PRENATAL DIAGNOSIS
Prenat Diagn 2003; **23**: 1086–1091.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/pd.762

ADAM12: a novel first-trimester maternal serum marker for Down syndrome

Jennie Laigaard[1,2,3,4], Tina Sørensen[2], Camilla Fröhlich[3], Bent Nørgaard Pedersen[2], Michael Christiansen[2], Kirsten Schiøtt[5], Niels Uldbjerg[5], Reidar Albrechtsen[3], Helle V. Clausen[4], Bent Ottesen[1,4] and Ulla M. Wewer[3]*

[1]*Department of Gynecology and Obstetrics, Rigshospitalet, University of Copenhagen, Denmark*
[2]*Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark*
[3]*Institute of Molecular Pathology, University of Copenhagen, Copenhagen, Denmark*
[4]*Department of Gynecology and Obstetrics, Hvidovre University Hospital, Copenhagen, Denmark*
[5]*Department of Gynecology and Obstetrics, Skejby Hospital, Aarhus, Denmark*

Objectives The concentration of bioavailable insulin-like growth factor (IGF) I and II is important to foetal growth. It is regulated by insulin-like growth factor binding proteins (IGFBP) 1 through 6. Proteolytic cleavage of IGFBP-3 takes place in human pregnancy serum; accordingly, IGFBP-3 serum levels decrease markedly during pregnancy. ADAM12 (A disintegrin and metalloprotease) is an IGFBP-3 and IGFBP-5 protease and is present in human pregnancy serum. The goal of this study was to determine whether ADAM12 concentration in maternal serum is a useful indicator of foetal health.

Methods We developed an enzyme-linked immunosorbent assay (ELISA) for the quantification of ADAM12 in serum. The assay range was 42 to 667 μg/L. Recombinant ADAM12 was used as the standard for calibration.

Results We found that ADAM12 was highly stable in serum. Serum concentration increased from 180 μg/L at week 8 of pregnancy to 670 μg/L at 16 weeks, and reached 12 000 μg/L at term. In 18 first-trimester Down syndrome pregnancies, the concentration of ADAM12 was decreased, thus the median multiple of mean (MoM) value was 0.14 (0.01–0.76). A detection rate for foetal Down syndrome of 82% for a screen-positive rate of 3.2% and a 1 : 400 risk cut-off was found by Monte Carlo estimation using ADAM12 and maternal age as screening markers.

Conclusion ADAM12 is a promising marker for Down syndrome. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: ADAM12; prenatal diagnosis; Down syndrome; IGFBP-3 protease; IGFBP-5 protease; PAPP-A

INTRODUCTION

The ADAMs (A disintegrin and metalloprotease) constitute a multi-domain glycoprotein family with proteolytic and cell-adhesion activities (Wolfsberg *et al*., 1995; Primakoff and Myles, 2000; Seals and Courtneidge, 2003). Human ADAM12 exists in two forms, ADAM12-L (long) and ADAM12-S (short), the latter being the secreted form of ADAM12. ADAM12-S differs from ADAM12-L at the C-terminal end in that it does not contain the transmembrane and cytoplasmatic domains (Gilpin *et al*., 1998). ADAM12-S binds to and has proteolytic activity against insulin-like growth factor binding protein (IGFBP)-3 and, to a lesser extent, IGFBP-5. *In vitro* cleavage of the 44-kDa IGFBP-3 by ADAM12-S yields several fragments of 10 to 20 kDa and is independent of insulin-like growth factor (IGF) I and II (Loechel *et al*., 2000). ADAM12-S has been detected by western blotting in pregnant serum, but not in non-pregnant serum (Shi *et al*., 2000; Loechel *et al*., 2000), and the mRNA for ADAM12-S is particularly abundant in placenta (Gilpin *et al*., 1998).

IGF I and II are proinsulin-like polypeptides that are produced in nearly all foetal and adult tissues. Lack of IGF I and II causes foetal growth retardation in mice (Powel-Braxton *et al*., 1993). The cleavage of IGFBPs into smaller fragments with reduced affinity for the IGFs reverses the inhibitory effects of the IGFBPs on the mitogenic and DNA stimulatory effects of the IGFs (Blat *et al*., 1994). Seventy-five percent of the IGFs are bound to IGFBP-3 in plasma (Jones and Clemmons, 1995).

