Author’s response to reviews

Title: Novel mutations of STXBP2 and LYST associated with adult haemophagocytic lymphohistiocytosis with Epstein-Barr virus infection: a case report

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Author’s Response to Editor

Title: Novel mutations of STXBP2 and LYST associated with adult haemophagocytic lymphohistiocytosis with Epstein-Barr virus infection: a case report

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Dear Editor,

We sincerely appreciate all your efforts and suggestions to strengthen the manuscript, and felt much encouraged by your positive feedback. To address the concerns of the editor, we have revised our paper and all the amendments are highlighted in red in the updated manuscript. We hope our efforts would be able to address your concerns.

Below, we provide a point-by-point response to the comments.

We are looking forward to your response.

Sincerely yours

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Response to editor

Editor Comments:

Data presentation and interpretation need to be further improved. specifically:

1. Please list all authors in correct order (First last) in the title page:
Author: Sheng Lingshuang1(*), Zhang Wei1, Gu Jia1, Shen Kefeng1, Luo Hui1, Yang Yang
Response: Your kind suggestion has been addressed (P1, L5-6).

2. * The first author. Don’t need *, if only one first author.
Response: Your kind suggestion has been addressed (P1, L5-6).

3. Insert page number; use line number continues from start to end
Response: Your kind suggestion has been addressed.

4. Delete in the discussion, L23-24: “These contents have been mentioned in Discussion section”
Response: Your kind suggestion has been addressed.

5. Move figure legend to the main text, before table 1. Write Figure 1; change “nature” to “natural”
Response: Your kind suggestion has been addressed.
6. Table 1:
LYST 1:235973288
NM_001301365
c.830A>T
p.His277Leu
0.493 Heterozygous
Not Applicable

Explain why not applicable, as it is a common SNP.

Add table 1 notes: add some information from your response 4 to reviewer 1. Source of feature.
table 2 note: +, mutation.

Response: We are so appreciated for your carefulness! The ID in dbSNP was automatically imported by software, and there might be problems given it was not updated to the latest version. Therefore, we have double retrieved the database and updated all the reference numbers. Besides, the notes to tables have been added as well.

7. Case presentation:

L5: 5 HLH (Table 1). Change to HLH (Table 1, Figure 1).
L7: conducted as well (Table 2). Change to conducted as well (Table 2, Figure 1).

Response: Your kind suggestion has been addressed (P4, L87; P4, L90).

8. Figure 1 M, N appear showing Sanger chromatogram sequences; if so, please indicate in the text and legend.

Response: Your kind suggestion has been addressed (P4, L88).
9. please add a detailed description of methods used for Whole-exome sequencing, e.g. library build, platform, length, company performed seq, analysis etc.

Add info from your response 1 to 4 and 9 to reviewer 2 to the manuscript

Response: Your kind suggestion has been addressed (in supplementary method).

10. many other info from your responses should be added to the manuscript.

Response: Your kind suggestion has been addressed (in supplementary method).

11. add the bioinformatically predicted pathogenicity potential of the identified mutations in text and to table 1

Response: Your kind suggestion has been addressed. We added bioinformatically predicted pathogenicity potential of the identified mutations in text (P3, L41-51; P5, L116-123) and to table 1 (Associated features).

12. L7 conducted as well (Table 2): describe and interpret the NK cell results

Response: Your kind suggestion has been addressed (P4, L90-94).

13. what is the role of EBV in HLH, in literature and in this case?

Response: Thanks for the good question you raised. We discussed the role of EBV (P7, L153-158), and add reference 24 to support our discussion. Besides, we added our concern about the role of EBV in this case (P7, L158-161).

14. what is the role of NK cell activity in HLH, in literature and in this case?

Response: Thanks for the good question you raised. We discussed the role of NK cell activity (P6, L129-137), and add reference 24 to support our discussion.

In this case, we evaluate NK cell activity to meet the diagnostic criteria, and to assess their internal ability of eliminating EBV infection.
15. as you stated: “Taken together, it is difficult to verify whether or not these mutated genes are all involved in this patient’s EBV infection and HLH. Whether this case follows a digenic or a polygenic model also remains a question.

However, it is certain that the major pathogenicity of this patient is inherited”

Remove “However, it is certain that the major pathogenicity of this patient is inherited”

Consider removing “digenic” from the title

Response: Your kind suggestion has been addressed. The title has been changed to “Novel mutations of STXBP2 and LYST associated with adult haemophagocytic lymphohistiocytosis with Epstein-Barr virus infection: a case report”.

16. STXBP2 (c.592A>C) and LYST (c.830A>T) mutations, have these mutations reported before?

What is novel? You mean “digenic” of two is novel?

Response: We believe “novel” means the variants have never been reported by any HLH academic papers or any major disease related databases, including Online Mendelian Inheritance in Man (OMIM, www.omim.org), Human Gene Mutation Database (HGMD, www.hgmd.cf.ac.uk), the Catalogue of Somatic Mutations in Cancer (COSMIC, https://cancer.sanger.ac.uk/cosmic) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar). Besides, the digenic mutations model of STXBP2 and LYST that possibly could lead to secondary HLH we brought forward have never been reported as well.

17. mother health condition was not described in reasonable detail. Given such low NK cell function, being healthy is worthy noting.

Response: Your kind suggestion has been addressed. We add more description about her mother’s health condition and our assumption in the manuscript (P4, L90-94; P6, L138-P7, L153).
18. When HLH results from an inappropriate immune response to Epstein-Barr virus or another viral illness, it may be due to a separate genetic condition called X-linked lymphoproliferative disease (XLP). XLP is caused by a mutation in the SH2D1A or XIAP gene (from https://rarediseases.info.nih.gov/diseases/6589/hemophagocytic-lymphohistiocytosis).

Please discuss XLP for differential diagnosis and indicate the mutation results for the SH2D1A or XIAP genes.

Response: I think you have raised a very good question and surely worth further discussion. It is certain that XLP is a secondary disease caused by immunodeficiency-mediated EBV infection. Individuals with XLP-1 are uniquely sensitive to diseases caused by EBV, which otherwise runs a fairly benign course in most healthy individuals. HLH represents 60% of all the disease clinical features while the age of onset is within the range of 0.5-40 years old [24]. The symptoms of HLH secondary to XLP is very similar to our case. However, the patient in our case cannot be diagnosed with XLP since we found that she and her parents had no SH2D1A or XLP1 mutations via WES and Sanger sequencing tests.

19. Variant allele frequency presented are likely wrong.

My checking: rs760187284 is C=0.00001 (1/119988, ExAC) rs776254567 is T=0.00019 (23/121140, ExAC)

I don’t understand why you didn’t make changes in table 1 as you have realized: Your question is much appreciated. The population based allele frequency of STXBP2 is 0.0001389 in east Asian population from GemoAD, LYST is 0.0001094, LRBA is 0.002860. While the frequency of AIRE is 0.0000 in both CHB (Han Chinese in Beijing) and CHS (Southern Han Chinese) from

Response: Your kind suggestion has been addressed. Here VAF is only used to judge zygosity, and we updated table 1 with population based allele frequency added in.

20. your key response should be put in the manuscript for readers.

Response: Your kind suggestion has been addressed. If there are any more information should be put in the manuscript from your point, don’t hesitate to contact us. Many thanks!