Author’s response to reviews

Title: Rare cause of Hemophagocytic Lymphohistiocytosis due to mutation in PRF1 and SH2D1A genes in two children – a case report with a review

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Author’s response to reviews:

Answer to comments from Reviewer-1:

Comment:

1. This manuscript describes novel PRF1 and XIAP HLH causable gene mutations.

   • The manuscript under submission talks about gene mutations of PRF1 (Perforin 1; Gene ID 5551) and SH2D1A (SH2 domain containing 1A; Gene ID 4068) genes only and not about XIAP gene mutations in HLH.

2. Unfortunately, only gene mutations were analyzed by NGS.

   • Analysis of gene mutations through NGS in a first step and then they were further confirmed by Sanger sequencing
3. As the authors mentioned, functional analysis like NK cell activity as well as PRF1/XIAP protein expression analysis by Western blot or flow cytometry are needed.

• The sentence(s) written from line 191 to 195 in discussion point towards a suggestion that ‘functional analysis’ may yield better insight into the immune competence of patients. We agree with the reviewer suggestions but are unable to carry out this study due to financial constraints.

4. There are a lot of papers about PRF1 and XIAP gene mutations, therefore not so much valuable information would be included in the draft.

We agree with the reviewer’s view, but in India very few cases are reported and present study has also identified novel mutation causing XLH. Thus our study will add up in the database and better understanding of the disease and its clinical progression

Answer to comments from Reviewer-2:

I recommend revisions to the manuscript as described in detail below:

1. In the abstract, for case 1, it is listed that a pathogenic variant in PRF1 was identified, and that parents and the fetus were found to be a carrier of this variant. This gives the impression that the affected fetus was a heterozygote, like the parents, whereas the text states that the fetus was homozygous. Please revise the abstract to reflect that the parents were heterozygous carriers, while the affected fetus was homozygous.

• Necessary changes have now been made in the revised abstract as advised.

2. In the background section, paragraph 2 (line 63), it is stated that primary HLH is further classified into FHL and lymphoproliferative disorder. I am not familiar with this classification, and a reference needs to be provided. As the author themselves discover, this separation is not of any practical or clinical significance, and thus SH2D1A can be considered as an FHL gene. Thus this sentence can even be deleted.
3. For case 1, fibrinogen was reported as decreased, but a value is missing. Since values for all other biochemical parameters were provided, this should be included as well. Please also include the alternative nomenclatures for the transaminases (ALT for SGPT, and AST for SGOT).

4. For case 2, EBV is reported as positive. What methodology was used - serology, PCR, in situ hybridization, or immunohistochemistry? And on what tissue - blood, bone marrow, CSF?

5. For case 2, how was aseptic meningitis suspected - data for cell counts, protein levels should be included.

6. For case 2, the trephine bone marrow reportedly did not show hemophagocytosis, however, it is later reported (line 146) that hemophagocytosis was one of the criteria used in reaching the diagnosis of HLH. This needs to be clarified.

• A reference (as Ref. no. 4) is added in the revised manuscript as suggested.

• Necessary changes are incorporated in the revised manuscript.

• Case-2: EBV was tested by serological test. This is mentioned in the revised manuscript.

• The clinical note mentioned aseptic meningitis. No further details are available.

• Case-2: We did find hemophagocytosis in the case of HLH (line 135-136) and then we continued saying on it in line 146. This may please be noted.
7. What did patient 2 die of at age 19 months? This information is important for clinicians who don't have access to all the diagnostic tools.

- ‘The proband died at the age of 19 months due to meningoencephalitis.’ This is added in the revised manuscript as suggested.

8. Algorithms such as polyphen and mutation taster can only suggest or predict if a given variant will be pathogenic, not confirm as stated on line 154. I suggest changing the word from confirmed to suggested or predicted.

- Suggested modification is incorporated in the revised manuscript.

9. I am not sure Table 1 adds significant information beyond what the opening paragraph of the discussion section states.

- Table-1 is now submitted as Supplementary File-2 in view of the comment by the reviewer.

10. IN the paragraph beginning on line 208, it is stated that a positive EBV test confirms the failure of immune system in protecting against EBV. This needs additional information as stated above, regarding what the test is. Simply being EBV positive is not an indicator of failure of the immune system. A persistent high viral load of the EBV virus on the other hand might be indicative of fulminant EBV infection or EBV-associated lymphoproliferation etc.0

- The sentence is changed in view of the comment giving an inference of ‘likely’ rather than ‘confirmed’.