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ORIGINAL ARTICLE

# A functional variant in the thrombospondin-1 gene and the risk of small for gestational age infants

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**Summary.** *Introduction:* Thrombospondin-1 (TSP-1) is a prothrombotic and anti-angiogenic glycoprotein expressed in the placenta. A functional single nucleotide polymorphism in the TSP-1 gene (*TSP-1 A2210G*) is a risk factor for familial premature myocardial infarction. Small for gestational age (SGA) infants are at increased risk of coronary artery disease in adult life and common genetic factors may underlie both conditions. We investigated the association of *TSP-1 A2210G* in SGA infants and their parents. *Method:* The 3234 nulliparous pregnant women, their partners and babies were recruited in Adelaide and Auckland to a prospective multicenter cohort study. Amongst 2123 Caucasian women, 216 (10.2%) delivered an SGA infant, defined as birth weight < 10th customized centile adjusted for maternal height, weight, parity and ethnicity, as well as gestational age at delivery and infant sex. Uncomplicated pregnancies served as controls ($n = 1185$). DNA extracted from peripheral/cord blood or buccal swabs was genotyped using Sequenom MassARRAY. Multivariable logistic regression was used to compare the odds of SGA between the genotype groups adjusting for potential confounders. *Results:* Paternal (adjOR, 1.4; 95% CI 1.0–2.0) and neonatal (adjOR, 1.8; 95% CI, 1.1–2.7) *TSP-1 A2210G* associates with SGA. The maternal polymorphism approaches significance for an association with SGA (adjOR, 1.3; 95% CI, 0.9–1.9). Maternal *TSP-1 A2210G* associates with a reduced maternal birth weight adjusted for gestational age at delivery ($P = 0.03$). *Conclusion:* The *TSP-1 A2210G* polymorphism, which is a risk factor for myocardial infarction, is associated with SGA pregnancies, suggesting that this polymorphism may associate with the risk of vascular disorders across the life course.

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## Introduction

Small for gestational age (SGA) infants are at increased risk of later life vascular disorders, including hypertension, coronary artery disease and stroke [1,2]. It is increasingly being recognized that an anti-angiogenic state is implicated in the pathophysiology of SGA pregnancies [3]. It is proposed that an angiogenic defect originating during the antenatal period may contribute to the risk of SGA and later-life vascular disorders. A recent study demonstrated that the angiogenic potential of cord blood endothelial colony forming cells was impaired and that the expression of thrombospondin-1 (TSP-1) was increased in low birth weight preterm infants [4].

Thrombospondin 1 (TSP-1) is a calcium binding glycoprotein expressed in many cells and is a major constituent of platelet α granules. TSP-1 is released from platelets in response to activation by thrombin and stabilizes platelet aggregation to injured endothelium through inhibition of ADAMTS13 degradation of ultra-large von Willebrand factor multimers [5,6]. In addition to its role in coagulation, TSP-1 is well known for its strong anti-angiogenic properties [7]. During pregnancy, TSP-1 is expressed in the placenta. Placental expression of TSP-1 mRNA and protein are increased in disorders of placental villous maturation, suggesting that over-expression of TSP-1 may be implicated in the pathogenesis of SGA pregnancies [8].

A single nucleotide polymorphism in the TSP-1 gene (*TSP-1 2210A/G*), which results in the substitution at residue 700 of a serine (Ser-700) for an asparagine (Asn-700), is found in 8–10% of European populations. The Ser-700 variant is associated with lower $Ca^{2+}$ binding capacity and conformational stability, enhanced interaction with fibrinogen on platelet surfaces and a higher rate and extent of platelet aggregation [9–11]. The expression of TSP-1 on the surface of platelets from carriers of the Ser-700 variant is also known to be increased [11]. The *TSP-1 2210G/A* polymorphism is reported to be a significant risk factor for familial premature myocardial infarction in both

homozygous and heterozygous carriers of the variant allele [12,13].

The association of an angiogenic imbalance in the pathophysiology of both small for gestational age pregnancies and coronary artery disease suggests that common genetic factors may underlie both these conditions [14]. A recent large study demonstrated that offspring birth weight (adjusted for gestational age at delivery) is inversely associated with cardiovascular mortality in parents, implying that shared genetic factors may contribute to these findings [15]. Familial correlations in birth weight have mostly been explained by fetal and maternal genetic factors [16]. However, there is growing evidence of associations of paternal characteristics with small for gestational age infants that suggest a significant contribution by paternal genes, which merits further investigation [17–20].

