Reviewer’s report

**Title:** LncRNAs regulating gene transcriptions may connect ubiquitin-proteasome system with infection-inflammation pathways in preterm birth and preterm premature rupture of membrane

**Version:** 2

**Date:** 8 June 2014

**Reviewer:** Elizabeth Bonney

**Reviewer’s report:**

The topic of the manuscript by Luo et al is timely and the work has high potential interest to readers in many fields. There are however several issues that need to be addressed.

Major Compulsory Revisions

1. Several of the sentences and phrases in the manuscript are grammatically incorrect or confusing. The use of present and past tense is also inconsistent.

Examples include:

- Line 82 (could say “may be involved’)
- Line 122 (could say “disruption in the expression of… has…”)
- Line 125 (tense)
- Line 128 (delete “the”)
- Line 134 (delete “The”)
- Line 146 , 147 (tense)
- Line 148 -152 (confusing… could read: “In addition, the… encoded by the opposite strand….can increase BACE1mRNA stability and protein at the ….as has been shown to be true in Alzheimer’s disease…” )
- Line 158 (exchange inflammation for inflammatory)
- Lines 161-165 (confusing, could read: “To test this we applied microchip technology to identify more than 2000 placental lncRNAs and used them to delineate 20 potential pathogenic pathways that are altered in PPROM…”)
- Line 174 (confusing, could read “Written informed consent was obtained from the pregnant women participating in this study)
- Line 175 (delete “ship”)
- Line 302-305 (cumbersome sentence. Could read “Because of PPROM’s relationship to perinatal morbidity and mortality, research in to the underlying pathogenic mechanisms has received greater recent interest” )
- Line 401 (tense)
- Line 499 (should read at the protein level)
2. The definition of the groups A-D is somewhat confusing. For example, group A is the PTB group, but it is not clear (although assumed) that this group delivered without rupture of membranes occurring before labor. However, were there any prolonged labors (with membranes ruptured for more than 18 ours?) For another example, B is the group which delivered at 39-40 weeks “without rupture of membranes” This group must have ruptured membranes at some point. Delivery without rupture of membranes does not occur that often. When did these mothers experience rupture of membranes—after contractions or cervical dilation occurred? Does this included mothers whose membranes were artificially ruptured? For yet another example, group C includes women who experienced PPROM at < 35 weeks gestation “without membrane rupture”. It is not clear who these women are. I assume these are women who ruptured at less than 35 weeks, but it is not clear how soon they delivered after membrane rupture, if any of them made it to term, or if they delivered spontaneously or with labor induction (e.g. pitocin). Group D it is assumed delivered at term but had membrane rupture before labor. How long was it between membrane rupture and delivery? Did these women need pitocin?

3. If the subject of the study heavily focuses on PROM/PPROM, why weren’t fetal membranes (chorion, aminon) collected? This is not to say that the study needs to be done again, I mean to suggest that the authors need to defend the choice and state the potential pitfalls of this choice with regards to the conclusions drawn.

4. The IncArray needs to be described in more detail or referenced in more detail. “collected….from.. many articles in the literature....” Is likely not appropriate.

5. The authors should investigate alternative methods of statistical analysis. Four groups are studied, and probably one way ANOVA and two tailed t-tests are probably not appropriate in this setting. The p values presented should also take into account repeated measures.

6. Line 270. Ten paired IncRNAs and their associated mRNAs were selected (I assume form qPCR). How and why were these selected? This is the same issue for the GeneMANIA analysis (starting at line 282). Why were these IncRNAs and mRNAs chosen for study. Line 291 says that “twenty-seven other related genes” also went in the analysis—what were they and why were they chosen?? The choice of IncRNA and mRNA might significantly bias the results obtained, and other choices might have lead to alternative conclusion. The read just needs to understand more about why certain choices were made in the course of this analysis.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published
**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I have no competing interests