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A Maternal Serum Screen for Trisomy 18: An Extension of Maternal Serum Screening for Down Syndrome

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Summary

The feasibility of extending second-trimester maternal blood screening for Down syndrome so as to include screening for trisomy 18 was examined using stored maternal serum samples collected for neural tube–defect screening. There were 12 samples from trisomy 18 pregnancies and 390 controls. The median maternal serum concentration of α-fetoprotein, free α-subunit human chorionic gonadotrophin, free β-subunit human chorionic gonadotrophin, intact human chorionic gonadotrophin, total estriol, unconjugated estriol, estradiol, human placental lactogen, and progesterone were lowered in those pregnancies affected by trisomy 18 when compared with unaffected pregnancies matched for racial origin, maternal age, gestational age, and sample-storage duration. At an estimated odds risk of 1:400, 83.3% of affected pregnancies were detected using an algorithm which combines the maternal age–related risk with the maternal serum concentrations of unconjugated estriol, free α-subunit human chorionic gonadotrophin, free β-subunit human chorionic gonadotrophin, estradiol, and human placental lactogen. The associated false-positive rate was 2.6%. At high risk odds of 1:10, the detection rate was 58.3%, with an associated false-positive rate of 0.3%. β-Subunit human chorionic gonadotrophin and unconjugated estriol were the most powerful discriminators. It is possible to incorporate into existing Down syndrome screening programs an algorithm for detecting trisomy 18 with high sensitivity and specificity.

Introduction

Several studies have noted an association between low maternal serum α-fetoprotein (AFP) and trisomy 18 (Merkatz et al. 1984; DiMaio et al. 1987). The chance observation by Merkatz et al. (1984) that a "below sensitivity" AFP sample was obtained from a trisomy 18–affected pregnancy, led to an investigation which demonstrated that low AFP was associated with trisomies 13, 18, and 21. Since the completion of that study and subsequent confirmation of the results, trisomy 21 has been the focus of attention. Trisomy 21 screening stategies have been developed which combine AFP results with prevalence rates of Down syndrome at different maternal ages (Cuckle et al. 1984; Baumgarten et al. 1985; Fuhrmann et al. 1985; Palomaki 1986; DiMaio et al. 1987; Knight et al. 1988). Further improvement in trisomy 21 detection strategies has occurred with the observation that there is an association between trisomy 21 and maternal serum concentrations of (1) human chorionic gonadotrophin (Bogart et al. 1987), (2) unconjugated estriol (Unc E3) (Canick et al. 1988; Wald et al. 1988*a*) and (3) specific β1 glycoprotein (SP-1) (Bartels and Lindemann 1988; Petrocik et al. 1990).

In contrast, screening strategies for trisomy 18 have not been fully developed, because of the lower incidence of this trisomy, its lethality, and, until recently, an apparently lower potential for detection. The usefulness of AFP, human chorionic gonadotrophin, and/or Unc E3 in trisomy 18 screening has been reported in several recent small studies (Canick et al. 1989; Bartels et al. 1990; Darnule et al. 1990; Nebiolo et al. 1990).

In South Australia a pilot Down syndrome antenatal

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maternal serum screening program (funded by the South Australian Health Commission) was implemented by the Department of Chemical Pathology, Adelaide Children's Hospital, on 1 January, 1991. The screening program is available to all South Australian expectant mothers. The biochemical indicators used to compute Down syndrome risk are free α-subunit human chorionic gonadotrophin (α-hCG) and free β-human chorionic gonadotrophin, (β-hCG), AFP, Unc E3, and human placental lactogen (hPL). In the present study we assess whether the measurement of several analytes in maternal serum in the second trimester can be used to screen for trisomy 18.

