Insulin family polymorphisms in pregnancies complicated by small for gestational age infants

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ABSTRACT

Being born small for gestational age (SGA) increases the risk for adverse perinatal outcomes and later life vascular and metabolic disorders. The insulin family plays a vital role in intrauterine growth. We investigated the association of functional SNPs in insulin (*INS*), insulin receptor (*INSR*) and insulin receptor substrate 2 (*IRS2*) with small for gestational age (SGA) pregnancies, uterine and umbilical artery Doppler and plasma insulin level. We conducted a nested case-control study of 1401 nulliparous Caucasian women, their partners and babies (216 SGA and 1185 uncomplicated). SGA was defined as a birth weight less than the 10th customized birth weight percentile adjusted for maternal height, weight, parity, ethnicity, gestational age at delivery and infant sex. Uterine and umbilical artery Doppler was performed at 20 ± 1 week gestation. The SNPs in the parent infant trios were genotyped using Sequenom MassARRAY. Plasma insulin was measured by double antibody RIA in 188 healthy non-pregnant adults to assess correlations between SNP genotypes and circulating insulin. Paternal [Odds Ratio (OR) (95% CI)=2.2 (1.3-3.9), p=0.005] and infant [OR (95% CI)=3.3 (1.7-6.2), p=0.0001] *INSR* rs2059806 AA genotype was associated with SGA. Infant *INSR* rs2059806 A allele was associated with abnormal umbilical artery Doppler [OR (95%CI) =1.3(1.0–1.7), p=0.04]. *INSR* rs2059806 AA homozygous individuals had lower plasma insulin compared to heterozygotes (p=0.03) and GG homozygotes (p=0.03). The *INSR* rs2059806 SNP previously associated with adult vascular and metabolic diseases is also associated with SGA pregnancies. This polymorphism may associate with the risk of vascular and metabolic disorders across the life course.

Key words: Insulin receptor, SNP, polymorphism, SGA, rs2059806

INTRODUCTION

Intrauterine growth is a complex process that is regulated by genetic, nutritional and hormonal factors. Diminished intrauterine growth is not only a cause of perinatal morbidity and mortality but also a risk for later life vascular and metabolic diseases including hypertension, ischaemic heart disease, stroke and type 2 diabetes mellitus (Barker *et al.* , 1990, Barker *et al.* , 1989, Lithell *et al.* , 1996, Putzker *et al.* , 2014). Therefore, identifying molecular mechanisms that regulate fetal growth is of importance.

The insulin family plays a vital role in glucose homeostasis and intrauterine growth. The insulin receptor (INSR) is a transmembrane receptor that is activated by insulin (INS), IGF1 and IGF2. The binding of these ligands to INSR results in structural changes within the receptor which facilitate the recruitment of specific adapter proteins including the insulin receptor substrate proteins (IRS1 and IRS2) which are fundamental for intracellular signalling (Lee and White, 2004). The insulin family is crucial for intrauterine growth with targeted deletion of the insulin family genes in mice resulting in intrauterine growth retardation. The severity of growth restriction in these models varies (22% for INS, 10% for INSR, 40% for IRS1 and 10% for IRS2) suggesting that the majority of the fetal growth promoting signals in mice are mediated through IRS1 (Araki *et al.* , 1994, Bueno *et al.* , 2010, Duvillie *et al.* , 1997, Withers *et al.* , 1998). However, in human placentas from pregnancies complicated by intrauterine growth restriction, the IRS2 protein level is reduced while IRS1 and INSR are unchanged compared to placentas from uncomplicated pregnancies suggesting that in humans, IRS2 may be the more important mediator of insulin family promotion of fetal growth (Laviola *et al.* , 2005). Together, these findings demonstrate that the insulin family plays a vital role in intrauterine growth.

Single nucleotide polymorphisms in the insulin family genes are associated with functional effects and in particular, the A allele of the *INSR* rs2059806 SNP is associated with adult onset vascular and metabolic diseases (Ouederni *et al.* , 2009, Schrader *et al.* , 1996, Thomas *et al.* , 2000, Wang *et al.* , 2012). The primary aim of this study was to investigate the association of functional single nucleotide polymorphisms (SNPs) in insulin (*INS*), insulin receptor (*INSR*) and insulin receptor substrate 2 (*IRS2*) with small for gestational age (SGA) pregnancies. The secondary aims were to investigate the association of the SNPs with uterine and umbilical artery Doppler as measures of placental function and plasma insulin as a measure of functional effects of the SNPs.

MATERIALS AND METHODS

We conducted a nested case control study where participants were recruited from the Screening for Pregnancy Endpoints (SCOPE) study. The SCOPE study is an international, multicenter, prospective cohort study with the aim of developing screening tests to predict preeclampsia, SGA infants and preterm birth across different populations (Andraweera *et al.* , 2011). Ethics approval was gained from local ethics committees (Australia REC 1712/5/2008 and New Zealand AKX/02/00/364). The SCOPE study is registered with the Australian and New Zealand Clinical Trial Registry (ACTRN12607000551493).

