Author’s response to reviews

Title: LncRNA-mRNA-miRNA expression variation profile in the urine of calcium oxalate stone patients

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Author’s response to reviews:

Dear editors,

Thank you for your email on January 27, 2019 that you would consider the revised version of our manuscript entitled “LncRNA-miRNA-mRNA expression variation profile in the urine of calcium oxalate stone patients” (ID: MGNM-D-18-00311). Here, we are very grateful for the opportunity to present you a revised version. We have revised our manuscript thoroughly and answered the reviewers’ comments point-by-point as fully as possible. The corrected parts with track changes are included in the revised manuscript last time and in this time a single clean version of revised manuscript without tracked changes was also uploaded as required. Other
requested “following corrections” were also revised to meet the journal's requirements. It is our hope that the revised manuscript is now satisfactory and suitable for publication. Thank you and all the reviewers for the kind advice.

Yours sincerely,

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Here below is our description on revision according to the comments.

Response to the reviewer’s comments:

Reviewer #1:

Reviewer Comment 1. Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

Author Response 1. Thanks for your advice. We try our best to response to the reviewers’ comments and the revised manuscript and figures will be uploaded to the submission system as required. However, to some extent, we may not understand these comments well and we guess that these comments may be got ready by the editors for the reviewers.

Reviewer #2:

Reviewer comment 1. The study seeks to identify biomarkers for the diagnosis of renal stone by testing urine from calcium oxalate (CaOx) stone patients. Although interesting observations and correlations were identified from the limited number of patients, the study doesn't provide robust biomarkers for renal stone diagnosis.
Author Response 1. Thanks for your constructive comments. We agree that the more profound relation between lncRNA, miRNA and formation of CaOx stones were still need to be further investigated. However, this is the first study to analyze and compare miRNA, mRNA and lncRNA microarray data either from the human urine or from kidneys of animal model (data from our previous study) and to explore the potential biomarkers of the renal stone. The mentioned miRNA, mRNA and lncRNA in the manuscript had the same variation in cell models, animal models and in the urine of patients, which could help to find robust potential biomarkers for early diagnosis of urolithiasis in the future.

Reviewer comment 2. Fig 3: It is unclear whether differentially expressed miRNAs were considered to build ceRNA network or miRanda was used to predict all possible miRNA-mRNA interactions? Further, the resolution of figure 3 is poor and unreadable.

Author Response 2. Thanks for your kindly comments. In order to make the description of this part clearer, the corresponding contents of the manuscript have been revised. CeRNA network was built by the miRNAs, lncRNAs and mRNAs with significantly difference. First, the targets of differentially expressed miRNAs were predicted by miRanda and pearson correlation coefficient. Then the predicted shared pairs of miRNA-mRNA and miRNA-lncRNA were selected for further analysis. Finally, the ceRNA regulatory network could be constructed and the ceRNA score could be calculated.

The resolution of the original image in Figure 3 was already 600 dpi before uploading to the submission system. It is probably that the image in the generated PDF manuscript were compressed after the figure being uploaded to the submission system, thus the low resolution of Figure 3 were caused. We have reproduced figure 3 in high-resolution version and uploaded the original 600 dpi figure.

Reviewer comment 3. Fig 4: Expression of some of the miRNAs, mRNAs and lncRNAs didn't validate in CaOx treated HK-2 cells. Could this be cell line specific and effect of CaOx should be tested in other kidney cell lines? HK-2 cells were treated with 1 mM NaOx for 24 hours. How was this time point selected and is 1 mM clinically relevant concentration? Were the oxalate levels measured in urine of the patients included in this study?

Author Response 3. It is a nice comment to address the above questions. The difference was existed among the urine of renal stone patients, animal model and cell models. Some potential biomarker, such as miR-30d-5p has been showed in all models. However, it is true that some of the miRNAs, mRNAs and lncRNAs were not fully matched in CaOx treated HK-2 cells and we have discussed it in the manuscript.
HK-2 cells were widely used in urolithiasis research, which is a proximal tubular cell line derived from normal human kidney, which has been used to investigate the expression of lncRNA and miRNA correlated to CaOx stone formation in previous studies [1, 2], so we also used HK-2 to verify the microarray result. In this study, we have also tried to use NRK-52E cell, a proximal tubular cell line derived from normal rat kidney, for the validation of differentially expressed miRNAs, mRNAs and lncRNAs. However, the primer of most of differentially expressed miRNAs and lncRNAs on rat species were failed to obtain from miRNA and lncRNA database.

The concentration of calcium oxalate for treating HK-2 was calculated according to the normal laboratory value for urinary oxalate in adults. The physiological concentration of urinary oxalate is approximately 0.75mmol/L, and 1mmol/L oxalate exposure was used to simulate a hyperoxaluria environment for reason [3]. For the time point selection, various previous similar studies investigating the CaOx stone formation have chosen 24 h as the treatment time point [1, 2]. Thus, we also selected 24 h as time point treatment for further validation.

