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*Reproductive Sciences* 2012 19: 547 originally published online 16 February 2012  
DOI: 10.1177/1933719111428520

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# Single-Nucleotide Polymorphisms in the KDR Gene in Pregnancies Complicated by Gestational Hypertensive Disorders and Small-for-Gestational-Age Infants

Reproductive Sciences  
19(5) 547-554  
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DOI: 10.1177/1933719111428520  
http://rs.sagepub.com  


Prabha H. Andraweera, MBBS<sup>1,2</sup>, Gustaaf A. Dekker, FRANZCOG, PhD<sup>1,3</sup>,  
Steven D. Thompson, BHthSc<sup>1</sup>, and Claire T. Roberts, PhD<sup>1</sup>

## Abstract

**Introduction:** Pregnancies complicated by preeclampsia and small-for-gestational-age (SGA) infants share placental vascular abnormalities and both disorders confer increased risk of later life coronary artery disease. Kinase insert domain receptor (KDR) is the main receptor for vascular endothelial growth factor A, a potent angiogenic factor which regulates the development of the placental vasculature. Two polymorphisms in KDR (-604T/C and Val297Ile) are known to be associated with coronary artery disease. We investigated the association of these polymorphisms with preeclampsia, gestational hypertension, and SGA infants. **Method:** Nulliparous pregnant women, their partners, and infants were recruited to a prospective cohort study (n = 1169). Doppler ultrasound of the uterine and umbilical arteries was performed at 20 weeks of gestation. Preeclampsia, gestational hypertension, and SGA were defined according to international guidelines. DNA extracted from peripheral venous or cord blood was genotyped using the Sequenom MassARRAY system. Multivariable logistic regression was used to compare the odds for the pregnancy complications between the genotype groups adjusting for potential confounders. **Results:** Among 937 Caucasian pregnancies, 427 (45.6%) were uncomplicated, 75 (8.0%) developed preeclampsia, 102 (10.9%) developed gestational hypertension, and 72 (7.7%) had SGA infants in the absence of maternal hypertensive disease. Paternal and neonatal KDR-604T/C was associated with preeclampsia (adjusted odds ratio [aOR] 1.6, 95% confidence interval [CI] 1.0-3.0 and aOR 2.2, 95% CI 1.0-4.4), SGA (aOR 1.9, 95% CI 1.1-3.3 and aOR 2.2, 95% CI 1.2-3.9), and SGA with abnormal Doppler (aOR 2.7, 95% CI 1.2-5.9 and aOR 3.2, 95% CI 1.2-5.9). **Conclusion:** Paternal and neonatal carriage of the KDR-604T/C polymorphism is associated with the risk of preeclampsia and SGA infants.

## Keywords

KDR, polymorphism, preeclampsia, small-for-gestational-age infants

## Introduction

Preeclampsia, gestational hypertension, and small-for-gestational-age (SGA) infants complicate approximately 15% to 20% of all nulliparous pregnancies and are leading causes of maternal and neonatal morbidity and mortality.<sup>1,2</sup> Preeclampsia and SGA pregnancies are associated with increased long-term risk of vascular disorders including coronary artery disease and stroke.<sup>3-7</sup> Early placentation defects including impaired trophoblast invasion and maternal spiral artery remodeling are demonstrated in many cases of preeclampsia and SGA pregnancies.<sup>8</sup>

Placental vascular development is regulated by many growth factors, of which vascular endothelial growth factor A (VEGFA) signaling represents a critical step in vessel growth and remodeling. The VEGFA exerts biologic effects through 2 high-affinity tyrosine kinase receptors: VEGF receptor 1 (VEGFR-1) also called fms-like tyrosine kinase 1 (Flt-1) and

VEGF receptor 2 (VEGFR-2) also called kinase insert domain receptor (KDR) in humans and fetal liver kinase 1 (Flk-1) in mice. Kinase insert domain receptor is the major mediator of the mitogenic, angiogenic, permeability enhancing, and endothelial survival effects of VEGFA.<sup>9</sup> The VEGFA acting through KDR is proposed to regulate early placental vasculogenesis, branching angiogenesis, and spiral artery remodeling.<sup>10,11</sup>

<sup>1</sup> Discipline of Obstetrics and Gynaecology, Robinson Institute, University of Adelaide, Australia

<sup>2</sup> Human Genetics Unit, Faculty of Medicine, University of Colombo, Sri Lanka

<sup>3</sup> Women's and Children's Division, Lyell McEwin Hospital, Elizabeth Vale, South Australia

