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Telomere length in white blood cell DNA and lung cancer: a pooled analysis of three prospective cohorts

Wei Jie Seow[1], **Richard M. Cawthon**[2], **Mark P. Purdue**[1], **Wei Hu**[1], **Yu-Tang Gao**[3], **Wen-Yi Huang**[1], **Stephanie J. Weinstein**[1], **Bu-Tian Ji**[1], **Jarmo Virtamo**[4], **H. Dean Hosgood III**[5], **Bryan A. Bassig**[1], **Xiao-Ou Shu**[6], **Qiuyin Cai**[6], **Yong-Bing Xiang**[7], **Shen Min**[1], **Wong-Ho Chow**[8], **Sonja I. Berndt**[1], **Christopher Kim**[1], **Unhee Lim**[9], **Demetrius Albanes**[1], **Neil E. Caporaso**[1], **Stephen Chanock**[1], **Wei Zheng**[6], **Nathaniel Rothman**[1,*], and **Qing Lan**[1,*]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland, USA [2]Department of Human Genetics, University of Utah, Salt Lake City, Utah, USA [3]Department of Epidemiology, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China [4]Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland [5]Department of Epidemiology & Population Health, Albert Einstein College of Medicine, Bronx, New York, USA [6]Division of Epidemiology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA [7]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China [8]Department of Epidemiology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA [9]Epidemiology Program, University of Hawaii Cancer Center, Honolulu, Hawaii, USA

Abstract

We investigated the relationship between telomere length and lung cancer in a pooled analysis from three prospective cohort studies: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, conducted among men and women in the United States, and previously published data from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial conducted among male smokers in Finland, and the Shanghai Women's Health Study (SWHS), which is comprised primarily of never-smokers. The pooled population included 847 cases and 847 controls matched by study, age, and sex. Leukocyte telomere length was measured by a monochrome multiplex quantitative PCR assay. We used conditional logistic regression models to calculate odds ratios (OR) and their 95% confidence intervals (CI) for the association between telomere length and lung cancer risk, adjusted for age and pack-years of smoking. Longer telomere length was associated with increased lung cancer risk in the pooled analysis (OR(95% CI) by quartile: 1.00; 1.24(0.90–1.71); 1.27(0.91–1.78); and 1.86(1.33–2.62); P-trend=0.000022). Findings were consistent across the three cohorts and strongest for subjects with very long telomere length, i.e., lung cancer risks for telomere length (OR(95% CI)) in the upper half of the fourth quartile were 2.41(1.28–4.52), 2.16(1.11–4.23) and 3.02(1.39–6.58) for the PLCO trial, the

Corresponding author: Wei Jie Seow, 9609 Medical Center Drive, Room 6E128, Rockville, MD 20850, telephone: 240-276-5310, weijie.seow2@nih.gov.
*These authors co-supervised this work.

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ATBC trial, and the SWHS, respectively. In addition, the association persisted among cases diagnosed more than six years after blood collection and was particularly evident for female adenocarcinoma cases. Telomere length in white blood cell DNA may be a biomarker of future increased risk of lung cancer in diverse populations.

Keywords

Leukocytes; Lung cancer; Prospective; Telomeres

Introduction

Telomeres shorten with each cell division, leading to loss of chromosomal and genetic integrity, and eventually either apoptosis, cellular senescence, or neoplasia (1). Shorter telomeres and telomerase inactivation are frequently observed in peripheral blood leukocytes of cancer patients for many different malignancies, including lung cancer (2–6). However, most of these findings were derived from retrospective case-control studies, which may have shown telomere length shortening as a consequence of tumor progression. In contrast, there is growing evidence that longer telomere length is associated with increased risk for cancers (7–13). It has also been suggested that the direction of the association between telomere length and cancer may vary by cancer types (14). Positive associations between telomere length and lung cancer were recently reported in two prospective cohort studies of Caucasian male smokers and Asian female never-smokers (15, 16).

To investigate this association in more populations, we carried out a pooled analysis of the new prospective Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, which was conducted among men and women in the United States and two previously published case-control studies nested in prospective cohorts: the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial conducted among male smokers in Finland and the Shanghai Women's Health Study (SWHS) cohort which is comprised primarily of female never-smokers (15, 16). Here we report the results from the pooled analysis as well as from each of the three studies individually.

