do carry a risk of error should the restriction enzyme digestion not be complete. The heteroduplex genotyping technique is straightforward to perform and produces gel patterns that are easily interpreted without ambiguity. In heterozygotes both alleles are visualized to the same extent. Heteroduplex analysis requires only three steps to obtain a result (DNA extraction, PCR, and electrophoresis). The speed, economy, and robustness of the method make it particularly suitable for screening studies requiring large sample throughput. Although this method requires the initial synthesis of the HG, once it is synthesized and cloned theoretically unlimited supplies are available. Whole EDTA blood or buccal cells are both suitable sources of DNA for genotyping. The recovery of cells from the cheek lining using a “cytobrush” is a convenient method for both patients and laboratory.

In a study of 605 healthy subjects from South Wales, the genotype frequency was TT, 73 (12.1%); CT, 242 (40.0%); and CC, 290 (47.9%). The gene frequency for the T allele (0.32 ± 0.03, 95% confidence interval) is similar to that described in other Northern European studies (9–11). This economical and rapid method for MTHFR genotyping will facilitate further research on the role of folate and homocysteine in human disease.

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References


Is Prostate-specific Antigen a Marker for Pregnancies Affected by Down Syndrome? Kevin Spencer and Paul Carpenter (Endocrine Unit, Clinical Biochemistry Department, Harold Wood Hospital, Gubbins Lane, Romford, Essex, RM3 0BE United Kingdom; *author for correspondence: fax 44 (0) 1708381486, e-mail Kevin_Spencer@ Compuserve.com)

Prenatal screening for trisomy 21 (Down syndrome) based on the analysis of biochemical markers in maternal serum during the second trimester of pregnancy is becoming an established part of obstetric practice (1, 2). Several markers have been investigated in the second trimester, and of these, a combination of α-fetoprotein and free β-human chorionic gonadotropin (hCG) has been shown in retrospective (3) and prospective studies (4, 5) to achieve detection rates of 65–75% (with a 5% false-positive rate) in routine practice. Similarly, combinations including α-fetoprotein, total hCG, and unconjugated estriol have been shown in prospective studies to perform almost as well, with detection rates on average of 65% (6).

Recent developments in this area of prenatal screening have focused on three specific areas. The first area is that of refinements to the risk algorithm, leading to improved detection efficiency by taking into account a variety of factors that influence the analyte concentrations, for example, multiple pregnancies (7), maternal weight (8–10), insulin-dependent diabetes mellitus (11, 12), gravity (13), ethnicity (9), previous pregnancy results (14, 15), and smoking (16). Correction for these factors seeks to reduce the between-patient variance, leading to reductions in the false-positive rate. The second area involves the investigation of possible new markers of trisomy 21, such as dimeric inhibin A, which has been reported to add anything from 3% (17) to 22% (18) to the detection rate using existing double or triple marker approaches. In addition, beta core in urine (19) and free β-hCG in urine (20) have both been proposed as possible markers of trisomy 21. Neither the urine approach nor the addition of dimeric inhibin A have yet been shown conclusively to be better than existing procedures, and indeed their use is still somewhat controversial. The third area of investigation is the development of screening programs in the first trimester, in which a combination of ultrasound (nuchal translucency) and the biochemical markers pregnancy-associated plasma protein A and free β-hCG enabled detection rates of ~90% (21).

Recently, Lambert-Messerlian et al. (22) in this Journal reported increased concentrations of prostate-specific antigen (PSA) in the maternal serum of second trimester pregnancies affected by trisomy 21. Although PSA was originally thought to be a marker specific to the prostate, a variety of biological fluids and extracts of female tissues has now been shown to contain and produce a material that reacts positively in sensitive PSA immunoassays. The original observation leading to the study of PSA in cases of trisomy 21 was a study in amniotic fluid (AF) that observed low values of PSA in four of six cases (23).

