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
We thank our reviewers for sparing time to review this manuscript and for providing their valuable input and suggestions. Based on their comments, the manuscript has been extensively revised. We believe inclusion of their suggestions has substantially improved the quality of this manuscript. Below we provide our point-by-point response to each critique.

Reviewer's report Referee #1

Major Compulsory Revisions
1. Because the US cohort included subjects with different origin, western and southern India, the combined analysis should be performed by a meta-analysis after evaluating heterogeneity, or by a logistic regression analysis including variables accounting for the origin. The former seems better way, since genotype distribution of each SNP in combined control tended to be deviated from HWE proportion, p = 0.0192, 0.0455, 0.0917 for rs2237892, rs2237895 and rs231362 respectively. Combined analysis for quantitative traits also should be performed by a meta-analysis.

In our previous submission, we perform combined analysis of Punjabi and US cohort using place of birth as covariate, as it was explained on page 9, line 17 under methods(old version). However, as suggested by the reviewer, we have now performed meta-analysis for analyzing the association of these SNPs with T2D as well as quantitative traits. Except some variation with the p values, the overall trend of association of these SNPs with T2D and quantitative traits (HOMA-B) remained the same. The association of rs231362 with T2D in combined (Punjabi and US cohorts) using meta-analysis showed significant association of this variant in fixed effect meta-analysis (p=0.0089) but not in random effect meta-analysis (p=0.390) despite of the fact that 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. However, meta-analysis results do suggest heterogeneity between the two populations by showing varying p values in fixed- effects (OR 1.15, p=0.009) and random-effects (OR 1.11, p=0.390) models (even though the Q p-value is non-significant). On the other hand, meta-analysis results of other two SNPs showed moderate significance in both fixed- and random- effect meta-analysis (see Table 2). QTLs meta-analysis reproduced similar results of association of rs2237895 with HOMA-B levels in meta-analysis (p=0.009) both for fix- and random-effects. We have included these changes in on Table 2 and Table 3 in our revised manuscript. These results have also been included on page 12, paragraph 1 under results and page 15,
2. The results of haplotype analyses added little information. In addition, the presented p values in this analysis seem something wrong (it is strange that haplotype with 95% CI of 0.96 – 1.48 has significant p value of 0.009, and this P value goes smaller after permutation). There are other similar strange results that haplotypes with 95% CI overlaid across 1.0 have significant P values (<0.05).

We thank our reviewer for pointing this out. The haplotype analysis was mistakenly performed by checking ‘single marker’ option instead of ‘haplotype block’, that is why p values were vague. We have now performed this analysis using PLINK and corrected the discrepancies in ORs. These updates are now included in Table 4 and also under results on page 13, paragraph 1. We have excluded individual haplotype analysis of US cohort because of the small size of this cohort.

Minor Essential Revisions

1. Power estimates should be based on ORs in previous reports and risk allele frequencies in the present population.

We have revised the power estimates based on ORs reported in previous studies and allele frequencies of our population. These changes are included on page 10, paragraph 2, under methods.

2. Footnote of table 2 is not match well with actual table 2, e.g. Position of SNPs on chromosome has been taken from NCBI (Genome Build 36.3) in the footnote, but not shown in the table.

We have deleted the footnote from Table 2.

3. KCNQ1 stands for potassium voltage-gated channel, KQT-like subfamily, member 1

Corrected

4. Line 6 in page 7, [non-Asian Asian] should be [non-Asian].

Corrected

5. Line 5 – 6 from the bottom of page 13, descriptions, [2% vs. 65%], [1% vs. 65%], are probably wrong.

Yes, we noticed that the minor allele frequency of East Asians was switched. The correct difference in our Asian Indian population vs.
East Asian populations for rs2237892 is 2-3% vs. 28-41%, and for rs2237897 is 1% vs. 28-39%, respectively. This information is now corrected in the manuscript on page 14, lines 4-5.