Several biochemical markers are under investigation as screening markers for Down syndrome (DS) and other chromosomal diseases in early pregnancy (Wald and Hackshaw, 2000). One that has come into routine use is an IGF-dependent IGFBP-4 and IGF-independent IGFBP-5 protease namely, pregnancy-associated plasma protein-A (PAPP-A) (Laursen *et al*., 2001; Cuckle and van Lith, 1999), which has also been shown to be of clinical importance as a marker of growth retardation and pre-term birth (Smith *et al*., 2002).

We hypothesised that ADAM12-S is an important indicator of foetal growth because ADAM12-S is an IGFBP-3 protease and IGFBP-3 is the most abundant IGFBP in serum. The proteolysis of IGFBP-3 would

*Correspondence to: Ulla M. Wewer, Institute of Molecular Pathology, University of Copenhagen, Copenhagen, Denmark.
E-mail: ullaw@pai.ku.dk

Received: 1 April 2003
Revised: 15 August 2003
Accepted: 29 September 2003

stimulate growth by increasing levels of bioavailable IGF I and II. Also, since PAPP-A and ADAM12-S are both IGFBP-5 proteases synthesised by the placenta, ADAM12 is a logical candidate for investigation as an indicator of foetal abnormalities.

In the present study, we developed an enzyme-linked immunosorbent assay (ELISA) for the quantification of ADAM12 and used it to assess ADAM12 as a first- and second-trimester marker for DS.

MATERIALS AND METHODS

Serum samples

Normal samples: Serum samples from first-trimester pregnant women ($n = 154$) were obtained as part of a routine prenatal screening programme for DS at Skejby University Hospital, Aarhus, Denmark. The programme is specifically for women who are 8- to 13-weeks pregnant, and includes ultrasound examination. Second-trimester serum samples ($n = 91$) were obtained through another routine prenatal screening programme for severe malformations and DS at Statens Serum Institut. This screening programme is restricted to women who are 14- to 20-weeks pregnant. All first- and second-trimester samples were taken in dry containers and were kept in cool storage (4 °C) until postage via the normal mail. Term samples ($n = 10$) were obtained from women participating in a pilot study for ADAM12 at Hvidovre University Hospital, Copenhagen. The women were 38- to 40-weeks pregnant and all had a normal pregnancy with no obstetrical complications prior to sampling. An apparently healthy 28-weeks pregnant employee at Statens Serum Institut donated blood for the storage temperature and blood container comparison. Term samples and samples for the storage temperature and blood container comparison were centrifugated and kept at −20 °C until the time of study and/or analysis.

DS samples: First-trimester DS samples ($n = 18$) consisted of samples from the Skejby screening programme ($n = 3$), which were identified as a result of the screening programme that is, in the first trimester, and samples from the ongoing quality-control programme at Statens Serum Institut ($n = 15$) that were diagnosed during second trimester ($n = 10$) or at birth ($n = 5$). Second-trimester DS samples ($n = 12$) were all from the ongoing quality-control programme at Statens Serum Institut and comprised of samples diagnosed in the second trimester ($n = 8$) or at birth ($n = 4$).

All DS diagnoses were established by karyotyping. Gestational age was determined by the date of the last menstrual period and, in most cases, confirmed by ultrasound examination.

Ethics

All samples were either collected for projects approved by the Scientific Ethics committees of Aarhus or Copenhagen County or were obtained as part of ongoing quality-control procedures of Statens Serum Institut.

Reagents

Recombinant ADAM12-S used for standardisation was obtained by transfecting human 293-EBNA cells with full-length cDNA encoding human ADAM12-S, and purified using cation-exchange and concanavalin A affinity chromatography as described by Loechel *et al.* (2000). Protein concentration was determined using the biconchonic acid (BCA) protein kit assay (Pierce, Rockford IL).

