

Journal Pre-proof

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PII: S2589-9333(22)00060-X
DOI: <https://doi.org/10.1016/j.ajogmf.2022.100618>
Reference: AJOGMF 100618

To appear in: *American Journal of Obstetrics & Gynecology MFM*

Received date: 28 November 2021
Revised date: 14 March 2022
Accepted date: 16 March 2022

Please cite this article as: Stephen Tong PhD, FRANZCOG , Susan P Walker MD, FRANZCOG , Emerson Keenan PhD , Teresa M MacDonald PhD, FRANZCOG , Rennae Taylor BScN, MHSc , Lesley M E McCowan MD, FRANZCOG , Tu'uhevaha J Kaitu'u-Lino PhD , Circulating SPINT1 in the second trimester is reduced among pregnancies that ends in low birthweight neonates: cohort study of 2006 pregnancies, *American Journal of Obstetrics & Gynecology MFM* (2022), doi: <https://doi.org/10.1016/j.ajogmf.2022.100618>

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Circulating SPINT1 in the second trimester is reduced among pregnancies that ends in low birthweight neonates: cohort study of 2006 pregnancies

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Financial support: This work was funded by the National Health and Medical Research Council of Australia (NHMRC Synergy Grant #1183854). NHMRC also provides salary support to ST and TK.

Conflicts of interest statement: S.T., S.P.W., T.M.M. and T.K.L. hold a provisional patent (PCT/AU2019/050516) relating to the use of SPINT1 as diagnostic markers in pregnancy. The remaining authors have no competing interests to declare.

OBJECTIVE: Serine peptidase inhibitor Kunitz- type-1 (SPINT1) is a circulating protein with possibly the strongest association with low birthweight and placental insufficiency yet reported. At 36 weeks' gestation low plasma SPINT1 was strongly linked with birth of a neonate <10th centile

birthweight ($p=2.7 \times 10^{-13}$; Australian cohort¹) as were levels at 26-34 weeks' gestation (validation cohorts in United Kingdom¹ and Singapore²). Its biomarker performance appeared to consistently outperform circulating placental growth factor¹. Furthermore, it is plausible that placental SPINT1 could be a disease driver of placental insufficiency: it is highly expressed in placenta, low levels are consistently associated with disease and *Spint1* deficiency in genetic mouse knockout studies is embryonically lethal (with severe placental abnormalities)³. Demonstrating that low SPINT1 levels from early pregnancy precedes low birthweight would further strengthen the case that it is a disease driver (temporality⁴). Therefore, we examined whether plasma SPINT1 concentrations at 15 and 20 weeks' gestation are associated with neonates with a birthweight <3rd or <10th centile.

STUDY DESIGN: We measured SPINT1 concentrations in plasma at 15 and 20 weeks' gestation from the Screening for Pregnancy Endpoints (SCOPE) cohort from New Zealand (see McCowan et al for details⁵). Plasma SPINT1 concentrations were measured by ELISA (Sigma-Aldrich, St Louis, Missouri, USA). We compared levels among pregnancies that ended with a neonate born at either <3rd, or <10th customized birthweight centile, versus those born >10th centile (controls). We also did a further analysis, comparing those born at these low birthweight cut-offs versus those born appropriate for gestational age (10th-89th birthweight centile). Analyses were carried out using Mann-Whitney U. We had ethics approval for this study and all participants provided written, informed consent (Auckland, New Zealand Regional Ethics Committee: AKX/02/00/364).

RESULTS: In women with plasma samples at 20 weeks' gestation there were 1756 controls and 189 who birthed <10th centile birthweight (among these, 48 were <3rd centile). Baseline clinical characteristics and pregnancy outcomes are shown in table 1. Compared to controls, SPINT1 levels were significantly reduced among pregnancies that ended with birth of neonates <10th centile birthweight ($P=0.006$; Figure 1a), or <3rd centile birthweight ($P=0.005$; Figure 1b).

Among those with plasma samples at 15 weeks' gestation there were 1807 controls and 199 who birthed <10th centile birthweight (among these, 54 birthed <3rd centile, See supplementary table 1 for baseline characteristics). Compared to controls, plasma SPINT1 levels were significantly

reduced among those who birthed <10th (P=0.02; Figure 1c) or <3rd birthweight centiles (P=0.004; Figure 1d).

Our findings were similar when we compared SPINT1 concentrations among pregnancies that ended with birth of neonates <3rd or 10th birthweight centile, compared to those that ended with birth of neonates with a birthweight appropriate for gestational age (supplementary table 2).

