ARIC Manuscript Proposal # 1702

PC Reviewed: 10/12/10	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Sequence variation in telomerase reverse transcriptase (*TERT*) as a determinant of lifespan and risk of cardiovascular disease: The Atherosclerosis Risk in Communities (ARIC) study

b. Abbreviated Title (Length 26 characters): Telomerase and heart disease

2. Writing Group:

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Other investigators welcome.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __x___ **[please confirm with your initials electronically or in writing]** JB

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3. Timeline: Statistical analyses: October 2010 –December 2010
Manuscript preparation: January 2011 –February 2011
Manuscript revision: March 2011
Manuscript submission: April 2011

4. Rationale: Telomeres are DNA-protein complexes that protect the ends of chromosomes. Telomerase maintains telomere ends during DNA replication by catalyzing the addition of short telomere repeats (TTAGGG). The enzyme is comprised of a protein with reverse transcriptase activity that is encoded by the telomerase reverse transcriptase (*TERT*) gene, and an RNA component which serves as a template for the telomere repeat after recognition of a single stranded G-rich primer[1]. The expression of telomerase is normally repressed in somatic cells leading to a gradual shortening of telomeres and cellular senescence with aging[2, 3]. Heritability of telomere length in humans has been reported to range from 36% - 90%[4, 5].

The relationship between telomere length and aging and longevity has been investigated previously including in several epidemiological studies. A negative correlation between telomere length and age has been consistently observed when examined in multiple tissues[3, 6-11]. In addition, in a sample of 901 Amish men and women who were recruited for the Amish Family Osteoporosis Study, telomere length was positively correlated with increased lifespan[11]. Further support for these observations was provided in a cohort of 143 unrelated individuals 60 years or older in which subjects with shorter telomeres showed a 3-fold and 8-fold increase in mortality rate from heart disease and infectious disease, respectively, when compared to those in the upper 50% of the distribution[12]. In contrast, Bischoff et al. found no correlation between telomere length and survival in a sample of 812 individuals from 3 different Danish study populations whose mean age was 81 years[13]. Similar results were reported in a study of participants in the population-based Health ABC Study of 3,075 healthy men and women aged 70-79 years in which neither overall survival or death from any underlying cause including cardiovascular disease was associated with telomere length[14].

Telomere length has also been shown to be associated with susceptibility to cardiovascular disease[15]. When mean telomere length was measured in 10 patients with severe coronary artery disease and compared to that observed for 20 controls, the size was significantly reduced and equivalent to that found in individuals without heart disease who were 9 years older[16]. Significantly shorter telomeres were also detected in leukocyte DNA from 203 subjects who had had a myocardial infarction before the age of 50 than in samples from 180 controls[17]and in 238 South Asian Indian coronary artery disease cases when compared to controls[18]. As evidence in favor of the hypothesis that chronic heart failure can be viewed as a condition in which there is accelerated biological aging, the median leukocyte telomere length was shown to be significantly shorter in 620 chronic heart failure patients in the MERIT-HF Study Group than in 183 age- and sex-matched controls[19]. Finally, in a prospective study in which it was possible to assess disease incidence, an inverse relationship between telomere length and diabetes and

fasting insulin level, as well as a 3-fold increased risk of myocardial infarction and stroke in individuals less than 73 years old was found in a subset of randomly selected participants from the Cardiovascular Health Study[20]. Taken together, these results suggest that telomere attrition may play a role in the risk and progression of cardiovascular disease.

The aim of this proposal is to determine whether sequence variation in the *TERT* gene is associated with length of the lifespan and risk of cardiovascular disease including incident coronary heart disease, heart failure, and stroke in participants in the large biracial population-based ARIC cohort.

