Mean Telomere Length and Risk of Incident Venous Thromboembolism: A Prospective, Nested Case-Control Approach

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Abstract

Background—Recent studies have shown telomere-length shortening as a risk predictor for cardiovascular disease. However, to date, no prospective data are available on its potential involvement in venous thromboembolism (VTE).

Methods—Using leukocyte DNA samples collected at baseline in a prospective cohort of 14,916 initially healthy American men, we examined the relationship of mean telomere repeat copy number to single gene copy number (T/S ratio), using a modified quantitative polymerase chain reaction protocol, amongst 108 white males who subsequently developed a first ever VTE event and amongst an equal number of age- and smoking-matched white males who remained free of vascular events during follow-up (controls).

Results—An inverse correlation between T/S ratios and age was observed in our controls (p=0.04). However, the T/S ratios were similar between cases and controls (p=0.31). Furthermore, in a multi-variable adjusted analysis, we found no evidence for an association of the observed T/S ratios with VTE risk. (odds ratio=1.20; 95%CI=0.58-2.52; p=0.62).

Conclusions—The present investigation found no evidence for an association of relative telomere length with risk of incident VTE.

Keywords

mean telomere length; VTE; risk factor

Introduction

Vascular disorders, including venous thromboembolism (VTE), are leading causes of mortality and morbidity in modern societies. The underlying patho-physiology is likely to be under the influence of both genetic and environmental factors. Telomeres are tandem repeats of DNA sequences —special chromatin structures— located at the ends of eukaryotic chromosomes. These structures are believed to protect the telomeric regions from recombination and

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degradation, thus avoiding a DNA damage cellular response (1). Recent evidence has demonstrated the relevance of telomere biology in human disorders including cardiovascular disease (1). In cross-sectional and case-control studies, shortening of telomere length has recently been associated with chronic heart failure (2), degenerative aortic valve stenosis (3), coronary artery diseases (4,5), and premature MI (6). However, to date, no studies have been conducted to examine the possible involvement of telomere length with risk of VTE. We prospectively examined the possible association of mean telomere length with VTE risk using a nested, matched case-control sample drawn from the prospective Physicians' Health Study (PHS) cohort.

Materials and Methods

Study Design

We employed a nested case-control design within the PHS, a randomized, double-blinded, placebo-controlled trial of aspirin and beta-carotene initiated in 1982 among 22,071 male, predominantly white (>94%), U.S. physicians, 40 to 84 years of age at study entry (7). Before randomization, 14,916 participants provided an EDTA-anticoagulated blood sample that was stored for genetic analysis. All participants were free of prior myocardial infarction (MI), stroke, transient ischemic attacks, deep venous thrombosis (DVT)/pulmonary embolism (PE) and cancer at study entry. Yearly follow-up self-report questionnaires provide reliable updated information on newly developed diseases and the presence or absence of other cardiovascular risk factors. History of cardiovascular risk factors, such as hypertension, diabetes or hyperlipidemia, was defined by self-report of diagnosis at entry into the study. For all reported incident vascular events occurring after study enrollment, hospital records, death certificates, and autopsy reports were requested and reviewed by an end points committee using standardized diagnostic criteria.

Diagnosis of DVT was confirmed by a positive report of venous ultrasound or venography, whereas the diagnosis of PE was confirmed in the presence of either a positive angiogram or a ventilation-perfusion scan with two or more mismatched defects (8). Idiopathic (primary/unprovoked) DVT/PE was defined as occurring in the absence of known malignancy or recent trauma or surgery.

For each case, a control matched by age±2 y, smoking history (never, past, or current) and length of follow-up were chosen among those subjects who remained free of vascular diseases. The present association study consisted of white men only: 108 idiopathic cases (DVT/PE; 60% and 40%, respectively) and 108 matched controls. The study was approved by the Brigham and Women's Hospital Institutional Review Board for Human Subjects Research.

Mean Telomere Length Determination

Unified Quantitative Polymerase Chain Reaction Assay—Genomic DNA was extracted from whole blood using the QIAmp Spin Column protocol (Qiagen, Chatsworth, CA). Telomere length was determined by a previously described, unified quantitative polymerase chain reaction (qPCR) protocol (9). In brief, two master mixes of PCR reagents were prepared, one for telomere reaction and one for single-copy gene reaction (*36B4* on chromosome 12). Telomere repeat copy number to single gene copy number (T/S) ratio was determined on an ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) in a 384-well format using the following PCR protocol: 95°C for 15 minutes to activate Taq polymerase; 40 cycles of denaturation at 95°C for 15 seconds, and annealing-extension at 54°C for 2 minutes. Each 15uL amplification reaction volume contained 1× Qiagen Quantitect Sybr Green Master Mix (Qiagen) and 10 ng of template DNA. The primer sequences used were described elsewhere (9,10). All samples for both the telomere and single-copy gene

amplifications were done in duplicate on the same 384-well plate. Ct-value assignment was carried out by two independent observers, and if necessary, a complete re-genotyping was performed. Duplicates of a no-template control were included in each run. Melting (dissociation) curve analysis was performed on every run to verify specificity and identity of the PCR products. The Ct values generated were used to calculate the T/S ratio for each sample using the equation: $T/S=2^{-\Delta Ct}$ (where $\Delta Ct=Ct_{single-copy gene}-Ct_{telomere}$). Results were scored blinded as to case-control status.

