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GENE*Note*

A functional variant in *ANGPT1* and the risk of pregnancies with hypertensive disorders and small for gestational age infants

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ABSTRACT

Pregnancies complicated by preeclampsia and small-for-gestational-age (SGA) infants demonstrate impaired placental vascular remodelling. Angiopoietin-1 is an angiogenic growth factor which regulates vascular integrity and remodelling. The TT genotype of angiopoietin 1 (*ANGPT1*) rs2507800 polymorphism has been associated with increased plasma angiopoietin-1 levels compared to the AA genotype. We aimed to investigate the association between *ANGPT1* rs2507800 polymorphism and pregnancies complicated by gestational hypertensive disorders and SGA infants. We also aimed to investigate whether the polymorphism was associated with abnormal uterine artery Doppler as a surrogate marker of impaired placental vascular remodelling. Genotyping data of 1361 nulliparous pregnant women, 1226 partners and 1190 infants were analysed. The prevalence of *ANGPT1* rs2507800 TT genotype was reduced in women with preeclampsia (p=0.01, aOR 0.5, 95% CI 0.3-0.9), hypertensive SGA (p=0.04, aOR 0.5, 95% CI 0.2-0.9) and SGA with abnormal uterine artery Doppler (p=0.009, aOR 0.4, 95% CI 0.2-0.8) compared to women with uncomplicated pregnancy. The prevalence of maternal *ANGPT1* rs2507800 TT genotype was reduced in women with increased uterine artery resistance index (p=0.03, aOR 0.7, 95% CI 0.5-0.9) and bilateral notching of the uterine arteries (p=0.004, aOR 0.6, 95% CI 0.4-0.9). These results remained significant after correcting for multiple testing. Maternal *ANGPT1* rs2507800 TT genotype is associated with a reduced risk for preeclampsia, hypertensive SGA and abnormal uterine artery Doppler. These findings suggest that the TT genotype may protect against these pregnancy disorders by increasing angiopoietin-1 production at the maternal-fetal interface. The *ANGPT1* rs2507800 polymorphism may have a potential role in screening women to predict the risk of these pregnancy complications.

Trial Registry Name: Screening nulliparous women to identify the combinations of clinical risk factors and/or biomarkers required to predict preeclampsia, small for gestational age babies and spontaneous preterm birth.

URL: https://www.anzctr.org.au

Registration number: ACTRN12607000551493

Key words: *ANGPT1*/ polymorphism/ preeclampsia/ SGA infants

INTRODUCTION

Preeclampsia, gestational hypertension and small for gestational age (SGA) infants complicate approximately 15-20% of all nulliparous pregnancies (Sibai *et al.* 2005; Hutcheon *et al.* 2011). Women who develop preeclampsia or deliver SGA infants are at increased risk for later life vascular disorders including coronary artery disease and stroke (McDonald *et al.* 2008).

The cause of these pregnancy complications remains largely unknown but impaired maternal spiral artery remodelling is implicated in the pathogenesis of preeclampsia and SGA pregnancies (Khong *et al.* 1986; Mayhew *et al.* 2004). These abnormalities result in increased vascular impedance in the uterine circulation which is detected as an increased uterine artery resistance index on Doppler velocimetry (Chen *et al.* 2002; Deurloo *et al.* 2009).

Many molecular pathways are involved in the regulation of maternal spiral artery remodelling, of which the angiopoietin family mediated angiogenic pathway is recognized as playing a key role (Geva *et al.* 2002). During pregnancy, angiopoietin-1 (ANG-1) and its receptor Tie-2 are expressed in many cells at the maternal-fetal interface (Seval *et al.* 2008; Schiessl *et al.* 2009). *Angiopoietin 1 (ANGPT1)* gene ablation in mice results in severe angiogenic defects and embryonic lethality further demonstrating that *ANGPT1* has a critical role in embryonic angiogenesis (Suri *et al.* 1996).

