ARTICLE

Association of Vascular Endothelial Growth Factor +936 C/T Single-Nucleotide Polymorphism With Pregnancies Complicated by Small-for-Gestational-Age Babies

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Objectives: To examine whether single-nucleotide polymorphisms (SNPs) in *VEGFA* (*–2578 C/A* and *+936 C/T*) associate with small-for-gestational-age (SGA) pregnancies and to identify their effects on first-trimester placental *VEGFA* expression.

Design: Multicenter prospective cohort study.

Settings: Adelaide, Australia, and Auckland, New Zealand.

Participants: A total of 3234 nulliparous pregnant women, their partners, and their infants.

Main Outcome Measures: The SNPs in the parent-infant trios and first-trimester placentae (n=74) were genotyped. Placental *VEGFA* messenger RNA expression was determined by quantitative reverse transcription–polymerase chain reaction. Small for gestational age was defined as a birth weight less than the 10th customized birth weight percentile adjusted for maternal height, weight, parity, and ethnicity and for gestational age at delivery and infant sex. Uterine and umbilical artery Doppler was performed at 20 weeks' gestation, and resistance indices greater than the 90th percentile were considered abnormal.

Results: Of 2123 pregnancies, 1176 (55.4%) were uncomplicated and 216 (10.2%) had SGA infants. Neonatal *VEGFA +936 C/T* SNP associates with SGA (adjusted odds ratio [aOR], 1.6; 95% CI, 1.0-2.3), SGA with abnormal Doppler findings (aOR, 3.5; 95% CI, 1.8-7.1), lower birth weight (*P*=.006), customized birth weight percentile (*P*=.049), and abnormal uterine artery Doppler findings (OR, 2.5; 95% CI, 1.2-5.4). Maternal *VEGFA +936 C/T* associates with abnormal umbilical artery Doppler findings (OR, 1.5; 95% CI, 1.1-2.2). *VEGFA +936 CT+TT* first-trimester placentae have 36% lower *VEGFA* messenger RNA expression compared with *CC* (*P*=.045).

Conclusion: Neonatal *VEGFA +936 C/T* associates with SGA, and the association is stronger for SGA with abnormal uterine or umbilical artery Doppler findings. The SNP also associates with reduced first-trimester placental *VEGFA* expression, suggesting that it may have a role in the pathogenesis of SGA.

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BEING SMALL FOR GESTATIONAL age (SGA) is an important predictor of health complications across the life course. Small-for-gestational age infants are at increased risk of neonatal complications, and approximately 40% of all stillborn infants are SGA at birth.[1] Babies who are SGA are also at higher risk for cognitive deficits and behavioral abnormalities in childhood and for lower academic achievement in adulthood.[2,3] A consistent association has also been demonstrated between SGA and adult-onset diseases, including increased risk of coronary artery disease and the related disorders of stroke, hypertension, and type 2 diabetes mellitus.[4-6] These associations are thought to be the consequences of "programming," whereby an insult at a critical period of development has lifelong effects.[7] 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.[8] Environmental and lifestyle factors along with the genetic makeup of both parents are implicated in determining how well the placenta develops and functions.[9]

Early placentation defects, including impaired maternal spiral artery remodeling and impaired placental villous vascularization, have been demonstrated in human pregnancies complicated by a growth-restricted fetus.[10,11] These abnormalities result in increased vascular impedance in the uterine and umbilical circulations that are detected during the antenatal period

by increased uterine and umbilical artery resistance indices (RIs) using Doppler velocimetry.[12,13] Many molecular pathways are involved in the pathogenesis of these vascular defects, of which the vascular endothelial growth factor (VEGF) family–mediated angiogenic pathway is recognized as having a key role.[14] There are 4 isoforms of VEGF, of which VEGFA is a potent angiogenic factor that is essential for development of the embryonic vasculature. Placental expression of VEGFA is known to be reduced in growth-restricted pregnancies,[15,16] and polymorphisms in the *VEGFA* gene may underlie this reduced expression.

