GENETICS

Metabolomic analysis for first-trimester trisomy 18 detection

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OBJECTIVE: The purpose of this study was to determine whether nuclear magnetic resonance—based metabolomic markers in first-trimester maternal serum can detect fetuses with trisomy 18.

STUDY DESIGN: This was a study of pregnancies between 11 weeks and 13 weeks 6 days' gestation. We analyzed 30 cases of trisomy 18 and a total of 114 euploid cases. Nuclear magnetic resonance—based metabolomic analysis was performed. A further analysis was performed that compared 30 cases with trisomy 18 and 30 trisomy 21 (T21) cases.

RESULTS: Metabolomic markers were sensitive for trisomy 18 detection. A combination of 2-hydroxybutyrate, glycerol and maternal age had a 73.3% sensitivity and 96.6% specificity for trisomy 18 detection, with an area under the receiver operating curve: 0.92 ($P <$ .001). Other metabolite markers, which include trimethylamine, were sensitive for distinguishing trisomy 18 from T21 cases.

CONCLUSION: This is the first report of prenatal trisomy 18 detection that has been based on metabolomic analysis. Preliminary results suggest that such markers are sensitive not only for the detection of fetal trisomy 18 but also for distinguishing this aneuploidy from T21.

Key words: metabolomics, screening, trisomy 18

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Metabolomics involves the comprehensive cataloging and characterization of the small molecules that are found in cells, organs, and tissues. Metabolomics has allowed scientists to develop an increasingly detailed picture of the biochemical activity of cells in both their normal and diseased states. The impact of external influences (eg, toxins, medications, and environmental changes) can be mapped based on metabolic profiles or metabolic signatures that have been measured by modern metabolomic techniques. Advances in instrumentation and improvements to bioinformatic tools are now permitting very extensive analysis of human biofluids with the use of these techniques.[1,2]

Metabolomics has shown significant promise for the discovery of new biomarkers for the detection of a number of complex clinical disorders.[3] However, the use of metabolomics in obstetrics is a relatively new phenomenon. Nevertheless, a number of recent studies have shown its value in the prediction of or detection of biomarkers for preeclampsia and preterm labor.[4-7] More recently, we identified a novel set of maternal serum metabolomic markers for the first-trimester detection of fetuses with Down syndrome.[8] Trisomy 18 is the second most common aneuploidy after Down syndrome. Maternal first-trimester screening for trisomy 18 detection has been an area of research interest for many years[9] and the standard of clinical care for more than a decade.[10,11]

Our objectives were, first, to determine whether maternal first-trimester serum from trisomy 18 pregnancies had a qualitatively or quantitatively distinct metabolomic profile relative to normal pregnancies. Second, we wanted to evaluate the sensitivity and specificity of serum metabolite markers for the detection of trisomy 18. Finally, we wanted to compare trisomy 18 with previously reported cases of Down syndrome to determine whether these 2 aneuploidies could be distinguished with the use of first-trimester maternal serum metabolomic analysis.[8]

Materials and Methods

Patients and specimen samples were obtained as part of an ongoing prospective study by the Fetal Medicine Foundation in London, England. The study was approved by the institutional review board of King's College Hospital, London, England. In this study, an average risk population of British women were screened and recruited prospectively from 2003-2009. Written consent was obtained from all participating women. The details of the study population, consent approval by institutional authorities, specimen collection, storage, transportation, and metabolomic analysis have been described in a previous publication.[7] Briefly, maternal demographic data, sonographic measurements, and karyotype data were entered prospectively in a database.

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TABLE 1
Comparison of ultrasound and demographic markers

Parameter	Trisomy 18 (n = 30)[a]	Control cases (n = 60)[a]	*P* value
Crown rump length, mm	58.7 ± 6.5	65.5 ± 8.8	< .001
Delta nuchal translucency, mm	4.0 ± 2.6	0.7 ± 0.3	< .001
Maternal age, y	36.7 ± 5.2	31.3 ± 6.4	< .001
Maternal weight, kg	69.7 ± 20.6	66.4 ± 13.7	.29
Maternal race, %			.001
White	27.0 ± 28.7	67.0 ± 71.3	
Nonwhite	3.0 ± 10.0	47.0 ± 41.2	
Parity: nulliparous, %	9.0 ± 30.0	52.0 ± 45.6	.149

[a] Data are given as mean ± SD.

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Nuchal translucency measurement was performed; pregnancy-associated plasma protein−A and free-β human chorionic gonadotropin measured as a part of routine clinical screening for aneuploidy. Delta nuchal translucency (ΔNT) values or the difference between the observed NT measurement and the median expected value based on the crown rump length (CRL) were calculated.[12] Maternal venous blood was collected in plain vacutainer tubes (Becton Dickinson UK Limited, Oxfordshire, UK), processed within 15 minutes of blood collection, and centrifuged at 3000 rpm for 10 minutes to separate serum from packed cells. The serum that was obtained was separated into 0.5-mL aliquots and stored at −80°C until subsequent analysis. None of the samples had been thawed and refrozen previously. The serum samples were transported by air courier to the laboratory in Alberta, Canada, for metabolomic testing; the samples were packed in dry ice and maintained in a frozen state at all times. Case and control specimens for the primary study were collected within 1 week of each other. The cases with trisomy 18 had a fetal karyotype confirmed based on chorionic villus sampling; control subjects were selected from women who delivered live born neonates at full term with a normal karyotype or a normal phenotypic examination.

