PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Cohort profile: the effects of environmental and genetic risk factors for salt sensitivity on blood pressure in northern China: the systemic epidemiology of salt sensitivity (EpiSS) cohort study
AUTHORS	Zhang, Ling; Qi, Han; Liu, Bin; Guo, Chunyue; Liu, Zheng; Cao, Han; Liu, Kuo; Sun, Weiping

VERSION 1 – REVIEW

REVIEWER	Bo Xi Shandong University, China
REVIEW RETURNED	17-Apr-2018

GENERAL COMMENTS	The EpiSS study adopted the systematic epidemiology approach with combination of molecular biology, epidemiology and bioinformatics methods and investigated the effects of environmental and genetic risk factors for salt sensitivity on blood pressure in northern China. This study basically described the baseline details of the cohort, especially the incidence of salt sensitivity in different population of northern China. The manuscript has the potential to become a valuable paper, however currently needs some rephrasing to improve clarity and impact. Suggested revisions are as follows: 1. Quality control is very important for a cohort study. How the quality control was conducted in the EpiSS study and whether a professional personnel was invited to evaluate the process of quality control? 2. Whether the determination method of salt sensitivity of blood pressure in this manuscript is widely used by other researchers? Why the authors choose this method? 3. In Table 1 and 2, it showed the inclusion and exclusion criteria for the study population. Please, explain the meaning of “unrelated individuals” in page 8, line 11? 4. Page 7, line 24/25: The selection of our study sites was based on the direction of the administrative regions. The sentence is not very clear. Please, specify how you have chosen the community health centers and whether the subjects selected could represent the general population. 5. Please explain in details how the sample size was calculated. 6. Are variables in Table 3 in normal distribution? To my knowledge at least, TG is not usually in normal distribution. Please also clarify other variables. 7. For the follow-up outcome, I suggest the authors consider measuring target organ damages such as cIMT, PWV, LVM, eGFR, etc. 8. Please also describe in details how the follow-up outcome will be analyzed.

	9. The proportion of females accounted for 75%, please expalain the reasons.

REVIEWER	Ferruccio Galletti Federico II University of Naples-Italy
REVIEW RETURNED	17-Apr-2018

GENERAL COMMENTS	The study by Han Qi at al. is the presentation of a protocol designed to discover the potential causes of salt sensitivity, including genetic factors. In my opinion, the topic is not novel, as some reviews have already been published in the last few years (the most relevant being The blood pressure-salt sensitivity paradigm: pathophysiologically sound yet of no practical value. Nephrol Dial Transplant 2016; 31:1386-91 - Genetics of salt-sensitive hypertension. Curr Hypertens Rep 2007;9:25-32 - Clock genes and salt-sensitive hypertension: a new type of aldosterone-synthesizing enzyme controlled by the circadian clock and angiotensin II. Hypertens Res 2016;39: 681-687 - Genomics and Pharmacogenomics of Salt-sensitive Hypertension. Curr Hypertens Rev. 2015;11: 49-56 - Mechanisms of Salt-Sensitive Hypertension. Curr Hypertens Rev 2015; 11: 14-21). In addition, the authors have forgotten to discuss and report the many papers published on the methods to assess SS. Lastly, other important points I feel should addressed are the following: - The authors must report the frequency distribution of SS in normotensive and hypertensive subjects - The reproducibility of the test used on a subsample should be reported - Given the lack of urinary creatinine values, the correctness of the urinary collections cannot be verified. - The authors must report the GFR of normotensive and hypertensive subjects My last question is: To suspend the treatment of hypertensive patients was considered possible by the ethics committee?

VERSION 1 – AUTHOR RESPONSE

Reply to Reviewer #1:

Q1: Quality control is very important for a cohort study. How the quality control was conducted in the EpiSS study and whether a professional personnel was invited to evaluate the process of quality control?

