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Telomere length variation: A potential new telomere biomarker for lung cancer risk

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Abstract

Objectives—In this report the associations between telomere length variation (TLV), mean telomere length in blood lymphocytes and lung cancer risk were examined.

Materials and Methods—The study design is case-control. Cases (N = 191) were patients newly diagnosed with histologically confirmed non-small cell lung cancer. Controls (N = 207) were healthy individuals recruited from the same counties as cases and matched to cases on age and gender. Telomere fluorescent in situ hybridization was used to measure telomere features using short-term cultured blood lymphocytes. Logistic regression was used to estimate the strength of association between telomere features and lung cancer risk.

Results—Telomere length variation across all chromosomal ends was significantly associated with lung cancer risk; adjusted odds ratios 4.67 [95% confidence interval (CI): 1.46 – 14.9] and 0.46 (95% CI: 0.25 – 0.84) for younger (age ≤ 60) and older (age > 60) individuals, respectively. TLV and mean telomere length jointly affected lung cancer risk: when comparing individuals with

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short telomere length and high TLV to those with long telomere length and low TLV, adjusted odd ratios were 8.21 (95% CI: 1.71 – 39.5) and 0.33 (95% CI: 0.15 – 0.72) for younger and older individuals, respectively.

Conclusions—TLV in blood lymphocytes is significantly associated with lung cancer risk and the associations were modulated by age. TLV in combination with mean telomere length might be useful in identifying high risk population for lung cancer computerized tomography screening.

Keywords

lung cancer; telomere length variation; telomere length; blood-based biomarker; risk prediction

INTRODUCTION

Telomeres, the nucleoprotein complexes at the end of eukaryotic chromosomes, are specialized structures that protect chromosome ends (1). Telomeres are composed of TTAGGG repeats and a specific associated protein complex termed shelterin (2), which regulates telomere protection and length. Telomerase is the key telomere maintenance enzyme. Most adult human cells have limited amount of telomerase so that telomere loss still occurs (3) and successive cell divisions lead to progressive telomere attrition due to the end-replication problems (4–6). Continued proliferation of cells with very short telomeres results in loss of telomere protection that ultimately leads to chromosomal instability (7–9). Since telomere shortening limits the lifespan of cells and prevents the onset of immortality, this mechanism has long been regarded as an important tumor-suppressive pathway (10). However, almost all cancer cells have found ways to escape from the normal replicative limitation through maintaining their telomeres, either by up-regulation of telomerase (11) or by an alternative lengthening of telomeres (ALT) mechanism (12, 13).

Although it is widely recognized that telomere dysfunction plays an important role in human carcinogenesis, the relationship between telomere function in somatic cells and the risk of developing lung cancer is not well defined. Two retrospective case-control studies (14, 15) reported that short average telomere length (TL) in blood leucocytes was significantly associated with a 2- to 3-fold increase of lung cancer risk; conversely, three prospective studies found that long average TL in blood leucocytes was significantly associated with an increased lung cancer risk (16–18). None of these previous studies evaluated other telomere features, i.e., telomere length variation, in relation to lung cancer risk.

Average telomere length was commonly used to assess the telomere function and cancer risk. However, average telomere length only represents an estimate of the abundance of telomere sequences in a given cell and does not provide any information on how the telomere sequences are distributed across all the chromosomal ends. Studies of human cancer cell lines and knockout mouse strains have shown that the shortest telomeres, not average telomere length, drove chromosome instability in cancer cells, and chromosomal arms possessing the shortest telomeres were more often found in telomere fusions, leading to chromosomal abnormality (19–21). Our recent data showed that telomerase, the key telomere maintenance enzyme, not only elongates short telomeres, but also shortens excessively long telomeres in normal and cancerous human cells (22). These dual functions

of telomerase indicate that keeping an optimal telomere length for each chromosomal end is a critical aspect of telomere maintenance and may be important for the protection of the genome. This notion is further supported by results from studying telomerase negative and ALT positive cancers. Telomerase negative and ALT positive cancer cells, i.e. osteosarcomas, are characterized by long average telomere length and striking telomere length heterogeneity (23, 24). Despite having long average telomere length, these types of cancers often exhibit increased chromosome instability and poor clinical outcome (24–26). Together, these previous data lead us to hypothesize that the variation in telomere lengths across all the chromosomal ends represents an important measure of telomere function and maybe a useful cancer risk biomarker. In the present study, in addition to average telomere length, we examined relationship between telomere length variation in blood lymphocytes and lung cancer risk.

