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ORIGINAL RESEARCH

The association of maternal *ACE A11860G* with small for gestational age babies is modulated by the environment and by fetal sex: a multicentre prospective case–control study

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ABSTRACT: We aimed to determine whether the *ACE A11860G* genotype is associated with small for gestational age babies (SGA) and to determine whether the association is affected by environmental factors and fetal sex. Overall, 3234 healthy nulliparous women with singleton pregnancies, their partners and babies were prospectively recruited in Adelaide, Australia and Auckland, New Zealand. Data analyses were confined to 2121 Caucasian parent–infant trios, among which 216 were pregnancies with SGA infants and 1185 were uncomplicated pregnancies. Women with the *ACE A11860G* GG genotype in the combined and Adelaide cohorts had increased risk for SGA [odds ratios (OR) 1.5, 95% confidence interval (CI) 1.1–2.1 and OR 2.0, 95% CI 1.3–3.3, respectively) and delivered lighter babies ($P = 0.02$; $P = 0.007$, respectively) compared with those with AA/AG genotypes. The maternal *ACE A11860G* GG genotype was associated with higher maternal plasma ACE concentration at 15 weeks' gestation than AA/AG genotypes ($P < 0.001$). When the Adelaide cohort was stratified by maternal socio-economic index (SEI) and pre-pregnancy green leafy vegetable intake, the *ACE A11860G* GG genotype was only associated with an increased risk for SGA (OR 4.9, 95% CI 1.8–13.4 and OR 3.3, 95% CI 1.6–7.0, respectively) and a reduction in customized birthweight centile ($P = 0.006$ and $P = 0.03$) if superimposed on maternal SEI <34 or pre-pregnancy green leafy vegetable intake <1 serve/day. Furthermore, the associations of maternal *ACE A11860G* with customized birthweight centile observed among Adelaide women with SEI <34 or pre-pregnancy green leafy vegetable intake <1 serve/day were female specific. The current study identified a novel association of maternal *ACE A11860G* with SGA. More interestingly, this association was modified by environmental factors and fetal sex, suggesting *ACE A11860G*–environment–fetal sex interactions.
Trial Registry Name: Screening nulliparous women to identify the combinations of clinical risk factors and/or biomarkers required to predict pre-eclampsia, SGA babies and spontaneous preterm birth.
URL: http://www.anzctr.org.au.
Registration number: ACTRN12607000551493.

Key words: *ACE A11860G* / small for gestational age / socio-economic index / pre-pregnancy green leafy vegetable intake / fetal sex

Introduction

Small for gestational age (SGA) babies are usually defined as those with birthweight less than the 10th centile for gestational age (Alkalay *et al.*, 1998; Lee *et al.*, 2003). Being born SGA not only increases the risk of morbidity and mortality in the perinatal period (de Courcy-Wheeler *et al.*, 1995; McCowan and Horgan, 2009), but is also associated with an increased risk of the metabolic syndrome in later life, in particular type II diabetes mellitus, cardiovascular disease and hypertension (Barker *et al.*, 1989, 1990, 2007; McKeigue *et al.*, 1998). To date, the

exact cause of SGA pregnancies remains largely unknown; however, impairments in maternal plasma volume expansion (Duvekot *et al.*, 1995; Salas *et al.*, 2006) and spiral artery remodelling in the uterus (Khong *et al.*, 1986) have been implicated.

The renin–angiotensin system (RAS), well-known for its role in regulating blood pressure, fluid and electrolyte balance (Griendling *et al.*, 1993), is one of the first hormone systems to 'recognize' pregnancy (Broughton Pipkin, 1992). Its activation is thought to be induced by decreased systemic vascular resistance and contributes to the plasma volume expansion by conserving sodium (Schrier and Niederberger, 1994). The existence of a local RAS in the uteroplacental unit has been documented by several studies (Morgan *et al.*, 1998; Cooper *et al.*, 1999; Anton *et al.*, 2009; Marques *et al.*, 2011). Evidence from *in vitro* and genetic association studies has suggested that angiotensin II, one of the main effectors of the RAS, in the uteroplacental unit inhibits trophoblast invasion and spiral artery remodelling (Morgan *et al.*, 1997, 1999; Xia *et al.*, 2002).

Given the role of the RAS in plasma volume expansion and spiral artery remodelling, genetic polymorphisms in the RAS component genes, which modulate encoded RAS protein level or activity, are likely to modulate women's risk for SGA. One of such polymorphisms is *ACE A11860G* (rs4343), located in exon 16 of the angiotensin-converting enzyme (ACE) gene on chromosome 17. It has been shown to be in near perfect linkage disequilibrium with the well-known 287 bp insertion/deletion polymorphism (i.e. *ACE I/D*) (Abdollahi *et al.*, 2008) and appears to be a stronger predictor of plasma ACE concentration than *ACE I/D* (McKenzie *et al.*, 2005). Furthermore, *ACE A11860G* has also been shown to associate with plasma ACE activity in hypertensive cohorts (Chung *et al.*, 2010).

The current study aimed to determine the association of *ACE A11860G* in mothers, fathers and babies with SGA in a nested case–control study of the prospective Screening for Pregnancy Endpoints (SCOPE) cohort. Paternal genotypes were considered because epidemiological evidence suggests that fathers also contribute to the risk for SGA. Specifically, fathers who were born SGA, have a 3.5-fold greater risk of fathering an SGA pregnancy (Jaquet *et al.*, 2005) and we have previously shown that paternal obesity is an independent risk factor for SGA (McCowan *et al.*, 2010a). In addition, since identifying gene–environment interactions is becoming an increasingly important aspect of genetic association studies (Wang *et al.*, 2002; Tsai *et al.*, 2008; Roberts, 2010), we also aimed to establish whether any association of *ACE A11860G* with SGA is affected by common risk factors for SGA. Furthermore, given sex differences in fetal growth and hence in risk for intrauterine growth restriction (Clifton, 2010), we also determined whether any association of *ACE A11860G* with SGA is affected by fetal sex.

