Author's response to reviews

**Title:** Race-Ethnic Differences in the Association of HbA1c-Associated Genomic Loci with HbA1c Levels and Mortality in U.S. Adults: the Third National Health and Nutrition Examination Survey (NHANES III)

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**Version:** 2 **Date:** 16 February 2012

**Author's response to reviews:** see over
February 16, 2012

Dear Editor,

Thank you for the helpful and constructive comments from reviewers for our paper, “Race-Ethnic Differences in Association of Genetic Loci with HbA\textsubscript{1C} Levels and Mortality in U.S. Adults: the Third National Health and Nutrition Examination Survey (NHANES III)”. We feel that our manuscript has improved with their valuable input and we are submitting our revision to be considered for publication in BMC Medical Genetics. Thank you for considering this manuscript for publication.

We have made adjustments (changes in red where possible) to our manuscript and have addressed each of the reviewers’ comments below in red.

Please contact us if you have questions.

Best wishes,

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Reviewer's report

Title: Race-Ethnic Differences in the Association of Loci with HbA1c Levels and Mortality in U.S. Adults: the Third National Health and Nutrition Examination Survey (NHANES III)

Version: 1 Date: 19 December 2011

Reviewer: Andrew D Paterson

Reviewer's report:
This is a clearly written paper on HbA1c risk allele frequencies across 3 race/ethnic groups in the US and their association with HbA1c and mortality.

Minor Essential Revisions:

The title is a little awkward, perhaps 'Hba1c-associated genomic' could be removed without loss of clarity.

We have removed “HbA1c-Associated Genomic” from the title and changed it from

“Race-Ethnic Differences in the Association of HbA1c-Associated Genomic Loci with HbA1c Levels and Mortality in U.S. Adults: the Third National Health and Nutrition Examination Survey (NHANES III)” to

“Race-Ethnic Differences in the Association of Genetic Loci with HbA1c Levels and Mortality in U.S. Adults: the Third National Health and Nutrition Examination Survey (NHANES III)”

It is not clear whether red cell traits were collected in NHANES. Even if the authors do not analyze them here, I think discussion of whether this would be possible would be useful in the discussion.

Red cell traits were collected in NHANES. We did not carry out an analysis with blood cell traits in the current study as this has already been carried out in a much larger sample set by Soranzo et al. (2010). Soranzo et al. studied associations of HbA1c loci with red cell traits (including hemoglobin, mean corpuscular volume, corpuscular hemoglobin, iron and transferrin) in a subset of four studies including NHANES III. They also conducted conditional analyses of HbA1c levels as a function of HbA1c SNPs and red cells traits, showing that some HbA1c associations are likely mediated by RBC biology, not glycemia per se. We added to the Discussion, page 7:

“Adjustment of models of these common variants predicting HbA1c levels for levels of hemoglobin concentration or mean corpuscular volume attenuate SNP-HbA1c relationships, suggesting mediation of HbA1c variation by elements of erythrocyte biology [7]”

In the Methods, the authors say that 704 with diabetes were excluded. It would be helpful to be clear how these were proportionately contributed by each of the 3 major ethnic groups studied.

We added this information in the methods:
“Of 3,894 individuals with complete data for analysis, we excluded 149 who were not of NHB, MA or NHW race-ethnicity and 704 with diabetes (293 NHW, 167 NHB and 244 MA), leaving 901 NHB, 909 MA, and 1,231 NHW individuals in the analysis”.

There is great genetic heterogeneity within each of the populations studied, especially African Americans. It would be useful to state this.

A statement was added to the discussion (see statement below).

Is there other genetic data that could be used to estimate the heterogeneity within each group, and whether that heterogeneity is associated with HbA1c.

We agree that heterogeneity within populations may influence variability in HbA1c levels. However, no ancestry markers are presently available in NHANES to evaluate heterogeneity within the three ethnic groups. Therefore, we added this as a limitation of our study in the Discussion Page 7:

“Furthermore, greater heterogeneity exists in NHB, and this heterogeneity may have influenced variability in HbA1c levels. Since there are no ancestry markers available in NHANES to evaluate genetic heterogeneity within populations, we were unable to evaluate substructure within ethnic groups, and for the purposes of this study, assumed little to no intra-population substructure”.