MATERIALS AND METHODS

Study Population

The study population accrual and eligibility criteria were described previously (27, 28). This analysis focuses on a subset of subjects to whom the chromosome preparations from blood lymphocyte were available. Lung cancer patients were recruited from seven hospitals in the Metropolitan Baltimore area between 1998 and 2004. All cases ($n = 191$) had histologically confirmed non-small cell primary lung cancer. Population controls ($n = 168$) were recruited from the same Maryland counties as the lung cancer cases by screening information obtained from the Motor Vehicle Administration (MVA), which allowed us to obtain a random sample of controls frequency-matched to the cases by gender, race, and age. Hospital controls ($n = 39$) were cancer-free patients recruited from the same hospital as cases, and were frequency-matched to the cases by gender, race, age, and smoking status.

Eligibility criteria for case and control selection: Eligible subjects had to be either Caucasian or African-American, free of known diagnosis of HIV, HCV and HBV; born in the United States; a resident of Baltimore City and adjacent counties of Maryland or the Maryland Eastern Shore; able to speak English well enough to be interviewed; non-institutionalized; currently not taking antibiotics or steroid medications; never being interviewed as a control for the study (for cases only). Subjects who had undergone any systemic treatment, i.e., chemotherapy or radiation therapy, were excluded from the study, and those who had undergone surgery provided a blood sample either before the surgery or three months after the surgery. Chemotherapy and radiation therapy are known to affect the telomere length in blood cells, and so we excluded such subjects to maximize the validity of the results.

The study was approved by the Institutional Review Boards of Georgetown University-Medstar Oncology, the National Cancer Institute, University of Maryland, the Johns Hopkins University School of Medicine, Sinai Hospital, MedStar Research Institute, and the Research Ethics Committee of Bon Secours Baltimore Health System. All participants signed an informed consent. Socioeconomic characteristics and epidemiological were collected through a structured, in-person interview.

Chromosome preparation from short-term culture of blood lymphocyte

Blood was obtained by trained phlebotomists in heparinized tubes and blood cultures were set up within 48 hours after the blood draw, following a standard cytogenetic protocol as previously described (27). Briefly, one ml of fresh whole blood was added to 9 ml of blood culture medium supplemented 1.5% of phytohemagglutinin. The blood lymphocytes were cultured at 37°C for 4 days and harvested using standard cytogenetic protocol. The chromosome preparation were kept at -20°C for future assays.

Measurement of telomere features

Telomere length at each of the chromosomal ends were measured by telomere quantitative fluorescent *in situ* hybridization (TQ-FISH) as previously described with modification (29). Chromosome preparations were dropped onto clean microscopic slides and hybridized with 15 µl of hybridization mixture consisting of 0.3 µg/ml Cy3-labeled telomere-specific peptide nucleic acid (PNA) probe, 1 µl of cocktails of FITC-labeled centromeric PNA probes specific for chromosomes 2, 4, 8, 9, 13, 15, 18, 20 and 21, 20 µg/ml of Cy3-labeled centromeric PNA probes specific for chromosome X, 50% formamide, 10 mM Tris-HCl, pH 7.5, and 5% blocking reagent. Slides were then placed in a Hybex microarray hybridization oven where the DNA was denatured by incubating at 75°C for 5 mins, followed by hybridizing at 30°C for 3 hours. After hybridization, the slides were sequentially washed 10 min each at 42°C: once in 1 × SSC, once in 0.5 × SSC, and once in 0.1 × SSC. The slides were then mounted in anti-fade mounting medium containing 300 ng/ml 4'-6-diamidino-2-phenylindole (DAPI).

