PRENATAL DIAGNOSIS
Prenat Diagn 2007; **27**: 611–615.
Published online 30 April 2007 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/pd.1750

ADAM 12 as a second-trimester maternal serum marker in screening for Down syndrome

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Background ADAM 12 is a placenta-derived glycoprotein that is involved in growth and differentiation. The maternal serum concentration of ADAM 12 is a potential first-trimester maternal serum marker of Down syndrome (DS). Here we examine the potential of ADAM 12 as a second-trimester maternal serum marker of DS.

Materials and Methods The concentration of ADAM 12 was determined in gestational week 14–19 in 88 DS pregnancies and 341 matched control pregnancies. Medians of normal pregnancies were established by polynomial regression and the distribution of $\log_{10}$ MoM ADAM 12 values in DS pregnancies and controls determined. Correlations with alpha-fetoprotein (AFP) and free β-human chorionic gonadotrophin (free β-hCG) were established and used to model the performance of maternal serum screening with ADAM 12 in combination with other second-trimester serum markers.

Results The ADAM 12 maternal serum concentration was significantly increased with a median MoM of 1.85 and a mean $\log_{10}$ MoM (SD) of 0.268 (0.2678) compared to a mean $\log_{10}$ MoM (SD) of 0.013 (0.4318) in controls. ADAM 12 correlated with maternal weight and ethnicity (with the serum concentration increased in Afro-Caribbeans), but neither with maternal age nor gestational age, and only marginally with AFP (r(DS) = 0.078, r(controls) = 0.093) and free β-hCG (r(DS) = 0.073, r(controls) = 0.144. The increase in detection rate—for a false positive rate of 5%—by adding ADAM 12 to the double test (AFP + free β-hCG) was 4%, similar to that of adding uE3 to the double test.

Conclusion ADAM 12 is an efficient second-trimester marker for DS. Further studies should be conducted to determine whether it may be a useful additonal or alternative marker to those currently used in the second-trimester.

KEY WORDS: prenatal screening; PAPP-A; free β-hCG; growth factors; integrins; ADAM12; aneuploidy; trisomy 21

INTRODUCTION

Maternal serum screening for chromosomal disorders in the second-trimester is a well established technique (Benn, 2002) and is commonly offered either as the triple test (employing the markers alpha-fetoprotein (AFP), total human chorionic gonadotrophin (hCG) or the free β-form of hCG (free β-hCG) and unconjugated estriol (uE3)) or the quadruple test where the triple test is supplemented with inhibin A (Benn *et al*., 2003). Second-trimester markers are also offered as part of integrated screening (Wald *et al*., 1999a) and contingent (Christiansen and Larsen, 2002; Benn *et al*., 2005) and sequential screening programes (Benn, 2002; Benn *et al*., 2005). The identification of new second-trimester markers will make it possible to reduce the false positive rate of screening, and thus, the number of invasive procedures associated with a 1% risk of aborting an otherwise healthy fetus (Tabor *et al*., 1986).

The short and secreted spliceform of ADAM 12—A Disintegrin And Metalloprotease 12-S (Gilpin *et al*., 1998) (in brief ADAM 12)—is a glycoprotein synthesized by the placenta (Gilpin *et al*., 1998; Shi *et al*., 2000) and found in increasing concentration in maternal serum through gestation (Laigaard *et al*., 2003). ADAM 12 has proteolytic activity against insulin-like growth factor binding proteins 3 (IGFBP-3) (Shi *et al*., 2000) and insulin-like growth factor binding proteins 5 (IGFBP-5) (Loechel *et al*., 2000) and is probably responsible for at least part of the increased IGFBP-3 and IGFBP-5 protease activity seen in pregnancy (Giudice *et al*., 1990; Hossenlopp *et al*., 1990). ADAM 12 is involved in cell adhesion (Huang *et al*., 2005), myogenic and adipogenic transformation (Kawaguchi *et al*., 2003; Lafuste *et al*., 2005; Yi *et al*., 2005), apoptosis (Kveiborg *et al*., 2005), and cell fusion, and modulation of the local effect, and bioavailability of IGF-I and IGF-II, and other growth factors (Rosenfeld and Roberts, 1999). ADAM 12 is probably of importance for placental and fetal growth.

