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Telomere Length and Ischemic Stroke in Women: A Nested Case-Control Study

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Abstract

Background—Telomere shortening has been implicated in cardiovascular disease. However, prospective data on the association between relative telomere length (RTL) and ischemic stroke are scarce and inconclusive.

Methods—We used a nested case-control design among women participating in the prospective Nurses' Health Study. Participants provided blood samples in 1990 and were followed through 2006. Women with confirmed incident ischemic stroke were matched to controls by age, smoking, postmenopausal status, and postmenopausal hormone use. Quantitative PCR was used to determine RTL in genomic DNA extracted from peripheral blood leukocytes. Conditional logistic regression was used to determine the risk of ischemic stroke associated with RTL, using RTL quartiles and as dichotomous according to the median.

Results—Data on RTL were available from 504 case-control pairs. Results did not suggest an association between RTL and ischemic stroke. The odds ratio for ischemic stroke was 0.82 (95% confidence interval [CI] 0.52–1.32) comparing lowest to the highest RTL quartile and 0.90 (95% CI 0.65–1.24) comparing RTL below the median to RTL above the median. Associations were unchanged after additional adjustment for cardiovascular risk factors. Further analyses suggested an association between RTL and fatal ischemic stroke (54 case-control pairs; lowest vs. highest quartile OR=1.99, 95%CI 0.26–14.9); however, results were statistically insignificant.

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Conflicts of interest

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Conclusion—In this large nested case-control study among women RTL was not associated with ischemic stroke. In light of the varying study results in the literature on the association between telomere length and stroke additional research is warranted.

Keywords

telomere length; ischemic stroke; nested case-control study; epidemiology

Introduction

Stroke is a major cause of death and disability worldwide [1] carrying a high individual and societal burden. Many modulators of stroke risk have been identified, including environmental and inherited factors [2]. Among the most striking risk factors, associated with a dramatic rise in stroke, is age [1]. The physiological process of aging is under genetic control and the telomere system plays a pivotal role in aging [3].

Telomeres are nucleoprotein complexes located at the ends of chromosomes and are responsible for maintenance of chromosomal integrity [3]. During each cell cycle telomeres shorten, a process that is further accelerated by oxidative stress, and excessive telomere attrition leads to premature cell senescence [4, 5]. In addition, cell senescence has been linked to changes in the vasculature implicated in atherosclerosis [6, 7]. Thus, telomere length shortening, a biological aging marker, may be associated with cardiovascular disease (CVD) and stroke.

Several studies have investigated the association between telomere length and CVD risk. Seven studies [8–14] had a prospective and one study each had a retrospective [15], a case-control [16], and a nested case-control design [17]. With respect to myocardial infarction all available studies reported a positive association, with shorter telomeres being associated with greater risk for myocardial infarction [10, 12, 14, 15]. In contrast, results from studies investigating stroke are less convincing. Four studies found an association between shorter telomeres and ischemic stroke [10–12, 16], while one study did not [13]. However, three of the positive studies had very small numbers of cases with stroke [10–12] and additionally one study only investigated fatal strokes [11]. Further, these studies differed in study design and ethnicity of the population.

We sought to further investigate the association between telomere length and incident ischemic stroke in a large cohort of women participating in the Nurses' Health Study.

Methods

Study population

The Nurses' Health Study is a prospective cohort study comprising 121,700 female nurses aged 30–55 years from 11 US states at enrollment in 1976 [18]. Detailed information on medical history and lifestyle factors have been collected with self-administered questionnaires at enrollment and every 2 years thereafter. Blood samples were collected from 32,826 women between 1989 and 1990, and participants were followed through June 2006. Women with incident ischemic stroke were matched to a control by age at blood draw (± 1 year), date of blood draw (± 3 months), menopausal status (yes, no), use of postmenopausal hormone (current or non-user), ethnicity (white or other), and smoking status (past, current, or non-smoker). The study was approved by Brigham and Women's Hospital Human Subjects Committee Review board.