A single nucleotide polymorphism (SNP) located in the microRNA-211 (miRNA-211) target site in the 3' UTR of *ANGPT1* is found in approximately 58% of Caucasian populations (*ANGPT1* rs2507800, NCBI db SNP database). The A allele is known to suppress angiopoietin-1 translation by facilitating miRNA-211 binding while the T allele is resistant to miRNA -211 induced reduction in translation. The TT genotype is also associated with higher plasma angiopoietin-1 levels compared to the AA genotype (Chen *et al.* 2010). The TT genotype was recently shown to be associated with a reduced risk of stroke in two independent large cohorts (Chen *et al.* 2010).

Considering the role of angiopoietin-1 in placental vascular remodelling, the consistent association of *ANGPT1* rs2507800 polymorphism with stroke and the data showing that women who develop preeclampsia or deliver SGA infants are at a higher risk of stroke, we aimed to investigate the association between the *ANGPT1* rs2507800 polymorphism and pregnancies complicated by

preeclampsia, gestational hypertension and SGA infants. We also aimed to investigate the association of this polymorphism with abnormal uterine artery Doppler as a surrogate marker of impaired spiral artery remodelling.

MATERIALS AND METHODS

Study population

We conducted a nested case control study where participants were recruited from the SCOPE (Screening for Pregnancy Endpoints) study. The SCOPE study is an international, multicenter, prospective cohort study with the aim of developing screening tests to predict preeclampsia, SGA infants and preterm birth across different populations. Ethics approval was gained from local ethics committees and all participants provided written informed consent.

Nulliparous women with singleton pregnancies were recruited between November 2004 and September 2008 in Adelaide, Australia and Auckland, New Zealand. Women considered at high risk of preeclampsia, SGA infants or preterm birth because of underlying medical, obstetric or gynaecological conditions were not eligible (Andraweera *et al.* 2011). Women were interviewed and examined by a research midwife at 15 ± 1 and 20 ± 1 weeks of gestation. Maternal data on demographics, medical history, previous obstetric history, family history of obstetric and medical disorders as well as current pregnancy data on any complications during pregnancy, diet, smoking, alcohol and the use of recreational drugs were collected. Maternal height, weight and blood pressure were measured at 15 weeks gestation. Proteinuria in a mid stream urine specimen was measured by dipstick or a protein:creatinine ratio.

If the woman was certain of the identity of the infant's father, the father was invited to participate in the study. Consenting male participants were interviewed at either the 15 ± 1 or 20 ± 1 weeks' visit. Paternal data collected included age, ethnicity and birthweight. Paternal height and weight were also measured.

All women were followed prospectively and Doppler ultrasound of the uterine arteries was performed at 20 weeks gestation (Groom *et al.* 2009). Mean uterine artery resistance index (RI) was calculated from the left and right uterine RI. Notching of each uterine artery was also recorded. A mean uterine

artery RI >90th centile was considered abnormal. Pregnancy outcome data and measurements of the infant were recorded within 72 hours of birth.

The primary outcomes were *gestational hypertension* defined as systolic blood pressure ≥ 140mmHg and/or diastolic blood pressure ≥90 mmHg on two or more measurements 6 hours apart after 20 weeks' gestation but before the onset of labour; *preeclampsia* defined as gestational hypertension or postpartum hypertension with proteinuria (24 hour urinary protein >300mg or spot urine protein:creatinine ratio ≥ 30mg/mmol creatinine or urine dipstick protein ≥++) or any multisystem complication of preeclampsia (North *et al.* 2011); *SGA* defined as a birth weight < 10th customised centile adjusted for maternal height, weight, parity and ethnicity, as well as gestational age at delivery and infant sex (McCowan *et al.* 2004); *normotensive SGA,* defined as birth of a SGA infant where the mother did not have hypertension; *hypertensive SGA,* defined as birth of a SGA infant where the mother had either gestational hypertension or preeclampsia, *SGA with abnormal Doppler* defined as SGA with mean uterine artery RI >90th centile. *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.

Peripheral blood samples were collected from the women. Peripheral blood, buccal sawbs or saliva samples were collected from partners. Cord blood, buccal swabs or saliva samples were collected from infants. The buccal swabs were applied to Whatman FTA cards (Whatman Inc, Piscataway, NJ, USA) immediately following sample collection and saliva was collected using Oragene kits (DNA Genotek Inc, Kanata, Ontario, Canada).

