Reviewer’s report

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

Version: 0 Date: 12 Dec 2018

Reviewer: Michael G Gravett

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MGNM-D-18-00236

Transcriptomic analysis of fetal membranes reveals pathways involved in preterm birth.

This manuscript presents a very small transcriptomics study of chorioamniotic membranes of women with and without preterm delivery by RNA sequencing. This is an especially important topic. Preterm birth complicates 15 million births annually in the world and is now the leading cause of childhood death at less than 5 years of age, accounting for 1 million childhood deaths a year. A plethora of genomic and transcriptomic manuscripts related to term and preterm labor have been published in the last decade, and generally reveal pathways associated with inflammation, immune function, coagulation, and extracellular matrix degradation. The strength of this manuscript is that it one of the very few that have used RNASeq to characterize the transcriptome, is from a population not previously well studied (South American), and correlates with QPCR.

In this manuscript, the major findings were that extreme preterm birth was associated with 252 genes that were differentially up-regulated and only 18 genes that were differentially down-regulated. Similar to other studies, up-regulated genes were found in a wide range of inflammatory and immune pathways. More interesting, were down-regulated genes that included a WNT family member and potassium-voltage-gated calcium channel members (known to be important in uterine contractility). Given the tight clustering of the down-regulated genes, one could speculate that a preterm birth "transcriptome" may exist.

The other important contribution of this manuscript is the observation that there was much tighter clustering of genes associated with term birth than those associated with preterm birth. This is in keeping with the hypothesis that term labor is a highly unified and coordinated activity, whereas preterm birth is multi-factorial with many etiologies that will not be easily defined.

The manuscript suffers from small sample sizes, with only 4 cases and 4 controls analyzed by RNASeq. The authors, however, correctly acknowledge this, and other limitations in the discussion. Specific criticisms that would strengthen the manuscript include:

1. The grammar is sometimes incorrect, and will require significant editing (e.g., p6,1109 "…tissues were obtained from no complicated pregnancies delivered after…". I think the authors mean to state that tissues were obtained from uncomplicated pregnancies)
2. How was the exact site of membrane rupture determined? This is especially difficult especially after a vaginal birth when the membranes are usually massively disrupted, and is important since the transcriptome may very depending upon where the membranes are samples.

3. Patients and controls included both those with and without premature rupture of the membranes (PROM). It would be interesting to state which patients, among the 8 analyzed, had PROM since the pathophysiology of PROM preceding labor may differ from that of labor without PROM. Could this account for the highly differential expression of genes seen in patient 1, Figure 1?

4. Table 1 is confusing, and probably unnecessary. Which groups were compared to which groups for the statistics? Total versus total and RNA versus RNA? It is not clear.

5. Figure 1, patient 1 had a very different gene profile than the other 3 patients with preterm birth (P02 - P04). How did this patient differ in clinical presentation?

6. In Figure 2, the order, from left to right, of the cases is changed from Figure 1. In Table 2, the lanes for P01 and P02 are reversed. Is this a simple mistake, or did the authors intend on this, and if so, why?

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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