Author's response to reviews

Title: Gestational tissue transcriptomics in term and preterm human pregnancies: A systematic review and meta-analysis

Authors:

Haley R Eidem (haley.eidem@vanderbilt.edu)
William E Ackerman IV (William.Ackerman@osumc.edu)
Kriston L McGary (kris.mcgary@vanderbilt.edu)
Patrick Abbot (patrick.abbot@vanderbilt.edu)
Antonis Rokas (antonis.rokas@vanderbilt.edu)

Version: 3
Date: 21 April 2015

Author's response to reviews: see over
Dear Dr. Sands,

Attached please find our revised manuscript entitled “Gestational tissue transcriptomics in term and preterm human pregnancies: A systematic review and meta-analysis” for consideration for publication in BMC Medical Genomics.

We were very pleased with the positive reception of our original manuscript as well as with the very constructive comments and suggestions raised by the two reviewers. In light of these comments, we have revised the manuscript accordingly, implementing all their changes and suggestions. We have provided a detailed point-by-point reply to all points raised by the reviewers in the pages that follow this cover letter.

Once again, we very much appreciate your consideration of this revised manuscript in BMC Medical Genomics and hope that you will find it suitable for publication.

Sincerely,

Antonis Rokas
Author’s responses are shown in bold, blue, and italicized font.

Editor’s comments: Your manuscript has now been peer reviewed and the comments are accessible in PDF format from the links below. Do let us know if you have any problems opening the files. We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns. Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted. We look forward to receiving your revised manuscript by 1 May 2015. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it. You should upload your cover letter and revised manuscript through http://www.biomedcentral.com/manuscript/login/man.asp?txt_nav=man&txt_man_id=3449750771542229. You will find more detailed instructions at the base of this email.

Authors’ response: Thank you for handling our manuscript and for your interest in our work. The constructive comments and suggestions are greatly appreciated. We have satisfactorily addressed all points made by both reviewers including altering PTB terminology, incorporating and commenting on additional suggested references, and clarifying methods and results. These changes are described in detail below.

Referee 1

Overall summary: The manuscript presents data based on a systematic review and meta-analysis to evaluate transcriptomic analyses for assessing preterm birth. The authors performed a targeted PubMed MeSH search followed by a systematic review of relevant articles. Preterm birth is a major problem and analyses of research in this area important. The current manuscript would be strengthened by some additional clarity and discussion.

Authors’ response: Thank you for your thorough assessment of our work and for your insightful suggestions. Specifically, we appreciate your careful attention to additional references that will strengthen the paper. All suggestions have been addressed as outlined below.

Major Compulsory Revisions

1. Please alter the language to be in keeping with the field of preterm birth and preterm labour. As currently written, parts of the introduction section are not clear. In general contrast to the field, the authors have described spontaneous preterm birth as iPTB for idiopathic preterm birth. The ‘i’ may possibly be interpreted as iatrogenic (medically indicated). In keeping with the terminology of the field I would suggest using, ‘spontaneous preterm birth (sPTB) either with or without preterm premature rupture of membranes (PPROM)’
Authors’ response: We very much appreciate your attention to this discrepancy and possible source of confusion. We have implemented the suggestion and changed all instances of ‘iPTB’ to ‘sPTB’ with or without PPROM.

2. To enhance clarity about the different types and incidence of preterm birth within the introduction, please include the following articles; Ananth and Vintzileos, 2006, Henderson et al, 2012, Moutquin JM 2003 and Myatt et al 2012 (see below for references).

Authors’ response: The papers by Ananth et al., Henderson et al., Moutquin et al., and Myatt et al. have been cited in the introduction (Line 75 and Line 91) in order to enhance clarity about PTB definitions and incidence.

3. Please include the recently published article, Manucke TA et al 2015 (see below for reference) and discuss, compare or contrast the relevance to the findings of the current paper.

Authors’ response: Thank you for the suggestion. We have added a paragraph in the discussion section that speaks to the need for more detailed meta-data that conforms to this new clinical phenotyping tool. The section (Lines 303-310) reads, “Furthermore, the recent publication of comprehensive phenotyping tools necessitates the connection of evidence-based phenotype knowledge with genomic data collection in order to make more targeted conclusions. It’s challenging to compare and contrast gene expression signatures between distinct subtypes without knowing whether the transcriptomes came from cases of sPTB due to maternal stress, uterine distention, or another subtype. Therefore, a greater focus needs to be placed on collecting the most detailed meta-data available regarding sPTB diagnosis as well as performing genome-wide studies of these newly described sPTB subtypes.”

