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ADAM 12 may be used to reduce the false positive rate of first trimester combined screening for Down syndrome

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Background ADAM12 has been shown to be an efficient maternal serum marker for Down syndrome (DS) in the first trimester; but recent studies, using a second generation assay, have not confirmed these findings. We examined the efficiency of a second generation assay for ADAM12.

Materials and Methods ADAM12 concentrations were determined in 28 first trimester DS and 503 control pregnancies using a novel Research Delfia^{R} ADAM12 kit. Log10MoM distributions of ADAM12 and correlations with other markers were established. Population performance of screening was estimated by Monte Carlo simulation.

Results ADAM12 was significantly reduced in the first trimester in DS pregnancies with a log10MoM of −0.1621 (equivalent to 0.68 MoM) ($p < 0.001$). The reduction decreased with advancing gestational age. ADAM12 used with PAPP-A+hCGβ+NT (CUB screening) increased the detection rate (DR) from 86% to 89% for a false positive rate (FPR) of 5%. When used for a fixed DR of 90%, the addition of ADAM12 resulted in a 25% reduction of the FPR.

Conclusion ADAM12 is a moderately effective DS marker. It is not a cost-effective addition to CUB screening, but may be used to reduce the FPR in selected high-risk cases. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: Prenatal screening; first trimester; Monte Carlo simulation

INTRODUCTION

A disintegrin and metalloprotease-12 (ADAM) (ADAM12) is a multidomain and multifunction protein involved in adhesion, proteolysis, myoblast fusion and cellular signalling (Wewer *et al*., 2005). It has insulin like growth factor binding protein (IGFBP)-3 and -5 protease (Loechel *et al*., 2000) and heparin-binding EGF like growth factor (HB-EGF) sheddase activity(Asakura *et al*., 2002) and is thus part of the insulin like growth factor (IGF)- and EGF like growth factor (EGF)-axes. It is synthesised by placenta (Gilpin *et al*., 1998; Shi *et al*., 2000) and found in increasing concentrations in maternal serum during pregnancy (Laigaard *et al*., 2003a), whereas the concentration in non-pregnant serum is negligible (Gilpin *et al*., 1998; Shi *et al*., 2000).

The maternal serum concentration of the secreted form of ADAM12 has been shown to be reduced in the early first trimester in pregnancies with a Down syndrome (DS) (Laigaard *et al*., 2006a,b, 2003a), trisomy 18, trisomy 13 (Laigaard *et al*., 2005a; Spencer and Cowans, 2007), Turner syndrome or triploid fetus (Poon *et al*., 2009). In the second trimester the ADAM12 concentration has been found to be increased in DS (Christiansen *et al*., 2007) and trisomy 18 pregnancies (Spencer and Cowans, 2007). Furthermore, the first trimester maternal serum concentration of ADAM12 has been shown to be reduced in pregnancies with fetal growth disturbances (Cowans and Spencer, 2007; Pihl *et al*., 2008; Poon *et al*., 2008) and in pregnancies complicated by pre-eclampsia (Laigaard *et al*., 2005b). The latter finding could not, however, be confirmed by a later study (Poon *et al*., 2008).

The early findings - described above, demonstrating that ADAM12 is a very efficient DS marker, and that ultra-low ADAM12 MoM values are found in the early first trimester DS pregnancies - have not been confirmed by a couple of recent studies (Spencer *et al*., 2008; Poon *et al*., 2009). One reason for this discrepancy may be a difference in the assays employed for ADAM12 quantification. The first assay, first generation, developed for quantification of ADAM12 (Laigaard *et al*., 2003) was based on two monoclonal antibodies, 6E6 and 8F8, both reacting with the disintegrin domain of ADAM12 (Wewer *et al*., 2006) and recombinant ADAM12 was used as standard (Loechel *et al*., 2000). This assay was also formulated for use on the semi-automatic AutoDELFIA platform (Laigaard *et al*., 2005b). Presently the only commercially available assay, the AutoDELFIA/DELFIA Research ADAM12 kit utilises one of the previously used antibodies, but the other has been exchanged. This has increased robustness. However, due to the complicated structure of ADAM12

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with a non-covalently bound prodomain and evidence of dimerisation (Wewer *et al*., 2006) different assays could result in quantification of different conformational variants of ADAM12, and this could explain different discriminatory abilities of different assays.

