PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Maternal Cardiovascular Disease Risk Factors as Predictors of Preterm Birth in California: A Case Control Study
AUTHORS	Rohlfing, Anne; Nah, Gregory; Ryckman, KK; Snyder, Brittney; Kasarek, Deborah; Paynter, Randi; Feuer, Sky; Jelliffe, Laura; Parikh, Nisha I

VERSION 1 – REVIEW

REVIEWER	Jennifer Stuart Brigham and Women's Hospital and Harvard Medical School, USA
REVIEW RETURNED	19-Oct-2019

GENERAL COMMENTS	As with other pregnancy complications, such as hypertensive disorders of pregnancy (gestational hypertension and preeclampsia) and gestational diabetes, preterm delivery is increasingly and consistently associated with an increased risk of maternal cardiovascular disease later in life. Identifying pre-pregnancy predictors of preterm delivery creates an opportunity for prevention of the pregnancy complication itself as well as for both primordial and primary prevention of CVD risk factors and events. Previous studies have demonstrated that CVD risk in these women can be detected before pregnancy and that traditional CVD risk factors (that develop before pregnancy) are risk factors for preterm delivery. In the current study, Rohlfing, et al. conducted a case-control study among 457 women who delivered at term (>=37 weeks) and 411 women who delivered preterm (<37 weeks; n=249 late preterm [32-36 weeks] and n=162 early preterm [<32 weeks]) (n=868 total) to identify maternal CVD risk factors present before pregnancy that may serve as predictors of preterm delivery. The investigators used data from a California-based cohort of singleton births (July 2009-December 2010) and randomly selected among women with available demographic information and measured first trimester biomarkers. They found that traditional CVD risk factors were significantly associated with the risk of preterm birth. See below for specific suggestions and questions: General: • Given the association between preterm delivery and maternal CVD risk factors after pregnancy, it would be helpful to clearly state when the maternal CVD risk factors are occurring (pre-pregnancy and/or during pregnancy) throughout the manuscript text/title/tables/figure to prevent confusion for the reader Abstract: • “Primary Outcome” should list only “Preterm delivery status” • Given that the primary outcome of interest in the study was preterm delivery, the first sentence of the Results should focus on

	preterm delivery rather than preeclampsia. Further, as written, it's unclear what the phrase "pre-eclampsia spectrum" mean and what the aORs presented in the parentheses represent. Are they for 1) preeclampsia overall, 2) preterm preeclampsia (or just preterm overall), 3) late preterm preeclampsia (or late preterm overall), and 4) early preterm preeclampsia (or early preterm overall)? • Consider interpreting at least one OR in the abstract rather than presenting all numeric results parenthetically • "Diabetes" = pre-pregnancy T1/T2 and/or gestational diabetes? • Please more fully interpret the linear dose response describe between total and LDL with late and early preterm birth • Abstract results should state whether they are crude or multivariable adjusted (and, if the latter, what the models were adjusted for) Methods: • Please list the covariates that were adjusted for in the multivariable models and the ROC curves. Importantly, how many of the known risk factors for preterm birth that were described in the second paragraph of the introduction able to be adjusted for in the current data? It's important to understand not only the crude association of the traditional CVD risk factors with preterm birth but also the independent association not accounted for by other known preterm birth risk factors. • Can the authors provide any validation results for the ICD-9 and hospital discharge records used to ascertain many of the CVD risk factors? Results: • Page 9: please remove the "n=" used outside of parenthetical statements • Under "CVD Risk Factor Associations", please remove "not" from the sentence beginning with "Neither maternal age nor race/ethnicity…" • Do the authors have information on parity and/or history of preterm birth? What proportion of the pregnancies included were among nulliparous women? Discussion: • Please change the first sentence to "CVD risk factors as predictors" rather than "CVD risk factor predictors" for clarity • Please restate #4 – as currently written, it sounds like total and LDL cholesterol are the outcome rather than exposure variables ("there was a significant dose response and an up to two-fold increased risk observed between total cholesterol and LDL with both late and early preterm birth") • Please change "…pooled cohort ASCVD…" to "in the most recent Pooled Cohorts ASCVD Risk Score model" Table 1: • Overall this table was a bit hard to digest: consider 1) splitting the "Sample Size" column into 2 columns to more directly compare the number of births and number of preterm births across each study, 2) using bold instead of the asterisk to flag statistically significant relationships, and 3) including the reference groups for the OR/RRs presented • Please add a footnote to define all abbreviations used, and include units for BMI and lipids Table 2:

