A Topic: Obstetrics

Quantifying mRNA coding growth genes in the maternal circulation to detect fetal growth restriction

Clare L WHITEHEAD¹, MBChB; Susan P WALKER², MD; Ms Sonali MENDIS², BMedSci; Martha LAPPAS², PhD; Stephen TONG¹, PhD.

¹Translational Obstetrics Group, Department of Obstetrics and Gynaecology, Mercy Hospital for Women, Heidelberg 3084, Victoria, Australia ²Department of Obstetrics and Gynaecology, University of Melbourne, Mercy Hospital for Women, Heidelberg 3084, Victoria, Australia

The authors report no conflicts of interest.

Financial support: Royal Australian and New Zealand College of Obstetricians and Gynaecologists Arthur Wilson Scholarship, William Buckland Foundation Grant, Medical Research Foundation For Women and Babies Grant and an NHMRC Project Grant (#1028521). ML and ST are supported by NHMRC Career Development Fellowships.

Corresponding author: Dr Clare Whitehead.

Translational Obstetrics Group, Department of Obstetrics and Gynaecology, University of Melbourne Mercy Hospital for Women, 163 Studley Rd., Heidelberg 3084, Victoria, AUSTRALIA.

Ph: +613 8458 4355   Fax: +613 8458 4380   clarew@unimelb.edu.au
IGF RNA to detect FGR
Condensation: RNA coding growth genes in maternal circulation are differentially regulated in severe preterm FGR, and at 28 weeks in pregnancies destined for FGR at term.

Short title: RNA coding growth genes in maternal blood with FGR
Abstract

Objective: To examine whether mRNA circulating in maternal blood coding genes regulating fetal growth are differentially expressed in 1) severe preterm FGR and 2) at 28 weeks’ gestation in pregnancies destined to develop FGR at term.

Study Design: mRNA coding growth genes were measured in two independent cohorts. The first was women diagnosed with severe preterm FGR (<34 weeks gestation; n=20) and gestation matched controls (n=15), where the mRNA was measured in both maternal blood and placenta. The second cohort was a prospective longitudinal study (n=52) of women whom had serial ultrasound assessments of fetal growth. mRNA coding growth genes in maternal blood was measured at 28 and 36 weeks in pregnancies with declining growth trajectories (ending up with term FGR; n=10 among the 52 recruited) and controls who maintained normal growth trajectory (n=15).

Results: In women with severe preterm FGR there was increased expression of PGH (6.3 fold), IGFs (IGF1 3.4 fold, IGF2 5.0 fold), IGF receptors (2.1 fold) and IGF binding proteins (3.0 fold), and reduced expression of ADAM12 (0.5 fold) in maternal blood (and similar trends in placenta) compared to controls (p<0.05). Notably, at 28 weeks gestation there was increased IGF2 (3.9 fold), PGH (2.7 fold) and IGFBP2 (2.1 fold) expression in maternal blood in women destined to develop FGR at term (p<0.05).

Conclusion: Measuring mRNA coding growth genes in maternal blood may detect unsuspected severe preterm FGR already present in utero, and predict term FGR when measured at 28 weeks’ gestation.

Keywords: free RNA, maternal circulation, fetal growth restriction, placenta, biomarker.
Introduction

Fetal growth restriction (FGR) is a leading cause of stillbirth \(^1,^2\) and strongly associated with increased perinatal morbidity. There are no effective intrauterine treatments, and therefore the management relies on early detection and timely delivery\(^3\).

Unfortunately, clinical detection of FGR is suboptimal and many cases remain undiagnosed\(^4\). It is thought that unrecognized FGR represents an important cause of stillbirth and therefore a reliable screening test for FGR could significantly improve clinical outcomes\(^5\). There are two situations where a non-invasive biomarker test for FGR may be useful: 1) to identify cases of unsuspected preterm FGR that are already present in utero, or 2) predict cases of normally grown fetuses at risk of developing FGR.

