PRENATAL DIAGNOSIS
Prenat. Diagn. **19:** 905–910 (1999)

The Proform of Eosinophil Major Basic Protein: A New Maternal Serum Marker for Down Syndrome

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The proform of eosinophil major basic protein (proMBP), the most abundant protein in the eosinophil specific granule, is synthesized by the placenta and secreted into the maternal circulation, where it is found complex-bound to pregnancy-associated plasma protein-A (PAPP-A) and other proteins. We examined the potential of proMBP as a maternal serum marker for fetal Down syndrome (DS) by determining its maternal serum concentration (MSpMBP) in 25 Down syndrome (DS) pregnancies and 152 control pregnancies in the first trimester, and in 105 DS pregnancies and 156 control pregnancies in the second trimester. The median (95 per cent confidence interval) MSpMBP MoM in DS pregnancies ($n=15$) was 0.66 (0.49–0.79) in gestational weeks 5–9; 1.06 (0.71–1.97) in weeks 10–12 ($n=10$) and 1.62 (1.18–1.98) in weeks 14–20 ($n=105$). Using parameterized receiver operator characteristics analysis for proMBP as a single marker for DS, detection rates (DRs) of 22 per cent and 38 per cent, for false-positive rates (FPRs) of 5 per cent, were found in weeks 5–9 (using MSpMBP $\leqslant$ cut-off) and weeks 14–20 (using MSpMBP $\geqslant$ cut-off), respectively. When age and MSpMBP were used as markers in combination, a DR of 36.8 per cent for an FPR of 5.5 per cent was obtained in weeks 5–9 using a risk cut-off of 1:250. In weeks 14–20 the DR was 48.4 per cent for an FPR of 5.3 per cent using the same risk cut-off. This makes proMBP a marker comparable in diagnostic efficiency to human chorionic gonadotrophin (hCG), and exceeding that of alpha-fetoprotein (AFP) and unconjugated oestriol (uE3), in the second trimester. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS: eosinophil major basic protein; Down syndrome; prenatal screening; first trimester; second trimester; receiver operator characteristics

INTRODUCTION

The proform of the most abundant protein constituent of the specific granule of the eosinophil leukocyte, the major basic protein (MBP) (Gleich and Adolphson, 1986), is also synthesized by the placenta (Maddox *et al*., 1984; Bonno *et al*., 1994a,b) and secreted into the maternal circulation. MBP antigen has previously been determined immunochemically (Maddox *et al*., 1983; Wasmoen *et al*., 1987, 1989, 1991; Wagner *et al*., 1993). MBP is a 117 residue cationic polypeptide and the proform (proMBP) has a highly glycosylated 88 residue N-terminal extension (Oxvig *et al*., 1994a), making the proBMP far less cationic and abolishing the toxicity of MBP (Barker *et al*., 1988; Popken-Harris *et al*., 1995). ProMBP is also present in amniotic fluid, but in lower concentrations than in maternal serum (Vernof *et al*., 1992b). In pooled maternal serum proMBP is found complexed, partly 2:2 with pregnancy-associated plasma protein-A (PAPP-A) (Oxvig *et al*., 1993, 1994b), and partly 2:2 with angiotensinogen or 2:2:2 with angiotensinogen and complement C3dg (Oxvig *et al*., 1995).

The function of proMBP in pregnancy has not been established, but the association of proMBP with PAPP-A, a putative matrix Zn^{2+}-metalloproteinase (Kristensen *et al*., 1994), angiotensinogen, synthesized by the placenta (Paul *et al*., 1992), and complement C3dg, a strong immunomodulator (Dempsey *et al*., 1996), suggest that proMBP may function as a carrier/inhibitor of otherwise active molecules involved in tissue remodelling or differentiaton. Alternatively, PAPP-A and angiotensinogen may prevent the cleavage of proMBP to the cytotoxic MBP (Bonno *et al*., 1994a). ProMBP has been suggested to play a pathophysiological role in maternal floor infarction (Vernof *et al*., 1992a). One study showed a correlation between the number of placental septa and cysts and the maternal serum concentration of proMBP (Wasmoen *et al*., 1991). The relationship between these clinical parameters does not, however, seem to be straightforward (Wagner *et al*., 1993). The maternal serum concentration of PAPP-A in DS pregnancies has consistently been found to be reduced to 20–40 per cent of the level in maternal sera from normal pregnancies in the first trimester (Wald *et al*., 1992; Muller *et al*., 1993; Macintosh *et al*., 1994; Brambati *et al*., 1994; Spencer *et al*., 1994; Casals *et al*., 1996; Krantz *et al*., 1996; Qin *et al*., 1996, 1997a,b; Forest *et al*., 1997), and PAPP-A seems to be the most promising serum marker for DS screening in the first trimester (Wald *et al*.,

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Contract/grant sponsor: Danish Biomembrane Centre.
Contract/grant sponsor: Aarhus University.

