Reviewer’s report

Title: Discovery and Replication of SNP-SNP Interactions for Quantitative Lipid Traits in over 60,000 Individuals

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Reviewer: Daniel Rotroff

Reviewer’s report:

The manuscript by Holzinger et al. describes a discovery/replication approach for identifying SNP-SNP interactions related to lipid variation in a large cohort of 60,000 individuals. It is thought that SNP-SNP interactions play an important biological role, but finding them is difficult due to the large numbers of SNP combinations that require testing. The purpose of the manuscript was two-fold: 1) reveal novel insights into the genetic etiology of lipid levels, and 2) develop a pipeline for computationally efficient interaction analysis. The paper did a very good job of addressing the 2nd goal; however, I think additional attention should be paid to describing the novel insights into the genetic etiology, and how these findings fit into the existing literature. Overall, this manuscript is well written and the methods will be impactful to the field.

Major Revisions:

1. Page 11, line 268-269 of the methods, the authors state '…individuals of self-reported European ancestry, subsequently verified using principal component analyses.' How was this verified? Were the clustered with other mixed race cohorts or was this verified by only clustering this group?

2. Page 12, line 296, "SNPs with a main effect p<0.001 based on a previous GWAS regression…” This seems like quite a liberal threshold, how was this selected?

3. Page 13, lines 309-314, the authors describe covariates that were included in the model. It is not clear whether these were selected to be included in the model (e.g. backwards selection) or whether they were forced into model. If they were forced into the model, were they all significantly associated with the lipid phenotypes?

4. In the same section, it states that the top 10 PCs were included as covariates, was including 10 PCs necessary since these were all from self-reported European Ancestry (which was stated previously as being validated by PCA). How much variation was explained after the first PCs and are you wasting degrees of freedom by incorporating so may PCs?

5. Page 13, lines 317-318, "We adjusted the threshold based on the number of estimated independent models that were tested." How was this determined?
6. P 14, lines 326-331, The purpose of the 'proxy models' are not clear. Can you provide the rationale for why these were generated?

7. Were all the replication cohorts also of European ancestry?

8. Page 14, lines 346-347, "Briefly, each cohort tested for population stratification and relatedness, adjusting accordingly." First of all, this sentence should be reworded,? However, more importantly, how was this performed? Was this done the same as for the discovery set?

9. Page 16, line 387, Was the LD pruning only done for the main effect?

10. What are the biological/translational implications of these findings? The authors mention the top replicating SNPs, but do not address the biological plausibility of these findings. For instance, CETP has been previously documented as being associated with lipids, but this is not mentioned in the discussion. Were any of the replicated interactions in genes that were previously identified in single SNP association analyses? Are any of these interactions pointing to new biology that would not be detected in a single SNP GWA?

Minor Revisions:

1. Page 11, line 275, references Table 3. I believe this is correct to reference table 3, but this is the first table mentioned in the manuscript, so this should be reordered.

2. Page 12, lines 293-294, "missing genotype rate > 95%". Should this be SNPs were removed if had >5% missingness? It seems unlikely that SNPs missing 94% would have been included, but this should be clarified.

3. Page 14, line 346, I believe Table 1 is actually referring to Table 3. However, as described above, this should probably be reordered to be Table 1.

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