Materials and Methods

Study population

Data from the following three case-control studies nested in prospective cohorts were pooled: The PLCO Trial (403 lung cancer cases and 403 controls individually matched by age at baseline (±5 years), sex, race, PLCO study center, and date of baseline blood draw (±3 months)) (17), the ATBC trial (229 lung cancer cases and 229 controls individually matched on date of birth (±5 years)) and the SWHS (215 lung cancer cases and 215 controls individually matched on date of birth (±2 years) and date of blood sample collection (±3 month)), resulting in a total of 847 cases and 847 matched controls, individually matched by age, sex and study. Briefly, the PLCO Trial includes 77,500 men and 77,500 women aged 55 to 74 years who were recruited in the United States between September 1993 and July 2001. The average time between sample collection and diagnosis among cases was 7.41

years and only the screening-arm participants provided DNA (18). The ATBC trial includes 29,133 male smokers 50 to 70 years of age, who were recruited from southwest Finland from 1985 to 1988, with an average of 5.23 years between sample collection and diagnosis among cases (19). The SWHS recruited 74,942 Chinese women aged 40 to 70 years who were primarily never-smokers between 1997 and 2000, with an average of 4.27 years between sample collection and diagnosis among cases (20).

All study participants provided written informed consent prior to participation, and the study protocols were approved by institutional review boards of each study center and the National Cancer Institute.

Telomere length measurement

All blood samples were collected prior to diagnosis of lung cancer among cases. DNA was extracted using the phenol–chloroform method for the ATBC trial and the SWHS study, and using the ProMega ReliaPrep, a magnetic bead-based extraction method, for the PLCO Trial. Telomere length for all three studies was measured in the same laboratory using the same monochrome multiplex quantitative polymerase chain reaction (PCR) method (15, 16, 21). Briefly, PCR (10 μL) was done using 10 mM Tris–HCl pH 8.3, 50 mM KCl, 3 mM MgCl2, 0.2 mM each dNTP, 1 mM DTT, 1M betaine, 0.75x SYBR Green I, and AmpliTaq Gold DNA polymerase, 0.625 U. Four primers were used in each reaction: telg (at 100 nM), telc (at 900 nM), to amplify telomere sequences; and either hbgu (at 500 nM) and hbgd (at 500 nM) to amplify the beta-globin gene, or albugcr2 (700 nM) and albdgcr2 (500 nM) to amplify the albumin gene. Relative telomere lengths were measured as T/S (telomere/single copy gene) values, which are proportional to the average telomere length per cell as described elsewhere (15, 16, 21). The main advantage of this method is that it eliminates pipetting steps that might introduce variation.

Cases were plated next to their matched controls to increase precision. Blind quality controls (5% of batch size) were assigned to all batches in random locations to evaluate assay reproducibility. All samples were assayed in triplicate and the averaged data were used in the analysis. The intra-class correlation coefficient (ICC) of the assay was 87% for the PLCO Trial, 80% for the ATBC trial and 87% for the SWHS. The overall coefficient of variation (CV) was 7.3% for the PLCO Trial, 7.0% for the ATBC trial and 11% for the SWHS.

Statistical Analysis

We compared characteristics of cases and controls for each of the three studies and pooled data using Wilcoxon signed-rank test for continuous variables, and Fisher's exact test for categorical variables. Correlations for continuous variables were evaluated using Spearman correlation coefficients. Telomere length was log-transformed because telomere length was right-skewed, and analyzed as both continuous and categorical (based on quartiles among controls). We also further categorized the fourth quartile (longest) of telomere length based on the lower and upper half to evaluate the association with very long telomere lengths. Analyses by telomere length quartile that retained the initial quartile categorization used in each study (in order to minimize the influence of potential assay variation across studies),

and using a new categorization based on pooling the original telomere length data, produced similar findings and results are presented for the former. We found no evidence of heterogeneity across studies using the Cochran's Q-test (Q = 0.97, P = 0.62) and therefore we pooled data from the three studies. We used conditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association between telomere length and lung cancer risk. All models were adjusted for age, to account for residual confounding, and pack-years of smoking as continuous variables. Other potential confounders were evaluated and did not affect the findings significantly, therefore, they were excluded from the final models. Tests of trend were calculated by treating the level of telomere length as a continuous variable using the original telomere length values and adjusting for age and pack-years of smoking. We also stratified the analysis by lung cancer subtypes (adenocarcinoma or squamous-cell carcinoma), time between blood drawn and lung cancer diagnosis (≤ median of 6 years or > 6 years), sex, and smoking status (ever or never). Among cases, the association between lung cancer subtypes, time to diagnosis, and sex was evaluated using Pearson's chi-square test. Only adenocarcinoma and squamous-cell carcinoma were considered in the analysis as these two are the major subtypes (both subtypes consist >50% of cases) in all three studies, and the other subtypes were too small in numbers for meaningful analyses (Table S1).

