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Telomere Length and Parkinson's Disease in Men: A Nested Case-Control Study

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Abstract

Background—Telomere shortening has been implicated in neurodegenerative disorders. However, available data on the association between telomere length and Parkinson's disease (PD) are inconclusive.

Methods—We used a nested case-control design among men participating in the prospective Physicians Health Study. A large proportion of participants provided blood samples in 1997 and was followed through 2010. Men with self-reported PD were age-matched to controls in a 1:2 ratio. Quantitative PCR was used to determine telomere repeat copy number to single gene copy number ratio (TSR) in genomic DNA extracted from peripheral blood leukocytes. We used TSR as a measure for relative telomere length (RTL) in our analyses. Conditional logistic regression was used to determine the risk of PD associated with RTL.

Results—Data on RTL were available from 408 cases and 809 controls. Median TSR was shorter in controls than in cases (47.7 vs. 50.2; $p=0.02$). The age-adjusted odds ratio for PD was 0.66 (95% confidence interval [CI] 0.46–0.95; $p_{\text{trend over quartiles}}=0.02$) comparing the lowest to the highest quartile. The pattern of association was unchanged when comparing RTL below vs.

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above the median (age-adjusted OR=0.75; 95% CI 0.59-0.96). Associations were similar after additional adjustment for many covariates.

Conclusion—Contrary to the expected, in this large nested case-control study among men shorter telomeres were associated with reduced PD risk. Future research on the nature of this counterintuitive association is warranted.

Keywords

telomere length; Parkinson's disease; nested case-control study; epidemiology

Introduction

Telomeres are tandem repeats of DNA sequences located at the ends of eukaryotic chromosomes, protecting the telomeric regions from fusion and degradation [1]. The main regulator of telomere length is telomerase activity; however, genetic and environmental factors are also implicated [2]. Accelerated telomere shortening is regarded a sign of premature aging. Parkinson's disease (PD) is a steadily progressing neurodegenerative disorder primarily affecting men [3]. The clinical diagnosis rests on the four cardinal symptoms: resting tremor, bradykinesia, rigidity, and postural instability. The pathological hallmark is a loss of dopamine-producing neurons in the substantia nigra pars compacta. Current research suggests that neuroinflammatory mechanisms, involving activated glial and peripheral immune cells, result in oxidative stress and cytokine-receptor-mediated apoptosis, ultimately leading to cell death and disease progression [4]. Further, accumulation of alpha-synuclein aggregates in certain brain areas and the spinal cord appears to be involved [5].

The risk for various neurodegenerative disorders including Alzheimer's disease and dementia with Lewy bodies [6, 7] has been associated with telomere shortening. While such an association also appears plausible for PD, available studies of small to moderate size have yielded inconclusive results [8-12].

We sought to further investigate the association between telomere length and incident PD using a large nested case-control study among Caucasian men.

Methods

Study population

The Physicians' Health Study (PHS) is a completed randomized, placebo controlled trial of aspirin and beta-carotene for the primary prevention of cardiovascular disease and cancer among 22,071 US male physicians [13, 14]. All participants provided written informed consent and the Human Subjects Committee Review board of Brigham and Women's Hospital approved the PHS and this study. At baseline in 1982, the participants ranged in age from 40 to 84 years, were apparently healthy, had no history of cancer (exception: non-melanoma skin cancer), cardiovascular disease or other serious illnesses, and had no indication or contraindication for aspirin or other pain medication use. 92.2% of the participants identified their race as white. Baseline information was self-reported and collected by a mailed questionnaire that asked about many cardiovascular and cancer risk factors as well as lifestyle variables. Twice in the first year and yearly thereafter, participants were sent follow-up questionnaires asking about study outcomes and other medical information during the study period. Post-trial follow-up is ongoing [14]. About 70% of participants of PHS provided blood samples in 1997. DNA for further genetic investigations had been prepared and stored in a repository in liquid nitrogen at -70°C .

For this analysis we considered the time of blood draw in 1997 as baseline and excluded all participants in the PHS who had reported PD by then. Participants that reported incident PD after the blood draw in 1997 were matched to controls free of PD in a 1:2 ratio by the following criteria: (1) age at blood draw (± 1 year), (2) control must be free of PD at the time of the case index date and (3) control must be alive at the case index date and the control follow-up time must be longer than the case follow-up time.