Several single-nucleotide polymorphisms (SNPs) have been described in *VEGFA,* and some are reported to be associated with differential expression of *VEGFA* and production of VEGFA protein. The *VEGFA* polymorphisms *–2578 C/A* in the promoter region and *+936 C/T* in the 3′-untranslated region are associated with reduced plasma VEGFA levels.[17,18] The *T* allele of the *VEGFA +936 C/T* SNP has previously been shown to be associated with pregnancy complications that are also associated with SGA, such as preeclampsia[19] and spontaneous preterm birth.[20] The *A* allele of *VEGFA –2578 C/A* has been shown to be associated with early-onset preeclampsia.[21] To our knowledge, the *VEGFA +936 C/T* SNP has not previously been studied in SGA. In a white cohort, we aimed to evaluate the role of *VEGFA* (Entrez Gene ID 7422) *–2578 C/A* (rs699947) and *VEGFA +936 C/T* (rs3025039) polymorphisms in SGA infants with and without maternal hypertensive complications and in SGA associated with abnormal uterine and umbilical artery Doppler findings. Because it is important to establish that a polymorphism found to associate with a disease has a relevant pathophysiologic effect, we also aimed to determine whether these SNPs associate with uterine and umbilical artery Doppler abnormalities as surrogate markers of impaired placental blood flow as well as birth weight adjusted for gestational age. We also aimed to determine whether these SNPs affect first-trimester placental *VEGFA* messenger RNA (mRNA) expression and, thereby, provide mechanistic evidence for the association with SGA.

METHODS

This is a nested case-control study where participants were recruited from the Screening for Pregnancy Endpoints (SCOPE) study between November 1, 2004, and September 30, 2008, in Adelaide, Australia, and Auckland, New Zealand. 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.[22] Ethics approval was gained from local ethics committees.

STUDY POPULATION

Nulliparous women with singleton pregnancies, their partners, and their babies were recruited. Women considered at high risk for preeclampsia, SGA, or preterm birth because of underlying medical conditions, gynecologic history, or 3 or more miscarriages or terminations of pregnancy or who received medical or surgical interventions that could modify pregnancy outcome were ineligible. Recruited women were excluded for the following reasons: protocol violation, lost to follow-up, conceived with donor sperms or oocytes, miscarriage or termination, their partner did not participate, and not of white ethnicity.

Participants were interviewed and examined by a research midwife at a mean (SD) of 15 (1) and 20 (1) weeks of gestation. Data were collected at each time point on demographics, medical history, obstetric history, family history of obstetric complications and medical disorders, and participant's birth weight. Information on complications during the current pregnancy, diet, smoking status, and alcohol and recreational drug use was also obtained. A low fruit intake was defined as less than 1 portion per week. A low intake of green leafy vegetables was defined as fewer than 2 portions per week. Maternal and paternal physical measurements included height, weight, and blood pressure. Doppler ultrasound studies of the umbilical and uterine arteries were performed at 20 weeks' gestation.[23] The mean uterine artery RI was calculated from the left and right uterine RIs. Umbilical artery and mean uterine artery RIs greater than the 90th percentile were considered abnormal.

All the women were observed prospectively, and pregnancy outcome data and infant measurements were recorded by research midwives usually within 72 hours of birth. Recorded variables included the neonate's birth weight and customized birth weight percentile.

SPECIMEN COLLECTION

Peripheral blood samples were collected from the women and their partners. All the women provided blood samples. We collected buccal swabs or saliva samples from partners who were unwilling to undergo venipuncture. The buccal swabs were applied to Whatman FTA cards (Whatman Inc, Piscataway, NJ) immediately after sample collection, and saliva was collected using Oragene kits (DNA Genotek Inc, Kanata, Ontario, Canada). Cord blood was collected at delivery. If cord blood was not obtained at delivery, a buccal swab or saliva sample was collected from the baby.

DEFINITIONS OF PREGNANCY OUTCOMES

Small for gestational age was defined as birth weight less than the 10th customized percentile adjusted for maternal height, weight, parity, and ethnicity and for gestational age at delivery and infant sex.[24] *Small for gestational age with abnormal Doppler findings* was defined as SGA with abnormal uterine and umbilical artery RIs. *Uncomplicated pregnancy* was defined as a pregnancy with no antenatal medical or obstetric complications and resulting in the delivery of an appropriately grown, healthy baby at 37 weeks of gestation or older.

COLLECTION OF FIRST-TRIMESTER PLACENTAL TISSUE

First-trimester placental tissue (6-12 weeks' gestation, n=74) was collected from women undergoing elective termination of pregnancy at the Women's and Children's Hospital (Adelaide). Ethics approval was obtained from the University of Adelaide Human Research Ethics Committee and from the Women's and Youth Health Service Human Research Ethics Committee. Written informed consent was obtained from all the women. Women undergoing termination for medical reasons, including fetal genetic abnormalities, were excluded. Placental tissue was collected immediately after the termination procedure. Placental villous tissue was dissected and cut into pieces weighing approximately 100 mg and collected into individual, sterile, snap-lock 1.7-mL tubes and snap frozen immediately in liquid nitrogen. Samples were stored at –80°C until required.