In this study, we endeavored to reproduce these initial
observations, using a similarly sensitive commercially available PSA assay (24), and to investigate the potential value of a sensitive PSA assay in screening for trisomy 21 in the second trimester of pregnancy.

The study population consisted of cases of trisomy 21 in women who presented through the Neural Tube Defect and Trisomy 21 screening program in this laboratory during the period 1994–1998. The serum collected from each woman was stored as aliquots at −20 °C after being analyzed in routine screening with assays for α-fetoprotein and free β-hCG. Our local ethics committee approved the use of stored serum banks for research. The samples plus analytical and clinical data were collated on the basis of abnormal birth outcome, with karyotyping or cytogenetic confirmation after midtrimester amniocentesis. A total of 43 singleton pregnancies associated with trisomy 21 were identified with maternal serum samples taken between 14 and 17 weeks of gestation. Each case of trisomy 21 was matched for gestational age, length of storage (±14 days), and number of freeze-thaw cycles with five samples from unaffected singleton pregnancies (total, 215 controls).

Of the cases of trisomy 21, 32 of 43 were identified by the screening program to have a trisomy 21 term risk of <1 in 250, and 11 of 43 were identified as a result of a liveborn trisomy 21 baby. The mean maternal ages were 28.4 years (range, 17–43 years) for the controls and 34.2 years (range, 18–43 years) for the trisomy 21 cases. The median gestational age was 115 days (range, 98–125 days) for the controls and 108 (range, 98–120 days) for the trisomy 21 cases. The mean storage time was 30.2 months (range, 4–49 months) for samples from controls and 30.0 months (range, 4–49 months) for samples from trisomy 21 cases.

AF samples were identified from those in a previous study (25, 26). A total of 38 AF samples from cases of trisomy 21 with gestational ages from 16 to 20 weeks were available for analysis. A total of 110 control samples with gestational ages from 16 to 20 weeks were identified as matched for the number of freeze-thaw cycles and length of storage (±1 month).

PSA concentrations were measured with an Immulite automated third generation ultrasensitive immunoluminescent assay (Diagnostic Products Corp., Los Angeles, CA). The assay has been shown by Ferguson et al. (24) to have comparable sensitivity and clinical performance to the assay used in the initial studies with cases of trisomy 21 (22, 23). The detection limit of the assay in our hands analyzing 30 replicates of the assay zero diluent was 2 ng/L (+2 SD of the measurement of the zero diluent). The detection limit (CV) was 5.3% at 7786 ng/L, 2.6% at 2704 ng/L, and 3.5% at 32.4 ng/L. The precision and sensitivity are similar to those observed by Ferguson et al. (24).

To assess the suitability of using the Immulite third generation PSA assay to measure PSA concentrations in AF, which has ~1/10 of the protein concentration of serum, a series of dilution experiments were carried out in which five male serum samples were diluted in a both zero diluent and phosphate-buffered saline (to simulate the low protein concentrations in AF). The results of the assay validation showed parallel and almost identical 100% recovery in the buffer and protein matrix and indicated no problem with matrix effects when measuring samples with low protein concentrations.

Results for each analyte were expressed in multiples of the median (MoM) for unaffected pregnancies of the same gestational age, derived from the regressed log_{10} medians for the analyte when appropriate and from the overall control population median for analytes with concentrations unrelated to gestational age. Statistical analysis of data was performed using Astute, a statistical software add-in for Microsoft Excel 5 (DDU Software, University of Leeds, UK).

Table 1 shows the observed and regressed median data for AF PSA and the observed medians for maternal serum PSA across the second trimester period. For AF, the median values showed a statistically significant increase (P < 0.01, Mann–Whitney U-test) across the second trimester period, whereas for maternal serum there was no significant change (P >0.05) across this period. The overall control population median in maternal serum was 9 ng/L, with PSA 25- to 100-fold higher in AF. The median concentrations in AF are comparable with those observed by Melegos et al. (23). Lambert-Messerlian et al. (22) stated that the median concentrations in maternal serum varied with gestational age (by −20%); however, they showed no data. Our study cannot confirm a variation of maternal serum PSA with gestational age.