A reduced first-trimester maternal serum level of ADAM 12 has been described in pregnancies with fetuses with Down (Laigaard *et al*., 2003; 2006a,b) and

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Received: 26 January 2007
Revised: 13 March 2007
Accepted: 20 March 2007
Published online: 30 April 2007

Patau syndromes (Laigaard *et al*., 2005a, 2006a) and in pregnancies complicated by preeclampsia (Laigaard *et al*., 2005b). However, three studies have suggested that the discriminatory efficiency of ADAM 12 as a Down syndrome marker decreases towards the end of the first-trimester (Laigaard *et al*., 2003, 2006a,b) and the discriminatory efficiency in the second-trimester has not been established.

Here we examine the maternal serum concentration of ADAM 12 in second-trimester pregnancies in order to assess whether ADAM 12 can be used as a second-trimester marker for Down syndrome.

MATERIALS AND METHODS

Serum samples from 88 second-trimester DS pregnancies were retrieved from the biobank at Harold Wood Hospital along with retrieval of the relevant patient demographic and screening records from the prenatal screening database. As a control group, 341 serum samples from singleton pregnancies from across the gestational ranges 14 to 19 weeks were selected to cover the storage period of the DS samples. All samples were collected as part of routine second-trimester serum screening services for which patients gave written consent for excess diagnostic material to be used for research purposes. All pregnancies had previously undergone routine screening using the markers AFP and free β-hCG measured using the Kryptor analyzer (Brahms AG, Berlin). All pregnancies were dated either by first-trimester crown-rump length or second-trimester biparietal diameter. Patient-specific risks were calculated by a multivariate approach, as previously described (Spencer, 1999). Women with a risk of DS of $>1:250$ were offered invasive testing to determine the fetal karyotype. Data on pregnancy outcome were obtained from the Cytogenetic Centres, the National Cytogenetic Register, or the maternity unit in the hospitals in which the pregnancy was delivered. The number of DS cases that were identified by second-trimester screening was 62 and the number of live born cases with DS that were missed by screening was 26.

All serum samples had been stored as aliquots at $-20\,^{\circ}C$ within a maximum of 4 h after being tested for the second-trimester markers. Aliquots for serum ADAM 12 quantification had not previously been frozen and thawed.

ADAM12 was quantified by a semi-automated time-resolved immunofluorometric assay using the AutoDelfia platform (PerkinElmer, Turku, Finland) as previously described (Laigaard *et al*., 2005b). Briefly, recombinant human ADAM12-S was used for standardisation. A previously described monoclonal capture antibody, 6E6 (Gilpin *et al*., 1998), was coated onto the surface of microtitre plates. After prewashing the coated plates. 50 uL of sample and 50 uL of AutoDelfia multibuffer (Perkin-Elmer, Turku, Finland) were added to each well followed by a 2 h room temperature incubation and four cycles of wash with multibuffer. The biotinylated monoclonal antibody 8F8 (Gilpin *et al*., 1998) was used for detection. The biotinylated 8F8 was added to each well and incubated for 1 h at room temperature followed by a further four cycles of wash. Europium labeled streptavidin (Perkin-Elmer) was added to each well and incubated for 1 h at room temperature followed by three wash cycles and the addition of enhancement solution and counting of time-resolved immunofluoresence after 10 min. The analytical performance of this assay has been previously described (Laigaard *et al*., 2005b). Both antibodies react with the disintegrin part of ADAM 12 (Wewer *et al*., 2006).

Statistical analysis of data was performed using Microsoft Excell 2000 with the Analyze-It (Smart Software, Leeds, UK) statistical software package or S-Plus vers. 6.0 (Insightful Corp, USA). All ADAM 12 concentration values were converted to a multiple of the median (MoM) for unaffected pregnancies at the same gestational age using a previously defined third order polynomial of the form Median ADAM 12 ug/L = 0.0009 * GA^3−0.2206 * GA^2 + 14.721 * GA − 82.755 with GA being gestational age in days (Laigaard *et al*., 2006b). Correction of each MoM for maternal weight was performed by using a correction formula derived by log-regression. Goodness-of-fit to a Gaussian distribution of $\log_{10}$ ADAM 12. MoM in the control group and the Down group was confirmed using normal probability plots and the Shapiro–Wilk test. Marker correlations were determined using the Pearson correlation coefficient.

