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Short and long telomeres increase risk of amnestic mild cognitive impairment

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ABSTRACT

Peripheral blood telomere length has been associated with age-related conditions including Alzheimer's disease (AD). This suggests that telomere length may identify subjects at increased risk of AD. Thus, we investigated the associations of peripheral blood telomere length with amnestic mild cognitive impairment (aMCI), a putative precursor of AD, among Mayo Clinic Study of Aging participants who were prospectively followed for incident aMCI. We matched 137 incident aMCI cases (mean age 81.1 years, [range 70.9–90.8]; 49.6% men) by age and sex to 137 cognitively normal controls. We measured telomere length (*T*/*S* ratio) at baseline using quantitative PCR. Compared to the middle *T*/*S* quintile (Q3), the risk of aMCI was elevated for subjects with the shortest (Q1: HR, 2.85, 95% Confidence interval [CI] 0.98, 8.25; $p = 0.05$) and the longest telomere lengths (Q5: HR, 5.58, 95%CI, 2.21, 14.11; $p = 0.0003$). In this elderly cohort, short and long telomeres were associated with increased risk of aMCI. Our findings suggest that both long and short telomere lengths may play a role in the pathogenesis of aMCI, and may be markers of increased risk of aMCI.

1. Introduction

The persistent failure to develop effective disease-modifying therapies for Alzheimer's disease (AD) has shifted the focus of research to identifying biomarkers for early detection in the preclinical or early clinical phase (Albert et al., 2011; Sperling et al., 2011). The rationale is that early detection using biomarkers, combined with effective intervention and treatment (when they become available) will reduce the risk of mild cognitive impairment (MCI) or progression from MCI to dementia. Peripheral blood leukocyte telomere length, a reliable surrogate for telomere length in other tissues, is a potential biomarker for early detection (Brouilette et al., 2007; Friedrich et al., 2000; Takubo et al., 2002). Telomere shortening occurs with increasing age due to repeated incomplete replications over time, and in some studies has been associated with cellular aging, mortality, and with cognitive impairment (von Zglinicki and Martin-Ruiz, 2005). The association of telomere length with cognitive impairment is not established; some studies have reported significant associations with cognition (Honig et al., 2006; Martin-Ruiz et al., 2006; Valdes et al., 2010; Yaffe et al., 2011), whereas others have not (Devore et al., 2011; Mather et al., 2010; Zekry et al., 2010b). While some of these studies have reported associations of telomere length with AD, few investigators have examined the association of telomere length with amnestic MCI, a putative precursor of AD, either prospectively or in a population-based setting. The objective of this study was to investigate the associations of peripheral blood telomere length measured at baseline with incident amnestic MCI (aMCI) in a subset of participants from the prospective, population-based, Mayo Clinic Study of Aging (MCSA).

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2. Methods

2.1. Mayo Clinic Study of Aging

The details of the Mayo Clinic Study of Aging protocols have been previously published (Roberts et al., 2008). Briefly, we enumerated the Olmsted County, Minnesota population aged 70–89 years on October 1, 2004 and March 1, 2008. We randomly selected subjects using an age and sex stratified sampling scheme. We invited eligible subjects to an in-person evaluation that included a semi-structured interview by a nurse to assess memory and functional status and a clinical evaluation by a physician to assess cognition, cerebrovascular disease history, and a full neurological examination. Participants underwent neuropsychometric testing to assess performance in 4 cognitive domains: memory, executive function, language and visuospatial skills. Assessments for each individual were reviewed by the 3 evaluators and a diagnosis of normal cognition, MCI (amnestic MCI [aMCI] or non-amnestic [naMCI]) or dementia was assigned by consensus (Roberts et al., 2008).

2.2. Longitudinal follow-up

Follow-up of the study cohort was performed every 15–18 months using the same protocols as at baseline. To avoid bias, the evaluators are blinded to all previous clinical information when assigning a diagnosis at the follow-up visit.

2.3. Standard protocol approvals, registrations, and patient consents

All study protocols were approved by the Mayo Clinic and Olmsted Medical Center Institutional Review Boards. All subjects provided signed informed consent to participate.

2.4. Study sample

To avoid prevalence bias, we used a nested case-control design. From among 437 subjects who developed MCI during follow-up, we identified all incident aMCI cases who had DNA at baseline ($n = 137$). We individually matched each aMCI case by age at blood draw (±2 years), sex, number of follow-up visits (±2), and duration of follow-up (±2 years) to cognitively normal controls (1:1 matching) selected from 691 eligible controls. A control had to be alive at the diagnosis of aMCI in the case, have DNA at baseline, at least one follow-up, and no diagnosis of aMCI.

2.5. Covariates

Demographic information was assessed by interview. Potential confounders (type 2 diabetes, cardiac disease, hypertension and stroke) were abstracted from the medical record. Body mass index was computed from measured weight and height, and the frequency of moderate physical exercise in the previous year or in midlife was assessed by questionnaire at baseline (Geda et al., 2010). Apolipoprotein E (APOE) genotyping was performed at baseline. The APOE ε4 allele is the most established genetic risk factor for late onset AD.