Considering the anti-angiogenic potential of TSP-1, the strong association of the *TSP-1 2210G/A* polymorphism with familial premature myocardial infarction and the data showing that infants born small for gestational age are at increased risk of coronary artery disease, we investigated the association of the *TSP-1 2210G/A* polymorphism in small for gestational age infants (SGA) and their parents in a Caucasian cohort.

## 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 pre-eclampsia, SGA infants and preterm birth across different populations. Ethics approval was gained from local ethics committees (Australia REC 1712/5/2008 and New Zealand AKX/02/00/364) and 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 15 weeks' of gestation were invited to participate. Consenting women were recruited between November 2004 and September 2008 in Adelaide, Australia, and Auckland, New Zealand. Those considered at high risk of pre-eclampsia, SGA or preterm birth because of underlying medical conditions (including known pre-existing chronic hypertension, on hypertensive medication or blood pressure > 160 per 100 mmHg at 15 weeks of gestation), gynecological history, three or more miscarriages or terminations of pregnancy, or couples who received medical or surgical interventions that could modify pregnancy outcome, were not eligible. If the woman was certain of the identity of the infant's father, the father was invited to participate in the SCOPE study. Male participants who agreed to participate provided written informed consent.

Recruited couples were excluded for the following reasons: protocol violation, lost to follow-up, conceived with donor sperms or oocytes, miscarriage or termination and woman or partner not of Caucasian ethnicity.

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 birth weight and the gestational age at which she was born as well as the partner's birth weight were also recorded. Current pregnancy data included information on any complications during current pregnancy, diet, smoking, alcohol and the use of recreational drugs. A low fruit intake was defined as less than one portion per week. A low intake of green leafy vegetables was defined as less than two portions per week. Maternal and paternal physical measurements obtained at 15 weeks of gestation included height, weight and blood pressure.

All women were followed prospectively and pregnancy outcome data and infant measurements were recorded by research midwives, usually within 72 h of birth.

### *Specimen collection*

Peripheral 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 FTA (Whatman Inc, Piscataway, NJ, USA) immediately following sample collection and saliva was collected using Oragene kits Oragene kits (DNA Genotek Inc, Kanata, Canada). Cord blood was collected at delivery. If cord blood was not obtained at delivery, a buccal swab or saliva sample was collected from the baby.

### *Outcome measure*

The primary outcome was small for gestational age (SGA), defined as a birth weight below the 10th customized centile adjusted for maternal height, weight, parity and ethnicity, as well as gestational age at delivery and infant sex [21].

### *Definitions of pregnancy outcome*

Gestational hypertension was defined as systolic blood pressure of ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg, on at least two occasions, 4 h apart, after 20 weeks of gestation, but before the onset of labour. Pre-eclampsia was defined as gestational hypertension or post-partum hypertension with proteinuria (24 h urinary protein level of > 300 mg or a spot urine protein: creatinine ratio of ≥ 30 mg $mmol^{-1}$ creatinine or urine dipstick protein level of ≥ + +) or any multisystem complication of pre-eclampsia [22]. Normotensive SGA was defined as birth of an SGA infant where the mother did not have hypertension, and hypertensive SGA was defined as birth of an SGA infant where the mother had either gestational hypertension or pre-eclampsia. Severe SGA was defined as birth weight below the 5th customized centile. 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.

### *Genotyping*

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 for *TSP-1 A2210G* (rs2228262) single nucleotide polymorphism 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 ensure that the sex of the sample was correct [23]. Parental and neonatal genotyping data were checked for a Mendelian pattern of inheritance and those found to be inconsistent were excluded from the analyses.

### *Statistical methods*

SGA infants and their parents were compared with parent-infant trios from uncomplicated pregnancies in a nested case–control manner. Missing data were excluded from the analyses. The chi-squared test was used to test the genotypes at the polymorphic locus for Hardy–Weinberg equilibrium and to compare categorical variables. ANOVA was used to compare continuous variables between the three genotype groups with post-hoc Sidak test for pairwise comparisons. Multivariable logistic regression was used to compare the odds of SGA between carriers of the variant allele and the additive model (GA + GG) with the reference common genotype (AA), adjusting for previously established risk factors for SGA. The covariates for the logistic regression model included maternal age, BMI, birth weight, smoking at 15 weeks of gestation, pre-pregnancy low fruit intake, pre-pregnancy low green leafy vegetable intake, and paternal BMI and birth weight [20,24]. Adjusted odds ratios and 95% CIs were also calculated for heterozygous and GG homozygous infants < 50th, 40th, 30th, 20th, 10th, 9th, 8th, 7th, 6th and 5th customized centiles compared with those > 50th customized centile to identify any genotype correlation with the birth weight centile. All data analyses were performed using PASW version 17.02 (SPSS, Chicago, IL, USA). Results were reported as number and per cent [*n* (%)] or mean ± standard deviation (SD) where appropriate. $P < 0.05$ was considered statistically significant. On the basis of prevalence of *TSP-1 A2210G* polymorphism in 10% of Caucasians in the general population and a ratio of six control subjects to 1 case, 150 SGA infants and 932 control subjects 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 trios were included in this study. The exclusions are detailed in Fig. 1.

