Reviewer’s report

Title: Differential proteomic analysis of virus-enriched fractions obtained from plasma pools of patients with dengue fever or severe dengue

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Reviewer: MERITS Andres

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Manuscript "Differential proteomic analysis of virus-enriched fractions obtained from plasma pools of patients with dengue fever or severe dengue" by Romain Fragnoud and co-authors describe the work done for identification of host proteins co-purified with Dengue virus virions and association of these factors with severity of disease. Two proteins, OLFM4 and PF4, which have prognostic values, have been identified. Such data is interesting and may have practical value.

To my opinion two halves of the study - virion purification/proteomics and subsequent association of identified proteins with disease are rather loosely connected. Authors are aware of this (lines 550-555) and have correctly highlighted that there is no conclusive evidence that the OLFM4 or PF4 are indeed associated with virions. Paper still reads as two separate stories, one dedicated to development and use of new approach to analyze virion proteins and another to association of some markers (not really shown to associate with virions) with severity of disease. However, I agree that presenting these things together is reasonable, as it shows the way the research was planned and performed.

One can also rise a question about biological relevance of the first part of study. The method is not sensitive enough to detect major virus proteins from patient samples (only peptide for E protein was detected, line 305. No peptides from M, Pr of C were revealed. In virus particles these proteins are likely lot more abundant than host proteins. If these major components of virus are detected how sure one can be about identification of less abundant (and possibly loosely associated) host proteins?

Comments:

1. Real-time RT-PCR has been named/abbreviated differently at different places (lines 116m 124, 127). If it is one and the same method it is better to be consistent

2. Line 229: why were samples pooled before virion purification? Can authors provide rational for this? The samples were both from DEN2 and DEN3 patients, can this possibly reduce specificity? In subsequent analysis also DEN1 patients were included (all Cambodian group was DEN1 infected). This is somewhat confusing
3. Line 271, also FIG 1C. Authors provide EM image of particles from mid-stage of purification process. Why do not they show final preparation - the one used directly for proteomics (after Viraffinity polymer purification). It is still possible that final treatment damages particles, so the image of these particles would be more relevant.

4. It would also be interesting to discuss why other two markers (CP1 and C1R) behaved differently between Columbia and Cambodian patients. Could it also be due to the difference of Dengue genotype? There is also interesting difference in viral loads. In Columbia group (table 3) the viral load is higher in SD patients (about 10-fold in average). It is completely different in Cambodian group (Table 4) where DS patients have in average 3.5 fold lower viral loads. Could this effect the results? Is there any explanation why DF patients in Cambodia have nearly 150-fold higher viral loads? How could this affect the results?

5. Line 848. KDa is molecular mass (not molecular weight) unit

6. Author could combine tables 3 and 4 - it will easier to see similarities and differences between patients groups. Please also use same units in both tables (even if they are separate tables). Currently total glycerol in table 3 is given in g/L while in table 4 it is mmol/L

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?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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