Antibodies

Several previously described antibodies against recombinant ADAM12 were tested in this study: monoclonal antibodies 8F8, 6E6, and 6C10; and polyclonal antibodies rb 122 and rb 134 (Gilpin *et al.*, 1998; Iba *et al.*, 1999; Kawaguchi *et al.*, 2002; Kronqvist *et al.*, 2002). On the basis of pilot studies comparing the effectiveness of all the antibodies in the ELISA, the antibodies 6E6 and 8F8 were used for coating and detection steps respectively.

Biotinylation

To generate biotinylated antibodies, 8F8 IgG was transferred to a labelling buffer consisting of 0.1M $NaHCO_3$ (pH 8.2) (Merck, Darmstadt, Germany), using NAP™ 5 columns (Amersham Biosciences, Sweden). The concentration was calculated from the absorbance at 280 nm using labelling buffer as a reference. A mixture of 100 mg of Biotin (Sigma 1759) dissolved in 2.5 mL Dimethylformamide (LabScan, Valby-Denmark) was added to the antibody (10 μL/mg). After mixing for 2 h at room temperature, biotinylated antibodies were purified by gel filtration using PD-10 columns (Amersham Biosciences, Sweden). The concentrations of biotinylated monoclonal antibody were calculated from the absorbance readings at 280 nm.

Standards and controls

Controls were prepared from a second-trimester serum pool diluted (83 μg/L, 164 μg/L, and 335 μg/L) in dilution buffer consisting of 1% (v/v) bovine serum albumin (BSA)(Sigma A 4503) and 0.05% (v/v) Tween 20 (Merck) in a 0.15 M phosphate-buffered saline (PBS) solution. We calibrated a third-trimester serum pool against recombinant ADAM12, and used the pool to generate a standard curve for determining ADAM12 concentrations. Standards ranging from 42 to 667 μg/L were prepared by diluting the serum in dilution buffer.

All standards, controls, and samples were analysed in duplicate.

ELISA procedures, optimisation, and testing

Microtiter plates (Nunc-Immuno™ Plate, MaxiSorp™ Surface, Nalge Nunc International-Denmark) were

coated with 0.41 μg/well monoclonal antibody 6E6 in 0.1 M carbonate buffer (pH 9.6). Plates were washed twice after overnight incubation at 4 °C. All washing steps were done with washing buffer consisting of 0.1 M PBS with 0.05% (v/v) Tween 20 (Merck 822184). A buffer consisting of 1% (v/v) BSA (Sigma A 4503) in wash buffer was then added to the plates (150 μL/well) to block non-specific binding. The plates were incubated in blocking buffer for 30 min at room temperature then washed three times. Standards, controls, and samples diluted in blocking buffer were added (100 μL/well), incubated for two hours at room temperature, then washed four times. Biotinylated monoclonal antibody 8F8 (0.5 μg/mL) was added and plates were incubated for 1 h at room temperature, then washed four times. Peroxidase-conjugated streptavidin (DAKO P397, Denmark) was added (100 μL/well), incubated for 1 h at room temperature, then washed three times. A colour reaction was obtained by adding 100 μL/well of a solution consisting of *ortho*-phenylene-diamine (OPD) tablets (Kem-En-Tec, Copenhagen, Denmark) and hydrogen peroxide dissolved in citric acid buffer (pH 5.0), and incubating for 30 min at room temperature. One hundred and fifty microlitres (10%v/v) of sulphuric acid was added to stop the colour reaction, and the reaction intensity was measured by spectrometry (490–620 nm) (Victor, PLS-Wallac, Turku, Finland). To assess the intra- and inter-assay variability, we analysed the same samples six times in the same run and repeated the same run for six days in a row.

Stability of ADAM12: To investigate the stability of ADAM12, we conducted repeated freeze–thaw tests. This study was conducted using the third-trimester serum pool, and individual aliquots were analysed twice. Recombinant ADAM12 was stored at −20 °C.

We also investigated the temperature stability of ADAM12 in serum. Samples were taken from one venipuncture of an apparently healthy employee at Statens Serum Institut in her third trimester of pregnancy. Serum was separated into aliquots immediately after centrifugation: 15 aliquots were kept at room temperature, 10 at 4 °C, and 10 at 37 °C. All samples were analysed in the same run.