Here we assess the performance of a second-generation ADAM12 assay, the AutoDELFIA/DELFIA Research ADAM12 kit, with respect to the ability to identify DS pregnancies. Furthermore, we estimate the population screening performance of ADAM12, determined with the second generation assay, in combination with other markers.

MATERIALS

Serum samples

Sera from 28 pregnancies, median age 36 years (range: 25–44 years) with a DS fetus, four of which ended with the birth of a DS child, were obtained from the Prenatal Registry at Statens Serum Institut, Copenhagen. The median gestational age of the sampling was 69 days (range: 56–98). All were singleton pregnancies and the diagnosis was verified from the national registry of cytogenetic diagnoses, Danish Cytogenetic Central Registry (DCCR). Sera from 503 pregnancies, median age 29 years (range: 17–45 years) with a normal course and a liveborn child were obtained from samples received from Holbæk Hospital for routine first trimester screening. The median gestational age of sampling was 67.5 days (range: 56–98 days).

Biochemical measurements

ADAM12 was quantified using the AutoDELFIA^R/Delfia ADAM12 Research kit 4025–0010 (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) on the 1235 AutoDELFIA^R automatic immunoassay system according to the manufacturer's instructions. The assay is a time-resolved solid-phase fluoroimmunometric sandwich assay using two monoclonal antibodies. The dynamic range of the assay was ca. 15 ng/mL–1500 ng/mL ADAM12 (depending on the actual calibration of the calibrators). Three controls, provided by the manufacturer (Sero, Oslo, Norway) were analysed with each run. The controls had the following values: 279, 6 ng/mL (SD: 8.6 ng/mL), 150, 3 ng/mL (SD:2.6 ng/mL) and 40.7 ng/mL (SD: 1.0 ng/mL). The standard curve of the assay is shown in Figure 1 with an imprecision profile based on 10 runs.

Stability analysis

A pool of first trimester pregnancy serum was aliquotted into separate vials that were stored for a variable time at +4 °C, +23 °C, +37 °C or +56 °C. A separate tube was used for each time point and temperature. ADAM12 measurements were performed in duplicate using either the AutoDELFIA® ADAM12 Research kit or a previously described time-resolved fluoroimmunoassay (Laigaard *et al*., 2003). ADAM12 was stable for ten freeze-thaw cycles using either assay (data not shown).

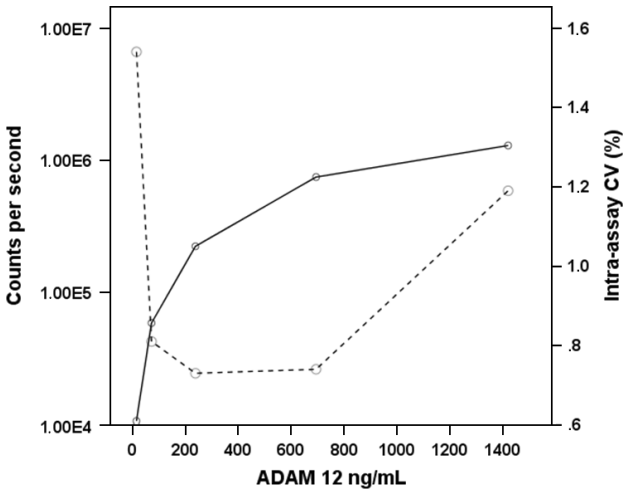

Figure 1—Standard curve (average of 10 runs) of the ADAM12 assay. The average intra-assay variation of ten duplicate determinations are shown (broken curve. CV = coefficient of variation)

Data analysis

Concentration values of ADAM12 were converted to multiples of the median (MoM) values by division with the regressed mean derived from a log-regression of ADAM12 concentrations on maternal weight and gestational age. Due to different relations between gestational age and ADAM 12 concentrations in earlyversus. late first trimester, the calculations were performed separately in two gestational age windows. Medians were compared by Mann–Whitney U-test. Correlations were calculated a.m. Pearson. Compatibility with the normal distribution was assessed by normal probability plots.

