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GENE NOTE

The interaction between the maternal BMI and angiogenic gene polymorphisms associates with the risk of spontaneous preterm birth

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ABSTRACT: Obesity is associated with an increased level of inflammation. Interactions between inflammatory and angiogenic pathways are implicated in the major pregnancy disorders. The aim of this study was to investigate whether functional polymorphisms in angiogenesis-regulating genes (*VEGFA* rs699947, *VEGFA* rs3025039, *KDR* rs2071559 and *ANGPT1* rs2507800) interact with the maternal BMI to modify the risk of a spontaneous preterm birth (sPTB). We conducted a nested case–control study of 1190 nulliparous Caucasian women (107 sPTBs and 1083 controls). Spontaneous PTB was defined as spontaneous preterm labour or a preterm premature rupture of membranes resulting in a preterm birth at <37 weeks of gestation. DNA was extracted from the peripheral blood and genotyped using the Sequenom MassAR-RAY system. Among overweight or obese women (BMI ≥ 25), the *VEGFA* rs699947 AA genotype was associated with a higher risk of sPTBs [odds ratio (OR) = 2.4, 95% confidence interval (CI): 1.4–4.6, $P = 0.001$] and a significant interaction between the BMI and the polymorphism was detected (OR = 4.2, 95% CI: 1.7–10.9, $P = 0.003$). Among women with a BMI <25, *ANGPT1* rs2507800 AA genotype was associated with a higher risk of sPTB (OR = 2.3, 95% CI: 1.2–4.4, $P = 0.02$) and a significant interaction between BMI and the polymorphism was detected (OR = 3.3, 95% CI: 1.1–9.3, $P = 0.02$). All results remained significant after adjusting for potential confounding factors. The maternal BMI interacts with angiogenesis-regulating gene polymorphisms to modify the risk of sPTBs.

Trial Registry Name: Screening nulliparous women to identify the combinations of clinical risk factors and/or biomarkers required to predict pre-eclampsia, small-for-gestational-age babies and spontaneous preterm birth (https://www.anzctr.org.au).

Registration number: ACTRN12607000551493.

Key words: *VEGFA/ANGPT1* / polymorphism / BMI / preterm birth

Introduction

Preterm birth (delivered before 37 weeks of gestation) accounts for ~75% of neonatal deaths (Goldenberg *et al.*, 2008). In addition to the immediate complications during the neonatal period, those born preterm are at an increased risk for neuro-developmental and behavioural disorders in childhood, and cardiovascular disorders and type 2 diabetes in adult life (Saigal and Doyle, 2008). Preterm birth may result from iatrogenic delivery for maternal or fetal concerns or following a spontaneous onset of preterm labour (PTL). A spontaneous preterm birth (sPTB) is known to be initiated by many factors including intrauterine infection, inflammation, utero-placental ischaemia or haemorrhage, uterine over-distension, stress and other immunological processes (Romero *et al.*, 1994, 2006). Impaired placentation is evident in ~15% of sPTBs. In a proportion of spontaneous preterm deliveries, the cause remains unknown and a genetic contribution is suggested with heritability in the range of 27–36% (Dolan *et al.*, 2010). There is growing evidence that polymorphisms in disease-susceptibility genes and gene-environment interactions may account for differences in the prevalence of preterm birth across populations (Tsai *et al.*, 2008, 2011; Romero *et al.*, 2010).