To determine the significance of the type of blood container used, we analysed serum/plasma from a single subject taken in EDTA, citrate, heparin, and dry blood containers. All samples were obtained in one venipuncture and handled under identical conditions.

Clinical assessment

Down syndrome: To investigate the value of ADAM12 concentration as a screening marker for DS, we compared the ADAM12 values from the pregnancies with confirmed DS with the median value for maternal serum ADAM12 concentration from non-DS pregnancies at the same gestational age. As only three of the first-trimester DS samples were recent and collected together with controls, whereas 15 DS cases were stored at −20 °C for up to several years, we examined the MoM values separately.

PAPP-A and beta human chorionic gonadotrophin (βhCG) versus ADAM12: To establish a relation between ADAM12, PAPP-A, and βhCG in first-trimester serum samples, we used 154 unaffected and 3 DS samples from the Skejby sceening programme for which PAPP-A and βhCG analyses had previously been performed (Schiøtt *et al*., in preparation). In addition, we used 15 DS samples from Statens Serum Institut where the same analysis for βhCG and PAPP-A had been performed.

ADAM12 throughout pregnancy: To assess the changes in serum levels of ADAM12 throughout pregnancy, we used the analysis of 154 first-trimester serum samples, 91 second-trimester serum samples, and 10 term-serum samples, that is, all the unaffected serum samples.

Statistics

Median serum ADAM12 concentrations were estimated by linear regression of the logarithm_{10} ADAM12 concentration on gestational age (days). Gestational age was determined according to the last menstrual period and confirmed by ultrasound (CRL or BPD) in most cases. All concentrations were transformed into multiples of the calculated medians (MoMs) of the unaffected pregnant women. Compatibility with the normal distribution was ascertained using normal plots. Correlations were performed by using Pearson's correlation coefficient. The screening efficiency of ADAM12 alone, or in combination with other markers, was assessed by the receiver–operator-characteristics (ROC)-analysis made by Monte Carlo simulation using published procedures (Larsen *et al*., 1998). A standardised age distribution of pregnant women was used (van der Veen *et al*., 1997). The *a priori*, age-related risk of giving birth to a DS child was taken from Cuckle *et al*. (1987). The distribution parameters for the markers PAPP-A, βhCG, and nuchal translucency (NT) were taken from a published meta-analysis (Cuckle and van Lith, 1999).

RESULTS

ADAM12 ELISA and stability

Figure 1A shows that coating with the monoclonal antibodies 6E6 and 8F8 resulted in the greatest absorbance. Background levels were high when 8F8 was used as the coating antibody; thus, we used 6E6 as catching antibody adsorbed on the polystyrene wells, and 8F8 as the biotinylated detector antibody. The ADAM12 ELISA has an assay range of 42 to 667 μg/L (Figure 1B). The intra- and inter-assay coefficients of variation were 5 and 13% respectively. Dilution curves of recombinant

