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The association of *AGT2R* polymorphisms with preeclampsia and uterine artery bilateral notching is modulated by maternal BMI

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ABSTRACT

Introduction: This study aimed to determine the association of *AGT1R* and *AGT2R* polymorphisms with preeclampsia and whether these are affected by environmental factors and fetal sex.
Methods: Overall 3234 healthy nulliparous women, their partners and babies were recruited prospectively to the SCOPE study in Adelaide and Auckland. Data analyses were confined to 2121 Caucasian parent-infant trios, among whom 123 had preeclamptic pregnancies. 1185 uncomplicated pregnancies served as controls. DNA was extracted from buffy coats and genotyped by utilizing the Sequenom MassARRAY system. Doppler sonography on the uterine arteries was performed at 20 weeks' gestation.
Results: Four polymorphisms in AGT1R and AGT2R genes, including *AGT1R A1166C*, *AGT2R C4599A*, *AGT2R A1675G* and *AGT2R T1134C*, were selected and significant associations were predominately observed for *AGT2R C4599A*. When the cohort was stratified by maternal BMI, in women with BMI $\geq$ 25 kg/m^2, the *AGT2R C4599A* AA genotype in mothers and neonates was associated with an increased risk for preeclampsia compared with the CC genotype [adjusted OR 2.1 (95% CI 1.0–4.2) and adjusted OR 3.0 (95% CI 1.4–6.4), respectively]. In the same subset of women, paternal *AGT2R C4599A* A allele was associated with an increased risk for preeclampsia and uterine artery bilateral notching at 20 weeks' gestation compared with the C allele [adjusted OR 1.9 (95% CI 1.1–3.3) and adjusted OR 2.1 (95% CI 1.3–3.4), respectively].
Conclusion: *AGT2R C4599A* in mothers, fathers and babies was associated with preeclampsia and this association was only apparent in pregnancies in which the women had a BMI $\geq$ 25 kg/m^2, suggesting a gene–environment interaction.

1. Introduction

Preeclampsia affects up to 7% of nulliparous pregnancies and is a major cause of maternal and perinatal morbidity and mortality worldwide [1,2]. To date, the exact cause of preeclampsia is still unknown. Since hypertension is both a risk factor and a symptom of preeclampsia, the renin angiotensin system (RAS), which plays an important role in blood pressure regulation, electrolyte and volume homeostasis [3], has been studied intensively for its contribution to the development of the disorder.

In third trimester preeclamptic women are reported to have reduced plasma renin activity [4], increased serum angiotensin converting enzyme (ACE) activity [4], reduced angiotensin II (ANG II) concentration [4] and increased responsiveness to ANG II [5,6] compared to women with normal pregnancy. The aberrant RAS levels/activities observed in preeclamptic pregnancies may indicate the involvement of RAS in the pathogenesis of preeclampsia. Therefore, genetic polymorphisms in the RAS components, which modulate RAS levels/activities, may potentially predispose women to preeclampsia.

Abbreviations: RAS, renin angiotensin system; *AGT2R*, angiotensin II type II receptor; SCOPE, **SC**reening f**O**r **P**regnancy **E**ndpoints; sBP, systolic blood pressure; dBP, diastolic blood pressure; BMI, body mass index.

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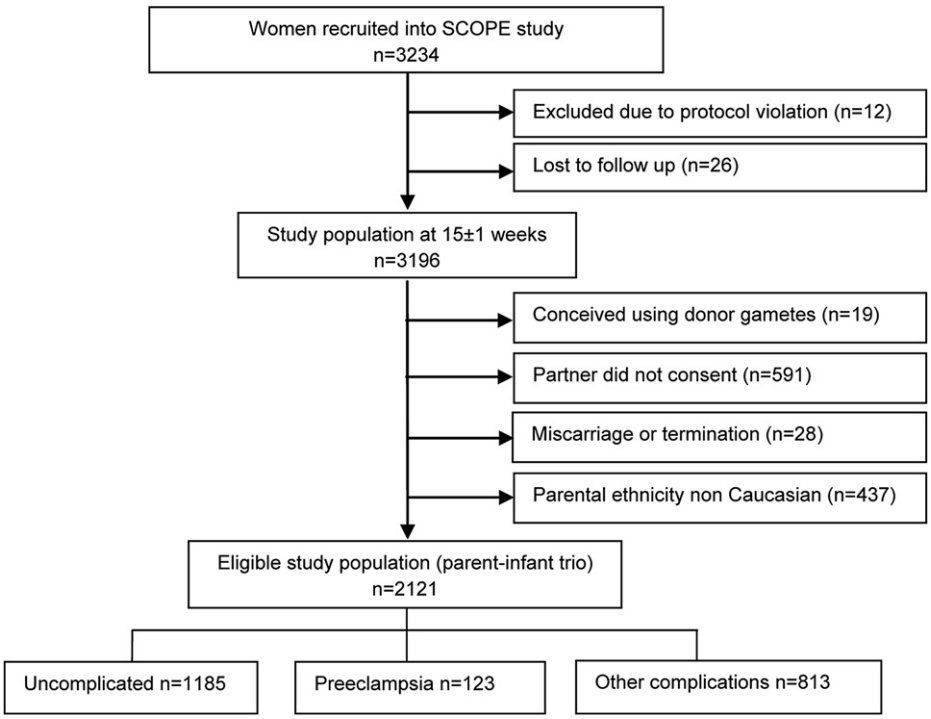

Fig. 1. Flow chart of participant recruitment.

Over the past decade, several polymorphisms in the AGT1R and AGT2R genes have been identified. *AGT1R A1166C* (rs5186) is located in the 3′ UTR of *AGT1R* on the chromosome 3. The *AGT1RA1166C* CC genotype is associated with greater ANG II responsiveness [7] and increases risk for coronary artery disease and myocardial infarction [8] compared with the AA genotype. *AGT2R C4599A* (rs11091046), *AGT2R A1675G* (rs1403543) and *AGT2R T1134C* (rs12710567) are located in the 3′ UTR of exon 3, intron 1 and the promoter region of the *AGT2R* gene on the X chromosome, respectively. The *AGT2R A1675G* G allele is associated with higher *AGT2R* expression compared with the A allele [9]. The functional effects of *AGT2R C4599A* and *AGT2R T1334C* on AGT2R have not been investigated previously. *AGT2R A1675G* and *AGT2R C4599A* have been shown to be in linkage disequilibrium in a Japanese population [10]. In a Chinese cohort, the *AGT2R T1334C* C allele is associated with an increased risk for essential hypertension compared with the T allele [11].