Materials and Methods

Participants

The current study is a nested case–control study within a large prospective multi-centre study, SCOPE. Healthy nulliparous women with singleton pregnancies were prospectively recruited to the SCOPE study between November 2004 and September 2008 in Adelaide, Australia and Auckland, New Zealand (McCowan *et al.*, 2007). The main aim of the SCOPE study is to develop screening tests to predict pre-eclampsia, SGA infants and spontaneous preterm birth. Overall, 3196 women, their partners and babies participated in the study. The study population for the current genetic study was confined to the 2121 Caucasian parent–infant trios (66%) (Fig. 1).

Participants were recruited to the SCOPE study prior to 15 weeks' gestation through hospital antenatal clinics, obstetricians, general practitioners, community midwives and self-referral in response to advertisements or recommendations of friends. Women were not eligible if they were considered at high risk of pre-eclampsia, SGA babies or spontaneous preterm birth due to underlying medical conditions, gynaecological history, three or more previous miscarriages or three or more terminations of pregnancy or those who had received interventions that might affect pregnancy outcome (McCowan *et al.*, 2007).

Women were interviewed and examined by research midwives at 15 $\pm$ 1 weeks' gestation. Maternal demographic and dietary information, including ethnicity, age, height, weight, birthweight, gestational age at birth, socioeconomic index (SEI: the New Zealand SEI of occupational status, a number between 10 and 90; it is a validated measure of the individual's socioeconomic status and derived from the specific occupation of the women. A higher score indicates higher socioeconomic status) (Davis *et al.*, 1999), smoking status at 15 weeks' gestation and pre-pregnancy green leafy vegetable intake among other variables as previously reported (McCowan *et al.*, 2010b), were recorded.

In addition, women's blood pressure at 15 weeks' gestation was measured twice consecutively. Paternal information, including age, birthweight, height and weight, was also recorded. Newborn parameters, including infant's gestational age at birth, body length, head circumference, mid-arm circumference, birthweight and customized birthweight centile, were measured and recorded by research midwives usually within 72 h of birth.

Sample collection

Whole blood was collected into EDTA tubes from women at 15 $\pm$ 1 weeks' gestation, from partners at some time during the woman's pregnancy and umbilical cord after delivery. Plasma and buffy coat were separated via centrifugation 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).

Pregnancy outcome definitions

SGA was defined as birthweight less than the 10th customized centile, adjusted for maternal height, booking weight, ethnicity, parity and infant gestation and sex (Gardosi *et al.*, 1995). Uncomplicated pregnancies were those without any pregnancy disorder and with delivery of an appropriately grown baby at term.

Genotyping assays

Maternal, paternal and neonatal 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 conducted by the Australian Genome Research Facility using the Sequenom MassARRAY system. Two quality controls were performed to ensure the accuracy of the genotyping data: (i) each sample was genotyped for Amelogenin, a sex-determinant gene (Sullivan *et al.*, 1993) and (ii) parental and neonatal genotyping data were checked for a Mendelian pattern of inheritance. Samples were excluded if an inconsistency between the sex of the sample and the corresponding Amelogenin genotype and/or non-Mendelian pattern of inheritance was