The study was limited to 11-13 weeks 6 days' gestation. An important advantage of metabolomic analysis is that a relatively large number of markers can be identified simultaneously that leads to the development of several different predictive algorithms. We looked at metabolomics-only algorithms and algorithms that combined metabolites with maternal demographic and other markers. In the primary metabolomic analysis, we conducted a case-controlled study of 30 cases with trisomy 18 and 60 matched euploid control specimens. Subsequently, to increase the study power and to facilitate the performance logistic regression analyses with an expanded number of predictive variables, an additional set of 54 euploid control specimens from a previously reported trisomy 21 study was also added for further analyses.[8] Only those measured metabolites that were common to each of the trisomy 18 and expanded control cases were considered for this particular analysis. In addition, the metabolomic analysis was limited to cases in which all demographic and sonographic measurements were available. This resulted in a total of 30 cases with trisomy 18 and 114 normal control subjects. For the sake of uniformity, the 30 cases with trisomy 18 and combined 114 control subjects were also used for TheGmax analyses (version 11.9.23; Available at: www.thegmax.com. Accessed April 30, 2013) so that the results of standard logistic regression and TheGmax analyses could be compared. Specimen collection, processing, and metabolomic analysis for the 54 control subjects who were used in the trisomy 21 study were performed with the same standards and were conducted at the same time.[8]

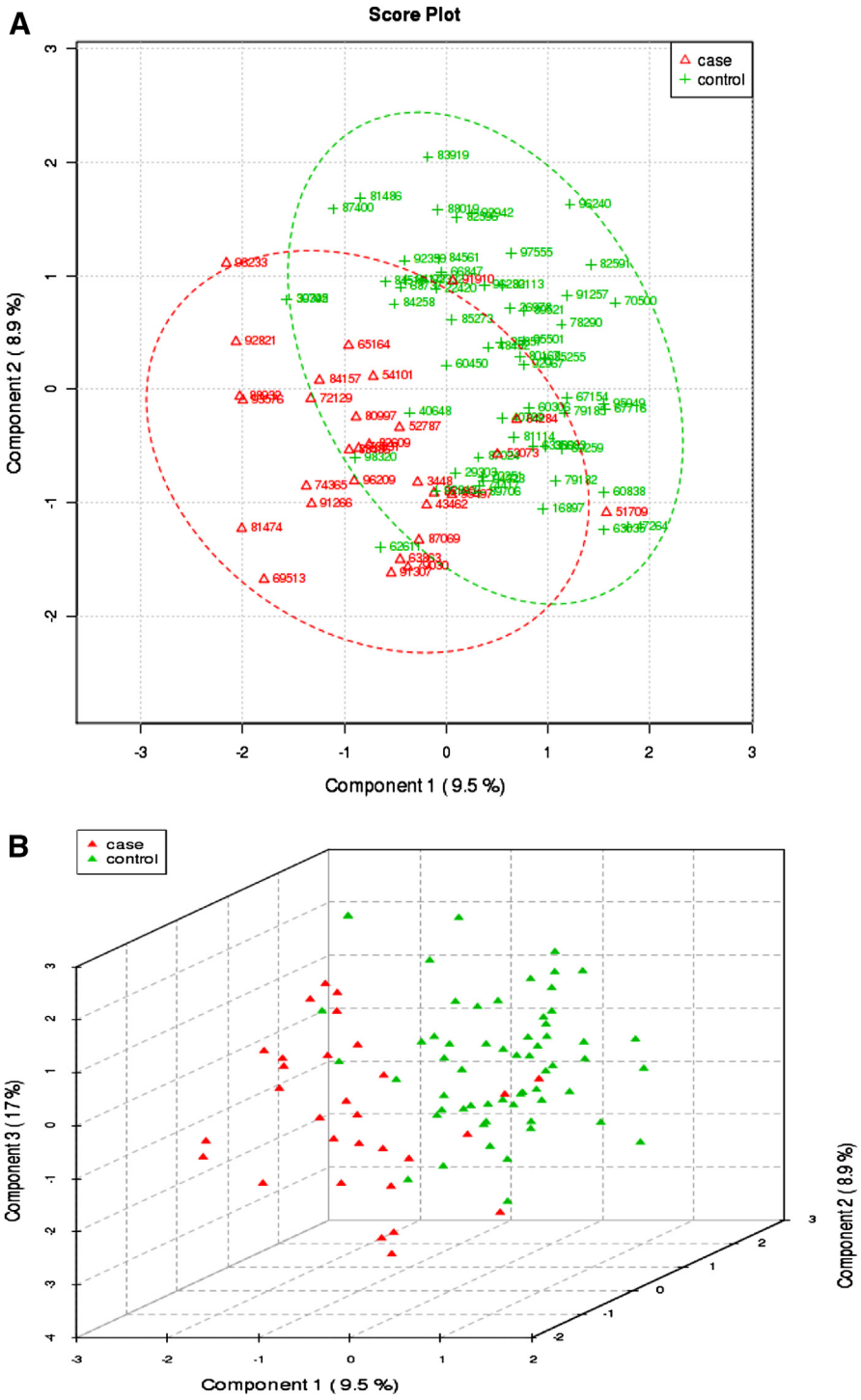
Metabolomic data collection was performed with the Varian Inova 500 MHz NMR (nuclear magnetic resonance) spectrometer (International Equipment Trading Ltd, Vernon Hills, IL) as previously described.[7] Plasma samples were filtered through 3-kd cutoff centrifugal filter units (Amicon Microcon YM-3; Sigma-Aldrich, St. Louis, MO) to remove plasma proteins. Aliquots (350 μL) of each plasma sample were transferred into the centrifuge filter devices and spun (10,000 rpm for 20 minutes) to remove macromolecules (primarily protein and lipoproteins) from the sample. If the total volume of the sample was <300 μL, an appropriate amount from a 50-mmol NaH_2PO_4 buffer (pH 7) was added until the total volume of the sample was 300 μL. Any sample that had to have buffer added to bring the solution volume to 300 μL was annotated with the dilution factor, and metabolite concentrations were corrected in the subsequent analysis. Subsequently, 35 μL of D_2O and 15 μL of a standard buffer solution (11.667 mmol disodium-2, 2-dimethyl-2-silcepentane-5-sulphonate, 730 mmol imidazole, and 0.47% NaN_3 in H_2O) was added to the sample.