In the methods 'SNP genotyping, the r2 of proxy SNPs is stated in CEU. Please add the r2 in other relevant HapMap populations.

We also intended to include the r^2 values for the other relevant populations when mentioning the proxies that we used. However, one SNP of each proxy pair is not available in SNAP for ASW and MEX populations, so obtaining these values was not possible. In Methods Page 4 we added:

“[r^2 for ASW and MEX populations not available]”.

In general it is not clear what the power is for each SNP in each ethnic group. A simple table describing the power to each locus for HbA1c in each group (based on the prior effect sizes) would be helpful. Are the mostly negative results expected due to universal low power?

We acknowledge that our lack of power probably results in some of the negative results, but we believe that the differences observed between ethnic groups remain of interest and are hypothesis generating. To show the power for each locus for HbA1c in each ethnic group, we constructed a Supplementary Table 3 (also below). We referred to this Supplementary Table in our Discussion: “Modest power given the relatively small sample size of NHANES III could also account for the relatively weak association of HbA1c SNPs with HbA1c in each race-ethnic group (Supplementary Table 3)”.
Supplementary Table 3. Power calculations for HbA1c at alpha=0.05 and alpha=0.05/11 (Bonferroni corrected) assuming similar effect sizes to those published by Soranzo et al. (2010).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Nearest Locus</th>
<th>NHW α=0.05</th>
<th>NHW α=0.05/11</th>
<th>NHB α=0.05</th>
<th>NHB α=0.05/11</th>
<th>MA α=0.05</th>
<th>MA α=0.05/11</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2022003</td>
<td>SPTA1</td>
<td>17.3%</td>
<td>3.4%</td>
<td>9.7%</td>
<td>1.5%</td>
<td>17.0%</td>
<td>3.3%</td>
</tr>
<tr>
<td>rs552976</td>
<td>ABCB11/G6PC2</td>
<td>52.3%</td>
<td>20.5%</td>
<td>28.3%</td>
<td>7.3%</td>
<td>49.0%</td>
<td>18.3%</td>
</tr>
<tr>
<td>rs1800562</td>
<td>HFE</td>
<td>19.3%</td>
<td>4.0%</td>
<td>11.1%</td>
<td>1.8%</td>
<td>14.8%</td>
<td>2.7%</td>
</tr>
<tr>
<td>rs179884</td>
<td>GCK</td>
<td>26.7%</td>
<td>6.7%</td>
<td>15.0%</td>
<td>2.8%</td>
<td>24.6%</td>
<td>5.9%</td>
</tr>
<tr>
<td>rs4737009</td>
<td>ANK1</td>
<td>18.9%</td>
<td>3.9%</td>
<td>13.2%</td>
<td>2.3%</td>
<td>17.1%</td>
<td>3.4%</td>
</tr>
<tr>
<td>rs6474359</td>
<td>ANK1</td>
<td>16.4%</td>
<td>3.2%</td>
<td>35.5%</td>
<td>10.6%</td>
<td>15.4%</td>
<td>2.9%</td>
</tr>
<tr>
<td>rs16926246</td>
<td>HK1</td>
<td>77.8%</td>
<td>45.4%</td>
<td>45.8%</td>
<td>16.2%</td>
<td>70.9%</td>
<td>37.0%</td>
</tr>
<tr>
<td>rs10830956</td>
<td>MTNR1B</td>
<td>21.6%</td>
<td>4.8%</td>
<td>13.2%</td>
<td>2.3%</td>
<td>17.2%</td>
<td>3.4%</td>
</tr>
<tr>
<td>rs282606</td>
<td>ATP11A / TUBGCI</td>
<td>16.8%</td>
<td>3.3%</td>
<td>16.1%</td>
<td>3.1%</td>
<td>17.8%</td>
<td>3.6%</td>
</tr>
<tr>
<td>rs1046896</td>
<td>FN3K</td>
<td>32.8%</td>
<td>9.3%</td>
<td>17.3%</td>
<td>3.4%</td>
<td>29.9%</td>
<td>8.0%</td>
</tr>
<tr>
<td>rs855791</td>
<td>TMPRSS6</td>
<td>23.4%</td>
<td>5.4%</td>
<td>9.4%</td>
<td>1.4%</td>
<td>21.3%</td>
<td>4.7%</td>
</tr>
</tbody>
</table>

Again, some statement about the expected power of the mortality analysis.

