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Association of Early Preterm Birth with Abnormal Levels of Routinely Collected First and Second Trimester Biomarkers

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Abstract

Objective—To examine the relationship between typically measured prenatal screening biomarkers and early preterm birth in euploid pregnancies.

Study Design—Included were 345 early preterm cases (< 30 weeks) and 1,725 controls drawn from a population-based sample of California pregnancies that all had both first and second trimester screening results. Logistic regression analyses were used to compare patterns of biomarkers in cases and controls and to develop predictive models. Replicability of the biomarker-early preterm relationships revealed by the models was evaluated by examining the frequency and associated adjusted relative risks (RRs^{adj}) for early preterm birth and for preterm birth in general (< 37 weeks) in pregnancies with identified abnormal markers compared to those without these markers in a subsequent independent California cohort of screened pregnancies (n = 76,588).

Results—The final model for early preterm birth included first trimester pregnancy-associated plasma protein A (PAPP-A) the 5th percentile, second trimester alpha-fetoprotein (AFP) the 95th percentile, and second trimester inhibin (INH) the 95th percentile (odds ratios 2.3 to 3.6). In general, pregnancies in the subsequent cohort with a biomarker pattern found to be associated with early preterm delivery in the first sample were at an increased risk for early preterm birth and preterm birth in general (< 37 weeks) (RRs^{adj} 1.6 to 27.4). Pregnancies with two or more biomarker abnormalities were at particularly increased risk (RRs^{adj} 3.6 to 27.4).

Conclusion—When considered across cohorts and in combination, abnormalities in routinely collected biomarkers reveal predictable risks for early preterm birth.

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The results HAVE NOT been presented at any scientific meetings.

Keywords/Phrases

Preterm Birth; Prenatal Screening; Biomarkers

INTRODUCTION

All biomarkers used in routine aneuploidy screening are directly or indirectly associated with placental function, pregnancy maintenance, and/or other processes that are closely tied to preterm birth (e.g. parturition, placental and trophoblast function, inflammation, immune system function).^{1–16} Thus, there is pathophysiological evidence that supports the findings of a number of investigators who have reported an increased risk of preterm birth when one or more routinely collected screening markers is abnormally high and/or low (first trimester nuchal translucency (NT), pregnancy associated plasma protein-A (PAPP-A), and human chorionic gonadotrophin (hCG), second trimester alpha-fetoprotein (AFP), hCG, unconjugated estriol (uE3), and inhibin (INH)).^{17–26} Despite these observations the standard of care for pregnancies with abnormal biomarkers is uncertain.²⁵ One challenge in creating a set of standards is the absence of well-defined population-scale data that have investigated preterm delivery by important clinical subgroups (e.g. early, spontaneous, medically indicated) in conjunction with biomarker patterns across trimesters.

Herein, we utilize data from the California Prenatal Screening Program^{27,28} and the California Perinatal Quality Care Collaborative (CPQCC)²⁹ to investigate whether preterm birth (overall and by medically indicated and spontaneous labor subgroups) is associated with single and multiple biomarker abnormalities. Two independent population-scale sample sets of euploid singleton pregnancies were used: one set was used to establish an association model and the second population set was used to determine whether the patterns could be recapitulated across cohorts.

MATERIALS AND METHODS

Evaluation of early preterm-biomarker relationships was undertaken in two independent datasets wherein one set was used for model building (the “training” study set) and one set was used for model testing (the “testing” study set). The training study set included 345 early preterm singleton cases (< 30 weeks gestational age (GA)) and 1,725 term singleton pregnancies (controls) with expected dates of delivery (EDDs) in September 2009 through December 2010. These cases and controls were drawn from 497,023 unique women who were participants in the California Prenatal Screening Program during this same time period. Cases and controls were restricted to pregnancies with ultrasound dating, maternal age between 12 and 60 years, non-missing information on race/ethnicity, and ‘sequential integrated’ screening results – that is, pregnancies with first trimester NT, PAPP-A and hCG measurements as well second trimester measures of AFP, hCG, uE3, and INH (n = 119,185). Cases and controls were also restricted to pregnancies with a linked newborn screening record (indicating a live birth between 20 and 44 completed weeks of gestation) without any history of diabetes or smoking, and without chromosomal or neural tube defects (NTDs) in registries maintained by GDSP.³⁰ We identified 643 case pregnancies that had resulted in early preterm birth between 22 weeks, 0 days and 29 weeks, 6 days and 83,039 control pregnancies with births at or after 37 completed weeks. The final case determination was made after linkage of the case group to the CPQCC dataset.²⁹ The CPQCC database stores clinical data on over 90% of all neonates who receive neonatal intensive care in California. All newborns with a GA between 22 weeks 0 days and 29 weeks 6 days qualify for inclusion in the CPQCC regardless of department of care within partner hospitals, as such, this set of early preterm pregnancies was ideal for more intensive analyses due to the availability of

extensive data on pregnancies. The CPQCC dataset was used to make additional exclusions from the early preterm case grouping (Figure 1). The final case-control set included the 345 cases after CPQCC linkage and exclusions and 1,725 controls selected randomly from the available 83,309 term pregnancies at a ratio of five controls for each case.