GENOTYPING

DNA was extracted from buffy coats from peripheral or cord blood, Whatman FTA cards, or saliva (Oragene DNA kits) and from first-trimester placental tissue according to the manufacturers' instructions. Genotyping was performed at the Australian Genome Research Facility (Brisbane, Australia) using the Sequenom MassARRAY system (Sequenom Inc, San Diego, California). As a quality control measure, 300 independent samples that were genotyped in-house for the same SNPs using reverse transcription–polymerase chain reaction were genotyped using the Sequenom MassARRAY system at the Australian Genome Research Facility. The concordance rate of the reverse transcription–polymerase chain reaction results and MassARRAY results was 100%. Each sample was also genotyped for amelogenin to ensure that the sex of the sample was correct.[25] Parental and neonatal genotype data were checked for a mendelian pattern of inheritance, and those found to be inconsistent were excluded from the analysis.

PLACENTAL *VEGFA* mRNA EXPRESSION

Total RNA was isolated from 100 mg of each first-trimester placenta using the TRIzol (Invitrogen, Carlsbad, California) method according to the manufacturer's instructions. For each sample, 2 µg of RNA was reverse transcribed to complementary DNA using random hexamer primers (GeneWorks, Adelaide) and SuperScript III (Invitrogen) according to the manufacturers' instructions. Quantitative reverse transcription–polymerase chain reaction was performed using a real-time polymerase chain reaction machine (Rotor-Gene 6000; Corbett Research, Sydney, Australia). All the reactions were set up using a liquid handling system (CAS-1200; Corbett Robotics, Brisbane, Australia). The primer sequences for VEGF were 5′-CTGGAGTGTGTGCCCACTGA-3′ (forward) and 5′-TCCTATGTGCTGGCCTTGGT-3′ (reverse). 18s Was used as the endogenous control for normalization of the raw data using the following primers: 5′-AGAAACGGCTACCACATCCAA-3′ (forward) and 5′-CCTGTATTGTTATTTTTCGTCACTACCT-3′ (reverse). All the reactions were performed in 10 µL of mixture containing 5 µL of SYBR Green PCR Master Mix (2X) (Applied Biosystems, Warrington, UK), 0.5 µL each of forward and reverse primer, 2 µL of complementary DNA, and 2 µL of sterile water for injection. The thermal cycling conditions were 10 minutes at 95°C, then with 40 cycles at 95°C for 15 seconds, 60°C for 10 seconds, and 72°C for 10 seconds. All the samples were assayed in triplicate, and a 6-point standard and an internal control were assayed in triplicate on each plate. Relative mRNA expression was determined by the standard curve method.[26]

STATISTICS

The SGA infants and their parents were compared with parent-infant trios from uncomplicated pregnancies in a nested case-control manner. The χ^2 test was used to test the genotypes at each polymorphic locus for Hardy-Weinberg equilibrium.

Missing data were excluded from the analyses. Categorical variables were compared using χ^2 or Fisher exact tests. Univariate analysis with post hoc Bonferroni adjustment or the *t* test was used to compare genotype data with continuous variables. Data deviating from a normal distribution were analyzed using nonparametric tests. Adjusted and nonadjusted odds ratios (ORs) were calculated for the genotype frequencies of SGA compared with controls using dominant and recessive genotype models by unconditional logistic regression analysis. The confounding factors for SGA in the logistic regression model included previously published risk factors for SGA as follows: maternal age, body mass index (BMI), birth weight, smoking status, and low fruit and vegetable intake and paternal age, BMI, and birth weight.[27-29] Because the SGA population comprised infants born to both normotensive and hypertensive mothers, the presence of preeclampsia and gestational hypertension were also included in the logistic regression model. All the data analyses were performed using a commercially available software program (PASW, version 17.02; SPSS, Inc, Cary, North Carolina). Results are reported as number (percentage) or as mean (SEM) as appropriate. $P < .05$ was considered statistically significant.