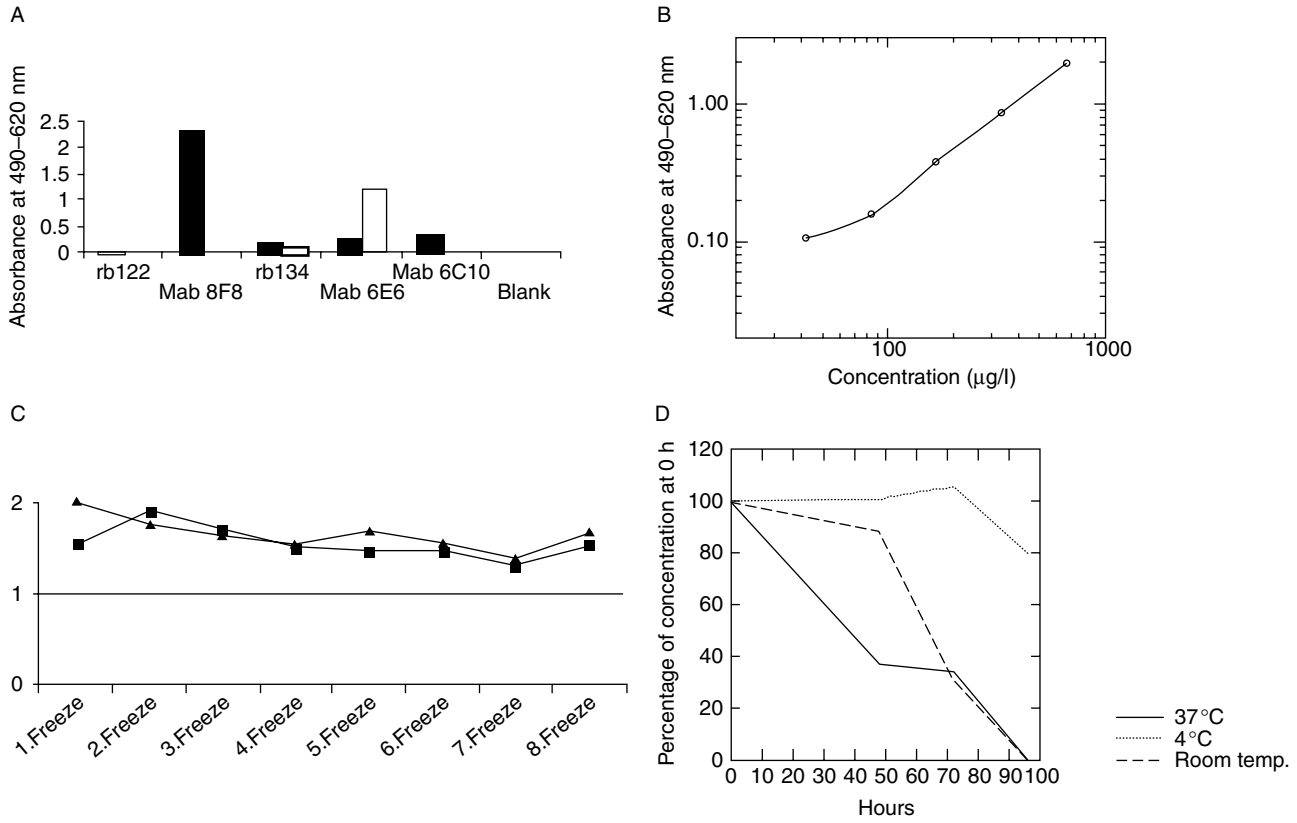

Figure 1—(A) Tests to determine the optimal coating antibodies. Monoclonal antibodies (Mab) 8F8, 6E6 and 6C10 polyclonal rabbit antibodies (rb) 122 and 134 were used to coat ELISA dishes. The biotinylated antibody used was 6E6 (black columns) or 8F8 (white columns). (B) Standard curve for ADAM12. Standard curves of absorbance at 490 to 620 nm using 6E6 for coating and 8F8 as the biotinylated antibody for detection. (C) Freeze thaw. Results of the analysis of serum samples after repeated freezing and thawing. (D) Stability of ADAM12 in serum. The stability of ADAM12 at different storage temperatures is shown over time after venipuncture and centrifugation

ADAM12 standards and pregnancy serum were linear and parallel.

Recombinant ADAM12 stored at −20 °C began to degrade after approximately four months. No degradation could be detected in ADAM12 in serum after six months of storage at −20 °C. We found that ADAM12 is stable through at least eight cycles of freezing and thawing (Figure 1C). Serum samples are stable for 42 h at room temperature, and storage at 4 °C prolongs the stability to four days (Figure 1D). The analysis of plasma samples taken in different blood containers showed that ADAM12 was barely detectable in plasma taken in EDTA containers. Heparin and citrate containers were equivalent to dry containers with regard to detectable serum ADAM12 levels.