After TQ-FISH, cells were analyzed using an epifluorescence microscope equipped with a charge-coupled device camera. Metaphase cells were captured with exposure times of 0.15, 0.25 and 0.05 second for Cy3, FITC and DAPI signals, respectively. Digitized metaphase images were analyzed using the Isis software (MetaSystems Inc. Boston, MA), which permits simultaneous measurement of telomere signals of 92 chromosomal ends after karyotyping. Telomere fluorescent intensity units (FIU) were recorded as an indirect measurement of telomere length. For each study subject, 30 metaphase cells were randomly selected from one or two slides and analyzed.

Several quality control steps were implemented in telomere measurement. Laboratory personnel who were responsible for the blood culture and telomere assay were blinded to the case-control status of the subjects. All new lots of reagents were tested to ensure optimal hybridization. A control slide containing cells with known telomere length was included in each batch of TQ-FISH to monitor the quality of the hybridization efficiency. Case and control samples were analyzed together in each batch and a total of 15 batches were run for the whole case-control set. Analysis of control slides from 15 batches showed that the CV of mean TL, TLV were 10.98% and 12.79%, respectively.

Definitions of telomere features are as follows: (1) mean telomere length (mean_TL) was determined by averaging the FIU of 2760 telomeres from 30 metaphase cells; (2) telomere length variation (TLV), defined as the coefficient of variation (CV) of all measured telomere lengths.

Statistical Analyses

The Chi-square test was used to examine the relationships between categorical variables and cases-control status. Student t-test was used to examine mean differences of numerical variables between cases and controls. Multivariable logistic regression was used to assess the relationships between lung cancer risk and telomere features, controlling for age, gender, race, smoking status (never, former, current), and pack-years of smoking. Interaction terms were included in the model if their significance level was at least 0.10. Age was dichotomized as ≤ 60 years of age and > 60 years of age. We initially stratified subjects into three age groups based on the tertiles of age distribution in the study population (≤ 60 , 61 – 74 and ≥ 75 years of age) and found that the direction and strength of association between telomere features and lung cancer risk in age groups of 61 – 74 and ≥ 75 years of age were identical. Therefore, these two age groups were combined for better statistical power. Smoking status was categorized into three groups: never smokers - individuals who had never smoked more than 100 cigarettes in their life; former smokers - individuals who had smoked more than 100 cigarettes in their life and had quit more than 12 months prior to their interview; and current smokers - individuals who had smoked more than 100 cigarettes in their life, were active smokers at the time of interview or had quit less than 12 months prior to their interview. Family history of cancer was defined as any cancer occurred among the first and second degree relatives. No significant differences were found when the means of telomere features were compared between population controls (N=168) and hospital controls (N=39); thus these two control groups were combined in the case-control analysis. All P values were two-sided. All analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC).

RESULTS

Characteristics of study population

Table 1 summarizes selected demographic characteristics of the case and control subjects. Lung cancer patients and controls were well matched on age, race, and gender. The lung cancer cases were significantly more likely than the controls to be smokers ($p < 0.001$), heavier smokers ($p < 0.001$) and had lower household income ($p < 0.001$). There were no statistically significant case-control differences in family history of cancer, marital status, education levels, and mean body mass index.

Telomere features and host factors

This part of analysis was restricted to control subjects only. Spearman correlation analysis revealed that average telomere length was moderately and inversely correlated with TLV [spearman correlation coefficient (r) = -0.54 , $p < 0.001$]. We found mean telomere length in blood lymphocytes was inversely correlated with age ($r = -0.47$, $p < 0.001$) and TLV in blood lymphocytes was also moderately correlated with age ($r = 0.35$, $p < 0.001$). The mean telomere length in African Americans was significantly longer than in whites ($p = 0.016$, Suppl table S1). There were no significant correlations between telomere features and gender, smoking status, pack-years, years since quitting smoking, family history of cancer, and body mass index (Suppl table S1).

