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

Authors:

Lingshuang Sheng (lssheng1122@163.com)
Zhang Wei (26060299@qq.com)
Gu Jia (1172639203@qq.com)
Shen Kefeng (skf@tjh.tjmu.edu.cn)
Luo Hui (luohui_tjh@163.com)
Yang Yang (eliteyoung@126.com)

Version: 1 Date: 12 Dec 2018

Author’s response to reviews:

Author’s Response to Editor and Reviewers

Title : Adult hemophagocytic lymphohistiocytosis associated with digenic novel mutations of STXBP2 and LYST with Epstein-Barr virus infection: a case report

MN: MGTC-D-18-00423

Version: 1

Date: 5-December-2018

Corresponding Author: Yang Yang, 0086-27-69378607, e-mail: eliteyoung@126.com
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 and reviewers, 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.

Besides, please be kindly noted that the first author's full name was mistaken. Lingshuang should be the first name and Sheng is the last name. It would be very appreciated if you can help revise it. Apologies for our carelessness!

Please do note that the title has already been changed to “Novel digenic mutations of STXBP2 and LYST associated with adult haemophagocytic lymphohistiocytosis with Epstein-Barr virus infection: a case report” according to the kind suggestion of referee 1.

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

We are looking forward to your response.

Sincerely yours

Yang Yang, MD, Ph.D
Department of hematology, Tongji Hospital
Tongji Medical College
Huazhong University of Science and Technology
Wuhan, Hubei, 430030, P. R. China
Phone: +86-27-69378607
eliteyoung@126.com
Response to Referees

For Referee #1

General comments

1. The paper needs to be assessed by a native English speaker.

Response:

We appreciate the reviewer’s good suggestion. The paper has been assessed by American Journal Experts (AJE), and the certification is attached below.

2. The title should be changed. The mutations are associated with a disease not the inverse.

Response:

We greatly appreciate the reviewer’s carefulness. The title has already been changed to “Novel digenic mutations of STXBP2 and LYST associated with adult haemophagocytic lymphohistiocytosis with Epstein-Barr virus infection: a case report”.

3. All the names of the genes reported should be written in italic.

Response:

We greatly appreciate the reviewer’s carefulness. All the names of the genes reported have been re-written in italic.
4. Table 1:

- first column: Mutation should be replaced by gene.

- Third column: authors reported in this column the frequency of the identified mutation. I assume this should be the depth of each mutation not at all the frequency. Otherwise these mutations became all frequent polymorphisms.

- in this table authors should add more details like for example the predictions of the pathogenicity of these mutations and if they are reported before or not and if they were linked to HLH before or not.

Response:

We greatly appreciated the reviewer’s deep insight into the study and helpful suggestions to improve the manuscript.

- In the first column, “Mutation” has already been changed to “Gene”.

- I suppose the reviewer meant the fourth column? In the fourth column, “Frequency” is variant allele frequency (VAF). To avoid misunderstanding, we have already changed “Frequency” to “Variant allele frequency” and re-presented the data with decimal form in Table 1.

Major comments

The two mutations listed by authors as associated with HLH are inherited from the mother only. Authors should discuss why the mother didn't develop any clinical sign.

Response:

Many thanks for all the question you raised. We entirely agree it is worth further discussing. Firstly, the patient's mother is heterozygote. Secondly, the patient inherited other potential pathogenic genes from her father as well, except for the 2 genes inherited from her mother that are most likely pathogenic. These contents have been mentioned in Discussion section. Last, we tested her mother's plasma EBV-DNA and the result was negative (1.35 x 102/L in peripheral blood mononuclear cell). Low titer EB virus is a possible reason of her absence of clinical manifestation, but it could be deduced that the mother falls under the high-risk population of secondary HLH. And we have added this part to “Discussion and conclusions”. (Page 5, line 19-28)

Authors assume that the mutations reported in table 1 are associated with the secondary HLH form in the patient. Authors should discuss more how did they pick up these genes and how did they related these genes to the disease.

Response:

Many thanks for your valuable comments. Considering HLH and CAEBV are all related to immunodeficiency, so our filtering strategy of the mutated genes were based on classification of primary immunodeficiencies compiled by the Primary Immunodeficiency Expert Committee (PID EC) of the International Union of Immunological Societies (IUIS) mentioned in response 4, and these mutated genes were further validated by Sanger sequencing.
1. What exome sequencing approach was used? On DNA isolated from what tissue?