Material and Methods

Case Descriptions

Maternal serum was available from 12 pregnancies resulting in the birth of a single child with trisomy 18. The diagnosis was confirmed by chromosome analysis in all cases. Eleven had regular trisomy 18, and one (case 1) had a translocation, 46,XX, + t(11;18). In addition, case 8 had a balanced Robertsonian translocation, 46,XX, + t(13;14), + 18. All cases other than cases 2, 5, 9, and 11 were given an autopsy. The major birth defects noted at autopsy and/or birth are listed in the appendix.

Samples

All maternal serum samples used in the present retrospective study were routinely collected during the operation of the South Australian Neural Tube Defects Screening Programme between 1980 and 1988. The samples had been stored frozen at −40°C since collection. Data detailing pregnancy outcome, sample condition, and storage history were available for all samples used.

The affected population comprised 12 samples of maternal serum obtained at gestational age 16–21 wk, from women 18–33 years of age whose pregnancies were affected by trisomy 18. There were seven full-term live born, two premature live born, two stillbirths, and one termination at 30 wk gestation after ultrasound detection. Three control samples from women whose pregnancies were unexceptional were selected for each test sample. These were matched for maternal race, maternal age (to within 1 year), gestational age (to within 1 wk), and date of sample collection (to within 1 wk). For trisomy 18 the mean gestational age was 17.7 wk (SD 1.56), mean maternal age was 331 mo (SD 57.5), and mean sample-storage duration was 39.2 mo (SD 24.9). For controls the mean gestational age was 17.5 wk (SD 1.55), mean maternal age was 330 mo (SD 52.4), and mean storage duration was 38.7 mo (SD 25.6). In addition, a further 354 unmatched control samples were available to confirm population parameter estimates and to validate inferences which arose during the investigation.

Analyses Performed

The analytes investigated were AFP, free α-hCG, free β-hCG, intact human chorionic gonadotrophin (I-hCG), total estriol (Tot E3), Unc E3, estradiol (E2), hPL, and progesterone (Prog). The specific and sensitive immunoassays used in the present study are commercially available and were performed as described by the manufacturers' instructions, except in those cases stated otherwise. The performance of each assay was determined by estimating 20 replicates for each of two different levels of QC material, and the assay imprecision was reported as the mean ± 1 SD. Measurement of AFP and I-hCG was carried out using the Pharmacia DELFIA solid-phase immunofluorometric system. For the determination of I-hCG, a dilution of 5 μl serum in 500 μl assay buffer was used, and the results were multiplied by 110 for sample size. Assay imprecision data for AFP and I-hCG were 21.7 ± 0.86, 101.9 ± 48 and 3.78 ± 0.66, 69.0 ± 8.64, respectively. The free α- and free β-hCG kits were obtained from Bio Clone Australia Pty. Ltd., and both are immunoradiometric assays. For measurement of free α-hCG a volume of 10 μl serum was used, and the results were multiplied by 5 for sample size. Assay imprecision data for free α- and free β-hCG were 49.2 ± 4.47, 46.8 ± 5.36 and 6.60 ± 0.94, 14.6 ± 1.25, respectively. The manufacturers' assessment of cross-reactivity states that the α-subunit monoclonal antibody has a 1.1% cross-reactivity with free β-hCG and a 0.18% cross-reactivity with I-hCG, whereas for the β-subunit monoclonal antibody there is no detectable cross-reactivity with free α-hCG and 0.36% cross-reactivity with I-hCG.

Assays for Tot E3, Unc E3, and hPL were obtained from Amersham (Australia) Pty. Ltd. and were all competitive radioimmune assays. Assay imprecision data for Tot E3 and Unc E3 were 11.2 ± 0.46, 48.1 ± 6.99 and 137.2 ± 7.1, 767.2 ± 83.2, respectively. For measurement of hPL, a 100-μl serum volume was used, and the results were multiplied by 0.2 for sample size. The assay imprecision data for this assay were 0.68 ± 0.06 and 3.02 ± 0.19.