In the current study, our primary aim was to determine if the aforementioned *AGT1R* and *AGT2R* polymorphisms in mothers, fathers and babies were associated with preeclampsia. Since assessing gene–environment interactions is becoming an increasingly important aspect of genetic association studies [12,13], our secondary aim was to determine whether the association of *AGT1R* and *AGT2R* polymorphisms with preeclampsia is affected by risk factors for preeclampsia, including maternal age [14,15], BMI [16], green leafy vegetable intake [17], fruit intake [18], socio-economic status [19] and smoking [20]. In addition, since RAS components are sexually dimorphic in adults [21], we explored our primary and secondary aims in pregnancies bearing female and male infants separately.

2. Materials and methods

2.1. Ethics approval

In Australia, ethical approval was obtained from the Central Northern Adelaide Health Service Ethics of Human Research Committee (study number: REC 1714/5/2008). In New Zealand, ethical approval was given by the Northern Region Ethics Committee (study number: AKX/02/00/364). All participants provided written informed consent. Australian clinical trial registry number: ACTRN 12607000551493.

2.2. Participants

The current study is a nested case control study embedded in a large prospective multicentre study, Screening for Pregnancy Endpoints (SCOPE). The participants were healthy nulliparous women with singleton pregnancies recruited to the SCOPE study between November 2004 and September 2008 in Adelaide, Australia and Auckland, New Zealand [22]. SCOPE is a prospective study with the main aim of developing screening tests to predict preeclampsia, small for gestational age infants and spontaneous preterm birth. Overall 3196 women, their partners and babies were recruited into the study. The population for this genetic study was confined to the 2121 Caucasian parent-infant trios (66%) (Fig. 1).

Women were recruited to the SCOPE study through hospital antenatal clinics, obstetricians, general practitioners, community midwives and self referral in response to advertisements or recommendations of friends. Women were excluded if they were judged to be at high risk of preeclampsia, small for gestational age babies or spontaneous preterm birth because of underlying medical conditions, gynaecological history, three or more previous miscarriages or three or more terminations of pregnancy or if they had received interventions that might modify pregnancy outcome [22].

Participants were interviewed and examined by a research midwife at 15 ± 1 weeks of gestation. Maternal demographic and dietary information was collected, including ethnicity, age, height, weight, birthweight, gestational age at birth, socio-economic index (SEI[1]) [23], smoking status at 15 weeks' gestation and pre-pregnancy green leafy vegetable intake. Two consecutive manual blood pressure measurements were recorded. Paternal information, including age, birth weight, height and weight, were also recorded. Newborn measurements were recorded by research midwives usually within 72 h of birth. The recorded parameters included infant's gestational age at birth, body length, head circumference, mid arm circumference, birth weight and customised birthweight centile. Ultrasound and Doppler studies of the umbilical and uterine arteries were performed at 20 weeks' gestation [24]. Bilateral notching is defined as the presence of early diastolic notching in the waveform of both uterine arteries [25].

2.3. Sample collection

Whole blood was collected in EDTA tubes from women at 15 ± 1 weeks of gestation, from partners at some time during the woman's pregnancy and umbilical cord after delivery. Blood samples were centrifuged and plasma and buffy coat

[1] The New Zealand socio-economic index of occupational status, a number between 10 and 90 and is an occupationally derived indicator of socio-economic status. It is a validated measure of an individual's socio-economic status and a higher score indicates higher socio-economic status.

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separated and stored within 3 h of collection. Buccal swabs or saliva samples were collected from partners who were unwilling to undergo venepuncture and babies whose cord blood was not obtained at delivery. The buccal swabs were applied to Whatman FTA cards (Whatman, USA) immediately following sample collection and saliva was collected using Oragene kits (DNA genotek, USA).

2.4. Pregnancy outcome definitions

Preeclampsia was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or both, on at least two occasions 4 h apart after 20 weeks' gestation but before the onset of labour or postpartum, with either proteinuria (24 h urinary protein ≥300 mg or spot urine protein: creatinine ratio ≥30 mg/mmol creatinine or urine dipstick protein ≥++) or any multisystem complication of preeclampsia [18].

Uncomplicated pregnancies were normotensive pregnancies with delivery of a healthy and appropriately grown infant at ≥37 weeks' gestation.

2.5. Genotyping assays

DNA was extracted from buffy coats isolated from peripheral or cord blood (QiAamp 96 DNA blood kit), Whatman FTA cards or from saliva (Oragene®DNA kits) following the manufacturers' instructions. Genotyping was performed by the Australian Genome Research Facility (AGRF) utilizing the Sequenom MassARRAY system. Two quality control procedures were in place to ensure the accuracy of genotyping data: 1) Each sample was genotyped for Amelogenin to assess the consistency between the sex of samples and the corresponding Amelogenin genotype [26]. 2) Parental and neonatal genotyping data were checked for a Mendelian pattern of inheritance. The samples with inconsistent results in either step were excluded from the analyses. In addition, some samples were excluded due to inadequate blood samples, low quality of DNA or failure to genotype. The sample sizes for the genotyping data are shown in the results tables.

2.6. Statistics

Chi-square test was used to test the genotypes at each polymorphic locus for Hardy–Weinberg Equilibrium (HWE). Independent samples *t* test (for continuous variables) and chi-square (for categorical variables) were used to compare characteristics between uncomplicated pregnancies and preeclampsia. The association of polymorphisms with preeclampsia and uterine artery bilateral notching was assessed by using logistic regression and odds ratios (OR) were generated. All data analyses were performed using PASW (SPSS, Chicago) version 17.02. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Study population

Of the 3234 recruited women, 1113 (34%) women were excluded due to one of the reasons shown in Fig. 1. The final analyses were conducted on 2121 Caucasian women, consisting of 1185 (55.9%) women with uncomplicated pregnancies, 123 (5.8%) preeclamptic women and 813 (38.3%) women with other complications.