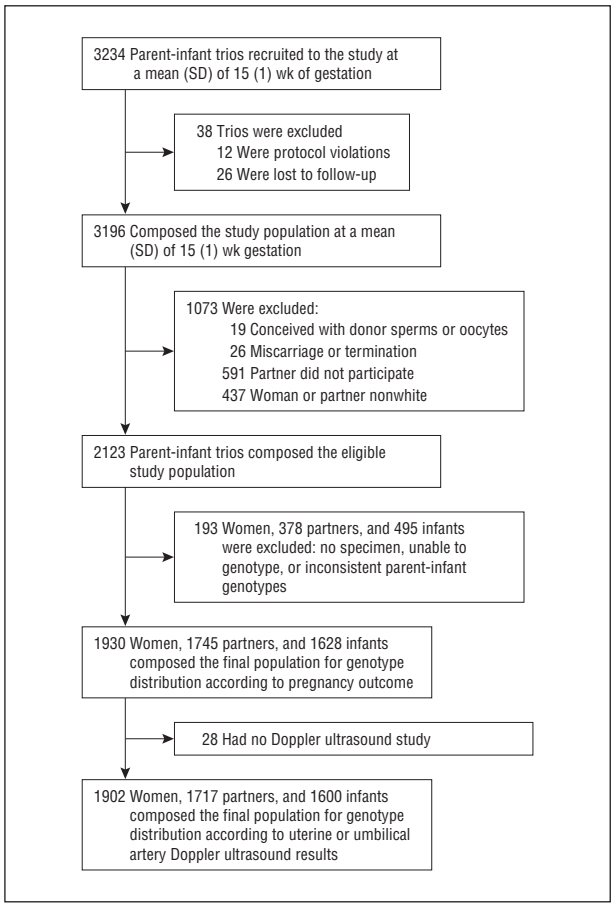

Figure 1. Study population.

RESULTS

Of the 3234 recruited parent-infant trios, 2123 were included in this study. The exclusions are detailed in **Figure 1**. Of 2123 pregnancies, 1176 (55.4%) were uncomplicated, 216 (10.2%) had an SGA infant, and the remaining 731 (34.4%) developed other obstetric, medical, or surgical complications during pregnancy. Of the 216 SGA infants, 158 (73.1%) were born to normotensive mothers and 58 (26.9%) were born to hypertensive mothers (preeclampsia [n=28] and gestational hypertension [n=30]). Sixty-one of the 216 SGA pregnancies (28.2%) had uterine or umbilical artery Doppler RIs greater than the 90th percentile at the 20-week scan. Of the SGAs with abnormal Doppler findings, 60.7% (n=37) had abnormal mean uterine artery RIs, 29.5% (n=18) had abnormal umbilical artery RIs, and 9.8% (n=6) had both abnormal uterine and umbilical artery RIs. The charac-

Table 1. Characteristics of the Study Population

Characteristic	Control Group (n=1176)	SGA Group (n=216)[a]	*P* Value[b]
Maternal characteristics			
Age, mean (SEM), y	28.2 (0.2)	28.5 (0.4)	.55
BMI, mean (SEM)	24.9 (0.1)	26.1 (0.4)	.002
Birth weight, mean (SEM), g[c]	3331 (16)	3167 (37)	<.001
Smoking continued after 15 wk of gestation, No. (%)	105 (8.9)	47 (21.8)	<.001
Paternal characteristics, mean (SEM)			
Age, y	30.7 (0.2)	31.1 (0.5)	.46
BMI[d]	26.6 (0.1)	27.2 (0.3)	.07
Birth weight, g[e]	3492 (17)	3313 (37)	<.001
Uterine and umbilical artery Doppler findings at 20 wk, No. (%)			
Abnormal mean uterine artery RI	65 (5.5)	37 (17.1)	<.001
Abnormal umbilical artery RI	83 (7.1)	18 (8.3)	.20
Abnormal mean uterine and umbilical artery RI	7 (0.6)	6 (2.8)	.008
Pregnancy outcome, mean (SEM)			
Neonatal birth weight (g)	3590 (12)	2587 (38)	<.001
Customized birth weight percentile	53.7 (0.7)	4.6 (0.2)	<.001
Gestational age at delivery, wk	39.7 (0.1)	38.4 (0.2)	<.001

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); RI, resistance index; SGA, small for gestational age.

[a] Born to normotensive women (n=158), women with preeclampsia (n=28), or women with gestational hypertension (n=30).

[b] By Pearson χ^2 or univariate analyses.

[c] Uncomplicated pregnancy (n=1144) and SGA (n=208).