Association of telomere length variation in blood lymphocytes and lung cancer risk

Overall, there was no significant difference in average TLV between cases and controls (Table 2). When stratified by age, average TLV was significantly higher in cases than in controls (mean $\pm$ SD = 61.5 ± 6.9 vs 58.7 ± 4.4 , $p = 0.010$) among the younger age group (≤ 60 years of age), while average TLV was lower in cases than in controls (63.7 ± 5.4 vs 65.1 ± 5.4 , $p = 0.035$) among the older age group (> 60 years of age, Table 2). No gender- or race-specific effects were observed in this stratified analysis; except the average TLV was significantly higher in cases than in controls among the current smokers.

Multivariate logistic regression analysis revealed that high TLV in blood lymphocytes was significantly associated with an elevated lung cancer risk in the younger age group, with an adjusted odds ratio (OR) of 4.67 (95% CI: 1.46–14.9, Table 3), after adjustment for age, race, gender, smoking status and pack-years. When the subjects were categorized into tertiles of TLV based on the control population, a significant trend of association between TLV and lung cancer risk was present ($P_{\text{trend}} = 0.001$, Table 3) in the younger age group. In contrast, high TLV in blood lymphocytes was significantly associated with a decreased lung cancer risk in the older age group (age > 60 years), with an adjusted OR of 0.46 (95% CI: 0.25 – 0.84, Table 3). When the subjects were categorized into TLV tertiles, a significant inverse trend of association between TLV and lung cancer risk was also present ($P_{\text{trend}} = 0.026$) in the older age group (Table 3).

Mean telomere length in blood lymphocytes and lung cancer risk

Overall, there was no significant difference in mean telomere length per telomere between cases and controls (Table 2). When stratified by age, mean telomere length was borderline significantly shorter in cases than in controls (mean $\pm$ SD = 2205 ± 490 vs 2409 ± 562 , $p = 0.04$) among the younger age group. In the older age group, no significant case-control difference in mean telomere length was seen ($p = 0.10$, Table 2).

Multivariate logistic regression analysis also revealed that short telomere length was associated with an elevated but not statistically significant lung cancer risk in the younger age group, with an adjusted OR of 2.33 (95% CI: 0.86 – 6.30, Table 3). In the older age group, short telomere length in blood lymphocytes was associated with a decreased lung cancer risk (OR = 0.52, 95% CI: 0.29 – 0.94), but the trend of association was not significant ($P_{\text{trend}} = 0.09$, Table 3).

Joint effects of telomere length and telomere length variation on lung cancer risk

Table 4 shows the joint effects of telomere length and TLV on lung cancer risk. In the younger age group, short telomere length and high TLV in blood lymphocytes jointly increased the risk of lung cancer by 8-fold compared with individuals who had long telomere length and low TLV; in contrast, short telomere length and high TLV jointly decreased risk of lung cancer by 67% compared with individuals who had long telomere length and low TLV among older subjects. There was no significant interaction between telomere length and TLV, and between telomere features and age.

DISCUSSION

To the best of our knowledge, this is the first study that evaluated the association between telomere length variation across all chromosomal ends in blood lymphocytes and lung cancer risk. Our results suggested that high TLV in blood lymphocytes were significantly associated with an increased risk of early onset lung cancer (defined as ≤ 60 years of age). In contrast, high TLV in blood lymphocytes was associated with a decreased risk of lung cancer among individuals older than 60 years of age. Interestingly, we found that the combination of TLV and mean telomere length in blood lymphocytes improved the risk stratification for lung cancer than mean telomere length or TLV alone.

TLV measures the overall variability of telomeric DNA distribution across all chromosome ends. Its value is driven by extreme values, such as very short or extremely long telomeres. We found that TLV is highly correlated with the frequency of very short telomeres (Spearman corr $r = 0.90$, data not shown) and individuals who had high TLV also had high numbers of chromosomal ends possessing very short, probably dysfunctional telomeres, even when the average telomere length seemed reasonable (within normal range).