For the 2121 Caucasian parent-infant trios, genotype data of up to 199 (9.4%) women, 470 (22.2%) partners and 578 (27.3%) infants could not be analysed for one of the following reasons: non availability of samples, genotyping failure or Mendelian inconsistencies in parent-infant genotypes. The available genotype data of each polymorphism for uncomplicated and preeclamptic pregnancies are shown in Table 2.

3.2. Characteristics of the population

Women who later developed preeclampsia were on average younger, heavier, had higher sBP and dBP at 15 weeks' gestation, were less likely to consume ≥1 serve/day of fruit and green vegetables prior to pregnancy and they themselves weighed less at birth than the women with uncomplicated pregnancies (Table 1). Partners who fathered a preeclamptic pregnancy on average were younger and heavier than those with uncomplicated pregnancies. Infants born to preeclamptic pregnancies were smaller (adjusted for gestational age where appropriate) in all neonatal measures than those born to uncomplicated pregnancies (Table 1). In addition, there was no difference in sex ratio between preeclampsia and uncomplicated pregnancy groups (Table 1).

Table 1
Demographic characteristics of the study population.

	Uncomplicated	Preeclampsia	*P*
Maternal characteristics	$n = 1185$	$n = 123$	
Age (yrs)[a]	28.2 (5.6)	26.8 (5.4)	***0.007***
BMI (kg/m^2)[a]	24.9 (4.5)	28.2 (7.2)	***<0.001***
sBP (mmHg)[a]	106.2 (9.9)	113.0 (10.1)	***<0.001***
dBP (mmHg)[a]	63.3 (7.6)	68.9 (8.1)	***<0.001***
Socio-economic index	41.9 (16.7)	36.5 (16.0)	***0.001***
Pre-pregnancy green leafy vegetable intake ≥1 serve/day (%)	615 (51.9%)	51 (41.5%)	***0.03***
Pre-pregnancy fruit intake ≥1 serve/day (%)	751 (63.4%)	66 (53.7%)	***0.03***
Smoking (%)[a]	111 (9.4%)	12 (9.8%)	0.9
Maternal gestational age (wks)	39.9 (1.9)	39.5 (2.2)	0.1
Maternal birth weight (g)	3334.6 (529.7)	3176.6 (543.6)	***0.02***[b]
Paternal characteristics	$n = 1182$	$n = 123$	
Age (yrs)	30.7 (6.3)	29.1 (5.6)	***0.005***
Height (cm)	179.6 (6.7)	179.2 (6.9)	0.5
BMI (kg/m^2)	26.6 (4.0)	28.3 (5.5)	***0.001***
Paternal birth weight (g)	3487.8 (571.4)	3506.5 (552.6)	0.7
Newborn characteristics	$n = 1185$	$n = 123$	
Gestational age at birth (days)	280.7 (8.1)	266.0 (17.7)	***<0.001***
Body length (cm)	51.0 (2.2)	48.4 (3.8)	***<0.001***[b]
Head circumference (mm)	35.2 (1.4)	33.8 (2.3)	***<0.001***[b]
Mid arm circumference (mm)	11.0 (0.9)	10.1 (1.5)	***<0.001***[b]
Birth weight (g)	3590.9 (393.8)	3078.4 (747.8)	***<0.001***[b]
Customised birthweight centile	53.7 (25.0)	44.8 (32.1)	***0.004***
Female babies (%)	584 (49.3%)	64 (52%)	0.6

Data are presented as mean (SD) or *n* (%). sBP: systolic blood pressure, the second measurement; dBP: diastolic blood pressure, the second measurement. Bold italics indicate significant difference.
[a] Measurements were taken at 15 weeks' gestation.
[b] Adjusted for gestational age.

3.3. The association of polymorphisms with preeclampsia and bilateral notching at 20 weeks' gestation

The analyses for the association of polymorphisms with preeclampsia were performed comparing the uncomplicated and preeclampsia groups (Fig. 1). The association of polymorphisms with uterine artery bilateral notching at 20 weeks' gestation were analysed in uncomplicated, preeclampsia and other complications group (Fig. 1). For subgroup analyses, the cohort was stratified by environmental factors, including maternal age (age <29 years versus ≥29 years), maternal BMI (BMI < 25 kg/m^2 versus ≥25 kg/m^2), SEI (SEI < 34 versus ≥34), pre-pregnancy green leafy vegetable intake (vegetable intake <1 serve/day versus ≥1 serve/day), pre-pregnancy fruit intake (fruit intake <1 serve/day versus ≥1 serve/day) and smoking status at 15 weeks' gestation (no smoking versus smoking).

Since *AGT2R* is on the X chromosome, male partners only have one allele of the *AGT2R* polymorphisms. Accordingly, analyses on partners were performed in the fashion of alleles. Male neonates also have one allele of the *AGT2R* polymorphisms, however, since sample size of male neonates was small, we grouped it with female neonates and data were analysed in the fashion of genotypes. Take the *AGT2R C4599A* polymorphism as an example, male neonates with C allele were allocated to the CC genotype group and those with A allele were allocated to the AA genotype group.

3.3.1. AGT1R A1166C and AGT2R T1334C

Since the frequency of maternal and neonatal *AGT2R T1334C* CC genotype was less than 3%, the CC and CT genotype were combined. *AGT1R A1166C* and *AGT2R T1334C* were not associated

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Table 2
The association of *AGT1R* and *AGT2R* polymorphisms with preeclampsia and uterine artery bilateral notching at 20 weeks' gestation.

