Peer Review File

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Reviewer A

Comment 1: In my opinion the term "molecular epidemiologic survey" is not well used in this context, it is not a survey. I suggest: genetic study.

Reply 1: Modification has been made accordingly.

Changes in the text: Page 1, line 1; Page 2, line 44.

Comment 2: It should be explained how the morbidity rate is obtained and its meaning.

Reply 2: We have explained how the morbidity rate is obtained and its meaning as advised.

Changes in the text: Page 8-9, line 172-178.

Reviewer B: Wei-Xia Lin et al reported allele frequencies of SLC25A13 in Shaanxi and Guangdong with two PCR assays which could detected four prevalent mutations in China. The allele frequency of the mutations in Guangdong was compatible with previous reports, while, in Shaanxi, it was not concordance in this study. The discordancy is probably due to mutation XIX. These PCR methods to detect the four mutations in SLC25A13 could help us to identify the four mutations which are prevalent in China.

I have the following concerns related to the manuscript:

Comment 1: The authors should include the data evaluated for the new PCR method (e.g. Sanger sequencing of the PCR product) in Results, because the method is the key in this study.

Reply 1: To evaluate the reliability of the new PCR methods in this study, the 4 prevalent *SLC25A13* mutations were detected in the 200 samples of Qingyuan city, and the results were completely consistent with those in the previous study by using HybProbe assay and HRMA approaches.

Changes in the text: Page 9, line 184-187; Figure 2.

Comment 2: Please describe the other limitations or constraints of this study in Discussion. For instance, the authors' assay could not detect any variants other than the four mutations, and the sampling of northern region was limited in Shaanxi province.

Reply 2: We have added the limitations of this study in Discussion as advised.
Changes in the text: Page 13, line 278-284.

Comment 3: The authors should mention the reference of SLC25a13 gene as NM_001160210 through the manuscript.
Reply 3: We have mentioned the reference of SLC25A13 gene as NG_012247.2(NM_014251.3) through the manuscript, and changed IVS16ins3kb to c.1751-5_1751-4ins(2684) accordingly.
Changes in the text: Page 3, line 55-56, 67-68; Page 5, line 88-89; Page 6, line 103; Page 7, line 146; Page 8, line 157; Page 9, line 195; Page 10, line 215; Page 11, line 227, Page 12, line 257, 267, 273.

Comment 4: L88-90: There is no reference or data in the 4 mutations which covered 82.9% in China. Please add the reference or the data.
Reply 4: We have added the reference Lin et al., 2016 accordingly. Data of 274 CD patients in this reference showed that the 4 mutations together had a relative frequency of 84.47% in China. Data of 184 new patients diagnosed from March 2016 to August 2020 are unpublished.
Changes in the text: Page 5, line 90.

Comment 5: L229: In this study, preciseness, time for the analysis, and cost-effectiveness of the authors' method were not surveyed, so this conclusive sentence would be an overstatement. The authors should add the data to support them. If you did not investigate them, please consider reconstructing the manuscript.
Reply 5: We have modified our text as advised.
Changes in the text: Page 11, line 232-235. Page 13, line 286-288.

Reviewer C
Comment 1: The authors examined frequencies of four prevalent SLC25A13 variants in Shaanxi and Guangdong provinces, as a molecular epidemiological survey for citrin deficiency. Establishment of experimental methods of variant detection and obtained data would be useful, however the scope of this study is very limited, and the results do not have enough scientific value for publication in this journal.
Reply 1: We concur with this view. However, we have found more than 60 pathogenic SLC25A13 variants in China. Substantial time and costs are required to support the detection of all the pathogenic variants, especially the large

insertion/deletion, in a larger population.

The authors should mention possible limitations of their strategy as molecular epidemiology based on the following points.

Comment 2: Page 3, lines 86-90: “From July 2005 to August 2020, our team had diagnosed 458 CD patients by SLC25A13 gene analysis from 29 different provinces, municipalities, and autonomous regions of China, and the 4 mutations c.852_855del, c.1638_1660dup, c.615+5G>A and IVS16ins3kb were on the top of the list, accounting for 82.9% of all mutated alleles.”
This means that surveys focusing on these four variants only would miss other pathogenic variants for CD at 17.1% probability.
Reply 2: We have added this limitation in Discussion.
Changes in the text: Page 13, line 278-280.

Comment 3: To examine frequency of pathogenic variants in a general population, it would be desired to detect reported and “predicted” pathogenic variants. This is because there may be unreported pathogenic variants in general human populations.
Reply 3: Modifications made accordingly on the manuscript title.
Changes in the text: Page 1, line 2.

Comment 4: Fisher’s exact test may be more appropriate than the Chi-square test in Table 3 because it contains small integers.
Reply 4: We have modified our text as advised.
Changes in the text: Page 3, line 60, 68; Page 9, line 180; Page 10, line 216; Table 3.

Comment 5: This manuscript needs English editing.
Reply 5: The manuscript has been checked by the AME Editing Service as advised.