In maternal serum, the median PSA MoM in the trisomy 21 group was 0.89 (95% confidence interval, 0.67–2.56); this was not significantly different from the controls (P = 0.2543, Mann–Whitney U-test). In the control group the 10th and 90th centiles were 0.22 and 4.56, respectively, with a 5th to 95th centile of 0.11–13.0. In the trisomy 21 group the 10th and 90th centiles were 0.22 and 7.22, respectively. The distribution of PSA MoM in the trisomy 21 group with gestational age is shown in Fig. 1B. The distribution of PSA MoM fitted a gaussian distribution after log_{10} transformation in the control group and the trisomy 21 group. The parameters of the distribution are mean log_{10} (controls = −0.003; trisomy 21 group = −0.104) and log_{10} SD (controls = 0.5635; trisomy 21 group = 0.5573).

In AF the median PSA MoM in the trisomy 21 group was 0.80 (95% confidence interval, 0.49–1.68); this was not significantly different from the control group (P = 0.4117, Mann–Whitney U-test). In the control group the 10th and 90th centiles were 0.23 and 4.15, respectively, with a 5th to 95th centile of 0.19–5.40. In the trisomy 21 group the 10th and 90th centiles were 0.16 and 7.18, respectively. The distribution of PSA MoM in the trisomy 21 group with gestational age is shown in Fig. 1A. The distribution of PSA MoM fitted a gaussian distribution after log_{10} transformation in both the trisomy 21 group and the controls. The parameters of the distribution are mean log_{10} (controls = 0.000; trisomy 21 group = −0.112) and log_{10} SD (controls = 0.4424; trisomy 21 group = 0.6722).

When the AF data in the trisomy 21 group were
analyzed by fetal sex, the median MoMs were 0.81 and 0.73 for female and male fetuses, respectively ($P = 0.3954$, Mann–Whitney U-test). No difference could be established in the control group.

In conclusion, we have been unable to confirm the previous report of Lambert-Messerlian et al. (22) that maternal serum PSA is increased in pregnancies with trisomy 21 fetuses. PSA concentrations in the previous study (22) were less than the detection limit of the assay in 22% of the control cases, whereas in our study only 10% of control cases were below the detection limit. It is possible, therefore, that the study design and this higher percentage of controls with undetectable PSA concentrations deflated the median value in the earlier study (22), resulting in the trisomy 21 MoMs being overestimated.

Previously, Melegos et al. (23) from the same group showed low concentrations of PSA in the AF of four of six cases of trisomy 21, and in our study we can confirm that AF contains PSA and that PSA concentrations on average tend to be lower in cases of trisomy 21. Moreover, AF PSA changes significantly with gestational age. The lowering of AF PSA in our study is mirrored by a lowering of maternal serum PSA in the trisomy 21 group. However, the amount of the lowering of maternal serum PSA and the width of the distribution are likely to mitigate against PSA being of value in a screening context.

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References

Table 1. Median AF and maternal serum PSA concentrations in the second trimester.

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<th>Regressed No.</th>
<th>Observed maternal serum PSA, ng/L</th>
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Fig. 1. Distribution of PSA in trisomy 21.
(A) AF PSA (MoM) in 38 cases of trisomy 21. (B) Maternal serum PSA (MoM) in 43 cases of trisomy 21.
Influence of Methodology on the Detection and Diagnosis of Congenital Analbuminemia, Andrew W. Lyon,1,2 Paul Meinerz,1,3 Garth A. Bruce,3,4 Victor A. Laxdal,1,2 and Mark L. Salkie1,2 (Departments of 1Pathology and 3Pediatrics, College of Medicine, University of Saskatchewan, and Departments of 2Laboratory Medicine and 4Pediatrics, Saskatoon District Health, Saskatoon, Saskatchewan, Canada) 7N OW8; * address correspondence to this author at: Department of Laboratory Medicine, Royal University Hospital, 103 Hospital Dr., Saskatoon, SK, Canada S7N OW8; fax 306-655-2223, e-mail andrew.lyon@usask.ca; poster presented at the 42nd Annual Conference of the Canadian Society of Clinical Chemists and the Canadian Association of Medical Biochemists, June 14–18, 1998, Ottawa, Canada)

