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Telomere length and risk of Parkinson's disease

Hao Wang, MD, PhD, Honglei Chen, MD, PhD, Xiang Gao, PhD, Monica McGrath, ScD, Dwayne Deer, BS, Immaculata De Vivo, PhD, MPH, Michael A. Schwarzschild, MD, PhD, and Alberto Ascherio, MD, DrPH

Department of Nutrition (Dr. Wang, Dr. Gao, Dr. Ascherio) and Epidemiology (Dr. McGrath, Dr. De Vivo, Dr. Ascherio), Program in Molecular and Genetic Epidemiology (Dr. McGrath, Mr. Deer, Dr. De Vivo), Harvard School of Public Health, Boston, Massachusetts; Epidemiology Branch (Dr. Chen), National Institute of Environmental Health Sciences, Research Triangle Park, NC; Channing Laboratory (Dr. McGrath, Mr. Deer, Dr. De Vivo), Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; Department of Neurology (Dr. Schwarzschild), Massachusetts General Hospital, Boston, Massachusetts;

Abstract

We investigated whether telomere length was associated with the risk of Parkinson's disease (PD) in a case-control study (96 cases and 172 age-matched controls) nested within the Health Professionals Follow-up Study. Relative ratio of telomere repeat copy number to single-gene copy number in peripheral blood leukocytes was determined by quantitative real time PCR. Men with shorter telomeres had a lower PD risk (multivariate adjusted relative risk for the lowest vs. the highest quartile 0.33; 95% confidence interval: 0.12–0.90). Our results suggest that, contrary to telomere attrition observed in several aging-related diseases, shorter telomeres are not associated with an increased risk of PD.

Keywords

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

Background

Telomeres form the ends of eukaryotic chromosomes, with major functions of keeping the chromosome intact upon replication and preventing end-to-end fusion.¹ It has been postulated that telomere length might be a possible biomarker of aging and aging-related diseases.² Epidemiological studies have shown that relatively short telomeres are associated with an increased risk of various age-related diseases including Alzheimer's disease^{3, 4} and dementia after stroke⁵, but there are no data on telomere length and Parkinson's disease (PD). We therefore examined whether telomere length is associated with the risk of developing PD among participants in the Health Professionals Follow-up Study (HPFS).

Methods

Study population

Briefly, in 1986 the HPFS recruited 51,529 male health professionals between the ages of 40 and 75 years.⁶ Every two years the participants completed a mailed questionnaire that collected updated information on disease history, diet, and lifestyle. We have identified incident PD

cases by means of mailed questionnaires through 2002. Between April 1993 and August 1995, we collected a blood sample from 18,018 participants (35%) in the HPFS cohort. Men with both a PD diagnosis and an available blood sample were included as cases in this study. PD cases of whom a blood sample was available had similar distributions to those without blood samples in terms of pack-years of smoking, body mass index (BMI), caffeine intake, alcohol drinking, and physical activity. The cases with blood sample tended to be slightly older at the recruitment compared to those without (mean 62 vs. 60 years). For each case, up to two controls matched on year of birth were randomly selected from the participants without PD and alive at the time of diagnosis of their matched case. Cases and controls with a history of cancer or stroke were excluded.

Case ascertainment

Ascertainment of PD cases was based on the information provided by their neurologists or by review of the medical records, as previously described.⁷ A case was confirmed if a diagnosis of PD was considered definite or probable by the treating neurologist, or if the medical record included either a final diagnosis of PD made by a neurologist, or neurological assessments with evidence of at least two of the four cardinal signs (rest tremor, rigidity, bradykinesia, and postural instability), along with the lack of evidence of non-responsiveness to levodopa and features suggesting other diagnoses. In a few cases (11.3%), the diagnostic information was obtained from the internist or primary care physician.

Telomere length measurement

Coded DNA samples were analyzed by personnel blinded to case-control status. Telomere length was measured by a modified quantitative real time polymerase chain reaction (PCR)-based assay.^{8, 9} Briefly, the telomere repeat copy number to single gene copy number (T/S) ratio was determined using an Applied Biosystems 7900HT thermocycler in a 384-well format. Five nanograms of buffy-coat derived genomic DNA was dried down in a 384-well plate and resuspended in 10μL of either the telomere or 36B4 PCR reaction mixture for 2 hours at 4°C. The telomere reaction mixture consisted of 1× Qiagen Quantitect Sybr Green Master Mix, 2.5mM of DTT, 270nM of Tel-1b primer (GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT), and 900nM of Tel-2b primer (TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA). The reaction proceeded for 1 cycle at 95°C for 5:00, followed by 40 cycles at 95°C for 15 seconds, and 54°C for 2 minutes. The 36B4 reaction consisted of 1× Qiagen Quantitect Sybr Green Master Mix, 300nM of 36B4U primer (CAGCAAGTGGGAAGGTGTAATCC), and 500nM of 36B4D primer (CCCATTCTATCATCAACGGGTACAA). The 36B4 reaction proceeded for 1 cycle at 95°C for 5 minutes, followed by 40 cycles at 95°C for 15 seconds, and 58°C for 1:10 minutes.

