First-trimester ADAM12 and PAPP-A as markers for intrauterine fetal growth restriction through their roles in the insulin-like growth factor system

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**Background** PAPP-A is a marker used as part of the most effective method of screening for chromosomal anomalies in the first trimester. ADAM12 is a recently discovered pregnancy associated member of the ADAM (a multidomain glycoprotein metalloprotease) family. Recently, ADAM12 has been shown as a potential marker for early screening for chromosomal anomalies. Both PAPP-A and ADAM12 have been identified as proteases to insulin-like growth factor binding proteins. In this role, they may have a regulatory function in controlling the amount of free bioactive insulin-like growth factor (IGF). We therefore wish to examine if the levels of either of these proteases are related to various growth related adverse pregnancy outcomes.

**Materials and Methods** PAPP-A and ADAM12 were measured in a subset of samples collected at 11 to 14 weeks as part of an OSCAR clinic screening for chromosomal anomalies. Follow-up of pregnancies screened between September 1999 and August 2003 identified 1705 pregnancies with an outcome of intrauterine fetal demise on or after 24 weeks, preterm delivery at 24–34 weeks or 35–36 weeks, very low birthweight (<1.5 kg), low birthweight (<2.5 kg), large birthweight (>4.5 kg), and birth weight below the 3rd or 5th or 10th centile for gestation. A series of 414 normal outcome pregnancies constituted the control group.

Marker levels were adjusted for gestation and maternal weight and the log MoM of the markers were compared using t-test of unequal variance between the control group and the various adverse outcome groups.

**Results** ADAM12 and PAPP-A concentrations were reduced in low for gestational age birth weights and in all births with weights below 2.5 kg. There was a linear relationship between the severity of the IUGR and the decrease in PAPP-A and ADAM12. In the larger babies, only ADAM12 was found to be significantly increased in babies above the 90th centile of weight for gestation.

**Conclusions** The results of our study are compatible with the proposed role of ADAM12 and PAPP-A in promoting growth and development by breaking down IGF binding proteins and causing the release of free IGF for uptake into cells to promote growth. In those cases that eventually result in poor fetal growth, levels of PAPP-A and ADAM12 at 11–14 weeks are significantly lower than normal—in this instance, lowered PAPP-A and ADAM12 would result in less free IGF being available for cell uptake and growth stimulation. Further studies may elucidate if screening using such modalities can lead to new potential treatments for poorly growing fetuses. Copyright © 2007 John Wiley & Sons, Ltd.

**KEY WORDS:** prenatal screening; growth factors; Adam12; PAPP-A; IUGR

INTRODUCTION

Intrauterine growth restriction (IUGR) occurs when the fetus fails to fulfil its expected growth potential, and it is one of the leading causes of perinatal morbidity and mortality. Further, there are links between IUGR and adult cardiovascular disease and diabetes (Barker et al., 1993; Rich-Edwards et al., 1999). If detectable, IUGR pregnancies may be carefully monitored and management practices for such pregnancies may be developed. The idea that a pregnancy is destined to result in IUGR from early on in gestation due to first-trimester impaired placental function is under investigation, and markers that would give the earliest indication of IUGR risk would be of most use to the obstetrician. As more is becoming understood regarding the pathology of IUGR, effective screening for the condition will eventually become a possibility.

Throughout pregnancy, the insulin-like growth factor (IGF) system is involved in fetal growth and development. IGF-I and IGF-II (IGFs) are small peptides, similar to insulin, which are usually bound to one of the six known IGF binding proteins (IGFBPs) in the circulation. When bound, the IGFBPs stabilise the growth factors, but they also inhibit their bioavailability. In addition, IGFBPs are also involved in IGF-independent mechanisms, binding to their own receptors (Oh et al., 1993; Andress, 1995). Knockout studies in mice have shown IGFs to be crucial for correct embryonic development and growth (DeChiara et al., 1990, 1991; Powell-Braxton et al., 1993).

