Leukocyte telomere length and risks of incident coronary heart disease and mortality in a racially diverse population of postmenopausal women

Materials and Methods

Study participants and data collection

The Women's Health Initiative (WHI) enrolled 161,808 post-menopausal women between the ages of 50 and 79 during 1993-1998 at 40 clinical centers nationwide.[1] The WHI design included multifaceted Clinical Trials (CT) and a prospective Observational Study (OS). Randomized CT were conducted to evaluate Hormone Therapy (HT), dietary modification (DM) to reduce total dietary fat, and calcium plus vitamin D supplementation (CaD) with follow-up through 2005 for disease outcomes.[2] Other women, including some who were ineligible or unwilling to participate in the CT, were enrolled in the OS. Women in the CT or OS were then re-consented for participation in the first WHI Extension Study (2005 through 2010); 77% of eligible women agreed to participate.

WHI participants completed an extensive baseline examination including data collection on demographics, vital signs, anthropometrics, medical history, health and lifestyle behaviors, socioeconomic status factors and medication use. Blood samples were collected and laboratory analyses of lipids and measures of inflammation were completed. DNA was collected from consenting participants and stored for future use.

Study outcomes

Evaluation of adjudicated incident events was performed during study follow-up through September 2010. CHD was defined as fatal or nonfatal MI, coronary revascularization (coronary artery by grafting and percutaneous coronary intervention), or CHD death. Participants reporting an event or hospitalization were contacted to provide detailed information and consent to obtain hospital records. Medical records and death certificates were reviewed by trained physician adjudicators to verify all CHD events and cause of death according to pre-specified criteria. The diagnosis of acute MI required overnight hospitalization and was defined according to an algorithm based on standardized criteria of clinical symptoms, cardiac enzymes and troponin levels, and ECG findings. CHD death was defined as death consistent with underlying cause of CHD plus one or more of the following: hospitalization for MI within 28 days prior to death, previous angina or myocardial infarction, death due to a procedure related to CHD, or a death certificate consistent with underlying cause of atherosclerotic CHD. Mortality events were identified using annual mailings and follow-up (proxy questionnaires, returned mailings), and National Death Index searches. Cause of death was ascertained from death certificates and National Death Index searches; the underlying cause of death was adjudicated by physicians. In the cause of death analyses, CVD death included death from definite and probable coronary heart disease, cerebrovascular, and other and unknown cardiovascular causes; cancer death included death from cancer at any site; and other death included all other known causes of death, as well as unknown causes of death that could not be adjudicated based on currently available information.

Sample eligibility and selection of participants for LTL measurement

A total pool of 26,369 white and AfAm women from the HT and OS were eligible for LTL measurement based on the following eligibility criteria: (1) provided consent for DNA research; (2) no self-report of prior myocardial infarction (MI) or revascularization at baseline; (3) had at least 6-months follow-up; and (4) had ≥ 7ug extracted DNA or buffy coat at baseline (or Y1 if baseline is not available, unless had a MI/revascularization event within 6 months after blood draw). Eligible participants were divided into three groups based on their outcome status as of

September 30th, 2010: (1) CHD case group (n=1,452), defined as having a non-fatal MI or revascularization; (2) non-case group (n=21,447), defined as free of MI or revascularization and still alive; and (3) death group (n=3,470). Participants in this study were randomly selected for LTL measurement, stratified by race (White or AfAm), from each group.

Measurement of LTL

DNA collected at baseline (or Year 1) from WHI participants and stored at -80°C was extracted by the 5-prime method (5 PRIME, Inc.; Gaithersburg, MD) and sent in batches over a 1-year period to the Center of Human Development and Aging laboratory at Rutgers for LTL measurement. Each batch included randomly selected case and control samples. The laboratory conducting the LTL measurements was blinded to all characteristics of participants. Quality control consisted of assessment of DNA integrity prior to LTL measurement.[3] DNA integrity was assessed visually after ethidium bromide-stained 1% agarose gel electrophoresis (200 V for 2 hours), and required that DNA appeared as a single compact crown-shaped band that migrated in parallel with the other samples on the gel. Telomere length in kilobases (kb) was measured by the mean length terminal restriction fragments using the Southern blot method as previously described.[3] Each sample was run in duplicate on different gels, and mean LTL was used for statistical analyses. The average inter-assay coefficient of variation for blinded pair sets was 2.0%. Individuals with LTL values exceeding 3 standard deviations from the sample mean were excluded from the analyses, n=3. A total of 1723 samples were sent; of these, 11 were found to have inadequate DNA available and 187 failed stringent quality control (DNA integrity or length >3SD) leaving 1525 samples for analysis.

Covariates

Data used as covariates in these analyses were obtained from the baseline or Year 1 (as appropriate) WHI clinical examination and blood draw. Height and weight were measured at the examination, and body mass index (BMI) was calculated in kg/m^2. Type II diabetes status was determined based on self-reported medication treatment. Hypertension was defined as measured systolic blood pressure above 140 mm Hg or diastolic over 90 mm Hg or use of anti-hypertensive medications. Current smoking status was self-reported, as were socioeconomic factors (education and household income). Lipid-lowering medication use was obtained from a medication inventory. Measurement of fasting lipids [high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), and triglycerides (TRI)], and glucose, C-reactive protein (CRP), and serum creatinine were performed at the University of Minnesota. Serum creatinine was used to estimate glomerular filtration rate (eGFR).[4] These CVD biomarker measurements were available in a subset (n=1135) of the total 1525 participants and include more than 96% of AfAm and 72% of whites.

Statistical Analysis

Primary analyses were performed stratified by race, and weighted using the reciprocal of the participant's sampling probability to account for the sampling design in WHI. Linear regression and analysis of variance were used to evaluate the associations between LTL and baseline participant characteristics. Cox proportional hazards regression was used to estimate the hazard ratio (HR) and 95% Confidence intervals (95%CI) of CHD or all-cause mortality associated with LTL as both as a continuous variable and categorized into race-specific quartiles. The assumption of proportional hazards was evaluated using Schoenfeld residual tests and time varying covariates. While some residual tests suggested potential deviation from proportional hazards for some of the predictors, these predictors were not found to vary significantly by time. 'Model 1' included adjustment for the baseline characteristics and traditional cardiovascular risk factors [except for lipids]: age, BMI category, hypertension, treated diabetes, current smoking, geographic region, and indicators of socioeconomic status

(years of education and household income). A second set of models, 'Model 2,' included additional adjustment for baseline biomarker levels, which were available in a subset (n=1135) of the entire sample--HDL, LDL, ln(TRI), ln(CRP), and in mortality models, eGFR--to evaluate LTL associations independent of cardiovascular risk factors and other biomarkers. Participants were censored at study drop out, event date (CHD or death) or September 2010. In sensitivity analyses in the sample of white women, we tested used Cox proportional hazards models stratified by HT treatment arm. Statistical significance was set at $p<0.05$. Power was estimated using Power Analysis and Sample Size software.[5]

References

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