Author's response to reviews

Title: ANGPTL4 variants E40K and T266M are associated with lower fasting triglyceride levels in Non-Hispanic White Americans from the Look AHEAD Clinical Trial

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Author’s response to reviews: see over
Dear Dr. Bluher,

We would like to thank the reviewers for contributing their time, expertise, and attention to our manuscript. Both reviewers felt the revised manuscript was substantially improved, however they requested modification and revision. We now submit a revised manuscript with additional experimental data included in the text and other important changes. We now submit the revised manuscript for consideration for publication in the *BMC Medical Genetics*.

We have made additional changes to meet the requirements of *BMC Medical Genetics*. We provide a version of the manuscript with the changes tracked to allow for easier review.

Our response to each review comment is listed below.

Sincerely,

Melissa C Smart-Halajko, Ph.D
Gordon Huggins, MD

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**Reviewer 1** “An article of importance in its field...The manuscript is substantially improved by the adaptation based on the reviewer comments... A few issues still should be considered:”

1) The authors should present the results from the association study in the subgroup of subjects without lipid-lowering medication in the manuscript.
We now present results from the association study on subjects not receiving a lipid-lowering medication at the bottom of Table 3 for E40K and to the bottom of Table 4 for T266M.

2) **Units should be consistently presented in SI-units.**

We now present the data in SI units.

3) **Genotypic means presented in the analysis adjusted by fasting glucose are slightly different than in table 4 of the manuscript. Please double-check.**

The genotypic means presented to the reviewers in the prior letter were adjusted for fasting glucose, which is why the values were slightly different than in Table 4 of the manuscript. We did not adjust for glucose in the manuscript. As there was no effect of glucose on the associations we did not adjust for glucose to keep the manuscript in line with the covariates set by the Look AHEAD committee. We have double checked our findings.

4) **Page 7/results section: please do not consider r2=0.05 as partial LD.**

We now state that there is no LD between E40K and T266M.

5) **Please include in the discussion that subjects with T2D on a heterogeneous antidiabetic medication were included in the analyses which could have impact on the association results.**

We thank the reviewer for this excellent suggestion. We have added to the “limitations” paragraph of our discussion the following: “While we included anti-diabetic medication use as a covariate in our analyses an additional limitation is that we were unable to control for the effect of specific anti-diabetic medications and doses, which may have different effects on triglyceride levels.”

6) **Needs some language corrections before being published**

We have further reviewed the manuscript and made corrections to the language.

**Reviewer 2** “An article of outstanding merit and interest in its field” The presented article by Melissa C Smart-Halajko describes effects of two ANGPTL4 non-synonymous variants E40K and T266M on triglyceride levels in T2D. The authors used for their investigations the Look AHEAD population and included in their study 2708 type 2 diabetes individuals. The revised manuscript benefits from a clear presentation of the working hypothesis and a well structured re-written discussion. The revised version is therefore now generally well-written. The authors answered most of the questions adequately. However, some issues need to be considered before publication.”

**Major comments:**

1. The reviewer is afraid the authors are confused about definition of linkage disequilibrium (LD). Since r2 is relevant for association studies r2 = 0.05 is no linkage at all. Therefore, it is not clear to the reviewer why the authors talk about partial linkage. Please correct this.
We now state there is no linkage disequilibrium between E40K and T266M.

2. **Table 2 still shows genotype frequencies, not allele frequencies as explained in legend.**

We have changed the Table 2 legend to read “genotype frequencies”.

3. **How many of the individuals are on lipid lowering medication?**

There were 1,424 subjects receiving a lipid lowering medication and 1,177 not receiving a lipid lowering medication. We have added to the results section the following sentence: “Of the 2601 study participants, 1,424 were taking a lipid lowering medication”.

**Minor comments:**

1. **Please present SI units throughout the entire manuscript.**

We have converted all data to SI units.