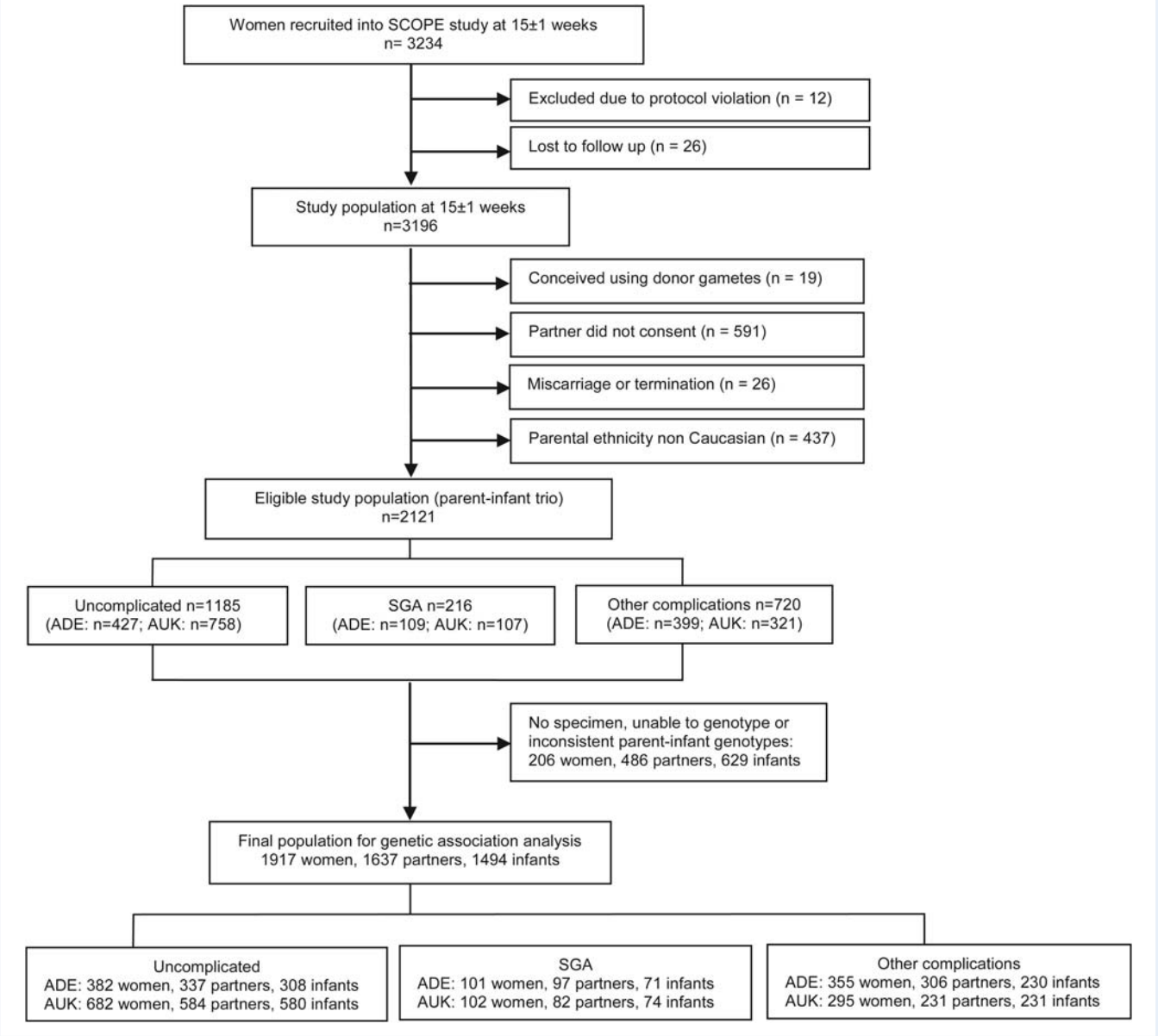

Figure 1 Flow chart of participant recruitment. ADE, Adelaide; AUK, Auckland.

observed. In addition, some samples were excluded due to inadequate blood samples, low quality of DNA or failure to genotype.

Plasma ACE concentration

A plasma ACE concentration was measured in 451 women with a range of pregnancy outcomes, who were selected from the Adelaide cohort, using a commercial ELISA kit (ACE duoset, R&D Systems, Minneapolis, USA). The sensitivity of the assay was 62.5 pg/ml. Optical density was determined using a microplate reader (BioRad, Benchmark) at 450 nm and corrected at 570 nm.

Statistics

The χ^2 test was used to test the *ACE A11860G* genotypes for Hardy–Weinberg equilibrium and to compare the categorical variables. Odds ratios (OR) were calculated by logistic regression to quantify the effects of genotypes and environmental factors on risk for SGA. The student's *t*-test or one-way analysis of variance with Dunnett's *post hoc* adjustment was performed to compare continuous variables. Covariate analysis was used to adjust for the effect of gestational age on baby length, birthweight and head circumference. The Mann–Whitney test was performed to compare the plasma ACE concentration. All data analyses were performed using PASW (SPSS, Chicago), version 17.02. P of <0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of the participants

Combined Adelaide and Auckland SCOPE

In the combined cohort, women who later delivered an SGA baby had a higher BMI ($P = 0.003$, Table I), higher systolic blood pressure (sBP) at 15

Table I Demographic characteristics of the study population.

