

Response to Reviewers #1

- 1. It is well established that symptoms and physiology of cardiac disease can be very different in females versus males. Since the majority of your subjects are males this could skew your results. At the very least mention of possible differences based upon gender should be addressed for future studies.**

Response: Thank you for your advice. In the discussion, we added some comments about the gender difference. The following words were added to line 251: Meanwhile, symptoms and physiology of cardiac disease have obvious differences between females and males, so the possible differences based upon gender should be considered in developing circRNA biomarkers.

- 2. There is a huge variation in the concentration of BNP in your HF population, does this correlate with the NYHA grade, and/or the presence of specific circRNA species?**

Response: Yes, the concentration of BNP in our HF population shows large variations. The concentration of BNP is only regarded as a reference factor to identify the grade of patients with HF. All the patients were strictly divided into four classes (I, II, III and IV) based on the New York Heart Association functional classification (NYHA). We did not analyse the correlation between the concentration of BNP and NYHA grade in our study. However, previous reports demonstrated that the mean of BNP is significantly correlated with NYHA class.¹ Our results showed that the expression of hsa_circ_0062960 correlated with BNP levels (Figure 4).

1. Sokhanvar S, Shekhi M, Mazlomzadeh S, Golmohammadi Z. The Relationship between Serum NT-Pro-BNP Levels and Prognosis in Patients with Systolic Heart Failure. *J Cardiovasc Thorac Res*. 2011;3(2):57-61.

- 3. What would the advantage be of detecting specific circRNAs versus BNP concentrations as markers of HF?**

Response: We believe that future protocol for the diagnosis of a disease should involve a series of biomarkers and not just one. More biomarkers can comprehensively assess various conditions of diseases and achieve a precise result. CircRNAs are stable and abundant in blood. We think that circRNAs can avoid some disadvantages of BNP, which we mentioned in the introduction section.

- 4. While the mean concentrations of selected circRNAs are significantly different between the entire cohort of HF patients and controls there is also significant overlap in the high values and low values in both groups (Figure 2) which would indicate that if used for diagnostic purposes there would be a large number of false positives and false negatives and conclusions about the role of circRNAs in the pathogenesis of HF more difficult.**

Response: Yes. These data are only a preliminary comparison of expression levels between patients and control groups. Our data demonstrated that the mean concentrations of selected circRNAs significantly differed between the entire cohort of patients with HF and controls. The receiver operating characteristic (ROC) curve is a standard graphic tool to characterize a biomarker's classification accuracy. The area under the ROC curve (AUC) has been commonly used to gauge and compare a biomarker's performance. In our data, ROC analysis showed that some circRNAs are potential biomarkers to diagnose HF. However, if these markers are applied to clinical settings, a standard expression level should be studied in a larger group to reduce false positives and false negatives as much as possible.

- 5. In lines 229,230 it is stated that "hsa_circ_0062960 showed a strong correlation with the**

serum BNP". Where is the data?

Response: In Figure 4, the data showed some correlation between the expression of hsa_circ_0062960 and the serum BNP.

6. - Line 94 "differentially circRNA" is not proper English.

Response: In the revised manuscript, 'differentially circRNA' was replaced by 'differential circRNA'.

7. - Line 117. **One normally uses standard deviations OR standard error, not both in data manipulations. Also, what method of reporting (SD or SE) was used for your different data sets was not indicated.**

Response: We used standard error in our data manipulations. We have corrected some errors in the revised worksheets.