[d] Uncomplicated pregnancy (n=1148) and SGA (n=210).

[e] Uncomplicated pregnancy (n=1099) and SGA (n=197).

teristics of the participants are given in **Table 1**. In the SGA group, mothers had a higher BMI, and both parents had a lower birth weight. Smoking was more prevalent in women who were delivered of an SGA infant compared with women who had an uncomplicated pregnancy. The prevalence of smoking was even higher in SGA with an abnormal uterine or umbilical artery Doppler finding (n=16 [26.2%]) compared with uncomplicated pregnancy (n=105 [8.9%], $P<.001$).

Genotype data from 193 women (9.1%), 378 partners (17.8%), and 495 infants (23.3%) could not be analyzed due to nonavailability of samples, genotyping failure, and mendelian inconsistencies in parent-infant genotypes. Genotype data were available for 157 SGA infants and 958 infants from uncomplicated pregnancies for *VEGFA +936 C/T* SNP and for 156 SGA infants and 940 infants from uncomplicated pregnancies for *VEGFA –2578 C/A* SNP.

GENOTYPE DISTRIBUTION ASSOCIATED WITH SGA AND SGA WITH ABNORMAL DOPPLER FINDINGS

Both SNPs were in Hardy-Weinberg equilibrium in cases and controls. The prevalence of neonatal *VEGFA +936 C/T CT+TT* was increased in SGA infants compared with infants born after an uncomplicated pregnancy (adjusted OR, 1.6; 95% CI, 1.0-2.3; $P=.03$ for the dominant genotype model) (**Table 2**). In SGA with abnormal uterine or umbilical artery Doppler findings at 20 weeks' gestation, there was a stronger association with neonatal *VEGFA +936 C/T* SNP (adjusted OR, 3.5; 95% CI, 1.8-7.1; $P<.001$ for the dominant genotype model) (Table 2). Neonatal *VEGFA +936 C/T* SNP was also associated with a lower birth weight adjusted for gestational age at delivery ($P=.006$) and a lower customized birth weight percentile ($P=.049$) (**Table 3**). Maternal and paternal *VEGFA +936 C/T* SNPs were not associated with the birth of an SGA infant, SGA with an abnormal uterine or umbilical artery Doppler waveform, infant birth weight, or customized birth weight percentile. *VEGFA –2578 C/A* SNP was not associated with any of the outcome measures.

GENOTYPE DISTRIBUTION ASSOCIATED WITH ABNORMAL UTERINE AND UMBILICAL ARTERY DOPPLER FINDINGS

Maternal *VEGFA +936 C/T* SNP was more prevalent in those who had abnormal umbilical artery Doppler findings (OR, 1.5; 95% CI, 1.1-2.2; $P=.01$ for the dominant genotype model) (**Table 4**). Association of maternal *VEGFA +936 C/T* SNP with abnormal uterine artery Doppler findings approached significance for the dominant genotype model (OR, 1.3; 95% CI, 0.9-1.8; $P=.051$) (Table 4). Neonatal *VEGFA +936 TT* was more prevalent in pregnancies with abnormal uterine artery Doppler findings (OR, 2.5; 95% CI, 1.2-5.4; $P=.01$ for the recessive genotype model) (Table 4). The ORs (95% CIs) of the previous results adjusted for maternal smoking and the presence of hypertension during pregnancy were similar to the crude results. Maternal and neonatal *VEGFA –2578 C/A* SNP was not associated with abnormal uterine or umbilical artery Doppler findings (data not shown).

GENOTYPE DISTRIBUTION ASSOCIATED WITH FIRST-TRIMESTER PLACENTAL *VEGFA* EXPRESSION

Placental *VEGFA +936 CT+TT* (dominant model) was associated with a 36% reduction in first-trimester placental *VEGFA* mRNA levels compared with the *CC* genotype (**Figure 2**). Placental *VEGFA –2578 C/A* was not associated with first-trimester placental *VEGFA* mRNA levels (data not shown).

COMMENT

The present study demonstrates the association of *VEGFA +936 C/T* SNP with SGA and its pathogenesis. *VEGFA +936 C/T* SNP in neonates is associated with SGA, SGA with abnormal uterine or umbilical artery Doppler findings, lower birth weight, lower customized birth weight percentile, and abnormal uterine artery Doppler findings. Maternal *VEGFA +936 C/T* SNP is associated with abnormal umbilical artery Doppler findings and approaches a significant association with abnormal uterine artery Doppler findings. We also found this SNP in the placenta to be associated with reduced first-trimester placental *VEGFA* mRNA expression.