Nulliparous women with singleton pregnancies attending hospital antenatal clinics, obstetricians, general practitioners or community midwives before 15weeks' of gestation were invited to participate in the SCOPE study (Andraweera *et al.* , 2012a). Women were recruited between November 2004 and September 2008 in Adelaide, Australia and Auckland, New Zealand. Those considered at high risk of preeclampsia, SGA or preterm birth because of underlying medical conditions (including known pre-existing chronic hypertension on

hypertensive medication or with a blood pressure >160/100 mmHg at 15 weeks of gestation), gynaecological history, three or more miscarriages or terminations of pregnancy or couples who received medical or surgical interventions, which could modify pregnancy outcome were not eligible. If the woman stated that she was certain of the identity of the infant's father, the father was also invited to participate in the SCOPE study. All women and partners who participated in the study provided written informed consent.

Recruited couples were excluded from the present analyses if any of the following reasons applied: protocol violation, lost to follow up, conceived with donor sperm or oocytes, miscarriage or termination and woman or partner not of Caucasian ethnicity (Figure 1). Couples who agreed to participate were interviewed and examined by a research midwife at 15 ± 1 and 20 ± 1 weeks of gestation. Data were collected at each time point and included demographic information, medical history, previous obstetric history, family history of obstetric complications and medical disorders. The woman's birthweight and the gestational age at which she was born as well as the partner's birthweight were also recorded. Current pregnancy data included information on any complications during current pregnancy, diet, smoking, alcohol and the use of recreational drugs. Maternal and paternal physical measurements obtained at 15 weeks of gestation included height, weight and blood pressure. Uterine and umbilical artery Doppler was performed at 20 weeks gestation. Uterine and umbilical artery resistance indices above the 90th centile were considered abnormal. All women were followed prospectively and pregnancy outcome data and infant measurements were recorded by research midwives usually within 72 hours of birth.

Definitions of pregnancy outcomes

Small for gestational age (SGA) was defined as a birth weight below the 10th customised centile adjusted for maternal height, weight, parity and ethnicity, gestational age at delivery

and infant sex (McCowan *et al.* , 2004). *Normotensive SGA* was defined as birth of a SGA infant where the mother did not have hypertension. *Hypertensive SGA* was defined as birth of a SGA infant where the mother had either gestational hypertension (defined as systolic blood pressure of ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg, on at least two occasions, four hours apart, after 20 weeks of gestation, but before the onset of labour) or preeclampsia (defined as gestational hypertension or post-partum hypertension with proteinuria or any multisystem complication of preeclampsia (Andraweera *et al.* , 2012b). *Uncomplicated pregnancy* was defined as a pregnancy with no antenatal medical or obstetric complications and resulting in the delivery of an appropriately grown, healthy baby at ≥ 37 weeks of gestation.

Sample collection and genotyping

Peripheral venous blood samples were collected from the women and partners. All women provided blood samples. Buccal swabs or saliva samples were collected from partners who were unwilling to undergo venepuncture. 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). Cord blood was collected at delivery. If cord blood could not be obtained at delivery, a buccal swab or saliva sample was collected from the baby. DNA was extracted from buffy coats from peripheral or cord blood, Whatman FTA cards or from saliva (Oragene®DNA kits) according to the manufacturers' instructions. Genotyping using allele-specific primers (Supplementary Table I) was performed at the Australian Genome Research Facility (AGRF) using the Sequenom MassARRAY system. For quality control, each sample was also genotyped for Amelogenin to confirm that the sex of the sample matched recorded data (Sullivan *et al.* , 1993).

General population genotyping and plasma insulin measures

Plasma insulin measurement and genotyping were performed in 188 healthy, non-pregnant adults recruited from the general population in Adelaide, South Australia (Gatford *et al.* , 2014). Ethics approval was gained from the University of Adelaide Human Research Ethics Committee (H-021-2005) and all participants provided informed consent. Inclusion criteria were age between 18-60 years and not taking regular medication other than the oral contraceptive pill. First degree (siblings, parent-child) and second degree (cousins) relatives were excluded (n = 12). Non-fasting blood samples were collected by venepuncture into EDTA tubes and placed on ice, before centrifugation at 2400*g* for 10 min at 4 ºC. Plasma and buffy coats were harvested and stored at -80 ºC for subsequent analyses. DNA was extracted from buffy coats and genotyped as described above. Concentrations of plasma insulin were measured by RIA (HI-11K, Merck Millipore, Darmstadt, Germany). The intra-assay coefficient of variation (CV) for the insulin assay were 1.8% and 2.6%, and inter-assay CV were 15.0% and 8.5% for QC samples containing 16.0 and 55.7 µU/ml insulin respectively (n = 2 assays).