4. Please include the following articles; Conde-Agudelo et al 2011, Kacerovsky et al 2014 and Menon et al 2011 (see below for reference) and discuss, compare or contrast the relevance of these additional meta analyses and systematic reviews for preterm birth to the findings of the current paper.

Authors’ response: Thank you for bringing these papers to our attention. We have incorporated these data into a new table (Table 2) that overlaps the published biomarkers with the results of our meta-analysis. We have also included a paragraph in the Discussion section (Lines 294-301) that discusses the findings of these past studies in the context of our study. Briefly, as Table 2 shows, there is considerable overlap in the data, with 12/19 (63%) of the published biomarkers appearing as candidate genes in 4 or more studies in our meta-analysis. We appreciate your suggestion and think that its addition has strengthened our study; despite considerable overlap, there are still 7/19 (37%) biomarkers that weren’t replicated in our meta-analysis and, conversely, thousands of candidate genes that haven’t been identified as biomarkers. Therefore, this systematic review and meta-analysis can both confirm previous results as well as point to promising new candidate genes potentially contributing to the pathogenesis of PTB.
Minor Essential Revisions

5. The introduction section is long and there are many cases when the points could be more concise, for example, line 67-69 is identical to line 22-24 in the abstract. Please revisit and revise both abstract and introduction for clarity and flow.

Authors’ response: We have removed Lines 67-69 in our original manuscript to eliminate repetition. Generally, the introduction section has been revised and edited to increase clarity and flow as well as to decrease repetition.

6. Please revisit all sections for grammar, clarity and flow. A number of sentences are cumbersome and difficult to discern. Please see below for some suggested examples: Line 27 – Methods: remove the word “all” Line 30 – Please amend sentence for clarity. Suggestion - “Our search yielded 2,362 studies on gestational tissues that included; placenta, decidua, myometrium, maternal blood, cervix, fetal membranes (chorion and amnion), umbilical cord, fetal blood and basal plate” Line 33-34 – change “genetic elements identified 96, 21, and 21 gene expression, microRNA and methylation studies, respectively” to “genetic elements identified 96 gene expression, 21 microRNA and 21 methylation studies” Line 34

Authors’ response: We have implemented all of these changes. The word ‘all’ has been removed in Line 27. Lines 30-32 have been amended and now reads, “Our search yielded 2,362 studies on gestational tissues including placenta, decidua, myometrium, maternal blood, cervix, fetal membranes (chorion and amnion), umbilical cord, fetal blood, and basal plate.” Lines 32-35 have been amended and now read, “Selecting only those original research studies that measured transcription on a genome-wide scale and reported lists of expressed genetic elements identified 93 gene expression, 21 microRNA, and 20 methylation studies” (the total number of studies was slightly revised after addressing the first reviewer’s point #7 and the second reviewer’s point #2).

7. I may have missed the rationale, but, I found it difficult to identify all the references, which were included in one set of analyses versus another, and compared to those that were cited within the reference list at the end of the article. In Line 144 – 146. The authors state the ‘138 genome-wide transcriptomic studies in human gestational tissue samples were, based on a number if selection criteria, deemed eligible for systematic review (Additional File 1) [12-129]’ The additional file contains 138 examples, but many of these are repeats. Could the authors please clarify whether the stated 138 genome-wide transcriptomic studies equate to 138 distinct references? Or multiple studies as defined by the authors within a reference? For example, Sood et al 2006 is listed in the additional file multiple times (a through to g), is this reference indicative of 7/138 genome-wide transcriptomic studies or 7 articles? I am not sure what to suggest, but would appreciate a clearer explanation to address this query.

Authors’ response: We apologize for not clearly defining our 138 ‘studies’ in the original manuscript. To address the reviewer’s point (as well as the second reviewer’s point #2) we re-examined all studies included in our meta-analysis. From 116 distinct references, we identified
134 studies that comprise our meta-analysis. This is because 14 of the references performed multiple comparisons that were separated into 33 distinct studies for the purposes of our meta-analysis. For example, Sood et al. published an analysis of gene expression patterns across different phenotypes (birth weight, delivery method, and healthy pregnancy) and study methods (expression and methylation). After re-examining the studies in our meta-analysis, we have now separated Sood et al. into 4 studies (a-d; expression/healthy/placenta, methylation/healthy/placenta, methylation/delivery method/placenta, and expression/birth weight/placenta). Sood et al. therefore represents 1/116 references and 4/134 genome-wide transcriptomic studies. We have clarified this classification method at the beginning of the results section and in the methods section of the manuscript. Lines 139-142 now read, “These 134 studies were identified from a total of 116 distinct publications; this is so because 14 publications reported multiple comparisons that were separated into 33 distinct studies for the purpose of this analysis” and Lines 372-374 now read, “116 references met all inclusion criteria and, due to multiple comparisons or analyses in 14 of these references, a total of 134 distinct studies were summarized (Additional File 1).”