Fetal growth restriction is associated with early placentation defects, including inadequate remodeling of

Table 2. Distribution of Neonatal *VEGFA* SNPs in SGA, SGA With Abnormal Doppler Findings, and Uncomplicated Pregnancy

Neonatal SNP	Uncomplicated Pregnancy, No. (%)	SGA, No. (%)	OR (95% CI)	aOR (95% CI)[a]	SGA With Abnormal Doppler Findings, No. (%)	OR (95% CI)	aOR (95% CI)[a]
VEGFA +936 C/T	(n=958)	(n=157)			(n=39)		
CC	729 (76.1)	108 (68.8)	1 [Reference]	1 [Reference]	18 (46.2)	1 [Reference]	1 [Reference]
CT	198 (20.7)	43 (27.4)	1.4 (1.0-2.1)[b]	1.5 (1.0-2.3)[b]	18 (46.2)	3.2 (1.5-6.8)[b]	3.4 (1.7-7.0)[b]
TT	31 (3.2)	6 (3.8)	1.3 (0.5-3.2)	1.5 (0.6-3.8)	3 (7.7)	4.9 (1.3-17.9)[b]	4.6 (1.2-17.4)[b]
Dominant model							
CC	729 (76.1)	108 (68.8)	1 [Reference]	1 [Reference]	18 (46.2)	1 [Reference]	1 [Reference]
CT+TT	229 (23.9)	49 (31.2)	1.4 (1.0-2.1)[b]	1.6 (1.0-2.3)[b]	21 (53.8)	3.7 (1.9-7.1)[b]	3.5 (1.8-7.1)[b]
Recessive model							
CC+CT	927 (96.8)	151 (96.2)	1 [Reference]	1 [Reference]	36 (92.3)	1 [Reference]	1 [Reference]
TT	31 (3.2)	6 (3.8)	1.5 (0.4-4.9)	1.5 (0.6-3.7)	3 (7.7)	1.0 (0.9-1.1)	3.0 (0.8-10.8)
VEGFA –2578 C/A	(n=940)	(n=156)			(n=40)		
CC	241 (25.6)	42 (26.9)	1 [Reference]	1.0 [Reference]	12 (30)	1 [Reference]	1 [Reference]
CA	466 (49.6)	75 (48.1)	0.9 (0.5-1.3)	0.8 (0.5-1.3)	18 (45)	0.9 (0.4-2.3)	0.6 (0.3-1.5)
AA	233 (24.8)	39 (25.0)	0.9 (0.6-1.5)	0.9 (0.6-1.6)	10 (25)	1.0 (0.4-2.7)	1.0 (0.4-2.7)
Dominant model							
CC	241 (25.6)	42 (26.9)	1 [Reference]	1 [Reference]	12 (30)	1 [Reference]	1 [Reference]
CA+AA	699 (74.4)	114 (73.1)	1.01 (0.9-1.1)	0.9 (0.6-1.3)	28 (70)	1.0 (0.9-1.3)	0.8 (0.4-1.6)
Recessive model							
CC+CA	707 (75.2)	117 (75)	1 [Reference]	1.0 [Reference]	30 (75)	1 [Reference]	1 [Reference]
AA	233 (24.8)	39 (25)	0.9 (0.7-1.3)	0.9 (0.6-1.4)	10 (25)	1.0 (0.8-1.2)	1.2 (0.5-2.5)

Abbreviations: aOR, adjusted odds ratio; OR, odds ratio; SGA, small for gestational age; SNP, single-nucleotide polymorphism.
[a] Adjusted for maternal factors: age, body mass index, smoking, low fruit intake, and low green leafy vegetable intake; paternal factors: age, body mass index, and birth weight; and pregnancy outcomes: presence of preeclampsia or gestational hypertension.
[b] Statistically significant.

uterine spiral arteries[10] and impaired placental vascularization.[12,30,31] The molecular mechanisms that control spiral artery remodeling are still not clear, but it is known that during invasion, trophoblasts lose their epithelial characteristics and acquire an endothelial phenotype.[32] This transition process is called *pseudovasculogenesis* and is known to be regulated by angiogenic growth factors, including VEGFA.[33] We showed that the placental *CT+TT* genotypes of the *VEGFA +936 C/T* SNP associate with reduced first-trimester placental *VEGFA* mRNA expression. The same genotypes in the infant were associated with increased mean uterine artery RIs at 20 weeks' gestation. Because the infant's genotype is likely to represent the placental genotype, we can hypothesize that these genotypes are associated with abnormal uteroplacental vascular adaptation in early pregnancy, before the fetus becomes SGA.