The discovery that nucleic acids circulate in the maternal blood where they can be quantified offers a novel avenue to identify biomarkers of placental function\(^6,^7\). RNA can be extracted reliably and subjected to molecular analyses with precision. In contrast, the detection of proteins relies on the existence of high quality monoclonal antibodies. Importantly, it is possible circulating mRNA in the maternal circulation may be derived from the placenta and reflect trends in differential transcript expression in the distant placental transcriptome \(^8,^9\). If true, then this approach could also provide unique insights into placental pathology previously not attainable whilst the fetus remains in-utero.

The insulin like growth factor (IGF) system is essential for fetal and placental growth and development. It includes IGF1 and IGF2, which can bind to one of six IGF binding proteins (IGFBPs) in order to modulate their bioavailability. The IGFs exert their metabolic actions by interacting with cell surface tyrosine kinase receptors...
IGF RNA to detect FGR

(IGF1R, IGF2R), which selectively bind the IGFs and insulin. Notably, these IGF ligands, receptors and binding proteins are expressed in the placenta, and the IGFs are released into the maternal circulation from early pregnancy \(^{10}\). Placental growth hormone (PGH) and ADAM12 are recently discovered placental derived growth factors that also modulate their effects via the IGF system. There is mounting evidence that abnormalities in the IGF system play a crucial role in the pathogenesis of FGR. However, the literature has been conflicting as to whether FGR is associated with changes in circulating IGF proteins in maternal blood.

Therefore in this study, we investigated whether mRNA of IGF1, IGF2, IGFBP-2, IGF1R, PGH and ADAM12 were dysregulated in the maternal blood in pregnancies affected by severe preterm FGR, and whether the circulating mRNA might reflect placental gene expression. We also studied a second cohort where we prospectively collected serial maternal samples from 28 weeks’ gestation in a low risk population and followed fetal growth until delivery. We identified cases with well-grown fetuses at recruitment (as determined by ultrasound) but who exhibited a decline in growth trajectory, developing FGR at term. We measured mRNA coding these six growth factors in maternal blood from this cohort at 28 and 36 weeks’ gestation to determine their potential to predict term FGR.
Materials and Methods

Clinical cohorts and recruitment of participants: Subjects were recruited from Mercy Hospital for Women and Monash Medical Centre, Melbourne between 2008-2011. Written informed consent was obtained and the study protocol was approved by both institutions’ Human Research and Ethics Committees.

There are two cohorts: a case–control study investigating severe preterm FGR, and a prospective longitudinal study investigating term FGR.

In the first cohort, cases of severe preterm FGR were defined as FGR (customized birthweight <10th centile) requiring iatrogenic delivery <34 weeks’, with evidence of uteroplacental insufficiency (asymmetrical growth + abnormal umbilical artery Doppler velocimetry, +/- oligohydramnios or abnormal fetal vessel velocimetry). FGR due to infection, chromosomal or congenital abnormalities, and multiple pregnancies were excluded. Both maternal blood and placenta was collected (n=20) and those with or without preeclampsia (ACOG guidelines 11) were included.

Preterm control blood samples were collected from women (n=20) with an appropriately grown fetus, at a gestation matched to the preterm FGR cases, and who subsequently delivered appropriately grown fetuses at term without obstetric complications (birthweight 20-80th centile). Preterm placental samples (n=8) were collected from women delivering preterm (<34 weeks) an appropriate grown fetus, in the absence of hypertensive diseases of pregnancy or chorioamnionitis. Term placental samples (n=8) were collected from uncomplicated, appropriately grown (birthweight 20-80th centile) singleton pregnancies at >37 weeks’. All placental samples were collected at pre-labour caesarean section.
In the second cohort, low risk healthy pregnant women were recruited at 28 weeks’ gestation. At the time of recruitment, none of the participants had obstetric, medical or surgical complications. Ultrasound assessment of fetal growth was performed at 28 weeks gestation, and only those with a well-grown fetus were included. Maternal blood samples were collected at 28 and 36 weeks gestation. Ultrasound assessment of fetal growth was repeated at 36 weeks gestation and final birthweight recorded. After birth the subjects were stratified into 3 cohorts (Fig. 3A):

1) Appropriate for gestational age fetuses (AGA, n=15), defined as fetuses with an estimated fetal weight 20-95th centile at 28 weeks who maintained their growth trajectory to term (change in centiles between the 28 weeks and birth <30%).