Amongst 2123 Caucasian pregnancies, 1185 (55.8%) were uncomplicated, 216 (10.2%) had SGA infants and the remaining 722 (34.0%) pregnancies developed other obstetric,

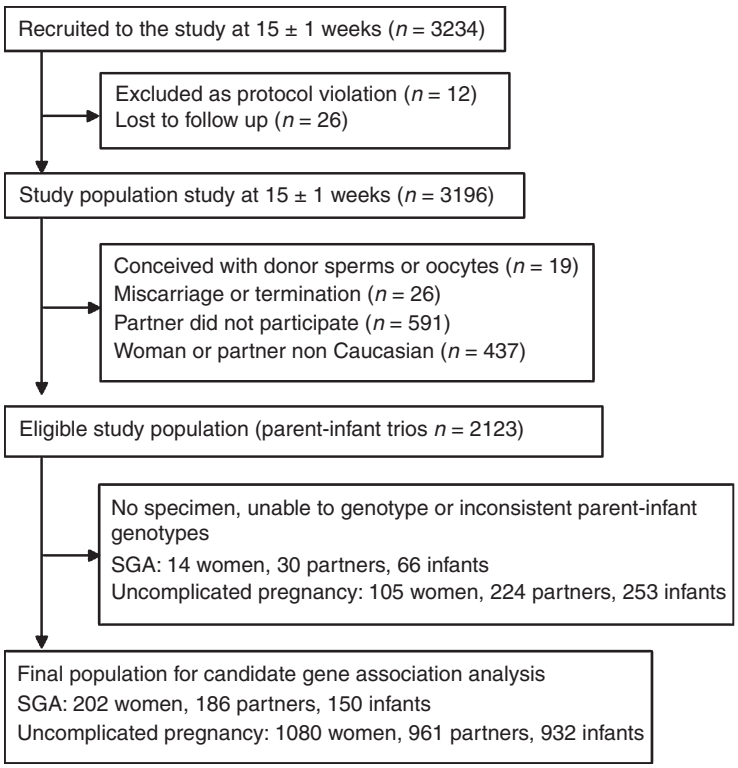


**Fig. 1.** Study population.

medical or surgical complications during pregnancy. One hundred and eleven infants (5.2%) had a birth weight < 5th customized centile. Of the 216 SGA infants, 158 (73.2%) were born to normotensive mothers and 58 (26.8%) were born to hypertensive mothers (pre-eclampsia [$n = 28$], gestational hypertension [$n = 30$]). The characteristics of the participants are shown in Table 1. In the hypertensive SGA group both parents had a higher body mass index (BMI) compared with the uncomplicated pregnancy group. Both parents had lower birth weights in the normotensive, as well as in the hypertensive, SGA group compared with the uncomplicated pregnancy group. Smoking was more prevalent among normotensive and hypertensive women who delivered an SGA infant compared with women who had an uncomplicated pregnancy.

Genotype data of 14 (6.5%) women, 30 (13.9%) partners and 66 (30.6%) infants in the SGA group and 105 (8.9%) women, 224 (18.9%) partners and 253 (21.3%) infants in the uncomplicated pregnancy group could not be analysed due to non-availability of samples, genotyping failure and Mendelian inconsistencies in parent-infant genotypes. Genotype data were available for 202 women, 186 partners and 150 infants in the SGA group and 1080 women, 961 partners and 932 infants in the uncomplicated pregnancy group.

