Author's response to reviews

Title: Association Analysis of ADPRT1, AKR1B1, RAGE, GFPT2 and PAI-1 gene polymorphisms with chronic renal insufficiency among Asian Indians with type-2 diabetes

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Version: 4 Date: 16 February 2010

Author's response to reviews: see over
To

Danielle Burgess, PhD
Assistant Scientific Editor
BioMed Central

Sub: Point by point reply to the reviewers’ comments

Dear Dr. Burgess,

Thank you very much for having our Ms. titled “Association of ADPRT1, AKR1B1, RAGE, GFPT2 and PAI1 gene polymorphisms with chronic renal insufficiency among Asian Indians with type-2 diabetes” reviewed by the experts in this area of work and for the very valuable comments and suggestions made by the reviewers. Please note that out of two reviewer’s that were appointed for this manuscript, we managed to satisfy all the queries raised by one of the reviewer’s, Dr. Goisa Trynka, in the first round of revision itself. The second reviewer, Dr. Ilja Nolte, however, had few more queries. In this second round of revision, we have been able to address all the issues raised by the reviewer and this has undoubtedly enhanced the quality of our Ms. Corrections/Modifications made according to the reviewers’ comments are marked in blue in the revised Ms.

We hope that you find our Ms. worthy of publication in your esteemed journal.

Thank you

Sincerely

Thelma B.K.

Professor

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Point by point reply to Reviewer: Ilja Nolte

Major Compulsory Revisions:

Please note that the revisions made according to the reviewer’s comments are marked in blue in the revised Ms.

Major Compulsory Revisions:

1. After multiple testing none of the associations remained significant. The title should therefore be changed to 'No association of ...' or 'Association analysis of ...'.

Reply: The title has been changed to read as “Association analysis of AKR1B1, RAGE, GFPT2 and PAI1 gene polymorphisms with chronic renal insufficiency among Asian Indians with type-2 diabetes”.

2. Although the authors agreed on weakening their statements on the significance of the associations because of multiple testing issues, they still maintained a significance level of 0.05 (p9, line 167) and hence claim to have found significant associations. They mention briefly that 'None of these association observed in the study withstood the Bonferroni correction' (p9, lines 188-189), but they didn't change their conclusions accordingly throughout the manuscript.

Reply: Considering reviewer’s comment on weakening statements on the significance of the associations, we have modified our sentences at various places including abstract, results and discussion in the revised Ms. The observed univariate associations are now called as marginal associations in the modified Ms.

3. Table 3 shows that there is no power for most of the SNPs investigated in this study. Therefore the authors should try to increase sample size or select other more frequent SNPs (see also remark 5).

Reply: we agree with the reviewer. However, the present report constitutes a pilot study. Fresh sample recruitment to increase the sample size is going on in our laboratory. Genotyping of these SNPs (after excluding tightly linked and monomorphic SNPs) along with all other analysed polymorphisms that we investigated in our previous reports will be carried out after sample collection and DNA isolation.

4. Prasad et al. 'attempted to identify putative pathological epistatic interactions between genes from multiple pathways using a combined data set from this study together with data from all our previous studies'. With this in mind it is strange that they only looked at two combinations of genes. This suggests
that they looked at more combinations. The choice of which combinations being analyzed and others not should be stated more clearly.

Reply: An attempt to clarify selection criteria for SNP combinations has been made in the modified Ms.

In the first step all the SNPs were put through MLR (a*b) interaction analysis where in pair wise interactions for all the possible combinations for all the SNPs from all the pathways were analysed by backward MLR method using (a*b) option in SPSS. No statistically significant epistatic interaction was observed for any of the SNP pairs in this analysis.

In addition, in an independent analysis, based on available biological evidences/reports, we combined the genotypic data on GFPT2 and PAI-1 polymorphisms from this study together with genotypic data on two SNPs from our previously published studies on RAAS and chemokine pathways, and tested pair-wise interactions between D allele of ACE gene and homozygous Del (4G/4G) marker of PAI-1; and between TGF \( \beta \)1 and GFPT2 genotypes using additive model in backward MLR.

5. The description of the selection of SNPs is still vague (p8, lines 154-157). Why were only functional SNPs selected? Most of them have low MAFs and hence low power (see also remark 3). A tagSNP approach would enable more firm conclusions. In particular when functional SNPs are not associated, the gene cannot be ruled out as a candidate gene.

Reply: We agree with the reviewer that absence of association of functional SNPs does not completely rules out role of a candidate gene. However, at the same time association, if present, of functionally relevant SNPs is more meaningful and therefore, given precedence over SNPs present in intronic or intergenic regions.

6. Furthermore concerning the selection of SNPs, in their reply Prasad et al. mention that they did not select the ADPRT1 SNP Leu54Phe because of low MAF but the MAF of the SNP they did select (Arg940Lys) was only slightly higher. How about LD between these SNPs? If LD is weak there is no reason not to type Leu54Phe (other than power, but that didn't bother them for the other SNPs either). By the way heterozygosity can not be 0.42 when the MAF is 0.03-0.08. Likely this is a typo and it must be 0.042, right?

Reply: Accepting the reviewers’ suggestion, genotyping for SNP Phe54Leu was carried out in 100 control chromosomes. The SNP is largely monomorphic (with Phe54 predominantly present) in our population and therefore cannot be investigated in case-control samples. Method used for genotyping this SNP has been added at appropriate place in the revised Ms.

Sorry for the typing error. Heterozygosity for Arg940Lys SNP is actually 0.042.
7. Prasad et al. mention in their reply that HWE was indeed calculated using the control sample only. They should also state this in the manuscript.

Reply: Thank you for drawing our attention to this oversight. A sentence in this regard has been added in the revised ms under “Methods (statistical analysis)” section.

8. The p-values of the Fisher's exact test differ much from the p-values of the chi-square test. You should check this, because that must be incorrect.

Reply: Sorry for the mistake. Fisher’s exact p value for one of the SNP in GFPT2 gene (3’UTR, C>T ; rs7725) was in correct. However, it has been corrected in the revised Ms. Please see Table 3.

Discretionary Revisions:

1. Results p10-11, lines 210-217: this is a copy from the introduction. I would suggest to remove it.

Reply: Done