**Author's response to reviews**

**Title:** Genetic Polymorphisms of Angiotensin-2 Type 1 Receptor and Angiotensinogen and Risk of Renal Dysfunction and Coronary Heart Disease in Type 2 Diabetes Mellitus

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**Author's response to reviews:** see over
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To BMC Nephrology:

We would like to thank the editors and three reviewers for their comments and suggestions about our manuscript entitled “Genetic Polymorphisms of Angiotensin-2 Type 1 Receptor and Angiotensinogen and Risk of Renal Dysfunction and Coronary Heart Disease in Type 2 Diabetes Mellitus” including the recognition of the importance and interest of the findings.

We have now included the abstract in the main body of the manuscript as well as inserted a “Competing Interests” and “Authors’ Contribution” sections as requested by the editorial office.

Point-by-point responses to the reviewers’ queries and suggestions are given below. Changes in the manuscript are given in tracked red underlined font in the revised version submitted.

REVIEWER: Fatini (Minor essential revision)

1. Several inaccuracies concerning both the English syntax and genetic terms are present throughout the text; the AGT1R may be replaced as AGTR1, in the aim of the study the AGT A(-6)G polymorphism has been omitted.

Although either abbreviation is acceptable, the term “AGT1R” appears to be more commonly used to refer to the angiotensin II type 1 receptor. A PubMed search for the term “AGT1R” yielded 551 articles whereas the term “AGTR1” yielded 121 articles. We would like to keep the terminology throughout our manuscript unless the editors feel strongly that we should replace it with “AGTR1” as suggested by the reviewer.

We did assay for the AGT A(-6)G SNP as stated in the Methods at the bottom of page 5, but because results for AGT A(-6)G were essentially identical to those for AGT M235T (because they were identified to be in complete linkage disequilibrium after the initiation of our
study), we state that we chose to present results for AGT M235T only on page 7 in the first sentence of the second paragraph of the Results section.

We are uncertain about what is meant by the “inaccuracies concerning both the English syntax and genetic terms” generally referred to by the reviewer and would be very happy to work with the editorial office in resolving any of these issues.

2. In the Discussion Authors state to examine an additive genetic model, nevertheless no results are present in the results section, please clarify. Page 10, line 21 the “CC variant must be replaced as CC genotype”.

We stated in the Results section on page 8 that “For the outcome of eGFR < 60 ml/min/1.73 m² … No significant associations were seen in recessive or additive models in the combined dataset.” and “For the outcome of CHD….No recessive models were significant for men, and all additive models were null for both groups.” In addition in the Results section on page 9, we stated that “In the combined data set, no significant associations were observed in recessive or additive models for CHD.” Because all the additive model results were null, we chose to summarize these results rather than present actual odds ratios for each of the several analyses.

We have replaced the term “CC variant” with “CC genotype” (now on page 11) as recommended by the reviewer.

3. Table 1. Due to the relationship between AGTR1 gene and angiotensin II AT1 receptor antagonists, Authors should provide information concerning this therapy in the clinical characteristics of patients.

We agree that this is a potentially important issue, however, as these are cross-sectional analyses of HPFS men in 1994 and NHS in 1990, angiotensin receptor blocker (ARB) medications were not widely used at that time; losartan was first approved for clinical use in 1995, for example. In fact, even ACE-inhibitor medication use was reported by these participants with type 2 diabetes at relatively low rates (8.2% and 9.7% for HPFS and NHS respectively) as the captopril randomized trial by Lewis et al. in type 1 diabetes showing renal benefit was published in the New England Journal of Medicine in 1993 and ACE-inhibitor therapy in diabetes was not yet established in the management of nephropathy in diabetes. Therefore, ARB medication use was considered to be 0% for both cohorts at the times of the blood collections used for plasma creatinine assessments.

4. Table 2. should be improved

We have added a footnote to the table to clarify definitions of renal dysfunction and CHD used for these analyses.
1. The authors need to give detailed inclusion criteria for CKD and CHD as this is the pertinent group(s).

As stated on page 6 of the Methods, “Coronary heart disease was defined as history of confirmed myocardial infarction, or self-reported coronary revascularization or angina. These self-reported outcomes have been previously validated by medical chart review 23.” and “Moderate renal dysfunction was considered to be GFR < 60 ml/min/1.73 m$^2$” based on the 4-variable MDRD equation. We have added this information to the bottom of Table 2 to provide additional clarity of our definitions for CKD and CHD.

2. How many patients had both CKD and CHD? What was the genotypic association in this group?

Only 38 (5%) participants in HPFS and 42 (5%) participants in NHS had both eGFR<60 ml/min/1.73m$^2$ and CHD; we did not examine for associations with our candidate SNPs for those with both because the low power would not likely provide meaningful informative results. We did add the following sentence to the first paragraph of the RESULTS section on page 8 to address this question, however:

“Only 38 (5%) participants in HPFS and 42 (5%) participants in NHS had both eGFR<60 ml/min/1.73m$^2$ and CHD.”

3. Not much detail has been provided about the phenotyping of patients in terms of Kidney function assessment. Urinary Albumin excretion is an important parameter which needs to be assessed. A more accurate measure of GFR involves the one depending on Serum Creatinine values (as per the flowing reference: Stevens LA, Coresh J, Greene T, Levey AS: Assessing kidney function: Measured and estimated glomerular filtration rate. N Engl J Med 354: 2473-2483, 2006).

We agree that data on urinary albumin is important; unfortunately, no data on urinary albumin were available in the vast majority of these participants as stated in the second sentence of the paragraph addressing limitations of the study on page 12. We have added the following sentence to the limitations paragraph as well:

“Kidney function was not directly measured but estimated by a single measurement of plasma creatinine in each participant.”

4. What was the power of the study for the SNPs genotyped for CKD and CHD? The number of sample with CKD or CHD is rather small and may lack adequate power for genetic studies.

We agree that some of the analyses may have lacked adequate power because of the relatively low numbers of eGFR<60 ml/min/1.73m$^2$ or CHD; we allude to this in the limitations paragraph with the sentence “The relatively small sample sizes and few numbers of participants meeting criteria for kidney dysfunction has resulted in wide confidence...”
intervals for many of the estimates for eGFR < 60 ml/min/1.73 m$^2$.” Low power would not affect our statistically significant findings reported here, however.

5. *The paper is well written and discussed. Limitations of the study are acknowledged.*

We thank the reviewer for these positive comments.

We hope that we have adequately addressed this issues raised by the editors and reviewers and that you find the revised manuscript is now suitable for publication in *BMC Nephrology*. We look forward to hearing from you in the near future.

Sincerely,

Julie Lin, M.D., M.P.H., F.A.S.N.