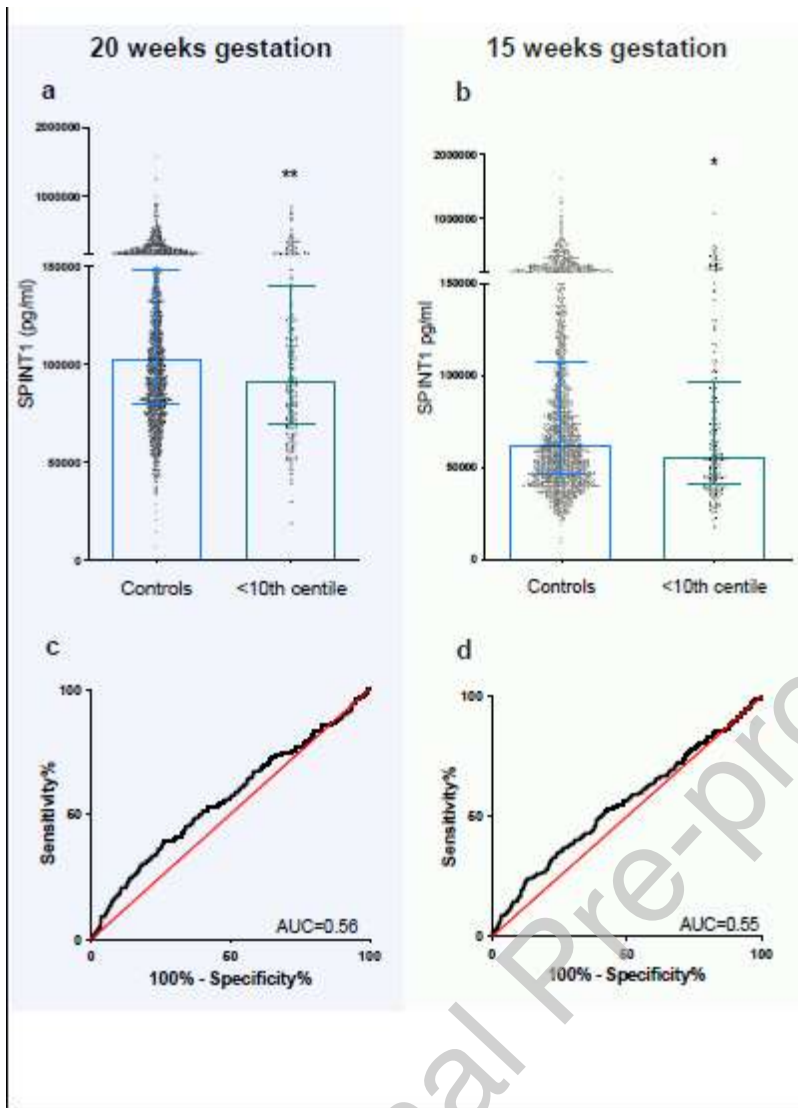
CONCLUSION: Circulating levels of SPINT1 are low at 15 and 20-weeks' gestation preceding low birthweight deliveries. As birthweight may reflect fetal growth restriction in utero, our findings lend further evidence that fetal growth restriction may have its pathogenic origins as early as the first half of pregnancy. When considering our findings together with what is known about SPINT1¹, low levels across pregnancy may play a mechanistic role driving placental insufficiency. Given there is a consistent association between low SPINT1 across pregnancy^{1,2} and low birthweight, SPINT1 merits further investigation as a clinical biomarker, perhaps combined with ultrasound or other circulating factors.