2) Term FGR (n=10), defined as fetuses appropriately grown at 28 weeks (20-95th centile) who subsequently exhibited a fall in growth trajectory resulting in term FGR (serial fall in estimated weight centiles from 28 weeks to 36 weeks gestation, with birthweight <10th centile)

3) The remainder of the pregnancies (n=27), had variable growth trajectories and could not be clearly classified into either of the above groups. Samples from this cohort were not analyzed.

Estimated fetal weight was calculated using the Hadlock equation\textsuperscript{12}. The customized centiles for estimated fetal weight and birthweight were generated using the Australian dataset of the GROW software (www.gestation.net) which takes into consideration maternal height, weight, ethnicity, parity and fetal gender\textsuperscript{13}. All ultrasounds were performed by one operator, trained and experienced in fetal biometry.

Sample collection and laboratory analysis: Maternal peripheral whole blood samples (2.5ml) were collected in PAXgene blood RNA tubes (PreAnalytix, Hombrechtikon,
Switzerland) and processed as per manufacturer’s instructions and stored at -80°C until processing.

Placental biopsies were obtained immediately after delivery by caesarean section. Placental biopsies were taken from the maternal side of the placenta, to a depth of 2/3 of the placenta, avoiding the decidua. The biopsies were clear of obvious infarction or calcification. Placental biopsies were washed in sterile PBS to remove contamination by maternal blood, snap frozen and stored at -80°C until processing.

RNA was extracted from peripheral whole maternal blood using the PAXgene Blood miRNA kit (PreAnalytix) according to manufacturer’s instructions, as previously described\textsuperscript{14}. Placental RNA was extracted using the mirVana Isolation Kit (Ambion, Austin TX) according to manufacturer’s instructions. Genomic DNA was removed using DNAsel treatment, and total RNA eluted and stored at -80°C if not used immediately. RNA concentration and purity were measured using a NanoDrop ND1000 spectrophotometer (Thermo Scientific, Pittsburgh, PA).

Real time quantitative RT-PCR analysis was performed after reverse transcription of 200ng RNA using Superscript Vilo (Invitrogen, Carlsbad, CA) according to manufacturers instructions. Quantitative gene expression analyses were performed with commercially available Taqman Gene Expression Assays (Applied Biosystems, Carlsbad, CA) for IGF1, IGF2, IGF1R, IGFBP-2, PGH and ADAM12. The RT-PCR was performed in triplicate, with multiple negative controls (including no template and no RT controls), on the CFX 384 (BioRad, Foster City, CA), with the following cycling conditions: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 sec, 60°C for 1 min, and 72°C for 30 sec.

Relative quantification was determined by the comparative CT method. Gene expression was normalized against the mean expression of GAPDH, GUSB and B2M.
to increase the stringency of the comparison, and calibrated against the mean expression level of the preterm control group.

Statistical analysis: All data was statistically analyzed using Graphpad Prism v 5 (GraphPad Software Inc., San Diego, CA). Differences in RT-PCR gene expression was assessed using Mann–Whitney U or Kruskal–Wallis test, or where the data was considered normally distributed the t-test or ANOVA. Patient characteristics were compared using $\chi^2$ where appropriate. Data was presented as mean +/- SEM. Significance was defined as $p<0.05$. 

IGF RNA to detect FGR
Results

mRNA coding growth genes are differentially expressed in placenta from cases of severe preterm FGR.

We first examined the expression of IGF1, IGF2, IGF1R, IGFBP2, PGH and ADAM12 in the placenta in severe preterm FGR (n=20), and compared them with preterm (n=8) and term (n=8) controls. The clinical characteristics are described in Table 1.