## Corresponding Author:

Claire T. Roberts, Discipline of Obstetrics and Gynaecology, Robinson Institute, University of Adelaide, Australia  
Email: claire.roberts@adelaide.edu.au

Homozygous gene mutations in Flk-1 in mice result in abnormalities in formation of mature endothelial cells with early embryonic death, suggesting that this receptor is crucial for the development of the embryonic vasculature.<sup>12</sup>

Two single-nucleotide polymorphisms (SNPs) in *KDR* (*Val297Ile* and *-604T/C*) are known to have potential biological functions and are also associated with coronary artery disease and stroke.<sup>13,14</sup>

Considering the role of *KDR* in placental vascular remodeling, the association of *KDR-604T/C* and *Val297Ile* polymorphisms with coronary artery disease and stroke, and the data demonstrating that preeclamptic women and SGA infants are at increased risk of vascular disorders, we aimed to investigate the association of *KDR 604T/C* and *Val297Ile* polymorphisms in pregnancies complicated by preeclampsia, gestational hypertension, and SGA infants.

## Materials and Methods

This is a nested case-control study where participants were recruited from the Screening for Pregnancy Endpoints (SCOPE) 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.<sup>15</sup> The participants were recruited at the Lyell McEwin Hospital to the Adelaide cohort of the SCOPE study. Ethics approval was gained from the Central Northern Adelaide Health Service, Ethics of Human Research Committee and the Human Ethics Committee of the University of Adelaide (REC 1712/5/2008).

Nulliparous women with singleton pregnancies attending hospital antenatal clinics, obstetricians, general practitioners, or community midwives before 15 weeks of gestation were invited to participate. Consenting women were recruited between September 2005 and September 2008. Exclusion criteria included women considered at high risk of preeclampsia, SGA infants, or preterm birth because of underlying medical conditions (chronic hypertension requiring antihypertensive drugs, diabetes, renal disease, systemic lupus erythematosus, antiphospholipid syndrome, and sickle cell disease), 3 or more miscarriages or terminations of pregnancy, previous cervical cone knife biopsy, interventions which could modify pregnancy outcome (such as aspirin and cervical suture), or known major fetal anomaly or abnormal karyotype.

Women were interviewed and examined by a research midwife at  $15 \pm 1$  and  $20 \pm 1$  weeks of gestation. Maternal data collected included demographic information, medical history, previous obstetric history, family history of obstetric complications, and medical disorders. The woman's birth weight and the gestational age at which she was born were obtained. Current pregnancy data included information on any complications during current pregnancy, diet, smoking,<sup>15</sup> alcohol, and the use of recreational drugs. Maternal physical measurements obtained at 15 weeks of gestation included height, weight, and blood pressure. Two consecutive manual blood pressure measurements (using mercury or aneroid sphygmomanometer, with

a large cuff size if the arm circumference was  $\geq 33$  cm and Korotkoff V for diastolic blood pressure) were recorded. Proteinuria in a midstream urine specimen was measured in all women 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 SCOPE study. Male participants who agreed to participate provided written informed consent and were interviewed at either the  $15 \pm 1$  or  $20 \pm 1$  weeks' SCOPE visit. Paternal data collected included age, ethnicity, socioeconomic index, birth weight, and history of medical disorders. Paternal height, weight, abdominal circumference, and blood pressure were measured by the research midwife.

All women were followed prospectively, and ultrasound and Doppler studies of the umbilical and uterine arteries were performed at 20 weeks of gestation.<sup>16</sup> Mean uterine artery resistance index (RI) was calculated from the left and right uterine artery RI. If only a left or right uterine artery RI was available, this was used as "mean RI." Both umbilical artery RI and mean uterine artery RI  $> 90$ th centile were considered abnormal. Pregnancy outcome data and measurements of the infant were recorded by research midwives usually within 72 hours of birth.