### *Genotype distribution associated with SGA*

The *TSP-1 A2210G* polymorphism was in Hardy–Weinberg equilibrium in cases and controls. There were no sporadic mutations detected in the genotyped infants in this study cohort. The prevalence of paternal and neonatal *TSP-1 A2210G* SNP was increased in the SGA group compared with the uncomplicated pregnancy group ($P = 0.03$ for the additive model for the paternal SNP and $P = 0.02$ for the additive model for the neonatal SNP, Table 2). The prevalence of

**Table 1** Characteristics of the study population

| Characteristic | Control ($n = 1185$) | NSGA ($n = 158$) | *P* | HSGA ($n = 58$) | *P* |
|---|---|---|---|---|---|
| Maternal characteristics | | | | | |
| Age (years) | 28.2 ± 5.6 | 28.6 ± 6.0 | 0.4 | 28.1 ± 6.6 | 0.9 |
| BMI (kg $m^{-1}$) | 24.9 ± 4.5 | 25.5 ± 5.1 | 0.1 | 27.9 ± 6.2 | **< 0.001** |
| Birth weight (g)* | 3331 ± 530 | 3171 ± 553 | **0.001** | 3154 ± 455 | **0.014** |
| Smoking at 15 weeks gestation | 105 (9%) | 36 (22.8%) | **< 0.001** | 11 (18.9%) | **0.014** |
| Paternal characteristics | | | | | |
| Age (years) | 30.7 ± 6.3 | 31.4 ± 6.6 | 0.2 | 30.2 ± 6.8 | 0.5 |
| BMI (kg $m^{-1}$)† | 26.6 ± 4.0 | 26.9 ± 4.5 | 0.2 | 27.7 ± 4.7 | **0.041** |
| Birth weight (g)‡ | 3492 ± 571 | 3305 ± 528 | **< 0.001** | 3336 ± 504 | **0.05** |
| Pregnancy outcome | | | | | |
| Neonatal birth weight (g) | 3590 ± 394 | 2640 ± 559 | **< 0.001** | 2442 ± 539 | **< 0.001** |
| Customized birth weight centile | 54 ± 25 | 5 ± 3 | **< 0.001** | 4 ± 3 | **< 0.001** |
| Gestational age at delivery (weeks) | 39.7 ± 1.2 | 38.6 ± 3.7 | **< 0.001** | 37.7 ± 3.0 | **< 0.001** |

NSGA, normotensive SGA; HSGA, hypertensive SGA. Data are either *n* (%) or mean ± SD. Comparisons with controls using Pearson chi-squared or Student's *t*-test. *Controls $n = 1144$, NSGA $n = 156$, HSGA $n = 56$. †Controls $n = 1148$, NSGA $n = 152$. ‡Controls $n = 1099$, NSGA $n = 144$, HSGA $n = 53$. *P* values in bold are significant.

**Table 2** Distribution of maternal, paternal and neonatal *TSP-1 A2210G* SNP in SGA and in uncomplicated pregnancy

| *TSP-1 A2210G* | Uncomplicated pregnancy (*n* %) | SGA < 10th centile *n* (%) | OR (95% CI) | aOR (95% CI) | SGA < 5th centile *n* (%) | OR(95% CI) | aOR (95% CI) |
|---|---|---|---|---|---|---|---|
| Maternal | $n = 1080$ | $n = 202$ | | | $n = 104$ | | |
| AA | 835 (77.3) | 145 (71.8) | 1.0 (ref) | 1.0 (ref) | 75 (72.1) | 1.0 (ref) | 1.0 (ref) |
| GA | 231 (21.4) | 53 (26.2) | 1.3 (0.9–1.9) | 1.3(0.9–1.9) | 28 (26.9) | 1.3 (0.9–2.1) | 1.4 (0.8–2.3) |
| GG | 14 (1.3) | 4 (2.0) | 1.7 (0.6–5.5) | 1.5 (0.4–5.5) | 1 (1.0) | 0.8 (0.1–6.1) | 0.8 (0.1–6.7) |
| GA + GG | 245 (22.7) | 57 (28.2) | 1.3 (0.9–1.2) | 1.3(0.9–1.9) | 29 (27.9) | 1.3 (0.8–2.1) | 1.3 (0.8–2.2) |
| Paternal | $n = 961$ | $n = 186$ | | | $n = 97$ | | |
| AA | 761 (79.2) | 135 (72.6) | 1.0 (ref) | 1.0 (ref) | 66 (68.0) | 1.0 (ref) | 1.0 (ref) |
| GA | 190 (19.8) | 46 (24.7) | 1.4 (0.9–1.9) | 1.3(0.9–2.1) | 29 (29.9) | **1.8 (1.1–2.8)** | **1.6 (1.0–3.1)** |
| GG | 10 (1.0) | 5 (2.7) | 2.8 (0.9–8.3) | **4.2 (1.2–14.7)** | 2 (2.1) | 2.3 (0.5–10.8) | 1.9 (0.8–14.5) |
| GA + GG | 200 (20.8) | 51 (27.4) | **1.4 (1.0–2.1)** | **1.4(1.0–2.0)** | 31 (32.0) | **1.8 (1.1–2.8)** | **1.5 (1.0–2.6)** |
| Neonatal | $n = 932$ | $n = 150$ | | | $n = 74$ | | |
| AA | 728 (78.1) | 105 (70.0) | 1.0 (ref) | 1.0 (ref) | 52 (70.2) | 1.0 (ref) | 1.0 (ref) |
| GA | 194 (20.8) | 39 (26.0) | 1.4 (0.9–2.1) | **1.6 (1.0–2.4)** | 19 (25.7) | 1.4 (0.8–2.4) | 1.4 (0.8–2.5) |
| GG | 10 (1.1) | 6 (4.0) | **4.2 (1.5–11.7)** | **5.9 (1.9–17.8)** | 3 (4.1) | **4.2 (1.1–15.8)** | **7.0 (1.7–28.5)** |
| GA + GG | 204 (21.9) | 45 (30.0) | **1.5 (1.0–2.2)** | **1.8 (1.1–2.7)** | 22 (29.8) | 1.5 (0.9–2.5) | 1.6 (0.9–2.7) |