The vascular endothelial growth factor family consisting of VEGF-A, placental growth factor (PlGF) and the receptors fms-like tyrosine kinase-1 and kinase-insert domain (KDR) along with the angiopoietins (ANG-1, ANG-2 and Tie-2 receptor) are the major angiogenic growth factors expressed at the maternal–fetal interface. As regulators of spiral artery remodelling, these molecules have a key role in placentation. Polymorphisms in these angiogenic genes regulate the transcriptional activity of the genes as well as protein production. We recently reported that functional polymorphisms in *VEGFA*, *KDR* and *ANGPT1* were associated with pregnancies complicated by pre-eclampsia and small-for-gestational age (SGA) infants (Andraweera *et al.*, 2011a, b, 2012). As in pre-eclampsia and fetal growth restriction, a failure in maternal spiral artery remodelling has been reported in sPTBs following both PTL with intact membranes and preterm premature rupture of membranes (PPROM) (Kim *et al.*, 2002, 2003). Therefore, polymorphisms in angiogenesis-regulating genes may also contribute to the risk of sPTBs.

Recently, there has been a significant increase in the number of women of reproductive age who are either overweight or obese with resultant increased rates of adverse pregnancy outcomes including pre-eclampsia and gestational diabetes mellitus. The literature regarding the association of maternal obesity with preterm birth is controversial with reports of increased (Adams *et al.*, 1995), decreased (Goldenberg *et al.*, 1998) and no risk (Hauger *et al.*, 2008). However, a recent review incorporating data from developed as well as developing countries reported that overweight and obese women were at an increased risk of sPTBs (McDonald *et al.*, 2010). Further, obesity is known to be associated with an alteration in angiogenic factors including VEGF-A and ANG-1 (Dallabrida *et al.*, 2003; Hindle *et al.*, 2010; Siervo *et al.*, 2011).

The aim of this study was to investigate whether functional single nucleotide polymorphisms in angiogenesis-regulating genes (*VEGFA* rs699947, *VEGFA* rs3025039, *KDR* rs2071559 and *ANGPT1* rs2507800) interact with the maternal BMI to modify the risk of sPTBs.

Materials and Methods

Study population

The SCOPE study is an international, multi-centre, prospective cohort study with the aim of developing screening tests to predict pre-eclampsia, SGA infants and preterm births across different populations (McCowan *et al.*, 2009). Ethics approval was gained from local ethics committees and all participants provided written informed consent.

Nulliparous women with singleton pregnancies being attended to by hospital antenatal clinics, obstetricians, general practitioners or community midwives before 15 weeks of gestation were invited to participate. Consenting women were recruited between November 2004 and September 2008 in Adelaide, Australia and Auckland, New Zealand. Women considered at a high risk of pre-eclampsia, SGA infant or preterm births because of underlying medical conditions (chronic hypertension requiring antihypertensive drugs, diabetes, renal disease, systemic lupus erythematosus, antiphospholipid syndrome and sickle cell disease), three or more miscarriages or terminations of pregnancy, previous cervical cone knife biopsy, interventions that could modify the pregnancy outcome (such as aspirin, cervical suture) or a known major fetal anomaly or abnormal karyotype were not eligible. Recruited women were excluded for the following reasons: protocol violation, lost to follow-up, conceived with donor sperm or oocytes, miscarriage or termination and woman or partner

not of Caucasian ethnicity. The final study population comprised 1190 nulliparous Caucasian women (107 sPTBs and 1083 controls).

Women were interviewed and examined by research midwives at 15 ± 1 and 20 ± 1 weeks of gestation. Maternal data on demographic information, medical history, previous obstetric history, family history of obstetric complications and medical disorders were collected (North *et al.*, 2011). Ethnicity was verified at the interview. Current pregnancy data included information on any complications during current pregnancy, diet, smoking, alcohol and the use of recreational drugs. Maternal physical measurements obtained at 15 weeks of gestation included height, weight and blood pressure. The BMI was calculated as weight (kg)/height (m^2). Obesity was defined as a BMI ≥ 30 and overweight was defined as a BMI ≥ 25 and < 30.

If the woman was certain of her last menstrual period (LMP), the expected date of delivery (EDD) was calculated from the LMP. The EDD was adjusted only if either a scan at < 16 weeks of gestation found a difference of > 7 days or a scan at 20 weeks of gestation found a difference of 10 or more days between the scan gestation and that calculated by the LMP. If the LMP was not known, the scan dates were used to estimate the EDD. All women were followed prospectively and pregnancy outcome data and measurements of the infant were recorded by research midwives within 72 h of birth.