## Outcome Measures

The primary outcomes were gestational hypertension defined as systolic blood pressure  $\geq 140$  mm Hg or diastolic blood pressure  $\geq 90$  mm Hg (Korotkoff V), or both, on at least 2 occasions 4 hours apart after 20 weeks of gestation but before the onset of labor; preeclampsia defined as gestational hypertension or postpartum hypertension with proteinuria (24-hour urinary protein  $> 300$  mg or spot urine protein-creatinine ratio  $\geq 30$  mg/mmol creatinine or urine dipstick protein  $\geq ++$ ) or any multisystem complication of preeclampsia.<sup>17</sup> Multisystem complications included any of acute renal insufficiency; effects on liver including raised aspartate transaminase and/or alanine transaminase concentration and severe right upper quadrant or epigastric pain or liver rupture; neurological effects including eclampsia, imminent eclampsia, or cerebral hemorrhage; and hematological effects including thrombocytopenia, disseminated intravascular coagulation, or hemolysis; SGA defined as a birth weight  $< 10$ th customized centile adjusted for maternal height, weight, parity, and ethnicity, as well as gestational age at delivery and infant sex<sup>18</sup>; SGA with abnormal Doppler defined as SGA with uterine and/or umbilical artery RI  $> 90$ th 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  $\geq 37$  weeks of gestation.

## Definitions of Other Pregnancy Complications

Normotensive SGA (NSGA) was defined as birth of an SGA infant where the mother did not have hypertension. Hypertensive SGA (HSGA) was defined as birth of an SGA

infant where the mother had either gestational hypertension or preeclampsia.

### Gene Variants Selection

*KDR-604T/C* (rs2071559) and *Val297Ile* (rs2305948) SNPs were selected based on their potential biological functions. The *KDR-604T/C* promoter variant may affect transcriptional factor E2F binding to the region, which may alter *KDR* expression.<sup>14</sup> The variant allele of *KDR-604T/C* is also shown to be associated with lower *KDR* protein levels.<sup>13</sup> The *KDR* exonic variant (exon\_7) rs2305948 results in a nonsynonymous amino acid change at *Val297Ile*. The amino acid is located at the third extracellular Ig-like domain that is important for ligand–receptor binding.<sup>13,14</sup>

### Genotyping

Peripheral blood samples were collected from the women and partners, and cord blood was collected at delivery. DNA was extracted from buffy coats separated from peripheral venous or cord blood. Genotyping for the *KDR-604T/C* (rs2071559) and *Val297Ile* (rs2305948) polymorphisms was performed at the Australian Genome Research Facility (AGRF) using the Sequenom MassARRAY system. As a quality control measure 300 independent samples which were genotyped in house for the same polymorphisms using quantitative real-time–polymerase chain reaction (qRT-PCR) were genotyped using the Sequenom MassARRAY system at AGRF. The concordance rate of the qRT-PCR results and MassARRAY results was 100%. The primer sequences for genotyping using the Sequenom MassARRAY system as well as the primer sequences and cycling conditions for qRT-PCR are shown in Supplementary Tables 1 and 2. Each sample was also genotyped for Amelogenin to ensure that the sex of the sample was correct.<sup>19</sup> Parental and neonatal genotype data were checked for a Mendelian pattern of inheritance and those found to be inconsistent were excluded from the analyses.

### Statistics

Women, partners, and infants in the adverse pregnancy outcome groups were compared with women, partners, and infants in the uncomplicated pregnancy group in a nested case–control study design. Missing data were excluded from the analyses. Chi-square test was used to test the genotypes at each polymorphic locus for Hardy–Weinberg equilibrium and to compare categorical variables. Analysis of variance was used to compare continuous variables. Adjusted and nonadjusted odds ratios were calculated for the genotype frequencies in adverse pregnancy outcome groups compared to controls using dominant and recessive genotype models by unconditional logistic regression analysis. The covariates for the logistic regression models for preeclampsia and gestational hypertension included maternal age, body mass index (BMI), mean arterial pressure at 15 weeks of gestation, smoking at 15 weeks of gestation, and