Re: We have set up a quality control group and made some relevant regulations to control the quality. To ensure the quality of the whole procedures, a community work manual was distributed to all personnel. Prior to the field survey, we had a unified technical training for all staff members which included the purpose and significance of this study, blood pressure measurement methods, anthropometric measurement methods, survey methods of questionnaire with electronic form, special requirements in collection, storage, transportation of blood and urine samples, etc. Moreover, all

personnel involved in blood pressure measurement must undergo additional training to ensure the accuracy of blood pressure.

During the whole test, subjects were asked to lie in sitting position to ensure that blood pressure was measured in the resting state and avoid any intense activity which could result in fluctuation of BP which would influence the BP change. To ensure equal sodium intake in the process of MSAOSL-DST, participants were allowed to eat one unified custom unsalted-bread. However, smoking, drinking and drugs intake were prohibited until the end of the test.

The questionnaire survey was conducted with electronic questionnaire, which could guarantee the data integrity and logically true. As for the non-electronic original data, which are used in EpiData 3.0 software, double input by independent staff and after double verification the general database is entered. The discrepancy data would be rechecking and correcting according to the original data.

For the follow-up, we could contact the participants through the health records of community centers or the telephone numbers in our databases to reduce the number of lost. There is at least one contact person in each center in charge of the recalling of participants.

The corresponding content has been added in the section of "Quality control"(page 13, line 22-28 and page 14, line 1-7).

Q2: Whether the determination method of salt sensitivity of blood pressure in this manuscript is widely used by other researchers? Why the authors choose this method?

Re: In EpiSS study, we used the modified Sullivan's acute oral saline load and diuresis shrinkage test (MSAOSL-DST) to evaluate the salt sensitivity of blood pressure. This method has the advantages of ease of implementation, short time and high accuracy, which has been widely used by the researchers to determine the salt sensitivity of blood pressure [1-7]. Other determination methods include chronic salt loaded and acute intravenous infusion of 2 L saline over 4-hour period. These methods often suffer of poor patient compliance to dietary instructions, and not suitable for large-scale epidemiological field investigation.

References in which used MSAOSL-DST to evaluate the salt sensitivity of blood pressure:

[1]. Sullivan JM. Salt sensitivity. Definition, conception, methodology, and long-term issues. Hypertension 1991; 17 (Suppl): I61–I68.

Hypertension 1991; 17 (Suppl): I61–I68.

[2]. Mu Jianjun LZ, Dingyi Yang, Xianglin Xu, Jixin Hu, Yuming Li, Wang Zhexun.

Erythrocyte sodium-lithium countertransport and urinary kallikrein excretion in children with hypertension. Chin J Hypertens 1993; 1: 76–79.

[3]. Wang RH, Lin F, Chen SQ, Pu XD. The Effect of Amlodipine in treatment of Hypertensive Patients with Salt Sensitivity. China Prac Med, 2013;8:149-150.

[4]. Long JH, Zhou QH. Influence of sodium chloride to curative effect of captopril in treatment of salt-sensitive hypertension. China Journal of Modern Medicine, 2006;16:1540-1541.

[5]. Huang Y, Yang J, Wang Z. Determination of plasma atrial natriuretic peptide, angiotensin II, aldosterone and efficacy of benazepril in patients with salt sensitive essential hypertension. Chinese Journal of Cardiology, 1999. 27, 372-375.

[6]. Liu Z, Qi H, Liu B, et al. Genetic susceptibility to salt-sensitive hypertension in a Han Chinese population: a validation study of candidate genes. Hypertens Res, 2017, 40 (10):876-884.

[7]. Liu K, Liu Z, Qi H, et al. Genetic Variation in SLC8A1 Gene Involved in Blood Pressure Responses to Acute Salt Loading. Am J Hypertens, 2018, 31 (4):415-421.

Q3. In Table 1 and 2, it showed the inclusion and exclusion criteria for the study population. Please, explain the meaning of “unrelated individuals” in page 8, line 11?