Power calculations for the mortality analysis are now provided in Supplementary Table 5 (and below). We also mentioned the lack of power in our Discussion (p. 7): “Another explanation for a lack of association of the HbA1c genetic risk score with mortality is the lack of statistical power due to small sample size within each ethnicity (Supplementary Table 5).”

Supplementary Table 5. Power calculations for mortality at alpha=0.05 and alpha=0.05/11*.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Nearest Locus</th>
<th>NHW α=0.05</th>
<th>NHW α=0.05/11</th>
<th>NHB α=0.05</th>
<th>NHB α=0.05/11</th>
<th>MA α=0.05</th>
<th>MA α=0.05/11</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2022003</td>
<td>SPTA1</td>
<td>5.02%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs552976</td>
<td>ABCB11/G6PC2</td>
<td>5.09%</td>
<td>0.47%</td>
<td>5.1%</td>
<td>0.47%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs1800562</td>
<td>HFE</td>
<td>5.03%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs179884</td>
<td>GCK</td>
<td>5.04%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs4737009</td>
<td>ANK1</td>
<td>5.03%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs6474359</td>
<td>ANK1</td>
<td>5.02%</td>
<td>0.46%</td>
<td>5.1%</td>
<td>0.47%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs16926246</td>
<td>HK1</td>
<td>5.17%</td>
<td>0.48%</td>
<td>5.1%</td>
<td>0.47%</td>
<td>5.1%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs10830956</td>
<td>MTNR1B</td>
<td>5.03%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs282606</td>
<td>ATP11A / TUBGCI</td>
<td>5.02%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs1046896</td>
<td>FN3K</td>
<td>5.05%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
<tr>
<td>rs855791</td>
<td>TMPRSS6</td>
<td>5.04%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
<td>5.0%</td>
<td>0.46%</td>
</tr>
</tbody>
</table>

* In our mortality power computation we used the estimate of a risk increase of 1.26 per 1 unit of HbA1c (average of male [1.24] and female [1.28]).


Ref # 7 should be updated with the published paper.

This reference is now updated with the published information.
Table 2, the SE for some SNPs are specified to too many significant digits - it will help reading to make them consistent.

Table 2 was adjusted and now contains fewer digits for SE.

Table 4. The 3 right-most columns are presumably for the 11SNP score, but the fact that the results occur on the line for the first SNP could potentially be misleading. Perhaps a separate supplementary table would be the best place for this part of the table. In the same table there's a strange green triangle in the 4\textsuperscript{th} last column - probably some Excel effect.

Table 4 was revised and is now less confusing. Another row was added above the first SNP in each group so that genetic risk score values do not line up with any single SNP. The green triangles (excel artifact) were removed from Table 4.

Fig 1. It would be helpful to add the name of the risk allele for each SNP.

Risk alleles for each SNP were added to Fig 1 (on the x-axis next to each SNP name).

In supplementary Table 1. in cell 'B4' the SNP name has been removed by error.

This cell in Supplementary Table 1 has been corrected.

What is the cause of the $p=0.002$ for HWE for HFE in NHW (supplementary Table 2).

It is likely that in the case of the HFE locus (rs1800562), there are more heterozygotes than expected. In NHANES III, the percentage of individuals who are homozygous for the non-risk allele (A) is extremely low (0.1\%) in all three race groups. When examining allele frequencies at this locus in HapMap for NHW, MEX, and ASW (and even 6 other race groups with data available in HapMap), 0 individuals in HapMap are homozygous for the non-risk allele (A/A). There may be slight undercalling of heterozygotes in our study which may explain the observance of the few A/A homozygotes in our populations. The cause of the significant HWE $p$ value observed in NHW is likely due to the fact that the population size of NHW is the largest of the three, providing enough power for a significant $p$ value.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Reviewer's report**

