntley BK, Heublein DM, Sandberg SM, McKie PM, Martin FL, Jougasaki M, Burnett JC Jr. Corin is present in the normal human heart, kidney, and blood, with pro-B-type natriuretic peptide processing in the circulation. *Clin Chem* 2011; **57:** 40–47.
42. Chung S, Moon JI, Leung A, Aldrich D, Lukianov S, Kitayama Y, Park S, Li Y, Bolshakov VY, Lamonerie T, Kim KS. ES cell-derived renewable and functional midbrain dopaminergic progenitors. *Proc Natl Acad Sci USA* 2011; **108:** 9703–9708.
43. Ono Y, Nakatani T, Sakamoto Y, Mizuhara E, Minaki Y, Kumai M, Hamaguchi A, Nishimura M, Inoue Y, Hayashi H, Takahashi J, Imai T. Differences in neurogenic potential in floor plate cells along an anteroposterior location: midbrain dopaminergic neurons originate from mesencephalic floor plate cells. *Development* 2007; **134:** 3213–3225.
44. Soloff MS, Jeng YJ, Izban MG, Sinha M, Luxon BA, Stamnes SJ, England SK. Effects of progesterone treatment on expression of genes involved in uterine quiescence. *Reprod Sci* 2011; **18:** 781–797.
45. Zhou Y, Wu Q. Role of corin and atrial natriuretic peptide in preeclampsia. *Placenta* 2013; **34:** 89–94.
46. Cootauco AC, Murphy JD, Maleski J, Blakemore KJ, Slodzinski MK. Atrial natriuretic peptide production and natriuretic peptide receptors in the human uterus and their effect on myometrial relaxation. *Am J Obstet Gynecol* 2008; **199:** 429 e421–426.
47. Dos Reis AM, Fujio N, Dam TV, Mukaddam-Daher S, Jankowski M, Tremblay J, Gutkowska J. Characterization and distribution of natriuretic peptide receptors in the rat uterus. *Endocrinology* 1995; **136:** 4247–4253.
48. Gililland JL, Tseng YC, Troche V, Lahiri S, Wartofsky L. Atrial natriuretic peptide receptors in human endometrial stromal cells. *J Clin Endocrinol Metab* 1992; **75:** 547–551.
49. Itoh H, Sagawa N, Hasegawa M, Nanno H, Kobayashi F, Ihara Y, Mori T, Komatsu Y, Suga S, Yoshimasa T. Expression of biologically active receptors for natriuretic peptides in the human uterus during pregnancy. *Biochem Biophys Res Commun* 1994; **203:** 602–607.
50. Vaillancourt P, Omer S, Deng XF, Mulay S, Varma DR. Differential effects of rat pregnancy on uterine and lung atrial natriuretic factor receptors. *Am J Physiol* 1998; **274:** E52–E56.
51. Chan JC, Knudson O, Wu F, Morser J, Dole WP, Wu Q. Hypertension in mice lacking the proatrial natriuretic peptide convertase corin. *Proc Natl Acad Sci USA* 2005; **102:** 785–790.
52. Zaki MA, El-Banawy SE-DS, El-Gammal HH. Plasma soluble corin and N-terminal pro-atrial natriuretic peptide levels in pregnancy induced hypertension. *Pregnancy Hypertens* 2012; **2:** 48–52.
53. Poulsen H, Sjoberg NO, Stjernquist M, Zia E. Atrial natriuretic peptide antagonizes the contractile effect of angiotensin II in the human uterine artery. *Hum Reprod* 1994; **9:** 1939–1943.
54. Holcberg G, Kossenjans W, Brewer A, Miodovnik M, Myatt L. Selective vasodilator effects of atrial natriuretic peptide in the human placental vasculature. *J Soc Gynecol Investig* 1995; **2:** 1–5.
55. Hutchinson HG, Trindade PT, Cunanan DB, Wu CF, Pratt RE. Mechanisms of natriuretic peptide-induced growth inhibition of vascular smooth muscle cells. *Cardiovasc Res* 1997; **35:** 158–167.
56. Itoh H, Pratt RE, Dzau VJ. Atrial natriuretic polypeptide inhibits hypertrophy of vascular smooth muscle cells. *J Clin Invest* 1990; **86:** 1690–1697.
57. Itoh H, Pratt RE, Ohno M, Dzau VJ. Atrial natriuretic polypeptide as a novel antigrowth factor of endothelial cells. *Hypertension* 1992; **19:** 758–761.
58. Lara-Castillo N, Zandi S, Nakao S, Ito Y, Noda K, She H, Ahmed M, Frimmel S, Ablonczy Z, Hafezi-Moghadam A. Atrial natriuretic peptide reduces vascular leakage and choroidal neovascularization. *Am J Pathol* 2009; **175:** 2343–2350.
59. Kuhn M, Volker K, Schwarz K, Carbajo-Lozoya J, Flögel U, Jacoby C, Stypmann J, van Eickels M, Gambaryan S, Hartmann M, Werner M, Wieland T, Schrader J, Baba HA. The natriuretic peptide/guanylyl cyclase-a system functions as a stress-responsive regulator of angiogenesis in mice. *J Clin Invest* 2009; **119:** 2019–2030.