In preterm FGR there was a substantial increase in placental mRNA expression of the following compared to preterm controls (Fig. 1A): IGF1 (2.3 fold, p<0.05), IGF2 (3.0 fold, p<0.01), IGF1R (2.6 fold, p<0.01), IGFBP2 (3.0 fold, p<0.05) and PGH (5.6 fold, p<0.05). In contrast, ADAM12 was significantly down-regulated in preterm FGR (p<0.001). Interestingly, only IGFBP2 significantly increased across gestation in the controls, with a 3-fold increase in term controls compared to preterm controls (p<0.05).

mRNA coding growth genes in maternal blood are differentially expressed in cases of severe preterm FGR, and mirror trends seen in the placenta.

To investigate whether circulating mRNA in the maternal circulation might reflect changes in the placental transcriptome, the expression of these six genes were measured in maternal blood (Fig. 1B). The expression of all six genes in maternal blood with severe preterm FGR closely correlated with the trends in the placental transcriptome (compared with Fig. 1A). Interestingly, the degree of fold change was generally higher in maternal blood compared to placenta. Thus, in preterm FGR there was a 3.4 fold increase in IGF1 (p<0.001), 5.0 fold increase in IGF2 (p<0.01), 2.1 fold increase in IGF1R (P<0.05), 6.3 fold increase in PGH (p<0.01) and 3.0 fold increase in IGFBP2. Like placenta, ADAM12 expression was down-regulated in the maternal blood in preterm FGR compared to controls (p<0.05).
Expression mRNA coding growth genes in maternal blood correlates with the severity of disease, in severe preterm FGR.

The severity of preterm FGR is characterized by increased placental resistance, which can be assessed by Doppler ultrasound of placental and fetal vessels. The mildest abnormality is reflected by an increase in the umbilical artery resistance (pulsatility index), whereas absent and reversal of end diastolic flow in the umbilical artery (AREDF) is associated around 30-70% destruction of the placental villous tree, and strongly correlated with fetal hypoxia/acidemia. We therefore examined expression of mRNA coding growth genes in maternal circulation was further dysregulated with increasing severity of FGR.

Fig. 2 demonstrates increased expression in both the placenta (Fig. 2A) and maternal blood (Fig. 2B) for IGF1, IGF2 and PGH with AREDF compared to increased resistance but positive end diastolic flow (Increased PI; p<0.05). Again, the fold changes in the maternal blood were far greater than that seen in placenta. There was a 2-fold increase in IGF1 in the placenta compared to a 4.8 fold increase in the maternal blood with AREDF. However, there was no difference in expression of IGFBP2, IGF1R or ADAM2 in either the placenta or maternal blood in FGR, stratified according to the severity of umbilical artery Doppler resistance.

mRNA coding growth genes in maternal blood gestation are differentially expressed at 28 and 36 weeks in pregnancies destined to develop term FGR.

We next investigated whether mRNA coding growth genes in the maternal circulation at mid-pregnancy can identify fetuses that are appropriately grown at 28 weeks gestation, but develop late placental insufficiency evidenced by a decreased growth trajectory, and term FGR.
We recruited 52 healthy pregnant women at 28 weeks, performing an ultrasound to confirm apparent normal fetal growth (EFW >20th centile) at the time of recruitment. We then followed participants longitudinally, collecting maternal blood at 28 and 36 weeks gestation and tracking growth trajectory by performing another ultrasound at 36 weeks, then noting final birthweight (Fig. 3 depicts how participants were recruited).

Of the 52 participants, we identified 15 appropriate grown controls (AGA) where the fetus clearly maintained growth trajectory (Fig. 3B is an example of a clinical growth chart plot where fetal growth was maintained) and 10 cases where growth trajectory serially declined and the pregnancy ended up with FGR at term (birthweight <10th centile; Fig. 3C). Clinical characteristics of this second cohort are shown in Table 2. None of the individuals in the second cohort developed pre-eclampsia. We then compared the mRNA coding growth transcripts in the maternal blood among the AGA controls and term FGR cases (Fig. 4).

Firstly we examined the expression of the growth transcripts across gestation in the AGA controls. There was a non-significant increase in the expression of IGF1, IGF2 and PGH between 28 and 36 weeks, but no change across gestation in IGFBP2, IGF1R and ADAM12.