	• Provide a more detailed title (ex: expand to include details of the analytic population)

REVIEWER	Mekhala Dissanayake Department of Epidemiology, University of North Carolina, Chapel Hill, USA Department of Obstetrics & Gynecology Oregon Health and Science University
REVIEW RETURNED	28-Nov-2019

GENERAL COMMENTS	This study focuses on an interesting, biologically plausible, and important association. Usage of biomarkers is a clear strength of the study and the statistical analysis appears to be appropriate. However, I don't believe the data source was explained thoroughly in terms of what was available (just demographics from OSHPD?) and what was used. I also have major concerns on the sampling method given the characteristics of the sample. -- The data source is unclear to me. If this is OSHPD birth cohort data, why was birth certificate information not available? It seems that only demographic information was abstracted from this but further information (e.g., information on hypertensive status and diabetes) could have supplemented information coming from discharge records. While these records would likely have high sensitivity for the conditions listed, they probably have very low specificity. -- I'm very concerned about the random sample drawn from this cohort. The very low (1%) sample of black women and the high prevalence of pre-eclampsia (12%) both give pause on the interpretation of these results. Given that the association between black race and preterm birth is very consistent, it is clear that this analysis was underpowered to detect this effect. While the authors address this in their limitations, I would limit my inference from these findings given that this sample, by chance, appears to be non-representative of the cohort at large. It is also not clear to me why the full cohort wasn't used in the first place. I think if the authors could clarify 1) why they used a sample and 2) how their sample differs from the full cohort, these results would be more useful. The general trends they show are interesting but I am not convinced these results provide accurate estimates of the effects they are interested in.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1, Comment (Abstract): "Primary Outcome" should list only "Preterm delivery status." Given that the primary outcome of interest in the study was preterm delivery, the first sentence of the Results should focus on preterm delivery rather than preeclampsia. Further, as written, it's unclear what the phrase "pre-eclampsia spectrum" mean and what the aORs presented in the parentheses represent. Are they for 1) preeclampsia overall, 2) preterm preeclampsia (or just preterm overall), 3) late preterm preeclampsia (or late preterm overall), and 4) early preterm preeclampsia (or early preterm overall)? Consider interpreting at least one OR in the abstract rather than presenting all numeric results parenthetically. "Diabetes" = pre-pregnancy T1/T2 and/or gestational diabetes? Please more fully interpret the linear dose response describe between total and LDL with late and early preterm birth. Abstract results should state whether they are crude or multivariable adjusted (and, if the latter, what the models were adjusted for).

Author Response: We have clarified the primary outcome by listing only preterm delivery status (page 2, line 9). In addition we have simplified the results section to start by including "In the multivariate adjusted model, all categories of hypertension led to increased risk of preterm birth, but particularly significant associations for the pre eclampsia group" (page 2, lines 10-11), and have removed the word "spectrum" in order to highlight we mean the entire pre-eclampsia category with relation to all preterm births. All of the AORs have been clarified to state how they are related to preterm birth as the primary outcome, and we have included that diabetes means both pregestational and gestational with interpreation of that specific aOR (page 2, lines 11-15). The dose response for cholesterol has been further elaborated on in the abstract as well to now state "A significant and linear dose response was found between total and LDL cholesterol and aORs for late and early preterm birth, with increasing cholesterol values associated with increased risk" (page 2, lines 16-18).

Reviewer #1, Comment (Methods): Please list the covariates that were adjusted for in the multivariable models and the ROC curves. Importantly, how many of the known risk factors for preterm birth that were described in the second paragraph of the introduction able to be adjusted for in the current data? It's important to understand not only the crude association of the traditional CVD risk factors with preterm birth but also the independent association not accounted for by other known preterm birth risk factors. Can the authors provide any validation results for the ICD-9 and hospital discharge records used to ascertain many of the CVD risk factors?