PAPP-A is a marker used as part of the most effective method of screening for Down syndrome: a combination of the ultrasound nuchal translucency thickness marker and the maternal serum free beta hCG and PAPP-A biochemical markers, at 10–14 weeks (Spencer et al., 2007).
ADAM12 is a recently discovered pregnancy associated member of the ADAM multidomain glycoprotein family, with cell adhesion and metalloprotease properties, and occurs in a long membrane bound form (ADAM12-L) and a shorter secreted form (ADAM12-S) that lacks the transmembrane and cytoplasmic domains (Gillpin et al., 1998). Recently, ADAM12-S has been shown to be of potential use in the detection of chromosomal anomalies (Laigaard et al., 2003, 2005a, 2006a,b) and preeclampsia (Laigaard et al., 2005b).

PAPP-A binds to and breaks down IGFBP-3 and IGFBP-4, and ADAM12 acts in the same way on IGFBP-3 and IGFBP-5 (Lawrence et al., 1999; Loechel et al., 2000). After being broken down into smaller fragments, the binding proteins have considerably reduced affinity for the IGFs (Blat et al., 1994). Therefore, ADAM12 and PAPP-A perhaps reverse the inhibitory effects of the IGFBPs, contributing to fetal growth.

In this study, we aim to evaluate the levels of the IGFBP proteases ADAM12 and PAPP-A at 10 to 14 weeks of pregnancy in a variety of growth related adverse pregnancy outcomes.

METHODS

Maternal serum samples were collected at a routine first-trimester prenatal screening clinic at Harold Wood Hospital (Essex). Upon collection, samples were analysed immediately for PAPP-A using the KRYPTOR random access immunoanalyser (Brahms AG, Berlin) as part of a one-stop clinic for assessment of risk (OSCAR) for fetal anomalies. The analytical performance of this system has been previously outlined (Spencer et al., 1999). Ethical approval and patient’s consent were obtained for research to be carried out on the remaining excess serum, which was immediately frozen at −20 °C until ADAM12 concentrations were determined. Serum ADAM12 levels were analysed using a newly developed Dissociation Enhanced Lanthanide Fluoro-Immunoassay (DELFIA) kit (Perkin Elmer, Turku), based on previously described ELISA and AutoDELFIA assays (Laigaard et al., 2003, 2005b). Briefly, standards, controls, and samples were loaded in duplicate onto microtiter plates coated with anti-ADAM12 6E6 monoclonal antibody, and then incubated at 4 °C with slow shaking for 3 h with europium labelled anti-ADAM12 8F8 monoclonal antibody. After washing, an enhancement solution was added, which dissociates the europium ions from the labelled antibody, where they form highly fluorescent micellar chelates with enhancement solution components. The plates were read for fluorescence in a Victor 1420 Multilabel counter (PerkinElmer (Wallac), Turku, Finland). The analytical range of the assay is 2 to 820 ng/mL, and samples above the higher cut off were diluted and rerun. The sensitivity of the assay defined as two standard deviations above the single at zero dose measured over 10 replicates was 0.02 ng/mL. All samples were analysed blind to the researcher.

Raw PAPP-A and ADAM12 marker concentrations were converted into multiples of the median (MoM) by dividing each result by the expected median marker level in normal pregnancies at the same gestational age, calculated from ultrasound crown–rump length (CRL). PAPP-A MoM was calculated at the time of screening based on a published median regression curve (Ong et al., 2000), whereas ADAM12 MoM was calculated using the control group medians from this study population. To account for haemodilution, corrections were made for maternal weight. In the case of PAPP-A, this was carried out according to a recognised log-linear procedure (Neuveux et al., 1996; Spencer et al., 2003). For ADAM12, the control group was divided into 10 kg bands by maternal weight, and the median log ADAM12 MoM for each group was plotted against the median weight for each group to derive an equation to determine the weight-corrected MoM, as outlined in Spencer et al., 2003.