First trimester PAPP-A and total hCG were measured in serum samples drawn between 10 weeks, 0 days and 13 weeks, 6 days of gestation. Second trimester AFP, hCG, uE3, and INH were measured in serum samples drawn between 15 weeks, 0 days and 20 weeks, 0 days of gestation. NT measurements were done between 11 weeks, 2 days and 14 weeks, 2 days of gestation by practitioners credentialed by the Nuchal Translucency Quality Review Program³¹ or Fetal Medicine Foundation.^{28;32} All serum samples were sent to one of seven regional laboratories in California for testing using fully automated equipment (Auto DELFIA, Perkin Elmer Life Sciences, Waltham, MA). As part of routine prenatal screening, all biomarker levels were converted to biomarker multiple of the medians (MoMs) to adjust for GA using log-linear or non-linear regression methods as appropriate wherein median analyte values were regressed against GA and were adjusted for maternal weight (as a proxy for blood volume), and self-reported race/ethnicity

Our analyses used logistic regression to calculate odds ratios (ORs). We estimated the odds associated with specific maternal characteristics in three early preterm case groups ('Spontaneous Labor', 'Medically Indicated', and a 'Combined' groupings) compared to those in the term control grouping. Preterm groupings were based on information from the CPQCC. Maternal characteristics included self-reported White, Hispanic, Black, Asian, and "Other" race/ethnicity, maternal age < 18, 18–34, and > 34 years, and an obesity proxy variable (maternal weight by race/ethnicity grouping at weeks of gestation at initial first trimester serum screen > the 95th percentile). Such a proxy was needed for this dataset given the absence of data on height and/or body mass index (BMI). Weight percentiles were computed using the full sample of pregnancies with screening during the same period (n = 119,185).

Logistic regression models were also used to compare the odds associated with first and second trimester biomarker abnormalities in early preterm cases relative to term controls and to build final predictive models. For NT, a measurement at or above 3.5mm was considered abnormal,¹² whereas for serum markers, MoMs the 5th or the 95th percentile were considered abnormal based on the distribution of markers in the full sample of screened pregnancies (n = 119,185). All maternal characteristics and biomarker groupings found to be associated with early preterm birth (by ('Spontaneous Labor', 'Medically Indicated', and a 'Combined' grouping) in crude analyses (p < 0.10) were entered into the full model. Final models included maternal characteristics and biomarkers that remained significant (p < 0.05) for early preterm birth when backward stepwise methods were applied.

The testing study set was used to test replicability of the biomarker-early preterm relationships revealed by the models in the training study set. The testing study set included all California singleton pregnancies with ultrasound dating that received screening from January through September 2011 for whom there was no indication of diabetes, smoking, aneuploidy, or NTD in GDSP records (n = 76,588). Other detailed information about obstetric risks and reasons for early preterm birth was not available for this population. As such, evaluation was restricted to a focus on the Combined early preterm model. Generalizability was evaluated using logistic binomial regression methods to examine the frequency and associated adjusted relative risks (RRs^{adj}) and 95% confidence intervals (95% CIs) for early preterm birth and all preterm births (< 37 weeks GA) in pregnancies with identified abnormal markers compared to those without these markers. The adjusted models

included all characteristics found to be significantly more or less frequent in the combined early preterm grouping compared to the term grouping.

The analyses were done using Statistical Analysis Software (SAS) version 9.2 (Cary, NC) and were based on data received at GDSP by September 1, 2012. The methods and protocols were approved by the Committee for the Protection of Human Subjects within the Health and Human Services Agency of the State of California and the Institutional Review Board of Stanford University.

RESULTS

Early preterm cases and term controls in the training study set were mostly Hispanic (43.2% and 40.5% respectively) and were between 18 and 34 years of age (72.8% and 72.1%). Pregnancies resulting in early preterm birth were more likely than term controls to be of Black race/ethnicity regardless of early preterm grouping (spontaneous labor, medically indicated, or combined) (ORs 2.5 to 4.3)(Table 1).

Cases in the training study set (with spontaneous or medically indicated preterm delivery) were significantly more likely than controls to have first trimester PAPP-A MoMs the 5th percentile and second trimester AFP and/or INH MoMs the 95th percentile (Table 2). Medically indicated cases were more likely to have hCG MoMs in the first and second trimester that were the 95th percentile and second trimester uE3 MoMs the 5th percentile (Table 2).

Employing a backward stepwise approach, cases in the training study set (regardless of spontaneous or medically indicated) were more than twice as likely to have PAPP-A MoMs the 5th percentile (ORs 2.0 to 2.4) and more than three times as likely to have AFP MoMs the 95th percentile (ORs 3.2 to 4.5). INH MoM the 95th was statistically dropped from the spontaneous labor model but remained in the medically indicated and combined models (ORs 6.4 and 3.2 respectively) (Table 3). The findings across models remained when cases were limited to those without PROM, hypertension, or small for gestational age (SGA) (with ORs after exclusion of 2.1 to 6.1).