Congenital analbuminemia is a rare recessive inherited disorder characterized by an absence or very low concentrations of serum albumin (1, 2). The diagnosis of analbuminemia is suggested by persistent hypoproteinemia with adequate hepatic function and a lack of evidence for either a renal or gastrointestinal protein loss. The diagnosis is typically confirmed by serum protein electrophoresis that depicts an absence of an albumin band (3). Rapid diagnosis of analbuminemia is appealing because patients can be subjected to extensive and prolonged testing to ascertain the cause of their apparent protein-losing disorder. Unfortunately, the diagnosis of analbuminemia may be delayed by clinically misleading laboratory results generated by tests of analytes that are usually bound to albumin, e.g., calcium (4) and thyroxine (5), and tests sensitive to the concentration of albumin in the matrix, e.g., some free thyroxine immunoassays (6, 7). In particular, rapid diagnosis of analbuminemia would be delayed if the method for serum albumin falsely reports the presence of albumin.

The most commonly used serum albumin assays in clinical laboratories rely on dye-binding techniques (8). Characteristically, these methods accurately measure the concentration of serum albumin near the limits of the reference range. In conditions such as nephrotic syndrome, the serum albumin and the albumin:globulin ratio are low. Under these conditions, results of the bromcresol green (BCG) methods can be falsely inflated (9–15) and bromcresol purple-binding methods prone to falsely lowered results (16), although reagent manufacturers have adopted many variations of the dye-binding albumin methodology in an attempt to assure accuracy.

Dye-binding albumin methods would likely be used to initially assess albumin status in undiagnosed patients with analbuminemia. Dye-binding albumin methods have falsely indicated the presence of albumin in analbuminemia in a rat model (17) and in a case report (18); however, few analytical details were reported. Here we compare the ability of two BCG methods to detect the absence of serum albumin in two new cases of congenital analbuminemia and comment on the role of methodology in previous studies.

Patient DN was admitted as a newborn with hypoglycemia and apnea; investigations revealed apparently low serum albumin (15 g/L). His serum albumin concentration remained low despite 24-h urine protein excretion rates within reference values. He was readmitted on three additional occasions in his first 6 months of life with respiratory distress and was eventually started on treatment for chronic asthma. There was no identifiable cause for the patient’s low serum albumin and no evidence of a protein-losing enteropathy, based on a 99Tc-labeled albumin scan.

Patient KB was admitted at 2 days of age for treatment of cellulitis and was subsequently readmitted three times over the next 6 months for respiratory distress involving upper respiratory tract infections and pneumonia. The reported serum albumin remained low (10–15 g/L), and there was no identifiable source of protein loss.

Serum protein electrophoresis performed at 10 weeks (patient DN) and 12 weeks (patient KB) revealed an absence of an albumin band (0–3 g/L), and the diagnosis of congenital analbuminemia was considered. Both neonates had no symptoms directly attributable to the lack of albumin, and both presented with recurrent respiratory tract symptoms related to bronchial asthma or pneumonia, which may be a coincidental observation (19–21).

In each case serum albumin >10 g/L was reported from an Ektachem 700XR analyzer at an early stage of clinical investigation. Reported albumin concentrations were variable, with no corresponding clinical changes but with method-dependent variability (Table 1). By immunoassay, albumin was <0.001 g/L (assay detection limit) for patient DN. BCG assays are prone to positive bias (9–15); however, the two BCG assays produced different results with analbuminemic sera (Table 1). Moreover, the Ektachem 700XR analyzer did not detect that the concentration of albumin was substantially below the lower limit of assay linearity. These observations prompted an evaluation of the dynamic range for each albumin assay.