Author’s response to reviews

Title: Relationship between Alterations of Urinary Microbiota and Cultured Negative Lower Urinary Tract Symptoms in Female Type 2 Diabetes Patients

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Author’s response to reviews:

1. Textual overlap
   We note that the current submission contains some textual overlap with other previously published works, in particular:
   This overlap mainly exists in the Methods (page 7-8).
   While we understand that you may wish to express some of the same ideas contained in these publications, please be aware that we cannot condone the use of text from previously published work.
   Please re-phrase these sections to minimise overlap.

   Answer: Changes have been made as suggested.
   (Methods section, line 144-172, page 7-8):
   Bioinformatics analysis
   Quantitative Insights Into Microbial Ecology (QIIME, version 1.80) was applied to create an operational taxonomic units (OTUs) table at a default similarity level of 97% based on the 16S rDNA
sequence data [15,16]. Subsequently, chimera detection was performed through the UCHIME method [17]. The representative sequence of each OTU was aligned to Silva and Greengenes database using Ribosomal Database Project Classifier [18].

Five alpha diversity was calculated by mothur software (version 1.31.2) indexes in QIIME including the Observed Species, Chao 1 index, ACE index, Shannon index, and Simpson index. The difference of alpha diversity was evaluated by Mann-Whitney U test in R (version 3.03). Observed Species, Chao1 index, and ACE index represented bacterial richness, while Shannon index and Simpson index were quantitative measures of bacterial diversity that reflecting both species richness and evenness.

To compare microbial composition between groups, principal coordinate analysis (PCoA) was applied on Bray Curtis, weighted UniFrac and unweighted UniFrac distance metrics to generate three-dimensional plots in QIIME. Multiple Response Permutation Procedure (MRPP, a nonparametric test which applied to test beta diversity values between groups) was performed to test the differences of Bray Curtis, weighted UniFrac, unweighted UniFrac distance metrics between groups in QIIME.

Significant bacteria on the relative abundance was tested by Metastats Test. Initial p-value was corrected with the method of Benjamini-Chochberg in R [19]. False discovery rate (FDR) represented the corrected p-value and FDR < 0.05 was considered significant. To identify significantly different bacteria as biomarkers between groups, taxa summaries were reformatted and inputted into Linear discriminant analysis effect size (LEfSe) via the Huttenhower Lab Galaxy Server [20]. Firstly, the Kruskal-Wallis rank sum test and Mann-Whitney U test were applied to identify the significantly different bacteria. Subsequently, linear discriminant analysis (LDA) was used to score the effect size of the different bacteria. Only taxa with logarithmic LDA score greater than 2 were considered significantly enriched.

2. Ethics approval and consent to participate

Please also include a status of consent to participate in this section. Please also specify whether consent to participate was given verbally or in written format. If verbal consent was used please provide a reason why verbal consent to participate was used rather than written consent. If consent was not required, please provide details of why this was waived and the institution/committee who approved this.

Answer: Changes have been made as suggested.

(Declarations section, line 522-524, page 24):
Ethics approval and consent to participate: This study was approved by the Ethical Committee of Southern Medical University; all co-authors consented to participate in the job in written format.

3. Authors’ contributions

We note that two authors have the same initials (YC) and three have the same initials (JZ). To distinguish between them in the Authors’ Contributions section please designate them as YC1 and YC1, with YC1 corresponding to Ying Cao, chosen because this name is furthest up the author list. Please distinguish three authors with the initial JZ in the same manner.

Please also ensure that all authors have read and approve of the final version and add a statement confirming this.

Answer: Changes have been made as suggested.

(Declarations section, line 534-539, page 25):
Authors' contributions: PW (Peng Wu), JC (Jiawei Chen) and JZ1 (Jie Zhao) were involved in study design; JC, YC1 (Ying Cao) and GZ (Guihao Zhang) processed the samples and carried out the molecular techniques; JC, JZ2 (Jialei Zhong) and YC2 (Yang Chen) performed the bioinformatics and taxonomic analysis; PW, JC and JZ1 drafted the manuscript; PW, GZ and WH (Weina Huang) performed the critical revision of the manuscript; JZ3 (Jiarong Zeng) performed the statistical analysis.
4. Role of funding body
The role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.
Answer: Changes have been made as suggested.
(Declarations section, line 529-533, page 25):
Funding: Supported by the Science and the Technology Planning Project of Guangdong Province (grant no. 2014A020212195), the Natural Science Foundation of Guangdong Province (grant no. 2016A030313605) and the National Natural Science Foundation of China (grant No.31401499). The funding body support our job in the design of the study.

5. Duplicate tables
Please remove duplicate tables from the file inventory if these were attached in the manuscript file.
Answer: Changes have been made as suggested.

6. Clean copy
On uploading your revisions, please remove any tracked changes or highlighting and include only a single clean copy of the manuscript.
Answer: Changes have been made as suggested.

7. Please address reviewer#3's comments as below.

Andrea Ticinesi (Reviewer 3): The authors have responded satisfactorily to all my previous comments, and have made a commendable effort for improving the quality of the manuscript. I just advise to re-check the quality of the English language throughout the paper, since some minor elements could be improved.
Answer: Changes have been made as suggested.