Author's response to reviews

Title: Variants in KCNQ1 increase type II diabetes susceptibility in South Asians
A study of 3,310 subjects from India and the US

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Author's response to reviews: see over
Once again, we thank our reviewers for sparing time to review this manuscript and also accepting most of the modifications included in the manuscript per their suggestions. We are also thankful for the comments and suggestions provided by the Section Editor. We have again analyzed our data based on these comments, and have provided our answers in a point-by-point response below:

Reviewer: Sung-Hoon Kim

Minor Essential Revisions
In reference number 11, the year of publication should be included. Please check again.
We apologize, this has happened due to Endnote problem. The year of publication is now included in the reference #11.

Section Editor’s Comments:
"I would like the authors to discuss the following issue so that the Editor in charge of the manuscript can definitively assess the validity of the reported results.
In table 2, while the rs231362-G allele is more frequent in T2D patients than in NG controls from the Punjabi cohort (0.775 vs. 0.733), the opposite effect is observed in US cohort (0.748 vs. 0.755). However, adjusting for different covariates modified the direction of the association in the US cohort as the G allele is then associated with slightly increased risk (OR =1.04 compared to the unadjusted OR of 0.965). Combining these two cohorts may not be completely correct and could explain the heterogeneity observed using RE model. Is there any specific variable used in the adjusted model that could explain this phenomenon.
Looking deeply to this table revealed that the allele frequency in T2D cases strongly between the two cohorts while in control the frequency are fairly homogeneous. Do the authors have any comments on that?

Because the US cohort included subjects with different origin from North-western and Southern India, the combined analysis was performed by a meta-analysis after evaluating heterogeneity, as suggested by the Reviewer 1. The cohort heterogeneity could not be confirmed as Cochrane statistics did not reveal any significant heterogeneity in these two Asian Indian data sets (p=0.114), perhaps, because only few data sets were meta-analyzed.
Yes, we agree with the Editor that the ‘G’ allele is more frequent in subjects with T2D in Punjabi cohort and not in US cohort. Even the genotype frequency of GG+GA carriers were similar among controls of Punjabi(92%) and US cohorts(92%), while it was higher in
Punjabi (94%) compared to the Us cohort (92%). One reason for this variation could be the small size of our US sample in addition to cohort heterogeneity. We agree that the OR in US cohort increases from 0.97 to 1.04 when adjusted with covariates. To check if there any specific variable used in the adjusted model that could explain this phenomenon, we adjusted our analysis with gender, age, BMI and place of birth separately as well as combined. However, with the exception of gender, all other covariates (age, BMI, and place of birth) increased the OR in this direction ranging from 1.01-1.04. Apparently, there was a larger difference in the mean age in US cohort. US patients (56.2 ± 11.0) were 11 years older in mean age than the controls (45.2 ± 13.4). Whereas in Punjabi cohort, the difference in the mean age between patients (55.4 ± 11.2) and controls (50.8 ± 14.4) was 4.6 years. Similarly, US T2D patients had higher BMI (28.6 ± 3.9) compared to US controls (26.2 ± 4.4) while mean BMI varied only by one unit (27.3 T2D vs. 26.3 controls) in Punjabi cohort. Thus, controlling for these variables might have increased the OR in this direction. Nevertheless, this association of rs231362 with T2D was not statistically significant in US cohort. We have now included ORs and p-values derived from logistic regressions analysis in the data un-adjusted and adjusted for covariates in Table 2 for clarity.

Conversely, the allele frequencies for the rs2237895 are fairly homogeneous in the two cohorts. One could have expected a stronger association for this SNP, but this was not the case. Could adjustment explain this?

We completely agree with the Editor that the allele frequencies for the rs2237895 are in the same direction in two cohorts; therefore stronger association would be expected in combined cohort. As shown in Table 2, we did observe a strongly significant association in the combined analysis without adjusting for covariates (1.15, p=0.006) but the p-value became moderately significant after controlling for the effects of age, BMI, gender, and place of birth (1.15, p=0.011), thus controlling the confounding effect of covariates explains why we have not seen stronger association of rs2237895 in combined cohort.

Finally, since it is said that both cohort exhibit different LD intensity for the genotyped SNPs, it should be extremely valuable to report in Table 4 the haplotype frequencies distribution in the US cohort, even if its size is modest. Besides, in this table, it seems that the rs231362-G allele is at risk for T2D only when it is on the GCC haplotype (0.34 vs. 0.30) and not on the GCA haplotype (0.44 vs. 0.44). If this is also observed in the US cohort, this would suggest a specific haplotype derived from rs231362 and rs2237895 SNPs and this should be emphasized.

As suggested, we have now included haplotype analysis of US cohort in Table 4. Yes, we observe similar trend in the haplotype frequency distribution in US cohort as seen in Punjabi and combined cohort. The G allele of rs231362 was associated with T2D risk when it was on GCC
haplotype and not in GCA haplotype, rather the effect of GCA was in the opposite direction (0.41 in T2D vs. 0.46 in controls) in the US cohort compared to Punjabi cohort (0.44 T2D vs. 0.44 controls), although it was statistically non-significant in the US cohort (p=0.176). On the other hand, ACA haplotype showed similar distribution (0.11 T2D and 0.14 controls) as seen in Punjabi cohort (0.12 T2D and 0.14 in controls). This suggests that T2D risk in KCNQ1 SNPs is derived from ‘G’ and ‘C’ alleles of rs231362 and rs2237895, respectively and ‘G’ allele was ‘at risk’ allele only in the presence of ‘C’ allele of rs2237895. We have emphasized this point in discussion on page 15, paragraph 2.

Minor Essential Revisions
* Page 10: bonferroni correction consist in dividing 0.05 by the number of tests performed, not the number of observations.

Changed as suggested.

* In reference number 11, the year of publication should be included."