References:

1. Morin, G. B. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell 59, 521-9 (1989).
2. Harley, C. B., Futcher, A. B. & Greider, C. W. Telomeres shorten during ageing of human fibroblasts. Nature 345, 458-60 (1990).
3. Hastie, N. D. et al. Telomere reduction in human colorectal carcinoma and with ageing. Nature 346, 866-8 (1990).
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15. Serrano, A. L. & Andres, V. Telomeres and cardiovascular disease: does size matter? Circ Res 94, 575-84 (2004).
16. Samani, N. J., Boultby, R., Butler, R., Thompson, J. R. & Goodall, A. H. Telomere shortening in atherosclerosis. Lancet 358, 472-3 (2001).
17. Brouilette, S., Singh, R. K., Thompson, J. R., Goodall, A. H. & Samani, N. J. White cell telomere length and risk of premature myocardial infarction. Arterioscler Thromb Vasc Biol 23, 842-6 (2003).
18. Mukherjee, M., Brouilette, S., Stevens, S., Shetty, K. R. & Samani, N. J. Association of shorter telomeres with coronary artery disease in Indian subjects. Heart 95, 669-73 (2009).
19. van der Harst, P. et al. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. J Am Coll Cardiol 49, 1459-64 (2007).
20. Fitzpatrick, A. L. et al. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. Am J Epidemiol 165, 14-21 (2007).

5**. Main Hypothesis/Study Questions**:

The aims of the study are:

Aim 1: To estimate the frequency distributions of the alleles and genotypes for 10 SNPS in or near the *TERT* gene in the ARIC cohort.

Aim 2: To determine if SNPS in or near the *TERT* gene are associated with time to death in the ARIC cohort. Gender will be included in all analyses as a covariate. Analysis models will also be adjusted for 1) age and gender and 2) BMI, lipid variables, smoking, diabetes status, and hypertension status.

Aim 3: To determine if SNPs in or near the *TERT* gene are determinants of susceptibility to cardiovascular disease by evaluating the association between each genetic variant and time to first myocardial infarction (MI), occurrence of hospitalized heart failure (HF), or stroke. Gender will be included in all analyses as a covariate. Analysis models will also be adjusted for 1) age and gender and 2) BMI, lipid variables, smoking, diabetes status, and hypertension status.

Aim 4: To determine whether the proportion of individuals who do not have either prevalent stroke, CHD, HF or type 2 diabetes at the baseline examination (visit 1) varies by genotype for each of the SNPs in or near the *TERT* gene.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Dependent Variables:

The incidence of CHD, stroke, hospitalization for heart failure, and time of death has been assessed in the entire ARIC cohort.

Incident cardiovascular events will be ascertained by annual telephone contact and surveillance of hospital and death records. Heart failure will be defined as the first HF hospitalization (ICD-9 code 428 in any position), or any deaths where the death certificate included an HF code (code 428, ICD-9 or 150, ICD-10, in any position). Exclusion for HF will be based on reported current medication use for HF, or having manifest HF as defined by Gothenburg criteria stage 3. The follow-up data in this study used to determine incident heart failure will include events from 1987 through December 31, 2004 (variable IN04ISC). CHD will be defined as a definite or probably myocardial infarction, a silent myocardial infarction detected by electrocardiographic interval changes consistent with an intercurrent ischemic event, death due to CHD, or a coronary revascularization procedure. The follow-up data in this study used to determine incident CHD will include events from 1987 through December 31, 2005 (variable IN_05SP). Stroke is defined as a hospital discharge diagnosis that included a cerebrovascular disease code (codes 430-438, ICD-9), a hospitalization for which a cerebrovascular procedure was mentioned in the discharge summary, or if the CT or MRI report showed evidence of acute cerebrovascular disease. The follow-up date in this study used to determine incident stroke will include events from 1987 through December 31, 2004 (variable INCHF04).

Deaths were ascertained through annual phone calls or through ongoing surveillance of health department death certificate files (variable DEAD04). The follow-up time interval is defined as the time between the baseline visit and the date of all-cause death, the date of last contact if lost to follow-up, or the end of follow-up, whichever came first.