ADAM12 in pregnancy

We found an approximate 60-fold increase of ADAM12 in serum throughout pregnancy. The median at 8 weeks' gestation was 180 µg/L, while the median at term (after 38 full weeks) for a normal pregnancy was 12 000 µg/L. The 154 first-trimester serum samples showed a significant increase with gestational age. Since the slope of the increase with gestational age that occurs in the first trimester decreased after week 10, we performed two log-linear regressions: one for samples with gestational age under or equal to 70 days, and another for gestational age above 70 days. Log-linear regression resulted in median values of 180 µg/L at 8 weeks, 262 µg/L at 9 weeks, 383 µg/L at 10 weeks, and 450 µg/L at 11 weeks (Figure 2). The analysis of second-trimester serum samples ($n = 91$) and linear regression showed a slower rate of increase with median values of 592 µg/L at 14 weeks, 630 µg/L at 15 weeks, 670 µg/L at 16 weeks, and 712 at 17 weeks. Residuals were normally distributed. Analysis of term serum ($n = 10$) showed a median of 12 000 µg/L. We observed a significant correlation ($r = 0.25$, $p < 0.01$) between log MoM ADAM12 and log MoM PAPP-A, and between log MoM ADAM 12 and log MoM βhCG ($r = 0.16$, $p < 0.05$) in the first trimester, but no correlation between log MoM ADAM12 and maternal age ($r = 0.05$, $p > 0.05$) or NT ($p > 0.05$). The first-trimester log MoM distribution of ADAM12 concentrations was compatible with a Gaussian distribution with a mean log MoM of 0.00 and standard deviation of 0.28.

ADAM12 in Down syndrome pregnancies

The analysis of first-trimester serum samples from pregnancies with confirmed DS ($n = 18$) showed

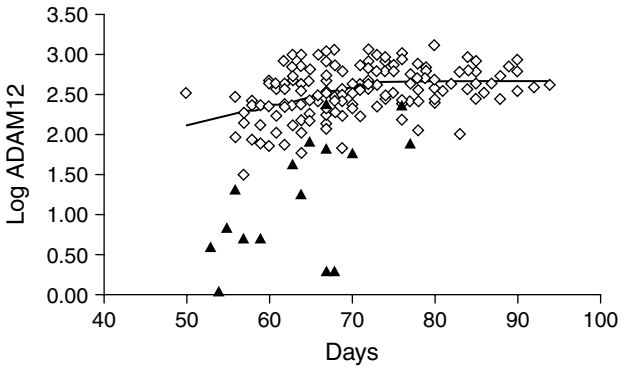

Figure 2—(A) First-trimester scatter plot of log 10 ADAM12 versus days. Black triangles are values for Down syndrome

decreased ADAM12 concentrations, that is, between 0 and 246 μg/L (Figure 2). The median MoM value was 0.14 (0.01–0.76). The mean MoM of the stored supplementary first-trimester samples was 0.17 and the mean MoM of the samples from the first-trimester screening programme was 0.29. The corresponding median MoM PAPP-A value was 0.36 (0.02–1.04). In affected pregnancies ($n = 18$), the mean log MoM ADAM12 of -1.28 and standard deviation of 0.78 was significantly lower ($p < 0.001$) than in normal pregnancies.

Table 1 demonstrates a high estimated screening performance of ADAM12 in combination with other first-trimester screening markers at risk cut-offs of 1 : 200 and 1 : 400 for giving birth to a DS child. In the second trimester, ADAM12 in DS pregnancies did not differ from ADAM12 in normal pregnancies. Thus, no further analysis of diagnostic efficiency was made for ADAM12 in the second trimester.

DISCUSSION

We have confirmed that ADAM12 is a pregnancy-associated protein (Shi *et al*., 2000; Loechel *et al*., 2000) and have successfully developed an ELISA method to measure ADAM12 throughout pregnancy. The concentration of ADAM 12 in maternal serum increases 60-fold during gestation, and is markedly decreased in the first trimester in pregnancies with foetal Down syndrome. Table 1 shows that ADAM12 appears to be an efficient maternal serum marker for DS. The discrimination is better than with any other established first-trimester marker.