We also found that TLV is highly correlated with frequency of excessively long telomeres ($r = 0.84$, data not shown). While the detrimental effects of short telomeres on human health have been intensively studied, the health consequences of excessively long telomeres remain to be illustrated. Excessively long telomeres in ALT cells suffer a decreased the saturation of shelterin proteins, leading to reduced compaction of telomeric chromatin and increased telomere fragility as suggested by previous studies (30, 31). This shelterin protein unsaturation or “intermediate status” of telomeres resulted in impaired chromosome end protections that are susceptible to DNA damage response, leading to genomic instability (32). Recent data by our group demonstrated that telomerase, a key telomere maintenance enzyme, not only elongates short telomeres, but also shortens excessively long telomeres in human cells, indicating maintaining telomere homeostasis is critical for a normal cellular function (22). TLV, as defined in the present study, measures the combined effects of very short and excessively long telomeres and represents a novel aspect of telomere function and might be a useful biomarker for cancer risk.

We also observed that mean telomere length in blood lymphocytes was moderately associated with lung cancer risk, and the direction of the association was modulated by age. Previous lung cancer studies reported the association of an increased lung cancer risk with both short (14, 15) and long telomere length (16–18) or no significant association (33). The reported opposite direction of the associations has puzzled the field of telomere research and generated skepticism regarding the usefulness of telomere length as cancer risk assessment tools. Three of these four early reports that focused on lung cancer did not take age into account as a modulator for the association. It is interest to note that, in the report by Shen *et al* (17), the analysis was stratified by duration of cigarette smoking, and longer telomere length was associated with an elevated lung cancer risk only among males who smoked > 38 years, but not in males who smoked ≤ 38 years. Longer smoking duration maybe a surrogate for older age. However, in a report by Jang *et al* (15), short telomere length was associated with an increased lung cancer risk in both younger (< 61 years of age) and older age groups

(< 61 years of age), although the association was stronger in the younger age group. Our results suggest that short mean telomere length is associated with an increased risk for early onset lung cancer, but is associated with a decreased lung cancer risk among older individuals.

One striking observation in the present study is that age was a significant modulator of the associations between telomere features and lung cancer risk. Sub-optimal telomere function, characterized by high TLV and relative short mean telomere length in blood lymphocytes, was strongly associated with early onset lung cancer risk. In contrast, Sub-optimal telomere function is inversely associated with lung cancer risk among older individuals (> 60 years of age). The underlying mechanism of the observed age differences in this association is currently unknown. One speculative explanation could be the role of telomerase and telomere length in aging. Telomere length and telomere length variation represent complex phenotypes that likely has both genetic and environmental components. It has been shown that genetic factors, i.e. genetic mutations in telomere maintenance gene, and environmental exposures, i.e. oxidative stress, affect telomere length (34–38). Short telomere length has been shown to be associated with older age in healthy individuals (39, 40).

Telomerase-deficiency could lead to sub-optimal telomere function, i.e., short telomere length and high telomere length variation, and accelerated biological aging, which in turn could increase cancer risk among young individuals. Strong evidence indicated that telomerase-deficient human diseases due to mutations in telomerase component genes typically result in accelerated-aging phenotypes and high incidence of early onset malignant tumors (41–43). In contrast, old individuals who have high level of telomerase activity in their cells may face some unwanted consequences. In addition to telomere maintenance, telomerase has other extra-telomeric functions, such as pro-cell proliferation activities (44–46). High cellular turnover/proliferation may drive genomic instability in old individuals whose capacity of DNA repair or maintaining genomic integrity have suffered aging-related decline (47–49), and thus increases risk of developing cancer. This notion is supported by a study of genetically engineered mice that demonstrated that high expression of mouse TERT gene resulted in high telomerase activity and promoted cancer development in old mice (50). Short telomere length, when coupled with low telomerase activity, may play a protective role of cancer development among old individuals by limiting cell proliferation. However, direct evidence in human studies to support this notion is currently lacking. We recommend cautious in interpreting our observed associations in older age groups, given the moderate sample size and potential misclassification due to assay errors. Clearly, future larger studies are needed to delineate the true underlying mechanism of the age differences in the associations between telomere function and lung cancer risk.