Demographic, biochemical, and ultrasound data for a range of patients with a singleton pregnancy screened between 11 and 14 weeks of gestation were extracted from the fetal database (ViewPoint, Weßling, Germany). Pregnancy outcome data were obtained from the cytogenetics laboratories, national chromosomal anomaly register, the patients themselves, their general practitioners or the maternity unit. Data was also obtained from the hospital midwifery and patient administration and matched to the prenatal screening records by a locally derived record linkage software.

The study group was created by searching the database for first-trimester karyotypical normal single pregnancies screened between September 1999 and August 2003 in which one or more of the following pregnancy complications occurred: intrauterine fetal demise (IUFD) at or after 24 weeks, preterm delivery (sub classified as between 24 and 34 weeks and between 35 and 36 weeks), very low birth weight (<1.5 kg), low birth weight (<2.5 kg), large birth weight (>4.5 kg), birth weight below the 3rd centile, birth weight below the 5th centile, birth weight below the 10th centile, birth weight above the 90th centile and birth weight above the 95th centile (Yudkin et al., 1987). One control in which the pregnancy resulted in the delivery of a karyotypical normal baby, unaffected by any of the conditions under investigation, was selected for every four test cases, matched for length of sample storage and gestational age with the cases.

In total, 2119 samples were analysed, which had complete first-trimester screening, maternal and birth outcome data. Of these, 1705 (80.5%) fell into one or more of the adverse outcome categories and the remaining 414 (19.5%) acted as controls.

The stability of ADAM12 was investigated by pooling ~3 mL of first-trimester serum that was kept at room temperature or 4 °C. Aliquots were removed at regular intervals for a period of up to two weeks and frozen at −40 °C until they were run on the DELFIA assay. Additionally, in a separate study, the effect of leaving blood samples for 2, 24, and 48 h before centrifugation and removal of serum was studied in 16 blood samples unrelated to the main study.
Statistics

ADAM12 and PAPP-A MoMs were $\log_{10}$ transformed, and the distributions for each control population were confirmed to be gaussian using the Kolmogorov–Smirnov goodness of fit test. $\log_{10}$ transformed MoMs between the different outcome groups and the controls were compared using the $t$-test, assuming unequal variance. All statistical analyses were performed using Microsoft Excel, Analyse-It (Analyse-IT Ltd, Leeds) and SPSS (SPSS Inc., Chicago, IL).

RESULTS

Demographics

Table 1 shows demographic data for the control and each case group, with $p$ statistics for Mann–Whitney or Chi squared tests. Not all demographic data were available: maternal age was documented on all records, parity in 39.5% of the records, BMI in 36.4% of the records, and smoking status in 98.4% of the records.

Gestational age and maternal weight corrections

There was an approximately 1.5-fold increase in median ADAM12 concentration throughout the gestational window of 11 to 14 weeks. A quadratic equation was found to best fit this change ($r^2 = 0.939$), shown in Figure 1. MoM values were calculated using the expected ADAM12 concentration at each gestational age derived from the following equation:

$$y = 38.986x^2 - 885.864x + 5379.264$$

Where $y$ is the expected normal ADAM12 concentration and $x$ is the gestational age at screening in decimal weeks.

ADAM12 MoMs were found to be related to maternal weight, shown in Figure 2, and a log-linear equation was used to correct for this:

$$Corrected\, MoM = \frac{MoM}{10^{0.42 - 0.006317 \times Weight\, (kg)}}$$

Previously determined PAPP-A MoMs were calculated corrected for gestational age and maternal weight, as described in the Section on Methods.

Adverse outcomes

The median MoM and $p$-values from the $t$-test for each test group versus the controls’ log MoM for PAPP-A and ADAM12 are shown in Table 2, along with the number of pregnancies in each group.