The plasma sample (350 μL) was then transferred to a standard microcell NMR tube (Shigemi, Inc, Allison Park, PA) for subsequent spectral analysis. All ^{1}H-NMR spectra were collected on a 500-MHz Inova (Varian Inc, Palo Alto, CA) spectrometer equipped with a 5-mm ITCN Z-gradient PFG cold-probe. The singlet produced by the disodium-2, 2-dimethyl-2-silcepentane-5-sulphonate methyl groups was used as an internal standard for chemical shift referencing (set to 0 ppm) and for quantification. All ^{1}H-NMR spectra were processed and analyzed with the Chenomx NMR Suite Professional Software package (version 7.1; Chenomx Inc, Edmonton, Alberta, Canada) that allows for qualitative and quantitative analysis of an NMR spectrum by manually fitting spectral signatures from an internal database to

the observed spectrum. Specifically, the spectral fitting for all detected metabolites was done with the use of the standard Chenomx 500 MHz metabolite library. Typically, 90% of the visible peaks were assigned to a compound, and >90% of the spectral area could be fit routinely with the use of the Chenomx spectral analysis software. It has been shown previously that this fitting procedure provides absolute concentration accuracy of ≥90%. Each spectrum was processed and analyzed by at least 2 NMR spectroscopists to minimize compound misidentification and misquantification. Metabolomic data analysis was performed with MetaboAnalyst, which is a web-based server that supports a wide range of univariate and multivariate statistical techniques.[13] Log normalization of the metabolomic data was performed before further analysis. Principal component analysis (PCA) is a multivariate statistical technique that we used to identify combinations of metabolites (principal components) that best distinguish trisomy 18 from euploid cases.[14] On the PCA plot, the first principal component, which is the most important discriminator, is represented on the x-axis; the second and third principal components, in order of discriminating power, are plotted on the y- and z-axis in the 2- and 3-dimensional PCA plots, respectively.

Partial least squares discriminant analysis (PLS-DA) further rotates the principal components that are identified on the PCA plot to find the combination of metabolites that optimally distinguishes cases from control subjects.[14] Permutation testing was performed with random relabeling of the metabolomic data and the rerunning of the PLS-DA to minimize the probability that any observed separation between the 2 groups could be due to chance. A total of 2000 repeated relabelings were performed; the probability value that corresponded to the probability that the observed group separation was due to chance was determined. MetaboAnalyst was used for both PCA and PLS-DA. A variable importance in projection (VIP) plot, which indicates the contribution of individual metabolites in the separation

FIGURE 1
Partial least squares discriminant analysis



A, 2-dimensional and **B,** 3-dimensional plots of maternal serum metabolites from trisomy 18 and control cases. Cases with trisomy 18 are in *red*; control cases are in *green*. Clustering and segregation of the 2 patient groups indicate that significant discrimination of groups is achieved with metabolomics (from the primary analysis: trisomy 18, 30 cases; euploid, 60 control cases). We performed 2000 permutations or resamplings ($P = .0095$).