Response:

We greatly appreciate your carefulness and thoughtfulness. For Whole Exome Sequencing (WES), the genomic library of the proband was recovered for exome enrichment with Agilent Sure Select Human Exon v7 and was sequenced by Illumina HiSeq2500 with an average 300x coverage. The Broad Institute’s Genome Analysis Toolkit was applied during the data analysis. Reads were aligned with the Illumina Chastity Filter and the Burrows Wheeler Aligner. Variants were identified by the GATK UnifiedGenotyper module. We described our filter strategy in response 9. Besides, DNA was isolated from peripheral blood.

2. What quality control measures were applied to the exome sequence data? What data quality metrics were used to filter variants?

Response:

Many thanks for the great details you bring forward. For quality control measures, coverage per base was 351x, and Q30 percentage was 92.71%.

3. Were the parents of the probands also exome sequenced? Or were the specific variants of interest genotyped based on the findings in the proband?

Response:

Thanks so much for your detailed questions. Your understanding is perfectly correct. WES study was not performed on the proband’s parents. Only potential pathogenic mutations in Primary Immunodeficiency (PID) associated genes (Table 1) of the proband were identified in the proband’s parents using Sanger sequencing.
4. It is not clear how the authors came up with the list of genes in which they report observed variants. Did they restrict their search to only a list of candidate genes? If so, those should be clearly defined and justified.

Response:


5. Table 1 lists the frequencies for the observed variants as being extremely common- if what they are referring to is a population frequency, which I think can't be right. My guess is that maybe they are referring to the proportion of reads that support the alternate allele? If so, this is not a particularly useful metric without knowing the total number of reads mapping to the locus.

Response:

We greatly appreciate the reviewer’s carefulness. Same as your assumption, “Frequency” is variant allele frequency(VAF), and it’s only for judging zygosity. To avoid misunderstanding, we have already changed “Frequency” to “Variant allele frequency” and re-presented the data with decimal form in Table 1. The population based allele frequencies are given in the next response.
6. I would actually like to see the population based allele frequencies given for each putatively pathogenic variant in the Han population (this can be readily looked up in the ExAC database (http://exac.broadinstitute.org) or dbSNP or similar.

Response:

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 1000 Genomes Project of dbSNP, IRF8 is 0.0000 in both CHB and CHS.

7. "undetermined genetic mutations" - should be something like "variants of unknown significance"?

Response: We greatly appreciate the reviewer’s carefulness. The term “undetermined genetic mutations” means the variants are associated with PID but the significance remains unknown with HLH, and it has been revised to “variants of unknown significance with HLH” that is more precise. (Page4, line 4-5)

8. The authors claim that the variants are "novel", but it's not clear what they mean: "Two-generation pedigree analysis showed that the mutations were inherited from her parents" - novel? The variants are all inherited, so they are not de novo. If the mean that the variant has never been seen before, they should report what resource they base that assertion on, eg novel to what database/population?

Response:

Many thanks for your insightful comments. We believe the "novel" in the manuscript mainly has 2 points: firstly, 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.
9. There is no discussion of variant filtering for pathogenicity. How were variants annotated and filtered? Were non-coding pathogenic variants considered? Such as splice site variants or covered promoter regions?

Response:

We greatly appreciate the reviewer’s carefulness. Filtering strategy: First, generated variants were locally annotated with Annovar software under Linux system. Next, only mutations affecting amino acid sequence were filtered for further analysis (including missense, nonsense, frameshift/non-frameshift insertion/deletion, splice-site mutation and other complex mutations). Afterwards, variants with frequency in Han population no less than 0.01 were filtered out. Then variants failed to pass quality control (reads<20, quality<30, or variants with significant strand bias) were filtered out. The mutations in genes listed in response 4 were finally selected for Sanger sequencing validation. Splice site variants were taken into consideration in this filter strategy while promoter regions were not.

10. The authors propose that heterozygous hits to two genes resulted in the phenotype, but the mom’s NK cell function looks worse than the proband's; it would aid readers if the authors gave some additional background on what has been seen before as associated with pathogenic variants in these genes.

Response:

Many thanks for the question you raised, and I think it’s very professional. We entirely agree it is worth further discussing. Firstly, the patient's mother is heterozygote. Secondly, the patient inherited other potential pathogenic genes from her father as well, except for the 2 genes inherited from her mother that are most likely pathogenic. These contents have been mentioned in Discussion section. Last, we tested her mother's plasma EBV-DNA and the result was negative (1.35 x 10^2/L in peripheral blood mononuclear cell). Low titer EB virus is a possible reason of her absence of clinical manifestation, but it could be deduced that the mother falls under the high-risk population of secondary HLH. And we have added this part to “Discussion and conclusions”. (Page 5, line 19-28)