Placental expression of VEGFA is intense during early pregnancy,[34] and VEGFA is known to be a potent regulator of early placental villous vascularization.[14] Gene ablation studies have shown that even $VEGFA^{+/-}$ mice have major vascular abnormalities resulting in early embryonic death, demonstrating that VEGFA is essential for early fetal development.[35,36] Abnormal placental villous vascular development is known to be associated with abnormal umbilical artery blood flow, as assessed by Doppler ultrasonography.[30] We found that the *CT+TT* genotype of the maternal *VEGFA +936 C/T* polymorphism was associated with abnormal umbilical artery RIs at 20 weeks' gestation, indicating that carriers of this SNP were at higher risk for abnormal placental villous vascular development.

Heterozygosity for *VEGFA +936 C/T,* as well as *CT+TT* in the dominant genotype model, was more prevalent in SGA infants, and these genotypes were also associated with reduced birth weight (adjusted for gestational age) and customized birth weight percentiles compared with those homozygous for the *C* allele. The SNP showed a strong association with SGA in which uterine or umbilical artery Doppler abnormalities were detected at 20 weeks, suggesting an increased effect of this SNP in SGA in the presence of impaired placental blood flow.

Table 3. Distribution of Neonatal *VEGFA +936 C/T* SNP in Birth Weight and Customized Birth Weight Percentile[a]

VEGFA +936 C/T Genotype	Birth Weight, g (n=1628)[b]	Customized Birth Weight Percentile (n=1628)[c]
CC	3450 (12)	48.9 (0.8)
CT	3382 (22)	45.7 (1.5)
TT	3326 (62)	41.8 (4.2)
P value	.006	.049

Abbreviation: SNP, single-nucleotide polymorphism.
[a] Data are presented as mean (SEM).
[b] Univariate analysis using the post hoc Bonferroni test and adjusted for gestational age.
[c] Kruskal-Wallis test.

To our knowledge, this is the first study to evaluate the role of *VEGFA +936 C/T* SNP in SGA. Bányász et al[37] studied the *VEGFA –2578 C/A* SNP in low-birth-weight infants and reported that the SNP was not associated with being born with a low birth weight, which is consistent with the present findings in SGA. One previous study[19] has reported the association of the *T* allele of *VEGFA +936 C/T* polymorphism with preeclampsia in Korean women, and another study[38] has shown the association of the *T* al-

Table 4. Genotype Distribution of Maternal and Neonatal *VEGFA +936 C/T* SNP in Uterine and Umbilical Artery Doppler[a]

SNP	Normal Ut.RI	Abnormal Ut.RI	OR (95% CI)	Normal Umb.RI	Abnormal Umb.RI	OR (95% CI)
Maternal *VEGFA +936 C/T*	(n=1717)	(n=185)		(n=1742)	(n=160)	
CC	1280 (74.5)	127 (68.6)	1 [Reference]	1302 (74.7)	105 (65.6)	1 [Reference]
CT	406 (23.6)	52 (28.1)	1.3 (0.9-1.8)	404 (23.2)	54 (33.8)	1.7 (1.2-2.3)
TT	31 (1.8)	6 (3.2)	1.9 (0.8-4.8)	36 (2.1)	1 (0.6)	0.3 (0.05-2.5)
Dominant model						
CC	1280 (74.5)	127 (68.6)	1 [Reference]	1302 (74.7)	105 (65.6)	1 [Reference]
CT+TT	437 (25.5)	58 (31.4)	1.3 (0.96-1.8)	440 (25.3)	55 (34.4)	1.5 (1.1-2.2)[b]
Recessive model						
CC+CT	1686 (98.2)	179 (96.8)	1 [Reference]	1706 (97.9)	159 (99.4)	1 [Reference]
TT	31 (1.8)	6 (3.2)	0.5 (0.2-1.3)	36 (2.1)	1 (0.6)	3.3 (0.4-24.6)
Neonatal *VEGFA +936 C/T*	(n=1455)	(n=145)		(n=1475)	(n=125)	
CC	1100 (75.6)	102 (70.3)	1 [Reference]	1112 (75.4)	89 (71.2)	1 [Reference]
CT	318 (21.9)	34 (23.4)	1.1 (0.8-1.7)	323 (21.9)	30 (24.0)	1.2 (0.8-1.8)
TT	37 (2.5)	9 (6.2)	2.6 (1.2-5.6)[b]	40 (2.7)	6 (4.8)	1.9 (0.8-4.5)
Dominant model						
CC	1100 (75.6)	102 (70.3)	1 [Reference]	1112 (75.4)	89 (71.2)	1 [Reference]
CT+TT	355 (24.4)	43 (29.7)	1.3 (0.8-1.9)	363 (24.6)	36 (28.8)	1.2 (0.8-1.9)
Recessive model						
CC+CT	1418 (97.5)	136 (93.8)	1 [Reference]	1435 (97.3)	119 (95.2)	1 [Reference]
TT	37 (2.5)	9 (6.2)	2.5 (1.2-5.4)[b]	40 (2.7)	6 (4.8)	0.5 (0.2-1.3)