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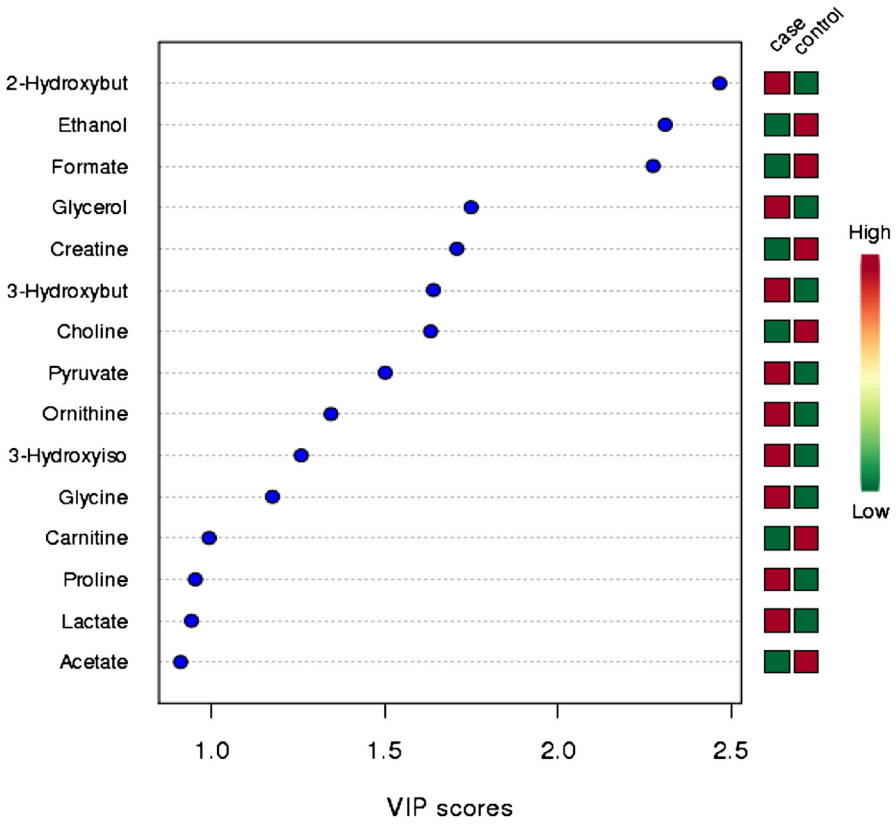
FIGURE 2
VIP plot of cases with trisomy 18 vs euploid cases



The most discriminating metabolites for distinguishing trisomy 18 vs euploid cases are shown in the upper right hand corner of the plot. The legend to the right of the plot indicates whether the metabolite is increased or decreased in the case and control samples (early trisomy 18, 30 cases; control, 60 cases).

VIP, variable importance in projection.

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of cases from control subjects was also constructed.[14]

Finally, we determined whether serum metabolite markers were able to distinguish pregnancies with trisomy 18 from pregnancies with trisomy 21. PCA and PLS-DA were performed with the use of the metabolomic data for 30 cases with trisomy 18 and for the 30 maternal serum specimens with trisomy 21 that had been reported in a previous study.[8] As noted previously, the specimens for trisomy 18 and 21 were collected during the same period from the same patient population and underwent metabolomic analysis at approximately the same time in the same laboratory. A VIP curve was also generated to determine the metabolites that best distinguished pregnancy specimens with trisomy 18 from specimens with trisomy 21.

The means and standard deviations of all measured serum metabolite concentrations were compared between the trisomy 18 and control groups. Using logistic regression, we determined the individual maternal risk of having a pregnancy with trisomy 18 based on metabolites, demographics, and ultrasound measurements. Receiver operator characteristic curves along with areas under the receiver operator characteristic curve (AUC) were determined. The sensitivity and specificity of several different algorithms for trisomy 18 detection were also calculated. In all cases, a probability value of $< .05$ was considered statistically significant.

Algorithms for trisomy 18 prediction were also generated with the use of genetic programming along with their corresponding sensitivity, specificity, AUC, and probability values. Genetic programming mimics the principle of evolutionary genetics (ie, selection, recombination, and mutation) to develop rules for the optimal prediction of an outcome of interest with the use of multiple and diverse prediction variables. The principles of genetic programming have been summarized in some of our previous publications.[7,15] Genetic programming analysis is a robust technique and is not limited by a lack of normality (ie, a Gaussian distribution) in the data, by sample size, or by missing data and is used currently in diverse fields from the pharmaceutical industry to banking. We used a commercial computer program called TheGmax. We also tested for a possible correlation between maternal demographic characteristics, gestational age, and significant metabolites in both trisomy 18 and normal cases.

TABLE 2
Metabolites for trisomy 18 detection—matched study and control cases[a]: logistic regression method

Model	Sensitivity, %	Specificity, %	Area under the curve (95% CI)	*P* value
2-hydroxybutyrate, glycerol	53.3	84.6	0.78 (0.68–0.88)	$< .001$
2-hydroxybutyrate, glycerol, maternal age[b]	73.3	96.6	0.92 (0.85–0.98)	$< .001$

CI, confidence interval.

[a] Trisomy 18, 30 cases; matched normal control, 60 cases; [b] Dataset used for this particular calculation.

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RESULTS

In our initial metabolomic analysis, the 30 cases with trisomy 18 were compared with 60 matched normal control subjects. Table 1 gives a comparison of the maternal demographic characteristics of trisomy 18 and healthy euploid cases. As seen from these data, CRL was lower in the affected cases, and ⊿NT and mean maternal age were higher in the affected cases. There was a significant difference in maternal racial heritage in the 2 groups with a disproportionately high percentage of white pregnancies among the euploid group. There were no significant differences in the frequency of smokers and medical disorders between the 2 groups (not shown). In Figure 1, the 2- and 3-dimensional PLS-DA plots provide significant visual evidence of clustering or separation of the 30 trisomy 18 vs the 60 matched euploid control subjects. Permutation analysis with 2000 resamplings was performed to determine whether the observed separation was due to chance. The results of the permutation analysis indicated that the probability that the observed separation or discrimination between trisomy 18 and normal control specimens was due to chance was .0095.

The VIP plot in Figure 2 indicates that 2-hydroxybutyrate was the most discriminating metabolite for the separation of cases with trisomy 18 from euploid control specimens in the primary analyses. The heat map on the right of the Y-axis indicates that 2-hydroxybutyrate was elevated in trisomy 18 compared with the control specimens.