Assays for E2 and Prog were obtained from Diagnostic Products Corporation and were both coated-antibody-tube radioimmune assay methods. Assay imprecision data for E2 and Prog were 0.39 ± 0.04, 3.35 ± 0.28 and 2.76 ± 0.85, 59.3 ± 13.2, respectively.

Data Analysis

Data analysis had three objectives: (1) to determine the most statistically significant combination of maternal serum analytes which could discriminate between affected and unaffected pregnancies, (2) to determine the optimum screening strategy by using the current range of analytes proposed and implemented for Down syndrome screening in South Australia (i.e., AFP, α-hCG, β-hCG, Unc E3, and hPL), and (3) to incorporate the trisomy 18–detection algorithm within the locally developed computer software for Down syndrome screening.

The data analysis involved transformation of biochemical data, isolation of the significant biochemical discriminator variables, construction of multivariate normal distributions for the set of feasible combinations of the significant biochemical variables, estimation of maternal age–specific incidence, computation of individual odds risk for trisomy 18, derivation and validation of detection rates and false-positive rates, development of an optimum screening algorithm, and sensitivity analysis of the optimum screening algorithm.

The method of data analysis was similar to that described elsewhere for Down syndrome (Wald et al. 1988*b*). Differences included (*a*) the use of several alternative statistical test procedures to cater for the small sample size, (*b*) the substitution of more reliable control-population parameters, derived from a total sample of 390 unaffected pregnancies, where there was no conflict with parameter estimates derived from the 36 matched control samples, and (*c*) the relaxation of endpoint adjustments for extreme analyte observations.

Although there were several extreme values encountered in the data, no observations were either discarded as outliers or modified in any way. It was assumed that in practice such observations would occur with low frequency. It will be shown that observations occurring in the lower tail of particular analyte distributions are pointers to the presence of trisomy 18. This situation can be contrasted with Down syndrome screening (Wald et al. 1988*b*), in which extreme values have been replaced by endpoint values.

Transformation of biochemical data. — The data analysis commenced with an examination of the correlation matrices and underlying univariate distributions of the biochemical variables for both the control and test samples. An examination of the correlation matrices revealed that (*a*) gestational age was pairwise correlated with most biochemical variables, (*b*) maternal age and sample-storage duration could be considered independent of any variable, and (*c*) pairwise correlation existed between most biochemical variable combinations. An examination of the underlying univariate control distributions of the biochemical variables revealed that they were positively skewed. To simultaneously remove these effects both the control and affected data were expressed as natural logarithmic gestational age–specific multiples of the median (MoM).

Since there was no statistical difference between the control medians obtained from the matched sample of 36 observations and those of the 354 unmatched control observations, the gestational age–specific MoMs derived from the total sample of 390 unaffected pregnancies were used. Prog was the only biochemical variable not correlated with gestational age and was expressed in natural logarithmic MoM form by pooling all gestational ages.

Isolation of significant biochemical discriminator variables. — Several regression-model functional forms were estimated, to isolate the significant trisomy 18 predictor variables. Iterative methods of variable selection were employed. These methods included stepwise regression using the forward-selection procedure and backward-elimination procedure. During this stage of the study, care was exercised to consider the substitution effects of those biochemical variables that exhibited a significant pairwise correlation. All iterative-regression procedural methods implied, in order of relevance, that β-hCG, Unc E3, E2, α-hCG, and hPL were the statistically significant analytes at the $P < .05$ level. At this stage of the analysis, AFP, Tot E3, and Prog were eliminated from any further consideration, and there was doubt concerning the relevance of α-hCG and hPL.

Construction of multivariate normal distributions. — Both a control distribution and an affected multivariate normal distribution were constructed for each feasible combination of the significant analytes.

Estimation of maternal age–specific incidence. — Information concerning maternal age–specific incidence of trisomy 18 at 16–21 wk gestation in South Australia was inadequate for the purpose of the present study. Consequently, the trisomy 18 maternal age–specific

incidences at 16 wk gestation for maternal ages 33 years and above, derived by Hook (1983), were used. For maternal ages under 33 years the incidence was linearly interpolated between 0.2/1,000 at maternal age 16 years and 0.6/1,000 at maternal age 33 years.

