Author’s response to reviews

Title: Serum Protein Gamma-Glutamyl Hydrolase, Ig Gamma-3 Chain C Region, and Haptoglobin are Associated with the Syndromes of Pulmonary Tuberculosis in Traditional Chinese Medicine

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Author’s response to reviews: see over
Dear Editor:

We have revised the manuscript in accordance with the reviewers’ comments. Here below is our description on revision according to the reviewers’ comments:

**Reviewer: Karen M. Dobos**

**Comment 1:** Is the question posed by the authors well defined? Yes, the question is well defined and TCM and subsequent syndromes are well described.

**Response 1:** The reviewer confirmed our experimental design.

**Comment 2:** Are the methods appropriate and well described? The methods are adequate. However, the sample size and technical replicates are way too small to be useful in this type of study. In addition, detail is lacking to assist the reviewer in determining if the proteomics mass spectrometry analysis conformed to the Minimum information about a Proteomics Experiment (MiAPE), and also lacked detail as to how peptide quantitative differences within a protein compared, how this was handled statistically, and how this intra-data set behaved (deviation, etc). Without this type of analysis one cannot accurately interpret iTRAQ data sets.

**Response 2:** Because a pure TCM syndrome was not easy to be found, we recruited only 71 cases of PYD syndrome, 79 cases of HFYD syndrome, and 64 cases of DQY syndrome in 3 years. And we measured all the samples eligible for ELISA analysis. The study sample provided at least 83.57% power to identify significant differences between TCM syndromes at a statistical support level of $\alpha=0.05$ with an effect size of 0.6 applying a two tails model calculated by Gpower3.0.5. In the present study, we used two technical replicates in the process of iTRAQ-2DLC-MS/MS analysis. Meanwhile, the similar sample size and technical replicates have also been used in other published studies, ‘*Analyst. 2012; 137*(2):490-5’, ‘*Proteomics 2014, 14*:322-331’.

We performed iTRAQ-2DLC-MS/MS analysis according to the steps described in ‘*Proteomics 2014, 14*:322-331’ and ‘*J Proteomics. 2012*, 77:480-91’. For iTRAQ quantitation, the peptide for quantification was automatically selected by Pro Group
algorithm to remove redundant hits and comparative quantification. Furthermore, we added the iTRAQ ratio value, unused ProtScore, and the coverage of peptide with the 95% confidence during the process of MS/MS analysis by two technical repeats to additional file 2 in order to enrich the results of iTRAQ-2DLC-MS/MS analysis.

Comment 3: Are the data sound? This question cannot be accurately addressed based on the lack of information provided (see response to question #2). Regardless, the sample # and technical replicates, and grouping information suggests that the data may not be complete.

Response 3: The sample and technical replicates are answered in response 2. The grouping information of the clinical symptoms and signs of TB cases with PYD, HFYD and DQY syndromes has been added in Additional file 1.

Comment 4: Do the figures appear to be genuine, i.e. without evidence of manipulation? Yes.

Response 4: The reviewer confirmed our figures.

Comment 5: Does the manuscript adhere to the relevant standards for reporting and data deposition? No (see response to question #2).

Response 5: We have added Tukey post-hoc test to find out the differences between PYD, HFYD, and DQY cases. In addition, clinical symptoms have also been added in Additional file 1.

Comment 6: Are the discussion and conclusions well balanced and adequately supported by the data? No—the authors over-interpret the significance of their findings. For example, the authors conclude that “GGH is associated with Mycobacterium proliferation” in their patient samples (and in general?) without any clear physiological and/or biochemical evidence to support such conjecture. In addition, the authors state that there is a bona fide relationship between DTH and the presence of “degenerative lesions” of Mycobacterium; with no evidence (and no
mention of the millions of DTH positive persons in the world with no evidence of lung lesions—with some studies identifying these persons as having LTBI). Another example is the statement correlating hypoxia as infection limiting—this is totally taken out of context, has not been shown, and hypoxia, in any in vivo event, has been associated with the granuloma environment and potentially organisms and an environment that contributes to drug tolerance.

**Response 6:** The discussion and conclusion have been revised according to the suggestions, and over-interpreted sentences are deleted.

1) The conclusion “GGH is associated with Mycobacterium proliferation” was not correct, so we revised it as “GGH may be associated with folic acid intake of Mycobacterium in PYD patients”.