> *Author Response:* We have specified in the Statistical Methods subsection that the multivariate models and ROC curves were adjusted for all the risk factors described in our study and included in Table 2 (page 6, lines 17-18). As mentioned below without data on those factors mentioned in the introduction (such as fetal fibronectin levels or maternal infections) those factors were not included, and with the extremely low percent of nulliparous and medically indicated preterm births in this dataset we did not include these either as risk factors. To our knowledge the ICD-9 codes in hospital discharge records have not been validated specifically for our dataset; however, the direction and strength of the associations found in our paper are similar to those found in direct studies in the literature (Table 1) and prior papers have used and validated similar ICD-9 categorizing (Appendix I) to study preterm birth risk (Bateman BT, et al. *Am J Obstet Gynecol.* 2012; 206(2): 134) or general cardiovascular risk factors with high specificity (Birman-Deych E, et al. Med Care. 2005; 43(5): 480).

Reviewer #1, Comment (Results): Page 9: please remove the "n=" used outside of parenthetical statements. Under "CVD Risk Factor Associations", please remove "not" from the sentence beginning with "Neither maternal age nor race/ethnicity…" Do the authors have information on parity and/or history of preterm birth? What proportion of the pregnancies included were among nulliparous women?

> *Author Response:* We deleted the "n=" as used in the Study Population subsection paragraph (page 7, lines 6-7) and the "not" in the CVD Risk Factor Associations subsection (page 8, line 4). We did have information on nulliparity available in the dataset, and have included that now in the results (page 7, lines 8-9). Unfortunately, we do not have access to data on prior preterm birth or further birth history, although we agree this would be valuable additional information given the multiple factors involved in preterm birth.

Reviewer #1, Comment (Discussion): Please change the first sentence to "CVD risk factors as predictors" rather than "CVD risk factor predictors" for clarity. Please restate #4 – as currently written, it sounds like total and LDL cholesterol are the outcome rather than exposure variables ("there was a significant dose response and an up to two-fold increased risk observed between total cholesterol and LDL with both late and early preterm birth"). Please change "…pooled cohort ASCVD…" to "in the most recent Pooled Cohorts ASCVD Risk Score model."

Author Response: The suggestions were made as suggested to the first paragraph of the discussion, with point 4 revised to state "increasing total and LDL cholesterol values led to an up to two-fold dose response for late and early preterm births" (page 9, lines 5-

14). The Pooled Cohorts sentence was also revised as suggested (page 13, lines 13-14).

Reviewer #1, Comment (Table 1): Overall this table was a bit hard to digest: consider 1) splitting the "Sample Size" column into 2 columns to more directly compare the number of births and number of preterm births across each study, 2) using bold instead of the asterisk to flag statistically significant relationships, and 3) including the reference groups for the OR/RRs presented. Please add a footnote to define all abbreviations used, and include units for BMI and lipids.

Author Response: We appreciate the comments on formatting of the Table 1 and have removed the formatting of sample size to simply have the number of births followed by the number/type of preterm births in italics, have bolded the significant relationships for ease of viewing, and adding a footnote to include referent group and abbreviations as well as units for BMI and lipids (pages 19-21).

Reviewer #1, Comment (Table 2): Provide a more detailed title (ex: expand to include details of the analytic population).

Author Response: We have relabeled the title of Table 2 as "Maternal Characteristics by Gestational Age Among California Singleton Births" (page 22).

Reviewer #2, Comment (1): The data source is unclear to me. If this is OSHPD birth cohort data, why was birth certificate information not available? It seems that only demographic information was abstracted from this but further information (e.g., information on hypertensive status and diabetes) could have supplemented information coming from discharge records. While these records would likely have high sensitivity for the conditions listed, they probably have very low specificity.

Author Response: We have clarified in the methods section under the Study Population subsection that the data is indeed from the OSHPD birth cohort, which uses both birth certificates and discharge records for mother and baby from 1 year before to birth to 1 year after (page 5, lines 5-6).