Computation of individual odds risk.—A computerized screening algorithm was developed which combines the maternal age–specific risk estimate with the biochemical risk estimate to produce a total trisomy 18 odds risk for each pregnancy. The probability of a trisomy 18 fetus, based wholly on the biochemical screening variables, was determined by the method of maximum likelihood for each feasible combination.

The total odds risk of a trisomy 18 fetus, based on any feasible analyte combination and maternal age, was computed by direct multiplication of maternal age probability and biochemical screening-variable likelihood ratio to obtain a final probability assessment for each pregnancy. This probability was then expressed as an odds risk.

Derivation and validation of detection rates and false-positive rates.—The detection rates and false-positive rates, for each feasible analyte combination were derived using numerical integration. The areas obtained were then proportioned and summed over the maternal age distribution of confinements in South Australia, by using single-year intervals over the range 16– to 50 years, to obtain approximate detection and false-positive rates. The detection and false-positive rates were assessed at various odds-risk cutoff values ranging between a high risk of 1:10 and a low risk of 1:400.

Development of an optimum algorithm.—The optimum algorithm sought was one which would provide the maximum detection rate with a low false-positive rate and which used the analytes implemented for Down syndrome screening in South Australia. The optimum algorithm was developed using sensitivity analysis. The optimization criteria employed in the present study assumed that chromosome analyses would be undertaken for all cases determined to be positive by the screen. The optimum algorithm was selected on the criteria of the minimum expected number of chromosome analyses required per trisomy 18 detection.

Results

The analyte concentration results, expressed in gestational age–specific MoMs for unaffected pregnancies, for the 12 trisomy 18–affected cases appear in table 1. Despite the small number of affected cases available to the present study, there is strong statistical evidence to suggest that the presence of a trisomy 18 fetus is associated with lowered concentrations of eight of the nine investigated indicators of fetoplacental function. An equivalence-of-means test was conducted to compare the mean (and median) obtained from the control sample data with the mean (and median) obtained form the trisomy 18–affected sample data. An equivalence relationship was rejected (P = .05) for all biochemical variables except α-hCG.

An examination of the correlation matrices revealed that many pairwise correlations were significant. In particular, the pairwise correlation between β-hCG and I-hCG for the affected correlation matrix (P = .94) indicated that these analytes had similar power for discriminating between trisomy 18 and normal pregnancies, and the control correlation matrix (P = .77) indicated a strong positive substitution effect. Other biochemical variables which were significantly pairwise correlated included (*a*) Unc E3 and AFP, Tot E3, E2, and hPL; (*b*) β-hCG with α-hCG; and (*c*) hPL with α-hCG, E2, and Tot E3.

The relevant summary statistics for three selected regression equations were calculated as follows (values in parentheses are standard errors, and values in square brackets are t values (absolute value):

Model 1: y = .0219(.0074)[2.944] + .0437αhCG (.0232)[1.886] − .0749βhCG(.0104)[7.202] − .0853 UncE3(.0213)[4.004] − .0408 E2(.0167) [2.439] − .0348 hPL(.0211)[1.650], where y = 1 if trisomy 18 affected and 0 if unaffected. Model 1 is the basic model resulting from both the elimination of irrelevant variables by using the backward-elimination procedure and the inclusion of relevant variables by using the forward-selection procedure. The implied order of biochemical variable relevance was β-hCG, Unc E3, E2, α-hCG, and hPL.

Model 2: y = .0183(.0075)[2.434] − .0757IhCG-(.0116)[6.553] − .0952UncE3(.0204)[4.668] − .039-3E2(.0169)[2.334]. Model 2 is the regression model obtained when β-hCG is replaced by I-hCG to test the degree of substitutability indicated by their pairwise positive correlation. The regression model obtained suggested that hPL was no longer a statistically significant addition to the equation if I-hCG was included as the substitute predictor. The regression was therefore estimated with hPL deleted from the equation.