CCC 0197–3851/99/100905–06$17.50

Received 30 June 1998
Revised 5 May 1999
Accepted 10 May 1999

1996). It is very likely, that proMBP is perturbed in some periods in gestation in DS pregnancies, and a diagnostic use of this could lead to an improvement of established maternal serum screening practices.

In this study we (i) examined whether the maternal serum concentration of proMBP (MSpMBP) was influenced by the presence of a Down syndrome fetus, (ii) evaluated the potential of MSpMBP as a screening marker for Down syndrome in the first and second trimesters, and (iii) examined the performance characteristics of a serum screening programme using proMBP in combination with age as risk markers.

MATERIALS AND METHODS

First-trimester maternal serum samples were obtained through a maternal serum screening programme for syphilis at Statens Serum Institut, Copenhagen, from 25 pregnant women with a Down syndrome fetus (DS pregnancies) and 156 women with a normal pregnancy outcome. Second-trimester maternal serum samples, 105 DS pregnancies and 151 normal pregnancies, were obtained through a screening programme for Down syndrome and fetal malformation at Statens Serum Institut, Copenhagen. All diagnoses were verified by karyotyping. Gestational age was determined from last menstrual period, and in most cases confirmed by ultrasound. All serum samples were stored at -20°C until use. Controls and Down syndrome maternal serum samples were matched for length of storage (± 0.5 years) and had only been frozen and thawed once before analysis.

The serum concentration of proMBP was determined by a two-site immuno-radiometric assay as previously described (Wagner *et al.*, 1993). Briefly, serum samples were reduced and alkylated (Maddox *et al.*, 1983) and added to microtitre wells coated with the catching mouse monoclonal antibody J13-6B6. Following incubation and washing, bound proMBP was detected with ^{125}I-labelled mouse monoclonal antibody J14-8A2. As a calibrator, a pool of pregnancy serum was used, previously calibrated against purified MBP (Wasmoen *et al.*, 1989).

Medians of serum proMBP were estimated for each gestational week by linear regression of the logarithm to proMBP concentrations on gestational week (weighted log-regression). All concentrations were transformed into multiples of the median (MoMs) of the unaffected pregnant women of the same gestational age. Groups were compared by the Mann–Whitney U-test. Compatibility with the log-normal distribution was assessed by normal plots supplemented with Shapiro–Wilk's test (using $p=0.001$ as significance level due to the extreme sensitivity to the presence of outliers) (Altman, 1991). Correlations were performed by the Spearman rank test. Estimates of discriminatory efficiency were obtained by receiver–operator characteristics analysis.

For the estimation of detection rates and false-positive rates of proMBP in combination with age for different risk cut-offs, published age-specific risks for Down syndrome at term were used (Cuckle *et al.*, 1987) together with a recently suggested standardized aggregate age distribution of women giving birth (Van der Veen *et al.*, 1997). This distribution is very similar to the Danish distribution from 1992 (Danmark Statistik, 1993). Detection rates and screening performance was assessed by a Monte Carlo based approximation procedure using an S-plus programme described in detail elsewhere (Larsen *et al.*, 1998). The upper and lower truncation limits of log MoM MSpMBP were chosen to be 1.0 and -1.0, respectively.