Estimated risks (ORs and 95% CIs) associated with a PAPP-A MoM the 5th percentile, an AFP MoM the 95th percentile, and/or an INH MoM the 95th percentile in cases in the training study set (ORs 2.4 to 3.2) were similar in magnitude to their counterparts (RRs and 95% CIs) observed in the testing study set which measured risks of early preterm birth in pregnancies with one or more of these target biomarker patterns compared to pregnancies without any of these patterns (RRs 2.4 to 3.6) (Table 4) (Figure 2). Pregnancies with these patterns (considered as a combined grouping or in isolation or in combination with other markers) were also found to be at increased risk of preterm birth occurring before 37 weeks (RRs 1.6 to 9.2, 95% CIs 1.3 to 12.3) (Table 4). The one exception was for pregnancies with an isolated first trimester PAPP-A MoM the 5th percentile (without second trimester AFP and/or INH MoMs at or above the 95th percentile). These pregnancies were not at increased risk for preterm birth < 30 weeks ($RRs^{adj} = 1.4$ (95% CI 0.7–3.0)) (Table 4).

Pregnancies with two or more predictive biomarker patterns were found to be at particularly increased risk for early preterm birth and for preterm birth in general (< 37 weeks GA) ($RRs^{adj} 3.6$ to 27.4) (Table 4). Pregnancies with all three abnormal biomarker patterns (first trimester PAPP-A the 5th percentile, second trimester AFP and INH 95th percentile, $n = 49$) were at the greatest increased risk (were at more than a 24-fold increased risk of early preterm birth (RR (crude) = 24.4, 95% CI 12.8–58.4) and at a more than 9-fold increased risk of preterm birth (< 37 weeks GA) ($RR^{adj} = 9.2$, 95% CI 5.9–14.3)).

Examination of maternal and infant characteristics and diagnoses in the training study set (the set with more detailed clinical data available) indicated that several factors were significantly more frequent among groups of pregnancies with ≥ 2 compared to 0 or 1 abnormal biomarkers (e.g. Black race/ethnicity, > 34 years of age at testing, and hypertension). There were also some characteristics and diagnoses that were significantly less frequent among pregnancies with ≥ 2 compared to 0 or 1 abnormal biomarkers (e.g. PROM and intraventricular hemorrhage) (Table 5).

COMMENT

This study explored population-level screening and clinical data from two separate population cohorts to investigate whether preterm birth (overall and by medically indicated and spontaneous labor subgroups) was associated with single and multiple biomarker abnormalities. This study showed increased risks for early preterm birth when first trimester PAPP-A was low and/or when second trimester AFP and/or INH were high, irrespective of whether the preterm birth was spontaneous or medically indicated. In addition the study showed that risks are predictable across cohorts and increase substantially when more than one abnormality is present. With respect to predictability, it is of note that nearly identical biomarker-early preterm (combined over spontaneous labor and medically indicated) patterns of risk were observed across cohorts for the overall biomarker groupings (low PAPP-A, high AFP, and/or high INH) (risks across cohorts ~ 2 to 3 fold). When only one biomarker abnormality was present the risk of early preterm birth was about 1 to 3 fold and was between 5 and 28 fold when there were two or more biomarker abnormalities present.

This study and others indicate a pattern of risk for certain biomarkers. For example, in this study as well as in population-based studies by Dugoff and colleagues,¹⁷ Smith and colleagues,²⁰ and by Spencer and colleagues,²⁴ pregnancies with first trimester PAPP-A the 5th percentile were at two to three-fold increased risk for preterm birth (both early and < 37 weeks GA) compared to pregnancies with higher levels (ORs 1.87 to 2.99). Findings of increased risk when second trimester AFP and/or INH levels were above the 95th percentile as well as the rise in risk when at least two markers were present are also consistent between this and other studies.^{17;20} Discrepancies between our findings and others include our null finding with respect to early preterm birth and increased nuchal translucency^{17;22;33} and also the numerous reports of an association between preterm birth and second trimester abnormalities in hCG and uE3 – including patterns reported by some from our group.^{21;26;34} While we suspect that the differences between our study and others with respect to NT may be due to our having too few pregnancies with increased NT in our study to be able to explore this relationship in detail, the difference between our study and other studies with respect to second trimester hCG and uE3 may be a result of our ability to consider both first and second trimester markers together.