Independent variables:

Nine (9) SNPs in or near the *TERT* gene have been genotyped in the entire ARIC cohort at the Broad Institute of MIT and Harvard and will be used as independent variables in this analysis. These SNPs were among a panel of nearly 49,000 SNPs in 2,100 genes previously associated with cardiovascular and metabolic phenotypes selected for inclusion on the custom IBC (ITMAT-Broad-CARe) array as part of the shared Candidate Gene Association Resource (CARe) funded by the National Heart, Lung, and Blood Institute[21]. The association of genetic variation in each of the SNPs and 1) time to death or 2) incident CHD, stroke, or HF over an 18-year period will be analyzed individually. The association between haplotypes in the *TERT* gene and all of the outcome variables will also be examined. For these analyses, haplotypes will be inferred using PHASE software that was designed based on the statistical method developed by Stephens et al. for population-based samples[22]. A codominant model will be assumed. These analyses will be performed by Jan Bressler under the supervision of Eric Boerwinkle; a signed data distribution agreement has been completed. A table showing a list of these polymorphisms is found below:

db SNP ID	Genome Build	Coordinate
rs2242652	36	1333028
rs2736100	36	1339516
rs2736122	36	1310621
rs2853668	36	1353025
rs34062885	36	1313715
rs34094720	36	1346767
rs4246742	36	1320356
rs4975605	36	1328528
rs6863494	36	1322006

Data Analytic Plan:

Caucasian and African-Americans will be evaluated separately by self-reported racial groups. For statistical analysis, comparison of risk factor levels between individuals with the three possible genotypes will be performed using contingency chi-square tests for categorical variables, and t-tests for comparison of group means for continuous variables. Cox proportional hazards models will be used to test the hypothesis that the time until death (Aim 1), or the incidence of CHD, HF, or stroke (Aim 2) does not differ between individuals with different genotypes for each of the SNPs in or near the *TERT* gene. Hazard ratios (HRs) based on the regression coefficients from Cox proportional hazards modeling will be reported. In Aim 4, the frequency of individuals in whom a set of defined co-morbidities is absent in each of three categories based on genotype for each SNP in or near the *TERT* gene will be compared using contingency chi-square tests. Genotype classes will be coded as the number of copies of the minor allele of each SNP assuming an additive genetic model.

Inclusion/Exclusion:

We will exclude by DNA restriction, ethnic group (as appropriate to each field center), and missing data. Other exclusion criteria will include prevalent stroke or TIA, prevalent HF, and prevalent CHD at the initial visit for the analyses of incident disease. For the analysis of time of death, traumatic deaths (accidents, suicide, and violence) will be excluded from the outcome.

Other variables of interest:

In both aims 2 and 3 above, we will determine whether any observed relationships are independent of cardiovascular risk factors and potential confounding factors. These factors will be taken from the baseline examination and will include but are not limited to:

Visit 1- age, gender, exam center, systolic BP, diastolic BP, fasting glucose, lipid variables (total cholesterol, LDL-c, HDL-c, triglycerides), smoking status, use of antihypertensive medications, use of medication to control diabetes, BMI, and alcohol consumption, diabetes case status.

References:

21. Keating, B. J. et al. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS One 3, e3583 (2008).
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7.a. Will the data be used for non-CVD analysis in this manuscript? _x_ Yes __ No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? _x_ Yes _ No (This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? __x__ Yes ____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = "No use/storage DNA"?
__x__ Yes ____ No

9.The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php ___x___ Yes ____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

#1472 Genome-wide association study of age at menarche (Lead author: Ellen Demerath, Ph.D., Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota)

#1474 Genome-wide association study of age at natural menopause and related phenotypes: the CHARGE consortium (Lead author: Ellen Demerath, Ph.D., Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota)

#1511 Genome-wide association of event-free survival and other longevity-related phenotypes: the CHARGE consortium (Lead author: Nora Franceschini, M.D.,

Department of Epidemiology, UNC Gillings School of Global Public Health, Chapel Hill, North Carolina)

There are no other manuscript proposals in ARIC investigating polymorphisms in the *TERT* gene and their relationship to either longevity or risk of cardiovascular disease.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __ __ Yes ___x_ No

11.b. If yes, is the proposal

_x_ A. primarily the result of an ancillary study (list number*)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire. Agree**.**