Author’s response to reviews

Title: Plasma metabolites and lipids associate with kidney function and kidney volume in hypertensive ADPKD patients early in the disease course

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Editor Comments:

Reviewer #1: This study by Kim et al. examines HALT-PKD Study A participants' baseline plasma samples for the potential predictive role of metabolites and lipids in a non-targeted platform to predict the measured eGFR and ht-TKV with the hope that certain identified species will correlate with these parameters and potentially inform on disease progression and prognosis. They find that a limited set of 12 metabolites significantly correlated with eGFR and 2 triglycerides significantly correlated with baseline ht-TKV at FDR q-values <0.05. As ht-TKV has been reported to correlate with disease severity and potential for disease progression, the authors propose that metabolites or lipids that correlate with ht-TKV and eGFR may be predictive of progression, although this is not tested in this study. Another limitation is that only Caucasian patients are used, which may limit the generalizability of the study to all patients and should be mentioned in the Discussion. Nevertheless, the study provides a useful foundation or baseline for potential future work. I have a number of additional specific comments and queries for the authors to address to strengthen the study.

1. In addition to plasma metabolic changes, urinary biomarkers may also be useful and potentially more specific to study, and this point should be mentioned by the authors in the Discussion.
Response: While we did not analyze HALT urine by non-targeted metabolomics, we agree with the reviewer that urine is a potentially high yield biofluid and have added this to the discussion with some of the references on the topic from our laboratory.

2. It would be useful for the authors to make the point that the identification of new blood or urine biomarkers would be potentially less costly and more convenient than ht-TKV.

Response: We appreciate this reviewer pointing out this fact, and we have added it to the Discussion.

3. p. 7, line 31: Should say "data".

Response: We thank the reviewer for pointing this out and it has been corrected.

4. p. 7, line 60: It says that the concentration of each internal standard is found in Table S1, but that information does not appear to be there.

Response: This was an error (a sentence from an earlier manuscript), and it has been removed. We apologize for the confusion.

5. p. 8, lines 49-53: The authors mentioned that there was one sample that was markedly dispersed from the rest of the samples and removed. More detail about the criteria used to judge and remove outlier(s) would be beneficial to include.

Response: We apologize for this lack of clarity. In the resubmission, we have now elaborated on the criteria used to remove outliers.
6. Table 1: For urine creatinine, potassium and sodium levels, the units given are in mEq/L/day, but it should likely be mEq/L if it is based on a spot collection or mEq/day if it is a 24-h collection. Please address.

Response: We thank this astute reviewer for catching this error and it has now been corrected to mEq/day.

7. p. 12, lines 44-47: Would revise to say "association was generally reversed by sex...". As the authors note later in the paragraph, creatinine exhibited negative associations for both males and females (Fig. 3 and Table S1).

Response: We agree with this reviewer that the statement in question was inaccurate and we have now corrected it.

8. All figure labels (writing) need to be made larger and more legible. The Figure 2 heat map is particularly difficult to read, thereby limiting its usefulness.

Response: Figure 2 has been improved per the reviewer’s suggestions.

9. p. 14, line 13: The authors should refer to Supplemental Table S3 rather than "data not shown", as the data are present in Table S3.

Response: We apologize for the oversight and agree with this reviewer that the Table should have been cited with that sentence. We have made this correction.

10. Much of the data shown is not significant when corrected by Storey's false discovery rate (FDR). The authors should consider mainly or only focusing on those with significant q values. Discussing the data with "significant" p values but insignificant q values may be misleading because there is really no reason to believe that these values have any significance.
Response: We thank the reviewer pointing out this critical issue of multiple testing. Compared to p-values, q-values give more accurate indication of the level of false positives for a given cut-off value of 0.05. However, when doing many tests as in this metabolomics experiment, it is more intuitive to interpret p- and q-values together by looking at the entire range of values rather than looking at each one independently and only those significant at a q-value of 0.05. This being a discovery study, we decided in the interest of full disclosure to present all results significant at a p- and q-value of 0.05; we reasoned that these data will be useful as a reference for investigators who wish to use our data in future studies.

11. Re. the unknown metabolite (191801), given the very highly significant p and q values, the authors should work it up further (as they probably plan to). One issue with these studies is that the data are biased toward the most abundant metabolites, which may not have the best significance. Identifying lower abundance metabolites that are highly discriminative would be impactful and potentially very useful clinically.

Response: We agree with the reviewer that the identity of that unidentified metabolite 191801 would have been extremely useful for this study, however it was not identified in this study due to insufficient quantity and funds to carry out this operation. However, we now provide more a website for more detailed analytical and species information on this metabolite. From that website, it can be seen that the species in which it is most highly expressed in are: 1 wine, 2 aspergillus, 3 microalgae broth, 4 bee intestines, 5 yeast, 6 yeast, 7 beetle, 8 plant flowers/leaves

Reviewer #2: The study by Kim et al reports a non-targeted GC-TOF/MS-based metabolomics approach to investigate the predictive role of plasma metabolites on hypertensive ADPKD patients. The authors identified 12 metabolites that were significantly associated with eGFR and 2 triglycerides that were significantly associated with ht-TKV. None of the metabolites and triglycerides showed significant effect modification by sex or genotype. Interestingly, these metabolites were altered in individuals with preserved renal function, suggesting that early metabolic derangements occur before significant loss of renal function and might prove helpful for the development of diagnostic and/or prognostic tools.
Although further investigation is needed to identify metabolites that can be used as prognostic indicators of slow and rapid disease progression, these data indicate that analysis of plasma metabolites in ADPKD can reveal predictors of early kidney dysfunction.

The paper is well written and the conclusions are well supported by the analysis. Some minor concerns need to be addressed.

1. The background section is missing references to the disease genetics, progression and environmental factors that can affect it.

Response: We have rewritten these sections of the introduction. The altered text is underlined in the revised manuscript.

2. Altered metabolism has emerged as a key feature in ADPKD and many papers have been published in recent years reporting such alterations. The discussion would benefit from more extensive comparison to other metabolomics data already published.

Response: We have added two paragraphs to the discussion about other metabolic disorders that have been found in ADPKD and including 3 additional references to studies that have made use of metabolomics in ADPKD (not many exist; e.g. Rowe et al 2013, Menezes et al 2012 and Menezes et al 2016).