Ascertainment of Parkinson's disease cases and validation

We identified all participants of the PHS who reported a new diagnosis of PD on their annual survey between 1982 and 2010. Among the PHS participants providing blood samples in 1997, a total of 408 reported incident PD after this date. For this analysis we only considered these incident 408 participants as PD cases.

The accuracy of the physicians' self-reported PD diagnosis was previously validated in a study among 73 participants who self-reported PD and for whom we had medical records collected for endpoint evaluations [15]. The clinical diagnosis of PD was considered valid if medical record review by trained neurologists revealed one or more of the following: a) PD as validated cause of death; b) Current use of PD medications such as Levodopa or a dopamine receptor agonist; c) Neurological examination with physical findings consistent with parkinsonism (at least two of the following: resting tremor, rigidity, bradykinesia or postural instability) with no evidence of a secondary cause such as stroke, history of encephalitis, brain tumor or neuroleptic treatment in the year before disease onset. Patients who developed dementia or severe dysautonomia within the first year of PD diagnosis were also not considered valid cases of PD; d) Patients followed by a neurologist or a movement disorders specialist for PD.

The overall validation rate was 90%. In 7%, criteria for a clinical diagnosis of parkinsonism was present, but a secondary cause could not be ruled out. The diagnosis was found to be incorrect in only 3%.

Ascertainment of socio-environmental factors, co-morbidities, and biomarkers

A large number of personal characteristics, medical history, demographics, and lifestyle factors have been collected in the PHS on the baseline and follow-up questionnaires. Further, information on specific validated co-morbidities including prevalent cardiovascular diseases and cancer during follow-up is available. This was used to calculate a modified Charlson Comorbidity Index, as described previously [16]. In addition, biomarkers have already been measured among participants that provided blood in 1997 (i.e., total cholesterol, HDL, non-HDL, CRP).

Determination of relative leukocyte telomere length (RTL)

Telomere length was determined using a validated 'unified' version [17] modified from the quantitative polymerase chain reaction (qPCR) protocol initially described by Cawthon [18]. The qPCR assay determines the telomere repeat copy number to single gene copy number ratio (TSR) on an ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) in a 384-well format. All samples for both the telomere and single-copy gene amplifications were performed in duplicate on the same 384-well plate along with duplicate of a no template control, and a reference DNA calibrator per each run. Standard curves with serial dilution were also performed as additional quality control. The threshold (Ct)-value assignment was carried out by two independent observers. Melting (dissociation) curve analysis was performed on every run to verify specificity and identity of the PCR products. Ct values were determined in the exponential phase. The Ct values generated were then used to calculate the TSR for each sample using the equation: $TSR = 2^{-\Delta Ct}$ (where

$\Delta Ct = Ct_{\text{single-copy gene}} - Ct_{\text{telomere}}$). The coefficients of variation of the telomere, single-gene, and TSR duplicate assays were all <3%, respectively [17, 19, 20].

Statistical analysis

For this analysis we used TSR as the measure for RTL.

To compare RTL and characteristics of cases and controls we used the Wilcoxon rank-sum test for continuous variables and the chi-square test for categorical variables.

We then used Spearman's correlation analysis to assess the effects of age, body mass index, smoking, blood pressure, biomarkers, etc. on RTL.

Conditional logistic regression was used to investigate the association between RTL and PD and to calculate odds ratios (OR) and 95% confidence intervals (CI) as the measure of association. We considered RTL as quartiles (based on the distribution in controls), but also investigated RTL below vs. above the median. We performed age- and multivariable-adjusted analyses. For the multivariable-adjusted analyses we built a model controlling for age (continuous), body mass index ([BMI], <25 kg/m², 25 – 29.9 kg/m², 30 kg/m²), smoking (never, past/current), randomized vitamin E assignment (yes, no), randomized vitamin C assignment (yes, no), systolic BP (<120 mmHg, >120-140 mmHg, >140 mmHg), diastolic BP (<80 mmHg, >80-85 mmHg, >85 mmHg), physical activity (less than 1 day/week, 1 day/week), alcohol consumption (daily, weekly, less than weekly), history of diabetes (yes, no), parental history of myocardial infarction prior to age 60 (yes, no), Charlson Comorbidity Index (continuous 0-10). We further explored a model that additionally controlled for log_e_total cholesterol (continuous), log_e_HDL (continuous), log_e_CRP (continuous).