An important objective of the present study was to develop an optimum screening strategy by restricting the range of analytes to those implemented for Down

Table 1

Analyte Concentrations for Trisomy 18–Affected Pregnancies, Expressed in Multiples of Median for Unaffected Pregnancies

	Concentration of (MoM)								
Sample	AFP	α-hCG	β-hCG	I-hCG	Unc E3	Tot E3	E2	hPL	Prog
1	.72	.69	.53	.72	.56	.62	.63	.44	.56
2	.69	.45	.16	.20	.48	.55	.25	.39	.60
3	.53	.54	.17	.23	.41	.48	.31	.49	.40
4	.47	.73	.05	.07	.51	.34	.31	.85	.63
5	.82	1.52	1.00	.93	.60	.68	.37	.91	.95
6	.66	.71	.15	.14	.63	.97	.41	.45	.43
7	1.06	1.01	.39	.56	.73	.69	1.51	.81	1.41
8	.56	1.25	.32	.20	.25	.35	.48	.36	.68
9	.51	1.54	.29	.45	.25	.32	.17	.55	.67
10	1.41	.99	.91	.97	.76	.72	.57	.74	.58
11	.78	1.06	.61	.54	.59	.58	.70	.62	1.04
12	.49	.52	.02	.08	.55	.61	.89	.56	.31
Median	.68	.86	.31	.34	.55	.60	.45	.55	.62

Note.—Data are adjusted for gestational age.

syndrome screening in South Australia. Although β-hCG was selected ahead of I-hCG, the substitution of I-hCG for β-hCG will result in minimal loss of predictive ability. The manufacturers' assessment of cross-reactivity of β-hCG with I-hCG is 0.36%, indicating that β-hCG accounts for virtually all of the free-β signal. In these data there was insufficient evidence to discriminate between I-hCG and β-hCG. Further statements concerning β-hCG apply equally to I-hCG.

Model 3: $y = .0221(.0075)[2.963] - .0757\beta hCG(.0101)[7.489] - .1118UncE3(.0183)[6.104]$. Model 3 is the regression model obtained when E2 is eliminated from consideration. The statistically significant regressor variables were α-hCG, β-hCG, Unc E3, and hPL. However, when only β-hCG and Unc E3 were considered, the predictive ability of the model did not change by any statistically significant amount. The effect on the false-positive rate was negligible. This could be evidenced by noting that (*a*) the adjusted R^2 statistics remained stable, (*b*) the effects of hPL were absorbed by the coefficient of Unc E3 (significantly pairwise correlated), (*c*) the effects of α-hCG were absorbed by β-hCG (significantly pairwise correlated), and (*d*) there was no statistical difference in the constant-term estimates. The minimal change in predictive ability was expected, since (*a*) both β-hCG and Unc E3 were highly significant ($P = .0001$), whereas α-hCG and hPL only attained significance at the $P = .05$ level, and (*b*) the pairwise correlations were known a priori. The inclusion of E2 raised the theoretical detection rate from approximately 70% for the β-hCG and Unc E3 combination (when an odds-risk cutoff value of 1:200 was used to approximately 72% when all three analytes were used.

Despite the favorable regression results implying that hPL and α-hCG were statistically significant ($P = .05$), it is suggested that they both be confirmed at a higher level of statistical significance prior to being formal used. There are two main reasons for this conservative approach. First, the sample size of 12 affected cases is small, and there remains insufficient evidence to suggest that the α-hCG concentration obtained from a trisomy 18–affected pregnancy differs from that obtained for an unaffected pregnancy (table 1). Second, there were more unexplainable extreme values encountered in the control data with these two analytes than with most other analytes. The regression-model parameter estimates were shown to be sensitive to these extreme observations. This was evidenced by noting that the omission of three extreme control observations led to α-hCG and hPL failing to reach significance at the $P = .05$ significance level.