8. Line 243 add ‘of PTB’ at the end of the subheading

Authors’ response: We have implemented the change and the subheading now reads, “Although gene expression profiles are available for 29 distinct phenotypes, PTB research is dominated by studies focused on select phenotypes of PTB.”

9. Please revisit the discussion (major compulsory revisions #1 and #2 above) and expand critical comparison of the current paper and other recent reviews / papers. How will the information from the current manuscript help change how the field moves forward?

Authors’ response: We have expanded our discussion section to include two new paragraphs: 1) a comparison of our meta-analysis with results from the previously mentioned biomarker studies and 2) a comment on the need to utilize new phenotyping tools to strengthen the detail of genomic meta-data. See lines 294-310.

10. Please add specific exclusion criteria to the methods. For example in the additional information 12, what is meant by the term ‘too early’?

Authors’ response: We apologize for the lack of clarity. We have clarified our specific exclusion criteria (e.g., “too early” was changed to “tissue collected before the third trimester of pregnancy”) and added the full list of the exclusion criteria used to the methods section. Lines 356-365 now read, “Furthermore, studies were excluded when the study data was not accessible (the number of gene candidates was reported but the list of candidate genes was not), the study data was not reported (the number of candidate genes was not reported and a list of candidate genes was not provided), the data was unclear, there were no significant gene candidates, the study was not genome-wide, the study was not human-specific, the study was not relevant, the study was not single-gene based (i.e., was focused on pathways or gene sets), the study used data from proteomics, the study was performed on cell line rather than in an in-
vivo tissue, the study’s supplement was not available, or when the study’s tissue was collected before the third trimester (Additional File 12).”

11. Additional references to include:

Authors’ response: We greatly appreciate your close attention to important literature previously missing from our reference list. We have added all of the abovementioned references to the manuscript.

Referee 2

Overall Summary: The authors have performed a search of genome-wide transcriptomic studies on gestational tissues. They have identified and selected gene expression studies with available differential expression results as well as microRNA and methylation studies. The authors broadly define gestational tissues as placenta, decidua, myometrium, cervix, fetal membranes (chorion and amnion), umbilical cord, basal plate and include maternal and fetal blood. As the authors state, the phenotypes studied were diverse and the comparison not straight forward, resulting in that the performed meta-analysis including 96 gene expression studies across 9 distinct gestational tissues and 29 clinical phenotypes mainly focused on preeclampsia in placenta (n=23), labor in myometrium (n=9) and PPROM in fetal membranes (n=4). Even though the results showed limited overlap of genes identified as differentially expressed across studies, a compelling aspect of this study is the consensus of GO term of function and previous research.
Authors’ response: Thank you for your interest in our work and for your constructive comments. All suggestions have been addressed as outlined below.

Major Revisions

1. The authors claim two key findings:

   Line 261-265: “Examination of our results identifies two key findings. First, the correspondence between PTB subtype prevalence and proportion of transcriptomic research devoted to these subtypes is weak. Second, the overlap between differentially expressed genes identified in different studies is quite small, even on studies aimed on the same phenotypes and tissues”.

Addressing the first find: in line 172-181 the authors states that: PTB research focus does not reflect PTB subtype epidemiological prevalence substantiated by the results “…To evaluate whether the proportion of transcriptomic studies devoted on different PTB subtypes reflects their clinical prevalence, we compared the frequencies of the three major clinical etiologies (iPTB at 45%, PPROM at 25%, and medically indicated PTB at 30%) to the frequency of transcriptomic studies devoted to these etiologies (Figure 4). We found that although only 30% of all PTB cases are due to medical indications, such as PE, IUGR, or GDM, 124/138 (90%) of the studies in our systematic review focused on them; 40/138 (29%) of these studies focused on PE alone. In contrast, although iPTB is responsible for 45% of all cases, only 10/138 (7%) of the studies in our systematic review studied this clinical subtype…. In supplement 1 there are correctly 40 studies of preeclampsia from different tissues all in all. However the gestational age seems not only preterm but equally often term. Could the authors please clarify if the term studies are included and counted as preterm (PTB) in the above results (Line 172-181; 40/138; 29%).