We next examined whether the expression of the growth transcripts in maternal blood was different between AGA and term FGR. There was a 2.2 fold increase in IGF1 and IGFBP2 (p<0.05), a 2.7 fold increase in IGF2 (p<0.01) and a 3.0 fold increase in PGH (p<0.01), in pregnancies on the way to developing FGR at term, but with an estimated fetal weight still above the 10th centile on ultrasound at 36 weeks (Fig. 4A). If these women had only had an ultrasound assessment of fetal size at 36 weeks, it is unlikely term FGR would have been predicted. In contrast, in cases with evolving term FGR, there was already evidence of differential expression of mRNA.
coding growth genes at 36 weeks. This suggests a blood test performed at 36 weeks’
gestation may potentially identify more cases of term FGR than an ultrasound at the
same gestation.

Finally, we examined the expression of the growth transcripts in maternal blood at 28
weeks gestation. We confirmed on ultrasound that all fetuses were well grown at the
time of blood sampling and there were no risk factors for FGR. In contrast, the
expression of IGF2 (3.9 fold, p<0.01), PGH (2.7 fold, p<0.05) and IGFBP2 (2.1 fold,
p<0.05) were already significantly increased in pregnancies that would subsequently
develop FGR at term (Figure 4B). Thus these transcripts were altered more than 10
weeks prior to the clinical manifestation of FGR.
Comment

FGR is strongly associated with severe perinatal morbidity and mortality, possibly accounting for up to 50% of unexplained stillbirths. Infants with FGR that survive the neonatal period are also at risk of delayed childhood neurodevelopment including cerebral palsy, as well as adult metabolic and cardiovascular disease. Unfortunately, there are no effective intrauterine treatments for FGR, and the goal of management is early detection and timely delivery. Current methods of clinical detection of the growth-restricted fetus have poor sensitivity and specificity. Some have estimated as many as 75-85% of FGR cases are missed antenatally.

Furthermore, there are no good cost effective biomarkers for FGR that can be applied to a low risk population to either predict FGR or identify FGR already present in utero.

We have investigated levels of mRNA circulating in maternal blood that code major growth genes in pregnancy using two approaches: detection of severe preterm FGR already present in-utero, and early prediction of FGR at term. In the severe preterm FGR study, we found mRNA expression of six growth factors in the maternal blood were dysregulated with severe preterm FGR, and mirrored trends seen in the placental transcriptome. In addition, they were further dysregulated with increasing severity of disease as determined by umbilical artery Doppler velocimetry. In our second study, we recruited low risk women at 28 weeks and tracked fetal growth longitudinally, and found mRNA coding growth genes in maternal circulation were dysregulated as early as 28 weeks gestation in those women who subsequently developed FGR. Therefore circulating growth factor RNA in the maternal blood may be a promising method to both detect established preterm FGR and predict FGR at term.
Interestingly, the trends in mRNA expression of all six growth genes in maternal blood not only mirrored those seen in placenta but the fold changes were generally much greater in maternal blood. We hypothesize the reason is that the origin of most of the circulating mRNA is shed particles from the syncytiotrophoblast layer, the same tissue layer that expresses the growth transcripts\textsuperscript{18}. As such, mRNA in maternal blood might be naturally enriched with IGF mRNA transcripts from the syncytiotrophoblast. If correct, this lends support to the promise of exploiting free circulating RNA of placental origin as a clinical biomarker.

Importantly, a strength of our study is the inclusion of both severe preterm FGR cases and term FGR, diseases that are managed very differently in the clinic. Preterm FGR occurs less frequently but has a strong association with perinatal mortality and neonatal morbidity due to prematurity. Therefore identifying these pregnancies to optimize timing of delivery is clinically important\textsuperscript{5}. Term FGR is a substantial contributor to perinatal mortality and notably, the incidence of stillbirth due to FGR is at its peak after 38 weeks. Therefore, induction of labour in these pregnancies is likely to yield the greatest clinical benefit with minimal harm.