Ascertainment of ischemic stroke

Women who reported a stroke were asked for permission to access medical records, which were reviewed by a physician. Stroke was classified according to criteria of the National Survey of Stroke [19], which requires evidence of a neurological deficit with sudden or rapid onset that persisted for >24 hours or until death. Cerebrovascular pathology caused by infection, trauma, or malignancy was excluded, as were silent strokes discovered only by radiological imaging. Strokes were classified as follows: ischemic stroke (thrombotic or embolic strokes), hemorrhagic stroke (subarachnoid or intraparenchymal hemorrhage), or stroke of undetermined subtype. Deaths were identified through information provided by the next of kin or postal authorities or by systematic searches of the National Death Index. Classification of fatal stroke was confirmed by review of hospital records or autopsy reports. All ischemic stroke cases included in the current analysis were confirmed by medical records review.

Determination of relative telomere length (RTL)

Genomic DNA was extracted from peripheral blood leukocytes using the QIAmp (Qiagen) 96-spin blood protocol. PicoGreen quantitation of DNA was performed using a Molecular Devices 96-well spectrophotometer. The ratio of telomere repeat copy number to a single gene copy number (T/S) was determined by a previously described modified, high-throughput version [20] of the quantitative PCR telomere assay [21]. Triplicate reactions of each assay were performed on each sample. Matched samples were run on the same 384-well plate. Relative telomere length is reported as the exponentiated sample T/S ratio corrected for a reference sample. Telomere and single-gene assay coefficients of variation (CV) for triplicates were 0.74% and 1.09%, respectively. The CV for relative telomere length of blinded quality control samples was 22%.

The experiments were performed in two batches with different comparison standards. This resulted in the following batch specific distribution of RTL among controls: Batch 1—median RTL: 0.409, quartile 1: RTL 0.319, quartile 2: 0.319<RTL 0.409, quartile 3: 0.409<RTL 0.503, quartile 4: RTL>0.503; batch 2—median RTL: 0.540, quartile 1: RTL 0.470, quartile 2: 0.470<RTL 0.540, quartile 3: 0.540<RTL 0.635, quartile 4: RTL>0.635. To ensure comparability for analyses we grouped samples from both batches according to their batch-specific RTL quartiles as well as according to their distribution above and below the batch-specific median.

Statistical analysis

RTL was analyzed as quartiles (based on the distribution in controls) and as a binary variable according to the median, after removal of experimental outliers. The Wilcoxon rank-sum test or the Kruskal-Wallis test for continuous variables and the chi-square test for categorical variables were used to compare characteristics of cases and controls as well as across quartiles of RTL in the combined sample.

Conditional logistic regression was used to investigate the association between RTL and ischemic stroke and to calculate odds ratios (OR) and 95% confidence intervals (CI) as the measure of association. We performed crude and multivariable-adjusted analyses. For the multivariable-adjusted analyses we built a first model (Model 1) controlling for history of elevated cholesterol (yes, no), history of hypertension (yes, no), history of diabetes (yes, no), history of coronary heart disease ([CHD], yes, no), alcohol consumption (none, >0 up to <15g/day, 15g/day), aspirin use (<1 tab/wk, 1 tab/wk), body mass index ([BMI], <25 kg/m², 25–29.9 kg/m², 30 kg/m²), and physical activity (continuous, measured as metabolic equivalents). We also built a second model (Model 2) that additionally controlled for total

cholesterol/HDL ratio (continuous), $\log_e(\text{HbA1c})$ (continuous), $\log_e(\text{C-reactive protein [CRP]})$ (continuous), and healthy dietary score (4 categories).