An estimate of the performance of various marker combinations in screening for DS was established using standard statistical Monte Carlo modeling techniques (Larsen *et al*., 1998). The analysis was performed using the S-Plus vers. 6.1 statistical program. Briefly, using the observed population parameters for ADAM 12 and those for AFP and free β-hCG from (Cuckle, 1995), a series of random MoM were selected from the distributions in unaffected and affected pregnancies. These values were then used to calculate likelihood ratios for the combinations. The likelihood ratios were subsequently used together with the maternal age-related risk of Down syndrome at birth (Cuckle *et al*., 1987) to calculate the expected detection rate of affected pregnancies at various false positive rates, in a population with a standardized distribution of maternal ages (van der Veen *et al*., 1997).

RESULTS

The median values across the second-trimester of pregnancy are shown in Table 1 along with the regressed medians based on the number of controls per gestational week. These results are significantly lower (by 6-fold) than that found in the earlier work of Laigaard *et al*. (2003) using a different assay incorporating the same calibrant and same monoclonal antibodies. However the rather flat profile in this current study is similar to that found in the earlier Laigaard *et al*. (2003) study—indicating an insensitivity to gestational dating errors.

The distribution of ADAM 12 MoM values in Down syndrome pregnancies is shown in Figure 1. The ADAM

DOI: 10.1002/pd

Table 1—Gestational age variation of ADAM 12

Median gestational day	Number of cases	Observed median ADAM 12 (ug/L)	Regressed median ADAM 12 (ug/L)
101	83	83	81
107	63	68	69
115	62	70	62
121	74	61	64
129	74	82	77

12 MoM values were significantly higher (1.85) in DS pregnancies than in normal pregnancies ($p < 0.001$). There was no significant correlation between the $\log_{10}$ MoM ADAM 12 values in DS pregnancies and gestational age ($r = 0.1378$), Figure 1. The distribution was log normal as demonstrated by the normal probability plots of control and DS pregnancies, shown in Figure 2(a) and (b), respectively, and the nonsignificant Shapiro–Wilk tests ($p = 0.4$ DS cases and $p = 0.6$ controls). The mean and standard deviation of the $\log_{10}$ MoM ADAM 12 distributions in normal and DS pregnancies were 0.013 and 0.4318, and 0.268 and 0.2678 respectively.

ADAM 12 was found to correlate marginally with maternal weight and a weight-corrected MoM (using the formula: Log_{10} MoM ADAM $12_{\text{corr}} = \text{Log}_{10}$ MoM ADAM $12 - 0.2505 + 0.0036^*$ maternal weight (in kgs)) was used in the comparison of different ethnic groups. The weight-corrected mean (SD) $\log_{10}$ MoM ADAM 12 was 0.250 (0.2556) in DS pregnancies.

The log MoM ADAM 12 values correlated with ethnicity as Afro-Caribbeans had a significantly higher mean $\log_{10}$ MoM ADAM 12 compared to Caucasians (Median ADAM 12 MoM 1.68 v 0.82) or other ethnic groups ($\chi^2 = 25.7$, d.f. $= 3$, $p = 0.00001$), see Table 2. However, the difference between Log_{10} MoM ADAM 12 in DS and normal pregnancies seem to be the same as for nonAfro-Caribbean pregnancies, Table 2.

The log MoM values of ADAM 12 (after removal of outliers outside +/− 3SD) did not correlate significantly with either the log MoM values of AFP (r(DS) $= 0.078$, r(controls) $= 0.093$) or free β-hCG (r(DS) $= 0.073$, r(controls) $= 0.144$.

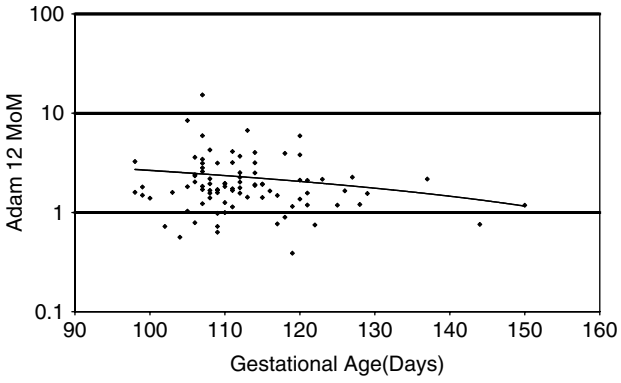

Figure 1—ADAM 12 MoM values as a function of gestational age in DS pregnancies. The regression line was: $y = -0.0299^*X + 5.6391$. As R^2 was 0.019, the contribution of gestational age to variation of ADAM 12 is less than 2%