We performed analyses in the primary study group (trisomy 18, 30 cases; euploid, 60 control subjects) to assess the performance of metabolomic markers in this smaller dataset in which cases were exactly matched with control subjects. The purpose of this exercise was 2-fold: First, we wanted to minimize any unrecognized bias that would affect the results (the smaller number of subjects in this group limits the number of predictive variables that can be considered in each regression analysis); second, we wanted to determine whether the findings in this matched group were

TABLE 3
Maternal first-trimester serum metabolite concentrations

	Concentration, μmol/L[a]		
Metabolite	**Trisomy 18 (n = 30)**	**Control cases (n = 114)**	***P* value**
2-hydroxybutyrate	26.96 ± 13.11	16.93 ± 8.17	< .001
3-hydroxybutyrate	54.57 ± 64.53	32.9 ± 53.42	< .004
3-hydroxyisovalerate	7.38± 4.18	5.24± 3.84	.009
Acetate	28.26 ± 12.83	22.9 ± 31.703	.79
Acetoacetate	19.56 ± 10.63	16.15 ± 9.18	.08
Acetone	22.19 ± 10.86	15.98 ± 9.44	< .001
Alanine	293.4 ± 83.44	258.62 ± 85.38	< .015
Arginine	110.97 ± 26.59	122.82 ± 39.7	< .04
Asparagine	39.13 ± 16.35	31.89 ± 13.6	< .04
Betaine	26.51 ± 11.92	25.27 ± 10.03	.98
Carnitine	26.04 ± 10.5	24.3 ± 11.7	.256
Choline	10.2 ± 2.2	31.6 ± 100.6	.875
Citrate	77.6 ± 19.1	66.1 ± 22.9	.008
Creatine	30.3 ± 12.8	35.6 ± 15.15	.054
Creatinine	55.9 ± 14.0	53.7 ± 14.6	.496
Ethanol	58.8 ± 72.3	63.7 ± 54.1	.346
Formate	19.1 ± 21.3	18.2 ± 11.2	.483
Glucose	4133.0 ± 903.7	3848.99 ± 783.5	.124
Glutamine	292.9 ± 77.3	250.2 ± 77.3	.007
Glycerol	281.8 ± 111.7	194.3 ± 157.44	< .001
Glycine	261.6 (99.4)	209.8 (74.9)	.001
Isobutyrate	6.77 ± 2.5	5.6 ± 4.7	.003
Isoleucine	41.1 ± 10.7	38.6 ± 16.6	.54
Lactate	1247.6 ± 900.3	946.4 ± 451.0	.03
Leucine	72.95 ± 24.0	71.6 ± 40.6	.04
Malonate	14.8 ± 7.5	15.0 ± 8.5	.54
Methionine	20.15 ± 7.05	26.09 ± 11.26	.007
Ornithine	36.83 ± 13.32	28.20 ± 10.61	.001
Phenylalanine	74.91 ± 34.67	58.24 ± 28.67	.003
Proline	135.31 ± 42.98	125.42 ± 48.53	.141
Propylene-glycol	9.66 ± 4.10	7.94 ± 3.69	.019
Pyruvate	74.66 ± 35.11	59.70 ± 23.3	.04
Serine	118.06 ± 37.23	132.03 ± 50.64	.067
Succinate	6.90 ± 6.33	6.50 ± 10.83	.018
Threonine	104.34 ± 48.09	108.80 ± 42.53	.36
Tyrosine	56.9 ± 15.2	55.82 ± 19.6	.46
Valine	121.9 ± 44.00	119.6 ± 38.1	.53
1-methylthistidine	51.2 ± 17.6	43.4 ± 22.3	.018

[a] Data are given as mean ± SD; 30 cases of trisomy 18 and 114 normal control cases (latter group includes 54 cases from a previous study[8]).

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TABLE 4
Metabolites alone and combined with delta nuchal translucency for trisomy 18 detection—expanded study group[a]: logistic regression method

Model	Sensitivity, %	Specificity, %	Area under the curve (95% CI)	*P* value
2-hydroxybutyrate	53.3	86.0	0.80 (0.73–0.89)	< .001
2-hydroxybutyrate, maternal age	63.3	95.6	0.89 (0.83–0.95)	< .001
2-hydroxybutyrate, delta nuchal translucency	90.0	100.0	0.96 (0.90–1.0)	< .001

CI, confidence interval.

[a] Trisomy 18, 30 cases; normal controls, 114 cases.

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consistent with that of the expanded study group in which a larger number of predictive markers can be considered simultaneously in the regression analysis and be presented subsequently. As shown in Table 2, the combination of 2-hydroxybutyrate and glycerol was sensitive for trisomy 18 detection. Maternal race did not contribute to trisomy 18 prediction when considered with these markers.