Table 1—Screening performance of different screening markers at the cut-off levels 1 : 200 and 1 : 400

	Risk > 1 : 200		Risk > 1 : 400	
Markers	DR	SPR	DR	SPR
ADAM12 and age	77.7	1.5	81.5	3.2
PAPP-A and age	52.3	5.1	66.2	11.2
βhCG and age	42.4	5.1	59.9	12.9
NT and age	67.4	2.8	74.3	5.9
ADAM12 and Age and βhCG	82.8	1.5	86.3	3.1
ADAM12 and age and βhCG and PAPP-A	85.4	1.6	88.7	3.0
ADAM12 and age and βhCG and PAPP-A and NT	92.4	0.8	94.1	1.5

The finding that the serum concentration of IGFBP-3 decreases markedly from six weeks' gestation to term, and that the pregnancy-specific proteolytic cleavage results in 29 to 30, 19 and 15 kDa fragments (Guidice *et al*., 1990; Hossenlopp *et al*., 1990; Bang and Fielder, 1997), is consistent with the finding that ADAM12-S cleaves IGFBP-3 into 10 to 20 kDa fragments (Loechel *et al*., 2000) and thus may be one of the putative IGFBP-3 proteases in pregnancy serum. Furthermore, our finding that the concentration of ADAM12 increases 60-fold during pregnancy adds to the explanation of the decrease in IGFBP-3 concentration. Additional support is provided in the results reported by Irwin *et al*. (2000) showing that human placental trophoblasts secrete a disintegrin and metalloprotease that cleaves IGFBP-3, is active at neutral and alkaline pH, and is sensitive to *o*-phenanthroline. The protease secreted by trophoblasts could be ADAM12 because mRNA for ADAM12 is particularly abundant in the placenta (Gilpin *et al*., 1998; Loechel *et al*., 1998), and ADAM12 has the same apparent characteristics (Loechel *et al*., 2000).

The finding by Langford *et al*. (1995) showing elevated levels of IGFBP-3 protease in third-trimester gestational serum in pregnancies with utero-placental insufficiency, and the suggested role of the IGFBP-5 protease, PAPP-A, as a predictor of intrauterine growth retardation (Smith *et al*., 2002; Morssink *et al*., 1998) make ADAM12 an interesting candidate for future studies exploring its role as a predictor of adverse pregnancy outcomes in addition to DS.

Our stability tests showed ADAM12 to be highly stable with routine handling; thus, we conclude that ADAM12 is an attractive analyte for clinical use. While further prospective studies are clearly needed, the data presented here suggest that ADAM12 is a potentially valuable marker for use in prenatal screening.

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance of Bente Bjoernskov. This study was supported by the Faculty of Health Sciences, University of Copenhagen (J.L.) and by the Danish Cancer Society and the Neye Foundation (U.W.).

REFERENCES

Bang P, Fielder PJ. 1997. Human pregnancy serum contains at least two distinct proteolytic activities with the ability to degrade insulin-like growth factor binding protein-3. *Endocrinology* **138**: 3912–3917.

Blat C, Villaudy J, Binoux M. 1994. In vivo proteolysis of serum insulin-like growth factor (IGF) binding protein-3 results in

increased availability of IGF to target cells. *J Clin Invest* **93**: 2286–2290.

Cuckle HS, van Lith JM. 1999. Appropriate biochemical parameters in first-trimester screening for Down syndrome. *Prenat Diagn* **19**: 505–512.

Cuckle HS, Wald NJ, Thompson SG. 1987. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. *Br J Obstet Gynaecol* **94**: 387–402.

Gilpin BJ, Loechel F, Mattei MG, Engvall E, Albrechtsen R, Wewer UM. 1998. A novel secreted form of human ADAM12 (meltrin alpha) provokes myogenesis in vivo. *J Biol Chem* **273**: 157–166.

Guidice LC, Farrel EM, Pham H, Lamson G, Rosenfeld RG. 1990. Insulin-like growth factor binding proteins in maternal serum throughout gestation and in the puerperium: effects of a pregnancy-associated serum protease activity. *J Clin Endocrinol Metab* **71**: 806–816.

Hossenlopp P, Segovia B, Lassarre C, Roghani M, Bredon M, Binoux M. 1990. Evidence of enzymatic degradation of insulin-like growth factor-binding proteins in the 150K complex during pregnancy. *J Clin Endocrinol Metab* **71**: 797–805.