The estimated individual odds risks for the 12 affected cases, when two different analyte combinations of interest are used, appear in table 2. Analyte "combination 1" is the five-analyte combination estimated to have maximum trisomy 18 detection capability—namely, β-hCG, Unc E3, E2, α-hCG, and hPL. "Combination 2" is the five-analyte combination implemented for trisomy 21 detection in South Australia. It is noticeable that the estimated trisomy 18 odds risks are similar for the two combinations. The similarity

Table 2

Trisomy 18 Odds Risk Estimates Computed by Screening Algorithm

Sample	Maternal Age (years/mo)	Odds Risk for Combination of Analytes 1	Odds Risk for Combination of Analytes 2
1	33/2	198	213
2	21/0	1	1
3	32/5	1	1
4	27/1	1	1
5	30/5	1,716	2,199
6	23/7	1	2
7	29/6	393	385
8	18/1	1	1
9	32/6	1	1
10	30/4	8,802	11,840
11	27/4	307	331
12	25/6	3	1

of odds can be attributed to the dominance of the combination of β-hCG and Unc E3. The majority of the trisomy 18–affected cases had β-hCG samples more than 3 SD below the median obtained for unaffected pregnancies and had Unc E3 samples more than 1.5 SD below the median obtained for unaffected pregnancies. Both of these combinations are a marginal improvement over the combination of Unc E3 and β-hCG. The combination of Unc E3 and β-hCG had estimated detection and false-positive rates of 57.9% and 0.3%, respectively, at a maternal odds risk of 1:10, increasing to 80.4% and 3.1% at a maternal odds risk of 1:400. All feasible combinations containing these two analytes produced similar odds estimates, detection rates, and false-positive rates. These two particular analytes are clearly the dominant biochemical indicators examined in the present study.

As shown in table 2, six of the 12 cases have an estimated odds risk of 1:1. The screening algorithm rounds the computed odds risk to the nearest integer. Hence, a computed odds-risk estimate of 1:1 indicates at least a .4 probability of being affected. Most of these unrounded odds-risk estimates implied that the pregnancy was more likely to be a trisomy 18–affected pregnancy than a non–trisomy 18–affected pregnancy.

The detection rate will depend on the odds-risk cutoff level selected, the management protocol applied to the individual sample-specific odds risk, and family attitudes toward testing and pregnancy termination. The computed odds risks for samples 2–4, 6, 8, 9, and 12 would clearly be sufficient for offering counseling to the family with a view to detailed ultrasound examination and amniocentesis. The computed odds risks for samples 5 and 10 would be insufficient to indicate that any further action is warranted. The management of samples with computed odds risks similar to samples 1, 7, and 11 would depend on the selection of an appropriate odds-risk cutoff level to signal a "positive case" and on the development of an appropriate management strategy. Factors affecting selection of the cutoff level will include the pre- and post-natal lethality of trisomy 18, the detection and false-positive rates, the capacity of cytogenetic services to perform additional tests, and the risk of miscarriage after amniocentesis.

Table 3 is a summary of the estimated detection and false-positive rates, at selected odds-risk levels, for the two analyte combinations examined in table 2. These estimates were derived by considering the multivariate normal analyte distributions and the current maternal age distribution of the South Australian population.