For the second series of analyses, the 30 cases with trisomy 18 were compared with an expanded number of euploid control subjects (n = 114). As noted earlier, the purpose of evaluating an expanded patient group is to strengthen the power for logistic regression analyses. Table 3 compares the metabolite concentrations between these 2 groups. Of a total of 38 metabolites that were evaluated in this expanded analysis, 21 metabolites were present in significantly different maternal serum concentrations between the 2 groups. Several logistic regression analyses were performed on this expanded dataset. The independent variables that were used in the logistic regression analysis were chosen on the basis of the most discriminating metabolite that was indicated by VIP analysis and important maternal demographic markers (such as age, race, and fetal gestational age). These are used currently in standard clinical screening. These were used to develop prediction algorithms from which AUC curves along with the sensitivity and specificity were calculated. As shown in Table 3, a metabolite-only algorithm that was based on 2-hydroxybutyrate had a 53.3% sensitivity and 86% specificity for trisomy 18 detection. When this metabolite was combined with maternal age, the sensitivity increased to 63.3% and specificity to 95.6%. Maternal race did not contribute significantly to trisomy 18 prediction in these analyses. When 2-hydroxybutrate was combined with ΔNT, a sensitivity of 90.0% and a specificity of 100.0% were achieved (Table 4).

Genetic programming analyses were performed on the expanded patient group of 30 cases with trisomy 18 and 114 euploid cases. Table 5 shows the diagnostic performance with the use of a limited combination of metabolites. Finally, with an expanded number of metabolites (glycine, 2-hydroxybutyrate, ornithine, lactate, pyruvate, and glutamine), maternal age, weight, and ethnicity explained 94.4% of the difference between cases of euploid and cases with trisomy 18. A 73.3% sensitivity and 100% specificity were achieved for trisomy 18 detection, with an AUC of 0.94 ($P < .00001$).

A logical question to ask is whether serum metabolomics can distinguish trisomy 18 from trisomy 21 in first-trimester pregnancies. Towards this end, a total of 30 trisomy 21 pregnancy samples were compared with 30 trisomy 18 specimens. As emphasized previously, these specimens were collected, processed, and stored and underwent metabolomic analyses with identical protocols and at essentially the same time. Our results show that NMR-based metabolomics successfully discriminated first-trimester trisomy 18 from trisomy 21 pregnancies. The clustering and segregation of the 2 groups can be seen in the PCA and PLS-DA plots in Figures 3. Permutation analysis that used 2000 resamplings showed that the observed separation between the 2 aneuploidies had a probability value of < .0005 of being by chance. Based on the VIP plot (Figure 4), trimethylamine appears to be the most important or informative metabolite for distinguishing trisomy 18 from Down syndrome specimens, followed by threonine, creatine and formate. Trimethylamine was elevated in cases with trisomy 18; the other 3 metabolites were reduced compared with trisomy 21 pregnancies.

Based on Spearman's rho, *t*-test, and Mann-Whitney *U* testing, there was no significant correlation between the

TABLE 5
Metabolites and trisomy 18 detection expanded study group[a]: TheGmax (parsimonious model)

Model	Sensitivity, %	Specificity, %	Area under the curve (95% CI)	*P* value
2-hydroxybutyrate	66.7	80.7	0.80	< .001
Acetone and glucose	43.8	98.2	0.81	< .0001
Delta nuchal translucency and glucose	100.0	100.0	1.0	< .00001

CI, confidence interval.

[a] Trisomy 18, 30 cases; normal controls, 114 cases.

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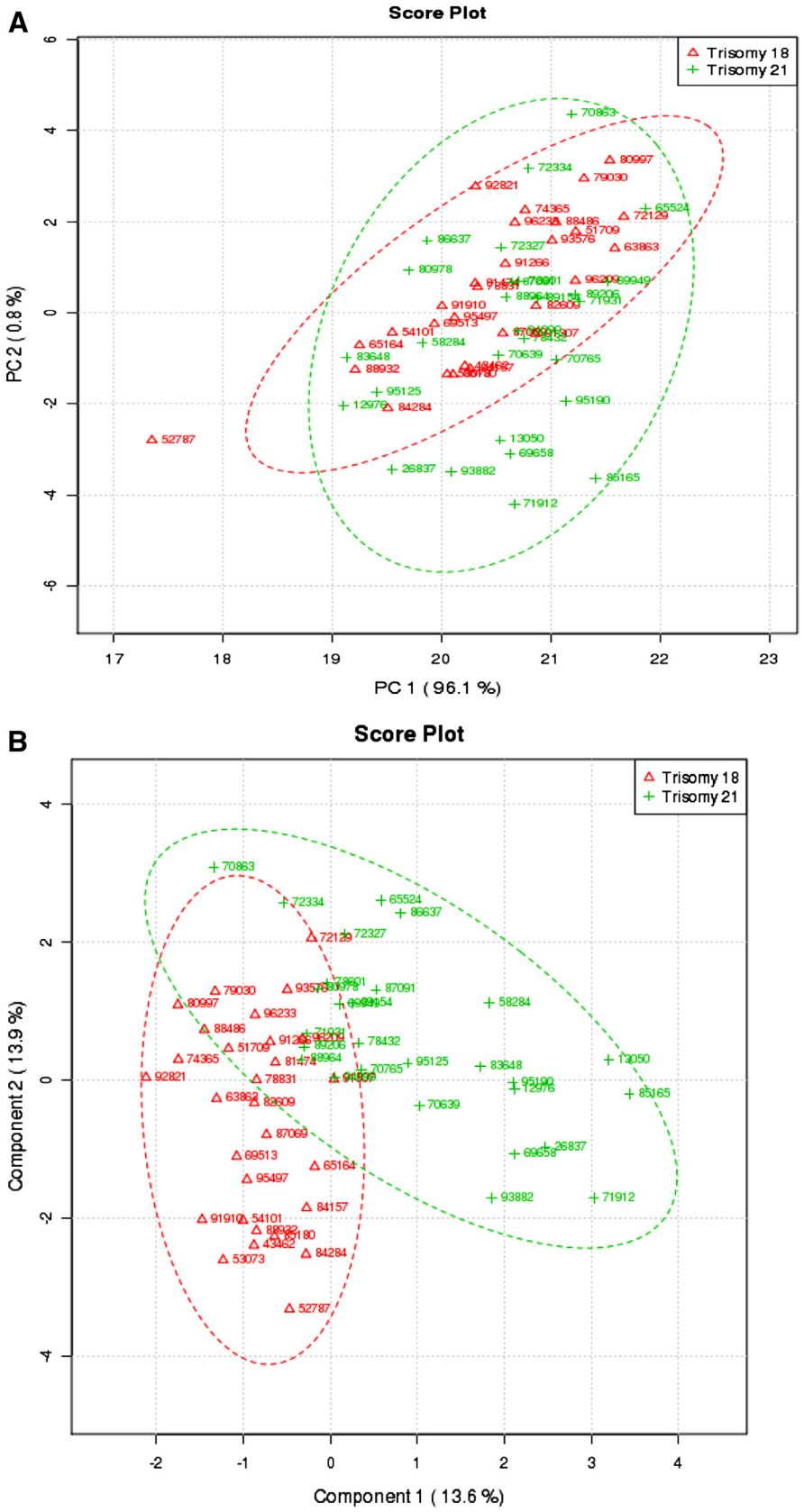
FIGURE 3
Discrimination of trisomy 18 from trisomy 21 pregnancies