To evaluate prior reports of possible effect modification [10, 22], we also performed stratified analyses by age (above and below median: ≤ 63 yrs vs. >63 yrs), smoking (current vs. never/past), and body mass index (BMI) (<25 kg/m² vs. ≥ 25 kg/m²), based on information available from the time of or closest to the time of blood draw. We also calculated the p-value for the interaction term between RTL (below vs. above the median) and each of these stratifying variables.

All analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC). We considered a p-value <0.05 as statistically significant.

Results

Association between RTL and incident ischemic stroke

Baseline characteristics for the 504 women with (cases) and 504 women without (matched controls) incident ischemic stroke available for this analysis are summarized in Table 1. The mean age was 61.4 years for both cases and controls. Cases were significantly more likely to report a history of hypertension and diabetes and also had higher total cholesterol/HDL ratios and CRP levels than controls. All other baseline characteristics were equally distributed among cases and controls. The vast majority was white (cases: 97.6%, controls: 98.4%). The mean time between blood collection and incident ischemic stroke among the 504 cases was 9.3 years.

The distribution of baseline characteristics in the combined sample of cases and controls across quartiles of RTL did not differ for any of the characteristics (Table 2). However, there was a suggestive trend for history of CHD being more common among women with shorter telomeres than among those with longer telomeres ($p=0.09$).

In logistic regression analyses, conditional on matching factors, RTL quartile 3 appeared to be associated with lower risk of incident ischemic stroke (OR=0.56, 95% CI 0.37–0.85; Table 3) compared to quartile 4 (longest RTLs); however, this was not apparent for even shorter RTLs in quartiles 2 and 1 (lowest vs. highest quartile: OR=0.82, 95% CI 0.52–1.32; Table 3). Results did not suggest an association with incident ischemic stroke when we compared RTL below the median to RTL above the median (OR=0.90, 95% CI: 0.65–1.24). The results did not change in multivariable-adjusted models accounting for factors associated with ischemic stroke (Model 1) and additionally accounting for biomarkers for CVD and diet (Model 2). Adding triglyceride levels to Model 2 did not further alter the results (data not shown).

Further exploratory analyses stratified by age, body mass index, and smoking, did not suggest effect modification of the relationship between RTL and ischemic stroke by these factors (all p-values for interaction >0.5 ; Table 4).

When we restricted our analyses to fatal ischemic stroke (54 cases and 54 matched controls) there was some suggestion that shorter RTL (in quartiles) is associated with an increased risk of fatal ischemic stroke. Compared to quartile 4 (longest quartile) the crude OR were as follows: quartile 3—OR=0.50, 95% CI 0.10–2.61; quartile 2—OR=1.66, 95% CI 0.21–13.34; quartile 1—OR=1.99, 95% CI 0.26–14.9. However, the number of cases and controls was limited and the results were not statistically significant, obviating firm conclusions for this subgroup. When we considered RTL as dichotomous according to the median there was no

indication that women with RTL below the median had an altered risk for fatal ischemic stroke compared to women with RTL above the median (OR=0.33, 95%CI 0.07–1.65).

Discussion

Telomere attrition has been linked to cell senescence and aging [4, 5] and has been posited as a sign of biologic aging that might be linked to multiple chronic diseases. However, the results of our analysis do not indicate that telomere length is associated with subsequent ischemic stroke in women. Several studies have investigated the association between telomere length and CVD and the results have varied depending on the end-point investigated. Two studies report an increased risk for the combined end-point of CVD events [12, 17], while one did not [9]. Data have more consistently shown that shorter telomere length is associated with an increased risk of myocardial infarction [10, 12, 14, 15]; however, this was not seen for cardiac death [11].