For clinical covariates we incorporated a missing value indicator in the multivariable-adjusted models if the number of participants with missing information was greater or equal to 100. We assigned participants with missing values to the covariate reference category if the number of missing information was less than 100. For laboratory covariates we did not impute missing values.

We evaluated effect modification by performing stratified analyses by age (above and below median: <71.4 yrs vs. >71.4 yrs), smoking (never vs. past/current), and cancer diagnosis (no, yes). We further statistically investigated the significance of effect modification by building models that included interaction terms between RTL (above and below the median) and these covariates and evaluating the respective p-values.

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

Results

Baseline characteristics for the 408 cases and 809 matched controls available for analysis are summarized in Table 1. The mean age was 70.7 years for both cases and controls. RTL was shorter among controls than cases. All baseline characteristics were equally distributed among cases and controls.

The mean time of follow-up for cases until PD diagnosis was 5.7 (±3.3) years, while the mean time of follow-up for controls was 5.5 (±3.3) years.

We observed an inverse relation between RTL and age in the combined sample of cases and controls (Spearman correlation= -0.0975, p=0.0007); however, there was no correlation

between RTL and systolic or diastolic blood pressure, BMI, Charlson Comorbidity Index, log_e total cholesterol, log_e HDL, or log_e CRP (data not shown).

In age-adjusted conditional logistic regression analyses shorter RTL was associated with reduced risk of incident PD (lowest vs. highest quartile: OR=0.66, 95% CI 0.46-0.95; p for trend over quartiles=0.02; Table 2). The pattern of association was unchanged when we compared RTL below vs. above the median (OR=0.75, 95% CI: 0.59-0.96). The results were similar in multivariable-adjusted models accounting for clinical variables (Table 2). Additional adjustment for biomarkers showed a similar pattern of association (lowest vs. highest quartile: OR=0.63, 95% CI 0.37-1.07; p for trend over quartiles=0.01; below vs. above the median: OR=0.56, 95% CI: 0.39-0.79).

Further exploratory analyses stratified by age (crude model $p_{\text{interaction}}=0.46$), smoking (age-adjusted model $p_{\text{interaction}}=0.92$), and cancer diagnosis (age-adjusted model $p_{\text{interaction}}=0.54$) did not suggest effect modification of the relationship between RTL and incident PD (Table 3).

Discussion

The results of our analysis suggest that men with shorter RTL have a reduced risk for incident PD. This association did not differ in stratified analyses by median age, smoking status, and absence or presence of a cancer diagnosis.

These findings are contrary to the expected, since shorter telomeres are markers of oxidative stress and oxidative stress is considered to increase the risk for PD [21]. Hence, similar to other neurodegenerative disorders like Alzheimer's disease [7] and dementia with Lewy bodies [6] one would expect telomere attrition to be associated with increased risk for PD.

Available studies on the association between telomere length and PD are inconclusive. This may in part be explained by differences relating to design, country of origin, sample size, age of the study population as well as methods and tissue in which telomere length is investigated. A nested case-control study from the US among participants of the Health Professionals Follow-up Study (96 PD patients, 172 controls) investigated RTL in peripheral blood leukocytes and found a statistical significant risk reduction for PD among carriers of the shortest telomeres compared to carriers of the longest telomeres [11]. When restricting the analyses to the 74 patients with PD diagnosis after blood draw the direction of the association remained the same, albeit statistically insignificant. In contrast, we excluded participants with prevalent PD at blood draw, ensuring a clear temporal relationship with a mean time of follow-up of 5.7 years until incident PD. A more recent case-control study from the UK found the same pattern of association when investigating telomere length in peripheral blood monocytes (109 PD patients, 99 controls) [10]; however, there was no association when telomere length from substantia nigra neurons was investigated (28 PD patients, 17 controls) [10]. Additional studies also used a case-control design and investigated telomere length in peripheral blood leukocytes. One study from Japan (28 PD patients, 27 controls) [8] and one from Finland (131 PD patients, 115 controls) [9] did not find an association between telomere length and PD risk. The authors of a small study in German PD cases (n=20) and controls (n=15) are the first to report shorter telomeres among PD patients, however, this result was not significant [12]. While in most studies the mean age was 65+ years, in one study from Japan the age range was 51-68 years among controls and 47-69 years among cases [8].