All statistical analyses were conducted using R, version 3.0.1 (http://www.R-project.org/), and SAS, version 9.3 (SAS Institute Inc., Cary, NC, USA). All statistical tests were conducted as two-sided, and a P-value of < 0.05 was considered significant.

Results

The pooled study population consisted of 847 incident lung cancer cases (mean age = 61.4 years, standard deviation (SD) = 6.5) and 847 matched controls (mean age = 61.1 years, SD = 6.4) (Table 1). Cases and controls were significantly different in their BMI (25.6 kg/m^2 among cases and 25.9 kg/m^2 among controls, P = 0.004), smoking status (71.9% smokers among cases and 54.5% smokers among controls, P < 0.001), and smoking pack-years (mean of 35.0 years among cases and 7.0 years among controls, P < 0.001). Telomere length was inversely correlated with age (Spearman correlation r = −0.19 in cases and −0.17 in controls, both P < 0.0001) and pack-years (Spearman correlation r = −0.38 in cases and -0.34 in controls, both P < 0.0001). Among controls, telomere length in the SWHS was longer than in the other two studies (median (Interquartile Range)): 1.50(0.34) in SWHS, 1.08(0.31) in ATBC and 1.13(0.31) in the PLCO Trial) (Tables 1, S2). This is due at least in part to women in the SWHS being almost all non-smokers and younger on average than subjects from one (PLCO Trial) of the other two cohorts (Table 1). Telomere length was significantly longer in cases compared to controls in the pooled population (Table 1).

Individuals with longer telomere length had significantly higher risk of lung cancer compared to individuals with shorter telomere length (OR (95% CI) by increasing quartile: 1.00; 1.24 (0.90–1.71); 1.27 (0.91–1.78); and 1.86 (1.33–2.62); P-trend = 0.000022) (Table 2). Findings were consistent across the three cohorts and particularly evident for subjects with very long telomere length, i.e., lung cancer risks for telomere length (OR (95% CI)) in

the upper half of the fourth quartile were 2.41 (1.28–4.52), 2.16 (1.11–4.23), and 3.02 (1.39–6.58) for the PLCO Trial, the ATBC trial, and the SWHS, respectively (Table 2).

We found in the pooled study population that longer telomere length was associated with increased risk of lung cancer among both smokers and non-smokers, and among both males and females (Table 3). Further stratification by tobacco use and gender showed similar associations, although the number of non-smoking males was too small to analyze.

The association between telomere length and lung cancer persisted for cases diagnosed more than six years from time of blood draw to lung cancer diagnosis (Table 4). Further, the association was most apparent for adenocarcinoma cases (OR (95% CI) by quartile: 1.00; 1.54 (0.84–2.83); 1.16 (0.64–2.09); and 2.52 (1.38–4.60), P-trend = 0.0029) vs. the null finding for squamous cell cancer cases (P = 0.026 for difference in the association for adenocarcinoma vs. squamous cell carcinoma for highest vs. lowest telomere length quartile) (Table 4).

A significant association between telomere length and lung cancer was also found among adenocarcinoma patients diagnosed more than six years from time of blood drawn to lung cancer diagnosis (OR (95% CI) by quartile: 1.00; 3.12 (1.17–8.30); 2.08 (0.84–5.12); and 4.61 (1.81–11.8), P-trend = 0.0019). Further, the association appeared to be stronger among female adenocarcinoma patients (OR (95% CI) by quartile: 1.00; 1.46 (0.65–3.31); 1.01 (0.46–2.24); and 3.08 (1.39–6.84), P-trend = 0.0090) compared to male cases (P = 0.0081 for difference, highest vs. lowest telomere length quartile) (Table 5).

Discussion

Our findings demonstrate a consistent, positive, and statistically significant association between longer leukocyte telomere length and risk of lung cancer - overall and among both smokers and non-smokers. This paper adds new analyses from two previously published cohorts (ATBC trial and the SWHS (15, 16)) and new data from a third cohort (the PLCO Trial). The novel pooled analyses of the combined data show strong and highly consistent effects across the three prospective cohorts and demonstrate for the first time that risks are particularly high for individuals who have extremely long telomere length. Further, we make the novel observation that the effect of long telomere length occurs well in advance (i.e., more than six years) of diagnosis so that it is unlikely that disease bias could influence the association. Finally, our findings demonstrate that the effect of long telomere length and lung cancer is particularly evident for adenocarcinoma, and especially among females.