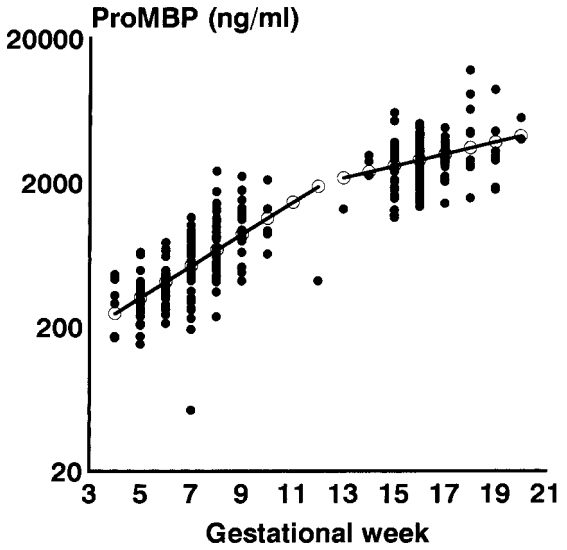

Fig. 1—The distribution of maternal serum concentrations of proMBP (MSpMBP) as a function of gestational age in pregnant women with a normal fetus ($n=308$). The open circles mark the regressed medians

RESULTS

The distribution of maternal serum concentrations of proMBP (MSpMBP) as a function of gestational age in normal pregnancies is shown in Fig. 1. MSpMBP increased with gestational age, and more so in the first trimester than in the second. A linear regression was performed on the logarithm of values of MSpMBP for first and second trimester separately, and the residuals were normally distributed. The medians produced from the log-regression are also shown in Fig. 1. All log-regressed medians were within the 95 per cent confidence interval of the empirical median.

All MSpMBP values were converted to multiples of the regressed median (MoM) and the MoMs obtained in Down syndrome (DS) pregnancies are shown in Table 1 and Fig. 2. The MoM values in DS pregnancies were markedly lower than one in early first trimester and clearly higher than one in the second trimester. In weeks 5–9, the median MoM (95 per cent confidence interval) in DS pregnancies ($n=5$) was 0.66 (0.49–0.79), significantly lower than in controls ($p=0.003$). In weeks 10–12, the median MoM in DS pregnancies was 1.06 (0.71–1.97) ($n=10$), not different from one. In weeks 14–20, the median MoM in DS

Table 1—Distribution of proMBP MoM values in DS pregnancies. In the first trimester, individual MoM values are given

Week	*N*	MoM	Median MoM
5	3	1.52	0.79
		0.65	
		0.79	
6	2	0.58	0.51
		0.43	
7	1	0.39	0.39
8	4	0.73	0.51
		0.36	
		0.66	
		0.66	
9	5	1.13	0.69
		3.11	
		0.67	
		0.69	
		0.49	
10	4	1.12	0.92
		1.53	
		0.71	
		0.74	
11	2	1.99	1.50
		1.00	
12	4	0.88	1.33
		1.81	
		1.97	
		0.48	
13	0	—	
14	7		1.22 (0.64–5.04)[a]
15	32		1.25 (0.80–1.74)[b]
16	22		1.47 (1.01–2.40)[b]
17	18		1.88 (1.45–2.52)[b]
18	15		2.10 (1.14–3.09)[b]
19	8		2.01 (0.60–2.73)[b]
20	3		0.61 (0.53–1.42)[a]
Weeks 5–8	15		0.66 (0.49–0.79)[b]
Weeks 10–12	10		1.06 (0.71–1.97)[b]
Weeks 14–20	105		1.62 (1.18–1.98)[b]

[a]Range.
[b]95 per cent confidence interval of median.

pregnancies ($n=105$) was 1.62 (1.18–1.98), significantly higher than in controls ($p<10^{-6}$). There was no significant correlation between maternal age and MoM MSpMBP either in controls or in DS pregnancies.

The MoM MSpMBP values in normal controls and DS pregnancies in weeks 5–9 were log-normally distributed, $p=0.27$ and $p=0.035$, respectively (Shapiro–Wilk's test). Likewise, the logarithms of MSpMBP values were normally distributed in weeks 14–20 in both controls and DS pregnancies, $p=0.12$ and $p=0.0012$, respectively (Shapiro–Wilk's test). The low p-value obtained in the group of DS pregnancies in weeks 5–9 was caused by a single high-level outlier, and in the group of DS pregnancies in weeks 14–20 by significant negative kurtosis, with a normalized derivative (kurtosis/standard error of kurtosis) of -2.1 ($p<0.05$). The normal plots in controls and DS pregnancies in both parts of gestation were clearly compatible with a log-normal distribution (data not shown).