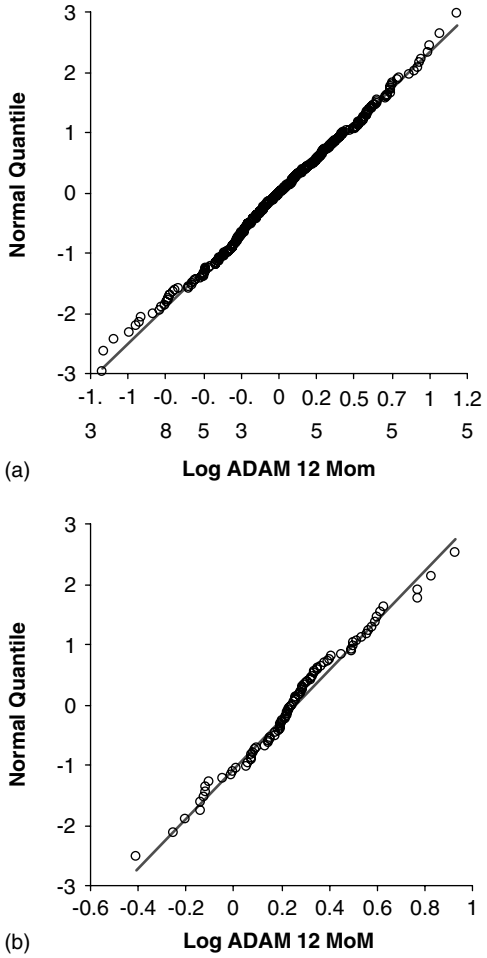

Figure 2—Normal probability plots of (A) Log_{10} MoM ADAM 12 in control pregnancies, and, (B) Log_{10} MoM ADAM 12 in DS pregnancies. Both plots are compatible with a normal distribution

In our analysis of the performance of ADAM 12 we have made no correction for smoking or ethnicity. The performance of ADAM 12, and ADAM 12 in combination with other markers, was modeled and the detection rates (DRs) and false positive rates (FPRs) for three different clinically relevant risk cutoff levels are shown in Table 3. For comparison, the DR obtained for a fixed FPR of 5% are also given for the different combinations with ADAM 12 and the combination of AFP and free β-hCG and the double test (AFP and free β-hCG).

DISCUSSION

The median values observed in this study across the second-trimester of pregnancy using a different assay incorporating the same calibrant and same monoclonal antibodies are significantly lower (by 6-fold) than that found in the earlier work of Laigaard *et al.* (2003) However, the rather flat profile in this current study is similar to that found in the earlier Laigaard *et al.* (2003)

DOI: 10.1002/pd

Table 2—The distribution of $\log_{10}$ MoM ADAM 12 (weight corrected) in different ethnic groups and the difference between controls and DS pregnancies. Afro-Caribbeans had significantly elevated values in both controls and DS pregnancies

	Controls			T21			Difference	
Ethnicity	Mean	SE	n	Mean	SE	n	Difference	SE
Afro-Caribbean	0.2254	0.0560	49	0.5611	0.1400	3	0.3357	0.1514
Caucasian	−0.0880	0.0314	175	0.2161	0.0310	63	0.3041	0.0441
Asian	0.0207	0.0563	51	0.3816	0.0648	15	0.3609	0.0860
Other	0.0615	0.0549	66	0.2384	0.1084	7	0.1769	0.1215

Table 3—Estimated performance, i.e. DR and FPR, of different marker combinations including maternal age for different risk cutoffs. The risk is the risk of giving birth to a DS child. The last column gives the DR for a fixed FPR of 5%

	Risk cutoff						
	1 : 100		1 : 25		1 : 400		5% FPR
Combination	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)
Maternal age	14.7	1.2	33.2	6.3	43.5	11.8	28.2
ADAM 12	22	1.7	40	6.5	53	12.9	36
ADAM 12 + AFP	32	2.1	53	7.6	65	13.4	45
ADAM 12 + Free β-hCG	47	2.2	64	6.5	72	10.8	59
ADAM 12 + AFP + Free β-hCG	55	2.4	70	6.3	78	9.8	67
AFP + Free β-hCG	50	2.2	66	6.1	74	9.8	63

study—indicating an insentivity to gestational dating errors. The wide SD of the controls dataset cannot therefore be explained by inaccuracy in gestational dating, but may point to the need for a closer examination of the stability of ADAM 12.