Reviewer #2, Comment (2): I'm very concerned about the random sample drawn from this cohort. The very low (1%) sample of black women and the high prevalence of pre-eclampsia (12%) both give pause on the interpretation of these results. Given that the association between black race and preterm birth is very consistent, it is clear that this analysis was underpowered to detect this effect. While the authors address this in their limitations, I would limit my inference from these findings given that this sample, by chance, appears to be non-representative of the cohort at large. It is also not clear to me why the full cohort wasn't used in the first place. I think if the authors could clarify 1) why they used a sample and 2) how their sample differs from the full cohort, these results would be more useful. The general trends they show are interesting but I am not convinced these results provide accurate estimates of the effects they are interested in.

Author Response: We agree with the reviewer that the small sample and its racial/ethnicity distributions are specific to this dataset; the study is therefore underpowered to detect effect of race as included in our discussion on limitations and mentioned in the article summary at the very beginning of the paper. While the sample is small it is indeed random and has been used previously in published articles by our co-author which we have cited in this manuscript as references 26 and 27 (Jelliffe-Pawlowski LL, et al. *BJOG*. 2015; 122(11): 1494; Jelliffe-Pawlowski LL, et al. *J Perinatol*. 2018; 38(8): 963). The small size was unfortunately necessary due to limitations of financial resources given the cost of testing serum samples and linking them with chart records. While the sample does slightly underrepresent the race/ethnicity distribution in the whole OSHPD cohort (thousands of births), overall the cohort is unique for its smaller proportion of black women at a prevalence of only 5.4% and so we feel that our sample does not differ greatly from the cohort with a rate of 1.8%.

VERSION 2 – REVIEW

REVIEWER	Jennifer Stuart

	Brigham and Women's Hospital and Harvard Medical School
REVIEW RETURNED	27-Jan-2020

GENERAL COMMENTS	As with other pregnancy complications, such as hypertensive disorders of pregnancy (gestational hypertension and preeclampsia) and gestational diabetes, preterm delivery is increasingly and consistently associated with an increased risk of maternal cardiovascular disease later in life. Identifying pre-pregnancy predictors of preterm delivery creates an opportunity for prevention of the pregnancy complication itself as well as for both primordial and primary prevention of CVD risk factors and events. Previous studies have demonstrated that CVD risk in these women can be detected before pregnancy and that traditional CVD risk factors (that develop before pregnancy) are risk factors for preterm delivery. In the current study, Rohlfing, et al. conducted a case-control study among 457 women who delivered at term (>=37 weeks) and 411 women who delivered preterm (<37 weeks; n=249 late preterm [32-36 weeks] and n=162 early preterm [<32 weeks]) (n=868 total) to identify maternal CVD risk factors present before pregnancy that may serve as predictors of preterm delivery. The investigators used data from a California-based cohort of singleton births (July 2009-December 2010) and randomly selected among women with available demographic information and measured first trimester biomarkers. They found that traditional CVD risk factors were significantly associated with the risk of preterm birth. See below for specific suggestions and questions: General: • Given the low numbers of black (1.8%), nulliparous (n=3, 0.7% of preterm and 0.4% term), and smoking (3% for preterm) women included, there are concerns about the representativeness of the sample included in the analysis. Further, given the large proportion of preeclampsia among preterm deliveries (16%), it was surprising to also see such a small number of medically indicated preterm births (n=9, 2% among preterm births); as the authors point out in the background, we would expect roughly 1/3 of the preterm births to be indicated. • In this revision, “pre and interpregnancy” has been added before “maternal CVD risk factors”. “Interpregnancy” typically refers to between pregnancies (e.g., “interpregnancy interval”) so the use of this word was confusing. Consider using “maternal CVD risk factors before and during pregnancy” or “prenatal maternal CVD risk factors” instead. • As highlighted by Table 1, a number of previous studies have been conducted to identify predictors or preterm birth. The impact of this paper would be strengthened if the authors more clearly stated the gap in the literature being addressed by the current study. • Since all pregnancies included in this study were drawn from California, it may be more specific to say “in California” than “in the US” in the title Abstract: • Please change “Diabetes (all types, pregestational and gestational)” to “Diabetes (types 1 and 2 and gestational)” or something similar to be more explicit about which types were included. Did the authors examine type 2/GDM (cardiometabolic conditions) separately from type 1 diabetes (autoimmune disorder)? • Line 11: please change “but particularly significant associations for the preeclampsia group” to “with the strongest magnitude of risk observed with preeclampsia” (or something similar).

