Reviewer’s report

Title: Transcriptomic analysis of fetal membranes reveals pathways involved in preterm birth

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Reviewer: Allison Momany

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In this study examining differential expression profiles in preterm birth, 4 cases (GA<33 weeks) were matched with 4 controls (GA>37 weeks) on maternal age, sex, and SES. Samples were taken from a Uruguayan population. Tissue was collected from the chorioamnion near the site of membrane rupture and RNA was extracted using a Trizol protocol. Typical RNA measures were employed to ensure RNA quality. In an effort to reduce false positives, genes were identified as DE if the absolute log 2-fold change was greater than 2 and the FDR-adjusted p-value was less than 0.05 for multiple testing (BH method). Results from RNA-Seq were validated by qRT-PCR in 15 term and 9 preterm samples (including the 8 samples that underwent RNA-Seq procedures). Results indicate 252 genes that were upregulated and 18 genes that were downregulated. None of the GO terms related to the downregulated genes survived correction for multiple testing. The GO terms for the upregulated genes were related to immune and inflammatory response. Seven of the upregulated genes were previously found to be upregulated in an independent study. Overall, this study contributes to the current literature on the pathophysiology of preterm birth and the experimental methods appear to be rigorous. The authors are transparent about their methods, results, and study limitations. The manuscript is organized, clear in most places, and thorough in discussing what is already known about this topic (introduction) and the new knowledge that is gained from this study (discussion).

INTRODUCTION

1. Overall, very clear and strong introduction. There are a few sentences that are somewhat awkward and would benefit from editing. For instance, "Within the group of idiopathic or spontaneous PTB (sPTB), approximately 45% of cases may occur without preterm rupture of membranes, while the remaining 25% is a consequence of the preterm premature rupture of membranes." I believe the 45% and 25% refer to percentages of ALL preterm births (45+25=70%, and medically-indicated = 30%), but this sentence presents it as 45% and 25% of idiopathic preterm births specifically.

2. The authors clearly state the goal of the experiment; however, it would be beneficial to also state hypotheses. Going into the experiment, what was expected? Up-regulation and down-regulation? Specific genes involved given previous research? Although I recognize that any type of "omics" is often regarded as "hypothesis-generating", some hypotheses can still generally be stated, particularly if previous transcriptomic studies related to pregnancy have been completed.
METHODS

1. The authors very clearly articulate the methods used to extract RNA and derive the DE profile. This appears to follow typical guidelines for RNA sequencing (liquid nitrogen or RNAlater, trizol extraction, etc.).

2. The authors have admirably already deposited data in the NCBI Sequence Read Archive.

3. At the end of page 8, the authors state "Genes were identified as DE with the following criteria: absolute logarithm fold change > 2 and FDR-adjusted p-value < 0.05 for multiple hypothesis testing (method = "BH"). With the aim of reducing false-positive hits, we required a gene to be selected by all 3 methods to be considered as DE." Given these 2 sentences, it appears that there are only 2 requirements for being considered to be DE (log fold change and FDR-adjusted p-value). Please clarify.

4. How were the 6 DE genes used for qRT-PCR selected? Why was there not at least one down-regulated gene included?

RESULTS

1. In the methods section, the authors reference "absolute logarithm fold change > 2 and FDR-adjusted p-value < 0.05", but throughout the results section, this is often >/= and </= instead of just > and <. Please determine which it is and use consistently.

DISCUSSION

1. The authors are thorough in their discussion of the genes and pathways identified in the study and are transparent about the limitations of the study, which is appreciated.

2. There are a few sentences that require editing. For example: "Several pathways and genes are involved in labor and most of them would be either turn on or off during pathological conditions." (page 17).

3. The difference in the number of genes found to be up-regulated (252) vs. down-regulated (18) is very large. Was this expected? Is there a hypothetical explanation for this difference?

TABLES AND FIGURES

1. Table 1 needs to be edited so that it column width is uniform and decimal points are used throughout instead of commas. This table should also include the Student's t-test of Chi-square test values and corresponding p-values.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
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Are the conclusions drawn adequately supported by the data shown?
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