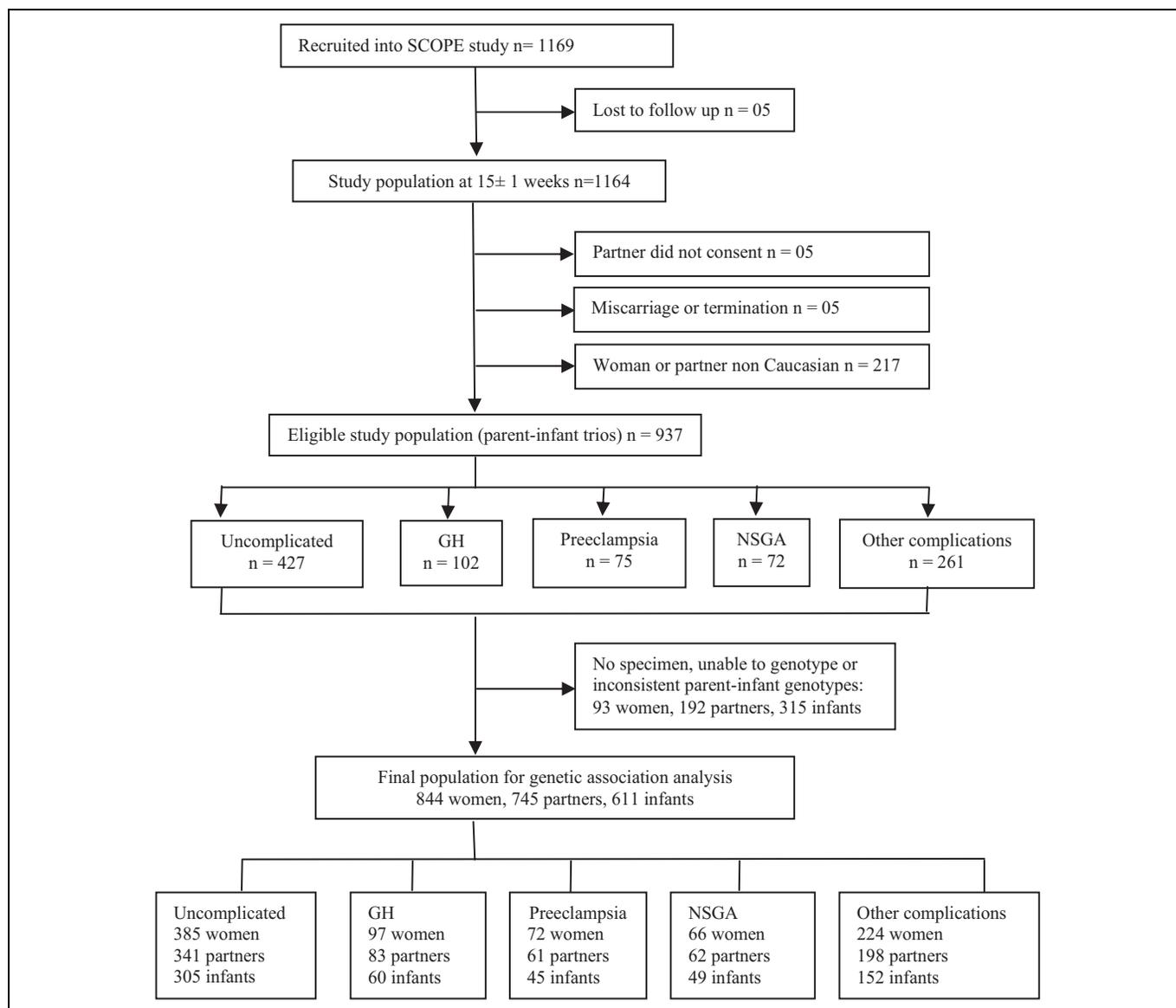
paternal age and BMI.<sup>20,21</sup> The covariates for the logistic regression model for SGA included maternal age, BMI, birth weight, smoking, and paternal BMI and birth weight.<sup>22–24</sup> All data analyses were performed using PASW version 17.02 (SPSS, Inc, Cary, North Carolina). Results were reported as number and percentage (n [%]) or mean  $\pm$  standard deviation (SD) where appropriate.  $P < .05$  was considered statistically significant.

### Results

Of those recruited, 620 women, 547 partners, and 459 infants were included in the case–control study. The exclusions are detailed in Figure 1. Among 937 eligible Caucasian pregnancies, 427 (45.6%) were uncomplicated, 75 (8.0%) developed preeclampsia, 102 (10.9%) developed gestational hypertension, 72 (7.7%) had SGA infants in the absence of maternal hypertensive disease, and the remaining 261 (27.8%) pregnancies developed other obstetric, medical, or surgical complications during pregnancy. Of the 75 preeclamptic women, 18 (24.0%) developed preterm preeclampsia requiring delivery before 37 weeks of gestation and 57 (76.0%) developed term disease. Sixty-one percent (n = 11) of the women who had preterm preeclampsia, 12.3% (n = 7) of the women who had term preeclampsia, and 18.6% (n = 19) of the women who developed gestational hypertension delivered an SGA infant. Maternal and paternal characteristics, Doppler results, and pregnancy outcome data in relation to adverse pregnancy outcomes are detailed in Table 1. Genotype data of 3 (4.0%) women, 14 (18.7%) partners, and 20 (26.7%) infants in the preeclampsia group; 5 (4.9%) women, 19 (18.6%) partners, and 42 (41.2%) infants in the gestational hypertension group; 2 (7.3%) women, 11 (10.9%) partners, and 40 (36.7%) infants in the SGA group; and 42 (4.9%) women, 83 (19.4%) partners, and 122 (28.6%) infants in the uncomplicated pregnancy group could not be analyzed due to nonavailability of samples, genotyping failure, and Mendelian inconsistencies in parent–infant genotypes. Both *KDR-604T/C* and *Val297Ile* polymorphisms were in Hardy–Weinberg equilibrium in cases and controls.