Statistical Analysis

As previously noted, the observed telomere-repeat copy number to single gene copy number ratios (T/S ratios) had a skewed distribution, the data were log_e-transformation. The T/S ratios between cases and controls were compared using the paired t-test. Spearman's correlation analysis was used to assess the effects of age on relative T/S ratios amongst the controls. Relative risk of DVT/PE associated with log_e-transformed T/S ratios were calculated by conditional logistic regression analysis, matched on age, smoking status, and length of follow-up since randomization, and further controlling for randomized treatment assignment, history of hypertension ($\geq$140/90 mmHg or on anti-hypertensive medication), presence or absence of diabetes, bodymass index (BMI) and hyperlipidemia ($\geq$240 mg/dl). All analyses were carried out using SAS 9.1 package [SAS Institute Inc., Cary, NC]. A two-tailed *p*-value of 0.05 was considered a statistically significant result.

Results

Baseline characteristics of the study participants are shown in Table 1. In concordance with published data, an inverse relationship between the observed T/S ratios and age in our controls was found (Spearman correlation=−0.198 *p*=0.040). However, the observed T/S ratios were similar between cases and controls (*p*=0.31; Table 1). Furthermore, no association of mean T/S ratios with risk of incident VTE was found in the regression analysis (adjusted odds ratio=1.205, 95%CI=0.575-2.519, *p*=0.621; Table 2).

Discussion

The present prospective, nested, matched case-control investigation is the first to examine the relationship of mean telomere length with risk of incident VTE, and we found little evidence for an association with risk of incident VTE. In concordance with previous reports, the present study found an inverse correlation between T/S ratios and age.

Recent animal and human studies have implicated the importance of leukocyte telomere biology in arterial disease (1). Human telomere length shortening has also been implicated in cerebrovascular and neurodegenerative conditions (11,12) including post-stroke mortality (13) and dementia (14). As no prospective, epidemiological data of mean telomere length on risk of VTE are available, a direct cross-reference comparison cannot be made on the present null findings in relation to VTE risk. Nonetheless, the present investigation suggests that telomere biology may not play a role in the underlying pathophysiology of VTE.

The nature of the present investigation in which the determination of a case status was based solely on the subsequent development of disease rather than on any arbitrary selection criteria designed by the investigators, greatly reduce the possibility of bias and confounding. Nonetheless, our study population consists of white males so the data cannot be generalized to other ethnic groups, women, and populations with different socioeconomic status. In our study, we had the ability to detect, based on the present sample size, assuming 80% power, at an alpha of 0.05, a true difference in the log_e-transformed mean telomere length ratio of <-0.203 or >0.203. As noted previously, telomere biology represents a new and challenging research

field, continuous development of qPCR technique with comparison to the gold-standard Southern Blot method is worthwhile.

In conclusion, in this prospective, nested case-control study of middle-aged white US men, no association was found between leukocyte telomere length and risk of incident VTE; however, our present findings require confirmation/replication in future prospective studies.

Acknowledgments

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Table 1

Baseline characteristics of study participants.

	DVT/PE-Controls (N=108)	DVT/PE-Cases (N=108)	*p*
Age (y)	58.4±0.9	58.5±0.9	m.v.
Smoking Status (%)			m.v.
Never	49.0	49.0	
Past	45.4	45.4	
Current	5.6	5.6	
Body-mass Index (kg/m^2)	24.7±0.2	25.8±0.3	0.004
Hyperlipidemia ≥240 mg/dl (%)	8.2	6.2	NS
Hypertension (%)	16.7	22.9	NS
Diabetes (%)	4.6	0.0	--
Aspirin use (%)	47.2	43.5	NS
Family History of Premature CAD <60 years (%)	8.6	6.5	NS
Log$_e$-transformed T/S ratio	3.18±0.04	3.12±0.06	NS

Mean±SE unless otherwise stated.

DVT/PE=deep venous thrombosis/pulmonary embolism, CAD=coronary artery disease, T/S=Telomere repeat copy number to single gene copy number.

Continuous and categorical variables were tested by paired *t*-test and McNemar's test, respectively.

m.v.=matching variable.

Table 2

Conditional logistic regression analysis of shortening of $\log_e$-transformed mean telomere length.

T/S ratio	Crude	Adjusted
	OR, 95%CI, *p*	OR, 95%CI, *p*
DVT/PE	1.408; 0.718-2.762; 0.320	1.205; 0.575-2.519; 0.621

Crude=conditional on age, smoking status, time of follow-up.

Adjusted=further controlling for randomized treatment group, body-mass index, hypertension, diabetes, and hyperlipidemia.

DVT/PE=deep venous thrombosis/pulmonary embolism.