Author’s response to reviews

Title: Identification of New Biomarkers for Acute Lung Injury by Expression-based Genome-wide Association Study

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Author’s response to reviews: see over
Dear BMC Pulmonary Medicine Editorial Team,

Please find attached our revised manuscript, which we would like to resubmit to BMC Pulmonary Medicine for publication. We have extensively reviewed the manuscript in accordance to the suggestions of the reviewers and feel that their suggested revisions have strengthened this work. Our responses to the reviewer’s comment are addressed in point by point fashion. This manuscript was proofread by Dr. Heruth, who is a native English speaking author. We also addressed the editor comment on the ethics statement of human studies. We believe that this revised manuscript is now acceptable for publication in BMC Pulmonary Medicine.

Sincerely,

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Responses to reviewers.

Reviewer 1

Comment 1: Hardly any readers will be familiar with eGWAS. I think the methods section needs to not just reference the DM PNAS paper from Atul Butte’s group, but go into more detail about how this was done. How were the probes across species, etc. put together.

Response 1: We thank reviewer for this thoughtful comment and expanded our Method section on the method of Butte’s group.
Lines 126-130 in the revised manuscript.

Comment 2: Table 2 would mean more to me if, instead of listing prior evidence, the columns showed the log2 changes and significance in each of the experiments, or some sort of summary of this (= # of significant models, significant in humans, etc.)
**Response 2:** We thank reviewer for this suggestion and generated additional Supplemental Table 3, which provides this information. The readers are referred to this Table on: Line 214-215.

**Comment 3:** I feel like the discussion section is really scanty. I think the authors could talk much more about the overlap with prior findings, and talk about the 2 genes they chose to validate: what are those genes? Any speculation on function?

**Response 3:** We thank reviewer for this thoughtful comment and have elaborated on the description of our candidates in light of the latest publications and added references 14 and 15. Lines 234-243 Lines 452-459

**Comment 4:** The N of 97 control and 122 ALI samples don’t fit with the N in the rest of the paragraph (= 11 mechanical ventilation, 8 LPS, etc., in rows 150-153; these models don’t add up to 219 samples). Clearer in supplement, would at least refer to it.

**Response 4:** We thank reviewer for the noticing this confusing description of sample representation. We broke our complex statement into two separate sentences, elaborated on the textual sample description and refer readers to the Supplemental Table 1. Lines 200-205

**Comment 5:** Need more details on the validation experiment. = How many animals? What probes for qPCR?

**Response 5:** We thank the reviewer for noticing these oversights and fixed them accordingly. The detailed description of CLP model and real time PCR were added to the revised version of the manuscript. Lines 145-170

**Comment 6:** Minor essential revisions: 1) 169 has a typo/incomplete thought; 2) Intro lines 56-58 about own work seems superfluous

**Response 6:** The sentence on line 169 of original manuscript was restructured. Lines 222-224 in the revised manuscript We also have add required details to our own work in the Introduction section Lines 60-61

**Reviewer 2**

**Comment 7:** The distant lung injury model (ischemia-reperfusion kidney injury via the bilateral clamping) and the model of sepsis (cecal ligation and puncture) should be described in more
detail. In addition, data confirming the presence of lung injury should be presented (histology and lung injury score at the minimum).

**Response 7:** The first concern of the reviewer has been already addressed in the **Response 5**. In response to the second part of this thoughtful comment we performed histological study of lung tissue, created **Figures 2A, 2B**; and described this study in the corresponding Materials and Methods, Results and Discussion, and Figure legend sections.

Lines 156-159  
Lines 224-225  
Lines 358-363

**Comment 8:** Only real time PCR was used to validate expression of CD300LF and CLEC4E and data presented in the graph form. The actual results should be included. Further, Western blot or histochemistry should be employed to analyze protein levels.

**Response 8:** We thank the reviewer for this valuable advice and performed Western blot analysis. The **Figure 3B** was created and corresponding description was added to the Materials and Methods, Results and Discussion, and Figure legend sections of the revised manuscript.

Lines 172-179  
Lines 238-239  
Lines 377-380

**Comment 9:** Is there more information about CD300LF and CLEC4E in other inflammatory conditions? How about expression levels in different organs?

**Response 9:** We thank the reviewer for this thoughtful comment and already addressed this in the **Response 3**.