Several small case-control studies have reported shorter telomeres to be associated with lung cancer (2, 22, 23), whereas results from our three prospective cohort studies have found the opposite effect (15, 16). At the same time, our findings contrast with a Danish prospective study that found a null association with lung cancer (14). It is possible that findings from case-control studies were driven by reverse causation bias (24). In our study, we observed a similar association among cases diagnosed more than six years after blood draw and it is highly unlikely that telomere length in these subjects was altered by disease.

Our studies are ethnically diverse, including both Caucasians of European descent and Asians, and the positive association between longer telomere length and lung cancer was consistent in all three cohorts. Telomere length in the three studies was measured in the same laboratory, which developed the original assay and has extensive experience conducting analyses for epidemiologic studies. Assays were done in triplicate, cases were plated next to their matched control, and the measurements were precise with high ICC values and low CVs determined through the use of blinded quality control samples.

Both shorter and longer telomere lengths are biologically plausible in driving carcinogenesis (25). Longer telomere length may increase cancer risk by promoting immortality of the cells, leading to aberrant cell proliferation and tumor formation (26, 27). Consistent with recently published mechanistic evidence showing that longer telomeres may be associated with genomic instability (28), we found very long telomere length measured in peripheral white blood cell DNA to be associated with increased risk of lung cancer. Telomere length in blood leukocytes is a relatively good surrogate for telomere length in tissues (29) and may be used as a biomarker for lung cancer risk and potentially a screening tool for early cancer detection (30). Telomere shortening signifies replicative senescence whereas telomere elongation signifies cell immortalization and unlimited cellular proliferation (31).

The effect of telomere length may differ according to cancer types and subtypes (14, 32). In our study, we also found the association between telomere length and lung cancer to vary by lung cancer subtype. Studies have reported differential gene expression, suggesting different tumorigenesis pathways between adenocarcinoma and squamous-cell carcinoma (33, 34). Adenocarcinoma is more commonly found in women than men and is the most common lung cancer subtype among lifelong non-smokers (35, 36). Consistent with this, our findings showed that the association within the adenocarcinoma patients appeared to be stronger among females than males. We also observed women to have longer telomere lengths than men, especially non-smoking women. Moreover, genetic studies have demonstrated relative telomerase activity and polymorphisms of the telomerase reverse transcriptase gene *TERT*, which is instrumental for telomere replication, to be associated with higher risk of adenocarcinoma and longer telomere length, suggesting that adenocarcinoma patients may be affected by telomerase activity and therefore, telomere length (37–39). Limitations of this study include the relatively small sample size in the lung cancer subtype analyses, which necessitates replication of our results by a larger study.

In summary, we found a statistically significant positive association between longer telomere length and lung cancer risk and evidence that this association persisted among cases diagnosed more than six years after blood collection and differs by lung cancer subtype. Telomere length in white blood cell DNA may be an important biomarker of future increased risk of lung cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of lung cancer cases and controls from the PLCO, ATBC and SWHS studies