	Uncomplicated	Preeclampsia	OR (95% CI)	No bilateral notching	Bilateral notching	OR (95% CI)
Maternal *AGT1R A1166C*	$n = 1068$	$n = 115$		$n = 1716$	$n = 202$	
AA	525 (49.2%)	59 (51.3%)	Ref	839 (48.9%)	99 (49.0%)	Ref
CA	445 (41.7%)	50 (43.5%)	1.0 (0.7–1.5)	736 (42.9%)	78 (38.6%)	0.9 (0.7–1.2)
CC	98 (9.2%)	6 (5.2%)	0.6 (0.2–1.3)	141 (8.2%)	25 (12.4%)	1.5 (0.9–2.4)
Paternal *AGT1R A1166C*	$n = 951$	$n = 101$		$n = 1510$	$n = 178$	
AA	443 (46.6%)	50 (49.5%)	Ref	715 (47.4%)	88 (49.4%)	Ref
CA	412 (43.3%)	43 (42.6%)	0.9 (0.6–1.4)	660 (43.7%)	71 (39.9%)	0.9 (0.6–1.2)
CC	96 (10.1%)	8 (7.9%)	0.7 (0.3–1.6)	135 (8.9%)	19 (10.7%)	1.1 (0.7–1.9)
Neonatal *AGT1R A1166C*	$n = 912$	$n = 90$		$n = 1366$	$n = 172$	
AA	451 (49.5%)	51 (56.7%)	Ref	677 (49.6%)	77 (44.8%)	Ref
CA	381 (41.8%)	32 (35.6%)	0.7 (0.5–1.2)	576 (42.2%)	81 (47.1%)	1.2 (0.9–1.7)
CC	80 (8.8%)	7 (7.8%)	0.8 (0.3–1.8)	113 (8.3%)	14 (8.1%)	1.1 (0.6–2.0)
Maternal *AGT2R C4599A*	$n = 1074$	$n = 117$		$n = 1727$	$n = 206$	
CC	280 (26.1%)	24 (20.5%)	Ref	457 (26.5%)	49 (23.8%)	Ref
CA	545 (50.7%)	59 (50.4%)	1.3 (0.8–2.1)	884 (51.2%)	99 (48.1%)	1.1 (0.7–1.5)
AA	249 (23.2%)	34 (29.1%)	1.6 (0.9–2.8)	386 (22.4%)	58 (28.2%)	1.4 (0.9–2.1)
Paternal *AGT2R C4599A*	$n = 974$	$n = 101$		$n = 1540$	$n = 174$	
C allele	508 (52.2%)	47 (46.5%)	Ref	814 (52.9%)	80 (46.0%)	Ref
A allele	466 (47.8%)	54 (53.5%)	1.3 (0.8–1.9)	726 (47.1%)	94 (54.0%)	1.3 (1.0–1.8)
Neonatal *AGT2R C4599A*[a]	$n = 951$	$n = 88$		$n = 1419$	$n = 180$	
CC	358 (37.6%)	24 (27.3%)	Ref	531 (37.4%)	66 (36.7%)	Ref
CA	232 (24.4%)	24 (27.3%)	1.5 (0.9–2.8)	371 (26.1%)	46 (25.6%)	1.0 (0.7–1.5)
AA	361 (38.0%)	40 (45.5%)	1.7 (1.0–2.8)	517 (36.4%)	68 (37.8%)	1.1 (0.7–1.5)
Maternal *AGT2R A1675G*	$n = 1084$	$n = 119$		$n = 1732$	$n = 207$	
AA	277 (25.6%)	24 (20.2%)	Ref	442 (25.5%)	50 (24.2%)	Ref
AG	544 (50.2%)	61 (51.3%)	1.3 (0.8–2.1)	888 (51.3%)	94 (45.4%)	0.9 (0.7–1.4)
GG	263 (24.3%)	34 (28.6%)	1.5 (0.9–2.6)	402 (23.2%)	63 (30.4%)	1.4 (0.9–2.1)
Paternal *AGT2R A1675G*	$n = 931$	$n = 98$		$n = 1482$	$n = 166$	
A allele	479 (51.5%)	46 (46.9%)	Ref	760 (51.3%)	79 (47.6%)	Ref
G allele	452 (48.5%)	52 (53.1%)	1.2 (0.8–1.8)	722 (48.7%)	87 (52.4%)	1.2 (0.8–1.6)
Neonatal *AGT2R A1675G*[b]	$n = 917$	$n = 87$		$n = 1384$	$n = 163$	
AA	330 (36.0%)	26 (29.9%)	Ref	509 (36.8%)	51 (31.3%)	Ref
AG	225 (24.5%)	25 (28.7%)	1.4 (0.8–2.5)	350 (25.3%)	44 (27.0%)	1.3 (0.8–1.9)
GG	362 (39.5%)	36 (41.4%)	1.3 (0.8–2.1)	525 (37.9%)	68 (41.7%)	1.3 (0.9–1.9)
Maternal *AGT2R T1334C*	$n = 1085$	$n = 119$		$n = 1735$	$n = 207$	
TT	1011 (93.2%)	108 (90.8%)	Ref	1620 (93.4%)	192 (92.8%)	Ref
CT&CC	74 (6.8%)	11 (9.2%)	1.4 (0.7–2.7)	115 (6.6%)	15 (7.2%)	1.1 (0.6–1.9)
Paternal *AGT2R T1334C*	$n = 994$	$n = 104$		$n = 1568$	$n = 179$	
T allele	964 (97.0%)	98 (94.2%)	Ref	1524 (97.2%)	171 (95.5%)	Ref
C allele	30 (3.0%)	6 (5.8%)	2.0 (0.8–4.8)	44 (2.8%)	8 (4.5%)	1.6 (0.8–3.5)
Neonatal *AGT2R T1334C*[c]	$n = 961$	$n = 93$		$n = 1444$	$n = 176$	
TT	914 (95.1%)	88 (94.6%)	Ref	1374 (95.2%)	167 (94.9%)	Ref
CT&CC	47 (4.9%)	5 (5.4%)	1.1 (0.4–2.9)	70 (4.8%)	9 (5.1%)	1.1 (0.5–2.2)

Data are presented as *n* (%). Ref: referent; OR: odds ratio.

[a] CC genotype = female neonatal CC genotype + male neonatal C allele; CA genotype = female neonatal CA genotype; AA genotype = female neonatal AA genotype + male neonatal A allele.

[b] AA genotype = female neonatal AA genotype + male neonatal A allele; AG genotype = female neonatal AG genotype; GG genotype = female neonatal GG genotype + male neonatal G allele.

[c] TT genotype = female neonatal TT genotype + male neonatal T allele; CT&CC genotype = female neonatal CT & CC genotype + male neonatal C allele.

with preeclampsia nor with uterine artery bilateral notching at 20 weeks' gestation (Table 2).