Both PAPP-A and INH are produced by the placenta and therefore observed associations with abnormalities in these markers may implicate the placenta in preterm birth. It is unclear however if these patterns are indicative of an antecedent or are a consequence of a cascade of events leading to early preterm birth. It is possible that these patterns point to some abnormality in trophoblast function in the subset of pregnancies with the observed abnormalities given that both markers have been implicated in this pathway which is also closely tied to preterm birth.^{4;5;7;9;14} The observed association between early preterm birth and high AFP lends additional support to the idea that these biomarker risks might reflect a pathway suggestive of placental involvement given the relationship between high AFP and placental dysfunction and damage.^{6;35;36} With or without direct placental involvement, the pathway that links the observed biomarker abnormalities with early preterm birth may be related to inflammation given an association with these markers^{37–40} and with preterm

birth.^{3;8;15} Our finding that early preterm pregnancies with two or more biomarkers abnormalities were more likely to be hypertensive supports the idea that observed patterns may be linked to placental function and inflammatory factors.^{41–43} This possible link may also explain the stronger biomarker-early preterm associations observed in the medically indicated grouping given that nearly half of all of the pregnancies in that grouping had “hypertension” listed as the indication. In addition, our finding that early preterm pregnancies with two or more biomarkers abnormalities were more likely to have a head circumferences < the 10th percentile, were more likely to be SGA, and were less likely to have intraventricular hemorrhage suggests that the pathways related to these biomarker abnormalities and preterm birth may have been in place early in pregnancy given the relationship between these anthropometric measures and adaptive mechanisms aimed at protecting against brain injury.^{44–47}

Although the total percent of pregnancies resulting in preterm birth with the biomarker abnormalities noted is relatively modest (29.9% of those resulting in early preterm birth and 24.3% of all preterm births in the 2011 cohort), given the magnitude of the risks associated with biomarker abnormalities occurring in isolation and in combination, the results indicate that some pregnancies might benefit from increased clinical scrutiny or care when first trimester PAPP-A levels are found to be unusually low or when second trimester AFP or INH levels are found to be unusually high. This may be particularly true for pregnancies with at least two biomarker abnormalities. The risks identified here also point our epidemiologic lens somewhat for investigating potential etiologic factors for preterm birth – a condition that continues to be evasive in terms of etiologic discovery.⁴⁸

We examined whether abnormal biomarker results *across* trimesters could predict a risk of preterm birth. Future efforts may benefit from a longitudinal approach to risk prediction where modification of risks based on first only *and then* combined trimester results are considered. Similarly, future studies may benefit from consideration of risk patterns that might emerge if other biomarker cut-points are considered (e.g. biomarker MoMs < 0.5 or > 2.0) and if biomarkers are considered as continuous rather than categorical variables. Pursuit of these questions would also be well served by the inclusion of a broad number of clinical factors in final models. In the present study clinical data on, for example, the presence of hypertension or chorioamnionitis was only available for cases. Although this allowed for exclusion of these case pregnancies from analyses, these factors could not be included logistic models. While we do not believe our results were affected greatly by the lack of this information on controls (given the likelihood that these characteristics would have been more likely to pull results towards a null finding), such consideration might allow for the refining of predictive models moving forward. It should also be noted that information of previous preterm birth was not available for this study. Moving forward evaluation of how the risks observed in the present study might be related to reoccurring preterm birth may be of particular interest. It should also be noted that while these patterns were found to replicate from one California cohort to another and therefore are likely generalizable to that large population, evaluation of patterns in other populations with differing distributions with respect to maternal characteristics (e.g. age, weight, race/ethnicity).

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IMPLICATIONS

- Results of the study indicate that some pregnancies might benefit from increased clinical scrutiny or care when first trimester PAPP-A levels are found to be unusually low or when second trimester AFP or INH levels are found to be unusually high;
- Increased clinical scrutiny or care may be especially warranted when two or more biomarker abnormalities are present (low first trimester PAPP-A and/or high second trimester AFP and/or INH);
- Risks identified point our epidemiologic lens somewhat for investigating potential etiologic factors for preterm birth that may be related to observed biomarker abnormalities.

Figure 1. Selection of cases and controls for singleton pregnancies with expected delivery in 2009 or 2010.

^aExclusions based on screening and registry data included 197 mother-infant pairs with chromosomal defects, 10 with NTDs, 1,093 with a stated history of smoking, and 715 with diabetes. ^bAdditional exclusions based on CPQCC/NICU data included 55 mother-infant pairs with other critical birth defects, 55 additional pairs with reported diabetes during or before pregnancy, 13 additional pregnancies with reports of smoking, 10 pregnancies with preeclampsia, and 9 pregnancies with oligio- or polyhydramnios.

Figure 2.

Observed associations between target biomarker and early preterm birth (combined over spontaneous labor and medically indicated) in the training study set (2009–2010 cohort) and in the testing study set (2011 cohort).

OR, Odds Ratio; RR, Relative Risk; 95% CI, Confidence Interval; PAPP-A, pregnancy associated plasma protein A; AFP, alpha-fetoprotein; INH, inhibin; MoM, multiple of the median

Figure 3.
Percent of preterm pregnancies by biomarker pattern: Singleton pregnancies with expected delivery in 2011.