All samples for both the telomere and single-copy gene reactions were performed in duplicate, and the threshold value for both reactions was set to 0.5. In addition to the samples, each 384-well plate contained an 8-point standard curve from 1.25ng to 50ng using pooled buffy-coat derived genomic DNA. The purpose of the standard curve is to assess and compensate for inter-plate variations in PCR efficiency. The slope of the standard curve for both the telomere and 36B4 reactions was -3.2, and the linear correlation coefficient value for both reactions was 0.98 and 0.99, respectively.

The relative telomere length was calculated as telomere repeat copy number to single-copy gene copy number (T/S ratio) in the study subjects as compared with that of a reference DNA sample. The coefficients of variation (CV) within duplicates of the telomere and single-gene assay were 0.9% and 2.4%, respectively. The relative T/S ratio estimated by real time PCR has been confirmed in previous work to be consistent with the Southern blot assay.⁸

Statistical analyses

Spearman rank correlations were used to investigate associations between telomere length and age, pack-years of smoking, caffeine intake (mg/day), alcohol drinking (g/day), body mass index (BMI in kg/m²), and physical activity (in METs/week). Non-dietary covariates were taken from the 1992 questionnaire, which was the most recent questionnaire before blood collection. Consumptions of caffeine and alcohol were obtained from the 1990 survey as diet was not measured in 1992. Self-reported history of high blood pressure, heart disease and diabetes was collected first in 1986, and then in 1988, 1990 and 1992. Conditional logistic regression was used to obtain relative risk (RR) and 95% confidence intervals (CI) for the association between relative telomere length (in quartiles according to the control distribution) and risk of PD with and without adjustment for potential confounders. Tests for trend were conducted by including telomere length as a continuous variable in the conditional logistic regression models. Interactions between telomere length and the covariates (pack-years of smoking, BMI, caffeine intake) were explored by including multiplicative terms in the conditional logistic regression models. All analyses were carried out using SAS software version 9.1 (SAS Institute, Cary, NC).

Results

Ninety-six cases of PD and 172 matched controls were included in this analysis. The mean age at PD diagnosis was 70.2 years (range, 53 to 85 years). The median relative telomere length was 0.50 (range, 0.04–1.15) for cases and 0.46 (range, 0.07–1.55) for matched controls ($p = 0.09$). Of the 96 cases, 74 had their blood collected before PD diagnosis. The mean interval between blood collection and PD diagnosis among these cases was 4.1 years. For 62 of the cases, the blood was collected before the onset of neurological symptoms attributable to PD (mean, 4.6 years; range: 0.2–8.9 years), whereas for 12 of the cases the blood samples were collected between the onset of symptoms and the diagnosis of PD. The distribution of covariates among the controls by quartile of relative telomere length is given in Table 1. As expected, the relative telomere lengths was inversely correlated with age (Spearman correlation $r = -0.09$; $p = 0.22$), and with pack-years of smoking (age-adjusted Spearman correlation, $r = -0.12$, $p = 0.10$), albeit non-significantly.

In analyses adjusted for age and pack-years of smoking, the RR for PD, contrary to expectation, decreased with shorter relative telomere lengths (Table 2). Participants whose telomeres were within the lowest quartile had approximately three-fold lower risk of PD than did those within the highest quartile. No significant interactions were found with age at blood collection, BMI, pack-years of smoking, or caffeine intake. Among men who never smoked (52 cases and 75 controls), there was no significant association between telomere length and PD risk (RR for below the median versus above the median = 0.87; 95% CI 0.39–1.95), but interpretation of this finding is limited by the small sample size. Results were similar when we restricted the analysis to cases with blood collection before the diagnosis (RR for the lowest vs. the highest quartile = 0.33, 95% CI 0.10–1.06), before disease onset (corresponding RR = 0.43, 95% CI 0.12–1.54), or at least two years before onset (corresponding RR = 0.31, 95% CI 0.07–1.39), although none of these associations were statistically significant due to diminishing sample sizes.