Re: The unrelated individuals mean the independent individuals with no kinship. We had corrected the "unrelated individuals" into "independent individuals" in Table 1 and Table 2.

Q4. Page 7, line 24/25: The selection of our study sites was based on the direction of the administrative regions. The sentence is not very clear. Please, specify how you have chosen the community health centers and whether the subjects selected could represent the general population.

Re: To avoid the influence of different districts and ensure the representative of the population, we choose the community centers stratified by the geographical districts. The community centers in each district that had the ability to undertake the survey and cooperated well would be selected as the study sites. The corresponding content had been added into the manuscript (page 7, line 22-25).

Q5. Please explain in details how the sample size was calculated.

Re: According to the sample size equation for the cohort study, we calculated the sample size with hypertensive patients for exposure group and normotensive patients for control group. The sample size equation for the cohort study as follows:

$$n = \frac{\left(z_{\alpha}\sqrt{2\overline{pq}} + z_{\beta}\sqrt{p_0 q_0 + p_1 q_1}\right)^2}{(p_1 - p_0)^2}$$

Studies have shown that the prevalence of SSBP in hypertensive and normotensive patients is 50% and 42%, respectively [1]. So we defined P_1=0.50 and P_0=0.42. The study required a minimum of

814 subjects for the two groups, with α=0.05 and β=0.10. The total sample size was 1791 and considered a 10% loss to follow-up.

Reference:

[1] Elijovich F, Weinberger MH, Anderson CA, et al. Salt Sensitivity of Blood Pressure: A Scientific Statement From the American Heart Association. Hypertension 2016; 6: e7-e46.

Q6. Are variables in Table 3 in normal distribution? To my knowledge at least, TG is not usually in normal distribution. Please also clarify other variables.

Re: Thanks for your comment. The variables are in abnormal distribution, such as age, waist circumference, hip circumference, BMI, WHR, FBG, TC, TG, LDL-C, HDL-C, SBP, DBP, MAP, sodium concentration, potassium concentration, urine volume, sodium excretion, potassium excretion, estimated salt intake. The analysis was used by Mann-Whitney U test and the footnote was shown in Table 3. We have modified the results from mean±SD to quartile.

Q7. For the follow-up outcome, I suggest the authors consider measuring target organ damages such as cIMT, PWV, LVM, eGFR, etc.

Re: Thanks for your valuable suggestions. We will consider measuring target organ damages such as cIMT, PWV, LVM, eGFR and so on for the follow-up outcome.

Q8. Please also describe in details how the follow-up outcome will be analyzed.

Re: We plan to follow up all participants for two times, every two years by telephone with the help of community health centers. The biannual follow-up will include an interview-based questionnaire survey, laboratory measurements and anthropometric measurements to assess whether there has been any cardiovascular event (for those indicated as hypertensive at baseline) or if there has been the development of hypertension (for those indicated as normotensive at baseline). Cox proportional hazard model and competing risk model will be used to analyze the influence of salt-sensitivity to cardiovascular disease (page 13, line 18-20).

Q9. The proportion of females accounted for 75%, please explain the reasons.

Re: Firstly, there were more retired females in communities than males. Secondly, females were more willing to participant in the free health examination. Lastly, female had more patience to complete the 4-hour of salt sensitive test, so the proportion of females were higher than males. We are planning to recruit more males to participate in the follow up survey.

Reply to Reviewer #2:

Q1. The study by Han Qi at al. is the presentation of a protocol designed to discover the potential causes of salt sensitivity, including genetic factors.