3.3.2. AGT2R C4599A

AGT2R C4599A in mothers, partners and neonates was not associated with preeclampsia nor with uterine artery bilateral notching at 20 weeks' gestation (Table 2). However, when the cohort was stratified by maternal BMI using 25 kg/m^2 as the cut-off point, among women with BMI $\geq$ 25 kg/m^2, maternal *AGT2R C4599A* AA genotype and paternal *AGT2R C4599A* A allele were associated with an increased risk for preeclampsia with OR 2.1 (95% CI 1.0–4.2) and OR 1.9 (95% CI 1.1–3.2), respectively (Table 3). In neonates, *AGT2R C4599A* CA and AA genotype both increased the risk for preeclampsia in women with BMI $\geq$25 kg/m^2 with OR 3.5 (95% CI 1.6–7.9) and OR 3.0 (95% CI 1.4–6.5), respectively (Table 3). In addition, the paternal *AGT2R C4599A* A allele was also associated with an increased risk for uterine artery bilateral notching at 20 weeks' gestation [OR 2.1 (95% CI 1.3–3.4)] (Table 3).

All these associations remained after adjusting for the potential confounding factor, maternal SEI, which is closely related to BMI where women with low SEI are more likely to have a higher BMI than women with high SEI (Table 3).

3.3.3. AGT2R A1675G

AGT2R A1675G was not associated with preeclampsia nor with uterine artery bilateral notching at 20 weeks' gestation (Table 2). When the cohort was stratified by maternal BMI, among women with BMI $\geq$ 25 kg/m^2, neonatal *AGT2R A1675G* AG genotype was

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Table 3
The association of *AGT2R C4599A* with preeclampsia and uterine artery bilateral notching at 20 weeks' gestation, stratified by maternal BMI.

Maternal BMI		*n*	Uncomplicated	Preeclampsia	OR (95% CI)	Adj OR (95% CI)[b]	*n*	No bilateral notching	Bilateral notching	OR (95% CI)	Adj OR (95% CI)[b]
Maternal *AGT2R C4599A*											
BMI < 25 kg/m^2	CC	153	143 (93.5%)	10 (6.5%)	Ref	Ref	236	211 (89.4%)	25 (10.6%)	Ref	Ref
	CA	333	308 (92.5%)	25 (7.5%)	1.2 (0.5–2.5)	1.1 (0.5–2.4)	490	431 (88.0%)	59 (12.0%)	1.2 (0.7–1.9)	1.2 (0.7–1.9)
	AA	152	141 (92.8%)	11 (7.2%)	1.1 (0.5–2.7)	1.1 (0.4–2.6)	214	184 (86.0%)	30 (14.0%)	1.4 (0.8–2.4)	1.4 (0.8–2.5)
BMI ≥ 25 kg/m^2	CC	151	137 (90.7%)	14 (9.3%)	Ref	Ref	270	246 (91.1%)	24 (8.9%)	Ref	Ref
	CA	271	237 (87.5%)	34 (12.5%)	1.4 (0.7–2.7)	1.4 (0.7–2.7)	493	453 (91.9%)	40 (8.1%)	0.9 (0.5–1.5)	0.9 (0.5–1.5)
	AA	131	108 (82.4%)	23 (17.6%)	***2.1 (1.0–4.2)***	***2.1 (1.0–4.2)***	230	202 (87.8%)	28 (12.2%)	1.4 (0.8–2.5)	1.5 (0.8–2.6)
Paternal *AGT2R C4599A*											
BMI < 25 kg/m^2	C allele	294	272 (92.5%)	22 (7.5%)	Ref	Ref	430	379 (88.1%)	51 (11.9%)	Ref	Ref
	A allele	282	267 (94.7%)	15 (5.3%)	0.7 (0.4–1.4)	0.7 (0.4–1.4)	402	360 (89.6%)	42 (10.4%)	0.9 (0.6–1.3)	0.9 (0.6–1.3)
BMI ≥ 25 kg/m^2	C allele	261	236 (90.4%)	25 (9.6%)	Ref	Ref	464	435 (93.8%)	29 (6.3%)	Ref	Ref
	A allele	238	199 (83.6%)	39 (16.4%)	***1.9 (1.1–3.2)***	***1.9 (1.1–3.3)***	418	366 (87.6%)	52 (12.4%)	***2.1 (1.3–3.4)***	***2.1 (1.3–3.4)***
Neonatal *AGT2R C4599A*[a]											
BMI < 25 kg/m^2	CC	184	170 (92.4%)	14 (7.6%)	Ref	Ref	276	241 (87.3%)	35 (12.7%)	Ref	Ref
	CA	141	135 (95.7%)	6 (4.3%)	0.5 (0.2–1.4)	0.5 (0.2–1.4)	215	188 (87.4%)	27 (12.6%)	1.0 (0.6–1.7)	1.0 (0.6–1.7)
	AA	233	216 (92.7%)	17 (7.3%)	1.0 (0.5–2.0)	1.0 (0.5–2.0)	311	272 (87.5%)	39 (12.5%)	1.0 (0.6–1.6)	1.0 (0.6–1.6)
BMI ≥ 25 kg/m^2	CC	198	188 (94.9%)	10 (5.1%)	Ref	Ref	321	290 (90.3%)	31 (9.7%)	Ref	Ref
	CA	115	97 (84.3%)	18 (15.7%)	***3.5 (1.6–7.9)***	***3.3 (1.5–7.5)***	202	183 (90.6%)	19 (9.4%)	1.0 (0.5–1.8)	1.0 (0.6–1.8)
	AA	168	145 (86.3%)	23 (13.7%)	***3.0 (1.4–6.5)***	***3.0 (1.4–6.4)***	274	245 (89.4%)	29 (10.6%)	1.1 (0.7–1.9)	1.1 (0.7–1.9)

Data are presented as *n* (%). Bold italics indicate significant difference. Ref: referent; OR: odds ratio; Adj OR: adjusted odds ratio.
[a] CC genotype = female neonatal CC genotype + male neonatal C allele; CA genotype = female neonatal CA genotype; AA genotype = female neonatal AA genotype + male neonatal A allele.
[b] Odds ratio is adjusted for maternal socio-economic index.

associated with an increased risk for preeclampsia with OR 2.5 (95% CI 1.2–5.4). There was a trend for maternal GG genotype, paternal G allele and neonatal GG genotype of *AGT2R A1675G* to associate with an increased risk for preeclampsia (Table 4). In fact, after adjusting for maternal SEI, the paternal G allele was associated with an increased risk for preeclampsia with OR 1.9 (95% CI 1.0–3.1) (Table 4). In addition, among women with BMI ≥ 25 kg/m^2, paternal *AGT2R A1675G* G allele increased the risk for uterine artery bilateral notching [OR 1.6 (95% CI 1.0–2.7] (Table 4). Moreover, neonatal *AGT2R A1675G* GG genotype also tended to associate with an increased risk for uterine artery bilateral notching among women with BMI ≥ 25 kg/m^2 (Table 4).

