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

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Abstract

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. 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. When the cohort was stratified by maternal BMI, in women with BMI≥25kg/m^2, the *AGT2R C4599A* AA genotype in mothers and neonates was associated with an increased risk for preeclampsia compared with the CC genotype [OR 2.1 (95% CI 1.0-4.2) and OR 3.0 (95% CI 1.4-6.5), 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 [OR 1.9 (95% CI 1.1-3.2) and OR 2.1 (95% CI 1.3-3.4), respectively]. *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≥25kg/m^2, suggesting a gene-environment interaction.

Key words: *AGT2R C4599A*; *AGT2R A1675G*; polymorphism; BMI; preeclampsia; uterine artery bilateral notching

Abbreviation: 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

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.

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], socioeconomic 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.

Materials and Methods:

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

Participants

The participants were healthy nulliparous women with singleton pregnancies recruited to the Screening for Pregnancy Endpoints (SCOPE) study between November 2004 and September 2008 in Adelaide, Australia and Auckland, New Zealand [22]. SCOPE is a prospective, multicentre cohort 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%) (Figure 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, birth weight, 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 hours 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].

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 separated and stored within 3 hours 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).

Pregnancy outcome definitions

[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 socioeconomic status and a higher score indicates higher socio-economic status.

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

Uncomplicated pregnancies were those without any pregnancy complication and with delivery of an appropriately grown baby at term.

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.

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.

Results:

Study population

Of the 3234 recruited women, 1113 (34%) women were excluded due to one of the reasons shown in figure 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.

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).

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 (Figure 1). The association of polymorphisms with uterine artery bilateral notching at 20 weeks' gestation were analysed in uncomplicated, preeclampsia and other complications group (Figure 1). For subgroup analyses, the cohort was stratified by environmental factors, including maternal age (age <29 years versus ≥29 years), maternal BMI (BMI <25kg/m^2 versus ≥25kg/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.

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 with preeclampsia nor with uterine artery bilateral notching at 20 weeks' gestation (Table 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 25kg/m^2 as the cut-off point, among women with BMI≥25kg/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 ≥25kg/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).

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≥25kg/m^2, neonatal *AGT2R A1675G* AG genotype was 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 addition, among women with BMI≥25kg/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≥25kg/m^2 (Table 4).

Discussion:

In the current study, in women with BMI≥25kg/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≥25kg/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.

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

238 polymorphism in fathers was also associated with uterine artery bilateral notching, an
239 indication of high uterine artery resistance and inadequate trophoblast invasion [25], the
240 placenta is likely to be involved. The expression of *AGT2R* in the placenta has been
241 documented across gestation [30, 31], however, its role in placentation is poorly understood.
242 Since AGT2R has been shown to induce apoptosis in various cells types [32-34] and
243 preeclampsia is characterised by an increased rate of trophoblast apoptosis [35, 36], it is
244 tempting to speculate that trophoblast apoptosis may hold the key to the association of
245 *AGT2R C4599A* with preeclampsia. Furthermore, since *AGT2R A1675G*, known to be in
246 linkage disequilibrium with *AGT2R C4599A* [10], associates with *AGT2R* expression *in vitro*
247 [9], one would expect such an association for *AGT2R C4599A*, that is, the A allele of *AGT2R*
248 *C4599A* is associated with higher *AGT2R* expression. Taken all together, the A allele or AA
249 genotype of *AGT2R C4599A* in parent-infant trios, which may associate with higher *AGT2R*
250 expression in the placenta, potentially links to an increased rate of trophoblast apoptosis and
251 consequently leads to an increased risk for preeclampsia.

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

262 women at risk for preeclampsia or uterine artery bilateral notching if superimposed on
263 adverse effects associated with elevated BMI such as chronic inflammation [38].

264 The strength of this study is its large multicentre prospective design. In addition, the outcome
265 data of these cases were reviewed by highly skilled SCOPE clinicians to ensure accurate
266 diagnosis. The weakness of the study is the missing genotypes of some participants, which
267 reduced our sample size and may potentially introduce bias into our results. However, there
268 are no systematic reasons for missing genotypes identified. In addition, in order to strengthen
269 the reliability of our results, independent cohorts are required to replicate our findings.