Characteristics[a]	PLCO (N=806)			ATBC (N=458)			SWHS (N=430)			Pooled (N=1694)		
	Cases	Controls	*P*[b]	Cases	Controls	*P*[b]	Cases	Controls	*P*[b]	Cases	Controls	*P*[b]
N	403	403	-	229	229	-	215	215	-	847	847	-
Sex												
Male	244 (60.55)	244 (60.55)	-	229 (100)	229 (100)	-	0 (0)	0 (0)	-	473 (55.84)	473 (55.84)	-
Female	159 (39.45)	159 (39.45)		0 (0)	0 (0)		215 (100)	215 (100)		374 (44.16)	374 (44.16)	
Age, years												
Mean (SD)	64.07 (4.97)	63.64 (4.74)	0.21	58.69 (5.00)	58.44 (4.79)	0.57	59.18 (8.32)	59.3 (8.34)	0.93	61.37 (6.53)	61.12 (6.35)	0.42
BMI, kg/m^2												
Median (IQR)	26.34 (4.60)	26.68 (5.08)	0.073	25.43 (4.63)	25.82 (5.14)	0.062	24.34 (4.45)	24.9 (4.87)	0.18	25.57 (4.87)	25.90 (5.16)	**0.0037**
Smoking status												
Never	39 (9.68)	180 (44.67)	**< 0.001**	0 (0)	0 (0)	-	199 (92.56)	205 (95.3)	0.31	238 (28.10)	385 (45.45)	**< 0.001**
Ever	364 (90.32)	223 (55.33)		229 (100)	229 (100)		16 (7.44)	10 (4.7)		609 (71.90)	462 (54.55)	
Pack-years												
Median (IQR)	46.00(44.00)	5.50 (34.00)	**< 0.001**	40.00 (21.00)	33.00 (23.00)	**< 0.001**	0 (0)	0 (0)	-	35.00 (51.00)	7.00 (34.00)	**< 0.001**
Adenocarcinoma	161 (39.95)	-	-	34 (14.85)	-	-	93 (43.26)	-	-	288 (34.00)	-	-
Squamous-cell carcinoma	79 (19.60)	-	-	74 (32.31)	-	-	10 (4.65)	-	-	163 (19.24)	-	-
Years to diagnosis												
Mean (SD)	7.41 (2.69)	-	-	5.23 (3.04)	-	-	4.27 (2.10)	-	-	6.02 (2.98)	-	-
Telomere length												
Median (IQR)	1.14 (0.37)	1.13 (0.31)	0.48	1.13 (0.30)	1.08 (0.31)	**0.053**	1.51 (0.38)	1.50 (0.34)	**< 0.001**	1.21 (0.43)	1.18 (0.39)	**0.0012**

Abbreviations: PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial; SWHS, Shanghai Women's Health Study; SD, standard deviation; IQR, Interquartile range; *P*, *P*-value

[a] Data shown are mean ± SD or median (IQR) for continuous variables and n (%) for categorical variables.

[b] *P*-values obtained from t-test for normally distributed continuous variables, Wilcoxon signed-rank test for non-normally distributed continuous variables and Fisher's exact test for categorical variables; *P*-values < 0.05 are in bold.

Table 2

Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for telomere length in circulating white blood cell DNA and lung cancer risk in PLCO, ATBC, SWHS studies and pooled data

Telomere length[a]	PLCO		ATBC		SWHS		Pooled	
	Ca/Co	OR (95% CI)[b]	Ca/Co	OR (95% CI)[b]	Ca/Co	OR (95% CI)[b]	Ca/Co	OR (95% CI)[b]
1st Quartile (shortest)	107/102	1.00	43/58	1.00	43/54	1.00	193/214	1.00
2nd Quartile	92/104	1.11 (0.65–1.92)	48/57	0.99 (0.56–1.74)	61/54	1.68 (0.90–3.14)	201/215	1.24 (0.90– 1.71)
3rd Quartile	91/98	1.20 (0.66–2.15)	72/57	1.64 (0.96–2.79)	36/53	1.05 (0.54–2.03)	199/208	1.27 (0.91– 1.78)
4th Quartile (longest)	113/99	**1.83 (1.05–3.19)**	66/57	1.53 (0.86–2.72)	75/54	**2.34 (1.16–4.74)**	254/210	**1.86 (1.33– 2.62)**
P-trend[c]		**0.011**		**0.034**		**0.0042**		**0.000022**
4th Quartile - lower	38/48	1.21 (0.60–2.44)	20/28	0.94 (0.45–1.97)	26/27	1.65 (0.73–3.74)	84/103	1.22 (0.80–1.86)
4th Quartile - upper	75/51	**2.41 (1.28–4.52)**	46/29	**2.16 (1.11–4.23)**	49/27	**3.02 (1.39–6.58)**	170/107	**2.51 (1.70–3.69)**

Abbreviations: PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial; SWHS, Shanghai Women's Health Study; Ca, Cases; Co, Controls

[a] Telomere length was categorized into four categories, in increasing order, based on quartiles among controls in each study. Range of telomere length for each quartile by study is PLCO (1st: < 0.99, 2nd: 0.99–1.13, 3rd: 1.13–1.30, 4th: >1.30, 4th lower: 1.30–1.42, 4th upper: >1.42); ATBC (1st: < 0.94, 2nd: 0.94–1.08, 3rd: 1.08–1.25, 4th: > 1.25, 4th lower: 1.25–1.35, 4th upper: >1.35); SWHS (1st: < 1.30, 2nd: 1.30–1.50, 3rd: 1.50–1.64, 4th: >1.64, 4th lower: 1.64–1.80, 4th upper: >1.80).

[b] Models were adjusted for continuous age and pack-years.