The primary outcome was a sPTB defined as spontaneous PTL or PPROMs resulting in a preterm birth at < 37 weeks. PTL was defined as spontaneous uterine contractions occurring > 6 per hour (at least every 10 min) for > 1 h, with or without ruptured membranes at < 37 weeks. The PPROM was defined as a confirmed rupture of membranes in the absence of labour and the time between the rupture of membranes to delivery is at least 6 h greater than the combined first and second stage of labour (URL: https://www.anzctr.org.au).Uncomplicated 'pregnancy' was defined as a pregnancy with no antenatal medical or obstetric complications and resulting in the delivery of an appropriately grown, healthy infant at ≥ 37 weeks of gestation.

Genotyping

Peripheral blood samples were collected from the women and DNA was extracted from buffy coats according to the manufacturers' instructions. Genotyping for the polymorphisms was performed at the Australian Genome Research Facility (AGRF, Brisbane, Australia) using the Sequenom MassARRAY system. As a quality control measure, each sample was also genotyped for Amelogenin to ensure that the sex of the sample was correct (Sullivan *et al.*, 1993). The primers used for genotyping are detailed in Supplementary data, Table SI.

Statistics

Women in the sPTB group (cases) were compared with women in the uncomplicated pregnancy group (control subjects) in a nested case–control study design. Missing data were excluded from the analyses. The χ^2 test was used to test the genotypes at each polymorphic locus for Hardy–Weinberg equilibrium and to compare categorical variables. An analysis of variance or Student's *t*-test was used to compare continuous variables. We used multivariable logistic regression models to estimate the individual and combined associations of the maternal BMI and the genotypes of the polymorphisms in relation to sPTBs with and without adjustments for the following maternal variables: age, smoking and alcohol consumption at 15 weeks gestation, birthweight, mean arterial blood pressure at 15 weeks of gestation, born preterm and family history of PTB. In our preliminary analysis, we evaluated three possible genetic models: dominant, recessive and additive. The BMI was analysed as a continuous variable as well as a dichotomous variable grouping as a BMI < 25 and BMI ≥ 25. We grouped the overweight and obese women together to detect clinically significant

results as the sample size in each category was small. A false discovery rate (FDR) correction was performed to adjust for multiple comparisons controlling the FDR at 15% (Benjamini et al., 2001). All data analyses were performed using PASW version 17.02 (SPSS Inc., Cary, NC, USA). Results were reported as the number and percent [n (%)] or mean $\pm$ standard deviation where appropriate. $P < 0.05$ was considered statistically significant.

Results

Of those recruited, a total of 1190 Caucasian women (107 in the sPTB group and 1083 in the uncomplicated pregnancy group) were included in the study. The exclusions are detailed in Fig. 1. Of the women in the sPTB birth group, 44 (41.1%) had PPROM and 63 (58.9%) had a spontaneous onset of labour with intact membranes. The characteristics of the participants are shown in Table I. Smoking at 15 weeks of gestation ($P = 0.007$), being born preterm ($P = 0.015$) and a positive family history of preterm birth ($P = 0.002$) were more prevalent among women who had an sPTB compared with women with an uncomplicated pregnancy (Table I). A higher maternal BMI was also demonstrated in the sPTB group ($P = 0.02$, Table I) compared with the uncomplicated pregnancy group. The distribution of maternal BMI groups in the study population is shown in Table II.