	Combined SCOPE			Adelaide SCOPE			Auckland SCOPE			ADE versus AUK	
	Uncomplicated *n* = 1185	SGA *n* = 216	*P*	Uncomplicated *n* = 427	SGA *n* = 109	*P*	Uncomplicated *n* = 758	SGA *n* = 107	*P*	Uncomplicated *P*	SGA *P*
Maternal characteristics											
Age (years)[a]	28.2 (5.6)	28.5 (6.2)	0.5	23.6 (5.0)	24.3 (5.3)	0.1	30.8 (4.1)	32.7 (3.8)	*<0.001*	*<0.001*	*<0.001*
BMI (kg/m^2)[a]	24.9 (4.5)	26.1 (5.5)	*0.003*	26.0 (5.6)	27.5 (6.2)	*0.01*	24.3 (3.7)	24.7 (4.3)	0.3	*<0.001*	*<0.001*
sBP (mmHg)[a]	106.2 (9.9)	109.5 (11.1)	*<0.001*	107.9 (8.9)	111.6 (11.2)	*0.002*	105.3 (10.3)	107.5 (10.8)	*0.05*	*<0.001*	*0.006*
dBP (mmHg)[a]	63.3 (7.6)	66.0 (8.9)	*<0.001*	63.5 (7.5)	66.0 (8.9)	*0.007*	63.2 (7.7)	66.1 (8.9)	*0.002*	0.5	1.0
Socioeconomic index (%)											
≥34	735 (62.0)	104 (48.1)	*<0.001*	80 (18.7)	9 (8.3)	*0.009*	655 (86.4)	95 (88.8)	0.7	*<0.001*	*<0.001*
<34	450 (38.0)	112 (51.9)		347 (81.3)	100 (91.7)		103 (13.6)	12 (11.2)			
PPGLV (%)											
≥1 serve/day	615 (51.9)	92 (42.6)	*0.012*	120 (28.1)	25 (22.9)	0.3	495 (65.3)	67 (62.6)	0.6	*<0.001*	*<0.001*
<1 serve/day	570 (48.1)	124 (57.4)		307 (71.9)	84 (77.1)		263 (34.7)	40 (37.4)			
Smoking (%)[a]											
Non-smokers	1074 (90.6)	166 (76.9)	*<0.001*	337 (78.9)	61 (56.0)	*<0.001*	737 (97.2)	105 (98.1)	1.0	*<0.001*	*<0.001*
Smokers	111 (9.4)	50 (23.1)		90 (21.1)	48 (44.0)		21 (2.8)	2 (1.9)			
Gestational age at birth (weeks)[b]	39.9 (1.9)	39.7 (2.1)	0.4	39.5 (1.9)	39.4 (2.3)	0.6	40.0 (1.8)	40.1 (1.7)	0.9	*<0.001*	*0.02*
Maternal birthweight (g)[c]	3335 (530)	3167 (527)	*<0.001*	3287 (549)	3136 (544)	*0.01*	3361 (517)	3197 (511)	*0.003*	*0.02*	0.4
Paternal characteristics											
Age (years)	30.7 (6.3)	31.1 (6.7)	0.5	26.4 (6.1)	27.6 (6.3)	0.054	33.2 (4.9)	34.6 (5.0)	*0.005*	*<0.001*	*<0.001*
Height (cm)[d]	179.6 (6.7)	177.6 (6.9)	*<0.001*	178.5 (6.7)	176.3 (7.2)	*0.002*	180.3 (6.7)	179.0 (6.4)	0.06	*<0.001*	*0.005*
BMI (kg/m^2)[d]	26.6 (4.0)	27.2 (4.6)	0.07	26.9 (5.0)	27.3 (5.0)	0.4	26.4 (3.3)	27.1 (4.1)	0.1	0.07	0.7
Paternal birthweight (g)[e]	3488 (571)	3314 (521)	*<0.001*	3462 (593)	3347 (558)	0.09	3502 (559)	3283 (485)	*<0.001*	0.2	0.4
Newborn characteristics											
Gestational age at birth (days)	280.7 (8.1)	271.6 (24.5)	*<0.001*	280.6 (8.0)	271.9 (23.2)	*<0.001*	280.7 (8.1)	271.4 (25.9)	*<0.001*	0.8	0.9
Body length[f] (cm)	51.0 (2.2)	47.1 (4.5)	*<0.001*	50.0 (1.8)	46.2 (3.9)	*<0.001*	51.5 (2.2)	48.0 (4.8)	*<0.001*	*<0.001*	*0.003*
Head circumference[f] (mm)	35.2 (1.3)	32.9 (2.5)	*<0.001*	34.9 (1.3)	32.8 (2.3)	*<0.001*	35.3 (1.4)	32.9 (2.8)	*<0.001*	*0.001*	0.7
Mid-arm circumference[f] (mm)	11.0 (0.9)	9.4 (1.1)	*<0.001*	11.0 (0.9)	9.2 (1.2)	*<0.001*	11.1 (0.9)	9.5 (1.0)	*<0.001*	0.2	0.2
Birthweight[f] (g)	3591 (394)	2587 (560)	*<0.001*	3575 (390)	2554 (536)	*<0.001*	3600 (396)	2621 (583)	*<0.001*	0.3	0.4
Customized birthweight centile	53.7 (25.0)	4.6 (2.9)	*<0.001*	53.9 (24.9)	4.0 (2.8)	*<0.001*	53.6 (25.1)	5.1 (2.9)	*<0.001*	0.9	*0.004*
Sex of babies (%)											
Males	601 (50.7)	107 (49.5)	0.8	210 (49.2)	49 (45.0)	0.4	391 (51.6)	58 (54.2)	0.7	0.4	0.2
Females	584 (49.3)	109 (50.5)		217 (50.8)	60 (55.0)		367 (48.4)	49 (45.8)			

Data are presented as the mean (SD) or *n* (%). sBP, systolic blood pressure; the second measurement; dBP, diastolic blood pressure; the second measurement; PPGLV, pre-pregnancy green leafy vegetable intake; 15 weeks, 15 weeks' gestation; ADE, Adelaide; AUK, Auckland. Italic values indicate significant difference.

[a]Measurements were taken at 15 weeks' gestation.

[b]Numbers for women's gestational age at birth were 1166, 208, 419, 105, 747 and 103, for each group, respectively.

[c]Numbers for women's birthweight were 1153, 208, 411, 104, 742 and 104, for each group, respectively.

[d]Numbers for partner's height or BMI were 1157, 210, 422, 108, 735 and 102 for each group, respectively.

[e]Numbers for partner's birthweight were 1107, 197, 385, 94, 722 and 103 for each group, respectively.

[f]Adjusted for gestational age at birth.

weeks' gestation ($P < 0.001$, Table I) and they weighed less at birth ($P < 0.001$, Table I) than those without any complication. The proportion of women with a socioeconomic index <34, smoking at 15 weeks' gestation and with pre-pregnancy green leafy vegetable intake <1 serve/day was significantly higher in pregnancies complicated by SGA than those in uncomplicated pregnancies ($P < 0.001$, $P < 0.001$ and $P = 0.012$, respectively, Table I). Partners, who fathered an SGA baby, on average were shorter ($P < 0.001$, Table I) and weighed less at birth ($P < 0.001$, Table I) than those who fathered an uncomplicated pregnancy. All newborn measurements were lower in SGA pregnancies compared with those in uncomplicated pregnancies (Table I).

Adelaide SCOPE versus Auckland SCOPE

In both uncomplicated and SGA pregnancies, Adelaide women were younger, had higher BMI and higher sBP at 15 weeks' gestation than Auckland women (Table I). The proportions of women who continued to smoke cigarettes at 15 weeks' gestation and those with SEI <34 and pre-pregnancy green leafy vegetable intake <1 serve/day were also significantly higher in the Adelaide cohort than those in the Auckland cohort (Table I). In both uncomplicated and SGA pregnancies, Adelaide fathers were shorter and younger than Auckland fathers (Table I). The characteristics of neonates were similar between the Adelaide and Auckland cohort except that Adelaide neonates were shorter than Auckland neonates (Table I).

Association of ACE A11860G *with SGA and newborn growth parameters*

Maternal, paternal and neonatal *ACE A11860G* genotype distributions in uncomplicated and SGA pregnancies were in Hardy–Weinberg equilibrium in the combined, Adelaide and Auckland SCOPE pregnancies (data not shown). In the combined SCOPE cohort, the genotype distribution of maternal *ACE A11860G* is: AA: 21.8% (uncomplicated) and 21.7% (SGA), AG: 51.3% (uncomplicated) and 42.9% (SGA) and GG: 26.9% (uncomplicated) and 35.5% (SGA); paternal *ACE A11860G* is: AA: 20.2% (uncomplicated) and 17.3% (SGA), AG: 50.8% (uncomplicated) and 54.2% (SGA) and GG: 29.0% (uncomplicated) and 28.5% (SGA); neonatal *ACE A11860G* is AA: 21.5% (uncomplicated) and 20.7% (SGA), AG: 48.9% (uncomplicated) and 48.3% (SGA) and GG: 29.60% (uncomplicated) and 31.0% (SGA).