Studies investigating stroke do not allow drawing convincing conclusions with respect to an association with telomere length. Four studies suggest an overall increased risk for any stroke and ischemic stroke among individuals with shorter telomeres [10–12, 16]. In contrast, a prospective study among men did not find such an association for ischemic stroke [13]. Our study agrees with the null result. Several factors may in part account for the differing results of the previous studies. First, some studies had a prospective design [10–12], while one study had a case-control [16], and one a nested case-control design [13] like our study. Second, the available prospective studies vary by number of stroke endpoints. Our study contains a large number of endpoints (n=504), while the Physician's Health Study had 259 strokes [13], the Bruneck Study had 46 [12], and the Cardiovascular Health Study had only 42 strokes [10]. The most recent study investigated the largest number of ischemic strokes (n=1309); however, it had a case-control design [16]. Third, the reporting of follow-up time differed between the prospective studies, ranging from seven to ten years and was thus shorter than for our study with a maximum follow-up of 16 years. Fourth, the end-point definitions for stroke have varied with one study looking at total stroke [10], two at ischemic stroke [12, 13], and one at fatal ischemic stroke [11]. Only one study that investigated total stroke also presented results for ischemic stroke and intracranial hemorrhage [16]. Results from our study do not suggest an association between RTL and overall ischemic stroke. The effect estimates for fatal ischemic stroke suggest a possible increased risk when comparing extreme RTL quartiles. However, results are based on a limited sample size and are statistically insignificant; hence, they must be interpreted with great caution. Fifth, one study suggests that stroke risk may be modified by age; in that study risk was apparent for those less than age 73 years but disappeared with increasing age [10]. This has also been reported for other outcomes including the composite CVD end-point [12], myocardial infarction [10], and mortality [23, 24], possibly reflecting a survivor effect. However, participants in our study as well as in the other negative study [13], were on average more than 10 years younger and results from both studies do not suggest an increased risk for ischemic stroke. In addition, our data do not suggest a differential association with ischemic stroke in stratified analysis according to median age (63 years). Finally, the method of telomere length measurement differed among the studies with some measuring the mean length of terminal restriction fragments (TRF) [10] and others using a PCR-based technique to determine the T/S ratio [13, 16]. However, the PCR assays have been found to correlate well with the Southern blot assays [21].

Our study has several strengths. First, the nested study design employed combines the efficiency of a case-control study with the advantages of a cohort study. This includes the ability to select cases and controls from the same clearly defined underlying population. In addition, it ensures that all ischemic stroke events were incident cases, i.e. occurred after the

baseline blood draw, facilitating establishment of a direction of association. Second, the underlying cohort, the Nurses' Health Study, is a large well-characterized cohort with ample information on personal characteristics, co-morbidities, life-style habits, and laboratory parameters, which allow accounting for potential bias and confounding. Specifically, we have used this information to match cases to controls and also to control for many additional covariates in the multivariable-adjusted regression models. Third, DNA for genetic analyses is available from a large proportion of the study participants. Combined with the high-throughput capability of the RTL assay, we were able to investigate our hypothesis in a very efficient way.

However, the following limitations should be considered. First, stroke ascertainment relied on self-report in a first step, which was then confirmed by medical record review. We cannot exclude that some strokes might be missed due to non-reporting. However, we believe that such misclassification is negligible since stroke is a severe condition that is hardly missed and all study participants are health care professionals expected to report conditions accurately. Second, telomere length measured at just one time point may show large inter-individual variability and only reflects a snapshot, while change in telomere length over time may be a better marker for disease development. However, data on change of telomere length were not available to us. This should be the focus of targeted investigations in future large independent studies. Third, our study population consists entirely of women. The association between telomere length and ischemic stroke may vary by gender. For example, women have longer telomeres than men and it has also been shown that telomere attrition manifests at a faster rate among men than women [25]; thus, potential associations may be more difficult to detect among women than men. However, our results agree with those from a study among men [13]. Fourth, the vast majority of our study population was white; hence, results may not be generalizable to non-white populations. Finally, the PCR-based assay had acceptable, but not excellent CVs for RTL blinded quality control samples (22%) which may render it more difficult to detect a true association were one present.