[c] P-values for trend were calculated by using telomere length as a continuous variable and adjusted for continuous age and pack-years; *P*-values < 0.05 are in bold.

Table 3

Adjusted odds ratios (OR) and 95% confidence intervals (CI) for telomere length in circulating white blood cell DNA and lung cancer risk in pooled data, stratified by smoking status (smokers, non-smokers), sex (male, female), and sex stratified by smoking status (smoking male, smoking female, non-smoking female)

Telomere length[a]	Pooled		
	Ca/Co	OR (95% CI)[b]	P-trend[c]
By smoking status:			
Smokers			
1st Quartile (shortest)	147/118	1.00	
2nd Quartile	138/120	1.09 (0.71–1.68)	
3rd Quartile	161/111	**1.60 (1.04–2.45)**	
4th Quartile (longest)	163/113	1.49 (0.96–2.32)	**0.0092**
Non-smokers			
1st Quartile (shortest)	46/96	1.00[d]	
2nd Quartile	63/95	1.60 (0.86–2.98)	
3rd Quartile	38/97	0.87 (0.46–1.67)	
4th Quartile (longest)	91/97	**2.14 (1.09–4.19)**	**0.011**
By sex:			
Males			
1st Quartile (shortest)	117/127	1.00	
2nd Quartile	109/121	1.14 (0.74–1.76)	
3rd Quartile	129/114	**1.57 (1.01–2.43)**	
4th Quartile (longest)	118/111	1.50 (0.96–2.35)	**0.012**
Females			
1st Quartile (shortest)	76/87	1.00	
2nd Quartile	92/94	1.38 (0.84–2.28)	
3rd Quartile	70/94	1.00 (0.59–1.71)	
4th Quartile (longest)	136/99	**2.33 (1.37–3.98)**	**0.00044**
Smoking males			
1st Quartile (shortest)	112/98	1.00	
2nd Quartile	106/99	1.10 (0.68–1.76)	
3rd Quartile	126/89	1.66 (1.05–2.62)	
4th Quartile (longest)	114/92	1.41 (0.87–2.29)	**0.026**
Smoking females			
1st Quartile (shortest)	35/20	1.00	
2nd Quartile	32/21	1.30 (0.41–4.18)	
3rd Quartile	35/22	1.42 (0.41–4.91)	
4th Quartile (longest)	49/21	1.82 (0.59–5.59)	0.19
Non-smoking females			

Telomere length[a]	Pooled		
	Ca/Co	OR (95% CI)[b]	P-trend[c]
1st Quartile (shortest)	41/67	1.00[d]	
2nd Quartile	60/73	1.81 (0.95–3.46)	
3rd Quartile	35/72	1.00 (0.51–1.96)	
4th Quartile (longest)	87/78	**2.41 (1.19–4.88)**	**0.0065**

Abbreviations: PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention; SWHS, Shanghai Women's Health Study; AC, adenocarcinoma; SCC, squamous cell carcinoma; Ca, Cases; Co, Controls

[a] Telomere length was categorized into four categories, in increasing order, based on quartiles among controls in each study. Range of telomere length for each quartile by study is PLCO (1st: < 0.99, 2nd: 0.99–1.13, 3rd: 1.13–1.30, 4th: >1.30); ATBC (1st: < 0.94, 2nd: 0.94–1.08, 3rd: 1.08–1.25, 4th: > 1.25); SWHS (1st: < 1.30, 2nd: 1.30–1.50, 3rd: 1.50–1.64, 4th: >1.64).

[b] Models were adjusted for continuous age and pack-years.

[c] P-values for trend were calculated by using telomere length as a continuous variable and adjusted for continuous age and pack-years, P-values < 0.05 are in bold.

[d] Models were adjusted for continuous age.

Table 4

Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for telomere length in circulating white blood cell DNA and lung cancer risk in PLCO, ATBC, SWHS studies and pooled data, stratified by years to diagnosis and lung cancer histology