In an attempt to confirm these results, the screening algorithm was tested against the sample of 36 matched controls and a further 354 unaffected pregnancies. The average maternal age of these 390 unaffected pregnancies exceeds the current average maternal age in the South Australian population by approximately 3 years. Table 4 is a summary of the detection and false-positive rates at selected odds-risk levels obtained from the sample. Given the small sample of 12 affected cases and 390 unaffected cases available to the study, the detection and false-positive rates obtained in tables 3 and 4 are not dissimilar. The false-positive rates are generally higher for the sample of 390 observations (table 4) when compared with their

Table 3

Theoretical Detection and False-Positive Rates for Trisomy 18 Detection Algorithm at Various Levels of Maternal Risk

	Rates for			
	Combination of Analytes 1		Combination of Analytes 2	
Odds Risk	Detection (%)	False Positive (%)	Detection (%)	False Positive (%)
1:10	60.5	.3	58.5	.3
1:100	68.3	.5	65.8	.5
1:200	74.9	1.0	72.1	1.1
1:300	80.1	1.7	77.4	1.9
1:400	84.7	2.6	81.9	3.0

theoretical rates (table 3). This either could be due to the inherent maternal age bias of the sample data or may simply reflect sampling variation.

On completion of the study it was noticed that, if the sum of the β-hCG and Unc E3 MoMs for each of the trisomy 18–affected cases are calculated and if these sums are then arranged in order of increasing magnitude, the result was the identical arrangement achieved by rearranging the samples in order of computed risk (unrounded) according to the trisomy 18 screening algorithm. The trend was also apparent for the 390 unaffected cases. This implies three important generalizations: (1) a screening protocol can be developed on the basis of the ad hoc approach of summing the two analyte MoMs (these two analytes are pairwise uncorrelated); (2) the results confirm the dominance of Unc E3 and β-hCG in trisomy 18 detection; and (3) the contribution of maternal age in risk derivation is minor when compared with the results of the biochemical screening variables.

Discussion

We have estimated an individual pregnant woman's risk of having a trisomy 18 fetus at 16–21 wk gestation, on the basis of her maternal age and the measurement, in maternal serum, of biochemical indicators of fetoplacental function. Further, the biochemical indicators have been restricted to those implemented for Down syndrome screening in South Australia, and it has been possible to incorporate a separate numerical algorithm into the locally developed software for Down syndrome screening.

The results obtained from the present study suggest that a strategy involving the offer of definitive chromosomal analysis for computed trisomy 18 odds risks exceeding 1:10 would be cost efficient and effective. Some 60% of trisomy 18 cases could be detected at 16–21 wk gestation in South Australia. One case of trisomy 18 would be detected for every 12 chromosome analyses performed specifically for this disorder.

Table 4

Screening Algorithm Performance against a Sample of 390 Unaffected and 12 Affected Pregnancies at Various Levels of Maternal Risk

	Rates for			
	Combination of Analytes 1		Combination of Analytes 2	
Odds Risk	Detection (%)	False Positive (%)	Detection (%)	False Positive (%)
1:10	58.3	0/390	58.3	0/390
1:100	58.3	(4/390) 1.0	58.3	(4/390) 1.0
1:200	66.7	(9/390) 2.3	58.3	(10/390) 2.6
1:300	66.7	(12/390) 3.0	66.7	(13/390) 3.3
1:400	83.3	(14/390) 3.6	83.3	(15/390) 3.8

This corresponds to detection of one live-born trisomy 18 for every 30–40 chromosome analyses, since 60%–70% of trisomy 18 pregnancies at this stage of gestation will die during pregnancy.

There was a significant positive pairwise correlation between Unc E3 and E2, for both the trisomy 18–affected and the unaffected samples. The relative importance of E2 may be overstated. The effect on detection and false-positive rates that is implied by the addition of E2 to the Unc E3 and β-hCG combination was relatively minor.

It should also be noted that the 12 trisomy 18–affected cases investigated in the present study either were live born or succumbed at advanced gestational ages. There is a clear need for a study to determine whether the concentrations of the biochemical screening variables for those trisomy 18 fetuses spontaneously aborted in the second trimester are similar to those from fetuses that either approach full term or survive to birth. It is possible that the results obtained in the present study only apply to those trisomy 18–affected pregnancies expected to reach full term.

