Maternal serum ADAM12s as a potential marker of trisomy 21 prior to 10 weeks of gestation

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Background ADAM12s (a disintegrin and metalloprotease) is a placenta-derived glycoprotein that is involved in growth and differentiation and has been shown to be a potential first-trimester and second-trimester marker of trisomy 21 and other aneuploidies. Maternal ADAM12s concentrations show a considerable temporal variation with gestational age and in the initial study levels were found to be significantly reduced in the early first trimester. Here we study the levels prior to 10 weeks of gestation to establish further the effectiveness or otherwise of ADAM12s as an early screening marker.

Materials and Methods Samples collected as part of routine first-trimester screening were retrieved from storage. In total, ten samples from singleton pregnancies with trisomy 21 were identified and were collected between the 8th and 9th weeks of gestation—of these 80% had been identified by combined first-trimester screening. A series of 62 gestational age-matched samples from singleton pregnancies collected during the same period formed the control group. ADAM12s was measured by a new DELFIA assay incorporating two monoclonals (6E6 and 8F8). Results were expressed as multiples of the median (MoM).

Results The median MoM ADAM12s at a median gestation of 9.3 weeks was 0.61 which was significantly lower than in the controls (p = 0.011) when compared by the Mann–Whitney test. The corresponding median pregnancy associated plasma protein (PAPP-A) was 0.30 and free beta-human chorionic gonadotropin (β-hCG) 2.02.

Conclusions Combining the data from this study and from the only other published study with data prior to 10 weeks suggests that ADAM12s may have the potential as an early screening marker for trisomy 21, but may not be as reduced as first thought. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: aneuploidy; screening; growth factors; PAPP-A; free β-hCG

INTRODUCTION

ADAM12s (a disintegrin and metalloprotease) (Gilpin et al., 1998) has been shown to have proteolytic activity against insulin-like growth factor binding proteins 3 and 5 (Loechel et al., 2000; Shi et al., 2000) and is thought to be part of a mechanism controlling foetal growth during pregnancy (Cowans and Spencer, 2007). In pregnancies, with trisomy 21, reduced levels of ADAM12s have been found in the early first trimester (Laigaard et al., 2003, 2006a,b) with levels increasing above normal in the second trimester (Christiansen et al., 2007). Similar patterns have been observed for trisomy 18 (Laigaard et al., 2005a, 2006a; Spencer and Cowans, 2007) and also with other rare aneuploidies (Spencer et al., 2007). Laigaard et al., 2006a and Spencer et al. (2007) have both concluded that it is unlikely that ADAM12s will be of value in screening during the 11 to the 13 week window—a time commonly used for screening using the combined test of nuchal translucency (NT), pregnancy associated plasma protein (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG).

The early work of Laigaard et al. (2003) with samples collected predominantly prior to 10 weeks suggested very low levels of ADAM12s in cases with trisomy 21. In this study, we seek to understand better the likely clinical performance of ADAM12s prior to 10 completed weeks of pregnancy.

MATERIALS AND METHODS

First-trimester screening for Down Syndrome was performed in a sequential (2-step) method as reported elsewhere (Gyselaers et al., 2005). Briefly, blood was sampled between 8–10 weeks of pregnancy and analysed for PAPP-A and free-β HCG using the Kryptor analyser (Brahms AG, Berlin, Germany). The performance of this system has been previously described (Spencer et al., 1999) NT was measured between 11–13 weeks of pregnancy (CRL 45–85 mm) and a combined risk was calculated. The risk cut-off level was 1 : 300.

In total from this routine screening program 72 samples from singleton pregnancies with known outcome, collected as part of routine prospective screening and which had not previously had ADAM12s measured were selected for further analysis. Of the trisomy 21, three
cases out of ten resulted in live-born infants with trisomy 21 and of the ten cases, two were missed by the screening program. In one live-born case, the mother refused further diagnosis.

As a control group, we used gestational age-matched data from 62 first-trimester samples collected during the same time period and stored under the same conditions. In all first-trimester cases, gestational age at sample collection (median controls = 65.1 days; median cases = 65.8 days) was determined by CRL measurement. Samples had on average been frozen and thawed as aliquots once (range 0–2). The control group had a median maternal age of 29.1 years, while that for the group of cases was 35.04 years. The maternal weight of the two groups was similar (median controls = 62.0 Kg; median cases was 61.8 Kg). The group was exclusively of Caucasian origin. An informed consent for use of leftover material is not obligatory in Belgium, as screening for Down Syndrome is the standard procedure in the follow-up of pregnancies.

Serum ADAM12s was measured blindly to clinical outcome, using a newly developed manual DELFIA assay (PerkinElmer Life & Analytical Sciences, Turku, Finland) incorporating the monoclonal antibodies 6E6 and 8F8, which was based on previously described enzyme-linked immunosorbent assay (ELISA) and AutoDELFIA assays (Laigaard et al., 2003, 2005b) as previously described (Cowans and Spencer, 2007). PAPP-A and free β-hCG results were available from routine analysis in which a Kryptor was used.

Regression analysis was used to establish the median ADAM12s marker concentrations across the narrow window for 8 to 10 weeks and the concentrations were converted into multiples of the median (MoM) by dividing each result by the expected median marker level from the control pregnancies at the same gestational age as derived from this equation.

**RESULTS**

Although the cases had a maternal age greater than the controls, previous studies have shown ADAM12s concentration to be unrelated to maternal age (Laigaard et al., 2003, 2006b). Figure 1 shows the regression equation for ADAM12s concentration in the controls against the gestational age alongside the concentrations of the ten trisomy 21 cases. Converting the ADAM12s concentration in the cases to MoM using the regression equation results in a median MoM of 0.61 at a median gestation of 9.3 weeks, this was significantly lower than in the controls (Mann–Whitney test p = 0.0116). Previous studies have shown the need for log10 transformation of ADAM12s MoM in order to fit a gaussian distribution (Laigaard et al., 2003, 2006b). The mean log10 MoM was −0.2624 with an sd of 0.2460. The median PAPP-A MoM in the same series was 0.30 and that for free β-hCG was 2.02 with log10 sd’s of 0.3081 and 0.2544, respectively. With only ten cases, it was felt inappropriate to provide estimates of correlation with these other markers. In the controls, the correlation with free β-hCG and PAPP-A were 0.051 and 0.285, respectively, similar to that in a previous larger series (Laigaard et al., 2006b). Figure 2 shows the distribution of ADAM12s MoM with gestation and shows the suggestion of an increase of MoM with gestational age.

**DISCUSSION**

The results of this small study have shown that maternal serum levels of ADAM12s are reduced in pregnancy prior to 10 weeks but not to the levels observed previously in the study by Laigaard et al. (2003). In the Laigaard et al. (2003) study, the median MoM ADAM12s prior to 10 weeks in 13 cases was 0.04.

While the present data might support a possible role for ADAM12s prior to 10 weeks, the early promise from the Laigaard et al. (2003) study has not been realised.
Further studies are required of cases prior to 10 weeks to assess the value of ADAM12s at this time.

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REFERENCES