Telomere length[a]	PLCO		ATBC		SWHS		Pooled[b]	
	Ca/Co	OR (95% CI)[c]	Ca/Co	OR (95% CI)[c]	Ca/Co	OR (95% CI)[c]	Ca/Co	OR (95% CI)[c]
By years to diagnosis:								
≤ 6 years								
1st Quartile (shortest)	41/35	1.00	28/27	1.00	34/41	1.00	103/103	1.00
2nd Quartile	22/31	0.49 (0.13–1.85)	24/34	0.58 (0.27–1.27)	47/43	1.58 (0.79–3.18)	93/108	0.91 (0.57–1.44)
3rd Quartile	23/21	1.29 (0.35–4.79)	39/34	1.06 (0.53–2.10)	27/40	1.00 (0.48–2.07)	89/95	1.02 (0.64–1.61)
4th Quartile (longest)	34/33	1.44 (0.47–4.44)	39/35	1.08 (0.52–2.27)	60/44	**2.23 (1.01–4.94)**	133/112	1.45 (0.90–2.32)
P-trend[d]		0.19		0.37		**0.045**		**0.023**
> 6 years								
1st Quartile (shortest)	66/67	1.00	15/31	1.00	9/13	1.00	90/111	1.00
2nd Quartile	70/73	1.36 (0.74–2.50)	24/23	1.79 (0.74–4.34)	14/11	2.25 (0.49–10.3)	108/107	**1.67 (1.06–2.65)**
3rd Quartile	68/77	1.17 (0.60–2.30)	33/23	**2.98 (1.22–7.29)**	9/13	1.51 (0.29–7.70)	110/113	1.62 (0.99–2.65)
4th Quartile (longest)	79/66	**2.06 (1.06–4.00)**	27/22	2.23 (0.86–5.80)	15/10	2.98 (0.62–14.4)	121/98	**2.41 (1.47–3.97)**
P-trend[d]		**0.028**		**0.047**		**0.046**		**0.00032**
By histology:								
Adenocarcinoma								
1st Quartile (shortest)	35/40	1.00	6/9	1.00	17/22	1.00[e]	58/71	1.00
2nd Quartile	33/37	1.24 (0.50–3.04)	7/4	4.15 (0.43–40.0)	22/21	1.70 (0.61–4.73)	62/62	1.54 (0.84–2.83)
3rd Quartile	41/46	1.19 (0.48–2.99)	9/11	1.25 (0.33–4.68)	13/21	1.07 (0.38–3.00)	63/78	1.16 (0.64–2.09)
4th Quartile (longest)	52/38	**2.82 (1.16–6.85)**	12/10	1.36 (0.23–7.99)	41/29	2.65 (0.92–7.60)	105/77	**2.52 (1.38–4.60)**
P-trend[d]		**0.011**		0.77		0.096		**0.0029**
Squamous-cell carcinoma								
1st Quartile (shortest)	29/21	1.00	15/10	1.00	0/3	1.00[e]	44/34	1.00
2nd Quartile	16/20	1.65 (0.41–6.73)	10/25	**0.25 (0.067–0.96)**	4/3	-	30/48	0.72 (0.32–1.61)

Telomere length[a]	PLCO		ATBC		SWHS		Pooled[b]	
	Ca/Co	OR (95% CI)[c]	Ca/Co	OR (95% CI)[c]	Ca/Co	OR (95% CI)[c]	Ca/Co	OR (95% CI)[c]
3rd Quartile	16/16	1.61 (0.43–6.03)	27/14	1.31 (0.37–4.66)	2/0	-	45/30	1.89 (0.81–4.42)
4th Quartile (longest)	18/22	1.57 (0.44–5.56)	22/25	0.66 (0.21–2.07)	4/4	-	44/51	1.14 (0.53–2.45)
P-trend[d]		0.27		0.96		0.15		0.28

Abbreviations: PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial; SWHS, Shanghai Women's Health Study; Ca, Cases; Co, Controls

[a] Telomere length was categorized into four categories, in increasing order, based on quartiles among controls in each study. Range of telomere length for each quartile by study is PLCO (1st: < 0.99, 2nd: 0.99–1.13, 3rd: 1.13–1.30, 4th: >1.30); ATBC (1st: < 0.94, 2nd: 0.94–1.08, 3rd: 1.08–1.25, 4th: > 1.25); SWHS (1st: < 1.30, 2nd: 1.30–1.50, 3rd: 1.50–1.64, 4th: >1.64).

[b] *P*-values from the chi-square test comparing the distribution of cases in the top to the bottom telomere length quartile are as follows: ≤ 6 vs > 6 years prior to diagnosis, $P = 0.83$; adenocarcinoma vs squamous cell carcinoma, $P = 0.026$.

[c] Models were adjusted for continuous age and pack-years.

[d] *P*-values for trend were calculated by using telomere length as a continuous variable and adjusted for continuous age and pack-years; *P*-values < 0.05 are in bold.

[e] Models were adjusted for continuous age.

