Reviewer’s report

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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Reviewer: Ashish Kumar

Reviewer's report:

In this case report, Sheth et al describe two cases of HLH from India. They identified mutations in genes known to be associated with HLH. However, the specific mutations they describe are rare, and given the rarity of this severe disease, such a report is valuable. 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.

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 - blod, bone marrow, CSF?

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

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.

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.

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

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.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**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

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
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Not relevant to this manuscript
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Needs some language corrections before being published

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