2.6. Measurement of telomere length

DNA was extracted from peripheral blood obtained at baseline using Gentra AutoPure with Puregene chemistry. Relative telomere length (*T*/*S* ratio) was measured in peripheral blood DNA using the quantitative PCR method and primers to the telomeric hexamer repeats developed by Cawthon to amplify telomeric DNA (Cawthon, 2002). Two master mixes of PCR reagents were prepared using the *T* and the *S* primer pairs. 17 μL of *T* master mix was added to sample well, control well, and standard curve well of the first plate and 17 μL of *S* master mix was added to sample, control and standard curve well of the second plate. For each sample, triplicates of the DNA sample (5 ng/μL) were added to Plate 1 and to the same well position in Plate 2. For each standard curve, one reference DNA sample was serially diluted in TrisEDTAbuffer (TE) by 1:2-fold per dilution to produce 8 concentrations of DNA ranging from 0.78 to 50 ng/mL. Two microliters of each concentration was distributed to the standard curve wells on each plate. The plates were centrifuged for analysis using the ABI 7900 HT instrument. The *T* and *S* PCRs were prepared identically with the exception of the oligonucleotide primers. The final concentrations of the reagents in the PCR were 15 mmol/L Tris–HCl, 0.2 mmol/L each dNTPs, 2.0 mmol/L $MgCl_2$, 1% dimethyl sulfoxide, 150 nmol/L ROX dye, 0.2× SYBR Green I (Molecular Probes), 5 mmol/L DL-dithiothreitol (DTT), 1.25 U AmpliTaq Gold DNA polymerase (Applied Biosystems). The final telomere primer concentrations were tel 1b, 600 nmol/L; tel 2b 900 nmol/L. DNA was quantitated with PICO green. The *S* control gene (*B2-globin* on chromosome 11) concentrations were B-2 globin forward primer (hbg1) 300 nmol/L; B-2 globin reverse primer (hbg2) 700 nmol/L. The primer sequences (5′–3′ were: tel 1b CGGTTTGTTTGGGTTTGGGTTTGGGTTT-GGGTTTGGGTT;

tel 2b GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT;

hbg1 GCTTCTGACACAACTGTGTTCACTAGC;

hbg2 CACCAACTTCATCCACGTTCACC. All PCRs were run on the ABI fast real-time 7900 HT (Applied Biosystems). Other details have been previously described (Cawthon, 2002; Skinner et al., 2012).

The average telomere length for a sample was determined by comparing the intensity of the sample's telomere signal (*T*) to that of a single-copy gene (*S*) of a reference DNA sample. The *T*/*S* ratio was computed using the median *T* and *S* of triplicates for each sample. Samples with a coefficient of variation greater than 10% were re-assayed in triplicate.

2.7. Inter- and intra-assay variability

We determined intra and inter-assay variability using stock DNA from one individual. For intra-assay variability, we compared results from the same specimen on which 25 telomere length measurements were performed on one PCR plate (one plate for telomere PCR, one plate for a standard hemoglobin (HBG) gene PCR). To determine inter-assay variability, 25 telomere length measurements from the same specimen were determined from one pair of telomere and HBG PCR plates and compared to a separate set of 25 telomere length measurements determined from a separate pair of telomere and HBG PCR plates performed on the same specimen. Our intra- and inter-assay variability was 3% and 3.5%, respectively, consistent with other studies.

2.8. Southern blots

We measured telomere lengths in 13 subjects (10 cases with MCI and 3 cognitively normal controls) using Southern blot TRF assays. MCI cases with short or long telomeres based on qPCR displayed heterogeneity in base pair length. There was consistency in telomere length by qPCR and TRF assays. Persons classified as having long telomeres by qPCR (samples 10 through 17) had longer average telomere lengths by Southern blot compared to those classified as having short telomeres (eFigure).

2.9. Statistical analyses

We categorized baseline *T*/*S* ratio into quintiles based on the distribution for the controls. We examined the associations of *T*/*S* quintiles with aMCI using conditional logistic regression models stratified on matched pairs, and adjusted for education (age and sex were matching variables). We also used unconditional logistic regression models that included age at blood draw, sex (both matching variables), and education (base model), to account for any residual confounding not captured by the conditional analyses. We examined potential confounding by APOE ε4 allele, obesity, diabetes, frequency of moderate exercise (≥1 vs. none per week) (Geda et al., 2010), cigarette smoking, cardiac disease, and stroke, with each variable added separately. Variables that were significantly associated with aMCI or *T*/*S* were simultaneously included in the full models. We also examined interaction of *T*/*S* ratio quintiles with age, sex, APOE ε4 allele, type 2 diabetes, and exercise.

Proportional hazards models: We also used proportional hazards models for nested case-control designs stratified on the matched pairs to take into account the prospective component of the study. To account for sampling of controls and to enhance representativeness of matched controls to the total eligible control cohort, we assigned a weight of 1 to cases and a weight equal to the inverse of the sampling fraction to controls (Ganna et al., 2012). We also performed analyses without stratifying on matched pairs (but included age at blood draw and sex as covariates) as was done for the logistic models to account for any residual confounding.

Finally, we used general additive models with a spline to test for a non-linear association of *T*/*S* ratio with aMCI case-control status (Nelder and Wedderburn, 1972; Stone, 1985). We used the log2 transformation of *T*/*S* ratio for all the analyses, given the skewed data and back-transformed the estimates where necessary. Associations were considered significant at a p value < 0.05; and analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary NC).

3. Results

Table 1 shows the characteristics of aMCI cases and matched cognitively normal controls. In this elderly 71–90 year old cohort, there was no difference in log2 *T*/*S* in cases vs. controls ($p = 0.44$). There was no correlation of *T*/*S* ratio with age in aMCI cases and controls (combined; $r = -0.089$, $p = 0.14$; in controls: $r = -0.112$, $p = 0.19$; or in cases; $r = -0.079$, $p = 0.36$). There was also no sex difference in log2 *T*/*S* ratio (mean [range]) in men (−0.46 [−2.78–2.99]) vs. women (−0.46 [−3.03–2.51]; $p = 0.84$). Generalized additive models demonstrated a significant non-linear association between *T*/*S* and log odds of aMCI in a model adjusted for age, gender, education, APOE ε4 allele, and type 2 diabetes ($p = 0.04$; Fig. 1).