Table 1—Demographic data in the control and adverse outcome groups and the significance ($p$) from controls (* denotes statistical significance)

<table>
<thead>
<tr>
<th>Control</th>
<th>Median maternal age</th>
<th>$p$</th>
<th>Median parity</th>
<th>$p$</th>
<th>Median BMI</th>
<th>$p$</th>
<th>Smoking (%)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUFD 11–14 weeks</td>
<td>28.75</td>
<td>n/a</td>
<td>1.00</td>
<td>n/a</td>
<td>24.2</td>
<td>n/a</td>
<td>15.46</td>
<td>n/a</td>
</tr>
<tr>
<td>24–34 weeks</td>
<td>29.81</td>
<td>0.0992</td>
<td>0.50</td>
<td>0.7649</td>
<td>24.1</td>
<td>0.3858</td>
<td>29.51</td>
<td>0.0131*</td>
</tr>
<tr>
<td>35–36 weeks</td>
<td>30.00</td>
<td>0.0013*</td>
<td>0.00</td>
<td>0.8990</td>
<td>25.2</td>
<td>0.1035</td>
<td>22.43</td>
<td>0.0172*</td>
</tr>
<tr>
<td>&lt;1.5 kg</td>
<td>30.36</td>
<td>0.0038*</td>
<td>1.00</td>
<td>0.3207</td>
<td>24.2</td>
<td>0.8277</td>
<td>18.10</td>
<td>0.1863</td>
</tr>
<tr>
<td>&lt;2.5 kg</td>
<td>29.29</td>
<td>0.2502</td>
<td>0.00</td>
<td>0.8306</td>
<td>23.55</td>
<td>0.2344</td>
<td>25.41</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>&gt;4.5 kg</td>
<td>29.76</td>
<td>0.6221</td>
<td>0.00</td>
<td>0.4473</td>
<td>29.6</td>
<td>0.0468*</td>
<td>0.00</td>
<td>0.6282</td>
</tr>
<tr>
<td>&lt;3rd centile</td>
<td>29.03</td>
<td>0.3037</td>
<td>0.00</td>
<td>0.2074</td>
<td>22.85</td>
<td>0.0096*</td>
<td>28.90</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>&lt;5th centile</td>
<td>28.51</td>
<td>0.9159</td>
<td>0.00</td>
<td>0.0803</td>
<td>22.9</td>
<td>0.0050*</td>
<td>27.15</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>&lt;10th centile</td>
<td>28.69</td>
<td>0.7339</td>
<td>0.00</td>
<td>0.0878</td>
<td>23</td>
<td>0.0048*</td>
<td>25.27</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>&gt;90th centile</td>
<td>30.40</td>
<td>0.0003*</td>
<td>1.00</td>
<td>0.3835</td>
<td>25.85</td>
<td>0.0162</td>
<td>16.30</td>
<td>0.9137</td>
</tr>
<tr>
<td>&gt;95th centile</td>
<td>30.40</td>
<td>0.0013*</td>
<td>1.00</td>
<td>0.4344</td>
<td>26.65</td>
<td>0.0043*</td>
<td>17.28</td>
<td>0.9137</td>
</tr>
</tbody>
</table>

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First-trimester mean log ADAM12 maternal serum levels in pregnancies that resulted in birth weight in the lowest 10th, 5th, and 3rd centiles for their gestational age at birth were significantly and progressively lower in all three sub classifications (Figure 3 and Table 2). Low birth weight babies (<2.5 kg), regardless of their gestational age, also had significantly lower ADAM12 levels, although very low birth weight babies (<1.5 kg) were not significantly different from controls. At the opposite end of the scale, pregnancies that resulted in birth weights in the top 10th centile (>90th centile) for their gestational age at birth had significantly larger first-trimester ADAM12 concentrations. There was, however, no significant difference between the top 5th centile (>95th centile) and controls.