Statistics

The genotype and allele frequencies of SGA infants and their parents were compared with those of infants and parents in the uncomplicated pregnancy group. The wild type genotype or allele was used as the referent. Genotype frequencies are commonly used to present associations between SNPs and clinical phenotypes, and allele frequencies are often used to demonstrate functional effects of SNPs. Since our study investigates both above parameters, we have used both genotype and allele frequencies in our analyses. Participants with missing data were excluded from the analyses. Chi squared test was used to test Hardy-Weinberg Equilibrium of genotypes for polymorphic loci and to compare categorical variables.

ANOVA was used to compare continuous variables. Multivariable logistic regression was used to adjust for previously established risk factors for SGA by including maternal age, BMI, birthweight, smoking at 15 weeks of gestation, and paternal BMI and birthweight as covariates in the logistic regression model (McCowan *et al.* , 2010a, McCowan *et al.* , 2010b). All data analyses were performed using PASW version 17.02 (SPSS, Chicago, IL). Results are reported as number and percent [n (%)] or mean ± standard deviation (SD) as appropriate. $P < 0.05$ was considered statistically significant. On the basis of prevalence of a polymorphism in 10% of Caucasians in the general population and a ratio of 6 control subjects to 1 case, 150 SGA infants and 932 control subjects in the current study has 80% power to detect an odds ratio of 2.0 ($\beta = 80\%$, $\alpha = 0.05$).

RESULTS

Of the 3234 eligible parent-infant trios 2123 Caucasian trios were included in this study. The exclusions are detailed in Figure 1. Amongst 2123 Caucasian women, 1185 (55.8%) had uncomplicated pregnancies, 216 (10.2%) had SGA infants and the remaining 722 (34.0%) developed other obstetric, medical or surgical complications during pregnancy. Of the 216 SGA infants, 158 (73.2%) were born to normotensive mothers and 58 (26.8%) were born to hypertensive mothers [preeclampsia (n=28), gestational hypertension (n=30)]. The characteristics of the participants according to pregnancy outcome are shown in Table I. Maternal BMI was greater in the SGA group compared to the uncomplicated pregnancy group. Maternal and paternal birthweight were lower in the SGA group compared to the uncomplicated pregnancy group. Maternal smoking after 15 weeks gestation was more prevalent in the SGA group compared to the uncomplicated pregnancy group (Table I).

Genotype prevalence in uncomplicated and SGA pregnancies

All polymorphisms were in Hardy-Weinberg Equilibrium in cases and controls. The prevalence of the paternal *INSR* rs2059806 AA genotype [p = 0.005, OR (95% CI) = 2.2 (1.3 - 3.9)] and A allele [p = 0.04, OR (95% CI) = 1.3 (1.0 - 1.7)] were increased in the SGA group compared to the uncomplicated pregnancy group. The prevalence of the infant *INSR* rs2059806 GA [(p = 0.004, OR (95% CI) = 1.4 (1.0 – 2.1)], and AA [p = 0.0001, OR (95% CI) = 3.3 (1.7 - 6.2)] genotypes and A allele [p = 0.0003, OR (95% CI) = 1.6 (1.2 - 2.1)] were increased in the SGA group compared to the uncomplicated pregnancy group (Table II). These results remained significant after adjusting for maternal age, BMI, birthweight and smoking at 15 weeks gestation; paternal BMI and birthweight and gestational age at delivery and sex of the infant. In contrast to the paternal and infant associations, the maternal *INSR* rs2059806 was not associated with SGA (Table II). Genotypes and allele prevalence for the maternal, paternal and infant *INS* rs3842752, *INSR* rs1051690, *IRS2* rs1805097 and *IRS2* rs1865434 SNPs were not associated with SGA (data not presented).

As a post-hoc analysis, we subcategorised SGA into normotensive and hypertensive groups. The prevalence of the paternal and infant *INSR* rs2059806 AA genotype and A allele were greater in the normotensive and hypertensive SGA groups compared to the uncomplicated pregnancy group (Table III). However, only the comparison between normotensive SGA and uncomplicated pregnancy group was significant, probably due to the lower sample size of the hypertensive SGA group.

Genotype prevalence according to uterine and umbilical artery Doppler outcome

The infant *INSR* rs2059806 A allele was more common in the group with abnormal umbilical artery Doppler compared to those with normal umbilical artery Doppler at 20 weeks of gestation [(p = 0.04, OR (95%CI = 1.3 (1.0 – 1.7), Table IV]. The maternal, paternal and

infant *INS* rs3842752, *INSR* rs1051690, *IRS2* rs1805097 and *IRS2* rs1865434 were not associated with umbilical artery Doppler (data not presented). None of the SNPs was associated with uterine artery Doppler (data not presented).