All polymorphisms were in Hardy–Weinberg equilibrium. No significant individual association was detected between the polymorphisms and sPTB ($P > 0.05$; Table III). After adjusting for confounding factors, a maternal BMI ≥ 25 was not associated with the risk of sPTB compared with a BMI < 25 [adjusted odds ratio (aOR) = 1.3, 95% confidence interval (CI): 0.9–1.9, $P = 0.1$; Table IV]. When the VEGFA rs699947 polymorphism was considered, the association between the maternal BMI and sPTB differed by the phenotype. Among women with a BMI < 25, the VEGFA rs699947 polymorphism had no significant association (Table IV). Among women with a BMI ≥ 25, the VEGFA rs699947 AA genotype was associated with a higher risk of an sPTB (OR = 2.4, 95% CI: 1.4–4.6, $P = 0.001$, Table IV). The maternal BMI had a significant interaction on the association between the VEGFA rs699947 polymorphism and the risk of sPTB (OR = 4.2, 95% CI: 1.7–10.9, $P = 0.003$; Table IV). The interaction was also significant when the maternal BMI was considered as a continuous variable and when adjusted for confounding factors (Table IV).

A significant interaction effect was also found between the maternal BMI and the maternal ANGPT1 rs2507800 polymorphism in the risk for an sPTB. Among women with a BMI < 25, the ANGPT1 rs2507800 AA genotype was associated with a higher risk of an sPTB (OR = 2.3, 95% CI: 1.2–4.4, $P = 0.02$, Table V). The maternal BMI had a significant interaction on the association between the ANGPT1 rs2507800 polymorphism and the risk of an sPTB (OR = 3.3, 95% CI: 1.1–9.3, $P = 0.02$; Table V). The interaction was also significant when maternal BMI was considered as a continuous variable and when adjusted for confounding factors (Table V).
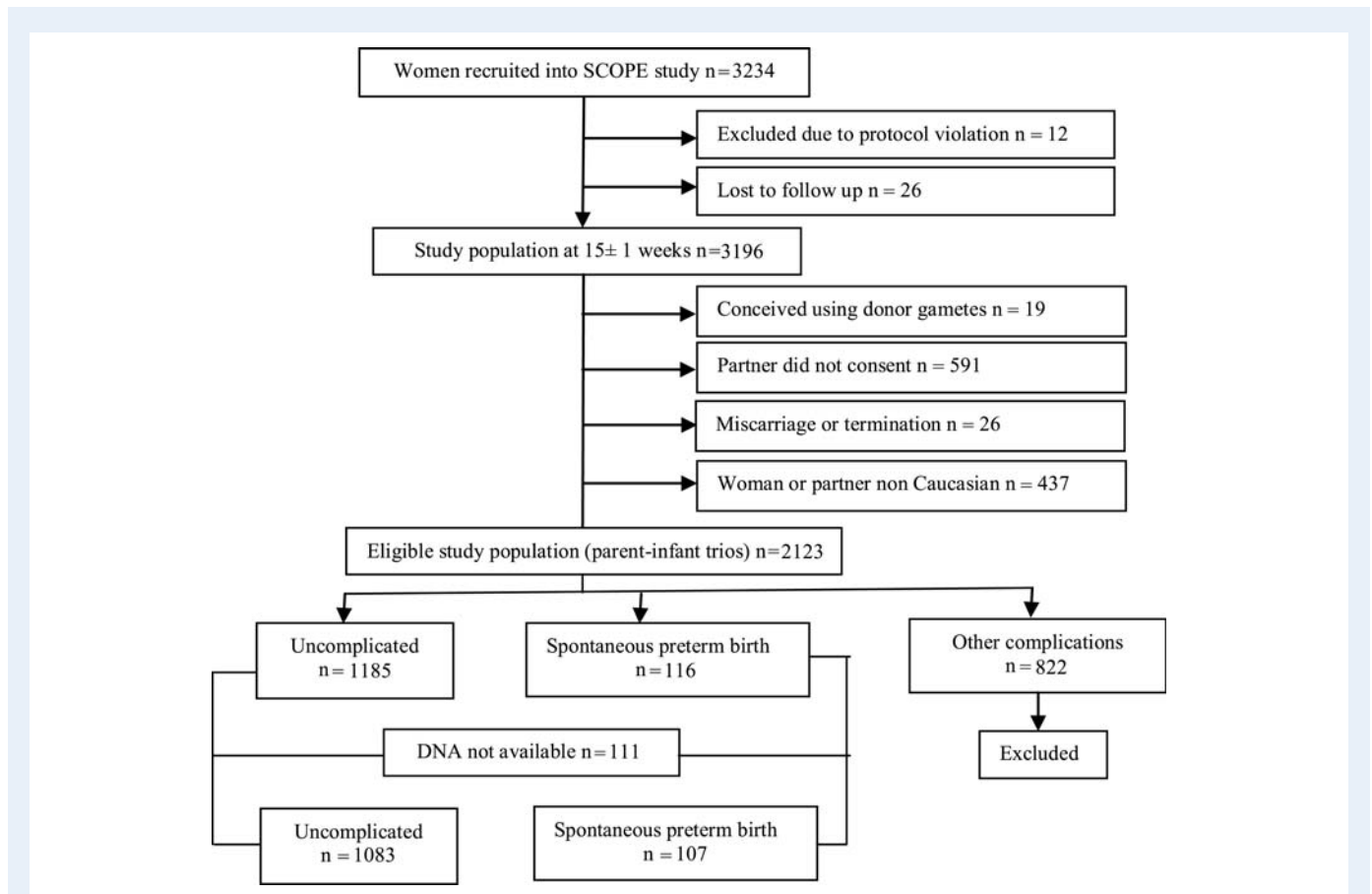
Figure 1 Study population.

