**Author's response to reviews**

**Title:** Common variants of the TCF7L2 gene are associated with increased risk of type 2 diabetes mellitus in a UK-resident South Asian population

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**Author's response to reviews:** see over
To whom it may concern,

Please find enclosed our responses to the reviewer’s comments and the revised manuscript ‘Common variants of the TCF7L2 gene are associated with increased risk of type 2 diabetes mellitus in a UK-resident South Asian population’. We hope these responses and revisions are acceptable.

Kind regards,

Simon Rees
Author’s response to reviewer’s comments

Title: ‘Common variants of the TCF7L2 gene are associated with increased risk of type 2 diabetes mellitus in a UK-resident South Asian population’

Authors:
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Response to referee 1

Major points:

1. It is unclear whether unrelated type 2 diabetes patients were used in the study or not.

Response:
No information regarding the relatedness of individuals within this study was collected, as the UKADS project was initially set up as an intervention trial without a genetic component. This has now been stated in the methods section.

2. The authors do not provide power calculation which gives an estimation of the effect sizes their study is powered to detect. That will help in the interpretation of the data.

Response:
We had not initially included an estimate of statistical power as all SNPs displayed a significant association with type 2 diabetes and power is the probability that a test will not make a type II (false negative) error. We have now included a small paragraph in the results section addressing our power calculations.

3. Please include more details on genotyping accuracy in Methods, e.g.
genotyping success rate.

Response

_Genotyping success rate has been added to the methods section_

4. One of the snps, rs12255372 didn’t follow the multiplicative genetic model (in disagreement with the original observation of Grant et al as well as with the previous study in the Indian population by Chandak et al.) The controls were not in HW (p=0.02). The authors have performed extra genotyping using the exactly same method, to exclude genotyping mistakes and did not find any inconsistence with the original results. They concluded that the departure from HW was due to the chance sampling.

Since the authors observe too many homozygotes for the minor allele, this observation may also point to a systematic genotyping error. It would be helpful to retype the SNP in ALL control samples with a completely different method to rule out this possibility, or to sequence a subset of the alleles.

Response:

_As mentioned above, we re-genotyped all individuals for this SNP to rule out genotyping errors as a reason for the observed excess of minor allele homozygotes (TT). We then re-genotyped all the putative rare allele homozygotes (along with a number of heterozygotes and common allele homozygotes for comparison) by RFLP, to rule out a systematic error with the TaqMan system. The rationale behind this was that any individual falsely genotyped as TT by the TaqMan method would be genotyped as CT or CC by the RFLP method. The RFLP method was in complete concordance with the TaqMan method. We therefore believe that the possibility of a systematic error has been ruled out. A more comprehensive explanation of our methods and rationale has been included in the manuscript._

5. In Table 2 the results of genotyping for rs7901695 in controls is: 226+169+42 =437 which is in disagreement with total number of 436 controls used in the study.

Response:

_The actual number of control subjects analysed was 437. Stating 436 was an error made by the first author. This has been amended in the manuscript._

**Minor Points:**

1. Please include the 95% confident interval in the Abstract.

Response:

_This has been added to the manuscript_

2. Methods, page 5. “Mendelian consistency” should be changed to “Hardy-Weinberg equilibrium”.
3. Results. At page 7 the authors state: “To further explore the effect of including young control subjects on the strength of association, we compared the allelic ORs calculated for each SNP using subsets of our control group defined by different minimum age thresholds. When increasing minimum age thresholds were applied, there was a corresponding increase in OR up to an age cut-off of 46 years (Figure 2). When the minimum age was increased beyond this point, the relationship with OR deteriorated, although regression analysis still revealed a significant positive relationship ($p < 7.11 \times 10^{-3}$) for all variants when the minimum age threshold was increased to 65 years (data not shown). “ It would be helpful to rephrase the statement as it is difficult to follow the author’s argumentation.

Response:
The text “although regression analysis still revealed a significant positive relationship ($p < 7.11 \times 10^{-3}$) for all variants when the minimum age threshold was increased to 65 years (data not shown)” has been removed from the above paragraph. We hope the removal of this text clears up any confusion regarding the paragraph.