### Genotype Distribution Associated With Preeclampsia and Gestational Hypertension

Homozygosity for the variant allele of the *KDR-604T/C* polymorphism was increased in fathers and infants in the preeclampsia group compared to fathers and infants in the uncomplicated pregnancy group (Table 2). Maternal *KDR-604T/C* polymorphism approached significance for an association with preeclampsia ( $P = .07$ ; Table 2). Maternal, paternal, and infant *KDR Val297Ile* polymorphism was not associated with preeclampsia (Supplementary Table 3). *KDR -604T/C* and *Val297Ile* polymorphisms in father, mother, and the infant were not associated with gestational hypertension (Table 2 and Supplementary Table 3).



**Figure 1.** Study population.

### Genotype Distribution Associated With SGA

Homozygosity for the variant allele of the *KDR-604T/C* polymorphism was increased in fathers and infants in the SGA group compared to fathers and infants in the uncomplicated pregnancy group (Table 2). Homozygosity for the variant allele of the *KDR-604T/C* polymorphism was increased in fathers and infants in the SGA group with abnormal uterine and/or umbilical artery Doppler, compared to fathers and infants in the uncomplicated pregnancy group (Table 2). Maternal *KDR-604T/C* was not associated with SGA and approached significance for an association with SGA with abnormal uterine and/or umbilical artery Doppler ( $P = .07$ ; Table 2). Maternal, paternal, and infant *KDR Val297Ile* was not associated with either SGA or SGA with abnormal Doppler (Supplementary Table 3). Post hoc analysis on the SGA subgroups demonstrated that the

paternal and infant *KDR-604T/C* SNP was associated with hypertensive SGA but not with NSGA (data not presented).

### Interaction Between Maternal *KDR-604T/C* Polymorphism and Smoking

As a post hoc analysis, we stratified the cohort by maternal smoking at 15 weeks of gestation and investigated the association between the maternal *KDR-604T/C* polymorphism and adverse pregnancy outcomes. The prevalence of the maternal *KDR-604T/C* CC genotype was increased in preeclampsia (Table 3), SGA, and SGA with abnormal Doppler (Table 3) in women who did not smoke at 15 weeks of gestation. Maternal *KDR-604T/C* polymorphism was not associated with any of the adverse pregnancy outcomes in women who continued to

**Table 1.** Characteristics of the Study Population<sup>a</sup>

	Uncomplicated pregnancy (n = 427)	Preeclampsia (n = 75)	P	GH (n = 102)	P	SGA (n = 109)	P
<b>Maternal characteristics</b>							
Age, years	23.6 ± 4.9	24.1 ± 4.3	.4	24.3 ± 5.0	.1	24.3 ± 5.3	.1
BMI, kg/m <sup>2</sup>	25.9 ± 5.6	29.4 ± 8.0	<b>&lt;.001</b>	30.5 ± 6.7	<b>&lt;.001</b>	26.3 ± 5.7	<b>.01</b>
Birth weight	3287 ± 549	3198 ± 562	.2	3268 ± 611	.7	3136 ± 544	<b>.01</b>
Mean arterial pressure at 15 weeks	78.3 ± 6.9	82.8 ± 7.9	<b>&lt;.001</b>	85.1 ± 8.1	<b>&lt;.001</b>	81.2 ± 8.6	<b>&lt;.001</b>
Smoking at 15 weeks	90 (21.1%)	11 (14.7%)	.2	19 (18.6%)	.6	48 (44.0%)	<b>&lt;.001</b>
<b>Paternal characteristics</b>							
Age	26.4 ± 6.1	26.8 ± 5.1	.5	28.3 ± 6.6	<b>.004</b>	27.6 ± 6.3	.057
BMI, kg/m <sup>2</sup>	26.9 ± 4.9	28.7 ± 6.1	<b>.005</b>	27.6 ± 5.1	.2	27.3 ± 5.0	.4
Birth weight, g	3462 ± 593	3490 ± 522	.7	3385 ± 700	.3	3347 ± 558	.08
<b>Uterine artery Doppler at 20 weeks</b>							
Abnormal mean uterine artery RI	37 (8.8%)	16 (21.3%)	<b>&lt;.001</b>	11 (11.0%)	.5	27 (25.0%)	<b>&lt;.001</b>
Abnormal umbilical artery RI	54 (12.8%)	14 (20%)	.2	14 (14.0%)	.7	18 (16.7%)	.3
<b>Pregnancy outcome</b>							
Neonatal birth weight, g	3575 ± 390	3075 ± 754	<b>&lt;.001</b>	3337 ± 520	<b>&lt;.001</b>	2554 ± 536	<b>&lt;.001</b>
Customized birth weight centile	54 ± 25	43 ± 32	<b>&lt;.001</b>	39 ± 30	<b>&lt;.001</b>	4 ± 3	<b>&lt;.001</b>
Gestational age at delivery, weeks	39.7 ± 1.2	37.7 ± 2.4	<b>&lt;.001</b>	39.2 ± 1.4	<b>&lt;.001</b>	38.4 ± 3.4	<b>&lt;.001</b>