Discussion

In this nested case-control study, we found that men with relative shorter telomeres had a lower risk for PD. This runs contrary to what we had expected, because shorter telomeres are considered a marker of oxidative stress¹⁰, and oxidative stress has been proposed as an important contributor to PD pathogenesis¹¹. Furthermore, short telomeres have previously been found to be associated with a higher risk of other age-related degenerative diseases, including dementia.^{3–5, 12} Nevertheless, other puzzling epidemiological findings have been

observed for PD: for example, smoking, an important risk factor in many aging-related disease, has been consistently shown to be inversely associated with PD¹³, and PD patients appear to have on average a lower incidence of cancer.¹⁴

An important strength of the present study is its prospective design, which minimizes artifacts due to the effect of PD and its treatment on telomere length. Further, cases and controls were selected from the same well-characterized cohort, and analyses were adjusted for smoking and other potential confounders using prospectively collected and detailed information on the relevant covariates. The main limitation was the moderate sample size, which reduced the statistical power in analyses restricted to never smokers or the analyses that excluded men who already had symptoms of PD at the time of blood collection.

We did not find a significant correlation between age and telomere length. The modest correlation may be due in part to intra-individual variability, because telomere length varies widely even among individuals of the same age.^{5,9} In agreement with the study by Valdes et al,¹⁵ our results showed an association of smoking with telomere loss. Cigarette smoking is associated with shorter telomere length and a low risk of PD, and is a confounder of the association between telomere length and PD risk. In our analysis, adjustment for pack-years of smoking only slightly attenuated of the RRs of PD across quartiles of telomere length, suggesting that confounding by smoking is an unlikely explanation of the relation between telomere length and PD risk. On the other hand, we cannot exclude the possibility that telomere length is a biomarker for some effect of cigarette smoking that is important for PD protection.

A biological explanation for our findings is not readily available. Telomere length is determined by several biological factors, which are under genetic and environmental influence. These factors include, among many others, division rates of somatic cells, oxidative stress, and telomerase activity.¹⁶ One possible explanation for the relatively longer telomeres in PD is the activation of telomerase (normally inactive in somatic cells), although other proteins that regulate telomere function and length (e.g., TRF: telomere repeat binding factor 1 and 2; and TERC: telomerase RNA component) have been identified and linked to human diseases.¹⁷ Alternatively, the longer telomeres in individuals with PD may reflect a higher rate of apoptosis and thus a shorter lifespan of senescent cells (which have shorter telomeres) in peripheral blood. It is possible that besides endogenous factors, unknown environmental or lifestyle exposures that were not accounted for in our analysis influenced our results.

In summary, in this prospective study, we found no indication that there might be an increased risk of PD in men with shorter telomeres. Rather, unexpectedly, men with shorter telomeres had a lower risk of PD than those with longer telomeres. In spite of the modest sample size, restriction to men, and the other limitations discussed above, this finding is intriguing and warrants further epidemiologic studies to confirm or refute it.

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Table 1

Select baseline (1992) characteristics of PD cases and controls in HPFS

	Cases (n=96)	Controls (n=172)	P-value*
Variables			
Age at blood collection (1993–95), mean (SD)	67.6 (7.9)	67.0 (8.0)	0.78
BMI (kg/m ²), mean (SD)	25.2 (3.1)	25.0 (3.4)	0.76
Pack-years of smoking, mean (SD)	8.2 (15.7)	15.9 (19.7)	0.001
Caffeine intake (mg/day), mean (SD)	155.6 (172.9)	173.0 (179.1)	0.49
Total alcohol intake (g/day), mean (SD)	8.4 (11.4)	11.9 (8.4)	0.04
Physical activity (METs/week), mean (SD)	33.2 (27.8)	43.4 (62.9)	0.13
Relative telomere length, median (range)	0.50 (0.04–1.15)	0.46 (0.07–1.55)	0.09
Relative telomere length, mean (SD)	0.51 (0.17)	0.49 (0.20)	

* Age-adjusted

Table 2

Relative risk of Parkinson’s disease associated with telomere length

	4 th quartile	Quartiles of relative T/S ratio			P trend
		3 rd quartile	2 nd quartile	1 st quartile	
No. of cases	33	26	21	16	
Age adj.	1.0 (ref)	0.66 (0.33–1.33)	0.49 (0.23–1.04)	0.34 (0.14–0.84)	0.01
Age & smoking adj.	1.0 (ref)	0.59(0.29–1.24)	0.51 (0.23–1.11)	0.34 (0.13–0.88)	0.02
Multivariate adj. ¹	1.0 (ref)	0.56 (0.25–1.23)	0.48 (0.21–1.21)	0.33 (0.12–0.90)	0.03

¹ Adjusted (adj.) for age at blood draw, pack-years of smoking, BMI, alcohol, and caffeine intake and history of heart disease, hypertension and diabetes.