With first-trimester PAPP-A, the number of small for gestational age birth weight babies in the 10th, 5th, and 3rd centiles (Figure 3 and Table 2) and babies born below 2.5 kg were found to be significantly reduced, so also with ADAM12. Babies born between 35 and 36 weeks also had significantly lower PAPP-A compared to the controls, which was not found with ADAM12. Large for gestational age births (>90th centile and >95th centile) did not have a significantly higher PAPP-A.

Table 3 shows the detection rates for adverse outcomes where more than 100 cases were available.

### Relationship between ADAM12 and PAPP-A

A small but significant positive correlation was found between log ADAM12 MoM and log PAPP-A MoM in the control group (Pearsons: $r = 0.25, p < 0.0001$)

<table>
<thead>
<tr>
<th>FPR</th>
<th>ADAM12 (%)</th>
<th>PAPP-A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.00</td>
<td>5.00</td>
</tr>
<tr>
<td>24–34 weeks</td>
<td>0.62</td>
<td>3.43</td>
</tr>
<tr>
<td>35–36 weeks</td>
<td>1.68</td>
<td>4.29</td>
</tr>
<tr>
<td>&lt;1.5 kg</td>
<td>0.00</td>
<td>6.45</td>
</tr>
<tr>
<td>&lt;2.5 kg</td>
<td>1.27</td>
<td>6.48</td>
</tr>
<tr>
<td>&lt;3rd centile</td>
<td>1.30</td>
<td>10.39</td>
</tr>
<tr>
<td>&lt;5th centile</td>
<td>1.34</td>
<td>8.60</td>
</tr>
<tr>
<td>&lt;10th centile</td>
<td>1.52</td>
<td>7.16</td>
</tr>
<tr>
<td>&gt;90th centile</td>
<td>0.74</td>
<td>4.44</td>
</tr>
</tbody>
</table>

### Table 2—Median marker values and interquartile range (IQR) in a range of adverse outcomes showing the significance ($p$) from controls (denotes statistical significance)

<table>
<thead>
<tr>
<th>n (%)</th>
<th>ADAM12 median MoM (IQR)</th>
<th>p</th>
<th>PAPP-A median MoM (IQR)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>414 (19.54)</td>
<td>1.009 (0.775–1.299)</td>
<td>0.9860</td>
<td>0.972 (0.581–1.528)</td>
</tr>
<tr>
<td>IUFD</td>
<td>61 (8.88)</td>
<td>0.986 (0.725–1.417)</td>
<td>0.4210</td>
<td>1.000 (0.599–1.544)</td>
</tr>
<tr>
<td>24–34 weeks</td>
<td>321 (15.15)</td>
<td>1.031 (0.763–1.362)</td>
<td>0.6759</td>
<td>0.952 (0.656–1.327)</td>
</tr>
<tr>
<td>35–36 weeks</td>
<td>536 (25.29)</td>
<td>1.015 (0.773–1.251)</td>
<td>0.3722</td>
<td>1.003 (0.555–1.549)</td>
</tr>
<tr>
<td>&lt;1.5 kg</td>
<td>124 (5.85)</td>
<td>0.988 (0.671–1.219)</td>
<td>0.0040*</td>
<td>0.872 (0.565–1.327)</td>
</tr>
<tr>
<td>&lt;2.5 kg</td>
<td>787 (37.15)</td>
<td>0.941 (0.712–1.208)</td>
<td>0.7668</td>
<td>0.974 (0.826–1.327)</td>
</tr>
<tr>
<td>&gt;4.5 kg</td>
<td>6 (0.28)</td>
<td>0.823 (0.663–1.115)</td>
<td>&lt;0.0001*</td>
<td>0.814 (0.554–1.251)</td>
</tr>
<tr>
<td>&lt;3rd centile</td>
<td>308 (14.54)</td>
<td>0.856 (0.664–1.096)</td>
<td>0.0001*</td>
<td>0.855 (0.777–1.263)</td>
</tr>
<tr>
<td>&lt;5th centile</td>
<td>523 (24.68)</td>
<td>0.885 (0.688–1.134)</td>
<td>0.0001*</td>
<td>0.893 (0.614–1.318)</td>
</tr>
<tr>
<td>&lt;10th centile</td>
<td>921 (43.46)</td>
<td>0.924 (0.709–1.184)</td>
<td>0.0001*</td>
<td>1.024 (0.798–1.540)</td>
</tr>
<tr>
<td>&gt;90th centile</td>
<td>135 (6.37)</td>
<td>1.117 (0.881–1.343)</td>
<td>0.0482*</td>
<td>1.052 (0.812–1.424)</td>
</tr>
<tr>
<td>&gt;95th centile</td>
<td>81 (3.82)</td>
<td>1.092 (0.882–1.277)</td>
<td>0.3868</td>
<td>1.052 (0.812–1.424)</td>
</tr>
</tbody>
</table>
Relationship between markers and maternal age