Abbreviations: GH, gestational hypertension; SGA, small for gestational age; BMI, body mass index; RI, resistance index; SD, standard deviation; ANOVA, analysis of variance.

<sup>a</sup>Data are either N (%) or mean ± SD; comparisons using Pearson chi-square or ANOVA; SGA includes both normotensive and hypertensive SGA; P values in boldface are significant.

**Table 2.** Distribution of Maternal, Paternal, and Neonatal *KDR-604T/C* Polymorphism in Adverse Pregnancy Outcomes and Uncomplicated Pregnancy<sup>a</sup>

<i>KDR-604T/C</i> variant	Genotype, n (%)			Dominant model CT + CC versus TT		Recessive model CC versus TT + CT	
	TT	CT	CC	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
<b>Maternal <i>KDR-604T/C</i></b>							
Uncomplicated pregnancy	102 (26.5)	189 (49.1)	94 (24.4)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Preeclampsia	14 (19.4)	34 (47.3)	24 (33.3)	1.5 (0.8-2.7)	1.5 (0.8-2.9)	1.1 (0.9-1.3)	1.6 (0.9-2.8)
Gestational hypertension	32 (33.0)	38 (39.2)	27 (27.8)	0.7 (0.5-1.2)	0.7 (0.4-1.2)	1.2 (0.7-1.9)	1.3 (0.7-2.4)
SGA	21 (20.8)	50 (49.5)	30 (29.7)	1.3 (0.8-1.9)	1.3 (0.9-1.9)	1.1 (0.9-1.2)	1.2 (0.9-1.7)
SGA with abnormal Doppler	6 (15)	20 (50)	14 (35)	2 (0.8-5.0)	1.9 (0.8-5.0)	1.7 (0.8-3.3)	1.7 (0.9-3.1)
<b>Paternal <i>KDR-604T/C</i></b>							
Uncomplicated pregnancy	93 (27.3)	172 (50.4)	76 (22.3)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Preeclampsia	12 (19.7)	28 (45.9)	21 (34.4)	1.5 (0.8-3.0)	1.7 (0.8-3.4)	<b>1.8 (1.0-3.3)</b>	<b>1.6 (1.0-3.0)</b>
Gestational hypertension	17 (20.5)	40 (48.2)	21 (31.3)	1.3 (0.7-2.4)	1.5 (0.8-2.8)	1.3 (0.7-2.3)	1.3 (0.7-2.3)
SGA	23 (23.4)	38 (38.8)	37 (37.8)	1.2 (0.7-2.1)	1.2 (0.9-2.4)	<b>2.1 (1.3-3.4)</b>	<b>1.9 (1.1-3.3)</b>
SGA with abnormal Doppler	6 (15.4)	17 (43.6)	16 (41)	2.1 (0.8-5.1)	3.2 (0.9-11.1)	<b>2.4 (1.2-4.8)</b>	<b>2.7 (1.2-5.9)</b>
<b>Neonatal <i>KDR-604T/C</i></b>							
Uncomplicated pregnancy	87 (28.5)	154 (50.5)	64 (21.0)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Preeclampsia	12 (26.7)	17 (37.8)	16 (35.6)	1.1 (0.6-1.8)	1.2 (0.6-1.7)	<b>2.1 (1.1-4.1)</b>	<b>2.2 (1.0-4.4)</b>
Gestational hypertension	15 (25.0)	27 (45.0)	18 (30.0)	1.2 (0.6-2.3)	1.4 (0.7-2.8)	1.6 (0.9-3.0)	1.5 (0.7-3.0)
SGA	12 (17.4)	32 (46.4)	25 (36.2)	1.6 (0.9-2.8)	1.4 (0.9-2.9)	<b>2.1 (1.2-3.8)</b>	<b>2.2 (1.2-3.9)</b>
SGA with abnormal Doppler	2 (7.7)	12 (46.2)	12 (46.2)	<b>4.8 (1.1-20.7)</b>	<b>4.3 (1.0-18.9)</b>	<b>3.2 (1.4-7.3)</b>	<b>3.2 (1.2-5.9)</b>