Table 1

Maternal characteristics within preterm case group (< 30 completed weeks of gestation) compared to that for term controls in training study set (2009–2010 cohort).

	Early Preterm Birth (< 30 Weeks GA)			Term Controls
	Spontaneous Labor^a	Medically Indicated^b	Combined	
	n = (%) OR (95% CI)	n = (%) OR (95% CI)	n = (%) OR (95% CI)	
All	222	123	345	1,725
<u>Race/Ethnicity</u>				
White	57 (25.7)	40 (32.5)	97 (28.1)	598 (34.7)
	Reference	Reference	Reference	
Hispanic	95 (42.8)	54 (43.9)	149 (43.2)	699 (40.5)
	1.4 (1.0–2.0)	1.2 (0.8–1.8)	1.3 (1.0–1.7)	
Black	29 (13.1)	12 (9.8)	41 (11.9)	71 (4.1)
	4.3 (2.6–7.1)	2.5 (1.3–5.0)	3.6 (2.3–5.5)	
Asian	23 (10.4)	13 (10.6)	36 (10.4)	207 (12.0)
	1.2 (0.7–1.9)	0.9 (0.5–1.8)	1.1 (0.7–1.6)	
Other	18 (8.1)	4 (3.3)	22 (6.4)	149 (8.6)
	1.3 (0.7–2.2)	0.4 (0.1–1.1)	0.9 (0.6–1.5)	
<u>Maternal Age at Term (Years)</u>				
< 18	4 (1.8)	--	4 (1.2)	9 (0.5)
	3.2 (0.9–10.6)		2.2 (0.7–7.2)	
18–34	170 (76.6)	81 (65.9)	251 (72.8)	1,243 (72.1)
	Reference	Reference	Reference	
> 34	47 (21.2)	42 (34.2)	89 (25.8)	
	0.7 (0.5–1.0)	1.4 (0.9–2.0)	0.9 (0.7–1.2)	472 (27.4)
<u>Maternal Weight (Percentile)^c</u>				
< 5 th	11 (5.0)	1 (0.8) ^d	12 (3.5)	78 (4.5)
	1.1 (0.6–2.2)		0.8 (0.4–1.4)	
5–95 th	195 (87.8)	111 (90.2)	306 (88.7)	1,568 (90.9)
	Reference	Reference	Reference	
> 95 th	15 (6.8)	10 (8.1)	25 (7.3)	79 (4.6)
	1.5 (0.9–2.7)	1.8 (0.9–3.6)	1.6 (1.0–2.6)	

GA, gestational age; OR, Odds Ratio; 95% CI, Confidence Interval

^aIncludes 88 pregnancies with premature rupture of the membranes (PROM);

^bIncludes pregnancies with indication of “fetal distress” (n=25), maternal hypertension (n = 60), placental problems (n = 29), cardiac disease (n = 1). HELLP syndrome (n = 2), prolapsed cord (n = 3), fetal bradycardia (n = 1), pulmonary edema (n = 2) “one month UTI with chorioamnionitis” (n = 1) and “severe IUGR” (n = 1). PROM was indicated in 13 of these pregnancies;

^cWeight percentile by race/ethnicity grouping at weeks gestation at initial testing;

^dORs not computed where cell frequency < 3.

Table 2

Results of crude logistic regression analyses examining the association between early preterm birth and first and second trimester maternal serum biomarkers compared to term controls in training study set (2009–2010 cohort).

	Early Preterm Birth (< 30 Weeks GA)			Term Controls
	Spontaneous Labor	Medically Indicated	Combined	
	n=(%)	n=(%)	n=(%)	
	OR (95% CI)^a	OR (95% CI)^a	OR (95% CI)^a	
All	222	123	345	1,725
<u>First Trimester Biomarkers</u>				
<u>NT</u>				
3.5 mm ^b	1(0.5) ^d	--	1(0.3) ^d	2(0.1)
<u>PAPP-A(MoM Percentile)^c</u>				
5 th	20 (9.0)	17 (13.8)	37 (10.7)	81 (4.7)
	2.1 (1.3–3.5)	3.2 (1.8–5.6)	2.5 (1.6–3.7)	
6 th –94 th	189 (85.1)	102 (82.9)	291 (84.4)	1,562 (90.6)
	Reference	Reference	Reference	
95 th	13 (5.9)	4(3.3)	17 (4.9)	82 (4.8)
	1.2 (0.7–2.3)	0.7 (0.3–2.1)	1.1 (0.6–1.8)	
<u>hCG (MoM Percentile)^c</u>				
5 th	7(3.2)	8(6.5)	15 (4.4)	98 (5.7)
	0.5 (0.3–1.2)	1.2 (0.6–2.6)	0.7 (0.4–1.3)	
6 th –94 th	203 (91.4)	104 (84.6)	307 (89.0)	1,549 (89.8)
	Reference	Reference	Reference	
95 th	12 (5.4)	11 (8.9)	23 (6.7)	78 (4.5)
	0.9 (0.4–2.2)	2.1 (1.1–4.2)	1.6 (1.0–2.6)	
<u>Second Trimester Biomarkers</u>				
<u>AFP (MoM Percentile)^c</u>				
5 th	8(3.6)	6(4.9)	14 (4.1)	95 (5.5)
	0.6 (0.3–1.3)	1.1 (0.5–2.6)	0.8 (0.5–1.4)	
6 th –94 th	189 (85.4)	84 (68.3)	307 (89.0)	1,556 (90.2)
	Reference	Reference	Reference	
95 th	25 (11.3)	33 (26.8)	58 (16.8)	74 (4.3)
	2.8 (1.7–4.6)	8.3 (5.2–13.2)	4.5 (3.1–6.5)	
<u>hCG (MoM Percentile)^c</u>				
5 th	7(3.2)	6(4.9)	13 (3.8)	109 (6.3)
	0.5 (0.2–1.0)	0.9 (0.4–2.1)	0.6 (0.3–1.1)	
6 th –94 th	200 (90.1)	95 (77.2)	295 (85.5)	1,543 (89.5)
	Reference	Reference	Reference	
95 th	15 (6.8)	22 (17.9)	37 (10.7)	73 (4.2)
	1.5 (0.9–2.8)	4.7 (2.8–8.0)	2.6 (1.7–4.0)	

