Author’s response to reviews

Title: Increased circulating bioactive C-type natriuretic peptide is associated with reduced heart rate variability in patients with chronic kidney disease

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Author’s response to reviews:

We sincerely thank the editor and the reviewers for your valuable time and efforts in improving our manuscript. The detailed point-by-point responses to the comments are as follow.

Editor Comments:

1. Since haemoglobin (Hb) and blood pressure (Bp) may affect the HRV, the association between Hb, Bp and HRV should be analysed.

Responses: In fact, we have evaluate the association of Hb, BP and HRV before performing the regression analysis. However, we found only a modest correlation between Hb and lg-TrianIndex (r=0.25, p=0.04). To further account the potential modifying effect of the two variates, we have included Hb and BP as covariates in the multiple regression analysis in the revision (Methods, Paragraph 8).

2. It will improve the quality of the manuscript if normal control samples (non-CKD) can be added into the manuscript.

Responses: We acknowledge in the revision that lack of healthy control is a limitation of the current study (Discussion, Paragraph 6 in the Revision). Before initiating this study, we just
wanted to examine that whether circulating bioactive CNP level increased as GFR decreased and tried to focus on the relationships between CNP and several major related CV parameters. Therefore, we had not recruited any healthy control and are unable to carry out additional recruitment at this time, which makes the comparing impossible in the current analysis.

3. Author should pay an attention to the statistic method and give more information for the multivariate analysis in the revised manuscript.

Responses: We have added detailed information about covariate selection in the methods section (Table 3 and Paragraph 4 in Methods).

Reviewer reports:

Chun Zhang (Reviewer 1):

This study explored the association of circulating CNP with cardiovascular alterations in CKD. They found that there were no associations between plasma CNP level and eGFR. The CNP levels between patients with or without endothelial dysfunction were not significant difference. Plasma CNP was negatively associated with time-domain HRV parameters. But this study still has one problem: 1. The normal control samples or patients without CKD as control samples are needed in the study.

Responses: Thanks for your comments. We acknowledge in the revision that lack of healthy control is a limitation of the current study (Discussion, Paragraph 6 in the Revision). Before initiating this study, we just want to examine that whether circulating CNP level increased as GFR decreased and try to focus on the relationships between CNP and several major related CV parameters. Therefore, we had not recruited any healthy control and are unable to carry out additional recruitment at this time, which makes the comparing impossible in the current analysis. Your advice will have a great influence on our future work.

Jing Hong Zhao (Reviewer 2):

1. This study aimed to explore the association of circulating CNP with cardiovascular alterations in CKD, and found a negative association between plasma CNP and time-domain HRV parameters. Therefore, the authors declare that there has a possible link between circulating CNP and autonomic dysfunction in CKD patients. The results are interesting, but it is not sufficient to define the role of CNP in cardiovascular disease of CKD patients. Major comments: 1. Whether
patients with congenital heart disease or other organic heart diseases are included in the study, because this may affect the results.

Responses: Detailed information about previous CVD history have been added in the revision (Table 1). No patient had congenital heart disease. There were 9 patients with coronary artery disease (including myocardial infarction). To account for the potential confounding effect, we have already included CVD as a covariate in the regression analysis. To further clarify the results, we additionally performed a sensitivity analysis excluding the 9 patients and the associations of CNP with HRV remained essentially unchanged (Results, Paragraph 4).

2. Previous study indicate that plasma CNP level is much higher in renal failure patients than healthy volunteer and correlated with plasma creatinine (Obineche et al. Kidney Int. 2006; 69(1):152-6.), but in this study, plasma CNP level was not increased with the eGFR reduction. The authors should discuss the different results.

Responses: We have added the reference the comment suggested into the revision for discussion. As we discussed in the paper, the major difference leading to the results maybe that Obineche et al. and other groups measured NT-proCNP while we measured the bioactive form of CNP (CNP-22) in the circulation. The possible explanation to this discrepancy may be that bioactive CNP is cleared much more rapid in the circulation compared to NT-proCNP in the circulation (Discussions, Paragraph 4).

3. Anemia and hypertension may have a greater impact on HRV. Thus, hemoglobin and blood pressure should be evaluated as corrective factors or involved in multiple linear regression analyses.

Responses: As the comment suggested, we have included Hb and SBP as covariates in the multiple linear regression analyses (Table 3 and Paragraph 4 in Methods).

4. A lot of study reports that CNP is closely related to left ventricular function, if the authors can add the correlation analysis of CNP and the evaluation index for left ventricular function, it will be better to confirm the key role of circulating CNP in cardiovascular disease of CKD patients.

Responses: Thank you for your valuable and thoughtful comments, but echocardiography were not performed in the current study. We realized that this is a important limitation of the study and stated it in the manuscript (Discussions, Paragraph 6). We will try to check it in our following work.
A Jaroszynski (Reviewer 3):

1. To verify chronicity, and thus to diagnose CKD two creatinine measurements at an interval of at least 3 months should be performed. Have all the measurements performed in studied patients been confirmed in the second test?

Responses: We recruited patients who referred to our hospital for CKD management. The diagnosis was made by the clinical physicians and was conform to the definition proposed by the K/DOQI guideline. At enrollment, study staffs have reviewed their medical docs to confirm the diagnosis. Corresponding explanation have been added in the revision (Methods, Paragraph 1).

2. The multivariate analysis should be described in detail in the methods section. What were the criteria for selecting covariates included in multivariate analysis?

Responses: The covariates were selected because there are sufficient evidences indicating their effect on HRV (Methods, Paragraph 8).

3. 42.1% of the studied patients had a previous history of cardiovascular disease. How the Authors defined the presence or absence of cardiovascular disease?

Responses: The definition of previous CVD have been added (Methods, Paragraph 7).

4. The prevalence of coronary artery disease, myocardial infarction as well as heart failure should be depicted in Table 1.

Responses: Corresponding revision has been made (Table 1).

5. Growing evidence suggests a link between CNP and myocardial infarction, which can be, in turn, accompanied with HRV abnormalities. Owing to this fact, the relation between CNP and HRV may be due to myocardial infarction. The Authors should check it in multivariate analysis.

Responses: There were 9 patients with coronary artery disease (2 of myocardial infarction). We performed a sensitivity analysis excluding these patients and found that the results remained essentially unchanged (Results, Paragraph 4).
6. Though some authors did not observe an association between plasma CNP and renal function, others, however, found such a relation (Clin Chem 2017; 63: 316-324). This issue should be shortly discussed.

Responses: The paper by Prickett et al. mentioned by the comment has been added as a reference for discussion. In their study, they found no correlation of CNP to serum creatinine but a significant association of NT-proCNP with creatinine. This is in line with other studies as well as ours. We measured bioactive CNP (CNP-22) in our study. The possible explanation to this discrepancy may be that bioactive CNP is cleared much more rapid in the circulation compared to NT-proCNP in the circulation (Discussions, Paragraph 4).