Statistical modelling

The population performance of screening programs employing different sets of parameters was assessed using the Monte Carlo simulation as described elsewhere (Larsen *et al*., 1998). Using the observed parameters of the log10MoM ADAM12 distributions and empirical correlations with other markers, in the examined gestational age windows, and published parameters for PAPP-A, hCGβ and NT (Cuckle and van Lith, 1999; Spencer *et al*., 2002), a series of random log10MoM values were selected from the distributions in unaffected and affected pregnancies. These series were used to calculate likelihood ratios for the different combinations of markers. The likelihood ratios were combined with the maternal age associated risk of giving birth to a DS child (Cuckle *et al*., 1987) and a standardised maternal age distribution (van der Veen *et al*., 1997) enabling the calculation of the expected DRs of affected pregnancies at various false positive rates. Truncation limits for

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Table 1—Distribution of log10MoM ADAM values in controls and DS pregnancies in different gestational age windows

Controls (n)	113	390	503
Mean log10MoM ADAM12	−0.0019	−0.0009	−0.0011
SD log10MoM ADAM12	0.1877	0.1494	0.1586
DS pregnancies (n)	10	18	28
Mean log10MoM ADAM12	−0.1928	−0.1451	−0.1621
SD log10MoM ADAM12	0.2542	0.1993	0.2170

all parameters were 0.8 log10 MoM (upper) and −0.8 log10MoM (lower), respectively.

RESULTS

The ADAM12 concentration increased with gestational age, but clearly more in the early first trimester (≤63 days of gestation) than in the late first trimester (>63 days), Figure 1. For gestational age >63 days a log-regression of ADAM12 on gestational age and weight in control pregnancies resulted in the regression line: log10 ADAM12 = 0.0121 × gestational age (days) −0.0038 × weight (kg) + 1.9052. A log-regression line: log10 ADAM12 = 0.013 × gestational age (days) + 1.5769 not involving maternal weight, was constructed-for use in cases where weight was not available. The regression lines were used to convert all ADAM12 concentrations to log10MoM values and the distributions are given in Table 1.

Prior to gestational day 64 a log-regression line: log10 ADAM12 = 0.0575 × gestational age (days) −1.249 was constructed by connecting the end of the regression line for the gestational age interval >63 days with the point defined by the average gestational age (60.27 days) and the average log10 ADAM12 (2.2162)Figure 2. The log-regression line was used to construct the log10MoM ADAM12 distributions shown in Table 1.

The empirical Pearson correlation coefficients between log10MoM ADAM12 and log10MoM PAPP-A and log10MoM hCG values in each gestational age window are given for control and DS pregnancies in Table 2. The empirical correlation between log10MoM PAPP-A and log10MoM hCGβ was 0.2136 (95% confidence limit: 0.117–0.306) in controls. There was no significant correlation between log10MoM ADAM12 and maternal age.

The estimated population screening performance of ADAM12 in combination with other serum markers and NT is shown in Table 3. It is seen that adding ADAM12 to the 'double test', that is the combination of PAPP-A and hCGβ, increases the DR from 66% to 74% for a FPR of 5%. If ADAM12 is added to the CUB screening, an increase in DR from 86% to 89% is seen for a FPR of 5%. The latter difference is so small that it must be considered negligible. In order to assess whether ADAM12 might be more useful in reducing the FPR than increasing the DR we estimated the effect on the FPR for different fixed DRs by adding ADAM12 to the CUB screening, Table 4. Interestingly, the fractional reduction of the FPR is from 17%–25%.

