Author's response to reviews

Title: Uromodulin Concentrations are not Associated with Incident CKD Among Persons with Coronary Artery Disease

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Author's response to reviews: see over
Dear Dr. Norton,

Thank you for the opportunity to revise our manuscript, and for forwarding the helpful comments from the reviewers. We have responded carefully to the reviewer’s concerns, and we believe that the paper has been improved as a result.

Below, I will respond to the individual comments of the reviewers. I hope this revision is pleasing to the editors, but we would be happy to address future concerns as well.

With best regards,

Michael G. Shlipak, MD, MPH
REVIEWER #1 COMMENTS:
The manuscript deals with an important question, namely if uromodulin genotypes and urine levels are associated with incident and progressive CKD. The authors confirm previous results of genotype distribution and association with uromodulin excretion. In contrast to a previous study they did not find an association of high urinary uromodulin excretion with incidence and progression of CKD. Substudies 1 and 2 of the manuscript are well performed and described. I do, however, have some concerns about study 3, especially selection of cases.

Thank you for your remarks. We have addressed your concerns below in a point by point response.

Major Compulsory revisions
1. One major point of concern is the selection of incident CKD cases based on CrCl measurements. According to Table 3 cases lost on average 40 ml/min of CrCl within 5 years. This is quite unusual for individuals starting with a normal CrCl and without albuminuria. The cases seemed to have a rather aggressive form of renal disease. What were the clinical diagnoses in these cases?

Thank you for your interesting comment. We do not have information on the clinical diagnoses of CKD in this study. Most of the participants would not have been aware of their CKD, as it was only detected at the 5-year follow-up visit. Since the entire cohort had coronary artery disease and many had heart failure, we hypothesized that most of the CKD is due to vascular disease and hypertension.

2. In addition, a reduction of CrCl of almost 50% would cause a doubling of serum creatinine. Was this really observed in the cases?

This is a very good question. We selected cases to be “extreme progressors” and they represented the decile with the fastest rate of kidney decline as defined by CrCl. Because we used creatinine clearance as the criteria for selection, these differences are naturally larger than differences observed in creatinine or eGFRcr. Nevertheless, the cases had greater progression rates by creatinine and eGFR as well (see data below).

We have added the data in the table below to Table 3 in the manuscript, which shows the change in serum creatinine (0.36 vs. 0.10) and eGFRcr (19 vs. 10) in cases compared with controls. We have also added the following statement in the Results section that explains this:

“Cases also had a more than 3-fold increase in serum creatinine and a near doubling in eGFR decline compared with controls.” (pg. 11, paragraph 2)

We do not have follow-up cystatin C measures.

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<td>Pre</td>
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<tr>
<td>Serum creatinine</td>
<td>1.08</td>
<td>1.44</td>
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<tr>
<td>eGFRcr</td>
<td>74.16</td>
<td>55.34</td>
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I would feel much more confident if the case selection had been based on eGFR calculated from serum creatinine (for example with the CKD-EPI formula) or from cystatin C levels. Although the efforts of the authors to get accurate 24 hour urine collections are acknowledged, this method seems much more vulnerable to collection errors.

We agree with you that this method is potentially open to collection errors; however, our methods for urine collection in the Heart and Soul Study are as rigorous as possible. We have added a statement in the Discussion section (page 14, paragraph 1) that reflects this limitation:

“A third limitation could be errors in the urine collections leading to biased estimates of CrCl; however, cases had much larger changes in creatinine than controls, as well.”
3. There are many factors causing CKD progression. Some of these factors such as BMI or diabetes were included in the calculations. Some other important factors should be available in the study. I suggest to include the following in study 3: blood pressure at baseline; smoking status; blockers of the renin angiotensin aldosterone system; statins. A difference in these factors between cases and controls could also explain disease progression. Thank you for your suggestion. We agree with you that it is a good idea to add these important factors to the study. We have added them to Table 3, but have not included them in our multivariate model because there were no observed differences in uromodulin concentrations in the univariate or demographic adjusted analyses.

Discretionary revision
One interesting question that could be answered by the data of the study is whether the proportion of progressors differed between the G/G, G/T and T/T groups. I acknowledge that the statistical power may be limited by the small number of T/T patients. This is indeed a very interesting question. We initially did not include this in the paper because the study was under-powered to find an effect due to the very low frequency of the T/T genotype. However, it is interesting to note that the protective T/T genotype was more common in controls than in cases (5% vs. 2%), albeit non-significant. We have added this finding to Table 3 in the paper. In addition, we have added a statement in the Results section (page 12, paragraph 1):

“Finally, we compared the UMOD genotypes among cases and controls. There was no significant difference in the distribution (p-value=0.33), but the protective T/T genotype appeared less frequent in cases than controls (2% vs. 5%).”

REVIEWER #2 COMMENTS:
In their manuscript Shlipak and colleagues investigated on the association of the level of urinary uromodulin with incident CKD among patients with CAD. In their study the authors confirmed the association of the protective allele of UMOD SNP rs12917707 with better kidney function and decreased uromodulin urinary levels. However, no significant differences in the uromodulin concentrations were observed between case/control groups. These data suggest that uromodulin levels are not associated with CKD among CAD patients and that other factors more strictly related to CAD, e.g. atherosclerotic damage, could play a role. As the authors underlined in the Discussion section, the strength of the study lies on the unique attributes of the patient cohort and the fact that this is the second study to date assessing the role of urinary uromodulin concentration in the prediction of CKD. On the other hand, the main weakness of the study is the small sample size. Thank you for your comments.

Major Compulsory Revision:
- Although studies #1 and #2 are clear, the results of study #3 are somewhat difficult to understand. The baseline CrCl is different between cases and controls, even though not statistically significant. As CrCl is different among all participants according to their genotype (Table 1), one wonders if the difference in the case/control subsets is contributed by the UMOD polymorphism. What are the genotype frequencies in the case/control sets? This is an interesting question and point. We have added the genotype frequencies in Table 3. Also, please see our response to Reviewer 1’s comment above for a further explanation.

Minor Essential Revisions:
- the number of individuals in the case/control study is not the same at page 2, 11 (99 cases and 92 controls) and at page 7 and in Table 3 (102 cases and 94 controls) - Some of the references at page 16 look incomplete and should be corrected, e.g. #1 and #4.
You are correct; there is a discrepancy in cases and controls between page 2 and Table 3. This was a mistake on our part and we have corrected the numbers on page 2 as those were incorrect. Thank you again for finding this error.

Thank you for also pointing out the incomplete references. We have updated #1, but #4 is complete as listed. We have also updated other references that were incomplete.