aOR(95% CI) adjusted for maternal factors: maternal age, BMI, birth weight, smoking at 15 weeks gestation, pre-pregnancy low fruit intake, pre-pregnancy low green leafy vegetable intake. Paternal factors: BMI and birth weight. GA + GG, additive genetic model is compared with the reference AA genotype; ref, referent. OR (95% CI) values in bold are significant.

paternal and neonatal *TSP-1 A2210G* SNP was increased in the severe SGA group compared with the uncomplicated pregnancy group ($P = 0.01$ for the additive model for the paternal SNP and $P = 0.02$ for GG homozygosity for the neonatal SNP, Table 2). The maternal SNP approached a significant association with SGA ($P = 0.06$ for the additive model, Table 2).

Forest plots of the association of the neonatal *TSP-1 A2210G* polymorphism with infants < 50th, 40th, 30th, 20th, 10th, 9th, 8th, 7th, 6th and 5th customized centiles compared with infants > 50th customized centile are shown in Figs 2 and 3. A significant association was evident from below the10th customized centile for infants homozygous for the G allele (Fig. 3).

As a post-hoc analysis we subcategorized SGA into normotensive and hypertensive groups. SGA subgroup analysis demonstrated that the prevalence of the neonatal *TSP-1 A2210G* SNP was increased in SGA neonates born to normotensive women compared with neonates born to women with uncomplicated pregnancies ($P = 0.02$ for the additive model, Table 3). The prevalence of the GA and GG genotypes of the maternal and paternal *TSP-1 A2210G* was increased in the normotensive SGA group compared with the uncomplicated group but the results were not significant ($P = 0.08$ for the additive model for the maternal SNP and $P = 0.08$ for the additive model for the paternal SNP, Table 3). Maternal, paternal and neonatal *TSP-1 A2210G* SNP was not associated with hypertensive SGA although the frequency of GA and GG genotypes was increased in the hypertensive SGA group compared with uncomplicated pregnancy (Table 3).

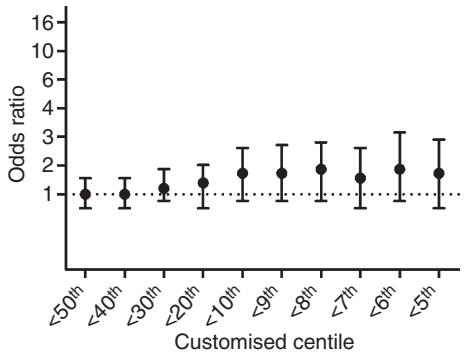


**Fig. 2.** Forest plots show the association between neonatal heterozygosity for *TSP-1 A2210G* polymorphism and infants < 50th customized centile; data are presented as aOR (95% CI).