Genotyping

DNA was extracted from buffy coats from peripheral or cord blood, Whatman FTA cards or from saliva (Oragene®DNA kits) according to the manufacturers' instructions. Genotyping for the *ANGPT1* rs2507800 polymorphism was performed at the Australian Genome Research Facility (AGRF) using the Sequenom MassARRAY system. The primer sequences were , 1st PCR primer 5'-ACGTTGGATGGGGAGAAAAATTGGCAAAAC-3', 2nd PCR primer 5'-ACGTTGGATGTTCTTAGTCAGGTGACTATG -3' and extension primer 5'-

GGCAAAACTATTATATGTAAGGGA-3'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).

Statistics

Women, partners and infants in the adverse pregnancy outcome groups (cases) were compared with women, partners and infants in the uncomplicated pregnancy group (control subjects) in a nested case control study design. Missing data were excluded from the analyses. The Chi-square test was used to test the genotypes at the polymorphic locus for Hardy-Weinberg Equilibrium and to compare categorical variables. ANOVA or Student's t test was used to compare continuous variables. Adjusted and non-adjusted odds ratios were calculated for the genotype frequencies in the adverse pregnancy outcome groups compared to the uncomplicated pregnancy group using additive, dominant and recessive genotype models by unconditional logistic regression analysis adjusting for previously established risk factors (McCowan *et al.* 2010; McCowan *et al.* 2011; North *et al.* 2011). 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, North Carolina). Results were reported as number and percent [n (%)] or mean ± standard deviation (SD) where appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

After the exclusion criteria detailed in figure 1 (and excluding patients with 'other complications'), genotyping data of 1361 women, 1226 partners and 1190 infants were analysed. Maternal and paternal characteristics, Doppler results and pregnancy outcome data in relation to adverse pregnancy outcomes are detailed in table 1. The *ANGPT1* rs2507800 polymorphism showed no deviation from Hardy Weinberg equilibrium. The TT genotype of the maternal *ANGPT1* rs2507800 polymorphism was associated with a reduced risk for preeclampsia (adjusted OR, 0.5; 95% CI, 0.3-0.9, $p = 0.01$, recessive model, table 2), hypertensive SGA (adjusted OR, 0.5; 95% CI, 0.2-0.9, $p = 0.04$, recessive model, table 2), and SGA with abnormal Doppler (adjusted OR, 0.4; 95% CI, 0.2-0.8, $p = 0.009$, dominant model, table 2), Maternal *ANGPT1* rs2507800 was not associated with gestational

hypertension or with normotensive SGA (p > 0.05, table 2). Paternal and neonatal *ANGPT1* rs2507800 were not associated with any of the adverse pregnancy outcomes (data not presented).

The maternal *ANGPT1* rs2507800 TT genotype was also associated with a higher infant birthweight which was adjusted for gestational age at delivery and maternal smoking at 15 weeks gestation (p = 0.03, recessive model, figure 2).

The maternal *ANGPT1* rs2507800 TT genotype was associated with a reduced risk for abnormal uterine artery Doppler (OR, 0.7; 95% CI, 0.5-0.9, p = 0.03, dominant model, table 3) and bilateral notching of the uterine artery waveform (OR, 0.6; 95% CI, 0.4-0.9, p = 0.004, dominant model, table 3). When adjusted for maternal smoking at 15 weeks gestation the results remained unchanged (Table 3). All results remained significant after correcting for multiple testing.

DISCUSSION

To our knowledge this is the first study to investigate the *ANGPT1* rs2507800 polymorphism in pregnancy complications. Our study demonstrates that the maternal *ANGPT1* rs2507800 TT genotype is associated with a reduced risk for preeclampsia, hypertensive SGA and SGA with abnormal uterine artery Doppler. The maternal *ANGPT1* rs2507800 TT genotype is also associated with a reduced risk for abnormal uterine artery Doppler.