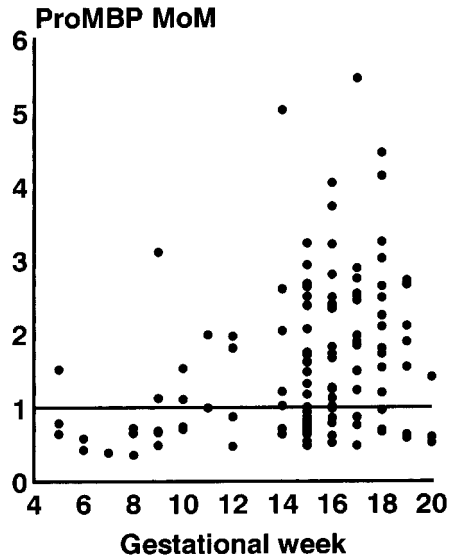

Fig. 2—MSpMBP MoM values in DS pregnancies as a function of gestational age. The line passing through MoM=1 represents the median of normal pregnancies

As the slight deviations from log-normality would tend to increase the discrimination between normal and DS pregnancies, we have not attempted to make corrections. As a consequence, the screening performance will be slightly underestimated. In weeks 5–9 log MoM MSpMBP is distributed with a mean of 0.0023 and a standard deviation of 0.2048 in controls, and a mean of -0.14444 and standard deviation of 0.2396 in DS pregnancies. In weeks 14–20 the same parameter was distributed with a mean of 0.0014 and standard deviation of 0.1569 in normal pregnancies, with a mean of 0.1713 and standard deviation of 0.2611 in DS pregnancies.

The discriminatory efficiency of MSpMBP MoM values was assessed in parametrized receiver–operator characteristics (ROC) analysis, based on the log-normal distributions given above. The detection rate for DS pregnancies, for a false-positive rate of 5 per cent was found to be 22 per cent in weeks 5–9 (using MoM MSpMBP $\leqslant$ cut-off) and 38 per cent in weeks 14–20 (using MoM MSpMBP $\geqslant$ cut-off). There was good agreement between parametrized ROC curves and empirical ROC curves (data not shown).

The expected detection rate and false-positive rates for a maternal serum screening programme based on age alone, proMBP alone, and the combination of age and proMBP, for the risk cut-off values of (for having a DS child at birth) 1:100, 1:250 and 1:400 are given in Table 2 for weeks 5–9 and 14–20, respectively.

DISCUSSION

This is the first demonstration of the usefulness of proMBP as a marker for Down syndrome in maternal serum screening. The gestational age dependence of the MSpMBP MoMs in DS pregnancies is very similar to that found for Schwangerschaftsprotein1 (SP1) (Qin

Table 2—Performance of age and proMBP+age in screening for Down syndrome

Marker(s)	Risk >1:400		Risk >1:250		Risk >1:100	
	DR	FPR	DR	FPR	DR	FPR
Age	43.3%	11.8%	32.9%	6.3%	19.8%	1.6%
5–9 weeks:						
Age+proMBP	49.7%	11.3%	36.8%	5.5%	19.0%	1.3%
14–20 weeks:						
Age+proMBP	57.1%	9.4%	48.4%	5.3%	33.4%	1.5%

et al., 1997c), whereas it differs from that of pregnancy-associated plasma protein-A (PAPP-A) where the optimal diagnostic window in the first trimester is from weeks 7–12 (Qin *et al*., 1996).

In early first trimester (weeks 5–9), the discriminatory potential of proMBP is not as good as that of SP1 (Qi *et al*., 1997c), and the performance assessment is based on only 15 cases, so it is not possible to make firm statements as to the performance of this marker so early in pregnancy, but proMBP should be included in prospective trials assessing the possibilities of screening at such an early time in pregnancy. Even though chorionic villus sampling (CVS) cannot be performed prior to week 10, due to the risk of inducing fetal defects (Firth *et al*., 1994), and a definitive fetal diagnosis thus cannot be established prior to weeks 11–12, an early (prior to week 10) supplementary screening, based on proMBP and or SP1 (Qin *et al*., 1997c), could give information that might be used by itself, or in combination with 10–12 week screening so as to increase the efficiency of the total screening programme.

