**Author's response to reviews**

**Title:** Association of the apolipoprotein A5 gene -1131 T>C polymorphism with fasting blood lipids: a meta-analysis in 37859 subjects

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**Author's response to reviews:** see over
List of changes

Referee: 1

1. We agree the referee 1’s first question that “Association with TG (and partially LDL) is highly heterogeneous, not so with TC. Can the authors speculate on the reasons (biological or otherwise) of this heterogeneity? Could this due to more sensitivity to lab measurement errors for some measurements, unexplained environmental perturbations or other effects? From Table 1, the association with TG shows remarkably similar associations and effect sizes in all groups tested, while TC is clearly more associated in East Asians - so what is heterogeneity reversed in this case? Finally, are there local differences in LD patterns that can explain such heterogeneity.”

The changes were as follows:

(1) Line 337-340 of page 16: added “The different effects of the -1131 T>C polymorphism on TC, TG, HDL-C, and LDL-C suggest that pathogenesis may different in different lipid fractions and the -1131 T>C polymorphism may exert varying effect.”

(2) Line 342-352 of page 16: added “Significant heterogeneity was respectively found across the studies for TG, LDL-C, and HDL-C. Prominent sources of heterogeneity include: ethnic heterogeneity, different study design, gender difference, and healthy status of the subjects included in the meta-analysis, etc. To explore the potential source of the observed heterogeneity, we performed subgroup analyses stratified by the characteristics of the subjects. For TG, the heterogeneity was effectively decreased or removed in the subgroup analyses. For LDL-C and HDL-C, significant heterogeneity was still observed in some subgroups. The sources of heterogeneity were further evaluated by Galbraith plot. Outlier studies were identified as the main contributors of heterogeneity by using the plot. The heterogeneity was effectively removed or decreased after exclusion of these outlier studies.”.

2. We agree the referee 1’s second question that “What have the more recent waves
of GWAS added to knowledge of this locus? particularly, has the exhaustive analysis of the locus through GWAS arrays that are more comprehensively representative of this locus revealed additional variants with less heterogeneity/more significant evidence for association?”.

Because a meta-analysis for GWAS of APOA5 gene has been published (Willer CJ, Sanna S, Jackson AU, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet* 2008, 2:161-169.), I did not perform the meta-analysis for the GWAS of APOA5 gene in this meta-analysis. But I provided data from existing GWAS meta-analyses to support my findings in the part of the discussion of this meta-analysis.

The changes were as follows:

(1) Line 331-333 of page 16: added “Genome-wide association studies has suggested that the polymorphisms in or near the APOA5 gene are among the strongest known genetic determinants of triglyceride concentration [48, 49].”.


3. We agree the referee 1’s third question that “Page 6 last sentence - what is the minimum sample size for the subgroup analyses?”.

Meta-analysis is a powerful tool for summarizing the results from different studies by producing a single estimate of the major effect with enhanced precision. It can overcome the problem of small sample size and inadequate statistical power of genetic studies of complex traits, and provide more reliable results than a single case-control study. We did not limit the minimum sample size for subgroup analyses. But every study included in this meta-analysis has at least 20 subjects.

**Referee: 2**

1. We agree the referee 2’s first question that “Please provide some sense of the power involved in the analyses so that in particular effects in small sample sizes can be more readily interpretable. This is of particular import where the authors perform subgroup analyses and analyses for LDL. What kind of effect sizes are they powered to find?”.

The statistic software (Review Manager 5.0 software and MIX 1.7 software) used for this meta-analysis have not the function for statistic power analysis. We have not other software to perform this analysis. So we can not provide the results of statistic power analysis, which is a deficiency of this meta-analysis. We have discussed this as the limitation of this paper in the part of the discussion.

The changes were as follows:

(1) Line 195-197 of page 9: added “34193, 20961, 19860, and 29588 subjects were included in comparing the difference in blood TG, TC, LDL-C, and HDL-C, respectively (Table S2 of additional file 2).”.

2. We agree the referee 2’s second question that “The additive model should be relatively well powered to pick up associations if the underlying genetic model is not necessarily dominant. Did analysis of the data using the additive model make any difference in the results? If so this would be important to note even if they think it is power based as many groups use an additive model for analysis and thus the results presented in the paper may be more interpretable to others if the dominant and additive model gave essentially similar results. Would emphasize in the discussion that the dominant model was chosen for analysis simply for power otherwise the reader may over interpret this to mean this is the genetically proven mechanism of action at this locus.”.

Because the frequencies of the minor allele homozygous were low, most of the studies included in this meta-analysis only provided mean blood lipid levels of all allele C carriers and did not respectively provide mean blood lipid levels of homozygote CC and heterozygote CT. In the 37 eligible studies, mean TC, TG, LDL-C, and HDL-C level of homozygote CC and heterozygote were respectively provided only in 8, 12, 8, and 10 studies. The frequencies of CC in some of these studies are 0. So we only employed a dominant model for this meta-analysis. We think the data is not enough to perform other genetic model analyses, such as additive model analysis.