Table I Characteristics of the study population.

Characteristic	Uncomplicated pregnancy (n = 1083)	sPTB (n = 107)	P-value
Maternal characteristics			
Age (years)	28.2 ± 5.6	27.9 ± 6.2	0.7
BMI (kg/m^2)	24.9 ± 4.5	25.9 ± 5.4	0.02
Smoking at 15 weeks of gestation	111 (10.2)	20 (18.7)	0.007
Participant born preterm	110 (10.2)	19 (17.8)	0.015
Family history of preterm birth	139 (12.8)	25 (23.4)	0.002
Pregnancy outcome			
Birthweight (g)	3591 ± 394	2394 ± 743	<0.001
Customized birthweight centile	54 ± 25	50 ± 31	<0.001
SGA infants	0	13 (12.1)	<0.001
Gestational age at delivery (weeks)	39.7 ± 1.2	33.6 ± 3.4	<0.001

sPTB, spontaneous preterm birth.
Data are either n (%) or mean ± SD, comparisons using Pearson's χ^2 or Student's t-test.

Table II Distribution of maternal BMI groups in the study population.

BMI category (kg/m^2)	Uncomplicated pregnancy	Spontaneous preterm birth
Underweight (<18.5)	16 (1.5)	1 (0.9)
Normal weight (≥18.5 and <25)	635 (58.6)	51 (47.7)
Overweight (≥25 and <30)	300 (27.7)	33 (30.8)
Obese (≥30)	132 (12.2)	22 (20.6)

Data are n (%).

Significant interactions were not demonstrated between the maternal BMI and the *VEGFA* rs3025039 and *KDR* rs2071559 polymorphisms (data not presented). All results remained significant after correcting for multiple testing.

Discussion

This is the first study to report gene-environment interactions in angiogenesis-regulating genes associated with an sPTB. We report that the BMI has a significant interaction on the association between the *VEGFA* rs699947 and *ANGPT1* rs2507800 polymorphisms and the risk of an sPTB.

Table III Genotype distribution of angiogenic gene polymorphisms in sPTB and uncomplicated pregnancy.