The important finding here is that the maternal serum concentration of ADAM 12 is elevated in the second-trimester in DS pregnancies. With the performance data reported in Table 3, it can be concluded that we have demonstrated that ADAM 12 is a second-trimester maternal serum marker for Down syndrome with a clinically relevant discriminatory efficiency. The lack of a significant second-trimester increase in ADAM 12 in a previous small study (Laigaard *et al*., 2003) is most likely explained by the small number of samples.

As maternal ADAM 12 is reduced particularly in early first-trimester DS pregnancies (Laigaard *et al*., 2003, 2006a,b) and increased in second-trimester DS pregnancies, it can be concluded that ADAM 12 as a single marker, in analogy with, e.g. SP1 (Qin *et al*., 1997; Brizot *et al*., 1995; Wald *et al*., 1999b), has a 'window of uselessness' (Brizot *et al*., 1995) located somewhere between 11 to 13 weeks.

It can be concluded from the simulation studies in Table 3, that the use of ADAM 12 in combination with AFP and free β-hCG, is at least as efficient as the conventional triple test (Benn, 2002). It is seen, that the addition of ADAM 12 to AFP and free β-hCG increases the DR from 63 to 67%. This increase is slightly higher than the increase seen by adding uE3 to AFP and free β-hCG (Benn, 2002). The effect of adding ADAM 12 to the triple test and exchanging inhibin A or uE3 with ADAM 12 could not be tested in the present study as the correlations between ADAM 12 and the two markers uE3 and inhibin A were not available. The fact that ADAM12 has some value in second-trimester screening means, that screening laboratories performing both first- and second-trimester screening could use ADAM 12 as a marker in both parts of gestation. With the development of semiautomated assays (Laigaard *et al*., 2005b) this is an attractive option.

The final assessment of the role of ADAM12 in second-trimester screening must await studies where uE3 and inhibin A are also included, as these markers have gained general acceptance as elements in the Triple test (AFP + free β-hCG + uE3) and the Quadruple test (Triple test + Inhibin A), respectively. Furthermore, the use of ADAM12 in integrated, sequential and contingent screening protocols with different other marker combinations should be assessed. As ADAM 12 is decreased in first- and elevated in second-trimester the performance of a 'repeated measures' (Wright & Bradbury, 2005) screening, using ADAM 12 in both trimesters should also be examined.

The ethnic differences seen in Table 2 have also been confirmed in the first-trimester (Laigaard *et al*., 2006b) and these differences should be taken into account when organizing the risk algorithm, but even though the analysis is based on only three DS pregnancies among Afro-Caribbeans, it seems as though ADAM 12 still discriminates between DS and normal pregnancies. In this regard, the size of the increase in ADAM 12 in Afro-Caribbean pregnancies is very similar to that seen with PAPP-A (Spencer *et al*., 2005).

In conclusion, ADAM 12 is a novel maternal serum marker for Down syndrome with an interesting dual applicability in the first- and second-trimester. But several issues remain unsolved. A clear definition of the window of uselessness is of importance as this may disclose whether ADAM 12 can be included in 'gold

DOI: 10.1002/pd

standard' first-trimester combined screening (Spencer *et al*., 2003) or whether some alternative protocol may be more applicable. It will also be of interest whether the finding in the first-trimester that other chromosomal diseases, such as, trisomies 13 and 18 (Laigaard *et al*., 2003, 2006a) can be identified using ADAM 12, and whether this can be extended into second-trimester screening.

However, the final assessment of the marker will depend on the availability of routine assays and the results of prospective clinical trials.

ACKNOWLEDGEMENT

This study was supported, in part, by a grant from NHS R&D (RF4: Risk Assessment in Pregnancy) to Professor Kevin Spencer.

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