Author’s response to reviews

Title: SLC5A2 mutations, including two novel mutations, responsible for renal glucosuria in Chinese families

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Author’s response to reviews:

Dear editors,

Thank you for your letter dated 6th November 2019 and for the reviewer’s and editorial comments concerning our revised manuscript entitled “SLC5A2 mutations including two novel mutations responsible for Chinese renal glucosuria families” (BNEP-D-19-00559R2). We do appreciate greatly for the comments and suggestions by the Reviewers and the Editorial Board. We revised the manuscript and responded to the reviewer’s and editorial comments point-by-point. Although the improvements to the English language within our manuscript have not been requested, we still thought the English writing needs to be revised. Therefore, Nature Research Editing Service (http://bit.ly/NRES_BS) which was recommended, was used for professional help in revising this manuscript.

All changes to the manuscript are indicated in the text by highlighting and using track changes. The revised manuscript which conforms to the journal style, has been read and approved by all of the authors.

With the extensive revision, we think we have addressed all the issues raised by the Reviewers and the Editorial Board. We hope that the revised manuscript could be accepted and published in BMC Nephrology.

Sincerely yours,
Response to Referee 1

Q1. Regarding the nomenclature of variants, the use of the word "mutation" should be avoided.

A1: We do appreciate the valuable comments. We avoided the use of the word "mutation" as much as possible. Furthermore, we revised the English writing by using the expert English language editing service of Nature Research Editing Service for professional help in revising the manuscript.

Q2. According to current human gene nomenclature guidelines, protein (that not confirmed by functional study) nomenclature should use brackets.

A2: Thank you for your nice comments. We used brackets for the amino acids sites in the manuscript.

Q3. There is no mention of which reference has been used in the Methods (page 5 line 5-6).
A3: Thank you for your nice question. The genomic DNA reference sequences of SLC5A2 (NG_012892.1, Gene ID: 6524) and protein reference sequences of SGLT2 (NP_003032.1) were acquired from the Entrez gene and protein database, respectively.

We have added these informations in the Methods section.

Q4. Page 5, line 48-49- "Fifty-five" should be "fifty-five".

A4. Thank you very much for your detailed comment. This mistake was corrected in manuscript.

Q5. Page 5, line 38-42- Spaces in "IVS-16 C&gt;A", "c.1540 C&gt;T" and "c.1152-63 del 12" should be removed, and in the rest of the manuscript. Besides, the description of "c.1152-63 del 12" is not standardized. All your mutation nomenclature should be consistent with the HGVS nomenclature guidelines. (http://varnomen.hgvs.org).

A5: We do appreciate the valuable comments and nice suggestions. Spaces in "IVS-16 C&gt;A", "c.1540 C&gt;T" and "c.1152-63 del 12" had been removed in the manuscript. All of mutation nomenclatures were rechecked in the manuscript, and mutation nomenclatures has been consistent with the HGVS nomenclature guidelines in revised manuscript.

Nine different mutation nomenclatures: IVS1-16C&gt;A, c.305C&gt;T/p.(A102V), c.395G&gt;A/p.(R132H), c.736C&gt;T/p.(P246S), c.886(-10_-31)delGCAAGCAGGTGACTTGAGCCC, c.1152_1163delGGTCATGCTGGC/p.(Val385_Ala388del), c.1222G&gt;T/p.(D408Y), c.1496G&gt;A/p.(R499H) and c.1540C&gt;T/p.(P514S).

Q6. The authors state that the IVS1-16C&gt;A and c.886(-10_-31)del were reported in different ethnic origins in previous studies. the mutation frequency of c.886(-10_-31)del in Chinese population was as high as 32%, [see: Identification of ten novel SLC5A2 mutations and determination of the renal threshold for glucose excretion in Chinese patients with familial renal glucosuria.] but the authors did not find these variants in their previous study, I propose that the authors should rescreen them in their FRG patients of previous studies.

A6: It’s a very nice suggestion. In previous study, the mutation frequency of c.886(-10_-31)del in Chinese population was as high as 32%, but we didn’t find it in our previous studies. Therefore, we rescreened the variant in identified twenty-two Chinese renal glucosuria families in our previous and current studies. Unfortunately, we didn’t find the mutation of c.886(-10_-31)del in others renal glucosuria families. We found the mutation of c.886(-10_-31)del only in two renal glucosuria families in current study.
We have added these informations in the Discussion section.