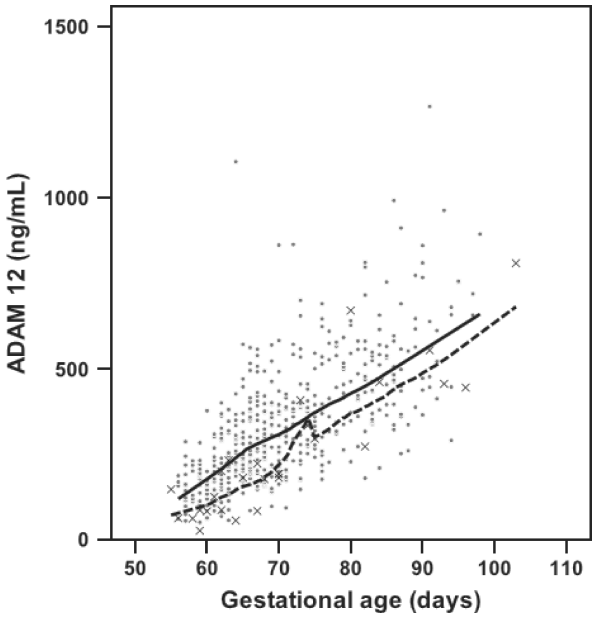

Figure 2—ADAM12 concentrations in DS pregnancies (x) and controls (●) as a function of gestational age. The broken line represents the LOESS regression line for DS pregnancies and the unbroken the LOESS regression line of controls. It is seen that the increase of ADAM 12 with gestational age is steeper prior to day 64 than later in pregnancy

We compared the temperature stability of the ADAM12 analyte for the Research ADAM12 kit with the first generation ADAM12 assay and it was seen, Figure 3, that the Research kit was considerably more robust.

DISCUSSION

We have shown that the maternal serum concentration of ADAM12 is significantly reduced in DS pregnancies

Table 2—Correlations, Pearson's rho and 95% confidence intervals, between serum markers and ADAM12 in different gestational age windows. All marker values examined were converted to log10MoM values

	≤63 days	>63 days	56–98 days
Controls (n)	113	390	503
PAPP-A	0.404 (0.24–0.55)	0.562 (0.49–0.63)	0.510 (0.44–0.57)
hCGβ	0.166 (−0.02–0.34)	0.214 (0.12–0.31)	0.199 (0.11–0.28)
DS pregnancies (n)	10	18	28
PAPP-A	0.754 (0.18–0.95)	0.667 (0.24–0.88)	0.713 (0.44–0.73)
hCGβ	0.128 (−0.59–0.73)	0.665 (0.23–0.88)	0.459 (0.07–0.73)

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Table 3—Estimated performance of ADAM12 alone or in combination with other markers in screening for DS

	Risk cut-off						Fixed FPR		
	1 : 100		1 : 250		1 : 400		1%	3%	5%
Markers	FPR(%)	DR(%)	FPR(%)	DR(%)	FPR(%)	DR(%)	DR(%)	DR(%)	DR(%)
ADAM12	1.6	30	5.9	47	11	57	25	39	45
PAPP-A + hCGβ	2.0	54	5.4	68	8.6	74	46	60	66
ADAM12 + PAPP-A + hCGβ	1.8	60	4.7	73	7.3	79	53	67	74
PAPP-A + hCGβ + NT	1.1	75	2.9	82	4.5	86	74	82	86
ADAM12 + PAPP-A + hCGβ + NT	1.1	78	2.8	84	4.2	87	76	85	89

Table 4—False positive rate of combined serum and ultrasound screening for DS by the addition of ADAM12 as a marker

	Fixed DR		
	85%	90%	95%
PAPP-A + hCGβ + NT	4%	8%	18%
PAPP-A + hCGβ + NT + ADAM12	3%	6%	15%
Decrease in FPR (%)	25%	25%	17%

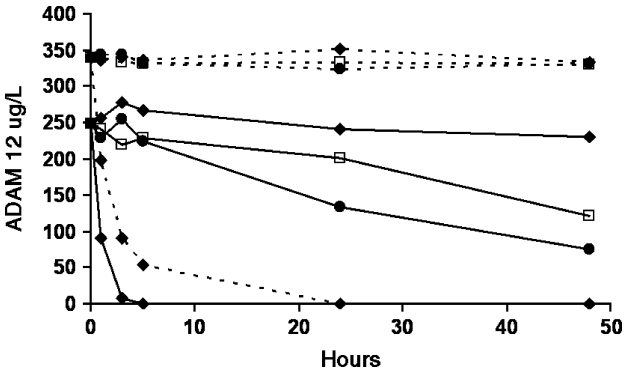

Figure 3—Stability of the ADAM12 analyte determined by the Delfia^{R} assay (broken lines) and an older manual ADAM12 assay (full lines) as a function of time and temperature. ♦: 56 °C; ●: 37 °C; □: 23 °C; ◆: 4 °C