most discriminating metabolites (ie, 2-hydroxybutyrate, ethanol, or formate concentrations) and CRL, maternal race, or maternal age in either the trisomy 18 or normal groups. CRL, which is the most accurate measure of gestational age, did not persist in either the logistic regression analyses or genetic programming analyses as a significant independent predictor of trisomy 18, despite the significant reduction of CRL values in the aneuploidy group (Tables 4 and 5). Thus, differences in metabolite concentrations between the case and control groups could not be explained by gestational age or by any maternal demographic characteristics in either the primary or extended analysis groups. Also, as noted previously, there was no significant difference in maternal weight between the groups (Table 1). The genetic programming analyses considered all metabolomic, maternal demographic, and ultrasound markers simultaneously and in an unbiased fashion; however, maternal weight still did not show up as a significant contributor to the prediction algorithms.

Comment

This is the first report to have shown that metabolomic markers in first-trimester maternal serum can be used to distinguish trisomy 18 from normal pregnancies. Unlike conventional screening, metabolomics has the power to detect a large number of predictive markers simultaneously that makes it possible to develop multiple different

Two-dimensional **A,** principal component analysis and **B,** partial least squares discriminant analysis plots of maternal serum metabolites for first-trimester trisomy 18 vs trisomy 21. Cases with trisomy 18 are in *red*; trisomy 21 cases are in *green*. Clustering and segregation of the 2 patient groups are demonstrated. Permutation analysis indicates that that the observed separation has a probability value of .0005 of being due to chance (30 trisomy 18 cases; 30 trisomy 21 cases).

PC, principal component.

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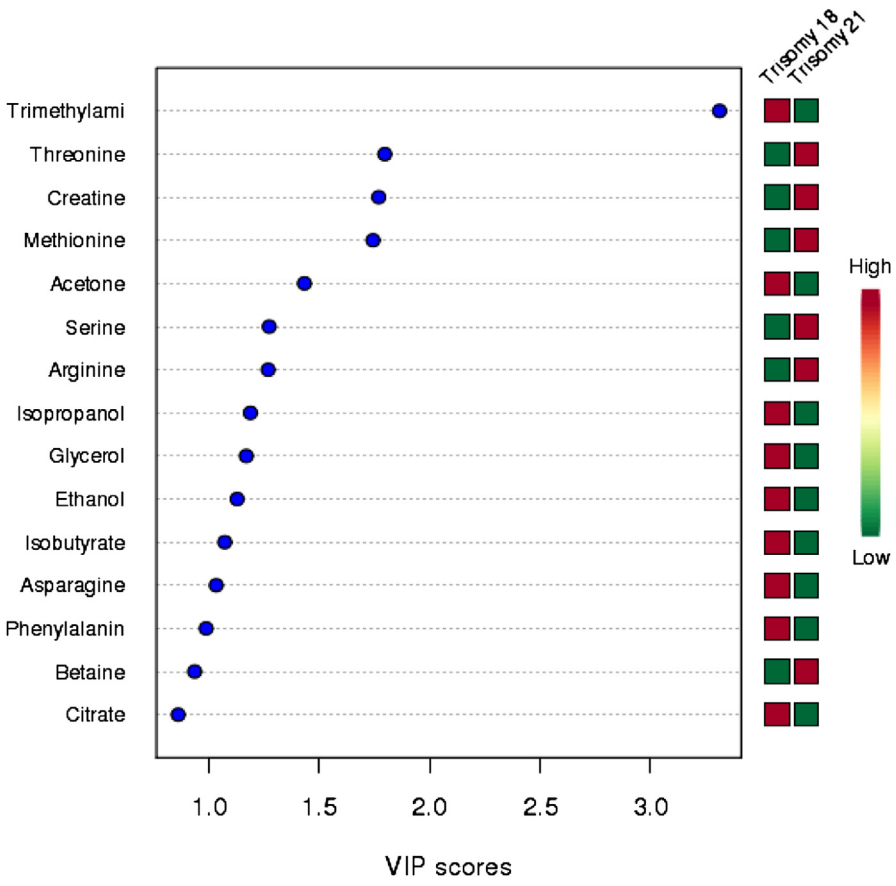
FIGURE 4
VIP plot of trisomy 18 vs trisomy 21 cases