Maternal variant	Uncomplicated pregnancy n (%)	sPTB n (%)	P
VEGFA rs699947 (n = 11)			
CC	270 (25.1)	33 (31.4)	
CA	537 (50)	46 (43.8)	0.3
AA	267 (24.9)	26 (24.8)	
VEGFA rs3025039 (n = 10)			
CC	793 (73.9)	75 (70.1)	
CT	257 (24)	29 (27.1)	0.7
TT	23 (2.1)	3 (2.8)	
KDR rs2071559 (n = 12)			
TT	274 (25.6)	25 (23.6)	
CT	522 (48.7)	57 (53.8)	0.6
CC	276 (25.7)	24 (22.6)	
ANGPT1 rs2507800 (n = 01)			
AA	170 (15.7)	22 (20.8)	
AT	514 (47.5)	49 (46.2)	0.3
TT	399 (36.8)	35 (33)	

sPTB, spontaneous preterm birth.
Genotyping failure; results are expressed as n (%); comparisons are with uncomplicated pregnancy.

In overweight and obese women, the *VEGFA* rs699947 AA genotype was associated with an increased risk of an sPTB, whereas this polymorphism was not associated with sPTBs in women of normal weight. Although the direct mechanism leading to this increased risk is not clear at present, previous studies suggest a plausible explanation. As individuals become obese, their adipocytes enlarge and the adipose tissue undergoes molecular changes leading to increased production of pro-inflammatory cytokines (Wang and Nakayama, 2010). Therefore, obesity is proposed to be associated with a chronic low-grade inflammation. Interaction between inflammatory and angiogenic pathways have been recognized in the pathogenesis of many human diseases. A recent study revealed that in the setting of inflammation, women experiencing sPTB had a low serum PlGF (a member of the VEGF family of angiogenic growth factors) (Bastek *et al.*, 2010). The *VEGFA* rs699947 AA genotype is associated with lower transcriptional activity and plasma VEGF-A levels (Shahbazi *et al.*, 2002). Our findings demonstrate that overweight or obese women with genotypes associated with low VEGF-A production may be at an increased risk of an sPTB.

We also found that, in women with a BMI <25, the *ANGPT1* rs2507800 AA genotype had an increased risk of an sPTB and that an interaction exists between the BMI and the polymorphism. The ANG-1 rs2507800 single nucleotide polymorphism is located in the microRNA-211 (miRNA-211) target site in the 3′ untranslated region of *ANGPT1* and results in an A to T base change. The A allele is known to suppress ANG-1 translation by facilitating miRNA-211 binding, while the T allele is resistant to miRNA-211 induced reduction in translation. The AA genotype is also associated with lower plasma ANG-1 levels compared with the TT genotype (Chen *et al.*, 2010). Adipocytes express ANG-1, and obesity is

Table IV Association of *VEGFA* rs699947 polymorphism with sPTB according to maternal BMI status.

BMI group	Genotype	No	PTB (%)	P-value	OR (95% CI)	aP-value	aOR (95% CI)
BMI <25		695	7.5		1.0		1.0
BMI ≥25		484	11.2	0.04	1.5 (1.0–2.2)	0.1	1.3 (0.9–1.9)
BMI <25	CC/CA	518	8.1		1.0		1.0
	AA	177	5.1	0.2	0.6 (0.3–1.3)	0.9	0.9 (0.6–1.6)
BMI ≥25	CC/CA	358	8.4		1.0		1.0
	AA	126	19	0.001	2.4 (1.4–4.6)	0.005	2.3 (1.3–4.3)
Interaction[a]				0.003	4.2 (1.7–10.9)	0.008	3.8 (1.4–10.2)
Interaction[b]				<0.001	1.2 (1.1–1.3)	<0.001	1.2 (1.1–1.3)

aP-value and aOR (95% CI), adjusted for maternal age, smoking and alcohol consumption at 15 weeks of gestation, birthweight, mean arterial blood pressure at 15 weeks of gestation, born preterm and family history of PTB.
[a]Interaction, BMI as a categorical variable.
[b]Interaction, BMI as a continuous variable.

Table V Association of *ANGPT* rs2507800 polymorphism with sPTB according to maternal BMI status.