270 In summary, we have shown that *AGT2R C4599A* in mothers, fathers and neonates is
271 associated with preeclampsia. The association was further strengthened by its association
272 with uterine artery bilateral notching at 20 weeks' gestation, an indication of poor placental
273 blood flow. More interestingly, these associations were modulated by maternal BMI and only
274 observed in women with BMI≥25kg/m^2, suggesting an *AGT2R* polymorphism-BMI
275 interaction.

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

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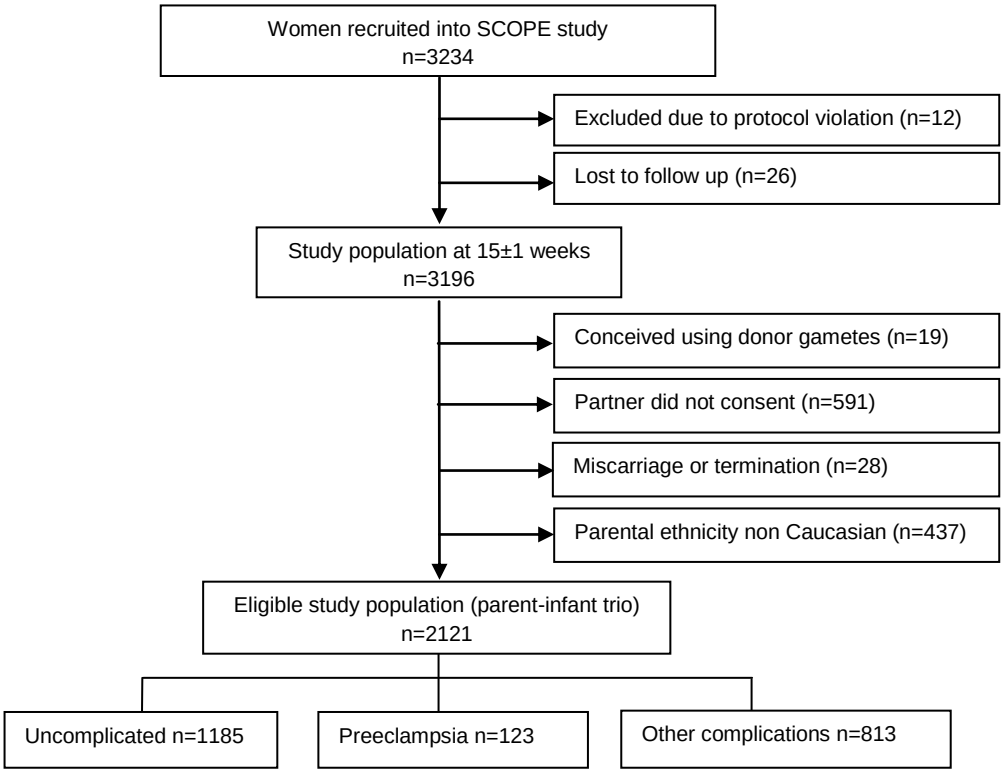

Figure 1 Flow chart of participant recruitment.

Table 1 Demographic characteristics of the study population.

	Uncomplicated	Preeclampsia	
Maternal characteristics	n=1185	n=123	P
Age (yrs)*	28.2 (5.6)	26.8 (5.4)	***0.007***
BMI (kg/m2)*	24.9 (4.5)	28.2 (7.2)	***<0.001***
sBP (mmHg)*	106.2 (9.9)	113.0 (10.1)	***<0.001***
dBP (mmHg)*	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 (%)*	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****
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/m2)	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****
Head circumference (mm)	35.2 (1.4)	33.8 (2.3)	***<0.001****
Mid arm circumference (mm)	11.0 (0.9)	10.1 (1.5)	***<0.001****
Birth weight (g)	3590.9 (393.8)	3078.4 (747.8)	***<0.001****
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 (%).*measurements were taken at 15 week's gestation. **adjusted for gestational age. sBP: systolic blood pressure, the second measurement; dBP: diastolic blood pressure, the second measurement. Bold italics indicate significant difference.

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**	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***	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***	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 (%).*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. **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. ***TT genotype = female neonatal TT genotype + male neonatal T allele; CT&CC genotype= female neonatal CT & CC genotype + male neonatal C allele.

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

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

Data are presented as n (%). Bold italics indicate significant difference. *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.

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

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

Data are presented as n (%). Bold italics indicate significant difference. *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.

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