In the combined and Adelaide cohorts, the maternal *ACE A11860G* GG genotype was associated with an increased risk for SGA (OR 1.5, 95% confidence interval (CI) 1.1–2.1 and OR 2.0, 95% CI 1.3–2.3, respectively, Table II). No associations of paternal or neonatal *ACE A11860G* with SGA were found in the combined, Adelaide and Auckland SCOPE cohorts (Table II).

In the combined and Adelaide cohorts, the maternal *ACE A11860G* GG genotype was associated with a mean 50 and 94 g reduction, respectively, in birthweight adjusted for gestational age (−1.5%, $P = 0.02$ and −3%, $P = 0.007$, respectively, Table III). Maternal *ACE A11860G* GG genotype in the Adelaide cohort was also associated with a 5.6% reduction in customized birthweight centile ($P = 0.02$, Table III).

Gene–environment and gene-fetal sex interactions

Since the association of maternal *ACE A11860G* with SGA and neonatal growth parameters was observed in the Adelaide cohort but not in the

Table II Maternal, paternal and neonatal *ACE A11860G* genotype distribution in uncomplicated pregnancies and SGA pregnancies, stratified by cohorts.

	Combined SCOPE			Adelaide SCOPE			Auckland SCOPE		
	Uncomplicated (%)	SGA (%)	OR (95% CI)	Uncomplicated (%)	SGA (%)	OR (95% CI)	Uncomplicated (%)	SGA (%)	OR (95% CI)
Maternal *ACE A11860G*	*n* = 1064	*n* = 203		*n* = 382	*n* = 101		*n* = 682	*n* = 102	
AA/AG	778 (73.1)	131 (64.5)	Ref	295 (77.2)	63 (62.4)	Ref	483 (70.8)	68 (66.7)	Ref
GG	286 (26.9)	72 (35.5)	*1.5(1.1–2.1)*	87 (22.8)	38 (37.6)	*2.0 (1.3–3.3)*	199 (29.2)	34 (33.3)	1.2 (0.8–1.9)
Paternal *ACE A11860G*	*n* = 921	*n* = 179		*n* = 337	*n* = 97		*n* = 584	*n* = 82	
AA/AG	654 (71.0)	128 (71.5)	Ref	237 (70.3)	72 (74.2)	Ref	417 (71.4)	56 (68.3)	Ref
GG	267 (29.0)	51 (28.5)	1.0(0.7–1.4)	100 (29.7)	25 (25.8)	0.8 (0.5–1.4)	167 (28.6)	26(31.7)	1.2 (0.7–1.9)
Neonatal *ACE A11860G*	*n* = 888	*n* = 145		*n* = 308	*n* = 71		*n* = 580	*n* = 74	
AA/AG	625 (70.4)	100 (69.0)	Ref	224 (72.7)	48 (67.6)	Ref	401 (69.1)	52 (70.3)	Ref
GG	263 (29.6)	45 (31.0)	1.1(0.7–1.6)	84 (27.3)	23 (32.4)	1.3 (0.7–2.2)	179 (30.9)	22 (29.7)	1.0 (0.6–1.6)

Data are presented as *n* (%). Ref, reference. Italic values indicate significant difference.

Table III The association of maternal *ACE A11860G* with newborn growth parameters, stratified by cohorts.

	Combined maternal *ACE A11860G*			Adelaide maternal *ACE A11860G*			Auckland maternal *ACE A11860G*		
	AA/AG	GG	*P*	AA/AG	GG	*P*	AA/AG	GG	*P*
Body length[a] (cm)	*n* = 1371 50.1 (3.2)	*n* = 529 49.9 (3.2)	0.2	*n* = 616 49.1 (3.5)	*n* = 219 48.9 (3.0)	0.4	*n* = 755 50.9 (2.8)	*n* = 310 50.6 (3.2)	0.08
Birthweight[a] (g)	*n* = 1383 3394 (607)	*n* = 532 3344 (584)	*0.02*	*n* = 617 3363 (644)	*n* = 219 3269 (626)	*0.007*	*n* = 766 3419 (574)	*n* = 313 3399 (546)	0.5
Customized birthweight centile	*n* = 1382 47.6 (28.1)	*n* = 531 45.5 (29.3)	0.2	*n* = 617 47.8 (28.7)	*n* = 219 42.3 (30.4)	*0.02*	*n* = 765 47.4 (27.6)	*n* = 312 47.7 (28.3)	0.9

Data are presented as the mean (SD). Italic values indicate significant difference.
[a]Adjusted for gestational age at birth.