Our study agrees in essence with some previous reports; however, the counterintuitive finding that shorter telomeres are associated with a reduced risk for PD deserves further consideration. First, telomere length is determined by both genetic and environmental

factors. Telomerase is an enzyme that protects telomeres from shortening [1]. While inactive in healthy cells, pathological conditions may alter genetic control of telomerase leading to activation. If such a process took place in PD, this could explain the finding of longer telomeres in PD. Further, smoking has been associated with shorter telomeres [22] and decreased risk of PD [23], hence may confound an association between telomere length and PD. However, neither our nor a previous study [11] suggest confounding (crude vs. multivariable-adjusted models) nor effect modification (stratified analyses) by smoking. Second, telomere length also displays cell specific variation, which may explain the finding of shorter telomeres in proliferating blood leukocytes compared to postmitotic substantia nigra neurons [10]. However, the lack of association between telomere length in substantia nigra neurons and PD has only been shown in one small study and awaits confirmation [10]. Third, senescence alters patterns of gene expression in aged cells [24]. Hence, the value of information from telomere length may differ between older and younger age. Finally, the lack of an expected association with shorter telomeres has also been described for other conditions. For example, relative telomere length was not linked with mortality in an elderly population aged 70-79 [25]. While the median age of onset for PD is 60 years [5], most of the studies investigating PD were performed in populations of an age range of 65+ years, similar to our study, which raises the possibility of a “survivor effect”. If shorter telomeres were to increase the risk of PD, patients might be expected to die earlier, since PD is associated with increased mortality [16]. In this scenario only PD patients with relatively longer telomeres would remain in an elderly population. While our analyses stratified by median age did not suggest a differential association, we cannot completely exclude an interaction by age as power may have been limited.

Several strengths distinguishing our study from previous ones deserve appreciation. First, the sample size of 408 PD cases and 809 controls is by far the largest study investigating telomere length in PD. Second, the PHS, which gave rise to our cases and controls, is a large well-characterized cohort with ample information, which allows accounting for potential bias and confounding. Specifically, we have used this information to match cases to controls and also to control for additional covariates in the multivariable-adjusted regression models. Third, the nested study design 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 population. It also ensures that all patients with PD were incident cases, facilitating establishment of a direction of association.

However, the following limitations need to be addressed. First, PD diagnosis in the PHS relies on self-report; hence we cannot exclude potential misclassification of PD patients and controls. However, a validation study among participants self-reporting PD by physician medical record review showed excellent agreement of 90% [15]. In addition, as misclassification would likely be unrelated to telomere length, we would likely underestimate any effect. Second, telomere length may show large interindividual variability and a single measurement may not adequately account for telomere attrition. Hence, change in telomere length over time may be a better marker for disease development. However, such data were not available in our study. Third, our study population consists entirely of men and an association between telomere length and PD may vary by gender. For example, it has been shown that telomere attrition manifests at a faster rate among men than women [26]. However, no gender specific results are available from previous studies [9, 10, 12]. Finally, we used a PCR-based technique to determine the T/S ratio. While most other studies employed the same method of telomere length measurement [9-11], some measured the mean length of terminal restriction fragments [8]. However, this is unlikely to have a major impact on the results, since the PCR assays have been found to correlate well with the Southern blot assays [18].