In the control data, a small but insignificant positive correlation was found between log ADAM12 MoM and maternal age (Pearsons: \( r = 0.09, p = 0.0723 \)), and no such correlation was found with log PAPP-A MoM and maternal age (\( p = 0.4427 \)).

ADAM12 stability

ADAM12 in serum had poor stability. When stored at room temperature, over 50% of the immunoreactivity was lost within 3 days. Stability was improved when stored at 4\(^\circ\)C, such that 50% of the immunoreactivity was lost within 9 days (Figure 4). Blood that was left to clot for 2 or 24 h before centrifugation and serum removal had unchanged levels of ADAM12. However, when left for 48 h before centrifugation, the ADAM12 concentration fell by 11.0%, on average, compared with freshly spun blood (\( p = 0.0453 \)). These results clearly hint at some of the issues regarding the stability of ADAM12 or its immunoreactivity. In our present study, sample stability should not be an issue because samples were collected in the One-Stop clinic and separated, analysed, and stored at 4\(^\circ\)C prior to archiving at −20\(^\circ\)C at the end of the working day.

Smoking

Maternal smoking status was known for 2084 of the 2119 mothers (98.35%). Of these, 1634 (78.41%) claimed to be non-smokers and the remaining 450 (21.59%) said they were smokers. The mean birth weight centile (the rank percentage that a birth weight appears in babies born at that gestational age) was significantly lower in smokers (25.66) compared to non-smokers (33.11) (\( p < 0.0001 \)). The crude birth weights were also significantly lower in smokers (2.57 kg) compared to non-smokers (2.70 kg) (\( p = 0.0004 \)).

Prevalence of smoking was higher in younger women, the median age of the smoking population was 27.0 and that of non-smoking was 29.6 (\( p < 0.0001 \)).

The control population was studied to see the effect of smoking on marker levels. Non-smokers had a median weight-corrected ADAM12 MoM of 1.024, which decreased to 0.917 in smokers (mean log MoM 0.00147 versus −0.04315; \( p = 0.03 \); SD = 0.177 versus 0.153). Median weight-corrected PAPP-A MoM reduced from 1.024 to 0.952 from non-smokers to smokers (mean log MoM 0.02709 versus −0.02216; \( p = 0.0577 \); SD = 0.226 versus 0.248).

DISCUSSION

Maternal age was significantly higher in the pregnancies with the following adverse outcomes: 24–34 weeks delivery, <1.5 kg birth weight, and babies born in the < 90th or < 95th centiles of birth weight for gestational age. A recent review found that increased maternal age is associated with preterm birth and small for gestational age babies (Newburn-Cook and Onyskiw, 2005). We found no previous reports of increased maternal age being associated with large for gestational age babies. Median BMI was significantly higher in < 95th centile babies and >4.5 kg babies, and significantly lower in babies born in < 3rd, 5th and 10th centiles of birth weight for gestational age, as previously associated (Wolfe et al., 1991). Incidence of smoking was significantly higher in IUFD, early preterm delivery (24–34 weeks), low birth weight (<2.5 kg), and small for gestational age (all groups), as expected (Bernstein et al., 2005; Vielwerth et al., 2006).