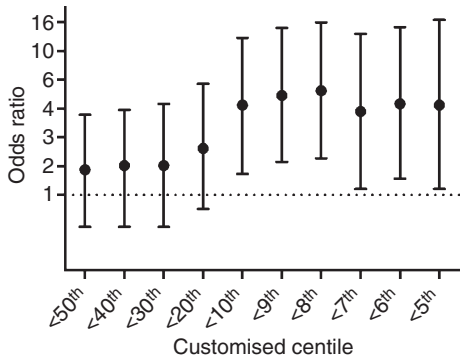


**Fig. 3.** Forest plots show the association between neonatal homozygosity for the G allele of the *TSP-1 A2210G* polymorphism and infants < 50th centile; data are presented as aOR (95% CI).

### *Genotype distribution associated with maternal birth weight*

The mother's own birth weight adjusted for gestational age at birth was on average 232 g lower in women with the *TSP-1A2210G* GG genotype compared with those with the AA genotype ($P = 0.03$, Table 4). We were not able to investigate the association between paternal *TSP-1 A2210G* SNP and paternal birth weight adjusted for gestational age at birth as we did not collect paternal data relating to gestational age at delivery.

### *Genotype distribution in parent-infant trios*

The prevalence of the polymorphism in both the infant and either of the parents was increased in SGA pregnancy trios compared with trios from uncomplicated pregnancies. The effect was similar (OR 1.9) in maternal, as well as in paternal, carriage of the variant allele if the infant was a carrier of the polymorphism (Table 5).

## Discussion

To our knowledge this is the first study to investigate the prevalence of *TSP-1 A2210G* polymorphism in small for gestational age infants and their parents. Our data demonstrate the association of the neonatal and paternal polymorphism with SGA. Our data also show that the association with SGA is stronger for neonatal homozygosity for the polymorphism and that the effect is similar in maternal as well as in paternal carriage of the variant allele. As the infant's genotype is likely to represent the placental genotype our data suggest that the polymorphism may have an effect through the placenta.

A successful pregnancy requires the development of an adequate utero-placental circulation and impaired placental villous vascularization is demonstrated in the pathophysiology of small for gestational age pregnancies [3,25]. The angiogenic potential of cord blood endothelial colony forming cells (ECFC) is impaired and the expression of both TSP-1 mRNA and protein is increased in low birth weight preterm infants [4]. Silencing TSP-1 is shown to restore the angiogenic properties of ECFC in LBW infants, suggesting that TSP-1 may be implicated in the pathophysiology of impaired placental vascularization demonstrated in growth-restricted pregnancies. TSP-1 inhibits angiogenesis by interacting with vascular endothelial growth factor (VEGF) and indirectly by inhibiting the VEGF receptor KDR [26].

In addition to its role in inhibiting angiogenesis, TSP-1 has prothrombotic properties. TSP-1 is released from activated platelets, binds to the platelet surface in a $Ca^{2+}$-dependant

**Table 3** Distribution of maternal, paternal and neonatal *TSP-1 A2210G* SNP in normotensive and hypertensive SGA and in uncomplicated pregnancy

| *TSP-1 A2210G* genotype | Uncomplicated pregnancy | NSGA *n* (%) | OR (95% CI) | aOR (95% CI) | HSGA *n* (%) | OR (95% CI) | aOR (95% CI) |
|---|---|---|---|---|---|---|---|
| Maternal | *n* = 1080 | *n* = 148 | | | *n* = 54 | | |
| AA | 835 (77.3) | 106 (71.6) | 1.0 (ref) | 1.0 (ref) | 39 (72.2) | 1.0 (ref) | 1.0 (ref) |
| GA | 231 (21.4) | 40 (27) | 1.4 (0.9–2.0) | 1.8 (0.8–2.0) | 13 (24.1) | 1.2 (0.6–2.3) | 1.2 (0.6–2.4) |
| GG | 14 (1.3) | 2 (1.4) | 1.1 (0.3–5.0) | 1.2 (0.4–5.0) | 2 (3.7) | 3.1 (0.7–13.9) | 3.5 (0.7–17.6) |
| GA + GG | 245 (22.7) | 42 (28.4) | 1.3 (0.9–1.9) | 1.8 (0.8–1.9) | 15 (27.8) | 1.3 (0.7–2.4) | 1.3 (0.7–2.6) |
| Paternal | *n* = 961 | *n* = 131 | | | *n* = 55 | | |
| AA | 761 (79.2) | 96 (73.3) | 1.0 (ref) | 1.0 (ref) | 39 (70.9) | 1.0 (ref) | 1.0 (ref) |
| GA | 190 (19.8) | 31 (23.7) | 1.3 (0.8–1.9) | 1.7 (0.7–1.9) | 15 (27.3) | 1.5 (0.8–2.9) | 1.6 (0.8–3.3) |
| GG | 10 (1.0) | 4 (3.1) | 3.2 (0.9–10.3) | 4.6 (0.9–19.4) | 1 (1.8) | 1.9 (0.2–15.6) | 1.9 (0.2–21.2) |
| GA + GG | 200 (20.8) | 35 (26.7) | 1.4 (0.9–2.1) | 1.3 (0.9–2.1) | 16 (29.1) | 1.6 (0.9–2.9) | 1.7 (0.9–3.3) |
| Neonatal | *n* = 932 | *n* = 113 | | | *n* = 37 | | |
| AA | 728 (78.1) | 78 (69.0) | 1.0 (ref) | 1.0 (ref) | 27 (73.0) | 1.0 (ref) | 1.0 (ref) |
| GA | 194 (20.8) | 30 (26.5) | 1.4 (0.9–2.3) | 1.6 (0.9–2.6) | 9 (24.3) | 1.3 (0.6–2.7) | 1.4 (0.6–3.0) |
| GG | 10 (1.1) | 5 (4.4) | **4.7 (1.6–14)** | **7.8 (2.4–25.9)** | 1 (2.7) | 2.7 (0.3–21.8) | 2.9 (0.4–32.7) |
| GA + GG | 204 (21.9) | 35 (31) | **1.6 (1.0–2.5)** | **1.8 (1.1–2.8)** | 10 (27.0) | 1.3 (0.6–2.8) | 1.4 (0.3–3.1) |