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; SGA, small for gestational age; CI, confidence interval; BMI, body mass index; ref, referent.

<sup>a</sup>Adjusted OR (95% CI) for preeclampsia and gestational hypertension are adjusted for maternal age, BMI, mean arterial pressure at 15 weeks gestation, smoking at 15 weeks gestation, and paternal BMI; aOR (95% CI) for SGA and SGA with abnormal Doppler are adjusted for maternal age, BMI, birth weight, smoking at 15 weeks gestation, and paternal BMI and birth weight; OR (95% CI) values in boldface are significant.

smoke at 15 weeks of gestation (data not presented). Maternal *KDR-604T/C* polymorphism was not associated with gestational hypertension in either smokers or nonsmokers (Table 3).

Associations between paternal and neonatal *KDR-604T/C* polymorphisms with adverse pregnancy outcomes were unaffected by maternal smoking (data not presented).

**Table 3.** Distribution of Maternal *KDR-604T/C* Polymorphism in Adverse Pregnancy Outcomes and Uncomplicated Pregnancy in Nonsmokers at 15 Weeks of Gestation<sup>a</sup>

Maternal <i>KDR-604T/C</i> variant	Genotype n (%)			Dominant model CC + CT versus TT	Recessive model CC versus TT + CT
	TT	CT	CC	OR (95% CI)	OR (95% CI)
Uncomplicated pregnancy	85 (28.1)	144 (47.7)	73 (24.2)	1.0 (ref)	1.0 (ref)
Preeclampsia	12 (19.7)	27 (44.2)	22 (36.1)	1.6 (0.8-3.2)	<b>2.1 (1.1-3.8)</b>
Gestational hypertension	25 (31.6)	31 (39.2)	23 (29.2)	0.8 (0.5-1.3)	1.3 (0.7-2.2)
SGA	8 (14.3)	26 (46.4)	22 (39.3)	<b>2.4 (1.1-5.2)</b>	<b>2.0 (1.1-3.7)</b>
SGA with abnormal Doppler	3 (13.0)	10 (43.5)	10 (43.5)	2.6 (0.8-9.0)	<b>2.4 (1.0-5.7)</b>

Abbreviations: ref, referent; CI, confidence interval; SGA, small for gestational age.

<sup>a</sup>Odds ratio (OR) (95% CI) values in boldface are significant.

## Comment

To our knowledge, this is the first study to investigate the association of *KDR-604T/C* and *Val297Ile* polymorphisms in pregnancy complications. Our preliminary data demonstrate that homozygosity for the C allele of the *KDR-604T/C* polymorphism in both the father and the infant is associated with preeclampsia and small-for-gestational-age pregnancies.

There is growing evidence that the father plays a significant role in the causation of both these pregnancy complications. The risk of fathering a preeclamptic pregnancy is increased among men who fathered a preeclamptic pregnancy with a different partner<sup>25</sup> and among men who were themselves the product of a preeclamptic pregnancy.<sup>26</sup> A paternal contribution to SGA has also been previously suggested by a positive correlation of paternal birth weight with infant birth weight<sup>22,27,28</sup> and that men who were SGA at birth are more likely than those with a normal birth weight to parent an SGA infant.<sup>22,29</sup> In our study, the paternal *KDR-604T/C* polymorphism remained associated with both preeclampsia and SGA after adjusting for established maternal and paternal risk factors for both disorders, demonstrating an independent association between the CC genotype of the *KDR-604T/C* polymorphism in the father with both pregnancy complications.