in the early first trimester when ADAM12 is determined with a new, second generation, assay. However, the reduction of ADAM12 is much smaller than found using the original antibody employing disintegrin domain-reactive monoclonal antibodies (Laigaard *et al*., 2003). The molecular localization of the epitope of the new antibody has not been disclosed. The mean ADAM12 MoM in DS pregnancies (0.68 MoM) is nearly identical to that reported by others studying early first trimester samples (Spencer *et al*., 2008). We have noted a significant increase in the log10MoM ADAM12 value with gestational age through the first trimester, and this is compatible with previous findings (Laigaard *et al*., 2006a,b, 2003; Spencer *et al*., 2008) with the first generation assay. This decrease in performance with increasing gestational age, which is also seen for PAPP-A (Cuckle and van Lith, 1999; Spencer *et al*., 2002; Christiansen and Jaliashvili, 2003), can explain why the most recent study on ADAM12 as a DS marker in gestational week 11–13 (Poon *et al*., 2009) did not find that ADAM12 differed between DS and control pregnancies. Except for the absence of ultra-low ADAM12 values in the early first trimester (Laigaard *et al*., 2003), the results obtained with the second generation ADAM12 assay are compatible with previous findings.

The greater stability of ADAM12 when assayed by the second generation assay compared to the first generation assay could explain the greater reproducibility of measurements. For the first generation assay it is seen that the stability is so poor that variation in normal handling of samples in the lab, as well as preanalytical variations in handling, may become significant. Alternatively, the difference in analyte stability may be a result of the use of different epitopes for immunochemical quantification and this may mean that the two assays are quantifying different ADAM12 complexes or variants. This could, by analogy to the situation with PAPP-A and ProMBP (Qin *et al*., 1997b; Christiansen *et al*., 2000) mean, that the assays may differ in discriminatory ability due to the fact that they quantify different analytes that are differentially perturbed by the presence of a DS fetus.

Another reason for the difference in discriminatory ability for DS pregnancies between the old assay and the new, second generation, assay is the finding that ADAM12 correlates strongly with PAPP-A, Table 2, using the second generation assay. This was not the case with the old assay (Laigaard *et al*., 2003, 2006b). However, this may also be a consequence of the instability of the analyte when quantified by the first generation assay.

A relative advantage of the use of ADAM12 is the indifference to the mode of conception, that is. it does not matter whether the pregnancy was conceived naturally (Laigaard *et al*., 2009); this as opposed to PAPP-A (Gjerris *et al*., 2009). However, this effect is so small that it is not seen in small sample series (Wojdemann *et al*., 2001) and it cannot alone justify the introduction of ADAM 12 in CUB screening.

The reduction of the false positive rate by using ADAM 12 is considerable, Table 3, and this could be an argument to introduce ADAM 12 as a new marker, particularly in high-risk women. However, other markers, for example hPL(Christiansen *et al*., 2007a), SP1(Qin *et al*., 1997a), ProMBP (Christiansen *et al*., 1999), inhibin A (Christiansen and Norgaard–Pedersen, 2005), and placenta growth factor (Poon *et al*., 2009) could probably confer the same or even a higher

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reduction in FPR. So, ADAM12 may be used to reduce the FPR in high-risk pregnancies and this may be the rational indication for having it as an 'opt-in' in prenatal screening, but the cost-effectiveness of this approach will have to be compared with that of other strategies.

The improvement in screening performance associated with the addition of ADAM12 to conventional CUB screening, however, is so small that it does not outweigh the increased cost and increased variability of the risk estimate (Christiansen and Larsen, 2001). This conclusion, together with other reports(Spencer *et al*., 2008; Poon *et al*., 2009) using the second generation assay now becoming available, makes it clear that the promises of ADAM 12 as a revolutionarily good marker in first trimester will remain unfulfilled.

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