4. Discussion

In the current study, in women with BMI ≥ 25 kg/m^2, maternal, paternal and neonatal *AGT2R C4599A* was associated with preeclampsia. In the same subset of women, a similar non-significant trend was also observed for maternal, paternal and neonatal *AGT2R A1675G*, which has previously been shown to be in linkage disequilibrium with *AGT2R C4599A* [10]. Furthermore, in women with BMI ≥ 25 kg/m^2, paternal *AGT2R C4599A* A allele and paternal *AGT2R A1675G* G allele were associated with an increased risk for uterine artery bilateral notching at 20 weeks' gestation.

Table 4
The association of *AGT2R A1657G* with preeclampsia and uterine artery bilateral notching at 20 weeks' gestation, stratified by maternal BMI.

Maternal BMI		*n*	Uncomplicated	Preeclampsia	OR (95% CI)	Adj OR (95% CI)[b]	*n*	No bilateral notching	Bilateral notching	OR (95% CI)	Adj OR (95% CI)[b]
Maternal *AGT2R A1675G*											
BMI < 25 kg/m^2	AA	149	139 (93.3%)	10 (6.7%)	Ref	Ref	233	208 (89.3%)	25 (10.7%)	Ref	Ref
	AG	333	308 (92.5%)	25 (7.5%)	1.1 (0.5–2.4)	1.1 (0.5–2.4)	489	433 (88.5%)	56 (11.5%)	1.1 (0.7–1.8)	1.1 (0.7–1.8)
	GG	163	151 (92.6%)	12 (7.4%)	1.1 (0.5–2.6)	1.0 (0.4–2.5)	225	193 (85.8%)	32 (14.2%)	1.4 (0.8–2.4)	1.4 (0.8–2.5)
BMI ≥ 25 kg/m^2	AA	152	138 (90.8%)	14 (9.2%)	Ref	Ref	259	234 (90.3%)	25 (9.7%)	Ref	Ref
	AG	272	236 (86.8%)	36 (13.2%)	1.5 (0.8–2.9)	1.5 (0.8–2.9)	493	455 (92.3%)	38 (7.7%)	0.8 (0.5–1.3)	0.8 (0.5–1.3)
	GG	134	112 (83.6%)	22 (16.4%)	1.9 (1.0–4.0)	1.9 (0.9–3.9)	240	209 (87.1%)	31 (12.9%)	1.4 (0.8–2.4)	1.4 (0.8–2.5)
Paternal *AGT2R A1675G*											
BMI < 25 kg/m^2	A allele	276	255 (92.4%)	21 (7.6%)	Ref	Ref	395	347 (87.8%)	48 (12.2%)	Ref	Ref
	G allele	267	253 (94.8%)	14 (5.2%)	0.7 (0.3–1.4)	0.7 (0.3–1.4)	390	349 (89.5%)	41 (10.5%)	0.9 (0.6–1.3)	0.9 (0.5–1.3)
BMI ≥ 25 kg/m^2	A allele	249	224 (90.0%)	25 (10.0%)	Ref	Ref	444	413 (93.0%)	31 (7.0%)	Ref	Ref
	G allele	237	199 (84.0%)	38 (16.0%)	1.7 (1.0–2.9)	***1.9 (1.0–3.1)***	419	373 (89.0%)	46 (11.0%)	***1.6 (1.0–2.7)***	1.6 (1.0–2.6)
Neonatal *AGT2R A1675G*[a]											
BMI < 25 kg/m^2	AA	170	157 (92.4%)	13 (7.6%)	Ref	Ref	253	225 (88.9%)	28 (11.1%)	Ref	Ref
	AG	137	130 (94.9%)	7 (5.1%)	0.7 (0.3–1.7)	0.7 (0.3–1.7)	203	177 (87.2%)	26 (12.8%)	1.2 (0.7–2.1)	1.2 (0.7–2.1)
	GG	236	220 (93.2%)	16 (6.8%)	0.9 (0.4–1.9)	0.9 (0.4–1.9)	318	279 (87.7%)	39 (12.3%)	1.1 (0.7–1.9)	1.1 (0.7–1.9)
BMI ≥ 25 kg/m^2	AA	186	173 (93.0%)	13 (7.0%)	Ref	Ref	307	284 (92.5%)	23 (7.5%)	Ref	Ref
	AG	113	95 (84.1%)	18 (15.9%)	***2.5 (1.2–5.4)***	***2.4 (1.1–5.0)***	191	173 (90.6%)	18 (9.4%)	1.3 (0.7–2.5)	1.3 (0.7–2.5)
	GG	162	142 (87.7%)	20 (12.3%)	1.9 (0.9–3.9)	1.9 (0.9–3.9)	275	246 (89.5%)	29 (10.5%)	1.5 (0.8–2.6)	1.5 (0.8–2.6)

Data are presented as *n* (%). Bold italics indicate significant difference. Ref: referent; OR: odds ratio; Adj OR: adjusted odds ratio.
[a] AA genotype = female neonatal AA genotype + male neonatal A allele; AG genotype = female neonatal AG genotype; GG genotype = female neonatal GG genotype + male neonatal G allele.
[b] Odds ratio is adjusted for maternal socio-economic index.

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The observed association of maternal *AGT2R C4599A* with preeclampsia is consistent with a recent Romanian study [27], in which women bearing the *AGT2R C4599A* AA genotype were at an increased risk of developing preeclampsia with OR 3.8 (95% CI 1.1–12.5). The novelties of the current study include 1) paternal and neonatal association of this polymorphism with preeclampsia and 2) modulation of these associations by maternal BMI.