In summary, our results do not show an association between peripheral blood leukocyte telomere length and incident ischemic stroke. Given the differences among the available studies with respect to design, RTL measurement, and size, additional studies in well-defined cohorts using standardized information and methodology are needed to clarify the nature of the association between telomere length and ischemic stroke.

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Table 1

Characteristics of cases with ischemic stroke and controls in the Nurses' Health Study

Characteristic	Cases (n=504)	Controls (n=504)	p-value *
<i>Age, yrs, mean (SD)</i>	61.4 (5.9)	61.4 (5.9)	matched
<i>Smoking, %</i>			
<i>Never</i>	40.8	40.4	
<i>Past</i>	42.2	43.6	
<i>Current</i>	17.1	15.9	matched
History of hypertension, %	50.4	34.3	<0.0001
History of diabetes, %	14.1	6.8	0.0001
History of elevated cholesterol, %	48.4	47.6	0.80
Body mass index, %			
<25 kg/m ²	48.2	54.2	
25–<30 kg/m ²	33.3	30.0	
30 kg/m ²	18.5	15.9	0.16
<i>Postmenopausal hormone use, %</i>	42.1	41.7	matched
<i>Postmenopausal status, %</i>	89.1	89.3	matched
Alcohol use, %			
None	40.8	42.4	
<15 g/day	48.2	47.0	
15 g/day	11.0	10.6	0.88
Physical activity, METS, mean (SD)	14.9 (17.5)	15.4 (17.9)	0.36
History of CHD, %	5.0	6.4	0.34
Aspirin use >1tabl/wk, %	47.9	50.0	0.51
Family history of MI, %	33.9	30.4	0.22
HbA1c, %, median	5.58	5.55	0.11
Total cholesterol/HDL ratio, median	4.03	3.76	0.004
CRP, mg/L, median	2.34	1.83	0.004

* p-value from chi-square test for categorical variables and from Wilcoxon rank-sum test for continuous variables.

Matching variables are in italics. METS, metabolic equivalents; CHD, coronary heart disease; HbA1c, hemoglobin A1c; CRP, C-reactive protein.

Distribution of characteristics according to quartiles of relative telomere length* of cases with ischemic stroke and controls in the Nurses' Health Study

Table 2

Characteristic	Quartile 1 (n=272)	Quartile 2 (n=244)	Quartile 3 (n=216)	Quartile 4 (n=276)	p-value [†]
<i>Age, yrs, mean (SD)</i>	62.2 (5.4)	61.0 (6.2)	60.8 (6.2)	61.5 (5.8)	0.10
<i>Smoking, %</i>					
<i>Never</i>	35.4	41.4	42.8	43.3	
<i>Past</i>	45.4	40.2	41.4	44.0	
<i>Current</i>	19.2	18.4	15.8	12.7	0.26
History of hypertension, %	44.9	42.2	40.3	41.7	0.77
History of diabetes, %	10.7	12.3	8.8	9.8	0.64
History of elevated cholesterol, %	51.8	48.8	46.3	44.9	0.40
Body mass index, %					
<25 kg/m ²	50.7	45.5	56.0	52.9	
25–<30 kg/m ²	33.1	34.8	27.3	30.8	
30 kg/m ²	16.2	19.7	16.7	16.3	0.40
<i>Postmenopausal hormone use, %</i>	44.5	43.0	39.4	40.2	0.62
<i>Postmenopausal status, %</i>	91.2	89.8	86.6	88.8	0.43
<i>Alcohol use, %</i>					
None	42.6	38.0	38.5	46.2	
0 – <15 g/day	46.0	52.7	49.3	43.6	
15 g/day	11.4	9.4	12.2	10.2	0.43
Physical activity, METS, mean (SD)	15.1 (16.3)	14.5 (17.6)	16.7 (22.2)	14.5 (15.0)	0.88
History of CHD, %	8.09	6.56	3.70	3.99	0.09
Aspirin use > 1 tab/ wk, %	49.6	45.5	47.4	52.5	0.42
Family history of MI prior to age 60, %	31.6	30.7	33.3	33.0	0.92
HbA1c, %, median	5.56	5.57	5.55	5.58	0.81
total cholesterol, median	226.0	226.5	220.0	225.0	0.86
Total cholesterol/HDL ratio, median	3.92	3.96	3.99	3.70	0.54
CRP, mg/L, median	2.15	2.29	1.67	2.14	0.08

Matching variables are in italics.