Impaired maternal spiral artery remodelling is implicated in the pathogenesis of the aforementioned pregnancy complications (Khong *et al.* 1986). Angiopoietin-1 and its receptor Tie-2 are expressed at the maternal-fetal interface and are of critical importance in placental vascular remodelling (Dunk *et al.* 2000; Geva *et al.* 2002; Seval *et al.* 2008; Schiessl *et al.* 2009). The A allele of the *ANGPT1* rs2507800 polymorphism is known to suppress angiopoietin-1 translation by facilitating miR-211 binding. The T allele is resistant to miR-211 induced reduction in translation, and subjects carrying the TT genotype have higher plasma angiopoietin-1 levels than those with the AA or AT genotypes (Chen *et al.* 2010). We observed that the frequency of the TT genotype was reduced in women who developed preeclampsia, hypertensive SGA and SGA with abnormal uterine Doppler. We hypothesise that the TT genotype may contribute to increased angiopoietin-1 production at the maternal-fetal interface.

Consistent with this, we found that the frequency of the TT genotype was reduced in women who had abnormal mean uterine RI as well as in those with bilateral notching of the uterine artery waveform at the 20 weeks Doppler scan. As smoking is known to influence the expression of angiogenic growth factors and also impacts on uterine artery Doppler indices possibly through this mechanism, we included maternal smoking at 15 weeks of gestation in our logistic regression model (Pons *et al.* 2011). The protective effect of *ANGPT1* rs2507800 TT genotype was independent of maternal smoking.

We did not find a significant association of the polymorphism and either gestational hypertension if not complicated by SGA or normotensive SGA. This is consistent with the theory that utero-placental vascular remodelling is unaffected in gestational hypertension not associated with SGA (Khong *et al.* 2011).

A history of preeclampsia is associated with increased long term risk for vascular diseases including coronary artery disease and stroke (McDonald *et al.* 2008). The relationship between preeclampsia and later life vascular disease involves shared risk factors for both, including chronic endothelial dysfunction (Chambers *et al.* 2001; Bushnell *et al.* 2011). Furthermore, familial segregation of preeclampsia, as well as cardiovascular and cerebro-vascular disease, suggest that these diseases share a common genetic predisposition that interact with the environment and may predispose individuals to vascular disorders which manifest at different time points throughout the life course. The TT genotype of *ANGPT1* rs2507800 polymorphism which is associated with higher plasma angiopoietin-1 was recently shown to be associated with a significant reduced risk for both haemorrhagic and ischaemic stroke in two large independent cohorts (Chen *et al.* 2010). Angiopoietin-1 acting through Tie-2 receptor plays a critical role in maintaining the integrity of the vascular endothelium and in suppressing endothelial permeability (Thurston *et al.* 2000). The protective effect of the TT genotype of *ANGPT1* rs2507800 polymorphism on preeclampsia and haemorrhagic stroke suggests that the associations may be due to its contribution in reducing endothelial dysfunction.

The strengths of our study include a large prospective cohort and collection of data on a large number of clinical variables. The clinical variables used in our multivariable logistic regression models were previously shown to be associated with preeclampsia and SGA pregnancies in our study cohort

(McCowan *et al.* 2010; McCowan *et al.* 2011; North *et al.* 2011). Our data show that the association of the maternal *ANGPT1* rs2507800 polymorphism with preeclampsia, hypertensive SGA and SGA with abnormal Doppler is independent of these clinical risk factors. The availability of uterine artery Doppler ultrasound prior to the development of pregnancy complications also enables us to comment on the potential mechanistic role of the polymorphism in the pathogenesis of these pregnancy disorders.

We acknowledge the following limitations in our study. Given the sample size of our cohort and a prevalence of *ANGPT1* rs2507800 TT genotype in 36.4% of women in the study cohort with complete genotyping information (n = 1361), the preeclampsia, gestational hypertension and normotensive SGA groups had 80% power to detect an OR of 0.5 (β = 80%, α = 0.05) but our groups of hypertensive SGA and SGA with abnormal uterine artery Doppler were relatively small. Hence, replication in other independent cohorts as well as investigating the effects of genotype on plasma angiopoietin-1 during pregnancy will be beneficial.