In my opinion, the topic is not novel, as some reviews have already been published in the last few years (the most relevant being The blood pressure-salt sensitivity paradigm: pathophysiologically sound yet of no practical value. Nephrol Dial Transplant 2016; 31:1386-91 - Genetics of salt-sensitive hypertension. Curr Hypertens Rep 2007; 9: 25-32 - Clock genes and salt-sensitive hypertension: a new type of aldosterone-synthesizing enzyme controlled by the circadian clock and angiotensin II. Hypertens Res 2016; 39: 681-687 - Genomics and Pharmacogenomics of Salt-sensitive Hypertension. Curr Hypertens Rev. 2015; 11: 49-56 - Mechanisms of Salt-Sensitive Hypertension. Curr Hypertens Rev 2015; 11: 14-21). In addition, the authors have forgotten to discuss and report the many papers published on the methods to assess SS.

Re: Thank you for your comment. We have once read these five literatures carefully, which are very contributable to salt sensitivity. Similar articles also include the AHA statement of Salt Sensitivity of Blood Pressure (SSBP) that published in hypertension [1], and "Incidence of hypertension in individuals with different blood pressure salt-sensitivity: results of a 15-year follow-up study"[2]. SSBP has received much attention since the positive association between blood pressure and salt was proved by the famous Intersalt study. It has been confirmed that SSBP was affected by genetic factors. However, the mechanism of SSBP is still unclear. In addition, the assessment method of SSBP lack a uniform standard. Thus, a prospect cohort study is important to uncover the risk factors of SSBP, reduce the incidence of cardiovascular disease of salt-sensitive ones and give rational medical recommendations for salt-sensitive hypertensive patients. Our research team have performed a network meta-analysis and found that patients with salt-sensitive hypertension can be effectively controlled their blood pressure by choosing calcium channel blocker [3].

To our knowledge, EpiSS is the largest salt sensitivity prospective epidemiology study in China that has used the modified Sullivan's acute oral saline load and diuresis shrinkage test (MSAOSL-DST). The acute saline loaded is easier to conduct in community centers and the short-term changes of blood pressure could be observed to understand the acute response of blood pressure to salt loaded. Besides, we aimed to discover the novel biomarkers of SSBP from the levels of genome and transcriptome to replace the traditional determination method of SSBP. As many researches believed that SSBP should be regarded as a continuous variable, the correlation between SSBP and other risk factors is also a major concern of EpiSS.

We had added the methods to assess SS in "Introduction" (page 6, line 2-5).

[1]. Elijovich F, Weinberger MH, Anderson CA, et al. Salt Sensitivity of Blood Pressure: A Scientific Statement From the American Heart Association. Hypertension, 2016, 68 (3):e7-e46.

[2]. Barba G, Galletti F, Cappuccio FP, et al. Incidence of hypertension in individuals with different blood pressure salt-sensitivity: results of a 15-year follow-up study. J Hypertens, 2007, 25 (7):1465-1471.

[3]. **Qi H**, Liu Z, Cao H, Sun WP, Peng WJ, Liu B, Dong SJ, Xiang YT, ***Zhang L****. Comparative efficacy of antihypertensive agents in salt-sensitive hypertensive patients: a network meta-analysis. Am J Hypertens, 2018 Jun 11;31(7):835-846. doi: 10.1093/ajh/hpy027

Q2. The authors must report the frequency distribution of SS in normotensive and hypertensive subjects.

Re: We had added the frequency of SS in normotensive and hypertensive subjects in Table 3.

Q3. The reproducibility of the test used on a subsample should be reported.

Re: We had done the secondary MSAOSL-DST for a subsample in the first follow-up. Two of 27 participants had different results of SSBP compared to the previous test. So the reproducibility of MSAOSL-DST is 92.6%.

Q4. Given the lack of urinary creatinine values, the correctness of the urinary collections cannot be verified.

Re: We had collected the time-point urine and 24-hour urine samples during the MSAOSL-DST and kept the urine samples in the -80℃, we would like to test the urinary creatinine in all samples and also on the subsequent follow-up.

Q5. The authors must report the GFR of normotensive and hypertensive subjects.

Re: Because it is difficult to test the GFR in community centers, we have not tested it in EpiSS study. We would like to test the GFR on the subsequent follow-up.