The pointers to the presence of trisomy 18 were clearly the "very low" concentrations of Unc E3, β-hCG (or I-hCG), and E2. All analyte concentrations were used at face value in the screening algorithm. It must be stated, however, that the accuracy of the estimated odds risks is highly dependent on the accuracy of the assays at low concentrations. If the assays were demonstrated to be imprecise at these levels, the previously mentioned ad hoc method of summing the two MoM scores for Unc E3 and β-hCG (or I-hCG) could be used, rather than the method of estimating individual odds risks. Both methodologies lead to similar differentiation between sample results, detection rates, and false-positive rates. A combined total of 0.8 MoMs for β-hCG (or I-hCG) and Unc E3, with the restriction that AFP and hPL are below normal, corresponds to an odds-risk level of approximately 1:10. Hence, the validity of the results obtained do not rely on the specificity of the assays at extreme concentrations. The results obtained confirm the results obtained by Canick et al. (1990) and further expand the set of potential indicator analytes useful in screening for trisomy 18 and other chromosomal defects.

The dominance of low concentrations of the combination of Unc E3 and β-hCG (or I-hCG) in the detection of trisomy 18 has also raised an important point concerning the maternal age component of trisomy 18 and trisomy 21 detection algorithms. Since maternal age and the results of the biochemical screening variables are considered jointly independent measures of risk, their contribution to the total odds-risk estimate are considered equivalent. The trisomy 18 results obtained have indicated that (*a*) more emphasis should be placed on the analyte concentrations relevant for the detection of trisomy 18, by weighting the results obtained for analyte concentrations and maternal age to reflect this apparent inequality and (*b*) an investigation of current trisomy 21 detection algorithms may be warranted, to determine whether they too should receive a weighting factor to reflect the relevance that these separate components have in the derivation of a total odds-risk estimate.

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Appendix

Trisomy 18 Case Descriptions

Case 1: Female, 40 wk gestation, birth weight 1,400 g. Hypoplastic fibulae, congenital heart disease (hypoplastic left heart, mitral valve atresia, persistent left superior vena cava draining into coronary sinus, double outlet right ventricle, and ventricular septal defect)

Case 2: Female, 40 wk gestation, birth weight 2,254 g. Microcephaly, cleft palate, hypoplastic thumbs, and congenital heart disease (ventricular septal defect).

Case 3: Male, stillborn at 40 wk gestation, birth weight 1,785 g. Hypoplastic thumbs, congenital heart disease (atrial septal defect and ostium secundum type).

Case 4: Male, 40 wk gestation, birth weight, 1,330 g. Malpositioned anus, absent appendix, and bladder-outlet obstruction.

Case 5: Male, 40 wk gestation, birth weight 1,800 g. Small ventricular septal defects and unilateral choanal atresia.

Case 6: Female, 36 wk gestation, birth weight 1,300 g. Hypoplastic thumbs, horseshoe kidney with hypo-

plastic left component, fused adrenals, and congenital heart disease (ventricular septal defect).

Case 7: Female, 30 wk gestation, birth weight 1,300 g. Holoprosencephaly, Klippel-Feil anomaly, four absent ribs, absence of radii and thumbs, exomphalos, bilateral duplex ureters, and congenital heart disease (ventricular septal defect).

Case 8: Female, 39 wk gestation, birth weight unknown. Microphthalmia and congenital heart disease (transposition of great vessels and ventricular septal defect).

Case 9: Female, 38 wk gestation, birth weight 1,000 g. Congenital heart disease (ventricular septal defect).

Case 10: Female, stillborn at 34 wk gestation, birth weight 1,305 g. Unilateral cleft lip and palate.

Case 11: Female, 36 wk gestation, birth weight 1,740 g. No malformations.

Case 12: Male, 40 wk gestation, birth weight 2,590 g. Malrotation of the bowel, horseshoe kidney, dilated left ureter and pelvis, and congenital heart disease (ventricular septal defect, hypoplastic left ventricle, aortic stenosis, and hypoplastic aorta).

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