Epidemiological studies have shown that the risk of preeclampsia is determined not only by maternal predisposition, but also by a paternal contribution. Men born to a preeclamptic pregnancy are twice as likely to father a preeclamptic pregnancy [28]. In addition, men who have fathered a preeclamptic pregnancy are nearly twice as likely to father a preeclamptic pregnancy with a different woman, regardless of whether she has already had a preeclamptic pregnancy or not [29]. The paternal and neonatal association of *AGT2R C4599A* with preeclampsia observed in the current study provides further evidence for the paternal genetic contribution to preeclampsia.

The mechanism behind the association of *AGT2R C4599A* with preeclampsia is yet to be determined. However, since the association was found in fathers and neonates and since the polymorphism in fathers was also associated with uterine artery bilateral notching, an indication of high uterine artery resistance and inadequate trophoblast invasion [25], the placenta is likely to be involved. The expression of *AGT2R* in the placenta has been documented across gestation [30,31], however, its role in placentation is poorly understood. Since AGT2R has been shown to induce apoptosis in various cells types [32–34] and preeclampsia is characterised by an increased rate of trophoblast apoptosis [35,36], it is tempting to speculate that trophoblast apoptosis may hold the key to the association of *AGT2R C4599A* with preeclampsia. Furthermore, since *AGT2R A1675G*, known to be in linkage disequilibrium with *AGT2R C4599A* [10], associates with *AGT2R* expression *in vitro* [9], one would expect such an association for *AGT2R C4599A*, that is, the A allele of *AGT2R C4599A* is associated with higher *AGT2R* expression. Taken all together, the A allele or AA genotype of *AGT2R C4599A* in parent-infant trios, which may associate with higher *AGT2R* expression in the placenta, potentially links to an increased rate of trophoblast apoptosis and consequently leads to an increased risk for preeclampsia.

Gene-environment interaction describes the phenomenon in which association of a genetic variant with a disease phenotype varies with the degree of exposure to an environmental factor or vice versa. In the current study, the associations of *AGT2R* polymorphisms with preeclampsia and uterine artery bilateral notching at 20 weeks' gestation were only observed among women with BMI $\geq$ 25 kg/m^2 but not among those with BMI $<$ 25 kg/m^2, suggesting an interaction between *AGT2R* polymorphisms and maternal BMI. Elevated BMI is a well established risk factor for preeclampsia [37]. In our SCOPE cohort, for every 5 units increment in maternal BMI, there is a 1.3-fold increase in risk for preeclampsia [18]. The *AGT2R*-BMI interaction observed in the current study may suggest that the adverse effects associated with *AGT2R C4599A* A allele or AA genotype are subtle and can only place women at risk for preeclampsia or uterine artery bilateral notching if superimposed on adverse effects associated with elevated BMI such as chronic inflammation [38].

The strength of this study is its large multicentre prospective design. In addition, the outcome data of these cases were reviewed by highly skilled SCOPE clinicians to ensure accurate diagnosis. The weakness of the study is the missing genotypes of some participants, which reduced our sample size and may potentially introduce bias into our results. However, there are no systematic reasons for missing genotypes identified. Furthermore, although we performed multiple comparisons, which would increase the likelihood of obtaining false positive results, our significant data were supported by the consistencies between mother, father and baby, between AGT2R polymorphisms in linkage disequilibrium and between preeclampsia and uterine artery bilateral notching, which makes it unlikely that our findings are due to chance.

In summary, we have shown that *AGT2R C4599A* in mothers, fathers and neonates is associated with preeclampsia. The association was further strengthened by its association with uterine artery bilateral notching at 20 weeks' gestation, an indication of poor placental blood flow. More interestingly, these associations were modulated by maternal BMI and only observed in women with BMI $\geq$ 25 kg/m^2, indicating an *AGT2R* polymorphism-BMI interaction. Finally, our data and those of others [12], demonstrate that genetic polymorphisms often have low penetrance and for complex disorders we recommend including clinical and lifestyle factors together with polymorphisms in the analyses to elucidate their associations more clearly [39].

Conflict of interest

None of the authors have any conflicts of interest to declare.

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References

[1] Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365(9461):785–99.
[2] Dekker G, Sibai B. Primary, secondary, and tertiary prevention of pre-eclampsia. Lancet 2001;357(9251):209–15.
[3] Jackson EK. In: Hardman JG, Limbird LE, Gilman AG, editors. Renin angiotensin system. New York: McGraw Hill; 2001. p. 809–95.
[4] Merrill DC, Karoly M, Chen K, Ferrario CM, Brosnihan KB. Angiotensin-(1-7) in normal and preeclamptic pregnancy. Endocrine 2002;18(3):239–45.
[5] Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. J Clin Invest 1973;52(11):2682–9.
[6] Dekker GA, Makovitz JW, Wallenburg HC. Prediction of pregnancy-induced hypertensive disorders by angiotensin II sensitivity and supine pressor test. Br J Obstet Gynaecol 1990;97(9):817–21.
[7] van Geel PP, Pinto YM, Voors AA, Buikema H, Oosterga M, Crijns HJ, et al. Angiotensin II type 1 receptor A1166C gene polymorphism is associated with an increased response to angiotensin II in human arteries. Hypertension 2000;35(3):717–21.
[8] Fatini C, Abbate R, Pepe G, Battaglini B, Gensini F, Ruggiano G, et al. Searching for a better assessment of the individual coronary risk profile. The role of angiotensin-converting enzyme, angiotensin II type 1 receptor and angiotensinogen gene polymorphisms. Eur Heart J 2000;21(8):633–8.

