Peer Review File

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Reviewer A

Comment 1: The prior studies examining kidney stone disease and cardiovascular risk reported differences in association by sex. See references 1 and 2 in manuscript. The authors should discuss this in the intro/discussion, and discuss how the study findings add and/or address this issue.

Reply 1: Thank you for Reviewer A's advice. In previous studies, KSD and cardiovascular diseases commonly coexist, and both age and gender were reported as risk factors of both of them. However, there is little evidence about the causality between KSD and these systemic diseases. So our aim in the recent study was to confirm the causality of these two clinical diseases through MR analysis. Furthermore, the MRBASE database didn't exhibit personal information such as age and gender. We agreed with Reviewer A, and we thought that the sentence "Kidney stone disease (KSD) and cardiovascular diseases are two common conditions, both of which show different prevalence rate in different age and gender." maybe pointed to a confusing direction. As suggested, we deleted the sentence in the revised manuscript.
Changes in the text: Please see page 4, line 68-70.

Comment 2: The authors in the Discussion should elaborate on how the study findings impact patient care.

Reply 2: Thank you for your advice. As suggested, we added elaborate on how the study findings impact patient care in the Discussion Section in our revised manuscript.
Changes in the text: Please see page 11, line 212-216: "*Based on these, we thought our findings were useful for patient care. We recommend that if patients with KSD, regular image logical examinations were suggested to monitor the probable risk of coronary atherosclerosis and cardiomyopathy. Meanwhile, for patients with hypertension, KSD was also need to be noticed in patient care.*"

Comment 3: Line 91 Methods - has Chinese text that translates to confusing text

Reply: Thanks a lot. In the revised manuscript, we deleted the text.

Changes in the text: Please see page 6, line 103.

Comment 4: Some minor grammatical errors need proofreading throughout text

Reply: Thank you for your advice. As suggested, we have tried our best to modify the minor grammatical errors in our revised manuscript.
Changes in the text: Minor grammatical errors were modified in the full text.

Reviewer B

The authors present an investigation of the relationship between kidney stones and cardiovascular disease using mendelian randomization to determine causality and whether a bidirectional relationship exists. The authors identified two independent populations, one with KSD, and another with CVD, and they determined that KSD is a causal factor in atherosclerosis and cardiomyopathy, while hypertension is a causal factor in KSD.

Comment 1: Intro
1. The authors appropriately justify the need for the study and identify that a causal relationship between KSD and CVD requires further investigation.
2. The authors appropriately justify the use of MR to investigate a bidirectional relationship.

Reply 1: We thank Reviewer B for the positive response.

Comment 2: Methods
3. Can the authors explain in further detail how the SNPs and genetic variants were identified? Were these based on the literature or are the genotypes and phenotypes labeled within MRBASE?

Reply 2: We thank Reviewer B for the valuable advice. As suggested, we add the details of instrumental SNPs selection in the Method Section.
Changes in the text: Please see page 6-7, line 116-123: "*All the SNPs used us instrumental variants were from GWAS studies above. For SNPs selection, first of all, genetic variants associated with exposure factors at genome-wide significance (P<5 x 10-5) and not in linkage disequilibrium (R2<0.01) were selected as instrumental SNPs. Furthermore, SNPs≥0.05 was defined as minor allele frequency. SNPs with MAF threshold was 0.01. Second, through sensitivity analysis, the nonspecific SNPs were removed. Third, through checking the allele and frequency information of SNPs in both the exposure and outcome groups, we removed the SNPs with inconsistent information.*"

Comment 3: Results/Tables/Figures

4. The authors appropriately include all results relevant to the paper's aims and all figures/tables are clear.

Reply 3: We thank Reviewer B for the positive response.

Comment 4: Discussion

5. Can the authors elaborate further on why they believe one group had a significant association with cardiomyopathy but the other did not? What are the known differences between the two groups analyzed?

Reply 4: Thank you for your advice. The results showed that KSD increased the risk of cardiomyopathy when we used ukb-b-13537 as exposure group. However, when we used ukb-b-8297 as exposure group, KSD didn't increased the risk of cardiomyopathy. We thought the source of samples in the two groups (ukb-b-13537 and ukb-b-8297) were different which cause to the different results. As suggested, we also added appropriate explain in the Discussion Section in our revised manuscript.

Changes in the text: Please see page 12-13, line 244-247: "*Of course, we need to pay attention to that we only observed the significance between KSD and cardiomyopathy when we used ukb-b-13537 as an exposure group. We thought the source of samples in the two groups (ukb-b-13537 and ukb-b-8297) were different which cause to the different results.*"

Comment 5: 6. The authors state that hypertension is a cause of KSD based on an OR of 1.001. Please justify whether the observed statistical significance is also clinically significant. If it is not clinically significant, this should be commented upon.

Reply 5: Thank you for your advice. In our study, the results showed that hypertension could increase the risk of KSD by using IVW method (Figure 3C, OR: 1.001; SE=±1.00, *P*=0.003) when we used ukb-b-8297 as exposure group. However, when we used ukb-b-13537 as exposure group, hypertension didn't increased the risk of KSD (Figure 3D, P>0.05). So we thought the observed statistical and clinically significance was not remarkably. As suggested, we added appropriate explain in the Discussion Section in our revised manuscript.

Changes in the text: Please see page 12, line 234-240: "*However, it was worth mentioning that in our recent study, we only observed that hypertension could increase the risk of KSD when we used ukb-b-8297 as an exposure group based on an OR of 1.001. When we used ukb-*

b-13537 as an exposure group, hypertension didn't increase the risk of KSD (P >0.05). Based on these results, we thought that the observed statistical and clinical significance was not remarkable. More independent analysis need to be performed to validate the findings in future studies."

Comment 6: 7. The authors provide appropriate references from the literature with proposed pathophysiological mechanisms for the associations they detected.

Reply: We thank Reviewer B for the positive response.

Reviewer C

Comment 1: Enjoyed reading the manuscript. However, authors have to clarify two points. Kidney stone disease encompass a variety of stone types with different etiologies. Uric acid stone formers are different from calcium oxalate and or calcium phosphate stone formers. All type of stones may not be associated with hypertension. Lumping all stone types in one group can lead to wrong interpretations. Authors must discuss this. I do not think that location of stone, kidney or ureter matters that much but type of stone, calcium oxalate or calcium phosphate or uric acid probably does.
Authors discuss the role of hypercalciuria as well as hyperoxaluria. I would like them to discuss how do these conditions may lead to hypertension or the other way around.

Reply 1: Thanks a lot for Reviewer C's advice. We very agreed with Reviewer C's viewpoint. In the manuscript, we used data from public database to perform the two-sample MR. For KSD, we selected the genetics variants in one European population from UK Biobank (version 2, n=462,933). We checked the population information carefully, and unfortunately we found that these KSD patients were only with a diagnosis of kidney stone/ureter stone/bladder stone without the specific type of stone. Therefore, we discussed the question in the discussion as our limitation.
Changes in the text: Please see page 13, line 260-265: "*Third, for KSD, we checked the population information carefully, and unfortunately, we found that these KSD patients were only with a diagnosis of kidney stone/ureter stone/bladder stone without the specific type of stone. Therefore, we could not confirm the specific type of KSD which could increase the risk of coronary atherosclerosis and cardiomyopathy. Based on these, we will collect another independent population to validate the findings in the future studies.*"