Reviewer's report Referee #2

Major Compulsory Revisions

1. Why did authors not choose to show corrected p-value in association of KCNQ1 SNPs with T2D? Authors do mention about them in discussion but they are not listed in tables. Same could be said for p-values obtained in association analysis between KCNQ1 SNPs and quantitative traits related to obesity and T2D.

We believe we presented corrected (covariate-adjusted) p values for the association of KCNQ1 SNPs with T2D in text as well as Tables in our previous submission. For instance, the values presented in Table 2 for association of SNPs with T2D were derived from logistic regression analysis and were corrected for age, BMI, and gender for individual cohorts and age, BMI, gender, and place of birth for combined (Punjabi+ US) cohorts. These details were also mentioned under methods on page 9, line 11 and 17 under statistical analysis (old version). Looks like, we also checked the association of rs231362 with T2D after controlling for WHR (mentioned only in discussion, now on 3 lines from bottom on page 14) could have confused the reviewer.

2. Authors have stated that the SNPs of KCNQ1 have little role on biology of T2D. However, in the similar studies with samples from East Asia, the p-values obtained were more significant, so authors should talk more about the genetic difference between the two?

We believe that the non-replication of association of rs223892, rs223897 in our Asian Indian cohort is due to difference in allele frequencies between East Asian and South Asian populations as detailed on our revised discussion on page 14: “Perhaps a significant ethnic difference in the allelic distribution could be the reason of non-replication as the MAF of these SNPs was significantly low in our sample as opposed to East Asian populations: 2-3% vs. 28-41% respectively for rs2237892 and 1% vs. 28-39%, respectively for rs2237897 [4-6, 8]. Given the low allele frequency of rs2237892 and rs2237897 in Asian Indians, the statistical power of this study to identify the association of these variants with T2D and related metabolic traits is low, which might explain the lack of association of these SNPs in our sample. Moreover, our data revealed a significant variation in LD patterns in these SNPs compared to East Asians; for instance, there was a strong LD between rs2237892, rs2237895 and rs2237897 SNPs in East Asians (D'=0.84-0.98, r^2= 0.20-0.66) [7] compared to our Asian Indian cohort (D'=0.51-0.65, r^2=0.0-0.41). These differences can also increase or decrease the disease risk; best example for this is the negative association of TCF7L2 SNPs with T2D in Chinese [20].”

Results of meta-analysis showed a previously non-significant SNP (rs2237892) to be moderately associated with T2D in combined cohort.
(p=0.030) both in fixed and random effect meta-analysis, which further suggests that the difference in allele frequencies is the reason of non-replicating this association with T2D in this cohort. Perhaps a very large sample size would be required to replicate these results of Japanese GWAS locus in this population.

3. Authors mentioned that “non-replication of association is only due to the low power of the US sample”. But this might not be the only reason because some of QTs associated with US cohort only, for instance, HOMA-B.

Apparently, our replication sample has low power to detect the association of KCNQ1 SNPs with discrete trait (T2D). On the other hand, we agree with the reviewer that the association of rs2237895 with HOMA B levels was only significant in US cohort and other factors could have been responsible for this difference. It is noteworthy that the Punjabi cohort showed a poor beta cell function as indicated by their significantly lower HOMA B levels compared to the US cohort (p = 4.64 x 10^-8). One reason could be that the US cohort is relatively younger (5.5 year) than the Punjabi cohort in addition to other reasons including of sample heterogeneity, and/or migration. Perhaps this difference could have resulted in genotype difference in affecting HOMA-B levels. We have included this information in discussion on page 15, paragraph 1.

**Minor Essential Revisions**
SNP rs number should all be italicized, and authors should decide on one way to write them. For example, in discussion, they used two different ways to write rs number.

*We have corrected this discrepancy in the entire manuscript.*

**Minor issues not for publication**

1. Last sentence of introduction may need a change. Change ‘further’ into ‘furthermore’.

   *Changed*

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

   *Updated*