	• Please introduce the maternal CVD risk factors of interest in the Methods of the abstract • Consider changing “In the multivariate adjustment model,…” to “Adjusting for the other maternal CVD risk factors of interest,…” to make clear what variables were included in the multivariable models. Methods: • Page 6, line 1: When is smoking status coming from (pre- or during pregnancy)? Did both race/ethnicity and smoking status come from hospital discharge records? • Page 6 line 3: In place of “pregestational”, please use “type 1” and/or “type 2” • Page 6, line 4: “gestational hypertension de novo or imposed on prior hypertension” – please remove “de novo or imposed on priori hypertension” as gestational hypertension refers to new onset high blood pressure during pregnancy by definition • Page 6, line 22-23: please include the BMI cutpoint(s) used to define obesity Results: please change this and other mentions of “rates” to “proportions” or something similar (“occurring among 16% and 2%, respectively). (Strictly speaking, a rate is the number of cases per person-time not the number of cases in a population.)◊• “The diagnosis of pre-eclampsia was also more prevalent in preterm versus full term births, occurring at rates of 16% vs 2%” • When interpreting the results of the logistic regression models (ORs), please use “odds” instead of “risk”. • Page 8, Line 15: What do the authors mean by “stepwise” multivariable logistic regression models? This is the only mention of it but, if the authors used stepwise regression to fit the models, this should be introduced in the methods. • Page 8, Line 20: please remove “spectrum” from “preeclampsia spectrum” (“significant associations of preeclampsia across all preterm gestational age categories”) Discussion: • Please reword #4: “higher total and LDL cholesterol values were associated with an up to two-fold increased odds of preterm birth” Table 1: the newly added footnote indicates that the reference group is “full term” but that is incorrect. For example, the reference group for the OR/RRs presented in the first row are likely age◊• The presentation of this table could be improved by: 1) splitting the “Sample Size” column into 2 columns to more directly compare the number of births and number of preterm births across each study/row, 2) removing the asterisks, 3) including the reference groups for the OR/RRs presented, and 4) providing the BMI and cholesterol units with the values presented in the table (not in the footnote) <35 nulliparous non-smokers and age <35 nulliparous smokers but it’s unclear what the reference group is for the second row (age <= 30?). Without the reference groups, it’s hard to give each OR/RR a proper interpretation. Table 2: • Please change the title from “Among California Singleton Births” to “Among Singleton Births in California”

VERSION 2 – AUTHOR RESPONSE

Reviewer #1, Comment (General): Given the low numbers of black (1.8%), nulliparous (n=3, 0.7% of preterm and 0.4% term), and smoking (3% for preterm) women included, there are concerns about the representativeness of the sample included in the analysis. Further, given the large proportion of preeclampsia among preterm deliveries (16%), it was surprising to also see such a small number of medically indicated preterm births (n=9, 2% among preterm births); as the authors point out in the background, we would expect roughly 1/3 of the preterm births to be indicated. In this revision, "pre and interpregnancy" has been added before "maternal CVD risk factors". "Interpregnancy" typically refers to between pregnancies (e.g., "interpregnancy interval") so the use of this word was confusing. Consider using "maternal CVD risk factors before and during pregnancy" or "prenatal maternal CVD risk factors" instead. As highlighted by Table 1, a number of previous studies have been conducted to identify predictors or preterm birth. The impact of this paper would be strengthened if the authors more clearly stated the gap in the literature being addressed by the current study. Since all pregnancies included in this study were drawn from California, it may be more specific to say "in California" than "in the US" in the title.