	Early Preterm Birth (< 30 Weeks GA)			
	Spontaneous Labor	Medically Indicated	Combined	Term Controls
	n=(%)	n=(%)	n=(%)	n=(%)
	OR (95% CI)^a	OR (95% CI)^a	OR (95% CI)^a	
uE3 (MoM Percentile)^c				
5 th	5(2.3)	16 (13.0)	21 (6.1)	84 (4.9)
	0.5 (0.2–1.2)	2.9 (1.6–5.1)	1.3 (0.8–2.1)	
6 th –94 th	201 (90.5)	103 (83.7)	304 (88.1)	1,562 (90.6)
	Reference	Reference	Reference	
95 th	16 (7.2)	4(3.3)	20 (5.8)	79 (4.6)
	1.6 (0.9–2.8)	0.7 (0.3–2.0)	1.3 (0.8–2.1)	
INH (MoM Percentile)^c				
5 th	10 (4.5)	3(2.4)	13 (3.8)	97 (5.6)
	0.8 (0.4–1.6)	0.6 (0.2–1.9)	0.7 (0.4–1.2)	
6 th –94 th	193 (86.9)	80 (65.0)	273 (79.1)	1,550 (89.9)
	Reference	Reference	Reference	
95 th	19 (8.6)	40 (32.5)	59 (17.1)	78 (4.5)
	1.9 (1.1–3.3)	9.9 (6.4–15.5)	4.2 (2.9–6.1)	

GA, gestational age; OR, Odds Ratio; 95% CI, Confidence Interval; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein A; MoM, Multiple of the Median; AFP, alpha-fetoprotein; hCG, human chorionic gonatotropin; uE3, unconjugated estriol; INH, inhibin.

^a Included in the models were Black race/ethnicity (all groups) and maternal weight (percentile) >than the 95th percentile ("Combined" group) (characteristics found to be more frequent in cases compared to controls (Table 1);

^b Referent group NT <3.5;

^c Biomarker cutpoints for MoM percentiles(the 5th and the 95th) were PAPP-A: 0.38 and 2.45, hCG (first trimester) 0.52 and 1.99, AFP: 0.59 and 1.68, hCG (second trimester): 0.42 and 2.24, uE3 0.62 and 1.36, INH: 0.54 and 2.05;

^d ORs not computed where cell frequency <3.

Table 3

Final predictive models for preterm birth < 30 weeks gestational age (overall and by subgroups) in training study set (2009–2010 cohort).

		OR ^a	95% CI
<u>Model #1: Spontaneous Labor</u>			
First Trimester PAPP-A MoM	5 th	2.0	1.2–3.5
Second Trimester AFP MoM	95 th	3.2	2.0–5.3
<u>Model #2: Medically Indicated</u>			
First Trimester PAPP-A MoM	5 th	2.4	1.2–4.9
Second Trimester AFP MoM	95 th	4.5	2.5–8.1
Second Trimester INH MoM	95 th	6.4	3.8–10.9
<u>Model #3: Combined</u>			
First Trimester PAPP-A MoM	5 th	2.3	1.4–3.6
Second Trimester AFP MoM	95 th	3.6	2.4–5.5
Second Trimester INH MoM	95 th	3.2	2.1–4.8

OR, Odds Ratio; 95% CI, Confidence Interval; PAPP-A, pregnancy-associated plasma protein A; MoM, Multiple of the Median; AFP, alpha-fetoprotein; INH, inhibin.

^aIncluded in the models were Black race/ethnicity (all groups) and maternal weight (percentile) > than the 95th percentile (“Combined” group) (characteristics found to be more frequent in cases compared to controls (Table 1).