In summary, the present study demonstrated that telomere length variation across all chromosomal ends in blood lymphocytes was significantly associated with lung cancer risk and this association was modulated by age. It is also worth noting that combination of TLV and mean telomere length improves risk stratification for lung cancer. In the younger age group, individuals who had short telomere length and high TLV in blood lymphocytes had 8-fold increased risk of lung cancer than those who have long telomere length and low TLV.

If confirmed by future studies, TLV and mean telomere length in blood lymphocytes may be incorporated into a panel of biomarkers for lung cancer risk assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Telomere length variation (TLV): a potential new telomere biomarker for cancer risk
- High TLV is significantly associated with increased risk of early onset lung cancer
- High TLV is associated with a decreased risk of lung cancer in older individuals
- TLV in combination with average TL improves the risk stratification for lung cancer

Table 1

Demographic characteristics of study subjects

	Cases N=191	Control N=207	p
	67.0	66.3	
Age, mean (SD)	(12.2)	(12.1)	0.52
Age distribution, N (%)			
40–50	21 (11.1)	22 (11.2)	
51–60	32 (16.9)	38 (18.5)	
61–70	51 (27.0)	58 (28.2)	
71–80	63 (33.3)	65 (31.6)	
81	21 (11.6)	22 (10.7)	0.99
Gender, N (%)			
Female	100 (52.4)	111 (53.6)	
Male	91 (47.6)	96 (46.4)	0.80
Race, N (%)			
African American	68 (35.6)	79 (38.2)	
Caucasian American	123 (64.4)	128 (61.8)	0.60
Smoking Status, N (%)			
Never	11 (5.8)	79 (38.2)	
Former	76 (39.8)	95 (45.9)	
Current	104 (54.5)	33 (16.0)	< 0.001
Pack-years among smokers, N (%)			
20	37 (20.7)	61 (48.0)	
21–40	60 (33.5)	34 (26.8)	
41–60	48 (26.8)	22 (17.3)	
>60	34 (19.0)	10 (7.9)	< 0.001
Education, N (%)			
High school or less	162 (89.0)	145 (86.8)	
College or higher	20 (11.0)	22 (13.2)	0.53
Income, N (%)			
Less than 10K	26 (15.9)	8 (5.1)	
10K–30K	62 (37.8)	48 (30.4)	
30K–60K	43 (26.2)	40 (25.3)	
Greater than 60K	33 (20.1)	62 (39.2)	< 0.001
Marital Status, N (%)			
Single, never married	11 (5.8)	13 (6.3)	
Married or has a partner	108 (56.5)	139 (67.2)	
Divorced, separated, or widowed	72 (37.7)	55 (26.6)	0.06
Family History of Cancer, N (%)			
No	44 (23.0)	47 (22.7)	
Yes	147 (77.0)	160 (77.3)	0.94

	Cases N=191	Control N=207	p
BMI ^a , mean (SD)	26.0 (5.0)	26.6 (5.2)	0.17

^aBody mass index

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Table 2

Case-control comparison of telomere features in blood lymphocytes, stratified by age, gender, race and smoking status