> *Author Response:* We have removed "interpregnancy" as a phrase and replaced with "before and during pregnancy" or "prenatal" as suggested to limit confusion (page 2, line 8; page 9, line 16; title of Table 3). We have also changed the title to focus on this specific population (page 1, line 2). With regard to the representativeness of the sample we agree and seek to highlight that as the major limitation throughout the manuscript (in the Article Summary, Strengths and Limitations, and Conclusions). As opposed to the studies shown in Table 1 this study is unique in its attempt to measure all of these risk factors in one patient population with information from both chart codes and serum measurements (again highlighted in the Article Summary; Introduction on pages 4-5, lines 18-23, 1-3; and Conclusions), filling in the gap of variation in those risk factors (page 4, lines 14-16).

Reviewer #1, Comment (Abstract): Please change "Diabetes (all types, pregestational and gestational)" to "Diabetes (types 1 and 2 and gestational)" or something similar to be more explicit about which types were included. Did the authors examine type 2/GDM (cardiometabolic conditions) separately from type 1 diabetes (autoimmune disorder)? Line 11: please change "but particularly significant associations for the preeclampsia group" to "with the strongest magnitude of risk observed with preeclampsia" (or something similar). Please introduce the maternal CVD risk factors of interest in the Methods of the abstract. Consider changing "In the multivariate adjustment model,…" to "Adjusting for the other maternal CVD risk factors of interest,…" to make clear what variables were included in the multivariable models.

> *Author Response:* As per the reviewer's suggestions, we have changed the wording in the abstract (page 2, lines 8-9, 11-13, 16), including the maternal CVD risk factors in its methods section. We did not consider type 2 separately from type 1, and have now highlighted that as well in our methods (page 6, line 1).

Reviewer #1, Comment (Methods): Page 6, line 1: When is smoking status coming from (pre- or during pregnancy)? Did both race/ethnicity and smoking status come from hospital discharge records? Page 6 line 3: In place of "pregestational", please use "type 1" and/or "type 2." Page 6, line 4: "gestational hypertension de novo or imposed on prior hypertension" – please remove "de novo or imposed on priori hypertension" as gestational hypertension refers to new onset high blood pressure during pregnancy by definition. Page 6, line 22-23: please include the BMI cutpoint(s) used to define obesity.

> *Author Response:* Smoking status (during pregnancy) and race/ethnicity information were from birth certificate data, and that has now been clarified in the methods section (page 5, line 22-23). We have again updated the labeling of pregestational to specify both type 1 and type 2 (page 6, line 1), deleted the phrase "de novo or imposed on prior hypertension" (page 6, line 2), and added the BMI cutpoints with units to the methods section as well (page 6, lines 4-5).

Reviewer #1, Comment (Results): "The diagnosis of pre-eclampsia was also more prevalent in preterm versus full term births, occurring at rates of 16% vs 2%" – please change this and other mentions of "rates" to "proportions" or something similar ("occurring among 16% and 2%, respectively). (Strictly speaking, a rate is the number of cases per person-time not the number of cases in a population.) When interpreting the results of the logistic regression models (ORs), please use "odds" instead of "risk". Page 8, Line 15: What do the authors mean by "stepwise" multivariable logistic regression models? This is the only mention of it but, if the authors used stepwise regression to fit the models, this should be introduced in the methods. Page 8, Line 20: please remove "spectrum" from "preeclampsia spectrum" ("significant associations of preeclampsia across all preterm gestational age categories").

> *Author Response:* To be more clear and accurate in our Results section we have revised the use of "rate" to now include "proportion" or not be used at all (page 8, lines 8-9), changed the phrase "risk" to "odds" (page 8, line 18; page 8, line 23), and removed the inadvertent use of "stepwise" (page 8, line 15). We have also removed "spectrum" per the suggestion in our results section discussing preeclampsia (page 8, line 19).

Reviewer #1, Comment (Discussion): Please reword #4: "higher total and LDL cholesterol values were associated with an up to two-fold increased odds of preterm birth."

> *Author Response:* We have revised the sentence as suggested (page 9, line 19-20).