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[9] Warnecke C, Mugrauer P, Surder D, Erdmann J, Schubert C, Regitz-Zagrosek V. Intronic ANG II type 2 receptor gene polymorphism 1675 G/A modulates receptor protein expression but not mRNA splicing. Am J Physiol Regul Integr Comp Physiol 2005;289(6):R1729–35.
[10] Jin JJ, Nakura J, Wu Z, Yamamoto M, Abe M, Chen Y, et al. Association of angiotensin II type 2 receptor gene variant with hypertension. Hypertens Res 2003;26(7):547–52.
[11] Zhang Y, Zhang KX, Wang GL, Huang W, Zhu DL. Angiotensin II type 2 receptor gene polymorphisms and essential hypertension. Acta Pharmacol Sin 2003; 24(11):1089–93.
[12] Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. JAMA 2002;287(2):195–202.
[13] Tsai HJ, Liu X, Mestan K, Yu Y, Zhang S, Fang Y, et al. Maternal cigarette smoking, metabolic gene polymorphisms, and preterm delivery: new insights on GxE interactions and pathogenic pathways. Hum Genet 2008;123(4):359–69.
[14] Saftlas AF, Olson DR, Franks AL, Atrash HK, Pokras R. Epidemiology of preeclampsia and eclampsia in the United States, 1979–1986. Am J Obstet Gynecol 1990;163(2):460–5.
[15] Bianco A, Stone J, Lynch L, Lapinski R, Berkowitz G, Berkowitz RL. Pregnancy outcome at age 40 and older. Obstet Gynecol 1996;87(6):917–22.
[16] Villar J, Carroli G, Wojdyla D, Abalos E, Giordano D, Ba'aqeel H, et al. Preeclampsia, gestational hypertension and intrauterine growth restriction, related or independent conditions? Am J Obstet Gynecol 2006;194(4):921–31.
[17] Brantsaeter AL, Haugen M, Samuelsen SO, Torjusen H, Trogstad L, Alexander J, et al. A dietary pattern characterized by high intake of vegetables, fruits, and vegetable oils is associated with reduced risk of preeclampsia in nulliparous pregnant Norwegian women. J Nutr 2009;139(6):1162–8.
[18] North RA, McCowan LM, Dekker GA, Poston L, Chan EH, Stewart AW, et al. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. BMJ 2011;342:d1875.
[19] Silva LM, Coolman M, Steegers EA, Jaddoe VW, Moll HA, Hofman A, et al. Low socioeconomic status is a risk factor for preeclampsia: the Generation R Study. J Hypertens 2008;26(6):1200–8.
[20] Conde-Agudelo A, Althabe F, Belizan JM, Kafury-Goeta AC. Cigarette smoking during pregnancy and risk of preeclampsia: a systematic review. Am J Obstet Gynecol 1999;181(4):1026–35.
[21] Komukai K, Mochizuki S, Yoshimura M. Gender and the renin-angiotensin-aldosterone system. Fundam Clin Pharmacol 2010;24(6):687–98.
[22] McCowan LM, North R, Taylor R. Australian New Zealand clinical trials registry, www.anzctrorgau/trialSearchaspx; 2007.
[23] Davis P, McLeod K, Ransom M, Ongley P. The New Zealand socio-economic index of occupational status (NZSEI). Wellington: Statistics New Zealand; 1997. Research Report No. 2.
[24] Groom KM, North RA, Stone PR, Chan EH, Taylor RS, Dekker GA, et al. Patterns of change in uterine artery Doppler studies between 20 and 24 weeks of gestation and pregnancy outcomes. Obstet Gynecol 2009;113(2 Pt 1):332–8.
[25] Cnossen JS, Morris RK, ter Riet G, Mol BW, van der Post JA, Coomarasamy A, et al. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. CMAJ 2008;178(6):701–11.
[26] Sullivan KM, Mannucci A, Kimpton CP, Gill P. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. Biotechniques 1993;15(4):636–8. 40-1.
[27] Procopciuc LM, Caracostea G, Zaharie G, Puscas M, Iordache G, Popa M, et al. Maternal/newborn genotype contribution of the renin-angiotensin system (Met235Thr, Thr174Met, I/D-ACE, A2350G-ACE, A1166C-AT2R1, C3123A-AT2R2, 83A/G-REN) to the risk of pre-eclampsia: a Romanian study. J Renin Angiotensin Aldosterone Syst 2011;12(4):539–48.
[28] Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, et al. Paternal and maternal components of the predisposition to preeclampsia. N Engl J Med 2001;344(12):867–72.
[29] Graham CH, Fitzpatrick TE, McCrae KR. Hypoxia stimulates urokinase receptor expression through a heme protein-dependent pathway. Blood 1998;91(9): 3300–7.
[30] Williams PJ, Mistry HD, Innes BA, Bulmer JN, Broughton Pipkin F. Expression of AT1R, AT2R and AT4R and their roles in extravillous trophoblast invasion in the human. Placenta 2010;31(5):448–55.
[31] Marques FZ, Pringle KG, Conquest A, Hirst JJ, Markus MA, Sarris M, et al. Molecular characterization of renin-angiotensin system components in human intrauterine tissues and fetal membranes from vaginal delivery and cesarean section. Placenta 2011;32(3):214–21.
[32] Horiuchi M, Hayashida W, Kambe T, Yamada T, Dzau VJ. Angiotensin type 2 receptor dephosphorylates Bcl-2 by activating mitogen-activated protein kinase phosphatase-1 and induces apoptosis. J Biol Chem 1997;272(30): 19022–6.
[33] Yamada T, Horiuchi M, Dzau VJ. Angiotensin II type 2 receptor mediates programmed cell death. Proc Natl Acad Sci U S A 1996;93(1):156–60.
[34] Grishko V, Pastukh V, Solodushko V, Gillespie M, Azuma J, Schaffer S. Apoptotic cascade initiated by angiotensin II in neonatal cardiomyocytes: role of DNA damage. Am J Physiol Heart Circ Physiol 2003;285(6):H2364–72.
[35] Huppertz B, Kingdom JC. Apoptosis in the trophoblast–role of apoptosis in placental morphogenesis. J Soc Gynecol Investig 2004;11(6):353–62.
[36] Ray J, Jurisicova A, Caniggia I. IFPA trophoblast research award lecture: the dynamic role of Bcl-2 family members in trophoblast cell fate. Placenta 2009; 30(Suppl. A(100)):S96–100.
[37] Bodnar LM, Ness RB, Markovic N, Roberts JM. The risk of preeclampsia rises with increasing prepregnancy body mass index. Ann Epidemiol 2005;15(7): 475–82.
[38] Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 2004;92(3):347–55.
[39] Roberts CT. IFPA award in placentology lecture: complicated interactions between genes and the environment in placentation, pregnancy outcome and long term health. Placenta 2010;31(Suppl(53)):S47–53.

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