BMI group	Genotype	No	PTB (%)	P-value	OR (95% CI)	aP-value	aOR (95% CI)
BMI <25	TT/TA	592	6.3		1.0		1.0
	AA	111	13.5	0.02	2.3 (1.2–4.4)	0.04	2.0 (1.0–3.9)
BMI ≥25	TT/TA	405	11.6		1.0		1.0
	AA	81	8.6	0.4	0.7 (0.3–1.7)	0.2	0.6 (0.2–1.4)
Interaction[a]				0.02	3.3 (1.1–9.3)	0.04	3.0 (1.1–8.8)
Interaction[b]				0.04	1.1 (1.0–1.2)	0.04	1.1 (1.0–1.5)

aP-value and aOR (95% CI), adjusted for maternal age, smoking and alcohol consumption at 15 weeks of gestation, birthweight, mean arterial blood pressure at 15 weeks gestation, born preterm and family history of PTB.
[a]Interaction, BMI as a categorical variable dropped out after adjusting for confounding factors.
[b]Interaction, BMI as a continuous variable.

known to be associated with up-regulation of *ANGPT1* (Dallabrida et al., 2003; Hindle et al., 2010). As non-overweight/obese women are unlikely to have the physiological up-regulation of *ANGPT1* expression, we can hypothesize that the AA genotype may increase the risk of sPTB in these women. We earlier reported that the *ANGPT1* rs2507800 polymorphism was associated with pre-eclampsia, SGA in the presence of maternal hypertensive disease and SGA with abnormal uterine artery Doppler findings (Andraweera et al., 2011a).

The increased risk in sPTB of the *VEGFA* rs699947 AA genotype when BMI was ≥25 and the *ANGPT1* rs2507800 AA genotype when the BMI was <25 group suggests that the modification in the risk of an sPTB with these polymorphisms is influenced by the BMI status. The controversial literature regarding the association of maternal obesity with an sPTB may partially be due to potential gene-environment interactions.

The strengths of our study include a large prospective cohort and collection of data on a large number of clinical variables. The association of the *ANGPT1* rs2507800 polymorphism with pre-eclampsia and SGA infants, as well as with an abnormal uterine artery Doppler in this cohort have been published before (Andraweera et al., 2011a). It has previously been proposed that the pregnancy complications of pre-eclampsia, SGA infants and preterm births

constitute a continuum of disorders with similar pathogenic mechanisms. Consistent with this theory, we found that the *ANGPT1* rs2507800 polymorphism, which was previously shown to be associated with other pregnancy complications, was associated with sPTB. Given the sample size of our cohort, the prevalence of a polymorphism in 25% of women and a ratio of 10 control subjects to one case, the sPTB group had 80% statistical power to detect an OR of 2.0 (β = 80%, α = 0.05). We acknowledge that grouping the overweight and obese women together in one group is a limitation of our study as different grades of obesity may interact differently with the polymorphism. Therefore, replication in an independent large cohort and investigating the association of the polymorphism with different levels of the BMI will be beneficial.

In summary, the findings of our preliminary study demonstrate that the maternal BMI interacts with angiogenesis-regulating gene polymorphisms to modify the risk of a sPTB.

Supplementary data

Supplementary data are available at http://molehr.oxfordjournals.org/.

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Authors' roles

P.H.A., G.A.D. and C.T.R. contributed to the concept and design of the candidate gene association study. G.A.D., L.M.E.M., R.A.N. and C.T.R. contributed to the design and supervision of the SCOPE study. S.D.T. provided administrative and technical support. P.H.A. conducted the statistical analyses and wrote the manuscript. All authors critically revised the manuscript for important intellectual content and approved the final version of this manuscript.

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Conflict of interest

R.A.N. has a consultancy relationship with Pronota and Alere. R.A.N. declares patent PCT number WO/2009/108073. None of the other authors have any conflicts of interest to declare.

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