The definition of a set of maternal serum markers useful in high efficiency screening for DS prior to week 9, would solve many of the inherent problems in late first-trimester and second-trimester screening, e.g. late termination with associated technical and psychological problems. However, very early screening may identify many DS cases that would have spontaneously aborted. Based on data available from weeks 9–14, it can safely be assumed that more than 50 per cent of the DS pregnancies diagnosed prior to week 10 will miscarry (Macintosh *et al*., 1995; Hook *et al*., 1988; Kratzer *et al*., 1992; Snidjers *et al*., 1994). The DS cases used in this study were identified either at CVS/amniocentesis or at birth, indicating that the DS cases identified through screening using proMBP will not solely be cases destined for abortion. The detection rates (DRs) given in this study thus reflect the reduction in disease prevalence, in the second trimester or at birth, to be expected if screening by proMBP is introduced.

In weeks 10–12, proMBP does not seem to discriminate between DS pregancies and normal pregnancies, but the number of cases in the present study is so small that further studies are necessary to reach a final conclusion. However, the existence of a 'gestational age window of uselessness' has been described for several placenta-derived markers for Down syndrome (Bersinger *et al*., 1995). Gestational weeks 10–12 seem to be the part of the first trimester where new screening is most likely to be introduced, as it is possible to perform serum screening based on PAPP-A and the free-β subunit of human chorionic gonadotrophin (free β-hCG) with a DR of 63 per cent for an FPR of 5.5 per cent with a risk cut-off at 1:300 (Wald *et al*., 1996). Furthermore, ultrasound screening with measurement of nuchal translucency is being advocated by centres mastering the technique (Pandya *et al*., 1995). A study group under the auspices of the Royal College of Obstetricians and Gynaecologists in London, recently issued a recommendation for the introduction of measurement of nuchal translucency into clinical practice. The study group was divided on the issue of whether the performance of serum screening in the first trimester had been sufficiently clarified, and a minority opinion found that prospective screening should not be initiated until more retrospective data had been acquired (Royal College of Obstetricians and Gynaecologists, 1997).

In weeks 14–20, the discriminatory performance of proMBP is similar to that of hCG or free β-hCG (Wald *et al*., 1994), where a DR of 48 per cent for an FPR of 5 per cent was found when the marker was used together with age. Based on a population model, it has been calculated that screening in the second trimester using the four markers, β-hCG, αhCG, AFP and unconjugated oestriol (uE3) would result in a DR of 65 per cent for an FPR of 5 per cent (Wald *et al*., 1994). Recently, it has been estimated that a screening using AFP, uE3, hCG and inhibin A could achieve a DR of 76 per cent for an FPR of 5 per cent (Wald *et al*., 1997). Whether this performance can be obtained in clinical practice or even improved by including proMBP or substituting proMBP for one or several markers remains to be seen. The possibility of substituting proMBP for hCG is an important option, particularly so, as patenting policies may make it important to be able to choose between different sets of markers.

In this study the DS pregnancies were identified either through a screening programme or diagnosed at birth. This has the advantage that the estimates of performance relates to the detection rates of DS cases that would survive until the second trimester or term, and thus provides information on the effect of screening on the prevalence of disease. The disadvantage of this method of selection is that it only makes it possible

to calculate the performance of screening among pregnant women already identified through another screening programme, so the results should be assessed through prospective screening, where the detection rate among otherwise screening-negative women can be ascertained.

The maternal serum concentrations of proMBP in normal pregnancies increase throughout the first trimester and level off in the second trimester (Oxvig *et al*., 1995). This is compatible with the results of a previous study (Wagner *et al*., 1993). The concentration of proMBP in pregnancy has been shown to correlate with maternal weight and gravidity (Wagner *et al*., 1993) and with the number of placental septa or cysts (Wasmoen *et al*., 1991). Whether these correlations will influence the performance of proMBP as a maternal serum marker for DS is not clear. It could not be tested in the present study.

The determination of proMBP concentrations is presently technically complicated, as it is necessary to reduce and alkylate the samples before analysis. If the reduction step is omitted, the measured concentrations will decrease by a factor of 100 (Maddox *et al*., 1983). The development of monoclonal antibodies reactive with complex bound proMBP may circumvent this problem and make the proMBP determination easy to include in automated assay systems.

A final conclusion on the clinical usefulness of proMBP, which will depend both on the performance of the marker itself and its relative performance compared with other established markers such as hCG, must be established in prospective clinical studies.

ACKNOWLEDGEMENTS

Lars Sottrup-Jensen was supported by the Danish Biomembrane Centre and a basic research grant from Aarhus University.

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