Q6. My last question is: To suspend the treatment of hypertensive patients was considered possible by the ethics committee?

Re: The hypertensive patients that included in EpiSS study were new-onset hypertensives or grade 1 hypertensives. The recommendation for them is the combination of antihypertensives drugs and the change of lifestyle habits. To avoid the effects of antihypertensives drugs to the blood pressure, we have to ask the patients for stopping the intake of antihypertensives drugs at least 24 hours considering

half-life of antihypertensive drugs, which followed voluntary principle. We recorded the situation of drug use and discontinuation on the day of the survey, and excluded those who took the medicine (page 8, line 2-6).

The whole process of EpiSS study was supervised by professional doctors. If there were any side effects happened during the suspend phase, the patients would get timely treatments from the doctors and withdraw the cohort. Besides, EpiSS obtained the approval of the Capital Medical University ethics committee (number 2013SY22) and all subjects signed written informed consent to participate in our study.

FORMATTING AMENDMENTS

-Authors must include a statement in the Methods section of the manuscript under the sub-heading 'Patient and Public Involvement'.

This should provide a brief response to the following questions:

-How was the development of the research question and outcome measures informed by patients' priorities, experience, and preferences?

-How did you involve patients in the design of this study?

-Were patients involved in the recruitment to and conduct of the study?

-How will the results be disseminated to study participants?

-For randomized controlled trials, was the burden of the intervention assessed by patients themselves?

-Patient advisers should also be thanked in the contributorship statement/acknowledgements.

Re: We had added a sub-heading "Patient and Public Involvement" in the Methods (page 14, line 8-15) section that answering the above questions.

VERSION 2 – REVIEW

REVIEWER	Bo Xi Shandong University
REVIEW RETURNED	14-Jul-2018

GENERAL COMMENTS	The authors have addressed my all questions.

REVIEWER	Prof. Ferruccio Galletti Dpt. of Clinical Medicine & Surgery Federico II University of Naples, Italy
REVIEW RETURNED	18-Jul-2018

GENERAL COMMENTS	The paper increased in this last version. However the authors did not reply to 2 important questions which could invalidate their conclusions: the authors stated that they prefer to test the parameters required in the next follow-up. Even if these parameters are important in the present paper. Q4. Given the lack of urinary creatinine values, the correctness of the urinary collections cannot be verified. Re: We had collected the time-point urine and 24-hour urine samples during the MSAOSL-DST and kept the urine samples in the -80℃, we would like to test the urinary creatinine in all samples and also on the subsequent follow-up. Q5. The authors must report the GFR of normotensive and hypertensive subjects. Re: Because it is difficult to test the GFR in community centers, we have not tested it in EpiSS study. We would like to test the GFR on the subsequent follow-up.

VERSION 2 – AUTHOR RESPONSE

Reply to Reviewer #1:

The authors have addressed my all questions.

Re: Thank you.

Reply to Reviewer #2:

The paper increased in this last version. However, the authors did not reply to 2 important questions which could invalidate their conclusions:

-the authors stated that they prefer to test the parameters required in the next follow-up. Even if these parameters are important in the present paper.

Q4. Given the lack of urinary creatinine values, the correctness of the urinary collections cannot be verified.

Q5. The authors must report the GFR of normotensive and hypertensive subjects.

RE: We completely agree with this comment. Thanks very much for your advice. It's really a limitation of our study, and we added and stated it in the manuscript (page 4, line 16-18). Since this is a protocol of a cohort study, due to logistical reasons and insufficient funds, these important parameters

were not examined in baseline survey. We will test these indicators using urine samples from the biobank, and also in the follow-up survey according to the advice of review (page 12, line 25-27).

The concentration of urinary Na+ and K+ would be affected by renal function. This study has excluded patients with kidney disease and other complications according to their history of diseases (exclusive criteria are in Table 1 and Table 2). We plan to test urinary creatinine and report GFR in the following process to monitor changes in renal function.