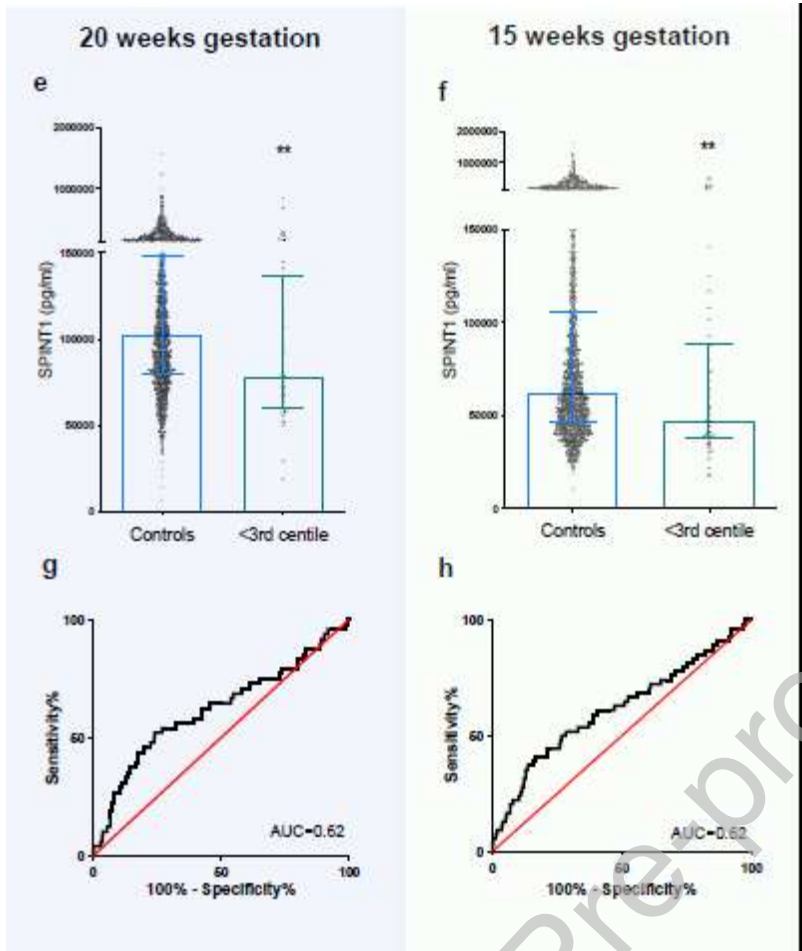
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Acknowledgements: We thank Ping Cannon and Vi Nguyen for performing the SPINT1 ELISA assays and for women enrolled in the SCOPE study for donating samples for research.

Figure 1: Plasma SPINT1 levels at 15 and 20 weeks' gestation. SPINT1 was significantly reduced in the circulation of women at 20 weeks' gestation who subsequently delivered an infant (a,c) <10th centile or <3rd birthweight centile. SPINT1 was also reduced at 15 weeks' gestation among those who subsequently delivered an infant (b,d) <10th centile or (f,h) <3rd centile birthweight. Shown are scatter plots (a,b,e,f) with median (blue and green boxes) and interquartile ranges; or receiver operated curves (c,d,g,h). AUC - Area under the curve. *P<0.05; **P≤0.006.





samples were collected at 20 weeks gestation (n = 1945)

	Controls: Over 10th centile birthweight N = 1756	Low birthweight: Under 10th centile birthweight N = 189	P =
Maternal Characteristics			
Ethnicity			0.67
Caucasian	1491 (85)	154 (81)	
Maori or Pacific Islander	90 (5)	10 (5)	
Asian	87 (5)	11 (6)	
Indian	63 (4)	10 (5)	
Other	25 (1)	4 (2)	
Primigravid	1323 (75)	129 (68)	0.04
Single	62 (4)	6 (3)	0.99
<12 years education	618 (35)	61 (32)	0.47
Smoking status at 15 weeks			0.90
Non-smoker	1551 (88)	167 (88)	
Ceased smoking before 15 weeks	139 (8)	16 (8)	
Current smoker	66 (4)	6 (3)	
Body Mass Index (BMI) category			0.03
<20.0	111 (6)	13 (7)	
20.0–24.9	959 (55)	93 (49)	
25.0–29.9	505 (29)	50 (26)	
≥30	181 (10)	33 (17)	
BMI (kg/m ²)	24.7 (4.1)	25.3 (5.0)	0.08
Maternal age (y)	30.4 (4.7)	31.3 (4.7)	0.02
Systolic BP (mmHg)	106.6 (10.6)	108.2 (10.6)	0.05
Diastolic BP (mmHg)	64.4 (8.2)	65.9 (8.8)	0.02
Pregnancy Outcome			
Birthweight (g)	3514.5 (485.3)	2611.3 (574.2)	<0.0001
Gestational age at birth (weeks)	39.7 (1.8)	38.7 (3.6)	<0.0001
Total preterm births (<37 weeks)	96 (5)	30 (16)	<0.0001
Admission to neonatal unit	95 (5)	32 (17)	<0.0001
Hypertensive pregnancy**	161 (9)	41 (22)	<0.0001

Results expressed as n (%) or mean (standard deviation SD).

*Preterm births were both spontaneous and indicated.

** Hypertensive pregnancy defined as preeclampsia, gestational hypertension or mild chronic Hypertension.