Reviewer #1, Comment (Table 1): The presentation of this table could be improved by: 1) splitting the "Sample Size" column into 2 columns to more directly compare the number of births and number of preterm births across each study/row, 2) removing the asterisks, 3) including the reference groups for the OR/RRs presented, and 4) providing the BMI and cholesterol units with the values presented in the table (not in the footnote) – the newly added footnote indicates that the reference group is "full term" but that is incorrect. For example, the reference group for the OR/RRs presented in the first row are likely age <35 nulliparous non-smokers and age <35 nulliparous smokers but it's unclear what the reference group is for the second row (age <= 30?). Without the reference groups, it's hard to give each OR/RR a proper interpretation.

> *Author Response:* As per the feedback on Table 1, we have split the Sample Size column into two columns with a birth/preterm birth split; due to consistency with study type and ability to compare sample size we have also removed reference 18. We have further revised the table to remove the asterisks and list referent groups for each RR/OR, as well as units for BMI and cholesterol as indicated. The footnote has been updated to remove the units and abbreviations now included in the table itself.

Reviewer #1, Comment (Table 2): Please change the title from "Among California Singleton Births" to "Among Singleton Births in California."

> *Author Response*: We have relabeled the title of Table 2 as suggested.

VERSION 3 – REVIEW

REVIEWER	Jennifer Stuart Brigham and Women's Hospital/Harvard Medical School, USA
REVIEW RETURNED	03-Mar-2020

GENERAL COMMENTS	Thanks to the authors for incorporating my previous suggestions and responding to my feedback in this second revision of their manuscript, which seeks to identify predictors of preterm birth in a case-control study among 457 women who delivered at term (>=37 weeks) and 411 women who delivered preterm (<37 weeks). Enthusiasm for the objective of the manuscript and the availability of

	measured biomarkers is diminished by the non-representative analytic sample with low numbers of black (1.8%), nulliparous (n=3, 0.7% of preterm and 0.4% term), and women who smoked (3% for preterm). The sample is particularly problematic for the current study given the role of these factors in contributing to the risk of preterm delivery and raises questions about how the case-control sampling was conducted. Were the 1,000 women initially sampled truly random and representative with regards to race, smoking, and parity? The authors address this limitation on page 13 (lines 20-23) of the revised manuscript, pointing to "small sample size" as a cause for the non-representative sample. However, the sample size is actually on the larger end for a study of directly measured biomarkers. The authors should more directly address this limitation, noting the potential for selection bias/lack of generalizability (not "validation" as stated on line 23).

VERSION 3 – AUTHOR RESPONSE

Reviewer #1, Comment (General): Enthusiasm for the objective of the manuscript and the availability of measured biomarkers is diminished by the non-representative analytic sample with low numbers of black (1.8%), nulliparous (n=3, 0.7% of preterm and 0.4% term), and women who smoked (3% for preterm). The sample is particularly problematic for the current study given the role of these factors in contributing to the risk of preterm delivery and raises questions about how the case-control sampling was conducted. Were the 1,000 women initially sampled truly random and representative with regards to race, smoking, and parity? The authors address this limitation on page 13 (lines 20-23) of the revised manuscript, pointing to "small sample size" as a cause for the non-representative sample. However, the sample size is actually on the larger end for a study of directly measured biomarkers. The authors should more directly address this limitation, noting the potential for selection bias/lack of generalizability (not "validation" as stated on line 23).

> *Author Response:* The 1000 women included were indeed a random sample, but given the valid concerns about limitations in its representation we have rewritten our Strengths and Limitations section as follows (and replaced the term "validation" for "generalizability"): "Our study was limited by its smaller sample size and a certain degree of selection bias. Because the sample was drawn randomly from all women participating in first and second trimester prenatal screening in the state of California, and who had an ultrasound dating prior to 20 weeks, this bias is particularly of concern to women who do not participate in prenatal screening or enter care after the first trimester. This is most notably seen in effects on race/ethnicity and smoking status. Subsequent studies will benefit from more focused testing of associations in group not well represented in this sample including Black women. Given its single geographic focus in the state of California, broader generalization of the results is also limited" (page 13, lines 21-23; page 14, line 1-5).