**Title:** Race-Ethnic Differences in the Association of HbA1c-Associated Genomic Loci with HbA1c Levels and Mortality in U.S. Adults: the Third National Health and Nutrition Examination Survey (NHANES III)

**Version:** 1 **Date:** 21 December 2011

**Reviewer:** David Meyre

**Reviewer's report:**

- Major Compulsory Revisions
In this report, JL Grimsby and colleagues assessed the ethnic differences in the association of HbA1c-associated loci with HbA1c levels and mortality in 3,041 black American, Mexican American and White American US adults. They conclude that minor allele frequency of some HbA1c-associated loci as well as their combined effect on Hba1c levels may vary according to the ethnic background. This is an interesting study that feeds the debate about the transferability of GWAS signals identified in Europeans to other ethnic backgrounds. The current version of the manuscript may however be significantly improved.

1-Please precise the study sample size in the abstract.

We added the sample size to the abstract:

“We studied 3,041 non-diabetic individuals in the NHANES (National Health and Nutrition Examination Survey) III.”

2-For the SNPs showing significant MAF differences according to the ethnic background, it may be useful to add a post-hoc test to know which specific ethnic background(s) significantly diverges from the other ones (NHW = NHB # MA, NHW # NHB = MA, NHW # NHB #MA) and to comment these results.

We believe that the data the reviewer is requesting is available in our Supplementary Table 1, which shows which of the three populations deviates from the others in risk allele frequency. We used Fisher’s P as a conservative test to determine which of the loci show significant differences in allele frequency by ethnicity. We have revised the statement in the Results (page 5) for greater clarity:

“Six out of 11 HbA\textsubscript{1c}–associated SNPs had risk allele frequencies that differed significantly across race-ethnic groups (Fisher’s \(p<0.0002\)). At five of these six loci, risk allele frequency of NHB was most divergent, including SNPs near \textit{ANK1} (two uncorrelated SNPs), \textit{MTNR1B}, \textit{ATP11A/TUBGCP3} and \textit{TMPRSS6}. At the SNP near \textit{SPTA1}, risk allele frequency differed most in MA. “

Given the strong selection pressure by infectious diseases on erythrocyte-related genes it would be especially interesting to investigate if the MAF differences are randomly or non-randomly distributed among the different ethnic groups.

We believe we addressed this in the Results, page 6., “Evidence of population differentiation …” and in the Discussion in the paragraph that continues on Page 7. Indeed, at some loci, allele frequency differences do not appear to be randomly distributed among the different ethnic groups.

Are the HbA1c increasing alleles systematically more frequent in certain ethnic backgrounds?

This is a good question. We checked in Supplementary Table 1 whether the HbA\textsubscript{1c} effect alleles are systematically the major (older and more frequent) allele or minor (newer and less frequent) allele in certain ethnic backgrounds. We added this information to the results page 5:

“The HbA\textsubscript{1c}–raising allele was the minor (more recent mutation) in all three ethnic groups for SNPs near \textit{SPTA1}, \textit{GCK}, \textit{MTNR1B}, \textit{FN3K}, and \textit{TMPRSS6}. The HbA\textsubscript{1c}–raising allele was the major (ancestral) allele at SNPs near \textit{ABCB11}, \textit{HFE}, \textit{ANKI} (rs6474359) and \textit{HK1}. At two loci,
ATP11A, and ANK1 (rs4737009), the HbA1c-raising allele was the minor allele in NHW and MA, but the major allele in NHB.

3-In my opinion the fact that the HbA1c genotype risk score (GRS) was significantly associated with HbA1c level in White and Mexican Americans but was not associated with HbA1c level in Black American does not signify that the average beta-values per additional risk allele significantly vary according to ethnicity. Indeed, the significance of the association depends on statistical power and sample size considerations. To properly demonstrate an ethnic heterogeneity in the impact of the HbA1c GRS on HbA1c value, the authors need to apply the following linear regression model in the whole U.S. sample: Hba1c level (outcome) = sex, age, GRS, ethnicity, GRS x ethnicity interaction. IF a significant (P < 0.05) GRS x ethnicity interaction on HbA1c level is found, the authors may conclude that the combined impact of HbA1c associated loci on HbA1c levels vary by ethnicity. If such interaction is demonstrated, it would be interesting to further discuss the potential reasons why the average beta-value per additional risk allele on HbA1C is 2-fold bigger in Mexican American than in European Americans (gene x environment interactions…). If true, this result is important because it suggests that GWAS signals derived from European populations may not only be transferable to other ethnic backgrounds but may be more explicative at least in certain ethnicities.