Routine ultrasound applied to low risk pregnancies has not been shown to improve perinatal outcome\textsuperscript{19}. In our longitudinal study, we have obtained encouraging evidence that measuring mRNA coding growth genes at 28 weeks gestation, even with a normal fetal size on ultrasound at the time of the blood test, may identify pregnancies at risk of term FGR. Furthermore, in our study, a number of these babies would have been missed by ultrasound at 36 weeks. This is of relevance clinically given an ultrasound is often timed at 36 weeks in suspected cases of FGR occurring near term. In contrast, the changes in mRNA expression in maternal blood in those destined to develop term FGR were not only still present at 36 weeks gestation, but more marked. While our findings in this longitudinal study are highly encouraging, they require validation in a larger prospective study.
In conclusion, we have confirmed that the expression of RNA transcripts for growth factors, circulating in the maternal blood may be novel predictive and diagnostic biomarkers for FGR. A predictive and diagnostic test for FGR would enable clinicians to identify pregnancies that would benefit from intensive monitoring and early delivery to reduce the morbidity, and potentially the rates of stillbirth, due to undiagnosed FGR.
References


Table 1. Clinical characteristics of patients in the severe preterm FGR study

<table>
<thead>
<tr>
<th></th>
<th>FGR Blood and placenta (n= 20)</th>
<th>Preterm Blood (n=20)</th>
<th>Preterm Placenta (n=8)</th>
<th>Term Placenta (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (yrs)</td>
<td>30.4 (5.9)</td>
<td>31.1 (1.5)</td>
<td>31.8 (1.7)</td>
<td>31.9 (1.1)</td>
</tr>
<tr>
<td>Parity (% primiparous)</td>
<td>65</td>
<td>60</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>29+5 (3)</td>
<td>40+2 (1.4)</td>
<td>29+4 (1)</td>
<td>38+3 (0.6)</td>
</tr>
<tr>
<td>Gestational age at sampling (weeks)</td>
<td>29+5 (3)</td>
<td>30+1 (2.1)</td>
<td>29+4 (1)</td>
<td>38+3 (0.6)</td>
</tr>
<tr>
<td>Birthweight (gms)</td>
<td>885 (273)</td>
<td>3565 (35)</td>
<td>1492 (203)</td>
<td>3400 (122)</td>
</tr>
<tr>
<td>Customised birth centile (%)</td>
<td>3 (3)</td>
<td>48 (6)</td>
<td>66 (7.5)</td>
<td>69 (4)</td>
</tr>
<tr>
<td>Perinatal death (%)</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pre-eclampsia (%)</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Umbilical artery Doppler velocimetry waveforms</td>
<td>REDF</td>
<td>AEDF</td>
<td>PI</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Data is presented as mean (SEM) or %.

REDF: reverse end diastolic flow. AEDF: absent end diastolic flow. PI: increased pulsitility index.
Table 2. Clinical characteristics of patients in the longitudinal term FGR study

<table>
<thead>
<tr>
<th></th>
<th>Appropriately grown at term (n=15)</th>
<th>Term FGR (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (yrs)</td>
<td>32.0 (1.2)</td>
<td>31.1 (1.8)</td>
</tr>
<tr>
<td>Parity (% primiparous)</td>
<td>27</td>
<td>60</td>
</tr>
<tr>
<td>Mode of delivery (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operative SVD</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>(weeks)</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Gestational age at 1st USS/ blood sampling (weeks)</td>
<td>28+1 (0.1)</td>
<td>28+1(0.1)</td>
</tr>
<tr>
<td>Gestational age at 2nd USS/ blood sampling (weeks)</td>
<td>35+4 (0.1)</td>
<td>35+3(0.1)</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>39+0 (0.3)</td>
<td>39+0(0.4)</td>
</tr>
<tr>
<td>Estimated fetal weight at 28 weeks (gms)</td>
<td>1266 (50)</td>
<td>1200 (42)</td>
</tr>
<tr>
<td>Customised centile at 28 weeks (%)</td>
<td>61(7)</td>
<td>51 (8)</td>
</tr>
<tr>
<td>Estimated fetal weight at 36 weeks (gms)</td>
<td>2839 (83)</td>
<td>2420 (60) **</td>
</tr>
<tr>
<td>Customised centile at 28 weeks (%)</td>
<td>57 (7)</td>
<td>28 (4) **</td>
</tr>
<tr>
<td>Birthweight (gms)</td>
<td>3580 (85)</td>
<td>2780 (120)***</td>
</tr>
<tr>
<td>Customised birth centile (%)</td>
<td>56 (6)</td>
<td>9 (2) ***</td>
</tr>
</tbody>
</table>