Table 5

Adjusted odds ratios (OR) and 95% confidence intervals (CI) for telomere length in circulating white blood cell DNA and lung cancer risk in pooled data, stratified by years to diagnosis, smoking status and sex.

Telomere length[a]	AC[b]			SCC		
	Ca/Co	OR (95% CI)[c]	P-trend[d]	Ca/Co	OR (95% CI)[c]	P-trend[d]
By years to diagnosis:						
≤ 6 years						
1st Quartile (shortest)	37/37	1.00		24/12	1.00	
2nd Quartile	31/33	0.89 (0.39–2.02)		10/27	**0.17 (0.043–0.70)**	
3rd Quartile	25/35	0.72 (0.31–1.67)		27/12	1.16 (0.32–4.18)	
4th Quartile (longest)	57/45	1.58 (0.67–3.70)	0.41	20/30	0.32 (0.097–1.07)	0.58
> 6 years						
1st Quartile (shortest)	21/34	1.00		20/22	1.00	
2nd Quartile	31/29	**3.12 (1.17–8.30)**		20/21	2.38 (0.74–7.70)	
3rd Quartile	38/43	2.08 (0.84–5.12)		18/18	2.25 (0.63–8.04)	
4th Quartile (longest)	48/32	**4.61 (1.81–11.8)**	**0.0019**	24/21	3.22 (0.92–11.2)	0.097
By smoking status:						
Non-smokers						
1st Quartile (shortest)	21/36	1.00[e]		0/11	1.00[e]	
2nd Quartile	28/34	1.84 (0.69–4.95)		4/12	-	
3rd Quartile	16/43	0.95 (0.36–2.51)		3/6	-	
4th Quartile (longest)	55/49	2.40 (0.90–6.41)	0.15	6/14	-	0.15
Smokers						
1st Quartile (shortest)	37/35	1.00		44/23	1.00	
2nd Quartile	34/28	1.48 (0.53–4.09)		26/36	0.42 (0.16–1.10)	
3rd Quartile	47/35	1.60 (0.67–3.80)		42/24	1.37 (0.55–3.44)	
4th Quartile (longest)	50/28	2.02 (0.80–5.11)	0.050	38/37	0.75 (0.32–1.76)	0.75
By sex:						
Males						
1st Quartile (shortest)	28/37	1.00		38/25	1.00	
2nd Quartile	30/25	1.67 (0.64–4.33)		22/41	0.54 (0.20–1.43)	
3rd Quartile	35/33	1.39 (0.56–3.45)		40/29	1.48 (0.58–3.79)	
4th Quartile (longest)	29/27	1.40 (0.53–3.74)	0.23	34/39	0.98 (0.41–2.38)	0.62
Females						
1st Quartile (shortest)	30/34	1.00		6/9	1.00	
2nd Quartile	32/37	1.46 (0.65–3.31)		8/7	1.91 (0.39–9.33)	
3rd Quartile	28/45	1.01 (0.46–2.24)		5/1	9.92 (0.55–180.5)	
4th Quartile (longest)	76/50	**3.08 (1.39–6.84)**	**0.0090**	10/12	2.05 (0.41–10.4)	0.19

Abbreviations: PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention; SWHS, Shanghai Women's Health Study; AC, adenocarcinoma; SCC, squamous cell carcinoma; Ca, Cases; Co, Controls

[a] Telomere length was categorized into four categories, in increasing order, based on quartiles among controls in each study. Range of telomere length for each quartile by study is PLCO (1^{st}: < 0.99, 2^{nd}: 0.99–1.13, 3^{rd}: 1.13–1.30, 4^{th}: >1.30); ATBC (1^{st}: < 0.94, 2^{nd}: 0.94–1.08, 3^{rd}: 1.08–1.25, 4^{th}: > 1.25); SWHS (1^{st}: < 1.30, 2^{nd}: 1.30–1.50, 3^{rd}: 1.50–1.64, 4^{th}: >1.64).

[b] P-values from the chi-square test comparing the distribution of cases in the top to the bottom telomere length quartile for stratified analyses that showed significant association in at least one strata are as follows: ≤ 6 vs > 6 years prior to diagnosis for AC cases, P = 0.24; males vs. females for AC cases, P = 0.0081.

[c] Models were adjusted for continuous age and pack-years.

[d] P-values for trend were calculated by using telomere length as a continuous variable and adjusted for continuous age and pack-years, P-values < 0.05 are in bold.

[e] Models were adjusted for continuous age.