The change was as follows:

(1) Line 303-307 of page 14: added “Because the frequencies of the minor allele homozygous were low, most of the studies included in this meta-analysis only provided mean blood lipid levels of all allele C carriers
and did not respectively provide mean blood lipid levels of homozygote CC and heterozygote CT. To ensure adequate statistical power, we employed a dominant model for this meta-analysis.

3. We agree the referee 2’s third question that “Please discuss more the possible causes of heterogeneity related to studies being outliers for analyses and if possible testing of those hypotheses like was done for small studies in the bias argument.”

The change was as follow:

(1) Line 342-352 of page 16: added “Significant heterogeneity was respectively found across the studies for TG, LDL-C, and HDL-C. Prominent sources of heterogeneity include: ethnic heterogeneity, different study design, gender difference, and healthy status of the subjects included in the meta-analysis, etc. To explore the potential source of the observed heterogeneity, we performed subgroup analyses stratified by the characteristics of the subjects. For TG, the heterogeneity was effectively decreased or removed in the subgroup analyses. For LDL-C and HDL-C, significant heterogeneity was still observed in some subgroups. The sources of heterogeneity were further evaluated by Galbraith plot. Outlier studies were identified as the main contributors of heterogeneity by using the plot. The heterogeneity was effectively removed or decreased after exclusion of these outlier studies.”

4. We agree the referee 2’s fourth question that “Please provide more analysis of whether the differences in effects in the subgroup analyses differ from the overall analysis. This might be of particular import as it may suggest instances where possible interactions with the effect exist and what the interaction variable might be and would increase the impact of this work.”

Any meta-analysis has its limitation. Our meta-analysis also has limitations. Lacking of the original data of the reviewed studies limited our further subgroup analyses in which potential interactions between gene-gene, gene-environment can be evaluated. We have discussed this question as the limitations in the part of the discussion of this meta-analysis: “Lacking of the original data of the included
studies limited our further evaluation of potential interactions because the interactions among gene-gene, gene-environment and even different polymorphic loci of the same gene may modulate blood lipid levels. For example, we did not perform the stratification analyses by environmental factors such as diet, exercise, and smoking. A more precise analysis could be conducted if more detailed individual data were available, which would allow for an adjusted estimate. Second, we did not perform a meta-analysis for haplotype analysis of the APOA5 gene, because a very limited number of studies were available.”

The change was as follow:

(1) Line 407-409 of page 19 added: “Second, we did not perform a meta-analysis for haplotype analysis of the APOA5 gene, because a very limited number of studies were available.”

Associate editor comments

1. We agree the Associate editor comments that “There seems to be minor issue raised by reviewers concerning primarily heterogeneity. Authors should also provide data from existing GWAS meta-analyses to support their findings. Authors should also cite and discuss their findings in light of a recent paper in the Lancet reporting a meta-analysis for -1131 T>C polymorphism lipid profile and cardiovascular diseases.”

The changes were as follows:

(1) Line 342-352 of page 16 added “Significant heterogeneity was respectively found across the studies for TG, LDL-C, and HDL-C. Prominent sources of heterogeneity include: ethnic heterogeneity, different study design, gender difference, and healthy status of the subjects included in the meta-analysis, etc. To explore the potential source of the observed heterogeneity, we performed subgroup analyses stratified by the characteristics of the subjects. For TG, the heterogeneity was effectively decreased or removed in the subgroup analyses. For
LDL-C and HDL-C, significant heterogeneity was still observed in some subgroups. The sources of heterogeneity were further evaluated by Galbraith plot. Outlier studies were identified as the main contributors of heterogeneity by using the plot. The heterogeneity was effectively removed or decreased after exclusion of these outlier studies.”.

(2) Line 331-332 of page 16: added “Genome-wide association studies has suggested that the polymorphisms in or near the APOA5 gene are among the strongest known genetic determinants of triglyceride concentration [48, 49].”.

(3) Line 333-340 of page 16: added “Our findings also are consistent the results from a meta-analysis by Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration, in which they reported that the APOA5 -1131 T>C polymorphism is associated with increased blood TG and lower HDL-C, and is not associated with increased LDL-C [50]. The different effects of the -1131 T>C polymorphism on TC, TG, HDL-C, and LDL-C suggest that pathogenesis may different in different lipid fractions and the -1131 T>C polymorphism may exert varying effect.”.


Other Changes

(1) Line 356 of page 17: changed “[48]” to “[51]”.

(2) Line 358 of page 17: changed “[48]” to “[51]”.

(3) Line 386 of page 18: changed “[49]” to “[52]”.

(4) Line 389 of page 18: changed “[49]” to “[52]”.


(6) Line 627 of page 29: changed “48” to “51”.

(7) Line 630 of page 29: changed “49” to “52”.