Data is presented as mean (SEM) or %. **p<0.01, *** p<0.001
**Figure Legends**

**Fig. 1.** mRNA expression of IGF1, IGF2, IGF1R, IGFBP2, placental GH and ADAM12 in placenta (A) and maternal blood (B) in severe preterm FGR compared to well grown preterm and term controls (A only for term controls). Normalized against GAPDH, GUSB and B2M. Data is presented as median +/- IQR. Mann-Whitney test was used for statistical comparison to preterm controls. *p<0.05, **p<0.01, ***p<0.001.

**Fig. 2.** Expression of IGF1, IGF2 and placental GH in placenta (A) and maternal blood (B) in severe preterm FGR stratified according to the severity of umbilical artery Doppler velocimetry. SDR: raised diastolic systolic ratio, positive end diastolic flow. AREDF: absent or reversed end diastolic flow. Normalized against GAPDH, GUSB and B2M. Data is presented as median +/- IQR. Mann-Whitney test was used for statistical comparison to preterm controls. *p<0.05, **p<0.01, ***p<0.001.

**Fig. 3.** Approach to select case and controls for the longitudinal study to predict term FGR. The control group (AGA) consisted of pregnancies where the fetus maintained its growth trajectory from 28 weeks to birth and remained well grown. The cases (term FGR) consisted of pregnancies where the fetus was initially well grown at 28 weeks but failed to maintain its growth trajectory, with a birthweight less than 10th centile. (B) is a growth chart plot, and depicts an example of a case (from AGA group) where the fetus maintained growth trajectory where (C) is an example where the fetus did not maintain growth trajectory and ended up as term FGR. Customised growth charts generated from GROW software (www.gestation.net) for example case and control.
**Fig. 4.** RNA expression of IGF1, IGF2, placental GH and IGFBP2 in maternal blood at (A) 36 weeks’ gestation and (B) 28 weeks gestation in women who subsequently delivered a baby with term FGR, compared to controls (cases where the fetus maintained growth trajectory). Normalized against GAPDH, GUSB and B2M. Data is presented as median +/- IQR. Mann-Whitney test was used for statistical comparison to preterm controls. *p<0.05, **p<0.01, ***p<0.001.
Figure 1
A: Placenta

B: Maternal blood
Figure 2

A: Placenta

B: Maternal blood
Figure 3

A

Recruited to study (n=52)

28 Weeks
Maternal blood sampling
Fetal growth assessment

36 Weeks
Maternal blood sampling
Fetal growth assessment

Birth
Fetal outcome data

AGA (n=15)
Maintained growth trajectory from 28 weeks gestation to birth

Term FGR (n=10)
Failed to maintain growth trajectory from 28 weeks to birth

Excluded (n=27)
Macrosomia/IDDM/HTN
Preterm birth
Inaccurate fetal biometry

B

C

CUSTOMISED FETAL GROWTH CHART v.7.06 (Australia)

CUSTOMISED FETAL GROWTH CHART v.7.06 (Australia)
Figure 4
A: 36 weeks

B: 28 weeks
Author/s:
Whitehead, CL; Walker, SP; Mendis, S; Lappas, M; Tong, S

Title:
Quantifying mRNA coding growth genes in the maternal circulation to detect fetal growth restriction

Date:
2013-08-01

Citation:

Publication Status:
Accepted manuscript

Persistent Link:
http://hdl.handle.net/11343/41903