Kinase insert domain receptor is the main receptor for VEGF. Homozygous *KDR* gene (FLK in mice) ablation in mice results in failure to form mature endothelial cells leading to embryonic death demonstrating that this receptor is essential for embryonic and placental angiogenesis.<sup>12</sup> Placental expression of KDR is intense during early gestation and VEGF acting via KDR plays a key role in regulating angiogenesis and spiral artery remodeling.<sup>10</sup> Impaired spiral artery remodeling and angiogenesis are demonstrated in many cases of both preeclampsia and SGA pregnancies.<sup>8</sup>

Our results demonstrate that the neonatal *KDR-604T/C* CC genotype is associated with both pregnancy complications and that the association is stronger for SGA with abnormal uterine and/or umbilical artery Doppler. As the infant's genotype is likely to represent the placental genotype, homozygosity for the C allele of the *KDR-604T/C* polymorphism may contribute to impaired placental vascular development.

In this study, the maternal *KDR-604T/C* polymorphism was not associated with preeclampsia or with SGA. This is an unexpected finding considering that KDR is not an imprinted gene. However, when the cohort was stratified by maternal smoking at 15 weeks of gestation, we found a significant association between the maternal *KDR-604T/C* CC genotype and preeclampsia, SGA, and SGA with abnormal Doppler in nonsmokers. This may be due to the increase in expression of angiogenic growth factors in smokers.<sup>30</sup>

We did not find a significant association of the *KDR-604T/C* polymorphism with gestational hypertension if not complicated by SGA or with SGA in the absence of maternal hypertensive disease. These are expected findings as the pathogenesis of these complications is different and placental insufficiency is not an established finding in these disorders.

Previous studies have shown an association between preeclampsia and SGA with later life vascular diseases including increased risk of developing coronary artery disease and stroke.<sup>3,5-7</sup> These associations are thought to be the consequences of "programming," whereby an insult during intrauterine life is predicted to have lifelong effects.<sup>31</sup> The placenta is considered a programming agent for future cardiovascular disease, and animal models have demonstrated that abnormal endothelial development in the placenta is associated with increased vulnerability to heart disease.<sup>32</sup> Homozygosity for the variant allele of the *KDR-604T/C* polymorphism was previously shown to be associated with increased risk of coronary artery disease.<sup>13</sup> Here, we have shown that homozygosity for the variant allele of the *KDR-604T/C* is increased in fathers and infants in both preeclamptic and SGA pregnancies, providing further evidence for potential shared genetic factors between these pregnancy complications and cardiovascular disease.

The strengths of our study include a prospective cohort with excellent follow-up and collection of data on a large number of variables which enabled us to adjust for potential confounders. A limitation of our preliminary study is that our adverse pregnancy outcome groups were small and hence the study was relatively underpowered. We also excluded a number of cases and controls due to nonavailability of genotype results, and it is possible that this may have introduced bias into our results. A parent-infant trio analysis may provide substantial support

for the apparent association seen in the case–control analysis. We were not able to perform a family-based association analysis due to the small sample size in our preliminary cohort. Previous studies have also demonstrated the association of other polymorphisms (Cys482Arg and Pro1147Ser) of the *KDR* gene with vascular diseases<sup>33,34</sup> which suggests that a more comprehensive evaluation across the *KDR* gene investigating the possibility of linkage disequilibrium between polymorphisms may be beneficial. The novel association of a polymorphism in both the father and the infant with preeclampsia and SGA in this study is an interesting finding which needs to be replicated in a larger cohort.

In conclusion, the *KDR-604T/C* polymorphism which is a risk factor for coronary artery disease is associated with preeclampsia and SGA infants, suggesting that this polymorphism may associate with the risk of vascular disorders across the life course. Our data also demonstrate that there is a significant paternal genetic association with both preeclampsia and SGA pregnancies.

### Acknowledgments

We wish to thank the SCOPE families who generously consented to participate in this study. We thank Denise Healy for coordinating the SCOPE study in Adelaide and the SCOPE midwives. We thank MedSciNet (Sweden) and Eliza Chan for support with the database. We thank AGRF for conducting the genotyping.

### Authors' Note

PA is the principal author and has contributed to the design of the candidate gene association study, performed the statistical analyses of the data, and drafted the manuscript. ST has provided administrative and technical support. GD and CR have contributed to the design of the SCOPE study and the candidate gene association study and obtained funding. All authors have critically reviewed the manuscript for important intellectual content and edited the manuscript to produce the final draft. 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.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: the SCOPE study was supported by the Premier's Science and Research Fund, the South Australian Government. Genotyping was funded by the National Health and Medical Research Council Australia and the Channel 7 Children's Research Foundation. PA holds an Australian Leadership Award funded by the Australian government. The study sponsors had no role in study design, data analysis, and interpretation or writing this report.

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