NSGA, normotensive SGA; HSGA, hypertensive SGA. aOR (95% CI) adjusted for maternal factors: maternal age, BMI, birth weight, smoking at 15 weeks gestation, pre-pregnancy low fruit intake, pre-pregnancy low green leafy vegetable intake. Paternal factors: BMI and birth weight. GA + GG, additive genetic model is compared with the reference AA genotype; ref, referent. OR (95% CI) values in bold are significant.

**Table 4** Distribution of maternal *TSP-1 A2210G* SNP in maternal birth weight

| *TSP-1 A2210G* genotype | Maternal birth weight* (*n* = 1875)† | *P* |
|---|---|---|
| AA | 3291 ± 538 | Ref |
| GA | 3269 ± 568 | 0.07 |
| GG | 3059 ± 503 | 0.03 |

Data are presented as mean ± SD. *ANOVA with post-hoc Sidak test and adjusted for gestational age at delivery. †After exclusion of 83 women with missing birth weight. Ref, referent.

manner and increases platelet aggregation [27]. The Ser-700 variant of the *TSP-1 A2210G* SNP exhibits higher affinities for platelets and fibrinogen and shows increased platelet surface expression compared with the Asn-700 variant [11]. Enhanced platelet aggregation and increased TSP-1 surface expression are both consistent with a prothrombotic phenotype.

Placentae from pregnancies affected by fetal growth restriction demonstrate thrombotic lesions within fetal and chorionic vessels, suggesting that placental thrombosis may contribute to fetal growth restriction [28]. Similar pathological changes underlie pregnancies complicated by SGA fetuses [29]. Our data show that heterozygosity for the *TSP-1 A2210G* SNP in infants is associated with an adjusted OR of 1.6 and homozygosity with an adjusted OR of 5.9 with SGA. As a potential pathophysiological mechanism, we hypothesize that the SNP may contribute to a prothrombotic and anti-angiogenic phenotype in the placenta.

Our data also demonstrate a borderline significant association between the paternal *TSP-1 A2210G* polymorphism and SGA. A paternal contribution to SGA has been previously suggested by a positive correlation of paternal birth weight with infant birth weight [18–20] and that men who were SGA at birth are more likely than those with a normal birth weight to parent an SGA infant [17]. In our study the paternal *TSP-1*

**Table 5** Distribution of the *TSP-1 A2210G* SNP in parent-infant trios

| | Control trios *n* = 690 (%) | SGA trios *n* = 119 (%) | OR (95% CI) |
|---|---|---|---|
| Mother-father-infant polymorphism absent | 413 (59.9) | 59 (49.6) | 1 |
| Father-infant polymorphism absent | 63 (9.1) | 5 (4.2) | 0.6 (0.2–1.4) |
| Mother polymorphism present | | | |
| Mother-infant polymorphism absent | 55 (8.0) | 10 (8.4) | 1.3 (0.6–2.6) |
| Father polymorphism present | | | |
| Father polymorphism absent | 67 (9.7) | 19 (16.0) | **1.9 (1.1–3.5)** |
| Mother-infant polymorphism present | | | |
| Mother polymorphism absent | 59 (8.6) | 16 (13.4) | **1.9 (1.0–3.5)** |
| Father-infant polymorphism present | | | |
| Infant polymorphism absent | 8 (1.2) | 3 (2.5) | 1.8 (0.4–1.4) |
| Mother-father polymorphism present | | | |
| Mother-father-infant polymorphism present | 25 (3.6) | 7 (5.9) | 1.9 (0.8–4.7) |

