ADAM12 A PROMISING NEW MATERNAL SERUM MARKER IN SCREENING FOR DOWN SYNDROME IN BOTH FIRST AND SECOND TRIMESTERS OF PREGNANCY

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Children, Youth and Women's Health Service

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AIM

To assess the ability of a new marker (ADAM12) to better detect Down syndrome affected pregnancies.

INTRODUCTION

ADAMs (A Distintegrin And Metalloprotease) are a family of more than 30 proteins of the metzincin superfamily of zinc-dependent proteases. ADAM12 is a multidomain glycoprotein with metalloprotease, cell adhesion and signalling activities. It consists of a prodomain, a metalloprotease domain, a disintegin-like domain, a cystein-rich domain, a trans-membrane domain, and a cytoplasmic tail. Human ADAM12 is expressed in two splice forms. The shorter soluble form, ADAM12-S, is found in serum while the long ADAM12-L is a transmembrane protein. ADAM12 is synthesized as a zymogen. The prodomain keeps the metalloprotease domain inactive through a cystein-switch mechanism. (Figure 1)
ADAM12 is synthesized in the placenta and is present in human pregnancy serum. It binds to and has proteolytic activity against insulin-like growth factor binding protein (IGFBP) 3 and 5. The proteolysis of IGFBP stimulates fetal growth by increasing the levels of bioavailable IGF (insulin growth factor) 1 and 11 (1, 2). ADAM12 has been shown as a potential new marker in two separate studies, one in first trimester and the other in second trimester (3, 4). We further assess this potential across both trimesters.

METHODS

The AutoDELFIA/DELFIA ADAM12 Research kit from PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku Finland was used in this study. The assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique in which two monoclonal antibodies are directed against two separate antigenic determinants on the ADAM12 molecule. Calibrators, controls and serum samples from pregnant women, containing ADAM12 are reacted with europium-labelled monoclonal antibody. The labelled antibodies are directed against different specific antigenic sites than the immobilized antibodies. Enhancement Solution dissociates europium ions from the labelled antibodies into the solution where they form highly fluorescent chelates with components of the Enhancement Solution. The fluorescence in each well is then measured. The europium fluorescence of each sample is proportional to the concentration of ADAM12 in the sample.

Levels of ADAM12 were determined retrospectively from -20C frozen maternal serum of < 2 years in 47 first trimester and 27 second trimester Down syndrome affected pregnancies and 306 unaffected matched controls. 60 false positive matched first and second trimester same pregnancy samples were also used to assess reductions in false positive rates if ADAM12 was included in the risk odds calculation. These 60 unaffected samples were initially screened at an increased risk for Down syndrome in first trimester using the combined nuchal and biochemical screen. These formed a study cohort where a second trimester blood sample was requested if the patient went on to have an amniocentesis. These second trimester study blood samples were collected before the amniocentesis procedure to avoid possible effects on biochemical markers from amniotic fluid "leakage".

Medians for ADAM12 were determined from the 306 unaffected controls using polynomial regression. Likelihood ratios derived from log transformed MoM (multiples of the population median) multivariate overlapping Gaussian distributions were used to calculate risk odds of an affected from maternal age risks at delivery. A risk odds cut off of ≥ 1 in 300 was used to assess performance for both first and second trimester protocols.
These data were compiled as part of two studies approved by the Women's & Children's Research Ethics Committee.

RESULTS

ADAM12 levels were significantly reduced ($p<0.001$, Median MoM = 0.82) in first trimester affected pregnancies but significantly elevated ($p<0.001$, Median MoM = 1.5) in second trimester affected pregnancies (Figure 2).

Of the 60 unaffected false positives, 21 were above the median line in first trimester and 28 were below the median line in second trimester (Figure 3).

By including ADAM12 into current first and second trimester algorithms, discriminant analysis showed reductions of 36% and 40% of the 60 unaffected false positive cases respectively.

New performances in pregnancies between 10 – 20 weeks gestation, with the inclusion of ADAM12 into current first and second trimester protocols were; first trimester 3% (3/99) false positives and 91.5% (43/47) detection, second trimester 4.9% (8/164) false positives and 70.4% (19/27) detection.

DISCUSSION

Retrospective testing on matched frozen 1st and 2nd trimester samples has revealed that the novel pregnancy marker, ADAM12, is suppressed in 1st trimester affected pregnancies (40/47) but elevated in 2nd trimester affected pregnancies (24/27) as shown in figure 2.

The switch from low to high appears to occur precisely at 14 weeks which is the cut off used between 1st and 2nd trimester screening (a fortuitous finding).

Current maternal serum markers measured are free beta hCG and Papp-A in 1st trimester and AFP, free beta hCG and unconjugated estriol (uE3) in the 2nd trimester screen. The addition of ADAM12 into these current screening protocols appears to significantly reduce false positive rates but also leads to improvements in detection rates. These new performances are consistent with the two published studies, also based on retrospective analysis of frozen samples (3, 4).

Despite measures taken to control for storage effects, the use of frozen samples is always problematic (sample deterioration, de-lipidation, matrix changes etc). Funding has been secured to conduct a 12 month prospective study on samples routinely submitted from South Australian patients for maternal serum screening in both first and second trimesters. From this prospective study we aim to establish population distributions for ADAM12 in pregnancies of normal karyotype and those affected with Down syndrome and other aneuploidies. We are expecting to observe 40 cases of Down syndrome from the first trimester cohort and 10 from the second.

We also intend to assess ADAM12 in different combinations with currently used markers to optimise screening performance for the detection of affected pregnancies with Down syndrome.

CONCLUSION

ADAM12 is a promising new maternal serum marker for the detection of Down syndrome affected pregnancies in both first and second trimesters, reducing false positives and improving detection. However prospective studies are required to confirm these findings.

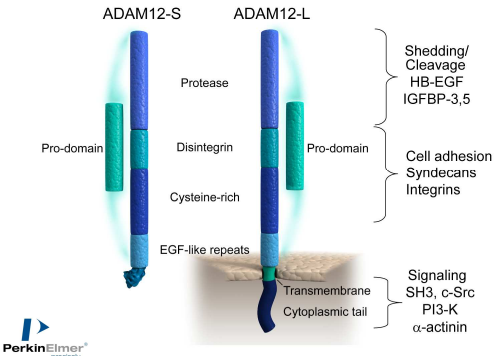

Figure 1

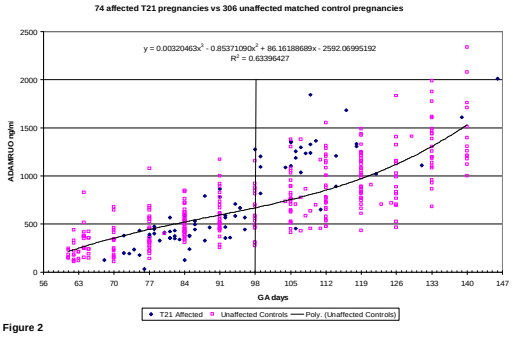

Figure 2

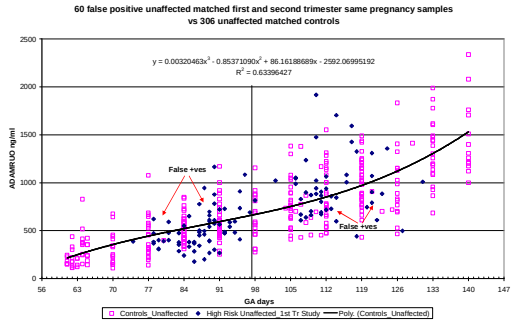

Figure 3

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