Author's response to reviews

Title: Triglyceride altering polymorphisms of the ApoAV gene have very different allele frequencies in India compared to Europeans

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Responses

**Reviewer 1**

**Major compulsory changes**

1. We thank the reviewer for pointing out that previous papers have quoted means and SE from untransformed data but performed statistical analyses on logged values. We have therefore changed Table 3, modified the legend, and added a sentence to the last paragraph of the methods to explain what we have done.

2. We have added the data on HDL, LDL and total cholesterol levels to table. We have also added the following sentence to the results section: “There were no associations between the two APOA5 variants and either HDL, LDL or total cholesterol in either the Pune Indian or UK white subjects, although this may be due to lack of power if the effects on these lipid parameters is less than the effects on triglycerides.”

**Minor essential revisions**

1. We thank the reviewer for pointing out that the triglyceride concentrations in the Indian subjects are not any higher than those in the Caucasian subjects from Plymouth EarlyBird study. However, the BMI is much lower in the Indian subjects. To clarify this, we state, on page 6, first paragraph, that “It is important to understand the genetic contribution to triglyceride metabolism in Asian Indians since they have higher triglyceride concentrations compared to Caucasians relative to their BMI and these differences can occur as early as 10 years old”

2. We thank the reviewer for pointing out the way we have used the terms “racial” and “North European”. We have changed “racial” to “ethnic” and “North European” to “Caucasian”. Also we have corrected the use of references 4, 11 and excluded reference 18.

**Discretionary revisions**

1. We have changed “rare” to “less common”. We agree this is more appropriate for common polymorphisms.

2. On page 8, second paragraph we have changed a < to = next to a p value.

3. We have now included glucose and insulin in table 1.

4. In line with the reviewer and other reviewers’ comments we have changed the title to: “Triglyceride associated polymorphisms of the ApoA5 gene have very different allele frequencies in Pune, India compared to Europeans”

**Reviewer 2**

**Major revisions**

1. We thank the reviewer for pointing out our misuse of “alters” in relation to the APOA5 alleles and have changed this to “associated” throughout the text. We have also reworded several situations where we had implied functionality rather than association. We have changed the title to: “Triglyceride associated polymorphisms of the ApoA5 gene have very different allele frequencies in Pune, India compared to Europeans”. This now reflects the fact that our subjects come
from only one part of the Indian subcontinent. We have also been more precise throughout the main text and referred to “Pune Indians” rather than “Asian Indians”.

2. As above we have changed the emphasis from “altered” to “associated” throughout the text including the abstract and title.

3. We have deleted the “triglyceride raising allele” part of the first sentence of the results section of the abstract.

4. We have added the 95% CI’s to the allele and carrier frequency estimates in table 2. This allows the reader to see more clearly how the frequencies are significantly different in the two populations. We thank the reviewer for this good suggestion.

5. The reviewer is correct to point out that we should use the correct gene nomenclature throughout. We have changed ApoAV to APOA5 in all places.

6. We have taken out the term “Mendelian randomization” from the abstract and discussion.

Background

7. We have modified the references in line with the reviewer’s comments.

Results and Discussion

8. We have referred to table 1 at the end of the first paragraph of the methods section. It is not meant to be a result but merely a descriptive table so that the readers can see at a glance the features of the two populations.

9. This sentence now reads: “The presence of the –1131C allele (TC and CC subjects combined) is associated with higher triglyceride concentrations in the Pune Indians by 0.25 standard deviations (95% CI, 0.12-0.39; p=0.0003) and in the Plymouth EarlyBird study UK whites by 0.5 standard deviations (95% CI, 0.11-0.89; p=0.01) relative to TT subjects.”

10. The “(19%)” and “(42%)” figures in the above sentence referred to the fact that 0.25 SDs and 0.5 SDs represented 19% and 42% higher triglyceride values in (TC+CC) individuals compared to TT homozygotes. We have now taken these values out to avoid confusion.

11. We thank the reviewer for pointing out that we need to be more consistent in naming our studies, and, in line with earlier comments, have changed “Asian Indians” to “Pune Indians” when we are referring to the Indian subjects in our study.