60. Tokudome T, Kishimoto I, Yamahara K, Osaki T, Minamino N, Horio T, Sawai K, Kawano Y, Miyazato M, Sata M, Kohno M, Nakao K, Kangawa K. Impaired recovery of blood flow after hind-limb ischemia in mice lacking guanylyl cyclase-A, a receptor for atrial and brain natriuretic peptides. *Arterioscler Thromb Vasc Biol* 2009; **29:** 1516–1521.
61. Khalil A, Rezende J, Akolekar R, Syngelaki A, Nicolaides KH. Maternal racial origin and adverse pregnancy outcome: a cohort study. *Ultrasound Obstet Gynecol* 2013; **41:** 278–285.
62. Wang W, Cui Y, Shen J, Jiang J, Chen S, Peng J, Wu Q. Salt-sensitive hypertension and cardiac hypertrophy in transgenic mice expressing a corin variant identified in blacks. *Hypertension* 2012; **60:** 1352–1358.
63. Wang W, Liao X, Fukuda K, Knappe S, Wu F, Dries DL, Qin J, Wu Q. Corin variant associated with hypertension and cardiac hypertrophy exhibits impaired zymogen activation and natriuretic peptide processing activity. *Circ Res* 2008; **103:** 502–508.
64. Nisell H, Carlstrom K, Cizinsky S, Grunewald C, Nylund L, Randmaa I. Atrial natriuretic peptide concentrations and hemodynamic effects of acute plasma volume expansion in normal pregnancy and preeclampsia. *Obstet Gynecol* 1992; **79:** 902–907.
65. Rizk DE. A study of α-human atrial natriuretic peptide in normal pregnancy and in pre-eclampsia. *J Obstet Gynaecol* 1997; **17:** 234–238.
66. Pouta AM, Hartikainen AL, Vuolteenaho OJ, Ruokonen AO, Laatikainen TJ. Midtrimester N-terminal proatrial natriuretic peptide, free β-hCG, and α-fetoprotein in predicting preeclampsia. *Obstet Gynecol* 1998; **91:** 940–944.
67. Ala-Kopsala M, Magga J, Peuhkurinen K, Leipala J, Ruskoaho H, Leppaluoto J, Vuolteenaho O. Molecular heterogeneity has a major impact on the measurement of circulating N-terminal fragments of A- and B-type natriuretic peptides. *Clin Chem* 2004; **50:** 1576–1588.
68. Katan M, Fluri F, Schuetz P, Morgenthaler NG, Zweifel C, Bingisser R, Kappos L, Steck A, Engelter ST, Müller B, Christ-Crain M. Midregional pro-atrial natriuretic peptide and outcome in patients with acute ischemic stroke. *J Am Coll Cardiol* 2010; **56:** 1045–1053.

69. Khaleghi M, Saleem U, Morgenthaler NG, Turner ST, Bergmann A, Struck J, Mosley TH, Kullo IJ. Plasma midregional pro-atrial natriuretic peptide is associated with blood pressure indices and hypertension severity in adults with hypertension. *Am J Hypertens* 2009; **22:** 425–431.
70. Hausvater A, Giannone T, Sandoval YH, Doonan RJ, Antonopoulos CN, Matsoukis IL, Petridou ET, Daskalopoulou SS. The association between preeclampsia and arterial stiffness. *J Hypertens* 2012; **30:** 17–33.
71. Khalil A, Cooper DJ, Harrington KF. Pulse wave analysis: a preliminary study of a novel technique for the prediction of pre-eclampsia. *BJOG* 2009; **116:** 268–276.
72. Khalil A, Cowans NJ, Spencer K, Goichman S, Meiri H, Harrington K. First-trimester markers for the prediction of pre-eclampsia in women with a-priori high risk. *Ultrasound Obstet Gynecol* 2010; **35:** 671–679.
73. Savvidou MD, Kaihura C, Anderson JM, Nicolaides KH. Maternal arterial stiffness in women who subsequently develop pre-eclampsia. *PLoS One* 2011; **6:** e18703.
74. Khalil A, Akolekar R, Syngelaki A, Elkhouli M, Nicolaides KH. Maternal hemodynamics at 11–13 weeks' gestation and risk of pre-eclampsia. *Ultrasound Obstet Gynecol* 2012; **40:** 28–34.
75. Franz MB, Burgmann M, Neubauer A, Zeisler H, Sanani R, Gottsauner-Wolf M, Schiessl B, Andreas M. Augmentation index and pulse wave velocity in normotensive and pre-eclamptic pregnancies. *Acta Obstet Gynecol Scand* 2013; **92:** 960–966.
76. Khalil A, Garcia-Mandujano R, Maiz N, Elkhouli M, Nicolaides KH. Longitudinal changes in maternal hemodynamics in a population at risk for pre-eclampsia. *Ultrasound Obstet Gynecol* 2014; **44:** 197–204.