Genotype distribution associated with plasma insulin

Plasma insulin concentrations were lower in individuals with the *INSR* rs2059806 AA genotype compared to GG ($p = 0.03$) and GA ($p = 0.03$) genotypes (Figure 2).

DISCUSSION

To our knowledge this is the first study to investigate the prevalence of insulin family gene polymorphisms in SGA infants and their parents. We observed that the *INSR* rs2059806 polymorphism was more common in SGA infants and their fathers. Furthermore, the SNP was associated with abnormal umbilical artery Doppler and lower plasma insulin levels.

The *INSR* rs2059806 SNP is an G-A variation on exon 8 of the *INSR* gene (Hanis and Bertin, 1990). The effect of this SNP on receptor function is yet to be determined but the SNP has been shown to be associated with many metabolic and vascular phenotypes including essential hypertension, type 2 diabetes and metabolic syndrome (Ouederni *et al.*, 2009, Schrader *et al.*, 1996, Thomas *et al.*, 2000, Wang *et al.*, 2012). Development *in utero* is recognised as a crucial determinant of later life disease susceptibility since the original theory was proposed by Barker and colleagues in 1990 (Barker, 1990). There is increasing evidence that being born SGA is a risk for later life vascular and metabolic diseases including hypertension, ischaemic heart disease, stroke and type 2 diabetes (Barker *et al.*, 1990, Barker *et al.*, 1989, Lithell *et al.*, 1996, Putzker *et al.*, 2014). One hypothesis suggested to explain the above association is that fetal programming in response to the intrauterine environment has consequences for later life metabolic and cardiovascular health (Barker *et al.* , 2002). A

second hypothesis is that common genetic factors contribute to both restricted fetal growth and later life disorders (Hattersley and Tooke, 1999). In support of the latter theory, we and others have previously demonstrated that polymorphisms in candidate genes that are associated with adult onset vascular and metabolic diseases are associated with reduced birthweight or SGA (Andraweera *et al.*, 2011, Andraweera *et al.*, 2012b, Hattersley *et al.* , 1998, Infante-Rivard *et al.* , 2003, Morgan *et al.* , 2010). The present study adds to these findings by demonstrating that the prevalence of the AA genotype and A allele of the *INSR* rs2059806 SNP previously shown to be associated with vascular and metabolic diseases (Ouederni *et al.*, 2009, Schrader *et al.*, 1996, Thomas *et al.*, 2000, Wang *et al.*, 2012) is increased in SGA infants.

The elevated risk of later life vascular disease is not confined to only SGA infants in these compromised pregnancies. Offspring birthweight adjusted for gestational age at delivery is inversely associated with cardiovascular mortality in parents, implying that parents of SGA infants may also be at risk (Smith *et al.* , 2005). This suggests that shared genetic, epigenetic and environmental factors contribute to cardiovascular disease risk in families with SGA infants. Familial correlations in birthweight are mostly explained by fetal and maternal genetic factors (Bukowski *et al.* , 2012, Lunde *et al.* , 2007). However, there is growing evidence of associations of paternal characteristics with SGA (Andraweera *et al.*, 2011, Jaquet *et al.* , 2005, Klebanoff *et al.* , 1998, Magnus *et al.* , 2001, McCowan *et al.*, 2010a). The present study demonstrates that the prevalence of the A allele of the *INSR* rs2059806 SNP is increased in men who father SGA pregnancies, which further suggests that common genetic factors may be implicated in the familial associations between SGA and later life vascular disease.

The association of the paternal and infant *INSR* rs2059806 and the absence of an association of the maternal SNP with SGA is an interesting finding. The *INSR* gene was recently reported to be imprinted with paternal expression in horse-donkey hybrid (mules and hinnies) placentae (Wang *et al.* , 2013). Imprinted genes are important in mammalian growth and development, with evidence generally showing that genes which are paternally expressed promote fetal growth, whilst maternally expressed genes supress fetal growth (Moore *et al.* , 2015). The effects of the paternal but not maternal *INSR* rs2059806 SNP are consistent with the pattern of imprinting of this gene in horse-donkey reciprocal matings, since a paternal SNP in an imprinted gene with paternal expression may restrict growth *in utero*, whereas a maternally-inherited allele is not expressed and will not affect fetal growth. Future studies to investigate the imprinting status and expression of *INSR* in human placentae are required to confirm this hypothesis.