Response to Referee 2

Q1. Table 1: Under glucose excretion the code "-" means not tested or not present. Please add this information in the legend.

A1: Thank you very much for your careful and nice suggestion. The code "-" means not present in qualitative test.

We added this information in the legend of table 1.

Q2. Are allele frequencies for the observed variants in the Chinese population (like ExAc and gnomAD) known? If so, please add this information.

A2: We do appreciate the valuable comments and nice suggestions. Unfortunately, the allele frequencies for the observed variants in the Chinese population are still unknown, but the allele frequencies for these variants could be found in East Asia in ExAc and gnomAD. So we added this information in table 2.

We have added these informations in the Results section.

Q3. Did the authors of the current manuscript etablish the effect of the observed splice site mutations in the lymphoblastoid cell lines. If yes, please add these data.
A3: Thank you for the nice suggestions. We really established permanent growing lymphoblastoid cell line in patients with FRG in our studies. In the current study, we found two splice site mutations of IVS1-16C&gt;A and c.1152-63del. But the effect of these two splice-site mutations had been verified in previous studies[Lei Yu, Ji-Cheng Lv, Xu-jie Zhou, Li Zhu, Ping Hou, Hong Zhang. Abnormal expression and dysfunction of novel SGLT2 mutations identified in familial renal glucosuria patients. Hum Genet. 2011; 129: 335-44. and Zhao X, Cui L, Lang Y, Liu T, Lu J, Wang C, et al. A recurrent deletion in the SLC5A2 gene including the intron 7 branch site responsible for familial renal glucosuria. Sci Rep. 2016; 6: 33920]. Therefore, we didn’t retest the effect of splice-site mutations in cDNA in current study. However, we still believed the method for establishment of permanent growing lymphoblastoid cell lines in patients with FRG was useful to maintain SLC5A2 gene information and more easily verify the effect of splice-site mutations in cDNA. We are very sorry that we didn't write clearly in the article, so we made modifications in the Discussion section.

We have added these informations in the Discussion section.

Q4. Did you check for the presence of the observed splice site mutations in every family of this manuscript (see table 1)?

A4: Thanks very much for the nice suggestion. We rescreened the observed splice site variants in every patient of the twenty-two Chinese renal glucosuria families which were found in our previous and current studies. Finally, none of the observed splice site variants was newly discovered in these renal glucosuria families.

We have added these informations in the Discussion section.

Q5. Why not develop (novel) specific primers to check for the presence of the observed splice site mutations on genomic DNA?

A5: We do appreciate the valuable comment. We believed specific novel primers should be developed to check for the presence of the observed splice site mutations on genomic DNA in future researches.

We have added these informations in the Discussion section.

Q6. Can you perform mutation prediction effect for the missense mutations with SIFT and Align (or some additional software programs)?
A6. Thanks a lot for the nice comments and suggestions. We performed mutation prediction effect for the missense mutations by SIFT and Align.

Amino acid substitutions were evaluated using the in silico prediction programs SIFT and PolyPhen-2. In addition, a comparative analysis of multiple amino acid sequences of SGLT2 was performed in different species by multiple sequence alignments of DNAMAN Version 6. The aligned reference sequences of Homo sapiens (NP_003032.1), Pan troglodytes (XP_009428973.2), Macaca mulatta (XP_001113206.3), Bos Taurus (NP_976236.1), Rattus norvegicus (NP_072112.2), Mus musculus (NP_573517.1), Danio rerio (NP_998091.1) and Xenopus tropicalis (XP_002940641.2) were used to evaluate the evolutionary conservation.

We have added these informations in the Methods, Results (Table 3 and Fig.2) and Discussion sections.

Q7. Please check the manuscript for typo's.

A7: Thank you for your nice suggestion. We had carefully rechecked the manuscript for typo's. Furthermore, Nature Research Editing Service (http://bit.ly/NRES_BS) was used for professional help in revising this manuscript.