METS, metabolic equivalents; CHD, coronary heart disease; HbA 1c, hemoglobin A 1c; CRP, C-reactive protein.

* Batch 1—quartile 1: RTL 0.319, quartile 2: 0.319<RTL 0.409, quartile 3: 0.409<RTL 0.503, quartile 4: RTL>0.503; batch 2—quartile 1: RTL 0.470, quartile 2: 0.470<RTL 0.540, quartile 3: 0.540<RTL 0.635, quartile 4: RTL>0.635.

† p-value from Kruskal-Wallis test for continuous variables and chi-square test for categorical variables.

Table 3

Association between relative telomere length and ischemic stroke in the Nurses' Health Study

RTL	Crude Model (n=1,008)		Multivariable-adjusted Model 1* (n=948)		Multivariable-adjusted Model 2† (n=784)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Quartile 4	Reference	---	Reference	---	Reference	---
Quartile 3	0.56 (0.37–0.85)	0.006	0.56 (0.35–0.88)	0.01	0.50 (0.29–0.84)	0.01
Quartile 2	0.77 (0.50–1.19)	0.23	0.77 (0.47–1.25)	0.29	0.76 (0.43–1.32)	0.32
Quartile 1	0.82 (0.52–1.32)	0.42	0.92 (0.55–1.55)	0.76	0.98 (0.54–1.79)	0.96
Above median	Reference	---	Reference	---	Reference	---
Below median	0.90 (0.65–1.24)	0.51	0.85 (0.59–1.23)	0.39	0.77 (0.51–1.16)	0.21

OR, odds ratio from conditional logistic regression analysis; CI, confidence interval.

Missing values in multivariable-adjusted models were not imputed; hence, the sample size is lower than in the crude model.

* adjusted for: history of elevated cholesterol, history of hypertension, history of diabetes, history of CHD, alcohol consumption, aspirin use, BMI, and physical activity.

† adjusted for: Model 1 plus total cholesterol/HDL ratio, loge(HbA1c), loge(CRP), healthy dietary score.

Association between relative telomere length (below the median vs. above the median [reference]) and ischemic stroke in the Nurses' Health Study stratified by age, BMI and smoking.

Table 4

	Crude Model		Multivariable-adjusted Model 1*		
	OR (95% CI)	p-value	OR (95% CI)	p-value	P _{interaction}
Age					
63yrs (n=507)	0.86 (0.55–1.34)	0.50	0.79 (0.47–1.32)	0.36	
>63 yrs (n=501)	0.79 (0.47–1.32)	0.36	0.74 (0.42–1.31)	0.30	0.61
BMI					
<25 kg/m ² (n=516)	1.06 (0.55–2.05)	0.87	1.05 (0.51–2.19)	0.89	
25 kg/m ² (n=492)	0.94 (0.49–1.83)	0.87	0.73 (0.33–1.61)	0.43	0.69
Smoking					
current (n=166)	0.92 (0.42–2.02)	0.84	0.74 (0.25–2.16)	0.58	
never/past (n=839)	0.92 (0.64–1.32)	0.64	0.92 (0.62–1.38)	0.70	0.55

BMI, body mass index; OR, odds ratio from conditional logistic regression analysis; CI, confidence interval.

* adjusted for: history of elevated cholesterol, history of hypertension, history of diabetes, history of CHD, alcohol consumption, aspirin use, BMI, and physical activity.