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Table 1
Characteristics of cases with Parkinson's disease and controls in the Physicians Health Study

Characteristic	Cases (n=408)	Controls (n=809)	p-value*
TSR; mean (SD)/median	112.1 (1120.9)/50.2	102.0 (1311.9)/47.7	0.02
Age at blood draw, yrs, mean (SD) [†]	70.7 (8.3)	70.7 (8.3)	0.99
Smoking, %			
Never	50.0	50.0	
Past	47.8	46.3	0.36
Current	2.2	3.7	
Systolic blood pressure, mmHg, mean (SD)	131.0 (14.8)	131.1 (12.7)	0.66
Diastolic blood pressure, mmHg, mean (SD)	78.3 (7.6)	77.9 (6.8)	0.19
History of diabetes, %	8.1	7.2	0.57
Body mass index, kg/m ² , %			
<25	42.7	44.5	
25-29.9	47.1	47.0	0.57
30	10.3	8.5	
Alcohol use, %			
daily	41.7	41.6	
weekly	36.3	36.4	1.00
monthly	5.4	5.6	
rarely	16.7	16.5	
Physical activity, %			
never	41.0	34.0	
<1 day/week	3.0	2.9	
1-2 days/week	15.3	14.5	0.08
3-4 days/week	23.7	30.7	
5-7 days/week	17.0	17.9	
Family history of MI prior to age 60, %	9.9	9.2	0.68
Randomized vitamin E assignment, %	46.5	51.0	0.25
Randomized vitamin C assignment, %	50.8	50.7	0.99
Charlson Comorbidity Index, mean (SD)	1.25 (1.36)	1.17 (1.32)	0.26
Total cholesterol, mg/dL, mean (SD)	199.9 (37.0)	205.6 (37.4)	0.05
HDL, mg/dL, mean (SD)	45.4 (16.1)	43.8 (14.8)	0.14
CRP, mg/dL, mean (SD)	2.63 (13.90)	1.81 (3.23)	0.68

[†] Matching variable.

TSR, telomere repeat copy number to single gene copy number ratio; SD, standard deviation; HDL, high density lipoprotein; CRP, C-reactive protein.

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

Table 2
Association between RTL and Parkinson's disease in the Physicians Health Study

RTL	Age-adjusted Model (n=1,217)		Multivariable-adjusted Model * (n=1,217)		
	OR (95% CI)	p-value	OR (95% CI)	p-value	P _{trend}
Quartile 4	Reference	---	Reference	---	
Quartile 3	1.08 (0.78-1.50)	0.63	1.12 (0.80-1.56)	0.51	
Quartile 2	0.90 (0.64-1.25)	0.51	0.91 (0.65-1.27)	0.57	0.02
Quartile 1	0.66 (0.46-0.95)	0.02	0.67 (0.46-0.96)	0.03	
Below vs. above median	0.75 (0.59-0.96)	0.02	0.75 (0.58-0.96)	0.02	----

RTL, relative telomere length (measured by TSR [telomere repeat copy number to single gene copy number ratio]); OR, odds ratio; CI, confidence interval both estimated from conditional logistic regression analysis.

* adjusted for age, BMI, smoking, randomized vitamin E assignment, randomized vitamin C assignment, systolic BP, diastolic BP, physical activity, alcohol consumption, history of diabetes, parental history of myocardial infarction prior to age 60, Charlson Comorbidity Index.

Association between RTL (below vs. above the median) and Parkinson's disease in the Physicians Health Study stratified by age, smoking, and cancer diagnosis.

Table 3

Characteristic	Age-adjusted Model		Multivariable-adjusted Model*	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age				
71.4 yrs (n=608)	0.83 (0.59-1.17)	0.28	0.82 (0.57-1.17)	0.27
>71.4 yrs (n=609)	0.69 (0.49-0.97)	0.03	0.73 (0.52-1.04)	0.08
Smoking				
Never (n=611)	0.76 (0.54-1.06)	0.11	0.71 (0.50-1.01)	0.05
Past/current (n=606)	0.75 (0.53-1.05)	0.10	0.74 (0.52-1.05)	0.09
Cancer diagnosis				
no (n=1084)	0.73 (0.57-0.94)	0.02	0.73 (0.56-0.94)	0.02
yes (n=133)	0.96 (0.47-1.99)	0.91	0.88 (0.37-2.13)	0.78

RTL, relative telomere length; OR, odds ratio from conditional logistic regression analysis; CI, confidence interval.

* adjusted for age, BMI, smoking, randomized vitamin E assignment, randomized vitamin C assignment, systolic BP, diastolic BP, physical activity, alcohol consumption, history of diabetes, parental history of myocardial infarction prior to age 60, Charlson Comorbidity Index.