In addition to the relationship with SGA, we demonstrated that the A allele in the infant was associated with abnormal umbilical artery Doppler in the present cohort. Since the infant's genotype is shared by the placenta, the association of the infant *INSR* rs2059806 A allele with abnormal umbilical artery Doppler suggests that this SNP may also perturb placentation and, in particular, placental angiogenesis. The insulin receptor is activated by insulin, IGF1 and IGF2, molecules that regulate the development of the early placental vasculature (White, 2003). Binding of insulin to the insulin receptor stimulates endothelial cell proliferation and vascular branching (Hiden *et al.* , 2009). In human umbilical vein endothelial cells (HUVEC), insulin activates endothelial nitric oxide synthase (eNOS) which stabilises hypoxia inducible factor 1 (HIF1) (Hiden *et al.*, 2009). HIF1 activation leads to the up-regulation of potent angiogenic stimulators including the vascular endothelial growth factor A (Hiden *et al.*, 2009). In addition to the above pathway, both IGF1 and IGF2 can bind and

activate downstream signalling from the insulin receptor. The IGFs play a crucial role in early placental angiogenesis, trophoblast invasion and transformation of the maternal spiral arterioles, mechanisms that are impaired in SGA pregnancies (Hiden *et al.*, 2009). Therefore, the association of the A allele of the *INSR* rs2059806 SNP with abnormal umbilical artery Doppler suggests that this variant may impair insulin and/or IGF signalling in the placenta.

In the second part of the study, we demonstrated in a non-pregnant adult population that the AA genotype of the *INSR* rs2059806 SNP is associated with reduced circulating insulin concentrations. This suggests another mechanism for the association between this SNP and fetal growth, since insulin directly stimulates intrauterine growth. The reduced insulin may lead to impaired placental vascularisation and intrauterine growth restriction. In sheep, fetal insulin deficiency induced by pancreatectomy reduces birthweight, crown rump length and limb lengths which can be restored to normal levels by insulin replacement (Fowden, 1992). In mice, targeted deletion of the *INSR* gene in the germ line induces intrauterine growth restriction (Novitskaya *et al.* , 2011). These findings suggest that a genotype that associates with low plasma insulin may contribute to the risk of SGA. The association of the *INSR* rs2059806 SNP with SGA in the present study and with type 2 diabetes in previous studies also suggest that this SNP may contribute a genetic component to the association between SGA and later life metabolic diseases (Ouederni *et al.*, 2009).

The strengths of our study include a large prospective cohort with excellent follow-up and rich metadata, which enabled us to adjust for potential confounders. Nevertheless, our study has a few limitations, which should be acknowledged. Although our prospective cohort was large our group of SGA pregnancies was relatively small. These novel findings of associations of infant and paternal A alleles of the *INSR* rs2059806 SNP with SGA and Doppler abnormalities therefore, need to be replicated in other independent cohorts. If

confirmed in an independent cohort, interactions of this SNP in the placenta with environmental factors may be important in determining which women whose partners and babies carry the SNP will actually deliver a SGA baby at risk of later cardiovascular and metabolic diseases.

Conclusion

This study demonstrates that the prevalence of the A allele of the *INSR* rs2059806 SNP which was previously shown to be associated with an increased risk of vascular and metabolic diseases is increased in infants and fathers of SGA pregnancies. Our findings suggest that this SNP may associate with the risk of vascular disorders across the life course.

Acknowledgements

The authors thank the SCOPE families who generously consented to participate in this study. We thank Denise Healy and Rennae Taylor for co-ordinating the SCOPE study in Adelaide and Auckland, respectively and the SCOPE midwives in both centres. We thank MedSciNet (Sweden) and Eliza Chan for support with the database. We thank AGRF for conducting the genotyping.

Authors' Roles: *Study concept and design:* Andraweera, Dekker, McCowan, Russell, Gatford and Roberts. *Acquisition of data:* Andraweera, Dekker, Thompson, Gatford and Roberts. *Analysis and interpretation of data:* Andraweera, Dekker, McCowan, Russell, Gatford and Roberts. *Drafting of the manuscript:* Andraweera. *Critical revision of the manuscript for important intellectual content:* Andraweera, Dekker, Gatford, Russell, McCowan and Roberts. *Statistical analysis:* Leemaqz. *Obtained funding:* Dekker, McCowan

and Roberts. *Administrative, technical, and material support:* Andraweera, Dekker, Thompson, and Roberts. *Study supervision:* Dekker, McCowan and Roberts.

Funding

The SCOPE study was supported by the Premier's Science and Research Fund, Government of South Australia in Australia, the Foundation for Research Science and Technology, Health Research Council and Auckland District Health Board Charitable Trust in New Zealand. Genotyping was funded by a National Health and Medical Research Council of Australia (NHMRC) project grant (GNT519225) and a Channel 7 Children's Research Foundation Grant, both awarded to CTR and GAD. CTR is supported by a NHMRC Senior Research Fellowship (GNT1020749). PHA is supported by a NHMRC Peter Doherty Postdoctoral Fellowship (GNT1090778). The study sponsors had no role in the study design, data analysis and interpretation or writing of this report.