ADAM12 concentrations increase from 10 to 14 weeks in normal pregnancies in a similar way to what has been previously demonstrated with PAPP-A (Ong et al., 2000), and reflect the temporal growth of

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**Figure 4**—Decrease in ADAM12, detectable by PE DELFIA assay, at room temperature and 4\(^\circ\)C as a percentage of \( t = 0 \)
the placenta producing these proteins. Although early studies with ADAM12 also showed an increase in its concentration with gestation (Laigaard et al., 2003), a more recent study (Laigaard et al., 2006b) showed a decrease, reaching a plateau at 115 days and then increasing again. We have no explanation for these differences. Maternal weight was correlated to ADAM12 concentrations in the way expected, by haemodilution and increase in blood volume.

ADAM12 and PAPP-A are produced by trophoblasts during pregnancy, (Gilpin et al., 1998; Guibourdenche et al., 2003) and are released into the fetal blood and cross the placenta into the maternal circulation. The concentrations of markers in maternal blood are assumed to reflect those in the fetal blood.

We have shown that ADAM12 and PAPP-A concentrations at 10–14 weeks of gestation are lower in pregnancies that resulted in low for gestational age birth weights and all birth weights below 2.5 kg. ADAM12, but not PAPP-A, was found to be higher at this gestational age range in the pregnancies that resulted in large for gestational age birth weights. These findings are compatible with ADAM12 and PAPP-A promoting growth and development by breaking down IGFBPs.

Approximately 75% of IGFs in circulation are latent, bound in a 150 kDa ternary complex with IGFBP-3 and an acyl labile subunit (ALS) (Baxter and Martin, 1989). While in this complex, IGFs will remain in the vasculature, possibly exiting into the tissues after the loss of the ALS by breakdown of this ternary structure by glycosaminoglycans on the endothelial cells lining the blood vessels (Baxter, 1990). A protease breaking down IGFBP-3 would free IGFs allowing them to bind to IGF receptors and instigate mitogenic signalling pathways. However, once free from the complex, IGFs have dramatically reduced half lives (Hodgkinson et al., 1989), therefore, if they are released far from their receptors they are more likely to get broken down before carrying out their biological effect. In this respect, IGFBPs have been speculated to promote the mitogenic effects of IGFs (Elgin et al., 1987), although the results from our study support the notion that the inhibitory actions of the IGFBPs are more expressive.

Further to modulating the actions of IGFs, it has been shown that the IGFBPs also carry out IGF-independent actions by binding to their own receptors (Villaudy et al., 1991). Protease of the IGFBPs would also reduce any IGF-independent actions the binding proteins perform.

These results support the view that ADAM12 and PAPP-A are required for the normal growth and development of the fetus. When ADAM12 or PAPP-A production is reduced at 10 to 14 weeks of pregnancy, the birth weight of the baby is lower than expected. This implies that the birth weight outcome at delivery is programmed from at least 10 to 14 weeks into the pregnancy.

PAPP-A has been shown to be a relatively specific protease to IGFBP-4 (Lawrence et al., 1999), and ADAM12 to IGFBP-3 and IGFBP-5 (Loechel et al., 2000), and the differences in their specificity may explain the differences in significance between the two markers and different pregnancy complications. However, ADAM12 and PAPP-A levels are significantly, but not strongly, correlated, suggesting that the expression of these placental proteins is linked. Further work needs to be carried out to determine whether ADAM12 can add value to a screening program containing the functionally similar PAPP-A.