12. Triglyceride levels are affected by factors such as age and gender in our study but genotype is not associated with age and gender (all p >0.05). Therefore p values and size effects only change very slightly when adjusting for these things. We have added a sentence to this effect in the results: “We also performed these analyses whilst correcting for age and sex but the p values and effect sizes changed very little because genotypes were not associated with age or sex.”

13. The reviewer raises an important point about Mendelian randomization. As the APOA5 -1131C allele is reported to be in strong LD with the APOC3 SstI variant, it clearly could be either gene, or a combination, that is resulting in differences in triglyceride levels. The combination of alleles does result in a net, genetically determined change in triglyceride concentrations, which may still mean these
variants can be used for Mendelian randomization studies, with the caveat that there may be additional effects of these variants on related measures such as HDL and APOA5 concentrations themselves. To avoid confusion however, we have excluded the sentence about MR in the discussion.

14. We thank the reviewer for pointing out that there is appreciable LD between APOA5 and APOC3 SNPs. The paper by Olivier et al. found that the APOA5 -1131C allele falls on the haplotype defined by the minor alleles of the APOC3 SstI, -482 and -455 SNPs 85% of the time. We therefore genotyped one of these; the APOC3 SstI variant, in 175 subjects. The D’ and r² values between APOA5 -1131TC and APOC3 SstI were 0.41 and 0.07 respectively. This suggests that our highly significant associations between APOA5 -1131TC and triglyceride concentrations are not driven by LD with the APOC3 SstI SNP. We do acknowledge that further work is needed to more comprehensively assess the role of variations in the APOC3 region in Indian subjects but we feel that this in depth work is a subject for a separate paper. We have added the following paragraph to the results section, page 10:

“We next assessed the extent of linkage disequilibrium between the APOA5 -1131T>C SNP and the APOC3 SstI SNP in Pune Indians and observed D’ and r² values between these two SNPs of 0.41 and 0.07 respectively. This suggests that the highly significant association between the APOA5 -1131T>C variant and triglyceride concentrations are not driven by LD with the APOC3 SstI SNP. Further work is needed to assess more comprehensively the role of other common variations in the APOC3 gene in Indian subjects”.

Reviewer 3
Major revisions
1. We thank the reviewer for pointing out the recent articles that show that the relationship between APOA5 alleles and triglycerides is more complicated than it at first appears because of the different effects on APOA5 levels. We have added in the appropriate references and the following sentence to the background: “Supporting this, the –1131C allele is associated with lower APOA5 concentrations and higher triglyceride concentrations whereas the 19W allele is associated with higher APOA5 and higher triglyceride concentrations, relative to subjects homozygous for the less common allele”. As outlined in the responses to reviewer 2 we have also analysed the LD structure between APOA5 -1131T>C and the APOC3 SstI variants and added the following paragraph to the results: “We next assessed the extent of linkage disequilibrium between the APOA5 -1131T>C SNP and the APOC3 SstI SNP in Pune Indians and observed D’ and r² values between these two SNPs of 0.41 and 0.07 respectively. This suggests that the highly significant association between the APOA5 -1131T>C variant and triglyceride concentrations are not driven by LD with the APOC3 SstI SNP. Further work is needed to assess more comprehensively the role of other common variation in the APOC3 gene in Indian subjects”.

"We next assessed the extent of linkage disequilibrium between the APOA5 -1131T>C SNP and the APOC3 SstI SNP in Pune Indians and observed D’ and r² values between these two SNPs of 0.41 and 0.07 respectively. This suggests that the highly significant association between the APOA5 -1131T>C variant and triglyceride concentrations are not driven by LD with the APOC3 SstI SNP. Further work is needed to assess more comprehensively the role of other common variations in the APOC3 gene in Indian subjects”.

Review
2. We thank the reviewer for pointing out that there are a large number of CC homozygote subjects in the Pune Indian subjects and so we have included details of these subjects as a separate column in table 3.

3. We have modified table 4 to include measures of HDL, LDL and total cholesterol.