Conflicts of interest: All authors declare no conflicts of interest

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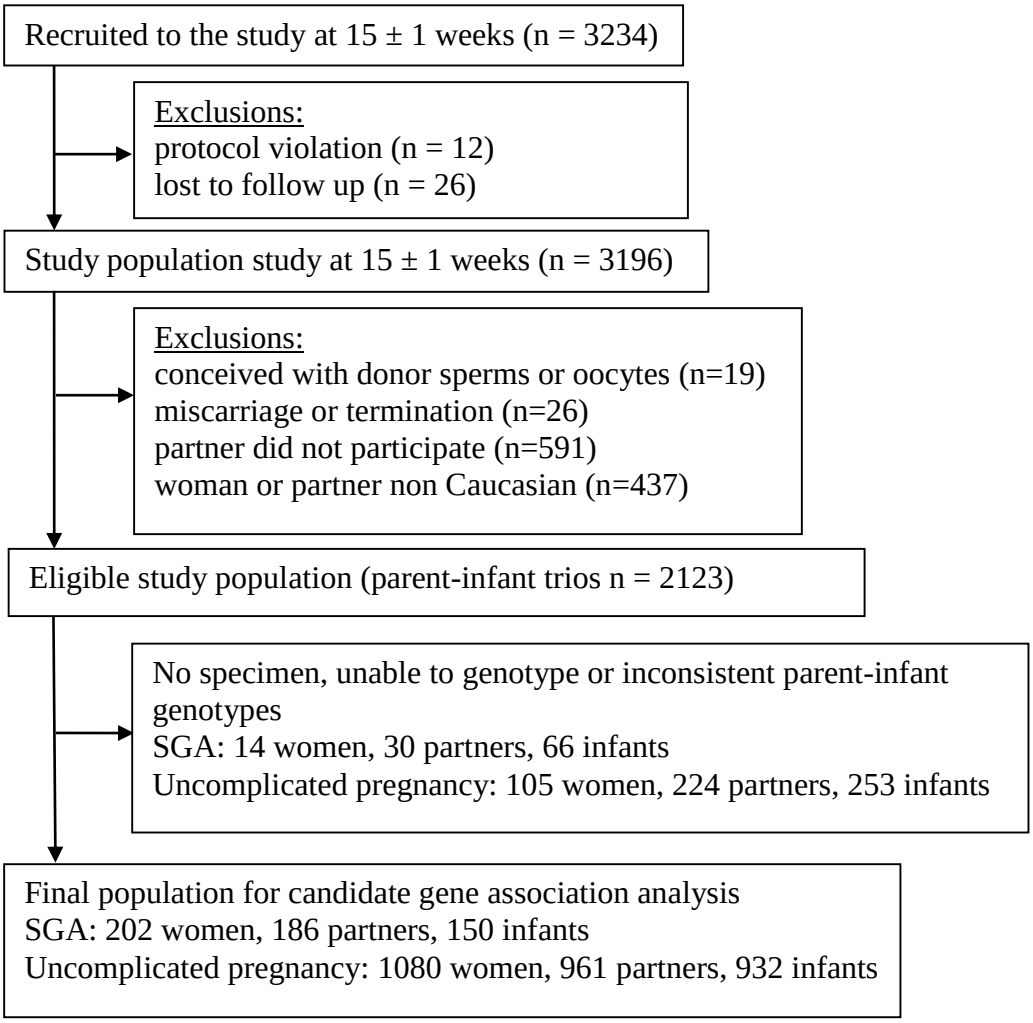
Figure legends

Figure 1. Flow chart to show the recruitment of the study population

Figure 2 Association of *INSR* rs2059806 with plasma insulin in healthy non-pregnant adults. The boxes include the interquartile range. The whiskers are within 1.5 interquartile range (Tukey boxplot)

Figure 1



SGA Small for gestational age.

Figure 2 Association of *INSR* rs2059806 with plasma insulin in healthy non-pregnant adults