At present literature on the predictive role of plasma angiopoietin-1 on pregnancy complications is limited, but it has been shown that the ratio of plasma angiopoietin-1 to angiopoietin-2 is reduced in preeclampsia (Bolin *et al.* 2009). However, angiopoietin-1 production is regulated by many factors and also the changes are detected only during the latter part of the pregnancy (Bolin *et al.* 2009; Hindle *et al.* 2010) making it an unsuitable marker for screening for pregnancy complications.

Considering the functionality of the polymorphism and the known association with stroke, our results are consistent with previous evidence. If similar genotype associations are demonstrated in a replication cohort, this polymorphism in combination with other clinical risk factors may have a potential role in early screening to predict the risk of these pregnancy complications.

In conclusion, our study demonstrates that the maternal *ANGPT1* rs2507800 polymorphism is associated with a reduced risk for preeclampsia, hypertensive SGA and SGA with abnormal uterine artery Doppler and that it may have a protective effect on the pathogenesis of these pregnancy complications.

Author contributions

PHA, GAD and CTR contributed to the concept and design of the candidate gene association study. GAD, LEMN, RAN and CTR contributed to the design and supervision of the SCOPE study. SDT contributed to acquisition of samples. PHA had access to all the required data, conducted the statistical analyses, drafted the manuscript and takes responsibility for the integrity of the data and the accuracy of the data analyses. All authors critically revised the manuscript for important intellectual content and approved the final version of this manuscript.