We carried out this interaction test as the reviewer suggested, and we saw no significant GRS x ethnicity interaction on HbA1c. However, given that we had somewhat low power for the main effect model, we anticipated that we would have even less power for interaction testing. Further, our principal aim was to examine associations within race strata. Nonetheless we conducted the combined analysis including first order ethnicity x genotype interaction terms. We added a statement in the Methods page 4 stating that we carried out this interaction test:

“To determine if a significant genetic risk score x ethnicity interaction effect on HbA1c exists, we also applied the following linear regression model on the whole sample: Hba1c level (outcome) = sex, age, genetic risk score, ethnicity, genetic risk score x ethnicity interaction. We added a statement in our results stating: “We observed no significant genetic risk score x ethnicity interaction on HbA1c level (p=0.68).”

We also added a statement to our discussion describing this limitation:

“No significant interactions were observed, also possibly due to low power”.

4-Do the authors have access to data relative to incident T2D events during the follow-up? IF the answer is yes, it may be interesting to test the impact of the HbA1c genotype risk score on incident T2D risk.

Unfortunately NHANES is not a longitudinal follow up study for disease events. Different people are examined at each NHANES cycle. The only follow up available is the mortality-linked cohort.

5-The authors have demonstrated that the mean HbA1C genotype score was varying significantly with ethnicity. Is the dispersion of the HbA1C genotype score also different according to the ethnic background (extreme GRS values, dispersion of GRS classes…)?

We found that the dispersion of the HbA1c genotype score is not different according to ethnic background. We added the following statement to the results section page 5 to include this information:
“Median genetic risk scores (unweighted) were 11.0 (SD = 2.2), 11.0 (SD = 2.3) and 11.0 (SD = 2.0) in NHW, NHB and MA, respectively, with distributions of genetic risk scores negatively skewed toward a lower score in all three ethnic groups.”

6-T2D has been diagnosed using fasting glucose value but no OGTT was performed. This may introduce some misclassification in the T2D status attribution, and may be listed as a limitation of the study.

We added this as a limitation in the Discussion:

“T2D diagnosis was based on fasting glucose with no OGTT which may have introduced misclassification in T2D status of study subjects.”

7-In order to provide a transparent quality control procedure please add a supplementary table with raw genotype counts and call rate values for each SNP in addition to HWE.

Raw genotype values are provided in Supplementary Table 2, which includes the % of individuals called for 0, 1 and 2 risk alleles for each locus and the total number genotyped (N).

8-Please describe the number of SNPs harboring a directionally consistent effect on HbA1c value in comparison with those from the recent GWAS for HbA1c published in Diabetes, not only in the European Americans but also in the two other ethnic subgroups. Discuss the results in the context of ethnic-specific LD structures, flip flop effects.

We added the following data to the results page 5:

“Though single-SNP associations are underpowered (Supplementary Table 3), we did observe that in NHW, eight of the 11 SNPs in NHW were consistent with Soranzo et al. (2010) in having a positive risk effect on HbA1c levels, with three of the SNPs used in our analysis (rs282606 [ATP11A], rs10830956 [MTNR1B], and rs2022003 [SPTA1]) serving as proxies for those in the MAGIC study. Beta coefficients were negative for three, three and four of the 11 SNPs in NHW, NHB and MA groups, respectively, but corresponding SNPs did not generate significant associations.”

9-Single SNP analyses: single SNP analyses in ethnic subgroups are clearly underpowered (only four out of 33 associations nominally significant) and must be discarded from the manuscript. To gain some statistical power in the analysis, I strongly recommend the following linear regression model in the whole U.S. sample: Hba1c level (outcome) = sex age genotype ethnicity genotype x ethnicity interaction. This may help to evidence inter-ethnic heterogeneity in the association of certain SNPs with Hba1c.