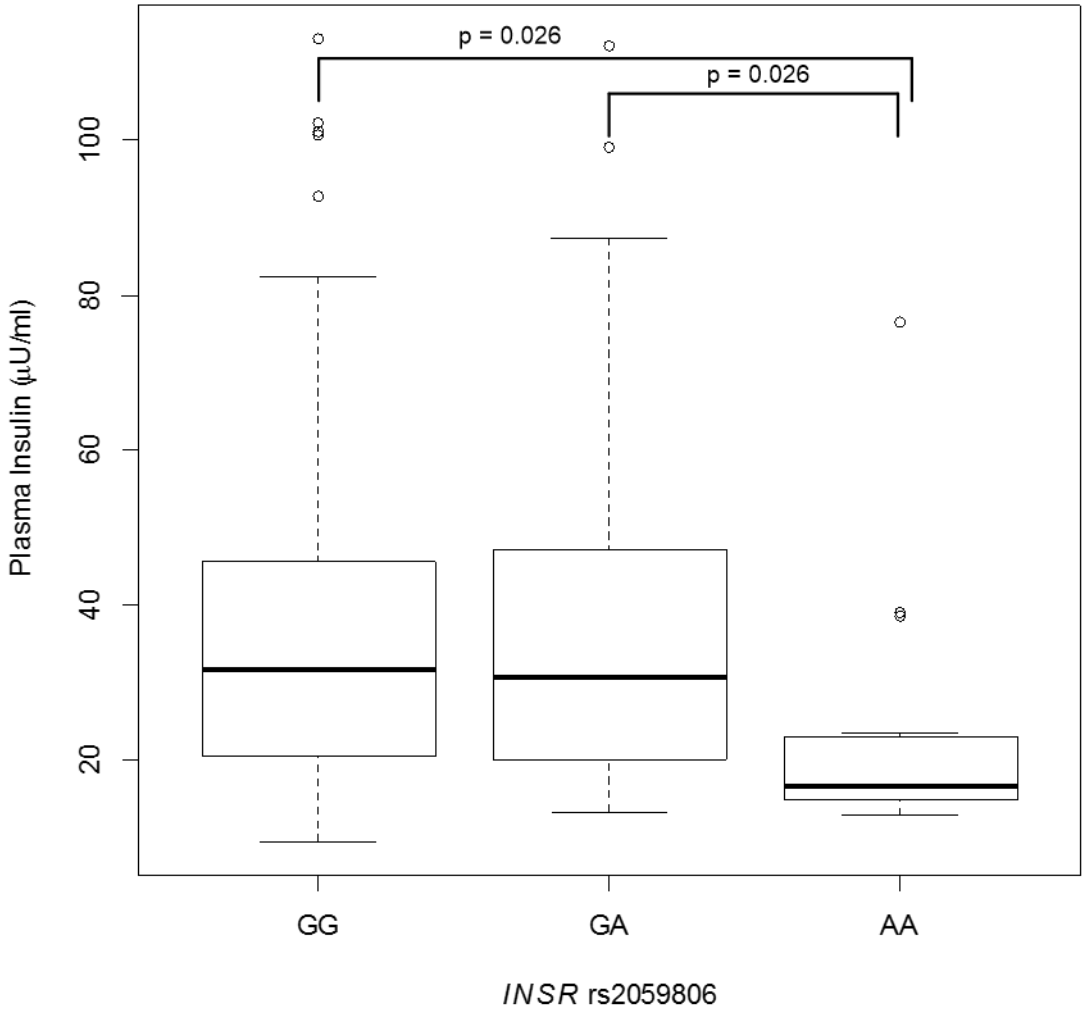


Table I. Characteristics of the study population

Characteristic	Uncomplicated pregnancy (n = 1185)	SGA (n = 216)	*p*
Maternal characteristics			
Maternal age (years)	28.2 ± 5.6	28.5± 6.2	0.505
Maternal BMI (kg/m)	24.9 ± 4.5	26.1 ± 5.5	**0.003**
Maternal birthweight (g)	3335 ± 530	3167 ± 527	**<0.001**
Maternal smoking continued after 15 weeks gestation	111 (9.4%)	50 (23.2%)	**<0.001**
Paternal characteristics			
Paternal age (years)	30.7 ± 6.3	31.1 ± 6.7	0.443
Paternal BMI (kg/m)	26.6 ± 4.0	27.1 ± 4.6	0.101
Paternal birthweight (g)	3488 ± 571	3314 ± 521	**<0.001**
Pregnancy outcome			
Neonatal birthweight (g)	3590 ± 394	2587 ± 560	**<0.001**
Customised birthweight centile	54 ± 25	5 ± 3	**<0.001**
Gestational age at delivery (weeks)	40.1 ± 1.2	38.8 ± 3.5	**<0.001**

SGA, Small for gestational age pregnancies; *p* values are for comparisons with uncomplicated pregnancy and the *p* values in bold are significant

Table II Distribution of maternal, paternal and neonatal *INSR* rs2059806 SNP genotypes and alleles in SGA and in uncomplicated pregnancy