The ability to detect ADAM12 in serum is new; however, several previous studies have looked at the effects of PAPP-A in the first trimester on predicting adverse outcomes later on in pregnancy. Many studies support the view that IUGR and decreased PAPP-A are associated (Ong et al., 2000; Smith et al., 2002; Yaron et al., 2002; Tul et al., 2003; Krantz et al., 2004), and a large study using the First- And Second-Trimester Evaluation of Risk (FASTER) trial data supported that low PAPP-A concentrations indicate low birth weight (Dugoff et al., 2004). One study claims no significant association between first-trimester PAPP-A values and consequent development of IUGR/SGA, despite finding that the PAPP-A MoM decreases from 0.98 in the control to 0.83 in SGA pregnancies (p = 0.08) (Morssink et al., 1998). Although we could not find a significant increase in PAPP-A in large for gestational age babies in our study, another study found PAPP-A MoM to increase from 1.01 MoM in the controls to 1.12 MoM (p = 0.036) (Tul et al., 2003), and we did find ADAM12 to be significantly higher in the > 90th centile group. Further evidence of the relationship of PAPP-A to fetal growth comes from the work of Leung et al., who showed that levels of PAPP-A were positively correlated with certain second-trimester ultrasound parameters of fetal growth, such as femur length (Leung et al., 2006). Decreased first-trimester PAPP-A levels in pregnancies that resulted in pre term delivery (PTD) have also been previously found (Ong et al., 2000; Smith et al., 2002; Dugoff et al., 2004; Krantz et al., 2004), although two articles stated that it did not change significantly (Morssink et al., 1998; Yaron et al., 2002). Stillbirth, or IUFD after 24 weeks, has been associated with decreased PAPP-A (Smith et al., 2002; Dugoff et al., 2004; Spencer et al., 2006), which we did not find in this study, perhaps due to our relatively small data set for this outcome (n = 61), but this has been shown by one of us in a much larger series (Spencer et al., 2006). Decreased maternal serum PAPP-A has also been linked with increased risks of miscarriage, pregnancy induced hypertension, and gestational diabetes (Ong et al., 2000; Smith et al., 2002; Dugoff et al., 2004), which we did not examine.

With regard to stability, ADAM12 in human serum has been found to be unstable. A previous study showed that ADAM12 would be immeasurable after four days at room temperature (Laigaard et al., 2003); we found ADAM12 to be more stable than this, having over 40% activity still remaining at this time.

Smoking has been linked to low for gestational age and low birth weight deliveries (Bernstein et al., 2005), and our data supports this. At the time of first-trimester screening, the incidence of smoking at different maternal ages followed the same downward trend previously reported (Spencer et al., 2004), although overall prevalence was higher in our sample population due to
smoking-linked adverse outcome case selection. Smoking was found to reduce both marker levels significantly, as previously reported with PAPP-A (Spencer, 1999; de Graaf et al., 2000; Niemimaa et al., 2003). Using a larger sample population, Spencer et al. (2004) found a more marked decrease in the median PAPP-A MoM of smokers compared to non-smokers, than was found in this study. Another recent study found that a decrease in PAPP-A was only found in women who smoked more than five cigarettes a day (Yigit et al., 2006), although dose-related PAPP-A/cigarette smoking relationships have been disputed previously (Spencer et al., 2004). A 13% reduction in levels of ADAM12 was also shown in a previous study of 70 smokers (Laigaard et al., 2006b). A putative relationship associating smoking with a decreased Down syndrome risk has been negated because the negative relationship between smoking and maternal age counteracts the positive relationship between maternal age and Down syndrome risk (Chen et al., 1999).

ADAM12 and PAPP-A are both potential markers for IUGR in pregnancy. PAPP-A is already being used as an important analyte in routine screening for fetal chromosomal anomalies, and the use of ADAM12 is likely to follow (Laigaard et al., 2006b). Therefore, these markers could establish themselves as valuable multipurpose markers in the prenatal screening clinic, allowing more information regarding the status of a pregnancy to be available at no additional cost.

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