Table IV The association of maternal *ACE A11860G* with SGA and customized birthweight centile in the Adelaide SCOPE, stratified by maternal SEI and PPGLV.

		n	Uncomplicated (%)	SGA (%)	OR (95% CI)	*n*	Customized birthweight centile	*P*
Maternal SEI	Maternal *ACE A11860G*							
SEI ≥ 34	—	74	66 (89.2)	8 (10.8)	Ref	181	50.1 (27.6)	Ref
SEI < 34	—	409	316 (77.3)	93 (22.7)	*2.4 (1.1–5.2)*	754	45.9 (29.6)	0.09
SEI ≥ 34	AA/AG	54	49 (90.7)	5 (9.3)	Ref	112	52.1 (27.4)	Ref
SEI ≥ 34	GG	20	17 (85.0)	3 (15.0)	1.7 (0.4–8.0)	42	46.4 (29.6)	0.6
SEI < 34	AA/AG	304	246 (80.9)	58 (19.1)	2.3 (0.9–6.1)	505	46.9 (29.0)	0.2
SEI < 34	GG	105	70 (66.7)	35 (33.3)	*4.9 (1.8–13.4)*	177	41.3 (30.5)	*0.006*
Maternal PPGLV	Maternal *ACE A11860G*							
≥1 serve/day	—	127	107 (84.3)	20 (15.7)	Ref	237	47.6 (29.5)	Ref
<1 serve/day	—	356	275 (77.2)	81 (22.8)	1.6 (0.9–2.7)	698	46.4 (29.2)	0.6
≥1 serve/day	AA/AG	94	82 (87.2)	12 (12.8)	Ref	149	49.7 (29.2)	Ref
≥1 serve/day	GG	33	25 (75.8)	8 (24.2)	2.2 (0.8–5.9)	63	44.9 (29.6)	0.6
<1 serve/day	AA/AG	264	213 (80.7)	51 (19.3)	1.6 (0.8–3.2)	468	47.3 (28.6)	0.7
<1 serve/day	GG	92	62 (67.4)	30 (33.3)	*3.3 (1.6–7.0)*	156	41.3 (30.7)	*0.03*

The data are presented as *n* (%) or mean (SD). SEI, socioeconomic index; PPGLV, pre-pregnancy green leafy vegetable intake; Ref, reference. Italic values indicate significant difference.

Auckland cohort, subsequent gene–environment and gene–fetal sex interaction analyses were only performed for the Adelaide cohort.

The factors selected included maternal age (<29 years versus ≥29 years), BMI (<25 versus ≥25 kg/m^2), pre-pregnancy green leafy vegetable intake (<1 serve/day versus ≥1 serve/day), SEI (<34 versus ≥34) and smoking status at 15 weeks' gestation (no smoking versus smoking). All these factors have previously been shown to affect risk for SGA and are also significantly different between Adelaide and Auckland SCOPE pregnancies in the current study. The selection of cut-off points for maternal age, pre-pregnancy green leafy vegetable intake and SEI was based on the fact that these points are at or close to the medians in the combined cohort.

Among these selected factors, only maternal SEI and pre-pregnancy green leafy vegetable intake were found to modulate the associations of interest. Specifically, the maternal *ACE A11860G* GG genotype was only associated with increased risk for SGA and reduction in customized birthweight centile if superimposed on maternal SEI <34 or pre-pregnancy green leafy vegetable intake (Table IV).

We further stratified the Adelaide cohort by fetal sex to investigate gene–fetal sex interactions. When the cohort was stratified by fetal sex, the associations of maternal *ACE A11860G* GG genotype with SGA and reduction in customized birthweight centile were specific to female-bearing pregnancies (Table V).

Plasma ACE concentration

In the uncomplicated and SGA pregnancies combined, women bearing the *ACE A11860G* GG genotype had higher circulating ACE concentration at 15 weeks' gestation than those bearing AA or AG genotypes ($P < 0.001$, Fig. 2). Subgroup analysis of women without any complication showed the same association ($P = 0.015$). In addition, women who delivered SGA infants had higher plasma ACE concentrations at

Table V The association of maternal *ACE A11860G* with SGA and customized birthweight centile in the Australian SCOPE cohort, stratified by maternal SEI, PPGLV and fetal sex.

		Female babies			Male babies		
	Maternal *ACE A11860G*	*n*	SGA (%)	*P*	*n*	SGA (%)	*P*
	AA/AG	188	33 (17.6)		170	30 (17.6)	
	GG	62	23 (37.1)	*0.003*	63	15 (23.8)	0.4
	Maternal *ACE A11860G*	*n*	Customized birthweight centile		*n*	Customized birthweight centile	
	AA/AG	323	48.1 (28.7)		294	47.6 (28.8)	
	GG	110	38.9 (29.5)	*0.004*	109	45.7 (31.0)	0.6
Maternal SEI	Maternal *ACE A11860G*	*n*	Customized birthweight centile		*n*	Customized birthweight centile	
SEI ≥ 34	AA/AG	63	52.9 (27.0)	Ref	49	50.9 (28.2)	Ref
SEI ≥ 34	GG	18	39.8 (31.4)	0.2	24	51.4 (27.8)	1
SEI < 34	AA/AG	260	46.9 (29.1)	0.3	245	46.9 (28.9)	0.7
SEI < 34	GG	92	38.8 (29.3)	*0.008*	85	44.1 (31.8)	0.4
Maternal PPGLV	Maternal *ACE A11860G*	*n*	Customized birthweight centile		*n*	Customized birthweight centile	
≥1 serve/day	AA/AG	74	49.7 (28.8)	Ref	75	49.7 (29.8)	Ref
≥1 serve/day	GG	35	42.5 (30.1)	0.5	28	47.9 (29.2)	1.0
<1 serve/day	AA/AG	249	47.6 (28.7)	0.9	219	46.8 (28.4)	0.8
<1 serve/day	GG	75	37.3 (29.3)	*0.03*	81	44.9 (31.7)	0.6

The data are presented as mean (SD) or *n* (%). SEI, socioeconomic index; PPGLV, pre-pregnancy green leafy vegetable intake; Ref, reference. Italic values indicate significant difference.

15 weeks' gestation than women with uncomplicated pregnancies ($P = 0.034$) and this relationship was specific to pregnancies bearing females ($P = 0.008$) (Fig. 3).

Discussion

In the current study, we have identified a novel association of maternal *ACE A11860G* with SGA in the combined Adelaide and Auckland cohorts. Specifically, women with homozygous *ACE A11860G* GG had increased risk for delivering an SGA infant. Consistent with this, we also found that babies delivered by women with the *ACE A11860G* GG genotype weighed less at birth, corrected for gestational age, than those born to women with *ACE A11860G* AA or AG genotypes.