We chose to include single SNP analyses in Table 2 but rather to emphasize in the manuscript that single SNP analyses are underpowered by stating in the Results page 5, as above:

“Though single-SNP associations are underpowered we did observe that...”.

As mentioned in our response to comment #3, we carried out the interaction model as described and did not observe any significant interaction.
10-The mortality rates reported in this study are very surprising: 24.1% in White Americans, 14.2% in Black Americans and 9.7% in Mexican Americans. Are these highly divergent mortality rates in line with data from the literature? Did the authors have explanations for these results?

We agree that the mortality data is not in line with expected results. Based on 2008 data from the U.S. Census Bureau, Statistical Abstract of the United States (2012: http://www.census.gov/compendia/statab/2012/tables/12s0109.pdf) we would expect lower mortality rates in whites compared to blacks and lowest mortality rates of all in MA. In the U.S. Census abstract death rates were 9.4% (age-adjusted) in Blacks, 7.7% (age-adjusted) in NHW, and 5.4% (age-adjusted) in people of Hispanic origin.

The initial mortality rates were calculated as number of deaths divided by the number of participants. However, given the survey design (over-sampling certain race groups, difference in mean age of participants, follow-up for the mortality status), it is better to present the race-standardized mortality rates per 1000 person years of follow-up and to age-standardize sample weights. The revised mortality rates are 12.8% in NHW, 19.3% in NHB and 14.5% in MEX. These fall closer to expected rates, at least with highest mortality rates in NHB. Table 4 has been revised accordingly.

The authors should provide the exact number of mortality events during the follow-up, in order to have a better idea about the statistical power of the mortality analyses.

We have provided an exact number of mortality events for each ethnic group during follow up. These results have been listed in the 10th column of Table 4. There are 143, 100 and 55 mortality events in NHW, NHB and MA, respectively.

11-Discussion: “Our analyses of differentiation and selection suggest that there may be some selection pressure at the ANK1, HK1, ATP11A, ABC11/G6PC2 and TMPRSS6 loci, all of which are erythrocyte-related loci.” This sentence is false; the ABC11/G6PC2 locus is related to glycemic control pathways.

We revised this statement appropriately to “Our analyses of differentiation and selection suggest that there may be some selection pressure at the ANK1, HK1, ATP11A, TMPRSS6 and ABC11/G6PC2 loci, the first four of which are erythrocyte-related loci (top paragraph p. 7).

12-Discussion: “Although hypothetical at this point, if race-specific selection pressures influence erythrocyte-associated SNPs, this could generate inter-race variation of SNP associations with HbA1c.” I do not necessarily share the view that selection processes may lead to inter-race variation of SNP associations with HbA1c level.

We removed this statement from the manuscript.

13-The authors found different alternative explanations to explain the lack of association between the HbA1c GRS and mortality, despite previous epidemiological association between HbA1c levels and mortality. In my opinion the main explanation for the lack of association between the HbA1c GRS with mortality is the lack of statistical power if analyses are done separately in the three ethnic subgroups. OR average values are directionally consistent with the epidemiological observations in the three subgroups, and a trend of association (P=0.09) is observed in the larger sample (White Americans). This alternative explanation has not been mentioned by the authors. In order to gain some statistical power, I recommend using the following logistic
regression model in the whole sample: mortality (outcome) = sex, age, GRS, ethnicity, GRS x ethnicity interaction.

We have added this alternative explanation to our discussion “Another explanation for a lack of association of the HbA1c genetic risk score with mortality is the lack of statistical power due to small sample size within each ethnicity (Supplementary Table 5). When pooling the entire sample and carrying out an interaction model we also observed no significant genetic risk score x ethnicity interaction on mortality.” (last full paragraph p. 7)

We added a statement to the methods stating that we also performed this pooled interaction analysis “We also applied the following logistic regression model in the whole sample: mortality (outcome) = sex, age, GRS, ethnicity, GRS x ethnicity interaction.” We also added a statement to the results: “We observed no significant genetic risk score x ethnicity interaction on mortality (p = 0.62).” (last paragraph p. 4)

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests.