Author's response to reviews

Title: Sequence variation in telomerase reverse transcriptase (TERT) as a determinant of risk of cardiovascular disease: the Atherosclerosis Risk in Communities (ARIC) Study

Authors:

Jan Bressler (jan.bressler@uth.tmc.edu)
Nora Franceschini (noraf@unc.edu)
Ellen W Demerath (ewd@umn.edu)
Thomas H Mosley (tmosley@umn.edu)
Aaron R Folsom (folsom@epi.umn.edu)
Eric Boerwinkle (Eric.Boerwinkle@uth.tmc.edu)

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Author's response to reviews: see over
Dear Dr. Pare:

My colleagues and I are submitting a revised manuscript entitled “Sequence variation in telomerase reverse transcriptase (TERT) as a determinant of risk of cardiovascular disease: the Atherosclerosis Risk in Communities (ARIC) Study” in response to the referees’ comments. We would like to thank the reviewers for their many helpful suggestions, and we have indicated our responses to the referees’ comment below organized by reviewer.

Response to Reviewer 1:

Minor Essential Revisions

1) The background section in the Abstract should be shortened.

The background section of the Abstract has been decreased by two lines.

2) More information should be added to the methods section in the Abstract: mention that SNPs were genotyped and how, add mortality to the outcomes.

The genotyping method has been added to the abstract, as well as mortality as an outcome of the study.

3) Add 95% confidence intervals to the hazard rate ratios in the Results sections of the Abstract and of the manuscript.

The confidence intervals have been added to the hazard rate ratios in the Results section of the Abstract, and also where they appear in the Results and Discussion sections of the manuscript.

4) Clarify the objectives at the of the Background section: precise why the 6 SNPs were selected (close to/on the TERT gene, including the one previously associated with telomere length…), add mortality to the list of outcomes.
The information that the *TERT* variants were selected as haplotype tagging SNPs, and mortality as an outcome has been added to the last paragraph of the Background section.

5) In the Methods section, precise the definition of stroke – it is not clear how ischemic strokes were identified/separated from hemorrhagic strokes (imaging?), what was the proportion of patient with imaging available?

The description of the method of ascertainment and classification of strokes in the ARIC study has been expanded and a reference added.

6) Clarify how the 6 SNPs were selected – were they all in the TERT gene, if not were they all within a certain distance? Were all SNPs within that distance included? (second paragraph, Methods section).

The location of the 6 SNPs within the *TERT* gene or proximal promoter, and their selection as haplotype tagging SNPs to capture the genetic diversity among four HapMap populations has been added to the Methods section.

7) What does “European Americans” mean in the Methods section, end of second paragraph? Was Hardy-Weinberg equilibrium verified in all ethnicities?

The term “European American” has been removed from the end of the second paragraph in the Methods Section. Hardy-Weinberg equilibrium was tested for all of the genetic variants separately by race as is now described in the statistical analysis section.

8) Describe the models chosen in the statistical analysis section – variables should be clearly stated and differences between outcomes should be explained (variables for model 2 are not the same in the table legends).

The models chosen for the Cox regression analyses have been added to the statistical analysis section. Model 2 now includes the same covariates for the analyses of incident coronary heart disease, incident stroke, and mortality as suggested by the Reviewer. As a result, some of the data displayed in Tables 5 and 6 has been slightly changed without any impact on the conclusions made in the manuscript.

9) In the last paragraph of the Results section, the first part of the sentence at line 221-222 should be in the Introduction or Methods section instead.

The sentence previously in the last paragraph of the Results section has been moved to the second paragraph of the Background section.

10) There is an error in the name of the SNP at line 250 (third paragraph of Discussion), it should be rs2853668 instead of rs285366.

The error in the SNP name has been corrected.
11) The authors should try to explain the discrepancy between their results and the WHG study for rs2853668 (protection vs increased risk) – related to the different ethnicity? Was this SNP linked to shorter or longer telomere length in any previous study?

Explanations for the reversal of the association of rs2853668 with incident ischemic stroke in the WHGS and ARIC studies have been added to the Discussion (lines 254-262). These include differences in linkage disequilibrium between whites and African-Americans that could lead to correlation between rs2853668 and a second protective or risk locus, respectively, if rs2853668 is not itself the true causal variant responsible for the association in either race. Other possibilities are a chance finding, or variation in other genetic or environmental factors associated with cerebrovascular disease in the two cohorts.

12) At the end of the Discussion, other genes associated with telomere length are mentioned, is there a reason why SNPs in those genes were not tested for association with CVD and mortality in the current study (were they available on the array)? Why choose only TERT?

Other genes involved in telomere biology were not included on the custom Illumina array.

13) In the Conclusion, it is speculative to imply that the study suggests that the genetic variations reported “may contribute to the etiology” of cardiovascular disease, “may be associated” would be a better formulation.

The sentence has been changed to “interindividual variation in a gene implicated in cellular aging may be associated with cardiovascular disease” as suggested by the Reviewer.

14) In Table 2, the third and the fourth SNPs were inverted for White – impairs readability.

The third and fourth SNPs have been reversed on Table 2.

15) The variable in model 2 are not the same depending on the outcomes (alcohol and cholesterol are only in some – legends of the tables), is that a mistake – why would the authors chose to adjust for different variables (see point 8)?

As indicated in the response to point 8 above, the variables in model 2 are now the same for the analyses of incident coronary heart disease, incident stroke, and mortality as suggested by the Reviewer. As a result, some of the data displayed in Tables 5 and 6 has been slightly changed without any impact on the conclusions made in the manuscript.

Discretionary Revisions

1) False-discovery rate analysis and power calculation could be performed to help in the interpretation of the results.

The power in the ARIC study to detect the moderate effect sizes for the variants identified in the WGHS has been added to the Discussion (lines 237-242).
2) The Discussion could be shortened, the second paragraph includes a part that is repeated in the Background and the fourth paragraph includes a lot of details for what appears to be a secondary outcome in the study (mortality).

The redundant information in the second paragraph of the Discussion has been removed, and the fourth paragraph has been streamlined.

3) The authors could mention in the limitations that telomere length was not measured, which limits the interpretation of the finding in terms of explaining the link with cardiovascular disease.

The absence of telomere length measurements in the ARIC study has been added as a limitation in the Discussion (lines 289-292).

Response to Reviewer 2:

Major Changes

Background

It is unclear why only TERT was focused on and not TERC? Both the reverse transcriptase and the RNA component are required to extend telomeres. RS10936599 (TERC gene) was the strongest association in the Codd et al 2013 paper. Please address why this was specifically selected.

Other genes involved in telomere biology such as TERC were not included on the custom Illumina array used to generate the genotyping data used in this manuscript.

Methods

A power calculation would be useful. Effect sizes are expected to be minute and perhaps the study was not powered enough to detect some associations.

The power in the ARIC study to detect the moderate effect sizes for the variants identified in the WGHS has been added to the Discussion (lines 237-242).

Discussion

The discordant findings between the Women’s Genome Health Study (WGHS) reported by Zee et al., (2011) and the present ARIC study warrant further explicit discussion. ARIC had many more events and a longer follow-up period, yet associations that were significant in WGHS were non-significant in ARIC.

The associations reported by Zee et al. (2011) for 154 polymorphisms in 11 telomere-associated genes and cardiovascular disease were not corrected for multiple comparisons, so it is possible that some proportion of the reported results may have been significant by chance. Since the associations found for the TERT variants in the ARIC study also did not survive Bonferroni
correction, this could be the source of the discordant findings. Another possibility is that the discrepancy could be due to the different case definitions used in the two studies for incident coronary heart disease. WGHS only included nonfatal myocardial infarction, while ARIC ascertained both myocardial infarction and fatal coronary heart disease. These possible reasons have been added to the Discussion.

Minor Changes:

General

Report confidence intervals with hazard ratio.

The confidence intervals have been added to the hazard rate ratios in the Results section of the Abstract, and also where they appear in the Results and Discussion sections of the manuscript.

Abstract

Line 54: How is the variant associated with mean telomere length? Is it associated with increased/decreased length?

The A allele of rs2736100 was associated with decreased telomere length. This is described in the last paragraph of the Background section.

Methods

Line 144: Should read “Six SNPs” rather than “Six (6) SNPs”.

This error has been corrected.

State whether the assumptions of the cox proportional hazards models were met.

The proportional hazards assumption was met for all of the TERT SNPs tested individually by race with the exception of rs2736122 (model 1) and rs4246742 (models 1 and 2) when analyzed in whites for association with incident CHD, and rs2853668 (model 1) in the analyses of mortality in whites. This has been added to the statistical analysis section.

Specify in manuscript text, report the variables adjusted for in the cox proportional hazards models.

The variables adjusted for in the two Cox proportional hazards models have been added to the statistical analysis section. Model 2 now includes the same covariates for the analyses of incident coronary heart disease, incident stroke, and mortality. As a result, some of the data displayed in Tables 5 and 6 has been slightly changed without any impact on the conclusions made in the manuscript.
Specify in manuscript text, the statistical tests used to generate p-values in the provided tables.

The statistical tests used to generate the p-values have been added to the table legends (Tables 1, 3, 4, 5, and 6). Comparison of continuous variables between groups by t-tests and categorical variables by chi-squared tests has been added to the statistical analysis section.

Results

Tables – 4,5,6 – please indicate the number of events in each ethnic group within the table.

The number of events has been added for each ethnic group to Tables 4, 5, and 6.

Discussion

A further limitation is the lack of a replication cohort. As the reported p-values are quite modest, replicating these findings with another population would strengthen the reported results.

This has been addressed in the Conclusion where it is stated that replication of the possible associations between TERT variants and cardiovascular disease in other cohorts is warranted.

Discretionary Revisions

Background

A brief comment regarding the bi-racial makeup of the ARIC study would be beneficial.

Relatively few studies have looked at telomerase variants in an African-American population and it is indeed a unique aspect of the report.

The absence of studies in which the association between telomerase variants and cardiovascular disease has been examined in an African-American population is now noted in the Background section.

Response to Reviewer 3:

Major Comments:

1- The weak association between rs2736122 and rs2853668 and myocardial infarction and stroke respectively and the fact that these associations did not remain significant after correction for multiple comparisons suggests that these associations could have been detected by pure chance or that the study lacks power to detect a true but weak effect of these SNPs. In both cases I believe that the findings of the manuscript would not generate sufficient interest in most readers of the BMC Medical Genetics journal.

We are in agreement that it is possible that the modest associations between the two TERT variants and coronary heart disease and stroke could be due to chance. The role of chance in the
findings of the manuscript has been addressed twice in the Discussion (lines 242-245, lines 260-262).

2- The materials and methods section should be shortened.

The genotyping section has been shortened, but the description of the Atherosclerosis Risk in Communities (ARIC) Study and the statistical analysis section has increased in response to points raised by Reviewers.

3- Please specify which risk factors were adjusted for in the second model.

The variables adjusted for in the two Cox proportional hazards models have been added to the statistical analysis section. Model 2 now includes the same covariates for the analyses of incident coronary heart disease, incident stroke, and mortality. As a result, some of the data displayed in Tables 5 and 6 has been slightly changed without any impact on the conclusions made in the manuscript.

Minor Comments:

4- The terms white and European Americans are used in the manuscript. The term European American would be preferable.

Only the term white is currently used in the manuscript.

Please feel free to contact me if you have any questions or require any additional information.

Sincerely,

Jan Bressler, Ph.D.
Assistant Professor
Human Genetics Center
School of Public Health
University of Texas Health Science Center at Houston
1200 Pressler Street
Houston, Texas 77030

Telephone: (713) 500-9919
Fax: (713) 500-0900
E-mail: jan.bressler@uth.tmc.edu