The most discriminating metabolites (trimethylamine, threonine, and creatine) for distinguishing trisomy 18 from trisomy 21 cases are shown in the upper right hand corner of the plot. The legend to the right of the plot indicates whether the metabolite is increased or decreased in the case and control samples (trisomy 18, 30 cases; 30 trisomy 21 cases).

VIP, variable importance in projection.

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algorithms with good to excellent diagnostic accuracies. Various combinations of metabolites, maternal demographic characteristics, and ΔNT measurements therefore were modeled in both the case control and expanded patient groups. VIP analysis indicated that 2-hydroxybutyrate was the most discriminating metabolite for distinguishing trisomy 18 from normal pregnancy. When 2-hydroxybutyrate was combined with glycerol and maternal age, we achieved a 73.3% sensitivity and a 96.6% specificity for trisomy 18 detection (Table 2). When glucose was combined with ΔNT measurements, a level of 100.0% sensitivity and specificity was achieved in the expanded patient group (Table 5).

Because trisomy 18 conventionally is combined with trisomy 21 screening for clinical prenatal aneuploidy assessment, an interesting question was whether metabolomics could distinguish between the 2 disorders. Our study found that significant discrimination between trisomy 18 and trisomy 21 cases was achievable. The most discriminating metabolites for this purpose were trimethylamine, threonine, and creatine.

Although not the primary objective of the study, we attempted to determine whether metabolomics could provide further insights into the pathophysiologic condition of pregnancies with trisomy 18. We reviewed the Human Metabolome Database (www.hmdb.ca; version 2.5)[16] which summarizes the role of most known metabolites in the human body. As noted earlier, 2-hydroxybutyrate was found to be increased in the maternal serum of pregnancies with trisomy 18. According to the Human Metabolome Database, 2-hydroxybutyrate is released as a by-product of the conversion of cystathionine to cysteine in the transsulfuration pathway of homocysteine metabolism. Cysteine is the substrate for the production of the antioxidant glutathione. The elevated 2-hydroxybutyrate levels could represent an antioxidant response to oxidative stress. The basis for such a metabolic response in pregnancies with trisomy 18 is unclear. The authors were unable to identify any previously published literature that addressed this issue.

The question may be posed about the necessity of the performance of metabolomic analysis of pregnancies with trisomy 18, given the high diagnostic accuracy currently being reported for noninvasive molecular testing in pregnancy. Metabolomics has the potential to address other scientifically intriguing questions that go beyond the detection of trisomy 18. For example, the mechanism by which the presence of an extra complement of chromosome 18 disrupts cell function remains unanswered. Although this was not our study objective, our preliminary data suggest that oxidative dysfunction could play a role at the cellular level. We could find no previous report in the literature that has addressed this issue. This observation is intriguing because our previous metabolomic analysis indicated that increased oxidative stress is a feature of Down syndrome,[8] which is another common aneuploidy. Metabolomics represents a paradigm shift to conventional screening. Multiple combinations of different metabolites can be identified, each with good accuracy for the prediction of the same disorder, which represents a proverbial excess of wealth. One of our algorithms had 100% sensitivity and 100% specificity in this preliminary study. Of course, a much larger number of cases and

controls, preferably obtained prospectively, would be required for precise sensitivity and specificity estimates. We did not analyze the additional impact of standard biochemical markers (such as free ß-human chorionic gonadotropin and pregnancy-associated plasma protein−A) if they were to be added to the predictive models. These conceivably could lead to additional biomarker combinations with high accuracy for trisomy 18. From a more utilitarian perspective, metabolomic analysis potentially could lead to the availability of accurate and affordable tests, particularly when cost is an issue.

Although this is an interesting question, we cannot say whether the observed changes in metabolite concentrations arose primarily from the fetal, placental, or maternal compartment. This was not an objective of our study, and the current study design precludes us from making such a distinction.

In conclusion, using both a case control and an expanded dataset, we report the identification of metabolomic markers that, by themselves or combined with maternal age or nuchal translucency measurement, can distinguish pregnancies with trisomy 18 from normal first-trimester pregnancies with accuracy. We used statistical methods that are recommended for both metabolomic data and standard biomarker analyses. We also used genetic programming, which is a powerful analytic and feature selection method, to confirm these associations. When metabolites were combined with NT measurements the detection rate for trisomy 18 was significantly increased. We did not evaluate the effect of combining these metabolites with established first-trimester protein biochemical markers (such as pregnancy-associated plasma protein−A and free β−human chorionic gonadotropin) for trisomy 18 prediction. Our preliminary assessment of the trisomy 18 maternal serum metabolic profile suggests the possibility of increased antioxidant response in pregnancies with trisomy 18. Finally, our analysis indicates that the first-trimester pregnancies with trisomy 18 and 21 can be distinguished potentially with the use of easily measureable maternal serum metabolite markers. ■

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