The *ACE A11860G*, located in exon 16 of the ACE gene, has previously been shown to associate with plasma ACE concentration (McKenzie *et al.*, 2005) and activity (Chung *et al.*, 2010). Consistent with this, in our pregnant cohort, mothers bearing the *ACE A11860G* GG genotype had a higher plasma ACE concentration at 15 weeks' gestation than those bearing AA or AG genotypes.

The *ACE A11860G* GG genotype has been shown to associate with various disease phenotypes, including nephropathy (Ahluwalia *et al.*, 2009), Alzheimer's disease (Zhu *et al.*, 2001; Meng *et al.*, 2006; Bruandet *et al.*, 2008; Helbecque *et al.*, 2009), coronary artery disease (Freitas *et al.*, 2008), hypertension (Alvi and Hasnain, 2009) and left ventricular hypertrophy (Wakahara *et al.*, 2007). To the best of our knowledge, no previous studies have investigated its association with SGA. For the first time we have demonstrated that *ACE A11860G* is associated with SGA, suggesting the involvement of the RAS in the pathogenesis of impaired fetal growth.

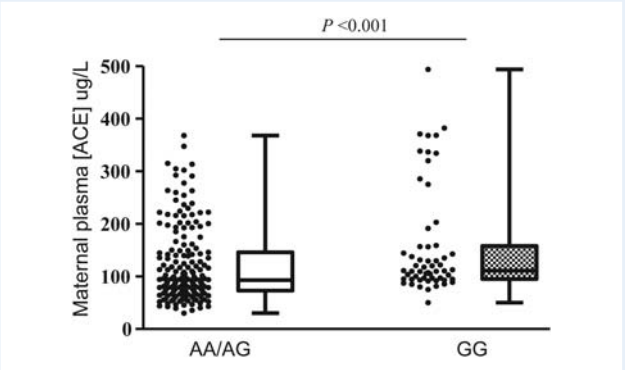

Figure 2 The association of maternal ACE A11860G with maternal plasma ACE concentration at 15 weeks' gestation in the Adelaide cohort. Mann–Whitney *U*-test, $P < 0.001$. $n_{AA/AG} = 183$, $n_{GG} = 57$.

The mechanism behind the association of maternal *ACE A11860G* with SGA is yet to be elucidated. We speculate that arterial stiffening in the maternal systemic vasculature may be involved. Specifically, women with the *ACE A11860G* GG genotype, who have a higher plasma ACE concentration, may chronically have stiffer arteries than those bearing AA or AG genotypes. As a result, they would be more likely to experience a deficit in plasma volume expansion and hence be at a higher risk of delivering an SGA baby. It has been shown that the DD genotype of *ACE I/D*, which is in linkage disequilibrium with *ACE A11860G* (Abdollahi *et al.*, 2008), is associated with higher arterial stiffness (Mattace-Raso *et al.*, 2004). In addition, increased arterial stiffness has previously been

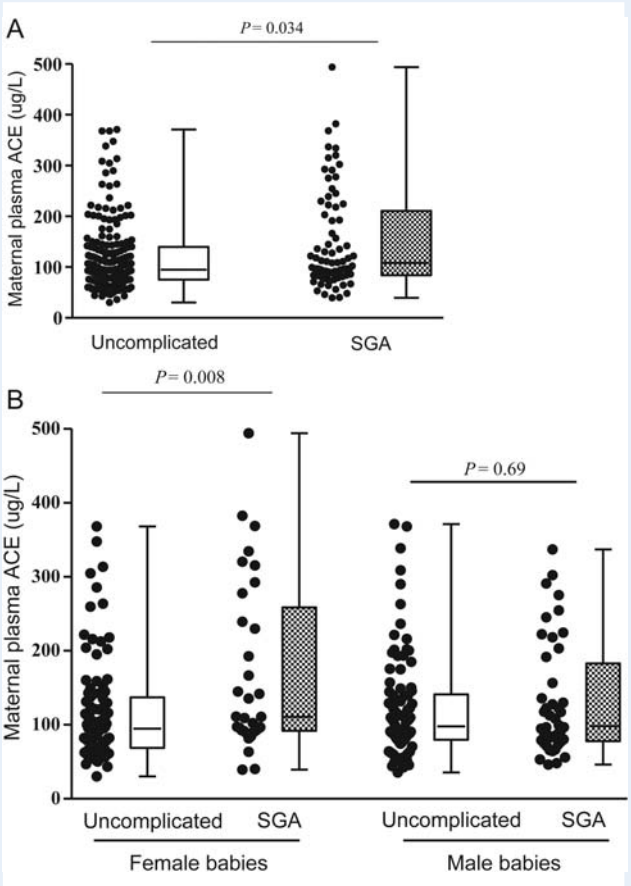

Figure 3 Maternal plasma ACE concentration at 15 weeks' gestation in Adelaide uncomplicated and SGA pregnancies. (A) All pregnancies irrespective of infant sex, Mann–Whitney *U*-test, $P = 0.034$, $n_{uncomplicated} = 190$, $n_{SGA} = 77$. (B) Pregnancies stratified by the sex of babies. For pregnancies bearing females, Mann–Whitney *U*-test, $P = 0.008$, $n_{uncomplicated} = 96$, $n_{SGA} = 33$; for pregnancies bearing males, Mann–Whitney *U*-test, $P = 0.690$, $n_{uncomplicated} = 94$, $n_{SGA} = 44$.

linked to a significant reduction in birthweight centile, potentially via diminished maternal plasma volume expansion and uteroplacental blood flow (Elvan-Taspinar *et al.*, 2005).