2) In order to avoid ambiguity to readers, the sentence “HFYD cases had highest incidence of degenerative lesions, suggesting strong DTH” and some other sentences are deleted to make the discussion more concise and suitable.

3) In clinical practice DQY cases have obvious symptoms of hypoxia, such as cyanosis. The sentence “hypoxic conditions can inhibit Mycobacterium infection” was not suitable for the context, so we have revised some sentences and the correlating cited references.

**Comment 7:** Are limitations of the work clearly stated? No.

**Response 7:** Limitations of this study have been added in the discussion as ‘In order to amplify the value of our study, a larger number of TB patients with DYY syndrome should be further tested. Further studies with treated patients are needed to confirm the importance of these results as potential biomarkers of PYD, HFYD and DQY syndromes’.

**Comment 8:** Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Not outside of TCM (see response to question #6).

**Response 8:** We carried out this research based on published works of our group, and
these published works have been added in the discussion. The PYD, HFYD and DQY syndromes of TB cases were classified as published work ‘J Ethnopharmacol 2014, 155:1322-31’, and the iTRAQ-2DLC-MS/MS method was based on ‘Proteomics 2014, 14: 322-31’.

**Comment 9:** Do the title and abstract accurately convey what has been found? Yes, but that data is questionable.

**Response 9:** Detail results of iTRAQ-2DLC-MS/MS analysis have been added in Additional file 2.

**Reviewer: Dr. Min Ho Cha**

**Comment 1:** In abstract, TCM divide pulmonary TB as four types according distinctive syndromes. But, only three types were showed in result. Describe in method why DYY syndrome was excluded in the study.

**Response 1:** ‘Only 5 patients with DYY syndrome were recruited in 3 years, which were not enough for further research’. The sentence has been added in the abstract.

**Comment 2:** In method, author divided patients according to the “Standard of disease diagnosis and curative effects of TCM”. But, which symptoms and signs are different among groups was not notified, not ref. and any of table explaining that. Add that in results as sentence or supplemental table.

**Response 2:** ‘Additional file 1 describing clinical symptoms and signs of TB cases with PYD, HFYD and DQY syndromes’ has been added.

**Comment 3:** In results (page 14, line 301), the sentence of “Statistical analysis was ---”, is included in method part. So, author make statistical analysis part in method.

**Response 3:** We have removed the following sentences from the method part and added them in the result part: “The demographic characteristics of the TB patients, treated-TB patients and healthy controls are shown in Table 1. There were no significant differences between the TB patients, treated-TB patients, and healthy
controls.”

**Comment 4:** In table 5, mark statistically different among groups after Post-Hoc test.

**Response 4:** Is figure 5 (there is no table 5), we have added Tukey post-hoc test to find out the differences between PYD, HFYD, and DQY cases.

**Minor comment**

**Comment 1:** Describing method was long. Especially, ELISA kit was purchased from company and ELISA might be performed by protocol provided from company. So, describe the method shorter.

**Response 1:** The description of method about ELISA has been shortened.

**A list of changes:**

1. Page 3, lines 61-62
2. Page 4, lines 72-74, lines 72 deleted, lines 74 deleted
3. Page 9, lines 189 deleted
4. Page 9, line 195
5. Page 13, lines 298-300
6. Page 14, lines 309-313, lines 313 deleted
7. Page 14, lines 316-320
8. Page 16, line 374
9. Page 16, line 388
10. Page 17, lines 407 to page 18, line 417
11. Page 18, lines 428
12. Page 20, lines 469, lines 469 deleted
13. Page 20, lines 474-475, lines 474 deleted, lines 475 deleted
14. Page 21, lines 501 deleted
15. Page 21, lines 504 deleted
16. Page 21, lines 505-507
17. Page 21, lines 509-510, lines 509 deleted, lines 510 deleted
18. Page 21, lines 514-517, lines 517 deleted
19. Page 22, lines 553 deleted, lines 554 deleted, lines 555 deleted
20. Page 22, lines 556-560
21. Page 22, lines 566 deleted
22. Page 22, lines 567-568, lines 567 deleted, lines 568 deleted
23. Added references: 23-24, revised references 25, 37-39
24. Page 33, line 887
25. Added additional files: additional file 1, additional file 2, the previous Additional file 1 was revised as Additional file 3.

Sincerely Yours,

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