SNP genotype and allele	Uncomplicated Pregnancy n (%)	SGA n (%)	*p*	OR (95% CI)
Maternal *INSR* rs2059806	(n = 1075)	(n = 200)		
GG	628 (58.4)	111 (55.5)	ref	1
GA	396 (36.8)	78 (39.0)	0.5	1.1 (0.8 - 1.5)
AA	51 (4.7)	11 (5.5)	0.6	1.2 (0.6 - 2.4)
G	1652 (76.8)	300 (75.0)	ref	1
A	498 (23.2)	100 (25.0)	0.4	1.1 (0.9 - 1.4)
Paternal *INSR* rs2059806	(n = 1000)	(n = 187)		
GG	587 (58.7)	101 (54.0)	ref	1
GA	363 (36.3)	67 (35.8)	0.7	1.1 (0.8 - 1.5)
AA	50 (5.0)	19 (10.2)	**0.005**	**2.2 (1.3 - 3.9)**
G	1537 (76.8)	269 (71.9)	ref	1
A	463 (23.2)	105 (28.1)	**0.04**	**1.3 (1.0 - 1.7)**
Neonatal *INSR* rs2059806	(n = 945)	(n = 156)		
GG	575 (60.8)	76 (48.7)	ref	1
GA	333 (35.2)	64 (41.0)	**0.04**	**1.4 (1.0 - 2.1)**
AA	37 (4.0)	16 (10.3)	**0.0001**	**3.3 (1.7 - 6.2)**
G	1483 (78.5)	216 (69.2)	ref	1
A	407 (21.5)	96 (30.8)	**0.0003**	**1.6 (1.2 - 2.1)**

SGA, small for gestational age; *p* values and OR (95% CI) in bold are significant

Table III Distribution of maternal, paternal and neonatal *INSR* rs2059806 SNP genotypes and alleles in normotensive and hypertensive SGA and in uncomplicated pregnancy

SNP Genotype and allele	**Uncomplicated pregnancy n (%)**	**NSGA n (%)**	**P value**	**OR (95% CI)**	**HSGA n (%)**	**P value**	**OR (95% CI)**
Maternal *INSR* rs2059806	n = 1075	n = 147			n = 53		
GG	628 (58.4)	84 (57.2)	ref	1	27 (50.9)	ref	1
GA	396 (36.8)	55 (37.4)	0.8	1.0 (0.7 - 1.5)	23 (43.4)	0.3	1.4 (0.8 - 2.4)
AA	51 (4.7)	8 (5.4)	0.7	1.2 (0.5 - 2.6)	3 (5.7)	0.5	1.4 (0.4 - 4.7)
G	1652 (76.8)	223 (75.9)	ref	1	77 (72.6)	ref	1
A	498 (23.2)	71 (24.1)	0.7	1.1 (0.8 - 1.4)	29 (27.4)	0.3	1.2 (0.8 - 1.9)
Paternal *INSR* rs2059806	n = 1000	n = 132			n = 55		
GG	587 (58.7)	70 (53.0)	ref	1	31 (56.4)	ref	1
GA	363 (36.3)	49 (37.1)	0.5	1.1 (0.8 - 1.7)	18 (32.7)	0.8	0.9 (0.5 - 1.7)
AA	50 (5.0)	13 (9.9)	**0.02**	**2.2 (1.1 - 4.2)**	6 (10.9)	0.07	2.3 (0.9 - 5.7)
G	1537 (76.8)	189 (71.6)	ref	1	80 (72.7)	ref	1
A	463 (23.2)	75 (28.4)	0.06	1.3 (0.9 - 1.7	30 (27.3)	0.3	1.2 (0.8 - 1.9)
Neonatal *INSR* rs2059806	n = 945	n = 117			n = 39		
GG	575 (60.8)	60 (51.3)	ref	1	16 (41.0)	ref	1
GA	333 (35.2)	44 (37.6)	0.3	1.3 (0.8 - 1.9)	20 (51.3)	**0.02**	**2.1 (1.1 - 4.2)**
AA	37 (4.0)	13 (11.1)	**0.0003**	**3.4 (1.7 - 6.7)**	3 (7.7)	0.1	2.9 (0.8 - 10.4)
G	1483 (78.5)	164 (70.1)	ref	1	52 (66.7)	ref	1
A	407 (21.5)	70 (29.9)	**0.004**	**1.6 (1.2 - 2.1)**	26 (33.3)	**0.01**	**1.8 (1.1 - 2.9)**

SNP, single nucleotide polymorphism; NSGA, normotensive SGA; HSGA, hypertensive SGA; *p* values and OR (95% CI) in bold are significant

Table IV Distribution of infant *INSR* rs2059806 SNP genotypes and alleles in normal and abnormal umbilical artery Doppler

SNP **Genotype and allele**	**Normal** **Umbilical artery** **Doppler** **n (%)**	**Abnormal** **Umbilical artery Doppler** **n (%)**	***p***	**OR (95% CI)**
Neonatal *INSR* rs2059806				
GG	834 (58.6)	71 (50.0)	ref	1
GA	514 (36.1)	60 (42.3)	0.08	1.4 (0.9 - 1.9)
AA	75 (5.3)	11 (7.7)	0.1	1.7 (0.9 - 3.4)
G	2182 (76.7)	202 (71.1)	ref	1
A	664 (23.3)	82 (28.9)	**0.04**	**1.3 (1.0 - 1.7)**

p values and OR (95% CI) in bold are significant