Iba K, Albrechtsen R, Gilpin BJ, Loechel F, Wewer UM. 1999. Cysteine-rich domain of human ADAM12 (meltrin α) supports tumor cell adhesion. *Am J Pathol* **154**: 1489–1501.

Irwin CJ, Suen L, Cheng B, *et al*. 2000. Human placental trophoblasts secrete a disintegrin metalloprotease very similar to the insulin-like growth factor binding protein-3 protease in human pregnancy serum. *Endocrinology* **141**: 666–674.

Jones JI, Clemmons DR. 1995. Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev* **16**: 3–34.

Kawaguchi N, Xu X, Tajima R, *et al*. 2002. ADAM12 protease induces adipogenesis in transgenic mice. *Am J Pathol* **160**: 1895–1903.

Kronqvist P, Kawaguchi N, Albrechtsen R, *et al*. 2002. ADAM12 alleviates the skeletal muscle pathology in mdx dystrophic mice. *Am J Pathol* **161**: 1535–1540.

Langford KS, Nicolaides KH, Jones J, Abbas AA, McGregor AM, Miell JP. 1995. Serum insulin-like growth factor binding protein-3 (IGFBP-3) levels and IGFBP-3 protease activity in normal, abnormal and multiple human pregnancy. *J Clin Endocrinol Metab* **80**: 21–27.

Larsen SO, Christiansen M, Nørgaard-Pedersen B. 1998. Calculation of roc curves in multidimensional likelihood ratio based screening with Down's syndrome as a special case. *J Med Screen* **5**: 57–62.

Laursen LS, Overgaard MT, Soe R, *et al*. 2001. Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP-5) independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. *FEBS Lett* **504**: 36–40.

Loechel F, Gilpin BJ, Engvall E, Albrechtsen R, Wewer UM. 1998. Human ADAM12 (meltrin α) is an active metalloprotease. *J Biol Chem* **273**: 16 993–16 997.

Loechel F, Fox JW, Murphy G, Albrechtsen R, Wewer UM. 2000. ADAM 12-S cleaves IGFBP-3 and IGFBP-5 and is inhibited by TIMP-3. *Biochem Biophys Res Commun* **278**: 511–515.

Morssink LP, Kornman LH, Hallahan TW, *et al*. 1998. Maternal serum levels of free beta-hCG and PAPP-A in the first trimester of pregnancy are not associated with subsequent fetal growth retardation or preterm delivery. *Prenat Diagn* **18**: 147–152.

Powel-Braxton L, Hollingshead P, Warburton C, *et al*. 1993. IGF-I is required for normal embryonic growth in mice. *Genes Dev* **7**: 2609–2617.

Primakoff P, Myles DG. 2000. The ADAM gene family: surface proteins with adhesion and protease activity. *Trends Genet* **16**: 83–87.

Seals DF, Courtneidge SA. 2003. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev* **17**: 7–30.

Shi Z, Xu W, Loechel F, Wewer UM, Murphy LJ. 2000. ADAM12, a disintegrin and metalloprotease, interacts with insulin-like growth factor-binding protein-3. *J Biol Chem* **275**: 18 574–18 580.

Smith GCS, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. 2002. Early pregnancy levels of pregnancy-associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *J Clin Endocrinol Metab* **87**: 1762–1767.

van der Veen J, Beekhuis JR, Cornel MC, Mantingh A, de Walle HE, de Wolf BT. 1997. A demographic approach to the assessment of Down syndrome screening performance. *Prenat Diagn* **17**: 717–724.

Wald NJ, Hackshaw AK. 2000. Advances in antenatal screening for Down syndrome. *Bailliereres Best Pract Res Clin Obstet Gynaecol* **14**: 563–580.

Wolfsberg TG, Primakoff P, Myles DG, White JM. 1995. ADAM, a novel family of membrane proteins containing a disintegrin and metalloprotease domain: multipotential functions in cell-cell and cell-matrix interactions. *J Cell Biol* **131**: 275–278.