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

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Figure legends

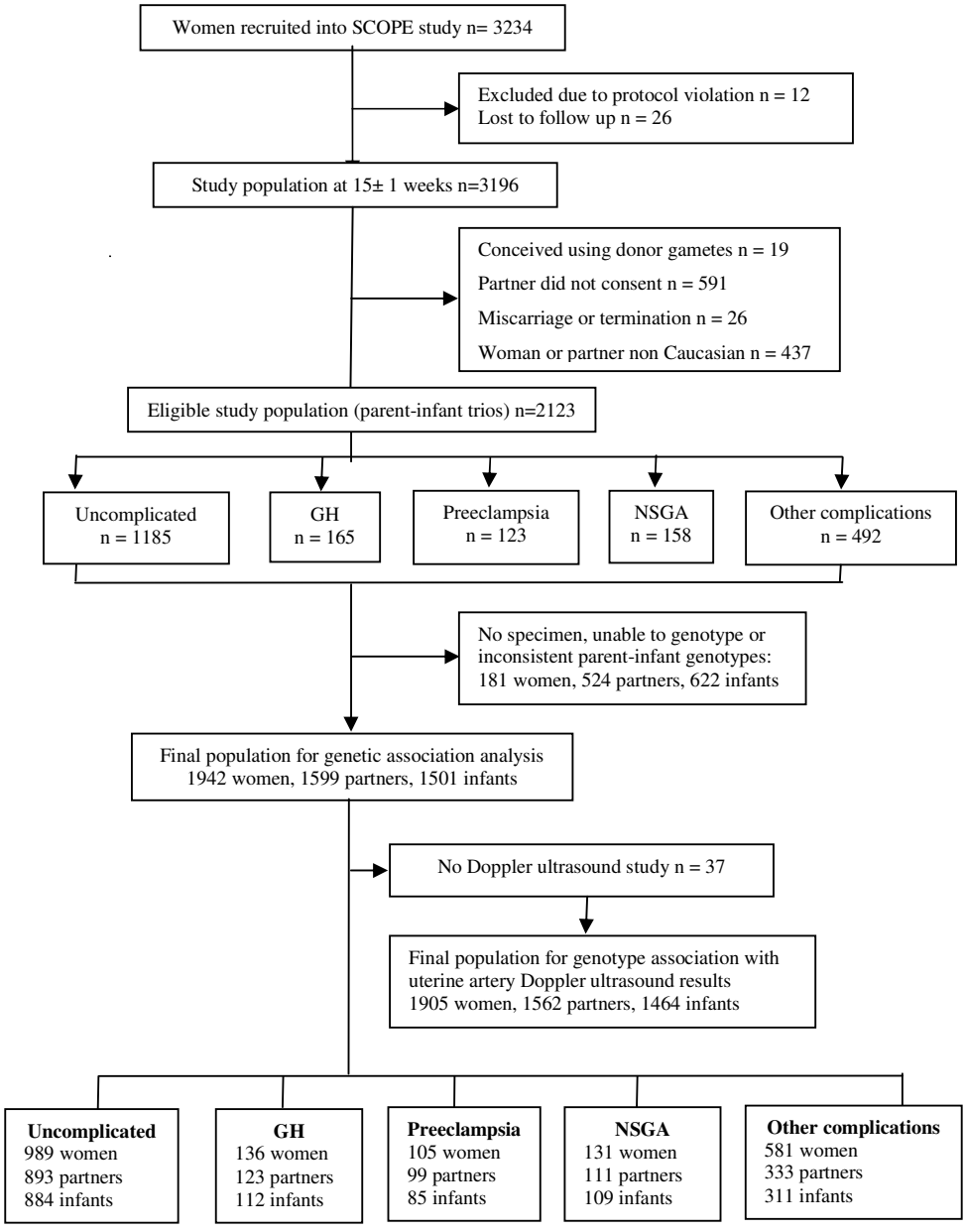
Figure 1 Study population

NSGA, Normotensive small for gestational age; GH, gestational hypertension

Figure 2 Distribution of maternal *ANGPT1* rs2507800 in neonatal birthweight

Neonatal birthweight is adjusted for gestational age at delivery and maternal smoking

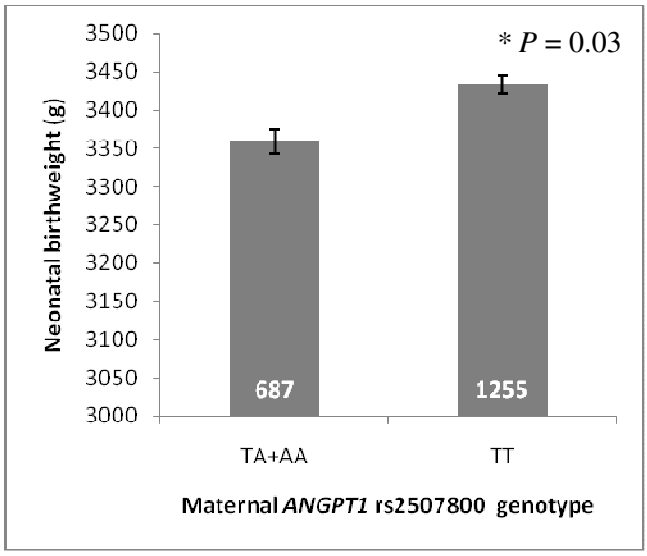
Figure 1 Study population



GH, Gestational hypertension; NSGA, Normotensive SGA

Figure 2

Distribution of maternal *ANGPT1* rs2507800 in neonatal birthweight



Neonatal birthweight is adjusted for gestational age at delivery and maternal smoking

Table 1 Characteristics of the study population

Characteristic	Uncomplicated Pregnancy (n = 989)	Preeclampsia (n = 105)	*P* value	GH (n = 136)	*P* value	NSGA (n=131)	*P* value
Maternal characteristics							
Age (years)	28.2 ± 5.6	27.3 ± 5.2	0.07	27.4 ± 6.4	**0.1**	28.9 ± 5.9	0.1
BMI (kg/m)	24.9 ± 4.5	28.4 ± 7.2	**<0.001**	28.9 ± 6.1	**<0.001**	25.3 ± 5.0	0.3
Birthweight (g)	3335 ± 530	3190 ± 551	**0.007**	3253 ± 605	**0.08**	3173 ± 533	**0.001**
Mean arterial pressure at 15 weeks	77.6 ± 7.4	83.6 ± 7.7	**<0.001**	84.8 ± 7.3	**<0.001**	78.3 ± 7.5	0.3
Smoking at 15 weeks gestation	91 (9.2)	9 (8.6)	0.9	20 (14.7)	**0.04**	27 (20.6)	**<0.001**
Socio economic index	42.2 ± 16.5	37.7 ± 16.3	**0.006**	36.2 ± 16.7	**<0.001**	39.9 ± 16.9	0.1
Paternal characteristics							
Age (years)	30.7 ± 6.3	29.3 ± 5.6	**0.01**	30.3 ± 6.5	0.4	31.4 ± 6.4	0.2
BMI (kg/m)	26.6 ± 4.0	28.4 ± 5.5	**<0.001**	27.7 ± 4.4	**0.002**	27.0 ± 4.5	0.2
Birthweight (g)	3488 ± 569	3489 ± 561	0.9	3419 ± 638	0.2	3287 ± 520	**<0.001**
Doppler studies at 20 weeks							