Telomere features	Cases mean (SD)	Controls mean (SD)	p
All subjects, N	191	207	
Mean_TL ^a	2087 (521)	2083 (529)	0.94
TLV ^b	63.1 (5.9)	63.1 (5.9)	0.91
Age ≤ 60, N	55	62	
Mean_TL	2205 (490)	2409 (562)	0.040
TLV	61.5 (6.9)	58.7 (4.4)	0.010
Age > 60, N	136	145	
Mean_TL	2039 (527)	1943 (448)	0.10
TLV	63.7 (5.4)	65.1 (5.4)	0.035
Male, N	91	96	
Mean_TL	1991 (498)	2069 (508)	0.29
TLV	64.2 (5.7)	63.4 (5.7)	0.36
Female, N	100	111	
Mean_TL	2174 (528)	2095 (548)	0.29
TLV	62.0 (5.9)	62.9 (6.1)	0.30
African American, N	68	79	
Mean_TL	2244 (548)	2186 (578)	0.53
TLV	63.0 (5.7)	63.5 (6.1)	0.64
Caucasian, N	123	128	
Mean_TL	2000 (486)	2019 (488)	0.75
TLV	63.1 (6.1)	62.9 (5.7)	0.83
Never smokers, N	11	79	
Mean_TL	2265 (295)	2078 (507)	0.10
TLV	60.3 (7.0)	63.3 (5.7)	0.11
Former smokers, N	76	95	
Mean_TL	2035 (509)	2006 (509)	0.71
TLV	62.9 (5.1)	63.8 (5.9)	0.33
Current smokers, N	104	33	
Mean_TL	2105 (545)	2310 (586)	0.07
TLV	63.4 (6.3)	60.9 (5.7)	0.039

^a mean telomere length,

^b telomere length variation.

Table 3

Risk estimation of association between telomere features in blood lymphocytes and lung cancer risk, for all subjects and stratified by age

Telomere length variation							Average telomere length				
		N					N				
All subject:	Case	Control	OR	(95% CI)	p		Case	Control	OR	(95% CI)	p
by median											
< median	96	103	ref				93	106	0.74	(0.44 – 1.22)	
median	95	104	0.80	(0.49 – 1.29)	0.36		98	101	ref		0.24
by quartile											
Q1	50	49	ref				50	49	0.60	(0.29 – 1.26)	
Q2	46	54	0.99	(0.51 – 1.93)			43	57	0.62	(0.31 – 1.24)	
Q3	51	49	0.81	(0.42 – 1.58)			48	53	0.71	(0.36 – 1.41)	
Q4	44	55	0.77	(0.39 – 1.52)	0.38*		50	49	ref		0.17 ^a
Age <= 60:											
by median											
< median	34	53	ref				21	14	2.33	(0.86 – 6.30)	
median	21	9	4.67	(1.46 – 14.9)	0.009		34	48	ref		0.10
by tertile											
Q1	27	43	ref				13	8	3.27	(0.86 – 12.4)	
Q2	13	16	1.24	(0.44 – 3.50)			15	18	0.99	(0.36 – 2.73)	
Q3	15	3	10.98	(2.02 – 59.8)	0.001*		27	36	ref		0.14 ^a
Age > 60:											
by median											
< median	62	50	ref				72	92	0.52	(0.29 – 0.94)	
median	74	95	0.46	(0.25 – 0.84)	0.012		64	53	ref		0.031
by tertile											
Q1	34	28	ref				51	60	0.52	(0.25 – 1.09)	
Q2	57	47	0.82	(0.38 – 1.78)			46	54	0.63	(0.30 – 1.36)	
Q3	45	70	0.44	(0.21 – 0.96)	0.026*		39	31	ref		0.09 ^a

odd ratios (OR) were adjusted for age, gender, race, smoking status and pack-years;

^a p-for-trend

Table 4

Joint effects of telomere length and telomere length variation on lung cancer risk

		N		OR	(95% CI)	p-for-trend
Mean_TL/TLV	Case	Control				
All subject:						
long/low	63	71	ref			
short/low	33	32	1.01	(0.48 – 2.12)		
long/high	35	30	1.10	(0.54 – 2.24)		
short/high	60	74	0.67	(0.36 – 1.24)		0.22
Age <= 60:						
long/low	26	43	ref			
short/low	8	10	1.22	(0.35 – 4.26)		
long/high	8	5	2.73	(0.60 – 12.43)		
short/high	13	4	8.21	(1.71 – 39.55)		0.007
Age > 60:						
long/low	37	28	ref			
short/low	25	22	0.78	(0.30 – 2.00)		
long/high	27	25	0.66	(0.27 – 1.64)		
short/high	47	70	0.33	(0.15 – 0.72)		0.004

ORs were adjusted for age, gender, race, smoking status and pack-years