Table 4

Population-level relative risks for preterm birth < 30 and < 37 weeks GA in complete sample of screened singleton pregnancies without any known history of diabetes, smoking, or known chromosomal defect in the offspring in testing study set (2011 cohort).

	Preterm Birth			
	<30 weeks		<37 weeks	
	n = (%)	RR ^{Adj} (95% CI) ^a	n = (%)	RR ^{Adj} (95% CI) ^a
Total Sample (n = 76,588)	422 (0.6)		4,734 (6.2)	
No Target Marker Pattern ^b (n = 66,113)	296 (0.5)	Reference	3,581 (5.4)	Reference
Any Target Marker Pattern ^b (n = 10,475)	126 (1.2)	2.7 (1.8–3.8)	1,152 (11.0)	2.1 (1.9–2.3)
Isolated Low PAPP-A (n = 3,385)	23 (0.7)	1.4 (0.7–3.0)	330 (9.8)	2.0 (1.6–2.4)
Any Low PAPP-A (n = 3,828)	42 (1.1)	2.4 (1.7–3.4)	430 (11.2)	2.2 (1.9–2.3)
Any High AFP (n = 3,772)	62 (1.6)	3.6 (2.7–4.8)	498 (13.2)	2.5 (2.3–2.7)
Any High INH (n = 3,828)	63 (2.1)	3.6 (2.7–4.8)	433 (11.4)	2.2 (1.9–2.4)
Isolated Low PAPP-A (n = 3,385)	23 (0.7)	1.4 (0.7–3.0)	330 (9.8)	2.0 (1.6–2.4)
Isolated High AFP (n = 3,048)	34 (1.1)	2.2 (1.2–4.2)	345 (11.3)	2.2 (1.8–2.6)
Isolated High INH (n = 3,156)	34 (1.1)	2.5 (1.4–4.5)	291 (9.2)	1.6 (1.3–2.0)
Low PAPP-A, High AFP Only (n = 232)	6 (2.6)	5.8 (1.5–2.9)	45 (19.4)	3.6 (2.2–5.8)
Low PAPP-A, High INH Only (n = 162)	7 (4.3)	10.0 (2.6–38.3)	34 (21.0)	4.2 (2.4–7.3)
High AFP, High INH Only (n = 443)	16 (3.6)	7.1 (2.7–18.7)	87 (19.6)	4.2 (3.0–6.0)
Low PAPP-A, High AFP and INH (n = 49)	6 (12.2)	27.4 (12.8–58.4) ^c	21 (42.9)	9.2 (5.9 – 14.3)

RR, Relative Risk; 95% CI, Confidence Interval; Adj, Adjusted; PAPP-A, pregnancy-associated plasma protein A; AFP, alpha-fetoprotein; INH, inhibin.

^aAdjusted for Black race/ethnicity;

^bAny risk based on final backward stepwise logistic model for preterm birth < 30 weeks (Black race/ethnicity, first trimester PAPP-A the 5th percentile (“Low PAPP-A”), second trimester AFP the 95th percentile (“High AFP”), second trimester INH the 95th percentile (“High INH”));

^cCrude relative risk.

Table 5

Maternal and infant characteristics associated preterm birth (< 30 completed weeks gestation) by no, single, and two abnormal biomarker(s)^a in training study set (2009–2010 cohort).

	Very Preterm Birth (< 30 Weeks)			P value ^b
	No Abnormal Biomarker ^a	Single Abnormal Biomarker ^a	2 Abnormal Biomarkers ^a	
	n = (%)	n = (%)	n = (%)	
Sample	229	83	33	
Maternal Characteristic				
Race/Ethnicity				
White	62 (27.1)	23 (27.7)	12 (36.4)	.085
Hispanic	95 (41.5)	40 (48.2)	14 (42.4)	
Black	30 (13.1)	4 (4.8)	7 (21.2)	
Asian	26 (11.4)	10 (12.1)	0	
Other	16 (7.0)	6 (7.2)	0	
Maternal Age at Term (Years)				
< 18	3 (1.3)	1 (1.2)	0	.064
18–34	177 (77.3)	54 (65.1)	20 (60.6)	
> 34	48 (21.0)	28 (33.7)	13 (39.4)	
Maternal Weight (Percentile) ^c				
< 5 th	10 (4.4)	1 (1.2)	0	0.245
5–95 th	205 (89.5)	71 (85.5)	30 (90.9)	
> 95 th	13 (5.7)	10 (12.1)	2 (6.1)	
Pregnancy Complication ^d				
Unknown	2 (0.9)	0	0	1.000
None	48 (21.2)	8 (9.6)	3 (9.1)	.026
Hypertension	39 (17.2)	31 (37.4)	22 (66.7)	<.001
PROM	73 (32.2)	24 (28.9)	3 (9.1)	.024
Bleeding	47 (20.7)	18 (21.7)	10 (30.3)	.459
Chorioamnionitis	25 (11.0)	3 (3.6)	0	.018
“Medically Indicated” PTB	61 (26.7)	38 (45.8)	24 (72.7)	<.001
Spontaneous Labor	168 (73.4)	45 (54.2)	9 (27.3)	<.001
Administration of Steroids/ Tocolytics				
Steroids	187 (81.7)	76 (91.6)	28 (87.9)	.087
Indomethacin	69 (30.1)	24 (28.9)	9 (27.3)	.935
Born at Hospital with “Approved” NICU ^e				
Yes	193 (84.3)	73 (88.0)	31 (93.9)	.277
No, Transferred in After Birth	36 (15.7)	10 (12.1)	2 (6.1)	
Infant Characteristics				
Sex				
Male	123 (53.7)	42 (50.6)	16 (48.5)	.791