Abnormal mean uterine artery RI	63 (6.4)	19 (18.1)	**<0.001**	15 (11.0)	**0.04**	22 (16.8)	**<0.001**
Bilateral uterine artery notching	95 (9.6)	20 (19.1)	**0.001**	15 (11.0)	0.5	17 (12.9)	0.2
Pregnancy outcome							
Birthweight (g)	3596 ± 393	3076 ± 714	**<0.001**	3342 ± 540	**<0.001**	2688 ± 482	**<0.001**
Customised birthweight centile	54 ± 25	44 ± 32	**<0.001**	41 ± 30	**<0.001**	5 ± 3	**<0.001**
Gestational age at delivery (weeks)	39.7 ± 1.2	37.6 ± 2.3	**<0.001**	38.9 ± 1.5	**<0.001**	38.9 ± 3.1	**<0.001**

GH, Gestational hypertension; NSGA, Normotensive SGA; Data are either N (%) or Mean ± SD, comparisons using Pearson chi-square or Student's t test, P values are for comparison with uncomplicated pregnancies and P values in bold are significant

Table 2 Associations between *ANGPT1* rs2507800 variant and pregnancy complications

	T allele		Genotype n (%)							
Pregnancy outcome	%	OR (95% CI)	AA	AT	TT	Model	*P* value	OR(95% CI)	a*P* value	aOR(95% CI)
Uncomplicated pregnancy	61	ref (1.0)	147 (14.9)	477 (48.2)	365 (36.9)			1.0 (ref)		1.0 (ref)
Preeclampsia	54.3	0.8 (0.6-1.0)	19 (18.1)	58 (55.2)	28 (26.7)	Additive 1	0.8	0.9 (0.5-1.6)	0.7	0.9 (0.5-1.6)
						Additive 2	0.09	0.6 (0.3-1.1)	0.2	0.6 (0.3-1.3)
						Dominant	0.7	0.8 (0.5-1.3)	0.8	0.9 (0.5-1.6)
						Recessive	**0.04**	**0.6 (0.4-0.9)**	**0.01**	**0.5 (0.3-0.9)**
Gestational hypertension	61.8	1.0 (0.8-1.3)	21 (15.4)	62 (45.6)	53 (39.0)	Additive 1	0.7	0.9 (0.5-1.5)	0.8	0.9 (0.6-1.4)
						Additive 2	0.9	1.0 (0.6-1.7)	0.7	1.1 (0.6-1.8)
						Dominant	0.8	0.9 (0.6-1.6)	0.7	0.8 (0.4-1.8)
						Recessive	0.6	1.1 (0.8-1.6)	0.5	1.2 (0.7-1.5)
Normotensive SGA	60.7	0.9 (0.7-1.3)	22 (16.8)	59 (45.0)	50 (38.2)	Additive 1	0.5	0.8 (0.5-1.4)	0.4	0.8 (0.6-1.6)
						Additive 2	0.7	0.9 (0.5-1.6)	0.9	1.0 (0.4-1.9)
						Dominant	0.6	0.9 (0.5-1.4)	0.7	0.9 (0.5-1.8)
						Recessive	0.8	1.1 (0.7-1.5)	0.7	1.1 (0.7-2.1)
Hypertensive SGA	52.2	0.7 (0.5-1.1)	7 (15.2)	30 (65.2)	9 (19.6)	Additive 1	0.5	1.3 (0.6-3.1)	0.2	0.5 (0.2-1.3)
						Additive 2	0.2	0.5 (0.2-1.4)	0.7	0.2 (0.4-3.9)
						Dominant	0.9	0.9 (0.4-2.2)	0.5	0.7 (0.3-1.8)
						Recessive	**0.02**	**0.4 (0.2-0.9)**	**0.04**	**0.5 (0.2-0.9)**
SGA + abnormal Doppler	47.7	**0.6 (0.4-0.9)**	13 (30.2)	19 (44.2)	11 (25.6)	Additive 1	**0.03**	**0.5 (0.2-0.9)**	**0.01**	**0.5 (0.2-0.9)**
						Additive 2	**0.008**	**0.3 (0.1-0.8)**	**0.009**	**0.3 (0.2-0.9)**
						Dominant	**0.003**	**0.4 (0.2-0.8)**	**0.009**	**0.4 (0.2-0.8)**
						Recessive	0.5	0.6 (0.3-1.2)	0.6	0.6 (0.4-1.6)