Abbreviations: Abnormal Umb.RI, umbilical artery RI greater than 90%; Abnormal Ut.RI, uterine artery RI greater than 90%; OR, odds ratio; RI, resistance index; SNP, single-nucleotide polymorphism.
[a] Data are presented as number (percentage).
[b] Statistically significant.

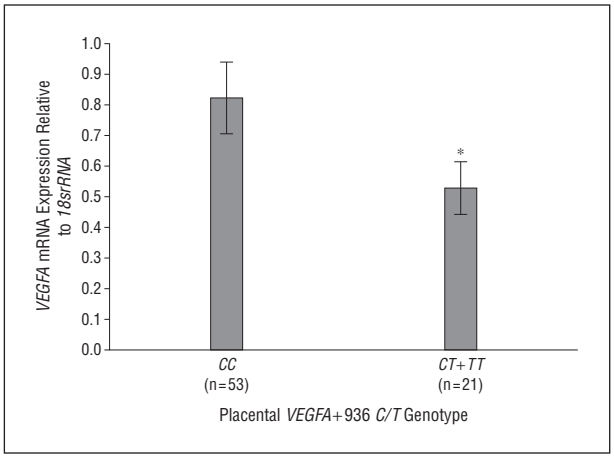

Figure 2. Genotype distribution of *VEGFA +936 C/T* single-nucleotide polymorphism in first-trimester placental *VEGFA* messenger RNA (mRNA) expression. Error bars represent SEM. *P=.045.

lele with severe preeclampsia in Greek women. The Korean study did not report the effects of growth restriction in preeclamptic women, and their preeclamptic population comprised infants with significantly lower birth weight compared with controls. The Greek study found no association of the SNP with preeclampsia, and the only association was with severe preeclampsia, which comprised only 20 women. This study also does not report the presence of growth restriction, which is likely found in their severe preeclamptic population. Therefore, the associations demonstrated in these studies may be due to the effects of growth restriction in their preeclamptic study population.

The present SGA population comprised SGA infants born to normotensive women and those born to women who had preeclampsia or gestational hypertension. We did not perform SGA subgroup analysis because the sample size in each category was not sufficient for adequate power to detect clinically relevant differences. Because previous studies have reported an association between *VEGFA +936 C/T* SNP and preeclampsia, we adjusted for the presence of hypertensive disease in pregnancy (preeclampsia or gestational hypertension) in the logistic regression model. The neonatal *VEGFA +936 C/T* SNP remained significantly associated with SGA independent of maternal hypertensive disease.

The strengths of this study include a large prospective cohort with excellent follow-up, inclusion of parent-infant trios, and defining SGA on customized birth weight percentiles. A limitation of this study was that we excluded several cases and controls owing to nonavailability of genotype results, and it is possible that this has introduced bias into the results. The availability of uterine and umbilical artery Doppler ultrasound before the development of SGA enables us to comment on the potential role of the SNPs in the pathogenesis of fetal growth restriction. Although this prospective cohort is large, the SGA group with uterine or umbilical artery Doppler flow abnormalities is relatively small, and a type I error may have occurred. These findings need to be replicated in other independent cohorts.

In conclusion, this study demonstrates that the neonatal *VEGFA +936 C/T* SNP associates with SGA and that the association is stronger for SGA with abnormal uterine or umbilical artery Doppler findings. The SNP is also associated with reduced first-trimester placental *VEGFA* expression, suggesting that it may have a role in the pathogenesis of SGA.

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