	Very Preterm Birth (< 30 Weeks)			P value ^b
	No Abnormal Biomarker ^a	Single Abnormal Biomarker ^a	2 Abnormal Biomarkers ^a	
	n = (%)	n = (%)	n = (%)	
Female	106 (46.3)	41 (49.4)	17 (57.5)	
Completed Weeks Gestation				
22–24	33 (14.4)	5 (6.0)	0	.007
25–29	196 (85.6)	78 (94.0)	33 (100.0)	
Weight < 1000 Grams	104 (45.4)	37 (44.6)	18 (54.6)	.586
Head Circumference < 10 th Percentile ^f	10 (4.4)	11 (13.3)	6 (18.2)	.002
SGA ^g	7 (3.1)	8 (9.6)	10 (30.3)	<.001
Symmetric ^h	5 (2.2)	6 (7.2)	6 (18.2)	<.001
Asymmetric ⁱ	2 (0.9)	2 (2.4)	4 (12.1)	.004
One-Minute Apgar < 4	58 (25.3)	21 (25.3)	9 (27.3)	.971
Infant Diagnoses				
Pneumothorax	9 (3.9)	2 (2.4)	2 (6.1)	.552
Patent Ductus Arteriosus	117 (51.1)	49 (59.0)	15 (45.5)	.323
Intraventricular Hemorrhage (Any)	59 (25.8)	25 (30.1)	3 (9.1)	.047
Grade 1	21 (9.2)	13 (15.7)	2 (6.1)	.199
Grade 2	15 (6.6)	5 (6.0)	0	.386
Grade 3	9 (3.9)	3 (3.6)	1 (3.0)	1.000
Grade 4	14 (6.1)	4 (4.8)	0	.447
Cystic Periventricular Leukomalacia	4 (1.8)	1 (1.2)	0	1.000
Retinopathy of Prematurity <(Any)	61 (26.6)	24 (28.9)	7 (21.2)	.699
Stage 1	21 (9.2)	7 (8.4)	3 (9.1)	1.000
Stage 2	21 (9.2)	8 (9.6)	2 (6.1)	.915
Stage 3	19 (8.3)	9 (10.8)	2 (6.1)	.665
Supplemental Oxygen at 36-Weeks	54 (23.6)	21 (25.3)	9 (27.3)	.875
Hospitalization at 40 Weeks	46 (20.1)	13 (15.7)	6 (18.2)	.674
Death in NICU (Any)	18 (7.9)	7 (8.4)	2 (6.1)	.674
< 30 Days	13 (5.7)	6 (7.2)	0	.346
30 Days	5 (2.2)	1 (1.2)	2 (6.1)	.344

PTB, preterm birth; PROM, premature rupture of membranes; NICU, Neonatal Intensive Care Unit

^aWhere abnormal biomarkers are considered to include first trimester pregnancy-associated plasma protein A (PAPP-A) the 5th percentile, second trimester alpha-fetoprotein (AFP) the 95th percentile, or second trimester inhibin (INH) the 95th percentile (based on logistic model for all early preterm births (< 30 weeks, Table 3).

^bBased on chi-square analyses comparing the frequency of characteristic/complication for all biomarker groupings (wherein characteristics/complications dichotomized as yes versus no in most instances except where all groups totaled 100% of the sample). Where the frequency of any cell was < 5, Fisher's Exact Test was used;

^dFrequencies and percents exclude pregnancies where information about complications indicated as "unknown" (n = 2);

^eWeight percentile by race/ethnicity grouping at weeks gestation at initial testing;

^f California Children's Services (CCS)-approved Intermediate, Community and Regional level NICU;

^g Head circumference < 10th percentile for gestational age (GA) and gender on smoothed norms;⁴⁹

^h Birth weight for GA < 10th percentile for GA gender on smoothed norms;⁴⁹

^h Birth weight for GA < 10th percentile and head circumference < 10th percentile for GA and gender on smoothed norms;⁴⁹

ⁱ Birth weight for GA < 10th percentile and head circumference < 10th percentile for GA and gender on smoothed norms.⁴⁹