Abbreviations: SGA, small for gestational age infant; T allele %, T allele frequency; Additive model 1; AT compared to AA; Additive model 2; TT compared to AA, Dominant model AT+TT compared to AA; Recessive model TT compared to AA+AT; a*P* value and aOR (95%CI) for preeclampsia and gestational hypertension adjusted for maternal factors; maternal age, mean arterial pressure at 15 weeks of gestation, body mass index, family history of preeclampsia,

family history of coronary artery disease, maternal birthweight, vaginal bleeding for at least five days during pregnancy, previous single miscarriage with the same partner, taking at least 12 months to conceive, low intake of fruit, cigarette smoking and alcohol use in the first trimester and paternal factors; age and body mass index; a*P* value and aOR(95%CI) for normotensive SGA, hypertensive SGA and SGA with abnormal Doppler adjusted for maternal factors; age, body mass index, birthweight, smoking, low fruit and vegetable intake, and paternal body mass index and birthweight; bold indicates significant values

Table 3 Associations of the *ANGPT1* rs2507800 variant with abnormal uterine artery Doppler findings

	T allele		Genotype n(%)							
Doppler finding	%	OR (95% CI)	AA	AT	TT	Model	*P* value	OR(95% CI)	a*P* value	aOR(95% CI)
Normal Doppler	60.1	ref (1.0)	275 (16.0)	823 (47.8)	623 (36.2)			1.0 (ref)		1.0 (ref)
Increased Uterine artery RI	52.9	**0.7 (0.6-0.9)**	41 (22.3)	91 (49.5)	52 (28.3)	Additive 1	0.1	0.7 (0.5-1.1)	0.1	0.7 (0.5-1.1)
						Additive 2	**0.008**	**0.6 (0.4-0.9)**	**0.008**	**0.6 (0.4-0.9)**
						Dominant	**0.03**	**0.7 (0.5-0.9)**	**0.03**	**0.7 (0.5-0.9)**
						Recessive	**0.03**	**0.7 (0.5-0.9)**	**0.03**	**0.7 (0.5-0.9)**
B/L notching	53.9	**0.8 (0.6-0.9)**	49 (23.7)	93 (44.9)	65 (31.4)	Additive 1	**0.01**	**0.6 (0.4-0.9)**	**0.01**	**0.6 (0.4-0.9)**
						Additive 2	**0.008**	**0.6 (0.4-0.9)**	**0.008**	**0.6 (0.4-0.9)**
						Dominant	**0.004**	**0.6 (0.4-0.9)**	**0.004**	**0.6 (0.4-0.9)**
						Recessive	0.2	0.8 (0.6-1.1)	0.2	0.8 (0.6-1.1)

Abbreviations: T allele %, T allele frequency; Additive model 1; AT compared to AA; Additive model 2; TT compared to AA, Dominant model AT+TT compared to AA; Recessive model TT compared to AA+AT; a*P* value and aOR(95%CI), adjusted for maternal smoking at 15 weeks gestation; bold indicates significant values