Data are *n* (%) using Pearson chi-squared or Fisher's exact test. Polymorphism absent, AA genotype. Polymorphism present, GA or GG genotype OR (95% CI) values in bold are significant.

*A2210G* SNP remained associated with SGA after adjusting for established maternal and paternal risk factors for SGA, demonstrating an independent association between the GG genotype of the *TSP-1 A2210G* SNP in the father and birth of an SGA infant.

We did not find a significant association between the maternal polymorphism and SGA but found that homozygosity for the G allele in the mother was associated with a reduced maternal birth weight adjusted for gestational age at delivery. Considering the consistent association of maternal birth weight with neonatal birth weight, the *TSP-1 A2210G* polymorphism may contribute to SGA through influences on the mother's own birth weight.

Although thrombophilias and angiogenic growth factor imbalances have been much studied in pregnancy complications, there is a paucity of literature on the potential role of TSP-1 in normal and complicated pregnancies. Topol and coworkers reported that the *TSP-1 A2210G* SNP was strongly associated with familial premature myocardial infarction in Caucasians homozygous for the G allele [12]. This was later confirmed in a large study which reported that the *TSP-1 A2210G* SNP was a risk factor for myocardial infarction in both homozygous and heterozygous carriers of the G allele [13].

A consistent association has been demonstrated between SGA and adult-onset diseases, including increased risk of developing hypertension, insulin resistance and coronary artery disease [1,2,30]. It is proposed that these associations result from fetal programming in response to the intrauterine environment, with long-term consequences for metabolic and cardiovascular function [31]. An alternative explanation is that common genetic factors may underlie both restricted fetal growth and adult-onset diseases [14]. In support of this theory, a few previous studies have identified polymorphisms in candidate genes that are associated with either reduced birth weight or SGA and adult-onset disorders. Hattersley *et al*. [32] reported that a mutation in the glucokinase gene that results in adult-onset impaired glucose tolerance was associated with a reduction in birth weight. Polymorphisms in genes associated with adult-onset diabetes are also associated with being born small for gestational age [33]. In agreement with this theory, Infante-Rivard *et al*. [34] demonstrated that a cardio-protective polymorphism in the Apo-lipoprotein gene was less prevalent among SGA infants. Here we have shown that the prevalence of the *TSP-1 A2210G* polymorphism, which was previously shown to be associated with myocardial infarction in two independent studies, was increased in infants and fathers of SGA pregnancies, providing further evidence for potential shared genetic factors between SGA and cardiovascular disease.

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. Our study has a few limitations that should be acknowledged. Although our prospective cohort was large our group of SGA pregnancies was relatively small, therefore the borderline significance of the paternal polymorphism may be due to the sample size. We also excluded a number of cases and controls due to no genotype available, and it is possible that this has introduced bias into our results. As this is a novel finding, our results need to be replicated in other independent cohorts.

## Conclusion

This study demonstrates that the prevalence of the *TSP-1 A2210G* polymorphism, which was previously shown to be a risk factor for familial premature myocardial infarction, is increased in infants and fathers of SGA pregnancies, suggesting that this SNP may be associated with the risk of vascular disorders across the life course. Our data also show that there is a paternal genetic association with SGA.

## Addendum

R. A. North, G. A. Dekker, L. E. McCowan, C. T. Roberts: SCOPE study concept, design and supervision of the clinical study. P. H. Andraweera, G. A. Dekker, C. T. Roberts: candidate gene association study concept and design. P. H. Andraweera, G. A. Dekker, C. T. Roberts, S. D. Thompson: conduct of candidate gene association study. P. H. Andraweera: statistical analysis of data and drafting of the manuscript. P.H Andraweera, G.A. Dekker, R.A. North, L.E. McCowan, C.T. Roberts: critical revision of the manuscript for important intellectual content.

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## Disclosure of Conflict of Interests

R. A. North has consultancy relationships with Pronota and Alere. R. A. North declares patent PCT number WO/2009/108073. The other authors state that they have no conflict of interest.

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