The Adelaide and Auckland cohorts have a similar distribution in maternal *ACE A11860G* genotypes, but striking differences in environmental risk factors for SGA. Of particular note, the Adelaide women were younger, ate fewer green leafy vegetables prior to pregnancy, had a higher BMI and lower socioeconomic status and were more likely to smoke during pregnancy than the Auckland women. In *post hoc* analyses when we analysed the data on the Adelaide and Auckland cohorts separately, the associations of maternal *ACE A11860G* with SGA and neonatal birthweight were only observed in the Adelaide SCOPE (i.e. the disadvantaged population), suggesting a gene–environment interaction.

We further investigated gene–environment interactions in the Adelaide cohort and observed that maternal SEI and pre-pregnancy green leafy vegetable intake modulated the associations of maternal *ACE A11860G* with SGA and customized birthweight centile. Specifically, the maternal *ACE A11860G* GG genotype was apparently harmless among women with SEI $\geq$34 or pre-pregnancy green leafy vegetable intake $\geq$1 serve/day, and it only became a risk factor for SGA and led to a reduction in customized birthweight centile when it combined with SEI $<$34 or pre-pregnancy green leafy vegetable intake $<$1 serve/day. It is worth noting that the combined effects of maternal *ACE A11860G* GG genotype with maternal SEI $<$ 34 or pre-pregnancy green leafy vegetable intake $<$ 1 serve/day were more profound than the effect of maternal SEI $<$ 34 or pre-pregnancy green leafy vegetable intake vegetable $<$ 1 serve/day alone. These data may indicate that the adverse effect of maternal *ACE A11860G* GG genotype on its own is subtle and can only put women at risk for SGA if superimposed on adverse environments. The mechanisms behind the observed gene–environment interactions are unclear. Vascular stiffness, which as proposed earlier, may be associated with maternal *ACE A11860G*, may well be involved, since both low socio-economic status and green vegetable consumption have previously been shown to associate with vascular stiffness (Thurston and Matthews, 2009; Aatola *et al.*, 2010).

Importantly, the current study has also demonstrated that the association of maternal *ACE A11860G* GG genotype with SGA and reduction in customized birthweight centile was specific to female-bearing pregnancies. If one considers a reduction in customized birthweight centile as a response to the adverse effect of *ACE A11860G* GG genotype, our data suggest that only female fetuses respond to this effect. Given the central role of the placenta in regulating fetal growth and survival (Clifton, 2010), the female-specific response observed in the current study may be attributable to sex-specific placental function. A study is under way to examine the sex differences in placentas from women with the *ACE A11860G* GG genotype. A female-specific response has previously been reported in pregnancies complicated by maternal asthma (Murphy *et al.*, 2003). Studies have also shown that placental global gene expression (Osei-Kumah *et al.*, 2011), microRNA expression (Clifton, 2010), proteomic profile (Osei-Kumah *et al.*, 2008) and placental structure (Mayhew *et al.*, 2008) vary between female and male placentas from pregnancies complicated by maternal asthma, indicating the underlying role of the placenta in the female-specific response. In summary, our data, together with those of others, suggest that there is a need for new investigations to define the molecular differences between the male versus female placenta.

Previously published genetic association studies for pregnancy complications have proved difficult to replicate. The gene–environment and gene–fetal sex interactions observed in the current study may shed light on this and suggest that conflicting results in the literature may be, in part, due to differences in environmental factors between study populations and failure to appreciate the significance of fetal sex.

Finally, in the current study, women who delivered an SGA baby had a significantly higher plasma ACE concentration at 15 weeks' gestation than those with an uncomplicated pregnancy. Interestingly, when the cohort was stratified by fetal sex, this SGA-related elevation of the plasma ACE concentration only remained in pregnancies bearing females. These results together provide functional support for the association of maternal *ACE A11860G* (a functional variant) with SGA and the interaction of this variant with fetal sex.

The strength of the current study is its large multi-centre prospective design. In addition, the outcome data of these cases were reviewed by highly skilled SCOPE clinicians to ensure the accuracy and consistency of diagnoses. 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 any systematic reasons for missing genotypes. Furthermore, the current study performed multiple comparisons. As a result, the likelihood of our significant results being false positive is increased. However, since our data were supported by functional data on plasma ACE concentration, it is unlikely we have made a false discovery. Finally, although our results on gene–environment and gene–fetal sex interactions were intriguing, they were derived from analyses of subgroups with low sample sizes. Further studies with larger sample size are required to validate these results.

In summary, we have shown that the maternal *ACE A11860G* was associated with SGA. More interestingly, the association was modulated by modifiable environmental factors (maternal SEI and pre-pregnancy green leafy vegetable consumption), as well as fetal sex, suggesting *ACE A11860G*–environment–fetal sex interactions. We recommend future genetic association studies should take into consideration clinical and lifestyle factors, as well as fetal sex, in order to elucidate the genetic associations more clearly.

Ethical approval

Ethical approval was gained from local ethics committees (Australia REC 1712/5/2008 and New Zealand AKX/02/00/364) and all women provided written informed consent.

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Authors' roles

G.A.D., C.T.R. and A.Z. had full access to all the required data and took responsibility for the integrity of the data and the accuracy of the data analysis. G.A.D., L.M.E.M. and C.T.R. had roles in SCOPE study conception and design. A.Z., G.A.D. and C.T.R. were involved in candidate gene association study conception and design. G.A.D., E.R.L., C.T.R. and A.Z. interpreted the data. G.A.D., E.R.L., L.M.E.M., C.T.R. and A.Z. took part in drafting and critical revision of the manuscript for important intellectual content. G.A.D., L.M.E.M. and C.T.R. obtained funding for the study. S.L., G.H. and S.D.T. provided statistical and technical support. G.A.D. and L.M.E.M. supervised the clinical study.

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Conflict of interest

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

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