4. It should be mentioned in Table 1 that BMI, waist circumference and blood pressure measurements were available for only 252 controls.

Response:
The text has been revised

5. “A case control study” can be omitted in the title of the paper.

Response:
The title has been revised
Response to referee 2

1. The results are as expected: moderately positive given the relative low frequency of the SNP T allele (SNP 146) in this population. Recent metaanalyses from Cauchy and also from Florez should be referred and discussed.

Response:
These papers have been referred to in the Introduction section.

2. The most interesting data are related to the minimal age of the controls in a population at high T2D prevalence. Authors should discuss these data with regard to the age related prevalence of T2D in their population. Multiple regression analysis can also be done taking into account age.

Response:
We believe that the most important point regarding the above issue is that not implementing appropriate minimum age cut-offs can produce an artificially low odds ratio, as young control subjects may develop type 2 diabetes later in life and are therefore essentially in the wrong group. From our findings it seems that this may lead to reduced statistical power and an increase in type II error (false negative results), which would be especially important when the OR of the variant in question is lower than that of TCF7L2. It is also important to note that the effect that young controls may have on association results will depend on the prevalence of the disease in the studied population. Prevalence of type 2 diabetes in UK-resident south Asian populations is high, probably accounting for the fairly marked effect reported in this manuscript. Populations of white Caucasian origin generally have a lower prevalence of the disease, and so young controls will have a smaller effect on association results. The last paragraph of the Discussion section in the original manuscript mentioned these points, but it has been reworded to more clearly convey our thoughts on this subject.

With regards to taking age into account using regression analysis, we do no believe that this fully addresses the problems illustrated here. For example, as we understand it, having age as a covariate in a logistic regression would only alter the output if age differed between the two categories of the dependent variable (i.e. disease status). It is possible for the average age of the two disease groups to be identical, leading to no alteration in the output of the regression test, and yet for there still to be young individuals within the control group who are not ‘true’ controls and may develop the disease later in life. We do not believe that using minimum age thresholds and including age in regression analysis has the same effect.

3. Recent papers from DECODE discussed the influence of TCF7L2 on BMI in diabetics and also in controls that should be analyzed in this population. Is there insulin values?
Response:
We have analysed the relationship between TCF7L2 variants and BMI within our cohort, but found no statistically significant result. We did observe a trend for all SNPs, within both case and control groups; the minor allele appeared to be associated with decreased BMI. In light of the DECODE paper, these non-significant results have been briefly mentioned in the manuscript.
We do not have insulin values to analyse.

4. Is the LD different from other populations?

Response:
The strength and pattern of the observed LD in our study was similar to that seen in the CEPH (CEU) HapMap samples, as was originally stated in the manuscript. It was also similar to previous publications involving white Caucasian cohorts, although most of these studies did not investigate all of the variants reported in this manuscript (most notably rs7901695 and rs11196205). References to a number of reports where some LD values were given have been included in the Results section.

5. These individuals have migrated from India to the UK. The environment is different in some respect. This aspect should be discussed especially if some data are available.

Response:
The issue of environmental differences between south Asian countries (India, Pakistan and Bangladesh) and the UK is a complex one. The incidence of type 2 diabetes is rapidly increasing on the Indian sub-continent. Many urban areas show similar levels of disease prevalence to UK-resident south Asian populations, but even in rural areas lifestyle transitions mean that the rural-urban divide is closing. In addition to this, strict adherence to cultural traditions can lead to relatively minor lifestyle changes after immigration to the UK. As we are not trying to compare our results with those of an indigenous south Asian population, we believe that it would be best not to include a discussion of this complex issue in this manuscript. Although environmental differences are bound to affect the prevalence of the disease, we hope that you will agree that this does not alter our findings that TCF7L2 is associated with type 2 diabetes in this UK-resident south Asian population.

6. As it is an intervention study the interaction between TCF7L2 and the intervention may be of interest (see the NEJM Florez paper and the recent Palmer C paper in diabetes on the effect of sulfonylureas).

Response:
It is our intention to investigate the possibility that genotype (of TCF7L2 and other genes) may mediate response to the intervention and/or the use of certain prescriptions,
with regards to improving cardiovascular risk factors. Currently, however, full data is still being collected and is not available for analysis.