Authors’ response: Thank you for the suggestion. We have changed the analysis to include only the studies of preterm tissue samples (54/134) instead of all studies. The section now reads, “We found that although only 30% of all PTB cases are due to medical indications, such as PE, IUGR, or GDM, 41/54 (76%) of the studies categorized as preterm in our systematic review focused on them; 21/54 (39%) of the preterm studies focused on PE alone. In contrast, although sPTB is responsible for 45% of all cases, only 10/54 (18%) of the preterm studies in our systematic review studied this clinical subtype”. We have also updated the associated figure (Figure 4) to reflect these changes.

2. It also seems that the PPROM phenotype has 3 preterm and one term study included and counted as preterm (PTB).

Authors’ response: Thank you for your careful attention to this data. After reviewing the table again, we re-categorized the aforementioned study (Nhan-Chang et al.) as ‘labor’ instead of ‘PPROM’ since it analyzed gene expression in fetal membranes at the site of rupture during term labor, not preterm. Additionally, we re-assessed all studies in the systematic review and
3. Some studies are from the same publication and the authors have suitably denoted these as a, b, c and so on, in suppl_1. However, the division into term and preterm deliveries is not clear outside of the supplement, in methods, results and discussion. It also follows that not all studies are independent but have similar control samples.

Authors’ response: 14 publications in our systematic review were separated into 33 studies based on the inclusion of multiple, separate analyses. For example, Mayor-Lynn et al. studied gene expression and microRNA expression changes in both preeclampsia and sPTB. We, therefore, split Mayor-Lynn et al. into Mayor-Lynn2011a and Mayor-Lynn2011b. Additional file 1 lists all data collected for all 134 studies and includes (but is not limited to) gestational tissue, phenotype, and gestational age of tissues assayed. Studies are either annotated as term (all tissues studied were collected at term), preterm (all tissues studied were collected preterm), or term and preterm (tissues studied were collected both at term and preterm). As mentioned above in the reviewer’s point #1, we selected only studies of preterm tissues for comparison with PTB epidemiological prevalence. However, studies of all gestational ages (preterm and term) were analyzed in both the systematic review and meta-analysis components to maximize data pertaining to specific gestational tissues and clinical PTB phenotypes.

Minor Revisions

4. Line 32-34: Consider rewording from “Selecting only those original research studies that measured transcription on a genome-wide scale and reported lists of expressed genetic elements identified 96, 21, and 21 gene expression, microRNA, and methylation studies, respectively”, to” Selecting only those original research studies that measured transcription on a genome-wide scale and reported lists of expressed genetic elements identified in total 96 gene expression, 21 microRNA and 21 methylation studies” or similar.

Authors’ response: We have implemented the change and the aforementioned text now reads, “Selecting only those original research studies that measured transcription on a genome-wide scale and reported lists of expressed genetic elements identified in total 93 gene expression, 21 microRNA and 20 methylation studies.”

5. Table 1: Please alter the name of Gene ID 93659 from CBG to CBG5 to distinguish it better. Please alter the name of Gene ID 94115 from CBG to CBG8 to distinguish it better.

Authors’ response: Thank you for suggesting this clarification. The official gene symbol for Entrez gene ID 93659 now reads “CGB5” and the official gene symbol for Entrez gene ID 94115 now reads “CGB8.”
6. Line 338: The method indicates gestational tissue, the protocol states placental tissue. Please clarify.

Authors’ response: Thank you for noticing this error. ‘Placental’ has been changed to ‘gestational’ in the list of inclusion criteria.

Discretionary Revisions

7. A more clear presentation of results could perhaps be obtained if the authors could decide to either focus on the 96 gene expression studies or more clearly incorporate additional microRNA and methylation results into the results and discussion section.

Authors’ response: We initially searched for all genome-wide gene expression studies of human gestational tissue including gene expression, methylation, and microRNA. 21/134 (16%) were microRNA studies, 20/134 (15%) were methylation studies, and the remaining 93/134 (69%) were gene expression studies. Since the vast majority of the data were differential gene expression results, it was most straightforward to focus the meta-analysis portion of our study on that data set. Given the lack of a considerable number of methylation or microRNA studies, we believe that, while very interesting, this analysis is something best left for a future study. We did think it was important to collect available data for both methylation and microRNA in the systematic review portion of our study, however, since both play significant roles on gene expression levels that could be important in PTB pathogenesis.